DRUGS AND THE PHARMACEUTICAL SCIENCES

DRUGS AND THE PHARMACEUTICAL SCIENCES

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1

Introductory Overview

C. T. RHODES

University of Rhode Island, Kingston, Rhode Island

1. STABILITY IS AN ESSENTIAL QUALITY ATTRIBUTE FOR DRUG PRODUCTS

> "There never was anything by the wit of man so well devized or so sure established which hath not in the continuance of time become corrupted..."
>
> Thomas Cranmer

Everything made by human hands—from the sublime Parthenon to the trivial milkshake—is subject to decay. Pharmaceuticals are no exception to this general statement. If there is any functionally relevant quality attribute of a drug product that changes with time, evaluation of this change falls within the purview of the pharmaceutical scientists and regulators who quantify drug product stability and shelf life.

The rate at which drug products degrade varies dramatically. Some radiopharmaceuticals must be used within a day or so. Other products may, if properly stored and packaged, retain integrity for a decade or more, although in many

jurisdictions the maximum shelf life that a regulatory agency will approve for a drug product is five years. (This restriction is hardly an onerous one, since even for a product with a five-year shelf life it is probable that over 95% of the product will be sold and used within thirty months of manufacture, providing all involved in the distribution process obey the first law of warehousing: FIFO—first in, first out.)

Since the evaluation of the stability of a drug product is highly specialized and esoteric in nature, reliance on the patient's suck-it-and-see organoleptic evaluation is of distinctly limited value. Thus governments in many parts of the world—most importantly in Western Europe, North America, and Japan—have, because of concerns about drug product safety, efficacy, and quality, found it appropriate to require some form of stability testing for drug products. However, it must be recognized that even before governments became active in this area many reputable companies were already giving attention to drug product stability and developing their own in-house approaches. The increasing intervention by regulatory agencies such as the FDA (U.S. Federal Food and Drug Administration) and the HPB (Canadian Health Protection Branch) stimulated standard approaches to stability testing in those parts of the world subject to their control (1). More recently, the process of globalization and harmonization has stimulated the development of world-wide standards. (This topic is further considered in Chapter 18.) It is now well accepted that stability is an essential property of drug products; thus the assignment of a shelf life is a routine regulatory requirement.

2. POTENTIAL ADVERSE EFFECTS OF INSTABILITY IN PHARMACEUTICAL PRODUCTS

There is a variety of mechanisms by which drug products may degrade, and thus a quite wide range of adverse effects that can occur.

2.1. Loss of Active

Obviously, loss of drug is of major importance in the stability studies of many pharmaceutical products. Unfortunately, one sometimes gets the impression that some regard this as the only adverse effect of drug product stability, This is, of course, not true, and for some products loss of active is not the critical variable that determines shelf life. However, it is certainly true that for many products loss of potency is of major importance. In general, we regard any product that contains less than 90% of label claim of drug as being of unacceptable quality. Therefore, for many drug products, determination of the time that elapses before the drug content no longer exceeds 90% (when the product is stored in conformance to label instructions) is an essential element in determining shelf life (2).

The essence of the conventional way of determining shelf life from loss of active is as follows. The potency of product stored at the appropriate temperature (25°C for products to be labelled "Store at Controlled Room Temperature") is determined as a function of time and the best straight line of potency as a function of time determined by least squares regression analysis (Fig. 1). Of course, because of analytical and sampling error there will normally be some scatter of the experimentally determined data points around the mean regression line. Thus in order to have a high comfort level about the shelf life that we will assign to the product, we use conventional

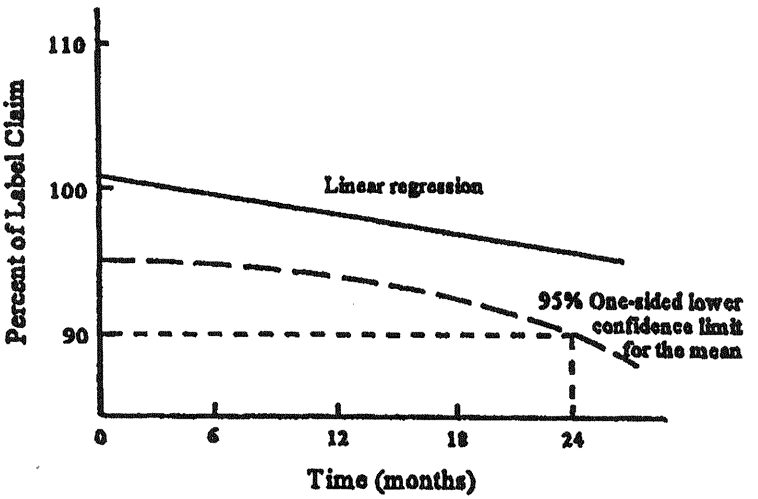

Figure 1 Least squares regression analysis.

statistical methods to calculate the 90% confidence zone of the regression line. This means that there is a 90% probability that the true regression line (of label claim potency as a function of time) is within the zone. Thus there is only a 5% chance that the true line is really below the 90% zone. It is apparent that any shelf life derived from the intersection of the lower 90% confidence bound and the 90% potency value has a 95% confidence level. In other words, there is only a 5% chance that our estimate of the shelf life will be too high. In fact, there is an additional safety margin built into most estimates of shelf life in that the period of time determined as described above (termed by A. J. Smith the *conformance period*) is usually significantly greater than the period defined as the shelf life. Suppose that for three separate pharmaceutical products we obtained 95% confidence estimates of the conformance periods of 13.2, 26.1, and 39.4 months. We would probably assign shelf lives of 12, 24, and 36 months to the three products. The difference between the conformance period and the assigned shelf life provides an extra stability reserve.

Those conversant with kinetic theory as outlined in Chapter 2 may wonder if the order of reaction affects the form of graph obtained as shown in Fig. 1. It might be felt that if the degradation process is governed by first-order kinetics, the plot would yield a curve rather than a straight line. In fact, since this type of plot only covers potency values from 100% down to 90%, both first and zero kinetic processes appear to be essentially linear. Yes, there are some degradation processes (3) that do produce nonlinear plots, but fortunately these are remarkably rare.

Shelf life values are normally assigned to a product rather than a batch. In order for such a practice to be legitimate, there must be reliable data that shows that, for at least three batches of the product, there is no significant difference in the slopes or the intercepts of the types of plot shown in Fig. 1. In those instances, where this level of batch similarity cannot be demonstrated, it might be possible to assign a shelf life based on a worst-case scenario (i.e., the shelf life is based on the worst-case batch). In this situation, we probably need more than three batches so that we can be comfortable that we have indeed identified the worst-case batch.

Even if no change of formulation, process, or site of manufacture is contemplated, there may be other reasons for additional stability studies. Unfortunately, it is not unknown for a new product that we believe to have been fully validated with respect to all quality attributes (including stability) to exhibit unexpected stability problems. These problems may progressively develop in a most insidious way, affecting all batches or, in some instances, only some batches intermittently. In either event, troubleshooting directed at identifying the cause and then taking appropriate remedial action is necessary.

Similarly, if complaints from patients, health professionals, or others involve stability problems, it is obviously important that stability group personnel should be involved in the evaluation of the problem and be consulted when it is decided if remedial action is required.

3.6. Product in the Channel of Distribution

It is not sufficient to restrict our concerns about drug product stability to the quality of the pristine, freshly manufactured material that we regard with justifiable pride as it waits in our warehouse for distribution after it has been cleared from quarantine by our QC/QA (Quality Control/Quality Assurance) department. Of course, it is normal to store some stability samples in our stability storage areas (retained samples). However, the evaluation of samples that have been stored under the utopian conditions in the manufacturer's stability storage areas is of limited value. Samples retained for stability testing are not dropped off the back of a truck; they are not left on a loading dock in the blazing sun; nor are they left in the freezing cold. Thus it is somewhat unrealistic to expect retained stability samples to reflect accurately the stability status range of products that are in the channel of distribution. As is discussed in Chapter 18, there is now increased concern about the stability status of products in the channel of distribution.

3.7. Product Under the Control of the Patient

There is good reason to believe that, in many instances, the conditions under which patients store their drug products is far removed from optimal. At one time some regulatory authorities were considering the possibility of requiring shelf lives that could be guaranteed right through to the time when the patient used the last dose of the product. It is now probably generally appreciated that this idea is not feasible. It certainly is, however, most appropriate that pharmacists should take time and trouble to counsel patients on the appropriate ways to store drug products.

3.8. *In Vivo* Stability

The final stability concern is the degradation of the drug *in vivo*. In particular, the hydrolysis of drug at the low pH conditions of the stomach can be particularly serious. The traditional answer to this particular problem is to enteric coat a tablet. In the past, the materials used as enteric coats were not always effective. The polymers now available for enteric coating are much more reliable (11).

4. REASONS FOR STABILITY TESTING

4.1. Our Concerns for Patients' Welfare

Obviously, our primary reason for stability testing should be our concern for the well-being of the patients who will use our products. Sometimes in the mad rush to comply with other requirements, this important fundamental may be discounted or forgotten. Indeed, sometimes one gains the impression that in some quarters stability is regarded as having little clinical relevance. Certainly, if a product that does not degrade to toxic decomposition products and that is not characterized by a narrow therapeutic ratio is present on the market at only 85% of label claim, one would not expect patients to be dropping dead in the streets because of this deficiency instability. However, this is not to say that stability problems can never have serious clinical consequences. For example, in the early 1980s a packaging stability problem with nitroglycerin tablets unfortunately resulted in some nitroglycerin tablets being available in the Midwest with potency values of less than 10% of label claim. Since nitroglycerin is used for the emergency treatment of a most serious cardiac condition, angina, there is unfortunately strong cause for concern that some patients may have died as a result of this stability problem.

Even if death is not likely because of stability problems with a particular drug product, the inconvenience, discomfort, and cost associated with the use of product that is subpotent or exhibits an unacceptably wide range of potencies may be a serious problem needing radical remedial response. For example, concerns about possible potency problem with L-thyroxene products were of considerable importance in stimulating the FDA to require, in August 1997, that all human L-thyroxene products that were on the U.S. market at that time could only remain on the market until August 2000, unless new regulatory NDAs (New Drug Applications) or ANDAs (Abbreviated New Drug Applications) were approved (12).

4.2. To Protect the Reputation of the Producer

We should all be jealous for the reputation that the stability of our pharmaceutical products—compounded or manufactured—enjoys. Thus a most important reason for conducting a stability testing program is to assure ourselves that our products will indeed retain fitness for use with respect to all functionally relevant attributes for as long as they are on the market.

4.3. Requirements of Regulatory Agencies

In many parts of the world, there are legal requirements that certain types of stability tests, as required by regulatory agencies, must be performed (13). Obviously, the law must be obeyed. However, it is wrong to abdicate from all scientific judgement and only conduct those stability tests that a regulatory agency is perceived as requiring. Indeed, there are occasions when any manufacturer with a true dedication to quality will perform stability tests that are over and above those required by regulation.

4.4. To Provide a Database That May Be of Value in the Formulation of Other Products

Data obtained in the stability evaluation of product X in 1999 may prove to be of value when, in 2003, we start developing product Y. There may be occasions,

although they are probably rare, when it will be worthwhile to continue stability testing on an R&D formulation that we know will never be marketed just because we are interested in the stability of a new excipient that we have included in the formulation.

5. MODES OF DEGRADATION

5.1. Chemical

Chemical degradation (solvolysis, oxidation, etc.) is common and is described in subsequent chapters of this book. Our knowledge of kinetics can be of material assistance in dealing with chemical degradation.

5.2. Physical

Physical degradation can be caused by a range of factors (e.g., impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing, or shearing). Physical testing is described in Chapter 10.

Unfortunately, in many instances, our knowledge of the exact mechanisms involved in physical degradation is incomplete. It is also unfortunate that a number of the physical test methods that could be used in evaluation of physical stability (e.g., tablet friability, tablet impact resistance, suspension redispersibility, or injection syringeability) are still nonofficial and variable. It is noteworthy that it was not until 1997 that official standardized test methods for the quantification of bulk and tap density were introduced into the USP, although such tests have value in helping to evaluate compressibility.

5.3. Biological (Especially Microbiological)

In North America, Japan, and Western Europe it is microbiological factors that are most likely to be involved in biological stability problems. However, in some parts of the world rats, roaches, ants, and other nonmicrobiological organisms can be responsible for stability problems.

5.4. Limitations of This Classification

Useful though the above tripartite classification of degradation mechanisms may be, there is a danger that its use may overcompartmentalize our approach to drug product stability. This can be dangerous. In fact, many stability problems involve more than one mechanism. For example, insufficient antioxidant in a rubber condom may result in oxidation of the device by a chemical mode. However, the effect that may be detected is loss of tensile strength, which is, of course, a physical parameter.

6. THE ESSENTIAL ELEMENTS OF A HIGH-QUALITY, COST-EFFECTIVE STABILITY PROGRAM

6.1. Commitment of the Organization to Quality

It is essential that throughout the organization responsible for the development and production of pharmaceutical products there be a *true* commitment to quality.

Absent this commitment, it is likely that a stability program will be regarded simply as a burdensome, nonproductive expense. If such an attitude pervades top or middle management—although such attitudes are rarely expressed directly in writing but rather transmitted by a nod and a wink—it is quite possible that the stability testing group will be starved of essential equipment and personnel. One sometimes visits companies where stability testing is two or three months, or even more, behind schedule. Top managers say they cannot understand how the problem has developed since, as everyone in the company knows, their personal dedication to product quality is second only to their commitment to God, the flag, and the family. Unfortunately, such individuals sometimes "talk big but spend little."

6.2. Firm Grasp of Underlying Scientific Theory

It should hardly need to be stated that stability testing of pharmaceuticals requires in-depth education in the science of pharmaceutical formulation, evaluation, analysis, and statistics. Unfortunately, there still are companies where personnel with such education are lacking.

6.3. Up-to-Date Knowledge of All Relevant Policies of Regulatory Agencies and Applicable Pharmacopoeial Standards

Official regulations and standard test methods continue to evolve. Thus it is important that at least one person in every company be charged with the responsibility of keeping up-to-date files on data from the FDA, the USP, or such other entities as may be relevant that impinge on any aspect of the design, execution, or interpretation of stability tests. Perusal of Pharmacopoeial Forum (*PF*), the journal in which the USP provides trailer-type information about possible new or modified test methods or monographs, should be mandatory in all companies for which USP standards may be of relevance.

6.4. Effective Communication Between R&D, Production, QC/QA, Complaints, and Regulatory Affairs

In order to have a successful stability testing program, it is important that there be clear, effective, and rapid communication between all the various organizational entities in a company that can provide useful input into the stability program.

6.5. A General Understanding of the Limitations of the Analytical Methods Used in the Stability Testing Program

Everyone with any degree of responsibility for decisions about a stability program—not just those performing the tests in the laboratory—should have a general understanding of the parameters that characterize the test methods used in stability testing (accuracy, precision, sensitivity, reproducibility, transferability, etc.). We do not require that everyone be expert at say HPLC (high-performance liquid chromatography) or ELISA (enzyme-linked imunosorbest assay), but we should expect that the decision makers be aware of the salient characteristics of the test methods, the results of which are used in decisions about stability.

6.6. Careful Monitoring of the Stability Budget

It is surprising that some companies have no stability budget. It is even more surprising to find that there are scientists designing stability protocols who select test method A instead of test method B (both of which might be technically satisfactory but of significantly different cost), who have knowledge of or interest in the relative costs of the two tests.

It is not easy to devise a mechanism for evaluating a stability budget such that we can be quite certain that we have accounted for all monies spent on the program. However, even though the budget that we estimate may be relative, rather than absolute, it still can be of substantial value.

6.7. Managerial Skills to Coordinate and Optimize the Program

The capstone of a high-quality cost-effective stability program must be managerial skills that nurture and coordinate the personal and professional skills of all involved with the program.

7. CONFORMANCE PERIODS, SHELF LIVES, AND EXPIRATION DATES

The conformance period of drug product is defined by the most vulnerable time-dependent quality attribute. As has already been indicated in Sec. 2.1 of this chapter, loss of potency is, for many products, the critical parameter. In those cases where some other attribute is more vulnerable, it will be that property that defines the conformance period. The same general approach as that shown in Fig. 1 should be followed; however, instead of plotting potency as a percentage of label claim on the y-axis, one plots the appropriate critical stability parameter. The conformance period is then determined from the intersection of the lowest (or highest) acceptable value of the parameter and the 95% confidence bound of the regression line. In the rather rare event that there are two stability attributes of about the same criticality, then both should be quantified and the lower conformance period used as the basis for the assignment of the shelf life of the product.

As has been previously indicated, the shelf life assigned to a product is equal to, or less than, the conformance and is usually a convenient round number (e.g., 7 days, 1 month, 1 year, 18 months, or 2, 3, or 5 years).

The expiration (or expiry) date placed on the label of any given batch indicates the date at which the shelf life ends for the batch. Thus if the product is stored in accordance with label instructions, it is expected that the product will retain fitness for use up to that date. With the exception of products that have very short shelf lives, it is conventional in many parts of the world to give only the month and year of the expiration date. It is expected that for such dates, e.g., May '03, the product should remain of acceptable quality until the *end* of the stated month.

When products have a 5-year shelf life, the practice of only giving expiration dates for the months of January or July seems to be becoming more common. This practice simplifies stock control, since there are fewer dates to deal with. This approach is used as follows: Suppose we have a product that has a five-year shelf life, and we manufacture batches of the product in February, April, June, August, and November of 2002. The first three batches would be dated January '07; the last two would be dated July '07.

Obviously, if a product is not stored in accordance with label instructions, the expiration date cannot necessarily be relied on.

8. SOME POSSIBLE STRATEGIES TO IMPROVE SHELF LIFE

It is fortunate that many drug substances and products are inherently stable; thus, with little difficulty, we can justify a shelf life of 3 years or more. However, there are drug substances that are very much more liable to degradation, and it may require much skill and hard work to develop a product with a shelf life that is commercially acceptable. Since this book is not focused on formulation *per se*, this section only outlines some of the general approaches that might be considered in efforts to improve shelf life.

8.1. Sampling and Analytical

Examination of Fig. 1 reveals that the more scatter that we have on a stability plot the wider the 90% zone of confidence will be. If we were somehow able to obtain experimental points that all fitted exactly on the regression line, then both the upper and lower 90% confidence bounds would also be on the mean regression line; thus our estimate of the conformance period would be given by the intersection of the regression line and the 90% potency line. Clearly, this would substantially extend the shelf life that we could legitimately claim. Of course, it is impossible to obtain such perfect data that the 90% confidence zone has no width whatsoever. However, anything that we can do to reduce its thickness will improve our shelf life.

There are two main causes for the fact that stability plots, such as that shown in Fig. 1, show scatter, *viz.*, sampling error and analytical error. Anything that we can do to reduce either or both of these errors will improve our shelf life without our having made any change to the formulation or process used for our product.

It is not often easy to see how sampling error could be reduced. Possibly the use of near-infrared spectroscopy for single-tablet assay (see Chapter 18) of the same known, individual tablets throughout the shelf life testing period, and averaging the data so obtained at each time point, might be a practicable method to reduce error due to content uniformity variation (14). Perhaps reduction of sampling error is one of the incentives that we have in making sure that all samples are tested on time.

In terms of analytical error, if we can improve precision and reproducibility we will slim the 90% confidence envelope and improve our shelf life. In some cases, it has been shown that the extra cost of a more sophisticated assay may be justified by the improvement in shelf life that results.

8.2. Statistical

If testing of samples is continued beyond the point at which degradation has reached the 90% confidence of the label claim value, we move the narrow "waist" of the 90% confidence zone to later times and thus improve our shelf life. This valid statistical approach was specifically mentioned in the 1984 FDA Stability Guidelines.

8.3. Process

Filling ampoules under nitrogen for solutions that are susceptible to oxidation is one example of a processing method that can improve stability. Of course, the most common process variable that is adjusted to control stability is selection of the package components and materials, and readers specifically interested in this topic are referred to Dixie Dean's chapter.

8.4. Formulation

The literature is replete with accounts of proven and potential methods of improving product stability and hence shelf life. All that is provided in this section are some general concepts that can, if appropriate, be explored in more detail.

In the past, stability overages,* which allowed a relatively easy method to improve shelf life, were quite common. Indeed, there are many drug products on the market in different parts of the world that contain a stability overage of up to 10% of label claim. However, a number of regulatory agencies, including FDA, are now showing much more reluctance than previously to approve such overages for drug products.† This reluctance to approve the use of stability overages probably stems from a number of causes.

First, there is concern about the possible increase in toxicity that might accompany the use of a stability overage. If a product for which compendial potency limits are 90–110% is released onto the market at 100% of label claim, then the maximum amount of any degradation product that could be present in the product up until the expiration date is 10%. However, if the product is released at 110% of label claim, then it is conceivable that in some instances there could be up to 20% of degradation product. If the degradation product, or part thereof, is toxic, use of a stability overage has *doubled* the potential hazard to which a patient is exposed.

Second, if stability overages are allowed, then the range of potencies to which a patient may be subjected is increased. For example, suppose that a patient who has a repeat prescription for drug X (which is known to have a relatively low range of acceptable therapeutic blood levels) finishes tablets of lot A101, which has a potency of 90%, and is then supplied with tablets from B103, which has a potency of 110%. Then (even neglecting degradation of drug while the tablets are under control of the patient and not considering content uniformity) we can see that the patient may experience a 20% variation in blood levels. In contrast, in the absence of a stability overage, the maximum potency variance would only be 10%. This substan-

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* Overages are of three types: container, manufacturing, and stability. A *container* overage is added to allow for the fact that it is not possible in some cases to remove all the contents from a container. Thus ampoules labeled 1.0 mL are normally filled with 1.1 mL. A *manufacturing* overage is added when it is known that relatively small and reproducible amounts of active are always lost during the manufacturing process although we are using modern equipment and facilities and well-trained staff. A manufacturing overage is, of course, dissipated by the time final product testing is completed.

† Vitamin products, which are classified by the FDA as food supplements (unless they are administered by the oral route or supplied under a doctor's prescription), still have substantial overages—sometimes up to 100% of label claim.

tial potency variation could lead to sub- or supratherapeutic blood levels and perhaps the need to retitrate the patient.

Third, and perhaps most important, there is a perception in some quarters that use of a stability overage is a cop-out that represents an easy Band-Aid approach to formulation that is quite unacceptable in modern pharmaceutical technology. It is thought that a more thorough investigation of the problem and a willingness to devote appropriate resources of time, personnel, and money might well allow the problem to be solved by other, more conventional, formulation approaches that do not require a stability overage.

Formulation approaches to reduce the problem of hydrolysis of drugs in solution have generally been of rather limited success. Recently, complexation of drugs with cyclodextrins has attracted considerable interest (15). Such complexes may show improved resistance to hydrolysis, faster dissolution, and better bioavailability. Of course, since most hydrolysis reactions are catalyzed by hydronium and hydroxyl ions, pH control might appear to have great value as a formulation approach to reducing hydrolysis. In practice, however, this approach has had rather limited success. For drugs liable to hydrolysis that are formulated into tablets, the use of a coating may be of value in improving stability.

In contract to hydrolysis, degradation by oxidation can often be successfully controlled by formulation approaches. There is a range of chelating agents and both oil- and water-soluble antioxidants that are used in products in various parts of the world. When a product contains an antioxidant, it is normal to monitor the amount (or concentration) of antioxidant as part of stability studies. In theory, it would be acceptable if all the antioxidant were used by the end of the shelf life period. In practice, most of us would feel rather uncomfortable if we did not have, say, 25% remaining at the end of the shelf life.

Antimicrobial preservatives, such as sodium benzoate, are commonly added to many pharmaceutical products. The amount (or concentration) of such components should be monitored during stability studies. Although chemical assay for antimicrobial preservatives may be acceptable at most time points, the testing performed at the last time point should be by a microbiological challenge test, such as that specified in the USP.

Perhaps the area where formulation approaches are particularly important in controlling stability problems is the field of protein drugs, an area of ever-increasing importance. Dr. Kottke and Dr. DiBiase give this topic specific attention in their chapter in this book.

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2

Solution Kinetics

JENS T. CARSTENSEN

Madison, Wisconsin

Stability is not synonymous with chemical kinetics, yet most of the rate-limiting phenomena are either associated with chemical reactions or are describable by some equation system that bears a resemblance to those encountered in chemical kinetics. It is, therefore, of importance to lay the proper kinetic foundation before discussing the actual phenomena encountered in dosage forms.

These fundamental principles are most conveniently described by solution kinetics. The simpler a system is, the easier it is to make it reproducible, and it is therefore not surprising that the largest number of pharmaceutical publications on the subject of kinetics deal with solution systems. Furthermore, the more dilute a system is, the more it will adhere to ideal laws, and hence the largest number of publications to be found deal with dilute systems. There are obviously pharmaceutical dosage forms that are solutions, viz. oral, parenteral, nasal, ophthalmic, and otic solutions. Of these, it is only the parenteral and ophthalmic solutions that are chemically fairly simple, i.e. contain only a few number of components. These are systems that would behave similarly to the patterns described in, for instance, the chemical literature. In oral solutions, there are many ingredients (sweeteners, solubilizers, etc.), so that, here, one would expect definite vehicle effects and interaction possibilities.

The Stability Guidelines make certain requirements on basic stability that are best elucidated (or only elucidated) through solution kinetics: First of all it is necessary to develop a stability-indicating assay. This is defined in lines 111 of the 1987 Guidelines as "Quantitative analytical methods that are based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured." The 1993 ICH Guidelines state,

> Analytical test procedures should be fully validated and the assays should be stability-indicating. The need or the extent of replication will depend on the results of validation studies (194–196).
>
> The focus may instead be on assuring the specificity of the assay ... of identified degradants as indicators of the extent of degradation via particular mechanisms (386–389).

This means that the assay must be capable of detecting quantitatively the amount of parent drug present, and identify, and to some degree quantitate, the decomposition products. Lines 265–277 of the 1987 Guidelines state, "When degradation products are detected, the following information about them should be submitted when available:

(a) Identity and chemical structure,
(b) cross-reference to any available information about biological effect and significance at the concentrations likely to be encountered,
(c) procedure for isolation and purification,
(d) mechanism of formation, including order of reaction...,
(e) physical and chemical properties,
(f) specifications and directions for testing for their presence at the levels or concentrations expected to be present."

Lines 141–144 further state that "the stability-indicating methodology should be

validated by the manufacturer (and the accuracy and precision established) and described in sufficient detail to permit validation by FDA laboratories."

In developing stability-indicating assay methodology, it is customary to deliberately decompose the drug in solution, so as to challenge the assay and insure its capability of separating the parent drug from decomposition products. It is obvious, also, that it is desired to establish the kinetic order of the decomposition.

1. THE ORDER OF A REACTION

The order of a reaction will be defined below, but in essence it determines how the degradation data are treated. That it is important to establish the order of a reaction is evident in that the 1993 ICH Guidelines specifically state,

> The nature of any degradation relationship will determine the need for transformation of the data for linear regression analysis. Usually the relationship can be represented by a linear, quadratic or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit of the data on all batches and combined batches (where appropriate) to the assumed degradation line or curve (138–143).

They also state (in respect to mass balance),

> This concept is a useful scientific guide for evaluation data but it is not achievable in all circumstances. The focus may instead be on assuring the routes of degradation, and the use, if necessary, of identified degradants as indicators of the extent of degradation via particular mechanisms (385–389).

Although the presentation modes outlined in this quotation are not (at least not in the case of the quadratic or cubic functions) of scientific bent, it is obvious that efforts must be made, before the formal stability program is started, to establish the order of the reaction.

Establishing the order is, furthermore, of financial importance, because the establishing of expiration periods (which will be discussed later) depends, to some degree, on the investigator's capability of extrapolating the concentration of drug beyond the last time point of testing. The 1993 ICH Guidelines further state,

> Limited extrapolation of the real time data beyond the observed range to extend expiration dating at approval time, particularly where the accelerated data supports this, may be undertaken. However, this assumes that the same degradation relationship will continue to apply beyond the observed data and hence the use of extrapolation must be justified in each application in terms of what is known about the mechanism of degradation, the goodness of fit of any mathematical model, batch size, existence of supportive data, etc. (149–155).

The longest possible expiration period is, of course, economically desirable, and many of the efforts of the stability programs of pharmaceutical companies are geared towards lengthening this period. As for definition of the order or a reaction, if

$$A + B \rightarrow E \tag{2.1}$$

then the reaction rate is given by

$$\frac{dC}{dt} = -k_{(n+m)}[A]^n[B]^m \tag{2.2}$$

where C is the concentration of the species being studied, brackets denote concentrations of A and B, and k denotes a rate constant, then the reaction is said to be of the order $n+m$. The rate constant, in this writing, will most often carry the subscript denoting the order of the reaction whenever reaction orders are discussed and being distinguished. (A notable exception is the notation in the section dealing with pH profiles). The most important orders of interest in the pharmaceutical sciences are integral orders, i.e. those in which the sum of n and m is 0, 1, or 2. (Orders of higher than two are rare.)

As alluded to above, knowledge of the order of a reaction is of great importance in stability determination of drug substances, in particular in solution. The problem is frequently to judge whether the concentration-time profiles are linear (zero order) or curved (first or other order). When large amounts of data are at hand (e.g., at different temperature, where the order does not depend on temperature), then a data-consolidation technique described by Carstensen and Franchini (1994), Carstensen (1997), and Franchini and Carstensen (1994, 1999). The technique has later been used by Shalaev et al. (1997) in the study of solid-state methyl transfer reactions. For instance, the data in Fig. 1, when plotted linearly, give *fairly* good plots, but since there are only a few points it is difficult to say with reasonable certainty whether the data are, indeed, linear or curve-linear.

A *fractional life* is the length of time it takes for a product or drug substance to decrease to the level indicated by the fraction: the half-life, t_{50}, of a substance is the length of time it takes to decrease the content of active compound to 50% of its value. If, in Fig. 1, a given fractional life (e.g., t_{90} as shown in the figure) is read

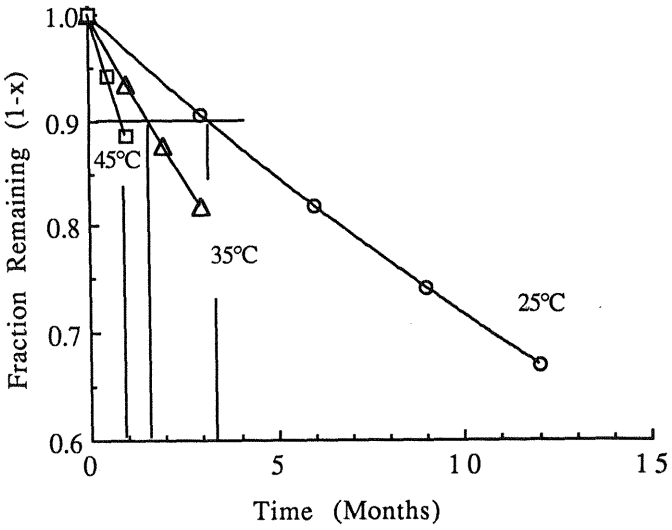

Fig. 1 Example of sparse data at three temperatures, such as is often encountered in early development of drug products.

Table 2.1 Example of Reduced Data Treatment in Kinetics

Time (Months)	Temperature (t_{90})	Fraction retained	Reduced time
0	25°C (3.5)	1.000	1.000
3		0.905	0.905
6		0.819	0.819
9		0.741	0.741
12		0.670	0.670
0	37°C (1.7)	1.000	1.000
1		0.935	0.935
2		0.875	0.875
3		0.800	0.800
0	45°C (0.95)	1.000	1.000
0.5		0.942	0.942
1		0.887	0.887

off the graphs, then the data may be consolidated. The t_{90} values shown are 45°C: 0.95; 35°C: 1.7; and 25°C: 3.5. The data in the first column in Table 1 are then reduced to the fourth column, and this is used as abscissa and the fraction retained (regardless of temperature) is used as ordinate in column 3, and the data are shown in Fig. 2.

The method allows better extrapolation tolerances, since the number of points is larger than for the individual temperatures. If, as in the unstable shown in Fig. 1, the t_{90} value is 3.5 months, then extrapolation could be made to $24/3.5 = 6.7$ half-lives, and the estimated potency after 24 months could be estimated with better precision than if only the 25°C data had been used.

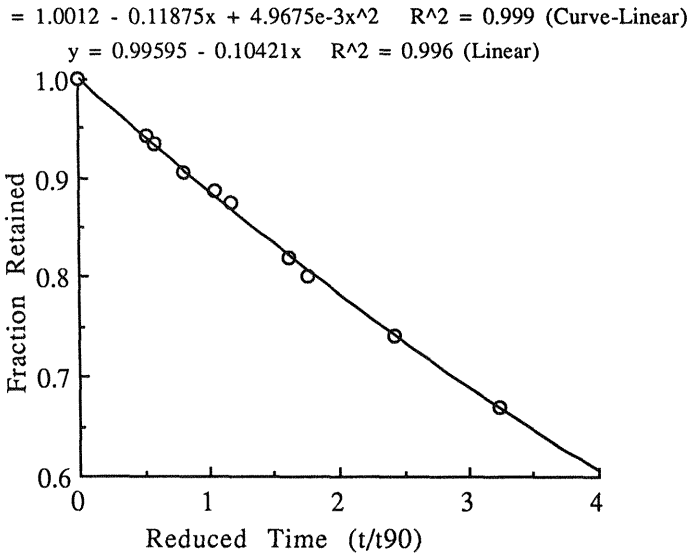

Fig. 2 Data from Table 1 plotted by reduced time treatment.

The fractional life should be *read off the graph* (Fig. 1) since at that point the reaction order is not known. Once the data are plotted, it is possible to execute curve fitting and estimate the best fit. In the case cited, the number of points is probably still too small to make a decision, but the indication is that the data are first order. Including data from even higher temperatures will help in this respect, but it is necessary that the order of reaction not change at the higher temperatures.

It has been mentioned that above 85% it is difficult to distinguish between different reaction orders. Li et al. (1998) report on apparent first-order plots for oxidation of a 4-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimidino[5,4-6][1,4] thiazin-6-yl)-(S)-ethyl-2,5-thenoyl-L-glutamic acid. It is to be noted (from their Fig. 6) that there is definite downward curvature in the plots, and that they probably are S-shaped, as is discussed further in the chapter on oxidation. Here again, using a method of fractional times will help in deciding on which orders are plausible, or if a certain order of reaction can be ruled out.

It should, finally, be mentioned that Mälkki-Lame and Valkeile (1988) have described a method for transforming regression curves to the determination of reaction order of given situations in stability studies, using the Box–Cox technique and the Link function transformations.

2. THE ZERO-ORDER REACTION

There are not many truly zero-order reactions in the pharmaceutical field. It will be shown at a later point that there are several types of reactions that will appear to be zero order, i.e., are pseudo-zero order. The equation for zero-order reactions is

$$\frac{dC}{dt} = -k_0 \tag{2.3}$$

where C is concentration, t is time, and k_0 is the zero order rate constant.

It is seen that the unit of k is concentration units per time unit, e.g. molar per second. The integrated form of Eq. (2.3) is

$$C = C_0 - k_0 t \tag{2.4}$$

or

$$C_0[1 - a] = kt_a \tag{2.5}$$

where a is the fraction remaining at time t_a.

A quantity often utilized is the half-time, $t_{1/2}$, which is given by

$$t_{1/2} = \frac{C_0}{2k_0} \tag{2.6}$$

It is noted that this is dependent on the initial concentration.

Zero-order data may be graphed on plain Cartesian graph paper, using concentration as ordinate and time as abscissa. An example (Higuchi and Rheinstein, 1959) is shown in Fig. 1.

3. FIRST-ORDER REACTIONS

In this case Eq. (2.2) takes the form

$$\frac{dC}{dt} = -k_1 C \tag{2.7}$$

which integrates to

$$\ln\left[\frac{C}{C_0}\right] = -k_1 t \tag{2.8}$$

It is noted that the a-fractional life is given by

$$\ln[a] = -k_1 t_a \tag{2.9}$$

The most common of the a-lives is the half-life and the t_{90} (i.e., the point where 90% of the original concentration is left), which adhere to Eq. (2.9) by the equations

$$k_1 t_{/2} = -0.693 \tag{2.10}$$

$$k_1 t_{0.9} = -0.105 \tag{2.11}$$

Fig. 3 and Table 2 show an example of a straight first-order reaction.

In stability situations it is required to monitor both the disappearance of the drug and the appearance of decomposition product(s). In most cases there is more than one decomposition product (and simple cases of this will be treated below). In the simplest case there is only one decomposition product. There are cases of this, e.g., aspirin in simple systems (Carstensen et al., 1985; Carstensen and Attarchi, 1988a, 1988b) decomposes, by a pseudo-first-order reaction, in a simple fashion, i.e. to salicylic acid and acetic acid. In such cases, if the assay of the decomposition product is fairly good, the decomposition can be monitored best by monitoring

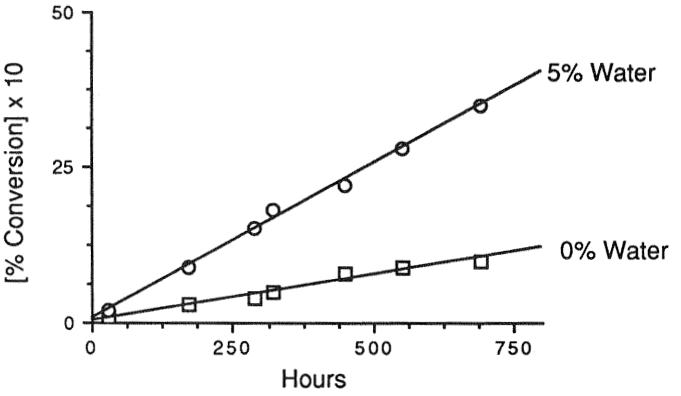

Fig. 3 Decomposition of vitamin A acetate (to anhydrovitamin A). The least squares fits are (with 5% water) $y = 0.48 + 0.015x$; (without water) $y = 0.75 + 0.05x$. (Figure constructed from data published by Higuchi and Rheinstein, 1959.)

Table 2.2 Decomposition of Decarboxymoxalactam

Time (Min)	% Retained	ln[% Retained]	% Decomposed
0	100	4.61	0
10	78	4.36	32
20	50	3.91	50
30	38	3.64	62
40	27	3.30	73
50	17	2.83	83

Source: Reconstructed from data published by Hashimoto et al. (1984).

the appearance rate of the decomposition product, which should follow the reaction

$$[B] = A_0[1 - \exp(-k_1 t)] \tag{2.12}$$

An example of this is shown in Table 2.

It should be noted that whenever this approach is taken, it is mandatory still to monitor the content of parent drug, because mass balance should persist throughout the reaction period. (If the molar quantities do not sum up to A_0 (within experimental error), then either the reaction is not simple A → B, or the analytical procedure fails in aged samples). The easiest way of plotting the data in Table 2 is obviously to subtract each [B] figure from A_0 and plot is as [A]. One might then argue that one might simply plot the experimental value of [A]. But for fairly stable systems, the values of [A] may not differ (decrease) much and may be masked by experimental error. The percentage change in [B], however, is substantial, as seen in the table, and plotting becomes more meaningful (see Fig. 4).

There are many reported instances of first-order reactions in solution. For instance, Jordan (1998) has shown that timolol and propanolol decompose by straight first-order kinetics at pH 7.4. Aso et al. (1997) have shown that aqueous solutions of cephalothin decompose by first-order kinetics and that they follow

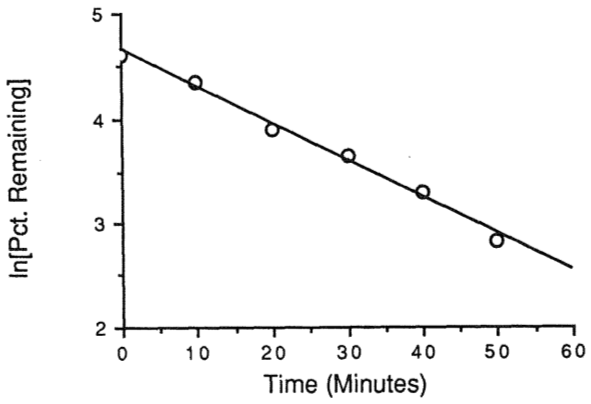

Fig. 4 Plot of first-order data from Table 1. (Graph constructed from data by Hashimoto et al., 1984.)

an Arrhenius equation. Hammad and Müller (1998) have found clonazepam to degrade first-order in phosphate buffers at pH 7.4 and to adhere to an Arrhenius equation.

Heat conduction *microcalorimetry* has been used as a method to evaluate stability and excipient stability by a series of researchers Angerg et al., (1988, 1990, 1993). Hansen et al. (1989), and Wilson et al. (1995) have described the general method and results interpretation. Oliyai and Lindenbaum (1991) have studied the decomposition of ampicillin in solution by means of microcalorimetry.

4. FIRST-ORDER REACTIONS WITH MORE THAN ONE END PRODUCT

The considerations above have assumed that the scheme is simply a reaction of type $A \rightarrow B$, but often there is more than one decomposition product.

4.1. Consecutive Reactions of The First Order

The 1993 ICH Guidelines state that mass balance (or material balance) is

> The process of adding together the assay value and levels of degradation products to see how closely these add up to 100 per cent of the initial value, with due consideration of the margin of analytical precision (382–384).

It is possible that the primary decomposition product itself is not stable, and in such cases the reaction scheme is

$$A \underset{k_1}{\rightarrow} B \underset{k_2}{\rightarrow} C \tag{2.13}$$

In other words, there will be more than one decomposition product. If all the products can be identified and quantitated, then it follows that *the number of moles of A, B, and C should always add up to the initial number of moles of A.* It is noted that it is the number of moles that must add up. Addition on a weight basis would be futile if there is a substantial difference between the molecular weights of the drug and the products. The guidelines recognize that it can be difficult, at times, to ascertain mass balance, partly due to analytical precision.

More often it is "unknowns" that cause the problem. If C were not identified, for instance, and was detected as a peak in a HPLC chromatogram, then its "content" is often stated as the area under the peak, using the drug as the unit of measure. But if, for instance, a UV detector is used, and C is lacking the amount of chromophores that A possesses, then the area under the C peak may grossly underestimate the amount of C.

An example of this is chlorbenzodiazepine, which hydrolyzes to the lactam form, and then further to the benzophenone (Carstensen et al., 1971). In fact in this reaction, for some of the benzodiazepines, C can progress further with the formation of the carbostyril and the acridone derivative, and some of the steps are associated with equilibrium conditions.

The rate equations governing scheme (2.13) are

$$\frac{d[A]}{dt} = -k_1[A] \tag{2.14}$$

$$\frac{d[B]}{dt} = -k_2[B] + k_1[A] \tag{2.15}$$

and

$$\frac{d[C]}{dt} = k_2[B] \tag{2.16}$$

These simultaneous differential equations may be solved by conventional means and yield the following results:

$$[A] = A_0 e^{-k_1 t} \tag{2.17}$$

$$[B] = A_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \tag{2.18}$$

and

$$[C] = A_0 - [A] - [B] \tag{2.19}$$

It is noted that the above expressions refer to molar quantities. An example of consecutive reactions is shown in Table 3 and Fig. 5. The table shows $C = 100 - [A] - [B]$. It often happens that one of the decomposition products is difficult to assay for, and in such a case, it may be obtained by difference, provided that mass balance is checked occasionally, e.g., in the early stages and at the end. Of course, there are reactions that have a multitude of end products, and in such cases it is conventional to assume that if e.g. a HPLC peak is less than 0.5% then it is considered negligible. This may be dangerous, because (especially if it is a constant wavelength peak), the actual molar content of the product(s) in the peak may be more than 0.5% (in which case mass balance would be lost).

To ascertain that the unidentified products are not toxic (and since they are unidentified, specific toxicity cannot be checked), it is conventional, as well, in such cases, to degrade a sample considerably and check its toxicity. It is worthwhile,

Table 2.3 Photolysis of Cefotaxime

Time (hours)	Cefotaxime A (% moles)	Anti-isomer, B (% moles)	*C* by difference[a]
0	100	0	0
0.25	82	10	8
0.5	70	15	15
0.75	55	18	27
1	43	19	38
1.5	28	18	54
2	20	15	65
3	10	10	80
4	5	5	90

[a] Column 3 not reported by Lerner et al. (1988). C may be more than one product.
Source: Constructed from data published by Lerner et al. (1988).

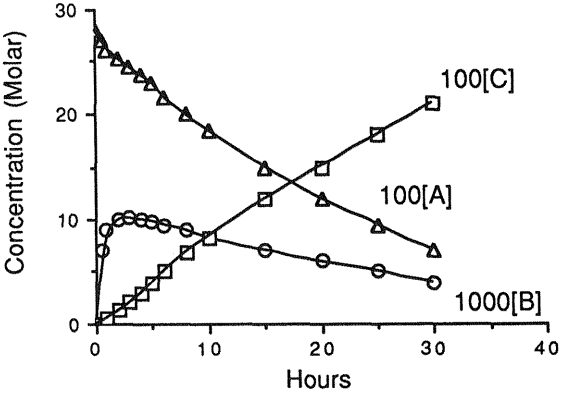

Fig. 5 The A → B part of cefatoxime photolysis. C_{total} has been obtained as 100-[A]-[B], but this does not account for the possibility of other reactions of C. (Graph constructed from data by Lerner et al., 1988.)

however, also to check the toxicity at intermediate points, because C might be toxic, but degrade into nontoxic products, and the toxicity of a partly degraded sample might be worse than that of a fully degraded sample).

The A → B → C reaction is rather common; for instance, it has been reported by Misra et al. (1993).

There continues to be, in present literature, reports of this type of reaction; for instance, Archontaki et al. (1998) reported on the decomposition of nordazepam and showed typical A–B–C plots with the A degradation being first order, the B profile having a maximum, and the C profile having the typical upswing. Burke et al. (1997) reported on the decomposition of theo-m-GLA and found it to be biexponential.

Buur and Bundgaard (1984) and Beal et al. (1993, 1997) reported that the hydrolyses of 3-acetyl- and 3-propionyl-5-FU were biexponential and found that an initial equilibrium of 3-acyl-5FU with O^2-acyl-5FU, which then hydrolyzed to 5-FU, explained this.

4.2. Parallel Reactions

If A can decompose into two species, B and C, then the reactions may be represented by:

$$A \rightarrow B \qquad \text{(rate constant } k_1\text{)} \tag{2.20}$$

and

$$A \rightarrow C \qquad \text{(rate constant } k_2\text{)} \tag{2.21}$$

The rate equation is

$$\frac{dA}{dt} = -k_1[A] - k_2[A] = -(k_1 + k_2)[A] \tag{2.22}$$

Table 2.4 Parallel Reactions (5-azacytosine decomposition)

Time (hours)	5-azacycytosine ($\times 10^4$ Molar)	5-azouracil ($\times 10^4$ Molar)	Nonchromophoric compounds ($\times 10^4$ M)
0	1.65	0	0
0.5	1.4	0.13	0.3
1	1.18	0.20	0.5
1.5	1.00	0.25	0.6
2	0.85	0.27	0.67
2.5	0.72	0.29	0.72

Source: Table constructed from data by Notari and de Young (1975).

which integrates to

$$\ln\left[\frac{A}{A_0}\right] = -[k_1 + k_2]t \tag{2.23}$$

At any given time the (molar) ratio of formation of B and C is given by

$$\frac{[B]}{[C]} = \frac{k_1}{k_2} \tag{2.24}$$

An example of this is shown in Table 4 and Fig. 6.

Other examples are those of Visconti et al. (1984), who have studied the degradation profile of cadralazine in aqueous solution. The reaction consists of four parallel reactions. Fabre et al. (1984) have shown that 3-acetoxymethylcephalosporin, cefotaxime sodium salt, in aqueous solution, decomposes by the scheme

$$\text{Cefotaxime} \xrightarrow{(k_2)} \text{lactone} \xrightarrow{(k_3)} \text{products}$$

$$\text{Cefotaxime} \xrightarrow{(k_2)} \text{products}$$

i.e., a combination of a parallel and a consecutive reaction.

5. EQUILIBRIA

Frequently a reaction will proceed and level off. In such cases there is often an equilibrium:

$$A \longleftrightarrow B \tag{2.25}$$

with an equilibrium constant, K, given by

$$\frac{[B]}{[A]} = K \tag{2.26}$$

Denoting the forward rate constant $k_>$ and the backwards rate constant $k_<$ it follows that when equilibrium has been achieved (at $t = \infty$), the amount going to the right in the reaction must equal the amount going to the left, i.e.

$$k_>[A] = k_<[B] \tag{2.27}$$

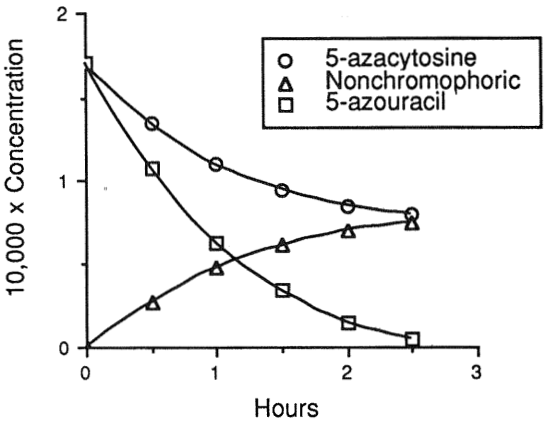

Fig. 6 Example of parallel reactions: decomposition of azacytosine. (Graph constructed from data published by Notari and deYoung, 1975.)

or

$$\frac{k_>}{k_<} = \frac{[\mathrm{B}]}{[\mathrm{A}]} = K \tag{2.28}$$

Denoting by A_∞ the infinity concentration of A (and hence by $A_0 - A_\infty$ the infinity concentration of B), Eq. (2.27) may be written

$$k_>[A_\infty] = k_<[A_0 - A_\infty] \tag{2.29}$$

which may be rewritten

$$A_\infty = \frac{A_0 k_<}{k_< + k_>} \tag{2.30}$$

The rate equation for Eq. (2.25) is

$$\begin{aligned} -\frac{dA}{dt} &= k_>[\mathrm{A}] + k_<[\mathrm{B}] = k_>[\mathrm{A}] + k_<[A_0 - A] \\ &= k_>[\mathrm{A}] + k_<[A_0] - k_<[\mathrm{A}] \\ &= [k_> + k_<]\{A - A_\infty\} \end{aligned} \tag{2.31}$$

which integrates to

$$\ln[A - A_\infty] = [k_> + k_<]t + \ln[A_0 - A_\infty] \tag{2.32}$$

or

$$\ln\left[\frac{A - A_\infty}{A_0 - A_\infty}\right] = -[k_> + k_>]t \tag{2.33}$$

The work regarding the hydrolysis of hydrocortisone butyrate by Yip et al. (1983) is an example of this type of decomposition combined with an $\mathrm{A} \rightarrow \mathrm{B} \rightarrow \mathrm{C}$

Table 2.5 Decomposition of Progabide in pH 1.75 Buffer

Time (min)	Concentration C (Molar)	ln[C-0.0055]
0	0.00896	−5.666
7	0.0076	−6.166
13	0.00700	−6.502
19	0.00660	−6.812
25	0.00612	−7.386
31	0.00600	−7.601

Source: Table constructed from data published by Farraj et al. (1988)

reaction, with the equilibrium occurring between A and B. Ghebre-Sellassie et al. (1984) have described the epimerization of benzylpenicilloic acid in alkaline media and shown it to be an equilibrium between 5R,6R-benzylpenicilloic acid with penamaldic acid (enamine).

Table 5 and Fig. 7 show data by Farraj et al. (1988) showing a leveling effect. If the data in Table 4 represent a simple equilibrium, then the equilibrium level could be obtained by iteration, by assuming different values for the equilibrium level and choosing the one giving best linearity in the form

$$\ln[C_\infty - C] = -kt + \ln[C_\infty - C_0] \tag{2.34}$$

In this case $C = 0.0055$ and the data are plotted in this fashion in Fig. 8, but it should be underscored that they have simply been used as an example. The alkaline hydrolysis of chlorambucil (Owen and Stewart, 1979) is another example of an equilibrium situation.

Beal et al. (1993) tested the hydrolysis of 3-acetyl-5-fluorouracil and showed equilibrium kinetics, as did Prankerd et al. (1992) in the case of rifampicin.

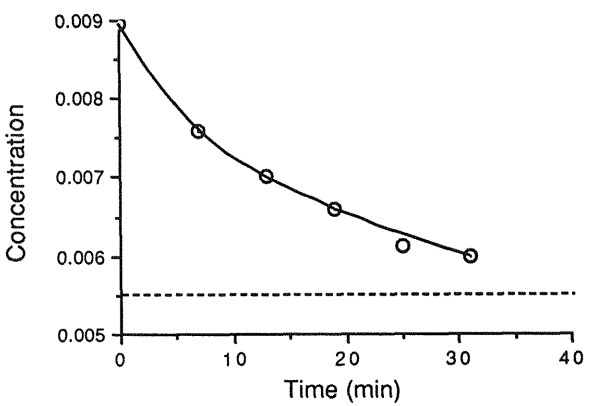

Fig. 7 Decomposition of progabide in pH 1.75 buffer. (Graph constructed from data reported by Farraj et al., 1988.)

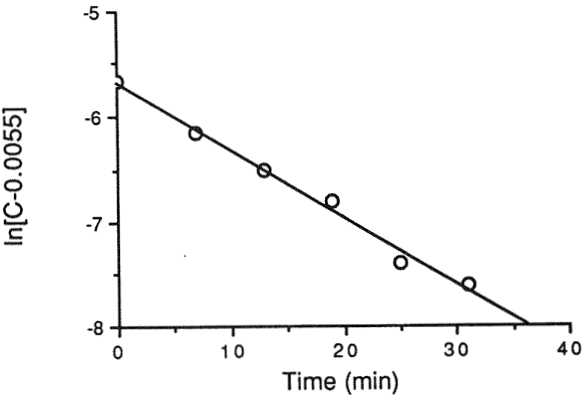

Fig. 8 Data from Fig. 6 treated using a niveau level of $C = 0.0055$. Least squares fit is $y = -0.764 = 0.0127x$ ($R = 0.97$). (Graph constructed from data reported by Farraj et al., 1988.)

5.1. Steady-State Situations

If a situation occurs where $A \rightarrow B \rightarrow C$ and the latter is fast, the kinetics can be simplified by assuming that [B] is "at steady state" throughout the time course. This, obviously cannot be true at the onset. The equations governing this situation are

$$\frac{d[A]}{dt} = -k_1[A] \tag{2.35}$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] = 0 \tag{2.36}$$

$$\frac{d[C]}{dt} = k_2[B] \tag{2.37}$$

where the steady state has been imposed by setting the expression in Eq. (2.36) equal to zero. Hence

$$[A] = A_0 e^{-k_1 t} \tag{2.38}$$

and since it follows from Eq. (2.36) that

$$[B] = \frac{k_1[A]}{k_2} \tag{2.39}$$

then Eqs. (2.38) and (2.39) inserted into Eq. (2.37) give

$$\frac{d[C]}{dt} = k_1 e^{-k_1 t} \tag{2.40}$$

which integrates to

$$C = C_\infty[1 - e^{-k_1 t}] \tag{2.41}$$

i.e., the reaction occurs as if B were not in the picture at all.

In general, if there is such a fast step in the first step of a complex reaction, it is not incorrect to consider it an A–B–C reaction.

The same arguments can be made in the case of A → B → C → D reaction where the B → C was much more rapid than the others, and in such a case it would be justified to think of this as an A → C → D reaction. To be more exacting, a steady-state approach would probably be better. It should be pointed out however, that the steady-state approach is a fundamental approximation, and if it is used, then the reasonableness of the approximation should always be checked.

The steady-state approach is often used, particularly in Michaelis–Menten type kinetics. Here, as an example, let us consider the situation, often occurring, that *many* low level decomposition products are encountered. There are different regulatory views on this, one being that no more than 1% of a product may be formed for it to be considered a minor decomposition product. The situation is hazy, at best, at all times, because often the compounds are unknown. In such cases the "amount" of the decomposition product in the small peak is estimated by the ratio of its area to that of the main peak. But if the decomposition product has a different λ_{max} then this estimate is incorrect, and this is likely to occur if, for instance, in an HPLC setup a single-wavelength UV detector is used.

A well documented and elucidated example is the case reported by Vilanova et al. (1994), who showed in alkaline hydrolysis of cefotaxime the presence of deacetylcefotaxime, the 7-epimer of cefotaxime, the 7-epimer of deacetylcefotaxime, the exocyclic methylene compound, and examine compounds. With such an array of decomposition products, it is important to establish the major products, and treat, in approximation, the decomposition in this light. In the simplest case, cefotaxime shows an A–B–C and A–D–E reaction, with two B curves and three C curves.

Another case that serves as such an example is the case of relaxin oxidation by hydrogen peroxide reported by Nguyen et al. (1993) shown in Fig. 9 where there are two intermediates (B and C) showing maxima and a final product, D, showing the monotonically increasing pattern.

6. PSEUDO-ZERO-ORDER REACTIONS

When only small amounts of decomposition occur, it is difficult to distinguish between zero- and first-order reactions. This is because for small values of x (< 0.15)

$$\ln[1 - x] \approx -x \tag{2.42}$$

where x is the fraction decomposed. If the initial amount of drug substance is A_0, then the fraction decomposed is

$$\frac{A_0 - A}{A_0} = 1 - \frac{A}{A_0} = x \tag{2.43}$$

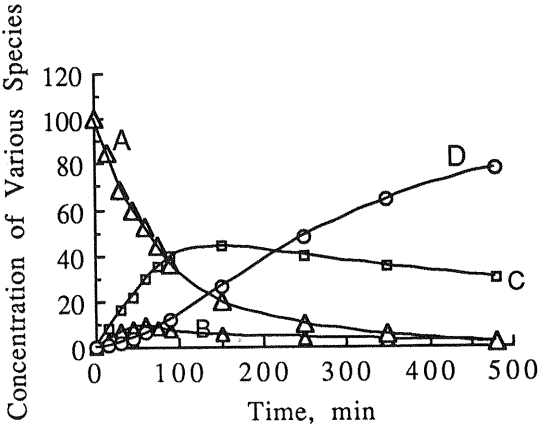

Fig. 9 Decomposition of an A → B; A → C; B → C → D reaction. (Graph constructed from data published by Nguyen et al., 1993.)

or

$$\frac{A}{A_0} = 1 - x \tag{2.44}$$

A first order reaction would require that

$$\ln \frac{A}{A_0} = -kt \tag{2.45}$$

but this may, via Eq. (2.37), be written

$$\ln[1 - x] \approx -x = -kt \tag{2.46}$$

or

$$x = 1 - \frac{A}{A_0} = kt \tag{2.47}$$

which may be written

$$A = A_0 - A_0 kt \tag{2.48}$$

i.e. a zero-order reaction. Since it actually was a first-order reaction [Eq. (2.45)] such a situation is referred to as a pseudo-zero-order reaction.

A set of data is shown in Table 6, treated in zero-order fashion in Fig. 10A and in first-order fashion in Fig. 10B; and it is seen that the fits are comparable. The least squares fit data are shown in Table 6.

It is noted that different time intervals are used for the different temperatures, and it is one of the tasks, before starting studies at higher temperatures, to establish what the time intervals should be. There is no sense in e.g. testing at 3, 6, and 9 months at 55°C, if all the drug is lost after 3 months' storage.

Table 2.6 Assays for an Arrhenius Study

	Potency (°C)				
Months	15	25	37	45	55
0	100	100	100	100	100
3		99.5	98.5	96.5	91
6		99	97	93	83
9		98.5	95.5		
12	99.5	98.5			
18		97.5			
24	99.0	96.5			
36	98.5				
48	98.0				
k_0 %/mo	0.042	0.0017	0.05	1.17	3
$\ln[k_0]$	−3.17	−1.77	−0.69	0.16	1.10

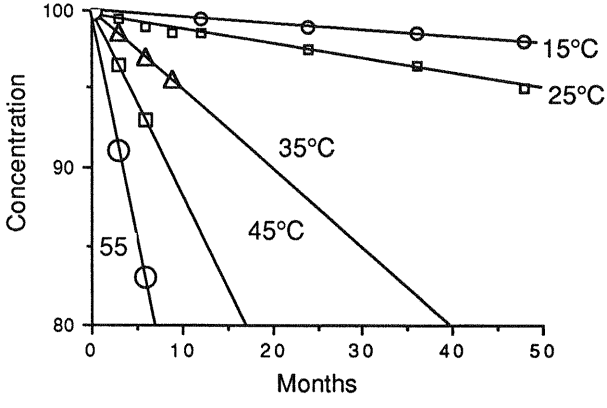

Fig. 10A Data from Table 5 treated by zero-order kinetics.

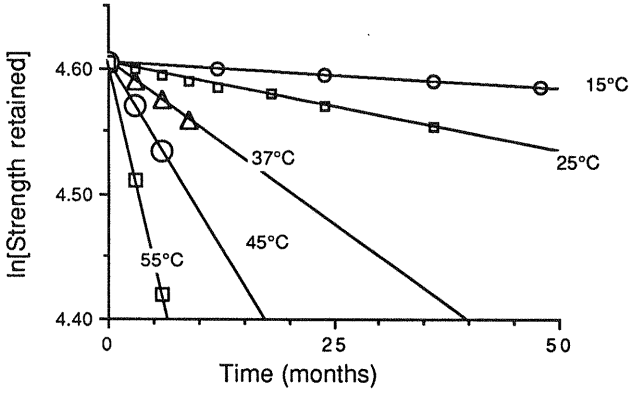

Fig. 10B Data from Table 5 treated by first-order kinetics.

7. THE ARRHENIUS EQUATION

Rate constants are, of course, a function of temperature, and the data shown in Table 5 are graphed in Figs. 10A and 10B.

If rapid results are desired for a given product, it is at times a practice to store it at elevated temperatures. The purpose of this is to force sufficiently large degrees of decomposition in a short time, so that they may be assessed with accuracy. The data in Table 6 are artificially precise, and with a bit of assay error, the 25°C data would not show a discernible loss after 6 months. Is it possible to get some idea of what the loss would actually be, and what it would be after 24 months, without having to wait too long? To get an answer to this (an estimate, not a precise answer) is one of the reasons that Arrhenius plotting is carried out for drug products. The method is actually quite precise in solution systems.

The temperature dependence of a chemical reaction (as long as it is the rate-determining rate constant that is being treated) follows the so-called Arrhenius equation given by

$$\ln[k] = -\frac{E_a}{RT} + \ln[Z] \tag{2.49}$$

or its antilogarithmic form,

$$k = Z \exp\left[-\frac{E_a}{RT}\right] \tag{2.50}$$

where E_a is the activation energy, R is the gas constant, and T is the absolute temperature (°K) obtained by adding 273.15° to the degrees Celcius (Centigrade).

Often the variable $1000/T$ is used (because the numbers then are between 2 and 4 rather than between 0.002 and 0.004 and hence are easier to handle). The slope of a plot according to Eq. (2.42) is still E_a/R, but E_a will now be in kCal (rather than in cal) per degree per mole.

An example of this type of treatment of the first-order data in Fig. 10A is shown in Table 7.

A similar table may be constructed for zero-order treatment, and the graphical presentation is shown in Fig. 10A. When the rate constants are plotted according to Eq. (2.49), then Fig. 11 emerges.

Table 2.7 Least Squares Fit Parameters from Fig. 10B

Temperature °C	Temperature °K	k (mo^{-1})	$1000/T$	$\ln[k]$
15	288.15	0.00042	3.473	−7.783
25	298.15	0.0014	3.356	−6.571
37	400.15	0.0052	3.224	−5.259
45	408.15	0.0118	3.145	−4.440
55	418.15	0.031	3.049	−3.471

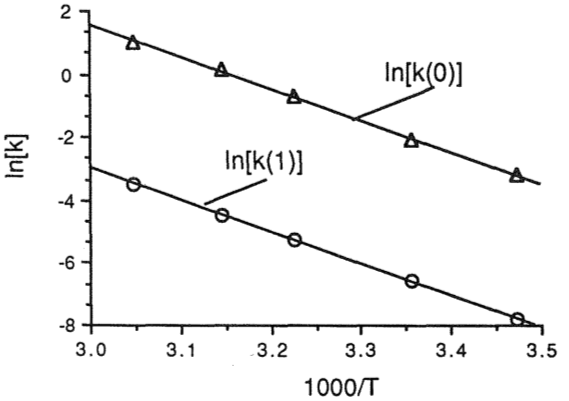

Fig. 11 Data from Figs. 10 A and B treated by the Arrhenius equation.

The Arrhenius equations for the two types of plotting are

$$\ln[k_0] = 31.63 - 10.03(1000/T) \tag{2.51}$$

$$\ln[k_1] = 27.631 - 10.2(1000/T) \tag{2.52}$$

It is noted that both plotting modes give good results and about the same activation energy. Using a value of $R = 1.99$ cal/degree-mole gives an activation energy of about 20 kCal/mole. *This is quite a common activation energy for many reactions.*

If only the data at the three high temperatures had been present, they could have been plotted as in Fig. 11 and extrapolated to 25°C (where $1000/T = 3.356$). Inserting this into Eq. (2.49) gives

$$[k_{25}] = 27.631 - 10.2 \times 3.356 = -6.601 \text{ or } [k_{25}] = 0.00136$$

which is close to the value in Table 7.

One can now construct a curve, as shown in Fig. 12, which is an extrapolated curve. The data points from Table 6 are shown for comparison. In general, extrapolations are not that good, but in solution systems they frequently approximate the curve that, after time has elapsed, is the actual curve. A case in point is flurogestone decomposition at pH 7.3 reported by Kabadi et al. (1984).

Arrhenius plotting can also be carried out by using t_{90} data. Since $k_1 t_{90} = -0.105$ it follows that

$$\ln[k_1 t_{90}] = \ln[t_{90}] + \ln[k_1] = \ln[t_{90}] - \frac{E_a}{RT} + \ln[Z] = -0.105$$

or

$$\ln[t_{90}] = \frac{E_a}{RT} - \ln[Z] - 0.105 \tag{2.53}$$

i.e. a plot of $\ln[t_{90}]$ versus $1000/T$ will be linear and the slope will be E_a/R, where E_a will be in kCal (rather than in cal) per degree per mole.

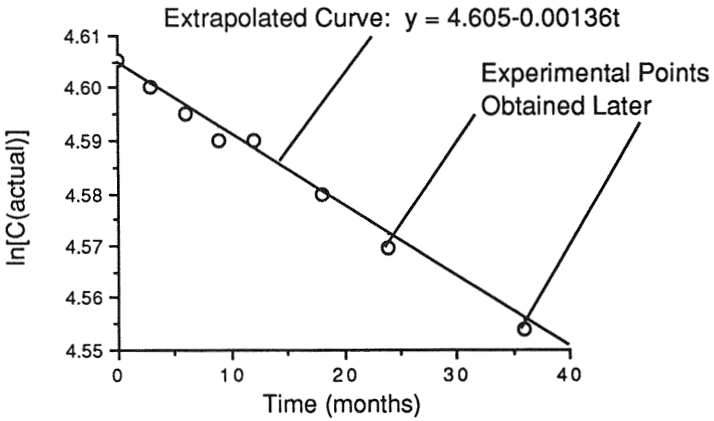

Fig. 12 Extrapolated decomposition curve of a solution obtained (e.g., in January 1993 after three months accelerated data) compared with the actual data accumulated in time and plotted 33 months later (i.e., in October 1995).

Table 2.8 Data from Table 6 Plotted by the t_{90} Method

Temperature	$1000/T$	k_1 (mo^{-1})	t_{90} (months)[a]	$\ln[t_{90}]$
55°C	3.049	0.031	3.40	1.233
45°C	3.145	0.0118	8.93	2.189
37°C	3.224	0.0052	20.26	3.009

[a] Calculated from k_1 values from the equation $kt_{90} = -0.1054$.

Table 8 shows the data from Tables 6 and 7 plotted by the t_{90} method. The data are shown in Fig. 13. The least squares fit equation in Fig. 13 is $\ln[t_{90}] = -29.695 + (10.142/T)$, and it is noted that the slope (activation energy) is the same as in Fig. 11 (where the slope is 10.153). The small difference lies in rounding off errors in calculating t_{90} from the k_1 values.

The t_{90} value at room temperature is determined to be 4.072 [Eq. (2.53)]. If plotted on semilogarithmic paper, the value will emerge directly, and this type of plotting is more easily understood by those not familiar with kinetics than plots of the type in Fig. 11.

7.1. Cyclic Testing

One advantage of testing at higher temperatures is that it is possible to construct decomposition profiles at nonconstant temperature. It should be pointed out that extended room temperature is defined in the USP, 1990, as between 15 and 30°C but is now 15–25°C. Stability studies are usually carried out isothermally, e.g., as mentioned, at 25°C. The rationale for testing temperatures will be discussed at a later point in the book; suffice it to say at this point that a product in the marketplace will never experience an isothermal shelf history.

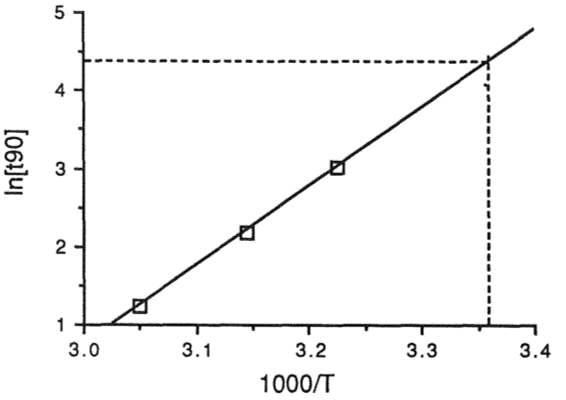

Fig. 13 Arrhenius plotting using t_{90}.

At best there will be daily fluctuations. This concept was investigated by Carstensen and Rhodes (1986), in the following fashion. Suppose a product is stored at 25°C with daily fluctuations of ±5°C. This means that the dependence of T on time, t (in days, if the cycle is one day), is given by

$$T = T_1 + T_2 \sin(2\pi t) \tag{2.54}$$

This is shown in Fig. 12.

Assume, as well, that the reaction is zero order, i.e.

$$C = C_0 - kt \tag{2.55}$$

Introducing Eqs. (2.50) and (2.54) into this and applying the situation to a differential time element dt, gives

$$dC = \left[Z \exp\left[-\frac{E}{R\{T_1 + T_2 \sin(2\pi t)\}}\right]\right] dt \tag{2.56}$$

so that to obtain the concentration after a time period t of cyclic storage, where $0 < t < 1$ day, is given by

$$C = C_0 - \int_0^t Z \exp\left(-\frac{E}{(R\{T_1 + T_2 \sin(2\Pi t)\}}\right) \tag{2.57}$$

These types of integrals can be solved by computer programs. The loss in e.g. one year will be 360 times that after one day, and so on.

The authors calculated the loss after 3 years of storage, using different activation energies, and using k_{25} (isothermal) = 0.01%, and using a daily cycle with a fluctuation of ±5°C. They arrived at the results in Table 9.

It is seen (Table 9, Fig. 14) that as long as the activation energy is less than 22 kCal per mole, the percent increase in the amount lost after a given storage period is less than 10%. For example, if a dosage form lost 5% after 3 years storage at static room temperature, it would lose 5.5% after 3 years of cyclic room temperature. This point will be of importance in the following section.

Table 2.9 Cyclic Versus Constant 25°C Data. $k_{25} = 0.01\%$ Per Day

E, kCal per mole	Loss after 3 years	Percent increase in loss
10	11.16	1.9
15	11.38	3.9
20	11.77	7.5
25	12.27	12.1
30	12.89	17.7

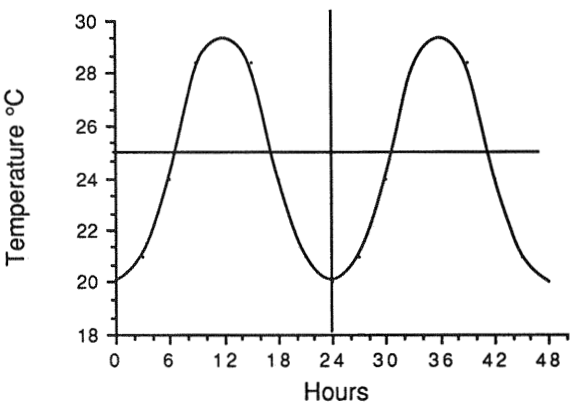

Fig. 14 Daily temperature fluctuations according to Eq. (2.54).

It is noted that any type of cycle can be used, e.g. seasonal cycles could be used as well. The problem of cyclic testing (for chemical stability) is raised from time to time. But to decide on a cycle is difficult, and it is much more rational to use the data from accelerated studies to produce the desired profile.

7.2. Nonisothermal Kinetics

It is possible, rather than studying a reaction at a fixed temperature (isothermally), to vary the temperature in a given fashion, and fit the data to Eq. (2.50).

For a zero-order reaction we can write

$$\frac{C_0}{C} = kt = \left[Z \exp\left(-\frac{E_a}{RT} \right) \right] \cdot t \tag{2.58}$$

We may allow T to vary in a given manner, e.g., in the simplest case as

$$\frac{1}{T} = a - bt \tag{2.59}$$

where a and b are the constants that we input into a programmable temperature

Table 2.10 Alkaline Decomposition of Riboflavin by Isothermal and by Several Nonisothermal Programs

Temperature program	Rate constant at 25°C	Activation energy (kcal/mole)	Reference
Isothermal	0.016	19.2	Cole and Leadbeater (1966)
Linear up	0.014	20.1	Guttman (1962)
Linear up	0.016	20.3	Rosenberg et al. (1984)
Log up	0.018	17.9	Madsen et al. (1974)
Log up[a]	0.015	20.9	Rosenberg et al. (1984)
Log down	0.015	18.9	Rosenberg et al. (1984)

[a] Triplicate experiments.
Source: Table constructed from data published by Rosenberg et al. (1984).

bath. By inserting Eq. (2.59) into Eq. (2.58), the concentration profile in time becomes

$$\frac{C_0}{C} = Z\exp\left[-\frac{E_a}{R}(a - bt)\right] \tag{2.60}$$

or, logarithmically,

$$\begin{aligned}\ln\left[\frac{C_0}{C}\right] &= \ln[Z] - \frac{E_a}{R}(a - bt) \\ &= \left[\ln\{Z\} - \frac{aE_a}{R}\right] + \left(\frac{bE_a}{R}\right)t\end{aligned} \tag{2.61}$$

This gives rise to a linear plot when $\ln[C_0/C]$ is plotted versus t, and (since a and b are the constants for the program we have chosen for our temperature bath), the slope (divided by b/R) will give us the value of E_a; and now $\ln[Z]$ can be obtained from the intercept.

The same procedure can be used for first-order reactions, although they are somewhat more complicated. In such a case a computer program is best, and curves can be generated to match the curve obtained experimentally.

Table 10 shows some of the investigations that have been carried out in the last 20 years, using this procedure.

7.3. Kinetic Mean Temperature

Since stability studies carried out in an industrial setting are isothermal, there has been a fair amount of discussion over the last 2 decades as to what the actual temperature of the study ought to be. Up to 1993, the FDA required stability studies to be carried out at 30°C for the approval of expiration periods. In contrast, the European community would consider the United States as an area where 25°C would be the appropriate temperature to require for isothermal testing.

The resolution of the problem in Europe came from by Futscher and Schumacher (1972), who established the climate zones, and Wolfgang Grimm (1985,

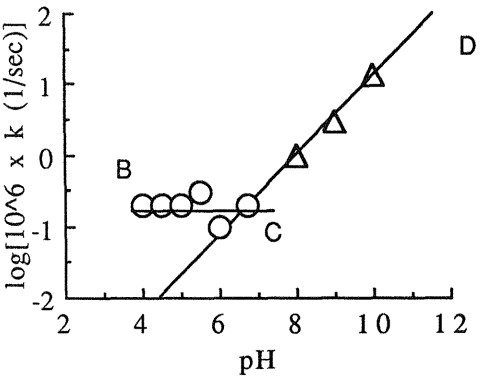

Fig. 25 Subtype BCD. Metranidazole in aqueous solution. (Graph constructed from data published by Wang and Yeh, 1993.)

Oxidation of captopril (Timmins et al., 1982; Kadin, 1982), pH 2–5, is of this type, but the slope is not −1 as in the decomposition of zenarestat 1-*O*-acylglucoronide in water at 37°C at pH 5, 6, 7.4, and 7.8, reported by Tanaka and Suzuki (1994).

Morphine oxidation (which may be better described by BCDE) is of this type and is described by

$$M + O_2 \rightarrow \text{products (rate constant } k_1) \tag{3.198}$$

$$MH^+ + O_2 \rightarrow \text{products (rate constant } k_2) \tag{3.199}$$

Echothiophate iodide (Hussain et al., 1968) pH 2–12, is of this type with slope 1 and is described by

$$k_{obs} = k_-[OH^-] + k_1 \tag{3.200}$$

Hydrocortisone-21-lysinate (Johnson et al., 1985) is of this type.

9.17. Subtype HJDE

Lee and Querijero (1985) report the rotational isomerism equilibration of *N*-thionaphthoyl-*N*-methyl glycinate to be of this type (schematized in Fig. 26).

This could typically be two noncatalytic reactions of two species of drug and one hydroxyl ion catalyzed reaction. Simpler systems exist, as will be seen in the following.

Methenamine (Tada, 1960) exhibits this type of profile, which may be ascribed to

$$BH^+ \rightarrow \text{products} \tag{3.201}$$

$$BH^+ + H^+ \rightarrow \text{products} \tag{3.202}$$

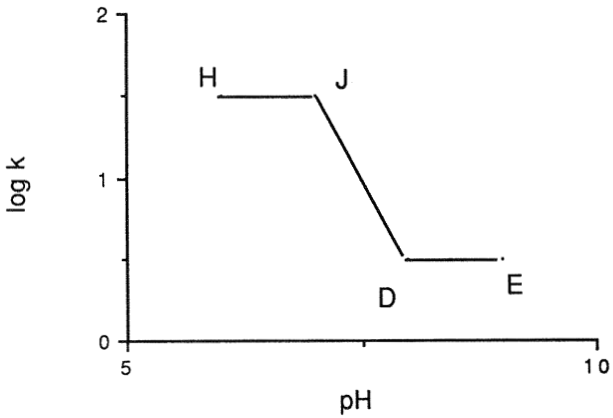

Fig. 26 Subtype HJDE. Units on both axes are arbitrary, and the graph is intended to be an *example* only.

i.e., with an observed rate constant of

$$k_{obs} = k + f_{HB} + [H^+] + k_1 f_{HB+} \tag{3.203}$$

Cycloserine (Kondrat'eva et al., 1971) follows this type of profile and is attributed to:

$$AH^{2+} \rightarrow \text{products} \tag{3.204}$$

$$AH \rightarrow \text{products} \tag{3.205}$$

$$A^{=} \rightarrow \text{products} \tag{3.206}$$

with an observed rate constant of

$$k_{obs} = \frac{k_{++}[H^+]^2 + k_+ K_{a1}[H^+] + k_1 K_{a1} K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1} K_{a2}} \tag{3.207}$$

Mitomycin C (Underberg and Lingeman, 1983 a,b) decomposition involves

$$AH^{2+} + H^+ \rightarrow \text{products} \tag{3.208}$$

$$AH + H^+ \rightarrow \text{products} \tag{3.209}$$

$$A \rightarrow \text{products} \tag{3.210}$$

$$A^- + OH^- \rightarrow \text{products} \tag{3.211}$$

9.18. Subtype HJDEF

Hou and Poole (1969a,b) have shown that ampicillin, in solution, exhibits this type of pH profile.

9.19. Subtype HJKLM

Van der Houwen et al. (1997) reanalyzed data published by Quigley et al. (1994) and showed propanolol acetate to be of this type or of type HJKLM. Van der Houwen et al. (1997) reanalyzed data published by Tu et al. (1989, 1990) and showed metronidazole to be of this type or of type HJKLM.

9.20. Subtype JDEN

Oliiyai and Borchardt (1994) have shown the pH profile for Val-Tyr-Pro-Asp-gly-Ala hexapeptide to be of this type.

9.21. Subtype JDENOP

Carbenicillin (Zia et al., 1974; Hou and Poole, 1969a,b, 1971) exhibits this type of profile as does ampicillin (Hou and Poole, 1969a,b). The latter is attributed to noncatalyzed and to general acid base catalyses of the two charged species of the drug, i.e., the set of reactions

$$AH_2 + H^+ \rightarrow \text{products} \tag{3.212}$$

$$AH_2 \rightarrow \text{products} \tag{3.213}$$

$$AH^- + H^+ \rightarrow \text{products} \tag{3.214}$$

$$A^- \rightarrow \text{products} \tag{3.215}$$

$$A^- + OH^- \rightarrow \text{products} \tag{3.216}$$

and the rate becomes

$$\begin{aligned} k_{obs} = {} & k_{1+}[H_2A+][H^+] + k_{2+}[HA^{\pm}][H^+] \\ & + k_3[HA^{\pm}] + k_4[A^-] + k_-[A][OH^-] \end{aligned} \tag{3.217}$$

Methotrexate (Chatterji and Galleli, 1978) has this type of profile and the rate equation is

$$\text{rate} = k_+[H^+] + k_1[HA] + k_-[OH^-][A^-] + k_2[A^-] \tag{3.218}$$

Carney (1988) reports closidomine to have a pH profile of this type. Kenley and Warn (1994) have reported on the acid catalyzed peptide bond hydrolysis (at 50°C) of recombinant human interleukin 11, as a profile to type JDENOP as shown in Fig. 27. (It should be pointed out that the authors consider this simply as ABCD profile, which indeed it may be.)

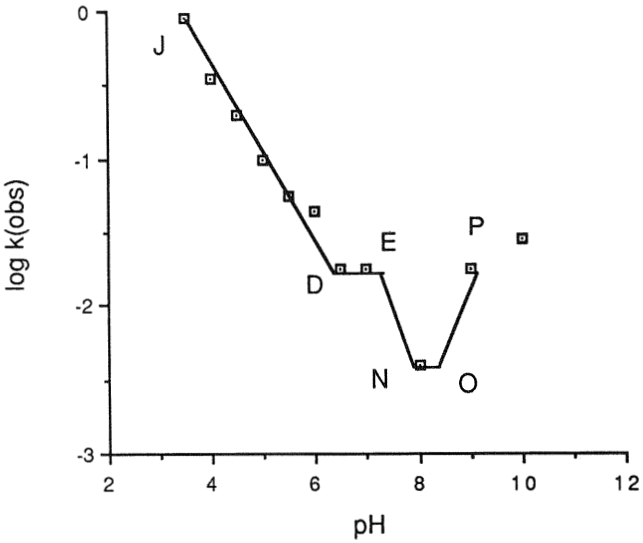

Fig. 27 JDENOP profile. (Graph constructed from data reported by Kenley and Warne, 1994.)

Ito et al. (1997) have shown that panipenem is first-order degraded in aqueous solution and that the rate constants follows this profile. They further demonstrated a lack of ionic strength effect.

10. EFFECT OF CONCENTRATION

Kinetic studies are usually carried out in quite dilute solutions. When solution kinetics are carried out as support for solid state stability work, then saturated solutions are employed. Here the pH profile may be quite different. Pralidoxime for instance at pH 1–3 exhibits a type AH profile with unity slope (Fyhr et al., 1986) as opposed to what was reported earlier, and aspirin (Carstensen and Attarchi, 1988a, 1988b; Carstensen et al., 1985) has a second-order hydrolysis rate constant virtually independent of acetic acid concentration from quite dilute to concentrated. *p*-aminosalicylic acid (Carstensen and Pothisiri, 1975) also exhibits a different kinetic profile in saturated solution.

As will be seen in the chapter dealing with disperse systems, drugs that form micelles will show, in general a shift in mechanism and rate above the critical micelle concentration.

11. EFFECT OF SOLVENT

It stands to reason that solvent will have an effect on the hydrolysis rate. Carstensen et al. (1971) demonstrated the effect of dielectric constant on the hydrolysis rate of benzodiazepines, and Hussain and Truelove (1979) showed the effect of dielectric constant on the hydrolysis rate of 2-tetrahydropyranyl benzoate (Fig. 28).

As mentioned, Brandl and Magill (1997) have reported on the pK values of ketorolac in isopropanol. They also showed the decomposition to be first order

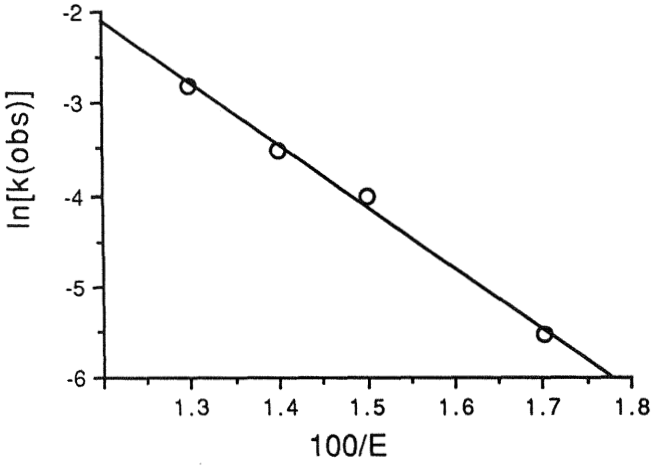

Fig. 28 Effect of dielectric constant on the hydrolysis rate of 2-tetrahydropyranyl benzoate. Least-squares fit $y = 5.98 - 6.74 \times (R = 1.00)$. (Graph constructed from data by Hussain and Truelove, 1979.)

in 35% isopropanol, although the acid form exists in equilibrium with its isopropyl ester and with its sodium salt.

Hou Poole (1969a,b) showed a linear effect of the reverse of the dielectric constant on the log of the rate constant for decomposition of ampicillin in solution.

12. KINETIC SALT EFFECT

Kinetic studies in solutions, as have been shown, should be carried out for at least two buffer concentrations, so that the pH profile can be constructed without confounding it with the catalytic effect of the buffer components. At times, however, the mere presence of an ionized substance may affect the kinetics of decomposition.

To investigate this, the ionic strength is varied by carrying out the decomposition in solutions of different concentrations of inert electrolyte. As mentioned, the definition of ionic strength is

$$\mu = 0.5 \cdot [\Sigma m_i z_i^2] \tag{3.219}$$

where m_i is the molarity of the ith species and z_i is its charge. One molar $CaCl_2$, for instance would be one molar in calcium ion and two molar in chloride ion, so that the ionic strength would be $[(1 \cdot 2^2) + (2 \cdot 129; 1^2)]/2 = 3$. For a 1:1 electrolyte the ionic strength will of course be the same as its molarity.

The decomposition reaction for a drug substance (A) interacting with an ionic solute (B) can be described as

$$A + B \rightarrow [AB^f] \rightarrow \text{products} \tag{3.220}$$

where $[AB^f]$ denotes a transition complex. In this case, the rate constant k, will be dependent on the ionic strength in concentrations below 0.01 M by the relation

$$\log k = \phi + 2.Q.z_A \cdot z_B \cdot (\mu^{1/2}) \tag{3.221}$$

where z denotes charge, and the vale of Q is given by

$$Q = 1.825 \cdot 10^6 \cdot \left[\frac{\rho}{(T\varepsilon)^3}\right]^{1/2} \tag{3.222}$$

where T is absolute temperature, ϵ is the dielectric constant of the solution, and ρ is its density. It is noted that at 25°C in aqueous solutions, the value of $2Q = 1.018$. This equation can only be expected to hold in the very dilute concentration range, and at higher concentrations of solute (up to ionic strengths of 0.1 molar), the Güntelberg equation rather than the Debye–Hückel equation is employed:

$$\log f_i = \frac{Q \cdot z_i \cdot (\mu^{1/2})}{1 + \beta(\mu^{1/2})} \tag{3.223}$$

where β is often 1, where i denotes a central ion and z_i its charge, and where Q is a constant. Figure 29 shows data by Wang and Yeh (1993) obtained by using Eq. (3.223). Brooke and Guttmann (1968) have found such linearity (but with a negative slope) for the decomposition of 3-carbomethoxypyridinium cation. The slope depends on whether the interacting ions are of the same sign or of opposite sign, as shown in Eq. (3.224).

Eq. (3.223) (Carstensen, 1970) when applied to rate constants, becomes

$$\log k = \phi + \frac{2 \cdot Q \cdot z_A \cdot z_B \cdot (\mu^{1/2})}{1 + \beta(\mu^{1/2})} \tag{3.224}$$

where β is a constant related to the ionic diameters of the solutions. Curve fits are relatively insensitive to the size of β, which is most often close to 1, so that most authors (e.g., Czapski and Schwarz, 1962) make this assumption—in fact the original Güntelberg equation made this assumption.

At even higher ionic strength the relationship becomes

$$\log k = \log k_0 + q \cdot \mu \tag{3.225}$$

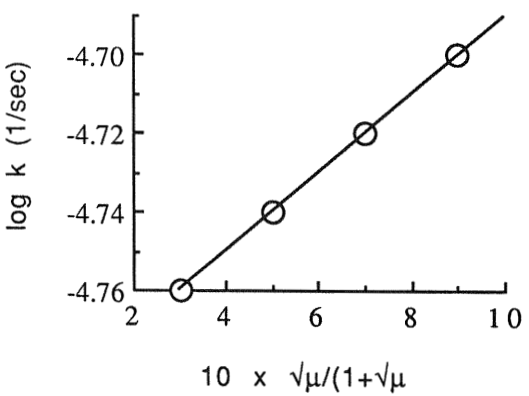

Fig. 29 Degradation metronidazole at pH 7.4. (Graph constructed from data published by Wang and Yeh, 1993.)

It is noted that in Eqs. (3.221) and (3.224) there will be a nonzero kinetic salt effect only if both A and B are charged. If either is uncharged, then the effect is zero. This is a useful tool, mechanistically, since it allows assessment as to the reaction species. If, for instance, a drug that can be either HA or A^- interacts with a species B^-, then a zero kinetic salt effect rules out $A^- + B^-$ as a possibility.

It is not to be expected that the slope of the kinetic salt effect lines will have the predicted slope. Since both species interacting must be charged, it follows that, for an acid for instance, the effect for HA is zero. The effect for A^- would be -1 if it were $[H^+]$ catalyzed and plus one if it were $[OH^-]$ catalyzed. However, depending on the pH, only part of the drug substance would be present as A^-. If this fraction is calculated, then the slope should equal either plus or minus that fraction.

There have been a small number of reports on kinetic salt effects in the pharmaceutical literature. Brooke and Guttmann (1968) reported on 3-carbomethoxy-1-methylpyridinium cation decomposition, Hussain et al. (1968) reported on echothiophate, Felmeister et al. (1965) on chlorpromazine, Finholt et al. (1962, 1963, 1965) on penicillin, Koshy and Mitchner (1964) on 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone, Szulczewski et al. (1964) on 4,6-diamino-1-(3,5)-dichlorophenyl-1,2-2-2-dimethyl-1,3-5-triazine, Windheuser and Higuchi (1962) on thiamine, Finholt and Higuchi (1962) on niacinamide, and McRae and Tadros (1978) have shown the kinetic salt effect on triclofos hydrolysis rates. Hashimoto et al. (1984) have reported a kinetic salt effect for the empimerization of moxalactam. The concentration range is, however, too high for Eq. (3.209) to hold, and it is possible that it is actually Eq. (3.210) that holds.

Kirsch and Notari (1984) have discussed the effect of the ionic atmosphere on the relative amount of general base attack and how and to what extent this can influence the kinetic salt effect.

The best presentation mode for kinetic salt data are in a form exponential to the forms stated in Eq. (3.224). This is repeated here for convenience:

$$\frac{\ln[k]}{2.303} = \phi + \frac{2 \cdot Q \cdot z_A \cdot z_B.(\mu^{1/2})}{1 + \beta(\mu^{1/2})} \tag{3.226}$$

The traditional conversion from non-Briggsian to natural logarithms has been carried out and the exponential form of this is

$$k = \exp[2.3\phi] \exp \frac{2 \cdot Q \cdot z_A \cdot z_B.(\mu^{1/2})}{1 + \beta(\mu^{1/2})} \tag{3.227}$$

An example of this is shown in (Fig. 30).

The advantage in this presentation mode is that it is not biased. On the contrary, lest-squares fitting of a log-square root plot would be biased because neither x and y values would be centered about their means.

As mentioned, the kinetic salt effect depends on the charges of the interacting ions, and if one of these is zero, then there is no such effect. For instance, Ito et al. (1997) showed that degradation of panipenem in aqueous solution exhibited first-order rate constants that showed a lack of ionic strength effect.

As mentioned, Brandl and Magill (1997) have reported on the pH values of ketorolac in isopropanol. They also showed the decomposition to be first order

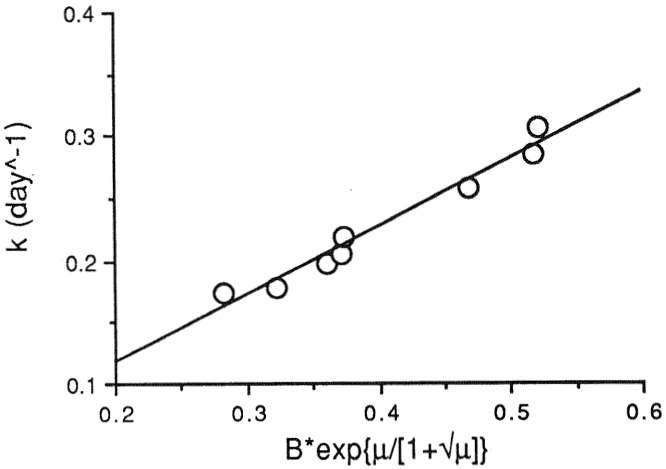

Fig. 30 Best presentation mode for kinetic salt effect data in the intermediate range. Least-squares fit is (with $R^2 = 0.967$) $k = 0.0085 + 0.55B\exp[\mu^{1/2}/(1 + \mu^{1/2})]$. (Plot constructed from data published by Franchini, 1992.)

in 35% isopropanol, although the acid form exists in equilibrium with its isopropyl ester and with its sodium salt.

Hou and Poole (1969a,b) showed a linear effect of the reverse of the dielectric constant on the log of the rate constant for decomposition of ampicillin in solution.

There are other, both recent and older, references to the kinetic salt effect. Hou and Poole (1969a,b) have shown that ampicillin is subject to a kinetic salt effect, which differs, based on the pH of the solution. Since a positive effect exemplifies reaction between charged species, they were able to establish a reaction mechanism. van Maanen et al. (1999) have demonstrated a kinetic salt effect in the aquous solution decomposition of thiotepa.

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4

Oxidation in Solution

JENS T. CARSTENSEN

Madison, Wisconsin

1. OXIDATIVE REACTIONS

The 1987 Stability Guidelines state that "It is also suggested that the following conditions be evaluated in stability studies on solutions or suspensions of the bulk drug substances.... High oxygen atmosphere."

Oxidation reactions are relatively rare in pharmaceutical dosage forms as a main reaction. Some oxidation probably takes place in many cases and results in small amounts of unidentified degradation products. When an oxidation reaction is one of the main reactions, then it is a serious matter, and formulating around such a problem can be difficult. Notable examples are liquid vitamin A products (although, strictly speaking, they are not solutions). Other examples will be cited shortly.

1.1. Oxidation Mechanisms

Oxidation, as the word implies, is an interaction between drug substance, A, and oxygen, and the net reaction would be

$$A + O_2 \rightarrow \text{products} \tag{4.1}$$

However, oxidation reactions are usually the sum of a series of reactions (at times chain reactions), and these start with one particular reaction (the initiation reaction), which usually does not involve molecular oxygen. Frequently, oxidations are catalyzed by metal ions M^{++}; an example of this is the oxidation mechanisms reported for captopril (Timmins et al., 1982). Captopril contains a thiol group and will be symbolized as ASH below:

$$2ASH + 2M^{++} \rightarrow 2\{AS\cdot\} + 2H^+ + 2M^+ \tag{4.2}$$

$$2\{AS\cdot\} \rightarrow AS \cdot SA \tag{4.3}$$

$$2M^+ + O_2 \rightarrow 2M^{++} + O_2^{2-} \tag{4.4}$$

$$O_2^{2-} + 2H^+ \rightarrow H_2O + 0.5O_2 \tag{4.5}$$

where $\{\cdot\}$ denotes free radical. Hence, the overall reaction is

$$2ASH + 0.5O_2 \rightarrow AS \cdot AS + H_2O \tag{4.6}$$

It is noted that there is no net consumption of metal ion. (This latter has been chosen as being divalent in the above but could have other valences depending on metal in question). There is, however, a consumption of oxygen (otherwise there would be no oxidation). Upon exhaustion of oxygen, the oxidation would cease.

If oxygen is abundant, then of course all the drug can decompose, and in this case, as will seen below, the kinetics can be simply first order, since $[O_2]$ becomes (virtually) constant.

When captopril decomposes without presence of metals, it undergoes an autooxidation:

$$2ASH \leftrightarrow 2AS^- + 2H^+ \tag{4.7}$$

$$2As^- + 2O_2 \rightarrow 2\{AS\cdot\} + 2\{O_2\cdot\}^- \tag{4.8}$$

$$2As^- + 2\{O_2\cdot\} \rightarrow 2\{AS\cdot\} + 2O_2^{2-} \tag{4.9}$$

$$2\{AS\cdot\} \rightarrow ASSA \tag{4.10}$$

$$O_2^{2-} + 2H^+ \rightarrow H_2O + 0.5O_2 \tag{4.11}$$

So the total reaction is

$$2ASH + 0.5O_2 \rightarrow ASSA + H_2O \tag{4.12}$$

Most oxidations in pharmaceutics occur in aqueous solution and are a function of the oxygen dissolved in the aqueous phase. In practice it is desired to minimize the decomposition, and it is a practice to remove oxygen from such systems by nitrogen flooding. Depending on the method used for the removal there can still be quite a bit of oxygen left in solution. Simple flooding is not effective, and to remove oxygen completely by boiling is rather difficult, and since oxygen has a low molecular weight, it can still be left present in sufficient (molar) excess to allow considerable oxidation. Hence, to free solutions of oxygen, nitrogen should be bubbled through them (the very best way of driving out dissolved oxygen). The use of antioxidants (bisulfite, ascorbic acid) is advocated where possible, in the case of oxygen-sensitive drug substances in solution.

In practice, complexing the heavy metals (e.g., by use of ethylene diamine tetraacetic acid) is often employed, since, as seen in the reactions sequences shown, metal ions are deleterious to drug stability in oxidative situations.

1.2. Quantitative Considerations in Oxidations in Two Phases

Most oxidation problems in the pharmaceutical sciences occur in systems (liquid or solid) that are in contact with a gas phase (usually air) that contains oxygen. As far as the methodology for following a decay, the monitoring of parent compound and decomposition products is what is usually carried out. If it is necessary to monitor oxygen concentrations, then Winkler's method (Winkler, 1888; Novaczyk et al., 1993) may be used, although it is cumbersome. Oxygen electrodes can also be used, but these are only good below 45°C. Finally, potentiometric titrations are possible. For work in the gas phase, Raman spectroscopy can be used. Most of what follows will concentrate on the disappearance of the parent drug.

Henry's law states, in the case of oxygen/water systems, that

$$P_{O_2} = K' X_{O_2} \approx K^* C \tag{4.13}$$

where C is the molar concentration and X the mole fraction of dissolved oxygen; K and K^* are Henry's law constants. For ordinary atmospheric conditions, $P_{O_2} = 0.22$ atm. At 25°C the value of K' for oxygen is $3.314\ 10^7$ atm, i.e., the mole fraction of oxygen dissolved water would be $0.22/(3.3 \times 10^7) = 6.6 \times 10^{-9}$. The molar concentration corresponding to this is given by

$$X_{O_2} = \frac{C_{O_2}}{55.5 + C_{O_2}} \approx \frac{C_{O_2}}{55.5}$$

i.e., C_{O_2} is about 3.7×10^{-7} molar. Equation (4.13) is often expressed in inverse form, i.e.,

$$C = KP \tag{4.14}$$

where $K = 1/K^*$; both presentation forms will be used in the following.

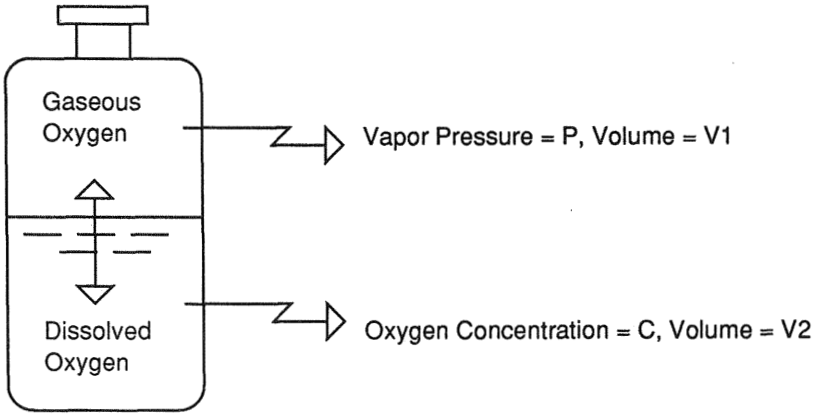

Fig. 1 Nomenclature used for kinetic schemes.

If a solution of a drug substance (A) is oxygen sensitive, then

$$A + O_2 \rightarrow B \tag{4.15}$$

where B represents oxidation products. In the first case considered, it is assumed that the system is closed (Fig. 1), that the head space is V_1 cm^3, and that the liquid volume is V_2 cm^3. The total number of moles of oxygen in the ensemble is the number of moles, n_1, in the gas phase plus the number of moles, n_2, dissolved.

The gas law gives the value for n_1 as

$$n_1 = \frac{PV_1}{RT} \tag{4.16}$$

where P in the following is understood to be the oxygen pressure in the atmosphere.

$$n_2 = V_2 C \tag{4.17}$$

so that the total number of moles, N, is given by

$$N = n_1 + n_2 = \frac{PV_1}{RT} + V_2 C \tag{4.18}$$

Introducing Eqs. (4.13) and (4.16) gives

$$N = \frac{PV_1}{RT} + V_2 K^* P = P\left(\frac{V_1}{RT} + V_2 K^*\right) \tag{4.19}$$

The oxidation is (a) simple or (b) catalytic or (c) autocatalytic.

1.3. Simple Oxidation in Solution in a Closed System

The simple case will be treated first (Franchini et al., 1993; Franchini and Carstensen, 1994). It follows from the general second-order reaction equation that

$$\frac{d[\mathrm{A}]}{dt} = -k_2[\mathrm{A}]C \tag{4.20}$$

where [A] is concentration of drug, k_2 is the second-order rate constant, and C is the oxygen concentration in solution at time t. If the initial concentrations are denoted by subscript zero, and the concentration of decomposition product is Y molar at time t, then

$$[\mathrm{A}] = A_0 - Y \tag{4.21}$$

and

$$d[\mathrm{A}] = -dY \tag{4.22}$$

so that now

$$\frac{dY}{dt} = k_2[A_0 - Y]C = k_2 A_0[1 - g \cdot Y]C \tag{4.23}$$

where

$$g = \frac{1}{A_0} \tag{4.24}$$

The number of moles decomposed is $V_2 Y$, so that the total number of oxygen molecules, $N(t)$, at time t is given by

$$N(t) = N_0 - V_2 Y \tag{4.25}$$

It is assumed that the distribution of oxygen between head space and liquid is rapid, so that at time t,

$$C = K P(t) \tag{4.26}$$

where $P(t)$ is the oxygen pressure remaining at time t. Mass-balancing of total oxygen gives

$$V_2 C + \frac{V_1 P(t)}{RT} = N_0 - V_2 Y \tag{4.27}$$

Introducing Eq. (4.26) into this gives

$$V_2 C + \frac{V_1 C}{RTK} = N_0 - V_2 Y \tag{4.28}$$

Solving for C gives the expression

$$C = q[1 - aY] \tag{4.29}$$

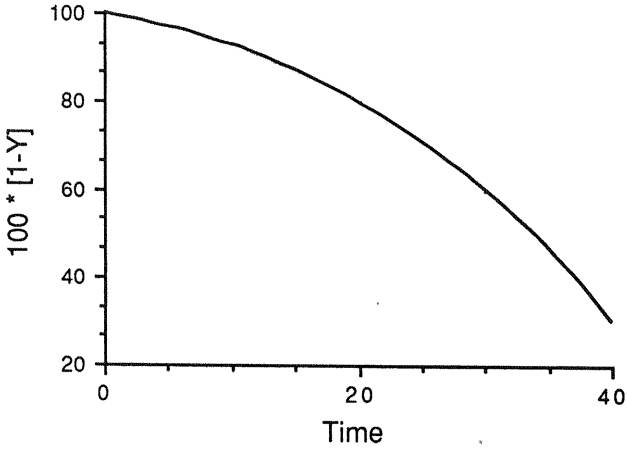

Fig. 2 Profile of $[1 - Y]$ versus t according to Eq. (4.32).

where

$$q = \frac{N_0}{V_2 + V_1/RTK} \tag{4.30}$$

and

$$a = \frac{V_2}{V_2 + V_1/RTK} \tag{4.31}$$

Introducing this into Eq. (4.23) gives

$$\frac{dY}{dt} = q \cdot k_2 A_0 [1 - gY][1 - aY] \tag{4.32}$$

This equation can be solved (in closed form as well as by graphical integration) and gives curves of the type shown in Fig. 2. Examples of this type of curve are the oxidation of promethazine at pH 3.2 (Underberg, 1978) and the oxidation of propildazine (Ventura et al., 1981). This latter is shown in Fig. 3.

If A_0 is large and fairly constant during the reaction, then

$$\frac{dY}{dt} \approx k_2 A_0 C \tag{4.33}$$

Introducing Eq. (4.29) then gives

$$\frac{dY}{dt} = k_2 A_0 q [1 - aY] \tag{4.34}$$

This can be rewritten as

$$d\left(\frac{1}{a} - Y\right) = a \cdot k_2 A_0 q \cdot dt \tag{4.35}$$

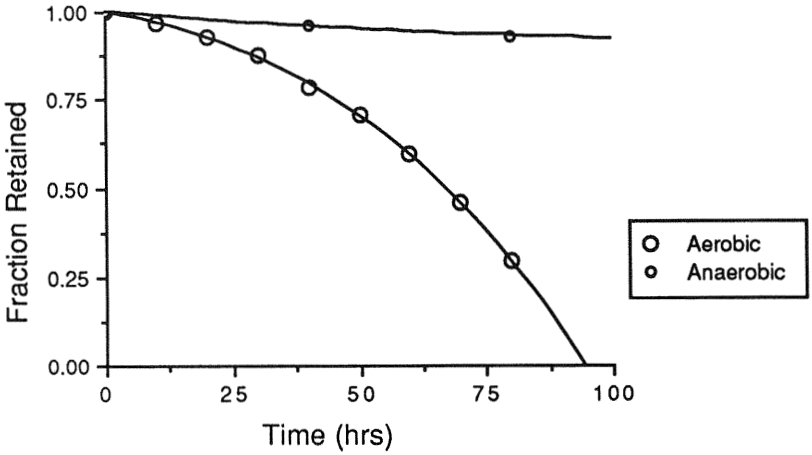

Fig. 3 Oxidation of propildazine. (Graph constructed from data by Ventura et al., 1981.)

which integrates to

$$\ln\left(\frac{1}{a} - Y\right) = -k_2 A_0 qat + \ln\frac{1}{a} \tag{4.36}$$

If a is close to unity, then this predicts first-order kinetics, as is for instance the case in work reported by Kassem et al. (1972) for ascorbic acid, and by Brown and Leeson (1969). The results of the latter are shown in Fig. 4.

It should finally be added that these are cases (which will be discussed in Chapter 7), where oxidation leads to zero-order profiles (Franchini and Carstensen,

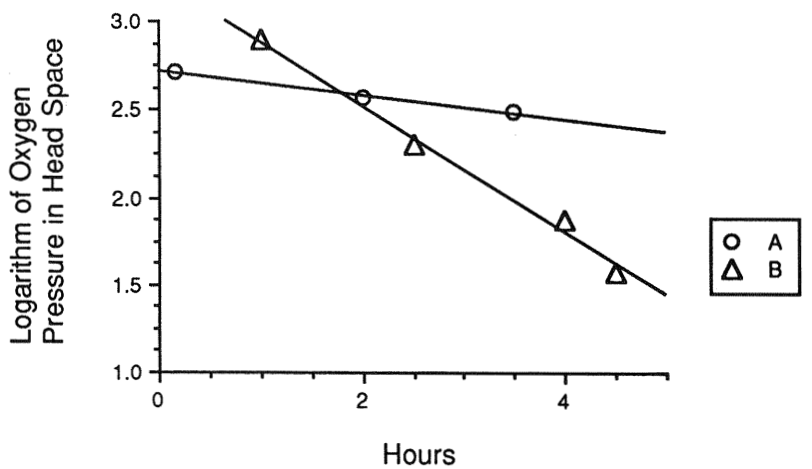

Fig. 4 Head space oxygen content versus time in (A) a system layered with nitrogen and (B) an unprotected system (which extrapolates to zero time at 21% oxygen as it should). (Graphs constructed from data published by Brown and Leeson, 1969.) The least squares fit lines are (A) $y = 2.71 - 0.067x$ and (B) $y = 3.24 - 0.36x$.

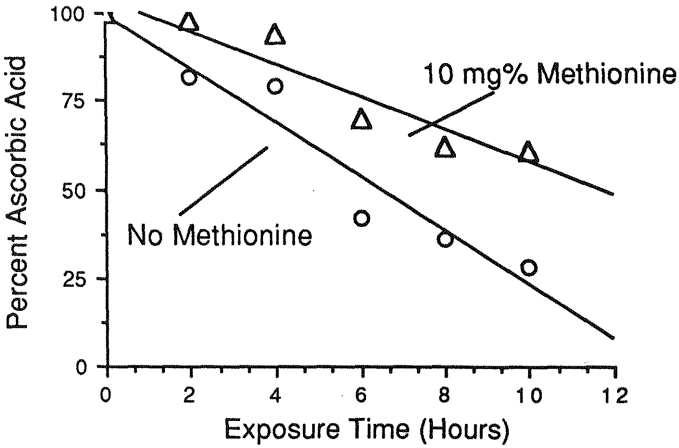

Fig. 5 Photolytic oxidation of ascorbic acid solutions. Least squares fit: 10 mg% methionine: $y = 99.38 - 7.64x$ ($R = 0.97$) and no methionine: $y = 104.19 - 4.67$ ($R = 0.95$). (Graph constructed from data by Asker et al., 1985.)

1995). These are cases where the drug is amphiphilic and where one of the non-micellar n-mers (A_n) oxidizes. In this case there is an excess of oxygen, and the concentration of the reaction species, A_n, is constant (since it will be replenished by the micellar species).

Cases where the oxidation is photochemically driven will often become zero order. An example of this is the work by Asker et al. (1985), whose data are shown in Fig. 5. It should be noted that the data are probably more suitably considered S-shaped curves, such as will be discussed shortly.

1.4. Oxidation in an Open System

It is always worthwhile determining k_2 in a separate experiment in an open system. This is done by bubbling oxygen through an aqueous solution of drug, so that C is the saturation concentration of oxygen (C_{sat}) at all times:

$$\frac{dA}{dt} = -k_2[\mathrm{A}]C_{sat} = -k_2[\mathrm{A}]KP \tag{4.37}$$

Since the amount of A present is the original amount of drug, $A_r m : o^*$ less the amount decomposed, Y^*, it follows that

$$\frac{dY^*}{dt} = k2[A_0^* - Y^*]KP \tag{4.38}$$

It is noted here that the A^* and Y^* values are V_2 times concentrations. Eq. (4.40) integrates to

$$\ln[A^* - Y^*] = -(k_2KP)t + \ln[A_0^*] \tag{4.39}$$

or

$$\ln\left[1 - \frac{Y^*}{A_0^*}\right] = \ln[\text{fraction retained}] = -(k_2KP)t \tag{4.40}$$

where the initial condition that $X=0$ at $t=0$ has been invoked. Equation (4.39) predicts (a) that the reaction is first order and (b) that the observed rate constant is

$$k_{obs} = k_2KP = k_2C \tag{4.41}$$

where C is the (now virtually constant) oxygen concentration in that aqueous phase. The situation described holds for any situation where the oxygen concentration changes so little that it becomes almost constant and the reaction is pseudo first order.

It has tacitly been assumed that the equilibrium between gaseous and dissolved oxygen is instantaneous, but at low agitation it may be diffusion controlled. A good example of this is the oxidation of sulfites reported by Schroeter (1963).

It can be seen from the data in Fig. 6 that at the lowest bubbling speed, the oxygen supply does not keep up with the decomposition. In general the observed rate constant should be a function of oxygen concentration, which in this model is constant. It can of course be varied by having different mixtures of oxygen and inert gas bubbling through the mixture, and in this case Eq. (4.41) becomes

$$\ln[k_{obs}] = \ln[C] + \ln[k_2] \tag{4.42}$$

Plotting either $\ln[k_{obs}]$ versus $\ln[C]$ or $[k_{obs}]$ versus C should give straight line plots. In the former case the slope would have to be unity. An example of this is (Fig. 7)

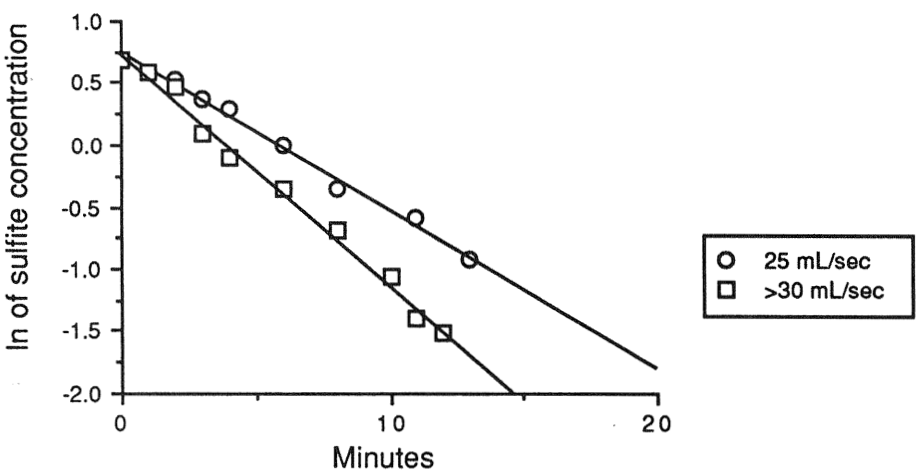

Fig. 6 Sulfite oxidation as a function of rate of bubbling. The least squares fit lines are 25 mg/mL: $y=0.75-0.13x$ and >30 mg/mL: $y=0.734-0.19x$. (Graph constructed from data by Schroeter, 1963.)

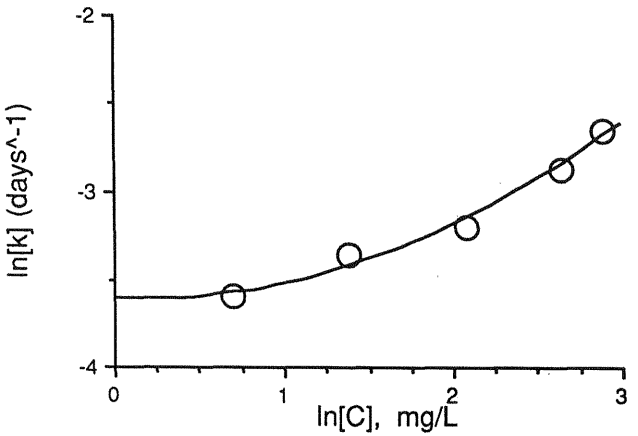

Fig. 7 Effect of oxygen concentration on oxidation rate. If plotted linearly the equation for the trace would be $y = -3.93 + 0.41x$. It is shown parabolically: $y = -3.6 - 0.04x + 0.125x^2$. (Figure constructed from data by Kassem, 1972.)

oxidation of ascorbic acid (Kassem et al., 1972) (where, however, the slope is not unity).

1.5. Oxidative Autocatalysis

In autocatalysis, the rate constant, k_2, is a function of Y. The most all-encompassing equation of this type is the Ng equation, Eq. (4.43) (Ng, 1975) which will be dealt with at another point. Eq. (4.23) assumed that the reaction rate was proportional to drug concentration and reactant concentration. Where this is in deviance from experimental results it may be assumed that it is dependent on powers of these, i.e.,

$$\frac{dY}{dt} = k_2 Y^n (1 - Y)^p \tag{4.43}$$

In many cases, the exponents are simply unity, and hence Eq. (4.43) becomes

$$\frac{dY}{dt} = k_2 \cdot Y[1 - Y] \tag{4.44}$$

where, here, Y denotes fraction decomposed. Profiles of this type are shown in Fig. 8.

There is an initial apparent lag time, and then a precipitous drop. Often, in real systems, it is difficult to duplicate the induction time point, t^*, at which the drop commences. The FDA has often quoted this situation as one that makes the Agency leery about extrapolations, because it is possible to get a good linear fit to the data at times $t < t^*$, and these would extrapolate to high retention values, which in the case cited would be incorrect. Eq. (4.44) is rearranged to

$$\frac{dY}{Y(1 - Y)} = k_2 t \tag{4.45}$$

To integrate Eq. (4.45) it is recalled that

$$\frac{1}{Y(1-Y)} = \frac{1}{Y} + \frac{1}{1-Y} \tag{4.46}$$

so that Eq. (4.45) becomes

$$d\ln[Y] - d\ln[1-Y] = k_2 t \tag{4.47}$$

which integrates to

$$\ln\left[\frac{Y}{1-Y}\right] = k_2(t - t_i) \tag{4.48}$$

where t_i is the point in time where the inflection point occurs. Eq. (4.48) may be expressed as

$$Y = \frac{\exp[k_2 t]}{1 + \exp[k_2 t]} \tag{4.49}$$

This type profile is comparable to a Henderson–Hasselbach curve, i.e., is an inverse *S*, and it would have the appearance shown in Fig. 8. If power functional dependence of k on Y is larger than 1, then the typical lag time curve will generate.

In the preamble to this section on oxidation, it was stated that oxidation was not the order of the day in pharmaceutical systems. This is exemplified by the number of publications. In J. Pharm. Sci., eg., for the period 1965 to 1990 there are only some 30 publications on the subject, and several of these do not deal with the kinetics but rather with the (very important) problem of proper assignment of degradation products. Some of the articles in the literature (beyond those quoted already) are listed below.

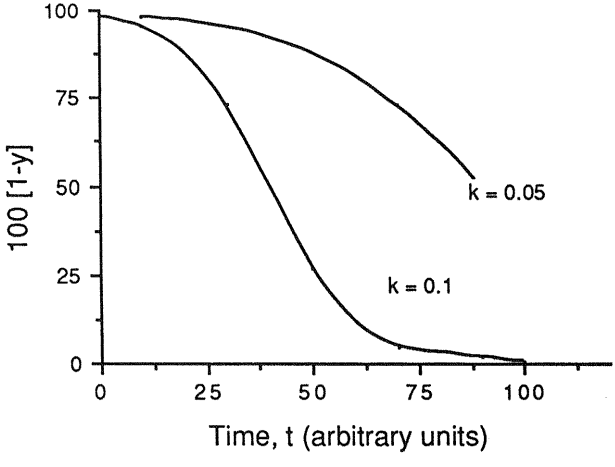

Fig. 8 Trace of Eq. (4.49), the function $\exp(-kt)/(1+\exp\{-kt\}]$ for various values of k_2. The profiles of the function have been generated for $k = 0.01$ and 0.005, and a starting value of 100.

Repta and Beltagy (1981) reported on the oxidation of 6-seleno guanosine in aqueous solution. Underberg (1978) reported on the oxidative degradation of promethazine. Duchene et al. (1986) have reviewed the effect of cyclodextrin complexes on drug oxidative stability. Szejtli et al. (1980) and Szejtli and Bollan (1980) demonstrated that for vitamin D_3 the cyclodextrine complex reacted less rapidly with oxygen. Swarbrick and Rhodes (1965) showed that the maximum oxidation rate of linoleic acid in aqueous systems containing the surfactant Brij 35 was a slightly decreasing linear curve.

Sadhale and Shah (1998) reported on the hydrolysis and oxidation of cefazolin at low (50 μg/g) and high (200 μg/g) concentrations. Under some of the conditions the plots are S-shaped or have downward curvature; in other cases the plots appear semilogarithmic. EDTA slows down the reaction, and if gels are present the degradation constants are 3–18 times lower. In the latter case, the reduced oxygen diffusion may be the reason. Similar results were obtained, although to a lesser degree, with cefuroxime.

2. MORE COMPLEX MODELS

A more general, semi-empirical equation for the treatment of autoxidative reactions (invoking some power dependence of Y and $(1 - Y)$ is

$$\ln\left\{\frac{Y^n}{(1-Y)^p}\right\} = -k'(t - t_i) \tag{4.50}$$

A more manageable form of this equation is

$$\ln\left\{\frac{Y^q}{1-Y}\right\} = -k(t - t_i) \tag{4.51}$$

where

$$k = \frac{k'}{p} \tag{4.52}$$

and

$$q = \frac{n}{p} \tag{4.53}$$

The problem exists as to how to treat Eq. (4.51) and obtain parameters from the untransformed Y versus t equation, since n and p and k are not known. The reason for recasting Eq. (4.50) is to reduce the number of iterants to one. Large number of iterants in a model are suspect, because even good but not quite rigorous first estimates may lead to secondary minima, and it is always a good practice to reduce the iterants to as few as possible. Equation (4.51) only requires one iterant, so that data treatment is not all that questionable.*

* Iteration with more than one iterant always leaves the question of whether the minimum obtained by iteration is a primary or a secondary minimum. An example of this is shown in Chapter 3.

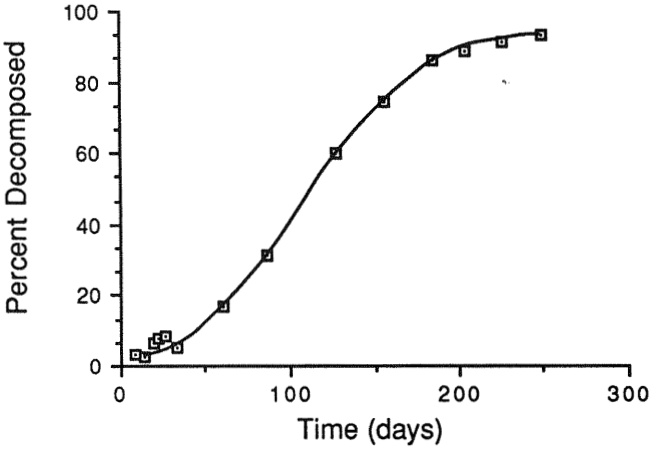

Fig. 9 Decomposition of retinoic acid at 37°C. (Graph constructed from data reported by Tan, Melzer and Lindenbaum, 1993.)

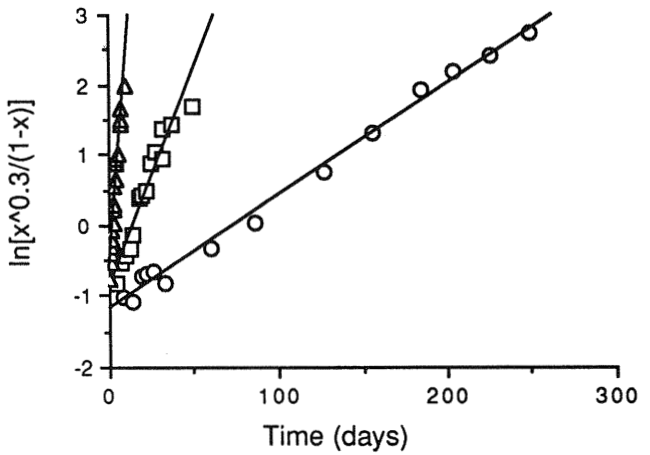

Fig. 10 Treatment of Equation (2.33) using $q = 0.3$. (Data reported by Tan et al., 1963).

But even then is it necessary to have a reasonable estimate of the iterant, q, before starting the iteration procedure. Such an estimate can be obtained (and in obtaining the estimate, often the iteration is unnecessary) by the considerations to follow. Consider the data (Fig. 9) reported by Tan et al. (1993).

Manual trial and error will often give reasonable estimates of iterants (in this case q), and the value $q = 0.3$, arrived at in a few tries, gives good results, as shown in Fig. 10. This value could then be used in computer iteration, as a first estimate.

The least squares fit lines are

$$37°C: \quad \ln\left[\frac{x^{0.3}}{1-x}\right] = -1.192 + 0.0160t \tag{4.54}$$

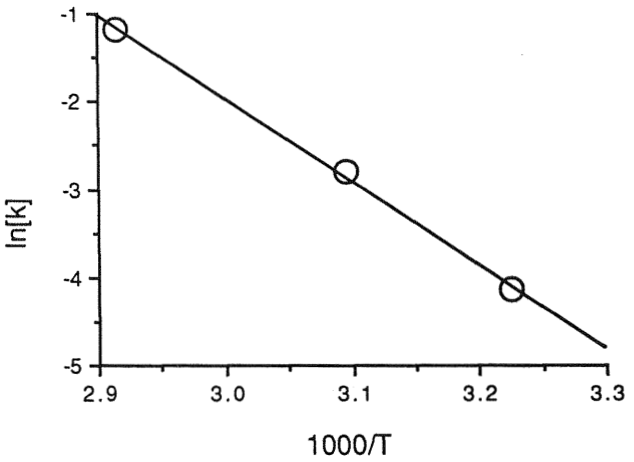

Fig. 11 Arrhenius plot of data in Fig. 4.10.

$$50°C: \quad \ln\left[\frac{x^{0.3}}{1-x}\right] = -0.881 + 0.0613t \tag{4.55}$$

$$70°C: \quad \ln\left[\frac{x^{0.3}}{1-x}\right] = -0.654 + 0.307t \tag{4.56}$$

When the slopes (the rate constants, in units of day^{-1}) are plotted versus absolute inverse temperature, a good Arrhenius fit is obtained as shown in Fig. 11. It is observed that the activation energy is about 18 kCal/mole, which is a reasonable figure.

2.1. Stability Prediction by Fractional Lives

It has been shown in Chapter 2 that for zero- and first-order reactions it is possible to assess and extrapolate stability from high-temperature data by use of fractional lives.

Tan et al. (1993) have extended this principle to oxidations, and Yoshioka et al. (1994) have extended it to complex reactions following neither of the more describable orders. They consider reactions where the fraction decomposed is a second-degree polynomial in time, t, and (more frequently) a triple exponential formation, i.e.,

$$x = A\exp(at) + B\exp(bt) + C\exp(ct) \tag{4.57}$$

or

$$x = a + bt + ct^2 \tag{4.58}$$

When treating decomposition of proteins by either of these equations, they obtain best parameters and hence can calculate the point in time where $x = 0.1$

(which is usually denoted t_{90}). They then show by this empirical mode that in the cases studied,

$$\ln[t_{90}] = \frac{Q}{T+q} \tag{4.59}$$

It is noted that the two presentation modes for the appearance of x with time do not contain what is usually thought of as a rate constant. Hence, unlike the treatment of zero- and first-order equations, there is no way of "invoking" a temperature dependence on k or t_{90}. The method has great practical value.

To the contrary, Tan et al. (1993) attempted to obtain an explanation for the fact that fractional lives (from $\beta = 0.1$ to 0.9) give linear plots, but they found that the lines change "activation energy". The argument put forth by Tan et al. (1993) is the following: the decomposition reaction may be characterized by the differential equation

$$\frac{dx}{\phi(x)} = k\,dt \tag{4.60}$$

where $\phi(x)$ is a function that is not "simple." If this is integrated (if it can be integrated), then the integral is denoted $F(x)$, i.e.,

$$F(x) = kt \tag{4.61}$$

or

$$F(\beta) = kt_{\beta} \tag{4.62}$$

where β is the fractional life. By now invoking that

$$k = Z\exp\left(\frac{-E_a}{RT}\right) \tag{4.63}$$

it follows that

$$F(\beta) = Z\left\{\exp\left(-\frac{E_a}{RT}\right)\right\}t_{\beta} \tag{4.64}$$

or

$$\ln[t_{\beta}] = \ln\left(\frac{F(\beta)}{Z}\right) + \frac{E_a}{RT} \tag{4.65}$$

i.e., pro forma, the linearity of $\ln[t_{\beta}]$ versus $(1/T)$ would appear to be proven. It should be noted that integrals of the type $dx/\phi(x)$ can rarely be solved in a manner such as to have only one "k-value."

If one takes the example by Yoshioka et al. (1994), where x is a polynomial in t, then one would have to express dx/dt as a function of x, not of t. Here

$$x = a + bt + ct^2 \tag{4.66}$$

If this is differentiated so that dx/dt is expressed as a function of x, not of t, then

$$\frac{dx}{dt} = b + ct \tag{4.67}$$

To eliminate t, Eq. (4.66) is solved with respect to t, and

$$t = -\frac{b}{2c} \pm \left[\frac{b^2}{4c^2} - \frac{a-x}{c}\right]^{1/2} \tag{4.68}$$

If

$$\left[\frac{a-x}{b}\right]^2 \ll \frac{b^2}{4c^2} - \frac{a-x}{c} \tag{4.69}$$

then

$$t \approx \frac{a-x}{b} \tag{4.70}$$

so

$$\frac{dx}{dt} = b + \left\{\frac{a-x}{b}\right\}c = A + Bx \tag{4.71}$$

where

$$A = b\left[1 + \frac{a}{c}\right] \tag{4.72}$$

and

$$B = -\frac{c}{b} \tag{4.73}$$

It is noted that even with a fairly simple function (a second-order polynomial) it is not possible to obtain an equation of the type of Eq. (4.60), where there is a definitive k. At best one might expect B to follow an Arrhenius equation.

Hence the method only has theoretical basis if $dx/\phi(x)$ can be presented in a form, and in such a way, that the rate constant, k, is a meaningful quantity.

The method, nevertheless, has great practical importance, because it allows extrapolations in a simple manner.

The data by Yoshioka are shown in Fig. 12. Again, the "activation energies" are high, but no theoretical importance should be placed on them.

3. ANTIOXIDANTS

Antioxidants work by consuming oxygen at a faster rate than the rate at which the drug substance reacts with oxygen; and in such cases they will protect the drug substance until they are completely used up.

This means that the use of antioxidants imposes a lag time upon the decomposition profile of the drug.

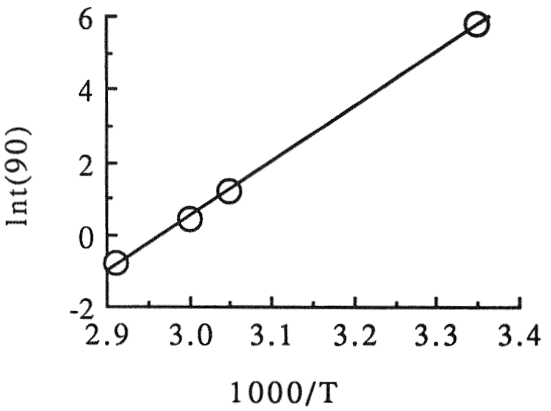

Fig. 12 Decomposition of *a*-chymotrypsin tablet. ln[t_{90}] plotted in Arrhenius fashion. The neat decomposition is not an integer order. (Graph constructed from data published by Yoshioka et al., 1994.)

An example is the work by Pudapeddi et al. (1992), who showed that sulfites act in a quantitative manner. Once the sulfites are consumed, the oxidation starts. Gonçalves et al. (1998) have reported on the antioxidant activity of 5-aminosalicylic acid in the presence of vitamins C and E of lipid peroxidation. They show typical S-shaped decomposition curves. Chakrabarti et al. (1993) have shown that hydroquinone, butylated deoxycholate, and ascorbyl palmitate stabilizes hyamycin (a polyene antifungal antibiotic).

Antioxidants can also act by interfering in e.g. the reaction schemes shown in Eqs. (4.2–4.11) so that the oxidative pathway is interrupted. In such a case they will themselves be regenerated, and will function in a manner independent of elapsed time. The most common antioxidants used are ascorbic acid, BHA, BHT, and sodium sulfite. The use of ethylene diamine tetraacetic acid as a chelator has already been mentioned.

4. OTHER WORK

The first step in oxidative (and any other) kinetic investigation is decomposition product identification. It is then possible later to study the kinetics of the system. For instance, Hooijmaaijer et al. (1999) have studied the peroxide catalyzed degradation of mocophenolate mofetyl in aqueous solution and have characterized the decomposition products.

As mentioned, until recently, the number of reports on oxidation in the pharmaceutical literature were scarce, but the gap is being filled. For instance, Bosca et al. (1992a) have described the oxidative decarboxylation of naproxen, and Bosca et al. (1992b) have described the photochemical byproducts. Vargas et al. (1992) have described the photochemical oxidation in light of nifedipine. Tereoka et al. (1993) have described the oxidation of ranitidine in acetate buffer.

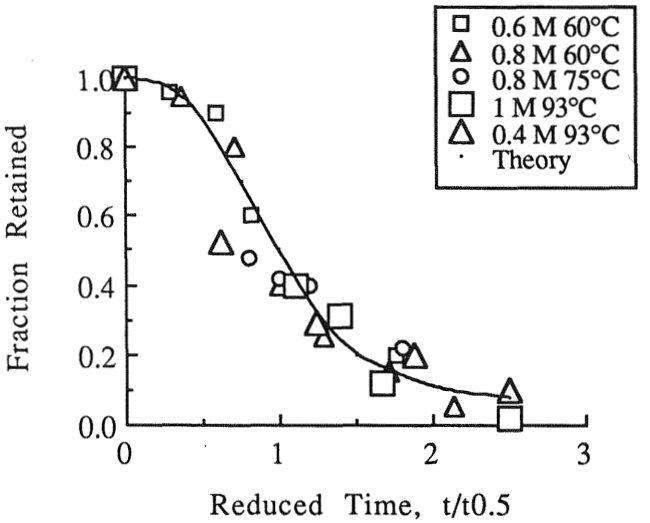

Fig. 13 In the legend *M* denotes molarity of phosphate buffer. The pH employed was 8.0 and the concentration of drug was 0.2 mg/mL. (Graph constructed from data published by Franchini and Carstensen, 1994.)

Franchini and Carstensen (1994) have studied the auto-oxidation (in the presence of trace quantities of heavy metals) of the oxygen-sensitive compound β-[2-(2-carboxyethyl)-thio-2]-[-8-2-phenylacetyl]-benzenepropanoic acid. This is denoted A in the following.

The degradation is an oxidation leading to a sulfoxide and a cinnamic acid. The mechanism that applies to the decomposition is

$$A + A \rightarrow A_2 \qquad \text{with equilibrium constant } K$$

and

$$A_2 + O_2 \rightarrow B \qquad \text{with rate constant } k_2$$

The ensuing rate equation is fourth order in A_{obs}. The authors studied the decomposition at a series of buffer concentrations and temperatures, and some of their data are shown in Fig. 4.13. They showed that the reaction was subject to kinetic salt effect, but that buffer catalysis was absent.

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5

Catalysis, Complexation, and Photolysis

JENS T. CARSTENSEN

Madison, Wisconsin

1. CATALYSIS

General and specific acid and base catalyses have been discussed in Chapter 3, and are one example of catalysis. When discussing catalysis, however, the metal induced decomposition is what comes to the pharmaceutical investigator's mind. In parenterals especially, great care is taken to exclude metals, because only slight decomposition caused by trace metals may cause sufficient discoloration to render the product unsatisfactory. Examples of this are thiamine hydrochloride injectables and ascorbic acid injectables.

Metals are most detrimental in oxidations, as shown in the previous chapter. Examples of metal catalyzed oxidation in pharmaceutical systems are cyanocobalamine (which is stabilized at very low concentrations, but destabilized at higher concentrations of ferrous ion), erythromycin (which is stabilized by such ions as mercuric, magnesium calcium, ferric, and aluminum, and destabilized by cobaltous, plumbic, zinc, and nickel) and (Kassem et al., 1969) ascorbic acid (which, in general, is destabilized by metal ions).

Figure 1 shows data by Kassem et al. (1969). Barcza and Lenner (1988) have shown that chloral hydrate forms hydrogen bonded complexes with halide ions

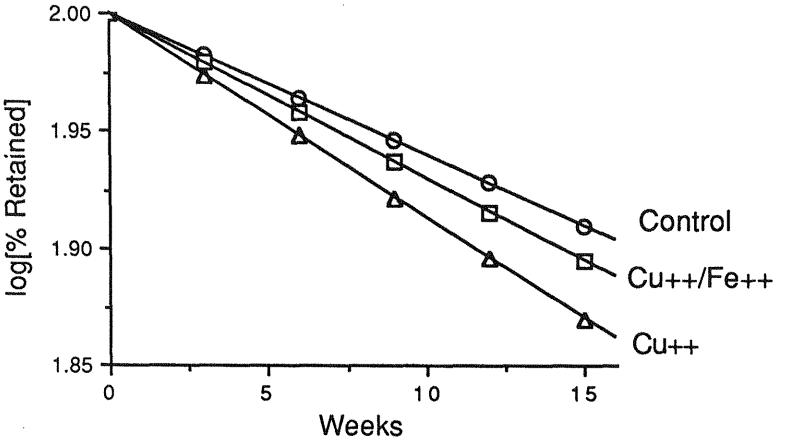

Fig. 1 Effect of metal ions on the decomposition of ascorbic acid. Least squares fit lines are (Control) $y = 2 - 0.006x$, (with copper and iron) $y = 2 - 0.007x$, (with copper alone) $y = 2 - 0.0087x$. (Figure constructed from data published by Kassem, 1969.)

in aqueous solutions. They are 1 : 1 complexes with relatively high stability constants. Upadrashta and Wurster (1988) used ethylene diamine tetraacetic acid to protect anthralin solutions from metal catalyzed oxidation. Tomida et al. (1987) showed that zinc ion increased the degradation of cephalosporins in tromethamine solution. The second-order rate constant, divided by $[Zn_o]$ plotted versus pH has unity slope from pH 7.5 to 8.5. They suggest the formation of a ternary complex (penicillin-Zn^{2+}-tromethamine).

Substances such as vitamin A and D are also prone to metal ion catalyzed decomposition.

Of other works in this area, McCrossin et al. (1998) reported on the effect of guanidine HCl on degradation of recombinant porcine growth hormone at alkaline pH and different concentrations and found it to be first order.

Fredholt et al. (1999) studied the catalytic effect of α-chymotrypsin on desmopressin decomposition and reported on the influence of concentration, pH, and cyclodextrin. The reaction is presumably A-B-C and the disappearance rate of the compound is first order. The pH profile is type AHJD with a maximum at pH 7.7.

2. COMPLEXATION

It is obvious that in many cases, drugs may complex with one or more of the ingredients in a solution dosage form. Sometimes this is intentional, e.g. bioavailability in certain instances may be improved (Levy and Reuning, 1964; Newmark et al., 1970). In other instances the stability of a drug is favorably affected by complexation, although in many cases the opposite is the case.

The basic principles of complex formation have been reviewed by Connors and Mollica (1964) and demonstrated by them as well. Only the formation and stability of 1 : 1 complexes will be covered here. For coverage of 1 : 2 and 2 : 1 complexes,

the reader is referred to the work by Connors and coworkers (Rosanske and Connors, 1980; Connors and Rosanske, 1980; Pendergast and Connors, 1984).

In the following, A will denote drug (substrate) and B will denote complexing agent (ligand). (It should be noted that either could be called either, and that there is no generally accepted nomenclature).

If A complexes with B by the scheme

$$A + B \leftrightarrow AC \tag{5.1}$$

then the complex is denoted 1 : 1. The terminology 1 : 2 or 2 : 1 is then obvious. The equilibrium (stability) constant of the complex AB is given by

$$K = \frac{[AB]}{[A][B]} \tag{5.2}$$

In a 1 : 1 complex (and other types as well), it would be fortuitous if the stability of both the drug and the complex were identical. In other words, in degrading, there will be two decomposition reactions:

$$A \rightarrow \text{products (rate constant } k) \tag{5.3}$$

and

$$AB \rightarrow \text{products (rate constant } k^*) \tag{5.4}$$

The analytically measured quantity is

$$C = [A] + [AB] \tag{5.5}$$

except if the study is carried out by other than chemical means, e.g., if one species is charged, then conductimetry might elucidate the concentration of one of the species.

The analytically measured rate is

$$-\frac{dC}{dt} = k[A][B] + k^*[AB][B] = \{k + k^*K[B]\}[A][B] \tag{5.6}$$

where use has been made of Eq. (5.2) for the last step.

The apparent rate with which the reaction proceeds is given by

$$\begin{aligned} -\frac{dC}{dt} &= k_{obs}[B]C = k_{obs}[B]\{[A] + [AB]\} \\ &= k_{obs}[B][A]\{1 + K[B]\} \end{aligned} \tag{5.7}$$

where Eq. (5.2) has been used for the last step. Equating Eqs. (5.6) and (5.7) then gives

$$\{k + k^*K[B]\}[A][B] = k_{obs}[B][A]\{1 + K[B]\} \tag{5.8}$$

Dividing through by [A][B] gives

$$k + k^*K[B] = k_{obs}\{1 + K[B]\} \tag{5.9}$$

Table 5.1 Effect of β-Cyclodextrin on Benzocaine Stability

Concentration of cyclodextrin (%)	k_{obs} (h^{-1} M^{-1})
0	0.666
0.25	0.358
0.5	0.299
1	0.129

Source: Data from Lach and Chin (1964a).

from which we obtain

$$k_{obs} = \frac{k + k^*K[B]}{\{1 + K[B]\}} = k + \frac{(k^* - k)K[B]}{1 + K[B]} \tag{5.10}$$

which may be expressed reciprocally as

$$\frac{1}{k_{obs} - k} = \frac{1}{k^* - k} + \frac{1}{K(k^* - k)} \cdot \frac{1}{[B]} \tag{5.11}$$

If the amount of [B] complexed in small, then [B] is synonymous with the amount of [B] added. Hence if kinetic studies were carried out in a series of systems with different concentrations of [B], then a reciprocal plot should be linear.

Example 5.1.

Lach and Chin (1964a,b) reported kinetic data for benzocaine, complexed with betacyclodextrin (Table 1). Calculate the equilibrium (stability) constant between benzocaine and betacyclodextrin, assuming a 1 : 1 complex.

Answer.

First of all the concentration units have to be consistent. Beta-cyclodextrine is taken to have a molecular weight of 1700, so that e.g. 0.25% = 2.5 g/L = $2.5/1700 = 1.47 \times 10^{-3}$ molar. This is shown in column 2 of Table 2. The sixth column lists the reciprocal of these figures, e.g., $1/0.00147 \times 680$ M^{-1}.

The values for $(k_{obs} - k)$ are listed in the fourth column, e.g., for the second entry, $0.229 - 0.666 = -0.43$.

The fifth column then lists $1/(k_{obs} - k)$, e.g. $1/(-0.537) = -1.862$.

The fifth column, $(k - k_{obs})$ is then plotted versus the sixth column, 1/[B]. This is shown in Fig. 2.

It is seen that the slope $= -0.0027$ (M^2h) and that the intercept is -1.3822 (Mh). Equation (5.11) predicts that the slope-to-intercept ratio is K, i.e.,

$$K = \frac{\text{slope}}{\text{intecept}} = \frac{-0.0027}{-1.3822} = 2 \times 10^{-3} M^{-1} \tag{5.12}$$

Table 5.2. Treatment of Data in Table 1. Effect of β-Cyclodextrin on the Decomposition of Benzocaine in Solution

Concentration of cyclodextrin					
%	$10^3 \times$ Molar	k_{obs} $h^{-1}M^{-1}$	$(k_{obs} - k)$	$1/(k - k_{obs})$	1/[B]
0	0	0.666	0		
0.25	1.47	0.368	−0.298	−3.247	680
0.5	2.94	0.299	−0.467	−2.288	340
1	5.88	0.129	−0.537	−1.862	170

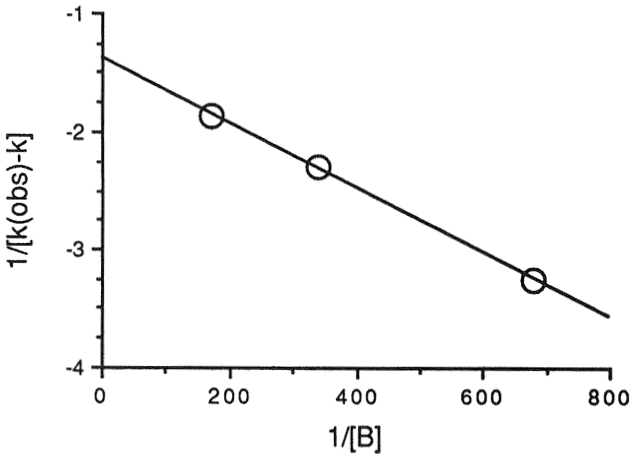

Fig. 2 Data from Table 2. (Graph constructed from data published by Lach and Chin, 1964.)

Complexation constants can also be deduced by determining the solubility of A in aqueous (or other) solutions of different concentrations of B. An example of this is the work by Chen et al. (1994).

Chen et al. (1994) have studied the complexation of adenine with a series of ligands. Their data from using caffeine as a ligand are shown in Fig. 3. The complexation constant is obtained by Eq. (5.13) by which

$$K = \frac{\text{slope}}{\text{intercept} \times (1 - \text{slope})} = \frac{0.26}{(0.0076 \times 0.74)} = 46.2\ (M^{-1}) \tag{5.13}$$

The pharmaceutical literature dealing with complexes is not abundant. One interesting example is urea including compounds, which were in vogue in the 1960s (and probably still are, although they are not as frequently published on), an example being the thiourea and urea inclusion compounds of alpha-lipoic acid methyl ester reported by Mima and Nishiwasha (1964). Other, presently quite researched, com-

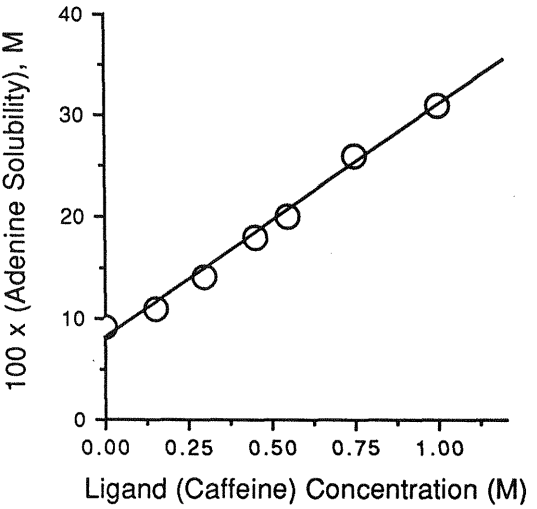

Fig. 3 Least squares fit: $y = 0.26x + 0.0076$, where x is solubility (rather than $100 \times$ solubility, as used in the figure). (Graph constructed from data published by Chen et al., 1994.)

plexes are those with cyclodextrins: for instance Connors and coworkers have published substantially in this area (Pendergast and Connors, 1984; Connors et al., 1982). Duchene et al. (1986) have reviewed the effect of cyclodextrin complexes on drug stability. Lach and Chin (1964a) have studied the complexes of cyclodextrins with benzocaine and found them to be 1 : 1 complexes. Higuchi and Lachman showed that benzocaine complexed with caffeine (1955). Lach and Chin (1964b) showed that a series of substituted benzoic acids complexed with cyclodextrin. Cyclodextrins are unique (alpha, beta, and gamma) in that they are doughnut shaped molecules that allow inclusion of the drug molecule. Other (non-macrocyclic) carbohydrate molecules also complex, for instance Gupta (1983) has shown that procainamide complexes with glucose, lactose, and maltose. All of these have a hemiacetal group, as opposed to sucrose and fructose. The complex formation was pH dependent.

Complexation constants may be obtained by spectrophotometric means as well. The complex can exhibit a specific maximum in the ultraviolet spectral range, so its concentration can be distinguished from that of the parent species such as in the case of the alendronate/Cu^{++} complex and the case of metal complexes of anhydrotetracycline (Siqueira et al., 1994).

2.1. Complexing Agents

Caffeine and polyvinylpyrrolidone were the most common complexing agents used in pharmaceutics for a series of years. In recent years the cyclodextrins have become of importance.

An example of this is the work by Van Der Houwen et al. (1994) dealing with the kinetics of 7-*N*-(*p*-hydroxyphenyl)mitomycin C (M-83) in the presence of γ-cyclodextrin. The pH profile of this is V shaped with a couple of extensions of

lesser slope and with a minimum at pH 7. Cyclodextrins have also been used in stabilization of monoclonal antibodies (Ressing et al., 1992).

Cyclodextrins (CDs) are powerful complexing agents, and much work has centered about their use in drug solubilization and stabilization. They are the subject of a fair amount of pharmaceutical research. For instance, Scalia et al. (1998) have described the complexation of butyl-methoxydibenzoilmethane with hydroxypropyl-β-cyclodextrin. Veiga and Ahsan have described the interaction between tolbutamide and β-cyclodextrins. The tolbutamide phase solubility diagram, in this case, shows a maximum in solubility at a concentration of β-CD of 0.008 M. Such maxima are usually ascribed to the limit of solubility of the complex. Ventura et al. (1998) have described the interaction between papaverine and modified and natural β-cyclodextrins. The phase-solubility diagram is such that the papaverin solubility increases monotonically (in a straight line or curved) with β-CD concentration. Ventura et al. (1997) have shown the increase in aqueous solubility of ursodesoxycholic and chenodesoxycholic acids by complexation with β-CDs.

Miyake et al. (1999) have demonstrated the inclusion compounds of itraconazolone with 2-hydroxypropyl-β-cyclodextrin in propylene glycol solution. Antoniadou-Vyza et al. (1997) have reported that the hydrolysis rate constant of methocarbamol is reduced almost 50% when complexed with hydroxypropyl-β-cyclodextrin. Vianna (1998) et al. showed that complexes of dexamethasone acetate with cyclodextrin showed marked improvement in aqueous solution stability and gave rise to first-order decomposition. Másson et al. (1998) have shown that chlorambucil and indomethacin have greatly improved (first-order) aqueous stability when complexed with a variety of cyclodextrins. Diazepam behaved in different manners depending on the particular cyclodextrin used.

Less common ligands such as dextran stabilize porcine pancreatic elastase (Chang et al., 1993).

3. PHOTOLYSIS

Attention is given to light stability in the 1993 ICH guidelines, although at the time of this writing, testing methods are not finalized. This is exemplified in lines 471–473:

> Light testing should be an integral part of stress testing. [The standard conditions for light testing are still under discussion and will be considered in a further ICH document.] (471–473).

In photolysis, light ($h\nu$) is absorbed by the solution and activates a species in it. ($h\nu$) here represents a quantum of light, where h is Planck's constant ($h = 6.626 \times 10^{-27}$ erg s) and ν (in units of s^{-1}) is the frequency of the light. The activated species (denoted by [*]) then returns to ground state, it either

1. Emits light (of a different frequency ν'); this is referred to as fluorescence or phosphorescence
2. Causes the activated species to decompose, in which case one deals with photolysis. The simplest sequences in photolysis would, therefore, be

$$A + (h\nu) \rightarrow [A^*] \tag{5.14}$$

$$[A^*] \rightarrow \text{products} \tag{5.15}$$

$$[A^*] + A \rightarrow 2A \tag{5.16}$$

At times a second component of the system, B, may preferentially absorb light, in which case photosensitization may occur:

$$B + (h\nu) \rightarrow [B^*] \tag{5.17}$$

$$[B^*] + A \rightarrow B + [A^*] \tag{5.18}$$

If only (5.17) predominates, and is followed by

$$[B^*] \rightarrow \text{products} \tag{5.19}$$

or

$$[B^*] \rightarrow B + (h\nu'') \tag{5.20}$$

then B is referred to as a screening agent. In the last case, [B] will not decrease with time and hence will protect the other photosensitive compounds in the preparation during the shelf life of the product.

When a photolysis experiment is carried out, one should know the quantum yield, i.e., the number of molecules decomposed as a function of the number of quanta absorbed. To measure the latter, use is made of an actinometer (which is a photosensitive system with known quantum yield). A simple and reliable actinometer is the chloroacetic acid actinometer, where the reaction is

$$ClCH_2COOH + H_2O + (h\nu) \rightarrow HOCH_2COOH + HCl \tag{5.21}$$

This has a quantum yield of 1.0 at concentrations of 0.3–0.5 molar. The use is simple. A product is placed in a container, e.g., a clear ampul, or a specially designed apparatus; then, directly before or after, a chloroacetic acid solution is studied under the same circumstances, for a given length of time, t, (e.g., 5 minutes). The hydrochloric acid formed is then titrated and the number of moles calculated, and this is then converted to number of molecules, N. If the drug substance subsequently is irradiated for t^* minutes, then the number of quanta absorbed (provided the absorbency of preparation and actinometer is the same) is given by

$$(h\nu) = \frac{t^* N}{t} \tag{5.22}$$

If the UV spectrum of the substance is known, and if the spectral distribution of the light source is known, then the absorbency (fraction f) of the drug substance solution in relation to the chloroacetic acid solution can be calculated, and the number of quanta are in this case

$$(h\nu) = \frac{ft^* N}{t} \tag{5.23}$$

The 1993 ICH Stability Guidelines promote the general philosophy of attempting to establish a reaction order.

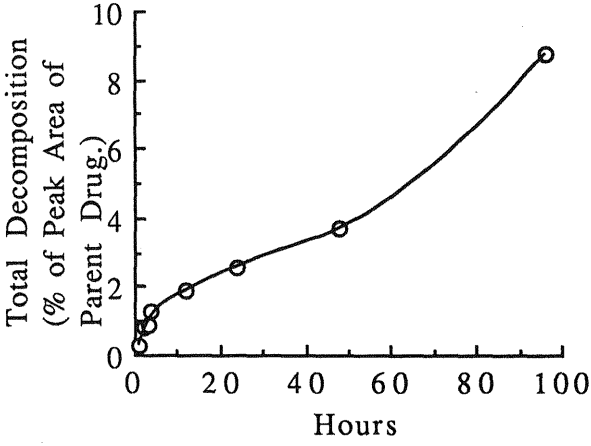

Fig. 4 Photolysis of ciprofloxacin. Irradiation with natural/artificial light. (Figure constructed by averaging of data from Table 2 of the publication by Tievenbacher et al. 1994.)

> The nature of the degradation relationship will determine the need for transformation of the data for linear regression analysis. Usually the relationship can be represented by a linear, quadratic or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit on all batches and combined batches (where appropriate) to the assumed degradation line or curve (303–308).

In pharmaceutical systems, most reported photolysis has been first order. Examples of this are cefatoxime photolysis (Lerner et al., 1988) and the work by Mizuno et al. (1994). They point out that the wavelength of the irradiating light plays an important part in photodecomposition. They showed the wavelength influence on the photodegradation of ethyl 2-[4,5-bis(4-methoxyphenyl)thiazole-2-yl]pyrrol-1-ylacetate in solution.

Another example of photodegradation is the work by Tievenbacher et al. (1994) dealing with a series of antimicrobial quinolones. Their data are shown in Fig. 4.

It is noted that here the reaction is not first order. More rarely is the reaction zero order except when it is an oxidation, in which case it often is zero order. The work by Asker et al. (1985) and Asker and Larose (1987), and the oxidation of chlorpromazine (Ravin et al., 1978; Felmeister et al., 1965) are examples of this.

In general the time frame in photolysis is different from that in usual kinetics, and this is also addressed in the 1993 ICH Stability Guidelines:

> Frequency of testing should be sufficient to establish the stability characteristic of the drug product. Testing will normally be every three months over the first year, every six months over the second year and then annually (271–273).

It is obvious that photolysis is a stress situation and that trimonthly time protocols would be unrealistic. For solution products, the testing should be carried out both in bulk, unprotected solution (and that is what elucidates the kinetic schemes and rates) and in the final package. This latter is more truly a package test. The 1993 ICH Stability Guidelines addresses this as follows:

The testing should be carried out in the final packaging proposed for marketing. Additional testing of unprotected drug product can form a useful part of the stress testing and pack evaluation, as can studies carried out in other related packaging materials in supporting the definitive pack(s) (276–279).

Some additional pharmaceutical examples of photolyses are the following: Fabre et al. (1993) have studied the photoisomerization kinetics of befurroxime axetil and found them to follow an A-B-C reaction. Matsuda and Masahara (1983) have shown that ubidecarenone is photochemically decomposed by a first-order process, and that the activation energy of a solution is different from that in solid state. Heat, light, and metal ions (e.g. copper) accelerate the oxidative decomposition of vitamin D3. This can be retarded and actually inhibited by the use of β-cyclodextrin (Szeijtli et al., 1980; Szejtli and Bollan, 1980). Vitamin A stability is also increased when complexed with cyclodextrins (Kyoshin, 1982). Asker and Larose (1987) have shown that uric acid increases the photostability of sulfathiazole sodium solutions, and Asker et al. (1985) have shown that dl-methionine increases the photostability of ascorbic acid in solution. Vandenbossche et al. (1993) have reported on the photostability of molsidomine in infusion fluids. Akimoto et al. (1985) have reported on the photostability of cianidanol in aqueous solution. In this case there is a leveling off, and the approach to the plateau is first order. Tønnesen et al. (1997) have reported on the photoreactivity of mefloquine hydrochloride in the solid state. Baertschi (1997) has discussed the quinine actinometry system embodied in the ICH guideline.

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6

Solid State Stability

JENS T. CARSTENSEN

Madison, Wisconsin

The stability of drugs in solid dosage forms is the most important, since solid dosage forms are more common than the other types, and because the first clinical trials are usually carried out in this type of dosage form.

The author was, for several years, in charge of the investigational stability program at Hoffmann-La Roche, Nutley, N.J., and in these years accumulated a feel for the manner in which such dosage forms behaved. Some of these behavior profiles were, at the time, explainable, others not. The ones not explainable formed the basis for a great deal of research in the university setting he later enjoyed.

The general pattern that emerges is that solid dosage forms decompose by either first- or zero-order profiles, after adjustment has been made for initial events. Before attempting to elucidate why, it is necessary to know what happens to a solid compound itself, when it is exposed to adverse storage conditions.

The 1993 ICH Stability Guidelines pay particular attention to the intrinsic stability of the drug substance, the bulk drug, as witnessed by the following lines of the Guidelines:

> DRUG SUBSTANCE
> General
> Information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation (36–39).
> Stress testing helps to determine the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products (41–43).

They formally introduce the concept of a re-test period for a bulk drug substance:

> Primary stability studies are intended to show that the drug substance will remain within specifications during the re-test period if stored under recommended storage conditions (46–48).
> The degree of variability of individual batches affects the confidence that a future production batch will remain within specification until the retest date (124–126).
> A re-test period should be derived from the stability information (165).

This requires that this be done on at least three batches of raw material manufactured at a minimum of pilot scale level:

> Stability information from accelerated and long term testing is to be provided on at least three batches. The long term testing should cover a minimum of 12 months duration on at least three batches at the time of submission. The batches manufactured to a minimum of pilot plant scale should be by the same synthetic route and use a method of manufacture and procedure that simulates the final process to be used on a manufacturing scale.
> The overall quality of the batches of drug substance placed on stability should be representative of both the quality of the material used in pre-clinical and clinical studies and the quality of material to be made on a manufacturing scale. Supporting information may be provided using stability data on batches of drug substance made on laboratory scale.
> The first three production batches of drug substance manufactured post approval, if not submitted in the original Registration Application, should be placed on long term stability studies using the same stability protocol as in the approved drug application (50–64).

It is not only the chemical but also pertinent physical parameters that must be monitored, such as possible polymorphic transformations:

> The testing should cover those features susceptible to change during storage and likely to influence quality, safety and/or efficacy. Stability information should cover as necessary the physical, chemical ... characteristics (66–69).

It also addresses the formation of decomposition products and their limits, a point frequently elucidated through kinetics:

> Limits of acceptability should be derived from the profile of the material as used in the pre-clinical and clinical batches. It will need to include individual and total upper limits for impurities and degradation products, the justification for which should be influenced by the levels observed in material used in pre-clinical studies and clinical trials (73–77).

The length of the studies are tied in with the anticipated use of the bulk drug:

> The length of the studies and the storage conditions should be sufficient to cover storage, shipment and subsequent use. Application of the same storage conditions as applied to the drug product will facilitate comparative review (79–81).

The ICH 1993 Stability Guidelines recommend a minimum set of testing conditions. It emphasizes that one of the virtues of accelerated testing is to ascertain that the effects of "excursions outside the label storage condition" such as might occur during shipping, can be assessed.

	Conditions	*Minimum Time Period at Submission*
Long term testing	25°C±2°C/60% RH ±5%	12 Months
Accelerated Testing	40°C±2°C/75% RH ±5%	6 Months

Where 'significant change' occurs during six months storage under conditions of accelerated testing at 40°C±2°C/75 percent RH ±5 percent, additional testing at an intermediate conditions (such as 30°C±2°C/60% RH ±5%) should be conducted for drug substances to be used in the manufacture of dosage forms tested long term at 25°C/60 percent RH and this information included in the Registration Application. The initial Registration Application should include a minimum of 6 months data from a 12 month study. 'Significant change' at 40°/75 percent RH or 30°C/60 percent RH is defined as failure to meet specification.

The long term testing will be continued for a sufficient period of time beyond 12 months to cover all appropriate re-test periods, and the further accumulated data can be submitted to the Authorities during the assessment period of the Registration application.

The data (from accelerated testing or from testing at an intermediate condition) may be used to evaluate the impact of short term excursions outside the label storage conditions such as might occur during shipping (90–109).

The frequency of testing is also specified:

> Frequency of testing should be sufficient to establish the stability characteristics of the drug substance. Testing under the defined long term conditions will normally be every three months, over the first year, every six months over the second year and then annually (111–114).

The containers are assessed as well, but in this respect the effect of oxygen and moisture, as covered in the next chapter, are probably more à propos.

> The containers to be used in the long term, real time stability evaluation should be the same as or simulate the actual packaging used for storage and distribution (116–118).

The 1993 ICH Guidelines address the evaluation of data as well. It is noted here that it is necessary to attempt to establish the "degradation relationship", i.e., attempt to assess the mechanism of degradation:

> The nature of any degradation relationship will determine the need for transformation of the data for linear regression analysis. Usually the relationship can be represented by a linear, quadratic or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit of the data on all batches and combined batches (where appropriate) to the assumed degradation line or curve (138–143).

There is the admission of limited extrapolation of data, but here again, it is necessary to know the degradation pattern:

> Limited extrapolation of the real time data beyond the observed range to extend expiration dating at approval time, particularly where the accelerated data supports this, may be undertaken. However, this assumes that the same degradation relationship will continue to apply beyond the observed data and hence the use of extrapolation must be justified in each application in terms of what is known about the mechanism of degradation, the goodness of fit of any mathematical model, batch size, existence of supportive data etc. (149—155).

The guidelines again emphasize the need for assessment of degradation products and "appropriate attributes":

> Any evaluation should cover not only the assay, but the levels of degradation products and other appropriate attributes (156–157).

Such "appropriate attributes" are most commonly, for a bulk drug substance, morphology, particle size, shape, and fractal dimension.

1. SIMPLEST DECOMPOSITION MODES OF PURE SOLIDS

If a solid is placed in a vacuum and exposed to temperatures at which it decomposes at a measurable rate, one of the following situations may arise:

I Solid → solid + solid
II Solid → solid + liquid
III Solid → liquid + liquid
IV Solid → solid + gas
V Solid → liquid + gas
VI Solid → gas + gas

Other schemes are theoretically possible, but not likely. Of the above, it is schemes IV and V which will be treated in some detail below, because they are the ones most investigated in the pharmaceutical sciences. It will later be shown that most pharmaceutical systems will not be of such a purist nature, but the experiences gathered from examining them will throw light on several important real-life situations.

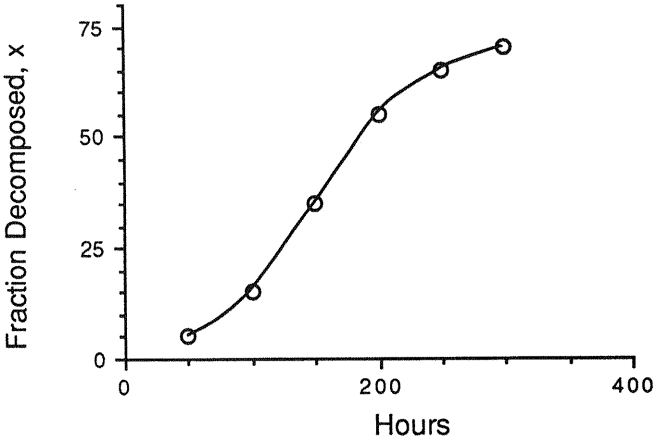

Fig. 1 Decomposition of *p*-aminosalicylic acid at 65°C in vacuo. (Figure constructed from data by Carstensen and Pothisiri, 1975.)

2. THE SOLID TO SOLID + GAS REACTION

This reaction has been investigated by Prout and Tompkins (1944) and later by Kornblum and Sciarrone (1964) and Carstensen and Pothisiri (1975). A typical example of such a reaction is that of p-aminosalicylic acid, shown in Table 1 and Fig. 1. It is noticed that the profile is S-shaped. The general explanation for this type of reaction is the following:

No solid has a smooth surface, i.e., there are always surface imperfections. These could be "steps" in the surface or they could be crystal defects. These sites are more energetic than the remaining sites. They are most likely to occur at surfaces, which in any event are populated with molecules that are unlike the molecules in the bulk of the crystal. For instance they have at least one less neighbor than bulk molecules. It is assumed that decomposition is more likely to occur at such "activated" sites (Fig. 2).

Once a molecule decomposes at an activated site it changes its geometry, and hence the neighboring molecules are more likely to decompose. There will then be a chain or plane of activated molecules forming, with a probability of a (second figure in Fig. 2). The rate of formation of activated molecules, N in number at time t, is dN/dt, and this is proportional to N. Initially this is then given by

$$\left[\frac{dN}{dt}\right]_0 = a \cdot [N + N_0] \tag{6.1}$$

Table 1 Decomposition of *p*-Aminosalicylic Acid

Time (hours)	0	50	100	150	220	260	325
Percent decomposed	0	4	18	36	60	70	77

Source: After Carstensen and Pothisiri, 1975.

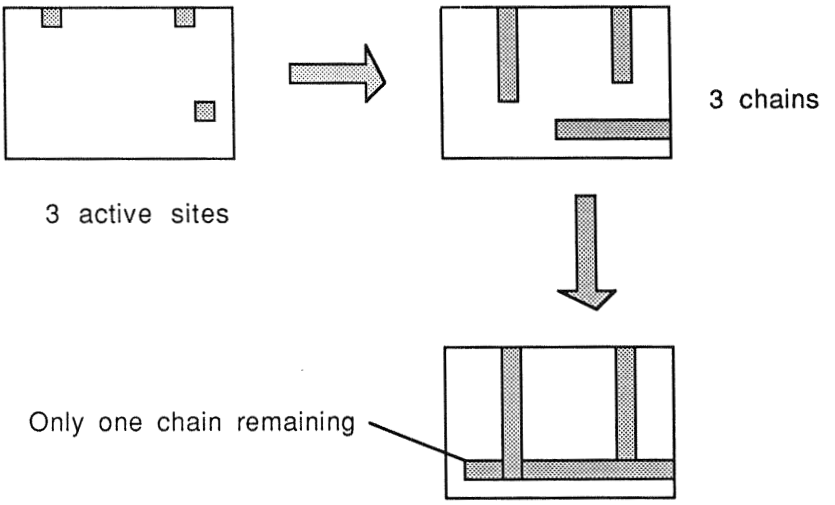

Fig. 2 The propagation of active site chains from three surface sites.

It is obvious that after even a short period of time N becomes much larger than N_0, so that this latter can be dropped at times even remotely larger than zero.

After a certain while (last figure in Fig. 2), planes will start to merge, and hence there will be a termination probability, b, so that at measurable times, Eq. (6.1) becomes

$$\frac{dN}{dt} = \{a - b\}N \tag{6.2}$$

Both a and b are functions of t (or what is equivalent, to the fraction decomposed x). It is reasonable to assume that

$$a = b \quad \text{at} \quad t = t_{1/2} \quad (\text{or } x = 0.5) \tag{6.3}$$

i.e., at the point in time where one half of the substance has decomposed. Also,

$$b = 0 \quad \text{at} \quad t = 0 \quad (\text{or } x = 0) \tag{6.4}$$

since there can be no termination probability at time zero. One (not necessarily the correct) function which satisfies this condition is

$$b = 2xa \tag{6.5}$$

When this is inserted into Eq. (6.2) one obtains

$$\frac{dN}{dt} = a[1 - 2x] \cdot N \tag{6.6}$$

The decomposition rate, dx/dt, is proportional to N, i.e., $dx/dt = k \cdot N$ or

$$N = \frac{1}{k}\frac{dx}{dt} \tag{6.7}$$

Equation (6.6) can now be written

$$\frac{dN}{dt} = \frac{a}{k}[1 - 2x]\frac{dx}{dt} \tag{6.8}$$

Chain differentiation of dN/dt gives

$$\frac{dN}{dt} = \frac{dN}{dx}\frac{dx}{dt} \tag{6.9}$$

Introducing Eq. (6.8) into Eq. (6.9) gives

$$\frac{dN}{dt} = \frac{dN}{dx}\frac{dx}{dt} = \frac{a}{k}[1 - 2x]\frac{dx}{dt}$$

dx/dt is cancelled out of the last part of this equation to give

$$\frac{dN}{dx} = \frac{a[1 - 2x]}{k} \tag{6.10}$$

which integrates to

$$N = \frac{a}{k}(x - x^2) \tag{6.11}$$

Equation (6.7) is now introduced to give

$$\frac{1}{k}\frac{dx}{dt} = \frac{a}{k}x(1 - x) \tag{6.12}$$

which integrates to

$$\ln\left[\frac{x}{1 - x}\right] = a(t - t_{1/2}) \tag{6.13}$$

The equations have a zero time problem, since the equation is not defined for $x = 0$. This is a consequence of neglecting N_0. Similar paradoxes exist in the scientific literature. The Gibbs adsorption isotherm, for instance, is not defined for $C = 0$, i.e., liquid without surfactant. In the case of solid state stability, it might be thought of in the vein, that as the material is being produced, i.e., at time zero (e.g., through recrystallization), it is already decomposing (however little).

Example 6.1.

A set of decomposition data for a sample of a 5.52 mmoles of a solid are as shown in Table 2. Obtain the rate constant and the value of $t_{1/2}$.

Answer.

The transformation of data into the form of Eq. (6.13) is shown in Table 3. These data are plotted in Fig. 3. The least squares fit of the line according to Eq. (6.13) is

$$\ln\left[\frac{x}{1 - x}\right] = 0.868(t - 4) \tag{6.14}$$

Table 2 Prout–Tompkins Data at 55.6°C

Time	0	1	2	3	4	4.5	5
Gas (mmoles)	0	0.48	0.86	1.56	2.77	3.71	4.96

Table 3 Data in Table 4.2 Treated According to Eq. (6.13)

Time	0	1	2	3	4	4.5	5
Gas (mmoles)	0	0.48	0.86	1.56	2.77	3.71	4.96
x	0	0.087	0.155	0.28	0.50	0.668	0.893
$\ln[x/(1-x)]$		−1.75	−1.17	−0.58	0	0.291	0.581

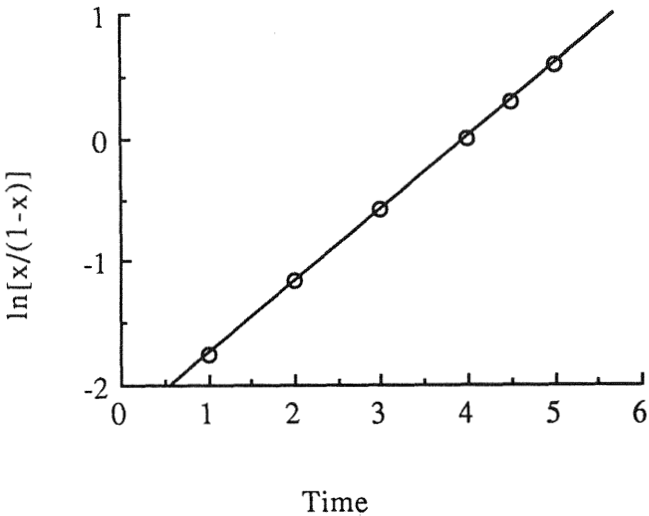

Fig. 3 Data from Table 2 plotted according to Eq. (6.13). The least squares fit is $\ln[x/(1-x)] = -2.334 + 0.5832t$ with an R value of 1.00.

This type of reaction embodies the dehydration of solid hydrates. Leung et al. (1998a,b) have shown that aspartame 2.5 hydrate cyclizes by Prout–Tompkins kinetics and that the rate constants follow an Arrhenius equation.

3. TEMPERATURE DEPENDENCE OF THE SOLID TO SOLID + GAS REACTION

The rate determining parameter in Eqs. (6.12) and (6.13) is a. In general, activation energies encountered in pharmaceutical systems are between 15 and 30 kCal/mole. However, the parameter a is a stoichiastic parameter and is not necessarily of this order of magnitude. Figure 4 shows the data from Table 3 extended to several temperatures. The least squares equation for the Arrehenius plot is

$$\ln[a] = 83.553 - 28.42\frac{1000}{T} \tag{6.15}$$

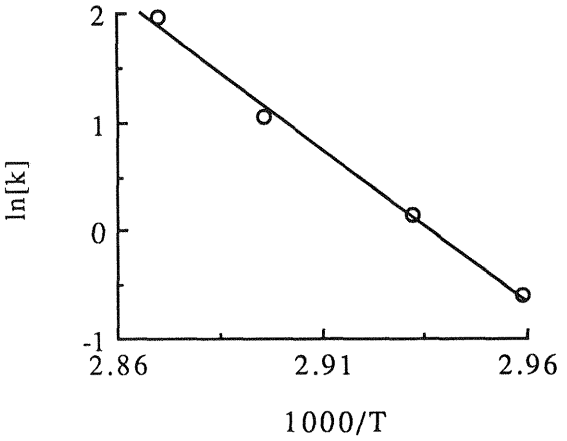

Fig. 4 Arrhenius plot of a values from data of the type shown in Table 2 carried out at 65 (Table 2), 68, 72, 75°C. Least squares fit: $\ln[k] = 83.553 - 28.421\{1000/T\}$ with $R = 0.997$.

and it is seen that $E_a/R = 28.42$ kCal, i.e., E_a is about 57 kCal. In most solid to solid + gas reactions the activation energy will be excessively high (up to 80 kCal/mole). This means that the decomposition will occur at a measurable rate over a narrow temperature range, T_1 to T_2. Below T_1 it is too slow to allow detection of the entire curve in a reasonable length of time, and above T_2 it is too fast to measure with precision. In the data in Fig. 4 the rate constants increase 10-fold in a 10° span. $T_1 - T_2$ is frequently denoted a *decomposition range*, under melting point, in chemical tables.

It should be pointed out that the solid to solid + gas reaction may be so only over a certain temperature range, or to a certain degree of decomposition. Fig. 5 shows the eutectic diagram of a compound with its solid decomposition product.

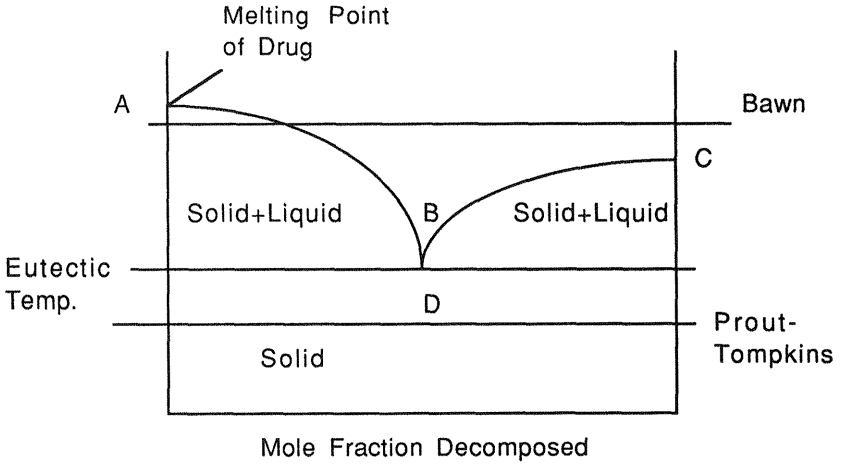

Fig. 5 Binary melting point diagram showing areas where Bawn kinetics apply and where Prout–Tompkins kinetics apply.

If the study is carried out at temperatures below the eutectic temperature, T^*, then the reaction will be solid to solid + gas. If above the eutectic temperature, then the reaction will be solid to solid + liquid + gas. The compounds reported in literature to be of the solid to solid + gas type are most often inorganic salts, e.g., potassium permanganate (Prout and Tompkins, 1944), silver permanganate (Goldstein and Flanagan, 1964), and some organic compounds, such as oxalic acid and *p*-aminosalicylic acid (Kornblum and Sciarrone, 1964; Pothisiri and Carstensen, 1975; Carstensen and Pothisiri, 1975).

Olsen et al. (1997) showed cefaclor monohydrate to decompose (as judged by related substances) by first-order kinetics. The rate constants could be plotted by Arrhenius plotting and were consistent with ambient rate constants. The reaction scheme, when amorphous material was present, was such that the rates were faster at early time points and then became equal to those of the crystalline modification. The conclusion was that the initial phase was decomposition of amorphous content parallel to conversion of amorphous to crystalline drug.

At times the solid state reaction cannot be completely specified yet can be descried in analytical terms. Tzannis and Prestrelski (1999) described the effect of sucrose on the stability of trypsinogen during spray-drying by plotting denaturation temperatures as a function of melting temperature and found a linear increase between residual activity after spray-drying and melting temperature. Adler and Lee (1999) have reported on the stability of lactate dehydrogenase in spray-dried trehalose.

4. THE SOLID TO LIQUID + GAS REACTION

Many more compounds seem to decompose by this reaction scheme than by the solid to solid + gas. The reaction kinetics are usually referred to as Bawn kinetics (Bawn, 1955). This situation at time t is as shown in Fig. 6, and as seen there will be a certain amount of liquid decomposition product. This amount corresponds to the amount of drug decomposed. However, the liquid decomposition product will dissolve parent compound to the extent, S (mole drug/mole decomposition product), to which it is soluble, so that the amount present in the solid state at time t is the original number of moles, A_0, minus the amount decomposed, A_0x, minus the amount dissolved, A_0Sx.

The rate of decomposition would be the sum of the rates of decomposition in the solid state (assumed first order with rate constant k_s, time^{-1} and in the dissolved state (assumed first order with rate constant k_1 time^{-1}. The rate equation, hence, is

$$\frac{dA}{dt} = -k_s[A_0(1-x) - A_0xS] - k_1[A_0xS] \tag{6.16}$$

Noting that

$$\frac{A}{A_0} = (1-x) \tag{6.17}$$

it follows, by division through by A_0, that

$$\frac{d(1-x)}{dt} = -k_s[1-x-xS] - k_1xS \tag{6.18}$$

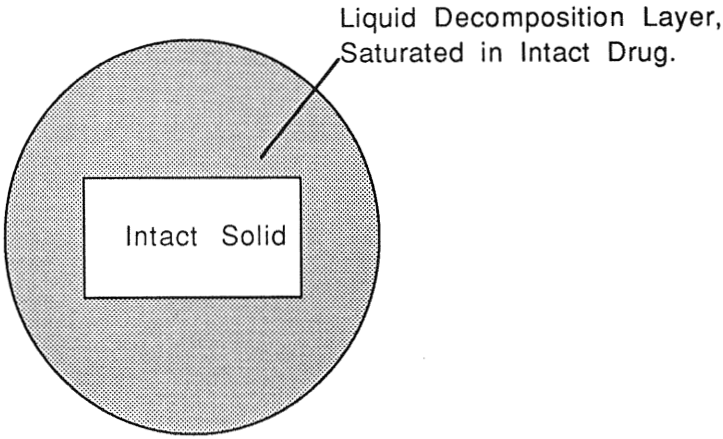

Fig. 6 Situation leading to Bawn kinetics.

or, noting that $d(1-x) = -dx$,

$$\frac{dx}{dt} = k_s[1 - x - xS] - k_1 Sx = k_s[1 + Bx] \tag{6.19}$$

where

$$B = \frac{k_1}{k_s} - 1 - S \tag{6.20}$$

Eq. (6.19) can be integrated, and it then yields

$$\ln[1 + Bx] = Bk_s t \tag{6.21}$$

Using B as an adjustable parameter, it is possible to find the value that makes the data profile through the origin, as dictated by Eq. (6.21), and also gives the best fit.

Figure 7 and Table 4 show an example of data from decomposition of *p*-methylaminobenzoic acid.

To plot this according to Eq. (6.21) it is necessary to assume values of B, plot the data, and assess the goodness of fit by some criterion. A different value of B is then chosen, and this process is repeated until a "best" value of B is arrived at. It can be shown that in general the sums of the squares of the deviations $[s^2_{yx} = \Sigma(y - \hat{y}\backslash\text{wedge})^2/(n-2)]$ of the points from the ensuing line can be used as a criterion. A different criterion is the correlation coefficient. In many cases this is also *not* a good criterion, and criteria for linearity (e.g., Durbin–Watson statistics) are the best. With data fitting to Eq. (6.21), the line must pass through the origin. Fitting the data in this fashion is shown in the table for three values of B (0.1, 0.85, and 2.0). It is, of course, best to do this by computer, and a simple program in BASIC is shown in Table 5.

The number of data points are inserted, the assumed value of B is inserted, and the program is run. One can then in three or four tries arrive at a "best" value for B.

In the case of Eq. (6.21), using the correlation coefficient is not a good parameter, because it simply increases with increasing values of B up to a very high

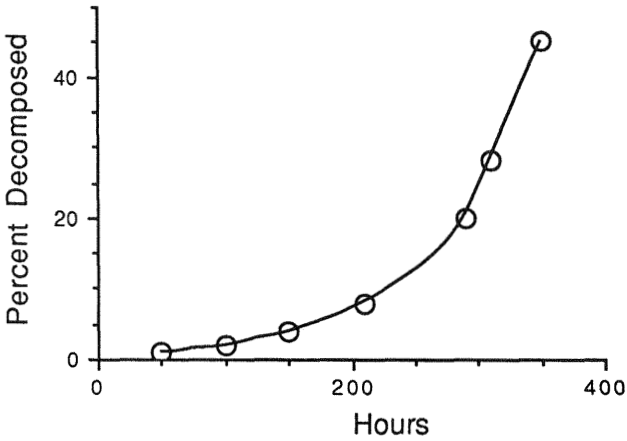

Fig. 7 Data from Table 4. Decomposition of *p*-methylaminobenzoic acid. (Graph constructed from data published by Carstensen and Musa, 1972.)

Table 4 Decomposition Data for *p*-Methylaminobenzoic Acid

Time (h)	0	50	110	150	210	290	310	350
% Decomposed	0	1	2	4	8	20.5	27.9	45

(unrealistic) value and also results in a very high intercept (see Table 6). All the correlation coefficients are good. The best criterion would be a criterion that dealt with curvature, but a simpler one, as stated, is simply to note the intercept, which should be zero (see Fig. 8).

Studies of this type are usually done on a vacuum rack. In this, the pressure is monitored as a function of time, and the sample can be observed. At a given point in time (which is quite reproducible), the last trace of solid will disappear. At this point in time, t^*, the amount not decomposed, $A_0(1-x)$, is just sufficient to dissolve the amount of liquid, A_0x, present, i.e., at time t^*:

$$S = \frac{1 - x^*}{x^*} \tag{6.22}$$

where x^* is the mole fraction decomposed at time t^*. Therefore Eq. (6.21) is valid from time 0 to time t^*. If $t^* = 350$ (as in the example used here), and $x^* = 0.45$ at this point, it follows that

$$S = \frac{0.55}{0.45} = 1.22 \text{ moles/mole} \tag{6.23}$$

The slope in the above case is 0.01 h^{-1}. Since the slope is $[B \cdot k_s]$ it follows that

$$k_s = \frac{\text{slope}}{B} = \frac{0.01}{0.85} = 0.012 \text{ h}^{-1} \tag{6.24}$$

Table 5 Program for Obtaining Best Values by Manual Iteration

```
100  PRINT "Type in data as x,y, in 400 block"
110  INPUT "Number of Data Points=";N1
120  INPUT "Iteration Parameter, B=";B
130  PRINT "T";SPC(6);"X";SPC(6);"LN(1+BX)
140  PRINT "----------"
200  READ A, C
210  X=A
220  Y=LOG(1+B*C)
230  X1=X1+X
240  X2=X2+(X^2)
250  Y1=Y1+Y
260  Y2=Y2+(Y^2)
270  Z1=Z1+(X*Y)
280  N2=N2+1
300  PRINT X;SPC(6);C;SPC(6);Y
310  IF N2=N1 goto 700
400  DATA 50,1
410  DATA 100,2
420  DATA 150,4
430  DATA 210,8
440  DATA 290,20
450  DATA 310,28
460  DATA 350,45
700  Z2=X2-((X1^2)/N2)
710  Z3=Y2-((Y1^2)/N2)
720  Z4=Z1-(X1*Y1/N2)
730  Z5=Z4/Z2
740  Z6=(Y1-(Z5*X1))/N2
750  PRINT
760  PRINT "Slope="; Z5
770  PRINT "Intercept="; Z6
780  Z7=(Z4^2)/(Z3*Z2)
790  Z8=(Z7)^(0.5)
800  PRINT "Correlation Coefficient=";Z8
810  Z9=(Z3-((Z5^2)*Z2))/(N2-2)
820  PRINT "syx^2=";Z9
```

k_1 is now calculated from Eq. (6.20):

$$0.86 = \frac{k_1}{0.012} - 1 - 1.22 \tag{6.25}$$

i.e.

$$k_1 = 3.08 \times 0.012 = 0.037\ \text{h}^{-1} \tag{6.26}$$

Beyond t^* the system is a solution system and should decompose by first-order kinetics. The density of the liquid will actually change with time, but it is assumed that both parent drug and decomposition product have approximately the same

Table 6 Data in Table 5 Treated by Eq. (6.21)

	$\ln[1+Bx]$		
Time (h)	$B=0.1$	$B=0.85$	$B=2$
50	0.095	0.615	1.099
100	0.182	0.993	1.610
150	0.334	1.481	2.200
210	0.588	2.054	2.830
290	1.099	2.890	3.710
310	1.335	3.210	4.040
350	1.705	3.677	4.510

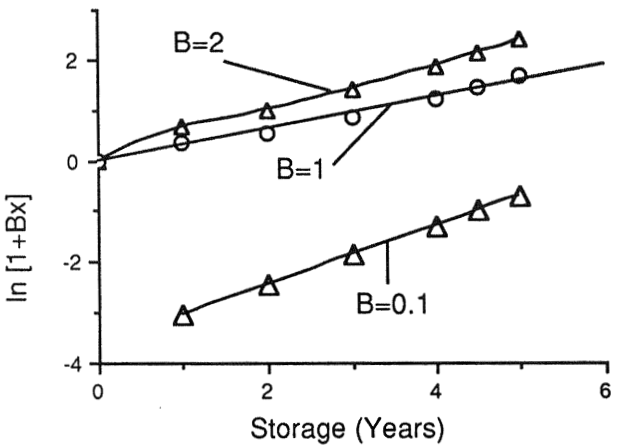

Fig. 8 Data from Table 4 treated by Eq. (6.21).

density. The common density is denoted ρ, and since there is a total number of A_0 moles, the volume of liquid is $A_0\rho$. The initial molar concentration (at time t^*) is, therefore, $A_0(1-x*)/[A_0/\rho] = (1-x*)/\rho$. The time is counted from $t=t^*$ and the concentration at time $t-t^*$ is $(1-x)/\rho$, so that

$$\ln\left[\frac{1-x}{\rho}\right] = -k_1 t + \ln\left[\frac{1-x^*}{\rho}\right] \tag{6.27}$$

or

$$\ln\left[\frac{1-x}{1-x^*}\right] = -k_1(t-t^*) \tag{6.28}$$

or

$$x = 1-(1-x^*)e^{-k_1 t} \tag{6.29}$$

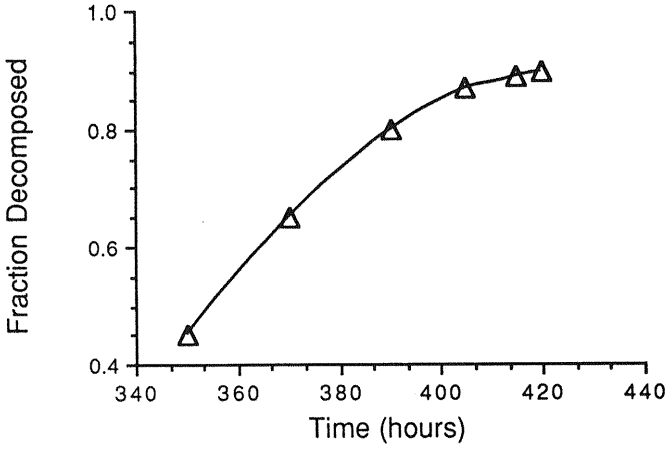

Fig. 9 Decomposition of *p*-methylaminobenzoic acid after t^* (350 hours) at which point $x = 0.45$ (i.e., $1 - x = 0.55$, as used in Fig. 10). (Graph constructed from data published by Carstensen and Musa, 1972.)

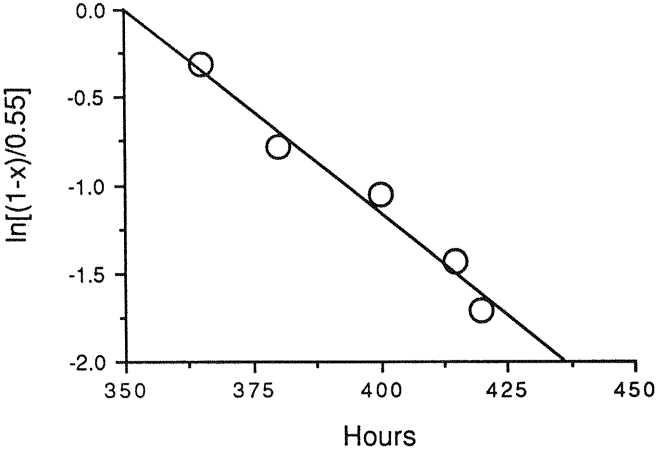

Fig. 10 Data in Fig. 9 treated according to Eq. (6.28). (Graph constructed from data published by Carstensen and Musa, 1972.)

Data of this type, for *p*-methylaminobenzoic acid, are presented in Figs. 9 and 10. It is seen that the data are quite first order. The first-order rate constant obtained from this plot is $k_1 = 0.040\ \text{h}^{-1}$ in quite good agreement with the value of 0.037 found from the first part of the curve.

When the total curve is plotted (i.e., when Fig. 7 and Fig. 9 are combined), then an S-shaped curve results. Unlike the Prout–Tompkins curve, the Bawn curve is a two-phase curve, one part relating to the phase where there is solid present, the other to the part where all solid has dissolved.

The values of x^* obtained at t^* will differ from temperature to temperature, since the solubility is a function of temperature. This is actually the value of the

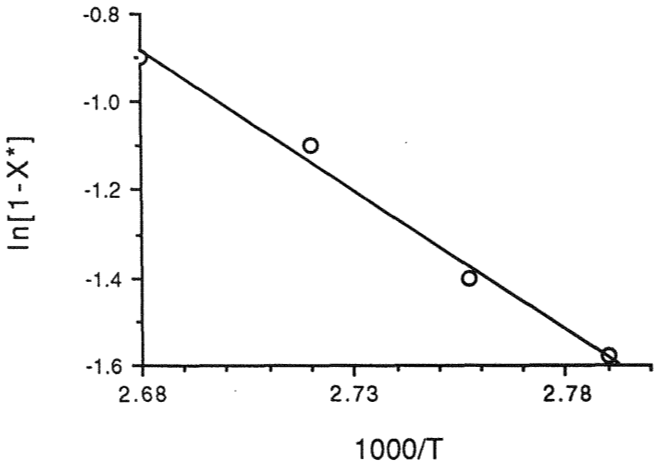

Fig. 11 $\ln[1-x^*]$ as a function of $1000/T$. Least squares equation: $y=16.19-6.37x$ ($R=1.00$). (Graph constructed from data by Carstensen and Kothari, 1983.)

liquidous line on a eutectic diagram. The melting point depression curve (Maron and Prutton, 1965) is given by

$$\ln(1-X^*)=\frac{\Delta H}{R}\left[\frac{1}{T_f}-\frac{1}{T}\right] \tag{6.30}$$

Such plots are quite linear, as shown in Fig. 11.

5. THE NG EQUATION

Ng (1975) suggested the following global equation for solid state decomposition:

$$\frac{dx}{dt}=x^n(1-x)^p \tag{6.31}$$

As pointed out earlier, a modification of this equation is

$$\ln\left\{\frac{x^n}{(1-x)^p}\right\}=-k'(t-t_i) \tag{6.32}$$

which may be written as

$$\ln\left\{\frac{x^q}{1-x}\right\}=-k(t-t_i) \tag{6.33}$$

where

$$k=\frac{k'}{p} \tag{6.34}$$

and

$$q = \frac{n}{p} \tag{6.35}$$

6. TOPOCHEMICAL REACTIONS

There are theories akin to the above, which simply, empirically state that (a) decomposition starts at the surface of the solid and works inwards. If, for instance, the solid were a cube originally with side a_0 cm, then, after a given time the side length, a, would be

$$a = a_0 - kt \tag{6.36}$$

i.e., it is assumed that the decomposition "front" progresses in a linear fashion. This is akin to physical phenomena such as crystal growth (the so-called McCabe law). At time t there will, therefore, be an amount undecomposed given by

$$N\rho a^3 = N\rho[a_0 - kt] \tag{6.37}$$

where N is the number of particles in the sample and ρ is the density of the solid. The original volume of the solid was Na_0^3, so that the fraction not decomposed, x, would be given by

$$x = \frac{N\rho a^3}{[N\rho a_0]^3} = \left[\frac{a}{a_0}\right]^3 = \left[1 - \left(\frac{k}{a_0}\right)t\right]^3 \tag{6.38}$$

It is noted from Eq. 38 that the rate constant (k/a_0) is particle size dependent. This property will be touched on frequently in the following.

An example of this type of decomposition pattern is aspirin in an alkaline environment (Nelson et al., 1974). This is shown in Fig. 12.

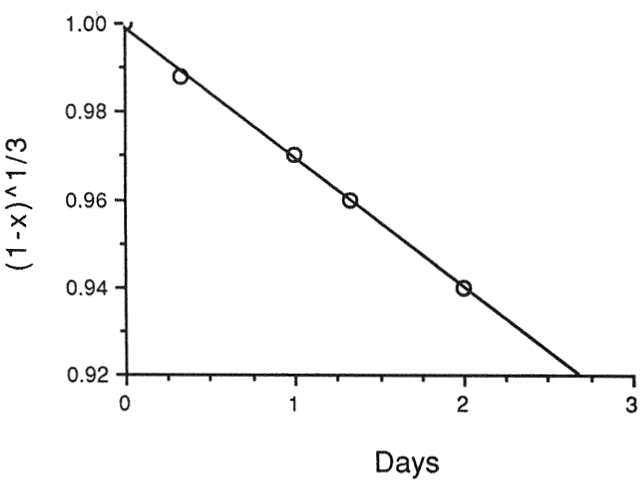

Fig. 12 Decomposition of aspirin in alkaline environment. Least squares fit: $y = 1.0 - 0.0295x$ ($R = 1.00$). (Graph constructed from data by Nelson et al., 1974.)

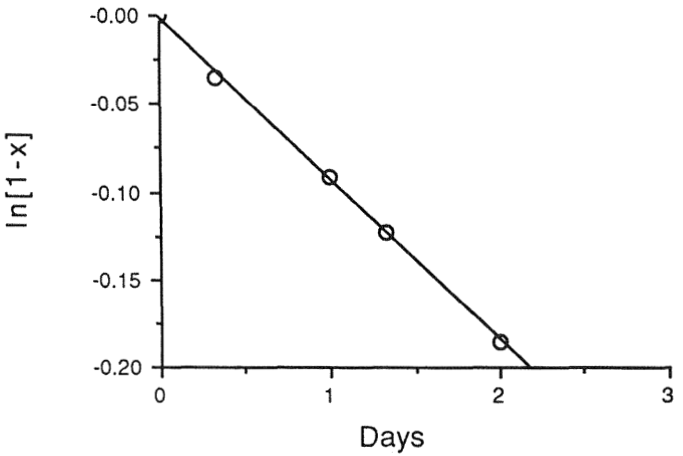

Fig. 13 Data in Fig. 12 treated as first order.

In general it is not possible to distinguish between a reaction of the type described by Eq. 38 and a first-order reaction. Only with excellent precision, with a fairly large number of assays, and with a sufficiently large decomposition will it be possible to distinguish between the two. The data in Fig. 12 are shown treated as first order in Fig. 13.

7. DIFFUSION CONTROLLED INTERACTIONS

Figure 14a shows a situation where a solid, A, is in contact with another solid, B. The contact area is assumed to be 1 cm^2. It is assumed that A can react with B in this situation, i.e.,

$$A + B \rightarrow C \tag{6.39}$$

As the reaction proceeds (Fig. 14b), decomposition product, C, will accumulate between A and B, and at a given time t, compound A must diffuse to the surface of compound B through a layer of compound C, h cm thick, in order for the reaction to take place. The density of B is denoted ρ. A layer of B h cm thick would contain $h\rho$ g of B, and hence

$$\frac{dB}{dt} = D\frac{dh}{dt} \tag{6.40}$$

By Fick's first law, dB/dt is inversely proportional to h, so that we may write

$$\rho\frac{dh}{dt} = \frac{q}{h} \tag{6.41}$$

or

$$h \cdot dh = \left[\frac{q}{\rho}\right]dt \tag{6.42}$$

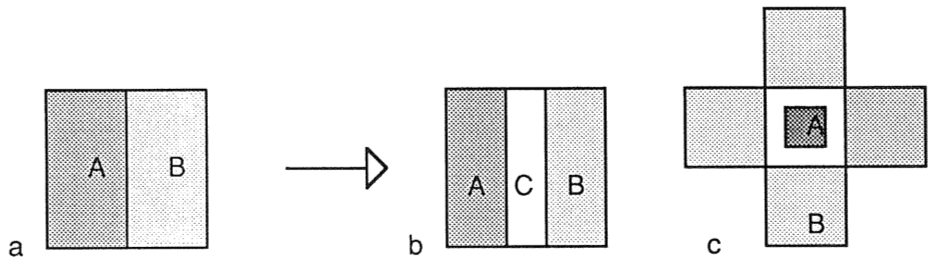

Fig. 14 Stages in Jander kinetics.

This may be integrated to

$$h^2 = \left[\frac{2q}{\rho}\right] t = k't \tag{6.43}$$

or

$$h = [k't]^{1/2} \tag{6.44}$$

where $k' = 2q/\rho$. If, as shown in Fig. 14, A and B are cubical, of side length a_0 initially, and a at time t, and if B is surrounded by A as shown, then

$$h = a_0 - a \tag{6.45}$$

The amount retained at time t is

$$\begin{aligned}(1-x) &= \left[\frac{a}{a_0}\right]^3 = \left[\frac{a_0 - a_0 + a}{a_0}\right]^3 \\ &= \left[1 - \frac{h}{a_0}\right]^3 = \left[1 - \frac{\{kt\}^{1/2}}{a_0}\right]^3\end{aligned} \tag{6.46}$$

or

$$\{1 - (1-x)^{1/3}\}^2 = \frac{kt}{a_0^2} \tag{6.47}$$

where x is fraction decomposed. It is seen that the rate constant is related to the particle size, i.e., the finer the particles the larger the rate constant. A system of this type is, again, the aspirin–sodium bicarbonate system, but at lower temperatures. At higher temperatures, the autodecomposition of aspirin is higher than the diffusion coefficient (related to q), and the reaction at higher temperatures then follows Eq. (6.40) (Nelson et al., 1974).

Recently it has become customary to compare polymorphic and pseudo-polymorphic transformation data with prevailing solid state equations, e.g. forms of the Ng-equation. Several such equations, some of them already alluded to, are listed in Table 7.

Table 7 Equations Relating to Decomposition in the Solid State

$\ln(x/(1-x)) = kt$	surface nucleation, Prout–Tompkins equation
$\{-\ln(1-x)\}^{1/n} = kt$	n-dimensional nuclear growth (Avrami)
	n-dimensional nucleus growth
$1-(1-x)^{1/n} = kt$	n-dimensional boundary reaction
$x^2 = kt$	diffusion in one dimension
$(1-x)\ln(1-x)+x = kt$	diffusion in two dimensions
$(1-(1-x)^{1/3})^2 = kt$	diffusion in three dimensions (Jander equation)

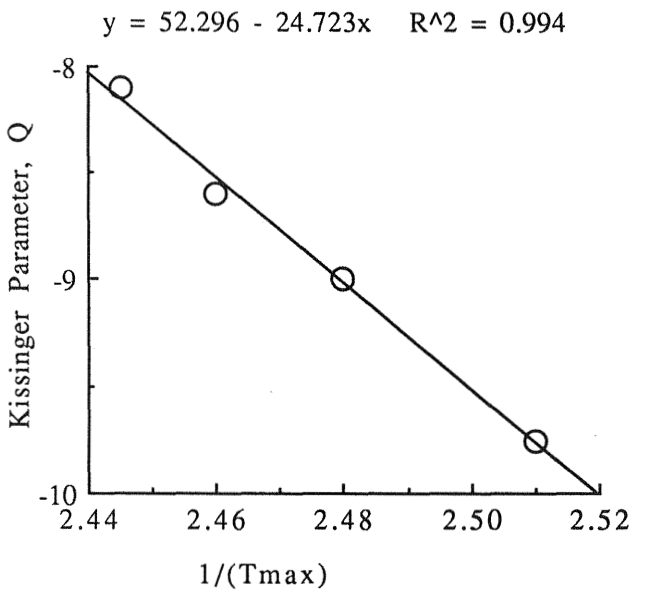

Fig. 15 Kissinger plot of polymorph II of glybuzole. (Plot constructed from data published by Otsuka et al., 1999.)

There has been a tendency in recent literature to simply fit data to several (or all) of these equation, and the equation that gives the "best fit" is then assumed to be the mechanism. Figure 15, for instance, shows a literature example of such data. It is claimed that this data best fits a Jander equation (and such treatment is shown in Fig. 16), but first of all the fit is not good, and secondly, it is obvious that the phase, C, in the Jander model (Fig. 14) cannot possibly apply to a polymorphic transformation where the reaction is simply $A \rightarrow B$, not $A + B \rightarrow C$.

It is emphasized here that sorting out mechanisms by statistical analysis can be dangerous.

Several recent investigations in this field have appeared in recent years. Fini et al. (1999) have studied the dehydration and rehydration of diclofenac salt hydrate at ambient temperature. Otsuka et al. (1999) investigated three forms of glybuzole

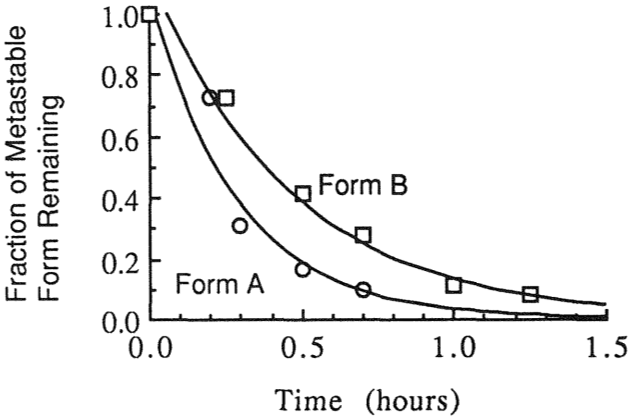

Fig. 16 Literature data dealing with two polymorphic transformations allegedly diffusional because it adheres (somewhat) to a Jander model.

(I, II, and amorphate) and found all to have fairly much the same solubility. Neither form I nor form II changed after storage at 40°C/75% and 0% RH for 2 months. DSC for form I showed no peak other than a sharp melting endotherm at 167.4°; form II showed a slight endotherm at 116.8°C and a sharp endotherm at 166.6°C. The amorphate showed a (slight) exotherm peaking at 81.5°C, presumably due to crystallization, and a sharp endotherm at 167.3°C. From this it would be reasonable to conclude that form II is stable at room temperature and transform to I at 116.8°C, this latter form being stable at the higher temperatures.

The authors estimated the polymorphic stability of form II by way of the Kissinger equation (Kissinger, 1956):

$$\partial\left\{\frac{\ln(\phi/T_{\max}^2)}{\partial(1/T_{\max})}\right\} = \frac{-E}{R} \tag{6.48}$$

where ϕ is the rate of heating, $T_{\max}$ is the temperature at the peak maximum in the DSC, E is the activation energy, and R is the gas constant. If the experiment is conducted at different heating rates, different $T_{\max}$ values result, and in the case of glybuzole there were four such values.

It can be seen from the graph that the activation energy is $24.723 \times 1.99 = 49.2$ kCal/mol. Otsuka et al. (1991, 1993, 1999) employed the Jander equation to explain crystallization rates of compounds, e.g., amorphous glybuzole. As mentioned above, however, the Jander equation is based on an assumption of a layer of "reaction product," and such a layer (i.e., such a model) cannot be conceived of in a polymorphic transformation. What would be the "reaction product"?

8. GENERAL INTERACTIONS IN DOSAGE FORMS

It is tempting to think of a tablet as an agglomeration of individual particles, independent of one another, but this cannot be the case. By their mere nature, particles

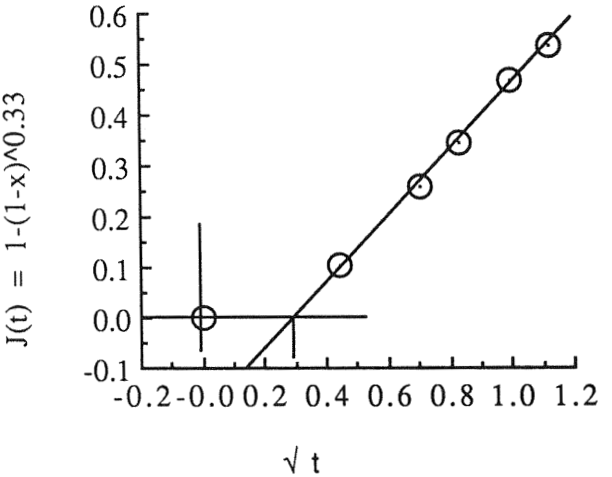

Fig. 17 Data from form B in Fig. 15 treated according to a Jander model. The curve follows the least squares fit equation: $J(t) = -0.194 + 0.652\sqrt{t}$.

are fused together (either by brittle fracture or by plastic deformation in tablets or tamping in capsules), and if the created contact area is between two different components of the tablet (one being the drug), then there is the possibility of interaction. It is highly likely that moisture plays a part in all of these. In fact, in one of the cases to be discussed below (tartaric acid + sodium bicarbonate) this is the case (although the tablet can, for all practical purposes, be anhydrous at the onset).

The most common type of interaction in solid dosage forms is actually between water and drug. This is a large topic in itself, and the following chapter is devoted to it. The topic discussed here will be of special cases where water is not the interactant (or the main interactant).

The following cases are illustrative examples:

1. Tartaric acid and sodium bicarbonate
2. Aspirin and phenylephrine
3. Aspirin and lubricants

In addition to the points made, note that in the curve in Fig. 17 a lag time has to be invoked for the data to linearize.

8.1. Tartaric Acid and Sodium Bicarbonate

This is a common combination in effervescent tablets. When the tablet is added to water, the acid and the base will react, forming carbon dioxide, which produces the desired bubble effect:

$$R_2(COOH)_2 + 2NaHCO_3 \rightarrow R2(COO^-)_2 + 2Na^+ + 2H_2O + 2CO_2 \qquad (6.49)$$

To be strictly correct, the left hand side should be written in ionic form as well.

Of course, it is necessary that this reaction not take place prior to the time it reaches the consumer, because if the reaction does occur in the solid state, then

(a) carbon dioxide will form in the container, (b) the tablet will become softer, and (c) upon "reconstitution" the bubble effect will be reduced to the extent that carbon dioxide was lost in storage.

The evolution of carbon dioxide would normally build up pressure in a glass bottle, but the tubes in which effervescent products used to be sold were not tight, and the carbon dioxide could escape. The same is true to a great extent in plastic bottles and in plastic blister packs, but the problem that the reaction (as will be demonstrated below) is catalyzed by moisture, in other words the fact that the container is not hermetic in this aspect, is a disadvantage. This is so sensitive that during manufacture extra precautions are taken to keep the relative humidity of the processing areas low. Hence one must also pack the products in hermetic containers, and aluminum foil has become a popular means of doing this. If, however, the initial moisture is not low enough, then the reaction will proceed, and in this case the internal pressure will cause the aluminum foil to balloon.

The solid-state reaction has been investigated by Usui and Carstensen (1985) and by Wright and Carstensen (1986). When the reaction occurs in the solid state, there are two questions that present themselves:

1. Is moisture important and if so in what sense?
2. What is the stoichiometry? Is it that of Eq. (6.48) or is it

$$R_2(COOH)_2 + NaHCO_3 \rightarrow R_2(COOH)COONa + H_2O + CO_2 \qquad (6.50)$$

Usui checked the weight loss of heated samples in hermetic containers, utilizing different ratios of acid and base, and established that the stoichiometry is that of Eq. (6.50), i.e., the mole-to-mole interaction of tartaric acid and sodium bicarbonate.

He next studied the weight loss in open containers and demonstrated that the tartaric acid did not lose weight, and that the sodium bicarbonate, and the mixture of sodium bicarbonate and tartaric acid, lost weight at a low rate, corresponding to that of the sodium bicarbonate itself. In other words, in an open container there was no interaction, simply decarboxylation of the bicarbonate itself.

He next studied the effect of compression on the decomposition of sodium bicarbonate. Characteristic curves are shown in Fig. 18. It is noted that the decomposition rate are a function of applied pressure. In the following it is assumed that the particles are isometric and that the reaction rate is proportional to the surface area of unreacted sodium bicarbonate. The following nomenclature is used: there are M g of unreacted sodium bicarbonate at time t, and M_0 initially. There are N particles each of area a, volume v, and density ρ. The surface area is proportional to the two-thirds power of the volume by the isometry factor Q, i.e.,

$$a = Qv^{2/3} = Q\rho^{-2/3}m^{2/3} \qquad (6.51)$$

The total area, A, hence is given by

$$A = NQ\rho^{-2/3}m^{2/3} = N^{1/3}Q\rho^{-2/3}M^{2/3} \qquad (6.52)$$

It follows that

$$A_0 = N^{1/3}Q\rho^{-2/3}M_0^{2/3} \qquad (6.53)$$

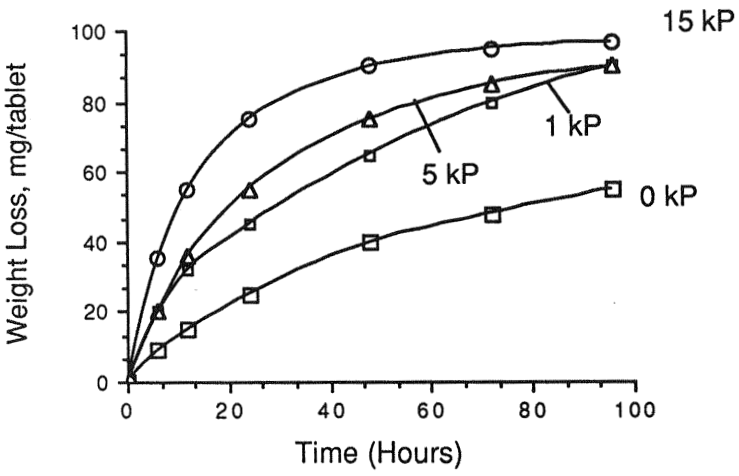

Fig. 18 Effect of tableting pressure on sodium bicarbonate decomposition at 70°C. (Graph constructed from data by Usui and Carstensen, 1985.)

The decomposition rate is proportional to the surface area at time t, i.e.,

$$\frac{dM}{dt} = -k''A = -k'M^{2/3} \tag{6.54}$$

where

$$k' = k''N^{1/3}Q\rho^{-2/3} \tag{6.55}$$

Rearrangement of Eq. (6.55) gives

$$\frac{dM}{M^{2/3}} = -k't \tag{6.56}$$

This can be integrated, and when initial conditions are imposed the following expression results:

$$\left(\frac{M}{M_0}\right)^{1/3} = (1 - X)^{1/3} = 1 - kt \tag{6.57}$$

where X is mole fraction decomposed, and where

$$k = \frac{k''N^{1/3}Q\rho^{-2/3}}{3M_0^{1/3}} \tag{6.58}$$

Eliminating N by inserting Eq. (6.55) into Eq. (6.58) gives

$$k = \frac{k''A_0}{3M_0} \tag{6.59}$$

The data should, therefore, plot by a cube root equation and Fig. 19 shows this, indeed, to be the case.

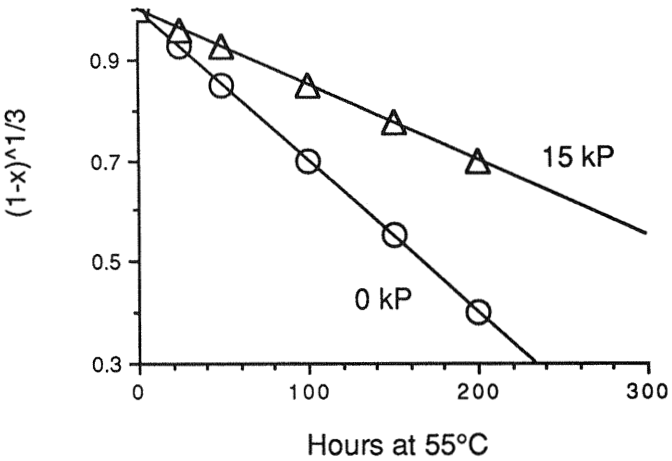

Fig. 19 Cube root plot of sodium bicarbonate decomposition at 55°C. Least squares fit equations: 0 kP: $y = 1 - 0.0015x$ ($R = 1.00$); 15 kP: $y = 1 - 0.003x$ ($R = 1.00$). (Graph constructed from data by Usui and Carstensen, 1985.)

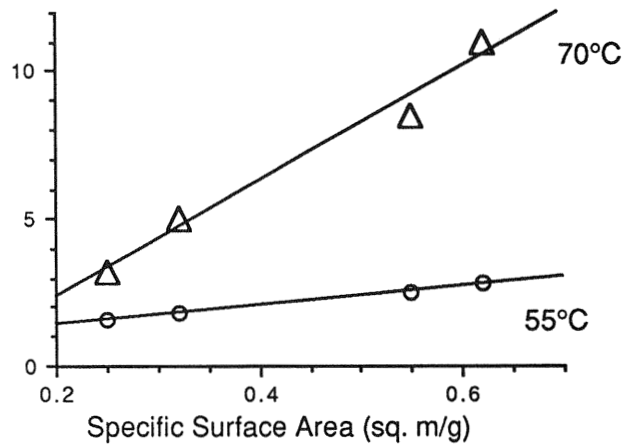

Fig. 20 Cube root constants from Fig. 19 versus specific surface areas. Least squares fits: 70°C: $y = -1.534 + 19.447x$ ($R = 0.99$); 55°C: $y = 0.788 + 3.188x$ ($R = 1.00$) (Graph constructed from data by Usui and Carstensen, 1985.)

The rate constants according to Eq. (6.59) should be proportional to the surface area at time zero (A_0/M_0). That this is the case is shown in Fig. 20. The rate constants follow an Arrhenius plot (Fig. 21) and are in line with the data reported by Schefter et al. (1974).

In a closed system there is a rapid interaction between the sodium bicarbonate and the tartaric acid in compressed tablets. Even though the system is supposedly dry, it is assumed that there is a very slight amount (z moles) of water present in the table initially and that the reaction starts in a dissolved stage. If this is the case, then, since water is produced in the reaction, there will be an acceleration.

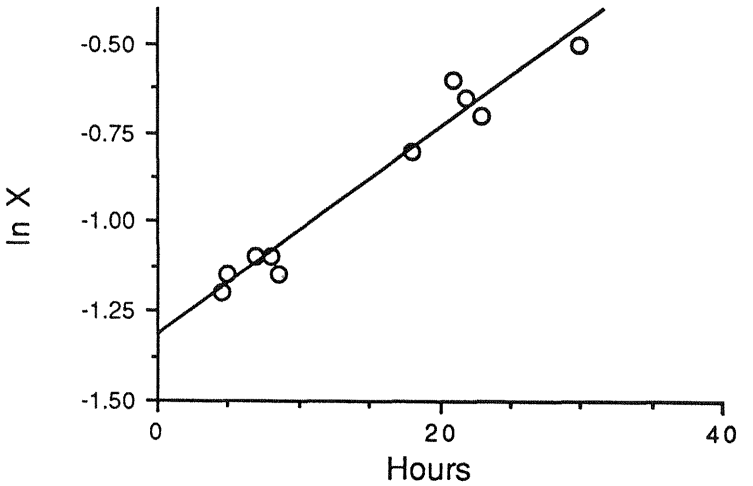

Fig. 21 Decomposition of tartaric acid plus sodium bicarbonate tablets at 55°C (5 kP force). Least squares fit: $\ln\{X\} = -1.3225 + 0.0291 \cdot t$ ($R = 0.98$). (Graph constructed from data by Usui and Carstensen, 1985.)

The data can be modeled in the fashion shown in the following. M' is the number of moles of sodium bicarbonate left at time t, M'_0 is the initial number of moles, S is its solubility in water, and C is the concentration in the water present at time t. S_1 is the solubility of the tartaric acid in water.

According to the reaction scheme the number of moles of water present at time t then is

$$(M'_0 - M' + z)\text{moles} = (M'_0 - M' + z)\ 0.018\ \text{liters} \tag{6.60}$$

The disappearance rate of sodium bicarbonate in solution is given by

$$\frac{-dC}{dt} = k_2 S_1 S \tag{6.61}$$

where k_2 is the second-order rate constant. To express this as number of moles decomposed, this figure is multiplied by the volume of water present, i.e., the expression in Eq. (6.60):

$$\frac{dM'}{dt} = -k^*(M'_0 - M' + z) \tag{6.62}$$

where

$$k^* = 0.018 k_2 S_1 S \tag{6.63}$$

Equation (6.62) can now be recast in the form

$$\ln(M'_0 - M' + z) = k^* t + \ln[z] \tag{6.64}$$

or employing X, the mole fraction decomposed:

$$\ln\left[X + \frac{z}{M_0}\right] = k^* t + \ln\left[\frac{z}{M_0'}\right] \tag{6.65}$$

Recalling that z is a small number, the term z/M_0 is small, and the Eq. (6.65) then simplifies to

$$\ln[X] = k^* t + \ln\left(\frac{z}{M_0'}\right) \tag{6.66}$$

Data are plotted in this fashion in Fig. 21. It is seen that the linearity is quite good. The value of z may be estimated from the intercept and comes to about 0.1 mg per tablet, which is a reasonable figure. This, in essence, shows that the theories suggested by Wright (1983) are correct.

It is obviously of pharmaceutical importance in most situations to slow down the reaction in the solid state and yet maintain the reactivity in the solid state. (An exception to this is when a reaction is purposely carried out during a granulation, for instance.) One way of retarding the reaction rate is to preheat the bicarbonate to 95°C for a certain length of time (White, 1963, Mohrle, 1980). This will react by the scheme

$$2NaHCO_3 \rightarrow Na_2CO_3 + H_2O \tag{6.67}$$

The water formed granulates the mixture and makes it easier to compress. But more importantly, the sodium carbonate formed can form double salts with the bicarbonate. These are dodecahydrates and act as moisture scavengers. They hence stabilize the acid/base mixture in the solid state (if reasonable moisture barriers are provided): any *small* amount of moisture created by a beginning reaction of the type of Eqs. (6.48) or (6.49) will react with a mixture of the carbonate and bicarbonate to form a double salt hydrate.

9. INCOMPATIBILITY PREVENTION TECHNIQUES

Frequently, interactions are particle size dependent. This stands to reason, because the finer a powder is, the more contact points there will be in the tablet mass, hence the larger the potential for interaction. Means of overcoming this are as follows.

In double granulation or pocketing techniques, one component is placed in one granulation, the other in another; keeping the granulations coarse will give fewer contact points, hence less interaction. This is a technique often used in vitamin granulations. Here the more famous incompatibilities are usually those involving (a) cyanacobalamine, iron, and ascorbic acid (b) vitamin A, (c) calcium pantothenate, and (d) tocopherol. The first of these cases is one where *pocketing* is used. This can be accomplished by actually coating (rather than just granulating) the iron salt (often ferrous fumarate) in order to separate it from the remaining ingredients. The other cases will be dealt with separately below.

Other means of separating incompatible ingredients is to make a compression-coated tablet. This consists of an inner tablet compressed in a coating granulation. This principle can be extended to a tablet within a tablet within a tablet

(Bicota™, Manisty). The machines used to make them are, however, slow. If a layer separation is necessary (and effective), then it is most often accomplished by triple-layer tablets. It should noted that these techniques are ineffective in the case of reactions that occur via the gas phase. (These types of reactions will be discussed in the following.)

As mentioned, coating is a special case of pocketing. Ferrous fumarate is sometimes coated, but the most famous case of coating is undoubtedly that of vitamin A esters. Prior to this technique, in the early 1950s vitamin A was added, with an antioxidant, to powder blends that were then encapsulated or tableted. The loss of the vitamin was excessive (frequently 50% in 6 months, plus a processing loss). In the early 1950s Hoffmann-La Roche and Pfizer (almost simultaneously) marketed a so-called beadlet. The coating of vitamin A was a bit different from that of other compounds, since the most common ester (acetate) is a liquid. The coating was therefore done by making an emulsion of the vitamin A in a solution of gelatin, spraying this onto an insoluble starch derivative (which rapidly absorbed moisture), and then further drying the beadlet. After drying, the starch derivative could be separate from the vitamin A by sieving. Later, the coated palmitate bead was introduced, and, with normal precautions, oxidation of the vitamin A (except for the droplets on or rather in the surface) was prevented. It follows from this that the finer the beadlet, the less stable will it be (because there will be more surface droplet of vitamin A). 40 mesh is about the coarsest than can be handled in tableting or encapsulation, and this mesh cut offers a good stability. Obviously compression will cause fracture of the beads to some extent and this is the actual stability problem in a dry tablet.

If moisture is present in the tablet, then the gelatin will soften and become more oxygen-permeable, and the stability will suffer. It is therefore always best to perform moisture stress tests in stability programs. At a point in development where enough tablets are available the following is done: four times the regular sample is taken, and this sample is subdivided into four equal portions, A, B, C, and D. A is placed on stability as is. B is exposed to water vapor in a desiccator, and removed and placed on stability when it has gained 0.5% in weight. (The tablets can be placed on Petri dished and weighed periodically.) The procedure is repeated with C to 1% and D to 2%. The information gained is valuable, because it aids in decisions of the following kind: (a) Should a desiccator bag be used? (b) What should the moisture specification on the product be? (c) If there is no effect of moisture, there would be less of a problem selecting plastic bottles for the product.

10. pH OF THE MICROENVIRONMENT

In the strictest sense, the term pH is not defined in a solid system. For it to have meaning, there must be some water mediation; tocopheryl acetate and calcium pantothenate are cases in point. The former is sensitive to high pH, the former to low pH. Calcium pantothenate is frequently admixed with magnesium oxide and granulated separately from the remaining ingredients. In this manner an alkaline microenvironment is created, which ascertains the stability of the vitamin.

In the case of tocopheryl acetate, the hydrolysis is accelerated by hydroxyl ions. Again it is noted that the reaction must be associated with some dissolution step in small amounts of water. The produced tocopherol is much less stable, and hence the hydrolysis and the presence of water are contraindicated. This is a particular

case where the use of alkaline excipients (e.g. hydroxyapatite) can be deleterious at higher temperatures. In the absence of (or at low levels of) moisture the reaction may not proceed. It is also characteristic that often, higher temperatures are not indicative of what will happen at room temperature.

If it is desired to control the pH of the microenvironment then citric, tartaric, and fumaric acids are the acids of choice. They are, however, all corrosive, and their pharmaceutical handling is far from ideal. In the case of alkali, sodium bicarbonate, sodium carbonate, and magnesium and calcium oxides are common. They are not as corrosive as the acids mentioned, but they are abrasive, and they, too, are not the most ideal substances to handle in a tablet or a capsule.

For certain compounds it is necessary to control the microenvironment in even more drastic fashion. Gu et al. (1990) report on drug excipient incompatibility studies of moexipril hydrochloride and demonstrate that (even "wet") adjustment of the microenvironmental pH (i.e., adding small amounts of water to a mixture of the drug with sodium bicarbonate or sodium carbonate) did not sufficiently stabilize the mix. But when the mixture *was wet granulated*, and when *stoichiometric amounts of alkali were used*, then stabilization resulted. This essentially means that in the solid state *the sodium salt is stable* as opposed to the acid. It might be argued that in such a situation the sodium salt should be manufactured and used as such. It might be argued that it should be claimed as the active ingredient (equivalent to a certain amount of free acid, or in the case of amphoteric substances, the acid addition salt), but often the salt is very soluble and hygroscopic (e.g., potassium clavulanate) and hence difficult to produce. The situation is referred to in the Federal Register as a *derivative drug*.

11. INTERACTIONS INVOLVING A LIQUID PHASE

At times an active ingredient or a decomposition product in a solid dosage form is a liquid, and this may interact with other ingredients in the dosage form. A typical example is the work by Troup and Mitchner (1964) dealing with aspirin and phenylephrine. The authors showed that the decomposition of phenylephrine was linearly related to the formation of salicylic acid. They showed that the decomposition of phenylephrine was an acetylation. This can be thought of in many ways. There has to be some moisture present to allow for the hydrolysis of aspirin. If the salicylic acid is formed by interaction of aspirin with traces of water, then the acetic acid formed may react with the phenylephrine ($R(OH)_3$), again liberating water, so that the moisture does not play a part quantitatively in the overall reaction; in other words,

$$C_6H_4(OCOCH_3) + H_2O \rightarrow C_6H_4(OH)COOH + CH_3COOH \tag{6.68}$$

$$CH_3COOH + 1/3\,R(OH)_3 \rightarrow 1/3\,R(OCOCH_3)_3 + H_2O \tag{6.69}$$

$$C_6H_4(OCOCH_3) + 1/3\,R(OH)_3 \rightarrow 1/3\,R(OCOCH_3)_3 \tag{6.70}$$

An alternate explanation would be that phenylephrine interacted directly with aspirin in an anhydrous solid state to transacetylate, which is not probable. The

question is whether the acetic acid (which has a sizable vapor pressure) interacts with the phenylephrine as a gas with a solid reaction (to be covered shortly) or as a liquid with a solid reaction.

There are other examples of the interaction of acetic acid with active ingredients, e.g., the work by Jacobs et al. (1966), in which acetylation of codeine in aspirin/codeine combinations was demonstrated. Again, whether the acetylation is achieved by acetic acid in the vapor phase or in the liquid state or (more unlikely) whether it is a direct solid to solid interaction, is not resolved at the present time. If it were the latter, then Jander kinetics should actually apply. But it is difficult to distinguish this and pseudo-first-order reactions. If it is an interaction in the liquid state, then it probably occurs by the phenylephrine dissolving in the acetic acid formed.

In more general terms, it is assumed that there are two drugs, A and D. A decomposes (e.g., by hydrolysis) to form a liquid decomposition product *C*. The reactions then are

$$A + H_2O \rightarrow B + C \qquad \text{(rate constant } k\text{)} \tag{6.71a}$$

$$D + C \rightarrow \text{decomposition} \qquad \text{(rate constant } k'\text{)} \tag{6.71b}$$

C is the species that is liquid. In this case a saturated solution (S moles/mole) of D in *C* is formed, and it is assumed that dissolution is fast. Let A be the number of moles of drug #1 present at time *t*, let *C* be the number of moles of acetic acid, and let M denote the molarity of the liquid decomposition product (e.g., for acetic acid at 25°C the density is 1.05 g/mL, so that, since its molecular weight is 60, *M* would be $1005/60 = 16.75$). The rate at which D disappears is the question. It is assumed that the disappearance rate of A is pseudo–first order, i.e.,

$$A = A_0 \exp(-kt) \tag{6.72}$$

The disappearance rate of D depends on how much C is present, so the equation for *C* must first be established and solved. C is created at a rate of kA, but it is consumed by D. The rate of the latter step is given by a second-order reaction term. The concentration of D is *S*, and the concentration of C is *M*. The amount of C at time *t* is *C*, so that (in terms of moles)

$$\frac{dC}{dt} = kA - k'SCM \tag{6.73}$$

Inserting Eq. (6.73), and using and denoting

$$k'SM = a \tag{6.74}$$

where *a* is constant, the following equation is arrived at:

$$\frac{dC}{dt} = kA_0 \exp(-kt) - aC \tag{6.75}$$

Laplace transformation, using **L** notation, gives

$$s\mathbf{L} - 0 = \frac{kA_0}{\mathrm{s}+\mathrm{k}} - a\mathbf{L} \tag{6.76}$$

or

$$\mathbf{L} = \frac{kA_0}{a-\mathrm{k}}\left[\frac{1}{\mathrm{s}+\mathrm{k}} - \frac{1}{\mathrm{s}+a}\right] \tag{6.77}$$

so by taking anti-Laplacians,

$$C = \frac{kA_0}{k'SM - k}\{\exp(-kt) - \exp(-k'SMt)\} \tag{6.78}$$

It follows that the decomposition rate of D is given by

$$\frac{dD}{dt} = k'SCM = aC \tag{6.79}$$

i.e., by integrating Eq. (6.79) and multiplying by a, we obtain

$$D = \frac{kaA_0}{k'SM - k}\left[\frac{\exp(-k'SMt)}{k'SM} - \frac{\exp(-kt)}{k}\right] \tag{6.80}$$

An example of this is shown in Fig. 22 using $A = 50$, $k = 0.2$, and $k'SM = 0.1$. A different situation arises when an insoluble component interacts with a drug in solution (or vice versa). An example of this is the interaction between microcrystalline cellulose (R′CHOHR″) and substituted furoic acids (RCOOH) (Carstensen and Kothari, 1983). The furoic acids decompose when heated by themselves, into a liquid decomposition product and carbon dioxide. In the presence of microcrystalline cellulose, however, the mixture forms carbon monoxide:

$$\mathrm{RCOOH} + \mathrm{R'CHOHR''} \rightarrow \mathrm{Q} + \mathrm{Q'} + \mathrm{CO} \tag{6.81}$$

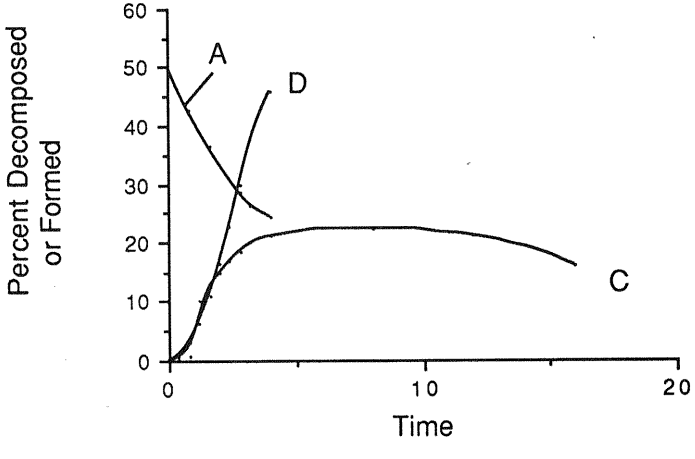

Fig. 22 Stability profile using $A = 50$, $k = 0.2$, and $a = 1$.

Q is a liquid that will dissolve furoic acid to the extent of its solubility and will spread over the microcrystalline cellulose. There will be a number of contact points, N, at which interaction can take place (essentially the "wetted" part of the microcrystalline cellulose). There will be a reaction probability, a, associated with each contact point. The reaction accelerates because the larger the extent it has reacted, the more liquid there will be to dissolve the furoic acid hence the more contact points. At a given point in time there will be overcrowding, since dissolved molecules will be next to contact points that have already reacted. Hence there is also a termination probability, b. But unlike the Prout–Tompkins model, this is finite at time zero.

It might be argued that the external surface of the microcrystalline cellulose would be insufficient to account for the total decomposition. There are however, two types of surface present in microcrystalline cellulose: nitrogen adsorption gives low surface areas (the external area), whereas for instance water isotherms give surface areas 100 times as large (Hollenbeck, 1978, Marshall et al., 1972, Zografi and Kontny, 1986).

By the decomposition at a contact point, it is assumed that the decomposition, creating one liquid decomposition molecule, will dislodge (dissolve) S molecules of furoic acid at the contact point. If the initial number of contact points is N_0 then

$$\frac{dN}{dt} = [-b + a(S-1)]N = qN \tag{6.82}$$

where $q = -b + a(S-1)$. The factor arises from the fact that when molecules react, aS new contact points are created and one (the one at which the reaction took place) is lost.

It follows then from integrating Eq. (6.82) (which can be done, since a and b are assumed constant), that

$$N = N_0 \exp(qt) \tag{6.83}$$

Since, at a given time t, the rate of decomposition is proportional to the number of contact points, then, L being the number of intact alkoxyfuroic acid molecules,

$$\frac{dL}{dt} = -gN \tag{6.84}$$

where g is a constant. From the definition of L it follows that the mole fraction, x, decomposed is given by

$$x = \frac{L_0 - L}{L_0} \tag{6.85}$$

or

$$\frac{dx}{dt} = -\frac{g}{L_0}\frac{dL}{dt} \tag{6.86}$$

Equation (6.84) inserted in this gives

$$\frac{dx}{dt} = \frac{1}{L_0} gN \tag{6.87}$$

Substituting Eq. (6.83) into this gives

$$\frac{dx}{dt} = \frac{gN_0}{L_0} \exp(qt) \tag{6.88}$$

This integrates to

$$x = \frac{gN_0}{L_0 q}[e^{qt} - 1] = A[e^{qt} - 1] \tag{6.89}$$

where the term $A = gN_0/L_0 q$ has been introduced for convenience. Equation (6.89) is equivalent to

$$\ln[1 + Ax] = qt \tag{6.90}$$

Fig. 23 shows data treated in this fashion.

12. CASES OF INTERACTION OF A LIQUID WITH A POORLY SOLUBLE DRUG

There are cases where there are liquids in a solid dosage form. An example is panthenol in a multivitamin tablet. Here it is customary to adsorb the liquid onto a solid carrier, and in the case of panthenol, magnesium trisilicate is used. At elevated temperatures (and at room temperature under compression as well) the panthenol will ooze out of the carrier and come into intimate contact with other solids. If interaction potentials exist, then separation techniques such as triple-layer

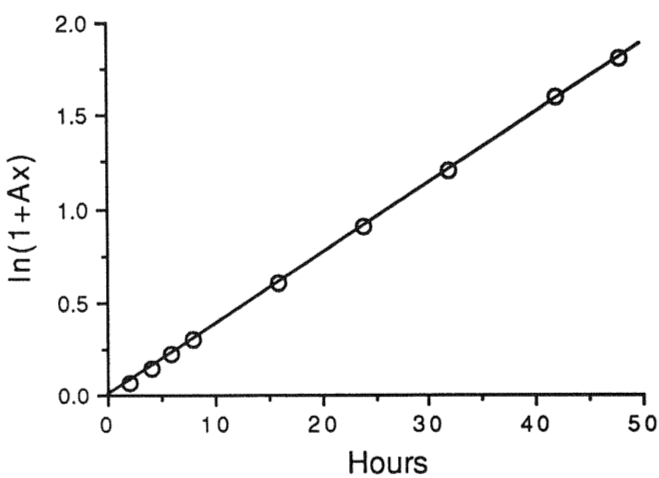

Fig. 23 Furoic acid data treated according to Eq. (6.90). (Graph constructed from data by Carstensen and Kothari, 1983.)

tablets (or compression coated tablets) are resorted to. In this case, the liquid will still ooze into the layer containing its interactant, but the process will be diffusion controlled. It can be shown (Jost, 1962) that the average concentration, C, of the liquid in the neighboring layer, with which it is in contact, is given by

$$\frac{C - C_f}{C_0 - C_f} = Qe^{-kt} \tag{6.91}$$

where C_f is the concentration at infinite time. The term on the right-hand side is actually the leading term of an infinite series.

13. REACTIONS VIA THE GAS PHASE

Sometimes the vapor pressure of a drug is sufficiently high that it may interact with other substances via the vapor phase. An example is ibuprofen (B). This is a Lewis acid, and it can interact with Lewis bases. Usual measures, such as e.g. triple-layer tablets, do not work in this case, since the interactant will be present in the gas phase.

If the reaction with another drug (D) is

$$\mathrm{D} + \mathrm{B} \rightarrow \text{decomposition} \tag{6.92}$$

then the initial reaction rate is given by

$$\frac{d\{D\}}{dt} = -kP_B[\mathrm{D}]A \tag{6.93}$$

where $\{D\}$ is the surface density of D molecules (number of molecules per cm^2) at time t, and A is the surface area. As long as there is no penetration into the crystals, the reaction will therefore be a first-order reaction, since Eq. (6.93) integrates to

$$\ln[C] = -kAP_B t + \ln[C_0] \tag{6.94}$$

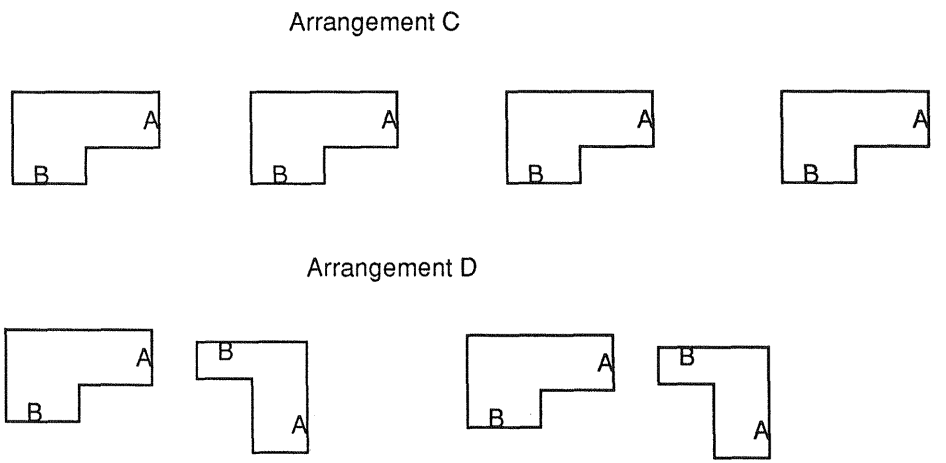

Fig. 24 Schematic of an example of molecular arrangement possibilities in a crystalline solid. If groups A and B can interact, then the situation in the upper arrangement is less prone to reaction, since A and B are at a greater distance.

where C_0 is the initial concentration. This will be true if only the surface of the solid interactant is affected. The extent of decomposition will be slight, because (unless the drug is extremely finely subdivided) only a small fraction of the molecules are on the surface. If, however, the ibuprofen penetrates the crystal, then Jander kinetics should prevail. A similar situation may be at work in the aspirin incompatibilities mentioned earlier.

14. AMORPHATES

As mentioned earlier, solids can occur either in crystalline form or as particulate amorphates. The chemical stability of the solid in crystalline form will differ from the same entity in amorphous form. In most cases the crystalline form, under the same conditions, will be more stable than the comparable amorphate.

The most interest and the largest body of work of amorphates is in the field of macromolecules. These usually possess a glass transition temperature*, T_g, and the states are referred to as "glassy" below† and "rubbery" above T_g.

Only a few articles have appeared on the subject of chemical stability of amorphates. In general, a compound is more stable in the crystalline state than in an amorphous state, but exceptions exist (Sukenik et al., 1975; O'Donnel and Whittaker, 1992; Stacey et al., 1959). There *are* cases that have been reported (Lemmon et al., 1958) where the crystalline state is less soluble than the molecule in solution, but they are rare.

In general, in a crystalline state, molecules are to a great extent fixed in position. In cases where the situation exists where a group from one molecule reacts with another group in a neighbor, the situation as shown in Fig. 24 arises.

Pothisiri and Carstensen (1975) have shown that in a situation such as the case of substituted benzoic acids the decomposition is between two groups in the same molecule.

Suppose parts A and B of the molecule depicted in Fig. 24 react. In such a case arrangement C would give better stability than arrangement D, because A would be further away from B in the former arrangement. Arrangement D can be more adverse than a random orientation as well, and if that is the case, then the amorphous form would be more stable than the crystalline (arrangement D). This is the exception rather than the rule.

In the presence of moisture, conversions from amorphous to crystalline modifications are promoted (Carstensen and Van Scoik, 1990; Van Scoik and Carstensen, 1990), and the material developed in the following all refers to anhydrous conditions.

In the work by Carstensen and Morris (1993), amorphous indomethacin was produced by melting a crystalline form of it to above melting (162°C) and recooling it to below 162°C. Amorphates made in this manner are morphologically stable down to 120°C‡ so that their chemical stability can be monitored. At a range of temperatures below this temperature, crystallization occurs too rapidly to allow for assessment of chemical stability. Amorphous samples were placed at several con-

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* More than one glass transition temperature may exist.

† The highest T_g in the case of multiple glass transition temperatures.

‡ If the temperatures are lowered rapidly, then stable amorphates can be formed at room temperature, but kinetics cannot be followed easily because of the slow reaction rate at room temperature.

stant temperature stations (145, 150, 155, 165, 175, and 185°C) and assayed from time to time. The content of intact indomethacin was assessed by using the USP method of analysis.

The decomposition curves of amorphous indomethacin and a melt of indomethacin at different temperatures is shown in Figs. 25 and 26. It is noted that the pattern is strictly first-order. Of the few reports in the literature dealing with the chemical stability of compounds in the amorphous state, amorphous cephalosporins (Pfeiffer et al., 1976; Oberholtzer and Brenner, 1979; Pikal et al., 1977) also adhere to a first-order pattern. One purpose of the following writing

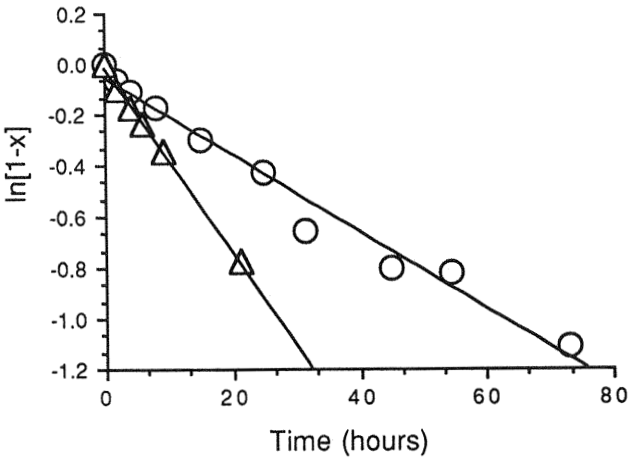

Fig. 25 Decomposition curves of solid, amorphous indomethacin at two of the three temperatures tested. ○: 145°C ($k = 0.015$ h^{-1}); △: 155°C ($k = 0.036$ h^{-1}).

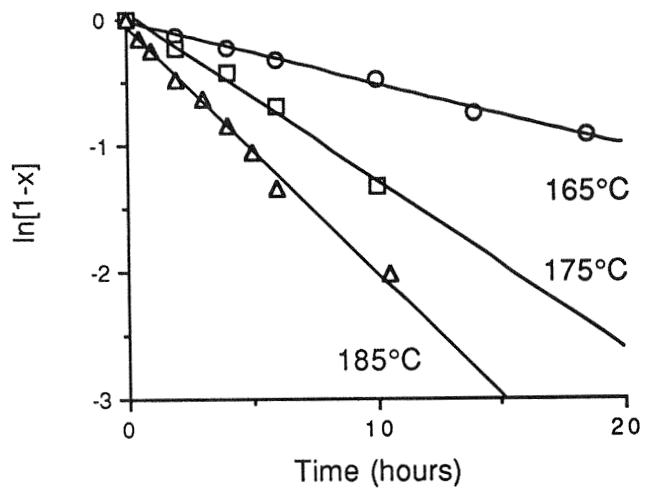

Fig. 26 Decomposition data of indomethacin in a molten state. Circles 165°C (rate constant 0.05 h^{-1}); squares: 175°C (rate constant 0.13 h^{-1}); triangles: 185°C (rate constant 0.19 h^{-1}).

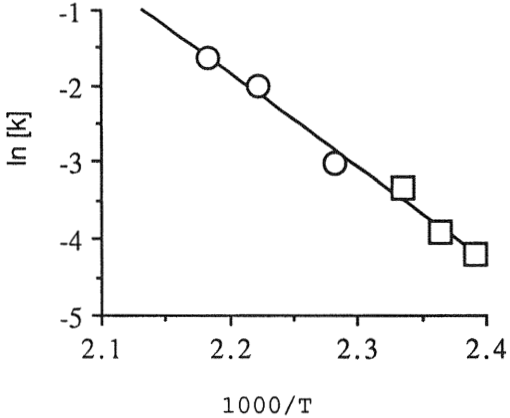

Fig. 27 Arrhenius plot of data from Fig. 24 and Fig. 25. Squares are melt and circles are amorphate. The least squares fit is $\ln[k] = 25.218 - (12{,}300/T)$.

is to seek an explanation for this pseudo-first-order (or indeed, truly first-order) pattern. The explanation must lie, in some manner, with the fact that in the rubbery state, the molecules can arrange in a random fashion, in a somewhat frozen (or much slowed) manner of that of the melt above the traditional melting point.

The results obtained from the melt are shown in Fig. 26, and against a first-order plot results. If an Arrhenius plot is drawn of the data from both Fig. 25 and 26, then Fig. 27 results.

It is seen that the Arrhenius plot of the amorphate continues into the Arrhenius plot of the melt. An attempt to explain this is made in the following.

If the substance in Fig. 24 were a crystalline solid, then the potential energy between molecules would be inversely proportional to a power function of their distance (the lattice constant) (Carstensen and Morris, 1993), i.e., would be akin to a Lennard-Jones potential (Lennard-Jones, 1931). However, in the amorphous state, if the decomposition is an intermolecular (rather than an intramolecular) reaction, then a group A in molecule I interacts with group B in the neighboring molecule II. The energy of the molecular pair will be dependent on the distance between the group A in one of the pair and group B in the other. These distances would be assumed to be randomly distributed, and a certain fraction $N_{>i}/N_0$ of the molecular pairs would be at or above a critical energy, E_i, necessary for reaction between A and B. The fraction of molecules that have this energy, E_i, is given by the Boltzmann distribution (Moelwyn-Hughes, 1961):

$$\frac{N_i}{N} = \frac{\exp(-E_i/RT)}{\sum_{k=0}^{k=\infty} \exp(-E_k/RT)} \tag{6.95}$$

where N is the total number of molecules and where the summation is over all energy levels. The fraction of molecules having energies in excess of $E_{>i}$ is then $N_{>i}$, given by

$$\frac{N_{>i}}{N} = \frac{\sum_{k=i}^{k=\infty} \exp(-E_k/RT)}{\sum_{k=0}^{k=\infty} \exp(-E_k/RT)} \tag{6.96}$$

There are several ways of approaching these summations, e.g., by considering the energy differences small and integrating. A discrete approach is to assume that the energy difference, ΔE, between adjoining energy states is constant. In this case Eq. (6.96) can be written:

$$\frac{N_{>i}}{N} = \frac{e^{-E_i/RT} + e^{-(E_i+\Delta E)/RT} + \ldots}{e^{-E_0/RT} + e^{-(E_0+\Delta E)/RT} + \ldots}$$
$$= \frac{e^{-E_i/RT}[1 + e^{-\Delta E/RT} + e^{-2\Delta E/RT} + \ldots]}{e^{-E_0/RT}[1 + e^{-\Delta E/RT} + e^{-2\Delta E/RT} + \ldots]} \tag{6.97}$$

i.e.,

$$\frac{N_{>i}}{N} = \frac{e^{-E_i/RT}}{e^{-E_0/RT}} = e^{-(E_i-E_0)/RT} \tag{6.98}$$

Alternatively, if the difference between energy levels is large compared to the ground state energy, one can simply approximate the series in the numerator and denominator of these equations with their leading terms. This leads to the same result:

$$\frac{N_{>i}}{N} = \frac{\sum \exp(-E_i/RT)}{\sum \exp(-E_0/RT)} = \exp\left[\frac{-(E_i - E_0)}{RT}\right] \tag{6.99}$$

If, in a time element dt, a fraction of the molecules (dN/N) reaching E_i (or higher) react, then, denoting this fraction q,

$$\left(\frac{1}{N}\right)\frac{dN}{dt} = q\left[\frac{N_{>i}}{N}\right] = q\exp\left[\frac{-(E_i - E_0)}{RT}\right] = -k_1 \tag{6.100}$$

where k_1 (by definition in differential form) is a first-order rate constant, i.e., by integrating Eq. (6.100) and imposing $N = N_0$ at time $t = 0$,

$$\ln\left[\frac{N}{N_0}\right] = -k_1 t \tag{6.101}$$

i.e., first-order, where the rate constant is given by

$$k_1 = q\exp\left[\frac{-(E_i - E_0)}{RT}\right] \tag{6.102}$$

or its logarithmic equivalent,

$$\ln[k_1] = \ln[q] - \frac{E_a}{RT} \tag{6.103}$$

i.e., an Arrhenius equation where the activation energy is given by

$$E_a = E_i - E_0 \tag{6.104}$$

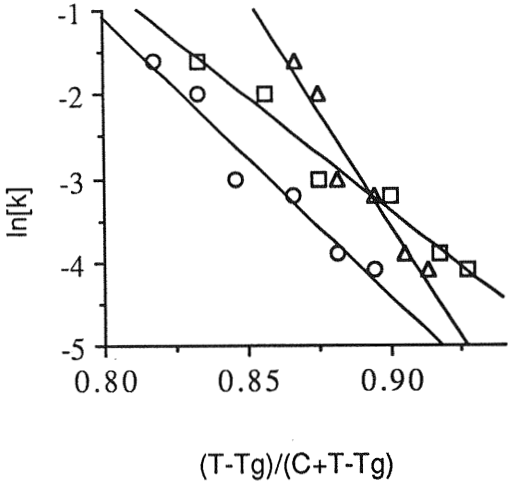

Fig. 28 Data from Fig. 26 plotted by the inverse function of the WLF equation. *Triangles:* $T_g = 80°$, $C_2 = 10$: $\ln[k] = 25.40 - 33.117\{T - T_g\}/\{C + (T - T_g)\}$; $R = 0.977$. *Circles:* $T_g = 100°$, $C_2 = 6$: $\ln[k] = 45.48 - 54.47\{T - T_g\}/\{C + (T - T_g)\}$; $R = 0.97$. *Squares:* $T_g = 120°$, $C_2 = 5$: $\{T - T_g\}/\{C + (T - T_g)\} = -0.771 \ln[k] + 0.0289$; $R = 0.982$.

The data in Figs. 24 and 25 demonstrate the correctness of Eq. (6.100), i.e., the expectancy of a first-order decomposition, and Fig. 27 demonstrates the correctness of Eq. (6.102).

There have been proposals (Moelwyn-Huges, 1961, Franks, 1989) that the stability of a compound near its T_g is best described in terms of the Williams-Landel-Ferry equation (Williams et al, 1955):

$$\ln[R] = \ln[R_g] + \left[\frac{c_1\{T - T_g\}}{\{C_2 + (T - T_g)\}}\right] \tag{6.105}$$

where C_2 and c_1 are constants. It is far from certain that this equation would apply to chemical reactions, but Fig. 28 shows its application to the data in Fig. 27. Several different values of C and T_g will give reasonable fits, as seen. It would seem intuitive that if the Arrhenius equation fits, then there would be values of C_2 that would make the WLF equation fit as well.

Schmitt et al. (1999) described the crystallization of amorphous lactose above the glass transition temperature to follow the Johnson–Mehl–Avrami (Johnson and Mehl, 1939; Avrami, 1939) equations:

$$[-\ln(1 - \alpha)]^{1/n} = k(t - t_i) \tag{6.106}$$

Pikal et al. (1977) employed solution calorimetry to determine the amorphous content of cephalothin sodium, cefazolin sodium, cefamandole naphtate, and cefamandole sodium. Since the amorphous forms are more energetic, they have a higher heat of solution, and the percent amorphate can be obtained if the heat of solution of amorphate and crystalline forms separately is known.

Lo (1976) showed that ampicillin trihydrate dehydrated to amorphous ampicillin, which had much poorer stability than the trihydrate. Upon storage the decomposition appears biphasic.

15. PSEUDO-POLYMORPHIC TRANSFORMATIONS

Dehydration, as mentioned above, may result in amorphous anhydrates, but it may also result in another crystalline phase (e.g., a lower hydrate or a crystalline anhydrate). These are, properly speaking, *pseudo-polymorphic transformations.* There are several steps in dehydration of a hydrate, and they can be summarized in the following manner, where S denotes solid, D denotes drug, V denotes vapor, and L denotes liquid (Han and Suryanarayanan, 1997).

$$D \cdot xH_2O \rightarrow D_S + xH_2O_L \qquad \text{(enthalpy of dehydration} = \Delta H_D)$$
$$D_S + xH_2O_L \rightarrow D_S + xH_2O_V \qquad \text{(enthalpy of vaporization} = \Delta H_V)$$

$$D \cdot xH_2O \rightarrow D_S + xH_2O_V \qquad \text{(enthalpy of transition} = \Delta H_T)$$

i.e.,

$$\Delta H_T = \Delta H_V + \Delta H_S \tag{6.107}$$

so that different results can be obtained in DSC experiments depending on whether a crimped or open pan is used.

Bray et al. (1999) have shown such a diagram for [2(S)-[p-toluenesulfonyl amino]-3-[[[5,6.7.8-tetrahydro-4-oxo-5-{5-[2-(piperidin-4-yl)ethyl]-4-H-pyrazolo[1, 5-a][1,4]diazepin-2-yl]carbonyl]amino]-propionic acid.

Suihko et al. (1997) have employed DSC to show that dehydration of theophylline monohydrate is a two-step process.

16. POLYMORPHIC TRANSFORMATIONS

Polymorphic transformation rates have become of importance of late, and an example is a recent article by Agbada and York (1994) dealing with the dehydration kinetics of theophylline. The article by Ng (1975) is similarly instructive in the sense that it reviews all the equations that have been developed for polymorphic transformation kinetics.

In most cases the transformation kinetics are S-shaped curves, and before any model is imposed on the data, the following model should be considered. (This is comparable to the model proposed by Carstensen and Van Scoik, 1990). If the phenomenon that governs the transformation is essentially the nucleation lag time, then the curves may be considered as representing either a normal or a log-normal error curve, and the mean would be the mean (or geometric mean) nucleation time. What this states is that each particle, in a sense, acts as its own entity, that there is a nucleation time (with an error or a variance attached to it) and that the particle will endure the nucleation time and then decompose, individually, very rapidly.

Table 8 Data (Fig. 16) from Which Fig. 29 Was Generated

	Fraction remaining		Standard normal deviate	
Time (h)	A	B	Z_A	Z_B
0	1.00	1.00		
0.2	0.698		0.50	
0.25		0.72		0.58
0.3	0.33		−0.43	
0.5	0.17	0.42	−0.92	−0.2
0.7	0.08	0.28	−1.5	−0.58
1.0		0.11		−1.18
1.25		0.08		−1.4

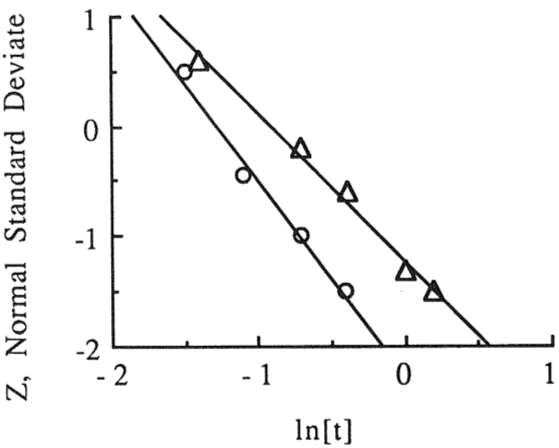

Fig. 29 Data from Fig. 16 treated as log-normally distributed in time.

The data from Fig. 16 are shown in tabular form in Table 8. These data are plotted log-normally in Fig. 29, and it is seen that there is excellent linearity. The model is much simpler and much more reasonable in the case of polymorphic transformations than other models relying on farfetched mechanistic assumptions.

The reason for the log-normal relationship is not difficult to understand. Solids are usually log-normally distributed. If the nucleation time is inversely proportional to size, then it too would be log-normally distributed.

Dehydration, at times, results in a morphic transformation. For instance, Lo (1976) showed that the transformation of crystalline ampicillin trihydrate to amorphous penicillin was primarily first order, it either was first order or followed a contracting cylinder model ($(1-x)^{1/2}$ being proportional to time).

16.1. Pseudo-Polymorphic Transformations. Dehydration Kinetics of Hydrates

A special case of polymorphism is pseudo-polymorphism which deals with hydrates. Anydrates and hydrates often crystallize in different crystal systems, but the mol-

ecule in solution is the same. In the solid state there is a difference in that water molecules form part of the matrix.

Dehydration kinetics of hydrates has had the attention of the pharmaceutical scientist for a while. As far back as 1967, Shefter and Kmack (1967) studied the dehydration of theophylline hydrate and found it to be first order. However, this is not the normal order of dehydration.

Byrn (1982) has developed a generalized kinetic theory for isothermal reaction in solids, and theophylline has been used as a model for several studies of this kind (Lin and Byrn, 1979; Suzuki et al., 1989; Agbada and York, 1994). In the recent work by Dudu et al. (1995), microcalorimetric methods were used and show a two-step process to take place. The predominant model used for this type of kinetics is the Avrami–Erofeev treatment leading to the equation

$$[-\ln(1-x)]^{0.25} = kt \tag{6.108}$$

and good adherence of the data for each step was found. This points to microcalorimetry as being a potent tool in the investigation of such projects.

There are vacuum/electrobalances on the market now, which have the capability of increasing the relative humidity and keeping the "new" relative humidity constant until the weight gain of a sample in the balance assembly has gained less than a preset quantity of water. When an anhydrate is placed in a vacuum in such a balance assembly and the relative humidity gradually raised, then the "up" and "down" portions of the curve will often have the profile shown in Fig. 30.

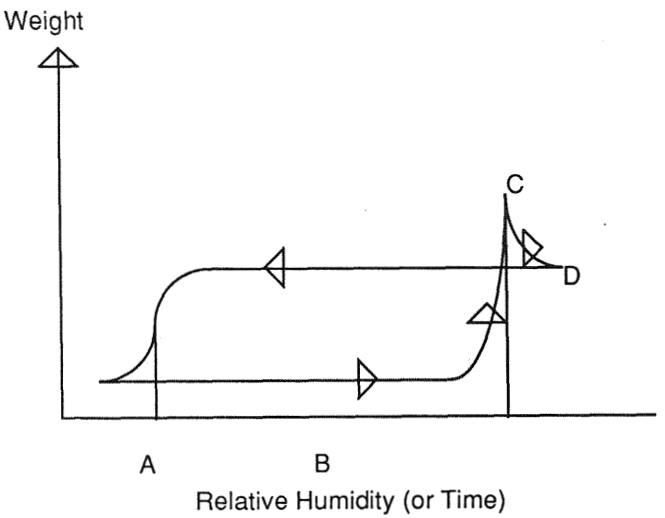

Fig. 30 A represents the relative humidity over the crystalline hydrate, and at this point hydration should actually occur. But a metastable situation usually occurs, so if the amorphate is exposed to higher humidities, then it is not until at B that it changes, and in this case it forms a (supersaturated) solution, which at C precipitates out as the crystalline hydrate D. The desorption isotherm then results in a constant composition until a lower humidity, where the hydrate starts dehydrating into the amorphous anhydrate.

It is to be expected that the weight of the anhydrate will not increase until the relative humidity of the salt pair is reached. If one only considers the part ABC, then this is logical.

It is noted that the abscissa is time as well as relative humidity, since this latter is changed as a function of time. The curve often goes beyond the weight of the anhydrate, and it is hypothesized that what actually happens is that the relative humidity at which the increase occurs is that of the saturated solution of the anhydrate. This is a metastable solution and will start precipitating out in time (point C) until the weight levels off at the theoretical weight of the hydrate. On the "down" curve, this hydrate then remains until the relative humidity of the salt pair is reached, and then it drops off.

So, if an experiment is carried out as shown in Fig. 30, it is not certain that the relative humidity at A is the equilibrium relative humidity of the hydrate/anhydrate salt pair, because as in a conventional isotherm, the "water channel" could act in the same fashion as a pore, and the "breakthrough" vacuum might be an indication of the effect of the Kelvin equation.

Only a few unreported studies have been carried out of this kind (Pudipeddi, 1995; Dali, 1995; Shlyankevich, 1995), but the method, in the sense of the preceding paragraph, could be of importance in assessing diameters of water channels and of interfacial tension between water and the organic and inorganic matrix molecule (via the Kelvin equation).

17. PHOTOLYSIS IN THE SOLID STATE

Not much systematic work has been reported on photolysis of solids. Lachman et al. (1961) pointed out that, most often, a solid tablet will decompose by photolytic decomposition only in the surface area, so that if one broke a "discolored," exposed tablet the color would be unaffected in the interior.

However, Kaminski et al. (1979) reported on a case where a combination of moisture and light caused an interaction between a dye and a drug that permeated the entire tablet.

Tønnesen et al. (1997) have reported on the photoreactivity of mefloquine hydrochloride in the solid state.

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7

Interactions of Moisture with Solids

JENS T. CARSTENSEN

Madison, Wisconsin

1. PREDOMINANT REACTION ORDERS

The ICH 1993 Stability Guidelines "do not accept the term 'room temperature'," yet it is so common that it will be used in this text in its intuitive sense. At room temperature, for a product to be marketable, decompositions are less than 15% (in fact a good deal less than 10%). Most products exhibit good content uniformity, and usually decompositions will appear zero order, i.e., be pseudo-zero-order. The mathematical approximation

$$\ln[1 - x] \approx -x \tag{7.1}$$

explains this. A reaction that is truly first order, but where x is small (less than 0.1 or at most 0.15), will, by way of Eq. (7.1), appear zero order. If M is drug present at time t and M_0 the initial amount, then it follows that

$$x = \frac{M_0 - M}{M_0} \tag{7.2}$$

hence

$$\frac{M}{M_0} = 1 - x \tag{7.3}$$

This means that the equation takes the form

$$\ln\left[\frac{M}{M_0}\right] = \ln[1 - x] \approx -x = -\frac{M_0 - M}{M_0} = -k_1 t \tag{7.4}$$

where k_1 is the first-order rate constant. One may write this

$$M = M_0 - \{M_0 k\}t = M_0 - k_0 t \tag{7.5}$$

i.e., the data will appear pseudo-zero-order with a rate constant of

$$k_0 = M_0 k_1 \tag{7.6}$$

There are other causes for pseudo-zero-order behavior, chemical and physical reasons. The effect moisture has on solid state stability is, in its simplest form, visualized by moisture being sorbed on the particles. In this sense the water would behave like a solution, a bulk layer, and it is assumed that this is saturated in drug. This is known as the Leeson–Mattocks model (Leeson and Mattocks, 1958; Li Wan Po and Mroso, 1984; Carstensen and Li Wan Po, 1993). The decomposition follows the equation

$$M = M_0 - k_0 t \tag{7.7}$$

where k_0 is the pseudo-zero-order rate constant given by

$$k_0 = k_1 SV \tag{7.8}$$

In this equation S denotes the drug solubility in the bulk aqueous phase and V denotes the volume of the layer. Most often one deals with hydrolysis and pseudo-first-order is assumed.

An assumption is that the moisture is in such excess that the term V does not change while the stability is being studied. Investigators (e.g. Kornblum and Sciarrone, 1964) have assumed that k_1 equals that from solution-kinetic studies. Quite often this is not so, because the model dictates that it is the kinetics in concentrated solutions that matters.

As seen in Chapter 9, anhydrous solids usually exhibit sigmoid profiles, i.e., Prout–Tompkins kinetics (Prout and Tompkins, 1944) or Bawn kinetics (Bawn, 1955). This is mostly not the case with decompositions when moisture is present in "large" amounts.

2. KINETICS IN THE DRY VERSUS MOIST STATE

Pothisiri (1975) and Carstensen and Pothisiri (1975) studied the decomposition of substituted *p*-aminosalicylic acids as a function of moisture content and attempted to extrapolate the pseudo-zero-order rate constants down to 0% (anhydrous). The extrapolated value (as well as the mechanism) differs from that observed in experiments where moisture is excluded (pseudo-zero-order versus sigmoid behavior).

It is therefore of interest to define the nature of the water in the transition between very low moisture contents (the anhydrous state) and the other extreme (the very moist state). The models just mentioned are extreme cases of very "dry" and very "wet." Various stages of "wetness" are depicted in Fig. 1.

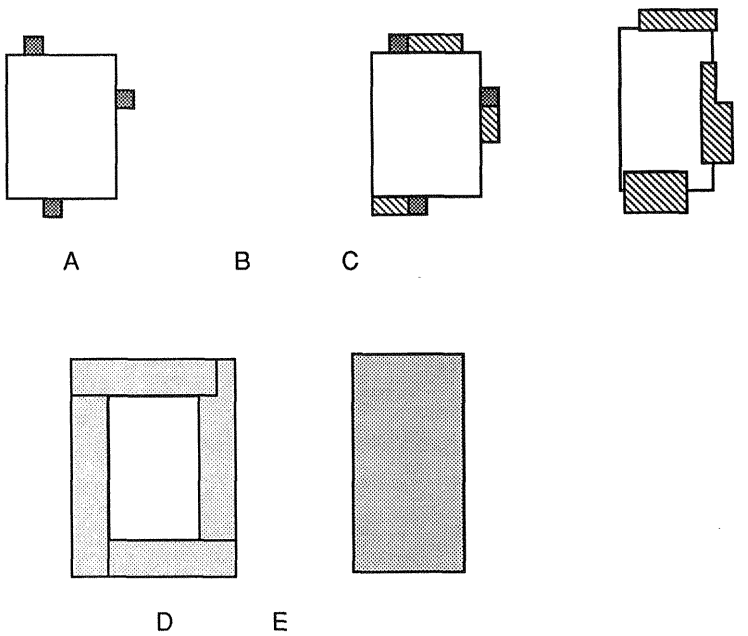

Fig. 1 (A) Anhydrous solid with active sites (cross-hatched); (B) solid with less than monolayer coverage of moisture; (C) solid with more than monolayer coverage, where the active sites have disappeared; (D) bulk sorbed moisture; and (E) moisture in an amount sufficient to dissolve the solid completely.

3. TYPES OF SURFACE MOISTURE

There are, broadly speaking, three types of situations: *Limited water*, where all the water is used up in the decomposition of the drug, but the amount is not enough to decompose all of the drug.

Adequate water, when there is enough moisture to decompose all of the drug substance.

Excess water, when the amount of water present is more than needed to dissolve the drug completely.

3.1. Excess Water

This is shown as E in Fig. 1, where the amount of water suffices to bring all of the drug into solution. This may not be applicable initially, but it occurs as the amount of parent drug decreases in time.

Examples of this are the work by Morris (1990), where the indomethacin/water system was studied in a closed system at 130°C. After a short period of time a eutectic consisting of indomethacin, decomposition products, and water is formed, and from this point in time the decomposition is first order as expected for solution kinetics (Fig. 2). The amount of time (t') required for the eutectic to form (for the mass to form a homogeneous liquid) is linear in water activity ($a = \mathrm{RH}/100$), i.e.,

$$t' = \beta - q'a \tag{7.9}$$

where β and q' are constants (Fig. 3).

Yoshioka and Uchiyama (1986a,b), Carstensen et al. (1987), and Yoshioka and Carstensen (1990a,b) have reported similarly in relationship to propantheline bromide. Yoshioka and Uchiyama (1986a) introduced *critical relative humidity* (CRH) as the point where the water activity just equals that of a solution saturated

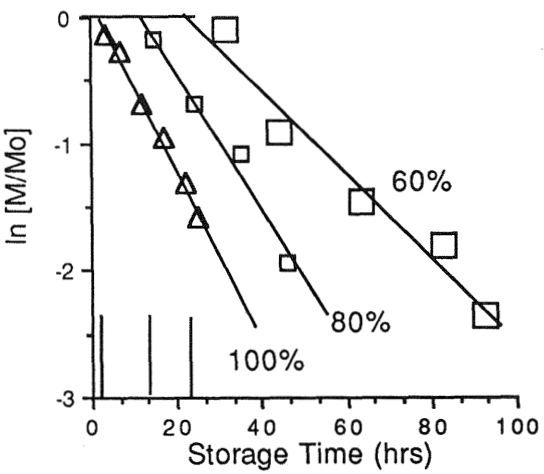

Fig. 2 Decomposition of indomethacin in the presence of moisture at 130°C. (Graphs constructed from data published by Morris, 1990.)

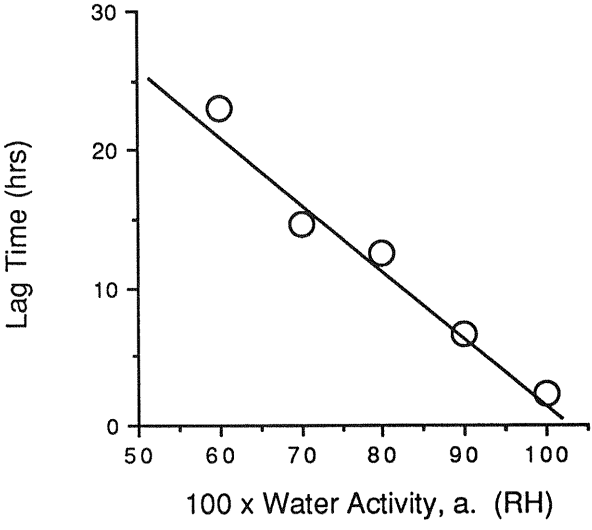

Fig. 3 Lag times from Fig. 2 plotted versus relative humidity. (Graph constructed from data published by Morris, 1990.)

in the drug (Carstensen, 1977) and they also showed that the mechanism changed at this point. At values higher than the CRH the degradation consists of (a) dissolution up to where dissolution is complete, after which (b) moisture condensation will continue until a concentration of the totally dissolved drug equals that of the RH of the atmosphere.

Koizumi et al. (1997) showed that the dependence of water concentration on the rate constant of decomposition of Lornoxicam tablets is log-log related to the log of the moisture content.

Carstensen et al. (1965) had shown this to be correct for vitamin A beadlets as well.

$$\frac{d[\mathrm{A}]}{dt} = -k[\mathrm{A}][\mathrm{H_2O}]^n \tag{7.10}$$

4. THE LEESON–MATTOCKS MODEL

This is the most frequently applicable model and it assumes that sorbed moisture forms a layer about the particles. It corresponds to situation D in Fig. 1. One might argue that such a layer (a so-called bulk sorbed moisture layer) could not be created until the moisture content is high enough, so that the RH of the atmosphere surrounding solid equals or is in excess of the RH of a saturated solution of the drug. One might then conclude that the Leeson–Mattocks model only holds at RH values in excess of the critical relative humidity (CRH). However *rather than that being true it holds below the CRH*. For a certain range of RH values less than the CRH, the Leeson–Mattocks model applies, and degradations are pseudo zero order. Phenobarbital when it decomposes at 80°C in the presence of phosphate buffer at pH 6.7 is an example of a case where, in the initial stages of decomposition, this

holds (Gerhardt 1990). Another case is that reported by Morris (1990) and Morris and Carstensen (1990a,b).

Equation (7.9) applies to the decomposition, hence one must know k_1, V, and S, which should allow for elucidation of the mechanism. This often holds true (Pothisiri, 1975; Pothisiri and Carstensen, 1975), but it has also been known to fail (e.g. Janahsouz et al., 1990).

Carstensen and Attarchi (1988) elucidated the discrepancy between the rate with which aspirin decomposes as a solid with water present and its behavior in saturated solution. If they presumed that the solubility in the moisture layer were three times that of the bulk solubility, then their calculated data corresponded to the experimental data. Whether it is possible that the condensed layers of water are so energetic that they would allow for such an increase is doubtful.

The speculation that the solubility might be increased in the sorbed moisture layer (Fig. 1D) might lead one to consider it akin to an amorphous state. An amorphous state would exhibit increased higher solubility over that of crystalline states, and would also possess a higher vapor pressure than would a crystalline form, the form that it would rest upon, posing the question of why and where the critical moisture content would exist.

A further extension of this, though, is that water dissolves into the solid. As mentioned, this happens for a wholly amorphous compound, but for a crystalline compound the crystallinity would have to be lost, an assumption that has no basis in fact. If the moisture molecules really created a "hot spot" of amorphous solid on the crystal surface, then at a certain given RH value the mass of moisture ad/absorbed should be equivalent to the composition of the amorphate/water in equilibrium at the RH in question.

Carstensen and VanScoik (1990) have demonstrated for small molecules (sucrose) that the water activity over this type of supersaturation of water in solid is simply an extrapolation of the RH values of saturated and unsaturated solutions at the other end of the diagram. One might consider this as water that is dissolved in the solid or as solid that is dissolved in the water, but in either view the important aspect is that (ideality assumed) the mass of water sorbed is linearly dependent on the RH. (For polymers of high molecular weight the isotherms are S-shaped, an example being microcrystalline cellulose reported by Hollenbeck et al., 1978, and by Marshall et al., 1972).

If an amorphate "hot spot" hypothesis were correct then the pseudo rate constants should be linearly related to the water activity (the relative humidity) used in the study. Figure 4 shows the profiles with which crystalline indomethacin decomposes at different water activities (RH values). Pseudo zero order obviously prevails, but the plot of rate constants versus water activities (RH values) is not a straight line (Fig. 5). As mentioned in the last chapter, one cannot "prove" a model by statistically comparing curve fittings, but one can eliminate models (Li Wan Po, 1984; Mroso et al., 1982) when a fit is lacking. An assumption made in Fig. 8.18 is, it is conceded, that ideality prevails, but for the "hot spot" model to hold, nonideality in the case would have to be drastic.

A different interpretation of the indomethacin data was forwarded by Morris (1990) and Morris and Carstensen (1990a,b), by demonstrating that the rate constants are related to a BET (or possibly to some other nonlinear) water adsorption

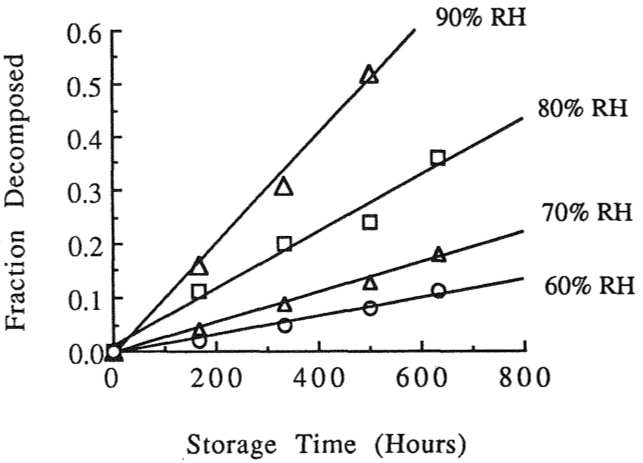

Fig. 4 Indomethacin decomposition at 115°C. This decomposition follows zero-order kinetics at the onset. (Graph constructed from data published by Morris, 1990, and Morris and Carstensen, 1990a,b.)

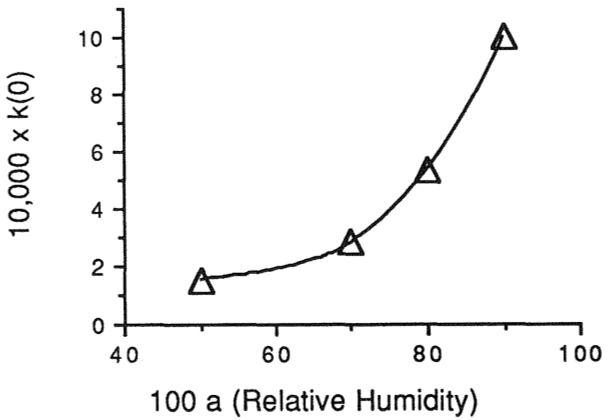

Fig. 5 Data from Fig. 4 of Chapter 10. Rate constants as a function of RH. (Graph constructed from data published by Morris, 1990, and Morris and Carstensen, 1990a,b.)

isotherm. The volume of water, V, adsorbed when a BET isotherm with high c value applies would be of the type

$$V = \frac{v_m}{[1-a]} \tag{7.11}$$

v_m is here a monolayer volume, and the symbol a is used to denote the water activity (RH/100). The k_0 value should, therefore, be linearly related to $[1-a]$. That this is the case is shown in Fig. 6.

It would therefore seem (at least in the case of indomethacin) that the amorphate "hot spot" model does not apply. In addition to this, Morris (1990) tested

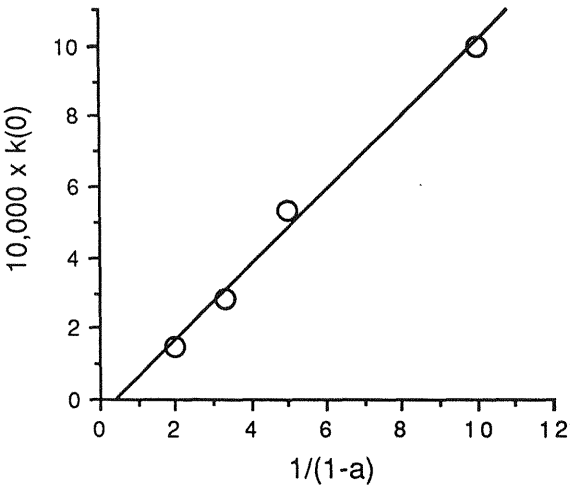

Fig. 6 Rate constants from graphs of the type shown in Fig. 2, plotted versus a BET-isotherm parameter $1/\{1-(1/a)\}$.

amorphous indomethacin with moisture present, but at high temperatures conversion to crystallinity was rapid to such an extent that kinetic data could not be obtained in reasonable time periods.

The "hot spot" theory is not new. In fact, work by Gluzman (1954, 1956, 1958) and Gluzman and Arlozorov (1957) postulated that "part of a surface of a solid was actually in a liquid like state"—in other words, in appearance being a solid, but with random molecular arrangement, and usually referred to as an amorphate.

Guillory and Higuchi (1962) hypothesized that if such a theory were correct, then the logarithm of the rate constant at a given temperature, T_d, of a series of analogous compounds in solid form should be inversely proportional to the inverse of the melting point, i.e.,

$$\ln[k] = -Q\left\{\frac{1}{T_d} - \frac{1}{T_m}\right\} \tag{7.12}$$

This has been found to be true in certain cases, e.g., for vitamin A esters at 55°C (Guillory and Higuchi, 1962) and substituted *p*-aminobenzoic acids (Carstensen and Musa, 1972), but in other cases, e.g., *p*-aminosalicylic acids, it does not hold well (Pothisiri and Carstensen, 1975).

More plausible than the "hot spot" amorphate theory is the hypothesis that the sorbed moisture layer acts as a solution layer and that degradation compounds (a) increase or decrease the drug solubility, (b) increase or decrease the kinetic parameter values of the drug, and (c) (noting that the degradants are solutes) cause a decrease in the water vapor pressure with which the moisture layer is in contact, so that in this manner the vapor pressure relationship is not violated. Gerhardt (1990) and Gerhardt and Carstensen (1989) have demonstrated that kinetic salt effects and salting-in of the drug into the moisture layer can explain the decomposition profiles exhibited by phenobarbital when moisture and buffers

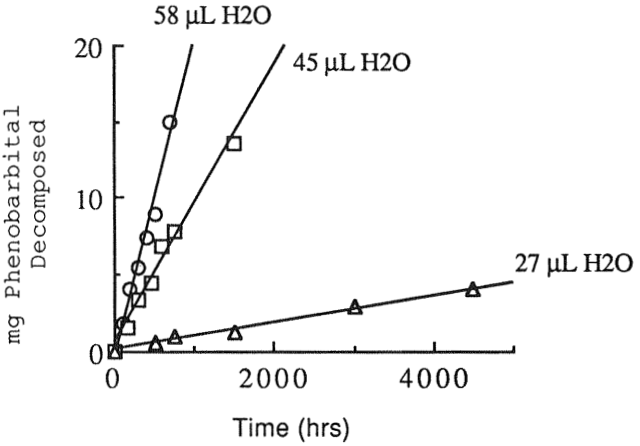

Fig. 7 Phenobarbital decomposition in the solid state at 80°C, with phosphate buffer present corresponding to a "pH" of 6.7.

are present. Carstensen and Pothisiri (1975) and Wright and Carstensen (1986) have done likewise.

In the case of very soluble drugs, e.g., ranitidine (Franchini and Carstensen, 1995; Carstensen and Franchini, 1995) the amount of moisture necessary to reach the CRH is small (i.e., the water activity (RH/100) over a saturated solution is of low magnitude). On the other hand, it is high for poorly soluble drugs.

5. KINETICALLY UNAVAILABLE (BOUND) WATER

Solid state rate constants often follow Eq. (7.2), in that they appear directly in proportion to the mass or volume of water the dosage form contains. Figure 7 presents data from the work of Gerhardt (1990) and Gerhardt and Carstensen (1989). The rate constants are pseudo zero order and are plotted versus moisture levels (Fig. 8). It is noted that the intercepts are nonzero, i.e.,

$$k_0 = k_1 S[V - w^*] \tag{7.13}$$

w^* is often called kinetically unavailable moisture or *bound water*. This is the case in many solid state reactions. The bound moisture, at times, is water of crystallization. For D,L-calcium leucovorin (Nikfar et al., 1990a,b), there are intermittent plateaus that correspond to a constant water activity (RH/100) for a series of water contents, i.e., akin to a salt pair. $[V - w^*]$ is denoted kinetically available, or more simply, *available moisture*.

Aso et al. (1997) have determined the decomposition rates of cephalotin in mixtures with pharmaceutical excipients and the effect of moisture. They found a linear relation between mobile water percentage and decomposition rate constants.

6. MICROENVIRONMENTAL pH

If a formulator is aware that a compound is more stable in an acid than in a neutral or basic environment one may often formulate it with solid acids (e.g., citric acid);

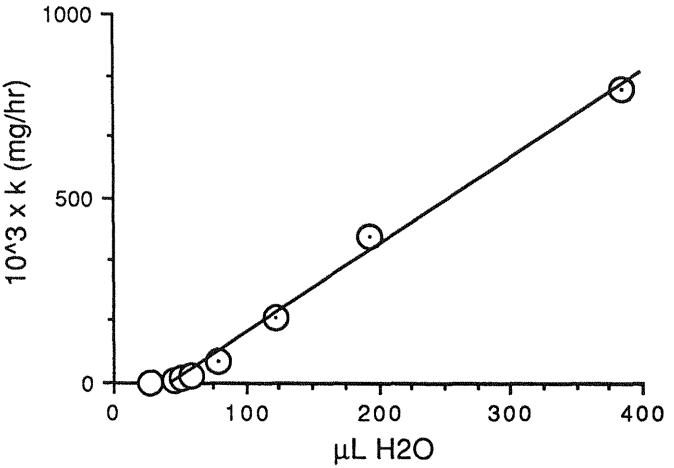

Fig. 8 Rate constants (pseudo zero order) from plots such as shown in Fig. 7 of Chapter 10, graphed versus added moisture. (Figure constructed from data published by Gerhardt, 1990, and Gerhardt and Carstensen, 1989.)

conversely, if it is acid-sensitive, one may employ bases (e.g., sodium carbonates) in an attempt to make an adjustment of "the microenvironmental pH." In the area shown as Fig. 1C, D, and E, if one may "buffer" a solid dosage form, Nikfar 1990, Nikfar et al. 1990a,b, Gerhardt 1990, Gerhardt and Carstensen 1989 have demonstrated the existence of a "solid pH-profile" that parallels (but is not identical with) the traditional pH profiles of the drug in solution. This is another piece of evidence of the sorbed moisture layer having solvent properties.

But how to define the microenvironmental pH? This a question that is not fully resolved yet. The shift in position of the kinetic pH profile in solution from the values obtained from solid state decomposition may be attributed to the fact that one assumes that the pH value of a saturated buffer solution is the same pH used for graphing of data from the moist solid. But the sorbed solution could be of a pH value displaced from that observed in solution.

There is also the possibility of a kinetic salt effect. It is seen from Fig. 9 (Nikfar, 1990; Nikfar et al. 1990a,b) that a displacement of 1.4 pH units applies to the rate constants in the solid state. The displaced values are symbolized by squares in Fig. 9, and if such a shift is made, then the data in solution would coincide with those in the solid state. In the work published by Gerhardt (1990) it would be necessary to force a 6 pH unit shift to obtain coincidence, so that are still unexplained factors at work.

7. VERY LOW MOISTURE CONTENTS

Such a case is shown in Figs. 1B and 1C. Nikfar (1990) and Nikfar et al. (1990a,b) suggested the term *immobile water* for cases such as the ones depicted in Fig. 1c. They have demonstrated that the decomposition in such a case translates into a pseudo-first-order profile (Fig. 10). At these levels of moisture the active sites in a Prout–Tompkins sense disappear by dissolution somewhat like what happens

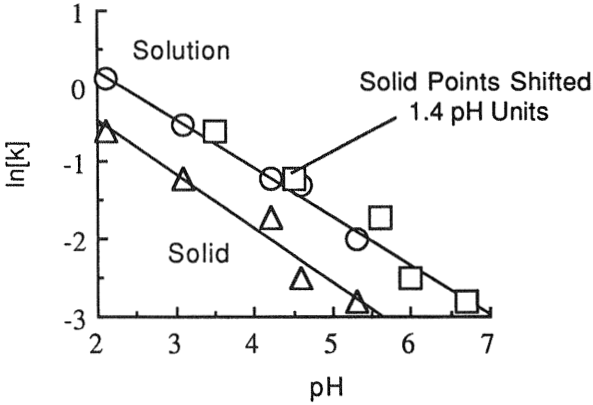

Fig. 9 pH-rate profile of first-order rate constants extracted from kinetics of decomposition of D,L-calcium leucovorin. The squares are points from solid-state decomposition shifted by 1.4 pH units.

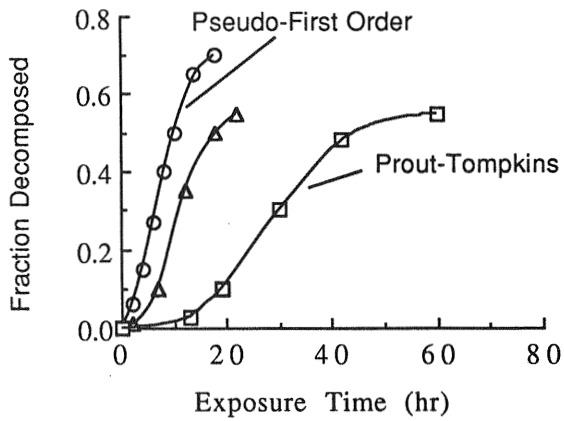

Fig. 10 D,L-calcium leucovorin with moisture and buffers added. ○: 5% water with a pH 2.2 buffer in the solid state (the buffer forms hydrates, and the water contents are percentages added and are not necessarily available moisture); △: intermediate moisture content; □: low moisture content.

in an etch-test of a metal. If one assumes that the aqueous solution is immobile, i.e., that only water molecules adjacent to intact drug molecules take part in the reaction, then first-order kinetics should prevail. One might also, at this level of moisture, consider the surface structure as amorphous, since amorphous substances in the presence of water degrade by first order (Pikal et al. 1977; Morris, 1990). Literature data are insufficient to distinguish if linearity or BET sigmoidness applies for rate constants when they are plotted as a function of relative humidity.

At even lower moisture contents (Fig. 10) the reaction profile takes on a sigmoid nature and can be explained by a surface-interaction model. The sigmoid profiles shown in Fig. 10 adhere well to Eq. (7.3). This can be explained by assuming the moisture to adsorb preferentially at the active sites (Fig. 1B and Fig. 11).

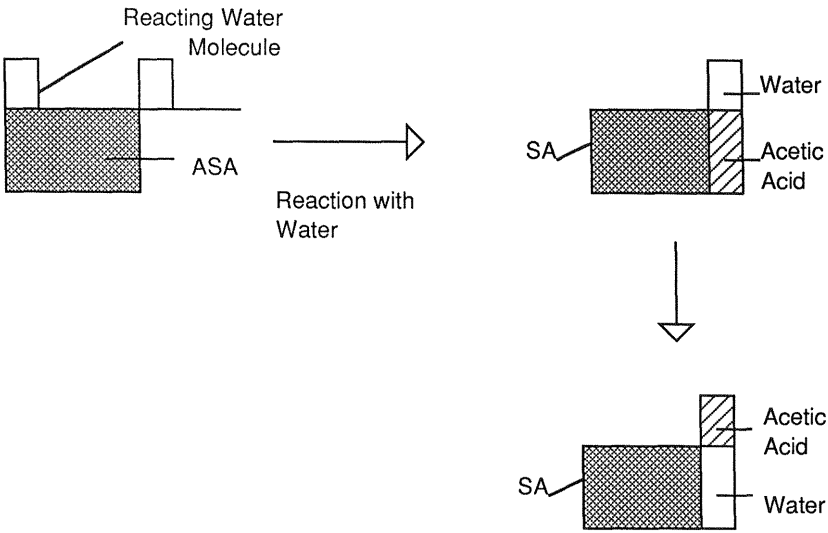

Fig. 11 Surface active site interaction using aspirin as an example. (Graph constructed from model proposed by Attarchi, 1984, and Attarchi and Carstensen, 1988.)

The amount of water does not suffice to "dissolve" the active sites, so the reaction is an interaction between moisture and drug at the activated site. The development of such a model has been published by Attarchi (1984) and Carstensen and Attarchi (1988). The applicable equation is Eq. (7.9):

$$\ln\left[\frac{x}{1-x}\right] = k(t - t_{1/2}) \tag{7.14}$$

The applicable model is presented in Fig. 11. Data plotted in this fashion is shown in Fig. 11, and the rate constants admirably follow an Arrhenius equation as shown in Fig. 12.

Obtaining the actual values of k and S in Eq. (7.2) is not as easy as might seem. As pointed out (and investigated) by Attarchi (1984) and by Carstensen and Attarchi (1988), both k and S are a function of amount of decomposition product. This was first pointed out by Pothisiri (1974), by Pothisiri and Carstensen (1975), and by Wright and Carstensen (1986). It is also a function of ionic strength, as pointed out by Gerhardt (1990) and by Gerhardt and Carstensen (1989), or simply a function of the composition of the sorbed moisture layer (Attarchi, 1984; Carstensen and Attarchi, 1988; Pothisiri, 1974).

8. DOSAGE LEVEL AND TOXICITY CONSIDERATIONS

In a great majority of cases the decomposition is zero order i.e., following Eq. (7.5). This means that the amount of decomposition product is linear in time.

If a product, for instance, is made in three dosage strengths, say 5 and 25 and 50 mg strengths, then after 3 years' storage at 25°C an amount of e.g. 0.075 mg has been decomposed, i.e., (assuming for simplicity equal molecular weights) 0.075 mg of decomposition product has formed (see Fig. 13). Since Eq. (7.5) is a

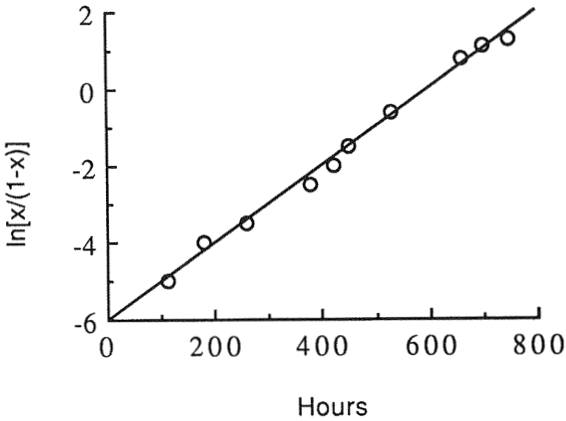

Fig. 12 Plot of aspirin decomposition data in the presence limited amounts (2.5%) of moisture. (Graph constructed from data by Carstensen and Attarchi, 1988.)

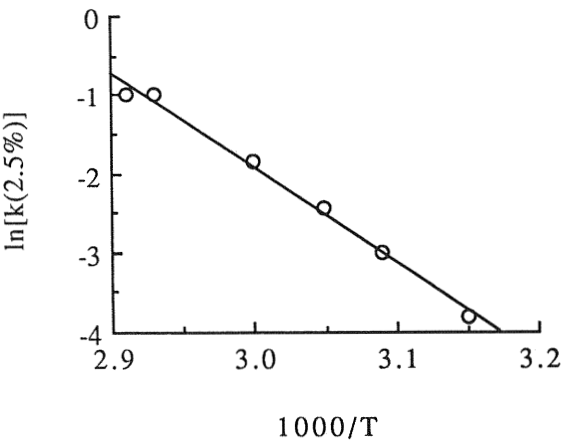

Fig. 13 Arrhenius plot of aspirin decomposition data in the presence of limited amounts of moisture. (Graph constructed from data by Carstensen and Attarchi, 1988.)

zero order reaction, this amount pertains to all the strengths, so that on a percentage basis, the 5 mg strength would have lost $100 \times 0.075/5 = 1.5\%$ of its original value, and hence (presuming an adequate precision and uniformity) would probably not meet the chemical requirement for a 3 year expiration period. The 25 mg and 50 mg strengths would have experienced 0.3% and 0.15% losses, respectively, and would be assumed to be satisfactory from a toxicity point of view.

However, often the decomposition product(s) is (are) unknown and as such are expressed as a percentage of the "main peak" if the method is HPLC (and this is usually the case). If, for instance, for toxicity reasons one assumes that the impurity peak may not exceed 1% of the area under the main peak, then in the above case, the 25 mg and 50 mg strengths would meet toxicity requirements, whereas the 5 mg would not. Nevertheless, the total amount of decomposition product is the

same in all cases. One would assume that it would be the absolute (total) amount of decomposition product that would be of importance, so that expressing the toxicity limitation as a percentage is not appropriate.

In cases where the Leeson–Mattocks model holds (but not in other cases of zero-order reactions), the above dilemma may be prevented by making the smaller dosage forms proportionally smaller, since the term V in Eq. (7.13) then becomes smaller as well.

Regulations are never immutable, and it may well be that at some future date such regulations will be modified to meet the stated need.

9. NONSTOICHIOMETRIC INTERACTIONS WITH WATER

It is sometimes a pharmaceutical practice to "coat" labile pharmaceuticals. One such example is vitamin A beadlets, which are an emulsion of vitamin A ester in a gelatin solution, which has then been converted into drops and dried. The beadlets are therefore a matrix of gelatin with droplets of oil in the interior. The protection offered is one against oxidation.

10. PARENTERAL SOLID PRODUCTS

When an injectable product is insufficiently stable in solution to allow marketing of a ready-made solution, there are still ways to develop a marketed product.

In the far past, there were so-called powder-filled products. Here a solid drug substance was made under exceedingly "clean" conditions so that it emerged from its synthesis as "completely" free of foreign material. In such a case it could be filled into a vial, sterilized by suitable means (heat, ethylene oxide (not used of injectables anymore), or γ-ray sterilization). Excipients used (sodium chloride, for instance) would have to be equally clean, and the practice is not, to the author's knowledge, used much any more.

Aside from the sepsis issue, there was also the problem with rate of dissolution, and from both these aspects, lyophilization offers a better (but probably more expensive) alternative.

10.1. Lyophilized Products

The process is one where a solution of the drug (+ excipients) is made and aseptically filtered. The solution is then aseptically filled into vials, which are loaded into a sterile lyophilization oven. This has cooling coils in its shelves and can be evaluated to very high vacuum.

The vials containing the solution are transferred to the oven, and coolant at very low temperature ($< 30°C$) is flowed through the tray coils. The solution freezes, and then a vacuum is applied of such magnitude (P_v torr) that it is lower than the vapor pressure of ice at the given temperature (P_i torr). This causes the ice to sublime, and then there remains a cake that either is crystalline and has an exceedingly high surface or is amorphous and also possesses a high surface area.

When this, at time of use, is reconstituted with water or diluent, the dissolution is, in both cases, rapid and the "original" solution is regained.

There are two stability issues in this case: (a) how stable is the lyophilized cake and (b) how stable is the solution after reconstruction?

10.2. Stability of Crystalline and Amorphous Lyophilates

It has been seen in Chapters 2 and 3 that a drug product in solution will possess an optimum pH. It is noted that this is accomplished by studying stability of the substance at different pH values, and that these latter are arrived at by the use of different buffers. If, for instance, the drug substance is a weak acid, then approximately speaking one may write

$$k_{obs} = k_0 + k_+[H^+] + k_-[OH^-] + k_{A^-}[A^-] + k_{HA}[HA] + k_{buffer}[HB] \tag{7.15}$$

where HB refers to buffer concentration and k_{HB} is the part of the rate constant attributable to the buffer. It is simplified, because k_{HB} is a combination of two terms, k_B and k_{HB}, but for this purpose it suffices to employ one (or at most two) terms. As discussed in Chapters 2 and 3, drugs mostly are protolytic and partly exist in ionized (A^-) and unionized (HA) form giving rise to the terms involving $k_{A^-}[A^-] + k_{HA}[HA]$, and k_0 is the part of the rate constant, which is neither acid nor base dependent. At lower pH, the term $k_-[OH^-]$ the term falls out, and $[A^-]$ and [HA] are dependent on the pH of the buffer used and of the $pK_{(a)}$ of the acid at the concentrations given. It is recalled that the $pK_{(a)}$ is also a function of ionic strength, the pK_a value being the value of $pK_{(a)}$ from which the ionic effect has been eliminated.

If such a substance in solution is allowed to cool down, then first water will freeze out as ice. The solution, hence, becomes more and more concentrated in both buffer and drug substance, and the pH changes as well. At the eutectic point (or the collapse temperature) all freezes out.

The stability of the substance as the concentrations change of course changes as well, because the buffer concentration changes, because the pH changes, and because the pK of the species in solution changes as well. Hence the optimum manufacturing pH is not the same as that of the corresponding solution. The experimental procedure to use is to make solutions of the desired concentrations of buffer and other excipients at several, say four, different pH values straddling the optimum solution pH, and then produce the lyophilized cake. The stability of this cake is then determined, and the optimum lyophilization pH determined in this manner.

10.3. The Labelling Dilemma of Parenteral Products

The FDA usually takes the strong positional stand that a different "salt form" constitutes a different drug substance and hence a new NDA is required. The drug on the label is the form of the drug in the dosage form. If for instance a product is made with a tetracycline base, then the label must state that this is the source of the antibiotic (as opposed to for instance the use of the addition salt, e.g., the hydrochloride).

But what about a lyophilized product? If one used tetracycline hydrochloride (RHCl) and buffered it at its pK value (at the given ionic strength), then, first

of all, the product would be present one half as positive ion (RH^+), one half as uncharged species (R). If the buffer is denoted HB, then, in concentrating a solution of this there would be two solubility products:

$$S_{RHCl} = [RH^+][Cl^-] \tag{7.16}$$

$$S_{RHB} = [RH^+][B^-] \tag{7.17}$$

aside from the solubilities S_{HB} and S_R. As the solution, hence, starts precipitating substances other than ice at the eutectic point, the species with the lowest S value or solubility product will at first precipitate out. This. for instance, could be RHB. As this precipitates out, both $[RH^+]$ and $[B^-]$ will decrease. At a given point R will start precipitating out. This will prevent further precipitation of RHB, because $[B^-]$ is now sufficiently low to be at the limit, had $[RH^+]$ not been affected. At a given point, because the amount of liquid water decreases as the process continues (freezing out of ice), the solubility limit of either HB or RHCl will be exceeded, and either species will then precipitate out until the remainder is left to freeze out as the last amount of water is solidified at the eutectic point.

The point is that the cake will contain four species: R, RHCl, HB, and RHB. And the question then is, under the present labelling policies, how does one properly label such a mixture?

11. OXIDATION

Oxidations are moisture mediated, as are hydrolyses. Often products that are oxidation sensitive are stored in glass rather than polymer bottles because, however good, these latter still allow permeation of oxygen.

In a glass bottle, if it is considered hermetic, and it often is, the oxygen in the head space will be consumed, and the amount of "initial" decomposition of the produce will tie in with the amount of oxygen available in the head space. It is a common phenomenon that solid dosage forms show an initial loss corresponding to the ratio between the amount of head space divided by the number of tablets in the bottle.

Often the oxygen is used up, and treatment of the data should be such that regression should be carried out on the data points after the *intial* drop.

Example 10.1.

A bottle contains 100 tablets and a head space of 25 mL of air. Each tablet contains 100 mg of drug substance of molecular weight 500. If the nonoxidative decomposition of the drug is 0.1% per month, how much would be expected, on the average, to remain after 3 years? Assume that one O_2 decomposes two drug molecules (i.e., $A + 1/2O_2 \rightarrow AO$).

Answer.

25 mL of air space at 25°C is $25/22.4 = 1.11$ moles of air, containing 22% of oxygen, so that the amount of available oxygen in the headspace is 0.22 millimoles.

This means that $0.22/100 = 2.44 \cdot 10^{-3}$ millimoles of oxygen will decompose an equal molar amount of drug *per tablet*. Each tablet contains $100/500 = 200 \cdot$

Over three years $36 \cdot 0.1 = 3.6\%$ of the drug will decompose by other means, so that a total of $2.4 + 3.6 = 6\%$ will decompose.

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8

Physical Characteristics of Solids

JENS T. CARSTENSEN

Madison, Wisconsin

1. STATES OF MATTER: CRYSTALLINITY AND AMORPHICITY

Prior to discussing the stability of drugs in the solid state, it is necessary to outline some characteristics of solids. A detailed discussion of the state of matter in regards to solids is outside the scope of this book. Suffice it here to say that solids may be characterized by being (a) crystalline or (b) amorphous. Crystalline solids are associated with a lattice, and amorphous solids are solids that are not crystalline. Some of the characteristics (those that apply to stability) of these two categories will be discussed in the immediate following.

There are seven crystal systems and two types of amorphates.

2. POLYMORPHISM

Inorganic (particularly ionic) solids usually are associated with one and only one crystal system. Well-known to all is that sodium chloride is cubic.

Organic solids, however, depending on how they are recrystallized, may occur in several different crystal modifications (polymorphs). There are two types of polymorphism, enantiotropes and monotropes. They are distinguished by their vapor pressure diagrams as shown in Figs. 1 and 2.

The situation referred to in Fig. 1 is one where there is a transition temperature, and DSC traces in such cases often have the appearance of either Fig. 2 or 3.

It is seen in Fig. 2 that two common situations may occur: first, the transformation may take place, so that there is an endotherm for the transformation followed by an endotherm for the melting. The melting point of form II (the room-temperature labile form) is recorded in this case as is the transition temperature.

The other possibility is that the transition is passed by, giving the melting point of the (now unstable) form I (lower trace). This forms an unstable melt, and often form II precipitates out, giving the exotherm shown in the lower graph followed by endotherm for the melting point of form II.

In some cases the exotherm is missing, and in such cases the melting endotherm of form II is also missing, i.e., the trace simply looks like the trace of melting of form

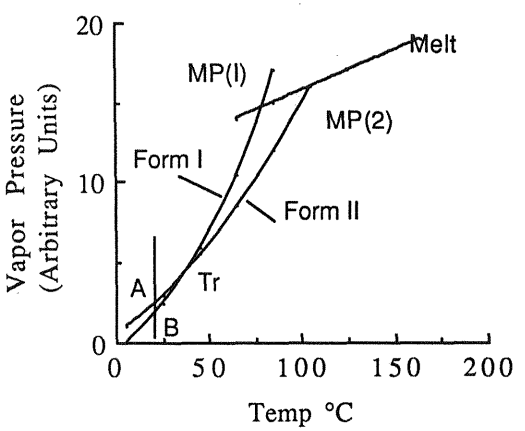

Fig. 1 Vapor pressure diagram of an enantiotropic pair.

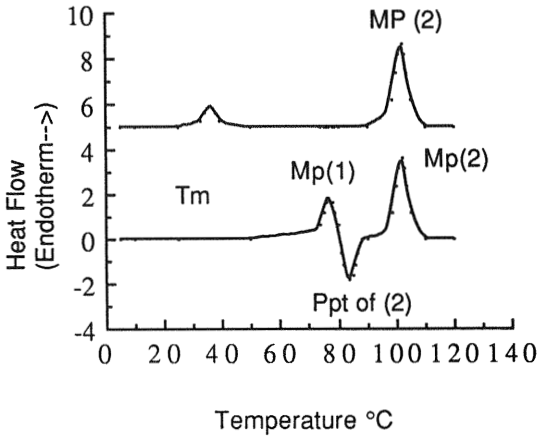

Fig. 2 Possible DSC traces resulting from heating of the room-temperature stable form of an enantiotropic pair.

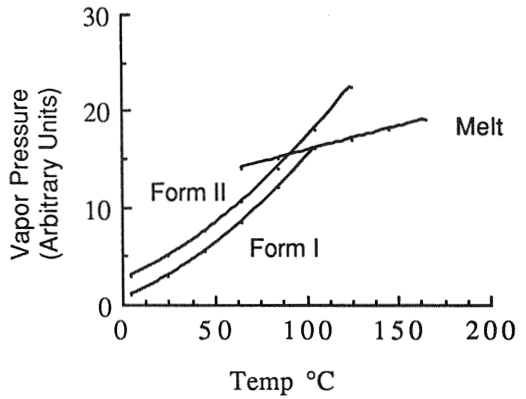

Fig. 3 Graph of vapor pressures for a monotropic pair.

I, as if no other form existed (similar to the bottom trace in Fig. 4). In such a case, if the compound is stable, it may be recooled, and the down direction melting point can be (and most often is) that of form II.

The other case is where one form (form II) is metastable throughout the melting range. This is exemplified in Fig. 3.

The DSC trace of such a pair may take one of several forms. The stable form will simply show up as a trace with one endotherm (the melting point of the stable form). Traces of the metastable form may either show up this way or as the lower trace in Fig. 4 .

As mentioned, if the compound is stable to melting, it is advisable to recool the mass and record the melting point on the down trace. Most often, however, decomposition of the solid and melt preclude conclusions from cooling curves.

The most powerful tool in polymorphic investigations, where it comes to determining whether two samples are of identical or different crystal systems, is

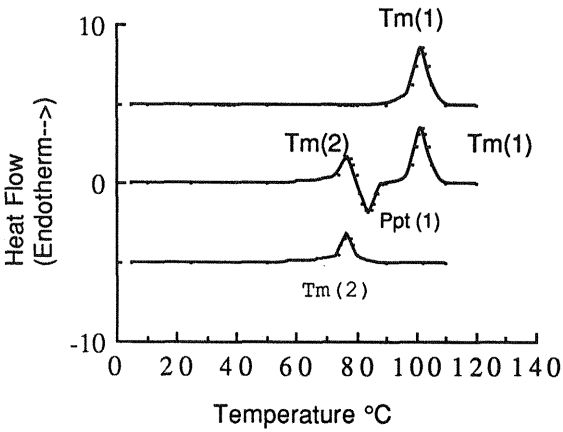

Fig. 4 Some possible DSC traces of the heating of polymorphs that are monotropic. The top trace is the heating of the stable polymorph in Fig. 3, and the two lower traces are the heating of the metastable polymorph, which may either simply melt (lower trace) or, as shown in the middle trace, melt and precipitate (exotherm) as the stable form I and then (second endotherm) remelt.

x-ray diffraction. Spacings in a crystal are related to the angle of the incoming beam (ϕ) by Bragg's law, which states that $2d\sin(\phi) = -k\lambda$, where λ is the wavelength of the x-rays used. The intensities are often used to monitor the amount of one form in another, or the amount present after a given time, t, when a conversion is taking place. An example of this is the work by Franchini and Carstensen on ranitidine (1994) where correlation was found between the content of form I in form II by the intensity at a 2ϕ value where form I did not "absorb" and where form II had a peak. Care should be taken in the interpretation of peak heights (or areas under the peak), since orientational factors can affect this. Orientation will, however, not affect the position of the peak.

It should be pointed out that in the strictest sense (Carstensen and Franchini, 1995, Martínez-Oharriz et al., 1994), there can only be true monotropism if the heats of solution are identical (and have the same temperature dependence). It is, therefore, advisable to perform heats of solution, calorimetrically, as was done for ranitidine by Franchini and Carstensen (1994) and for diffusinal by Martínez-Oharriz et al. (1993). If the heat of solution of the metastable polymorph in the pair is higher than that of the stable, the two curves may intersect at a temperature lower than the lowest temperature investigated. Of course, if this intercept is below absolute zero, then monotropism still prevails. If the heat of solution of the metastable polymoprh in the pair is lower than that of the stable one, then the two curves may intersect above the melting point, and in that case monotropism also prevails.

In the case of ranitidine, the two forms have identical solubilities (within experimental error), and what is denoted form I has a lower melting point. If the heats of solution are truly identical, this would then imply that form I is metastable over the entire temperature range and that it is a monotropic pair.

It follows from thermodynamics that the change in Gibb's energy by a path from metastable to stable form, ΔG, is given by

$$\Delta G = -RT \ln\left[\frac{P_{\text{metastable}}}{P_{\text{stable}}}\right] \tag{8.1}$$

It is negative, so the form with the highest vapor pressure at a given temperature is the least stable (metastable) compound. The term metastability (rather than instability) is used because under advantageous conditions the metastable compound may be "stable", i.e., not change for years or even decades.

3. SOLUBILITIES OF POLYMORPHS

It can be shown via Henry's law that solubilities are (approximately) linearly related to vapor pressures (actually activities as solubility are linearly related to fugacities). The graphs in Figs. 1 and 2 then become as shown in Figs. 5 and 6.

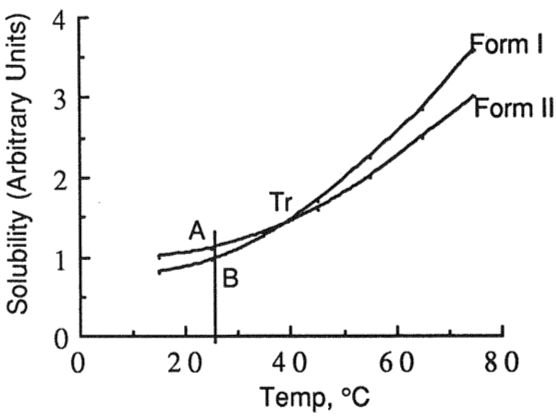

Fig. 5 Solubilities (in mass of solute per mass of solvent) of an enantiotropic pair.

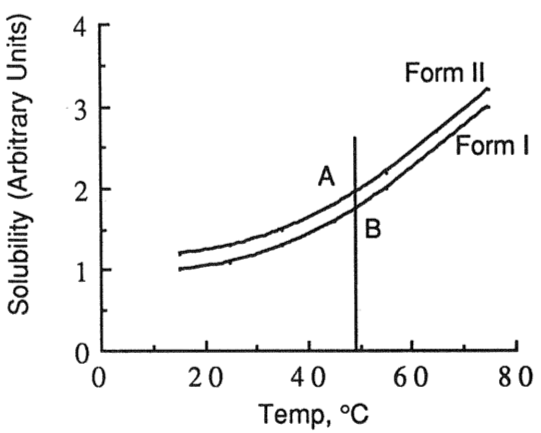

Fig. 6 Solubilities (e.g., in mass of solute per mass of solvent) of a monotropic pair.

If the Henry's law argument is applied to Eq. (8.1), then

$$\Delta G = -RT \ln\left[\frac{S_{\text{metastable}}}{S_{\text{stable}}}\right] \tag{8.2}$$

where S denotes solubility, R the gas constant, and T absolute temperature.

There are cases where the solubilities are close to one another over the entire temperature range, and in such cases it may be difficult to separate the two polymorphs in the final purification (recrystallization, reprecipitation), and there are cases where companies have been forced to suggest specifications that stipulate a minimum and a maximum of one polymorph in relation to another.

Increased solubility increases dissolution rates, and herein lay the initial interest in polymorphism in pharmacy. Shefter and Higuchi (1963) have shown the effect of solvates and hydrates on dissolution rates of several drug substances.

Pfeiffer et al. (1970) determined the solubility of cephaloglycin and cephalexin in binary mixtures and established that, depending on the composition of the medium, one or another polymorph would be stable (Fig. 7).

Poole and Bahal (1968) showed the differences in dissolution rates of anhydrous and dihydrate forms of ampicillin. The anhydrous form is amorphous, and hence would have a higher apparent solubility and hence a faster dissolution. Poole and Bahal (1970) have used Van't Hoff plots to show the conversion temperature between the anhydrous and dihydrate forms of an aminoalicyclic penicillin.

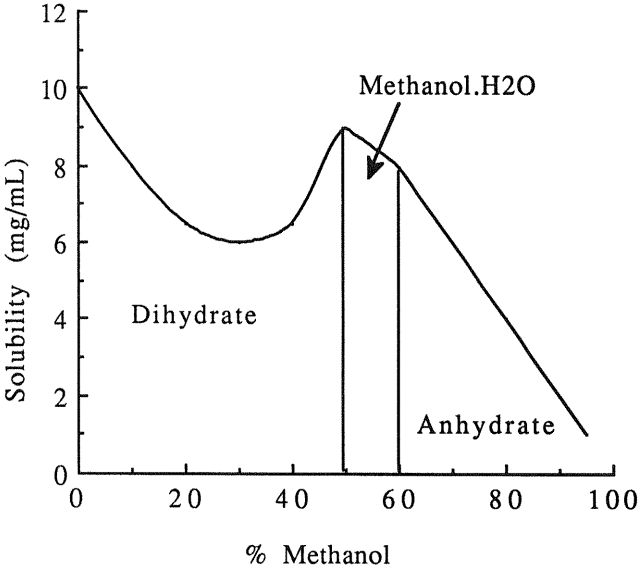

Fig. 7 The areas indicate the solid phase in which the solution is in equilibrium. (Graph constructed from data by Pfeiffer et al., 1970.)

4. RATES OF CONVERSION IN MOIST STORAGE

Good stability of a metastable compound can be achieved by (a) low temperature, (b) coarse crystals, and (c) dry storage. The moisture is the most significant contributor to conversion.

Moisture will condense onto the surface of the metastable form (II), which will then saturate the moisture layer to form a solution which is supersaturated in (I). This will eventually nucleate, and all of the II will convert to I.

The conversion rate is therefore a function of the nucleation rate in "solution," and it is well known (Mullin, 1961) that the nucleation rate, J, is inversely proportional to the viscosity of the solution and also to the supersaturation ratio, ΔS by the relation

$$J = A \exp\left[\frac{-q}{T^3 \ln \Delta S}\right] \tag{8.3}$$

For very soluble compounds, ΔS will be a very small number, and the tendency for one polymorph to change into another will be very small. An example of this is ranitidine.

5. EQUILIBRIUM MOISTURE CONTENT OF SOLIDS AND HYGROSCOPICITY

Hygroscopicity is the potential for moisture uptake that a solid will exert in combination with the rate with which this will happen. The condition of the atmosphere is an important factor as well, so a short, concise definition of hygroscopicity is not possible.

If a solid is placed in a room, moisture will condense onto it. If this moisture is simply a limited amount of adsorbed moisture, the substance is not hygroscopic under those conditions. These conditions exist if the water vapor pressure in the surrounding atmosphere is lower than the water vapor pressure over a saturated solution of the solid in question.

Often, however, the water vapor pressure in the atmosphere, P_a, is lower than that of the saturated solution, P_p. Then there will be a thermodynamic tendency for water to condense upon the solid. This is depicted in Fig. 8.

From a thermodynamic point of view, the situation shown dictates that moisture keeps on adsorbing until all solid has dissolved, and then continues until the solution is sufficiently dilute to have a vapor pressure of P_a. In this respect the moisture uptake curve differs from that of surface adsorption (polymers, and situations at atmospheric pressures below P_s), because these have asymptote at much lower levels.

The rate and extent of which moisture can condense on solids is usually collected under the term "hygroscopicity." In recent years a series of articles dealing with this phenomenon (e.g., Van Campen et al., 1980) have appeared in the pharmaceutical literature dealing with this subject. The purpose here is to derive a rational equation for the rate with which moisture is adsorbed onto a water-soluble solid.

As mentioned, if a solid is placed in an atmosphere that has a vapor pressure, P_a, higher than the vapor pressure, P_s, of the saturated solution of the compound,

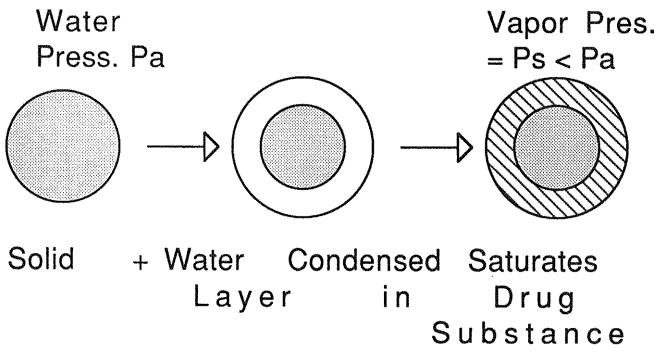

Fig. 8 Mechanism of moisture condensation.

the condensed water will dissolve solid. It will be assumed below that the sorbed solution is saturated at all times. The question is what sort of curve might be expected for the extent of moisture uptake with time (the moisture uptake rate curve, the MUR curve).

Assume that, at time t, a certain amount of moisture, w, has been adsorbed by a particular solid particle weighing m grams and of diameter, d_0, where the subscript denotes the condition prior to moisture adsorption. At time t, moisture will have adsorbed, some solid will have dissolved, and the diameter, d, of the solid itself will have decreased from its original value. The diameter of the ensemble, D, is the sum of the diameter of the remaining solid and the thickness, h, of the moisture layer.

It is assumed in the following that one gram of solid is studied and that the sample is monodisperse. Such a sample would consist of N particles, where

$$Nm = \frac{N\rho\pi d_0^3}{6} = 1 \tag{8.4}$$

The amount of solid present at time t is given by the original amount less the amount dissolved. If there are W grams of water adsorbed by one gram of solid (i.e. w gram dissolved per particle), then

$$N\left(\frac{\rho\pi}{6}\right)d^3 = N(m - wS) = 1 - WS \tag{8.5}$$

or

$$d^3 = \frac{1 - WS}{N(\rho\pi/6)} \tag{8.6}$$

The volume of liquid adsorbed by one solid particle has a volume of the total particle minus the solid particle, i.e.,

$$\begin{aligned} \frac{w}{\rho^*} &= \left(\frac{\pi}{6}\right)D^3 - \left(\frac{\pi}{6}\right)d^3 \\ &= \left(\frac{\pi}{6}\right)D^3 - \frac{\pi/6(1 - WS)}{N(\rho\pi/6)} \end{aligned} \tag{8.7}$$

where ρ^* is the density of the adsorbed liquid. Since it is assumed that it is always saturated, it is time-independent, and under ideal conditions it would be

$$\rho^* = (1 - x_s)\rho_0 + x_s\rho \tag{8.8}$$

where $1 - x_s$ and x_s are the volume fractions of liquid and solid, respectively, in the ensemble particle, and ρ_0 and ρ are the respective densities. It follows from Eq. (8.7) that the amount of moisture adsorbed per gram can be expressed in terms of diameters as

$$\mathrm{W} = \rho^* N\left(\frac{\pi}{6}\right)D^3 - \frac{\rho^*(1 - WS)}{\rho} = QD^3 - F + FSW \tag{8.9}$$

where

$$F = \frac{\rho^*}{\rho} \tag{8.10}$$

$$Q = \rho^* N\frac{\pi}{6} \tag{8.11}$$

Equation (8.9) may be written

$$F + (1 - FS)W = QD^3 \tag{8.12}$$

or

$$D = \left\{\frac{F + (1 - FS)W}{Q}\right\}^{1/3} \tag{8.13}$$

The area, a, of the particle (solid plus liquid) is, hence,

$$a = \pi\left\{\frac{F + (1 - FS)W}{Q}\right\}^{2/3} = B[E + W]^{2/3} \tag{8.14}$$

where

$$\mathrm{B} = \pi\left[\frac{1 - FS}{Q}\right]^{2/3} \tag{8.15}$$

$$\mathrm{E} = \left\{\frac{FQ}{1 - FS}\right\}^{2/3} \tag{8.16}$$

The rate of condensation (dW/dt) is proportional to the pressure gradient, i.e., the difference between the water vapor pressure, P, in the atmosphere and the vapor pressure, P_s, over a saturated solution. At a given atmospheric milieu, this gradient is a constant.

It is also proportional to the surface area, a, by a mass transfer coefficient, k, so that we may write

$$\frac{dW}{dt} = ka(P_a - P_s) = k(P_a - P_s)B[E + W]^{2/3} \tag{8.17}$$

where Eq. (8.14) has been used for the last step. This may be written

$$\frac{dW}{[E + W]^{2/3}} = 3G\,dt \tag{8.18}$$

where

$$3G = k(P - P_s)B \tag{8.19}$$

Eq. (8.18) integrates to

$$[E + W]^{1/3} = Gt + [E]^{2/3} \tag{8.20}$$

where the initial conditions, $W = 0$ at $t = 0$, have been imposed. Equation (8.20) can be solved by iteration.

As an example of this, VanCampen et al. (1980) studied the moisture pickup in a vacuum system by using a Cahn balance and exposing the evacuated head space to relative humidities created by salt baths. They also reported moisture uptake rates of choline chloride at room temperature and different relative humidities using a desiccator method. An example of their results obtained by the latter method is shown in Fig. 9.

Jakobsen et al. (1997) have employed a highly sensitive microcalorimeter to evaluate the hygroscopicity of hydrophilic drug substances, such as flupentixol dihydrochloride (solubility>1 g/mL) as well as hydrophobic substances (such as

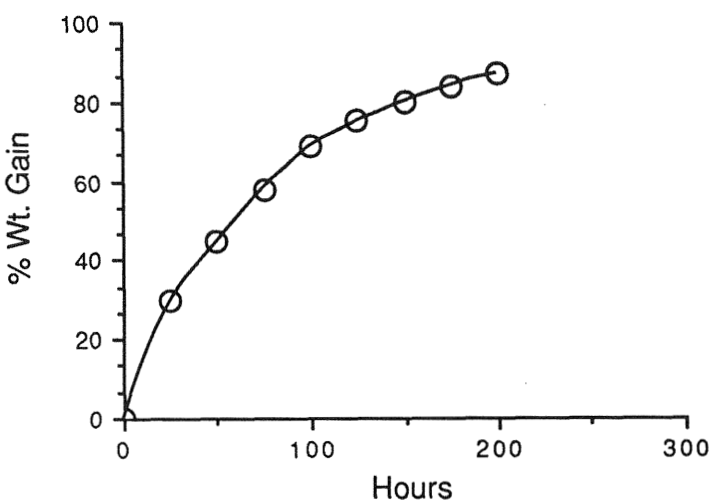

Fig. 9 Data for choline chloride moisture adsorption at 100% RH. (Drawn from data published by VanCampen et al., 1980.)

ertindole, solubility 10 μg/mL). Pinderre et al. (1997) have described coating powders with Eudragit to protect them against moisture uptake and have evaluated the coatings by way of moisture uptake rates.

6. CRITICAL MOISTURE CONTENT

There are humidities below which a solid will not adsorb (considerable amounts of) moisture, i.e., not form a "bulk-sorbed" layer. These are dictated by the solubility of the compound, as will be seen below.

Suppose a solid is placed in a room of a given RH, as shown in Fig. 10. If the RH were 30%, then it might pick up moisture at a given rate, at 50% RH at a higher rate, and at 80% RH at an even higher rate.

The rate with which it picks up moisture is determined by weighing the sample at given intervals, as demonstrated in Table 1. It is noted that there is a linear section of the curve (up to 6 days), as shown in Fig. 11 and 12. The slope of this linear segment is the moisture uptake rate (MUR). The actual uptake rates (determined from the linear portions) are shown in Table 2.

The uptake rates can simply be obtained by weighing the sample after a given time (6 days), but in such a case it is assumed that the moisture uptake is still in the linear phase. If, e.g., the weight gain is 5 mg per 10 g sample in 6 days, then the MUR is $5/10/6 = 0.083$ mg/g/day.

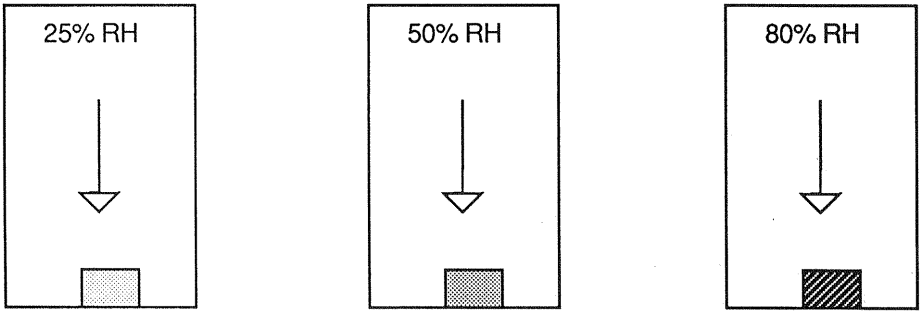

Fig. 10 Mechanism of moisture uptake.

Table 1 Moisture Uptake of a Water-Soluble Compound at 50% RH

Days stored at 50% RH	Moisture pickup (mg/g)
2	0.5
6	1.5
18	2.25
36	3.4
100	3.0
144	4.2
288	4.3

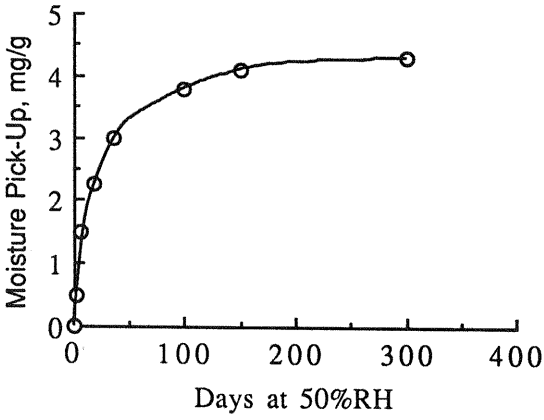

Fig. 11 Moisture uptake data from Table 1.

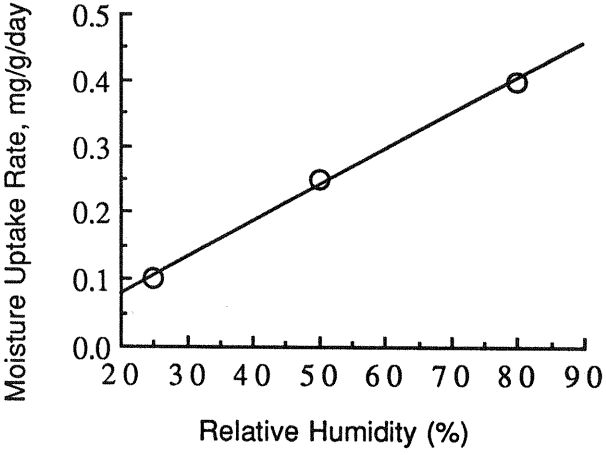

Fig. 12 Moisture uptake rate as a function of RH. Least squares fit is $y = -0.06264 + 0.006374x$, with $R^2 = 0.999$.

Table 2 Moisture Uptake Rate of Water-Soluble Compound

% RH	mg/g/day
25	0.1
50	0.25
80	0.45

If the MUR values are plotted versus RH, then a straight line results (Fig. 11). The curve intercepts the x-axis at 20% RH. This means that the compound can be stored without moisture pickup in atmospheres of less than 20% RH. In some cases the compound will dry out under such conditions (e.g., a hydrate), but in general

Table 3 Characteristics of Disodium Hydrogen Phosphate

Type	% Moisture in solid		$P(H_2O)$ (mm Hg)	Water activity (RH/100)
Anhydrous	0			
		Pair	9	0.38
Dihydrate	20			
		Pair	14	0.58
Heptahydrate	47			
		Pair	18	0.75
Dodecahydrate	60			
		Pair	22	0.92
Satd. solution (100 g water/4.5 g salt)				

the useful information reached from such a graph is the maximum RH that is satisfactory for storage of the products. 20% RH happens to be the relative humidity over a saturated solution of the compound (or over a salt pair, as will be discussed presently).

For inorganic compounds and hydrates, the curves are stepwise curves. For instance, for disodium hydrogen phosphate, the following situation exists: the compound can form three hydrates (2, 7, and 12) aside from being anhydrous. The percent of moisture in, e.g., the dihydrate, is calculated as follows: disodium hydrogen phosphate has a molecular weight of 142. The dihydrate hence has a molecular weight of $142 + 36 = 178$. Hence the moisture percentage is

$$100 \times \frac{36}{178} = 20\%$$

The moisture contents for the remaining hydrates are shown in Table 3.

7. EQUILIBRIUM MOISTURE CURVES FOR SALT HYDRATES

The previous section dealt with the *rate* with which moisture is taken up. As shown in Fig. 11, at longer time periods, the moisture level (the weight of the sample) will taper off and plateau at an equilibrium value. This equilibrium value is also a function of RH, and there are two types of curves that occur when equilibrium values are plotted against RH: salt pairs and continuous adsorption. The former will be discussed first.

It is seen in the table that the RH of the atmosphere above a mixture of anhydrous disodium hydrogen phosphate and the dihydrate is 9 mm Hg or $100(9/24) = 38\%$ RH. It is noted that any mixture of the anhydrous salt and the dihydrate will given this relative humidity. Hence disodium hydrogen phosphate containing from 0 to 20% moisture will have above it an atmosphere of 38% RH. Similarly, as shown in Table 3, the heptahydrate contains 47% moisture, and mixtures of di- and heptahydrate give rise to water vapor pressures of 14 mm Hg (58% RH). Proceeding in this fashion, a graph as shown in Fig. 13 results.

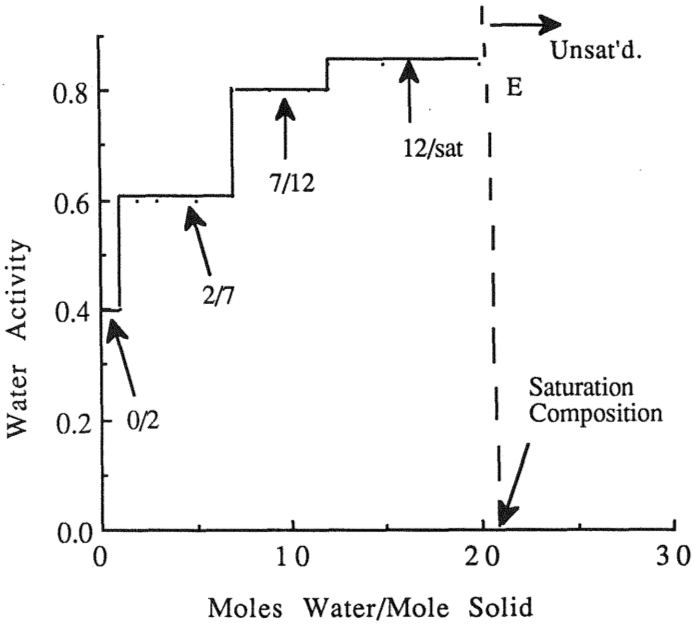

Fig. 13 Vapor pressure diagram of salt forming a dihydrate, a heptahydrate, and a dodecahydrate.

Two further points need to be mentioned. (a) If disodium hydrogen phosphate is stored at a relative humidity between 38 and 58% RH, it will not pick up moisture. Once the relative humidity is raised to (slightly above) 58%, then it will start picking up moisture until it has completely converted into the heptahydrate. (b) If the relative humidity is raised to (slightly above) 92% RH, then the dodecahydrate is converted to saturated solution. At higher RH values, the equilibrium will be dictated by the water vapor pressure over the now unsaturated solution.

At 100% RH the system in equilibrium is infinite dilution (pure water), and if a diagram such as this (and the following diagram for organic macromolecules) is carried out to 100% RH, then a sharply increasing curve should result at very high RH. The diagram in Fig. 12 is at a given temperature. Figure 13 shows a diagram of a dihydrate at different temperatures. At the temperature T_3, the line for the salt pair has caught up with that of the saturated solution. Essentially this means that the enthalpy of hydration for the solid is higher than the heat of vaporization of water from the saturated solution, since both have Clausius–Clapeyron type vapor pressures. Above T_3, therefore, the salt would have a higher vapor pressure than the saturated solution, but this is thermodynamically untenable, and T_3 is simply the highest temperature (and a triple point) where the dihydrate exists.

For a monohydrate as depicted in Fig. 14, the moisture content of the "salt hydrate" will increase drastically when the water vapor pressure is higher than that depicted by point H. Moisture keeps on condensing and converting the monohydrate to saturated solution, and this will continue until all is dissolved. After that the vapor pressure will increase so that it is always in equilibrium with the concentration in the (now) unsaturated solution.

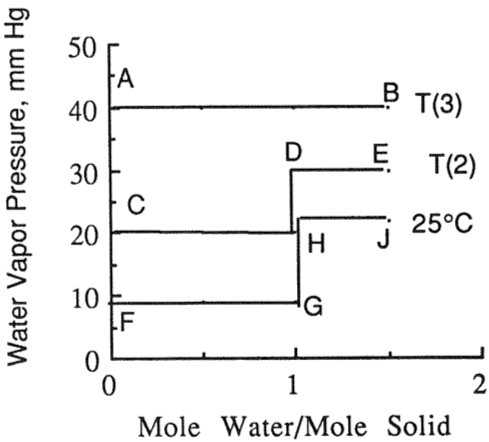

Fig. 14 Single salt pair (monohydrate) vapor pressures as a function of temperature. The line at point A has been drawn a mite to the left for graphical clarity. It occurs at 1 mole of water/mole of solid.

In general for an x-hydrate with a saturation concentration of $1/(x+y)$ moles of salt per mole of water, the reaction on the constant pressure isotherm is

$$\mathrm{B} \cdot x\mathrm{H_2O} + y\mathrm{H_2O} \rightarrow \mathrm{B} \text{ dissolved in } (x+y) \text{ moles of } \mathrm{H_2O} \tag{8.21}$$

The solubility of B in water is $1/(x+y)$.

Beyond the solubility concentration, there will be a total of z moles of water and 1 mole of solid, so that the mole fraction of water will be $z/(1+z)$. The vapor pressure of the now unsaturated solution would be given by Raoult's law, i.e., $a = P/P_0 = z/(1+z)$, or, since a here is plotted versus z, it would be given by

$$z = \frac{a}{1-a} \tag{8.21a}$$

However, many authors plot a versus z.

Example 8.1.

The diagram in Fig. 15 is a vapor diagram of a drug substance that forms a pentahydrate. Comment on the following statements: (a) The pentahydrate is stable between 10 and 45%. (b) If the hydrate is exposed to a relative humidity of 81%, then it will lose water and become anhydrous. (c) It neither loses nor picks up moisture at 81%. (d) It gains moisture and forms a saturated solution.

Answer.

(a) and (d) are correct, but (d) is strictly correct only for 80% RH. (It will form a very slightly undersaturated solution.)

As an example of research on hydrate forms, it should be mentioned that Allen et al. (1978) have shown that erythromycin exists in crystalline form as an anhydrate, a

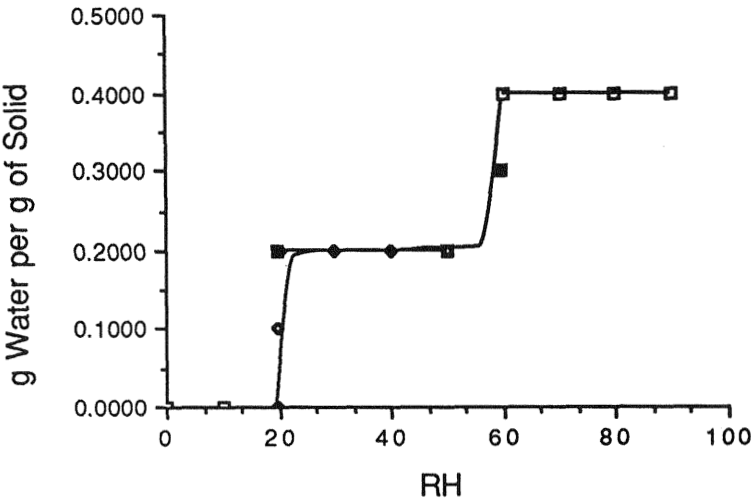

Fig. 15 Vapor phase diagram of an organic substance that forms a pentahydrate at 25°C (MW 360 + 90).

monohydrate, and a dihydrate. Shefter and Kmack (1967) showed that the dehydration kinetics of theophylline hydrate were first order.

Hemihydrates also exist. Wu et al. (1996) have reported on an anhydrous and a hemihydrate form of brequinar sodium. Both have fairly comparable solubilities. Loosely bound water is also present in the structure, and this is lost (in thermograms) at 90°C, and the water of hydration is released at about 175°C.

It should be mentioned that in some cases "bound" moisture is indeed held very tightly. Magnesium chloride tetrachloride is an example. Heating this substance to 80–100°C will remove two of the molecules of water. But further heating results in the removal of 2 moles of hydrochloric acid, leaving magnesium hydroxide behind.

8. MOISTURE EQUILIBRIUM CURVES OF A SMOOTH NATURE

There are substances such as gelatin and corn starch that give rise to moisture equilibrium curves of the type shown in Fig. 16. These are referred to as BET moisture isotherms.

As a dry sample is exposed to increasingly higher vapor pressures, P_u (u stands for "up"), moisture contents x_u will be in equilibrium with the sample. If the experiment is terminated at a pressure of P^*, and the vapor pressures in the atmospheres decrease, then, e.g. at P_d (d stands for "down") the moisture content will be x_d, i.e., higher than during the up curve. The hysteresis loops shown in Fig. 15 are exaggerated for graphical clarity. Such curves can be shown to be variants of the BET equation or the GAB equation (Guggenheim, Anderson, and deBoer) (Guggenheim et al., 1968; Zografi and Kontny, 1986; Grandolfi, 1986). It is noted that y_d is not an equilibrium condition. Obviously

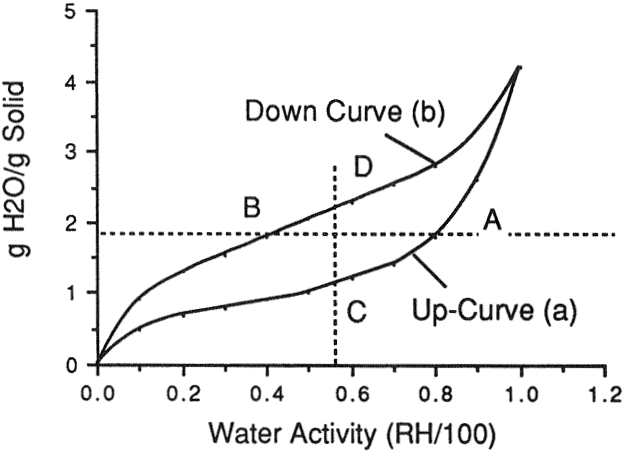

Fig. 16 BET up (adsorption) and down (desorption) moisture isotherm.

ΔG is negative in going from the down curve to the up curve, because

$$\Delta G = V \int_{P_u}^{P_d} V\, dP = V[P_u - P_d] < 0 \tag{8.22}$$

Several common tablet excipients give rise to Langmuir isotherms. An excipient study by Sangvekar (1974), when all the data are lumped together, gives an equation of the type

$$\frac{1}{y} = \frac{A}{P} + B \tag{8.23}$$

Usually, in pharmaceutical and engineering literature, the moisture equilibrium curves are shown in a sense opposite to that shown in Fig. 15, i.e.,

$$P = \phi(y) \tag{8.24}$$

The high RH tail of the curve is usually above 85% RH and therefore does not apply to most realistic pharmaceutical conditions, but it is applicable to one often-conducted test (40°C, 75%RH). Zografi and Kotny (1986) have described these types of moisture isotherms by either a BET equation or a GAB equation.

For routine isotherms, the high relative humidity tail is difficult to obtain with reasonable precision, and one approach (Carstensen, 1980) is to approximate them by Langmuir isotherms (i.e., not use the high-end portion).

9. AMORPHATES

Solids which are not crystalline are denoted amorphous. If one melts a (stable) solid and recools it, then it should crystallize when the melting point is arrived at.

This requires nucleation, and nucleation propensity is a function of the viscosity of the liquid in which it occurs. Materials that are viscous about their melting point are therefore prone to form supercooled solutions.

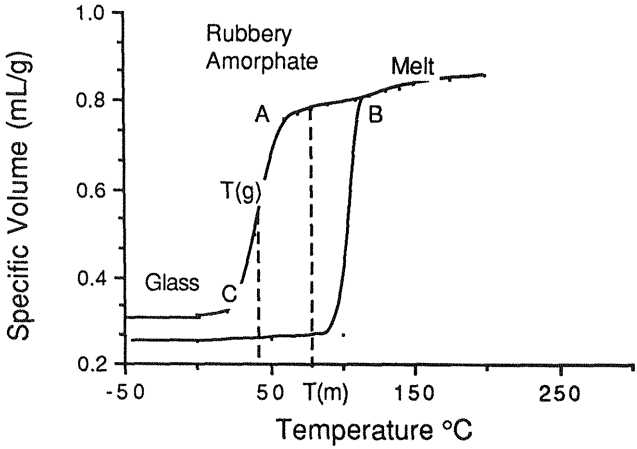

Fig. 17 Molecular volume as a function of temperature of a solid prone to forming an amorphate.

At a given high viscosity (attained at or below the melting point), the melt will have the appearance of a solid, and this is the type of material referred to as amorphous.

Right below the melting point, the molecules will have no specific orientation, and molecular movements will be random in direction and magnitude (within the limits of the system) as opposed to a crystalline material, where the molecules are arranged in lattices (ordered arrays), and where the orientation of each molecule is set.

At a temperature T_g, lower than the melting point, there will be a physical change in the amorphate. An example of this is shown in Fig. 17.

Between points A and B the properties of the amorphate are often like that of the melt. This is referred to as the "rubbery" state, and below C it is referred to as a glass.

10. WATER ABSORPTION "ISOTHERMS" INTO AMORPHATES

Amorphates are solids that are not crystalline. It is assumed at this point that the term "solid" is self-evident, although amorphates in the rubbery state (just below the melting point of the crystalline form of the compound) are actually highly viscous liquids. When exposed to humid atmospheres, they will pick up moisture in a fashion that is not like that of a BET isotherm (to be covered shortly). The moisture actually penetrates into the solid, which thus may be considered a "solution."

In an ideal situation, the water activity, a, will decrease linearly with $(1-x)$, where x is the mole fraction of solute. At a given point ($x=0.24$ in Fig. 17) the solution becomes saturated. (This concentration, of course, differs from compound to compound.) Beyond this concentration, the solution itself will be saturated, and the vapor pressure will not change with further addition of compound; rather, the composition will change, but the vapor pressure will stay constant. In this type of graph the coordinates are in the opposite direction of a usual isotherm.

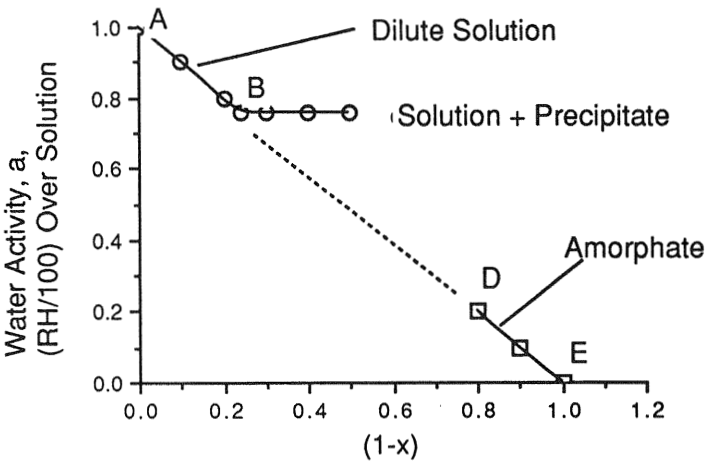

Fig. 18 Moisture isotherm for an amorphous solid. (Graph constructed from data published by Carstensen and VanScoik, (1990.)

If an amorphous form of the compound is produced and exposed to different relative humidities, then the "isotherm" is often quite linear, if the amount of water absorbed is expressed as a mole fraction (line DE in Fig. 18). As shown by Carstensen and VanScoik (1990) for amorphous sugar, this line is an extension of the solution vapor pressure line (AB in Fig. 18), and one may consider the moist amorphate as a highly concentrated, supersaturated "solution."

Due to the random arrangement and the mobility of the molecules in an amorphate as opposed to a crystalline modification, amorphates are usually less stable chemically than crystalline modifications (Carstensen et al., 1993).

Carstensen and VanScoik (1990) were the first to point out that for an amorphous substance, it is illogical to use the traditional moisture isotherms, because in this case it is probably not an adsorption, but rather an absorption, which is at play.

By exposing amorphous sucrose to various relative humidities, various moisture levels were reached. If these moisture levels were expressed as mole fractions of sucrose, then the vapor pressures fell in line with the vapor pressure curve of sucrose itself.

The fraction to the right of point B is the principle used for salt solutions to obtain constant relative humidity in desiccators. With electrolytes, the vapor pressure depression is larger (due to the 2- or 3-fold number of ionic particles, over that of the molarity of the salt), and the solubilities are often high, so that these are preferred for creating constant relative humidity in desiccators.

Zografi and Hancock (1993) have used this principle in their investigation of whether such an approach, i.e., solution theory, could be applied to macromolecules. To quote, "If one considers the absorption process to be completely analogous to the solution process, then it should be possible to use basic solution theories to model the data." Their data for PVP K30 are shown in Fig. 19.

First of all note that the "ideal solution" model advocated earlier is (probably) not applicable to macromolecules. (The concentrations, however, are not converted to mole fractions, but such a conversion would not make the plot linear.) The data

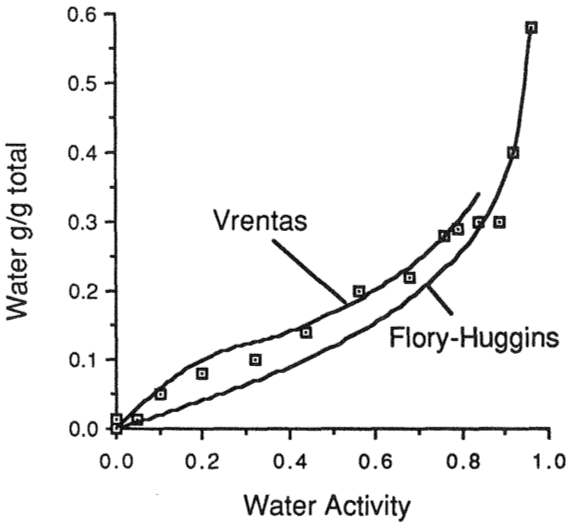

Fig. 19 Fit of vapor pressure data of aqueous solutions of PVP K30 at 30°C to the Flory–Huggins equation. The points are taken off Figs. 6 and 7 as accurately as possible, as is the trace of the Flory–Huggins equation. (Plot constructed from data published by Hancock and Zografi, 1993.)

obviously, fit the Vrentas equation better at low water activity, but the Flory–Huggins equation may be more applicable at high water activity. Data become slightly uncertain at such high humidities in any event.

It has been mentioned that one method of stabilizing a "solution" for marketing is to lyophilize it and thus increase its storage stability. Many lyophilizates are amorphous. The method for making a lyophilizate is first to make a solution, then to freeze it, and then to sublime off the moisture. In this process it is important that the solution stay sufficiently stable before and during freezing. Various lyoprotectants are used for such purposes, and Dekeyser et al. (1997), for instance, have shown that chymopapain is stabilized in the presence of different lyoprotectants such as maltodextrins.

Amorphates exhibit glass transition temperatures. These are a function of water content, as shown e.g. by Hancock and Dalton (1999) and in Table 4. These authors and others (e.g., Carstensen, 1995) compared moisture adsorption isotherms with the equations of Flory–Huggins, Vrentas, and Raoult.

Glass transition temperatures are always somewhat approximate. For instance, in contrast to the above, Hatley (1997) has reported the T_g of sucrose to be 64°C at 0.73% moisture.

11. MOISTURE EXCHANGE BETWEEN DOSAGE FORM INGREDIENTS

Gore and Ashwin (1967) were the first to report that for an excipient (in their case citric acid), "given a knowledge of the equilibrium moisture content for a particular moisture sensitive compound at the upper limit of its moisture specification, it would

Table 4 Glass Transition Temperatures of Water-Containing Sugar Amorphates

Water (%)	Lactose glass trans. temp. (°C)	Sucrose glass trans. temp. (°C)	Raffinose glass trans. temp. (°C)	Trehalose glass trans. temp. (°C)
0	112	74	103	115
1	102	60	92	101
2	94	50	83	90
3	85	32	75	80
4	80	<25	67	70
	71	<25	58	60

Source: Table constructed from data by Hancock and Dalton (1999).

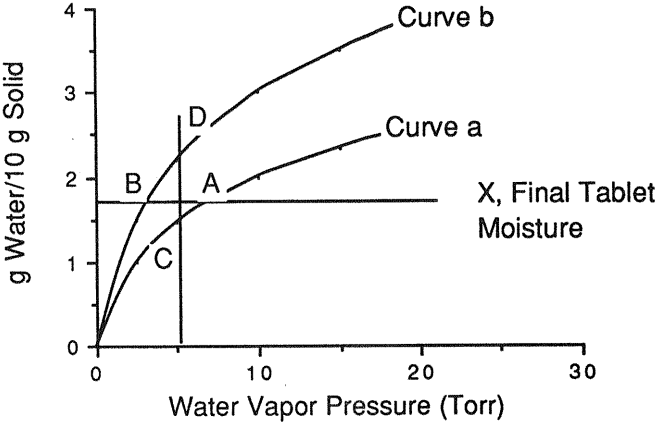

Fig. 20 Langmuir moisture isotherm presentation of initial part of a BET isotherm. From (Carstensen, 1980.)

be a simple matter to define moisture limits for citric acid in a particular formulation".

A solid dosage form (e.g., a tablet) is usually made to a given moisture content, e.g. (Fig. 20), 1.8 g per 100 g of solid. Since the drug and the excipients have different moisture isotherms, they will have different equilibrium RH values. There can, however, only be one RH condition in the pore space of the solid dosage form, so the result is that compound b will pick up moisture (move from B to D) and compound a will lose moisture (moving from A to C). The question is to estimate, quantitatively, where (at what RH) the line DC will be.

In Fig. 19, the two moisture equilibrium curves have (in an abbreviated fashion) been represented as Langmuir isotherms. This can be verified by inspection of Fig. 10, where lines OC and OB would both fairly well adhere to Eq. (8.25).

This may be used to estimate the moisture movement in a solid dosage form after it is manufactured. In consulting Fig. 20 and assuming that the up curve is that of drug (A) and the down curve that of excipient (B), there are m_A grams of A on an anhydrous basis, and A contains a fraction (on a dry basis) of q_A

moisture, i.e., a total of $m_A q_A$ grams of water. There are m_B grams of B on an anhydrous basis, and A contains a fraction (on a dry basis) of q_B moisture, i.e., a total of $m_B q_B$ grams of water.

The dry weight of the dosage form is therefore $mA + mB$, and as the dosage form (e.g., tablet) is made, it is made at a particular moisture content of a fraction (on a dry basis) of q moisture, i.e., a total of $mq = [m_B + m_A]q$ grams of water.

Since, as seen from the figure, the relative humidity (the vapor pressure, P) in the pore space must be one particular figure (P), it follows that A must give up moisture (from point A to point C) and B must take up moisture (from point B to point C).

The moisture isotherms are of the type

$$x(\mathrm{A}) = \frac{q_A m_A}{m_A} = q_A = Q_A P_A^{1/n_A} \tag{8.25}$$

and

$$x(\mathrm{B}) = \frac{q_B m_B}{m_B} = q_B = Q_B P_B^{1/n_B} \tag{8.26}$$

The values of n usually do not differ much (and the two isotherms can therefore be represented as only differing in the values of the Q's). It is noted that the areas have not been taken into account, and the isotherms apply to two samples of material. (To account for the area, plotting by BET would have to be done.)

Where a known amount of A, m_A, is mixed with a known amount of B, m_B, mass balance (assuming no loss of moisture) gives

$$x_C[m_A + m_B] = x_A m_A + x_B m_B \tag{8.27}$$

or

$$x_C = \frac{x_A m_A + x_B m_B}{m_A + m_B} \tag{8.28}$$

and the amount of moisture lost can then be gauged from

$$\text{moisture loss in A} = m_A(x_A + x_C) \tag{8.29}$$

and for B,

$$\text{moisture loss in B} = m_B(x_D - x_B) \tag{8.30}$$

Since x_C is known, P is then also known.

If for instance the two compounds are mixed together, moisture added (as in a granulation), and this then dried, then x_C is known. Mass balance about ACB in Fig. 20 then gives that the moisture loss experienced by A,

$$m_A(x_A - x_C) = m_A Q_A[P^{1/n} - P_C^{1/n}] \tag{8.31}$$

must equal the moisture gained by B, i.e.,

$$m_B(x_C - x_B) = m_B Q_B[P_C^{1/n} - P_B^{1/n}] \tag{8.32}$$

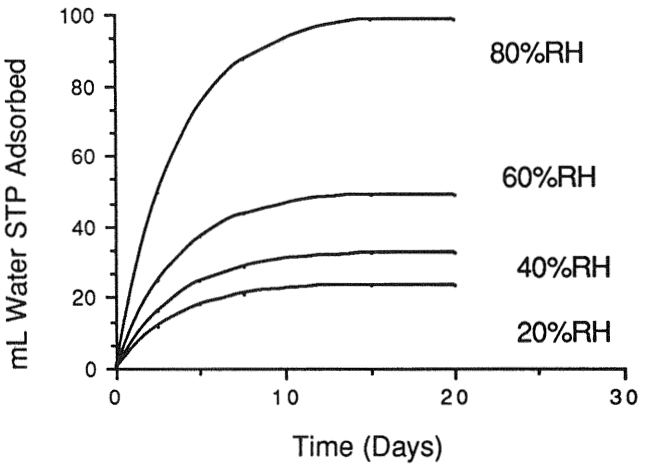

Fig. 21 Moisture uptake curves for a sample of silica gel at 20, 40, 60, and 80% RH.

All quantities are known, so that P [$= P_C = P_D$] can be calculated, i.e., both moisture losses and gains, and the final relative humidity can be calculated. In this latter case, the isotherms should be determined on samples that had been wetted and dried the same way the final mix had been wetted and dried (since the surface area changes).

12. EQUILIBRIUM MOISTURE CONTENTS FOR MACROMOLECULES

For an organic compound such as starch, a smooth equilibrium moisture curve will result. Here again there is a sharp upswing at very high relative humidities.

If experiments such as are exemplified in Table 1 and Fig. 11 are carried out on e.g. cornstarch, then curves of the *type* shown in Fig. 21 result. The figure shows moisture uptake rate curves at four different relative humidities: 20%, 40%, 60%, and 80%. When the moisture contents (x mg water/mg solid) of these levels are plotted as a function of relative vapor pressure, P/P^* (the relative humidity, divided by 100, the so-called water activity), then an isotherm results. This moisture isotherm has the shape shown in Fig. 16.

When $P/[x\{1-P\}]$ is plotted versus P, then a straight line results.

13. ADSORPTION ISOTHERMS OF SILICA

The curve in Fig. 21 eventually levels off. The equilibrium level is a function of the relative humidity at which the experiment is carried out. Table 5 shows an example of moisture uptake curves of a sample of silica, at various relative humidities. These levels are tabulated in the second column. It is customary in isotherm work to convert these adsorbed amounts to the volumes that would have been occupied at 0°C and 1 atm, and this can easily be done, e.g., for the first row. The number of moles is $n = (17.5 \times 10^{-3})/18 = 9.75 \times 10^{-4}$ moles. The volume of this at 25°C and 1 atm would be $V = nRT/P = 9.75 \times 10^{-4} \times 82 \times 298/1 = 23.8$ mL. These figures are shown in the third column and are denoted V.

Table 5 Data from Which Fig. 22 Was Constructed, and Conversion to BET Parameters

RH (%)	mg adsorbed	V (mL) (O°C, 1 atm)	RH/(V{100-RH})
20	17.5	23.8	0.01
40	23.9	32.5	0.021
60	36.1	49.2	0.030
80	72.6	98.9	0.040

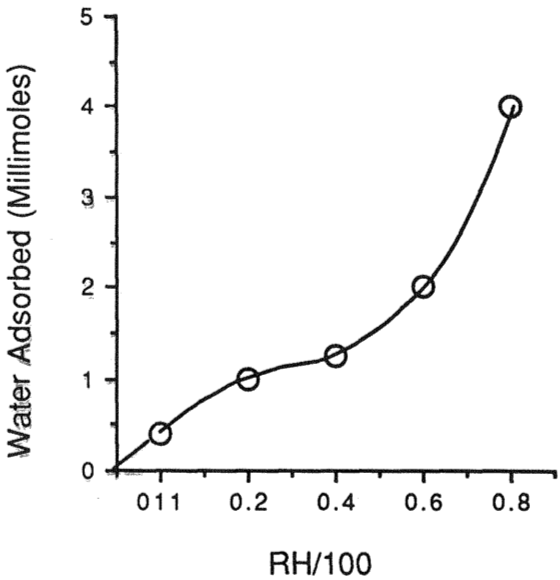

Fig. 22 The equilibrium levels in Fig. 20 plotted versus relative humidity.

The isotherms of this type are called BET isotherms. The data in the third column are shown in Fig. 22. It can be shown that such data follow the BET equation:

$$\frac{\mathrm{RH}}{V\{100-\mathrm{RH}\}} = \phi + \frac{1}{V_\mathrm{m}}\left[\frac{\mathrm{RH}}{100}\right] \tag{8.33}$$

Treatment by this equation is shown in Fig. 23. V_m is here the volume (0°C, 1 atm) of water that just constitutes one layer on the entire surface of the solid sample. RH/[V{100-RH}] has been calculated in the table (last column) and is plotted in Fig. 23 versus RH/100.

The slope of the line is $1/V_\mathrm{m}$, so

$$\frac{1}{V_\mathrm{m}} = 0.05 \qquad \text{or} \qquad V_\mathrm{m} = 20\,\mathrm{mL} \tag{8.34}$$

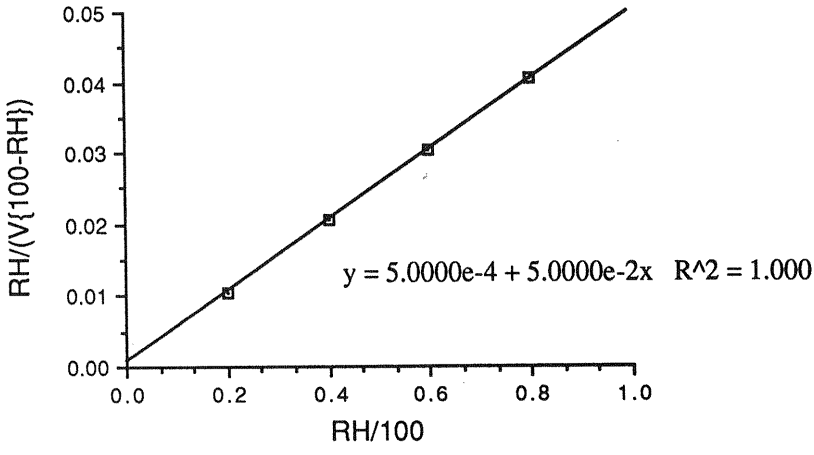

Fig. 23 Data from Table 5 treated by the BET equation.

This can be converted to moles (n) and then to molecules (N);

$$n_m = \frac{20PV}{RT} = 1 \times \frac{20}{82 \times 298} = 12.9 \cdot 10^{-4} \text{ moles}$$
$$= 6 \times 10^{23} \times 12.9 \times 10^{-4} = 77 \times 10^{19} \text{ molecules} \tag{8.35}$$

Water molecules in a monolayer will position themselves so that their cross-sectional area is $10\,\text{Å}^2 = 10 \times 10^{-16}\,\text{cm}^2$, so that in this case the entire surface area would be the number of molecules times the area of each molecule, i.e.,

$$77 \times 10^{19} \times (10 \times 10^{-16}) = 77 \times 10^4\,\text{cm}^2 = 77\,\text{m}^2$$

Most substances are not "hygroscopic" below 20% RH.

If a bag of silica is placed in a bottle with a dosage form, then, if there is a critical moisture content beyond which the dosage form becomes unstable, it is possible to calculate, from the isotherm of the dosage form, at which relative humidity this occurs. From the silica isotherm one can then calculate how much moisture is taken up by the silica bag at this point, and dividing this figure by the moisture penetration of the package, it is possible to calculate the length of time the product is good.

Moisture isotherms are of great significance in pharmaceutics. Cases in point are the moisture isotherms of PVP and of the complex of misoprostol and hydroxypropyl methylcellulose.

14. HYDROUS AMORPHATES

As mentioned, solids that are not crystalline are called amorphous. An important category of these are lyophilized cakes (for intravenous reconstitution). These are formed by freezing aqueous solutions. Upon such freezing (when part of the solid comes out as an atmosphere), ice will first freeze out, and then the remaining solution (which usually crystallizes as a eutectic) will supercool and will become

"solid." But in this case the "solid" is simply a very viscous solution. Fig. 24 is an example of this and is constructed from data published by Her and Nail (1994).

The "solid" is (when dried) referred to as a lyophilized cake. The glass transition temperature can usually be arrived at from thermal analysis, as shown in Fig. 25. The collapse temperature is a temperature dictated by mechanical pro-

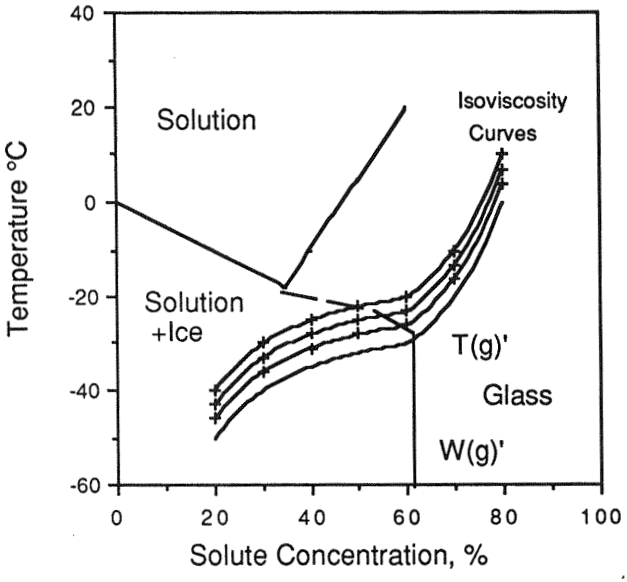

Fig. 24 Graph constructed from data published by Her and Nail (1994).

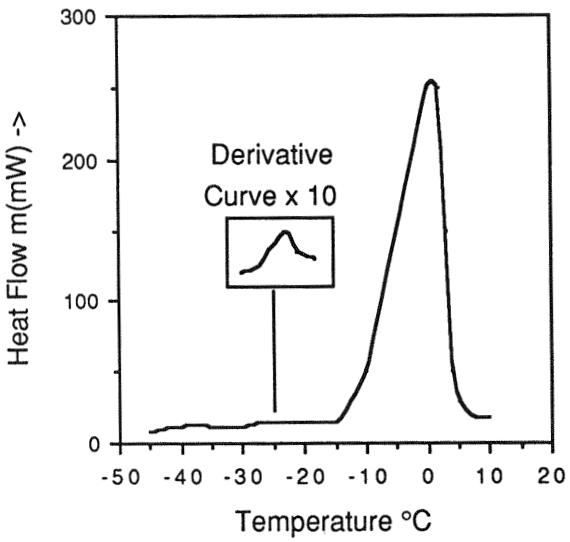

Fig. 25 Thermogram of aqueous solution of 10% PVP. The relative magnitudes of the endotherms for glass transition vis-à-vis melting is shown. (Graph constructed from data published by Her and Nail, 1994.)

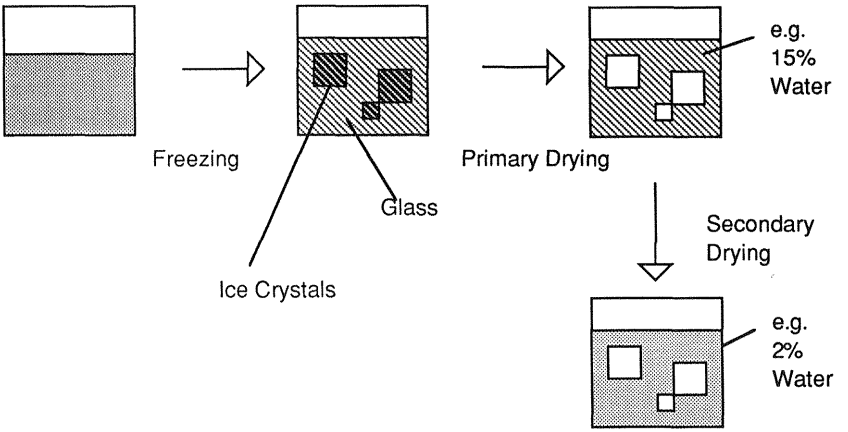

Fig. 26 Schematic of freeze-drying events.

perties. Just above the glass transition temperature, sucrose solutions, for instance, have viscosities of about 10^6 Pa/s, but below T_g this figure is 10^{12} Pa/s. The general sequence of events in freeze drying is shown in Fig. 26.

The primary drying (Fig. 26) consists of the evaporation of the crystalline ice, so that the cake is left with "holes" in it, and a glass of a water content in the range of 12–15% results. As mentioned, if the temperature is below the glass transition temperature, then this glass has a high viscosity and will dry slowly, since the diffusion coefficient, D, for evaporation of water, will be high.

If, after the primary drying, the initial freezing temperature were 240°K as shown in Fig. 26, and the solids content were 50%, then the composition would be at point C, Fig. 27, between the T_c and T_g curves. But if sublimation were continuously carried out at this temperature, then, at point B, the glass transition would be passed, and the viscosity would become very high, and sublimation would be very slow. The temperature is therefore continuously increased, so that the lyophilization temperature can stay within the bounds of the two curves.

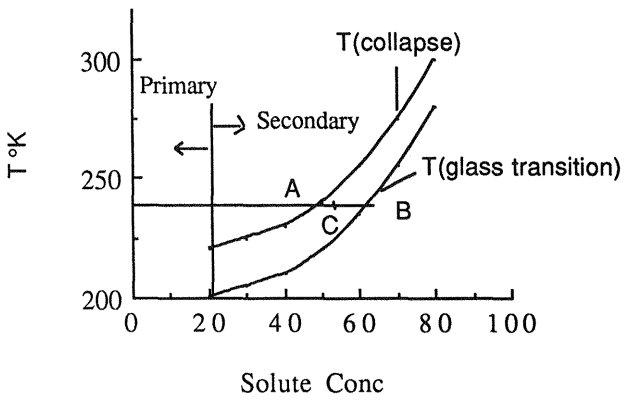

Fig. 27 Limiting phases in a lyophilization event.

Some proteins have stabilities that depend on the cooling rate, but this is primarily due to electrolytes (e.g., sodium chloride) and stabilizers (e.g., glycine) in the composition. These will crystallize out and give the cake structural strength so that T_s increases, but their presence, as well as the initial freezing rate, will modify the positions of the two curves, so that a slow cooling rate may provide a different (and sometimes worse) cake than when a fast cooling rate is employed.

These aspects have been discussed in detail by Franks (1990), Levine and Slade (1988), Mackenzie (1977) and Suzuki and Franks (1993).

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9

Preformulation

JENS T. CARSTENSEN

Madison, Wisconsin

Historically, preformulation evolved in the late 1950s and early 1960s as a result of a shift in emphasis in industrial pharmaceutical product development. Up until the mid-1950s, the general emphasis in product development was to development elegant dosage forms, and organoleptic considerations far outweighed such (as yet unheard of) considerations as whether a dye used in the preparation might interfere with stability or with bioavailability.

In fact, pharmacokinetics and biopharmaceutics were in their infancy, and although stability was a serious consideration, most analytical methodology was such that even gross decomposition often went undetected.

It was, in fact, improvement in analytical methods that spurred the first programs that might bear the name "preformulation." Stability-indicating methods would reveal instabilities not previously known, and reformulation of a product would be necessary. When faced with the problem of attempting to sort out the component of incompatibility in a 10-component product, one might use many labor hours. In developing new products, therefore, it would be logical to check, ahead of time, which incompatibilities the drug exhibited (testing it against common excipients). This way the disaster could be prevented in advance.

A further cause for the birth of preformulation was the synthetic organic programs started in many companies in the 1950s and 1960s. Pharmacological screens would show compounds to be promising, and pharmacists were faced with the task of rapid formulation. Hence they needed a fast screen (i.e., a preformulation program) to enable them to formulate intelligently. The latter adverb implies that some of the physical chemistry had to be known, and this necessitated determination of physicochemical properties, a fact that is also part of preformulation.

1. PREFORMULATION'S PLACE IN THE STABILITY FUNCTION

The approach of preformulation was so logical, indeed, that it eventually became part of the official requirements for INDs and NDAs (Schultz, 1984):

> New drug substances in Phase I submission. For the drug substance, the requirement includes a description of its physical, chemical or biological characteristics. We in the reviewing divisions regard stability as one of those characteristics. The requirement of NDA submissions ... of the rewrite stability information is required for both the drug substance and drug product. A good time to start to accumulate information about the appropriate methodology and storage stations for use in dosage form stations for use in dosage form stability studies, therefore, is with the unformulated drug substance.... Stress storage conditions of light, heat and humidity are usually used for these early studies, so that the labile structures in the molecule can be quickly

identified.... If degradation occurs, the chemical reaction kinetics of the degradation should be determined.... Physical changes such as changes from one polymorph to another polymorph should be examined.... With the drug substance stability profile thus completed, the information should be submitted in the IND submission.

2. TIMING AND GOALS OF PREFORMULATION

The goals of the program are therefore (1) to establish the necessary physicochemical parameters of a new drug substance, (2) to determine its kinetic rate profile, (3) to establish its physical characteristics, and (4) to establish its compatibility with common excipients.

To view these in their correct perspective, it is worthwhile to consider when, in an overall industrial program, preformulation takes place. The following events take place between the birth of a new drug substance and its eventual marketing (it is a fact, however, that most investigational drug substances never make it to the marketplace for one reason or another):

1. The drug is synthesized and tested in a pharmacological screen.
2. The drug is found sufficiently interesting to warrant further study.
3. Sufficient quantity is synthesized to (a) perform initial toxicity studies, (b) do initial analytical work, and (c) do initial preformulation.
4. Once past initial toxicity, phase I (clinical pharmacology) begins and there is a need for actual formulations (although the dose level may not yet be determined).
5. Phase II and III clinical testing then follows, and during this phase (preferably phase II) an order of magnitude formula is finalized.
6. After completion of the above, an NDA is submitted.
7. After approval of the NDA, production can start (product launch).

3. PHYSICOCHEMICAL PARAMETERS

Physicochemical studies are usually associated with great precision and accuracy, and in the case of a new drug substance would include studies of (a) $pK_{(a)}$ (if the drug substance is an acid or base), (b) solubility, (c) melting point and polymorphism, (d) vapor pressure (enthalpy of vaporization), (e) surface characteristics (surface area, particle shape, pore volume), and (f) hygroscopicity. Unlike in the usual physicochemical studies, an abundance of material is usually not at hand for the first preformulation studies: in fact, at the time this function starts, precious little material is supplied, and therefore the formulator will often settle for good estimates rather than attempt to generate results with four significant figures.

There is another good reason not to aim too high in the physicochemical studies of the first sample of drug substance. In most cases the synthesis is only a first scheme, and in later scale-up it will be refined; and in general the first small samples contain some small amount of impurities, which may influence the precision of the determined constants. But it is necessary to know, *grosso modo*, important properties such as solubility, pK, and stability. These are dealt with in order below.

3.1 pK_a and Ionizable Substances

The definition of $pK_{(a)}$ and pk_a have been discussed in chapter 2. For substances that are carboxylic acids (HA) it is advantageous to determine the pK_a, since this property is of importance in a series of considerations. For carboxylic acid the species A^- usually absorbs in the ultraviolet (UV) region, and its concentration can be determined spectrophotometrically (Underberg and Lingeman, 1983); HA on the other hand will absorb at a different wavelength.

The molar absorbances of the two species at a given wavelength are denoted ε_0 and ε_- (it is assumed that at the wavelength chosen $\varepsilon_0 < \varepsilon_-$), and it can be shown that if the solution is m_0 Molar in total A, then

$$\frac{A^-}{HA} = \frac{\varepsilon - \varepsilon_0 m_0}{m_0 \varepsilon_- - \varepsilon} \tag{9.1}$$

so that the ratio A^-/HA can be determined in a series of buffers of different pH. Hence the $pK_{(a)}$ can be found as the intercept by plotting pH as a function of $\log[(A^-)/(HA)]$ by Henderson–Hasselbach:

$$pH = pK_{(a)} + \log\left[\frac{A^-}{HA}\right] \tag{9.2}$$

If several buffer concentrations are used, extrapolation can be carried out to zero ionic strength, and the pK_a can be determined. For initial studies, however, a $pK_{(a)}$ in the correct range (i.e., +0.2 unit) will suffice, so that the determination above can be done at one buffer concentration only.

The conventional approach is to do titrations (Fig. 1), and this will yield graphs of fraction (neutralized (x) as a function of pH. Usually, the water is titrated as well (Parke and Davis, 1954), and what is presented in Fig. 1 is the "different." The $pK_{(a)}$ is then the pH at half neutralization (which is also the inflection point).

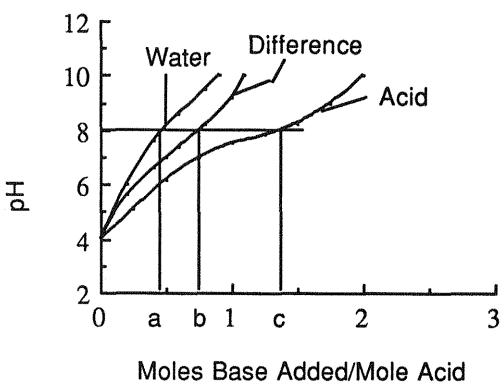

Fig. 1 Typical titration curves. The "water" curve indicates the amount of alkali needed to "titrate" the water, and the "acid" curve is a conventional titration curve. The difference curve is the horizontal difference between the "acid" and the "water" curve and is the adjusted titration curve. For example the point "b" is "c" minus "a". The $pK_{(a)}$ is the point of inflection, which is also the point where half of the acid is neutralized.

The pH solubility curve can now be constructed simply by determining the solubility of HA (at low pH) and A^- (e.g., of NaA) at high pH (e.g., at pH 10).

4. SOLUBILITY

One important goal of the preformulation effort is to devise a method for making solutions of the drug. Frequently, the drug is not sufficiently soluble in water itself to allow for the desired concentrations, for example for injection solutions. Solubilities are determined by exposing an excess of solid to the liquid in question, and assaying after equilibrium has been established. This usually is in the range 60 to 72 h, and to establish that equilibrium indeed has been established, sampling at earlier points is necessary. Unstable solutions pose a problem in this respect and will be dealt with in more detail later. Solubilities cannot be determined by precipitative methods (e.g., by solubilizing an acid in alkali and then lowering the pH to the desired pH) because of the so-called metastable (solubility) zone (Rodriguez-Hornedo, 1984). In the writing to follow, drug substances are subdivided into two categories: (1) ionizable substances, and (2) (virtually) nonionizable substances.

Solubility determinations are necessary both for stability reasons and for formulation reasons. It was noted, in the chapter dealing with stability of solids in the presence of water, that the solubility term becomes part of a rate constant. Since preformulation occurs in the early stage of development, the optimization of stability by way of compound selection (correct salt) is of importance, and often a drug product can be stabilized by keeping the solubility of the drug substance low. In the limit this, however, might affect bioavailability.

Probably among the most well-known examples of such stabilization are that of procaine penicillin and that of potassium clavulanate. In the latter case, the sodium salt, for instance, is unstable to such an extent that it cannot be utilized. The decrease in solubility of the potassium salt renders the product machinable (although low humidities must be observed in manufacturing).

4.1 Use of Salt Formation to Increase Solubility

It is noted that at a given pH the amount in solution in a solubility experiment is

$$S = S_{HA} + C_{A^-} \tag{9.3}$$

where S denotes solubility. The last term can be determined from knowledge of the pK_a, the pH, and the use of Eq. (2).

For drugs that are amines, the free base is frequently poorly soluble, and in this case the $pK_{(a)}$ is often estimated by performing the titration is a solvent containing some organic solvent (e.g., ethanol). By doing this at different organic solvent concentrations (e.g., 5%, 10%, 15%, 20%), extrapolation can be carried out to 0% solvent concentration to estimate the aqueous $pK_{(a)}$.

Usually, alkali metal salts of acids are more soluble than the free acids, and in the case of basic (e.g., amine type) drugs, the solubility of the acid addition salts are more soluble than the free bases. At times (e.g., in the case of enalipril) the compound is amphoteric. The acid addition salt is soluble, the free base is less soluble, and the sodium salt is, again, more soluble. In simple cases, the solubility curve

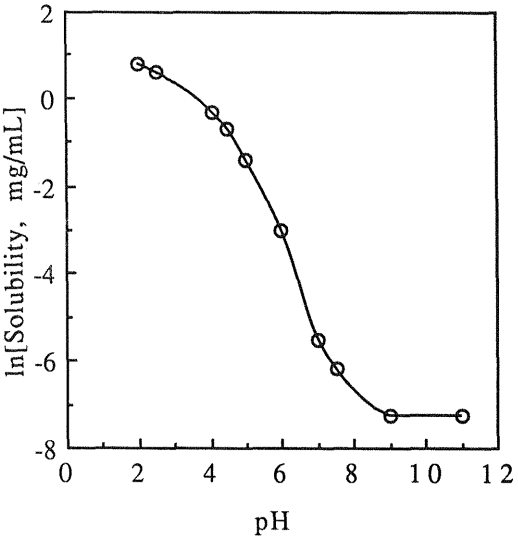

Fig. 2 pH solubility plot of imiquimod in water. (Graph constructed from data published by Chollet et al., 1999.)

will simply mimic the Henderson–Hasselbach equation. An example of this is imiquimod (Chollet et al., 1999), the pH solubility profile of which is shown in Fig. 2.

Streng and Yu (1998) have published a computer program for prediction of stability curves based on the pK_a value of the compound in question.

4.2. Nonionizable Substances

For hydrophobic, (virtually) nonionizable substances (i.e., those that show no ionic species of significance in the pH range 1 to 10, e.g., diazepam), solubility can usually be improved by addition of nonpolar solvents. Aside from solubility, stability is also affected by solvents either in a favorable or in an unfavorable direction (Bakar and Niazi, 1983). Theoretical equations for solubility in water (Yalkowsky and Valvani, 1983) and in binary solvents (Acree and Rytting, 1983) have been reported in the literature, but in general the approach in preformulation is pseudoempirical. Most often the solubility changes as the concentration of nonpolar solvent, C2, increases. For binary system, it may simply be a monotonical increasing function (Carstensen et al., 1971), as shown in Fig. 3.

The solubility is usually tied to the dielectric constant, and in a case such as that shown in curve A, the solubility is often log-linear when plotted as a function of inverse dielectric constant, ε, that is,

$$\ln S = -\frac{e_1}{\varepsilon + e_2} \tag{9.4}$$

where ε is the dielectric constant and the e terms are constants (Underberg and Lingeman,. 1983).

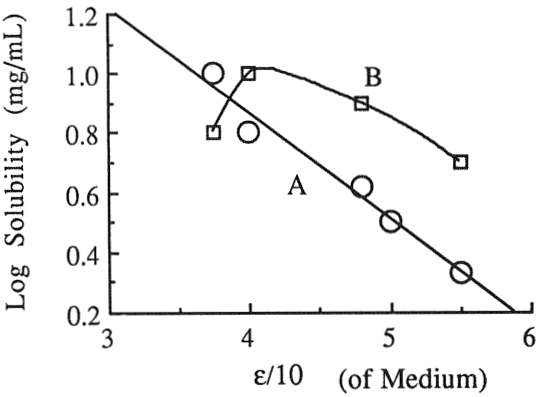

Fig. 3 (A) Solubility of 7-chloro-1,3-hydro-5-phenyl-2H-1,4-benzodiazepine-2-one-4-oxide in aqueous propylene glycol. (Data from Carstensen et al., 1971.) (B) Solubility of another benzodiazepine. (Unpublished data.)

Frequently, however, the solubility curve has a maximum (as shown in curve B in Fig. 3) when plotted as a function of C2 and ε (Paruta and Irani, 1964). In either case it is possible to optimize solubility by the selection of a solvent system with a given value of ε; that is, once the curve has been established, the optimum water/solvent ratio for another solvent can be calculated from known dielectric constant relationships (Cavé et al., 1979).

4.3. Ternary Systems and Optimization

Frequently, *ternary* solvent systems are resorted to. Examples are water–propylene glycol–benzyl alcohol or water–propylene glycol–ethanol. In such cases the solubility profile is usually presentable by a ternary diagram (Sorby et al., 1963). This type of diagram usually demands a fair amount of work; that is, the solubility of the drug substance in many solvent compositions must be determined. A priori, it would therefore seem that they would be out of place in a situation where only limited quantities of drug are available. However, their principle gives some validity to optimization procedures.

The diagram can be of one of two types, as shown in Figs. 4 and 5. In the first type, the solubility may be assumed to be of the type

$$S = a_{10} + a_{11}C_1 + a_{12}C_2 \tag{9.5}$$

where C denotes concentrations of nonaqueous solvents. An example of this is shown in Fig. 4. Here the subscripts to C denote the two nonaqueous solvents. Hence three solubility experiments would determine the relationship (with zero degrees of freedom). It is usual to do at least five, and determine possible curvature [i.e., inclusion of more terms in Eq. (9.6)].

In the second case, Fig. 5 each tie line will give a parabolic type curve as shown. Hence at a given concentration of C_2 the solubility can be approximated by

$$S = b_{10} + b_{11}C_1 + b_{12}C_1^2 \tag{9.6}$$

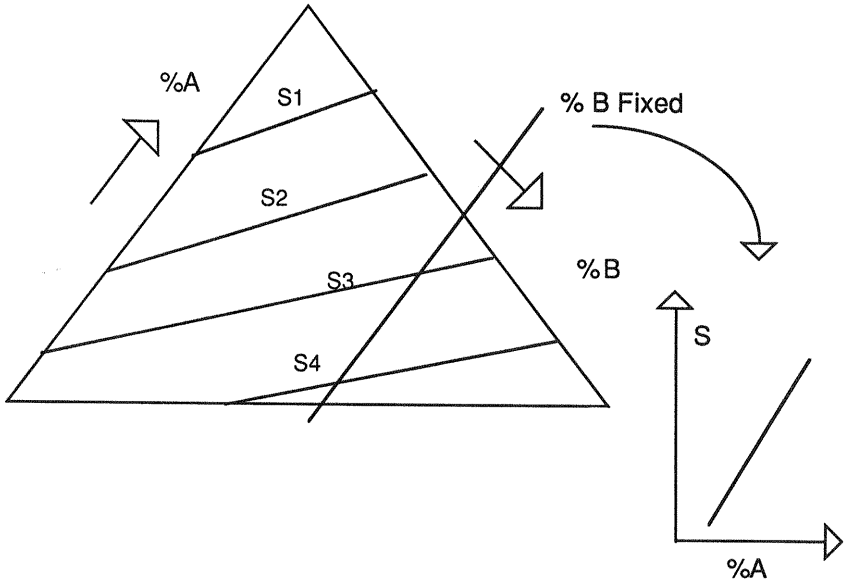

Fig. 4 Ternary diagram of solubility of a compound in a ternary mixture with linear solubility response. Inset: Concentration of drug in compositions with constant concentration of B. The composition of the solute is the constant concentration of B, the concentration of A in the abscissa, and the complement concentration of C (the third apex, not indicated in the figure). The drug solubility response is linear in the A concentration in this case.

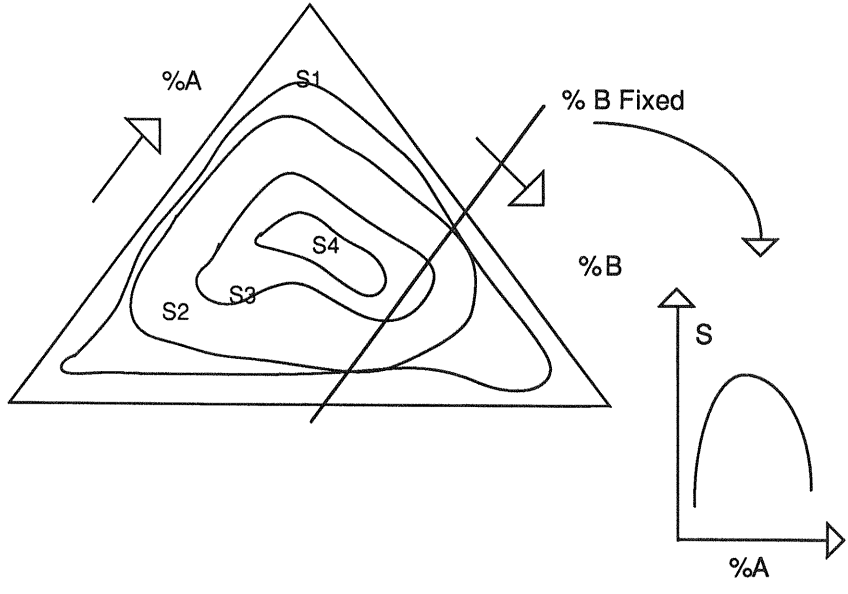

Fig. 5 Ternary diagram and tie line concentration in a nonlinear system.

where, in the simplest case,

$$b_{10} = C_{20} + C_{21}C_2 \tag{9.7}$$

Hence optimization can be achieved by five (or more experiments, with zero (or in general n-5) degrees of freedom.

4.4. Prediction of Solubility

It is advantageous, with a new drug substance, to be able to estimate what its solubility might be, prior to carrying out dissolution experiments. There are several systems of solubility prediction, e.g., the work by Amidon et al. (1974) and Yalkowsky et al. (1972, 1975). Their equation, for solubility of p-aminobenzoates in polar and mixed solvents, is a simplified two-dimensional analog of the Scatchard–Hildebrand equation and is based on the product of the interfacial tension and the molecular surface area of the hydrocarbon portion of a molecule.

More recently Bodor et al. (1989) have developed a semiempirical solubility predictor based on 14 variables (S = molecular surface in Å^2, I_a = indicator variable for alkanes, D = calculated dipole moment in Debyes, Q_n = square root of sum of squared charges on oxygen atoms, Q_o = square root of sum of squared charges on oxygen atoms, V = molecular volume in Å^2, S_2 = square of molecular surface, C = constant, MW = molecular weight, {O} = ovality of molecule, A_{bh} = sum of absolute values of atomic charges on hydrogen atoms, A_{bc} = sum of absolute values of atomic charges on carbon atoms, A_m = indicator variable for aliphatic amines, and N_h = number of N-H single bonds in the molecule).

The aqueous solubilities, W, of 331 compounds were found to follow the equation (with tolerances omitted)

$$\begin{aligned}\log W = &-56.039 + 0.32235D - 0.59143I_a + 38.443Q_n^4 \\ &- 51.536Q_n^2 + 18.244Q_n + 34.569Q_o^4 - 31.835Q_o^2 + 15.061Q_o \\ &+ 1.9882A_m + 0.15689N_h + 0.00014102S^2 + 0.40308S - 0.59335A_{bc} \\ &+ -0.42352V + 1.3168A_{bh} + 108.80\{O\} - 61.272\{O\}^2\end{aligned} \tag{9.8}$$

Of the parameters listed only the ovality and the indicator value for the alkanes I_a are unfamiliar entities that are obtained from the literature (Bodor et al., 1989).

5. DISSOLUTION

The importance of dissolution is such (from biopharmaceutical considerations) that it is now used throughout the USP and is required in NDAs on solid dosage forms. According to Noyes and Whitney (1897),

$$\frac{dm}{dt} = \frac{VdC}{dt} = -kA(S - C) \tag{9.9}$$

where m is mass not dissolved, V is liquid volume, t is time, k is the so-called intrinsic dissolution rate constant (cm/s), and A is surface area of the dissolving solid.

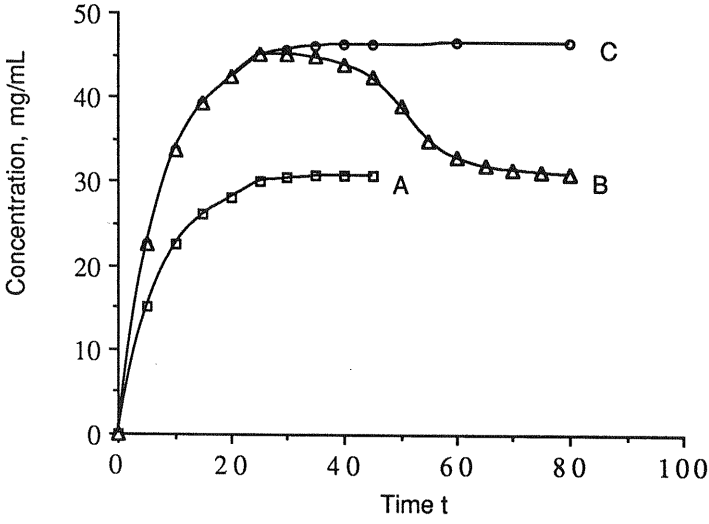

Fig. 6 Dissolution profiles obtained from the solubility determination of two polymorphic forms of the same drug substance. A is the stable form with solubility 31 mg/mL. B is the profile of the metastable form with solubility 46 mg/mL. This solubility, C, (circles) is not achieved in many instances, and precipitation of the stable form occurs at a point beyond the solubility of A, and the trace becomes B.

Many criticisms have been voiced against Eq. (9.9), but in general it is correct, and it will be assumed to be so in the following. Experimentation can be carried out with constant surface as when using a Wood's apparatus (Wood et al., 1963) or, with smaller amounts, making a small pellet and encasing it in wax and exposing only one face to a dissolution medium, or simply employing an excess of solid throughout the dissolution experiment. In such cases Eq. (9.9) may be integrated to give

$$\ln\left[1 - \frac{C}{S}\right] = -\frac{kA}{V}t \tag{9.10}$$

or

$$C = S\left[1 - \exp\left(-\frac{kA}{V}t\right)\right] \tag{9.11}$$

A typical curve following Eq. (9.11) is shown in Fig. 6.

In the critical time path for product development, solid dosage forms (tablets or capsules) must eventually be manufactured for the clinic (e.g., in clinical phase II). If possible, the drug substance per se is subjected to a dissolution test in a Wood's apparatus (Wood et al., 1963). This test is useful although it is quite dependent on hydrodynamic conditions. It consists of placing the powder in a special type of tablet die, compressing the tablet, and exposing the flat, exposed side of the tablet (with surface area A) to a dissolution liquid (usually water or N/10 HCl) in which it has a solubility S. Under these conditions (Carstensen, 1974), the intrinsic dissol-

ution rate constant (cm/s) can be obtained by Eq. (9.10), which under sink conditions (i.e., when C is less than 15% of S) becomes

$$C = \frac{SkA}{V}t \tag{9.12}$$

It has been suggested (Riegelman, 1979) that if k is obtained under sink conditions over a pH range of 1 to 8 at 37°C in a USP vessel by way of Eq. (9.12) at 50 rpm, then if the dissolution rate constant (kA/V) is greater than 1 mg min^{-1} cm^{-2}, the drug is not prone to give dissolution-rate-limited absorption problems. On the other hand, if the value is less than 0.1, such problems can definitely be anticipated, and compounds with values of kA/V of from 0.1 to 1 mg min^{-1} cm^{-2} are in a gray area. For compound selectivity it is frequently useful to express dissolution findings in terms of k (i.e., in cm/s).

For a small amount of powder, dissolution of the particulate material can often be assessed (and compared with that of other compounds) by placing the powder in a calorimeter (Iba et al., 1991) and measuring the heat evolved as a function of time. The surface area must be assessed microscopically (or by image analyzer), and the data must be plotted by a cube root equation (Hixson and Crowell, 1931):

$$1 - \left[\frac{M}{M_0}\right]^{1/3} = -\frac{2kS}{\rho r}t \tag{9.13}$$

where M is mass not dissolved, M_0 the initial amount subjected to dissolution, ρ true density, S solubility, and r the mean "radius" of the particle. The method is simply comparative, not absolute, because the hydrodynamics are different in the calorimeter from what it would be in a dissolution apparatus.

It is obvious that the dissolution rate is a function of the exposed surface area, but how this changes during dissolution is not quite obvious. Sunada et al. (1989) measured the change in surface area during dissolution of *n*-propyl-*p*-hydroxybenzoate and found dissolution rates proportional to surface area.

5.1. Solubility of Unstable Compounds

Quite often a compound is rather unstable in aqueous solution. Hence the long exposure to liquid required for traditional solubility measurements will cause decomposition, and the resulting solubility results will be unreliable. In this particular case Nogami's method may be used. If a solution experiment is carried out as a dissolution experiment with samples taken at equal time intervals, δ, it can be shown (Nogami et al., 1966) that when the amount dissolved at time $t+\delta$ is plotted versus the amount dissolved at time t, a straight line will ensure. The following relationship holds:

$$C(t+\delta) = S[1 - \exp(-k\delta)] + \exp(-k\delta)Ct \tag{9.14}$$

hence such a plot as shown in Fig. 7 will give k from the slope; inserting this in the intercept expression will give S. The advantage of the method is that it can be carried out in a short period of time, and reduce the effect of decomposition; the disadvantage is that it is not as precise as ordinary solubility determinations.

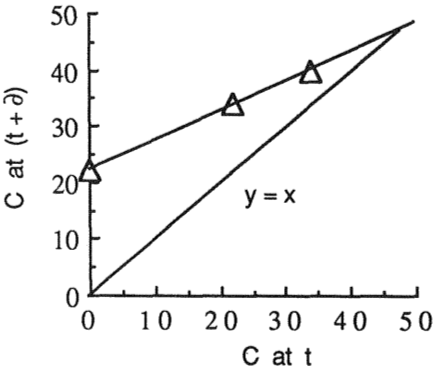

Fig. 7 The Nogami method applied to the upper curve in Fig. 5. The least-squares fit for the upper line is $y=27.081+0.53139x$, so that $y=x$ when $x=y=22.081/(1\text{-}0.53139)=47.3$.

5.2. Solubility of Metastable Polymorphs

Polymorphism is an important aspect of the physical properties of drug substances. One of the characteristics of a metastable polymorph is that it is more soluble than its stable counterpart. The dissolution profile of it will be as the upper curve shown in Fig. 6; A is the stable form with solubility 31 mg/mL. B is the profile of the metastable form with solubility* 46 mg/mL. This solubility (circles) is often not achieved, and precipitation of the stable form occurs at a point beyond the solubility of A, and the trace becomes B.

In such cases, the Nogami method can be applied to the early points curve (Fig. 7), and the solubility, S', of the polymorph can be assessed. One of the important aspects of metastable polymorphs in pharmacy is exactly their higher solubility, since the dissolution rate will also be higher [Eq. (9.9)]. Hence the bioavailability will be increased where this is dissolution rate limited (Shibata et al., 1983).

5.3 Polymorphism

As mentioned at an earlier point, solids may exist either as amorphous compounds or as crystalline compounds. In the latter, the molecules are positioned in lattice sites. A lattice is a three-dimensional array, and there are eight systems known. Compounds often have the capability of existing in more than one crystal form, and this phenomenon is referred to as polymorphism.

If a compound exhibits polymorphism, one of the forms will be more stable (physically) than the other forms; that is, of n existing forms, n-1 forms will possess a thermodynamic tendency to convert to the nth, stable form (which then has the lowest Gibbs energy; it should be noted that in the preformulations stage it is not known whether the form on hand is the stable polymorph or not).

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* It is noted that the "solubility" is not the equilibrium solubility. The solution is a supersaturated solution, but it is referred to as solubility, because conducting a solubility experiment on a metastable most often will give a reproducible figure. The supersaturated solution will eventually precipitate out as demonstrated in Fig. 5 of Chapter 12.

One manner in which different polymorphs are created is by way of recrystallizing them from different solvents, and at a point in time when sufficient quantities of material (and this need not be very much) are available, the preformulation scientist should undertake recrystallization from a series of solvents.

Knowledge of polymorphic forms is of importance in preformulation because suspension systems should never be made with a metastable form (i.e., a form other than the stable crystal form). Conversely, a metastable form is more stable than a stable modification, and this can be of advantage in dissolution [Eq. (9.11)].

6. VAPOR PRESSURE

In general, vapor pressures are not all that important in preformulation, but it should always be kept in mind that a substance may have sufficiently low vapor pressure to (a) become a lost to sufficient extent to cause apparent stability problems and content uniformity problems, and (b) exhibit a potential for interaction with other compounds and adsorption onto or sorption into package components (Pikal and Lukes, 1976).

Most drug substances are, substantially, not volatile. As an initial screen, it can be determined whether the drug is sufficiently volatile to cause concern, by placing a weighed amount of it in a vacuum desiccator and weighing it daily for a while. It is better to have a high-vacuum system for this, and the use of a vacuum electrobalance is best for this purpose. A good estimate of the vapor pressure can be obtained (Carstensen and Kothari, 1981) by using a pierced thermal analysis cell, placing it on a vacuum electrobalance, and monitoring the weight loss rate. A substance with known vapor pressure can then be used for calibration, the loss rates being proportional to the vapor pressures.

By using constant temperature TGA, graphs such as that shown in Fig. 8 will result. The weight rate (which should be established as due to evaporation of the compound) is given by

$$\frac{dW_a}{dt} = -kA'P_a \tag{9.15}$$

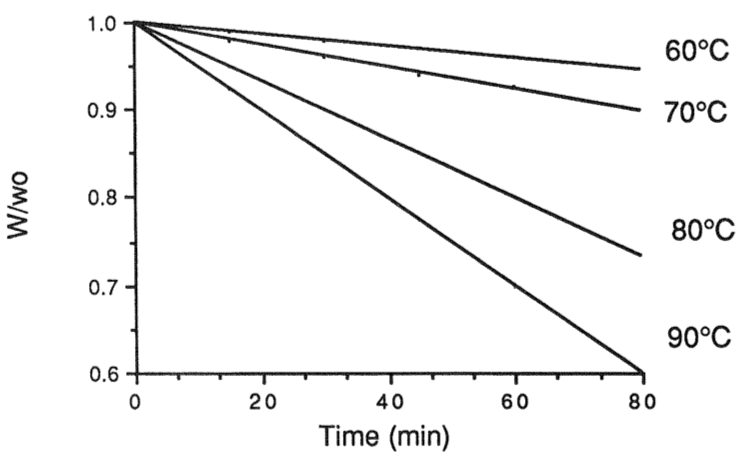

Fig. 8 Weight loss curves from constant temperature TGA.

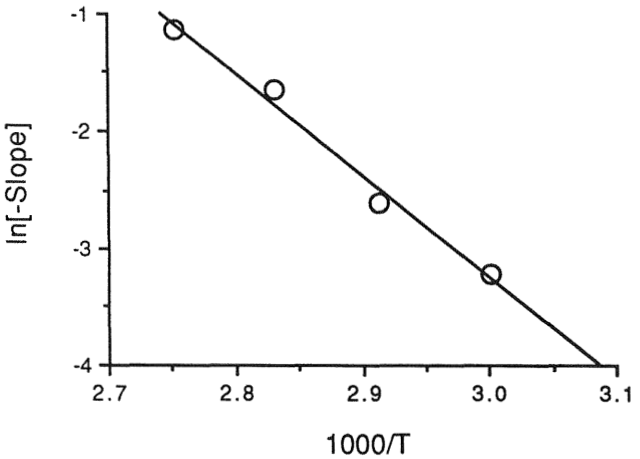

Fig. 9 Plot showing the Clausius–Clayperon treatment of the data in Fig. 7. The least-squares fit is ln[-slope] = 22.861-8.7(1000/T.)

where W is weight, t is time, k is a heat/mass transfer coefficient, A' is surface area, and P_a is the vapor pressure of the compound at the given temperature.

If the specific surface area is

$$A_a = \frac{A'}{W_{ao}} \tag{9.16}$$

where W_{ao} is the original weight of the sample, we inserted this into Eq. (9.5) and obtain

$$\frac{W_a}{W_{ao}} = 1 - kA_aP_at \tag{9.17}$$

An example of such a plot is shown in Fig. 8.

If a compound with known vapor pressure (e.g., benzoic acid, subscript b) is subjected to the same conditions, then it will exhibit a weight loss curve given by a similar equation:

$$\frac{W_b}{W_{bo}} = 1 - kA_bP_bt \tag{9.18}$$

The ratio of the slopes is A_bP_b/A_aP_a, so if the specific surface area is known for each, then (since P_b of the reference is known), P_a can be calculated.

The heat of sublimation, ΔH, can be obtained by plotting the negative of the slopes by a Clausius–Clayperon equation, as shown in Fig. 9. In the cited case, the negative of the slope of this plot is $\Delta H/R = 8.7$ kCal/mol, so that the heat of sublimation is $\Delta H = 17.4$ kCal/mol. For this, no reference is necessary; it is only necessary to know and adjust for the weights of the samples studied.

7. PARTITION COEFFICIENT

Partition coefficients between water and an alkanol (e.g., octanol) should be determined in preformulation programs (Yalkowsky et al., 1983). The partition coefficient of a compound that exists as a monomer in two solvents is given by

$$K = \frac{C_1}{C_2} \tag{9.19}$$

If its exists as an n-mer in one of the phases, the equation becomes

$$K - \frac{(C_1)^n}{C_2} \tag{9.20}$$

or

$$\log k = n \log C_1 - \log C_2 \tag{9.21}$$

The easiest way to determine the partition coefficient is to extract V_1 cm^3 of saturated aqueous solution with V_2 cm^3 of solvent and determine the concentration C_2 in the latter. The amount left in the aqueous phase is $C_1V_1 - C_2V_2 = \mathrm{M}$, so that the partition coefficient is given by

$$K = \frac{M}{V_1 C_1} \tag{9.22}$$

If it is assumed that the species is monomeric in both phases, the partition coefficient becomes the ratio of the solubilities, and it is simply sufficient to determine the solubility of the drug substance in the solvent (since it is assumed that the solubility is already known in water):

$$K = \frac{S_1}{S_2} \tag{9.23}$$

8. HYGROSCOPICITY

Hygroscopicity is, of course, an important characteristic of a powder. It can be shown for a fairly soluble compound that the hygroscopicity is related to its solubility (Carstensen, 1977, VanCampen et al., 1980), although it has been shown that the heat of solution plays an important part in what is conceived as "hygroscopicity" (VanCampen et al., 1983a,b,c). As mentioned in Chapter 8, a hygroscopicity experiment is carried out most easily by exposing the drug substance to an atmosphere of a known relative humidity (e.g., storing it over saturated salt solutions in desiccators). Each solution will give a certain relative humidity (RH), and the test simply consists of weighing the powder from time to time and determining the amount of moisture adsorbed (weight gained). This does not work with drug substances that decompose as, for instance, effervescent mixtures, which start losing weight due to carbon dioxide evolution (Carstensen and Usui, 1984).

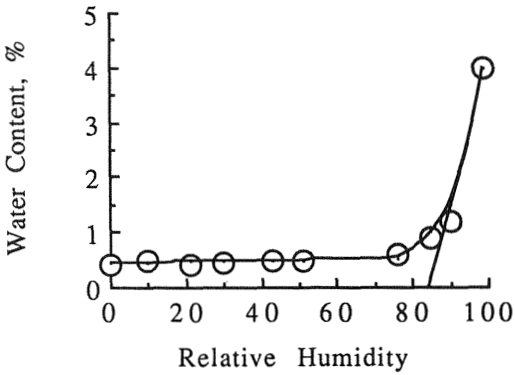

Fig. 10 Sorption isotherm of anhydrous droloxifene citrate. (Graph constructed from data published by Burger and Lettenbichler, 1993.)

It can be shown that if the air space is sufficiently agitated to prevent vapor pressure gradients, the initial uptake rate (g H_2O/g solid per hour) is related to the relative humidity by

$$L = a_{21}[\mathrm{RH} - \mathrm{RH}_0] \tag{9.24}$$

where RH_0 is the vapor pressure of a saturated solution of the drug substance in water. An example of this is shown in Fig. 10.

X_s can be estimated by an ideality assumption; that is, if the solubility is expressed as a mole fraction X_s, the vapor pressure over a saturated solution will be P' given by

$$P' = (1 - X_s)P_0 \tag{9.25}$$

where P_0 is water's vapor pressure at that temperature.

The experiments above are rather easy to carry out and should always be part of a preformulation program, since hygroscopicity can be so important that it will dictate whether a particular salt should be used. Dalmane, for instance, is a monosulfate, and is used as such since the disulfate, desirable in many other respects, is so hygroscopic that it will remove water from a hard-shell capsule and make it exceedingly brittle.

9. COMPATIBILITY TESTS

Prior to attempting the first formulation with a new drug, most research groups carry out compatibility testing (Carstensen et al., 1964). The principle is to make up reasonably ratioed mixtures of drug and excipient, to ascertain which excipients may be reasonably used with the drug. The original method used in the 1960s (Carstensen, 1964) consisted of visual observation of such mixtures, spectrophotometric assay, and TLC. The methods used nowadays have followed in step with analytical developments and are (a) chemical assay, (b) TLC, (c) HPLC, (d) DSC, and (e) microcalorimetric methods. The latter two have been of special interest in recent years and will be treated separately.

9.1. Use of DSC

Rustichelli et al. (1999) have employed DSC to obtain the phase equilibrium diagrams of the enantiomers of (a) verapamil HCl and (b) gallopamil HCl. In the former case the eutectic composition is at 90% (2S)-(-)-verapamil HCl and in the latter at 70% (2S)-(-)-gallopamil HCl.

Mura et al (1998) have used thermal analysis (DSC) to study compatibility of picotamid with common pharmaceutical excipients (palmitic acid, stearic acid, stearyl alcohol, PEG 20,000, and sorbitol) and showed that the interactions were primarily due to dissolution in the melted excipient.

9.2. Use of Microcalorimetry

Heat conduction microcalorimetry has been used as a method to evaluate stability and excipient stability by a series of researchers. Angerg et al. (1988, 1990, 1993), Hansen et al., (1989), and Wilson et al. (1995) have described the general method and results interpretation. For instance, Angberg et al. studied the oxidation of ascorbic acid in aqueous solution by microacalorimetry, and other researchers have used this method as well. Oliyai and Lindenbaum (1991) studied the decomposition of ampicillin in solution. Tan and Meltzer (1992) studied the solid state stability of 13-*cis*-retinoic acid by means of microcalorimetry and HPLC, and Pikal and Dellerman (1989) studied the kinetics of cephalosporin in the solid and solution states using the same method.

Seltzer et al. (1998) used the method to evaluate stability and excipient compatibility of (S)-(3-(2-4-(S)-(4-(amino-imino-methyl)-phenyl-4-methyl-2,5-dioxo-imidazolidin--1-yl)-acetylamino))-3-phenyl-propionic acid ethyl ester, acetate. The excipients used were potato starch, calcium hydrogen phosphate anhydrous, and colloidal silica.

They consider the reaction A + B→C + D and denote the concentration of C as x, the fraction decomposed at time t. The trace of heat evolved as a function of time is then characterized by

$$\frac{dx}{dt} = -k\{[A_0] - x\}^n \tag{9.26}$$

The amount decomposed is associated with (a usually exothermic) reaction enthalpy, ΔH, where the heat evolved is proportional to x, hence the heat flow is proportional to dx/dt and the proportionality constant is ΔH, so that

$$\phi = \frac{dQ}{dt} = \Delta H \frac{dx}{dt} \tag{9.27}$$

hence

$$\phi = \Delta Hk\left\{[\mathrm{A_0}] - \left(\frac{Q}{\Delta H}\right)\right\} \tag{9.28}$$

For a first-order reaction this becomes

$$\phi = \Delta Hk[\mathrm{A_0}] - kQ \tag{9.29}$$

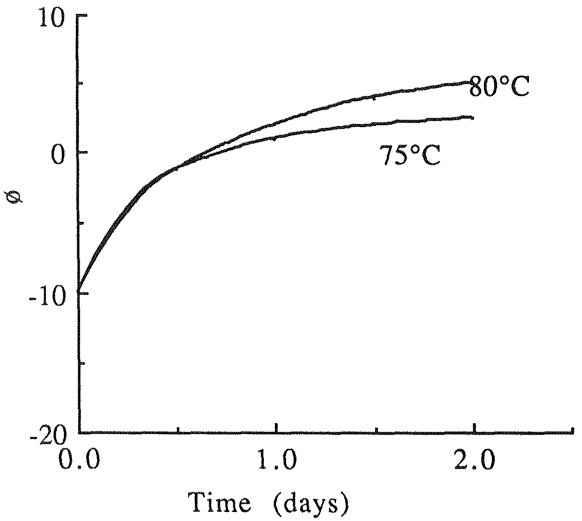

Fig. 11 Figure constructed from data published by Seltzer et al. (1998).

so that the terms k and ΔH may be deduced from nonlinear regression of a plot of heat flow versus time (Fig. 11).

If the reaction is zero order, then ϕ is simply a constant:

$$\phi = k\Delta H \tag{9.30}$$

This allows determination of kinetic profiles and Arrhenius plots of the studied reaction.

The value of Q at time t is obtained through integration (area under) of the curve from zero to t.

It should again be emphasized that at the onset of a new drug program, there are only small amounts of drug substance at hand. One of the first tasks for the preformulation scientist is to establish the framework within which the first clinical batches can be formulated. To this end it is important to know with which common excipients the drug is compatible. In the following, the distinction will be made between solid and liquid dosage forms.

The microcalorimetric methods give no direct information about the chemical nature of the reaction.

9.3. Compatibility Test for Solid Dosage Forms

It is customary to make a small mix of drug substance with an excipient, place it in a vial, place a rubber stopper in the vial, and dip the stopper in molten carnauba wax (to render it hermetically sealed). The wax will harden and form a moisture barrier up to 70°C. A list of common excipients characteristic of this type of test is shown in Table 1. At times it is possible to obtain quantitative relationships of excipient characteristics and interaction rates (Carstensen et al., 1964; Perrier and Kesselring, 1983). In addition to the test as described, a similar set of samples are set up where 5% moisture is added. A storage period of 2 weeks at 55°C is employed [except

Table 1 Categories for Two-Component Systems

			Worse		Total score	
		Identical	17–27 mo at 25°C	10 days at 55°C	25°	55°C
Drug per se	Dry	15	4	1	38	31
	5%H_2O	9	8	3	49	38
+ Magnesium stearate	Dry	16	3	1	34	30
	5% H_2O	15	4	1	43	35
+ Calcium stearate	Dry	13	4	3	37	32
	5% H_2O	12	5	3	38	35
+ Stearic acid	Dry	15	5	0	42	31
	5% H_2O	7	11	2	60	38
+ Talc	Dry	14	5	1	38	30
	5% H_2O	10	8	2	45	34
+ Acid-washed talc	Dry	12	8	0	44	31
	5% H_2O	10	9	1	49	35
+ Lactose	Dry	12	5	3	38	32
	5% H_2O	9	7	4	65	56
+ $CaHPO_4$, anhydrous	Dry	12	6	2	46	36
	5% H_2O	9	8	3	66	53
+ Cornstarch	Dry	12	5	3	39	34
	5% H_2O	10	5	5	40	37
+ Mannitol	Dry	10	7	3	39	31
	5% H_2O	8	7	5	47	45
+ Terra alba	Dry	14	6	0	41	28
	5% H_2O	11	6	3	50	45
+ Sugar 4 ×	Dry	12	6	2	41	34
	5% H_2O	9	7	4	63	61

Source: Constructed from data published by Carstensen et al. (1964).

for stearic acid, where 45°C is used, and dicalcium phosphate, where 37°C is used (Toy, 1980)], after which time the sample is observed physically for (1) caking, (2) liquefaction, (3) discoloration, and (4) odor or gas formation. It is then assayed by thin-layer chromatography (or HPLC).

It is noted that one of the samples set up is the drug by itself. This is done for several reasons, one of which is that it is now required by the FDA for IND submissions (Schultz, 1984). One more reason is that at the onset of a program, the organic synthesis of the compound may lack the refinement it will later have, and it is not uncommon that there will be several weak TLC spots (impurities) on a TLC chromatogram of a compound obtained by initial laboratory synthesis. Hence, in selecting the excipients with which the drug substance is deemed to be compatible, it is customary to use as criteria that (after accelerated exposure of a drug/excipient mix) no new spots have developed and that the intensity of the spots in the drug stored under similar conditions (2 weeks at 55°C is the same as in the acceptable excipient. This type of program is used by many companies

with good success (i.e., the formula developed based on the findings from the compatibility program is stable).

It should be noted that liquefaction at times occurs because of eutectic formation (e.g., often with caffeine combinations) and that this may not necessarily be associated with decomposition. On the other hand, discoloration (e.g., amines and sugars) usually is.

Finally, the reason for not forcing dicalcium phosphate (a very valuable formulation aid in direct compression) beyond 50°C is that at higher temperatures it converts to the anhydrate, a conversion that is, curiously enough, catalyzed by water. In other words, the dihydrate will be autocatalytic in this respect at elevated temperatures, and it should not be ruled out based on high-temperature findings.

Aside from magnesium stearate, dicalcium phosphate and lactose are the excipients that are the most often found incompatible with drugs. In the former case it is usually the pH effect, in the latter it is the formation of Schiff's bases with amines (and many drugs are amines), i.e.,

$$R_1CHO + H_2NR_2 \rightarrow R_1CH = NR_2 + H_2O$$

For instance, Eyjolfsson (1998) reported on the incompatibility of lisinopril with lactose.

Aso et al. (1997) have determined the decomposition rates of cephalotin in mixtures with pharmaceutical excipients and the effect of moisture. They found a linear relation between mobile water percentage and decomposition rate constants.

Several examples of this type of screening exist. Malan et al. (1997) have studied the compatibility of tablet excipients with albendazole and closantel. They prepared drug–excipient mixtures in a mixture and in 1 : 1 mixtures that were granulated with water and dried at 50°C. DSC and HPLC were used to evaluate the compatibilities. The excipients tested were colloidal silicon dioxide, microcrystalline cellulose, dibasic calcium phosphate monohydrate, starch, sodium starch glycolate, and magnesium stearate.

9.4. Compatibility with Containers

Compatibility studies may also include compatibility with container materials. Hourcade et al. (1997), for instance, reported that granisetron in concentrations of 1 mg/mL, when kept in polypropylene syringes, were quite stable, whereas dilutions with 0.9% NaCl or with 5% glucose resulted in unsatisfactory storage stability.

10. KINETIC pH PROFILES

pH profiles have been discussed in Chapter 3. Frequently, a broad screen of stability is performed on the initial small sample used for initial preformulation; this is frequently referred to as "forced decomposed studies" (Bodnar et al., 1983). In this the drug is exposed to "acid degradation," "base degradation," "aqueous degradation," "drug powder degradation," and "light degradation." More refined studies are eventually needed.

For any compound marketed by a pharmaceutical concern, at one time during its development, there should be a concerted project to establish a very exact pH profile. To do this correctly is a time-consuming undertaking. However, the information that can be gleaned from it is very important with regard to formulations, and it is therefore customary to carry out an approximate kinetic pH profile (Carstensen et al., 1992) early in the development stage. This will allow formulation of solutions for injections and for oral products as well, at a pH, and using buffers, that will give the best stability. Without it formulation is essentially guesswork.

11. LIQUID COMPATIBILITIES

The pH profile is the most important part of liquid compatibilities. However, two component systems are set up in aqueous (or other types of) solutions and treated as in Section 10 of Chapter 12. This is now required in the stability guidelines, which state that "it is suggested that the following conditions ... be evaluated in studies on solutions or suspensions of bulk drug substances: acidic and alkaline pH, high oxygen and nitrogen atmospheres, and the presence of added substances, such as chelating agents and stabilizers" and it is suggested "that stress testing conditions ... include variable temperature (e.g., 5, 50, 75°C)."

11.1. Aqueous Solution Compatibility

In general, such studies are carried out by placing the drug in a solution of the additive. These can be (and usually are) a heavy metal (with or without chelating agents present) or an antioxidant (in either oxygen or nitrogen atmosphere). Usually, both flint and amber vials are used, and in many cases an autoclaved condition is included. This will answer questions about susceptibility to oxidation, to light exposure, and to heavy metals. These are important questions as far as injectable compatibilities are concerned. Exposure to various plugs is frequently included at this point so that early injectable preparations can be formulated.

For preparations for oral use, knowledge of the desired dosage form is important, but compatibility studies with ethanol, glycerin, sucrose, corn syrup, preservatives, and buffers are usually carried out. This type of study also gives an idea of the activation energy, E, of the predominant reaction in solution. Arrhenius plots for compounds in solution are usually quite precise.

11.2. Nonaqueous Liquids

With transdermal dosage forms being of great importance of late, it is advisable to test for compatibilities with "ointment" excipients and with polymers (e.g., ethylvinyl polymer, if that is the desired barrier). In the case of transdermals, the dosage form is either directly placed in a stirred liquid or it is placed in a cell with an appropriate membrane (e.g., Cadaver skin) to estimate the release characteristics of the drug from the ointment (Chien et al., 1983).

It should be noted here that if the overall flux is J, then

$$\frac{1}{J} = \frac{1}{J_{\text{ointment}}} + \frac{1}{J_{\text{membrane}}} \tag{9.31}$$

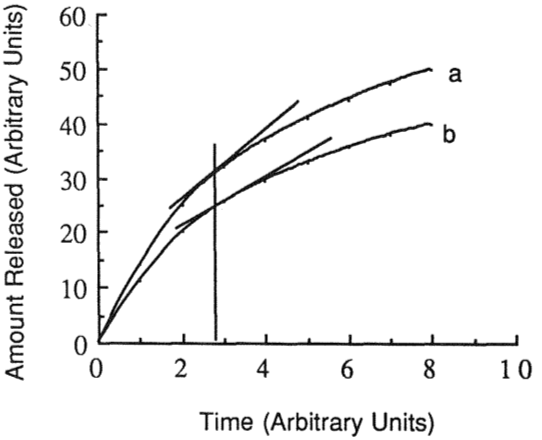

Fig. 12 Slope determination of flux of ointment release and release from ointment + membrane.

where subscripts refer to the respective phase J_{membrane} can be obtained from curves such as shown in Fig. 12 in the fashion that first the overall flux is obtained (with the membrane in place), giving the value of J, then the release is obtained without with membrane in place, giving J_{ointment}, that is

$$J = \frac{1}{A}\frac{dm_1}{dt} \tag{9.32}$$

and

$$J_{\text{ointment}} = \frac{1}{A}\frac{dm_2}{dt} \tag{9.33}$$

J_{membrane} is then obtained as the reciprocal of the difference.

In vivo testing is usually carried out by applying the dosage form to hairless rats followed by subsequent sacrifice. Since the skin consists of a number of layers with differing hydrophilicity, the overall fate of the drug is of importance.

11.3. Emulsions

In the case of emulsions, the preformulation studies become very formulation oriented. Williams and Mahaguna (1998) have described preformulation studies of Freund's incomplete adjuvant (FIA), which is a water-in-oil emulsion. This included measuring the critical micelle concentration of the formulations to be investigated. Using ovalbumin (a model antigenic protein) in the interface, the surface activity of mannide monooleate, in the interface between water and oil phases, was determined.

11.4 Gels

Wong et al. (1997) studied the stability of cefazolin in Pluronic F-127 gels and found the decomposition to be first order, and for all Pluronic concentrations used (20, 25, and 30%), Arrhenius plotting was satisfactorily linear.

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10

Physical Testing

JENS T. CARSTENSEN

Madison, Wisconsin

A great deal of space has been devoted to the subject of chemical testing. However, even if a product, chemically, is sufficiently stable to sustain e.g. a 3-year expiration date, physical changes may have occurred. In a solid dosage form, the dissolution may have slowed down to such an extent that the product is no longer as bioavailable

as it was at the time of manufacture, and more importantly, it may not meet the minimum required for efficacy. For a solution, a precipitate may have occurred. This may not affect the chemical content, but for a parenteral product it would, obviously, be quite unacceptable, and for an oral solution it would also be unsatisfactory, because the dispensing pharmacist would rightfully question the integrity of the product. The caking of a suspension impairs the dispensing of a known amount of drug in a teaspoon, and a separated or broken emulsion or cream obviously will not have the same emollient properties as would a proper product.

Physical stability will be treated by product category in the same order as in the case of chemical stability.

1. PHYSICAL STABILITY OF SOLUTIONS

Solutions are broadly divided into two categories: oral and parenteral solutions. Appearance, in both cases, is an important factor. In the case of oral solutions, organoleptic properties are also of great importance. Organoleptic evaluation is usually done subjectively, i.e., a tester (operator, technician), will judge the product and score it, either numerically or descriptively or both. In the case of appearance of solutions, there should always be a subjective statement (quantitative or subjective description) even if more quantitative instrumental parameters are recorded. A few words are therefore in order regarding organoleptic and appearance testing.

1.1. Organoleptic Testing

For organoleptic testing it is important to establish a test panel early in the stability program. (Or if a stability program is in place, but no such testing is carried out, a test panel should be selected at the first opportunity when a product with important taste or odor properties is placed on stability.) Many companies utilize just one tester for the task of organoleptic testing, but this can be shortsighted, because the tester may leave, go on vacation, or become ill, and in that case the logical solution is to assign someone else to the task. There may be an evaluational bias between the two testers, and this should be established at the onset.

First of all, the depth of organoleptic capacity should be tested. This can be done by asking the tester to taste serial dilutions of a bitter substance (e.g., quinine). Hence a sensitivity level can be established. A control of e.g. water or high dilutions should always be part of the protocol.

It should be noted that the technicians are not taste testers in the ordinary sense. That is, it is not necessary to match their "likings" to that of the general public. Rather, it is important that they can (a) duplicate their results and (b) remember them, since they will be asked to taste a preparation that they originally tested 3 or 6 months earlier. In so doing they would have to score the degree of flavoring, e.g., is it less than originally present, i.e., is the flavor being lost? They would also have to be able to describe the flavor well originally. For example, if the chemical is slightly anesthetizing, the duration of the anesthesia would be important. If there is interaction with a plastic bottle, are off flavors appearing in the product? Finally it is important to screen several testers to ascertain that they give the "same result."

In describing the flavor, several categories can be used (degree of sourness, degree of saltiness, level of flavor, type of flavor). Each of these may be assigned

to a level of e.g. 1–5. A flavor profile may hence be established, and this can then be reestablished at several time points in the room-temperature storage. It is not recommended to evaluate results from higher temperatures (although they may be carried out).

1.2 Subjective Appearance Testing

Solutions, particularly parenteral solutions, may have a tendency to discolor slightly. Often it is not possible, within analytical sensitivity, to establish either the source of the color or the level of the substance causing it. In this case it is a good practice to use a color standard to describe the "intensity" of the discoloration. Roche, for instance, uses the so-called Roche Color Standard (RCS), which uses a compound (the identity of which is a secret) that can be reliably reproduced and has exceptional color stability. Making up serial dilutions of this compound then gives solutions of different "slight" discolorations; they are denoted RSC#1, #2, etc., so that a solution can always be compared in this fashion. It is a bit like the old-fashioned Dubosque colorimeter (which can be used with advantage in this type of situation). The principle of the Dubosque colorimeter is to have a view of two test tubes from the top. One is the control, and the other is the solution being matched. It is possible to adjust the length of the light path in the second tube, and this is done until the intensity matches that of the standard. The length of the path is then an indication of the "concentration."

The RCS (and similar types of numbers) are difficult to analyze, but a Dubosque colorimeter gives numbers that follow Beer's law and are logarithmically proportional to concentration (although the proportionality factor cannot be known). In this fashion the "decomposition" could be represented simply as a first-order reaction, where the concentration, X, of the decomposition product would be given by

$$X = X_\infty[1 - \exp(-qt)] \tag{10.1}$$

or

$$\ln\left[1 - \frac{X}{X_\infty}\right] = -qt \tag{10.2}$$

where q is a constant, t is time, and X_∞ is found by iteration. This allows (from accelerated studies) a visual estimate of the worst appearance that a product could take on. The appearance of tablets can be treated differently and will be discussed later.

2. PARENTERAL SOLUTIONS

In parenteral solutions, physical stability includes interaction with a container and changes in chemical composition that give rise to physical changes. The latter will be discussed first.

One manifestation is slight discoloration. Thiamine hydrochloride solutions, for instance, may discolor slightly without showing detectable changes in content of parent compound. Such discolorations can be followed as described immediately

Table 1 Usual Concentrations of Antioxidants and Chelating Agents

Antioxidant	Usual concentration
Acetylcysteine	0.5%
Ascorbic acid	0.02–1%
BHT, BHA, and propyl gallate	0.005–0.02%
Citric acid (chelator)	Variable*
Sodium edetate (chelator)	0.01–0.075
Sulfites	0.1–0.15%
Thioglycerol	0.1–1.0%
Thiourea	0.5–1.0
Tochopherols	0.05–0.075

* Citric acid can be present in large amounts if it is present as a buffer (as well as present as a chelator).
Source: Table constructed from data published by Mendenhall (1984).

above, and at times they are detectable analytically. They are often oxidative in nature and metal ion catalyzed. Such a case in captopril (Lee and Notari, 1977).

Mendenhall (1984) has reviewed the stability aspects of parenteral products and has shown that discoloration is often either photochemical or oxidative. He has summarized the usually used antioxidants and chelating agents. These are shown in Table 1.

2.1. Swirly Precipitates

Often a parenteral solution will develop a swirly precipitate upon storage. This is most prevalent in vials and is usually an interaction with either the glass or the stopper. It may be difficult for the uninitiated to detect such slight changes, and the best person to use for this type of evaluation is a parenteral inspector. It is difficult to estimate the extent of the precipitate; it can be done by mechanical counting (e.g., with a Coulter counter), but the results are difficult to interpret. Often the count does not correspond to the "severity of the swirl." More to the point is how many swirls exist. If a box of e.g. 144 vials is placed on this type of stability, then the vials can be examined from time to time, and one may establish how many vials have become swirly. This number can then be treated in proper fashion to evaluate the severity of the problem, i.e., the stability parameter would be the number of swirly vials per box of 144.

Preferably there should be no swirls at all in the preparation, and if reformulation can be undertaken (which is wise), then an improved product would be the result. Otherwise, the stability program will establish the percentage probability of finding a vial with a swirl at the end of the expiration period. At times it is necessary to lyophilize products that are chemically stable, simply because the problem of swirls cannot be solved.

As mentioned, the occurrence of swirls is usually a container interaction, and a change in the stopper or the glass may often eliminate the problem. Vials should always be stored (a) upright, (b) on the side, and (c) upside down to check the interaction with the stopper. In this way primary evidence can be established as to the culpability of the closure.

2.2 Whiskers

McVean et al. (1972) reported on the case of a parenteral solution (morphine) where "whiskers" occurred at the tip of the ampul in a large percentage of ampuls upon room-temperature storage.

This is a defect that will occasionally occur in a product. It is due to pinholes in the glass. The solution wicks out, and the liquid evaporates on the outside. The solid that is formed serves to wick out more solution, and long crystals or "whiskers" may occur. One might ask why the pinholes have not been detected in the dye test used for autoclaved ampuls. There are two reasons. One is that the hole may be too small for detection (about 0.5 μm is the detection limit). The other is that the ampul was tight at the time of manufacture, but the heat sealing line was run too rapidly, or the flame temperature was incorrect, so that the glass did not have time to anneal properly, and the strain caused the crack during storage (not immediately after manufacture).

2.3 Cloud Times

Sometimes a cloud will appear in a product as the storage time progresses, and this is most often due to chemical changes in the system. If for instance an ester (e.g., polysorbate, which is a fatty acid ester) hydrolyzes, then the produced acid may be poorly soluble. If the solubility is denoted S, then the following holds: If the reaction in general is written

$$A + H_2O \rightarrow B \tag{10.3}$$

where A is a drug of initial concentration A_0 and B is the decomposition product with solubility S (which is assumed to be limited). Assuming first order, the concentration of B is then given by

$$[B] = A_0[1 - \exp(-kt)] \tag{10.4}$$

At time t^* the solubility will be exceeded, and t^* is what is denoted the cloud time. t^* is given by

$$S = A_0[1 - \exp(-kt^*)] \tag{10.5}$$

or

$$\ln\left[1 - \frac{S}{A_0}\right] = -kt^* \tag{10.6}$$

If $A_0 \gg S$ then this simplifies to

$$t^* = \frac{S}{kA_0} \tag{10.7}$$

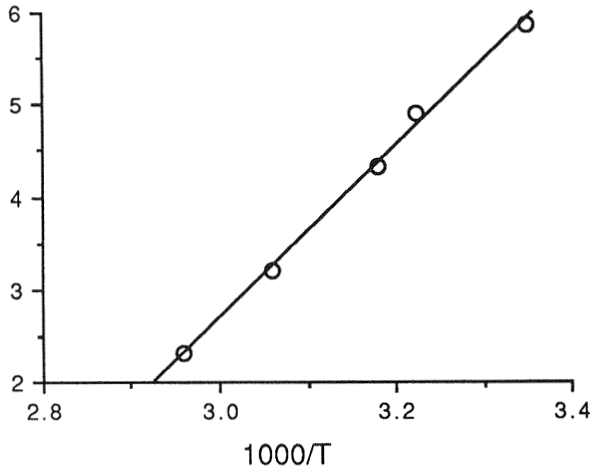


Fig. 1 Cloud times of a parenteral diluent containing polysorbate 80 and maleic acid. (Constructed from data published by Carstensen, 1972.)

Taking logarithms gives

$$\ln[t^*] = -\ln[A_0] + \ln\left[\frac{S}{k}\right]$$
$$= -\ln[A_0] + Q - \frac{\Delta H - E}{RT} = -\frac{\Delta H - E}{RT} + Q'' \tag{10.8}$$

where Q and Q'' are constants. This shows that the cloud times can be plotted by Arrhenius plotting. Such plotting is quite predictive, as is shown in Fig. 1.

The precipitation may also occur by the solubility product being exceeded, or from any situation leading to a product with limited solubility.

There are other causes for precipitation on storage, one being the original use of a metastable form, so that the solutions in question, in fact, are supersaturated solutions. It was the author's experience, at his tenure at Hoffmann–la Roche in 1965, that a product to be introduced (Taractan Injectable) was in this category. Several pilot batches had been successfully made, but the first production batches precipitated, a more stable polymorph crystallizing out. This necessitated reformulation to a lower strength (corresponding to the lower solubility of the stabler polymorph) and subsequent resubmission of data to the FDA. This points out the importance of careful preformulation studies of the solubility of compounds. Errors of the above type are costly, both in terms of resubmission and in lost market time. Even official products fall into this category.

Calcium gluceptate is used to treat calcium deficiency and (USP XX, 1980) is highly water soluble (up to 85%). Solutions, however, show a tendency to precipitate on standing at room temperature (Muller et al., 1979). The storage time required for precipitation is a function of the commercial source, as is pointed out by Suryanarayanan and Mitchell (1981). It was shown that the precipitate was a

sparingly soluble crystalline hydrate, and that the raw material was an amorphous (much more soluble) form of the drug. Seeds, and unfortuitous ratios of alpha and beta epimers of the calcium gluceptate, catalyzed the precipitation.

Precipitation is a nucleation and crystal growth phenomenon (Carstensen and Rodriguez, 1985, Rodriguez, Hornedo and Carstensen, 1985), and as such it can be impaired or prevented by inhibitors. These are often viscosity-impairing substances (carboxymethyl cellulose for instance), and hence the stability of the viscous component becomes important. The loss of this can be detected by following viscosity.

The viscosity of these agents is often Bingham bodies, i.e., they possess a yield value. The correct way of checking them is, therefore, with e.g. a cup-and-bob viscometer, so that a rheogram can be drawn. In this fashion it is possible to check both changes in yield value and slope of the rheogram (apparent viscosity). For very fluid solutions (dilute aqueous solutions) this is difficult, and most often it is best followed by the use of an Ostwald–Fenske pipette. Two pipettes (with different flow times) should be used in this case, because the difference in the measured viscosity is a measure of the yield value (although calculation of the yield value from the difference is a priori not possible). Both yield value and apparent viscosity are functions of concentration (Ben-Kerrour et al., 1980); in a multicomponent system there will usually be one main component responsible for viscosity, and it is the breakdown of this one compound that would be of importance. Often when drastic changes occur in viscosity, bacterial contamination can be suspected.

Precipitation is tied into solubility, as seen in the foregoing. Solubility can be augmented by various means. In the case of cloud times, the use of cosolvents (e.g., polyethylene glycol) will increase the value of S. Other methods are the use of a micellar approach and the use of complexation. A recent example of this latter is the work by Mehdizadeh and Grant (1984) on the complexation behavior of griseofulvin with fatty acids. Order of magnitude increases in solubility were reported.

2.4 Oral Solutions

The main types of changes in appearance of oral solutions (syrups, elixirs, etc.) are loss of dye, precipitation, and bacterial growth. Precipitation has already been dealt with to some degree, but some cases particular to oral solutions will be mentioned. Change in dye content will be treated below. Bacterial growth will be treated separately.

Scott et al. (1960) showed the loss of blue dye in a vitamin syrup, and showed that it could be treated exactly like a drug substance. Predictions by Arrhenius plotting are quite good in the case of degradation in solution, because the homogeneity is good. Figure 2 shows an example of this.

3. DISPERSE SYSTEMS

Disperse systems are suspensions and emulsions. The rationale for the physical tests carried out on these will be discussed below.

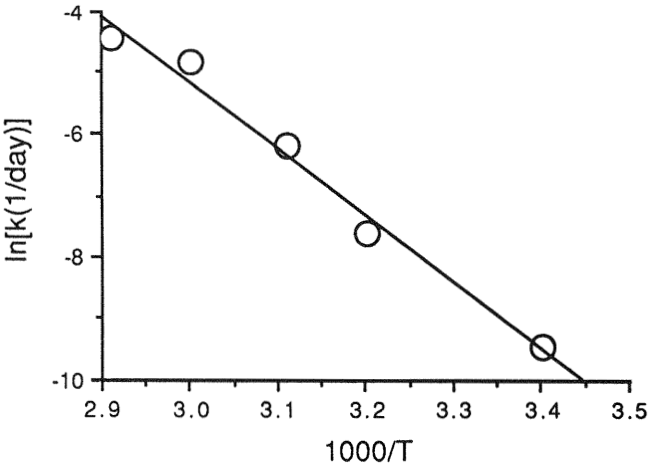


Fig. 2 Arrhenius plot of FDC Blue Dye #2 in a syrup. (Graph constructed from data by Scott et al., 1960.)

3.1 Suspensions

It would be desirable to have a suspension that did not settle (and there are such suspensions), but the general rule is that a suspension will settle, and therefore there are two parameters that are followed in this respect, namely sedimentation rate and sedimentation volume. When the sedimentation volumes are small, then there is a tendency for the suspension to cake, and hence various types of shaking tests are carried out.

Tests can be purely subjective, in that a tester notes that e.g. the suspension after three months' storage at 25°C was "difficult to resuspend, leaving some cake at the bottom." Such subjective tests should always be included in a program, but more quantitative means are desirable also. A typical quantitative test is to rotate the bottle under reproducible conditions. The type of setup used for solubility determinations is a good type apparatus for this purpose. The bottle is rotated x rotations, a sample of the supernatant is taken, and it is assayed. (This assay need not be stability indicating.) This is then repeated for twice the number of rotations, four times the number of rotations, and eight times the number of rotations. The time-relation of the assays is similar to that of a dissolution curve (although the phenomenon is redispersion), and it can often be represented by

$$Y = Y_\infty[1 - \exp(-kt)] \tag{10.9}$$

Y_∞, the asymptote value (found by iteration), should equal the dose, if caking has not occurred. The value of k is best found from the logarithmic presentation mode:

$$\ln\left[1 - \frac{Y}{Y_\infty}\right] = -kt \tag{10.10}$$

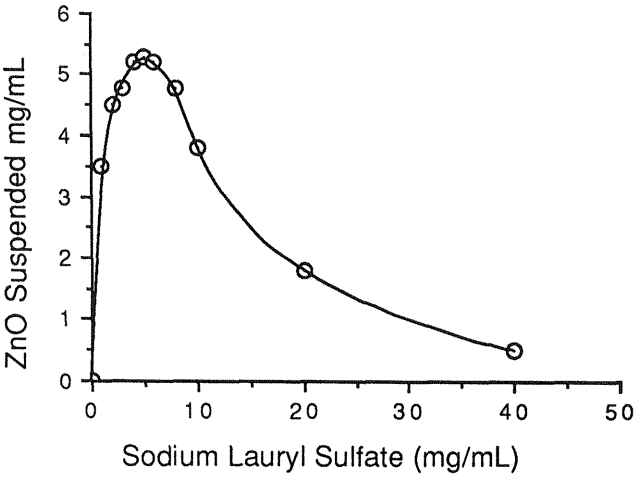


Fig. 3 Resuspension characteristics by controlled rotation. (Graph constructed from data published by Lemberger, 1967.)

k and Y_∞ can then be found by data treatment for extrapolated values or assessed in a room-temperature stability program to estimate the stability of the resuspendability parameter. Suspendability is also improved by the use of surfactants. Figure 3 shows a suspension isotherm (Moore and Lemberger, 1963) of the zinc oxide/sodium lauryl sulfate system. Such suspension isotherms should be carried out prior to the formulation of suspensions. They are in general not carried out in the preformulation effort, but rather by the formulator.

One way of accelerating the settling is to place the suspension product on a shaker at e.g. 37°C. This makes particle movement more rapid and allows the fine particles to slip into the interstices of the larger particles, hence promoting a close packing. This can then be used to judge qualitatively whether caking will take place.

It might be thought that centrifugation would be a good way in which to "accelerate" sedimentation, and the Stokes law indeed predicts this. However, it gives only an acceleration of the "initial settling rate," and the further settling, and the caking phenomena in which the formulator is interested, are not well predicted by this method.

Some caking is due to crystal growth, and this is accelerated by the use of freeze–thaw tests, i.e., alternating the temperature every 24 h from e.g. 25°C to −5°C (or some other low temperature above the freezing point of the product). The temperature cycle will promote crystal growth, and the effect of this on the product can be assessed. The freeze–thaw cycle has the advantage of emulating (and overstating) some real conditions to which the product could be exposed during shipping.

Zapata et al. (1984) have described the effect of freeze–thaw cycles on aluminium hydroxycarbonate and magnesium hydroxide gels. Coagulation after freeze–thaw cycles led to the formation of aggregates that were visible. These aggregates were particles in a primary minimum, and these were only reseparable by ultrasonic treatment. The freeze–thaw cycle affected content uniformity of both the gels, but the treatment did not alter the surface characteristics or the morphology

(as judged by x-ray powder diffraction). It did cause a reduction in the acid neutralization rate, and the rate of sedimentation increased. The effect was pronounced after the first cycle (and indeed most of the effect occurred at this point). The duration of freezing was not important, but the aggregate size grew inversely with the rate of freezing. The use of polymers in the suspensions reduced the effects of the freeze–thaw cycle.

Freeze–thaw cycles (aside from being a stability monitoring tool) can be used to screen products as well, the best of a series of suspensions or emulsions being the one that stands up best to the test. This on the surface may be logical, but without a theoretical basis it is difficult to judge the generality of such a statement.

3.2 Sedimentation Volumes

If a suspension is particulate, then the particles will (approximately) settle by a Stokes law relation, i.e., the terminal velocity, v, is given by

$$v = d^2 \cdot g \frac{\Delta\rho}{18\eta} \tag{10.11}$$

where the constant g is gravitational acceleration, $\Delta\rho$ is the difference in density between solid and liquid, η is the viscosity of the liquid, and d is the diameter of the particle. The final apparent volume of the sediment, provided it is monodisperse, would be given by the fact that in cubical loose packing a sphere of diameter d will occupy the space of its confining cube, i.e., the sedimentation volume will be

$$V = n \cdot d^3 \tag{10.12}$$

where n is the number of particles per cm^3 of suspension. Since their density is ρ g/cm^3, then (denoting the dosage level Q g/cm^3) the following holds:

$$Q = \frac{\rho \cdot n \cdot \pi d^3}{6} \tag{10.13}$$

so that, solving for n,

$$n = \frac{Q \cdot 6}{\rho \pi d^3} \tag{10.14}$$

which inserted in Eq. (10.12) gives

$$V = \frac{6Q}{\rho\pi} \tag{10.15}$$

In this view, each particle touches its neighbors. The potential diagram from two particles is as shown in Fig. 4.

When the particles touch, the potential energy becomes exceedingly large ($x = 0$), and from an equilibrium point of view they will be trapped in the primary minimum, which is the deep minimum at short distance in Fig. 4. Hence it becomes difficult to separate them, and the precipitate becomes a cake. This would prevent redispersion by shaking and would make proper dispensing impossible. It is a formulation goal to prevent this from happening, and this is done by adjusting the

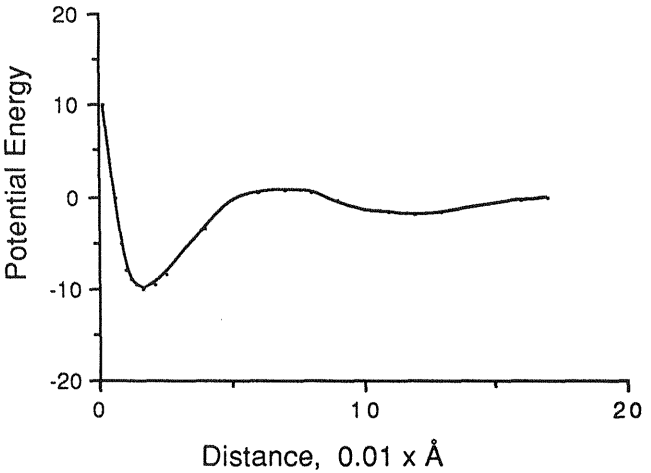


Fig. 4 Potential energy diagram for two particles.

zeta potential, as will be discussed shortly. From a formulation point of view, it is better to have the particles at larger distances, e.g., in the secondary minimum occurring at longer distances (Fig. 4).

A discussion of the connection between caking tendency and the so-called zeta potential is beyond the scope of this book. Suffice it to state the following: When particles are suspended in a liquid, they acquire a charge (and the liquid acquires a similar opposite charge, to maintain electroneutrality). The zeta potential is related to this charge, and caking is prone to happen if the charge potential is outside a range of −10 mV to +10 mV. If the zeta potential is high it can be lowered by the addition of negatively charged ions. Highly valent ions (e.g., citrate) are preferable. On the other hand, if the zeta potential is low, then it can be increased by the addition of positively charged ions (e.g., aluminium ions).

The zeta potential is measured with a zetameter. In this the particles are placed in an electrical field (between two electrodes, the voltage of which can be adjusted), they are tracked under a microscope, and their velocity is determined. The relation of velocity to voltage allows determination of the zeta potential.

It is worthwhile occasionally to check the zeta potential in a stability check of suspension (and emulsion) products. Counterions could be adsorbed and hence lose their capability of keeping the zeta potential close to zero, and this, in turn, could be the reason for subsequent caking.

When the zeta potential is close to zero, the suspension will be flocculated, i.e., the particles are positioned in the secondary minimum. The floccules are large and hence settle more slowly, but on the other hand the sedimentation volume is large. Since the particles are in the shallow minimum (small potential, i.e., easy to disrupt), they are easy to resuspend.

There are suspensions that do not settle. Here the yield value of the suspension is so large that the gravitational force does not exceed it. In this case it is very important to carry out complete rheological profiles at different time points in the stability program, to insure that the yield value is not changing. In such a system the yield value (Carstensen, 1973) is a function of the solids content and the viscosity of

the medium. If the viscosity imparting substance deteriorates, or if the flocculation characteristic (the "diameter" of the particles) changes, then the yield value may change, and what originally was not prone to cake might at a later time have such a propensity.

It has been stated elsewhere that for Bingham bodies, a yield diameter of the bottle can be calculated and below this bottle diameter there will be no settling.

3.3. Sedimentation Rates

The rational treatment of sedimentation rates has been described by Carstensen and Su (1970). Since the suspension, when placed on stability, has just been well agitated, the floccule size is not the same as it will be at equilibrium (it will be smaller). The first part of a settling curve is, therefore, governed by the reforming of the equilibrium floccule, and the latter part is governed by settling towards the equilibrium sedimentation volume. A typical plot of the final settling phase of kaolin suspensions is shown in Fig. 5. The intercept does not correspond to full height, because the settling is the final phase. The first phase, as mentioned, consists of reflocculation of the equilibrium floccule (which does not exist at time zero, because the suspension has been thoroughly shaken at that point).

The sedimentation curve is, therefore, two-phasic, and the equation for the settling curve is

$$Y - Y_\infty = A_0 \exp(-k_0 t) + A_1 \exp(-k_1 t) \tag{10.16}$$

and the curve can be deconvoluted by feathering, or by programmed four-parameter techniques.

3.4. Preservation Stability

Methyl, ethyl, propyl, and butyl esters of 4-hydroxybenzoic acid are used in various combinations in antacid suspension (and other pharmaceutical) products. The

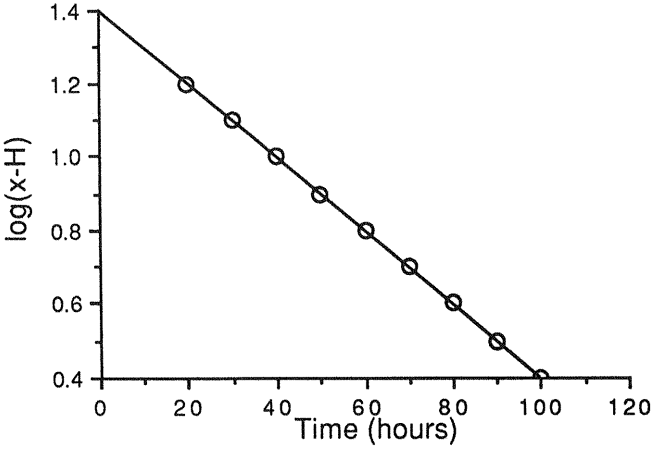


Fig. 5 Settling of kaolin suspensions. (Constructed from data published by Carstensen and Su, 1969.)

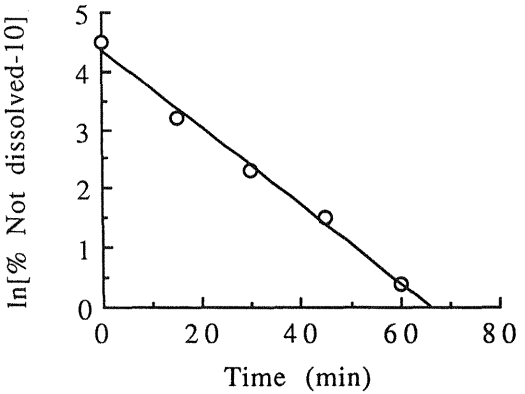


Fig. 6 The least squares fit equation is $y = 4.69 - 0.066x$ with $R^2 = 0.99$. (Graph constructed from data published by Cárdenas et al., 1994.)

antacids have high pH values, and hence hydrolysis of the esters occurs. The rationale for using several in combination is, exactly, to allow a certain amount to remain to retain preservative qualities of the suspension. An assay of the four esters and the parent acid (one of the decomposition products) in products where all occur has been described by Schieffer et al. (1984).

3.5. Dissolution of Suspensions

The 1987 Guidelines require testing of suspensions for dissolution. Cárdenas et al. (1994) have described the dissolution profiles from suspensions of benzoyl metronidazole, and a graph constructed from their published data is shown in Fig. 6. The curves should, by all rights, follow a cube root law without lag time, but they do not do so. If adjusted for amount not dissolved at the end (in the figure, 10%) they will adhere to a sigma minus plot.

3.6. Temperature Testing of Disperse Systems

A suspension is, as the name implies, a two-component system consisting of a solid and a liquid phase. (Gas phases are considered nonessential in this connection). Obviously, the solubility of the compound is a function of the temperature, and at a given temperature above 25°C this solubility will be reached. Testing about this temperature obviously has no meaning as far as suspension stability (neither physical nor chemical) is concerned. Prior to starting a program, this temperature should be established, so that unnecessary sampling stations can be avoided.

3.7. Semisolid Suspension Systems (Ointments, Suppositories)

Some semisolid systems (ointments and suppositories) are suspensions. Their testing is not different, in general philosophy, from what is described above, except that the rheology is checked differently. Davis (1987) has reviewed sophisticated means of checking the stability of such systems.

The factors checked for in stability programs of such products are the following:

1. Consistency, fell to the touch
2. Viscosity
3. Polymorphism

It is mentioned elsewhere that migration of a "disperse" phase within a semisolid product is quite possible when another phase is present. This situation may occur in the case of the use of benzocaine in, for instance, a suppository wrapped in aluminum foil coated with polyethylene. Polyethylene lining of aluminum wraps of suppositories is used to prevent contact between the metal and the suppository, and in most cases this has a positive effect.

However, a partitioning of drug or additive between the two phases may be possible if the drug or additive is suspended in the suppository. Denoting its solubility in the polyethylene S_p and the solubility in the suppository base S_s, the compound would disappear from solution in the suppository at a rate proportional to $S_p - S_s$, and "disappeared" compound would be replenished by dissolution from the solid phase.

The rate of disappearance would be governed in that the value of S_p would increase by a sigma minus relation (i.e., in the same manner as the appearance of decomposition product in a first-order reaction), and this then would be the overall "loss" of compound as a function of time. Since this is a first-order overall relationship, the "decomposition" would, initially, appear to be first order.

3.8. Ointments and Transdermals

Polymorphism can be followed by x-ray analysis and in some cases by thermal methods. There is, in fat systems, the possibility of trans esterification, and this can be tested for chemically.

The problem of morphology changes is often of particular importance and of particular frequency in the case of suppositories. In this type of product, it is also important to check for migration of suspended/dissolved substances. Often a substance is added to a suppository as a suspended particle, which is soluble in the suppository base to some extent. The phenomenon of dissolution will, of course, become evident by checking the particle size as a function of time. If a substance is soluble in the base, then it is preferable (if possible) to saturate the base with it at the onset. For this reason it is necessary to determine the solubility (S gm/gm) of the drug (or other) substance in the base. A Van't Hoff plot [solubility as a function of temperature (T°K), i.e., plotting $\ln[S]$ versus $1/T$] will allow extrapolation to room temperature. In manufacturing it is advisable to dissolve the drug (or other substance) to the extent of its solubility during the intermediate temperature phase of manufacturing (where the preparation is still quite fluid) and then suspend the rest at a lower temperature. An example is ascorbic acid, which is a good antioxidant in Carbowax bases. To exert its antioxidant action it must, however, be dissolved (and it is quite soluble in polyethylene glycols).

Dissolved drug (or other substance, e.g., benzocaine) will diffuse in the suppository base, and can, for instance, partition into polyethylene linings of the suppository wrap.

Release rates are important in many topical preparations, in particular in transdermal preparations. Here there are several investigational methods available. In-vitro methods involve placing the ointment on a membrane and measuring the appearance of drug in a receptor compartment on the sink side of the membrane. Hoelgaard and Møllgaard (1983) have, for instance, described the in-vitro release of linoleic acid through an in-vitro membrane. They mounted abdominal human skin in one case and skin from hairless rats in another to open diffusion cells. The dermal side was bathed with a receptor medium stirred at 37°C. The medium was 75 mL of 0.05 N phosphate buffer (pH = 7.4) which contained 0.05% Pluronic F68 and 0.01% butylhdroxytoluene, the latter two ingredients added in order to increase the lipid solubility. Linear, Ficksian diffusion curves were obtained. In a stability program, such tests are obviously useful and should be repeated periodically, but an "internal standard" or "calibrator" should be used, i.e., a stable test substance, the diffusion of which is known (e.g., salicylic acid). Other pseudo-in-vivo methods involve shaved or hairless rabbits, or cadaver skin. The interaction between ointment and container (patch) should also be part of the stability program.

Some of the testing applicable to semisolid emulsion systems is also applicable to ointment systems and will be discussed at a later point.

4. EMULSIONS

An emulsion should be thought of as a metastable system. In most cases the emulsion system (Fig. 7) is thermodynamically more energetic than the ground state system, which would simply be the totality of the two phases, separated. There will, therefore, always be the potential for oil droplets re-merging in an attempt to create the thermodynamically stable system.

Emulsion systems are taken orally (LipoGantracin™, Roche), parentally (as parenteral fat emulsions), and topically (creams).

4.1 The Emulsion Interface

The factors that stabilize the emulsion system are a layer of surfactant and protective colloid on the exterior of the droplet. The amount of these two must be such that they

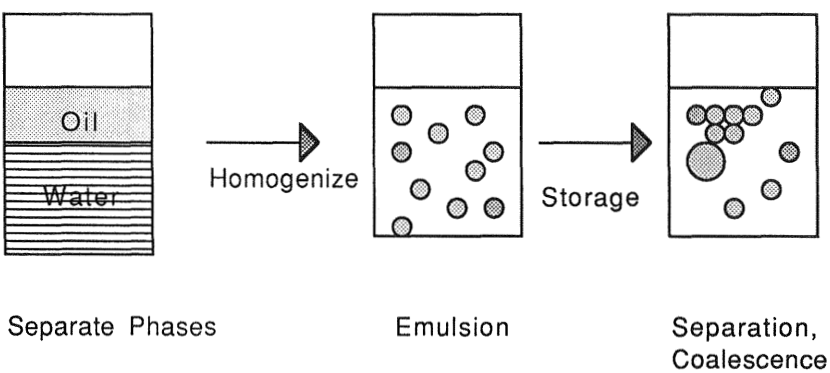


Fig. 7 Emulsion system.

cover the entire area of the droplets, otherwise coalescence will occur to the extent that the area, A, of the droplets will be reduced to such a point that it now will be completely covered by surfactant and protective colloid.

If, for instance, 1 g of emulsion contained W g of droplets of a size d μm and the oil had a density of ρ g/cm^3, then there would be n droplets per cm^3, where n is given by Eq. (10.14). Each particle has a surface area of πd^2, so that the total area is

$$A = n\pi d^2 = \frac{6Q}{\rho d} \tag{10.17}$$

Example 10.1.

If the density of the oil is 0.9 g/cm^3, the amount of oil phase per cm^3, is 0.75 g, and the diameter of the oil globules is 10 μm (10^{-3} cm) what is the surface area of the oil phase?

Answer.

$$A = \frac{0.75}{10^{-3}}\frac{6}{0.9} = 5 \cdot 103\,\text{cm}^2 \tag{10.18}$$

Example 10.2.

If a surface active agent of molecular weight 800 and cross-sectional molecular area of 30 Å is present in a concentration of 0.2% will that suffice to cover the surface in Example 10.1?

Answer

2 mg/cm^3 = 2/800 = $2.5 \cdot 10^{-3}$ millimoles = $2.5 \cdot 10^{-6}$ moles, which in turn equals $2.5 \cdot 10^{-6} \cdot 6 \cdot 10^{23} = 1.5 \cdot 10^{18}$ molecules = $30 \cdot 1.5 \cdot 10^{18}$ Å^2 = 4500 cm^2, this is the surface the surfactant could cover. This is slightly less than the 5000 cm^2 surface area of the oil, so that the entire surface of the oil globules cannot be covered by the surfactant.

The above calculations are oversimplified. They assume, for instance, that all the surfactant is adsorbed onto the oil, which is not the case. It is important, however, to check, originally, whether enough surface coverage of the oil is provided for. If not, there will be an initial shrinkage of surface area (increase in droplet size) attributable to this. Hence, if the coverage of the droplets with surfactants and/or protective colloid is incomplete at the time of manufacture, then the droplets will grow in size as time progresses. Rowe (1965) for instance demonstrated that the globule size decreases with increasing surfactant concentration, as shown in Fig. 8.

4.2. Globule Size and Viscosity

The breakage of suspensions will be dealt with shortly, but (Fig. 7) it might be suspected that breakage would be a function of Stoksian motion [Eq. (10.11)], i.e., the globules move and collide and hence coalesce. This is true in a sense, but

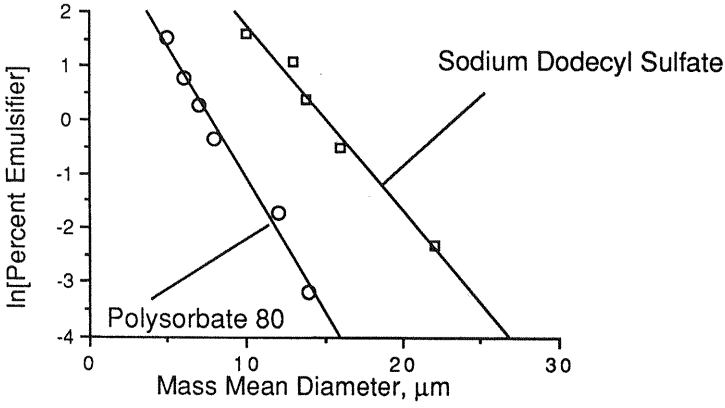


Fig. 8 Effect of emulsifier concentration of globule size. (Graph constructed from data by Rowe, 1965.)

the conclusion that might be drawn would then be that to increase viscosity [Eq. (10.11)] would reduce the severity of such impacts. However, Siragusa (1995) has demonstrated that although increased viscosity to some extent makes an emulsion more stable, the more important factor is the stability of the surfactant/protective colloid system at the interface. From a stability point of view, there is a correlation between the overall emulsion viscosity and the globule size. Figure 9 shows a typical example of viscosity as a function of droplet size and phase ratio (Sherman, 1964).

Hence, checking viscosity in the stability program, in a manner of speaking checks the globule size, which is the prime indicator of potential for progressing creaming and breaking. The viscosity is usually checked by a cup and bob method. The limitations of this will be discussed in the section dealing with semisolid emulsion systems.

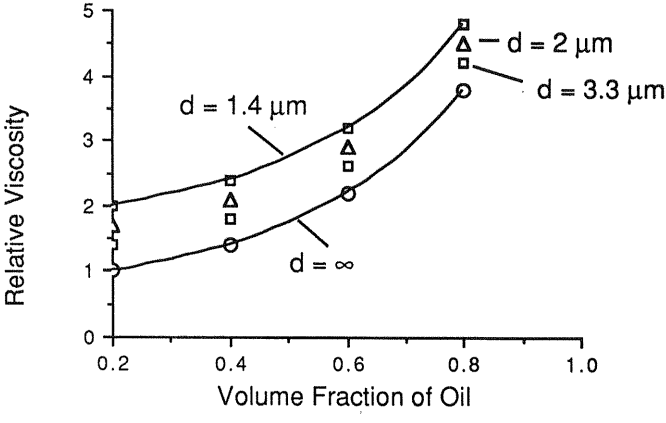


Fig. 9 Viscosity as a function of droplet size and phase ratio. (Constructed after data published by Davis, Sherman, 1964.)

Direct measurement of the droplet size can be accomplished in several ways: microscopy, electronic (Coulter) counters, photon correlation spectroscopy (for particles that are very small, Davis, 1967), diffuse reflectance spectroscopy (Akers and Lach, 1976) and the measurement of ultrasound (Rassing and Atwood, 1983). Davis (1987) points to the importance of choosing the proper techniques. He cites an example where a fat emulsion was tested for stability (as regards droplet size and distribution). The accelerated test used was a shaking test. The tests used were (a) microscopy (large globules), (b) electronic counting (medium size globule count) and (c) photon correlation spectroscopy (small particle count). Figure 10 shows the results.

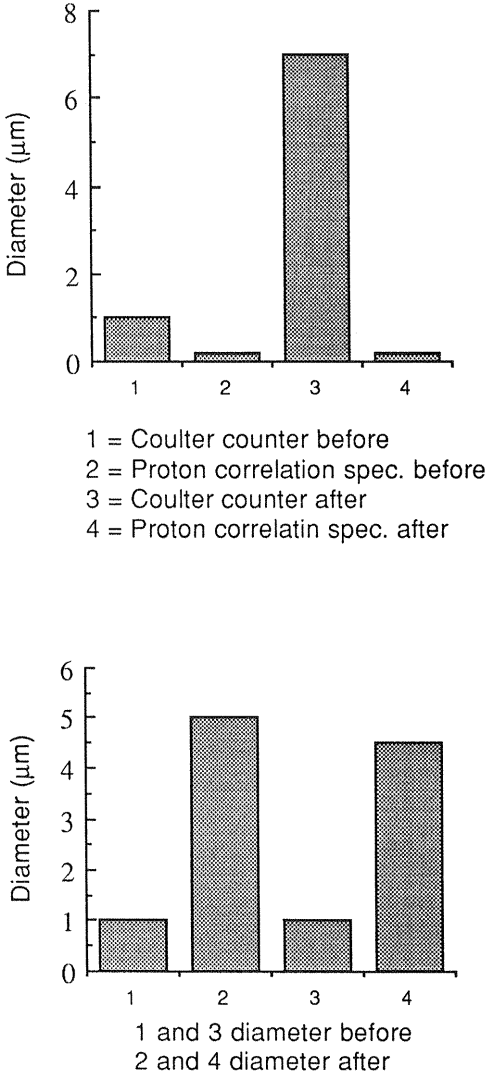


Fig. 10 Particle size analysis of accelerated test of emulsion system. (Constructed from data published by Davis, 1987.)

It is obvious that the small globule count does not change much, but that the intermediate count changes a lot. (The large globule count would then change in complement fashion to the intermediate count, and this was confirmed by the microscopy results.) What is important in this particular case (the system tested was a parenteral fat emulsion) is that there was formation of large oil droplets (not visible to the naked eye), and that these could have had a bearing on the toxicity of the product. This demonstrates that one method in itself is not enough, and that not one but several methods should be considered.

Reng (1984) has advocated electrical conductivity as an overall, common means of determining the state of dispersion of an emulsion system, and he shows that this parameter changes significantly over short periods of time, if the emulsion system is not satisfactory.

4.3. Stability of the Emulsifier/Protective Colloid System

The other phenomenon that may happen, which affects droplet size, is chemical breakdown in the surfactant. Nonionic surfactants are frequently used, and they are esters that may hydrolyze or interact with other components of the emulsion. Part of the formulator's job is, in independent experiments, to determine the pH profile and interaction potential of the surfactant (in a system simply consisting of the aqueous phase) with the other additives of the emulsion system. This can be done simply by cloud times (at accelerated temperatures) if the acid or alcohol from the hydrolysis or the interaction product is poorly soluble (as it is in the case of polysorbates and arlacels).

The problem with nonionic surfactant hydrolysis is exactly that it produces a fatty acid, which may become part of the oil phase and hence (aside from providing less coverage of the oil droplet) change the emulsion characteristics of the system.

In general the formulator also determines the HLB (hydrophilic/lyophilic balance) of the system he works with and matches it to the surfactant used [Atlas Chemical Company (now ICI Americas), 1963]. The HLB of the emulsifier can be adjusted by mixing two emulsifiers, e.g., arlacel 85 has an HLB value of 2.0 and polysorbate 80 one of 16.5. If an emulsion system required an HLB of 10 for instance, then the ratio of polysorbate (x) to arlacel ($1-x$) would be given by

$$2(1-x)+16.5x=10 \tag{10.19}$$

or

$$x=\frac{8}{14.5}=0.55 \tag{10.20}$$

4.4. Emulsion Type

In emulsion formulation, the type of emulsion is of concern. If it is desired to make an oil-in-water emulsion (o/w, i.e., oil is the discontinuous phase), then it is important that phase inversion not occur. Investigating this possibility must be a task in the stability program (and is usually carried out by the formulator, not the preformulator). Most often phase inversion is associated with creaming and separ-

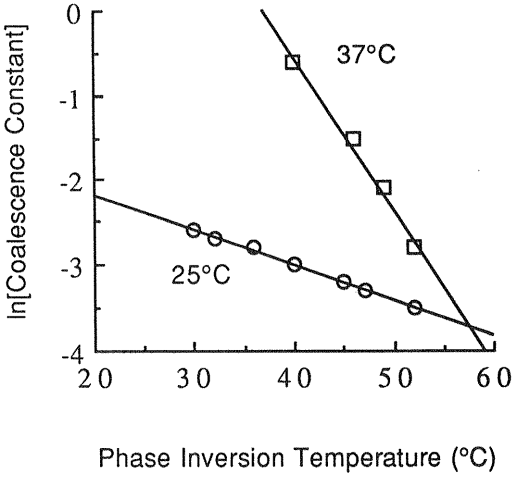


Fig. 11 Coalescence rate versus inversion temperature. (Graph constructed from data published by Enever, 1976.)

ation and will be noticed in the appearance testing of the emulsion. Such phenomena lead to graininess of feel. In some cases part of an emulsion will invert, another not, and then there is a distinct difference in appearances in various regions of the emulsion (creaming).

But the possibility for inversion should always be considered. It is the more likely the closer the system is to a close-packed system of spheres. In this connection, another of the formulator's tasks should be to determine the inversion temperature. (This is at times used to advantage in the manufacturing step, in that, in producing the emulsion, the inverse emulsion is produced at high temperature; this is then cooled, and at the inversion temperature, the "correct" type will result. Conversion in this manner gives rise to very small globules, and homogenization is then often not necessary.) If an inversion temperature exists, then accelerated testing above this temperature is meaningless. So preliminary testing is always advocated, if accelerated testing is contemplated, the philosophy being that there is no sense in testing a system above a temperature where it converts to a physical state that differs from that at room temperature (or recommended storage temperature). Enever (1976) has shown that there is a correlation between phase inversion temperature and the rate of coalescence (Fig. 11). It is possible to use a combination of sedimentation field flow fractionation and photon correlation spectroscopy to record droplet sizes in fat emulsions, and this would appear to be an excellent technique for studying the coalescence of finer spheres, and hence to obtain an extrapolatory tool early on in the storage of an emulsion system.

4.5. Rheological Properties

It has been mentioned that there is a gross correlation between viscosity and globule size. However, the rheological characteristics of an emulsion system in general depends on other factors as well (Sherman, 1955):

1. The viscosity of the internal phase

2. The viscosity of the external phase
3. The phase volume ratio
4. What emulsifiers are used and in what amount
5. The electroviscous effect
6. Distribution of particle sizes

4.6. Appearance of Emulsion Systems

The appearance of the emulsion will be a function of globule size, and Table 2 gives a gross correlation of these two factors. When an emulsion breaks, the hyponatant, rather than being a solution, will have one of the two first appearances in the table, i.e., will also be an emulsion, but with very fine droplets.

4.7. Breaking and Coalescence

It can be concluded from what has been mentioned that the reasons for breaking would include

1. Chemical incompatibility between the emulsifier and another ingredient in the emulsion system (Borax and gum acacia is a case in point)
2. Improper choice of surfactant pair (e.g., wrong HLB)
3. High electrolyte concentration
4. Instability of an emulsifier
5. Too low a viscosity
6. Temperature

As shown in the foregoing, breaking and creaming of emulsions are the typical defective criteria to be looked for in stability programs. Breaking implies that the emulsion separates into two distinct phases (Fig. 7). If this is a slow process, it often manifests itself in the appearance of small amounts of oil particles on the surface, and it then is referred to as *oiling*. When separation into two emulsions occurs (as described above), then the phenomenon is called *creaming*. A rapid test for this is to dip a finger into the preparation and notice if there are different "colors" present (Brown, 1953). Also, a creamed o/w emulsion will not drain off the skin with ease, and the converse holds for a creamed w/o emulsion.

A few words regarding the effect of ionic substances and the actual process of flocculation and coalescence are in order. Van den Tempel (1953) demonstrated that flocculation and coalescence are two different processes. Flocculation depends on electrostatic repulsion (and is akin to the zeta-potential considerations discussed previously). Coalescence depends on the properties of the interfacial film.

Table 2 Correlation Between Globule Size and Appearance of Emulsions

Globule size (μm)	Appearance
>0.005	Translucent (transparent)
0.005–0.1	Semitransparent, gray
0.1–1	Bluish-white emulsion
>1	Milky-white emulsion

Cations, as a whole, are less soluble in the oil phase than anions, and this gives rise to negatively charged droplets (akin to the creation of a zeta-potential in suspensions). The potential drop over the film depends on the nature of the electrolyte (and it should be noticed that there is a diffuse double layer in both liquids as opposed to the case of suspensions, where there is only one diffuse double layer).

Electrolytes may either improve or worsen the stability: If they eliminate the protection offered by the surfactant/protective colloid system then coalescence occurs. Most often electrolytes have the effect of reducing the emulsifying powers of surfactants and causing salting out or actually precipitating the surfactant. However, in some cases, electrolytes will favorably affect the potential drop over the two double layers, and in this case they may stabilize the suspension system.

4.8. Semisolid Dosage Forms

Semisolid emulsions (cold creams, vanishing creams) are not different, in general philosophy, from the above, except that the rheology is checked differently. Davis (1984) has reviewed sophisticated means of checking the stability of these types of systems. He lists the following properties as being important in stability programs for semisolid emulsions:

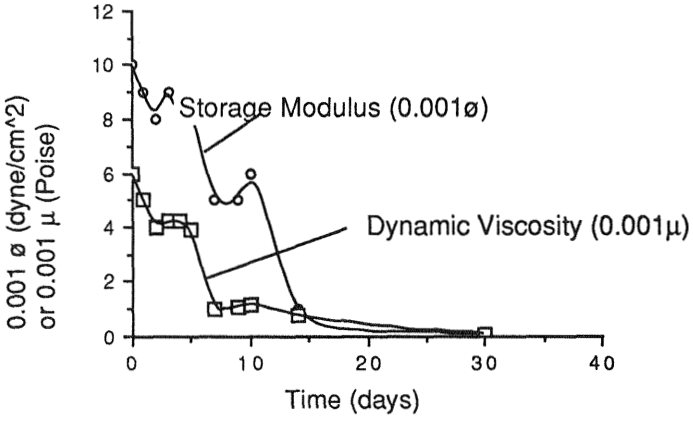
1. Particle size
2. Polymorphic/hydration/solvation states
3. Sedimentation/creaming
4. Caking/coalescence
5. Consistency
6. Drug release

Of these, particle size, sedimentation/creaming, caking-coalescence, and consistency have been discussed earlier.

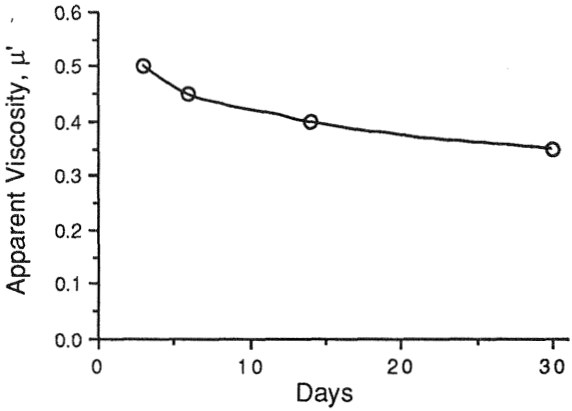
Following viscosity as a function of time is here of particular interest. The problem is how to measure the viscosity, and what viscosity in essence means. Davis (1987) points out that changes in viscoelastic properties are much more sensitive than simple continuous shear measurements (Barry, 1974). He demonstrates this via data published by Eccleston (1976). Here (Fig. 12) the variation of the dynamic viscosity (η) and the storage modulus (ϕ) are shown and compared with the same type of graph for apparent viscosity (μ') from continuous shear experiments. It is obvious that the two former measurements are much more sensitive.

4.9. Transdermals

The most important concern about transdermals is the release of drug substance from them and the stability of this property. Other properties (stickiness, appearance, etc.) are of importance as well, but the release characteristic is paramount. Kokobo et al. (1991) have described a means of checking this in vivo by using a single diffusion cell. The volume could be, for instance, 2.5 mL, and the diffusion area could be of the order of 1 cm^2. The matrix is placed, e.g., in contact with a 40% polyethylene glycol solution, which can be, e.g., removed in 500 μL quantities.



(a)



(b)

Fig. 12 (a) Dynamic viscosity (v) and storage modulus (ϕ) and (b) apparent viscosity (μ') as a function of storage time for cosmetic creams made from stearyl alcohol. (Graph constructed from data published by Eccleston, 1976.)

Kokobo et al. (1994) have reported on the interaction between pressure-sensitive adhesives and drug combinations used in transdermals. Their data are shown in Fig. 13. The data fit neither a diffusion equation (ln of retained versus time) nor a square root equation directly. It would appear that if one allows for either an initial dumping in the diffusion equation (or includes more than one term in the Barrer equation) or a lag time in a square root equation, then the data will

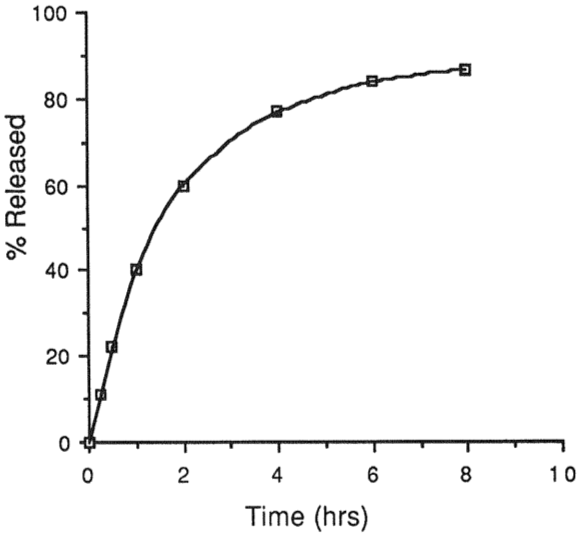


Fig. 13 Release of dipropylphthalate from 2-ethylhexylacrylate acrylic acid copolymer (2EHA/AA). (Graph constructed from data reported by Kokobo et al., 1994.)

fit either. A modified diffusion equation is probably the most likely. The authors suggest the use of the Williams–Landel–Ferry equation for fitting:

$$\{\log D\} - A = \frac{-896}{51.6 + (T - T_g)} \tag{10.21}$$

where D is the diffusion coefficient, A is a constant, and T_g is the glass transition temperature of the polymer.

5. ACCELERATED TESTING AND PREDICTION

Accelerated testing of physical properties of disperse systems is not as clear-cut as for instance chemical kinetics prediction. For instance, the stability of properties of semisolid materials is very difficult, for instance, for creams and ointments that give rise to bleeding there does not seem to be any reliable predictive test. Yet a series of stress tests are used for disperse systems. They include

Shaking tests
Centrifugal tests
Freeze–thaw tests
Elevated temperature tests

It should be cautioned that although these types of tests can be performed on a comparative basis (Is formula A better in one respect than formula B?), their interpretation, other than saying that A is better (or worse) than B, is uncertain, and predictive aspects are somewhat lacking, because the phenomena tested in the accelerated tests do not necessarily mimic what will happen in room temperature storage a/o shipping (Davis, 1987; Rhodes, 1979a and b).

For the freeze–thaw test, the question is what the minimum temperature should be, temperatures from −5° to +5°C being the most common. −5°C frequently gives rise to phase separation and irreversible changes that would not be seen in usual temperature ranges (Nakamura and Okada, 1976), but again, such tests may be used to select a "presumably best" formula from a series of preparations in product development. Results of a typical freeze–thaw cycle are shown in Fig. 10.

Centrifugation has been used by some investigators (Tingstad, 1964; Hahn and Mittal, 1979; and Ondracek et al., 1985). The general idea is that g can be increased in the terminal velocity predicted by Stokes's law (Eq. 11), but often the stresses caused by centrifugation may cause coalescence, which would not occur during normal collision stress.

Some investigators claim fair success in predictions by this means, but as Davis (1987) cautiously states, "as a general rule it can be stated that systems that withstand accelerated stress conditions should be stable under normal storage conditions. However the corollary is not necessarily true." That is, if the preparation fails the test it *may* still be all right, but if it passes the test it should be all right. Although this may be true overall, one can visualize that if a preparation is centrifuged right after manufacture, then the stress does not include the chemical changes (surfactant decomposition for instance) that occur on storage, and in this respect it may give too optimistic a prediction.

Buscall et al. (1979) have measured phase separation at several different centrifugal gs and have established from these data a so-called coalescence pressure. This (again recalling that the test does not account for chemical changes on storage) may be an appropriate parameter.

One predictive method in formulation is the correlation afforded by coalescence rates (Fig. 11), and this is rational in selecting the "best" of many formulations; in general the system with the highest phase inversion temperature is the best. The (nonchemical stability dictated) coalescence rate could theoretically be calculated prior to storage, and the difference between observed and calculated then attributed to chemical stability causes.

For emulsions, it should again be pointed out that rapid creaming and flocculation does not necessarily mean rapid coalescence. For emulsions there have been reports (Rhamblhau et al., 1977) that attempted to tie zeta-potentials to emulsion behavior on storage, but the generality of such an approach has been questioned (Davis, 1987).

The shaking test is usually carried out at 2–3 hertz (Davis, 1987), and the philosophy here is to intensify the collision frequency between globules (and to some degree also the intensity). This is therefore considered an accelerated test, but it actually is part of the product life (transportation). In any event, it should be included in protocols and simply reported.

6. AEROSOLS

Sciarra has reviewed pharmaceutical and cosmetic aerosols (1974). Aerosols are solutions, primary emulsions, or suspensions (i.e., suspensions in a suitable solvent such as ethanol) of active principle in chlorinated hydrocarbons, contained in a pressure can. Either a dip tube or a metering device connects the pressurized liquid contents to the valve. Upon activation of this, the internal pressure will force

the liquid through the valve orifice and atomize the suspension. The chlorinated hydrocarbon and the primary emulsion or suspension vehicle will evaporate, and the drug, in finely divided form, will be administered to the location of treatment (lung, skin).

In general the physical instability of aerosols can lead to changes in (a) total drug delivered per dose or (b) total number of doses that may be obtained from the container. It is intuitively obvious that the particle size range must be fine (i.e., the particles will have to pass through the valve).

In general the primary disperse system is filled into a seamless aerosol can, the valve assembly is attached, and the halogenated hydrocarbon is filled by pressure through the valve. The under-the-cap filling method has been described by Boegli et al. (1969). The halogenated hydrocarbon can, alternatively, be "liquid filled" at low temperature. For products that are moisture sensitive, this presents the problem of condensed ice and water in the product.

As far as "cleanliness of operation," aerosol lines are usually kept separate from conventional filling lines (Sciarra, 1974) (or the product is contract filled). Some attempts have been made to use ethylene oxide sterilization of the can (Joyner, 1969a, 1969b), and aseptic fillings (Harris, 1968; Sciarra, 1967) can be carried out.

6.1. Aerosol Testing

Some testing methods are official in the USP (XXI). The Chemical Testing Manufacturers Association has developed a series of tests described in the ASCM Handbook (Aerosol Guide, 1981).

Several test methods are used to detect physical aerosol instability, viz., (1) unit spray content, (2) color and odor, (3) rate of leakage, (4) moisture and trace catalytical substances, (5) particle size distribution, (6) spray characteristics, (7) moisture and trace catalytical substances, (8) pH, (9) delivery rate, (10) microbial limit tests, and (11) container compatibility.

Of the above, leak testing is official in the USP (XXI). This consists of obtaining the weight loss after at least 3 days of storage and converting it to loss per year. If plastic-coated glass containers are used, the test should be done at constant humidity. A faster method is to use an eudiometer tube described in the CSMA aerosol guide. This has the advantage of speed and also is advantageous in that it distinguishes between leakage from crimp versus leakage from valve gaskets.

For spray characteristics a qualitative measurement is to spray onto paper that is treated with a mixture of dye and talc, as described in the CSMA Aerosol Guide. There are also radiotracer techniques (Smith et al., 1984) and TLC graphic techniques (Benjamin et al. 1983). The Aerosol Guide, p. 77 also describes a method whereby the spray is sprayed through a pie shaped wedge onto a rotator.

Particle size analysis is the most important characteristic and hence the most important aerosol stability test. Sciarra states that particle sizes are between 1 and 10 μm and mostly between 3 and 5 μm. Particle size affects stability of delivery rate, effective dose, mass of drug delivered and of course the stability of the suspension itself. The methods used are microscopy, sedimentation methods, light scattering, cascade impactors, and liquid impingers. If the particle size distributions are determined by electronic methods (e.g., Coulter counter, Malvern), then allowance for solubility should be made.

Polli et al. (1969) have shown that the spray particle size is reduced by decreased drug particle size, by concentration of drug, and by the valve orifice size. Higher propellant temperature, vapor pressure, and using a surfactant in the formula also made the spray particle size smaller.

Particle sizes are important for reasons other than physical stability. For inhalation aerosols, for example, it should be recalled that particles larger than 20 μm do not go past the terminal bronchioles, and particles at 6 μm do not reach the lower alveolar ducts. Particles 0.5 to 5 μm reach the alveolar walls and are intermixed with alveolar fluid (Idson, 1970). The chance for a 1 μm particle depositing is less than 50%. There is therefore, particularly for inhalation aerosols, a very narrow particle size range of effectiveness.

Moisture testing is of importance, and except for foams, pharmaceutical aerosols are nonaqueous suspensions. Devices exist that will allow the transfer of the content of the can directly into a Karl Fisher apparatus. This is preferable over transfer by cutting the can open, since this method would allow for condensation of water into the product (which is chilled at the time of the opening of the can). A description of the can device for piercing the can is to be found in the CSMA Aerosol Guide; it allows direct sampling from the content of the aerosol. The moisture is measured by Karl Fisher titration, and there are a number of commercially available instruments that can accomplish this.

Pressure testing is also an official USP XXI method. A prepressurized gauge is placed on the valve stem, and the valve is actuated so that it is all the way open. In the CSMA Aerosol Guide, pressure testing is described. One method employs piercing the can; the other tests directly through the valve.

Microbial limits are described in the USP, e.g., betamethasone valerate topical aerosol. The microbial limits must meet the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under the Microbial Limit Tests.

Delivery rate is official in the USP XXI. The aerosol is allowed to temperature-equilibrate at 25°C. The weight is determined, the can is then actuated for 5 seconds, the weight is determined again, and the delivery rate is then calculated by difference. Delivery rates usually change on storage because of changes in elastomer hardness and gasket swelling. An apparatus is available from Peterson/Puritan Inc. that is accurate to four significant figures. In this assembly a solenoid can hold and actuate, and measure to 0.001 seconds by stop clock (Johnson, 1972).

Poiseuille's law applies to aerosol spray delivery rates: Fisher and Sheth (1973) have shown that delivery rate is linearly related to the container pressure and that it is inversely proportional to viscosity of the can content. Also for a satisfactory system, the delivery rate will not to any great extent be a function of how much of the can has been emptied out. Of course if a can is emptied in one fell swoop, then the cooling effect of expansion may slow down the rate. Also, fractionation of propellant mix occurs and may lead to increased variation of delivery rate.

Valve testing and evaluation should always be done on the final formula (i.e., not on selected solvent systems). A pure solvent will not fractionate, and hence the variation of spray rate may be smaller than with the final formula.

Finally there is the question whether there is an interaction between the can and the product. Can interaction and moisture content are closely related, since under

adverse conditions, the halogenated hydrocarbon will react with water and form a halogen acid that may corrode the can. Coating of cans can slow down the rate of this corrosion but not necessarily eliminate it. The control of moisture is therefore important not only for this reason but often also for the reason of chemical stability of the drug.

6.2 Sprays

These are mentioned here in distinction from aerosols; they are mostly nasal sprays. In testing these, the droplet size is important in metered-dose sprays, since small droplets can reach bronchi and alveoli, which would be undesirable, e.g., for delivery of corticosteroid treatment of rhinal disease. Yu et al. (1984) have described a simple experimental setup used for determining the droplet size of flunisolid nasal spray. It is a glass chamber with an air inlet and a plastic stopper that has a hole matching that of the spray unit. This is connected via a conical cavity to a cascade impactor and an appropriate flow meter. This can be done (more expensively) by laser holography (Yu et al., 1983). Such instrumentation may be used to follow possible changes in droplet size distribution as a function of time.

VanOort et al. (1994) and Byron (1990) emphasize that the size of the particles is one of the most important factors in the efficiency of deposition of solids from inhalation aerosols. The FDA has called for a sampling chamber size of 500 mL (Adams, 1989). VanOort et al. (1994) have modified the Anderson Impactor as shown in Fig. 14 and have shown that the chamber volume greatly affects the percentage respirable dose.

VanOort et al. (1994) also tested the effect of the chamber volume, as shown in Fig. 15. In an Andersen Sampler (Andersen Sampler Inc., Mark II 1 ACFM Nonviable Ambient Sampler), the manufacturer recommends that, at a flow rate of 28.3 L/min, the effective cutoff diameters (ECD) are 9, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 μm for stages 0 to 7.

7. POWDERS

Pharmaceutical powders are for reconstitution into either suspensions or solutions. A prescription example of the former is chloramphenicol palmitate, where the reconstitution is carried out by the pharmacist prior to dispensing. An example of the latter is Metamucil, where the customer reconstitutes the product (e.g., in orange juice). Examples of solutions are Achromycin IM (which is a parenteral powder, i.e., not a lyophylizate). Over-the-counter examples of oral solutions of this type are older products such as Vi Magna Granules (Lederle^{TM}). Analogies in the food area are fruit drink powders, which are sold in packets and reconstituted by the consumer to a certain volume.

The main physical concerns in this type of product are appearance, organoleptic properties, and ease of reconstitution. Only the latter will be treated here.

There are several reasons a powder may change dissolution time as a function of storage time. The most common reasons are (a) cohesion, (b) crystal growth, and (c) moisture sorption, which causes a *lumping up* of powders. The latter is simply due to the dissolution and bridge-forming that occurs and is akin to what happens in wet granulation.

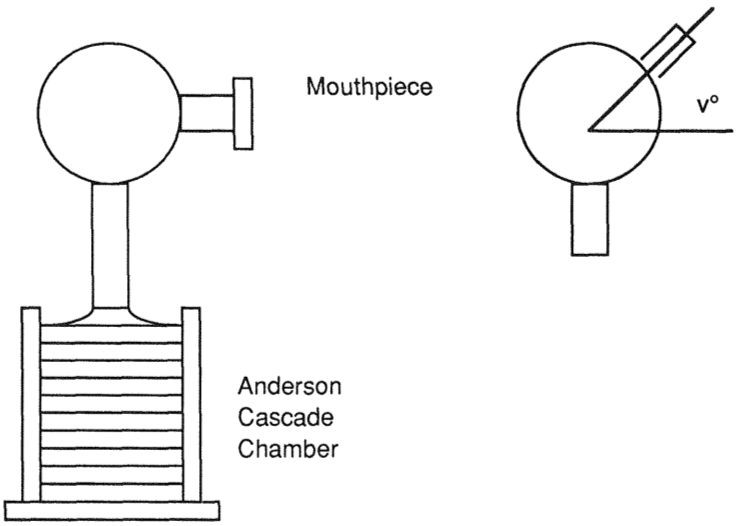


Fig. 14 Figure drawn from schematic published by VanOort et al. (1994).

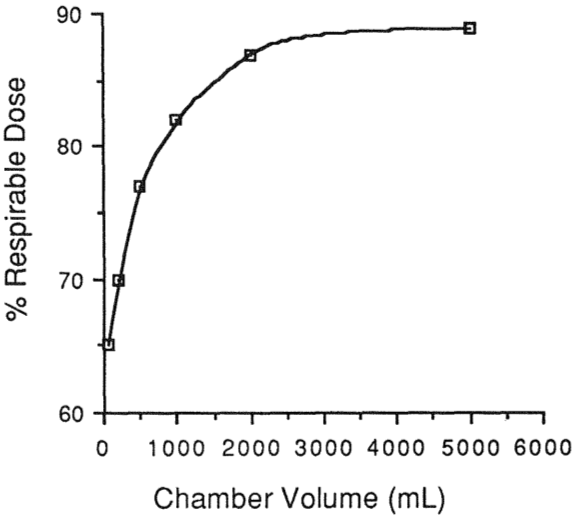


Fig. 15 Chamber volume vs. respirable dose. (Graph constructed from data published by VanOort et al., 1994.)

Cohesional force is the force between two particles, and *cohesion* in general is the stress (force per cm^2 of surface) that a particle experiences due to the surrounding particles. Problems due to cohesion are particularly predominant when a powder is fine, and great fineness of a powder is often required for dissolution reasons. Cohesional forces are inversely proportional to the square of the distance between the particles, so that in storage, where vibration, for instance, may consolidate the powder bed, these forces become large, and the powder "cakes up." This may give rise to problems in reconstitution.

There are two situations in *crystal growth*. One is due to the polymorphism. If the original product is either a metastable polymorph or amorphous, the conversion may occur in storage. For this to happen, some stress, e.g., the presence of moisture, must occur. The stress need not necessarily be moisture, conversion of a small amount of powder might occur in the filling head of the filling machine and then propagate in time.

If the content of the drug substance is such that there are no neighboring drug particles, then this conversion is limited. Particularly, contact points allow for propagation of conversions in situations where the spontaneous nucleation probability is low. The presence of moisture will accelerate conversions of this type, once a seed of the stable polymorph (or in the amorphate situation, once a crystal) has formed.

Crystal growth is, per se, not to be expected. It is true that, by the Ostwald–Freundlich equation, a larger crystal is thermodynamically favored over a smaller one; but the energy differences in the usual particle ranges is small and the activation energy high, so that the likelihood is rather low. If sufficient moisture is present so that the vapor pressure in the container exceeds that of a saturated solution, then some of the drug will dissolve in sorbed moisture. Fluctuations in temperature are never absent and would cause dissolution followed by precipitation, and this can lead to crystal growth. In cases where a drug substance is capable of forming a hydrate, and where an anhydrate is used, growth by way of hydrate formation is possible.

Ease of reconstitution is usually carried out subjectively, in that a tester carries out the reconstitution in the prescribed manner and records the length of time required to finish the operation. For this purpose it is important to have detailed directions on how the reconstitution is to be carried out, and to be sure that there is no operator-to-operator performance bias.

To insure the latter, a set of operators is usually selected for the operation at a point in the stability history. These operators will then be the test instruments for all testing of reconstitutability of oral powders.

The manner of screening operators could be as follows. A random sample is taken of a batch of a product. Random sets of four are taken from this random sample, and e.g. three operators tested. They are each given four samples to reconstitute on the first day, four on the second day, and four on the third day. It is a good policy to have two batches and mix them by day and operator, so as to carry out the test in a blind fashion. The results of such a screening could be as shown in Table 3.

Table 3 Screening of Operators for Reconstitution Testing. Reconstitution Time (min)

	Operator		
	1	2	3
Day 1	1.3±0.3	1.5±0.2	1.4±0.5
Day 2	1.7±0.5	1.4±0.4	1.5±0.3
Day 3	1.5±0.6	1.3±0.5	1.7±0.4

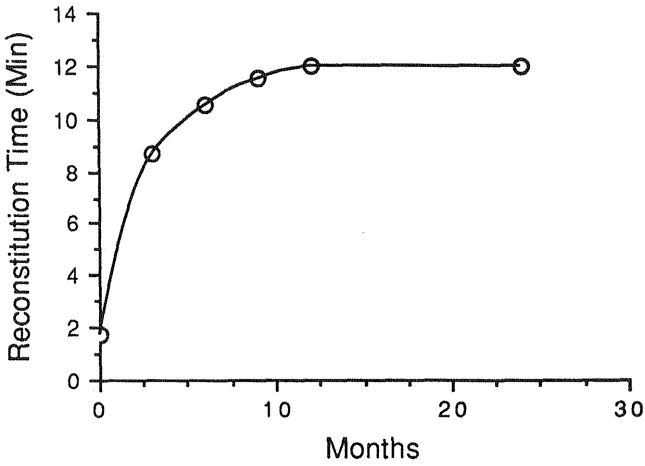


Fig. 16 Change of reconstitution time of a powder on storage.

The ranges shown denote standard errors of the mean. An F-test (Anova) will now fail to show a significant difference between operators. On storage, the reconstitution time could change as shown in Fig. 16.

As mentioned, the most common reason for increases in reconstitution time upon product storage is that the powder becomes more "lumpy" through cohesion developing over time or because it becomes coarser due to crystal growth. Both phenomena are associated with moisture content, and just as it is important to test the effect of the level of moisture content in the case of stability of a solid dosage form, so is it important to test it in the case of a powder.

If m is the number of mL of water adsorbed on one gram of powder, and if S is the solubility (in mg/mL) of all the soluble substances in the preparation, then, since the moisture layer is stagnant, the concentration of solubles at time t will be given by

$$C = S[1 - \exp(-qt)] \tag{10.22}$$

where q is the dissolution constant (kA/V).

The layer will have a higher viscosity (η), the more solid is dissolved, presumably by a power function:

$$\eta = \beta \cdot C^n \tag{10.23}$$

In analogy with the definition of viscosity, the force (F) needed to move two planes separated by a liquid is proportional to the viscosity. It would also be proportional to the amount of liquid, m, in the powder situation stated, so that combining this concept with Eqs. (10.22) and (10.23) gives

$$F = m \cdot \beta \cdot S^n \cdot [1 - \exp(-qt)]^n \tag{10.24}$$

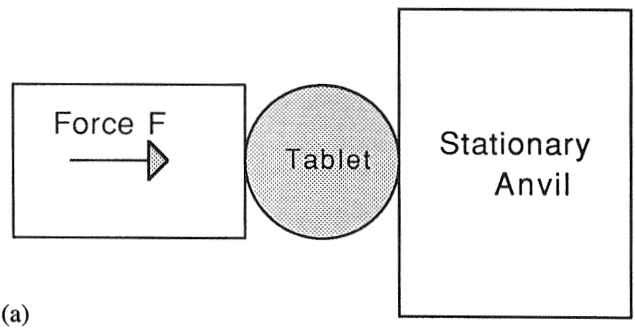
so the reconstitution time would be proportional to this. The data in Fig. 16 follow this pattern.

8. TABLETS

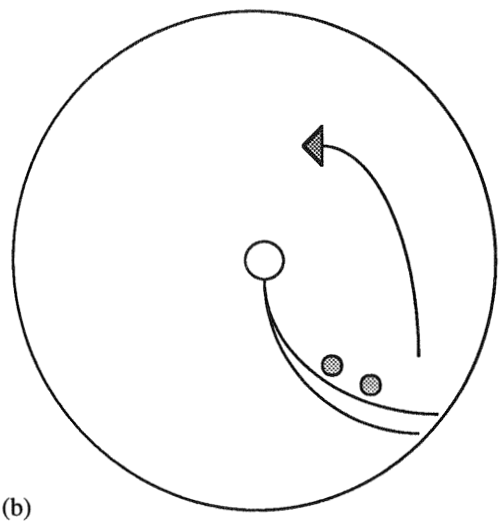
The physical properties associated with tablets are disintegration, dissolution, hardness, appearance, and associated properties (including slurry pH). For special tablet products (e.g., chewable tablets) organoleptic properties become important. These have been described earlier, but in the case of tablets, the chewability and mouth feel also become of importance. The properties will be discussed individually below.

8.1. Tablet Hardness

The "hardness" properties of a tablet are usually assessed by subjecting the tablets to a diametral failure test. The tablet is placed (Fig. 17) between two anvils, one of which is stationary. The other anvil is moved at constant speed against the tablet, and the force (as a function of time) is recorded. The force, at which the tablet breaks is denoted the "hardness" and is usually measured in kp (kilopond = kilogram



(a)



(b)

Fig. 17 (a) Hardness tester and (b) friabilator.

force). Other older units (Strong Cobb Units, SCU, or pound force) are used, usually when older instrumentation is used. Until recently, one limitation was that forces over 20 kp would simply register as F > 20 kp. Newer instrumentation allows for quantitation of higher forces. From a stability point of view this is important, since the better a parameter can be quantitated, the clearer the picture that emerges will be.

Tablets are made either by wet processing (wet granulation) or by dry processing (direct compression or slugging/roller compaction). In the former case a binder in solution is added to the powder mixture (or is contained in the powder mixture, and wetting then carried out). The binder forms soft bridges between particles, and when the granulation is dried then these bridges become hard. They form the bonds during the compression, and this is one of the reasons for the addition of the binder. The hardness of the tablet is tied in with the strength of the bond. The nature of the actual bond formation will be discussed presently.

In order for a bond to form, the particles or binder bridges must first be exposed to stresses (pressures) that exceed the elastic limit of the material. On failure, the material will either deform plastically or experience brittle fracture. A material that flows well and has a low elastic limit is, therefore, easy to transform into a tablet, and several such materials, known as direct compression ingredients, are used in the manufacture of pharmaceutical tablets. In these cases drug is simply mixed with the direct compression excipient (and other excipients), lubricated, and compressed. If the drug content is less than (approximately) 20% then the tablet will (generally) have the properties of the direct compression ingredient. At higher percentages, direct compression is usually only feasible if the drug substance itself is fairly compressible (i.e., has a low elastic limit).

The hardness of a tablet will be a function of the strength of the bond and the number of bonds. However, this is statistically oversimplified. If there are, for instance, many bonds in the bottom of a tablet and only a few in the top, then the tablet will break easily. Hence it is the average bond density and the standard deviation of the bonds that are really of importance. The same is true about the strength of the bond. Train (1957) has shown that the particle density in a tablet varies from spot to spot, and hence there is a variation in the density of the bonds (and probably in their strength as well).

If the hardness of a tablet is plotted versus the applied pressure, then a plot such as shown in Fig. 18 results. It is seen that the curve goes through a maximum. For good formulations, this maximum does not occur until very high pressures (outside the range of pressures used in pharmaceutical tableting). The maximum occurs because above the critical pressure, P^*, the tablet will laminate or cap, and a laminated tablet (Fig. 19) will contain strata of air and hence be thicker and weaker. Tablet thicknesses will respond in a manner opposite to the hardness, i.e., show a minimum (e.g., at 500 MPa in Fig. 18).

The reason for this phenomenon is the following: As applied pressure increases, the number of bonds, N, increases as well. But assuming that there is a maximum number of bonds, N^*, that can be formed, then the strength, H, of the tablet will asymptote as well. In a simplified manner the relations would be

$$N = N \cdot [1 - \exp(-qP)] \tag{10.25}$$

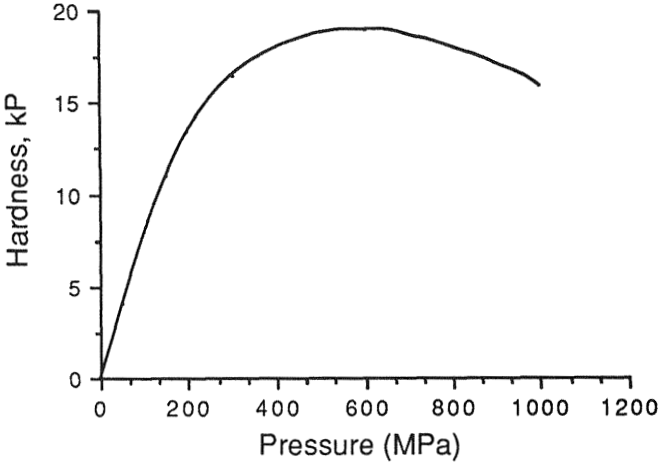


Fig. 18 Tablet hardness versus applied tableting pressure. (Graph constructed from data by Carstensen et al., 1986.)

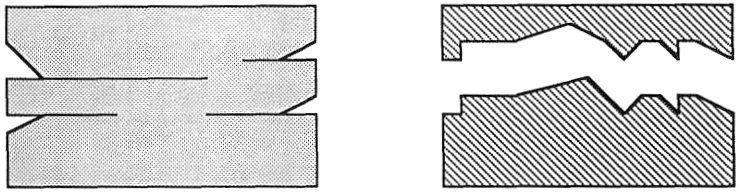


Fig. 19 Laminated and capped tablets.

If the hardness is assumed proportional to the number of bonds, then

$$H = \beta N = H \cdot [1 - \exp(-qP)] \tag{10.26}$$

where H is the capability of the tablet to withstand stress and β is a proportionality constant (Fig. 20).

During the tableting process, when the upper punch is released, a stress is exerted on the tablet, and this stress (S) is the larger, the larger the applied pressure, i.e.,

$$S = f(P) \tag{10.27}$$

At a given point, S becomes larger than H, and then fracture (lamination) occurs within the die, before the tablet is ejected.

There is a second type of stress that occurs during compression, and this happens upon ejection from the die. Here, many tablets expand, and this expansion is a stress that may also exceed H, i.e., laminated or capped tablets are formed.

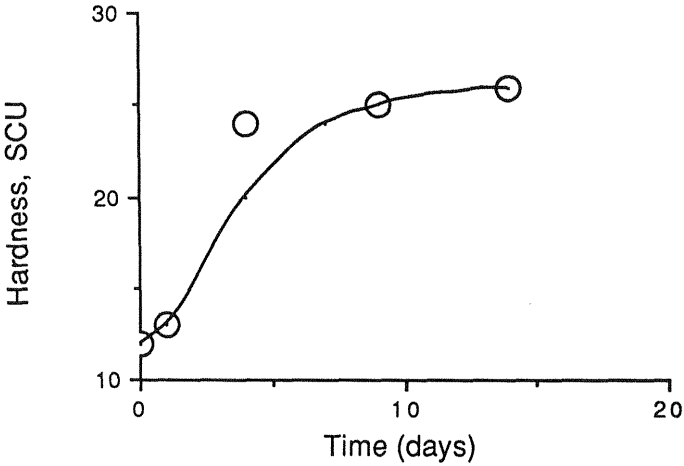


Fig. 20 Hardness as a function of time in pharmaceutical tablets. (Figure constructed from data published by Chowhan, 1979.)

On storage, this expansion can continue (Gucluyildiz et al., 1977), i.e., a tablet may become softer on standing for simple expansion reasons. Expansion is rarely checked as part of a stability program, and the cited article is one of the few published attempts to measure porosity as a function of time.

Frequently tablets will become either softer or harder within short periods of time after manufacture. Figure 20 shows hardness as a function of time for a series of tablets reported by Chowhan (1979).

Aside from the quoted instance of porosity changes and expansion, there are cases where crystallization of a soluble compound has occurred via the sorbed amounts of moisture in the tablet. This happens most often with very soluble compounds, and in such cases it is important to ascertain storage in a dry environment. A test that is now a requirement in the ICH Guidelines is storage in the final container at 40°C, 75% RH. During this test moisture is usually adsorbed by the tablets, and this can then cause softening of the binder bridge because of moisture uptake. At times, redrying will reinstitute the original hardness. Sometimes hardening occurs when the sorbed moisture causes recrystallization of a compound or excipient.

8.2. Softening

Softening can be associated with chemical interaction. Several furoic acids (Carstensen and Kothari, 1983), when tableted with microcrystalline cellulose, will cause a specific interaction leading to the formation of carbon monoxide (rather than decarboxylation of the acid). This interaction is not slow at 55°C, and it causes the tablets to crumble. At room temperature the effect is less pronounced yet significant.

Since a tablet, when produced, is not in equilibrium, there will be a redistribution of moisture. This could make the bonds of a lower or a higher moisture content, and there may for this reason be a change in hardness during a fairly short period of time after manufacture.

Table 4 Moisture Content of Selected Excipients

	Water content		
Excipient	TGA	Calcium carbide	Karl Fischer
Sta-RX 1500	10.8	9.7	10.4
Solca-Floc	5.8	4.6	6.4
CMC	9.2	3.7	14.9
Celutab	8.6	0	9.0
Microcryst. Cellulose	3.4	2.9	4.7
Polyvinyl-pyrollidone	5.4	2.9	6.4

Source: Table constructed from data published by Schepky (1974).

The moisture content of granules, when they are made initially, is a function of their particle size. Pitkin and Carstensen (1973) have shown that when granules are dried, each is associated with one given drying time, t^*. Since the drying (if it is countercurrent, or fluid bed) is a diffusional process, conventional diffusion theory predicts that the amount of moisture left in a granule, m, in relation to the initial amount, m_0, is given to a first approximation by

$$\frac{m}{m_0} = \exp\left[-\frac{D}{a^2}t\right] \tag{10.28}$$

where D is the diffusion coefficient of water in the granule and a is its diameter. The larger granules will hence, have a higher moisture content at the beginning, but the moisture will equilibrate, in most cases, on storage. However, Zoglio et al. (1975) have shown that in some cases (spray dried sucrose granules) there will be no redistribution of moisture between larger and smaller granules.

The moisture contents of various excipients have been reported by Schepky (1974) and are listed in Table 4.

In stability situations it is often the change in moisture as a function of storage time that is of importance. In such cases (Shepky, 1974), the thermogravimetric method may be of advantage.

It is of interest, in cases where moisture equilibrates and causes change in hardness on storage, to be able to assess the extent of moisture transfer within the tablet. As mentioned at an earlier point, the situation is that (in a simple case of a two-component tablet) the two components (I and II) have different moisture isotherms. These are approximated in linear fashion in Fig. 21. This is best illustrated by example.

Example 10.3.

A tablet is made of two components, I and II. It has a given moisture content, 10 mg of water per g of dry tablet weight. The moisture isotherms are as shown in Fig. 21. Calculate the final moisture contents of the two components after the moisture has equilibrated.

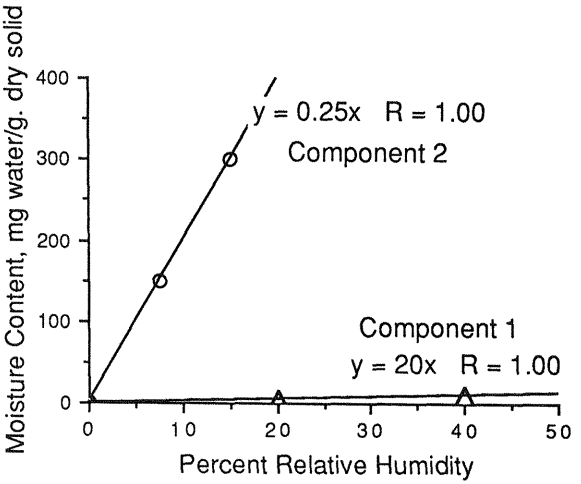


Fig. 21 Moisture exchange between ingredients.

Answer.

For component II, 10 mg of moisture per g of dry solid corresponds to a relative humidity of 40%, i.e., the equation for the isotherm for II is given by

$$y_2 = \frac{10}{40} x_2 = 0.25 x_2 \tag{10.29}$$

since it passes through the point (10, 40). The moisture isotherm for I has the equation

$$y_1 = \frac{300}{15} x_1 = 20 x_1 \tag{10.30}$$

since it passes through the point (15, 300). At the tablet moisture content (10 mg/g dry solid) it is in equilibrium with a gas phase of relative humidity

$$x_1 = \frac{10}{20} = 0.5\% \text{ RH} \tag{10.31}$$

Hence the situation is not an equilibrium situation, because there is no common vapor pressure over the solids. Component I will, therefore, give up (q grams of) water, and II will pick up (q grams of) water until a common vapor pressure (X^*) in the porous and external vapor space has been achieved.

The equilibrium relative humidity is given by

$$X^* = 0.25(10 - q) \tag{10.32}$$

and

$$X^* = 20(0.5 + q) \tag{10.33}$$

and equating the two right hand sides then gives

$$2.5 - 0.25q = 10 + 20q \tag{10.34}$$

or

$$20.25q = 12.5 \tag{10.35}$$

or

$$q = \frac{12.5}{20.25} = 0.6\,\text{mg} \tag{10.36}$$

It simplifies the computation that the moisture content is given in mg of moisture per g of dry solid (i.e., not in percent, which would be related to mg of moisture per g of total weight). It should be noted that this situation is simplified by assuming the isotherm to be linear.

8.3. Disintegration

Tablets (whether coated or not) are usually subjected to a disintegration test. The disintegration was the first in-vitro test used by the U.S.P. It is now not obligatory compendially (but is recommended); in an obligatory sense it has been replaced by the dissolution test. This latter, hence, is the more important test, but it will be seen that there often is a correlation between the two, and since the disintegration test is much more easily carried out, a stability program will check disintegration frequently, and dissolution less frequently, primarily due to labor intensity.

The apparatus used (U.S.P. XX, p. 958) is shown schematically in Fig. 22. It is an apparatus where six tubes are placed in holders on a circular screen, which is then raised and lowered between 29 and 32 times per minute through a distance of 5.3–5.7 cm in a 1000 mL beaker containing the disintegration medium (either water or N/10 hydrochloric acid). The wire mesh oscillates so that it is 2.5 cm (or more) below the surface at the upstroke and 2.5 cm (or more) from the bottom of the

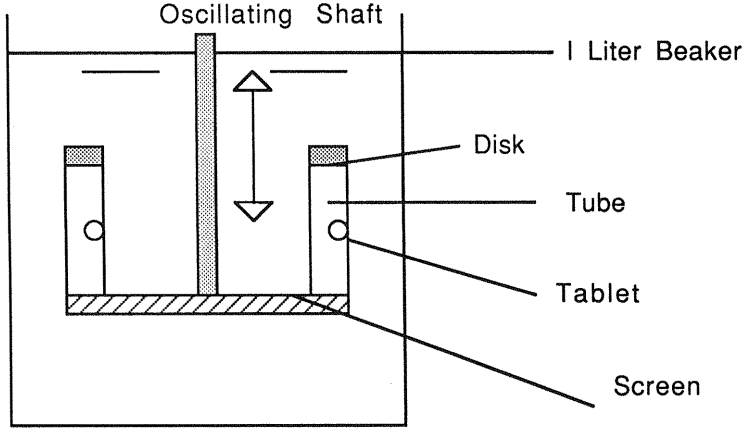


Fig. 22 Disintegration apparatus.

1000 mL beaker at the downstroke. The open-ended glass tubes are 17.75±0.25 cm long and have an inside diameter of 21.5 cm. The glass thickness is 0.2 cm.

Each tube is provided with a disk 95 mm thick and 20.7 mm in diameter, made of plastic of a specific gravity between 1.18 and 1.20 g/cm^3. There are five 2 mm holes in the cylinder (one of them in the axis). The disk also has notches in it and serves to keep the tablet within the tube and submerged during the stroke of the assembly.

To operate the apparatus, one tablet is placed in each of the six tubes, disks are added, and the apparatus is operated at 37°C in the immersion fluid. For quality control release purposes as well as for investigational purposes the time is noted when all tablets have disintegrated completely, and if not all tablets have disintegrated at the end of the specification limit, then the basket is removed and the tablets observed. If one or two tablets have failed, then 12 more tablets are tested, and these must all disintegrate within the limit. However, in stability testing it is important to note the time that each individual tablet disintegrates.

It should be pointed out that complete disintegration is defined as "that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus is a soft mass having no palpably firm core." There are apparatuses on the market that have a sensor attached to the disk and can determine this state automatically and record the time at which it occurred. Such an attachment is strongly recommended for stability studies, since it provides an easy means of recording the time of disintegration of each tablet.

There are relatively few articles in the pharmaceutical literature that deal with the subject of the change in disintegration and dissolution upon storage, yet these qualities are as important as the retention of potency of the active compound. If a product falls short of specifications during its shelf life, it becomes unsatisfactory, regardless of the particular parameter that is shortfalling.

One fairly systematic study of this is the work by Chowhan (1979). Here disintegration and dissolution times of e.g. dicalcium phosphate based tablets were studied for prolonged times at 25 and 37°C. The pattern is a sigma minus type of pattern as shown in Fig. 23.

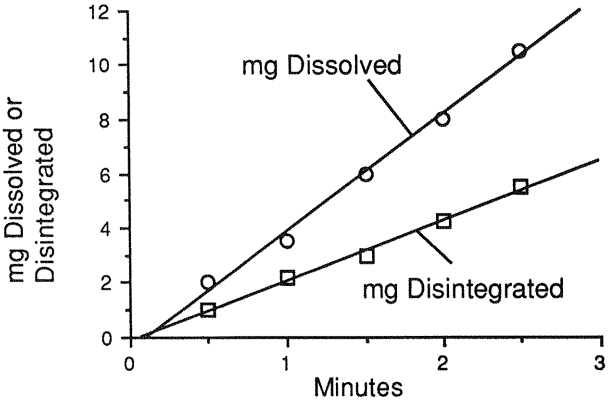
Carstensen et al. (1980a, 1980b) have shown that there often is a correlation between dissolution and disintegration, and Carstensen et al. (1978a, 1978b) have shown the theoretical basis for this. Figure 24 shows such a correlation of dissolution and disintegration times in a U.S.P. apparatus.

Couvreur (1975) has shown that the disintegration of a tablet is a function of several factors. If the tablet disintegrates by virtue of a disintegrant which expands, once it is wetted, then the most important attribute is the rate at which the disintegrating liquid penetrates the tablet, and hence the contact angle between the solid and the liquid is of importance.

8.4. Porosity of Tablets

Cruaud et al (1980) showed that there was a direct correlation between dissolution and porosity in a case where the correlation between disintegration and dissolution was not apparent.

There is one well documented case (Gucluyildiz et al., 1977) where the porosity was shown to change in a tablet as a function of time. What this indicates is that if



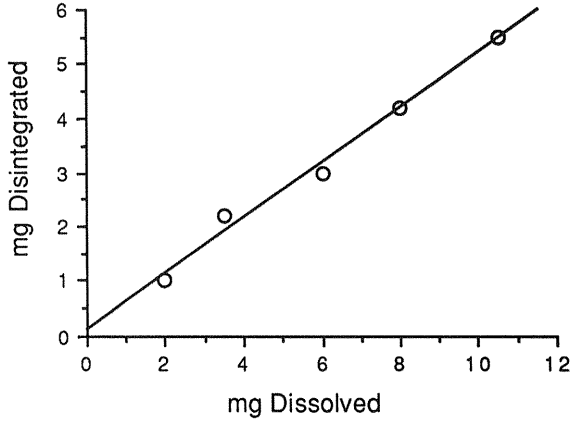


Fig. 23 Top figure: Correlation between dissolution and disintegration upon storage. (Graph constructed from data published by Carstensen et al., 1980a.) Bottom figure: Direct correlation between dissolution and disintegration.

dissolution and disintegration change on storage, then they may be functions of the change in porosity, if indeed porosity changes as a function of storage time.

A rational way of studying this would be to study mercury intrusion as a function of time in tablets of a drug, and to study simultaneously the disintegration and dissolution profiles.

There is a distinct effect of moisture uptake or equilibration on disintegration (and hence, indirectly, on dissolution). If the liquid penetrates an "average" pore, then it encounters, on its way, disintegrant particles. It is assumed that there are q disintegrant particles per linear length of pore. It is also assumed that N particles (per pore) must have been wetted before the tablet can break up (disintegrate).

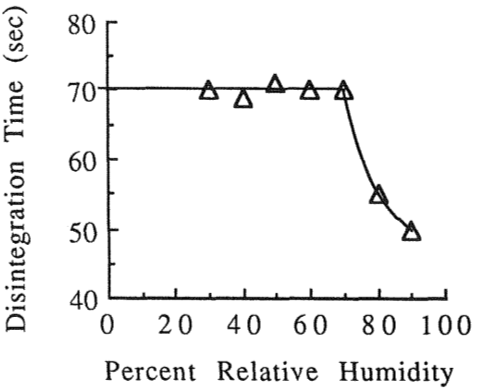


Fig. 24 Effect of storage at various relative humidities on disintegration time of an antidiabetic tablet. (Graph constructed from data published by Grimm and Schepky, 1980a.)

According to the Washburn equation (Washburn, 1921; Nogami et al., 1966; Carstensen, 1980), the length, L, of penetration of liquid at time t is given by

$$L^2 = \left(\frac{r \cdot f \cdot \cos \phi}{2\eta}\right) t = \beta \cdot dt \tag{10.37}$$

where

$$\beta = \frac{f \cdot \cos \phi \cdot r}{4\eta} \tag{10.38}$$

and where f is the interfacial tension, d is the average pore diameter, r is the pore radius, ϕ is the contact angle, and η is the viscosity.

The number of particles wetted n, is related to L by

$$q = \frac{n}{L} \tag{10.39}$$

or

$$L = \frac{n}{q} \tag{10.40}$$

where q is a proportionality constant. The disintegration time, t_N, occurs where $n = N$, so

$$LN = \frac{N}{q} \tag{10.41}$$

and

$$LN^2 = \beta \cdot t_N \cdot d \tag{10.42}$$

Combining these two equations gives

$$t_N = \frac{(N/q)^2}{\beta \cdot d} \tag{10.43}$$

Hence, the following hold for the disintegration time, t_N:

1. It is the larger the more disintegrating particles must swell to make the tablet disintegrate.
2. It is the longer the finer the pore (the smaller d is).
3. It is the smaller the larger the disintegrant concentration, q.
4. It is the smaller the larger the value of β (the smaller the contact angle and interfacial tension).

Of these, N may change, e.g., if the disintegrant becomes wetter, and partly expanded as a result of moisture uptake, this will affect the disintegration adversely. For instance, the Joel Davis test (40°C, 75% RH for three months) has an adverse effect on disintegration for this reason, although it is only true if the relative humidity of the testing station is above a certain critical moisture content (Grimm and Shepky, 1980a). This is demonstrated in Fig. 24.

8.5. Dissolution

The dissolution apparatuses used are usually USP Method I (basket apparatus) or USP Method II (paddle apparatus). Carstensen et al. (1976a,b) have pointed out that the hydrodynamics of the basket method is poor and results in highly different liquid velocities in different parts of the apparatus, and also causes a phenomenon known as coning: powder accumulates at the bottom of the dissolution vessel, where it is fairly stagnant and hence dissolves slowly. Most tests nowadays are therefore carried out with the paddle apparatus.

The assembly is described in USP XX p. 959 and is basically as shown in Fig. 25. The original apparatus could be operated at 50, 100, or 150 RPM, but the more up-to-date apparatus has a variable speed rheostat. In almost all instances the

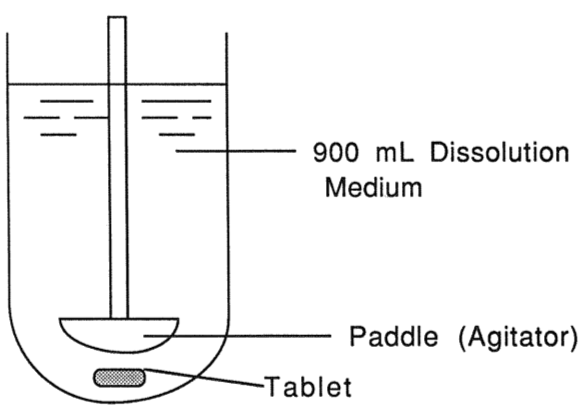


Fig. 25 USP dissolution apparatus.

FDA asks for 50 RPM (sometimes 100 RPM), but only rarely does it accept 150 RPM and insist on the test being "discriminating".

The apparatus should be subjected to a suitability test (USP XX, p. 959), using one tablet of the USP dissolution calibrator, disintegrating type, and one tablet of the USP dissolution calibrator, nondisintegrating type. The apparatus is satisfactory if the data are within the stated range of acceptability for each calibrator.

The dissolution medium is water, hydrochloric acid, or pH 7 buffer. These should be deaerated, since dissolved air may interfere with the dissolution rates.

The procedure used is to transfer usually 900 mL of the dissolution medium to the dissolution vessel and bring it to 37°C. After temperature is equilibrated, the thermometer is removed and one dosage unit is placed in the apparatus. Care is taken to exclude air bubbles from the surface of the tablet and to operate the apparatus right away. After given times, samples are removed from the supernatant and assayed, and the concentration is plotted as function of time. The results may be expressed as percentage of the highest possible concentration (D/V, where D is the dose and V is the volume of the liquid). Monographs specify, usually, a given time at which a certain percentage of label claim, Q, must be dissolved, and the term Q_{30} for instance is frequently used; it indicates the percent of label claim dissolved after 30 minutes. This is known as a one-point assay. For quality control purposes, this is acceptable, but for stability purposes, if rational graphing is contemplated, a dissolution curve rather than a one-point determination should be determined. This will allow determination of the dissolution constants, which will be discussed shortly.

It is often (particularly with slowly dissolving or sustained release products) of importance to have the value "at infinite time." This is usually imitated by increasing the rotational speed (e.g., to 150 RPM) and running the dissolution for an extra two hours. It is, in this scheme, assumed that all the drug will dissolve under such circumstances.

Shortcomings of the apparatus are still (a) that tablets made with excipients of high density will have a tendency to "cone," i.e., after disintegration accumulate in the dead spot just below the agitator; this gives false lows in dissolution rates, and (b) that capsules (and some tablet formula) may float. To avoid floating, a coil is usually placed about the capsule. It is interesting that some tablet formula with relatively small changes in composition (or compression pressure) will change their density, so that they float in one composition (or pressure) and sink in another (only slightly different) composition (or pressure). Expansion of tablets during storage may also change the density so that a table can change from a sinking to a floating composition which will give rise to an apparent slowing down of the dissolution rate.

It should be pointed out that dissolution testing of pharmaceutical products is carried out for several different reasons. In the early stages, the intent of dissolution testing is to get a feel for the comparative estimated bioavailability (on a rank order scale) of different formulations.

In preformulation, intrinsic dissolution rate constants are usually estimated. Although it is not possible, in a direct manner, to tie this in with an estimated bioavailability, it gives a feel for whether the drug substance will be exceedingly problematic, very problematic, problematic, or (in rare cases) not problematic. This feel is comparative with the intrinsic dissolution properties (obtained in a similar fashion) for other drug substances previously developed.

Table 5 Relative Rankings of Furosemide In-Vivo Versus In-Vitro (Random Cross-Over, 12 Patients)

	Rank dissolution	AUC (h-μg/mL)	C_{max} (mg/mL)	T_{max} (h)*
1	B	D	D	D
2	D	A	A=B=E	A=B=C=D
3	A=C	B=E	A=B=E	A=B=C=D
4	A=C	B=E	A=B=E	A=B=C=D
5	E	C	C	A=B=C=D

* Smallest T gets best rank, highest AUC and C gets best rank (i.e., lowest number in column 1).
Source: Table constructed from data published by McNamara et al. (1987).

In formulation, it is generally assumed that if e.g. three formulae, A, B, and C (Table 5) are developed, then the one that has the fastest dissolution rate should be the best. Whether this general statement is correct is debatable (Table 5), but lacking other criteria it is an accepted yardstick.

In postformulation, i.e. at the point where the new product is manufactured, and when the new product has become an established or old product, dissolution is in the domain of quality assurance. Here it is part of a specification, and the intent of conducting the test is to declare to the public (given the criterion that in-vitro dissolution within certain limits corresponds to in-vivo performance) that the product made on day X, year Y, is comparable to (and should perform in a manner similar to) the batches made year previously on day Z, year Q, when it was tested in the clinic.

If this premise were generally correct, then an in-vitro dissolution test would be universal for all formulae, and it will be seen below that that is an unwarranted extension. The question whether batches of the same formula fall under such a rank order rule is probably acceptable. In the history of a product, however, small changes are often made, and the question whether these small changes shift the in-vitro to in-vivo interrelation is, of course, not known a priori. What constitutes smallness is not clear (and actually is not determinable). Minor changes are defined, now, as changes that tighten specifications and do not involve change in procedure, equipment, or raw material.

If a "substantial" (major) formula change is made, then the bioequivalence between the clinical formula and the new formula must be established.

If subsequent formula changes are made (e.g., a bioequivalence study is carried out in year Y, then another in year Y + 1, etc.), then comparisons should be made with the original clinical formula, not with the previous formula. If the formulae are denoted A (clinical), B, C, etc., then if successive comparisons were made and performance were denoted P, then PB could be 0.8 times PA (and deemed equivalent), PC could be 0.8 times PB (and deemed equivalent), but PC would be $0.8^2 = 0.64$ times PA and hence no longer equivalent. Hence, equivalence testing should always test back to the formula used in the original clinical batches that were part of the medical scheme (and the results of which were approved by the Food and Drug Administration).

8.6. Percolation Thresholds

When a solid is compressed, then one might imagine that at "full" compression, the tablet would be similar to a perfect crystal, in that there would be no void space left in it. This is never achieved, however, and the fraction of void is called the porosity. This may be visualized as isolated pockets of void space or, as the porosity increases, strings of void, eventually terminating at the surface. The porosity at which this latter situation is achieved is denoted the threshold value.

Threshold values for a drug and its excipients in combination are important because they govern such properties as dissolution, hardness, and disintegration. For this purpose, percolation studies are often employed in pharmaceutical research.

Leuenberger and Leu (1992) and Leu and Leuenberger (1993) introduced the concept of drug percolation to the pharmaceutical sciences. By this, a pharmaceutical system is described as a bond/site system. In this concept, a cluster is defined as a group of nearest neighbor sites where all positions consist of the same component. There is a concentration where there is maximum probability that the clusters will start to percolate, and this is the percolation threshold. If the measured porosity of the tablet is denoted ε_m and (after dissolution) the porosity created by loss of dissolved matter is denoted ε^*, then the so-called β property is

$$\beta = -c\varepsilon_c + c\varepsilon \tag{10.44}$$

where $\varepsilon = \varepsilon_m + \varepsilon^*$ is the initial + developed (matrix) porosity, c is a constant, and ε_c is the critical porosity threshold for percolation. This ties in with the Higuchi type plot, the slope of which is b, and β is defined as

$$\beta = \frac{b}{[2A - \varepsilon S]^{1/2}} \tag{10.45}$$

where A is the drug load (g/cm^3 of total tablet) and S is solubility. When porosity is plotted versus β value, then a straight line ensues that cuts the x-axis at the percolation porosity.

The threshold for drug percolation may be obtained when more drug is available than that described in Chapter 9. Soriano et al. (1998) have described percolation methods that are done primarily by conducting dissolution studies with drug substance at various concentrations. They employ the method of Bonny and Leuenberger (1993) and Leuenberger and Leu (1992) for this purpose.

8.7. Multipoint Determinations

In post NDA testing, there is some reason for not carrying out dissolution at more than one time point, because of both human resources and equipment. In pre-NDA situations, however, as described e.g. by Prandit et al. (1994), the importance of carrying out multiple time points in dissolution cannot be stressed enough. Conclusions are difficult to reach if this is not done.

For instance Prandit et al. (1994) reported that aging affected the dissolution of nalidixic acid tablets and concluded that the effect was not attributable to an increase in disintegration time (as measured in a dissolution apparatus). Published data often

suffer from being *one point data*, so dissolution/disintegration correlations cannot be deduced from the reported figures.

8.8. Dissolution Media

There is always the problem of what dissolution medium to use. For poorly soluble drugs there are several approaches: cosolvents, micellar systems, and/or large dissolution volumes. Naylor et al. (1993) studied the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems by using a rotating disk and found the Levich equation to hold.

8.9. In-Vivo to In-Vitro Correlation

The problem of whether an in-vitro dissolution test generally measures in-vivo performance on a rank scale basis is still open to debate, when the problem is considered in general, i.e., if product A from manufacturer A has a dissolution rate curve "above" that of manufacturer B, will his product also have a better in-vivo performance as far as large magnitude (C_{max}) and short peak time (T_{max}) for the maximum of the blood level curve and high value for the area under the blood level curve (AUC)? The general premise is that the answer is yes, but as shall be seen below, this is not necessarily so. The correct general statement is that if two batches of the same product and formula are tested, then such a comparison is correct, i.e., that a "higher" dissolution curve implies at least one of the following: lower T_{max}, higher C_{max}, or higher AUC. An example of noncorrelation, when the formula is not the same, is the work by McNamara et al. (1987), in which furosemide from five manufacturers was tested against a solution. The relative rankings are shown in Table 5.

It is seen, then, that the best performer in vivo (D) is by no means the best performer in vitro, and that the worst performer in vitro (E) is not the worst performer in vivo.

The best and simplest method for correlation of in-vitro to in-vivo data would appear to be the mean residence time (MRT), and such comparisons have recently been described by Block and Banakar (1988). MRT is defined by many authors as shown in Fig. 26. The MRT factual definition is a measure of the "average" length of time a drug molecule is in the body (Fig. 26).

Mean residence time via statistical moment has also been described by Yamaoka et al. (1978). Podzeck (1993) has compared in-vitro dissolution profiles by calculating mean dissolution time and mean residence time.

Of late, deconvolution has been often reported and may form part of the 1995 USP. This method consists of comparing a blood level curve after solid dosage form administration with one after either solution or IV administration. The amount dissolved in the GI tract is then obtained by deconvolution. Sugawara et al. (1994) tested a series of controlled release preparations of prednisolone in alginate gel beads, all in a drug-to-alginate ratio of 1:4. As seen in Fig. 27, they were able to obtain in-vitro methods that "matched" the amount released in-vivo.

It is seen in the figure that for the fast releasing formulation (a), the in-vitro test, whether at pH 1.2 or at pH 6.8, follows the deconvoluted in-vivo results fairly well, but for the slow formula, it is only the pH 1.2 in-vitro test that correlates with the deconvoluted in-vivo dissolution test.

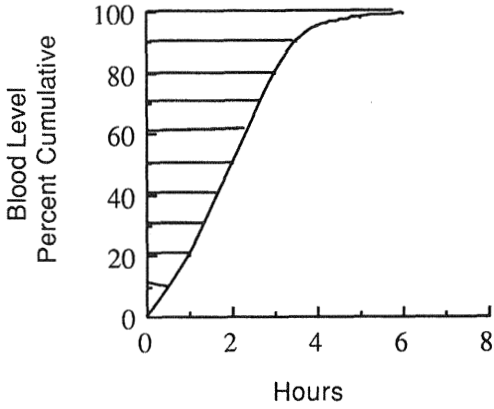


Fig. 26 Cumulative blood level curve (or urinary excretion curve, or dissolution curve).

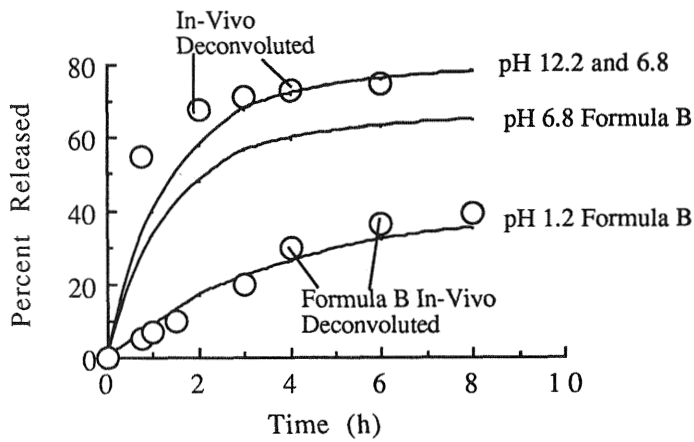


Fig. 27 Time vs. percent released. (Graph constructed from data published by Sugawara et al., 1994.)

The deconvolution method used was the one that has been described by Katori et al. (1991). Other, more recently developed methods are those of Gillespie and Cheng (1993). They first created a hypothetical clean curve with convolution. Then the absorption rates and cumulative amounts absorbed of the drug and metabolite were estimated by the proposed deconvolution method. For this purpose, polyexponential functions were fitted to the simulated data. The resulting parameters were compared by a multidimensional deconvolution program NDCREV (user-friendly IBM compatible).

8.10. Stability of Dissolution Curves

The problem from a stability point of view is that at times the dissolution curve will change as a function of storage time [as e.g. shown by Chafetz (1984) for hard shell capsules], but the bioavailability "stays the same." In such a case the in-vitro

MRT (or dissolution curve) would change but the in-vivo (either MRT or deconvolution curve) would not, so how can there be a correlation between the two?

The accelerated test in the ICH Guideline (40°C, 75% RH) is too severe a test for hard- and soft-shell capsules. Upon dissolution, a skin (a pellicule, or as some authors call it, a pellicle) will form around the capsule in the dissolution apparatus, and this will prevent dissolution. For instance, Dey et al. (1993) exposed etodolac capsules to the accelerated test so that they formed pellicules and showed that the dissolution was not affected when tested with enzymes, but that pellicules formed and dissolution decreased drastically on storage when nonenzymatic fluids were used. They showed that there was no difference in blood level curves of fresh, stored, and failed batches.

In the case of hard- and soft-shell capsules, gelatin can interact with substances in the fill. Gautum and Schott (1994) demonstrated an interaction of anionic compounds (substituted benzoic and sulfonic acid dyes) with gelatin. Capsule fills that contain, or on storage produce, keto groups will always show this phenomenon (Carstensen and Rhodes, 1993).

It should be pointed out that when disintegration of a dosage form changes on storage, it usually happens quite rapidly (usually within 12 weeks) at room temperature. Often, however, the tablet is not checked until 6 months after manufacture. There are then instances where it would seem to be logical to attempt an accelerated test at higher temperature. There has, to date, not been any convincing correlation between disintegration (and dissolution) profiles at higher temperature, vis-à-vis those at lower temperatures. Judging from the factors that affect these two properties, this is not surprising. But what is more to the point is that changes can usually be determined rapidly at room temperature. It is therefore more rational to determine disintegration at 4, 8, and 12 weeks at room temperature in stability programs, and to dispense with testing at higher temperatures.

Gordon et al. (1993) have reported on the effect of aging on the dissolution of wet granulated tablets containing superdisintegrants. Often the decay in dissolution efficiency is due to the lengthening of the disintegration time.

There is, obviously, a correlation between particle size and dissolution, and if the particle size changes as a function of storage time, there may be a correlation between accelerated temperature storage and dissolution. But in such a case the correlation should be established on the neat drug, as was done e.g. by Grimm and Shepky (1980b). Their data for oxytetracycline are shown in Fig. 28.

Dukes (1984) and Murthy and Ghebre Sellassie, (1993) have discussed storage stability of dissolution profiles in general, and Rubino et al. (1985) have described the specific storage stability of the dissolution of phenytoin sodium capsules. Carstensen et al. (1992) have discussed the mathematical basis for change in dissolution curves of dosage forms as a function of storage time. They employ the sigma minus model for dissolution, i.e.,

$$\frac{M}{M_0} = 1 - \exp[-k(t - t_i)] \tag{10.46}$$

where t is dissolution time, t_i is dissolution lag time, M_0 is initial amount in the dosage form, M is the amount left undissolved at time t, and k is a dissolution constant (time^{-1}). t_i is primarily a function of disintegration time.

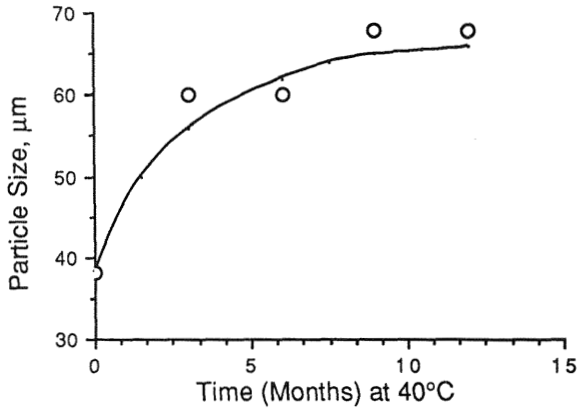


Fig. 28 Particle growth in accelerated storage of oxytetracycline. (Graph constructed from data published by Grimm and Schepky, 1980b.)

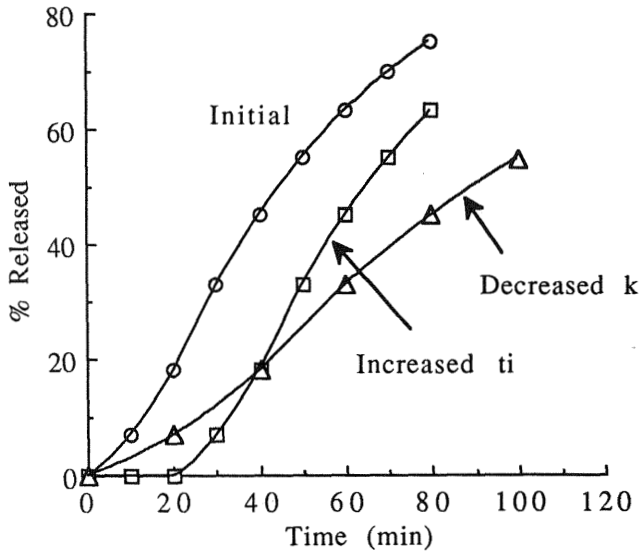


Fig. 29 Dissolution upon storage.

Frequently, upon storage, t_i may change, but k may not, in which case the dissolution curves simply move in parallel to higher and higher mean dissolution times. k, however, may change, and t_i may stay constant, in which case the curve becomes "flatter" (Fig. 29). Finally, both may change, giving rise to a flattening *and* a parallel displacement of the curves. If such parameters as t_{90} (the length of time for 90% to be dissolved) or Q_{45} (the amount dissolved at 45 minutes) are employed, then power function relationships result, and these are difficult to interpret. A better approach is to study and plot k and t_i as a function of storage time. If t_i on storage approaches 45, then the storage stability curve may have a shape as in Fig. 30.

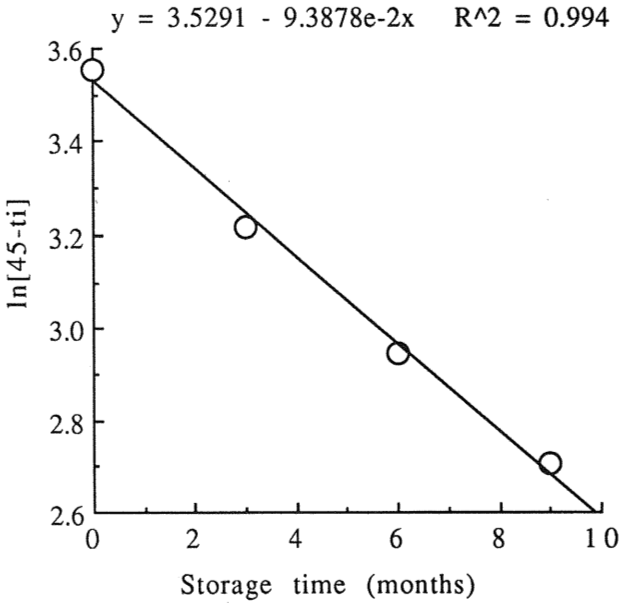


Fig. 30 Storage stability of lag time.

For instance, if a secondary parameter, such as the time needed for 50% dissolution, t_{50}, is followed, then

$$\ln 0.5 = -0.693 = -k(t_{50} - t_i) \tag{10.47}$$

or

$$t_{50} = \left(\frac{0.693}{k}\right) t_i \tag{10.48}$$

and if

$$t_i = t_i^{\infty}[1 - \exp(-q\phi)] + t_i^0 \tag{10.49}$$

where t_i^0 is initial lag time, t_i^{∞} is lag time at infinite storage time, ϕ is storage time, and q is the stability constant, then

$$t_{50} = \left(\frac{0.693}{k}\right)\{t_i^{\infty}[1 - \exp(-q\phi)] + t_i^0\} \tag{10.50}$$

If, now, the storage stability of k is of importance, the expression for t_{50} becomes even more complicated (but can obviously be deduced).

Jørgensen and Christensen (1996, 1997) have approached this problem by introducing a so-called Order Model. By this an order of reaction, n, is assigned to the dissolution curve, and the expression becomes

$$\frac{M}{M_0} = 1 - [1 - \{(1-n)k(t - f(t_0))\}]^{1/(1-n)} \tag{10.51}$$

where $f(t_0)$ is the lag time function given by

$$f(t_0) = t_0\left[1 - \exp\left(\frac{-t}{t_0}\right)\right] \tag{10.52}$$

t is, again, the dissolution time.

8.11 Appearance of Tablets and Capsules

A stability program should record the appearance of tablets as a function of storage time. This is most often done by subjective description, or by a rating index (0 for unchanged, 5 for vastly changed). Quantitative methods exist and are the following:

Comparison with color chips or charts (Rothgang, 1974)
Dissolving the dosage form and measuring the solution spectrophotometrically (Hammouda and Salakawy, (1971)
Photography (Armstrong and Marsh, 1974)
Reflection measurements (Matthews et al. (1974/75), Carstensen et al. (1964), Carstensen (1964), Goodhart et al. (1967), Turi et al. (1972), Wortz, R. B., (1967)

In the case of the second and fourth methods, a qualitative appearance description is always necessary, because the instrument will "average" the product. Comparison with chips can be used but is somewhat subjective. Such color charts have triangularly arranged chips, and the operator matches the object with a chip, which has a coordinate number. In fact the degree of whiteness (L), redness, (a) and yellowness (b) can be calculated from this, and it will be seen later on that this will allow for quantitative treatment of the change of the color of a pharmaceutical tablet or capsule.

Photography, of course, is relying on stringent adherence to conditions (exposure, aperture, and development) to insure that it is actually the tablets that are being compared, not the procedure for making the photograph.

Reflection measurements are often carried out in tristimulus meters and have been used quite extensively with varying degrees of success. If a tablet (or other surface) is placed in the meter, then reflectance values at three spectral regions are registered and recorded as x, y and z values. Rowe (1985) has reviewed these and points out that the whiteness index is $4(100Z/Z_0) - 3Y$, and the yellowness index is $100[1 - (100Z/\{Y \cdot Z_0\})$, where $Z_0 = 118.1$. In actuality, the degree of whiteness, L, the degree of redness, a, and the degree of yellowness, b, are given by the formula (for a Hunter tristimulus meter):

$$L = 100\left(\frac{Y}{100}\right)^{1/2}$$

$$a = 175\frac{(X/98.041) + (Y/100)}{(Y/100)^{1/2}}$$

$$b = 70\frac{(Y/100) - (Z/118.103)}{(Y/100)^{1/2}}$$

Table 6 Tristimulus Parameters for Some Colors

Color	x	y	z	L	a	b
White	82.3	84.3	101.1	91.8	−0.7	−1.0
Light yellow	65.0	69.9	7.0	83.5	−7.4	53.5
Yellow ochre	32.9	28.7	7.5	53.6	16.1	29.3
Scarlet	36.2	20.6	4.3	45.4	62.2	26.2
Magenta	23.9	13.0	23.9	36.0	55.2	−13.9
Turquoise	13.8	21.0	44.7	45.8	−26.5	−25.7
Emerald green	17.7	30.1	12.3	54.8	−38.3	25.1

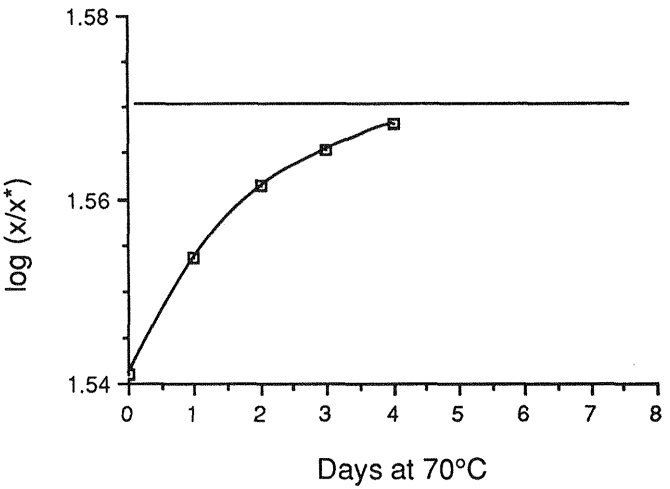


Fig. 31 Reflectance X values (with initially $X = X^*$) of vitamin C tablets as a function of storage time. (Graph constructed from data published by Carstensen et al., 1964.)

Komerup and Wanscher (1967) and Rowe (1983) give as an example the following values for some standard colors (Table 6).

The values calculated in the last three columns by Roe correspond quite well with those obtained from or listed in color charts (Komerup and Wanscher, 1967).

It should be pointed out first of all that reproducibility in reflectance meters is poor, and so results should always be obtained as averages of at least nine independent measurements. Since these are rapidly carried out, the labor is not all that intensive.

Changes in these values are difficult to interpret from a qualitative point of view, but the following procedure allows extrapolation, using x, y, or z (or composites).

Carstensen et al. (1964) have shown that the response values (Y) can be plotted as a function of storage time (t) to give graphs as shown in Fig. 31. This type of plot can be plotted as a sigma minus function:

$$Y = Y_\infty\{1 - \exp(-kt)\} \tag{10.53}$$

The k values can be plotted as an Arrhenius plot, i.e., one may, after short periods of time, at elevated temperature, calculate an extrapolated k value at room temperature. By sampling daily at 55°C, one can determine the Y value ($Y_{\text{lower limit}}$), which corresponds to the poorest appearance that is acceptable. Since k is known for room temperature (k_{25}), it is possible to calculate a "shelf life date" (t^*) based on appearance from inserting $Y_{\text{lower limit}}$ into Eq. (10.54):

$$\ln\left\{1 - \frac{Y_{\text{lower limit}}}{Y_\infty}\right\} = -kt^* \tag{10.54}$$

9. SUSTAINED RELEASE PRODUCTS

There are several types of sustained release principles used in pharmaceutical products, and a detailed description is beyond the scope of this book. What will be done here is simply to state the types of dissolution profiles that can be expected, and how the parameters could change with time.

9.1 Coated Beadlets and Granules

The coated nonpareil seed is the original sustained release form invented by SKF in the 1950s. Here a drug is applied (in the form of a sugar syrup) to monodisperse sugar crystals. Drying is carried out after each application step, so that the drug eventually is in a sugar matrix around the original seed. This beadlet is then coated with either a semipermeable film or an impermeable film with a soluble filler. The latter, upon exposure to dissolution medium, will allow the soluble filler to dissolve, so that pinholes are created in the film. Liquid then diffuses in through the film (or the holes in it), becomes saturated on the inside of the beadlet, and the dissolved drug then diffuses out. The diffusion takes place under an (approximately) constant concentration gradient (the solubility of the drug in the medium), as long as there is undissolved material inside the beadlet (and the concentration is low in the outside fluid creating sink conditions). Once the last drug has dissolved, the concentration inside the beadlet will decrease, and the diffusion slows down. It is, therefore, often, difficult to get the last 5–10% of material to release from this type (and other types of sustained release) dosage forms.

There are, obviously, three stages in the dissolution (Fig. 32):

$0 < t < t_i$: Penetration of liquid into the pellet. t_i is the time it takes for this to complete, and it is denoted the lag time.

$t_i < t < t_f$: t_f is the point in time where all the drug inside the pellet has dissolved.

$t > t_f$: This is the final period where dissolution is slower.

The general dissolution pattern in the period $t_i < t < t_f$ is

$$\ln\left[\frac{M}{M_0}\right] = -k(t - t_i) \tag{10.55}$$

M is the mass not dissolved (and M_0 is the dose) and is obtained by multiplying concentration with dissolution liquid volume and subtracting this (the amount dissolved) from M_0. k is the dissolution constant and will be the smaller (and t_i

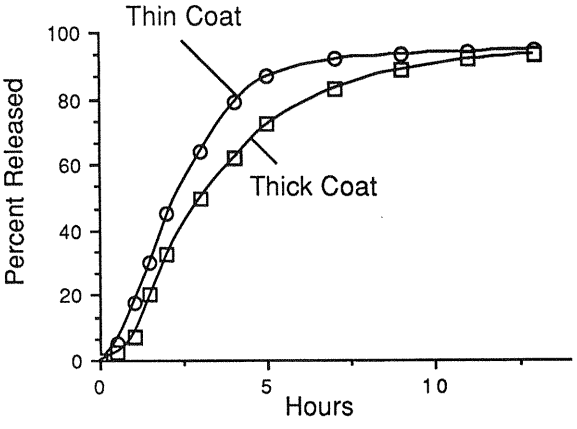


Fig. 32 Release patterns of thinly and thickly coated pellets.

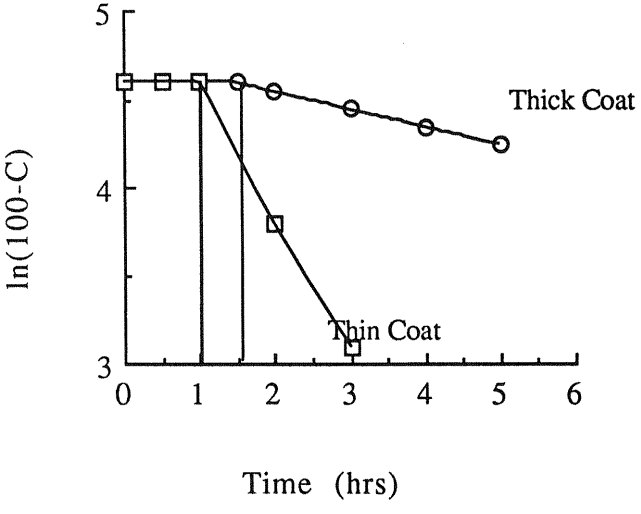


Fig. 33 Dissolution curve of sustained release beads. The thickness of the coat of II is 1.6 times that of I. The graph shows the decrease in slope and the increase in lag time with increased thickness. (Graph constructed from data published by Carstensen, 1973.)

the longer), the thicker the film and the lower the amount of soluble filler in the film. Figures 32 and 33 show this type curve (Carstensen, 1973). The first two points have been omitted, as have the last three points, i.e., t_i = about 1.5 hours and t_f = about 5 hours. The points omitted are such that the remaining points give the best linearity. It is seen from the graph that the least squares fit equations are

$$\text{I} \qquad \ln[M_1] = 5.09 - 5.04t \tag{10.56}$$

$$\text{II} \qquad \ln[M_2] = 4.8 - 0.29t \tag{10.57}$$

so that, as expected, the slopes are in a ratio of 5/3, i.e., the inverse of the ratio of thicknesses. The actual lag times are found by setting $\ln[M] = \ln[100]$, and they are $t_I = 0.67$ hours and $t_{II} = 0.96$ hours, i.e., again in the correct ratio. t_f is the point of inflection, i.e., occurs when all the drug inside the pellets will have dissolved (although not all will have diffused out).

In stability programs, t_i and k are the logical parameters to follow, i.e., complete dissolution curves should be determined. Again, it is wise to do this at room temperature storage at fairly short intervals at the onset (4, 8, and 12 weeks). Again, accelerated testing is not of much use.

9.2 Erosion Tablets

Tablets can be made of e.g. a waxy substance, which does not dissolve or disintegrate, but erodes away. The drug in the eroded portion will dissolve, and (in theory) the drug in the noneroded part will not have dissolved. There is, however, always some penetration of liquid into the waxy tablet, so that more than the eroded drug will often have dissolved. If pure erosion occurs, then the dissolution equation will be

$$M = M_0^{1/3} - K_e(t - t_i) \tag{10.58}$$

where K_e is an erosion constant (cube root dissolution rate constant) and t_i is the length of time of wetting. Both of these parameters can be calculated at different storage periods, and changes can be monitored in a logical fashion. Accelerated studies of this are not meaningful.

9.3 Insoluble Matrices

If a drug is enclosed in an insoluble matrix that is porous, then the release rate is given by the Higuchi square root law (Higuchi, 1963):

$$Q = K_i(t - t_i)^{1/2} \tag{10.59}$$

or

$$Q^2 = K_i^2 \cdot (t - t_i) \tag{10.60}$$

where

$$K_i^2 = a^2\left[2DS\varepsilon\left\{A - \frac{S\varepsilon}{2}\right\}\right] \tag{10.61}$$

a is here the surface area through which the diffusion takes place, ε is the porosity, and A is the loading, the amount of drug per cm^3 of dosage form. ε, the porosity, is the inherent porosity of the tablet plus the porosity created by the drug that has dissolved (i.e., A/ρ, where ρ is the density of the drug).

Eq. (10.61) applies to situations where the drug dosage, A, is larger than $S\varepsilon 2$. If this is not the case (Table 7), then the equation takes the form (Fessi et al., 1982)

$$Q^2 = a^2Dt \tag{10.62}$$

Table 7 Dissolution According to Eq. (10.62)

Time (min)	Amount released (mg)	Square root of time ($min^{1/2}$)	Amount released
0	0	0	0
8	12.3	2.87	151
15	19.8	3.87	392
45	43.4	6.71	1884
78	59.4	8.83	3528
96	67	9.80	4489
128	75.5	11.31	5700
164	80	12.8	6400
216	84.9	14.7	7209
276	87.7	16.6	7691

Source: Data from Fessi et al. (1982).

Certain products are not porous but depend on the dissolution of the drug to create the porosity. In such cases there is a minimum drug content necessary for creating a porous network, and some of the drug will be occluded, i.e., will never release. A practical minimum is about 20% drug is such cases.

The derivation of Eq. (10.59) is based on the assumption that the penetration of liquid is faster than the dissolution of the drug. If, e.g., the contact angle (wettability) changes with storage (e.g., due to moisture redistribution), then this assumption could be rendered false.

Equation (10.59) applies only as long as there is undissolved material in the matrix (and until liquid has penetrated into the center of the tablet) The parameters K_i and t_i may be monitored at various periods of room temperature storage time. In the case of insoluble matrices, accelerated studies might be possible in certain instances (i.e., when neither matrix nor drug changes physically at the higher temperatures). Table 8 gives an example of Eq. (10.62).

These data are depicted graphically in Fig. 34. It is seen that the least squares fit is given by

$$Q^2 = -2.55 + 0.477t \tag{10.63}$$

when the linear points are used. [These are, again, obtained by successively omitting terminal points (beginning and end) until the best linear fit is obtained.] It is noted that the first two and the last three points have been omitted, i.e., $t_i = 2.55/0.477 = 5$ minutes and t_f (from the best, high point omitted) is 128 minutes. Again, 75.5% are released at this point, and this is quite characteristic, and it calculates out well for most such dosage forms as the point dissolved at the time the tablet has filled up with dissolution medium.

Curing of the product is at times necessary. The work of Omelczuk and McGinnity (1993) has, for instance, shown that matrix tablets containing poly(DL-lactic acid) change release pattern if thermally cured. Drug release from

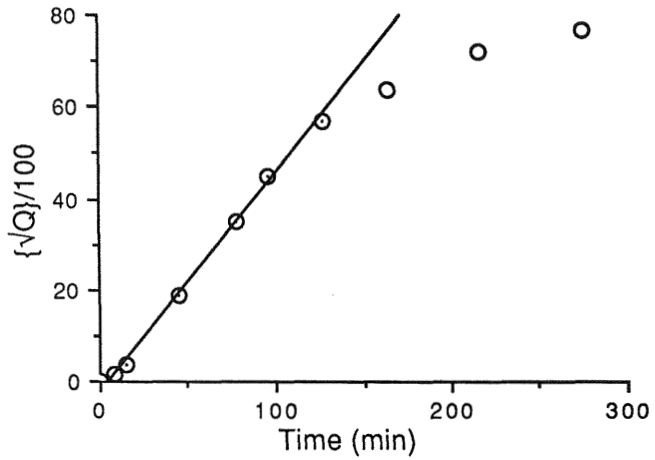


Fig. 34 Dissolution pattern in matrix dissolution. (Graph constructed from data published by Fessi et al., 1982.)

the tablets is neatly square root of time dependent as predicted by the Higuchi equation (Higuchi, 1962) shown in Eq. (10.58).

9.4. Osmotic Pump

The osmotic pump is a tablet coated with an impervious film, into which is (laser-)drilled a hole of exacting dimensions. Dissolution liquid will penetrate into the interior of the tablet, and a saturated solution will form. The excipients are chosen so that they have a given solubility and hence produce a given osmotic pressure. (The drug itself contributes to this as well.) This will be larger than the osmotic pressure in the outside liquid, and the difference between the osmotic pressure inside and outside will be the driving force by which liquid is being forced out through the hole. This gives rise to zero-order kinetics (which biopharmaceutically is an advantage), and the osmotic pump in many experimental situations, as well as in marketed situations, seems to be the dosage form that gives the most desirable release pattern, and also the one most likely to give in-vivo results that are predictable from in-vitro data. Dissolution data will therefore plot linearly, when amount released is plotted as a function of time. There may be a small nonzero (negative y-) intercept, i.e., a lag time. There will also be a point in time when there is no more solid drug inside the tablet, and deviations from linearity will occur from this point on.

There is no literature published on the stability pattern of this type of dosage form, but it is to be expected that it would be no more (and probably less) prone to change on storage than the other types mentioned.

9.5. Gel Forms

There are sustained release tablets that rely on gel-forming substances to accomplish the sustained release. In these cases the dissolution liquid will form a gel when it

encounters the surface of the tablet. Drug must dissolve and diffuse out through this gel layer. As time goes on, the gel layer gets thicker, and the diffusion path becomes longer. The data can be represented by Eq. (14.49) (Bamba et al., 1979). There is also the possibility of some "sloughing off" (i.e., erosion) of gel, and in this case the release becomes a hybrid between erosion and diffusion through increasing thickness of gel.

10. COATED TABLETS

Tablets are often film coated and, less frequently, they are sugar coated. Sugar coating, when properly applied, provides an excellent moisture and quite an adequate oxygen barrier. Film coating does the same, but not quite as effectively. For instance, vitamin A beadlets are more stable in a (properly made) coated tablet, less so (but yet quite stable) in a film coated tablet, of course provided there are no drastic incompatibilities in the core.

Film coating offers many advantages and often is the coat of preference, because (a) its application is much less labor intensive (cycle times being in hours for film coated tablets, in days for sugar coated tablets); (b) they also provide the advantage of allowing an engraving to "show through," i.e., identification requires no extra operation. On the contrary, sugar coated tablets, for identification, require a separate printing step; and (c) there is an inherent advantage in film coating in that it allows the appearance of a deep color without the use of much dye. If an uncoated tablet is colored, the dye is present throughout the tablet, whereas in a film coated tablet it is only present in the outer layer (the film itself).

Enteric coated tablets belong in the category of coated tablets and will be treated below as well.

10.1. Film Coated Tablets

Film coated tablets are produced either in a coating pan or by column coating (Wurster coating). Most coatings, nowadays, are aqueous film coats (hydroxymethyl cellulose, hydroxypropyl methylcellulose). There are several types of defects that can occur originally (orange peel effect for instance). All coatings, essentially, are such that each applied coat is not complete, so that there are overlaps, and in essence there is always an orange peel effect, except in a "good" tablet this cannot be seen. It is simply assumed in this writing, that the tablets placed on stability are not defective.

From a stability point of view there may be changes in appearance, mostly due to dislodging or rupture of the film. Sometimes these changes are first seen in the engraving. To properly record changes in appearance of the film, descriptive means can be used, but it is often a good idea to take a photomicrograph originally of all coated tablets (be they sugar or film coated). If defects show up in the coating as a function of time, then the question arises whether this is due to the formula (film and uncoated tablet) or to the way in which it was made (initial defective procedure, possibly not noticeable). Most often, these problems result in efforts in the formulation area, and recording (visually or photographically) at many intervals (3, 6, 9, 12 months) is therefore advisable. In this manner reformulation can be carried out as soon as the problem is identified.

One property that should be monitored, both for film and for sugar coated tablets, is their gloss. This is usually done subjectively. Rowe (1987) has described a glossmeter that assesses the gloss, but points out that there is still a great deal of subjectivity in the use of it.

As a problem-solving tool, scanning electron microscopy is advised, because of the augmented detail it offers, a detail that often pinpoints the individual problem.

In some formulation setups, it is possible (e.g., with an Instron tester) to measure the force necessary to strip a film from a substrate. If this substrate is the tablet surface, it is possible to evaluate films, moisture contents, effect of additives, etc., to ascertain which is the proper way in which to reformulate the film.

The actual appearance (i.e., the color) of the film coated tablet can be checked by means of a reflectance meter (or by diffuse reflectance), as described in the previous section.

Dissolution and disintegration are, of course, sensitive parameters, because any change in the film will be reflected in these properties.

10.2. Sugar Coated Tablets

Detailed descriptions of sugar coating procedures are beyond the scope of this writing. In brief, in sugar coated tablets there is applied (usually in a coating pan) first a barrier coat (frequently shellac or other polymer), then a subcoat (frequently terra alba/gelatin, with talc used as a conspergent), then a dye coat (consisting usually of sucrose syrup and lake dye), then a finishing coat (usually sugar syrup), and finally a polish coat (usually beeswax either dry or in solvent solution). The latter is carried out in a canvas coated coating pan.

The typical defects on storage are chipped tablets and tablets that split in the periphery. The former can be tested for by using a friabilator test. In such cases, a correlation with an actual shipping test should be attempted. In such a shipping test, tablets are sent by various routes (rail, truck, air) from the plant to several destinations and then back again. In so doing, it is possible to observe whether the artificial stress test is comparable to the actual transportation test.

When tablets split in the periphery it is usually due to trapped moisture (i.e., the tablet may not have been quite dry at the time one of the coats was applied). Very often it is due to an improperly applied barrier coat.

Again, photomicrography (and in problem cases, scanning electron microscopy) is advocated as a reference for changes in appearance. The actual appearance (i.e., the color) of the coat can be checked by means of a reflectance meter (or by diffuse reflectance), as described in the previous section.

Dissolution and disintegration are, of course, sensitive parameters, because any change in the film will reflect in these properties.

10.3. Enteric Coated Tablets

An enteric coat is an attempt "to administer two doses in one tablet." This is done by placing an acid resistant film (e.g., a polymer containing a carboxyl group with a pK of 4–6) on an uncoated tablet and then sugar coating it. The first dose is contained in the core, and the second dose is applied in the sugar coat, which should release the material immediately.

Enteric coating is a delicate operation, and often there is, in the production write-up, a statement that in-vitro dissolution must be carried out after e.g., the seventh coat. The e.g. eighth coat may then be applied or not depending on the outcome of the in-vitro test. This latter is usually the USP test that calls for placing one tablet in each of the six tubes of the basket in water at room temperature for 5 minutes. The apparatus is then operated without discs in simulated gastric fluid at 37°C. After one hour the basket is removed and the tablets are observed, and they should show no sign of disintegration, softening, or cracking.

Next a disk is added to each tube, and the apparatus is filled with simulated intestinal fluid TS at 37°C for 2 hours (or whatever the monograph or the in-vitro to in-vivo relation calls for). If all of the tablets have disintegrated at the prescribed endpoint time, then the batch is acceptable, but if one or two tablets fail, then it is retested sequentially by testing an additional 12 tablets, all of which must pass.

Enteric coats (e.g., cellulose acetate phthalate) have tendencies to polymerize. (Shellac is particularly vulnerable in this respect.) Hence disintegration on storage should be monitored at all intervals (3, 6, 9, 12, 18, 24, and 36 months).

It is noted that the initial dose (in the coat) should be available immediately, and a check should be made (one or two points) to assure that disintegration of the coat also results in dissolution of the drug (which it usually does).

The behavior at accelerated temperatures is not necessarily indicative of (nor extrapolable to) room-temperature characteristics.

11. HARD AND SOFT SHELL CAPSULES

Grimm and Schepky (1980a) have demonstrated how, depending on the sorption isotherms of the capsule fill, a capsule shell can lose moisture to the capsule fill and become brittle, or conversely under opposite sorption isotherm conditions can draw moisture out of the fill and become soft.

As mentioned earlier, dissolution rate of the gelatin decreases in water. HCl, and aqueous buffer solution on storage, but gastric juice containing enzymes might well eliminate such a problem. A thorough review of the problem with cross-linking of gelatin and the occurrence of pellicule formation has been discussed by Digenis et al. (1994).

Ofner and Schott (1987) have studied the swelling of gelatin (Fig. 35) and have applied Eq. (10.64) to their considerations. If W grams of aqueous buffer solution is absorbed by 1 gram of gelatin at time t, then

$$\frac{t}{W} = A + Bt \tag{10.64}$$

where A and B are constants. The effect of additives can then be studied.

Vastly different behavior of gelatin was experienced with different drugs. This, obviously, is a powerful preformulation tool (when combined with data regarding the hygroscopicity of the drug, as demonstrated e.g. in Example 10.3).

York (1981) has reported on the moisture isotherms of gelatins. Knowing the moisture isotherm of the powder mixture in the gelatin, it is possible (as shown in the previous section dealing with tablets) to calculate the shift in moisture from shell to powder mixture (or vice-versa).

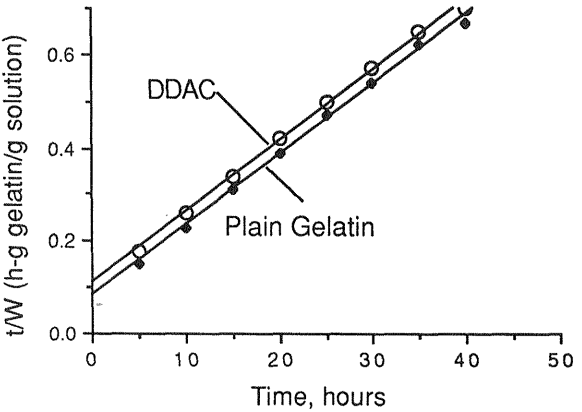


Fig. 35 Swelling isotherms of gelatin.

12. MICROCAPSULES

Microcapsules may decompose as a function of time. This has been reported by Makine et al. (1987) for the case of poly(L-lactide) microcapsules. Logical means of estimating the loss of intact polymer is (a) from the decrease in weight-averaged molecular weight, (b) by monitoring the loss in weight of polymer by gel permeation chromatography, and (c) by determining the amount of lactic acid formed. Figure 36 is an example of the decrease in weight-averaged molecular weight upon storage.

13. LIGHT SENSITIVITY TESTING

Both the ICH and the 1987 Guidelines advocate exposure of dosage forms to UV light, and although this might be instructive, it does not represent a test that simulates conditions in actual commerce (in general). There are exceptions: certain products are liable to be kept in handbags and kept out in the open, but these are the exception. In general products are considered to be kept in controlled plant environments, in warehouses or in controlled pharmacy conditions or in (short) transit.

To define a storage condition it is necessary to examine the actual conditions in the marketplace, and this has been done by Esselen and Barnby (1939), Lachman and Cooper (1959a, 1959b), and Lachman et al. (1960). They determined the spectral composition of light and light intensity in the typical American pharmacy, and in general it is assumed that the average foot-candles in a pharmacy is 5–15, and 10 is used as an average.

One could now proceed by checking a product for three years under such conditions, but rather than do that, it is desirable to accelerate the conditions so as to obtain an answer somewhat more rapidly. The guidelines' suggestion of using more energetic (UV) light is not good for such acceleratory attempts, because the more energetic light will (or may) give rise to reactions that would never take place in the light in a pharmacy (which is much more poor in ultraviolet light).

Lachman and Cooper determined that a #48 12 CWRS GE lamp 1.5″ in diameter and 48″ long produced a good average spectrum and produced 3250 lumens per

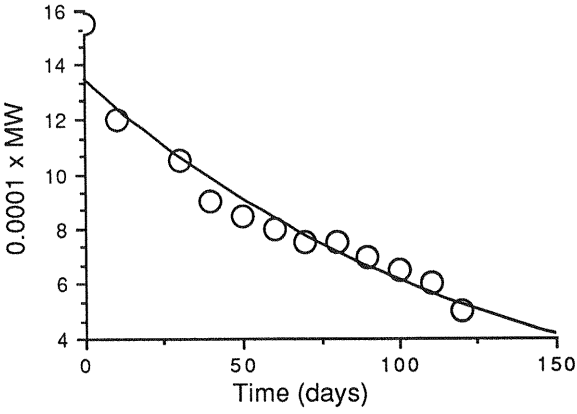


Fig. 36 Weight-averaged molecular weight of poly(L-lactide) microcapsules. (Figure constructed from data reported by Makine et al., 1987.)

Table 8 Appearance Change of a Tablet as a Function of Time in a Lachman–Cooper Light Cabinet

Storage (weeks)	Open dish	Amber bottle
0	0.302	0.302
4	0.212	0.315
8	0.188	0.312
12	0.190	0.309

60 watts. They suggested accelerating the test by increasing the lumen reached by the dosage form, and they increased this by placing it closer to the light source. The light quanta absorbed by the dosage form are inversely proportional to distance (d) of the dosage form from the light source squared, i.e., proportional to $1/d^2$. They describe a light cabinet, equipped with shelves close to the described light source, adequately ventilated so that the temperature does not rise substantially. Light meters on the shelves allow either movement of the shelf so that the light intensity is always the same, or simply serves as a device to control when the bulbs should be changed.

If used as described, 1 month in the light cabinet is equivalent to 24 months on the average pharmacist's average shelf. A typical set of data of the discoloration of a dyed tablet (as gauged by optical density) is shown in Table 8.

It is seen that the cabinet allows for evaluation of the means of preventing photoinduced changes or reactions to take place (use of the amber bottle). This could be accomplished by other means as well (coating of a tablet, using an opaque rather than a transparent capsule, using a capsule with a dye that screens out the part of the spectrum that causes the photoreaction).

In the case of uncoated tablets, most often the dye will discolor or fade in the upper layer only. If the tablet is broken, the color is still intact on the inside. If it fades all the way through, then this is indicative of photoinduced interaction. In the case of ethinyl estradiol and an FDC dye, Kaminski et al. (1979) showed

interaction, but this interaction was first noticed in a light test and was photocatalyzed.

The regulations on light testing suggest the use of xenon lamps. In these a very high intensity light is applied in a very short period of time (minutes), and the effect on the dosage form (and the drug and the drug product) is recorded. The correlation of this with "real time and exposure" is unknown, and a database will have to be established before it can be rationally analyzed.

14. DIAGNOSTIC PAPERS

There is very little in literature regarding the stability of diagnostic product. In the case of diagnostic papers, the reagent is adsorbed on filter paper. The adsorption is usually governed by a Langmuir isotherm; there will, however, be active sites on the paper, and these may bind part (often a large percentage) of the reagent. Hence there will be an initial "loss" of reagent, and this will have to be compensated for by excesses, since only the reagent that is not chemisorbed will be available for the reaction in the diagnosis.

Stability data are gauged on the basis of initial assay (not theoretical content). Usually the stability is evaluated in a semiquantitative manner by an in-use test, i.e., an operator will carry out the diagnosis initially. For example, if it is a test that monitors sugar content (e.g., in urine), the filter paper will be judged against sucrose solutions of various concentrations. The instructions may state that the reaction is "positive" if a certain color is achieved (i.e., the concentration of sucrose is above a level, L) and negative if the response level is below another concentration, L^*, where $L^* < L$. The area in between is then "doubtful," and this would call for a retest. Initially the test should evoke the correct response (since the batch is, presumably, quality control released). As time goes by there will be a certain decomposition, which will vary from strip to strip, so at time t, a fraction of all the strips, q, may give an incorrect response. The best parameter to follow, stability-wise, is this parameter q, which should be such that it could be said at the expiration date with 95% confidence that q is below 5%.

15. EXPIRATION PERIODS

It was shown in the chapter dealing with statistics and expirations periods that there are mathematical means of calculating expiration periods from chemical stability data. This is not directly possible with physical testing. The reason for this is often the difficulty that exists in quantitating the physical property. Davis et al. (1977) state broadly that "the physicochemical changes that can occur ... upon storage or after processing or other external influence, should not be such that they can alter the therapeutic efficacy of the product." This is a good guideline, but the only way to test it is somehow to transform the experimental data into some quantity that can be extrapolated.

For instance, a suspension may start caking, but there are degrees of caking, and if it still can be shaken up in a reasonable length of time, then it should be all right. Here, a criterion must be set by the investigator, the quality control group, and the regulatory group within an organization. Such a criterion can be set up much like a test panel. Several containers of different degrees of caking can be

evaluated, and the "worst acceptable" (akin to the lower acceptable quality limit in release philosophy) agreed to. The investigator now can do a rotation test on this in the fashion described by Moore and Lemberger (1963), and state e.g. that after 20 controlled rotations, an assay of the supernatant must not be less than L mg/mL. This type of test can then be carried out at various storage times at room temperature; L can be plotted versus time, and a usual statistical test performed on this number. In other words, for physical testing that has no number associated with it, it is important to attempt to find such a number.

In some cases this is not possible. A case in point is the particle size of an intravenous oil emulsion. Coalescence and formation of free oil will result in toxic manifestations, but it is next to impossible to determine an acceptable upper limit for droplet size (Davis, 1987).

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11

Development and Validation of HPLC Stability-Indicating Assays

DONALD D. HONG

Pharmaceutical Consultant, Raleigh, North Carolina

MUMTAZ SHAH

Trigen Laboratories, Salisbury, Maryland

Part I: Method Development

Part I: Method Development

1. WHAT IS A STABILITY-INDICATING METHOD?

According to the regulatory definition (1), a stability-indicating method is one of a number of

> Quantitative analytical methods that are based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured.

Therefore a stability-indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredient (API) from any degradation (decomposition) product(s) formed under defined storage conditions during the stability evaluation period. In addition, it must also be sufficiently sensitive to detect and quantify one or more degradation products. A corollary may be added that the analytical method must be also capable of separating or resolving any other potential interfering peak such as an internal standard. With these criteria, then, the discriminating "nature" of the method indicates the method to be *stability-indicating* as well as *stability-specific*. Later in the discussion we will see that other methods may be stability-specific but not stability-indicating. Stressed testing may be used (1,2) to expedite the decomposition pathway(s) to generate decomposition product(s) for the API. However, stressed testing under forced conditions of oxidation, photolysis, hydrolysis, and varying pH values may form some decomposition products that are unlikely to be formed under accelerated or long-term stability storage conditions. The products generated nonetheless may be useful in developing and validating a suitable stability-indicating analytical method for the analysis of the drug substance and the drug product, expediting the availability of the completed analytical method.

It is paramount that the chosen analytical method used for stability evaluation be validated and discriminating to ensure efficacy of the subsequent stability evaluation. Confidence in the stability data is predicative on time invested up front to ensure a viable procedure as well as to conform to legal and regulatory requirements (2).

2. STRATEGY OF METHOD DEVELOPMENT

Development of a stability-indicating method should be predicated on the method's intended application as well as selecting a suitable technique designed to assess

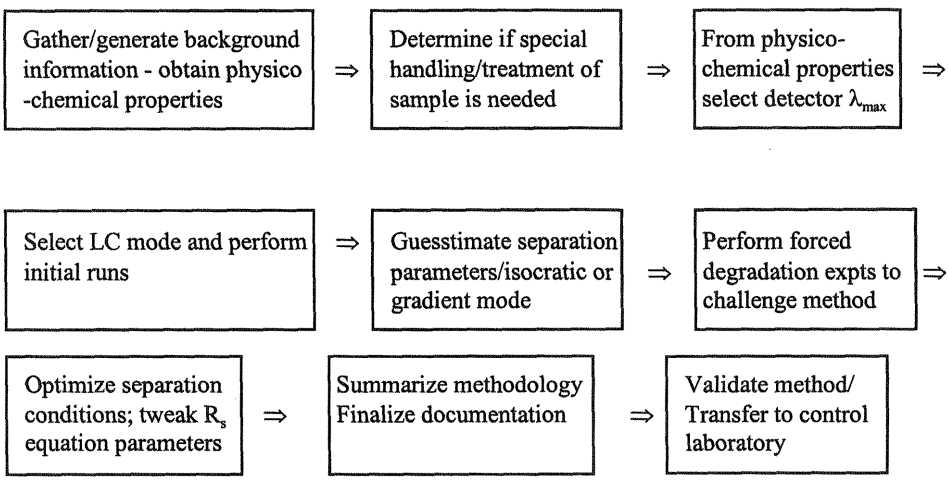
the API's stability requirements. Obviously the intended application of a stability-indicating method is for monitoring the stability of a given drug in a finished product and would require assessment of the method's stability-indicating properties. One specialty application of a stability-indicating method is cleaning validation testing, which would require assessment of its stability-indicating properties, as holding time (of the swaps) would be a critical factor. Other applications such as product release, performance testing (i.e., dissolution testing) and in-process testing do not require this assessment. Some pharmaceutical houses still (but now less commonly due to technological advances and overall industry practice) utilize a non-stability-indicating method such as UV, for product release, and an HPLC method for stability testing. However, whenever there is a hold time issue, common in dissolution or in-process testing, it would be prudent to assess the method for its stability-indicating properties before its intended application.

Other chromatographic separation methods, such as chiral chromatography (CC), thin-layer chromatography (TLC), gas chromatography (GC), and (increasingly) capillary electrophoresis (CE), are stability-indicating and stability-specific methods. Still the most prevalent technique is reversed-phase HPLC alone or coupled with ion-suppression, which accounts for 85% or more of the general pharmaceutical applications.

Nonchromatographic and spectroscopic techniques such as titrimetry, atomic absorption, UV spectrophotometry, and infrared spectroscopy, while precise, are not considered stability-indicating, and as such not suitable for stability assessment applications.

3. OVERVIEW OF THE METHOD DEVELOPMENT PROCESS

Before beginning with actual experimentation it would be advantageous to view method development from a broader perspective. The method development process can be visualized from a high-level process map perspective better to define the general steps encountered to achieving the end product, a stability-indicating method.



4. GETTING STARTED

It is probably best to approach method development with the intention of using the developed method for stability assessment as a final application, after the method has been validated. This approach entails determining the discriminating ability of the selected method up front before investing time and money in evaluating other analytical parameters prior to assessing the stability-indicating element of the method.

Reversed-phase HPLC is the method of choice for stability-indicating and stability-specific methods, although thin-layer chromatography (TLC), gas chromatography (GC), and capillary electrophoresis (CE) are also acceptable choices. Reversed-phase HPLC coupled with ionic suppression account for probably over 85% of stability-indicating methodologies for small molecular weight pharmaceutical entities. This combination is well suited for applications in release testing, in-process as well as stability testing. Additional applications may be in cleaning validation and performance testing. Other techniques such as titration and UV spectroscopy, while commonly used for release testing, are generally considered nonspecific and thus are not considered for stability assessment.

Invariably when one is faced with finding or developing a method, one or two routes may be used depending on the nature of the chemical entity: modification or development. Modification is used when there is information or a method already exists for a similar entity. In this case, the existing method is modified or tweaked to accommodate the new entity. This may or may not be suitable; if not, development (starting from scratch) is the way to go. The goals of the separation should also be considered at this point.

4.1. Background Information

Knowledge of physicochemical properties of the API is invaluable to the method development process. Information on the various properties has been collected, either through a systematic program of generating the appropriate information in support of drug discovery (organic chemistry synthesis) on the one hand, or on the other, from a search of the literature or from company drug profiles, spectral libraries, or reports. Information such as dissociation constants, partition coefficients, fluorescent properties (if any), chromatographic behavior, spectrophotometric properties, oxidation-reduction potentials, formulation stability studies, and solubility studies are all very useful and can expedite the development process.

Dissociation constant and partition coefficients can be used to develop efficient liquid/liquid extraction procedures, and data on fluorescence, spectrophotometric, chromatographic, and oxidation-reduction properties can be used to determine the best means of measuring and quantifying the analyte of interest. Stability studies are performed on the drug substance, in solution and mixed with pharmaceutical excipients as part of compatibility studies. Labile functional groups are identified, and the susceptibility of the drug to hydrolysis, oxidation, thermal degradation, etc. is determined. Compatibility studies are performed to assess the stability of the API when mixed with common excipients and lubricants as well as to determine any interaction between the drug and the (inactive) raw materials. Solubilities should

be determined in a number of solvents covering a range of polarities that are commonly used in method development.

Solubilities should be determined in aqueous and organic solvents, such as

Aqueous	*Organic*
Water	Ethanol/methanol
Buffers	Chloroform
0.1 N HCl	Cyclohexane
0.1 N NaOH	Acetonitrile
	Tetrahydrofuran

Spectral libraries are established, and information gleaned is useful for selection of initial conditions for an HPLC separation. On the other hand, however, sometimes this physicochemical information may not be known or available, so that an initial separation would have to be tried, based on prior experience, in order to determine a course of action for subsequent experimentation.

4.2. What Is Known About the Sample

Ideally, knowledge of the API's nature relative to composition and other properties would be beneficial. For example, information about the compound's synthetic route would shed light on any related product(s) and possible degradation product(s), as well as possible impurities; knowledge of the compound's chemical structure would reveal any possible stereoisomer which in turn would necessitate a different separation strategy, and so forth.

Table 1 shows typical information that would be helpful concerning the nature of the compound. The more information is available, the less empirical the approach to developing a separation method will be.

5. SEPARATION GOALS

To determine the separation goals, which should be clearly defined, a number of questions should be asked to help delineate the end purpose of the separation. Typical questions may include

> What is the overall purpose of the method—quantitative, qualitative, or for isolation/purification of a compound (i.e., content assay, stability, impurities, cleaning assay, or for purification application)?

Table 1 Useful Physicochemical/Related Information Concerning the Compound

Wavelength of absorption (λ_{max})
Identity/number of compounds present (i.e., stereoisomers/chiral centers?)
Chemical structure (functionality); amphoteric
Molecular weight
pK_a values of compounds
Salt form of the drug
Solubility of compound
Purity of compound

What level of accuracy and precision would be needed?
The method is designed for what type of matrix? How many types of sample matrices are encountered?
Is the method developed using certain equipment transferable to the control laboratory, which may not have the same equipment?
Will the method be used for a few samples or many samples?
What chromatographic parameters are needed?
How much resolution is needed?
What is a suitable/acceptable separation time?
What is a suitable column pressure?
How much sensitivity is required?
Is an internal standard needed?
Are there any detection issues? Most analytes absorb in the UV region of the spectrum.
Does integration use peak area or height?
Is the mode isocratic or gradient?

6. SELECTION OF THE CHROMATOGRAPHIC MODE

6.1. The Different Modes of Liquid Chromatographic Methods (HPLC)

While there are a number of HPLC methods available to the development chemist, perhaps the most commonly applied method is reversed-phase. Reversed-phase and reversed-phase coupled with ion-pairing probably account for more than 85% of the applications for a typical pharmaceutical compound. The typical pharmaceutical compound is considered to be an API of less than 1,000 daltons, either soluble in water or in an organic solvent. The water-soluble API is further differentiated as ionic or nonionic which can be separated by reversed-phase. Similarly, the organic soluble API can be classed as polar and nonpolar and equally separated by reversed-phase. In some cases, the non-polar API may have to be separated using adsorption or normal phase HPLC, in which case the mobile phase would be a nonpolar organic solvent. For those "special" compounds that do not fall into this category (API>1000 daltons [biopharmaceuticals], isomers or enantiomers), other chromatographic modes may be necessary for separation. These include ion-exchange and chiral chromatography. In this discussion of developing a stability-indicating HPLC method, only reversed-phase will be discussed.

6.2. Reversed-Phase Chromatography

Thus given the limited number of methods with stability-indicating properties, it is probable that the method selected would be HPLC. Two very advantageous characteristics of HPLC, its discriminating power and its ability to operate at room temperature or at low elevated temperature, would not contribute to the degradation of the analyte. It is further assumed that the API is of low molecular weight (<1000 daltons), organic in nature (versus inorganic), and not a biopharmaceutical. These restrictions apply to a large percentage of the pharmaceuticals and enable them to be readily separated using reversed-phase HPLC, and sometimes with the aid of an ion-suppression agent, in roughly 85%

of the applications. The next question, then, is whether the chromatographic mode would be isocratic or gradient (see Fig. 3, Sec. 15).

6.3. Chiral Chromatography

Within this decade, since 1992, the FDA has published a position paper on the development of new stereoisomeric drugs (3). Prior to this time the majority of chiral synthetic compounds were marketed as racemic mixtures. This is because, until recently, it was not technically possible or economically feasible to separate racemic mixtures into their individual enantiomers. Experience has indicated that the individual enantiomers may exhibit different therapeutic effects. For example, the R-enantiomer of sotalol is antiarrhythmic while the S-enantiomer is a beta-blocker (4); and the dextro isomer *d*-propoxyphene (Darvon®, Lilly) is analgesic while the levo isomer *l*-propoxyphene is antitussive (but never developed into a marketed product) (5). However, with the FDA's position paper and current technological advances such as large-scale chiral separation techniques and asymmetric syntheses, new chemical entities (NCEs) containing a chiral center must be resolved into the different enantiomers and each enatiomer characterized and the drug product be composed of only one enantiomer instead of a racemate.

Thus, as contained in the International Conference on Harmonization (ICH) draft guideline on drug/drug product specifications (6), the tests in the table must be satisfied for new drug substances that are optically active:

Drug substance	*Test/specification requirement*
Impurities	Similar to other impurities
Assay	Enantioselective procedure or achiral method with appropriate means to control enantiomeric impurity
Identity	Test(s) should discriminate the enantiomers
Drug Product	
Degradation products	Control of other enantiomer if that enantiomer is a degradation product
Assay	If enantiomer is not a degradation product, an achiral method is acceptable, but chiral assay is preferred, or alternatively, achiral assay plus means to control the presence of the enantiomer
Identity	Test to verify the presence of the correct enantiomer

As such, in the development of a chiral method, the regulatory requirements must be considered. The reader is referred to decision tree #5 (page 62903) of the same reference for a schematic guide to development strategy and to the Wozniak (7) paper to determine what additional analytical information is needed for the development of chiral drug products.

6.4. Gas Chromatography

Gas chromatography, while *stability-indicating*, is not as versatile as HPLC, as the drug substance may not be volatile. On the other hand, increasing the temperature

to effect volatility may cause degradation as well as effecting racemization. However, there may be a limited number of instances in which this technique would be useful, such as for small nonaromatic compounds that simple are not possible to separate by current HPLC and TLC techniques.

6.5. Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a mature chromatographic technique and is still widely used throughout the pharmaceutical industry in research as well as in the control laboratory. It is used throughout the drug development process for determining the purity of the drug substance, reference standards, and intermediates. It possesses many advantages including simplicity, low cost, and a short run time. It is cost effective. Its main disadvantage is variability. Constanzo (8) has proposed a three-point window approach to optimize resolution, and thus to minimize the variability, by controlling the mobile phase composition.

TLC (limit test) is used to complement a non-stability-indicating procedure as indicated in the FDA Guideline for Submitting Samples and Analytical Data for Methods Validation (2).

6.6. Capillary Electrophoresis and Capillary Electrochromatography

As sciences, both capillary electrophoresis (CE) and capillary electrochromatography (CEC) today are probably where HPLC was 10 years ago. CE is a separations technique based on the mobility of ions through a buffer-filled capillary in an electrically charged environment. This would provide a separation of charged species. When CE is coupled with a stationary phase and high pressure, it is known as CEC, in which the separation is based on electrophoretic migration and chromatographic partitioning enabling the separation of neutral species. Both techniques are more applicable to biological systems, in biopharmaceutical and other R&D applications, than in quality assurance/product specification applications. The techniques are very sensitive and well suited for separations of small amounts of expensive biopharmaceuticals. On the other hand, they have less utility as a product release or stability test methodology, especially in product specification applications of small molecular entities where there is an abundance of samples and where sensitivity is not an issue.

The utility of the technique, however, lies in its ability to achieve high sensitivity and resolution through high efficiencies with minimal peak dispersion. Moffatt et al. (9) have reported unusually high efficiencies of up to 2.5 million plates per meter in the capillary electrochromatographic analysis of partially ionized anionic-neutral pyrimidine compounds using a standard C_{18} stationary phase.

The number of manufacturers of CE/CEC equipment are not nearly as many as for HPLC equipment. Major manufacturers include Unimicro Technologies, Thermo Bioanalysis, Beckman Coulter, and Micro-Tech Scientific. The last company's model Ultra-Plus II has an integrated, gradient capillary HPLC/CE/CEC system. This combination of gradient elution and electrophoretic migration provides a rapid analysis with high resolution (10).

7. ROLE OF FORCED DEGRADATION

7.1. Regulatory Basis

The 1987 edition of the FDA stability guidance document (1) stipulates that the API be subjected to a number of forced degradation conditions to include acidic, basic, and oxidative conditions. Workers in the field have also included temperature and light (photostability). The current draft stability guide (11), while not yet official, specifically includes photostability and temperature cycling requirements; no mention of acidic, basic, or oxidative conditions were made, however. The current ICH guidances (Q2A and Q2B) also do not specify how degradation studies are to be conducted; this was left to the discretion of the responsible companies.

7.2. Scientific Basis

Forced degradation should be one of the activities performed early in the development process to ensure that the method is discriminating before a lot of time, effort and money have been expended. The guidance documents do not indicate detailed conditions, so the conditions and interpretations are left up to the development scientist. Suggested forced degradative conditions are summarized in Table 2. Trial and error are needed to find the proper combination of stress agent concentration and time to effect a degradation, preferably in the 20–30% range. Depending on the API, not every stress agent may effect a degradation, but each agent has to be evaluated to determine whether degradation results.

Additional comments are warranted.

Adequate k'. The initially developed method should achieve a suitably retained peak, with a k' of about 4 to 10. This range allows a suitable time space in the chromatogram for degradants to elute before or after the active (major) peak. Since the polarity of the degradants relative to the major peak is not known, the k' of the major peak eluting in the middle of the chromatogram adds some assurance that the degradants would elute on either side of the main peak.

Degradation conditions. Unfortunately this is a trial and error process. Typical degradative conditions involve hydrolysis, photolysis, acid/base reactions, and temperature. The goal is to obtain about 20–30% degradation and not complete degradation of the active compound. Achieving 100% degradation would be too strenuous and could possibly cause secondary degradation, giving degradation products of the degradation product(s), which are not likely to be formed under normal storage conditions. Depending on the API, not all of the degradation conditions effect degradation, and after a reasonable effort (varying concentrations and time) to produce a degradation product with no success, one can move on to the next condition. For example, when chlorhexidine digluconate, an antimicrobial agent in mouthwash, was subjected to each of the above conditions, only degradants were isolated from heat, acid and light (12). While it was impervious to the other conditions, this was not known up front, so each of the conditions had to be tried.

Acid/base. Generally the concentration of the API is doubled to enable the reaction solution to be neutralized before injecting into the HPLC system to prevent damage to the silica-based chromatographic column.

Controls. Refer to Table 2. It is important that corresponding matrices and appropriate controls be treated in a similar fashion to identify possible interferences.

Table 2 Suggested Outline for Performing Forced Degradation Studies

Decide/select matrix for degradation

		Degradation conditions					
Product/matrix	Degradation	Acid	Base	Peroxide	Bisulfite	Photostability	Temperature
Product	Yes	√	√	√	√	√	√
Placebo/vehicle	Yes	√	√	√	√	√	√
API/raw material	Yes	√	√	√	√	√	√
Internal standard	No	—	—	—	—	—	—
Controls							
Product	No	—	—	—	—	—	—
API/Raw material	No	—	—	—	—	—	—
Blank solution	No	—	—	—	—	—	—

Decide/select degradation conditions/agents

Medium	Conditions*
1 N HCl, 10 mL	Reflux 30 minutes, neutralize with base
0.1 N NaOH, 10 mL	Reflux 30 minutes, neutralize with acid
3% Hydrogen peroxide, 10 mL	Reflux 30 minutes
10% Sodium bisulfite, 10 mL	Reflux 30 minutes
Light	Light chamber, 1 lumens (92.9 lux = 1000 ft-candles), 7 days
Temperature (dry heat)	80°C, 7 days

* Strive for 20–30% degradation.

Internal standard. Should the analytical method utilize an internal standard, it is not recommended to degrade the internal standard, but its k' should not interfere (elute) at any of the possible eluting k' peaks.

Evaluation of the degradation mixture is generally performed using a photodiode array detector. Assessing the purity of the major peak is very important and could be difficult in light of possible peak inhomogeneity after the degradation process. One must be assured that there is no degradation peak (hiding) under or unresolved from the major peak of interest. The utility of the diode-array detector is that the analyst can select a whole wavelength range, say from 200 to 350 mm, with a bandwidth of 80 nm. With just one single chromatographic run, all compounds absorbing within this range will be detected. With only one wavelength selected using a conventional UV detector, for instance at 280 nm, any compound not absorbing at this wavelength will not be detected. Figures 1A and 1B depict diode-array chromatograms for assessing peak purity.

Refer to Section 17.4 for further discussion.

8. PEAK PURITY

There is always that nagging question of whether the peak of interest (the major analyte peak) is pure or homogeneous. This is a difficult question, and many investigators have tried to prove the homogeneity of the major peak under stressed conditions during the method development and validation process. Various techniques have been used to characterize peak homogeneity, such as spectral suppression, absorbance ratio, spectral overlay (13), electrospray mass spectrometry (14,15) and dual detection (16).

9. SAMPLE PREPARATION

Sample preparation is a critical step in the overall chromatographic process, and can affect the chromatography if not developed or treated properly. This step encompasses sample filtration, sample extraction as well as sample derivatization, although the latter is not commonly used in the pharmaceutical quality laboratory. The purpose of this step is to prepare the sample so that the drug substance can be readily chromatographed, separated from other materials. Thus, it is a step to remove any interferences, to enhance the detection of the drug substance as well as to protect or enhance the life of the analytical column.

The following considerations are noted:

What is the matrix?
Ensure complete dissolution of the analyte in mobile phase or weaker solvent.
Miscibility and solubility.
Does the analyte precipitate in the buffer?
Some typical treatment modes are
- Direct injection
- Dilution
- Sonication
- Shaking

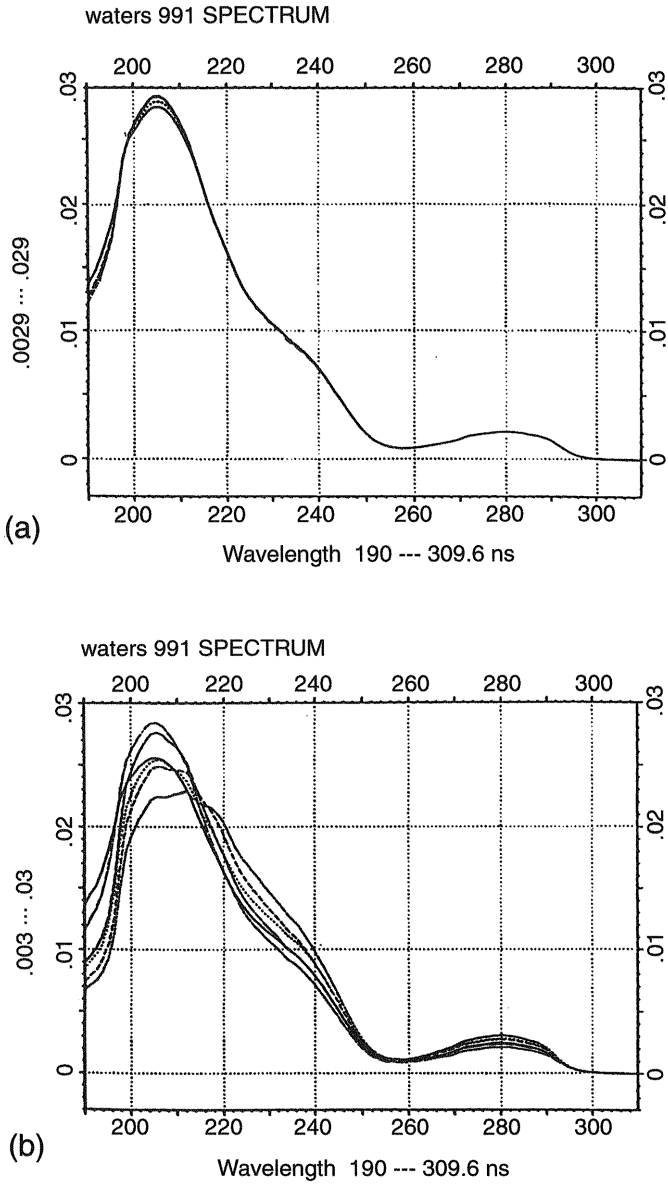


Figure 11.1 (a) No degradation (scans superimposable). (b) Presence of degradation (scans not superimposable).

Filtration/ultrafiltration
Extraction—Liquid or solid phase
Evaporation
Reconstitution
Derivatization
Heating/cooling

On rare occasions, the drug product, in a solution package form, may be injected directly or after an appropriate dilution. Typically, for a solid, such as a tablet or a capsule, the pretreatment would necessitate a comminution step, followed by extraction/sonication, filtration, and dilution. For example, an ointment may necessitate an extraction followed by evaporation, reconstitution, and dilution, or heating to dissolve the analyte/matrix, followed by cooling to precipitate the matrix, and then filtration.

While the typical dosage form—solid (tablet/capsule), semisolid (ointment/cream), or solution (cough syrup/ophthalmic solution)—utilizes a combination of the treatment modes mentioned earlier, solid-phase extraction (SPE) has become a recognized and viable technique for sample preparation methodologies, especially for biosamples and as an alternative to liquid-liquid extractions in many U.S. Environmental Protection Agency (EPA) methods. A recent supplement to *LC/GC* magazine was dedicated to advances in SPE (17).

It is very important that the sample preparation, prior to injection into the liquid chromatograph, be freed of particulate matter, through either filtration or centrifugation, and that the solvent be compatible with the HPLC system. If there is incomplete sample solubility or if the solvent is too polar, band distortions or tailing will result. Ensuring that the sample is completely dissolved in the proper solvent and then diluting the sample in mobile phase will eliminate these problems.

10. DEVELOPING THE SEPARATION—CHOOSING THE EXPERIMENTAL CONDITIONS

From Sec. 5, we assume that separation goals have been determined, such as resolution (at least baseline), reasonable run time (under 10 minutes), and ruggedness. These elements are further discussed below and developed in greater depth in Part II of this chapter under Validation. From Sec. 6.2 above, a case has been made that reversed-phase HPLC is suitable for our API of interest. The next step is to determine whether the API is typical. Referring to Secs. 6.1 and 6.2 above, let us further assume that the API is ionic and acidic. From a listing of generic separation conditions, see Table 3, conditions for an ionic and acidic compound are selected, and an initial exploratory run using gradient elution is made.

At this point, two options may be available to us before performing the exploratory run in the development of the desired stability-indicating procedure. First, there may be a method, either in-house or from the literature, already available for the same API or compound of interest. Useful information may be gleaned from here to modify to suit the specific compound on hand. On the other hand, sometimes established methods may not be optimal, so rather than modifying the method to suit our need, it may be better in the long run to develop a new method that is optimal and rugged.

Exploratory runs can be done manually or with computer software. Both are trial and error methods, but the latter is more systematic, quicker, and requiring fewer injections. When and after an initial exploratory run has been performed, the chromatogram is evaluated before proceeding with the next injection, and subsequent adjustments are made to the mobile phase composition. Each subsequent injection is thus based on the previous conditions, so that after a number of injections

Table 3 General Experimental Conditions for an Initial HPLC Run

	Initial Parameters		
Chromatographic variables	Neutral compounds	Ionic-acidic compounds (carboxylic acids)	Ionic-basic compounds (amines)
Column			
Dimension (length, ID)	25 cm × 0.46 cm	25 cm × 0.46 cm	25 cm × 0.46 cm
Stationary phase	C_{18} or C_8	C_{18} or C_8	C_{18} or C_8
Particle size	10 μm or 5 μm	10 μm or 5 μm	10 μm or 5 μm
Mobile phase			
Solvents A and B	Buffer-acetonitrile	Buffer-acetonitrile	Buffer-acetonitrile
% B (organic) isocratic	50%	50%	50%
% B (organic) gradient	20%–80%	20%–80%	20%–80%
Buffer			
Type	Phosphate	Phosphate	Phosphate
Concentration	50 Mm	50 mM	50 mM
pH	3.0	3.0 & 7.5 (gradient)	3.0 & 7.5 (gradient)
Modifier	10 mM triethylamine and 1% acetic acid, if needed	1% acetic acid	25 mM Triethylamine
Flow rate	1.5–2.0 mL/minute	1.5–2.0 mL/minute	1.5–2.0 mL/minute
Temperature	Ambient to 35°C	Ambient to 35°C	Ambient to 35°C
Sample size			
Volume	10 μL – 25 μL	10 μL – 25 μL	10 μL – 25 μL
Mass	<100 mcg	<100 mcg	<100 mcg

the proper conditions can be found (18). Refer to Sec. 12 below further discussion on software method development.

10.1. Key Variables—Resolution Equation Parameters

In reversed-phase/ion-pair chromatography, there are essentially 8–10 key variables that affect the separation, as depicted in the resolution equation, R:

$$R = \frac{1}{4} \cdot N^{1/2} \cdot (\alpha - 1) \cdot \frac{k'}{1 + k'}$$

where N, α and k' are referred to as the efficiency, selectivity, and retention (capacity) factors, respectively affecting the resolution of the analyte from other components in the separation. The efficiency is affected by the nature of the column, and both selectivity and retention are affected by the solvent. Column variables include length, particle size, and flow. Solvent variables are the nature of the sample, the mobile phase, and the column surface, i.e., bonded-phase (adsorbent type) such as C18, phenyl or cyano, etc.

These key variables include mobile phase strength, solvent type, column type/size, pH, temperature, ion-pair reagent (type and concentration), buffer, and mobile phase flow rate.

10.2. Isocratic or Gradient Mode

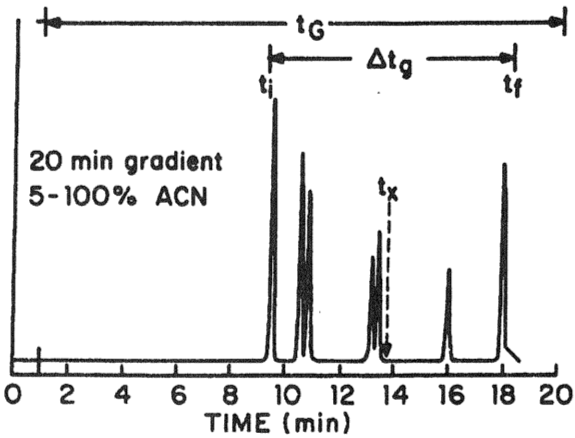
Either isocratic or gradient mode may be used to determine the initial conditions of the separation, following the suggested experimental conditions given in Table 3. Depending on the number of active components to be resolved or separated, the more complex the separation, the more gradient elution would be advantageous over isocratic mode, which is akin to a brute force application when trying to separate a complex mixture. When faced with developing a method to separate a complex mixture, the use of computer software is useful. This is further discussed in Sec. 12.

In deciding whether a gradient would be required or whether isocratic mode would be adequate, an initial gradient run is performed, and the ratio between the total gradient time and the difference in gradient time between the first and last component are calculated. When the calculated ratio is < 0.25, isocratic is adequate; when the ratio is > 0.25, gradient would be beneficial (19) as shown in Figure 2.

For complex mixtures (separations), when there are many degradation products, a long gradient run may be needed. In this case, a compromise may have to be made, using an isocratic method for product release and a gradient method for stability assessment. The isocratic method has generally a shorter run time, say under 15 minutes, and no degradation product would be monitored, assuming that none are formed initially. With time the degradation products are formed and must be monitored, which requires a gradient method to resolve completely the mixture (15 minutes and longer depending on the complexity of the degradation mix). The gradient method, then, would be the stability or regulatory method.

10.3. Role of pH

pH is another factor in the resolution equation that will affect the selectivity of the separation. In reversed-phase HPLC, sample retention increases when the analyte



$$t_x = (t_f + t_i)/2$$

$\Delta t_g/t_G > 0.25 \longrightarrow$ gradient

$\Delta t_g/t_G < 0.25 \longrightarrow$ isocratic

$$\Delta t_g/t_G = \frac{18.0-9.5}{20} = 0.425$$

$\therefore$ gradient required

Figure 11.2 Isocratic or gradient? (From Ref. 19.)

is more hydrophobic. Thus when an acid (HA) or base (B) is ionized (converted from the unionized free acid or base) it becomes more hydrophilic (less hydrophobic, more soluble in the aqueous phase) and less interactive with the column's binding sites.

$$\begin{aligned} HA &\Leftrightarrow H^+ + A^- \\ B + H^+ &\Leftrightarrow BH^+ \\ \text{Hydrophobic} &\quad \text{Hydrophilic} \\ \text{(more retained on column)} &\quad \text{(less retained on column)} \end{aligned}$$

As a result, the ionized analyte is less retained on the column, so that the k' is reduced, sometimes dramatically. When the $pH = pK_a$ for the analyte, it is half ionized, i.e., the concentrations of the ionized and unionized species are equal. As mostly all of the pH-caused changes in retention occur within ±1.5 pH units of the pK_a value, it is best to adjust the mobile phase to pH values at least ±1.5 pH units above or below the pK_a to ensure practically 100% unionization for retention purposes. The pH range most often encountered in reversed-phase HPLC is 1–8, normally considered as low pH (i.e., 1–4) and intermediate pH (i.e., 4–8). Generally, at low pH peak tailing is minimized and method ruggedness is maximized. On the other hand, operating in the intermediate range offers an advantage in increased analyte retention and selectivity. See Sec. 10.7 for further discussion. For a detailed

treatment of retention as a function of pH, the reader is referred to the works of Lewis et al. (20) and Schoenmakers and Tijssen (21).

As stated in Table 1, it is important to know the salt form of the drug substance of interest or whether it is amphoteric. This information is invaluable to the development of the analytical methodology because it will aid in the optimization of the method to effect better separation, resolution, and chromatography. If the drug is amphoteric, the pH can be selected whereby the compound exists as a single species and not a mixture of species. Mixed species will lead to poor separations. On the other hand, if the drug has different salt forms, say the hydrochloride and the napsylate (e.g., *d*-propoxyphene hydrochloride, Darvon®, and *d*-propoxyphene napsylate, Darvon-N®, both drug products marketed by Eli Lilly), the problem is not as critical, for

The salts represent different products and are marketed separately for different pharmacokinetic effects, i.e., different absorption profiles, with the hydrochloride being more soluble, and thus showing a faster absorption and distribution.

In solution, both salts will be dissociated from the organic propoxyphene moiety so that the final analytical methodology is appropriate for the separation and detection (or titration) of the analyte free base. For example, in the USP monographs for the two propoxyphene (hydrochloride and napsylate salts) APIs and their several products (22), the final analytical methods, be they titrimetric or chromatographic, all detect the analyte free base propoxyphene, and the assay percentage is calculated using a molecular weight correction factor.

10.4. Role of Solvent Type

Solvent type (methanol, acetonitrile, and THF) will affect selectivity similarly for ionic and neutral analytes. Hence changing a solvent would be a useful variable in the separation. The choice between methanol and acetonitrile may be dependent on the solubility of the analyte as well as the buffer used. While THF may be the least polar of the three, it has the highest solvent strength. If that property is not essential, its odor and potential peroxide formation may be a deterrent.

10.5. Role of Mobile Phase

The mobile phase composition (percent aqueous to organic) as well as the solvent strength will affect both α (solvent selectivity) and k' (solvent strength). The sample solvent will have a similar effect as well and may lead to peak distortion if the polarity between the mobile phase and the sample solvent is great. Thus, if at all possible, it is best to dissolve the sample in the mobile phase, if not, at least to make the final dilution in the mobile phase.

Chromatographic separations thus vary with solvent properties and are related to sample solubility, polarity, and solvent strength. Solvents that interact strongly with the sample will increase the sample solubility and decrease the chromatographic retention as more sample ions exist in the solvent and are not able to be in equilibrium with the adsorbent surface. Thus changing the organic solvent will change the selectivity. Polarity is the summation of dipole and hydrogen bonding

interactions, and in reversed-phase chromatography, less polar solvents exhibit greater solvent strength than polar solvents. The solvents water (most polar), methanol, acetonitrile, and tetrahydrofuran (THF) are placed in ascending order of polarity but reversed in their order of solvent strength.

These three organic solvents (methanol, acetonitrile, and THF) form the basis of the solvent selectivity triangle and exhibit differences in their relative interactions. They are also miscible with water and possess low viscosity and UV transparency. Collectively these three organic solvents along with water provide a four-solvent mobile phase optimization strategy. Each organic solvent in combination with water or water containing a buffer or additive(s) comprise the mobile phase. Sometimes the mobile phase may contain two organic solvents. The aqueous phase composition is commonly referred to % A and the organic phase as % B.

When the sample is eluted with a mobile phase of 100% B (organic), there is no separation, as the sample is eluted in the void volume. This is because the sample is not retained; but retention is observed when the mobile phase solvent strength is decreased to allow equilibrium competition of the solute molecules between the bonded phase and the mobile phase. When the separation is complex, that is, many components are to be separated, and when the solvent strength is decreased and there is still no resolution between two close peaks, another organic solvent of a different polarity or even a mixture of two organics may need to be tried to effect separation. Additionally, mobile phase optimization can be enhanced in combination with bonded phase optimization (i.e., substituting C18/C8 with cyano or phenyl). A goal for the band spacing of a solute (k') should be in the range of 4 to 9 and a run time of about 15 minutes or 20 minutes at most for most routine product release or stability runs.

10.6. Role of Buffer

When the mobile phase contains only water and organic solvent, it is recommended that the pH of the mobile phase be controlled by using a buffer to provide capacity. Thus when selecting a buffer for a given application, the following considerations are important:

The buffer capacity is dependent on pH, buffer pK_a, and buffer concentration.
UV absorbance—UV transparent to below or at the wavelength of the organic solvent.
Other properties, such as solubility and stability of the buffer and its reactivity to the analyte and hardware components of the chromatographic system.

The buffer concentration, or ionic strength, will affect the selectivity. An increase in the buffer concentration can lead to a decreased retention as the ionic interaction between the analyte and silanols are swapped out by the increased buffer concentration. When selecting a given buffer, additive, or even the solvent, sufficient regard for their compatibility with the analyte or HPLC system must be considered (23).

10.7. Role of the Ion-Pair Reagent

Initially, when deciding whether to select reversed-phase HPLC or reversed-phase HPLC with ion-pairing, a good rule of thumb is to consider the nature of the analyte

of interest. If the sample is neutral, begin with reversed-phase; and if the sample is ionic, use ion-pairing. Thus reversed-phase HPLC and reversed-phase HPLC with ion-pairing are similar except that the latter contains an ion-pair reagent in the mobile phase to improve the selectivity of ionic samples. The use of an ion-pair reagent is suggested only when separation is not adequate with reversed-phase HPLC. This is because using an ion-pair reagent introduces additional experimental parameters that need to be controlled, such as what ion-pair reagent to use and its concentration. Because of this added variable, reversed-phase HPLC should be utilized on any ionic analyte first before trying ion-pair reversed-phase HPLC.

The solubility of the ion-pair reagent may also be affected depending on the organic solvent used in the mobile phase. Methanol is generally preferred over acetonitrile or THF because it provides better solubility for the ion-pair reagent as well as for buffers and salts. In this case a suitable buffer is chosen, and at a concentration of about 25 mM, the pH and ion-pair reagent concentration are varied to provide optimal selectivity to the separation. These variables are not easily altered with commercial ion-pair "kits" as their pH and concentration have been standardized and ready to use.

Table 4 summarizes the types of ion-pair reagents and the conditions for their use.

The mechanism of retention imparted by the ion-pair reagent, such as an alkyl sulfonate, provides a change in the equilibrium between the ionized analyte and the ion-pair reagent that is attached to the silica adsorbent through the hydrophobic alkyl group and the negative charge of the sulfonate ion. The positively charged

Table 4 Listing of Types of Ion-Pair Reagents and Conditions of Use

Ion-pair reagent type and examples	Used for compound class	pH of mobile phase	Concentration of ion-pair reagent
Alkyl sulfonates (sulfonic acid alkyl salts)	Cationic samples (protonated bases)	3.5	0.005 M
Pentane sulfonate Hexane sulfonate Heptane sulfonate Octane sulfonate Examples PIC® B series (Waters) Q-Series (Regis)	Basic compounds		
Alkyl ammonium salts	Anionic samples (ionized acids)	7.5	0.005 M
Tetrabutylammonium phosphate Tetrabutylammonium hydrogen sulfate Examples: PIC® A series (Waters) Q-Series (Regis)	Acidic compounds		

(protonated) analyte ion competes for the negative site of the sulfonate ion. This altered equilibrium in effect imparts a change in the solubility of the analyte sample which in turn alters the retention as the analyte is now "attached" to the adsorbent so that it is eluted at a later time.

The pH of the mobile phase is closely associated with ion-pairing, whether the ion-pair reagent is positively charged (tetrabutylammonium, TBA^+) or negatively charged (C_5- or C_6- sulfonate/C_5- or C_6-SO_3^-), and dependent on whether the analyte is an acid or a base. Cationic samples (protonated base) or bases use the pentane, hexane, or a higher hydrocarbon sulfonate ion-pair reagent. Anionic samples (ionized acids) or acids commonly use tetraethylammonium or tetrabutylammonium hydroxide as the ion-pair reagent. Their optimization is pH dependent. For example, Waters® Chromatography ion-pair reagents operate in the low and intermediate pH ranges: PIC™ A (tetrabutylammonium phosphate) for acids operates at pH 7.5 and PIC™ B5 to B8 (pentane to octane sulfonic acid) for bases operate at pH 3.5.

For selection of the proper ion-pair reagent, alkyl chain lengths must be considered. The length of the alkyl chain enables selective separation of the analyte. The longer the chain, the more hydrophobic the counterion, and therefore, the greater the retention due to equilibrium between the counterion and the column adsorbent. Thus by selecting a reagent with a longer chain, selective solubility is obtained, enhancing the resolution.

10.8. Role of the Column

The HPLC column is the heart of the method, critical in performing the separation. The column must possess the selectivity, efficiency, and reproducibility to provide a good separation. All of these characteristics are dependent on the column manufacturer's production of good quality columns and packing materials. Properties of the silica (backbone) such as metal content and silanol activity produced in the manufacturing and bonding processes determine the properties of the finished bonded phase. A good silica and bonding process will provide the reproducible and symmetrical peaks necessary for accurate quantitation.

Commonly used reversed phases are C18 (octadecylsilane, USP L1), C8 (octylsilane, USP L7), phenyl (USP L11), and cyano (USP L18) (24). They are chemically different bonded phases and demonstrate significant changes in selectivity using the same mobile phase. Their properties vary from manufacturer to manufacturer, but given the state-of-the-art character of the vendor's manufacturing process, they show good quality control and provide batch-to-batch reproducibility. For example, no two L1 columns are the same, they vary from manufacturer to manufacturer relative to their pore volumes, pore sizes, surface areas, particle sizes (average range), carbon loads, whether end-capped or not, and the amount of bonded-phase coverage, as well as varying in their basicity and acidity characteristic. With state-of-the-art developments in column technology, most columns on the market exhibit good quality control and provide excellent column-to-column reproducibility and batch-to-batch reproducibility (25), and in some cases they give the chromatographer the option of using column selectivity as an alternative tool (besides mobile phase selectivity) to optimize the HPLC method development (26).

Column length also plays a role in the separation resolution. As column length changes, the efficiency (N) changes in direct proportion to the ratio of the column length (27). Resolution, as indicated in the resolution equation (vide supra), changes as a function of the square root of the change in N, and an estimate of the change in resolution as a function of column length can be approximated with the equation

$$R_{s2} = R_{s1} \cdot \left(\frac{L_2}{L_1}\right)^{1/2}$$

where R_{s1} is the resolution obtained from column 1 and R_{s2} is the estimated resolution with column 2.

Similarly the run time (RT) and column back pressure (P) will also change in direct proportion to a change in the column length by

$$RT_2 = RT_1 \cdot \frac{L_2}{L_1} \qquad P_2 = P_1 \cdot \frac{L_2}{L_1}$$

where RT_1 is the run time for column 1, RT_2 the run time for column 2, P_1 the pressure for column 1, and P_2 the pressure for column 2.

While most analytical columns are standardized to a 4.6 mm id, their lengths vary; they are available in lengths of 5 cm, 15 cm, and 25 cm, whereas the original Waters μBondapak® C18 column measures 30 cm × 3.9 mm id. A good selection of columns illustrating type and sizes can be found in most HPLC vendors' supply catalogs.

Claessens et al. (28) have reported on an extensive study on the effect of buffers on silica-based column stability in reversed-phase HPLC. As the analytical column has a silica-based backbone, it is not stable in alkaline pH. The authors reported that silica-based bonded phase packings variably degrade with buffers as a function of the type of anion, cation, pH, buffer type, and temperature.

10.9. Role of Temperature

While temperature is a variable that can affect selectivity, α, its effect is relatively small. Also, the k' generally decreases with an increase in temperature for neutral compounds but less dramatically for partially ionized analytes. Still, it may have some effect when there is a significant difference in shape and size between samples. Overall, it is better to use solvent strength to control selectivity than to use temperature; its effect is much more dramatic. Snyder et al. (29) reported that an increase of 1°C will decrease the k' by 1 to 2%, and both ionic and neutral samples are reported to show significant changes in α with temperature changes. Because of possible temperature fluctuations during method development and validation, it is recommended that the column be thermostated to control the temperature.

10.10. Role of Flow Rate

Flow rate, more for isocratic than gradient separation, can sometimes be useful and readily utilized to increase the resolution, although its effect is very modest. The slower flow rate will also decrease the column back pressure. The disadvantage is that when flow rate is decreased, to increase the resolution slightly, there is a corresponding increase in the run time.

11. OPTIMIZATION (OPTIMIZING THE SEPARATION)

Up to this point, efforts to develop a suitable stability-indicating HPLC method have revolved around the resolution equation (see Sec. 10.1). To optimize the method, the chromatographer must tweak the three variables in the equation. The capacity factor, k', can be affected with a change in the solvent. The efficiency factor, N, can be altered with a change in the column dimension, particle size, stationary phase, and flow rate. Lastly, the separation factor, α, can be modified with a change in the solvent, pH, ionic strength of the buffer, stationary phase, mobile phase additives, and temperature. These three factors need to be considered for optimizing the method, conveniently performed utilizing the Plackett–Burman design and computer software. The use of computer software for optimization is becoming more and more common. Refer to Sections 12 and 17.10 for further discussions on these topics.

11.1. Peak Area or Peak Height for Quantitation

The chromatographer can either select peak area or peak height for quantitation assuming that both modes have been properly calibrated and validated. It is suggested, however, that peak area be used for development and peak height for stability for the reasons stated in the table.

Development—Peak Area	*Stability Monitoring—Peak Height*
Suitable for simple, well-resolved mixtures	More accurate/sensitive than peak area
Less frequent standardization required	Requires less resolution of compounds
Generally more precise than peak height	More affected by instrumental variations
Generally best when simple equipment is used	Best suited for complex mixtures
Better suited for nonsymmetrical peaks	Use for trace analysis

11.2. Plackett–Burman Design

Often in method optimization it is necessary to consider various variables, such as environmental and experimental conditions, that affect the ruggedness of a given method. One such experimental design often used in ruggedness testing is the Plackett–Burman design named after the authors that first published their work more than a half a century ago. Refer to Sec. 17.10 in the *Validation* part of this chapter for further discussion on this subject.

12. COMPUTER SOFTWARE FOR METHOD DEVELOPMENT

The discussion in Sec. 10 presumes developing a method by manual trial and error, yet in a systematic manner. That is, the conditions for an initial run are noted, and, based on the outcome of the first run, modifications are made for the second run. Then based on the results of the second run, additional modifications are made for the third run, and so forth until a good separation is obtained. Thus a number

of these trial and error runs may be needed to obtain the desired separation, which may conceivably be time consuming.

In the last fifteen years or so, the use of software for method development in reversed-phased HPLC has increased dramatically, with the intended purpose of separating complex mixtures by shortening the development time and optimizing the resolution based on a limited amount of experimental retention data. A number of these computer systems are commercially available. Many reviews on the subject have been published (30), and many references to using the DryLab^{TM} have been reported (31). DryLab^{TM} is a widely used computer simulation program that after a limited number of actual injections at different conditions can predict an optimal condition or separation at other conditions.

13. OTHER APPLICATIONS

13.1. Analytical Method for Cleaning Assessment

Normally, production equipment is shared to manufacture different pharmaceutical products. Thus cleaning processes following production of pharmaceutical products are critical to prevent cross-contamination. The analytical method used to assess the effectiveness of the cleaning process is usually the same stability-indicating method used for product release and stability monitoring, with some adjustments to increase its sensitivity. How sensitive and specific the method has to be is commonly determined from a joint effort between the pharmaceutical engineer and the analytical chemist to establish the necessary cleaning limit. The method developed must be capable of being validated and rugged enough to meet predetermined specifications consistently. In addition to HPLC, total organic carbon (TOC) analysis has become a widely used method for analyzing cleaning residues, and the Compendia have dedicated General Chapter <643>, Total Organic Carbon, to the subject (32). TOC, however, is not as specific as HPLC. Conductivity has also been used. Generally HPLC is the most accurate, reliable, and specific of all the analytical cleaning methods.

13.2. Physicochemical Characterization Method (Dissolution Method)

A liquid chromatographic method developed for product release or stability monitoring can be adapted for use with a dissolution assay. An HPLC method for dissolution assay testing is optimized for speed and is not intended for determination of degradation products or process impurities. Instead, the real utility of this combination (dissolution with HPLC determination) is that it eliminates interferences from formulation excipients. Assuming that the HPLC method has been developed and validated, the development process is bridged over to developing the dissolution methodology. A preliminary dissolution test is developed very early in the pharmaceutical development process to support formulation development. Primary dissolution parameters for development include selection of the filter, the apparatus type, the rotation speed, and the dissolution medium. Once these parameters have been established, they are to be validated as part of the total validation effort for the HPLC dissolution methodology. The reader is referred to the article by Skoug et al. (33) for an overview of the subject.

13.3. Nonchromatographic Methods

Approaches and guidelines used to develop and validate a chromatographic method can be applied to develop nonchromatographic methods (not stability-indicating) as well. It is equally appropriate to follow the guidelines of USP 23 General Chapter <1225>, Validation of Compendial Method (34), selecting and validating those analytical elements that are needed for a rugged method. These nonchromatographic methods include UV spectrophotometry, atomic absorption, infrared spectroscopy, and titrimetry.

Additional discussions on the validation of various nonchromatographic methods are found in Sec. 19.3.

Part II: Method Validation

14. REGULATORY AND COMPENDIAL BASIS OF METHOD VALIDATION—WHERE TO START

Analytical methods including chromatographic and nonchromatographic techniques are used to generate reliable and accurate data during drug development and post approval of the drug products. The testing, in general, includes the acceptance of raw materials and the release of drug substances and finished products, in process testing, and analysis of stability samples for establishing expiration dating. Therefore test methods that are used to assess the compliance of pharmaceutical products with established acceptance criteria must meet proper cGMP standards of accuracy and reliability as set forth by the regulatory agencies (35).

According to Section 501 of the Federal Food, Drug, and Cosmetic Act, assays and specifications in monographs of the USP and NF constitute legal standards. Under the Food Drug, and Cosmetic Act, the FDA can enforce the USP/NF standards of strength, quality, purity, packaging, and labeling. Therefore for compliance purposes, every analytical method should be validated according to pharmacopeial standard, because each method could be included in a drug monograph.

Method validation is a regulatory requirement. The Food and Drug Administration and the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use have published a series of guidelines on the validation of analytical procedures (36,37). In USP 23/NF 18, General Chapter <1225> has been allocated for validation of compendial methods (34). This chapter describes in detail as well as in summary how to evaluate particular performance parameters. In general, it is assumed that this chapter is applied to chromatographic methods of analysis, and that for nonchromatographic procedures some alternate guidelines should be used. However, in USP 23/NF 18 no such distinction has been made. Therefore performance parameters given in General Chapter <1225> can be used to evaluate the performance of any analytical method. However, one needs to be careful in selection of performance parameters. Also, methods described in the current USP are not stability-indicating in nature. Therefore for monitoring of stability studies, guidelines given in General Chapter ⟨1225⟩ can be used to validate these methods.

What constitutes validation? The validation of analytical method is the process in determining the suitability of a given methodology by laboratory studies that the method in question can meet the requirements for the method's intended use. Method validation is not simply a measure of procedure; method validation is a measure of performance of the total analytical system. Sections 211.165(c) and 211.194(a)(2) of the cGMP for method validation specify that any method adopted at the product development stage be verified under actual conditions of use, and that subsequent variations on existing methodology are subjected to validation.

General Chapter <1225> states, "Validation is the process of providing documented evidence that the method does what it is intended to do." In other words, the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use.

The articles in the current revision of the Compendia are also recognized to be legal standards when determining compliance with the Federal Food, Drug, and Cosmetic Act. Regulated industries must perform method validation to comply with Compendial or other regulatory requirements, and the data generated becomes a part of the methods validation package submitted to the FDA.

Similarly, the general regulation, which is currently represented in 21 CFR 2.19, states, "Where the method of analysis is not prescribed in a regulation, it is the policy of FDA, in its enforcement programs to utilize the methods of analysis of AOAC as published in the current edition." Further, it is stated in the FDA's current Good Manufacturing Practices for Finished Pharmaceuticals regulations 21 CFR 211.165(e) and 21 CFR 211.194(a)(2) that if a firm is using AOAC-OMA or USP/NF methods of analysis, only minimal additional validation data is required (35).

15. VALIDATION PROTOCOL

While the text of Title 21 CFR Part 211, ICH Guidelines, and General Chapter <1225> all provide terms and definitions, there is no specific discussion of validation protocol and methodology. In ICH Guidelines (Q2B) on Method Validation Methodology, the applicant has been made responsible for the appropriate validation protocol and procedure suitable for their product. Therefore prior to initiating a validation study, a well-planned validation protocol is required. This protocol should consist of experimental design and elements required for validation of the proposed test method that have been reviewed for scientific soundness and completeness by qualified individuals and approved by appropriate company management authority. The validation protocol should include a detailed test procedure, basic experimental design, elements for validation, predefined acceptance criteria, reference of related methods, and management approval.

As mentioned earlier, description of the test method is very significant for successful validation. Therefore a test procedure is a description of the "analytical method" to be used as a guide in validating the method and serves as a basis for the preparation of the validation protocol. It should include

1. A listing of reagents, solvents, and other supplies
2. Instructions for the preparation of standards, samples, and solutions
3. A listing of equipment to be used or equivalents
4. Instrumental parameters and chromatographic conditions

5. System suitability requirements
6. Standard and sample analysis sequence
7. Calculation section to include results formatting

Prior to outlining the experimental design or protocol, however, it is necessary to make some basic assumptions as suggested by Swartz and Krull (38,39). These assumptions are that

Specificity or selectivity for the developed method has been demonstrated (i.e., forced degradation already performed).

The developed method has been optimized to the point where investing time and effort in validation is justified and feasible.

Evaluation of data generated by the developed method is performed by valid statistical approaches to remove some of the subjectivity of method validation.

Keeping in mind these assumptions, the current ICH methodology guidelines, and the requirements for validation depending on the type of analytical method, one can design a stepwise protocol. A typical protocol designed by Swartz and Krull is given in Fig. 3 (38,39).

As shown in Fig. 3, the first parameter to be evaluated is robustness. This parameter is usually evaluated during the method development stage, when the effect

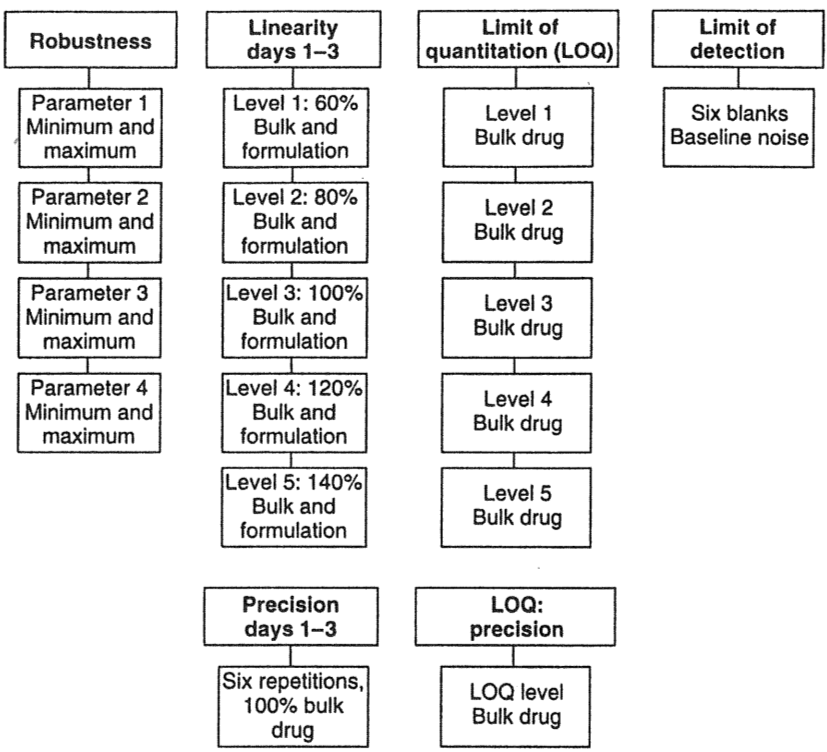


Figure 11.3 Sample method validation protocol. (From Refs. 38, 39.)

of different parameters on selectivity is studied. Method robustness can be evaluated in a stepwise univariate approach or as a part of an experimental design incorporating multivariate parameters.

Next, a linearity test over five levels for both the drug substance and the dosage form is performed. The range is determined according to the test method's intended use (34,36,38,39). Comparison of the results between the drug substance and the dosage form fulfills the accuracy requirements. A minimum of three measurements at each level should be made.

At the end of day 1, a minimum of six repetitions are performed at the 100% level of the drug substance for repeatability.

Steps 1 and 2 are repeated over additional days for intermediate precision. The detection limit (DL) and quantitation limit (QL) can then be determined if required. For calculation of these performance characteristics one can follow criteria given in the USP or ICH guidelines (Q2B). It is stated that this protocol is merely a generic example, and specific protocols or SOPs should be documented and followed for the particular method and its intended use.

16. VALIDATION PARAMETERS

Prior to conducting validation studies it is imperative to decide which parameters are required to be studied. These parameters are termed "analytical performance characteristics" or sometimes "analytical figures of merit." Most of these terms are familiar and are used daily in the laboratory. However, some may mean different things to different laboratory groups. Therefore a complete understanding of the terminology and definitions of these characteristics is important.

The selection of desired performance characteristics would depend on the type of analytical method and its intended use. For example, an assay method designed for finished product release should not be used for the determination of detection or quantitation limits of an active ingredient. However, if the method has been designed to monitor trace quantities of the active ingredient in cleaning validation samples, then knowledge of the detection and quantitation limits are appropriate and necessary.

Therefore, selection of validation parameters for each assay or test method should be made case by case, to ensure that parameters are appropriate for the intended use. This is even more important when validating stability-indicating methods, because such validations are complex, as these involve forced degradation studies, spiking of samples with known degradants and literature searches.

16.1. USP General Chapter <1225>, Validation of Compendial Methods

General Chapter <1225> (34) describes typical analytical performance characteristics, how they are determined, and which subset of data elements is required to demonstrate validity, based on the method's intended use. These performance characteristics can be referred to as the "Eight Steps of Method Validation." These analytical performance characteristics are

Accuracy
Precision
Specificity

Detection limit (DL)
Quantitation limit (QL)
Linearity and range
Ruggedness
Robustness

Compendial test and assay procedures vary significantly in type of analytical method used, and the type of information required for validation of a given analytical method will vary depending on the nature of the method. Consequently in General Chapter ⟨1225⟩ (34), the most common test and assay procedures have been divided into four categories for harmonization with the ICH guidelines:

Category I: Analytical methods for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished dosage forms
Category II: Analytical methods for determination of impurities in bulk drug substances or degradation products in finished dosage forms, including quantitative assays and limit tests
Category III: Analytical methods for determination of performance characteristics (e.g., dissolution, drug release)
Category IV: Identification tests

Analytical variables that are normally required for method validation in each of these categories are listed in Table 5.

An evaluation of performance characteristics shown in Table 5 indicate that for assays in Category I, determination of DL and QL is not required because the major component or active ingredient to be quantitated is present at high levels. All other parameters are evaluated to obtain quantitative information needed. Assays in Category II are further divided into quantitative and limit tests subcategories. If quantitative information is required, measurement for DL is not necessary, but the remaining parameters are evaluated. For limit tests, on the contrary, no quantitation is required. Thus it is sufficient to measure only the DL and demonstrate specificity and ruggedness. The parameters to be determined under Category III are dependent upon the nature of the test. Dissolution testing, for example, falls into this category.

Table 5 USP Data Elements Required for Assay Validation

Analytical performance parameters	Assay Category I	Assay Category II		Assay Category III	Assay Category IV
		Quantitative	Limit tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
DL	No	No	Yes	*	No
QL	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

* May be required depending on the nature of the specific test (Ref. 33).

16.1.1. Stability-Indicating Nature of USP Assays

A word of caution. Assays appearing in USP monographs are not always stability-indicating. They may be for the innovator product, as submitted by the innovator company for inclusion as a USP monograph, which then becomes the benchmark. If another company wishes to market the same product, as a generic version, that company must validate the assay according to the validation parameters discussed in USP General Chapter <1225>, because that product is different from the innovator product relative to the source API and formulation.

16.2. ICH Guidelines

ICH Guidelines Q2A (Text on Validation of Analytical Procedures) and Q2B (Validation of Analytical Procedures: Methodology) were developed within the Expert Working Group (Quality) of the Requirements for Registration of Pharmaceuticals for Human Use. These documents present a discussion of the characteristics for consideration during validation of analytical procedures included as part of registration applications submitted within the European Union, Japan, and the United States.

ICH Guidelines Q2A also provides descriptions of typical validation parameters, how these are measured, and which subset of each parameter is suitable for validation of the analytical method, based on its intended use. The discussion of the validation of analytical procedures has been divided into three common categories of analytical procedures:

Identification tests
Quantitative tests for impurity content—Limit tests for the control of impurities
Quantitative tests of the active moiety in bulk drug substance or drug product or other selected component(s) in the drug product

As per ICH Guidelines Q2A, the objective of the analytical procedure needs to be clearly understood since this will govern the validation characteristics that need to be evaluated. Typical validation characteristics, which should be considered, are

Accuracy
Precision
Repeatability
Intermediate precision
Specificity
Detection limit
Quantitation limit
Linearity
Range
Robustness
System suitability

Analytical variables that are normally required for method validation is summarized in Table 6.

Table 6 ICH Validation Characteristics Versus Type of Analytical Procedures

Type of analytical procedure	Identification	Impurity testing		Assay
		Quantitative	Limit tests	
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Intermediate precision	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
LOD	No	Yes	Yes	No
LOQ	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

Source: Refs. 35 and 37.

The difference in the USP and ICH terminology is for the most part one of semantics; however, there is one notable exception. In the ICH Guidelines, system suitability is part of validation, whereas the USP deals with system suitability under chromatography in the USP, called General Chapter <621> Chromatography (24). The FDA is already implementing the ICH Guidelines, and it is anticipated that the ICH definitions and terminology will become a part of the USP chapter on validation. It is probable that USP categories I and II will match the ICH categories of Assay and Impurity testing, respectively. The ICH has not yet chosen to address methods for performance characteristics (USP Category III) but has instead included analytical methods for compound identification. In this ICH category, it is only necessary to show that the method is specific for the compound being identified.

ICH Guidelines Q2B is complementary to ICH Guidance Q2A, which presents a discussion of characteristics that should be considered during the validation of analytical procedures. This guidance gives recommendations on how to consider the various validation characteristics for each analytical procedure. These recommendations will be discussed in detail under definition of validation parameters.

16.3. FDA Reviewer Guidance

The FDA Reviewer Guidance—Validation of Chromatographic Methods provides comprehensive description of typical validation parameters and how these are determined (40). This FDA guidance has similarities to the ICH Guidelines Q2A and Q2B, but has examples in form of tables or figures to demonstrate data representation for validation parameters. The purpose of this guidance is to present the issues to be considered when evaluating chromatographic test methods from a regulatory perspective. Examples of common problems, which can delay the validation process, have been included.

The validation characteristics to be evaluated according to this FDA guidance are

Table 7 Comparison of Analytical Parameters Required for Assay Validation

USP General Chapter <1225>	ICH Q2A Guidelines	FDA Reviewer Guidance
Accuracy	Accuracy	Accuracy
Precision	Precision	Precision
No	Repeatability	Repeatability
		Injection
		Analysis
No	Intermediate precision	Intermediate precision
No	No	Reproducibility
Specificity	Specificity	Specificity/selectivity
Detection limit	Detection limit	Detection limit
Quantitation limit	Quantitation limit	Quantitation limit
Linearity	Linearity	Linearity
Range	Range	Range
Ruggedness	No	No
Robustness	Robustness	Robustness
System suitability[a]	System suitability	System suitability
		Sample solution stability

[a] System suitability discussed separately in USP 23 General Chapter <621>.

Accuracy
Detection and quantitation limits
Linearity
Precision
Repeatability
Injection repeatability
Analysis repeatability
Intermediate precision
Reproducibility
Range
Robustness
Sample solution stability
Specificity/selectivity
System suitability specifications and tests

A comparative discussion of validation parameters given in the FDA and ICH guidelines will be made under Sec. 17, "Definition of Validation Parameters." Analytical parameters needed for method validation as described in the General Chapter <1225>, ICH Guidelines Q2A, and the FDA Reviewer Guidance are summarized in Table 7.

17. DEFINITION OF VALIDATION PARAMETERS

In the literature, there are many articles on definition and interpretation of validation parameters required for assay validation as published by Krull and Swartz (38,39,41,42). Persson et al. (43) have discussed the evaluation of method

validation in an article titled "How good is your method?" In Sec. 17, definition of validation parameters is based on requirements stipulated in the ICH Guidelines Q2A, Q2B, the FDA Reviewer Guidance, and USP General Chapter <1225>.

Though many types of chromatographic techniques are available, the most commonly submitted method in NDAs and ANDAs is reversed-phase HPLC with UV detection. Therefore this method is selected here to illustrate parameters for validation. The criteria for the validation of this technique can be extrapolated to other detection methods and chromatographic techniques. For acceptance, release, or stability testing, accuracy should be optimized, since the need to show deviation from the actual value is of great concern.

17.1. Accuracy

Accuracy is the measure of how close the experimental value is to the true value. It is measured as the percent of analyte recovered by assay or by spiking samples in a blind study. For the drug product, this is performed by analyzing synthetic mixtures (placebos) spiked with known quantities of drug. Accuracy should be established across the specified range (that is, line of working range) of the analytical procedure. For the assay of the drug substance, accuracy measurements are made by comparison of the results with the analysis of a standard reference material or to compare the results obtained from a second well-characterized independent procedure, the accuracy of which is stated and/or defined. For quantitation of the impurity, accuracy is determined by spiking drug substance or drug product with known amounts of available impurities. In case it is impossible to obtain impurities or degradation products, comparison of results to a second well-characterized independent method is acceptable. The response factor of the drug substance can be used. Another approach is to perform specificity studies by forced degradation. This will be discussed under specificity. It should be decided up front how the individual or total impurities are to be reported, e.g., percent weight/weight or area percent, in all cases relative to the major analyte.

The FDA recommends that recovery be performed at the 80, 100, 120% of label claim as stated in the Guideline for Submitting Samples and Analytical Data for Method Validation (2). Recovery data, at least in triplicate at each level (80, 100, and 120% of label claim) is recommended. The data should be calculated as percent label claim, and the mean of the replicates along with % RSD for each level is reported to demonstrate accuracy and sample analysis precision.

ICH Guidelines Q2B recommend assessment of accuracy at three concentration levels covering the specified range (i.e., three concentration levels and three replicates at each level of the total analytical procedure). The data should be reported as the percent recovery of the known amount added or as the difference between the mean and true values with confidence intervals.

17.2. Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. In USP 23/NF 18, General Chapter <1225>, precision is defined as "the degree of agreement among individual test results obtained by repeatedly applying the analytical method to multiple samplings of a homogeneous sample." Thus precision refers to the distribution

of individual test results around their average. Precision is usually expressed as percent relative standard deviation (% RSD) for a statistically significant number of samples. Both the FDA and the ICH recommend that precision be measured at three different levels. No such recommendation is given in the USP.

17.2.1. Repeatability

Repeatability expresses the results of the method operating over a short time interval under the same conditions. Repeatability is also termed intra-assay precision. According to the FDA Reviewer Guidance, repeatability is evaluated for injector performance and analysis of samples. For injector repeatability, there must be a minimum of 10 injections with an RSD of not more than ±1%. Similarly, with the methods for release and stability studies, an RSD of not more than ±1% for at least five injections for the active drug is desirable. For low-level impurities, higher variations in RSD may be acceptable. For analysis repeatability, determinations are made on multiple measurements of a sample by the same analyst under the same analytical conditions. The FDA recommends that the study be combined with accuracy.

The ICH recommends that repeatability should be determined from a minimum of nine determinations covering the specified range for the procedure (e.g., three levels, three replicates each), or from a minimum of six determinations at 100% of the test or target concentration. The target concentration is defined as the concentration of the compound of interest given in the analytical method.

17.2.2. Intermediate Precision

Intermediate precision expresses within-laboratory variations. This was previously evaluated as part of ruggedness. This attribute evaluates the reliability of the method in an environment different from that used during the method development phase. Depending on time and resources, the method can be evaluated on different days, with different analysts and equipment, etc. The FDA recommends performing accuracy on two separate occasions to indicate the intermediate precision of the test method. The ICH recommends using an experimental design (matrix) so that the effects, if any, of the individual variables on the analytical procedure can be monitored.

17.2.3. Reproducibility

Reproducibility is assessed by performing collaborative studies between laboratories. Multiple laboratories are desirable, if possible. According to the FDA Reviewer Guidance, reproducibility is not required if intermediate precision is achieved. The ICH recommends that reproducibility studies be performed for standardization of an analytical procedure, for instance, for inclusion of procedures to pharmacopoeias. The ICH also recommends that documents in support of each type of precision should include the standard deviation (S), the % RSD, the coefficient of variation, and the confidence interval.

17.3. Specificity/Selectivity

The terms specificity and selectivity are often used interchangeably. The term selectivity has been used in General Chapter <1225> of the 1990 edition of the

USP (44), whereas in the 1995 edition the term specificity has replaced selectivity. Specificity is generally used to express a method's response for a single analyte, whereas the term selectivity of a method is a measure of the extent to which the method can determine a particular compound in the analyzed matrices without interference from matrix components. However, as both the USP and the ICH currently use the term specificity, it will also be used here to avoid any confusion.

The USP defines specificity as the ability to measure accurately and specifically the analyte of interest in the presence of other components in the sample matrix. These components may include other active ingredients, excipients, impurities, and degradation products. According to the ICH, the validation procedure should be able to demonstrate the ability of the method to assess unequivocally the analyte in the presence of impurities, matrix components, and degradation products. Lack of specificity of an individual procedure may be compensated by other supporting procedure(s) such as TLC.

Specificity has been divided into two separate categories by ICH:

A. IDENTIFICATION. Specificity is demonstrated by the ability to discriminate between compounds of closely related structures, which are likely to be present. The other approach is by comparison of results to a known reference material.

B. ASSAY AND IMPURITY TEST(S). For assay and impurity tests, specificity can be demonstrated by the resolution of the two components which elute closest to each other. Chromatograms obtained should be appropriately labeled to show individual components. For nonspecific assays, overall specificity may be demonstrated by use of other supporting analytical procedures. For example, where a titration is adopted to assay the drug substance for release, the combination of the assay and a suitable test for impurities can be used.

The ICH has also addressed issues of specificity for impurities. The approach is similar for both assay and impurities. If impurities are available, then it must be demonstrated that the assay is unaffected by the presence of spiked materials such as impurities and/or excipients. For the impurity test, the discrimination may be shown by spiking drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix.

If the impurities or degradation product standards are not available, then specificity may be demonstrated by comparison of the test results to a second well-characterized pharmacopoeial or independent validated procedure. For the assay, the two results are compared. For the impurity tests, the impurity profiles are compared head to head.

For stability-indicating assays where potency and impurities are determined simultaneously mass balance must be taken into consideration. Any decrease in potency should be explained by mass balance. The following equation can be used to account for any loss of potency:

$$100\% = \text{Drug}\% + \text{Related substances}\% + \text{Water}\% + \text{ROI}\% + \cdots$$

In the FDA Reviewer Guidance, specificity/selectivity is established by showing that the analyte should have no interference from extraneous components and be well resolved from them. A representative chromatogram showing resolution of these

extraneous peaks from the main analyte peak is required for submission. The origins of extraneous peaks in drug substance are process impurities (which include isomeric impurities) from the synthesis process, residual solvents, and other extraneous components from extracts of natural origins. For the drug product, sources of extraneous peaks include any impurities, degradation products, interaction of the active drug with excipients, residual solvents from both the active drug substance and the excipient, and so on.

17.4. Forced Degradation

In previous sections, we have defined specificity as discussed by USP Chapter <1225>, the ICH Guidance, and the FDA Reviewer Guidance. The discussion was limited to specificity studies in the presence or absence of impurities and excipients. A question that arises if nothing (i.e., no extraneous peaks) is observed is, What approach one might use to show the specificity and stability-indicating nature of the proposed method?

Both the FDA and the ICH recommend forced degradation/or stress testing of the drug substance and drug product. For these studies, acid and base hydrolysis, temperature, photolysis, and oxidation are recommended. Neither the ICH nor the FDA guidelines specify how to perform these forced degradation studies. Experimental conditions and the design of these studies have been left to the discretion of pharmaceutical companies. A generic protocol for these studies is shown in Table 2.

To demonstrate that the analyte chromatographic peak obtained after forced degradation or stress studies is a single entity, peak purity tests are recommended by the FDA and the ICH. Photodiode array detection can be used to demonstrate peak purity. The spectra collected across a peak are compared mathematically to establish peak homogeneity.

It is generally recommended that about 20–30% of analyte degradation, at least, in one medium be achieved. For some compounds, severe degradation conditions may be required.

17.5. Detection Limit (DL)

The detection limit (DL) is the lowest concentration of the analyte that can be detected, but not necessarily quantitated, under the stated experimental conditions. It is a parameter of limit test and specifies whether or not an analyte is above or below a certain value. In the current USP General Chapter <1225>, determination of limit of detection is described for instrumental and noninstrumental methods. For instrumental methods, one determines the signal-to-noise ratio by comparing test results from samples with known concentration of analyte with those of blank samples and establishes the lowest concentration at which analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is required. Another approach is to calculate the standard deviation for analysis of a number of blank samples. The standard deviation multiplied by a factor, usually 2 or 3, gives an estimate of limit of detection.

For noninstrumental methods, DL is determined by the analysis of samples with known concentrations of analyte. The minimum concentration at which the analyte can be reliably detected is the limit of detection. The ICH has recognized

the signal-to-noise ratio convention but also lists several other approaches for determining DL, depending on whether the procedure is instrumental or noninstrumental. These approaches are as follows.

A. BASED ON VISUAL EVALUATION. Visual evaluation may be used both for instrumental and noninstrumental methods. It requires analysis of samples with concentrations of analyte and establishing the minimum level at which analyte can be reliably detected. Visual noninstrumental methods can include DL determined by techniques such as TLC or titration.

B. BASED ON THE STANDARD DEVIATION OF THE RESPONSE AND THE SLOPE. The detection limit may be calculated based on the standard deviation (SD) of the response and slope (S) of the calibration curve (a specific curve should be generated by using samples containing analyte in the range of detection limit), according to the formula

$$\text{Detection limit (DL)} = 3.3 \times \text{SD}$$

The SD of the response can be determined from the SD of the blank, the residual SD of the regression line, or the SD of the y-intercept of the regression line. The detection limit and method used to determine the detection limit must be documented and supported, and a suitable number of samples should be analyzed at the limit to validate it. The FDA is of the opinion that expression of the detection limit in terms of a signal-to-noise ratio of 2 or 3 is not very practical. The reason for this is attributed to differences in the noise level on a detector during the method development phase and when samples are analyzed on different detectors. Detector sensitivity can vary with the model number or manufacturer.

17.6. Quantitation Limit (QL)

The quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions of the method. This is a parameter of the quantitative assays for low concentrations of compounds in sample matrices such as impurities in bulk drug substances and degradation products in finished products.

In the current USP General Chapter <1225>, the quantitation limit, QL, which is similar to the detection limit, is expressed as the concentration of analyte in the sample, and precision and accuracy of the measurements are also reported. The QL is dependent on the type of procedure, i.e., instrumental or noninstrumental. For instrumental methods, sometimes a signal-to-noise ratio of 10 : 1 is used to determine the QL. However, it is pointed out that the determination of the QL based on signal-to-noise ratio criteria is a compromise between the concentration and the required accuracy and precision. In other words, as the QL concentration level decreases, the precision increases. For better precision, a higher concentration must be reported for the QL. This compromise is dependent on the analytical method and its intended use.

As with to the limit of detection, the ICH has recognized using a signal-to-noise ratio of 10 : 1 for quantitation. However, this approach can only be applied to analytical procedures that exhibit baseline noise. Again, as with the DL, the ICH lists the same two options that can be used to determine the QL. They are visual evaluation for both noninstrumental and instrumental methods; the latter method can

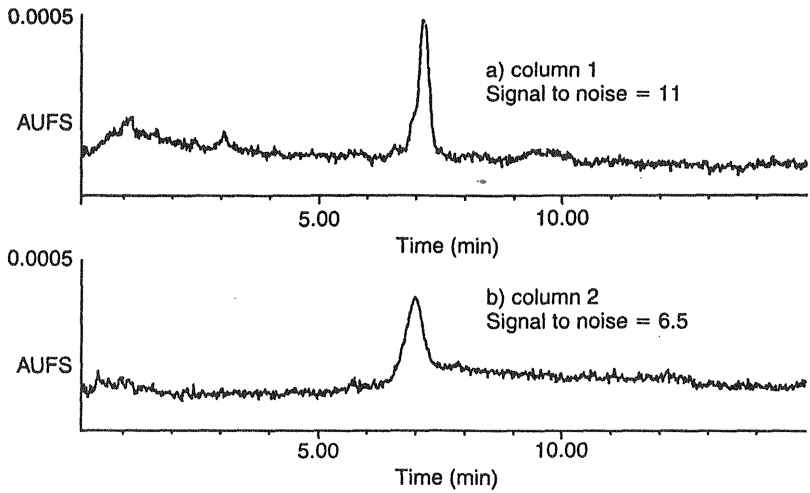


Figure 11.4 Effect of peak shape on LOD and LOQ. (From Ref. 38.)

be based on the standard deviation of the response and the slope. The formula is changed to

$$\text{SD} = 10 \times \frac{\text{SD}}{\text{S}}$$

Determination criteria and requirements for documentation are the same as described under DL in Sec. 17.5, as well as comments by the FDA on the subject. In addition, the FDA Reviewer Guidance recommends that data for analysis repeatability and injection repeatability at the quantitation limit be generated. Further, the Guidance recommends that the use of an additional reference standard at the quantitation limit level be incorporated in the test method.

Additional points regarding the detection and quantitation limits are warranted. These parameters are affected by chromatography. Figure 4 shows the effects of peak shape and efficiency on the signal-to-noise ratio. Sharp peaks will yield a higher signal-to-noise ratio, thus lowering both the DL and the QL. Therefore for the chromatographic determination of these parameters, the age and type of the column and the age of the detector lamp need to be considered. Thus periodic maintenance of the chromatographic detector to maintain optimal results is required.

Finally, the DL and the QL should not be confused with sensitivity. Sensitivity is defined as the slope of the calibration curve, and as such does not usually reference the actual limit of detection or limit of quantitation.

17.7. Linearity

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to the concentration of analyte in the sample within a given range. Linearity is generally reported as the variance of the slope of the regression

line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. The linear range of detection that obeys Beer's law is dependent on the compound analyzed and the type of detector used.

USP General Chapter <1225> gives general directions on the determination of linearity along with handling of the data. However, there are no concentration levels specified to monitor linearity. The ICH also has adopted an approach similar to that of the USP for the determination of linearity and data interpretation. The least squares method is recommended for evaluation of the regression line.

The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be reported. For linearity studies, a minimum of five concentrations is recommended. According to the FDA Reviewer Guidance, the linearity range depends on the intended use of the test method. For content assay, linearity should be performed between 80% and 120% of target concentration. The linearity range for the assay/impurities combination method based on area percent (for impurities) should be greater than 20% of the target concentration down to the limit of quantitation of the drug substance or impurity. A coefficient of correlation (r^2) value, an intercept, and a slope should be reported.

17.8. Range

The range of an analytical method is the interval between the upper and lower concentration levels of analyte (including these concentrations) for which the method as written has been shown to be precise, accurate, and linear. The range is usually expressed in the same units as test results obtained by the analytical method. According to USP General Chapter <1225>, the range of method is validated by verifying that acceptable precision and accuracy is obtained by the analytical method when actual analysis of samples containing analyte is performed throughout the intervals of the range.

The ICH recommends an approach similar to the USP for validation of range. It recommends specific ranges based on the intended use of the method, as follows.

1. For assay of a drug substance or drug product, the minimum specified range is 80% to 120% of the target concentration.
2. For content uniformity testing, the minimum range is 70% to 130%.
3. For the determination of impurity, the minimum range is from the reporting level of an impurity to 120% of the specification.
4. For a combination assay procedure for both active and impurity, where a 100% standard is used, linearity should cover the range from reporting level to 120% of the assay specification.
5. For dissolution testing, the recommended range is ±20% over the specified range of the test. That is, in the case of an extended release product dissolution test with a Q value of 20% after 1 hour, up to 90% in 24 hours, the range for validation will be 0 to 110% of the label claim.
6. For toxic or more potent impurities, the range should be commensurate with the controlled level. FDA recommendations for range are as discussed under the Linearity and Accuracy sections. These ranges can also be applied to other substances such as preservatives.

17.9. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in some parameters and provide an assurance of its reliability during normal usage. The robustness of the method is investigated by varying some or all conditions, e.g., organic composition of the mobile phase, pH, ionic strength, column temperature, age of column, column type. ICH guidelines recommend that robustness studies be performed during the method development stage. Also, if measurements are affected by variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the test method.

Robustness can also be partly assured by good system suitability specification. Therefore, it is important to set tight but realistic system suitability specifications.

17.10. Application of Plackett–Burman Design to Ruggedness Testing

Ruggedness is normally defined as the lack of influence on test results by operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under normal operational conditions from laboratory to laboratory and from analyst to analyst. According to ASTM Guidance E 1169-89, "Standard Guide for Conducting Ruggedness Tests" (45), it is necessary to monitor the effects of environmental and experimental factors on the results obtained using the test method to assure method accuracy. Furthermore, the purpose of ruggedness testing is to determine which variables the method is susceptible to and how to control it. Ruggedness testing does not determine the optimum operational conditions for the test method. To determine the ruggedness of the method, the ASTM guidance recommends use of the experimental design as reported by Plackett and Burman. This guidance discusses effects of change on two levels per variable, as this design is easy to use and provide useful information needed for improvement of the test method. An example of ruggedness testing for an HPLC method is given in Tables 8 through 10.

Table 8 shows the various factors and their high and low limits to be considered in ruggedness testing. Table 9 shows the factors and their high and low limits in a +/− format. Lastly, Table 10 summarizes the results obtained when each of the eight combinations (rows across the spreadsheet) are experimentally performed.

Table 8 Ruggedness Testing—Typical HPLC Factors

Factor	Low value	High value
A. pH	3.0	4.0
B. Temperature	35°C	40°C
C. Mobile phase composition	45/55	55/45
D. Buffer concentration	0.05 M	0.1 M
E. Particle size	3 micron	5 micron
F. Column length	3 cm	5 cm
G. Flow rate	1.0 mL/min	1.5 mL/min

Table 9 Ruggedness Testing—Typical HPLC Conditions

Excel	A	B	C	D	E	F	G	H	I
1	Run/factor	A	B	C	D	E	F	G	Result
2	1	−1	+1	+1	+1	−1	−1	+1	99.8%
3	2	+1	−1	+1	+1	+1	−1	−1	101.1
4	3	−1	+1	−1	+1	+1	+1	−1	98.9
5	4	−1	−1	+1	−1	+1	+1	+1	99.5
6	5	+1	−1	−1	+1	−1	+1	+1	99.9
7	6	+1	+1	−1	−1	+1	−1	+1	98.5
8	7	+1	+1	+1	−1	−1	+1	−1	98.0
9	8	−1	−1	−1	−1	−1	−1	−1	97.0
10	Effect	0.575	−0.575	1.025	1.675	0.825	−0.025	0.675	

Table 10 Ruggedness Testing—Typical HPLC Conditions

Excel	A	B	C	D	E	F	G	H	I
1	Run/factor	A	B	C	D	E	F	G	Result
2	1	3	40	55/45	0.1	3	3	1.5	99.8%
3	2	4	35	55/45	0.1	5	3	1.0	101.1
4	3	3	40	45/55	0.1	5	5	1.0	98.9
5	4	3	35	55/45	0.05	5	5	1.5	99.5
6	5	4	35	45/55	0.1	3	5	1.5	99.9
7	6	4	40	45/55	0.05	5	3	1.5	98.5
8	7	4	40	55/45	0.05	3	5	1.0	98.0
9	8	3	35	45/55	0.05	3	3	1.0	97.0
10	Effect	0.575[a]	−0.575	1.025	1.675	0.825	−0.025	0.675	

[a] Content of cell = SUM PRODUCT(B2:B9,$I2:I9)/4. This takes the difference between the average test results for the "+" runs and the average test results for the " − " runs. Conclusion: Eight experiments performed compared to 56 individual experiments. The cell with the "highest" effect value indicates the most variable factor. In this example, it is Factor D, the buffer concentration, followed by Factor C, the mobile phase composition.

Results obtained are placed in a spreadsheet, such as Excel, and the effect calculated. The highest effect (i.e., largest value) in the column listed would indicate that factor to be the most critical, and special attention is needed to control its variability.

For a detailed discussion of Plackett–Burman design experimentation, readers should consult the ASTM guidance (45) and Torbeck (46).

17.11. Stability of Sample and Standard Solutions

The FDA recommends that solution stability of the drug substance (used as sample or in-house standard) or drug product after preparation according to the test method should be evaluated. This is considered critical as most HPLC analyses are automated. For the duration of an analytical run, the standard or sample will stay in solution for hours in the laboratory environment before all the samples are com-

pletely tested. Therefore monitoring of sample or standard stability will ensure that there is no degradation occurring due to hydrolysis, photolysis, or adhesion to glassware over the course of the run period. The FDA recommends that data to support the stability of sample or standard solution under normal laboratory conditions for a minimum period of 24 hours should be generated.

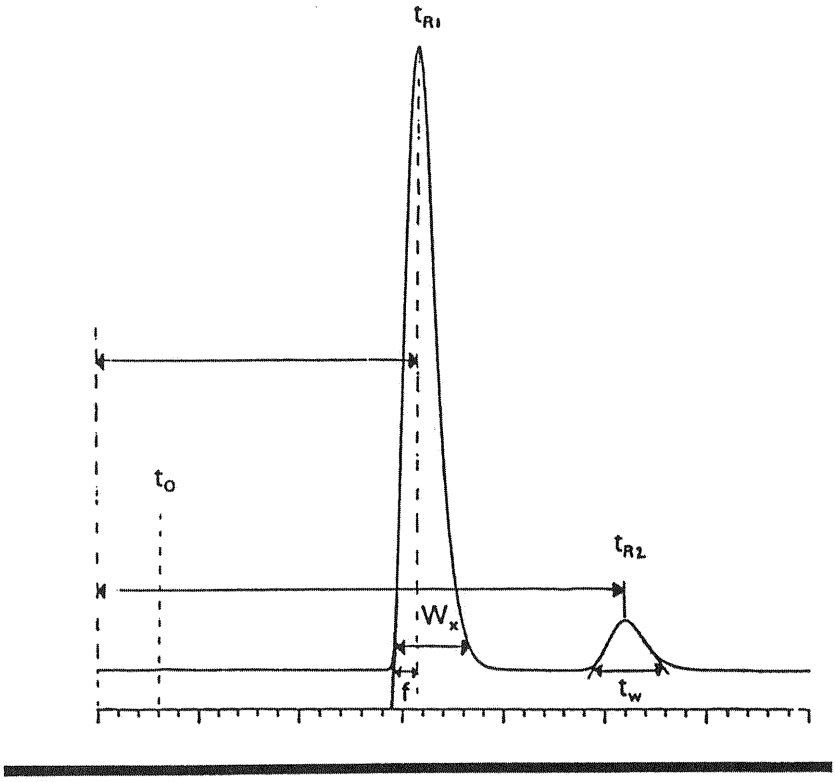
17.12. System Suitability Specifications and Tests

The accuracy and precision of HPLC data collected begin with a well-behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose. According to the ICH and the USP, system suitability testing is an integral part of chromatographic procedures. These tests are used to determine that the resolution and reproducibility of the system are adequate for the analysis to the performed. The basis for these tests is that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as a whole. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. In USP 23 General Chapter <621>, Chromatography, a section has been devoted to system suitability requirements. It is important to know what are regulatory requirements for system suitability tests and specifications for method validation. As stated earlier, system suitability involves checking a system to ensure it is performing adequately before or during the analysis of unknowns. To establish these required parameters [i.e., plate count, tailing factor, resolution (if by-products or impurity standards are available; otherwise a chromatogram from forced degradation studies may be used)], the reproducibility (% RSD) of five or six replicates is calculated and compared to predetermined specification limits. System suitability tests are performed prior to analysis of actual samples. These parameters are studied by analysis of a system suitability sample that is a mixture of main active drug and expected by-product or a known impurity. Table 11 summarizes the parameters to be measured and their recommended regulatory limits for the system suitability tests and specifications (38,40). Definition of terms for system suitability parameters is shown in Figure 5.

Table 11 System Suitability Parameters and Recommendations

Parameter	Recommendation
Capacity factor (k')	The peak should be well resolved from other peaks and the void volume, generally $k' > 2.0$.
Repeatability	RSD $\leq 1\%$ for $N \geq 5$ is desirable.
Relative retention	Not essential so long as the resolution is stated.
Resolution (R_s)	R_s of >2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.).
Tailing factor (T)	T of ≤ 2.
Theoretical plates (N)	In general should be >2000.

Source: Ref. 37 and 39.



Where
W_x = width of the peak determined at either 5% (0.05) or 10% (0.10) from the baseline of the peak height
f = distance between peak maximum and peak front at W_x
t_o = elution time of the void volume or non-retained components
t_R = retention time of the analyte
t_w = peak width measured at baseline of the extrapolated straight sides to baseline

Figure 11.5 Definition of terms for system suitability parameters. (From Ref. 40.)

For accuracy and precision of analysis, all system suitability parameters play a significant role. Therefore, a critical evaluation of these parameters and their effect on a chromatographic separation are required. As an example, the effects of peak tailing and different resolution values on quantitation are depicted in Figs. 6, 7, and 8.

Resolution is a measure of how well peaks are separated from each other. For reliable quantitation, well-resolved peaks are essential. This parameter is very useful in determining if peaks can interfere in individual quantitation. As shown in Fig. 6, with a small resolution, accuracy of analysis will decrease.

Tailing peaks affect quantitation. With an increase in peak tailing the accuracy of quantitation decreases due to improper peak integration (the area under the peak will not be accurate). The effect of peak tailing is shown in Figs. 7 and 8.

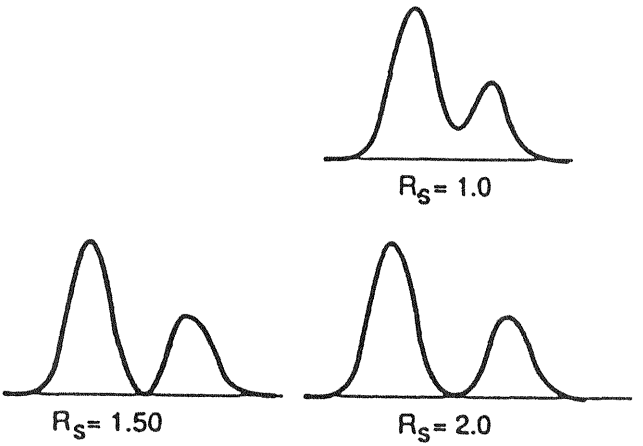


Figure 11.6 Separation of peaks as indicated by R. (From Ref. 40.)

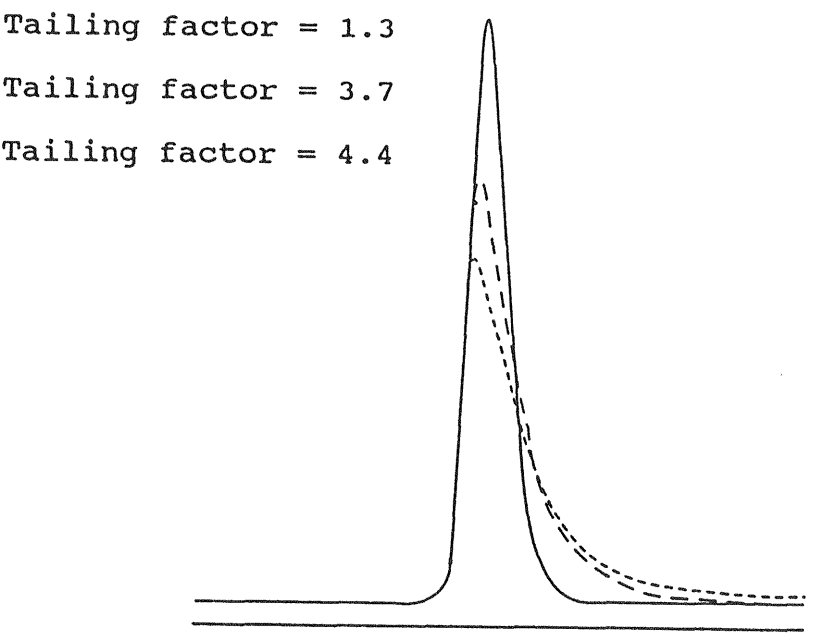


Figure 11.7 HPLC peak with various tailing factors. (From Ref. 40.)

18. POST VALIDATION ISSUES

The validation process does not end after experimental evaluation of the analytical parameters. Data must be evaluated to determine whether validation was successful or not. Does all the data generated meet the specified requirements or not? In the following subsections, steps required to finalize the validation process will be discussed.

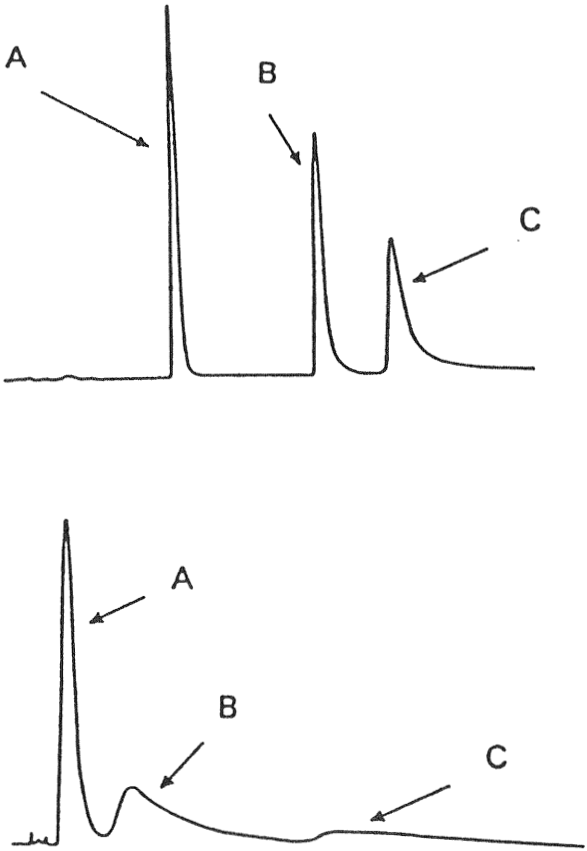


Figure 11.8 Effect of peak tailing on quantitation. (From Ref. 40.)

18.1. After the Laboratory Work

After completion of the laboratory work and documentation of the data in the analyst's notebook, it is very important that all data be carefully reviewed and audited by a qualified person. This process will ensure that data generated through the validation process is correct and meets all the requirements. Only after this step is completed can the next phase of the validation process be implemented.

18.1.1. Validation Report

The method validation report is a regulatory requirement and needs to be submitted to the FDA. The method validation report should be written by the method development group. The format for the report should be agreed upon at the onset of the validation process. This report must describe all the experimental procedures including equipment used, detector type, columns, information on reference standard, chemicals and composition of placebo for accuracy studies. All chromatograms and figures should be labeled properly. For forced degradation studies, conditions used and how this was performed must be explained.

18.1.2. Acceptance Criteria

For each step of the validation analytical parameters, acceptance criteria to determine success or failure of validation, are required. These acceptance criteria should be based on the intended use of the method. Also, regulatory implications should be taken into consideration. For acceptance criteria, it is imperative that responsible personnel with backgrounds in method development be involved. All validation steps should be evaluated against these acceptance criteria. Similarly, system suitability parameters should also accompany acceptance criteria. General acceptance criteria for each validation parameter have been discussed in Sec. 17.

18.1.3. Generation of the Test Method

Generation of the test method is the responsibility of the method's development group. The test method must include a detailed written procedure. For example, for a chromatographic procedure, preparation of mobile phase, column type, detector type, wave length, injection volume, flow rate, reference standard (USP/in-house), preparation of standard and sample solutions, reagent grade, and filters used for standard and sample solutions should be documented. If the method is designed to quantitate the main analyte and impurities simultaneously, then relative retention times for impurities should be given. If the main bulk active is light sensitive, then a precautionary note is required in the test method. Similarly, it should be reflected in the test method whether ambient or elevated temperature is required.

18.2. Revalidation

At some time during the lifetime of the method, for one reason or another, revalidation of the method may become necessary. For revalidation, reactive or proactive approaches may be used. Reactive validation will be required for changes in incoming bulk drug active, manufacturing batch changes, formulation changes, or other changes such as dilutions or sample preparation in the method. Recently, method change versus an adjustment has been the subject of discussion between regulatory agencies and industry (36,38,47).

This distinction is critical, as a process change requires method revalidation, whereas an adjustment does not. As a result of these discussions, limit changes for chromatographic changes do not require revalidation. Changes have been proposed under the following categories:

Aqueous buffer pH

1. Analytes without ionizable groups: ±1 unit
2. Analytes with basic or acidic groups and the buffer pH = pKa±2 units: ±0.2 unit
3. Analytes with basic or acidic groups and the buffer pH ⟨or⟩ pKa±2 units: ±1 unit

In each case a reference standard must be used to demonstrate that there is improvement in chromatography due to pH adjustment. No pH adjustment is allowed if standards are not available for all analytes of interest

Column dimensions

1. Length: ±70%
2. Inner diameter: ±25%, provided a constant linear flow velocity is maintained.

In addition, flow rate changes up to ±50% have been proposed. Work on limits for changes for mobile phase solvent ratios is underway. It is proposed that any method adjustment within these limits will not require additional validation. Proactive revalidation takes into consideration the availability of new technology or perhaps the automation of previously complex or time-consuming manual procedures. In such cases, revalidation may be more comprehensive, depending on the scope of the project.

18.3. Method Transfer

Method transfer is dependent on the intended use of a validated method. If other laboratories such as quality control or stability group are going to use this validated method, then a proper method transfer will be required. Under ideal conditions all laboratories involved should use an interactive approach to achieve method development, optimization, and validation goals in an efficient manner. If the end user has been involved in the development and validation process from the onset of this process as a participant or an observer, then it is convenient to place this method on line in a timely manner. Otherwise, reasonable time and effort will be required for the transfer process to be completed in a timely manner. Validation of a method demonstrates suitability of the method, whereas the method's evaluation and validity is approved by the end user.

The first step in a method transfer is to design a protocol, which is a document consisting of elements as outlined in the validation protocol, and other additional elements such as acceptance criteria, report format, and approval signatures of both the originating and the receiving laboratories. In addition, a detailed test procedure, design of experiments, sampling plan, analyst and equipment, interday and intraday ruggedness, and method transfer report form should also be included for method transfer studies.

Studies required for method transfer include system suitability, linearity, precision (day-to-day, within-day, analyst-to-analyst, analysis of multiple lots), collaboration of laboratories, developer user agreement on split sample results, and use of appropriate statistical standards, e.g., F-Test and t-Test, for evaluation of the method transfer process. The receiving laboratory should allocate enough time for the transfer, participate in interlaboratory studies, anticipate problems, and have a checklist ready of questions for the originating laboratory. For a successful method transfer, it is important to compare equipment or instrumentation in both laboratories. For example, for a chromatographic method, the age of the detector, the column, and the internal diameter of connecting tubing will play significant roles in the generation of comparable chromatograms.

Finally, where is method transfer required? In general, method transfer will be required for a new laboratory, a new method, new personnel, significant changes in a method, from company to a contract laboratory and from research and development group to quality control laboratory and stability group.

19. APPLICATION OF VALIDATION PRINCIPLES TO OTHER ANALYTICAL TECHNIQUES

19.1. Cleaning Method

To discuss cleaning validation in detail is beyond the scope of this work, as this has become an independent field. However, important steps required for cleaning validation will be briefly described. Readers are encouraged to research relevant publications on this subject. Recently, Kirsch has published an excellent review article on this subject (48). Cleaning validation is a regulatory requirement. The FDA has published a document titled "Guide to Inspections, Validation of Cleaning Processes" on this subject for field application. It is common industry practice to use the same equipment for production of a variety of products. The FDA has placed an increased emphasis on the cleanliness of the equipment to eliminate or minimize the risk of cross-contamination and adulteration of drug products.

Several analytical methods have been used by the pharmaceutical industry to demonstrate the cleanliness of process equipment surfaces. For low-level residues in rinse samples, the electronic conductivity technique are used. This technique is applicable to samples such as detergents and cleaning agents, which contain one or more ionic species. However, this technique is nonspecific and cannot be applied to neutral or highly polar compounds. Also, the FDA has specified a requirement that a correlation must be established between measurable conductance and concentration around the cleaning limit, which is a time-consuming process and not always possible for all analytes in a given formulation.

UV-visible spectrophotometry is another approach used for the detection of residue in rinse samples. This technique is sensitive but is dependent on the presence of a strong chromophore in the analyte for trace level determination. This is also a nonselective technique and not discriminating if more than one UV-active analytes are present in same sample.

Total organic carbon (TOC) has gained wide acceptance for cleaning applications (33,48). This technique is highly sensitive and specific for organic carbon-bearing analytes. TOC may be used in tandem along with conductivity, pH, and perhaps titrimetry to demonstrate the absence of both acid and alkaline detergents used for cleaning. TOC is only applicable to aqueous samples, and extra caution is required during sample acquisition and preparation to avoid bias in results due to carbon contamination.

High performance liquid chromatography (HPLC) has successfully been applied for cleaning residue samples. HPLC is sensitive for many pharmaceutical actives, and the necessary specificity can be obtained by this technique. For this technique, there are a variety of detection modes, such as spectrophotometric, electrochemical, fluorescence, and refractive index, to handle the diversity of pharmaceutical compounds.

Before the validation process begins, the appropriate predetermined level of cleanliness, i.e., at or below the limit at which equipment is considered clean, the final solvent used for the cleaning of equipment, and the type of swabs to be used should be chosen in consultation with the manufacturing group. It is critical that the limit agreed upon is practical and routinely achievable when an appropriate cleaning assay method is followed. Additionally, an acceptance limit that assures

that the next product manufactured on the same piece of equipment is not adulterated or contaminated to the extent that its fitness for use is compromised, must be established. For determination of cleaning residues by HPLC, an appropriate laboratory procedure must be developed using available methodology and validated to meet certain acceptance criteria. For validation, a written protocol will be required.

The method should be sensitive enough to detect the analyte of interest at levels below or above the acceptance limit. The following studies are suggested for validation of the cleaning assay method:

1. Linearity of response for a wide range depending upon cleaning limit. Generally 50% of the cleaning limit to 10 times this concentration.
2. Specificity or selectivity to prevent false passing or failing results.
3. Precision and accuracy to assure correct results.
4. Limits of detection and quantitation.
5. Analyte stability before and after extraction from swab or in rinse samples.
6. The solvent used in the final rinse should be compatible with the assay mobile phase.

Since cleaning validation is considered a limit test, validation requirements may be less rigorous than an HPLC method used for bulk drug active, finished product, and stability samples.

For accuracy, swab and surface recovery approaches should be utilized and evaluated. In swab recovery, an appropriate number of swabs (minimum three) are spiked with amounts of analyte in rinse solution equivalent to the amount of analyte that should theoretically be removed from a known surface area. The spiked analyte is allowed to dried on the swab followed by extraction in a known volume of extracting solvent. Recovery studies should be performed at least for three levels, i.e., limit level, 50% of limit level, and 100% of limit level. The percentage recovery is calculated by using an external standard prepared at limit level concentration. The percent recovery obtained should not be less than 80%.

In surface recovery, 316 stainless steel or inert glass plates are used. A known surface area is spiked with a known amount of analyte by uniformly spreading analyte in rinse solution over the known surface area. The plate is dried under ambient conditions. The drying time will depend on the solvent used. However, overnight drying is preferable. The dried analyte residue is then swabbed using a premoistened swab across the spiked area. Swabbing can be done by horizontal, vertical, and zigzag motion of the swab. For better recoveries more than one swab may be required for removing analyte from the plate surface. The swabs are then placed in a 50 mL tube and extracted with the extracting solvent specified in the method. Recovery calculations are done against an external standard prepared at limit level. Recoveries can be performed at limit level, 50% of limit level, and 100% of limit level. It is pointed out that in the surface recovery approach, recovery values obtained are usually about 70 to 80%. Loss in recovery value is attributed to analyte solubility, analyte–metal binding strength, reactivity of surface with analyte, and swabbing technique. Also, streaking effects encountered in swabbing surfaces are detrimental and result in loss of the active.

19.2. Physicochemical Characterization Method (Dissolution)

USP 23 General Chapter <1225> designates dissolution testing under Category III. The validation parameters recommended in the Compendia are precision and ruggedness studies. Other studies are left to the discretion of the end user. It is common industry practice to verify a USP dissolution method by performing studies such as linearity of standard solutions, placebo interference, capsule shell interference (if a capsule) and reproducibility of response at specified times(s) for release. However, for an in-house developed dissolution method, proper validation studies are required. These studies would include specificity (interference from placebo), precision, linearity, system suitability, filter adsorption, and sample and standard stability. For automated dissolution systems, in addition to filter adsorption, there should be evidence of nonadsorption to active tubing used for delivery throughout the system and carryover effects.

It is difficult to perform recovery studies for dissolution, as spiking of placebo in vessels is not practical. Placebo excipients have a tendency to float on top of the dissolution medium. In addition, it is difficult to make single tablets unless a hand-held press is used. Hand filled capsules lack uniformity, and the procedure is tedious. In another approach, placebo along with label claim amount of active are placed in a 900 mL volumetric flask. The flask is filled to volume with dissolution medium and a magnetic stir bar is used to stir this mixture on a magnetic plate for the specified time period. Calculations of recovery are done against an external standard prepared in the dissolution medium. Acceptance criteria for precision, specificity, system suitability, and linearity are similar to assay validation.

19.3. Nonchromatographic Methods

There are a variety of guidelines available for the validation of the chromatographic procedures, but comparatively little information is available on validation of nonchromatographic methods. In general, it is assumed that USP General Chapter <1225> on analytical validation is only applicable to chromatographic methods. This assumption is incorrect, as USP General Chapter <1225> does not state that the validation parameters given in this chapter cannot be used for nonchromatographic techniques. By careful selection of parameters, a validation protocol can be designed for validation of nonchromatographic methods. Brittain has discussed validation issues and data elements required for validation of nonchromatographic methods (49).

19.3.1. UV Spectrophotometry

For UV spectrophotometric methods for assay, one needs to study parameters such as precision, accuracy, specificity, and linearity (49). For precision, a sufficient number of individual sample preparations should be assayed to permit the calculation of a statistically valid relative standard deviation. Accuracy can be determined by spiking a mixture of excipients (placebo) with known amounts of drug active at different concentration levels. Spike levels are, in general, similar to the linearity range. Spiked samples are prepared by following the "sample preparation" procedure and assayed against an external standard at the target level concentration. The accuracy is calculated from the test results as the percentage

of analyte recovery by the assay. For specificity studies, intrinsic differences in chemical or physical properties are used to ensure accurate determination of analyte even in complex sample mixtures.

The purpose of a specificity study is to demonstrate that the method will yield reliable results even in the presence of interfering species. One approach to determine any possible bias in an assay is by comparison of results of assay value obtained in the presence of placebo excipients to assay value without placebo excipients. Assay bias is evaluated by calculating the percentage agreement between these two results by the formula (49)

$$\text{Percent agreement} = \frac{\text{TP}}{\text{TA}} \times 100$$

where TP = test results in the presence of placebo and TA = test results in the absence of placebo. A 100% agreement with show the absence of bias due to placebo or the potential interfering species. Agreement values >100% indicate positive bias, while agreement values <100% indicate negative bias in the assay procedure.

If standards for impurities or degradation products are not available, then the specificity can be determined by analyzing the samples containing the impurities or degradation products (from bulk drug) and comparing the results with those obtained by another independent and validated assay procedure. The independent assay is considered as the reference assay, and the degree of agreement between these two test results will dictate the specificity for the intended method. Calculations are similar to that described above. A percent agreement of 100% will be required for the absence of any bias in the intended method.

Linearity should be performed at least for five levels, including target level as 100%. Other concentrations should be 50%, 75%, 125%, and 150%. It is important that linearity responses obey Beer's law. Statistical evaluation of linearity is similar to that (as explained under linearity studies) for the chromatographic method validation.

19.3.2. Atomic Absorption Spectroscopy

Atomic absorption is used to determine heavy metals present in the drug substance. Heavy metals fall into the category of a limit test. Therefore rigorous validation may not be required. However, as these metals are present at trace levels, determination of limits for detection and quantitation is of significant importance for the validation of atomic absorption methods. Other validation parameters such as linearity, precision, specificity, and accuracy may be performed as described under Sec. 19.3.1.

The limits of detection and quantitation are determined by analyzing a number of samples prepared at low levels such as 2 ppm, 5 ppm, and 10 ppm. For each concentration level, multiple analysis is performed and standard deviation (SD) is calculated. All standard deviations are then averaged to calculate the mean standard deviation (MSD). To obtain an estimate of the noise level, the MSD is then divided by the slope of the calibration curve. For the detection limit the noise level obtained is multiplied by a factor of 3, whereas for the quantitation limit, a factor of 10 is used.

19.3.3. Infrared Spectroscopy (IR)

Infrared is used for identification of compounds. Currently in USP 23 there is a scarcity of monographs describing the use of IR for quantitation of analytes. For example, IR quantitation is used for the analysis of simethicon bulk drug active, tablets, oral suspensions, and capsules. As such, validation parameters required may be limited to interference studies. This interference may be due to the compound itself. For example, in an infrared spectrophotometric identification test, polymorphism may produce interference. Therefore, for compounds that exhibit polymorphism, it is critical that test samples and the reference standard have similar crystalline form. It then becomes obvious that for the infrared identification test, one should demonstrate that the method is insensitive to any polymorphic form of the material, or that the polymorphic effects have been taken into account. It is pointed out that unlike the chromatographic procedure, there are no official guidelines available on the validation of an infrared technique at present. An article by Ciurczak, "Validation of Spectroscopic Methods in Pharmaceutical Analyses," gives an overview of this subject (50).

19.3.4. Titration

USP 23 has several monographs that stipulate using titrimetry for release of bulk actives. These procedures are nonspecific and may not give accurate results in the presence of reactive impurities or degradation products. Therefore, for validation of these procedures an innovative approach will be required. The parameters to validate a titrimetric method include linearity, accuracy, blank determination, and insensitivity of the method to the amount of indicator used.

For linearity studies, different weights of the compound should be titrated, and the actual and theoretical results should agree. Alternatively, the titration could be done using a narrow range of compound weight, and then it should be stated in the method that the weight of the sample must be within this range. The accuracy should be studied by showing that the volumes of titrant for replicate titrations are very close to each other. In other words, small differences in volume of titrant required to reach an end or equivalence point does not introduce any significant error into the results.

As stated earlier, titrimetric procedures are nonspecific and cannot be used for simultaneous assay of active and impurities. In this case, impurities should be monitored by another independent validated procedure. For bulk active assay, comparison of results obtained by an alternate validated method and those obtained by the titrimetric procedure will demonstrate the validity of the titrimetric method.

19.4. General Considerations

The accuracies of chromatographic methods rely heavily on the purity of reference standards. Therefore a well characterized and highly pure standard is important. The FDA recognizes two categories of reference standards, i.e., compendial and noncompendial. The USP is the source of compendial standards. As these standards are well characterized, no further characterization is required. Noncompendial standards are also of high purity and can be obtained by reasonable effort and should be

thoroughly characterized to ascertain their identity, strength, quality, and purity. Testing requirements for the reference standards are more rigorous as compared to bulk drug substance. The purity correction factors for non-USP standards should be included in any method calculations.

Quantitation of actives in chromatographic methods is based on either external or internal standards. An external standard method is used when the standard is analyzed on a separate chromatogram from the sample. Quantitation in this case will be by comparison of the sample and reference standard responses, i.e., peak area or peak heights for HPLC and GC or spot intensity in TLC for a given analyte of interest. External standard methods are generally used for samples with a single target concentration and narrow concentration ranges (acceptance and release tests). Simple sample preparation procedures or longer run times for detection of extraneous peaks, e.g., impurity test, HPLC methods for stability, and TLC methods also use external standards. For internal standard methods, a compound of known purity is added directly to the sample. However, it must be ensured that the compound being used as an internal standard does not interfere with any analyte of interest or degradation products in the sample. The response ratio between internal standard and analyte of interest in the sample is compared to the ratio of the internal standard and the analyte in the standard that is used for quantitation purposes. Internal standard methods are widely used for quantitation in biological samples and for low and wide sample concentration ranges, e.g., in pharmacokinetics studies.

There are some basic points that should be addressed in the test method.

1. The sample and the standard should be prepared in the mobile phase. If this is not possible, then the level of organic solvent used in the preparation of the sample and the standard must be lower than that present in the mobile phase.
2. The sample and standard concentrations should be close to each other.
3. Sample preparations often require filtration prior to injection onto the system. Filtration removes particulate matter that may clog the column. However, analyte adsorption on the filter can take place. This adsorption effect is important for low-level impurities. Therefore, data to validate this aspect will be required.

In conclusion, method validation is a dynamic process and should not be considered a one-time situation. The design and validation of the method should be such that they ensure its ruggedness or robustness throughout the life of the method. The accuracy of the data is affected by variations in the manufacturing process, the preparation of samples in the laboratory, and the instrument performance. With a well-designed validation and tight chromatographic system suitability criteria, the reliability of the data can be significantly improved. Variations, except from the drug product manufacturing process, can and should be minimized. Good, reliable validated methods will generate data that is trustworthy.

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APPENDICES

1. List of Guidance Documents, CDER. http://www.fda.gov/cder/guidance/index.htm
2. Useful websites:
   www.pharmweb.net
   www.waters.com
   www.usp.org
   www.ich.org
   www.aoac.org

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Stability Testing of Clinical Trial Materials

WOLFGANG GRIMM

Biberach, Germany

1. INTRODUCTION

1.1. General Requirements for Stability Testing

The aim of stability testing is to ensure the quality, safety, and efficacy of drug products up to their expiration date. This means that all

Organoleptic
Physicochemical
Chemical
Microbial

test results must be within the shelf life tolerance ranges up to the end of the shelf life. Extensive studies are needed for this purpose. Stability testing accompanies the development of a medicinal product from the first preliminary trials with the drug substance up to continuous production. If the stability program is scientifically well founded, systematically structured and logically coordinated stability information will be continuously augmented and become increasingly reliable.

The overall stability program can be divided into six steps (1):

Step 1: Stress and acceleration tests with the drug substance
Step 2: Preformulation and formulation finding for
toxicological test samples
clinical samples
final dosage form
Step 3: Stress and acceleration tests with selected formulations
toxicological test samples
clinical samples
final dosage form
Selection of primary packaging materials
Step 4: Acceleration and long-term tests on drug substance and drug products up to marketing authorization
Step 5: Ongoing stability testing of drug substance, drug products
marketing authorization batches
production batches
Step 6: Follow-up stability tests on drug substance, drug products
continuous production
modifications during continuous production

Each stage covers eleven basic principles (2):

Selection of batches and samples
Test criteria
Analytical procedures
Specifications
Storage conditions
Testing frequency
Storage period
Number of batches
Packaging materials
Evaulation
Stability information

Some of these elements, such as selection of samples and batches, number of batches, storage conditions, testing frequency, and storage periods are firmly established, while others, such as test criteria, analytical procedures with validation, and specifications undergo further development. Combining the six stages and the eleven basic principles yields a systematically structured stability study schedule.

1.2. Specific Requirements for Stability Testing of Clinical Samples

Similarly to the requirements for proprietary medicinals, the aim of stability testing in this area of clinical samples is to maintain the quality and safety of these materials up to the end of phase I, II, and III clinical trials.

This means that all relevant test results must remain within the minimum shelf life specifications up to the end of the clinical trial.

In systematically structured stability programs, stability tests are carried out on clinical samples in steps 2 and 3.

Stability testing accompanies phase I to III clinical trials.

At first, neither the dosage formulation nor the dosage form is definitely established; they are gradually defined during the course of development.

The same applies to analytical procedures and specifications.

The effort and scope of stability testing must be tailored to the specific problem.

The stability program will always differ from that required to generate stability information (expiration date, etc.) for marketing authorization application documents for proprietary medicinals. This stability information is based on the results obtained with three representative pilot plant batches put into storage after the end of development. The stability information is then applicable to all production batches.

The stability information for clinical samples is required only for a small number of batches and for the duration of the respective clinical trial.

The shelf life determined is thus not a maximum shelf life ($\leq$5 years) at the end of which the acceptance criteria of the shelf life specifications for individual test parameters are reached, but a minimum shelf life at the end of which the tolerance limits usually have not been reached.

There are no defined official regulations stipulating effort, scope, and implementation, which are left to the manufacturer's discretion.

In the USA (6), in each phase of the investigation sufficient information should be submitted to ensure the proper identification, quality, purity, and strength of the investigational drug; the amount of information needed to achieve that assurance will vary with the phase of the investigation, the dosage form, and the amount of information otherwise available.

Therefore, although stability data are required in all phases of the IND to demonstrate that the new drug substance and drug product are within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation, if very short term tests are proposed, the supporting stability data can be correspondingly very limited. It is recognized that modifications to the method of preparation of the new drug substance and dosage form, and even changes in the dosage form itself, are likely as the investigation progresses.

In the USA and Japan, clinical samples are not required to be marked with an open expiration date, as the period of use (use-by date, expiry date, or re-test date). Therefore in an initial phase I CMC submission, the emphasis should generally be placed on providing information that will allow evaluation of the safety of subjects in the proposed study. The identification of a safety concern or insufficient data to make an evaluation of safety are the only reasons for placing a trial on clinical hold on the CMC section.

Information to support the stability of the drug substance during the proposed clinical study(ies) should include the following: a brief description of the stability study and the test methods used to monitor the stability of the drug substance and preliminary tabular data on representative material. Neither detailed stability data nor the stability protocol need to be submitted. When significant decomposition during storage cannot be prevented, the clinical trial batch of drug product should be retested prior to the initiation of the trial, and information should be submitted to show that it will remain stable during the course of the trial.

This information should be based on the limited stability data available when the trial starts. Impurities that increase may be qualified by reference to prior human or animal data.

Development of drug product formulations during phase II should be based in part on the accumulating stability information gained from studies of the drug substance and its formulations.

The objectives of stability testing during phases I and II are to evaluate the stability of the investigational formulations used in the initial clinical trials, to obtain the additional information needed to develop a final formulation, and to select the most appropriate container and closure. This information should be summarized and submitted to the IND during phase II. Stability studies on these formulations should be well underway by the end of phase II.

At this point the stability protocol for study of both the drug substance and the drug product should be defined, so that stability data generated during phase III studies will be appropriate for submission in the drug application.

In stability testing during phase III IND studies, the emphasis should be on testing final formulations in their proposed market packaging and manufacturing site based on the recommendations and objectives of the ICH Stability Guideline.

It is recommended that the final stability protocol be well defined prior to the initiation of phase III IND studies.

In this regard, considerations should be given to establishing appropriate linkage between the preclinical and clinical batches of the drug substance and drug product and those of primary stability batches in support of the proposed expiration dating period. Factors to be considered may include for example source, quality, and purity of various components of the drug product, manufacturing process of and facility for the drug substance and the drug product, and use of the same containers and closures (6).

Clinical samples in the EU are required to be marked with an open expiration date (7).

The corresponding information for this open expiration date must be available before the packaging of the clinical trial batches.

The base for the stability information concerning clinical samples forms the Stability Profile of the drug substance derived from the stress investigations in step

1 of the systematically structured stability schedule, the stress and accelerated testing with the drug substance.

These studies are undertaken to elucidate the intrinsic stability characteristics of the drug substance with reference to physicochemical and chemical properties, to establish the degradation pathway in order to identify the likely degradation products, and to validate the stability-indicating power of the analytical procedures used.

The investigations are of general nature and not specific to any particulate dosage form.

The additional influence of the excipients is investigated in step 2 "Preformlation and formulation finding for clinical trial samples." The results are summarized in a research report, "Formulation evaluation."

From now on two different procedures can be pursued to provide the stability information for the open expiration date:

1. Stress and accelerated tests and long-term testing for confirmation.

Stress and accelerated testing are performed with selected batches to derive the minimum shelf lives for the phases I, II, and III. These minimum shelf lives are applicable to all batches of the relevant phase.

The predictions are then supported and confirmed by long-term testing under storage conditions presenting climatic zone II, 25°C/60% r.h.

This procedure has the following advantages.

Instable formulations are recognized at an early stage and can be reformulated if required.

The continuous development of the drug product is guaranteed without substantial time loss due to long-term testing.

The stress and confirmation testing is performed in phases I, II, and III. Thereby an excellent linkage is given between the preclinical and clinical batches in support of the proposed shelf life for the registration batches.

A series of possible influencing factors on the formulation can be assessed.

The repeated confirmation of predicted minimum shelf lives by the results of long-term testing guarantees a high degree of reliability of the stability information.

The number of investigated batches is limited; a prediction of the required capacity can be established.

If the results of the stress investigations indicate decomposition but no distinctive temperature dependence, then a prediction is not possible and a corresponding long-term testing has to be performed.

2. Accelerated and long-term testing with the clinical trial batches.

The clinical trial batches are put on accelerated and long-term testing.

Only a limited prediction of the minimum shelf life, especially in the phases II and III, is possible, with the consequence that not the total period of the intended clinical investigation may be covered.

In the USA no open expiration date is required. The clinical samples may be stored at 25°C/60%. r.h. and analyzed in parallel with the clinical trial investigation.

If all data are within the specifications, nothing has to be done.

If the data, however, exceed the specification it may be necessary to replace the samples in the clinic by new ones. Therefore corresponding batches have to be kept in stock.

This is not easy to accomplish.

Therefore procedure 1 is preferred and consequently described in detail.

Stability information for clinical samples plays a very important role in the general assessment of the quality and safety of a medicinal product.

Continuous stability is assured for the transition from phase I to III, including pivotal and equivalence batches, to the finished drug product in the commercial form.

Stability programs for clinical samples now are based on the ICH Tripartite Guideline for Stability Testing of Drug Substances and Drug Products, although the guideline itself, as already mentioned, does not apply to clinical samples.

The basic principles of the ICH Tripartite Guideline correspond to the aforementioned eleven principles and now have to be adapted to deal with the specific problems encountered with clinical samples.

2. BASIC PRINCIPLES OF STABILITY TESTING APPLIED TO CLINICAL SAMPLES

2.1. Selection of Batches and Samples

The drug product is in the process of development. Several strengths are tested in clinical phase I and the formulation and dosage form are modified in the transition to II and III.

This developmental process has to be taken into account when selecting the batches. Especially in the initial phase of development, no representative batches are available. The following batches are put into storage with the aim of establishing the minimum shelf life for phases I to III:

Clinical phase I:	experimental batches
Clinical phase II:	clinical experimental batches
Clinical phase III:	clinical or pilot plant batches

2.2. Test Criteria

The criteria of the product are investigated

That are potentially subject to change during the course of storage

That have a particular bearing on the quality, safety, or acceptance of the product.

The relevant test criteria will become apparent during the course of development from phase I to phase III.

2.3. Analytical Procedures

The analytical procedures themselves undergo a process of development from phase I to phase III. The same applies for the validation.

Three steps of validation are differentiated:

Orientational
Preliminary
Complete

Table 1 lists the extent of validation for the three steps.

Table 1 Extent of Validation During Development

	Extent of validation		
Validation characteristic	Orientational	Preliminary	Complete
Specificity	x	x	x
Linearity	x	x	x
Quantitation limit	x	x	x
Detection limit[a]	x	x	x
Accuracy		x	x
Range		x	x
Repeatability		x	
Intermediate precision			x
Robustness	x	x	x
Validation report			x

[a] Only for semi-quantitative procedures instead of quantitation limit

2.4. Specifications

Fixing specifications is an evolving process that accompanies the development of the new drug substances and drug products.

It can be described as a four step-procedure; see Table 2.

For all four steps one has to differentiate between

Release specification
Shelf life specification

Table 2 The Four Steps of the Specification

Step of development drug substance, drug product	Specifications	Characterization
Preclinical Clinical phase I	Orientational	Target values
Clinical phases II/III Pivotal batches	Preliminary	Broader acceptance criteria, ranges, numerical limits
Pilot plant batches Registration batches	Registration	Acceptance criteria focusing on safety and efficacy
Production batches after marketing authorization	Post approval	Experience gained with manufacture of a particular drug substance or drug product

This concept of different acceptance criteria for release versus shelf life specifications applies to products only, not for drug substances.

The ICH Guideline requires this distinction between release and shelf life specification.

Release specifications describe the quality after manufacture and include

Analytical variability
Manufacturing variability

The variability is described by

RSD of repeatability or intermediate precision of the analytical procedure. Accordingly, validation has to be performed, preliminary or complete.
Data of ≥ 3 batches to describe the manufacturing variability.

Shelf life specifications describe the quality at the end of the shelf life and include

Tolerable changes during storage and shipment.

Therefore corresponding stability data are required with organoleptical, physicochemical, chemical, and microbial tolerable changes.

2.5. Storage Conditions

A basic distinction is drawn between stress, accelerated, and long-term storage conditions; see Table 3.

Table 3 Stress, Accelerated, and Long-Term Storage Conditions

Type	Condition
Stress	Temperature: 10°C higher than accelerated temperature of 40°C, e.g., 50°C, 60°C, 70°C ≥ -10°C Temperature cycle 5–40°C Open storage at 25°C/60% r.h., 30°C/70% r.h. and 40°C/75% r.h. Xenon lamp 48 hours
Accelerated	40°C/75% r.h. (30°C/70% r.h.)
Long-term	25°C/60% r.h. 30°C/70% r.h.

The conditions used in stress and accelerated tests are above those of the relevant climatic zones, allowing

The discriminatory power of the analytical procedure to be verified.
Weaknesses of a formulation to be identified.
Stability information to be generated.

The last-named aspect is particularly important in designing a stability program for clinical samples. To ensure continuous development, specific stress and acceleration tests are carried out, which are then verified by long-term tests.

If stress and acceleration tests are to be successful, two aspects must be paid special attention:

Clear separation between the tests for
organoleptic and physicochemical stability
chemical/microbial stability
Use of packaging materials impermeable to water vapor for stress tests at elevated temperatures, allowing application of the laws of reaction kinetics

The reaction mechanism may change with higher temperature if the samples are dried.

It is therefore necessary to use packaging materials that are impermeable to water vapor to prevent solid formulations becoming dehydrated at higher temperatures or the active ingredient concentration of liquid formulations increasing due to loss of moisture.

The laws of reaction kinetics cannot be used to make stability predictions for organoleptic and physicochemical changes.

This is the reason that solid dosage forms, for example, are stored without packaging at 25°C/60% r.h. This induces the maximum possible changes due to absorption or loss of water. Semisolid and liquid dosage forms are stored at ≥ -10°C, semiliquid at 5–40°C in order to detect irreversible changes.

Storage at 30°C/70% r.h. is not usually necessary because most clinical trials are performed in countries of climatic zones I and II.

2.6. Testing Frequency

The testing frequency is established to suit the problem being studied. Retest periods are different for stress, acceleration, and long-term tests.

2.7. Storage Period

The storage period depends on the required minimum shelf life. We can differentiate between stress, accelerated, and long-term storage period; see Table 4.

2.8. Number of Batches

With all the different strengths, dosage forms, and packaging materials examined during the development phase, it is not possible to provide three batches for each dosage form. Reliable information can be obtained nevertheless by applying

Table 4 Storage Periods

	Storage period (months)		
Clinical phase	Stress	Accelerated	Long-term
I		1.5	3
		3.0	6
II	3	6	12–18
III	3	6	24–36

scientifically based rationalization measures. This includes the expedients mentioned in the ICH Stability Guideline:

Bracketing
Matrixing

Both methods are based on the assumption that a reduced number of investigated samples is representative of the stability behavior of all samples.

In bracketing, only "limit samples" are tested, for example: the lowest and highest dosage, the smallest and largest container.

In matrixing, selection is performed according to a statistical procedure (random number).

A rational bracketing system for all dosage forms would be as in Table 5.

A rational matrixing system would be as in Table 6.

Table 5

Dosages	Samples tested
1–2	all
3–4	highest lowest
>4	highest middle lowest

Table 6

Test sample	Tests
Beginning, end	all
Intermediate values	1/3 or 2/3 design[a]

[a] At each testing point 1/3 or 2/3 of all samples are analyzed.

2.9. Packaging Materials

At higher temperatures, desorption and loss of moisture also occurs at higher relative humidities.

Unless packaging materials impermeable to water vapour are used for stress tests with solid dosage forms, the samples lose moisture at different rates in the temperature range 40–60°C, and the results are not suitable for a reaction kinetics calculation.

Packaging materials permeable to water vapor can however also result in a falsification of the results for semisolid and liquid dosage forms if varying degrees of weight loss occur that lead to differences in the active ingredient concentration or ion strength.

The use of inert standard packaging materials that are impermeable to water vapor is thus an important precondition for stress tests that are to be evaluated in terms of reaction kinetics, and on the results of which stability predictions are to be based.

An overview of the most important packaging materials for stability testing for clinical samples is given in Table 7.

2.10. Evaluation

A systematic approach should be adopted in the evaluation and presentation of the analytical results.

Tests for significant changes with the aid of statistics, reaction kinetics calculations, or linear regression analysis are valuable tools. The stress and accelerated test results are evaluated for each clinical phase taking into account the specific objective of the respective storage conditions.

On the one hand, there is the test for organoleptic and physicochemical stability, on the other hand for chemical or microbial stability. If, for example, discoloration, a decrease in hardness, an increase in dissolution, or phase separation is observed after 3 months's storage at 70°C, these changes will be recorded, but they are only of limited relevance for predicting stability. If there are no significant changes in the test for organoleptic and physicochemical stability, stability can be predicted by means of reaction kinetics calculations that are based mainly on the results obtained after storage at stress temperatures.

Considering these facts, all test criteria can be included in the stability prediction. A critical examination is conducted to determine whether relevant changes have occurred and whether the proposed minimum shelf life tolerance limits have been reached or exceeded.

Stability studies for clinical samples are based on stress and acceleration tests with the aim of speeding up, especially, chemical decomposition by storing samples at elevated temperatures. The results are then used to calculate the stability behaviour at 25°C/60% r.h. based on the laws of reaction kinetics.

The equations for a first-order reaction and the Arrhenius model are used. If decomposition levels are available for only one temperature (4,5), the expression ΔE: 83 kJ mol^{-1} is used for the activation energy.

Table 8 shows decomposition levels for 25°C/60% r.h. calculated from values obtained after storage at 40°C to 70°C. Reported in Table 6 are the decomposition determined after storage at accelerated and stress temperatures, and the decomposition for 25°C derived from these data.

After evaluating the data of one batch it is assessed whether the strengths or the dosage forms exhibit different stability behavior or whether the results of the batches can be combined to produce uniform stability information.

The packaging material and possible interactions have to be included in the evaluation.

The general stability information, the period of use, and if necessary storage instructions are based on

Primary data
: The results of the stress and accelerated investigations and later the results of the long-term testing for confirmation.

Supportive data
: Drug substance stability profile, which also includes orientational predictions regarding the chemical stability of the drug substance in solid, semisolid, and liquid dosage forms.

Table 7 The Most Important Packaging Materials for Stability Testing of Clinical Samples

Clinical phase	Dosage form, packaging material		
	Solid	Semisolid	Liquid
I Acceleration tests	Standard packaging material Glass container with twist-off closure	Standard packaging material Standard tube	Standard packaging material Ground-glass-stoppered flask or Glass ampoule or Injection vial with rubber stopper
Long-term test	Standard packaging material Glass container with twist-off closure PP tubes[a]	Standard packaging material Test packaging material	Standard packaging material Test packaging material
II Stress tests	Standard packaging material Glass container with twist-off closure	Standard packaging material	Standard packaging material
Acceleration tests	Standard packaging material Glass container with twist-off closure	Standard packaging material Testing packaging material	Standard packaging material Testing packaging material
Long-term test	Standard packaging material Glass container with twist-off closure PP tubes or Test packaging material: blister	Standard packaging material Test packaging material	Standard packaging material Test packaging material
III Stress tests	Standard packaging material Glass container with twist-off closure	Standard packaging material	Standard packaging material
Acceleration tests	Standard packaging material Glass container with twist-off closure	Standard packaging material Test packaging material	Standard packaging material Test packaging material
Long-term test	Standard packaging material Glass container with twist-off closure PP tubes or Test packaging material: blisters	Standard packaging material Test packaging material	Standard packaging material Test packaging material

[a] PP = polypropylene

Stability investigations during formulation findings for the clinical phases I–III, which provide specific information regarding the influence of excipients and the overall formulation on organoleptic, physicochemical, chemical, and microbial stability.

For phase II

Minimum shelf life for clinical phase I: prediction and confirmation.

For phase III

Minimum shelf life for clinical phases I and II: prediction and confirmation.

It is an important fact that the predicted minimum shelf lives will always be confirmed by the concurrently performed long-term tests.

Table 8 Reaction Kinetics Extrapolation

Clinical phase	Storage condition	Decomposition found/ extrapolated decomposition (%)					
I	40°C: 1.5 months	0.10	0.20	0.30	0.40	0.50	1.00
	25°C: 3 months	<0.10	<0.10	0.12	0.16	0.20	0.40
I	40°C: 3 months	0.10	0.20	0.30	0.40	0.50	1.00
	25°C: 6 months	<0.10	<0.10	0.12	0.16	0.20	0.40
II	60°C: 3 months	0.10	0.20	0.30	0.40	0.50	1.00
	25°C: 12 months	<0.10	0.18	0.27	0.35	0.44	0.89
	25°C: 18 months	0.13	0.27	0.40	0.53	0.66	1.30
II	40°C: 6 months	0.10	0.20	0.30	0.40	0.50	0.60
	25°C: 12 months	<0.10	<0.10	0.12	0.16	0.20	0.40
	25°C: 18 months	<0.10	0.12	0.18	0.24	0.30	0.60
III	70°C: 3 months	0.50	1.00	2.00	3.00	4.00	5.00
	25°C: 24 months	<0.10	0.10	0.20	0.30	0.40	0.50
	25°C: 36 months	<0.10	0.15	0.30	0.45	0.60	0.76

2.11. Stability Information

All the results and the stability information derived therefrom are complied in a stability report.

This contains

The batch information
The results
A critical assessment of the analytical procedures and of the results
The minimum shelf life
Storage instructions if necessary

Usually for each clinical phase a stability report is written.
Stability report:

Stress Testing and Long-Term Testing
Phase I
Phase II
Phase III

The results of stability tests on clinical samples are used to set minimum shelf lives and not expiration dates.

The duration of each clinical trial including logistics and provision of clinical supplies must be fully covered. In many cases, therefore, the minimum shelf life will not be determined by reaching a shelf life tolerance limit. In all these cases the expiration date can be extended, if necessary, on the basis of suitable studies.

The following minimum shelf lives are considered optimal:

Clinical phase I: 3–6 months
Clinical phase II: 12–18 months
Clinical phase III: 24–36 months

The established minimum shelf lives are valid for all the batches of the relevant clinical phase.

Since the product is in the process of development, the shelf lives are not based on three representative validation batches but on

batches comprising different dosages as tested by the bracketing procedure.
batches comprising different dosage forms obtained as development progresses.
batches of different origin and size. These are usually experimental and development batches.

If a critical examination reveals that the batches exhibit similar stability behavior, the result can be applied to the "same" batches of the relevant clinical phase with a high level of reliability.

It can be further stated that the factors batch size, technology, and equipment used in the manufacturing process affect primarily the quality of manufacture and not that of stability.

This means that they are identified in the analysis conducted immediately after manufacture that determines the quality in relation to the release specifications (Table 9).

Table 9 The Influencing Factors for the Individual Dosage Forms

Influencing factors	Possible influence on quality of the dosage forms		
	Solid	Semisolid	Liquid
Batch size	Appearance	Appearance	Appearance
Equipment	Content uniformity	Homogeneity	pH
Manufacturing process	Dissolution	Homogeneity within a container	Preservation
		Preservation	Chemical stability after manufacture
		Chemical stability after manufacture	

If all the analytical data obtained after manufacture are within the release specifications, the stability information obtained from the stress tests and acceleration tests can be considered generally applicable with a high degree of reliability.

It may be necessary to ensure compliance with the minimum shelf life by marking packs with storage instructions.

It is important to present this information unambiguously (Table 10).

Table 10 How Storage Instructions Should Be Worded

Storage instruction	Reason
Do not store above 30°C	Relevant changes were seen in the samples after storage at 40°C.
Do not store above 25°C	Relevant changes were seen in the samples after storage at 30°C/70%, but not after storage at 25°C/60%.
Store at ≤8°C in a refrigerator	Revelant changes were observed in the samples after storage at 25°C/60%.
2–8°C, store in a refrigerator, do not freeze	Relevant changes were observed in the samples after storage at 25°C/60% and −10°C.

2.12. Reliability of Minimum Shelf Lives

The shelf lives for the batches of clinical samples are established to cover the duration of the clinical trial plus a supplement to allow for logistics and the provision of clinical supplies. The shelf lives determined apply to all the batches of the relevant development stage, although only the batches in the final phase of development originate from a validated manufacturing phase and are therefore representative.

How reliable is shelf life and stability information?

This question can be answered as follows.

The shelf lives for clinical phases I and II (and in some cases III) represent a minimum shelf life, in other words, they still include a "reserve." A shelf life of 3 months for a first clinical trial does not mean that the batch may not be stable for longer periods. The shelf lives may be extended after appropriate storage and tests.

Minimum shelf lives are therefore associated with a lower risk than shelf lives at the end of which the sample may be unstable.

Furthermore, the principle of "semi-coverage" applies to clinical phase I, i,e., half the shelf life (3–6 months) is covered by storage at higher temperatures.

If there are several strengths, bracketing is performed, i.e., two to three strengths are tested simultaneously for stability.

If all two or three strengths exhibit the same stability behavior, a statement can be made regarding the reproducibility or the technological parameters.

If the stability information for two to three batches of different strengths or composition is identical, the information is naturally also applicable to identical batches.

As phases I through III progress, experience with analytical procedures and shelf life specifications increases. The results and stability information derived from them become steadily more reliable.

Finally, the stability program is designed in such a way that stability predictions are verified by the results of long-term tests.

There is thus still the possibility of replacing batches if necessary.

Like the overall system of stability testing, the stability program for clinical samples is systematically structured in such a way that the aggregate available information is continuously augmented (Table 11).

Table 11 Number of Dosages and Packaging Materials

Stage of development	Tests[a]	Number of dosages	Number of packaging materials	Total number	Derived information
Drug substance	Stress and acceleration tests	1	1	1	Retest date ≥2 years
Clinical phase I	40°C/1.5 months	≥2	2	≥4	3 months
	40°C/3 months	≥2	2	≥4	6 months
Clinical phase II	60°C/3 months	2[b]	2	4	12–18 months
Clinical phase III	70°C/3 months	1[b]	3	3	24–36 months
				≥16	

[a] A confirmatory long-term test is conducted concurrently with stress and acceleration tests.

[b] If more than 2 dosages are used in clinical phase II, ≥2 applies; if more than 1 dosage is used in clinical phase III, ≥2 also applies. This data in the table are therefore minimum limits.

2.13. Extension of the Derived Minimum Shelf Life

A minimum shelf life is determined for clinical trial samples and can be extended as necessary if the corresponding tests yield favorable results. Parallel to the stress and accelerated tests, samples are stored at 25°C/60% r.h. in order to confirm and support the predicted minimum shelf life.

If all the predicted data and the data confirmed in long-term tests are within the minimum shelf life limits, they can be extended if necessary. For this purpose, a new prediction is performed and samples are stored at 25°C/60% r.h. to confirm the new minimum shelf life (see Table 12).

3. PERFORMANCE

The performance of the stress and confirmation studies in phases I–III is described.

The base forms the Stability Profile of the NME, the corresponding drug substance.

Table 12 The Objective Is to Extend the Shelf Life by 30%

Derived minimum shelf life (months)	Extension by 30% to (months)
3	4
6	9
12	16
18	24
24	32
36	48

The Stability Report comprises the stability data of stress investigations with the active ingredient it represents the stability profile of the NME.

The following influencing factors were investigated: Moisture, temperature, moisture + temperature, moisture + temperature + drug substance concentration, pH, ionic strength, oxidation, and light.

The following stability information is derived.

For drug substance,

Test criteria for accelerated and long-term testing with the registration batches
Analytical procedures
Selection of packaging materials
Preliminary retest period
Storage instructions, if required

For drug product,

Solid, liquid and semiliquid dosage forms can be developed concerning chemical stability.

From the investigations of step 2, performulation and formulation finding for clinical trial samples, information is available on the additional influence of the excipients on the stability of the drug substance.

On the base of this comprehensive information, the stress and accelerated testing is planned and performed.

3.1. Solid Dosage Forms: Tablets, Capsules

3.1.1. Selection of Batches and Samples

Phase I: Experimental laboratory batches from the development laboratory
Phase II: Clinical batches, pilot scale from manufacturing clinical supplies
Phase III: Pilot plant batches, final formulation from pilot plant

3.1.2. Test Criteria

Organoleptic and physicochemical stability
Tablets: appearance, hardness, average mass, disintegration time, dissolution rate
Capsules: appearance, elasticity, average mass, average mass of content, average mass of filling, disintegration time, dissolution rate

Chemical stability

Tablets: appearance, hardness, disintegration time, dissolution rate, average mass, drug substance decomposition and assay

Capsules: appearance, elasticity, average mass, average mass of content, average mass of filling, disintegration time, dissolution rate, drug substance decomposition and assay

The organoleptic and physicochemical test criteria are included in chemical stability. The results may not be relevant to normal storage conditions.

3.1.3. Analytical Procedures

Stability specific information on degradation pathway is available from the stability profile. If possible the same analytical procedure is applied.

The validation is performed in three steps (Table 13).

If several strengths are investigated in phase I or II, the extent of validation can be reduced. If the final concentration of the analyte is the same after sample preparation, the validation is limited to one strength, usually the lowest.

Table 13 The Three Steps of Validation

Clinical phase	Step of validation	Validation criteria
At beginning phase I	Orientational	Specificity, linearity, quantitation limit ≙ reporting limit: 0.1%, robustness
At beginning phase II	Preliminary	In addition: accuracy, range, repeatability of assay and decomposition product, dissolution rate included
At beginning phase III	Complete	In addition: intermediate precision, complete robustness

3.1.4. Specifications, Table 14

Table 14

Clinical phase	Specifications
Clinical phase I	Orientational, target values
Clinical phases II/III	Preliminary

3.1.5. Storage Conditions, Storage Period, Testing Frequency

The same distinction is made for the storage conditions as for the test criteria.

For organoleptic and physicochemical stability testing, the samples are stored without packaging under the climatic conditions of long-term testing at 25°C/60% r.h. until equilibrium is reached. The maximum possible changes occur during this period.

The test for chemical stability is performed including also those samples that were stored without packaging at 25°C/60% r.h. until equilibrium was reached. With

this approach, the influence not only of the temperature but also of humidity on the stability of the product can be examined.

Parallel to the stress and acceleration tests, samples are stored at 25°C/60% r.h. representing the conditions of climatic zone II for confirmation. The storage period corresponds to the planned expiration date. These measures allow the stability prediction to be checked (see Tables 15 and 16).

Table 15 Storage Conditions for Organoleptic and Physicochemical Stability

Clinical phase	Packaging material	Storage condition	Storage period
I, II	Without, open	25°C/60% r.h.	Until equilibrium (2 weeks)
III	Without, open	25°C/60% r.h. 30°C/70% r.h. 40°C/75% r.h.	Until equilibrium (2 weeks)

3.1.6. Packaging Material

For stress testing, tight containers are required. 50 mL glass containers with twist-off closure are suitable or tight equivalent.

For long-term testing and clinical trial samples, on the basis of the results of the stress tests for solid dosage forms, the sensitivity to moisture can be determined, and suitable packaging materials can be selected.

As a rule, no interactions are to be expected.

If the final packaging material has been selected, and samples packed in the final packaging material are available, the investigation on photostability should be performed.

The samples with and without container are irradiated with a xenon lamp (Suntest 250 W/m^2) for 22 hours (ICH Guideline on Photostability Testing).

The test criteria are appearance, drug substance decomposition and assay.

3.1.7. Number of Batches

There should be one batch per clinical phase.

If more than one strength is required in phases I or II, bracketing is applied.

3.1.8. Evaluation

If all the results of the test for organoleptic and physicochemical stability are within the shelf life specifications, the stability prediction depends exclusively on the chemical stability. If the water content of the pretreated samples (open storage, 25°C/60% r.h.) has no influence, or if the influence is acceptable, there is no restriction on the choice of packaging materials.

If the results of the test for organoleptic and physicochemical stability are outside the tolerance limits, or if the water content influences the chemical stability to an unacceptable degree, a packaging material impermeable to water vapor must be selected, e.g., glass bottle with screw closure, polypropylene or polyethylene tubes, aluminum blister, or aluminum/aluminum.

Table 16 Storage Conditions for Chemical Stability

Clinical phase	Minimum shelf life	Packaging material	Pretreatment	Storage conditions (°C)	(%)	Storage frequency, storage period								
I	3	Twist-off[a]	None	40	—	0	2	4	6					weeks
	months	Twist-off	25°C/60%	40	—	0	2	4	6					weeks
		Twist-off	None	25	60					12				weeks
		PP tubes	None	25	60					12				weeks
I	6	Twist-off	None	40	—	0	1	2	3					months
	months	Twist-off	25°C/60%[b]	40	—	0	1	2	3					months
		Twist-off	None	25	60					6				months
		PP tubes	None	25	60					6				months
II	12–18	Twist-off	None	60	—	0	1	2	3					months
	months	Twist-off	None	40	—		1	2	3	6				months
		Twist-off	25°C/60%[b]	60	—	0	1	2	3					months
		Twist-off	25°C/60%[b]	40	—		1	2	3	6				months
		Twist-off	None	25	60						12	18		months
		PP tubes or test packaging material	None	25	60						12	18		months
III	24–36	Twist-off	None	70	—	0	1	2	3					months
	months	Twist-off	None	60	—		1	2	3					months
		Twist-off	None	50	—		1	2	3					months
		Twist-off	None	40	—		1	2	3	6				months
		Twist-off	25°C/60%[b,c]	70	—	0	1	2	3					months
		Twist-off	25°C/60%[b]	60	—		1	2	3					months
		Twist-off	25°C/60%[b]	50	—		1	2	3					months
		Twist-off	25°C/60%[b]	40	—		1	2	3	6				months
		Twist-off	None	25	60						12	18	24	36 months
		PP tubes or test packaging material	None	25	60						12	18	24	36 months

[a] 50 mL glass bottle with twist-off closure or corresponding tight container.
[b] If stability data of phase I indicate that moisture does not influence the stability, this investigation can be deleted.
[c] In phase III, samples that have adsorbed the highest amount of water during open storage at 25°C/60%, 30°/70%, 40°C/75% r.h. will be included in the stress testing.

3.1.9. Stability Information

All the results and the stability information are compiled in a Stability Report. Correspondingly three Stability Reports are available.

Stress Testing and Long-Term Testing phase I
Stress Testing and Long-Term Testing phase II
Stress Testing and Long-Term Testing phase III

If the data of the different stress investigations are comparable, it can be concluded that the quality of the clinical trial batches is comparable with the quality of the registration batches; the patient after marketing authorization will get the same quality as the patient during the clinical trials.

Furthermore, packaging materials can be recommended for the registration batches as in Table 17.

Storage instructions should be given if required.

Table 17

	Climatic zones		
Packaging material	I+II	III	IV
PVC/PVDC blister	x	x	—
Polypropylene tubes with polyethylene closure	x	x	x
Polyethylene bottle	x	x	x
Glass bottle with screw cap	x	x	x
Aluminum blister	x	x	x

3.2. Semisolid Dosage Forms: Creams, Ointments

3.2.1. Selection of Batches

This is as for solid dosage forms.

3.2.2. Test Criteria

Organoleptic and physicochemical stability; appearance, odor, homogeneity, consistency, pH, particle size (if active ingredient is in suspension), recrystallization.

chemical and microbial stability: Appearance, homogeneity, content uniformity within the container (tubes stored vertically are cut open, samples are taken from the beginning, middle and end of the tube and analyzed), drug substance decomposition and assay, preservative decomposition, assay

3.2.3. Storage Conditions, Storage Period, Testing Frequency, Packaging Material

Although the samples are stored differently for organoleptic/physicochemical and chemical/microbial stability, the tests overlap; see Tables 18 and 19.

Table 18 Storage for Organoleptic and Physicochemical Stability

Clinical phase	Packaging material	Storage condition	Storage period
I–III	Standard tube (aluminum tube with internal lacquering)	$\geq -10°C$	4 weeks
		5–40°C in a 24 hour cycle	2 weeks

Table 19 Storage Conditions for Chemical and Microbial Stability

Clinical phase	Minimum shelf life	Packaging material	Storage conditions temp. (°C)	rel. hum. (%)	Storage period, testing frequency
I	3 months	Standard tube	40	—	0 2 4 6 weeks
			25	60	12 weeks
	6 months	Standard tube	40	—	0 1 2 3 months
			25	60	6 months
II	12–18 months	Standard tube	40	—	0 1 2 3 6 months
			25	60	12 18 months
					months
		Intended for application	40	—	0 3 6 months
			25	60	12 18 months
					months
III	24–36 months	Standard tube	50	—	0 1 2 3 months
			40	—	1 2 3 6 months
			25	60	12 18 24 36 months
					months
		Intended for application	40	—	0 3 6 months
			25	60	12 18 24 36 months

The standard tubes in phases II and III (aluminum tube with inert internal lacquering) are also stored vertically with the neck of the tube pointing upwards. After 1 and 3 months the samples stored at 40°C are subjected to a threefold analysis for homogeneous distribution of the active ingredient (content uniformity) by testing material taken from the beginning, middle, and end of the tube.

3.2.4. Number of Batches

These are as for solid dosage forms.

3.2.5. Selection of Packaging Material for Semisolid Dosage Forms

Suitable tests have to be carried out.

Packing: Aluminum tube internally lacquered, plastic tubes.

Problems: Corrosion of metal tube; interaction with internal lacquering; sorption; permeation of water vapor, oxygen, aromas, and essential oils.

Testing packaging material—dosage form: To test for corrosion, the filled metal tubes are stored horizontally, upright, and inverted at 40°C for 3 months and are then investigated.

To test for permeation and sorption, the filled plastic tubes are stored for 3 months at 40°C.

When selecting the packaging material, the climatic zone in which the product is to be introduced must also be taken into account.

Because of the problems arising with plastic tubes, aluminum tubes are preferred.

If the final packaging material has been selected, the investigations on photostability are performed.

The samples with and without container are irradiated with a Xenon lamp (Suntest 250 W/m^2) for 24 hours.

The test criteria are appearance, drug substance decomposition and assay.

3.2.6. Evaluation

If the results of the test for organoleptic and physicochemical stability are within the shelf life specifications, the stability prediction is only determined by the chemical and microbial stability. Content uniformity within a container and possible interactions with the packaging material must also be considered. Generally, stability predictions are most difficult for semisolid dosage forms, and this applies particularly to the use of reaction kinetics. It may also be necessary to ensure compliance with the minimum shelf life by marking packs with storage instructions.

3.2.7. Stability Information

This is as for solid dosage forms.

3.3. Solutions, Ampoules

3.3.1. Selection of Batches

This is as for solid dosage forms.

3.3.2. Test Criteria

Organoleptic and physicochemical stability: Appearance, clarity, pH.

Chemical and microbial stability: Drug substance decomposition and assay preservative decomposition and assay.

3.3.3. Storage conditions, storage period, testing frequency, packaging material (Tables 20 and 21)

3.3.4. Number of Batches

This is as for solid dosage forms.

3.3.5. Selection of Packaging Material for Liquid Dosage Forms

Packaging: ampoule, injection vial with rubber stopper, glass bottle or plastic bottle with screw closure or pilferproof closure and liner.

Problems: pH, leakage, desorption, sorption, permeation, interaction with rubber stopper, interaction with liner.

Tests packaging material—dosage form: To test for sorption, permeation, pH, and leakage, the final formulation solution is filled in the container, and for desorption placebo solution is used. The samples are stored vertically and inverted

Table 20 Storage Conditions for Chemical and Microbial Stability

Clinical phase	Minimum shelf life	Packaging material	Storage conditions temp. (°C)	rel. hum. (%)	Storage period, testing frequency								
I	3 months	25 ml ground-glass-stoppered glass bottle or glass ampoule or injection vial with rubber stopper, plastic bottle	40	—	0	2	4	6					weeks
			25	60					12				weeks
	6 months	25 ml ground-glass-stoppered glass bottle or glass ampoule or injection vial with rubber stopper, plastic bottle	40	—	0	1	2	3					months
			25	60					6				months
II	12–18 months	25 ml ground-glass-stoppered glass bottle or glass ampoule or injection vial with rubber stopper, plastic bottle. Intended packaging material	60	—	0	1	2	3					months
			40	—		1	2	3	6				months
			25	60						12	18		months
			40	—	0			3	6				months
			25	60						12	18		months
III	24–36 months	25 ml ground-glass-stoppered glass bottle or glass ampoule or injection vial with rubber stopper, plastic bottle. Intended packaging material	70	—	0	1	2	3					months
			60	—		1	2	3					months
			50	—		1	2	3					months
			40	—		1	2	3	6				months
			25	60						12	18	24	36 months
			40	—				3	6				months
			25	60						12	18	24	36 months

Table 21 Storage Conditions for Organoleptic and Physicochemical Stability

Clinical phase	Packaging material	Storage condition	Storage period
I	Ground-glass-stoppered bottle Glass ampoules	5°C	1 week
II–III	Injection vial with rubber stopper Plastic bottle	≥−10°C	4 weeks

under the following conditions: 30°C/70% r.h., 40°C, and 50°C for up to 12 weeks. Testing intervals: 0, 1, 2, 3 months.

If the final packaging material has been selected, the investigations on photostability are performed.

The samples in colorless glass and the original packaging material are indicated with a Xenon lamp (Suntest 250 W/m^2) for 24 hours.

The test criteria are appearance (colour of solution), clarity of solution, drug substance decomposition and assay.

3.3.6. Evaluation

If the results of the organoleptic and physicochemical tests are within the shelf life tolerance limits, reaction kinetics prediction presents few problems.

The influence of the packaging materials also has to be considered, especially when elastomers are used.

3.3.7. Stability Information

This is as for solid dosage forms.

4. STABILITY INFORMATION FOR COMPARATOR OR REFERENCE PRODUCTS

When an investigational medicinal product is compared with a marketed product, attention should be paid to ensure the integrity and quality of the comparator product (final dosage form, packaging materials, storage conditions, etc.). If significant changes are to be made to the product, data should be available (e.g., stability, comparative dissolution, bioavailability) to prove that these changes do not significantly alter the original quality characteristics of the product.

Because the expiry date stated on the original package has been determined for the medicinal product in that particular package and may not be applicable to the product where it has been repackaged in a different container, it is the responsibility of the sponsor, taking into account the nature of the product, the characteristics of the container, and the storage conditions to which the article may be subjected to determine a suitable use-by date to be placed on the label. Such a date is not later than the expiry date of the original package. In the absence of stability data or if stability is not followed during the clinical trial, such a date should not exceed 25% of the remaining time between the date of repackaging and the expiry date

on the original manufacturer's bulk container or a six-month period from the date the drug is repacked, whichever is earlier (7).

According to the EU, GMP guideline stability data are necessary for comparator or reference drug products.

The following cases are differentiated:

The samples are repacked into packaging material that is as tight or tighter concerning moisture and light than the original packaging material. The original shelf life is used.

The samples are repacked into packaging material that is less tight than the original packaging material. Then the samples are tested for moisture sensitivity in the open at 25°C/60% r.h. and for photostability for 24 hours with the Xenon lamp (Suntest). Test criteria: average mass and appearance if no changes take place, the original shelf life is used; if changes take place, tighter or more protecting packaging material must be selected. Then the original shelf life is used.

Samples are reworked (tablets are ground and filled into capsules). There the stability protocol for phase I is applied with the difference that the samples are stored at 25°C/60% r.h. in the intended packaging material up to 18 months for phase II and 36 months for phase III (Table 22).

Table 22 Storage Conditions, Storage Period, and Testing Frequency for Reworked Comparators

Clinical phase	Minimum shelf life	Packaging material	Pretreatment	Storage conditions temp. (°C)	Storage conditions rel. hum. (%)	Storage frequency, storage period	
II	12–18 months	Twist-off	None	40	—	0 1 2 3 6	months
		Twist-off	25°C/60%	40	—	0 1 2 3 6	months
		Twist-off	None	25	60	12 18	months
		Intended packaging material	None	25	60	12 18	months
III	24–36 months	Twist-off	None	40	—	0 1 2 3 6	months
		Twist-off	25°C/60%	40	—	0 1 2 3 6	months
		Twist-off	None	25	60	12 18 24 36	months
		Intended packaging material	None	25	60	12 18 24 36	months

Testing specifications: Testing specification for release and stability testing of clinical samples.

5. STABILITY TESTING WITH PIVOTAL AND BIOEQUIVALENCE BATCHES

The stability information for a finished medicinal product is derived mainly from the primary data, i.e., the results obtained from the three registration batches. Usually these are representative pilot plant batches. After marketing authorization, three production batches are added.

Results from the development phase, supporting data, are also included in the application for marketing authorization to underpin the stability information.

The stability results obtained with clinical samples are a major factor for achieving a comprehensive assessment of the quality of a finished medicinal product. In this way it is possible to establish a link between the quality of clinical batches for phases I, II, and III and the quality of the finished drug product. If development is fully covered by stability data, then the quality, efficacy, and safety of the clinical batches will correspond to those of the finished medicinal product. Stability information gained by this broad-based approach thereby acquires a completely new dimension.

Pivotal and bioequivalence batches are also required for this comprehensive general strategy unless they are covered by batches from clinical phase III.

If this is not the case, pivotal and bioequivalence batches are included in the stability program.

Since the results are combined to produce a general statement, emphasis is placed not on the stress test but on the long-term test.

The stability program combines acceleration tests with long-term tests in accordance with the ICH Guideline. The storage period, however, is limited to 18 months (Table 23).

Table 23 Storage Conditions for Pivotal Batches

Storage conditions (°C)	Storage period and testing frequency (% rel. hum.) (months)						
25	60	0	3	6	9	12	18
30[a]	70[a]		3	6			
40	(75)		3	6			

[a] These conditions are only used if significant change occurs after storage at 40°C(/75%).

The test criteria, specifications, and analytical procedures are the same as those used for batches of clinical phase III and the marketing authorization batches.

6. TIME TO AVAILABILITY OF STABILITY INFORMATION

The stability program for clinical samples is designed to produce stability information as rapidly as possible. The time required until minimum shelf lives and stability information are available is an important factor for planning clinical trials and establishing the data of manufacture.

In phases II and III it we can differentiate between preliminary and final prediction. The preliminary is based only on the data of the stress investigation, whereas the final includes also the data of the samples stored at accelerated condition.

Table 24 gives an overview.

7. REQUIRED CAPACITY

In the course of the strategic planning it is important to estimate the required capacity for the different stress investigations. Since each development is different it will always be a range.

Table 24 Time to Availability of Prediction of Minimum Shelf Life

Stage of development	Storage period		Analysis and evaluation	Stability report	Total period	
	Stress[a]	Accelerated			Preliminary*	Final
Phase I	—	6 weeks	1 week	2 weeks	—	9 weeks
Phase I	—	12 weeks	1 week	2 weeks	—	15 weeks
Phase II	12 weeks	24 weeks	1 week	2 weeks	15 weeks	27 weeks
Phase III	12 weeks	24 weeks	1 week	2 weeks	15 weeks	27 weeks
Registration batches	12 weeks	24 weeks	1 week	2 weeks	—	27 weeks

[a] In clinical phases II and III a preliminary prediction can be made based on the data of samples stored at stress conditions.

In Table 25 estimations for phases I, II, and III are given.

Table 25 Estimated Capacities for the Stress Investigations of Clinical Samples

Test sample batch	Dosage form	Analytical procedures, validation (weeks)	Analysis of test samples no. of dosages			Total required capacity no. of dosages		
			1 (week)	2–4 (weeks)	>4 (weeks)	1 (week)	2–4 (weeks)	>4 (weeks)
Phase I	Solid	6	5	10	15	11	16	21
	Semisolid	3	2	4	6	5	7	9
	Liquid	3	1	2	4	4	5	7
Phase II	Solid	6	9	17	25	15	23	31
	Semisolid	3	4	8	12	7	11	15
	Liquid	3	2	5	7	5	8	10
Phase III, final formulation	Solid	7	16	31	47	23	38	55
	Semisolid	3	6	11	17	9	14	20
	Liquid	3	5	10	15	8	13	18

8. SUMMARY

The stability program for clinical samples as presented in this chapter is based on the principles of the ICH Guideline "Stability Testing for New Drug Substances and Drug Products."

However, these principles have been adapted to suit the complex circumstances arising during ongoing development, as exemplified by the transition from clinical Phase I to III.

Storage conditions, storage periods, and the derived minimum shelf lives correspond to the duration of clinical trials in Phases I to III.

Shelf lives are established on the basis of stress and acceleration tests. Only with this approach can shelf lives be established rationally and all batches provided with an open expiration date. By consistently separating the storage conditions for organoleptic, physicochemical, and chemical-microbial test criteria, all stability-indicating test criteria can be integrated in the stability information.

The number of analyses can be reduced by bracketing if phases I and II are performed using several dosages.

Stability predictions based on stress and acceleration tests are supported by long-term tests conducted under the storage conditions representing climatic zone II, i.e., 25°C/60% r.h. The packaging material planned for commercial use is always included.

The analytical procedures and the specifications to be derived from the results also undergo a process of development.

For example, at the outset the validation has a preliminary character and includes specificity, linearity, recovery, and limit of quantitation, whereas on completion there is the completely validated specification for clinical samples and stability testing. In the same way, the specifications initially serve as a general guide that becomes increasingly specific. This flexible approach makes it possible to obtain reliable stability information while ensuring the rational use of resources.

Not carrying out stress and acceleration tests and replacing them by long-term tests would either cause major delays in clinical development or make it impossible to state an open expiration date.

An alternative would be to include all batches in stability testing, running the serious risk of having to replace batches during the clinical trial and, in addition to the great analytical effort involved, of always having to keep up-to-date stable batches available in order to safeguard the continuity of the clinical trial.

Summarizing, it can be stated that the systematic approach of proceeding in logically coordinated steps represents the best way of supporting the clinical trial by stability testing.

Furthermore, stability testing of clinical samples is a central factor for generating comprehensive stability information, i.e., an overall assessment of the quality of the finished medicinal product.

By applying the same principles to the stability testing of clinical samples and the finished medicinal product, the marketed drug, it is ensured that the results of the clinical trial can be considered applicable to the finished medicinal product; both products have similar stability and therefore quality.

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13

A Rational Approach to Stability Testing and Analytical Development for NCE, Drug Substance, and Drug Products: Marketed Product Stability Testing

WOLFGANG GRIMM

Biberach, Germany

1. INTRODUCTION: THE STRATEGIC PLANNING

Big efforts are necessary to reduce the period of time from the start of development for a new chemical entity, drug substance, or drug product to registration application and finally marketing authorization.

The results of the analytical development and the stability testing fro a NCE form an important part of a registration application.

To reduce the period of time and to provide information to assess variation and changes, a procedure was developed for analytical development and stability testing: the strategic planning.

By a strategic planning it is possible to secure a successful marketing authorization in the shortest period of time and in the most efficient way. The strategy is based on the ICH Harmonized Tripartite Guidelines (1).

Stability Testing of New Substances and Products	Q1A
Photostability Testing of New Substances and Products	Q1B
Text on Validation of Analytical Procedures	Q2A
Extension of the ICH Text "Validation of Analytical Procedures"	Q2B
Impurities in New Drug Substances	Q3A
Impurities in New Drug Products	Q3B
Residual Solvents	Q3C
Specifications, Test Procedures and Acceptance Criteria for New Drug substances and New Drug Products: Chemical Substances	Q6A

It is also based on the Extension of the ICH Tripartite Guideline for worldwide marketing (2,6,7).

Thereby the strategy considers all aspects of analytical development and stability testing for a New Chemical Entity which are necessary for a registration application in the EU, Japan and the USA, and worldwide.

The overall development and stability program for the strategic planning has been divided into six decisive steps (3); see Table 1.

Furthermore, eleven basic principles have been established (4) that are decisive for stability testing and that are applicable to all stages of development, on all dosage forms.

The ICH tripartite stability guideline had taken over these principles and is likewise built upon them.

The 11 principles are as follows:

Selection of batches and samples

Table 1 Overall Development and Stability Program

Step	Analytical development and stability testing
1	Stress and accelerated testing with the drug substance
2	Preformulation and formulation finding for
	Toxicological samples
	Clinical trial samples
	Final dosage form
3	Stress and accelerated testing with selected formulations:
	Toxicological samples
	Clinical trial samples
	Final formulation
	Registration batches
	Selection of packaging material up-scaling, pilot-plant
4	Accelerated and long-term stability testing with registration batches up to registration application:
	Drug substance
	Drug product
	Transfer of analytical procedures to quality control
5	On-going stability testing with registration and production batches
	Drug substance
	Drug product
6	Follow-up stability testing
	Continuous production
	Variations and changes during continuous production

Test criteria
Analytical procedures
Specifications
Storage conditions
Storage period
Testing frequency
Number of batches
Packaging material
Evaluation
Stability information, statements

These principles apply to each of the six steps. At the same time they also pass through a process of development and have differing degrees of importance. Some of these principles, such as selection of samples and batches, number of batches, storage conditions, testing frequency, and storage periods are firmly established, while others, such as test criteria, analytical procedures with validation, and specifications undergo further development.

Combining the six steps and the eleven basic principles yields the systematically structured stability schedule as shown in Tables 2–4.

The big advantage of this systematic procedure is that the resulting data are in a logical relationship and can be put together like a puzzle. Therefore the information on the drug substance and drug product widens constantly.

Table 2 Systematically Structured Stability Schedule (Part 1)

Basic principles	Step 1 Stress and accelerated testing with the drug substance	Step 2 Preformulation and formulation finding	Step 3 Stress and accelerated testing with selected formulations, up-scaling	Step 4 Accelerated and long-term stability testing up to registration application	Step 5 On-going stability testing	Step 6 Follow-up stability testing
Selection of batches and samples	Experimental batch	Experimental laboratory batches	Experimental and clinical batches, representative pilot-plant batches	Representative pilot plant batches	Representative batches	Representative and experimental batches
Test criteria	Corresponding to objective	Corresponding to objective	Corresponding to objective	Corresponding to results of step 1–3	As for step 4	As for step 4
Analytical procedures	Stability indicating, preliminary validation	Stability indicating, orientational validation	Stability indicating, orientational and preliminary validation	Stability indicating, completely validated	As for step 4	Stability indicating completely validated or revalidation
Specifications	Orientational specifications	Orientational specifications	Preliminary	Release and shelf life specifications proposed for registration	Post approval, release and shelf life specifications	Post approval, release and shelf life specifications
Storage conditions			−10°C			
		5°C	5°C			
	25°C/60%	25°C/60%	25°C/60%	25°C/60%	25°C/60%	25°C/60%
	25°C/75%					
	30°C/70%	30°C/70%	30°C/70%	30°C/70%	30°C/70%	30°C/70%
	40°C/75%	40°C/75%	40°C/75%	40°C/75%	40°C/75%	40°C/75%
	40°C, 50°C, 60°C, 70°C	40°C, 60°C	40°C, 50°C, 60°C 70°C			

Table 3 Systematically Structured Stability Schedule (Part 2)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
Basic principles	Stress and accelerated testing with the drug substance	Preformulation and formulation finding	Stress and accelerated testing with selected formulations, up-scaling	Accelerated and long-term stability testing up to registration application	On-going stability testing	Follow-up stability testing
Testing frequency	0, 1, 2, 3 months	Depending on problem	Depending on problem 0, 1, 2, 3, (6) months	0, 3, 6, 9, 12, (18) months	(18), 24, 36, 48 60 months 0, 3, 6, 9, 12, 18, 24, 36, 48, 60 months	0, 12, 24, 36, 48 60 months 0, 1, 2, 3, 6 months
Storage period	Up to 3 months	Depending on problem	Up to 3 months Up to 6 months	Up to 12 or 18 months	Up to 60 months	Up to 60 months Up to 6 months
Number of batches	1	Depending on problem	Depending on problem 1	3	3 3	1 per year 1–3
Packaging material	Open, standard packaging material	Depending on problem Standard packaging material	Depending on problem Standard packaging material	Commercial packaging material	Commercial packaging material	Commercial packaging material Standard packaging material

Table 4 Systematically Scheduled Stability Schedule (Part 3)

Basic principles	Step 1 Stress and accelerated testing with the drug substance	Step 2 Preformulation and formulation finding	Step 3 Stress and accelerated testing with selected formulations, up-scaling	Step 4 Accelerated and Long-term stability testing up to registration application	Step 5 On-going stability testing	Step 6 Follow-up stability testing
Evaluation	Statistics reaction kinetics Stability report as drug substance stability profile Preliminary testing specification for stability testing of drug substance	Statistics reaction kinetic Research reports Orientational testing specifications	Statistics reaction kinetics Stability report and preliminary testing specification for release and stability testing of toxicological samples clinical trial samples final dosage form	Statistics reaction kinetics Stability report drug substance Testing specification for stability testing of drug substance Stability report drug product Testing specification for release and stability testing of drug product	Statistics Stability report	Statistics reaction kinetics Stability report
Stability information	Re-Test Period Storage instructions Selected test criteria for long-term testing Orientational shelf life predictions for drug products	Formulation selection	Period of use toxicological samples clinical samples phase I–III Shelf life prediction final dosage form Storage instructions Selection of packaging material Selection of test criteria for long-term testing	Shelf life prediction Storage instructions In-use stability Holding time for intermediates and bulk	Confirmation and extension of shelf life	Confirmation of shelf life Assessment of variations

The data are summarized in research reports and stability reports, testing specifications.

The stability reports of the different steps of development are built up and written in the same format. Thus all data can be cross-checked easily, and the final shelf life can be based on all these data.

By presenting all the available stability data in such a comprehensive way, considerable savings can be reached concerning different strengths, different packaging materials, and later with variations.

The derived stability information is based on a broad set of data and assures the quality of the drug product to the patient.

Table 5 gives an overview of the different documents that result during development.

The analytical procedures, the specification and the corresponding testing specifications are developed systematically in steps 1–4.

In step 4 they are transferred to quality control, which elaborates on this basis the testing specifications for quality control.

Therefore the registration application contains two types of testing specifications:

- Those that have been applied during development for release of clinical trial samples and for stability testing and will be applied for on-going stability testing
- Those that will be applied for quality control of running production and follow-up stability testing

The analytical procedures are usually not changed after the transfer into quality controls, but the format may be changed to consider the requirements of different countries.

After an overview of the required capacity (Table 6) and period for analytical statements (Table 7), each step is described in detail, practical examples are given, the required capacity calculated, and the period for analytical statements indicated.

2. Step 1: Stress and Accelerated Testing with the Drug Substance

2.1. Objective

- Elucidation of the intrinsic characteristics of the drug substance with reference to chemical properties (physical properties are investigated separately)
- Establishment of the degradation pathway, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedure
- Investigation of the following influencing factors: moisture, temperature, moisture + temperature, moisture + temperature + drug substance concentration, pH, ionic strength, oxidation, light

The tests with the drug substance are of general nature and are not specific to any particular dosage form. Consequently the results are generally applicable. These investigations are required by the ICH Stability Guideline. The performance, however, is up to the applicant's discretion.

Table 5 Overview of the Documents that Result During Development

	Step of development	Document	Needed for	ICH Guidelines
1	Stress and accelerated testing with the drug substance	Stability report as preliminary stability profile of drug substance	Predevelopment	
		Preliminary testing specification for stability testing of drug substance	CMC, Part II: IND, CTX, RA	Q2A, Q2B
		Stability report as stability profile of drug substance	CMC, Part II: IND, CTX, RA	Q1A, Q1B
2	Preformulation and formulation finding for toxicological samples clinical trial samples final dosage form	Orientational testing specification for toxicological samples clinical trial samples final dosage form		
		Research report	Development pharmaceutics, Development report	(Q1A)
		Formulation evaluation		
		Research report	Development pharmaceutics, CMC: NDA	Q6A
		Rationale for analytical procedure dissolution rate		
3	Stress and accelerated testing with selected formulation: toxicological samples clinical samples phase I–III final dosage form, representative batches	Stability report and preliminary testing specification for release and stability testing of		
		toxicological samples	GLP	
		clinical samples phase I	CMC, Part II: IND, CTX	(Q1A)
		clinical samples phase II	CMC, Part II: IND, CTX, RA	(Q1A)
		clinical samples phase III	CMC, Part II: IND, RA	(Q1A)
		final dosage form, representative batches	CMC, Part II: RA	(Q1A)
		Evaluation of packaging material	(Development pharmaceutics)	(Q1A)

RA: Registration application ≙ NDA.

	Step of development	Document	Needed for	ICH Guidelines
	Cleaning validation	Preliminary testing specification for cleaning validation	GMP	
	Scaling-up, pilot plant	Preliminary testing specification for release and stability testing	GMP	
	optimization	Testing specification for intermediate (IPC)	GMP (RA)	Q6A
	validation	Research report scaling-up	GMP, Development pharmaceutics	
4	Accelerated and long-term Testing with registration batches up to registration application:	Testing specification for stability testing of drug substance	CMC, Part II: RA	Q1A, Q2A, Q2B, Q3A
	drug substance	Rationale testing specification drug substance	CMC, Part II: RA	Q6A, Q3A
		Stability report of drug substance	CMC, Part II: RA	Q1A, Q1B
	drug product	Testing specification for release and stability testing of drug product	CMC, Part II: RA	Q1A, Q2A, Q2B, Q3B, Q6A
		Validation report, drug substance, drug product	CMC, Part II: RA	Q2A, Q2B
		Rationale testing specification drug product	GMP: (RA)	Q2A, Q2B
		Stability report of drug product	CMC, Part II: RA	Q6A, Q3B
			CMC, Part II: RA	Q1A, Q1B
5	On-going stability testing drug substance registration batches production batches	Stability report drug substance	CMC, Part II: RA	Q1A
	drug product registration batches production batches	Stability report drug product	CMC, Part II: RA	Q1A
6	Follow-up stability testing during continuous production	Stability report	GMP	
	variation and changes	Stability report	Regulatory authorities	

RA: Registration application ≙ NDA.

Table 6 Summarized Required Capacity in Steps 1–5

Step of development	Stage of development	Required capacity (weeks)	Required capacity (weeks × 1.3)[a]	Capacity per step/total capacity (weeks)	Capacity per step/total capacity (weeks × 1.3)[a]	Capacity per step/total capacity (years ≙ 42 weeks)[b]
1	Stress and accelerated testing with drug substance					
	Preliminary stability profile	8	10			
	Stability profile	9	12	17	22	0.53
2	Preformulation and formulation finding	23	30	23	30	0.71
3	Stress and accelerated testing					
	clinical phase I	24	31			
	clinical phase II	24	31			
	clinical phase III	32	42			
	final formulation	18	23			
	cleaning validation	5	7			
	scaling-up	23	30	126	164	3.90
4	Accelerated and long-term testing					
	drug substance	9	12			
	drug product	17	22			
	PAI preparation	6	8	32	42	1.00
5	On-going stability testing					
	drug substance	20	26			
	drug product	42	55	62	81	1.93
Total 1–4				198	257	6.1
Total 1–5				260	338	8.1

[a] To calculate the actual time span, the remaining work that is indirectly related with the NCEs has to be considered, such as SOPs, qualifications, literature. Therefore 0.75% of a week is used corresponding to the factor of 1.3.
[b] One year is calculated with 210 days ≙ 42 weeks.

Table 7 Stability Testing on the Critical Path

Step of development	Stability information	Needed for	Time to availability from start
1	Preliminary stability profile of drug substance	Start of predevelopment	6 weeks
	Stability profile of drug substance	Base for minimum shelf life phase I	16 weeks
3	Minimum shelf life toxicological samples	Release of toxicological samples	8 weeks
	Minimum shelf life clinical samples phase I	Release of clinical trial batch phase I	9, 15 weeks
	Minimum shelf life clinical samples phase II	Release of clinical trial batch phase II	15, 27 weeks
	Minimum shelf life clinical samples phase III	Release of clinical trial batch phase III	15, 27 weeks
4	Stability report for registration application	Filing data for registration application	(Release of 3d batch for accelerated and long-term testing) 15 months

The drug substance to be investigated should have been selected as the most suitable salt form.

2.2. Application of the Basic Principles

Selection of batches and samples: experimental batch; it must comply with the acceptance limits of the preliminary testing specification, as far as they are available. The impurity profile, particle size distribution, and the surface area are especially important.

Test criteria: appearance, physical properties, assay, decomposition.

Analytical procedure:

Specific for stability testing

Orientational validation at the beginning specificity, linearity, quantitation limit $\geq 0.05\%$ of the drug substance.

Preliminary validation at the end in addition: accuracy, range, repeatability, robustness for drug substance and decomposition products

Specifications: at the end: preliminary release specifications.

Storage conditions:

In open containers: 25°C/60%, 25°C/75%, 30°C/70%, 40°C/75%.

In standard packaging material: 40°C, 50°C, 60°C, 70°C.

Storage period: Until equilibrium is reached at open storage, < 3 months.

Testing frequency: ≤ 4.

Number of batches per investigation: 1.

Packaging material: flask with ground-glass stopper, glass container with twist-off closure, glass container lined with polyethylene foil.
Evaluation: statistics and reaction kinetics are applied in evaluating the results. Assessment of observed decomposition products, whether they may be formed under accelerated and long-term testing. Establishment of degradation pathway of selected decomposition products; elucidation of their structure. Assessment of applied analytical procedures. The data and the derived stability information are summarized in a stability report, as the stability profile of the investigated drug substance. The stability report is part of CMC for CTX, IND and for registration application.
Stability information: the stability report contains the following stability information:
Stability prediction drug substance
Preliminary prediction of the retest period
Storage instructions if required
Test criteria, packaging material, assessed analytical procedure and stability test protocol for accelerated and long-term testing of registration batches
Stability predictions drug product
Orientational prediction of the chemical stability of the drug substance in solid, semisolid, liquid dosage forms

2.3. Practical Examples

The influencing factors to be investigated are now illustrated by means of a practical example.

To start the predevelopment as soon as possible, the investigations are carried out in two steps:

Preliminary investigation
Complete investigation

2.3.1. Preliminary Investigation

The experiments are organized so that the preliminary stability profile is available within 6 weeks.

Necessary amount: about 25 g.

2.3.1.1. Moisture

Sample: Drug substance stored in open container for 1 week at 25°C/75% r.h
Test criteria: Appearance, mass, DTA. If the drug substance has absorbed water it is investigated further in 50 mL glass container with twist-off closure under 2.3.1.2

2.3.1.2. Temperature, Moisture

Sample: Drug substance with and without adsorbed water in 50 mL glass container with twist-off closure
Test criteria: Appearance, decomposition, assay, DTA at the end
Analytical procedure: Orientational validation at the beginning
Storage temperature: 70°C

Storage period: 4 weeks
Testing frequency: 0, 2, 4 weeks

2.3.1.3. pH

Sample: 1% aqueous solution or slurry in 0.1, 0.01 M HCl, in McIlvaine's buffers (0.1 M citric acid, 0.2 M disodium phosphate), pH 3, 4, 5, 6, 7, 0.01 M NaOH in volumetric flask with ground glass stopper
Test criteria: Appearance, decomposition, assay
Storage temperature: 60°C
Storage period: 3 weeks
Testing frequency: 0, 1, 3 weeks

2.3.1.4. Oxidation

Sample: 1% aqueous solution or slurry in 0.3% H_2O_2 solution in 25 mL glass flask with ground glass stopper
Test criteria: Appearance, pH, decomposition, assay
Storage temperature: 50°C
Storage period: 3 weeks
Testing frequency: 0, 1, 3 weeks

2.3.1.5. Photostability (Xenon Lamp, Atlas Suntest, 250 W/m²)

Test sample: Drug substance spread in colorless and brown glass across the container to give a thickness of not more than 3 mm
1% aqueous solution (or inert organic solvent) with and without N_2 gasing in colorless glass flask with ground glass stopper
1% aqueous solution (organic inert solvent) in brown glass flask with ground glass stopper as control
Test criteria: Appearance, decomposition, assay
Storage period: 48 hrs, xenon lamp
Testing frequency: 24, 48 hrs

The investigations are summarized and listed in the stability test protocol, preliminary investigations including the applied analytical procedures (Table 8).

If the drug substance decomposes fast (>10% after the first test point), storage at lower temperature should be considered.

If the drug substance is not wettable or does not dissolve at all, the stability information will be limited. The drug substance may appear very stable because it did not dissolve at all.

In these cases the procedure has to be modified accordingly to reach wettability and increase the solubility.

If an enantiomer is present, testing for racemization is performed during the course of stability studies.

The results are summarized in the Stability Report as Preliminary Stability Profile of Drug Substance.

2.3.2. Complete Investigation

The stability protocol based on the results of the preliminary investigations has to be adjusted accordingly.

Necessary amount: about 50 g.