Critical Review

Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis

Jorge G. Ibanez a,c, Alejandra Carreon-Alvarez b, Maximiliano Barcena-Soto a, Norberto Casillas a,c

a Chemistry Department, Universidad de Guadalajara, Blvd. Marcelino Garcia Barragan #1451, Guadalajara, Mexico CP 44430, Mexico
b Chemical Engineering Department, Universidad de Guadalajara, Blvd, Marcelino Garcia Barragan #1451, Guadalajara, Mexico CP 44430, Mexico
c Chemical Engineering and Sciences Department, Universidad Iberoamericana, Prol. Paseo de la Reforma 880, 01219 Mexico DF, Mexico

ABSTRACT

A critical review is offered concerning the different sources, effects, concentrations, removal methods, speciation, and analysis of metals (e.g., Al, Ca, Cd, Cu, Fe, Pb, Mg, Mn, Ni, K, Na, and Zn) present in a wide variety of alcoholic beverages.

1. Introduction

The concentration of metals in many alcoholic beverages can be a significant parameter affecting their consumption and conservation. This derives from the negative and positive effects caused directly or indirectly by the presence of metals. Negative effects include beverage spoilage and hazing, as well as sensorial and health consequences (Mayer et al., 2003; Green et al., 1997). Positive effects include the removal of bad odors and tastes (Green et al., 1997), participation in fermentative processes (Akrida-Demertzi and Koutinas, 1992), provision of pathways for dietary intake of some essential minerals (Mayer et al., 2003), and usefulness for authentication purposes (Kokkinofta et al., 2003).

Because of all this, many metals are carefully monitored and regulated, which has resulted in the development of a plethora of analytical techniques for their analysis. Since alcoholic beverages are complex aqueous matrices containing a wide variety of organic compounds, such techniques must be attuned to deal with their presence—usually by the inclusion of a pretreatment step. Metals in alcoholic beverages are often determined by atomic absorption or emission techniques (Pyrzyńska, 2007); however, the high cost of the instruments involved and the long sample-preparation times required often preclude their widespread use. Electrochemical methods represent a valuable option for such analyses. The aim of this paper is to review the various sources and concentrations of metals in alcoholic beverages (e.g., Al, Ca, Cd, Cu, Fe, Pb, Mg, Mn, Ni, K, Na, and Zn), their effects, speciation, removal methods, and key electrochemical alternatives for analysis.

2. Sources of metals in alcoholic beverages

Metals find their way into alcoholic beverages at different stages and through various sources including raw materials, brewing, process type and equipment, bottling, aging/storage, and adulteration as discussed below. (An updated, extensive review of the sources of metals in wine can be found elsewhere, Pohl, 2007b).

2.1. Raw materials

Several metal ions can be taken up from the surrounding soil by plants from which an alcoholic beverage is prepared (FSA, 1998). For instance, the type of soil (i.e., its geogenicity), its
products. Several examples follow.

2.4. Process equipment

2.3. Process type

several times more Ni than distilled beverages (e.g., brandy, wine, certain fermented beverages (e.g., wine and beer) contain among alcoholic beverages depending on their processing. In this regard (e.g., whiskies), since more acidic beverages tend to contain more Cu (Adam et al., 2002). Addition of fining and clarifying substances (e.g., flocculants) to reduce turbidity can bring about an increase in Na, Ca or Al in wine (Pohl, 2007b). Most of the Mg found in beer can be introduced with the malt (Weiner and Taylor, 1969). Cu in beer comes mainly from raw materials (Mayer et al., 2003); on the contrary, only a small percentage of the final Cu content in whiskey comes from the barely from which the spirit is distilled (Adam et al., 2002).

2.2. Substances added during brewing

Hops, acids, bases, silica gel, dilution water, flavoring agents, additives, and stabilizers are potential sources of metal ions in the brewing process (Mayer et al., 2003). For example, the main source of Cu in wine is the CuSO₄ added to remove sulfidic odors (Green et al., 1997). The acidity of the liquor to be distilled may be important in this regard (e.g., in whiskies), since more acidic beverages tend to contain more Cu (Adam et al., 2002). Addition of fining and clarifying substances (e.g., flocculants) to reduce turbidity can bring about an increase in Na, Ca or Al in wine (Pohl, 2007a).

2.5. Bottling process

Bottling water and equipment may also introduce metals in beverages. For example, the content of Ca, Mg, and Na in brandies depends on the quality of water used for dilution after distillation (Varju, 1972). The modification of certain imported alcoholic beverages “for the purpose of bottling and sale by the addition of distilled or otherwise purified water to adjust the beverage to a required strength” is sometimes allowed (DJC, 2005). It is noteworthy that when metallic capsules seal alcoholic beverage bottles, some Pb may be carried over (Mena et al., 1997).

2.6. Aging/storage

Possible effects caused by metals during these stages are multiple. For example, Fe(III) and Mn(II) affect the stability of old wines and modify the sensorial quality after bottling (Pohl, 2007a) since they are believed to activate molecular oxygen by forming reactive oxygen species (e.g., hydroxyl radicals) (Pohl, 2007a); this is possible due to their electronic configurations involving unpaired electrons that may interact quantum mechanically with the dioxygen triplet. Likewise, Fe catalyses the oxidation of polyphenolic substances and Mn facilitates acetaldehyde formation; the products of these reactions yield undesirable precipitates. Metal complex formation is also common at this aging/storage state, which may alter a significant number of beverage parameters (Pohl, 2007a). Customer-accessible containers for alcoholic beverages include metal cans, glass bottles, plastic containers, and paperboard cartons, and the containers themselves sometimes are a source of metal ions in the beverage. For example, Cu and Zn can be introduced into beer by welded cans (Mayer et al., 2003). In fact, the Zn concentration in a specific brand of bottled beer was measured at 0.33 ppm, whereas in canned beer it reached 0.87 ppm (Weiner and Taylor, 1969). On the contrary, the Ni content in canned beer as compared with glass-bottled beer is not higher (Dugo et al., 2004).

2.7. Adulteration

This term comes from the Latin word adulterare (i.e., to defile or falsify), and means to make something impure by the addition of extraneous, inferior, or improper ingredients. As far back as 1875, legislation has prohibited the adulteration of drinks (MacRae and Alden, 2002). Unfortunately, Pb and other metallic impurities can enter beverages during adulteration practices (Ijeri and Srivastava, 2001). For example, adulterated vodka has been found to contain an excess of Ca and Mn ions (Servadio et al., 1975).

- Contact of wine with process equipment, pipes, casks, and barrels is the usual source of Al, Cd, Cr, Cu, Fe, and Zn (Pohl, 2007b).
- The main sources of heavy metals in the production of an anise-type beverage are the bronze pot stills (Moutsatsou et al., 2003).
- Lead plumbing can add Pb to beverages (Green et al., 1997).

Metal addition from process equipment (e.g., stills) can be prevented by the use of high-quality steel or glass, although some organoleptic properties may be altered by the absence of certain metal ions added during distillation (e.g., the lack of minute amounts of Cu in tequila affects negatively its flavor).

The concentration of Cu coming from process equipment in vodka is twice as much that coming from the raw materials (Trawinska et al., 1979).

The main source of Cu in whiskey is the copper still used for its distillation (Adam et al., 2003). Corrosion of tequila-distillation equipment (made of Cu) provokes the presence of this metal in the final product (Carreon et al., 2001).

Storage of metal in metal containers (e.g., low-quality steel or Cu alloys) results in their corrosion with the concomitant introduction of metals into the liquor (Krasnopolski and Tishchenko, 1984; Trawinska et al., 1979). This is also the case with acidic wine vinegars (Guerrero et al., 1996).

The Zn, Fe, and Cu contained in home-produced alcoholic drinks can be essentially unrelated to the material fermented as it primarily depends on the vessel materials (Reilly, 1972).

The temperature in the distillate and the degree of still utilization affect the Cu content in whiskey (Reaich, 1998).

Extremely high concentrations of Cu, Zn, and Fe in home-produced beers and spirits can be largely traced to the use of galvanized metal fermentation drums, when these replace old clay and wooden vessels (Reilly, 1973).

The Fe-content in pulps and musts increases due to the Fe in concrete tanks used for the storage of raw materials (Trawinska et al., 1979).
3. Effects of metals present in alcoholic beverages

These effects can be classified according to the final subject they act upon.

3.1. Effects on the beverages

These include negative or positive aspects as described below.

3.1.1. Negative aspects

Minerals may generate irreversible turbidities in liquors; the formation of sediments is a long lasting process that may depend on the contents of all metals and substances present, the redox potential, pH, and temperature. Thus, the establishment of absolute limiting values for metal contents is rather difficult (Trawinska, 1977). Turbidity typically increases with metal concentrations. For example, maximum turbidity in soplica liquor was observed with the addition of Ca after more than 2 months of storage, whereas a smaller Ca addition (i.e., 20 mg Ca/L) barely changes a Winiak liquor’s clarity; Fe can likewise contribute to turbidity. Some metal ions promote the development of turbid colloids during cognac storage (Russu et al., 1985).

Small amounts of Cu (ca. 3 mg Cu/L) cause maximum turbidity after 5 months of storage of some beverages (Trawinska, 1977); it can also be a factor in the formation of hazes (called copper case) in wine and in beer (Mayer et al., 2003; Green et al., 1997). In the case of wine, the basis of this phenomenon is attributed to the Cu-promoted flocculation of negative hydrophobic colloids formed by protein-tannins moieties, and also to the interaction of these Cu cations with HS-groups resulting from protein denaturation (Esparza et al., 2007). Cu also contributes to the oxidation of beer and imparts a coppery, unpleasant metallic taste (Mayer et al., 2003); here, even small amounts of this metal (e.g., 0.15 mg Cu/L) can cause gushing (Mayer et al., 2003). Radical formation in sugar cane spirits depends mainly on the Cu content, as detected by electron spin resonance (Bettin et al., 2002).

Color changes in some alcoholic beverages (e.g., wine) can be attributed to different factors (Esparza et al., 2005): (a) complex formation of Fe, Cu, Al or Mg with anthocyanins and tannins, (b) the presence of metal–polyphenol complexes, (c) hyperchromic of batochrome effects originating from copigmentation processes, and (d) an exogenous contribution of selected metal cations that might result in favorable color modification.

Examples of additional color changes include the following. Cu can increase the rate of oxidative spoilage of wine, which ultimately results in its browning (Pohl, 2007b). There is a relationship between the color of a brandy and its Fe and Cu contents (Varju, 1972); if the concentration of metals in brandy reaches a critical limit, they promote precipitation (Varju, 1972). Although a significant correlation is not found between the content of metals (e.g., Fe, Cu, and Zn) and the color development in sake (a traditional Japanese drink) during the first 2 years of storage, more extensive darkening is observed after 3 years (Kondo, 1966).

Another effect involves the influencing of key organoleptic properties by high concentrations of certain metals. A case in point is the negative influence of Zn in wines (Salvo et al., 2003).

3.1.2. Positive aspects

Contrary to the above, some metals enhance the flavor of wine (Esparza et al., 2005). One plausible indirect pathway for this effect involves the binding of sulfur derivatives to reduce the sulfury flavor (e.g., Cu in whiskey, sugar cane spirits, and cognac), as confirmed by thermal desorption gas chromatography (Reich, 1998; Richter et al., 2001). Cu is also involved in the reduction and formation of congeners throughout the distillation of whiskey (Reich, 1998; Nedjma and Hoffmann, 1996). Key concentration ratios of K, Na, and Ca to Cu can speed up the fermentation kinetics of a solution composed of raisin extract and sucrose (Akirida-Demertzzi and Koutinas, 1992); this may be attributed to the increase of the glucose uptake rate. On the other hand a higher concentration of Ca superscions results in their uptake by Saccharomyces cerevisiae cells, which reduces cell growth (Akirida-Demertzzi and Koutinas, 1991). Yeasts consume Ca, Cu, Fe, K, Mg and Zn and therefore their concentrations tend to decrease (Pohl, 2007b); a substantial cofactor role occurs in some cases (Esparza et al., 2005).

Another useful aspect of the presence of specific metals in alcoholic beverages involves their use in quality analysis and in authentication (fingerprinting) due to their typical stabilities (Guerrero et al., 1996; Nuñez et al., 2000). Chemometrics and pattern recognition methods are used for the distinction of beverages according to their origin, quality, variety, type, and other features (Kokkinofta et al., 2003; Nuñez et al., 2000). For example, the Cu concentration in a malt Scotch whiskey is ca. 1.5–3.5 times greater than that in a blended (grain) Scotch whiskey (Adam et al., 2002). Another case in point is the metal content in zivania (a Cypriot traditional drink), which allows its differentiation from spirits produced in other countries (Kokkinofta et al., 2003). Likewise, the analysis of Na and Mg is often sufficient to discriminate among different wines; even the analysis of Mg alone may serve such a purpose (Kokkinofta et al., 2003). In the same manner, the metal profile of brandies depends on their elaboration process; accordingly, the content of metals is sometimes used as a chemical descriptor for classifying different kinds of brandies. Statistical techniques are most helpful here, since classification procedures based on artificial neural networks lead to a predictive ability up to 90% (Camean et al., 2001).

3.2. Effects on humans

Metal ion effects on humans can be quite varied, and their detailed coverage falls beyond the scope of the present review; key information can be found in toxicology treatises. To exemplify this issue, among the best known effects are those of the toxic Pb(II) and Cd(II) ions. In fact, Benjamin Franklin in the eighteenth century noticed that certain homemade rum was causing human paralysis; he successfully traced the problem to Pb-containing equipment (UMA, 2003). Chromium—specifically Cr(VI)—can also be toxic; fortunately, dangerous concentrations are not common in alcoholic beverages. For instance, in a wide sampling of beverages (including wine, beer, cider, brandy, rum, whiskey, gin, vodka, anisette, and liquors) its concentration was found to be below 0.025 mg/L (Lendinez et al., 1998).

On the contrary, the presence of certain metal ions in alcoholic beverages is beneficial in that it may provide an intake path for necessary nutrients in consumers (Mayer et al., 2003). For example, moderate wine consumption provides important necessary nutrients in consumers (Mayer et al., 2003). For example, moderate wine consumption provides important

4. Metal concentrations and limits

The concentrations of metals in alcoholic beverages can vary widely, as exemplified by the concentrations of Cu summarized in Fig. 1. Other metal concentrations are condensed in Table 1. As shown here, metals are sometimes present in alcoholic beverages at rather high concentrations. In fact, extreme metal ion concentrations in home-produced beer and spirits from different parts of Africa, India, Europe, and Canada revealed concentrations.
of up to 58, 68, and 245 mg/L of Cu, Zn, and Fe, respectively (Reilly, 1973). Homemade—but commercially available—alcoholic beverages in Tanzania can have up to twice the World Health Organization recommended maximum for Zn in drinking water (i.e., 5 mg/L), and one brewed beverage was found to contain toxic amounts of Mn (12.8 mg/L) (Nikander et al., 1991). These instances dramatically demonstrate that the establishment of limits is indispensable. Table 2 shows examples of metal concentration limits established by various regulatory agencies for selected beverages.

Note that limits imposed for alcoholic beverages are higher than those established for water utilized in human consumption. This reflects the lower intake of the former (Green et al., 1997). For example, alcoholic drinks usually contribute only marginally to typical dietary Cu intakes (∼0.05 mg/day) (Sadhra et al., 2007). Regrettably, marked absences in legislation do exist (Baldo and Daniele, 2005).

Metal ion concentrations in wastewaters of breweries and alcoholic beverage factories are typically below the limits established for the introduction of such concentrations into municipal treatment systems. Metals that are sometimes present in concentrations near the upper regulation limits include Hg and Cu (Koller and Sahlmann, 1987).

5. Metal removal

Methods for metal removal from alcoholic beverages can be exemplified by the following cases:

- Cu in whiskey may form complexes that bind to wood during storage and aging; Cu is thus removed from the liquor (Adam et al., 2002). On the contrary, the contents of Ca and K in sherry brandy increase with aging (Camean et al., 2000).
- An alternative metal removal scheme consists in raising the pH (e.g., in wine and brandy) with NaHCO₃ or CaCO₃ to ca. 4.5–5, then adding tannins or tannic acids and allowing the mixed substances to react for several days. Finally, gelatin and bentonite are added to react with the metal tannates and the mixture is stirred, decanted, and filtered. Large reduction factors in Cu, Fe, and Zn concentrations are thus obtained (Bakalov et al., 1989; Ionescu, 1980).
- Ion exchange resins decrease the metal content of certain distillates to the allowable limits (Hodejeu et al., 1972). (As a case in point, we have obtained up to 99.6% removal of Cu from tequila.)
- MgCO₃ and CaCO₃ can act as cationic exchangers to remove Cu(II) from sugar cane spirits (Almeida Neves et al., 2007).
- Cu is removed from spirituous beverages by precipitation with rubeanic acid (Peña et al., 2004).
- Some metal ions (e.g., Cu and Fe) are removed from alcoholic beverages by adding polymers that contain metal-binding groups (Detering et al., 1991; Kern, 1987).
- Electroless cementation (Ibanez et al., in press) is under testing for Cu removal from tequila by cementation on Fe. We have removed up to 60% of Cu with Fe (from a 10 ppm Cu solution).
- Electrolysis for Cu removal from a variety of matrices is widely practiced (Rajeshwar and Ibanez, 1997). (We are currently testing it for the removal of Cu(II) from tequila, whereby preliminary results indicate that ohmic drop losses prevent a removal higher than 50%.)
- Certain chelating agents (e.g., the trisodium salt of nitrilo tris-methyleneephosphonic acid) in wine and cognac can precipitate Fe, Al, and Cu within 1 week (Romantseva et al., 1982). The same compound can be used in brandy to precipitate Fe and Al (Cu does not precipitate here) (Mekhuzla et al., 1978).
- Potassium hexacyanoferrate (II) binds some metals and sulfides present in alcoholic beverages, although it cannot be used in applications where the Cu content exceeds that of Fe; besides, it may release poisonous HCN.
- A cross-linked, insoluble, powdery polymer may circumvent some of the problems described earlier. It is produced with...
### Table 1
Summary of sample metal concentrations in various alcoholic beverages

<table>
<thead>
<tr>
<th>Alc. beverage</th>
<th>Concentration, mg/L</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Al</td>
<td>Ca</td>
</tr>
<tr>
<td>Anisette</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>0.1–58</td>
<td>0.2–25</td>
</tr>
<tr>
<td>Beverage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spirits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brandy</td>
<td>ND–14.8</td>
<td></td>
</tr>
<tr>
<td>Cachaza</td>
<td>1.36–44.6</td>
<td>0.04–14.3</td>
</tr>
<tr>
<td>Cognac</td>
<td>6.0–11</td>
<td>0.005–5.31</td>
</tr>
<tr>
<td>Finnish berry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>liqueurs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gin</td>
<td>4.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Rum</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Scotch whiskey</td>
<td>0.02–1.58</td>
<td>0.5–4</td>
</tr>
<tr>
<td>Sherry brandy</td>
<td>ND–14.8</td>
<td>0.22–5.31</td>
</tr>
<tr>
<td>Sugar cane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spirits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Tequila       | 4.0
| 0.38–11.80 | 0.02–1.58 | 0.5–4 | 5.31–0.30 | 0.1–1.7 | 0.02–28 | <0.005 | 0.02–4 | 0.002–0.6 | 2–24 | 17.8–635 | 0.02–20 |        |
| Vodka         | 3.0    | 0.5    | ND     | ND–0.1 | 0.02–0.195| 1.0 | 0.5 |       |       |       |       |        |        |
| Whiskey       | 4.0    | 0.23–0.5| ND–0.1 | 0.02–0.195| 1.0 | 0.5 |       |       |       |       |       |        |        |
| (regular)     |        |        |        |        |        |        |        |        |       |       |       |        |        |
| Wine, fruit   | 0.017–14.3| 3.50–24I | ND–0.052| ND–7.62| ND–23.7| ND–1.125| 7.8–718 | ND–5.5 | 0.073–19.4 | 265–3056 | ND–310| ND–8.9 |        |
| wine, cocktails |        |        |        |        |        |        |        |        |       |       |       |        |        |

Note: ND = not detectable.
The chemical form in which an element, ion or compound is found in a given medium depends on the physicochemical conditions of the system. Thus, the distribution of species in an aqueous medium (e.g., an alcoholic beverage) is contingent on pH, composition, temperature, and the oxidation-reduction potential of the solution. These variables define precipitation, dissolution, redox, and complexation reactions. Biological phenomena (e.g., bioaccumulation) frequently depend on the chemical form or speciation of a metallic ion.

The redox environment can determine some properties of metallic and non-metallic species. For example, when arsenic is present in oxic (oxidizing) environments as As(V), its toxicity is very low; however, its reduced form, As(III) is highly poisonous. The opposite occurs with Cr(III). Solubility can also be severely affected by the redox environment. Examples of this are Fe(II) and Mn(II) species, typically soluble in aqueous solutions deficient in dioxygen, whose oxidized forms precipitate quite easily. Similarly, ligand speciation may drastically affect the nature and physicochemical properties of the metallic complexes they form. Bioavailability and toxicity of metal ions in aqueous systems are often proportional to the concentration of the free metal ion and thus decrease upon complexation. However, some metal compounds are more dangerous than the metallic element itself (e.g., methyl mercury vs. mercury) (Ibáñez et al., 2007).

Because of the aforementioned reasons, determination of the total concentration of a metal in a given matrix often does not adequately or effectively characterize it; as a result, speciation has gained considerable ground. Speciation is the process that yields evidence of the atomic or molecular form of an analyte. It can be defined either functionally (e.g., the determination of species that have certain specific functions), or operationally (e.g., the determination of the extractable forms of an element from a given matrix) (Ibáñez et al., 2007). A thorough review of the development of chemical speciation and fractionation analysis of metals in wine is available, including a description of the methods to identify ionic, labile and tightly bound species (Pyrzynska, 2007).

### Examples of the importance of speciation in alcoholic beverages include the following:

- Metals can be either in a state “bound” to the wine matrix, or “unbound” in solution. Some analytical methods (e.g., anodic stripping voltammetry, ASV see below) may not detect the total amount of the metal in the sample, but merely identify the unbound fraction. This problem has been circumvented by means of the standard addition method (SAM) used for their quantification (Akkermans et al., 1998). Nevertheless, one must be careful when interpreting results since the added metal ions may perturb the equilibrium distribution of species in the target matrix (Green et al., 1997). In fact, this method is not recommended for metal ions that strongly bind the alcoholic matrix (Green et al., 1997).

- The concentration of metals in distillates may be affected by complex formation in the stills, given that such complexes may be less volatile than the metal ions themselves and thus their final concentration in the distillate would be smaller than what could be predicted (Adam et al., 2002).

- Bioavailability is usually more important than the total concentration of a metal (Green et al., 1997). Labile metal complexes (ML) are normally more toxic because they are more easily absorbed by organisms (Arcos et al., 1993).

- In the case of Cu in wine, its chemical form—rather than the total concentration—may be a better indicator of its potential for spoilage, since Cu sometimes produces spoilage even at low concentrations (Green et al., 1997). Additionally, some Cu complexes are stable even at pH = 1 and their stability increases if the wine is young and the organic matter is not very oxidized (Arcos et al., 1993). Cu in white wine is mainly complexed by thiol groups (Wiese and Schwedt, 1997); in fact, this principle is used in determining it by differential pulse ASV (dpASV, see below) when forming a complex on the surface of a mercury drop (Ensafi et al., 2001). Tannins, peptide, and protein groups also highly influence the formation of Cu complexes in white wine (Wiese and Schwedt, 1997).

- Organic complexes of Cu are present in residues of the distillation process of Scotch whiskies (Adam et al., 2002). Cu and Zn form soluble complexes in the spent wash of whiskey distilleries. Cu is believed to be bound to an organic fraction containing carbohydrate and ninhydrin, whereas Zn is complexed by a lower molecular weight fraction containing hexose and phenolic moieties (Quinn et al., 1982).

- Red wine has a high complexation capacity for Pb, as it rapidly and strongly binds it (Green et al., 1997).

- Ultrafiltration removes the species that complex Pb ions in wine, which means that such species are macromolecules (e.g., polyphenols or polyuronides) (Green et al., 1997).

- Fe can complex certain triketones, the main bittering compounds in beer. In doing so, it may induce their deprotonation.
and thus contribute to changes in beer quality during ageing (Blanco et al., 2003).

- Metals can be present in beer as cations (e.g., Mn²⁺) or as complex anions (e.g., Fe(III)) with cyclic triketones (Blanco et al., 2003). Strong interactions between the metal cations and ligands in alcoholic beverages are believed to play a key role in some properties of the final product (Blanco et al., 2003).

- Metals in the waste from an alcoholic anis-type beverage facility have been found in complexed form in the solution. The existence of metals in the amorphous phase (as shown by SEM) indicates the presence of metal–organic complexes (as verified by HPLC). Complexation data can be used for selecting the proper wash treatment method. The formation of large molecules favors precipitation and chemisorption treatment methods (Moutsatsou et al., 2003).

Finally, some confusion in the literature as for the use of the terms labile or stable complexes should be noted. Traditional usage implies that the labile/stable status is defined on the grounds of kinetic stability (e.g., Cotton and Wilkinson, 1976), whereas recent publications use this distinction on the basis of thermodynamic stability (Wiese and Schwedt, 1997). Thus, one must be careful as to the meaning of these terms; in the present review we have maintained their meanings as they are used in each original publication.

### 7. Determination of metals in alcoholic beverages

Analytical methods frequently require sample preconcentration and/or pretreatment for the destruction of the organic matrix such as wet digestion, dry ashing, and microwave oven dissolution (Salvo et al., 2003; Dugo et al., 2004). Common analytical methods include atomic absorption spectrometry (AAS), atomic emission spectrometry (AES) and inductively coupled plasma–optical emission spectrometry (Camean et al., 2001). Ion chromatography is also used for the analysis of metals, for example in vodka (Obrezkov et al., 2000). The SAM can be used with some of these schemes; for example, Cu is determined in cachaza with SAM–AAS (Farias-Almeida et al., 2003). Another alternative involves a previous fractionation step of the metals from the beverage matrix using either ionic resins or adsorbents before analyzing previous fractionation step of the metals from the beverage schemes; for example, Cu is determined in cachaza with SAM–AAS.

Electrochemical methods are effective for metal speciation and/or pretreatment for the destruction of the organic matrix (e.g., Cotton and Wilkinson, 1976), whereas recent publications use this distinction on the basis of thermodynamic stability (Wiese and Schwedt, 1997). Quite interestingly, the catalytic properties of free and labile Cu(I) ions can be exploited for determining the element since the oxidation of an azine does not occur in the presence of stable Cu complexes and thus depends directly on the free metal ions (Wiese and Schwedt, 1997).

#### 7.2. Potentiometric methods

These are based on the potentiometric response of the target analytes. The PSA is the most important method in this group. The principles of two key potentiometric methods are depicted in Figs. 2 and 3.

In the PSA, reoxidation plays a key role since an oxidizing agent is necessary (see Fig. 2). For example, O₂ is used in the determination of Pb, Cd, and Cu in water and in some alcoholic beverages (Macca et al., 1997), and of free and total Cu in wines (Green et al., 1997). Hg(II) is also used as oxidizer in PSA (Mayer et al., 2003; Ensafi et al., 2001; Marken et al., 1997; Jager, 1978; Green et al., 1997; Barbeira and Stradiotto, 1996, 1998a).

In many cases, electrochemical measurements can be performed in the natural medium without sample pretreatment (e.g., in potentiometric stripping analysis, PSA) (Green et al., 1997).

- Organometallic complexes of many metal ions can adsorb onto Hg drops, and therefore they are—in principle—suitable for electrochemical analysis using Hg surfaces (Dugo et al., 2004).

Electrochemical methods are effective for metal speciation studies (Green et al., 1997).

Frequently all the metal ions need to be in the same form prior to analysis. An example for solving this problem before an electrochemical analysis is as follows: Cu is typically analyzed in alcoholic beverages by adding a high concentration of chloride ions to ensure that all the Cu is in the same form (i.e., CuCl₂) (Green et al., 1997). Quite interestingly, the catalytic properties of free and labile Cu(I) ions can be exploited for determining the element since the oxidation of an azine does not occur in the presence of stable Cu complexes and thus depends directly on the free metal ions (Wiese and Schwedt, 1997).

#### 7.1. Electrochemical methods: principles, advantages, challenges, and proposed solutions

Electroanalytical techniques are either passive or active (i.e., dynamic), depending on whether or not the process of excitation/response forces concentration changes at the electrode–electrolyte interface. In this way, potentiometric techniques (that measure the solution potential as a response to physicochemical variables) are passive, whereas voltammetric techniques (that observe redox behavior as a consequence of dynamic potential changes) are active (Rajeshwar and Ibanez, 1997).

Advantages of the electrochemical methods include the following:

- They are straightforward, fast, sensitive and inexpensive, and they tend to encompass simple requirements (Mayer et al., 2003; Barbeira and Stradiotto, 1996, 1998a).

- In many cases, electrochemical measurements can be performed in the natural medium without sample pretreatment (e.g., in potentiometric stripping analysis, PSA) (Green et al., 1997).

- Organometallic complexes of many metal ions can adsorb onto Hg drops, and therefore they are—in principle—suitable for electrochemical analysis using Hg surfaces (Dugo et al., 2004).

- Electrochemical methods are effective for metal speciation studies (Green et al., 1997).

Frequently all the metal ions need to be in the same form prior to analysis. An example for solving this problem before an electrochemical analysis is as follows: Cu is typically analyzed in alcoholic beverages by adding a high concentration of chloride ions to ensure that all the Cu is in the same form (i.e., CuCl₂) (Green et al., 1997). Quite interestingly, the catalytic properties of free and labile Cu(I) ions can be exploited for determining the element since the oxidation of an azine does not occur in the presence of stable Cu complexes and thus depends directly on the free metal ions (Wiese and Schwedt, 1997).

**Fig. 2.** Derivative potentiometric stripping analysis.

**Fig. 3.** Adsorptive stripping chronopotentiometric analysis.
Nan et al., 1994). However, besides the health threats posed by Hg (see below), this element does not allow the detection and adequate reproducibility for the quantitation of certain metal ions (e.g., Cu) (Macca et al., 1997; Daniele et al., 1989) due to their low solubility in Hg (Ostapezuk et al., 1997). Mercury-free, solid-state electrodes are useful alternatives (Wang and Tian, 1993).

The selection of the oxidizer depends on the supporting electrolyte, as in the case of high HCl concentrations in which O2 cannot be used (Green et al., 1997). If the deposition time is extended, the analytically useful response is enhanced (Ostapezuk et al., 1997). Proteins may interfere with the electrochemical processes involved (Mayer et al., 2003), which often prevents the use of PSA when determining metals in alcoholic beverages. Adsorptive stripping chronopotentiometry (AdSCP, see Fig. 3) is used in the analysis of tea, coffee, fruit juice, coca cola, white and red wines, beer, brandy, whiskey, and vodka (Dugo et al., 2004). Derivative anodic stripping chronopotentiometry is used for the determination of inorganic anions (Cl–, NO3, SO42–) and metal cations (Cd2+, Pb2+, Zn2+) in wine (Dugo et al., 2005).

Advantages of the potentiometric methods include the following:

- The analysis is not affected by the adsorption of organic compounds when the stripping is performed by a chemical oxidant (Green et al., 1997).
- They are suitable for direct measurement of labile metal ion concentrations in alcoholic systems with a high organic content (Green et al., 1997).
- Maintenance costs are low, and the methods are highly sensitive, accurate and precise; there is often no need for an extraction step, and disposable electrodes may be used (Dugo et al., 2004; Wang and Tian, 1993; Andruzzi et al., 1983).
- dPSA allows the direct determination of many metals in wines without any sample pretreatment (other than pH adjustment), because the glassy carbon-Hg film working electrode (GCMFE) remains inert to organic molecule interferences during the signal collection phase (Salvo et al., 2003).
- The optimum adsorption potential can be found by analyzing the response of the system at various applied adsorption potentials. For example, in the case of Ni analysis, H2O molecules and interfering ions can also be adsorbed at various potentials (Dugo et al., 2004).
- The transformation of E vs. t data into a E vs. dt/dE format (i.e., derivative dPSA) allows for the elimination of the capacitive current and reduces the influence of the adsorbed species, see Fig. 2 (Dugo et al., 2004). The area under the peaks then reflects the time required for the corresponding (faradaic) reduction processes, whereas the area under the baseline represents the charging time for the double layer (Dugo et al., 2004).
- dPSA allows the direct determination of Cd(II), Cu(II), Pb(II), and Zn(II) in acidified wine samples (Salvo et al., 2003).
- Analysis of Cu by PSA can yield essentially the same results as AA (Richter et al., 2001).

Challenges posed by these methods include the following:

- They require toxic Hg to plate the working electrode.
- The also toxic Hg(II) is often utilized for the chemical stripping (oxidation) step.
- The use of dioxygen as the chemical oxidizer is precluded in those cases where a high Cl– concentration is utilized, since this lowers the solubility of the gas in the stripping medium (Green et al., 1997).

7.3. Voltammetric methods

Here, the potential is changed at will and the system responds in the form of a measurable current; this offers a powerful tool for mechanistic and diagnostic studies. The most popular voltammetric method is cyclic voltammetry, where a potential ramp is applied to the system normally from the point of zero current up to a desired potential, limited by the decomposition of the solvent. In this way, the redox behavior of the system can be mapped to include oxidations and reductions of the analyte, coupled chemical or electrochemical reactions, electrode participation in redox processes, the kinetic facility of reactions involving analyte species (i.e., reversibility/sluggishness), the kinetics of charge transfer at the electrode/electrolyte interface, and the measurement of the concentration of analyte species (Rajeshwar and Ibanez, 1997).

Voltammetric methods have been widely used in the food industry for the analysis of metals, vitamins, additives, antioxidants, among others (PAR, 1982). For example, the fraction of labile ML to the total metal content in alcoholic beverages can be estimated electrochemically. This is done by analyzing the metal under the natural conditions of the beverage; then, the results are compared with those found after the addition of specific reagents that produce the necessary conditions for the rupture of virtually any other complex present (i.e., all the complexes, bound metal is liberated) (Salvo et al., 2003; Green et al., 1997; Daniele et al., 1989) and for the avoidance of insoluble oxide formation (Salvo et al., 2003). Typical reagents include acidic solutions of highly concentrated chlorides (Daniele et al., 1989), and a combination of HClO4, H2O2, and UV (Richter et al., 2001). This is exemplified below as

\[
ML + 2H^+ \rightarrow M^{2+} + H_2L
\]  

(1)

This step is of paramount importance for the analysis of the metal because at a higher pH and low Cl– concentration, for example, no useful Cu peaks are obtained in stripping voltammetry (Green et al., 1997). When identical results are obtained with and without such “uncomplexing” reagents, the conclusion is that the metal ion complexes are totally labile (Richter et al., 2001). Examples of labile metal fractions in an alcoholic beverage are given in Table 3.

Stripping voltammetry coupled to quantum mechanical calculations is a powerful tool in the elucidation of complex stoichiometries as well as structural, thermodynamic, and kinetic information on ML (Esparza et al., 2005). It is also used as a gauge of the vinification stage in certain wines (Esparza et al., 2007). Even though stripping voltammetry is more susceptible than stripping chronopotentiometry to interferences by electroactive organic molecules and by capacitive currents (Dugo et al., 2004), its use seems to be more widespread.

7.3.1. Polarography

Polarography is a branch of voltammetry where the working electrode is an Hg drop (Wang, 2000; Monk, 2001). It is simple and fast, and—by a judicious selection of the working conditions—it can also be made highly selective. Polarography is widely used in the determination of metals and sometimes in the determination of the ethanol content of an alcoholic beverage (Song et al., 2000; Dechsuwan and Vaneesorn, 1997). Other examples of

<table>
<thead>
<tr>
<th>Metal</th>
<th>% as labile metal content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>53</td>
</tr>
<tr>
<td>Cu</td>
<td>64–78</td>
</tr>
<tr>
<td>Cd</td>
<td>67</td>
</tr>
<tr>
<td>Zn</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 3 Labile metal fractions in wine (Green et al., 1997; Daniele et al., 1989)
metals analyzed in alcoholic beverages include Cd, Cu, and Pb in beer (Nguyen et al., 2003), Fe, Zn, Cu, and Pb in wine (Vasyutinskii et al., 1967; Fournier et al., 1998), and Pb in white spirits (Jin et al., 2001). Reported detection limits for Pb in the later case range from 0.021 to 0.1 \mu g/L, and for Cd it is given as 0.5 \mu g/L (Jin et al., 2001; Zhang et al., 2002).

Polarographic methods used in alcoholic beverage analysis—other than classical polarography—include:

- Differential pulse polarography in the determination of metal traces (Kobori and Sugiyma, 1995; Kobori and Matsuyama, 1994; Shukla and Pittre, 1998), and of ethanol (Dechsuwan and Vaneesorn, 1997), where a smaller standard deviation than that obtained with square wave voltammetry was evidenced.
- Adsorptive wave polarography for the determination of Mn (Liang and Li, 1992).
- Square wave polarography for the determination of Mn in sake (Kondo and Yamada, 1967).
- Anodic stripping polarography for the analysis of Cu, Pb, Cd, and Zn in wine (Cobo and Zamora, 1985).

Sample data for comparison with similar optical absorption analyses are given in Table 4.

The main advantages in using Hg as the working electrode include the following (Wang, 2000; Monk, 2001; Mikkelsen and Schrøder, 2003):

- Hg is an effective working electrode since it has a high overpotential towards hydrogen evolution as compared to other common electrodes, which provides a large potential window for its usage; as a result, many metals can be analyzed.
- Hg presents a renewed electrodis surface to the solution in each drop, which eliminates or minimizes contamination effects due to undesired adsorption phenomena. This avoids interference from possible residues from previous determinations.
- The use of fresh Hg drops eliminates the need to clean the electrodis surface with more chemicals.

The main disadvantages in using Hg include the following:

- As discussed earlier, Hg is highly toxic (Monk, 2001).
- Many metals cannot be analyzed since Hg is oxidized at positive potentials.
- The set up and its operation become complicated because of the need to house liquid Hg.

- Hg cleaning (normally by triple distillation) is costly.
- Due to the inconveniences mentioned above, mercury amalgams have been developed—although this does not completely preclude its associated toxicity (Lovrić et al., 1991).

7.3.2. Rotating disk electrode

Here, a rotating disk enhances mass transport for the electrodeposition of a target substrate. Advantages include mercury avoidance, simplicity, and reproducibility of results. An example is the analysis of Cu in tequila using a Pt rotating disk electrode with the SAM. The obtained results compare well to those with AAS (Carreon-Alvarez et al., 2008).

7.3.3. Stripping analysis

The quantitation of metals in alcoholic beverages may be achieved by stripping voltammetric analysis. Here, a judiciously selected potential is applied during a preselected time to reduce and deposit the metal from the liquid matrix onto the electrode surface. Then, a potential is applied such that the metal is reoxidized, see Fig. 4. The amount of metal reduced and then reoxidized is proportional to its concentration in the liquid (Wang, 2000; PAR, 1991; Bard and Faulkner, 2001).

Advantages of this technique include its sensitivity ($10^{-10}$ M in some cases), accuracy, cost effectiveness, and speediness; typically, no pretreatment of the sample is required. Quantitation is achieved by measuring the area under the oxidation curve. Metals commonly analyzed with this technique include Cu, Cd, Pb, Zn, and Ni, although Ti, Sn, Bi, and Sb have also been reported. Typical results compare well with those obtained by AAS.

Several techniques derive from this stripping analysis; the main differences involve the nature of the parameters monitored. The principal stripping techniques used for the quantitation of metals in alcoholic beverages include:

- PSA, used in the quantitation of metals and estimation of their labilities in cachaza, German wine, and white wine (Green et al., 1997; Richter et al., 2001; Ostapezuk et al., 1997; Mikkelsen et al., 2004).
- dPSA in beer, and white and red wines (Salvo et al., 2003; Mayer et al., 2003; Mikkelsen and Schrøder, 2002).
- ASV in wines and grappa (Baldo and Daniele, 2005; Arcos et al., 1993; Baldo et al., 1998).
- Sono-ASV in beer (Agra-Gutierrez et al., 1999).
- Differential pulse voltammetry in wines and in synthetic alcoholic beverages (Ijeri and Srivastava, 2000), and in cognacs (Barbeira and Stradiotto, 1998a).

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquor</td>
</tr>
<tr>
<td>Pb</td>
</tr>
<tr>
<td>White spirit</td>
</tr>
<tr>
<td>White spirit</td>
</tr>
<tr>
<td>Alc. beverages</td>
</tr>
<tr>
<td>Alc. beverages</td>
</tr>
</tbody>
</table>

Fig. 4. Anodic stripping voltammetric analysis.
● DPASV in white wine and in synthetic alcoholic beverages (Ijeri and Srivastava, 2001; Wiese and Schwedt, 1997; Daniele et al., 1989).

Stripping analysis is also used for metal determination in other foodstuffs such as Pb and Cu in butter (Szylk and Szyszkowska-Czerniak, 2004), in Sicilian olive oil (Pera et al., 2002), and in the determination of Cd, Cu and Pb in Coca Cola (Andruzzetti et al., 1983). A typical challenge when using stripping analysis is that sample decomposition is often required for the removal of interfering substances prior to measurement (Green et al., 1997).

There are several factors in ASV that affect the analysis of metals in alcoholic beverages. Representative examples are discussed below.

Sample pretreatment can in some cases increase the analytical response of a metal in the reoxidation step (Arcos et al., 1993); a case in point is wine digestion. However, when the sample is strongly acidified with HCl-or when it is diluted-the response to reoxidation is not the same. In the latter case such a response is probably due to a lowering of the metal concentration below its detection limit (e.g., Cd in wine) (Ostapezuk et al., 1997).

A variety of electrodes are in use for ASV, including the following:

● Hg drop—the most typical electrode—is used for Zn, Pb, and Cu in whiskey, rum, and cachaza (Barbeira et al., 1995; Barbeira and Stradiotto, 1997; Barbeira and Stradiotto, 1998b).

● Reticulated vitreous carbon (RVC) is covered with Hg prior to use in ASV (Mayer et al., 2003; Macca et al., 1997; Marken et al., 1997; Ostapezuk et al., 1997; Agra-Gutierrez and Compton, 1998).

● Pt microelectrodes (1 μm in diameter) covered with Hg increase mass transfer and reduce preconcentration time (Daniele et al., 1989).

● Dental amalgam electrodes are used for the analysis of metals in wines (Mikkelsen et al., 2004; Mikkelsen and Schroder, 2002; Mikkelsen and Schroder, 2000).

● Solid normal electrodes (e.g., Pt, Au, RVC) are used in the determination of Cu in alcoholic beverages (Barbeira and Stradiotto, 1999).

● Other working electrodes used for this purpose include GCMFE, chemically modified electrodes, CD-trodes, carbon disc microelectrodes, and Hg microelectrodes. Additional factors to be taken into account for the determination of metals in alcoholic beverages by this method include:

● Deposition time, τd: When this is increased, the reoxidation response also increases. Longer times are recommended when analyzing Cd since it is usually found at low concentrations (Ostapezuk et al., 1997). τd affects the peak potential, E, and the peak current, I, as both increase with τd (Arcos et al., 1993).

● pH: This may play a role in the different equilibria present in a given matrix. For example, when Cu and Pb are analyzed, the pH of the sample should be made rather acidic so as to obtain a larger response signal (Green et al., 1997).

8. Conclusions

Metals find their way into alcoholic beverages through many possible sources discussed here. Their effects on the beverages as well as on humans after consuming such beverages are quite varied. This then necessitates that metals be subject to regulations. Electrochemical techniques offer distinct advantages for their analysis.

Acknowledgements

JGI acknowledges financial support from Universidad Iberoamericana and Universidad de Guadalajara during a sabbatical leave. We are thankful to CONACyT for their support through Grant 62066 for the scholarship of Alejandra Carreon-Alvarez. This paper is dedicated to Prof. Margarita Watty (U. Iberoamericana) on the occasion of her 80th birthday.

References


Crane, J.V. 1972. Removal of off-odors, metals, and sulfur compounds from wine for hydrolysis. Instituto de Cercetari si Proiectari Alimentare (Romania) 9, 127–137.


