Voltammetric Determination of Trace Concentrations of Metals in the Environment

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In this article, the application of voltammetric methods for the determination of metals in various environmental matrices is reviewed. The advantages and disadvantages of such methods are discussed, and particular emphasis is placed on sample preparation and speciation.

One of the most serious problems facing the world today is the contamination of the environment by inorganic, organic, and organometallic species. One area of particular interest is the detection of heavy metals and metalloids in environmental matrices and the elucidation of their pathways through various environmental compartments (1–4). Ultimately, these toxic metals are incorporated into drinking water and various food chains. Since metals are biologically nondegradable, they tend to accumulate in various vital organs; therefore, even exposure to trace concentrations of various metal ions can lead to long-term toxic effects.

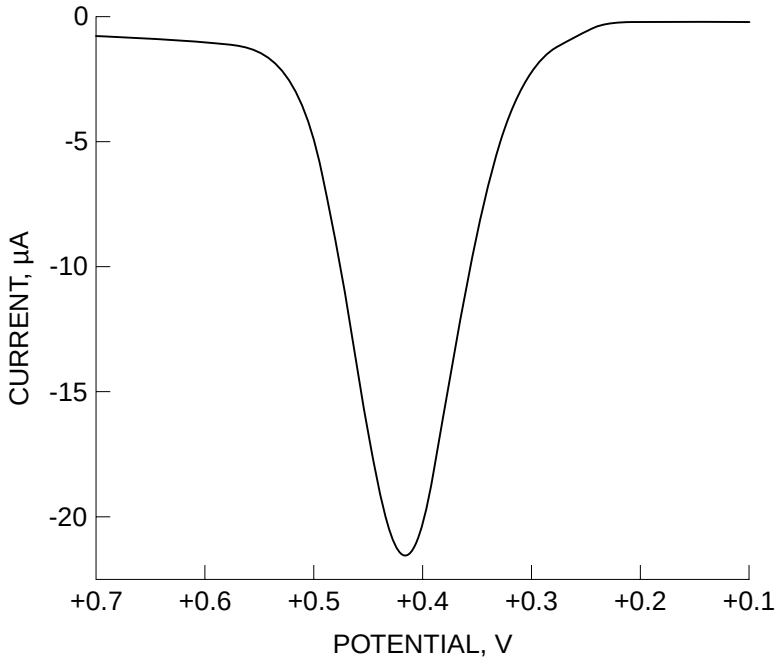
Metal and metalloid ions can be divided into three groups, based on their toxicity (2,4). The metal/metalloids of the first group are toxic at all concentrations and have no known biological functions; this group includes lead, cadmium, and mercury. The ions of the second group (which includes arsenic, bismuth, indium, antimony, and thallium) also have no known biological functions, but are less toxic; however, they are still toxic if present in more than trace concentrations. The final group includes essential metal/metalloids such as copper, zinc, cobalt, selenium, and iron, which are required for various biochemical and physiological processes, and are toxic above certain concentrations.

There are a number of requirements for an analytical technique to be useful in environmental analysis (2,4). These include sensitivity, precision, accuracy, dynamic range, ease of pretreatment/sample preparation, ease of automation, cost, suitability for studies in the field, applicability to a wide range of substances, and the capability of determining more than one species. In addition, there is often the added burden that a method be approved by regulatory authorities.

Voltammetric determinations are based on the current response of an analyte to an applied potential waveform (the current is plotted as a function of the applied potential, as shown in ***F1***). The magnitude of the peak current is proportional to the concentration of the analyte in solution. Voltammetry exhibits a wide dynamic range, from sub-ppb to ppm or higher. For the higher concentrations (ppm or high ppb), direct measurement by differential pulse voltammetry (DPV) or (Osteryoung) square-wave voltammetry (OSWV) is possible; for lower concentrations, these voltammetric techniques are combined with a preconcentration step (which may be electrochemical or nonelectrochemical). These latter techniques are referred to as stripping techniques (5,6). For many metal/metalloid ions (e.g., lead, cadmium, copper, zinc, antimony, and bismuth), Anodic Stripping Voltammetry (ASV) at a mercury electrode

F1

Typical current response for differential pulse voltammetry.



is used. For ASV, the preconcentration step involves reduction of the metal ions followed by amalgamation with the mercury electrode; the metals are then removed in the anodic stripping step by oxidation. If the redox potential of the metal/metalloid ion is more positive than that of mercury, a solid electrode (e.g., gold) must be used. There are some metal/metalloid ions that are not suitable for detection by ASV (e.g., cobalt and nickel); Adsorptive Stripping Voltammetry (AdSV) can often be used for such metals. In the preconcentration step, the metal ions are adsorbed as complexes at the surface of the electrode (typically mercury) by a nonelectrochemical process; the concentration of the complex can then be measured in the stripping step (which can be anodic or cathodic).

One major advantage of voltammetric methods is that they are substance-specific; that is, they can differentiate between the different chemical forms of the metal/metalloid ions (the significance of this is discussed in more detail below). However, the selectivity of voltammetric techniques is typically poor, and a separation step is often required; for example, determination of a number of electrochemically active analytes with similar redox potentials may not be possible by DPV or OSWV (or the analogous stripping techniques), but determination of all the analytes may be possible by liquid chromatography followed by electrochemical detection (LCEC) (7). Consequently, although many organic pollutants are electrochemically active and have been shown to be amenable to detection by AdSV (8), the poor selectivity of voltammetric techniques usually makes them inferior to LCEC for trace analysis of such species.

Sample Preparation

Although voltammetric techniques are inherently precise and accurate, the results obtained using these techniques may be invalidated due to contamination caused by poor sample handling and preparation. Therefore, stringent conditions should be routinely used for analyses, particularly when dealing with trace concentrations (9). For example, all reagents, standard solutions, etc., should be ultra-pure, and all glassware needs to be scrupulously cleaned. Similarly, stringent conditions should also be used for sampling and the pretreatment of samples; these two stages should be simplified as much as possible to minimize the potential for sample loss and contamination.

Sampling of sea water and similar environments must be done using a small rubber boat, due to the contamination caused by large ships due to engine exhaust, antifouling paint, etc. (10). Samples should be collected in polyethylene or Teflon bottles that have been scrupulously cleaned and preconditioned with pure water solutions of calcium and magnesium ions to prevent losses due to the adsorption of the analytes to the walls of the container (9,11). Since the vertical distribution of metal ions is also of interest, samples must also be taken from deeper water. This procedure requires a special all-Teflon sampler (the C.I.T. sampler), since other samplers are susceptible to contamination from rust, lead, and grease (12). Automated samplers have been developed for the collection of rain and snow. These samplers, controlled by humidity sensors, are open only during rain or snowfall (13). This prevents contamination of the sample by dust.

The first step in sample pretreatment is filtration through a 0.45-µm membrane filter to remove suspended and particulate matter (9) (however, it should be noted that some colloidal particles are small enough to pass through the filter). The filtrate is then acidified to pH 2 by the addition of ultra-pure hydrochloric acid (1). This prevents adsorption of the analyte ions on the walls of the container (9), and causes dissociation of metal ions from some complexes, thereby making these ions available for the analysis (11). The acidified samples can be stored at –20 °C. In some water samples, there is a substantial concentration of organic material, which can form stable complexes with the metal ions and can also adsorb onto the electrode surface. These interfering effects can be eliminated by destroying the or-

ganic material by either UV-irradiation or acid digestion (2,14,15). Acid digestion or low-temperature ashing can also be used for pretreatment of the suspended and particulate matter removed in the filtration (2,16).

These pretreatments do not necessarily have to be used for all environmental samples (2). For example, open ocean water and tap water usually do not have particulate matter or significant organic content, so the filtration and UV-irradiation steps are not necessary. These steps can often also be omitted for rain and snow samples (however, samples from areas with high levels of air pollution do require all the steps). The various pretreatments required for the different samples are summarized in ***F2*** (2,3).

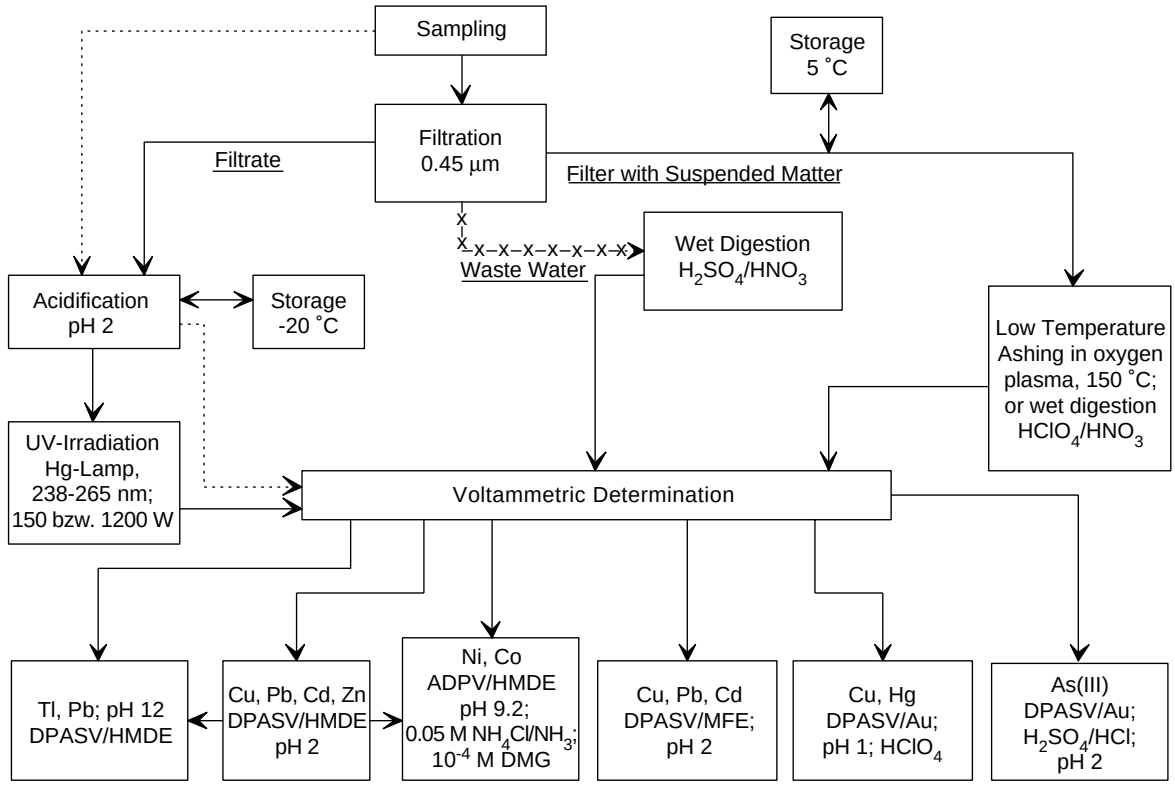
Speciation

One of the major applications of voltammetry in environmental analysis has been speciation studies (2–5,17,18). Speciation analysis is defined as the determination of the concentrations of the different physicochemical forms of the element which make up its total concentration in the sample. Metals can exist in a range of physicochemical forms in environmental samples, including hydrated metal ions, inorganic and organic complexes, and adsorbed on organic and inorganic colloidal particles. The toxicity of a metal ion varies with its physicochemical form, and the predominant reason for speciation studies is to measure the "toxic" fraction of the metal. Typically, the most toxic forms are the hydrated metal ions and labile complexes (i.e., dissociation can readily occur), and the least toxic forms are strongly bound metal complexes and metal adsorbed on colloidal particles (indeed, in unpolluted natural waters, metals are usually present in one of these latter two forms). Metal complexes with very lipophilic organic ligands can also be highly toxic due to the ability of these complexes to cross cell membranes (this group also includes organometallic alkyl compounds of lead, tin, and mercury).

One simple example of speciation is the measurement of the concentrations of inorganic lead and organometallic lead compounds. The latter species are considerably more toxic than the former. (The LD50 for rats for various lead compounds are as follows (7): Et_4Pb = 12–14 mg/kg, Et_3PbCl = 20 mg/kg, Et_2PbCl_2 = 120 mg/kg, and Pb(II) = 810 mg/kg.) In one study, the concentrations of inorganic lead, dimethyl- and trimethyllead were measured by varying the deposition potential in ASV (19). At a deposition potential of –1.4 V, all lead compounds were deposited, whereas only the inorganic lead and the dimethyllead were deposited at –0.6 V. Complexation of the inorganic lead with EDTA shifts the reduction potential of Pb(II) to more negative values, so it is no longer plated at –0.6 V; that is, only the

F2

Pretreatments required for various water samples. The filtration and irradiation steps can be omitted for samples that do not have particulate matter or significant organic content (e.g., tap water and open ocean water); this is represented by the dashed line. Adapted from reference 3.



dialkyllead is deposited at this potential.

The toxicity of some metal ions also varies with oxidation state. For example, As(V), Cr(III), Tl(III), and Sb(V) are nontoxic, whereas As(III), Cr(VI), Tl(I), and Sb(III) are toxic (20,21). However, these pairs of oxidation states can be distinguished electrochemically, since only one of each pair is electrochemically active (22). The concentration of the electroinactive oxidation state can be calculated by measuring the total metal ion concentration following conversion of the electroinactive oxidation state to the electroactive oxidation state. For example, the concentration of Sb(III) can be determined in 0.2–0.5 M hydrochloric acid, and the total Sb concentration can be determined following reduction of Sb(V) to Sb(III).

However, it is important to realize that it is generally impossible to measure the concentrations of individual species; for example, Cd^{2+}, $CdSO_4$, $CdCl^-$, and $CdCO_3$ cannot be distinguished by ASV. It is only possible to divide the species into groups based on gross behavioral differences (17,18,23). Furthermore, the distribution between the groups that is measured experimentally cannot be the same as that present in the original environment, since the measurement process (e.g., filtration, pH changes, the application of an electric field) alters the equilibria between the different forms of the species. The limitations apply not only to voltammetric techniques, but also to all techniques that are used for speciation studies. However, Florence has observed that the aim of speciation studies is not to provide a detailed species distribution, but rather to provide an estimation of the fraction of the metal present in a toxic form (18). Consequently, there is much useful information to be derived in spite of these limitations, although sample pretreatment should be kept to a minimum, not only to minimize the changes in the sample equilibria, but also to reduce the potential for contamination.

The simplest voltammetric speciation studies involve separation of the metal species into two groups—labile and inert. Labile species are species that can be detected by ASV, such as hydrated metal ions, and metal ions dissociated from weakly bound complexes or weakly adsorbed on colloidal particles (24). However, the composition of the labile fraction can vary with experimental conditions (e.g., pH, temperature, buffer composition, and the rate of stirring), and hence the exact conditions used must be specified. The inert fraction consists of the species that do not dissociate the metal under the conditions used. The total metal fraction can be measured following prolonged UV irradiation in an acidic medium (25) (which promotes dissociation of the metal from all types of ligands and colloids), and hence the inert fraction can be calculated from the two experiments.

It is assumed in this approach that metal complexes are not directly reduced during the deposition step; however, this assumption is not necessarily valid, particularly at very negative deposition potentials (26,27). The validity can be tested by using a range of deposition potentials, and plotting the anodic stripping current versus deposition potential (18). If only ionic metal is reduced, then the peak current increases from zero to a limiting value over a small potential range; however, if metal complexes are directly reduced, the peak current increases continuously as the deposition potential is made more negative. In order to avoid the possibility of directly reducing metal complexes, it is recommended that the minimum deposition potential be measured for each metal (i.e., just sufficiently negative to give the maximum anodic stripping current) and that the metals be determined in separate experiments using the appropriate minimum deposition potential (18).

One advantage of using ASV for speciation studies is that the "ASV-labile" fraction can correlate well with the toxic metal fraction (28,29) (obtained using bioassays). The ASV method can also be combined with other separation methods to subdivide the labile and inert groups (it should be re-emphasized that the results of these experiments will depend on the nature of the separation method; that is, they are *operationally defined*). One method reported by Florence and Batley combined ASV with a chelating resin separation and UV irradiation (30,31). The chelating resin (Chelex-100) was used to separate ionic metals from metals strongly bound in metal-ligand complexes or strongly adsorbed to colloidal particles (31,32). The UV-irradiation was applied to a solution at pH 4.8 (rather than in strongly acidic solution) and hence only caused dissociation of the metal from organic complexes or colloids (28,32). This method is shown schematically in ***F3*** (17), and the four separation steps are as follows:

1. Filtration through a 0.45-μm filter, which separates the metal into "dissolved" and "insoluble" fractions.
2. Passage of the "dissolved" fraction through a Chelex-100 ion-exchange column.
3. UV-irradiation of the "dissolved" fraction at pH 4.8.
4. Passage of the "dissolved" fraction through a Chelex-100 ion-exchange column followed by UV-irradiation at pH 4.8.

Both the "ASV-labile" fraction and the total metal content were measured after each of these four separation steps, and these eight measurements were used to quantify seven groups of metal-containing species. This method was used to analyze the speciation of copper, lead, cadmium, and zinc in a variety of different natural waters (31–34).

Generally, in unpolluted waters, copper was present either in stable organic complexes or associated with colloidal particles (31,34); that is, the concentration of the toxic "ASV-labile" copper was low (33). The concentration of ionic lead was also low in (unpolluted) natural water, and most lead was present either adsorbed to inorganic colloidal particles or as an inorganic molecular species (31,33). In contrast, cadmium was generally present in an "ASV-labile" form (31,33). This approach has been modified to include a step for the extraction of lipid-soluble complexes (32) (since such complexes can also be toxic).

A related method was developed by Figura and McDuffie for speciation studies of natural water (35) ***F4***. The "ASV-inert" fraction was divided into three groups, based on the rate of metal dissociation on Chelex columns: "moderately labile," "slowly labile," and "inert" (the "ASV-labile" fraction was described as "very labile"). It was found that cadmium and zinc existed predominantly in the "very labile" and "moderately labile" groups, whereas copper existed in the "moderately labile" and "slowly labile" groups, and lead existed in the "slowly labile" and "inert" groups. These results are consistent with the results reported by Florence (*vide supra*).

It was noted above when discussing studies that do not involve speciation that the presence of organic material can cause erroneous results in ASV experiments (surface-active molecules can adsorb to the electrode surface, and complexing agents can bind the ionic metal), and the destruction of organic matter in the sample pretreatment was recommended. However, this approach cannot be used in speciation studies, since the formation of complexes with naturally occurring ligands is an integral part of the speciation process. Various methods have been suggested for eliminating the undesirable effects of surface-active molecules and complexing agents, while retaining the ability to measure the concentration of metals bound to ligands. One method involves medium exchange; that is, the electrode is removed from the sample after the deposition step, and the stripping step is performed in a simple buffer, thereby eliminating the effect of surface-active molecules and complexing agents on the stripping step. Comparative studies showed that the medium exchange often led to a significant increase in the "ASV-labile" fraction (36,37). Another method uses pH variation of the deposition and stripping steps of the ASV experiment to eliminate the effect of complexing agents (37–39). The variation is as follows:

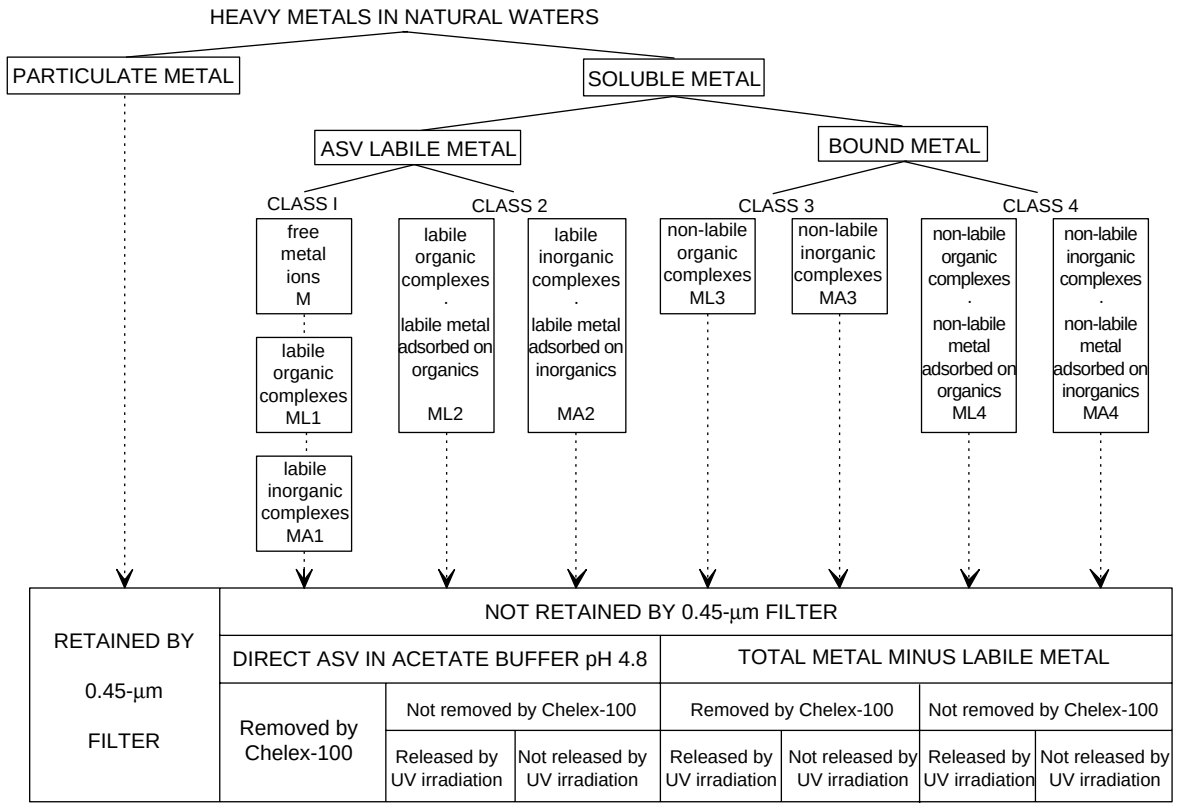
a)both deposition and stripping at pH 7

b)deposition at pH 7, stripping at pH 2 (i.e., the solution is acidified at the end of the deposition step)

c)both deposition and stripping at pH 2

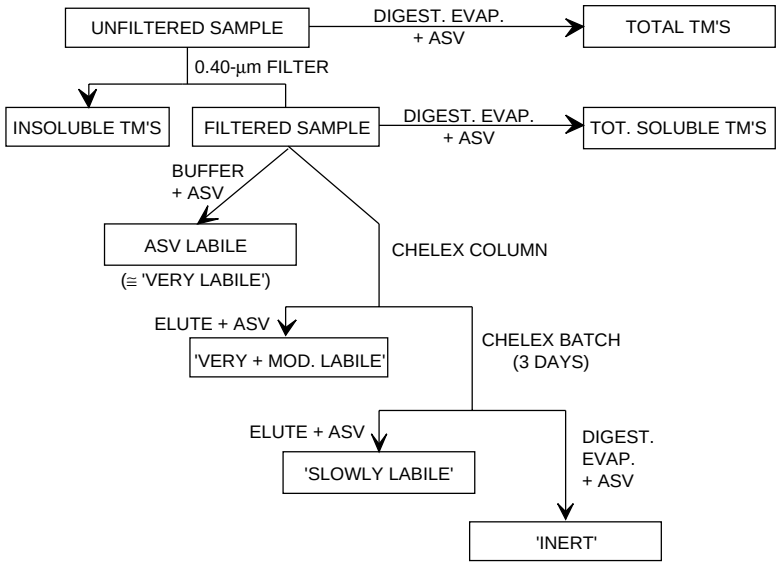
F3

Batley-Florence scheme for the chemical speciation of trace metal ions in natural waters. Adapted from reference 17.



F4

Figura-McDuffie scheme for the chemical speciation of trace metal ions in natural waters. Adapted from reference 35.



It is assumed in this method that metal-ligand complexes are stable at pH 7, but dissociate at pH 2. Therefore, the difference between the peak currents of (a) and (b) is due to the effect of complexing agents on the stripping step, whereas the difference between the peak currents of (b) and (c) is a measure of concentration of complexed metal in solution at pH 7. This "double-acidification" method was used in conjunction with the medium exchange method to study synthetic water samples consisting of copper, cadmium or lead and fulvic acid, humic acid (naturally occurring complexing agents), or Triton X-100 (a synthetic surface-active molecule) (39). The "toxic" fraction was assumed to be the difference between the total metal and the complexed metal (which is the difference between the peak currents of (b) and (c)), and was compared with the results from the "direct" measurement (a). The "toxic" fraction measured using this method was much higher than that determined using the "direct" measurement for all the samples.

As discussed above, the fraction of the metal ions that is incorporated into inorganic and organic complexes or adsorbed to colloidal material is typically not toxic. Therefore, the presence of appropriate organic and inorganic material in natural waters provides a mechanism for the "detoxification" of the metal ions (17,18). The ability to incorporate metal ions in non-toxic forms is referred to as "complexing capacity," which can be measured by a number of methods, including ASV titration (40). The ASV method involves titration of a water sample with Cu(II) ions, followed by an ASV determination. The endpoint of the titration is indicated by the presence of free Cu(II) ions in the solution (the slope of the peak current vs. Cu concentration increases to that found for free Cu(II)) (41). Typically, overnight equilibration of the added Cu(II) following each titration is recommended (18). However, it has been shown that ASV titration using Cu(II) is not a reliable method for determination of "complexing capacity" (by comparison with bioassay studies), due to dissociation of Cu complexes during the deposition step (18,42) and interference from adsorbed organic material (43). AdSV has also been used for "complexing capacity" determinations, using the competition between natural ligands and catechol or tropolone for Cu(II) (42,44). This method eliminates the problem due to dissociation of the Cu(II) from the complexes, but may still be prone to adsorption interferences.

Conclusion

In the light of the increased awareness (and legislation) concerning trace concentrations of toxic metal ions in environmental and biological matrices, there are likely to be increases both in the number of samples that require analysis and in the accuracy, precision, and detection limits required for these measurements. Although voltammetric methods have many features that make them suitable for such measurements, much work is required before voltammetry can be adopted as a routine analytical method that can rival techniques such as atomic absorption spectroscopy. One area which requires attention is the development of totally automated procedures. Unattended automation reduces labor costs and is viewed as essential in many analytical laboratories.

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