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ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 1984

Impact Factor: 5.64 · DOI: 10.1021/ac00277a031 · Source: PubMed

Critical Evaluation of Some Wet Digestion Methods for the Stripping Voltammetric Determination of Selenium in Biological Materials

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The suitability of wet digestion methods for the cathodic stripping voltammetric determination of selenium in biological materials is evaluated. Methods currently available for the determination of the element by other analytical techniques were found to be unreliable. However, a modified digestion procedure using a HNO_3 and H_2SO_4 mixture enables adequate digestion of the sample material and retention of selenium in a state amenable for determination and is suitable for cathodic stripping voltammetric determination of the element in most sample materials. Application of the proposed digestion procedure to a range of biological standard reference materials provides satisfactory data.

Increasing concern about the significance and toxicity of selenium has led to the development of numerous analytical methods for its determination in various biological materials. Stripping voltammetric methods (*1*, *2*) provide a simple, sensitive, and rapid approach for the determination of the element in many matrices. Unfortunately, direct determination of the element in biological materials is not feasible because of the matrix effect usually encountered at the relatively low concentration in such samples. Sample treatment, therefore, often has to be undertaken before the element can be determined accurately by stripping voltammetry and many other analytical methods (*3–18*).

The most widely used digestion methods for the determination of selenium involve decomposition with acid mixtures such as $HClO_4$ and HNO_3 (*3–12*), HNO_3 and H_2SO_4 (*13*, *14*), HNO_3 and H_2O_2 (*15*), and HNO_3, H_2SO_4, and $HClO_4$ (*16–18*). The preference for these wet digestion methods is based on the reduced danger of losses at the low temperatures at which they operate and the simplicity of the apparatus required for their operation. The methods of dry ashing, on the other hand, often result in great losses (*19–21*) unless an ashing aid is used to minimize or prevent losses (*1*, *22*). Losses may also occur during wet digestion depending upon the material and the selected digestion mixture (*23*). A recent report by the Analytical Methods Committee (*24*) indicates that digestion mixtures such as H_2SO_4 and H_2O_2, HNO_3 and H_2SO_4, and HNO_3, H_2SO_4, and $HClO_4$ gave very low recoveries of selenium for samples containing a high proportion of fats such as milk. It was indicated however that further investigation by radiochemical studies revealed that the selenium was not lost from the digestion vessel but rather was present in a state or form which was not detectable by the analytical technique employed. A similar observation has previously been made by Levesque and Vendette (*25*). In a more recent study Nygaard and Lowry (*26*) also indicated that digestion mixtures such as HNO_3 and H_2SO_4, H_2O_2 and H_2SO_4, and H_2O_2 and HNO_3 gave low recoveries of selenium for organo compounds but found that the same digestion mixtures gave ≥95% recovery of the element when present in the inorganic form in aqueous solution. Several other workers (*3*, *7–9*, *27*) reported that wet digestion methods employing various acid mixtures yield up to 100% recoveries of selenium in biological materials.

These few examples clearly demonstrate the conflicting views of various workers on the suitability of some wet digestion methods for the determination of selenium in biological materials. It appeared more likely that the reported inconsistencies may be partly due to the varying degree of adequacy of the wet digestion methods for the determination of selenium by various analytical techniques. Ideally, a digestion method that is suitable for all biological materials and the subsequent quantitative techniques would be preferred. However, this is quite difficult to achieve as the organic matrix composition may vary from one sample to the other. More importantly, the resulting sample digest may not present the selenium in the form that can be adequately detected by all analytical techniques. For example, a sample digest that proved adequate for the determination of selenium by atomic absorption spectrometry which incorporates an additional ashing step may not necessarily be as adequate for other techniques which rely on the presence of the element in a suitable state or form in solution for its accurate determination (*24*).

A survey of the literature revealed that the suitability of some wet and dry ashing methods for the determination of selenium in biological materials is frequently made for various analytical techniques such as atomic absorption spectrometry, spectrophotometry, and fluorometry. However, no similar investigation appeared to have been reported for the stripping voltammetric determination of the element.

The present study evaluates the suitability of some wet digestion methods for the cathodic stripping voltammetric determination of selenium in biological materials. The methods considered were those employing HNO_3 only, HNO_3 and H_2SO_4, and HNO_3 and $K_2S_2O_8$. Methods requiring the use of perchloric acid and other violent chemicals were not included as their use requires the installation of some special facilities such as a perchloric fume collector and in some cases use of these chemicals has been known to result in explosion and/or fire during digestion.

In the methods considered, an indication of the contribution of the digestion period to the overall analysis time is given. Also the adequacy of the sample digest for direct determination of selenium without additional modification of the matrix is examined.

EXPERIMENTAL SECTION

Reagents and Standard Solutions. All acids used were Aristar grade (B.D.H. Chemicals) purity while other reagents were of Analytical grade purity. A stock solution (1 g/L) of selenium(IV) was prepared by dissolving an appropriate amount of analytical grade selenium dioxide (Ajax Chemicals) in 0.1 M hydrochloric acid and storing in prewashed polyethylene bottles. The required standards were prepared daily by appropriate di-

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lution of the stock solution with 0.1 M hydrochloric acid. Distilled deionized water was used in all sample and solution preparations.

Instrumentation. An EG&G Princeton Applied Research microprocessor-based polarographic analyzer (PAR Model 384) equipped with a PAR Model 303 static mercury drop electrode and a PAR Model 305 stirrer was used to record all stripping voltammograms. The electrode compartment consists of a hanging mercury drop electrode (HMDE) a silver–silver chloride (saturated KCl), and a platinum wire as its working, reference, and auxiliary electrodes, respectively. Deposition of selenium(IV) onto the mercury electrode was achieved with a fast stirring rate and either a medium or large drop size with a surface area of 0.015 cm^2 or 0.024 cm^2, respectively.

Standard solutions of selenium were added to the polarographic cell with fixed volume Socorex micropipets with disposable tips.

Glassware. All glassware and polyethylene bottles were soaked in 2 M nitric acid for at least 7 days, washed three times with distilled deionized water, soaked in distilled deionized water, and finally soaked in 0.1 M hydrochloric acid until ready for use.

Biological Standard Reference Materials. Bovine liver, oyster tissue, and orchard leaves were obtained from the U.S. Bureau of Standards, Washington, DC, animal muscle was from the Analytical Quality Control Services of the International Atomic Energy Agency (I.A.E.A.) in Vienna (Austria). All materials were treated as recommended by the suppliers.

Digestion Vessel. All samples were digested in 125-mL Erlenmeyer flasks (Pyrex No. 5100) on a Corning hot plate–stirrer (Model PC-351). A glass funnel was inserted into the flask to maintain refluxing condition and minimize sample losses during the digestion. Acids and other reagents were added into the vessel through the funnel.

Initial Investigations. The suitability of some of the currently available digestion procedures utilizing HNO_3 (*28*), HNO_3 and H_2SO_4 (*14*), and HNO_3 and $K_2S_2O_8$ (*26*) for open digestion of biological materials and subsequent cathodic stripping voltammetric determination of selenium was initially investigated by using freshly collected urine samples. In all cases, the urine samples were digested within 30 min of collection. The digestion methods were subsequently modified for CSV as described below.

Proposed Digestion Procedures. Digestion with HNO_3 Only. (*a*) *Urine Samples.* Transfer 25 mL of urine, 10 mL (65%) of nitric acid, a precleaned glass bead, and a magnetic stirrer into the digestion vessel. Place on hot plate, stir continuously, and heat initially at medium rate for 10 min. Then heat on maximum setting until nitrogen oxide fumes are just given off and white residue is left. Leave the vessel to cool for about 2 min and repeat digestion with an additional 10 mL (65%) of nitric acid; this time heat until nitrogen oxide fumes cease to appear. Cool vessel again for about 2 min and then add 3 mL of 1:1 (37%) hydrochloric acid. Heat the mixture at medium rate for 20 min to convert selenium to the Se(IV) form. Cool to room temperature and make up to 10 mL with distilled deionized water.

(*b*) *Other Biological Samples.* Transfer 0.2–1 g of sample, 10 mL (65%) of nitric acid, a precleaned glass bead, and a magnetic stirrer into the digestion vessel. Place on hot plate and heat initially at medium rate for about 10 min or until the sample has completely dissolved. Start stirring and increase heat to maximum setting. Heat until a brown residue is left and nitrogen oxide fumes are given off. Leave the vessel to cool for about 2 min and repeat digestion in the same way with multiple additions of 10 mL (65%) nitric acid until a clear solution or white residue is left. Heat until nitrogen oxide fumes cease to appear. Cool the digest and add 3 mL of 1:1 (37%) hydrochloric acid. Heat mixture at medium rate for 20 min to convert selenium to the Se(IV) form. Cool to room temperature and make up to 25 or 50 mL with distilled deionized water.

Digestion with HNO_3 and H_2SO_4 Mixture. (*a*) *Urine Samples.* Transfer 25 mL of urine, 10 mL (65%) of nitric acid, 2 mL (98%) of sulfuric acid or 4 mL (24.5%) sulfuric acid, a precleaned glass bead, and a magnetic stirrer into the digestion vessel. Proceed with digestion as previously described for the HNO_3 only digestion procedure. Repeat digestion with multiple additions of 10 mL (65%) of nitric acid until a clear solution is left and nitrogen oxide fumes and sulfite mists cease to appear. Convert selenium to the Se(IV) form as described before and make up the final solution to 10 mL with distilled deionized water.

(*b*) *Other Biological Samples.* Transfer 0.2–1 g of sample, 10 mL (65%) of nitric acid, 4 mL (24.5%) of sulfuric acid, a precleaned glass bead, and a magnetic stirrer into the digestion vessel. Proceed with digestion as previously described for the HNO_3 only digestion procedure. Again repeat digestion with multiple additions of 10 mL (65%) of nitric acid until a clear solution is left and appearance of nitrogen oxide fumes and sulfite mist has ceased. Convert selenium to the Se(IV) form and make up to 25 or 50 mL with distilled deionized water.

Digestion with HNO_3 and $K_2S_2O_8$ Mixture. (*a*) *Urine Samples.* Transfer 25 mL of urine, 10 mL (65%) of nitric acid, 8 mL (4% w/v) of potassium persulfate, a precleaned glass bead, and a magnetic stirrer into the digestion vessel. Proceed with digestion as described for the HNO_3 and H_2SO_4 digestion procedure, this time until white residue or clear solution is left and nitrogen oxide fumes are no longer given off. Convert selenium to the Se(IV) form and make up the final solution to 10 mL with distilled deionized water.

(*b*) *Other Biological Samples.* Digest 0.2–1 g of sample with the same HNO_3 and $K_2S_2O_8$ mixture as described for the HNO_3 and H_2SO_4 digestion procedure. Repeat digestion with multiple additions of 10 mL (65%) of nitric acid until white residue or clear solution is left and nitrogen oxide fumes cease to appear. Convert selenium to the Se(IV) form and make up to 25 mL or 50 mL with distilled deionized water.

Stripping Voltammetric Determinations. Transfer an aliquot (5 mL) of the sample digest into the polarographic cell, deoxygenate for 15 min, and maintain under a flow of nitrogen during the experiment. Unless otherwise stated, the selenium was determined as Se(IV) under the conditions reported elsewhere (*2*) using differential pulse CSV. The peak potential, E_p, observed for the stripping peak is a function of pH. In 1 M acid a value of about −0.45 V vs. Ag/AgCl is found and obeys the relationship E_p = constant + $(RT/nF \ln [H+]$. (Note sign error in ref. 2). In the presence of other metals the peak potential can be shifted to considerably more negative potentials (*1*). Quantitation of selenium in the sample is made by standard addition technique within the linear region (*1*).

Working Area. This work was carried out in a class 1000 clean room controlled at a temperature of 22.5 ± 0.5 °C. This laboratory forms part of the Deakin University Trace Analysis Unit.

RESULTS AND DISCUSSION

Urine Samples. The unmodified HNO_3 (*28*), HNO_3 and H_2SO_4 (*14*), and HNO_3 and $K_2S_2O_8$ (*26*) digestion procedures adopted for open digestion of the urine samples which were adequate for gas chromatography (*28*) and atomic absorption spectrometry (*14*, *26*) proved to be inadequate for the cathodic stripping voltammetric (CSV) determination of selenium. The voltammograms in Figure 1 indicate that none of these digestion procedures present the element in a state adequate for quantitation by this technique. The most likely explanation for the poor responses obtained by these digestion procedures is that the organic matrix in the sample is not completely decomposed with respect to selenium compounds which of course may not matter for methods based on atomic absorption spectrometry.

The proposed digestion procedures are more vigorous and thus enable adequate destruction of the organic matrix in the urine samples and present the element in a state, as Se(IV), that can be determined by CSV. Figure 2 clearly illustrates that the three digestion procedures proposed gave quantitatively useful responses for selenium in the samples by this analytical technique. The lower sensitivity obtained for selenium with the HNO_3 and H_2SO_4 digestion procedure is probably indicative of the high residual acid in the sample digest obtained with this mixture. Generally, selenium peak current is known to decrease dramatically with increasing acid concentration beyond 0.5 M (*1*, *2*). Nevertheless the response obtained by digestion with the HNO_3 and H_2SO_4 mixture was still useful for quantitative determination of selenium and does not require a long deposition time for adequate detection and measurement of the peak current.

Table I. Concentrations of Selenium in Selected Biological Standard Reference Materials by DPCSV Using the Proposed Digestion Procedures

digestion mixture	amt of Se found, μg/g			
	bovine liver	animal muscle	oyster tissue	orchard leaves
HNO_3[a]	0.49 ± 0.09			
$HNO_3 + H_2SO_4$[a]	1.13 ± 0.03	0.288 ± 0.002	1.94 ± 0.07	0.078 ± 0.004
$HNO_3 + K_2S_5O_8$[a]	1.15 ± 0.08			
certified values[b]	1.1 ± 0.1	0.28 ± 0.08	2.1 ± 0.5	0.08 ± 0.01

[a] Based on triplicate determination, error is mean deviation. [b] Error is standard deviation.

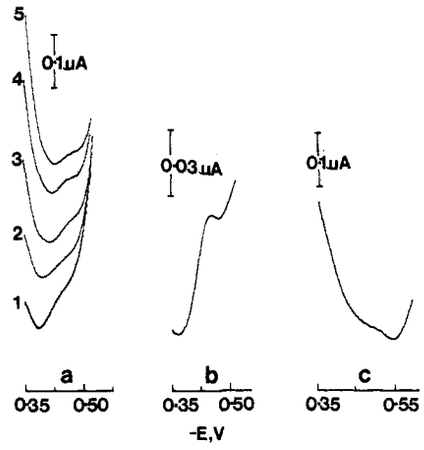

Figure 1. Determination of selenium in urine samples by DPCSV using the unmodified digestion methods: (a) HNO_3 only, total electrolysis time (t_e) (1) 30 s, (2) 60 s, (3) 120 s, (4) 180 s, and (5) 300 s; (b) HNO_3 and H_2SO_4 mixture, t_e = 120 s; (c) HNO_3 and $K_2S_2O_8$ mixture, t_e = 120 s; drop size, 0.024 cm^2; pulse amplitude, −50 mV; equilibration time, 15 s. Other conditions are the same as those given in ref 2.

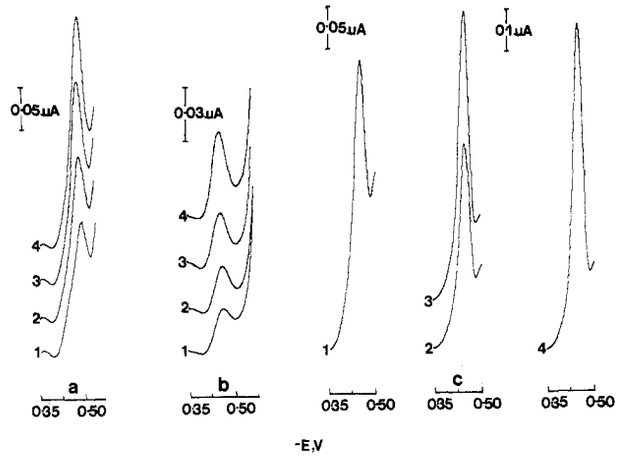

Figure 2. Determination of selenium in urine samples by DPCSV using the proposed digestion methods: (a) HNO_3 only, t_e = 120 s, (1) sample only, (2) +5 ng/mL, (3) +10 ng/mL, and (4) +15 ng/mL Se(IV); (b) HNO_3 and H_2SO_4 mixture, t_e = 60 s, (2) +5 ng/mL, (3) +10 ng/mL, and (4) +25 ng/mL Se(IV); (c) HNO_3 and $K_2S_2O_8$ mixture, t_e = 60 s, (2) +12.5 ng/mL, (3) +25 ng/mL, and (4) +37.3 ng/mL Se(IV). Other conditions are the same as those given in Figure 1.

Data obtained showed that both the HNO_3 and H_2SO_4 and the HNO_3 and $K_2S_2O_8$ digestion procedures proposed gave a comparable concentration for selenium in the urine samples. For example, 6.9 ± 0.3 and 7.2 ± 0.3, respectively, for the same sample based on triplicate determination with error expressed as a mean deviation. The low selenium concentration obtained with the HNO_3 only digestion procedure (5.2 ± 0.6 for same sample cited above) may be due to imcomplete destruction of some of the organic matrix. It appears that only between 64 and 81% of the selenium in the urine sample is presented by the HNO_3 digestion procedure in a state adequate for CSV determination. Recovery values also indicate that as well as producing low results, the precision of such digestion technique is inadequate for accurate determination of selenium in biological materials. Similarly, low recoveries and poor precision have been previously reported for the HNO_3 only digestion with other analytical technique (*28, 29*). With most electrochemical techniques, the adsorption of the incompletely digested organic matrix may inhibit the electrode process and distort the response (*30–32*). Complete destruction of the organic matrix with only HNO_3 may be somewhat limited by the low boiling temperature of this acid. Repeated digestion of the urine samples with additional nitric acid did not improve the recovery or precision of the HNO_3 digestion procedure.

Other Biological Samples. (*a*) *Bovine Liver.* The digestion of bovine liver with the HNO_3 and H_2SO_4 (Figure 3) and the HNO_3 and $K_2S_2O_8$ procedures was also adequate for the cathodic stripping voltammetric determination of selenium in the sample. In both of these procedures the use of the standard addition method only gave linear responses for selenium concentrations up to 5 ng/mL and peak potentials were more negative than expected as has been noted on other occasions (*1*). This linear range was however adequate, as shown in Table I, for quantitation of selenium in the sample

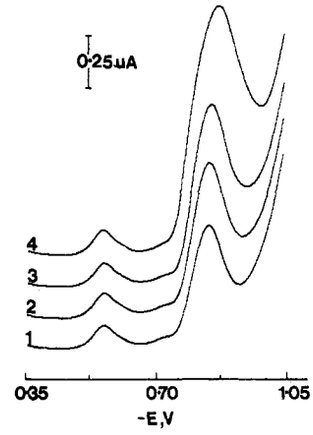

Figure 3. Determination of selenium in bovine liver by DPCSV using the proposed procedure for the HNO_3 and H_2SO_4 mixture: (1) sample only, (2) +1 ng/mL, (3) +2 ng/mL, and (4) +4 ng/mL Se(IV); t_e = 30 s; 0.2 g sample; final volume, 50 mL; drop size, 0.015 cm^2. Other conditions are given in Figure 1.

as results compare favorably with the certified value for the bovine liver sample. The high residual acid in the HNO_3 and H_2SO_4 sample digest requires dilution to 50 mL while dilution to 25 mL proved adequate for the HNO_3 and $K_2S_2O_8$, digest.

In contrast to the above methods the digestion with HNO_3 only was not adequate for determination of selenium in bovine liver. Deposition of the element onto the electrode at the selected deposition potential (−0.35 V) did not produce any selenium peak, even with longer deposition time or with addition of selenium standard (Figure 4a). However, the utilization of a more negative deposition potential (−0.70 V) revealed an additional peak at −0.97 V (Figure 4b). This peak increased with increasing addition of selenium and thus

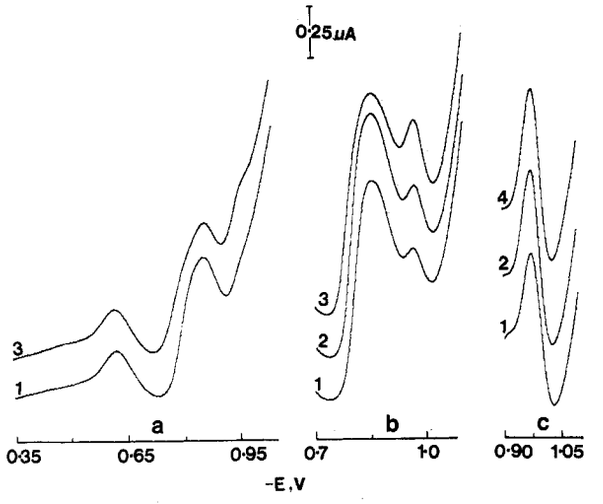

Figure 4. Determination of selenium in bovine liver by DPCSV using the proposed HNO_3 only procedure: (1) sample only, (2) +1 ng/mL, (3) +2 ng/mL, (4) +3 ng/mL Se(IV); deposition potential (a) −0.35 V, (b) −0.70 V, and (c) −0.90 V; t_e = 30 s; 0.2 g sample; final volume = 50 mL. Other conditions are given in Figure 3.

suggests that the selenium in the sample solution may have been suppressed by the presence of some undesirable matrix component. Attempts to use even more negative deposition potential (−0.90 V) to improve sensitivity were not successful for quantitating selenium in the sample as there was no linear relationship between the observed peak current and the amount of selenium added (Figure 4c). Quantitation of selenium in the bovine liver was therefore made with the peak obtained when a deposition potential of −0.70 V was used. The results in Table I indicate that only about 36–53% of selenium in the sample is presented by the HNO_3 digestion in a state adequate for determination by cathodic stripping voltammetry. Again the precision of this digestion procedure is unsatisfactory for accurate determination of selenium in biological materials. Other attempts made to ensure complete removal of the organic matrix in the HNO_3 only digestion did not improve the amount of selenium detected in the sample by CSV.

(*b*) *Animal Muscle.* The HNO_3 and H_2SO_4 digestion procedure also proved to be adequate for the cathodic stripping voltammetric determination of selenium in animal muscle (see Table I). Unlike in the bovine liver sample, the selenium peak, in this case, appeared at about the same peak potential observed for the element in the urine samples. This perhaps suggests that the organic and/or selenium matrix of the animal muscle sample is much less complex than that of the bovine liver sample. However, the HNO_3 and $K_2S_2O_8$ digestion procedure, was inadequate for the determination of selenium in this sample by CSV. No selenium peak is observed for the sample digested with this mixture even though the addition of selenium standard into the sample gave an additional peak which increased with increasing concentration of the element. Attempts made to enable adequate decomposition with the HNO_3 and $K_2S_2O_8$ mixture by additional nitric acid digestion and/or dilution did not improve the CSV determination of selenium in the animal muscle sample. The HNO_3 only digestion procedure was not considered for this sample owing to its inadequacy for accurate determination of selenium in the urine and bovine liver samples.

In view of the above successful use of the HNO_3 and H_2SO_4 digestion procedure, further investigation was undertaken to determine its suitability, as an ideal decomposition method, for the CSV determination of selenium in other biological material.

(*c*) *Orchard Leaves and Oyster Tissue.* Digestion of the sample, as proposed with the HNO_3 and H_2SO_4 mixture, to a clear solution but with slight yellow coloration gave a broad peak at about −0.66 V which was not useful for quantitating selenium. In contrast, digestion of the sample to a clear colorless solution produced a well-defined peak which increased with increasing addition of selenium into the solution and was adequate for quantitating selenium. The selenium peak observed in this case appeared at a more positive potential than that observed for the incompletely digested sample. Furthermore, the results obtained by using this peak for the quantitation of selenium in the orchard leaves agreed satisfactorily with the certified value (Table I). It seems evident, therefore, that the digestion is not completed until a clear colorless solution is obtained.

Table II. Contribution of the Proposed Digestion Procedures to the Overall Analysis Time

digestion mixture	digestion time[a] for various sample materials, min				
	urine	bovine liver	animal muscle	oyster tissue	orchard leaves
HNO_3	80	100			
HNO_3 + H_2SO_4	90	125	125	120	150
HNO_3 + $K_2S_2O_8$	130	125	125		

[a] Based on digestion of 25 mL of urine or 0.3 g of sample.

The results in Table I also show that the digestion with HNO_3 and H_2SO_4 mixture, as proposed in this study, was adequate for the determination of selenium in oyster tissue by CSV. In this case, the selenium peak appeared at even more negative potential (−0.95 V) than that observed for the bovine liver sample and was useful for quantitation by the standard addition technique. The more negative shift of the peak is perhaps indicative of the more complex nature of the oyster tissue matrix.

CONCLUSIONS

The determination of selenium in biological materials by cathodic stripping voltammetry requires a highly efficient digestion method. The proposed HNO_3 and H_2SO_4 digestion procedure enables adequate decomposition of the organic matrix and hence permits accurate determination of the element in various biological samples by this technique. The high residual acid of the sample digest created no problem and was amenable for direct determination after minimal dilution. Previous experiences with closed digestion methods clearly indicate that there is no significant difference between the results obtained for selenium in bovine liver and animal muscle samples by the proposed open digestion procedure and those obtained by closed digestion procedure which utilized a similar acid mixture (*2*). However, the open digestion procedure can reduce the analysis time considerably. The contribution of the digestion time (Table II) of the proposed procedure to the overall analysis time is quite minimal particularly when used for sample batches of three or more.

The shift of the selenium peak potential from one sample to the other may be due to the varying composition of the different samples. Previous work (*1*) has shown that the selenium peak may be shifted to a more negative potential in the presence of other metal ions such as copper, lead, and cadmium. However such problems can be overcome by use of a separation method (*2*) which enables more specific determination of the element at similar peak potential for all sample materials.

Registry No. Selenium, 7782-49-2.

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RECEIVED for review February 24, 1984. Accepted May 15, 1984.

Applications of Cyclic Voltammetry in the Characterization of Complexes at Low Ligand Concentrations

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A simple and general simulation for calculation of cyclic voltammetric curves for reversibly reduced complexes in the presence of low ligand concentration is described. Calculated curves are presented which demonstrate effects of ligand concentration and formation constants on the forms of curves for single complex and binary systems. Analysis of curve shape allows calculation of β_1 values for systems not accessible by conventional polarographic analysis. log β_1 values of 5.5 for the Cu(II)–oxalate system and 5.6 for the Cd(II)–propylenediamine system were obtained by this method. Both are in good agreement with values reported from potentiometric analysis.

Electroanalytical techniques have long found wide application in the characterization of complex ions in solution. Early treatments were limited by the assumptions of a reversible electrode reaction, a single complex in solution, and the presence of a large excess of ligand (*1*, *2*). DeFord and Hume (*3*) extended the treatment to include stepwise formation of complexes, and Schaap and McMasters (*4*) treated the case of mixed ligand complexes.

The limitations of the original DeFord–Hume treatment have been pointed out by a number of workers (*5*, *6*) particularly in regard to the accuracy required in $E_{1/2}$ measurements. The advantages of using least-squares curve fitting programs in processing the data as opposed to the originally proposed graphical procedure have been pointed out (*5*, *7*), and this is now standard practice. A comprehensive error analysis has been given by Klatt and Rouseff (*5*). These workers also addressed the question of necessary and sufficient conditions for detecting the presence of mixtures from polarographic data and pointed out that although polarographic and potentiometric determinations of stability constants depend on the same functional relationship the potentiometric method is generally more powerful because it allows studies over a wider concentration range.

Descriptions of current–potential curves in the absence of the restriction that a large excess of ligand be present have been presented by a number of workers (*8–12*).

In earlier work (*13*) we reported results of calculated linear scan (LSV) and cyclic (CV) curves in the absence of excess ligand and suggested possible applications of these results in characterizing single complexes and mixtures of two complexes in systems where the ligand is not present in excess. Subsequently it has been shown that these simulations reliably predict the form of LSV and CV curves for previously well-characterized systems with a single predominate complex (*14*).

These simulations were somewhat cumbersome and time consuming and were limited to the case with $\beta_q[X]^q \gg 1$ where β_q is the overall formation constant

$$\beta_q = \frac{[MX_q]}{[M][X]^q}$$

and [X] is the free-ligand concentration (charges omitted).

The purpose of this report is to present a much simpler and more versatile simulation and to compare calculated and experimental curves. In addition, applications of this approach in the determination of β_1 values for two complex systems in which the DeFord–Hume treatment is not applicable will be given.

EXPERIMENTAL SECTION

All current–potential curves were obtained by using an IBM EC/225 voltammetric analyzer with a 7424 M x-y recorder. A conventional three-electrode cell with a saturated sodium chloride calomel electrode (SSCE) was employed. The working electrode in linear scan and cyclic measurements was a PAR 9323 hanging mercury drop electrode (HMDE), and the electrode area normally

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