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Stripping Voltammetric Determination of Selenium in Biological Materials by Direct Calibration

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Specific and accurate determination of selenium in biological materials can be made by stripping voltammetric techniques following ion-exchange separation of the element from the bulk of the acid-digest matrix. The use of an anion-exchange method enables direct quantitation of selenium in the sample from calibration curves rather than by the normally recommended time-consuming standard addition method. The separation method described removes interferences usually encountered from other metal ions and also enables preconcentration of the analyte. Differential pulse anodic stripping voltammetry at a rotating gold disk electrode and differential pulse cathodic stripping voltammetry at a hanging mercury drop electrode gave comparable limits of detection for selenium. However the latter approach was adopted because of its inherently greater reproducibility. Application of the method to a range of biological standard reference materials proved to be very satisfactory and it is currently being used for biomedical studies. Selenium concentrations less than 1 ng/mL can be determined by this method.

The accurate determination of selenium in biological materials has become increasingly important due to the narrow difference between the concentration range at which it is considered essential (*1–5*) and that at which it is known to be toxic (*6–8*). The estimated (*9*) and recommended (*10*) safe daily intake of selenium for adult humans ranges between 50 and 200 μg; however the daily intake of the element in an average diet is estimated as 62 μg (*11*). Despite the regular intake of the element, its levels are generally low in human tissues and body fluids. Selenium concentration ranges of 4.8–46 μg/L in urine, 57–320 μg/L in whole blood, 98–327 μg/L in blood serum, 261–410 ng/g in liver, and 100–630 ng/g in kidney have been reported (*12*). These low concentrations of the element require a highly sensitive, specific, and dependable analytical method for their accurate determination.

To date, only a few methods (*13–21*) provide the necessary reproducibility and sensitivity required for the determination of selenium in biological materials at the trace to ultratrace levels commonly encountered in biomedical studies. The high sensitivity of the neutron activation technique makes it most attractive, but it is not frequently used because of the specialized techniques, skills, time, and costs involved (*2, 22–24*). The comparably high sensitivity of electrochemical techniques such as cathodic and anodic stripping voltammetry provides a far less expensive approach for the determination of selenium in most matrices. Unfortunately matrix effects associated with the determination of selenium in biological materials require that the time-consuming standard addition method is employed with these electrochemical techniques (*25–30*).

This paper describes a method which employs an anion exchange procedure and stripping voltammetry for the determination of selenium in biological materials after acid digestion. Complete removal of the matrix composition enables direct quantitation of selenium from a calibration curve rather than by the time-consuming standard addition method. The anion exchange–stripping voltammetric method is particularly suited for biomedical studies in which the concentrations of selenium in a large number of samples often have to be determined. The method is currently being used for routine determination of selenium in milk and blood samples. No previous application of stripping voltammetric techniques to the determination of selenium in these materials appears to have been reported, although numerous applications to other sample materials have been made (*31–36*).

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. Acetate buffer solution was prepared as described by Vogel (*37*) and stored in a prewashed polyethylene bottle. Stock solutions (1 g/L) of selenium(IV) and various other metal ions were prepared by dissolving their analytical grade salts in 0.1 M hydrochloric acid and were stored in prewashed polyethylene bottles. The required standards were prepared daily by appropriate dilution of the stock with chosen electrolyte. Distilled deionized water was used in all sample and solution preparations.

Instrumentation. (*1*) *Cathodic Stripping Voltammetry (CSV).* An EG and 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 consisted of a hanging mercury drop (HMDE), 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 by using a fast stirring rate and a medium size drop with a surface area of 0.015 cm^2. The solutions were degassed with highly purified nitrogen at the start of each experiment and a flow of nitrogen was maintained over the solution during the experiment to prevent oxygen interference. All experiments were performed at a constant room temperature of 22.5 ± 0.5 °C. Solution pH measurements were made on a Metrohm E520 pH meter. Standard solutions of the various metals were added to the polarographic cell with fixed volume Eppendorf micropipets with disposable tips.

(*2*) *Anodic Stripping Voltammetry (ASV).* The PAR microprocessor-based polarographic analyzer (Model 384) was also used with a three-electrode system for the anodic stripping voltammetric determinations. A standard Metrohm 20-mL cell assembly (Metrohm AG, Herisau, Switzerland) was used together with a Metrohm rotating gold disk electrode (RAuDE) assembly (E628,

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E618, EA289/3), a platinum wire auxiliary electrode, and a Metrohm silver–silver chloride reference electrode (EA437) filled with saturated potassium chloride solution. A salt bridge with a porous Vycor tip was used in conjunction with the reference electrode to minimize contamination of the test solution. The gold electrode was pretreated prior to each experiment by polishing the surface with alumina on a microcloth dampened with distilled deionized water, rinsed sequentially with doubly distilled water and acetone, and finally dried and polished with dry tissue paper. The electrode was then preconditioned over a period of 15 min in a deaerated solution of 0.2 M perchloric acid by applying alternate cycles of 30 s at +2.0 V and 10 s at 0.0 V. This preconditioning was essential when using the electrode for the first time or after long periods of idleness or after heavy contamination with selenium. After the preconditioning step, the electrodes were rinsed with distilled deionized water and a fresh solution was placed in the cell for the determination. The deposition of selenium(IV) onto the gold disk electrode was achieved by rotating the electrode at a preselected rotational velocity (1500 rpm). Solution deaeration, room temperature, pH measurement, and addition of standards into electrolytic cell were as previously described for the CSV determinations.

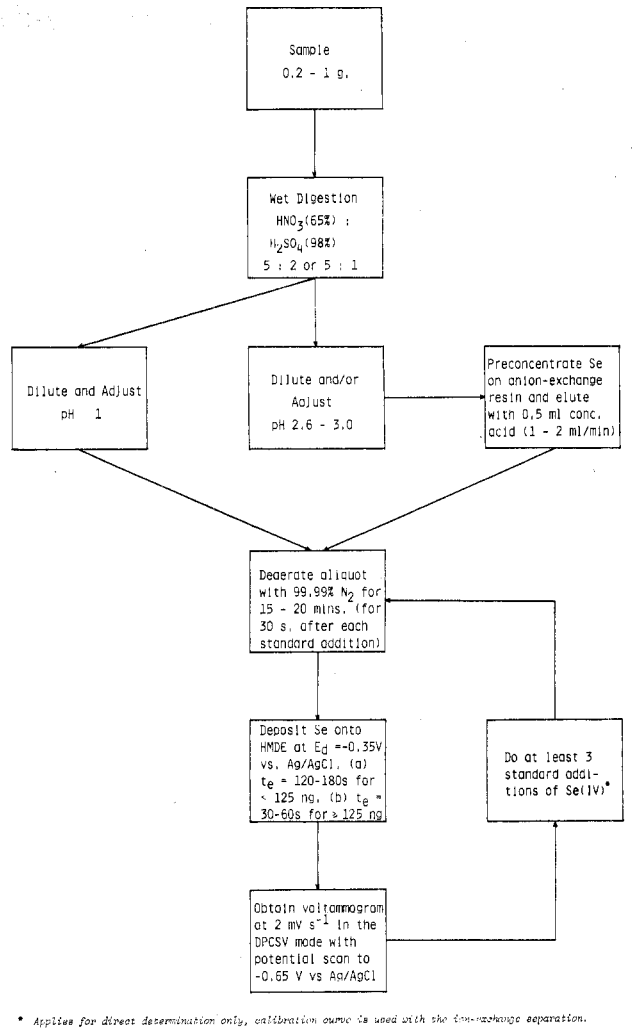
Glassware. All glassware and polyethylene bottles were soaked in 2 M nitric acid for at least 7 days, washed three times with distilled deionized water, stored in distilled deionized water, and finally soaked in the chosen electrolyte for at least 24 h to enable sufficient equilibration prior to use.

Ion Exchange Material. Amberlite IRA-400 strongly basic anion exchange resin (B.D.H. Chemicals) containing quaternary ammonium groups based on polystyrene matrix was used. The analytical grade of the chloride form has a particle size range of 14–52 mesh and it is thermally stable under both acid and alkaline conditions up to 75 °C. The resin was conditioned by washing successively with distilled deionized water, 3 M hydrochloric acid, and again with a large volume of distilled deionized water. Conversion of the resin to the acetate form was achieved by washing three times with 3 M acetic acid followed by numerous washings with distilled deionized water. The resin is stored, ready for use in the acetate form, in 0.5 M acetic acid. At the beginning of each experiment, a 15 cm × 1.5 cm i.d. glass column is packed with the resin to about 6 cm length and washed with at least 10 bed volumes of distilled deionized water prior to the actual separation. Regeneration between experiments is achieved by washing with 1–3 bed volumes of 3 M acetic acid followed by extensive washing with distilled deionized water.

Standard Reference Materials. Milk and animal muscle samples were obtained from the Analytical Quality Control Services of the International Atomic Energy Agency (IAEA) in Vienna (Austria); bovine liver was from the U.S. National Bureau of Standards, Washington, DC; Bowen Kale was from H. J. Bowen of the Department of Chemistry, Reading University, Whiteknights Park, Reading, Berks, U.K. All materials were treated as recommended by the suppliers.

Procedures. (*a*) *Sample Digestion.* The following procedure is generally recommended: to 0.2 g of the sample, add 4 mL (98%) of sulfuric acid and 10 mL (65%) of nitric acid plus two precleaned glass beads. Slowly heat the mixture until gently refluxing and maintain under this condition until all sample material has completely dissolved. Continue heating until nitrous fumes cease to appear. Reduce solution volume with increased heating until sulfite mist appears. Cool the digest, add 12.5 mL (37%) of hydrochloric acid, and boil for 30 min to convert all selenium to the Se(IV) form. Prepare blank and standards in the same way. For milk and blood samples further digestion with 5 mL of a 1:1 mixture of nitric acid (65%) and hydrogen peroxide (30%) was required prior to the conversion of selenium to the Se(IV) form. Direct determination can be made after adjusting the pH of the digest to approximately 1 but this approach is only useful when selenium concentration is <0.1 μg/L and provided that a linear response is obtained by the method of standard addition (*25*). For the more accurate method of determination described in this work, proceed with the separation of selenium from the matrix (Figure 1).

(*b*) *Anion Exchange Separation and Preconcentration of Selenium.* Depending on the pH of the sample digest, either dilute with distilled deionized water to obtain a final pH between 2.6 and 3.0 or adjust the pH to this range with acetate buffer. Equilibrate the resin by washing with 20 mL of 10^{-3} M HCl or acetate buffer (pH 3.0) depending on which of the above dilution procedures has been used. Pass the sample solution through the resin to adsorb selenium while eliminating other matrix composition (Figure 2). Wash the resin with 25 mL of distilled deionized water to remove residual matrix composition. For determination at the HMDE elute selenium with 0.5 mL (37%) of hydrochloric acid and collect eluate in a 25-mL flask. For the determination of selenium by ASV on the gold electrode elution was achieved with 0.5 mL (72%) of perchloric acid. Wash the resin with 20 mL of distilled deionized water to ensure complete removal of the eluted selenium. Make up to the 25-mL calibration mark with distilled deionized water before taking an aliquot for the selenium determination.



* *Applies for direct determination only, calibration curve is used with the ion-exchange separation.*

Figure 1. Flow diagram for the direct and ion-exchange determination of selenium in biological matrices.

Stripping Voltammetric Determinations. (*a*) *CSV.* An aliquot (5 mL) of the sample solution was transferred into the polarographic cells, degassed for 20 min, and maintained under a flow of nitrogen during the experiment. Unless otherwise stated, the selenium was determined as Se(IV) at the HMDE under the following conditions: operating mode, differential pulse stripping (DPCSV); deposition potential, −0.35 V vs. Ag/AgCl (saturated KCl); scan rate, −2 mV s^{-1}; duration between pulses, 1 s; modulation amplitude, −50 mV; deposition time, 15–165 s (stirred); equilibration period, 15 s (unstirred). t_e is the total electrolysis time prior to commencement of potential scan, that is, the sum of the equilibrium period and deposition time. The amount of selenium present in the sample was determined from a calibration curve prepared under the same conditions using the stripping peak which appeared at about −0.5 V vs. Ag/AgCl. Direct determination without chromatography was made by the standard addition method, where applicable, to provide comparative data.

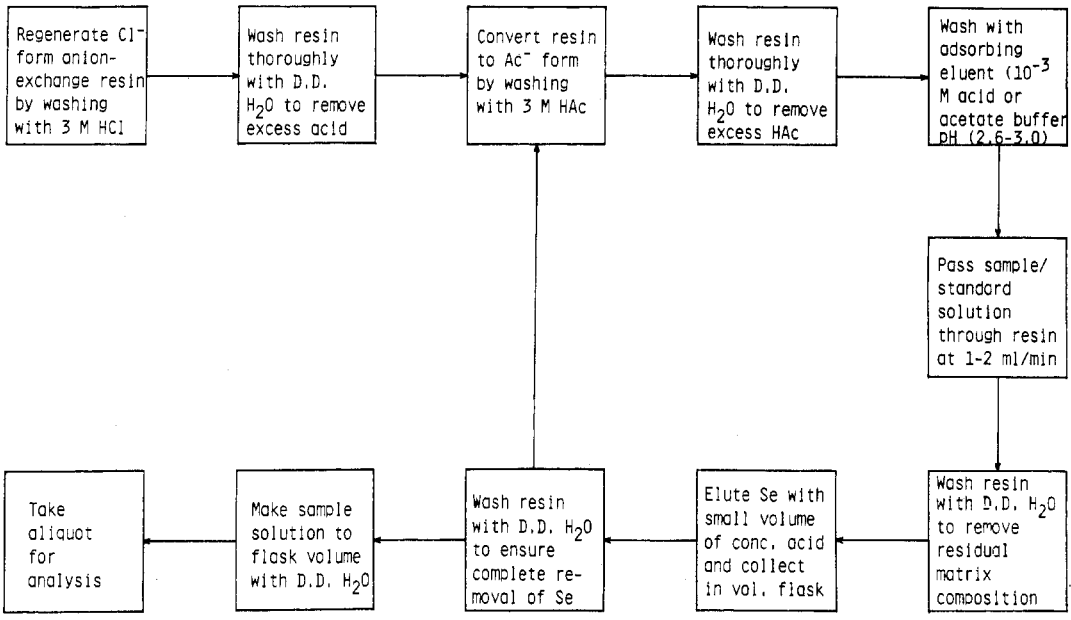


Figure 2. Flow diagram for the separation and preconcentration of selenium on anion exchange resin.

The presence of other metal ions in this case may shift the stripping peak to a more negative potential (*25*).

(*b*) *ASV.* An aliquot (10 mL) of the sample was transferred into the electrolytic cell and degassed and the selenium determination at the RAuDE was performed, unless otherwise specified, as follows: operating mode, differential pulse stripping (DPASV); conditioning potential, +0.90 V vs. Ag/AgCl (saturated KCl); conditioning time, 30 s; deposition potential, +0.15 V vs. Ag/AgCl (saturated KCl); scan rate, 2 mV s^{-1}; duration between pulses, 1 s; modulation amplitude, 50 mV; deposition time, 45 s (stirred), equilibration period, 15 s (unstirred).

The concentration of selenium in the sample was determined, after ion exchange separation of the possible interferants, by the standard addition technique, using the stripping peak which appeared at about 0.45 V vs. Ag/AgCl. Quantitation of the selenium from a calibration curve was not possible owing to the difficulty in obtaining reproducible gold electrode surface area from one experiment to the other.

Working Area. Most of the work reported here 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

(a) Cathodic Stripping Voltammetry. Selection of Deposition Potential. The conventional approach for the cathodic stripping voltammetric determination of selenium in acidic media (*27–29, 31–33*) is based on the use of a deposition potential, E_d, between +0.05 V and −0.05 V vs. SCE (approximately the same potential as the Ag/AgCl reference electrode used in this work). The determination of selenium at these deposition potentials is quite susceptible to interferences from other metal ions which often suppress the stripping peak (*28, 33, 38*). Figure 3 shows that the selenium peak was considerably suppressed in the presence of other metal ions when a deposition potential within the conventional range was used.

In view of known interferences, the influence of various deposition potentials on the determination of selenium in the presence and absence of interfering ions was investigated in order to determine the deposition potential where the least change in the peak height occurs. This procedure when coupled with chromatography to remove most of the potentially interfering species should produce the optimal approach. The results in Figure 4 show that the selenium peak height generally increases in the presence of copper when deposition potentials more negative than −0.05 V vs. Ag/AgCl are used.

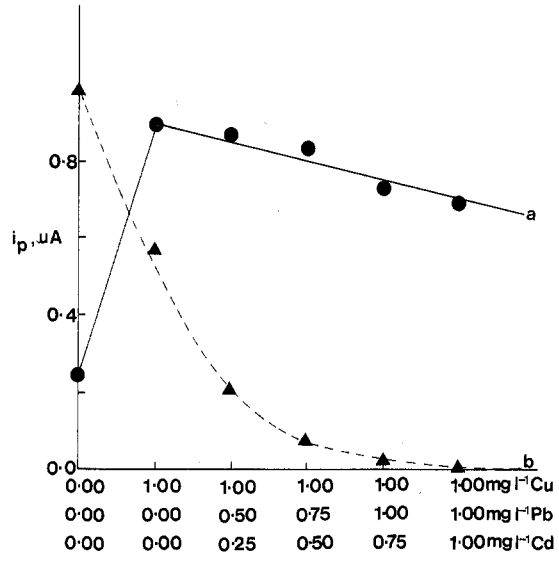


Figure 3. Influence of other metal ions and deposition potentials on selenium peak current: Deposition at (a) −0.3 V vs. Ag/AgCl and (b) 0.0 V vs Ag/AgCl; t_e = 90 s; electrode, HMDE; operating mode, dc-CSV; electrolyte, 0.1 M HCl; 200 ng of Se(IV)/mL.

This suggests that the suppression noted in the conventional situation can be avoided by selective deposition. The most favorable deposition potential for determining selenium was found to be −0.4 V vs. Ag/AgCl where the least change in the peak height was observed in the presence of copper(II) ions. However the contribution from other metal ions must also be taken into consideration. Comparison of parts a and b in Figure 3 indicates that the presence of increasing concentrations of lead(II) and cadmium(II) ions slightly suppressed the selenium peak current at a fixed concentration of copper(II) ions when a deposition potential of −0.3 V vs. Ag/AgCl is used. The magnitude of the observed suppression at this deposition potential was considerably lower than those observed with deposition potentials of about 0.0 V vs. Ag/AgCl. In summary, the use of the negative deposition potentials (−0.2 V to −0.4 V vs. Ag/AgCl) instead of values around 0.0 V is confirmed to be superior for determining selenium in samples containing relatively high concentrations of other metal ions (*25, 29*). This potential region also has the advantage of decreasing the scanning time in the stripping experiment

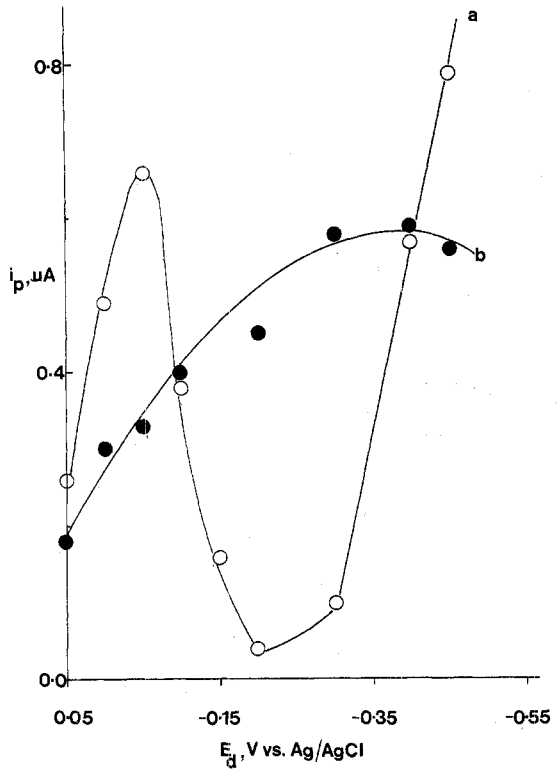


Figure 4. Dependence of selenium peak current on deposition potential (a) 100 ng of Se(IV)/mL and (b) with addition of 1 μg of Cu(II)/mL. Experimental conditions are the same as those given for Figure 3.

particularly when the differential pulse mode is employed.

The use of a deposition potential of −0.35 V vs. Ag/AgCl enables a good linear working range of 0–150 μg/L for selenium to be obtained in 0.1 M HCl using a total electrolysis time (t_e) of 60 s. This deposition potential was used for all analytical work.

Electrochemistry of the Stripping Process. It has been postulated (*25*) that the cathodic stripping voltammetric determination of selenium(IV) using a deposition potential more negative than −0.05 V vs. Ag/AgCl at a hanging mercury drop electrode involves

(1) Formation of mercuric selenide during the deposition step

$$H_2SeO_3 + 4H^+ + Hg + 4e^- \rightarrow HgSe + 3H_2O \quad (1)$$

(2) Reduction of the mercuric selenide during the stripping step

$$HgSe + mH^+ + 2e^- \rightarrow H_mSe^{(2-m)-} \quad (2)$$

This postulated stripping process (2) is identical with the one suggested by other workers (*38–41*) for the second reduction peak of selenium observed when deposition potentials within the conventional range were used. This view has now been verified by assuming Nernstian behavior and experimentally testing that eq 3 is valid (*42*)

$$\begin{aligned} E_p &= \text{constant} - \frac{RT}{2F} \ln \frac{[H^+]^m}{[H_mSe^{(2-m)-}]} \\ &= \text{constant} - \frac{2.303\,RT}{2F} \log \frac{[H^+]^m}{[H_mSe^{(2-m)-}]} \\ &= \text{constant} - \frac{0.059}{2} \log \frac{[H^+]^m}{[H_mSe^{(2-m)-}]} \text{ (at 25 °C)} \end{aligned} \quad (3)$$

A plot of peak potential, E_p, vs. −log [H^+] for 100 ng of Se(IV)/mL in hydrochloric acid solutions with t_e of 30 s gave a slope of 57 mV corresponding to an m value of 1.93 and is consistent with a two-electron reduction associated with two protons for the stripping process. Hence the suggested stripping processes (1) and (2) are valid even when deposition potentials more negative than the conventional range are used.

Interference from other metal ions may occur through the formation of an intermetallic compound with selenium as suggested by reaction 4

$$H_2SeO_3 + 4H^+ + Cu^{2+} + Hg + 6e^- \rightleftarrows Cu(Hg)Se + 3H_2O \quad (4)$$

The formation of such compounds could account for a negative shift of the peak potential (E_p) in addition to modification of the peak height (i_p). A shift of up to 100 mV or more has been previously reported (*25*, *29*) for the selenium peak in the presence of interfering ions. Thus, evidence for an interference free method in this work is that the peak position should be the same on unknown solutions as for the calibration solutions and this is an excellent test of the ability of the chromatography to remove interference.

Choice of Supporting Electrolyte. The form in which selenium is adsorbed onto a mercury drop electrode is dependent upon the composition of the chosen supporting electrolyte. Acidic electrolytes such as hydrochloric, hydrobromic, sulfuric, perchloric, and nitric acids gave much better sensitivity for selenium than neutral or basic electrolytes. While the higher sensitivity in acidic media is consistent with the literature (*31*, *33*), the relatively low sensitivity in alkaline solution may be indicative of the hydrogen ion dependence of the electrode processes (1) and (2). The observation of similarly low sensitivity at pH ≥5 indicates that some of the selenium at the higher pHs may exist in other form(s) that may be electrochemicaly inactive (*25*).

Hydrochloric acid was chosen as the appropriate electrolyte for this work because (i) it gave the best sensitivity for selenium, (ii) it enabled good resolution of the selenium peak, and (iii) it is often used to reduce selenium to its electroactive Se(IV) state after decomposition of the sample material. The optimum concentration of hydrochloric acid was determined by examining the concentration dependence of the selenium peak current and was found to be 0.1 M.

Electrolysis Time. The accountable electrolysis time (t_e) in stripping analysis includes the deposition time (t_d) and the equilibrium period (t_{eq}). Theoretically the observed peak current should be directly proportional to t_e (*43*); however, this is not often the case in practice. Figure 5 shows that the lower concentration of selenium(IV) examined (2 and 10 ng/mL) obeys this relationship for electrolysis time up to 240 and 120 s, respectively, while the response for the high concentration (100 ng/mL) was only linear with electrolysis time up to 60 s. It appeared that an equilibrium surface concentration is reached in the latter case when longer deposition times are used. However, results indicate that an electrolysis time of up to 240 s may be used for selenium concentrations within the range of 0 to 10 μg/L while only about 30 to 120 s of electrolysis time should be adequate for concentrations ≥10 ng/mL. The use of an electrolysis time of 60 s enabled determination of selenium within a linear working range of 0–100 ng/mL and with a detection limit of 0.25 ng/mL.

Scan Rate. Scan rates within the range of 1–4 mV s^{-1} gave adequate resolution for the selenium peak but a scan rate of 2 mV s^{-1} was chosen because it gave a linear response for increasing concentration of selenium and was sufficiently rapid for routine analysis. Scan rates exceeding 4 mV s^{-1} resulted in distorted and drawn out peaks. Christie et al. (*44*) have previously attributed this problem to the time constants in the PAR 174 sampling circuitry and to the large change in potential during the lifetime of the pulse. Similar explanations probably apply to the PAR 384 system used in this work.

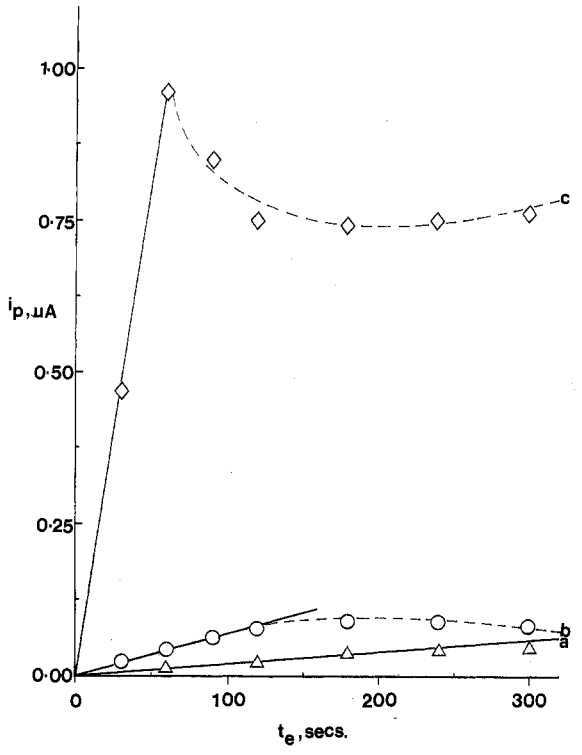


Figure 5. Dependence of selenium peak current at the HMDE on electrolysis time at various concentrations: (a) 2 ng of Se(IV)/mL; (b) 10 ng of Se(IV)/mL, and (c) 100 ng of Se(IV)/mL. Electrolyte was 0.1 M HCl. Other parameters are specified in the Experimental Section for DPCSV.

The observed peak current, at the scan rate of 2 mV s^{-1}, increased linearly with modulation amplitude over the range of 5 to 50 mV. At higher modulation amplitudes (>50 mV), the selenium peak became distorted from its normal symmetrical shape. The use of a modulation amplitude of 50 mV enabled adequate resolution and sufficient sensitivity for the selenium determination and is recommended for routine analytical work.

Ion Exchange Separation of Selenium from Complex Sample Matrix. The specific and accurate determination of selenium in most biological materials by stripping voltammetry requires separation from possible interferants (*26, 33, 36, 45, 46*). The anion-exchange procedure developed in this study (Figure 2) enabled complete retention of selenium(IV) on the resin while eliminating the other matrix composition. Investigation of the influence of various metal ions on the retention of the element on anion-exchange resin indicates that complete separation of selenium from some of the likely interferants in biological materials can be achieved by this procedure. The observed change in peak height when selenium was retained on the anion exchange resin in the presence of other metal ions was ≤2%. Complete removal of interfering species present in the matrix permits the use of a calibration curve for direct quantitation of selenium in the sample. The adequate use of this approach is however dependent upon certain critical factors such as (1) the resin condition, (2) flow rate, (3) instrumental variation, and (4) temperature. As the resin efficiency may deteriorate with time it is advisable to replace the resin on a daily basis to avoid any inconsistency that may arise through a change in the resin condition.

Flow rates of 1–3 mL/min enabled adequate retention of selenium on the resin and were sufficiently rapid for routine analysis. No problem of instrumental variation was experienced in this study as the PAR Model 384 microprocessor-based instrumentation used incorporates an autoranging system that eliminates the need for instrumental calibration. However if this approach is to be used with instrumentation which is susceptible to variation, a considerable amount of care must be exercised to ensure that the use of the calibration curve is adequate for quantitation of the element with such a system. The dependence on temperature also requires that the separation and determination are made under constant temperature conditions.

Table I. Recovery Efficiency of Acid Digestion Method for Selenium

selenium added, ng	selenium found,[a] ng	% recovery[a]
0	0	
250	252 ± 8	101 ± 3
500	483 ± 10	97 ± 2
1000	986 ± 19	99 ± 2

[a] Based on triplicate determination. Error is mean deviation.

The possible use of the ion-exchange procedure for preconcentration of selenium is limited if the desired hydrochloric acid concentration range (0.1 to 0.5 M) is to be used. To work within this range, the minimum volume in which the element can be retained is 12 mL. The use of 20 mL of water in washing the resin after the initial elution with 0.5 mL of concentrated acid and the subsequent dilution to a final volume of 25 mL gave a linear calibration curve for selenium from 10 to 6000 ng under the chosen conditions. Substantial preconcentration may however be achieved for selenium concentration ≤100 ng by eluting initially with 0.25 mL of concentrated acid and then washing the resin with sufficient water to make up to a final volume of 10 mL.

Application to Biological Materials. The accurate determination of selenium in biological materials by the combined ion exchange–stripping voltammetric method requires a resonably efficient digestion procedure that will adequately decompose the sample matrix while ensuring that little or no losses of the analyte(s) occur. Preference for wet-digestion procedures in trace metal analysis has increased considerably over the last decade because of the reduced danger of losses at the low temperatures at which they operate and the simple apparatus required for their operation (*47–51*). The suitability of a wet-digestion procedure for the determination of selenium at the low levels commonly found in biological materials was investigated in this study. Known amounts of selenium were taken through the digestion procedure, and the recovery was determined from the amount found after removal of the possible contaminants by the ion exchange procedure. The results in Table I indicate that the digestion procedure enabled almost full recovery of the element in most cases. The percentage recovery was based on the mean deviation for triplicate digestion and determinations. A minimum of 95% recovery was achieved by this wet-digestion procedure for selenium. Although these results do not account for any problems with organoselenium compounds which may be present in biological materials, the data however indicate that little or no loss of "free" soluble selenium will occur by this digestion procedure. Application of the general method to biological materials proved to be satisfactory, except that the digestion of milk and blood samples required further decomposition with a mixture of nitric acid (65%) and hydrogen peroxide (30%). Addition of 5 mL of a 1:1 mixture of these reagents enabled complete digestion of these materials. The results in Table II indicate that the use of a calibration curve was adequate for quantitating selenium in the biological materials since results agree favorably with the certified values. The significance of the ion-exchange procedure is well illustrated by the voltammograms shown in Figure 6. The presence of relatively large amounts of interfering metal ions in the Bowen Kale sample suppresses the selenium peak when

Table II. Concentrations of Selenium Found in Selected Biological Standard Reference Materials

ref material	supplier	no. of detns	DPCSV value,[a] μg/g	DPASV value,[a] μg/g	certified value,[b] μg/g
milk	IAEA (Vienna)	8	0.035 ± 0.005	0.037 ± 0.005	0.034 ± 0.007
Bowen Kale	H. J. Bowen (Reading)	6	0.138 ± 0.005		0.133 ± 0.021
bovine liver	NBS (Washington)	4	1.08 ± 0.01	1.09 ± 0.01	1.1 ± 0.1
animal muscle	IAEA	4	0.273 ± 0.005		0.28 ± 0.08

[a] Error is mean deviation. [b] Error is standard deviation.

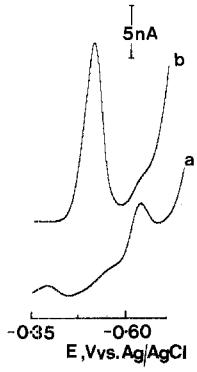


Figure 6. Significance of ion-exchange separation on the determination of selenium in Bowen Kale sample: (a) direct determination (pH 0.8) and (b) after ion exchange separation. t_e = 120 s. Other parameters are as specified in the Experimental Section for DPCSV at the HMDE.

direct determination (Figure 6a) is attempted, but the peak becomes more pronounced after removal of the interferants by the ion exchange procedure.

(b) Comparison between Anodic and Cathodic Stripping Voltammetric Determinations of Selenium. The voltammetric deposition of selenium(IV) and its subsequent anodic stripping as elemental selenium requires the use of a solid electrode as the working electrode. Rotating gold disk electrode (RAuDE) and gold-plated glassy carbon electrodes (*30*, *34*, *35*) have been successfully used for the ASV determination of selenium. The gold disk electrode enables better detection limits than the gold-plated glassy carbon electrode owing to the relatively large residual current contribution from the glassy carbon electrode.

On a gold electrode, selenium can be deposited in three major forms: (i) bulk selenium, (ii) adsorbed selenum, and (iii) an intermetallic Au–Se compound of unknown stoichiometry. Andrews and Johnson (*34*) have indicated that only the adsorbed form is useful for quantitative determination of selenium. Hence the electrode process can be made more specific and simple by adequate selection of a deposition potential that will prevent or minimize the formation of the other forms of selenium.

Examination of the influence of deposition potential on the ASV determination of selenium in this study indicated that the selenium peak current decreased as the deposition potential became more positive (Figure 7). A deposition potential of 0.15 V vs. Ag/AgCl was found to be more appropriate than the deposition potentials between −0.4 and −0.2 V used in the previous studies as it will only enable formation of the adsorbed selenium on the gold electrode, in addition to providing sufficient sensitivity and enabling rapid quantitation of the element, particularly in the differential pulse mode. Moreover this deposition potential reduces the extent of codeposition of other elements such as copper and lead which are often present in biological materials.

The sensitivity of the DPASV determination of selenium can also be influenced by the electrolysis time, rotational velocity of the electrode, conditioning potential, conditioning time, and scan rate. Figure 8 shows that the selenium peak current increased linearly with electrolysis time until the

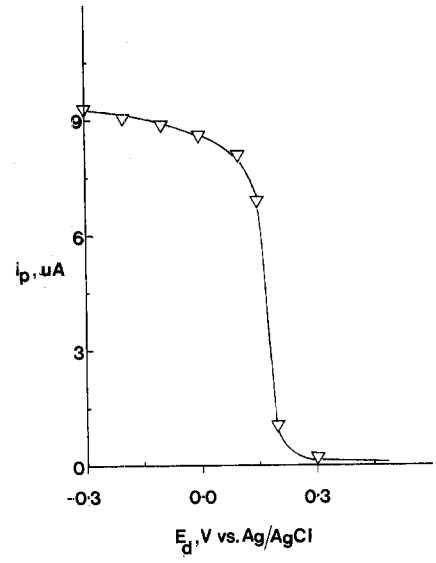


Figure 7. Influence of deposition potentials on selenium peak current at the RAuDE in 0.1 M $HClO_4$: 40 ng of Se(IV)/mL; ω = 1500 rpm; t_e = 60 s. Other parameters are as specified in the Experimental Section for DPASV.

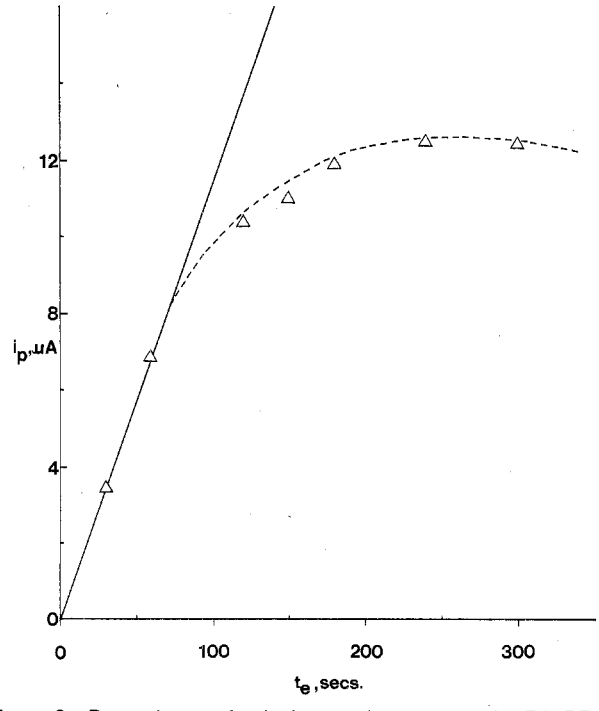


Figure 8. Dependence of selenium peak current at the RAuDE on electrolysis time. E_d = 0.15 V vs. Ag/AgCl. Other conditions are the same as those for Figure 7.

electrode surface became saturated. An electrolysis time of 60 s provides adequate sensitivity for selenium determination and allows considerable reduction in the total analysis time. Optimum sensitivity was obtained at a electrode rotational velocity of 1500 rpm. Beyond this velocity the proportionality

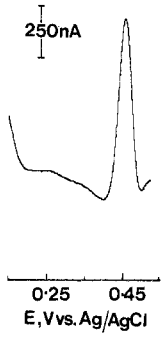


Figure 9. Typical voltammogram obtained for selenium at the RAuDE: 5 ng of Se(IV)/mL; t_e = 60 s. Other conditions are the same as those for Figure 8.

of the stripping peak current to selenium concentration was lost. Conditioning of the electrode at 0.9 V vs. Ag/AgCl for 30 s enables reasonable reproducibility for successive scans. The use of a slow voltage scan rate of 2 mV s^{-1} gave adequate sensitivity and favorable resolution for the selenium stripping peak. In addition the use of the differential pulse mode (DPASV) gave better sensitivity and a well-resolved peak than those obtained in the previous studies (*30*, *34*, *35*) where the dc stripping mode (dc-ASV) was used for selenium determination.

The conditions established in this study enable detection of as little as 0.5 ng of Se/mL with only 60 s deposition. Figure 9 shows a typical voltammogram obtained for selenium under those conditions. The selenium peak appeared at 0.45 V vs. Ag/AgCl which corresponds to the known standard reduction potential of 0.43 V vs. SCE for the H_2SeO_3/Se couple in 0.1 M H^+ (*52*). The linear working range for selenium with this technique is 0–80 ng/mL which is considerably lower than the 0–150 ng/mL range attained by the CSV technique (prior to ion exchange separation). Additionally the ASV technique requires frequent mechanical pretreatment (polishing) of the electrode surface which is neither simple nor convenient (*30*, *35*) for obtaining a reproducible electrode surface. Variation of up to 10% or more may occur depending upon how well the electrode is prepared and this, in particular, does not permit the use of a calibration curve method with the DPASV technique. The use of standard addition method with this technique, following ion-exchange separation of the interferant, however, gave comparable results to those obtained by the CSV calibration curve method (Table II) but was more time-consuming.

The ASV approach was considered to be unsuitable for routine determination of selenium in a large number of samples having biomedical significance because of its relatively poor degree of reproducibility from one experiment to the other. The CSV approach was adopted because of its ease of attaining renewable and reproducible surface, particularly with the PAR static mercury drop electrode system. Consequently, the CSV technique enables the use of calibration curve after the ion-exchange separation, reducing the total analysis time considerably.

CONCLUSION

The combined ion exchange-stripping voltammetric method provides a simple and adequate approach for determining selenium at the sensitivity and reproducibility required for biological materials. The separation of potential interferants such as copper, lead, cadmium, and zinc enabled specific and accurate determination of the element while permitting the use of calibration curves for direct quantitation. This method enabled reproducibility with a relative standard deviation (n = 10, 1 ng/mL) of 1.4% for the determination of selenium.

Registry No. Selenium, 7782-49-2.

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