Talanta, Vol. 41, No. 1, pp. 53–58, 1994
Printed in Great Britain.

0039-9140/94 $6.00 + 0.00

MICROWAVE DIGESTION OF FISH TISSUE FOR SELENIUM DETERMINATION BY DIFFERENTIAL PULSE POLAROGRAPHY

WEI GUANG LAN and MING KEONG WONG*

Department of Chemistry, National University of Singapore 10 Kent Ridge Crescent, Singapore 0511, Republic of Singapore

YOKE MIN SIN

Department of Zoology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Republic of Singapore

(*Received* 23 *March* 1993. *Revised* 14 *June* 1993. *Accepted* 16 *June* 1993)

Summary—In KIO_3–NH_3–NH_4Cl medium, the selenium complex $Se(O)SO_3^{2-}$, resulted from the reaction of selenite and sulphite in acid solution, gave a catalytic wave, which was applied to the determination of selenium in fish by differential pulse polarography. The sample was decomposed using the $HNO_3/H_2SO_4/H_2O_2$ digestion mixture in a closed PTFE digestion vessel with microwave heating. The detection limit was 0.06 $\mu g/dm^3$. The calibration curve was linear up to 8 $\mu g/dm^3$. Selenate present was reduced with hot hydrochloric acid to selenite. The recoveries of the selenite and selenate in two spiked samples investigated ranged from 91 to 104%. The NIES CRM No. 6 mussel was analyzed and the results obtained agreed well with the reference value (reference value: 1.5 $\mu g/g$; found: 1.43 ± 0.05 $\mu g/g$). The results obtained by differential pulse polarography were in good agreement with those found by hydride generation atomic absorption spectrometry.

Selenium is an interesting element in biological materials because of its dual role as both an essential nutrient at low concentration levels and a toxic substance at higher concentration levels. The concentration range between selenium as an essential nutrient and a toxic substance is, moreover, rather narrow. This interest has created a need for reliable analytical methods for the determination of selenium in various biological materials where sub-$\mu g/dm^3$ levels are encountered. The accurate determination of selenium in biological materials, however, is still a major challenge for analysts.

The polarographic reduction wave of selenium was first studied many years ago by Schwaer and Suchy,[1] later by Lingane and Niedrach[2] and, by Christian *et al.*[3] As recently as the late 1980s, many studies[4–7] have applied polarographic reduction wave of Se(IV) in acidic medium for the determination of selenium in a variety of matrices. However, these methods were limited by detection limit or troublesome separation technique. In a number of the most recent publications,[8–9] a polarographic method using the catalytic wave of $Se(O)SO_3^{2-}$–KIO_3 in NH_3–NH_4Cl medium was described. The method is relatively simple, rapid, and highly sensitive for the determination of selenium in biological materials. As in many other analytical methods, the assay of selenium in biological materials by polarographic method requires the destruction of organic matrix.[10] However, in the polarographic determination of selenium reported,[8–9] the time-consuming conventional wet digestion method was used. Moreover, perchloric acid was employed to destroy the organic matrix in digestion step. The use of perchloric acid has been known to result in an explosion and/or fire during digestion if the mixture, sample/perchloric acid, becomes dry.[11]

Recently, there has been an increasing interest in using microwave digestion method to speed up the dissolution of a variety of biological materials.[12–16] The merits of pressurized acid digestion in a closed PTFE vessel with microwave heating, particularly the increased speed

*To whom correspondence should be addressed.

and reduced losses of volatile elements, are widely recognized.[14–16] Currently, several commercial microwave systems are being marketed, but their cost has limited their acquisition by budget-constrained laboratories.

In recent years, the tendency in the wet digestion method is to replace perchloric acid with other acid mixtures.[11,17–19] The use of an inexpensive domestic microwave oven instead of a commercial microwave digestion system is also becoming popular.[16,20] However, no similar studies have been applied to the polarographic determination of selenium in biological materials.

In this study, we propose a microwave digestion method without perchloric acid, combined with differential pulse polarography (DPP) for determining selenium in fish tissue. An inexpensive domestic microwave oven instead of a commercial microwave digestion system was used.

EXPERIMENTAL

Apparatus

Differential pulse polarography was performed with an EG & G Princeton Applied Research Polarographic Analyzer (PAR Model 174A), equipped with a PAR Model 303 static mercury drop electrode (SMDE) and a Graphtec wx 2400 xy recorder. The reference electrode was Ag/AgCl–KCl (satd.). The parameters chosen for the determination are as follows: (1) 174A polarographic analyzer: initial potential, −0.5 V; scan-rate, 5 mV/sec; scan direction, negative; scan-range, 1.5 V; modulation amplitude, 50 mV; low-pass filter, 0.3 sec. (2) model 303 SMDE: mode, DME; drop size, small; purge time, 4 min. (3) xy recorder: x-axis, 0.1 V; y-axis, 1 V.

The hydride generation atomic absorption spectrometric data were measured by an atomic absorption spectrometer (Perkin-Elmer Model 280) equipped with a selenium hollow cathode lamp, in conjunction with a hydride vapour-generation accessory (Perkin-Elmer MHS-10 system).

An unmodified Delonghi MW-155 domestic microwave oven (made in Italy, purchased from local department store) with 15–100% full power (600W) capability in 15–25% increments was used for digestion. Sample digestion was carried out in closed PTFE vessels, which were donated by Xiamen University, P. R. China. A wide-mouthed plastic box with a lid was used as it protects the microwave oven from the corrosive vapour released.

Reagents

All reagents used were of analytical grade, unless otherwise stated. All-glass apparatus was used to produce doubly distilled water, which was used throughout the study.

Standard solutions. Stock selenium solution (1000 μg/cm^3 Se, atomic absorption standard, BDH) was diluted daily as required. Stock selenate solution (1000 μg/cm^3 Se) was prepared by dissolving 0.2393 g of anhydrous sodium selenate in 100 cm^3 of 1 mol/dm^3 hydrochloric acid. Further standards were obtained by diluting this solution before use.

Buffer solution (pH 10.2) was prepared by dissolving 40.2 g of ammonium chloride and 5 g of EDTA-2Na in 50 cm^3 of distilled water and mixing with 150 cm^3 of concentrated aqueous ammonium solution.

Preparation of selenium-free sulphuric acid. Concentrated hydrobromic acid (15 cm^3) was added to 100 cm^3 of sulphuric acid. The solution was heated on an electric heater until fumes appeared and then for 30 min longer, stored in a glass bottle after cooling.

Mixed-acid digestion solution. Se-free $H_2SO_4 + HNO_3$ (1:3 v/v); sodium sulphite solution (1.3 mol/dm^3) was freshly prepared within 1 week; potassium iodate solution (0.20 mol/dm^3); gelatin solution (0.08% w/v).

Biological standard reference material. The National Institute for Environmental Studies (NIES, Japan) certified reference material (CRE) No. 6 mussel, with a selenium reference content of 1.5 μg/g, was used.

Digestion procedure

The microwave digestion of fish tissue or reference material was carried out using four replicates of about 100 mg of the dried samples weighed into dried cleaned PTFE vessels. The sample digestion procedures were also applied to the blank determination.

A 5 cm^3 aliquot of mixed-acid digestion solution was added to each digestion vessel. Eight tightly capped digestion vessels, together with one small plastic beaker filled with 30 cm^3 of water, used to avoid damage to the magnetron, were placed in a lidded plastic box, which was then placed in the oven. The digestion programme consisted of three heating stages as follows. (1) The plastic box was heated for 6 min at 330 W (55% power), and then removed from

the microwave oven. After cooling to room temperature, the PTFE vessels were opened to release the pressure. (2) An aliquot of 0.2 cm^3 of hydrogen peroxide (30%) was added to each of the PTFE vessel, recapped tightly, and then placed in a plastic box, followed by heating for 4 min at 450 W (75% power). Then, the cooling and pressure release sequence were repeated as in Step 1. (3) Another portion of 0.2 cm^3 of hydrogen peroxide (30%) was added and the content heated for another 4 min at the highest power setting (600 W) of the oven.

Reduction of the selenate to selenite

The digest obtained from the procedure described above was transferred into a glass tube, 2 cm^3 of 4 mol/dm^3 hydrochloric acid was added and placed in a boiling water-bath for 20 min. to reduce any selenate to selenite. After cooling to room temperature, the solution was quantitatively transferred into a 25 cm^3 volumetric flask. For DPP method, the solution in which was diluted with distilled water to the mark. However, for HG–AAS technique, 2.5 cm^3 of 10% potassium ferricyanide was added and made up to volume with 1 mol/dm^3 HCl.

Determination of the selenium by DPP

Calibration. A series of known amounts of standard selenite solutions were pipetted into 25 cm^3 volumetric flasks, 0.50 cm^3 of perchloric acid (70%) and 3.0 cm^3 of 1.3 mol/dm^3 sodium sulphite were added and mixed well and allowed to stand for 20 min at room temperature. Then 5.0 cm^3 of the buffer solution (pH 10.2), 2.0 cm^3 of potassium iodate solution and 1.0 cm^3 of gelatin solution were added and mixed thoroughly. The solution was diluted with distilled water to the mark for calibration without the blank matrix. Oxygen was purged from the solution by bubbling with pure nitrogen. The cathodic sweeping differential pulse polarogram was recorded from −0.50 to −0.84 V. The peak potential of the catalytic wave was at −0.79 V *vs* Ag/AgCl–KCl (satd.). The calibration with the blank matrix was made in the same manner as that described above but with the addition of 10 cm^3 of the blank matrix, which was prepared by boiling the fish sample solution with hydrobromic acid according to the method described by Yi *et al.*[21] before the addition of known amounts of standard selenite solutions.

Sample analysis. Sample analysis was done in the same way as that described for the measurement of calibration curve without the blank matrix except that a 10 cm^3 aliquot of the prepared sample solution instead of known amount of standard selenite solution was used. Quantification of selenium in the sample was calculated by calibration curve with the blank matrix or made by standard addition technique within the linear region.

Determination of the selenium by HG–AAS

A 10 cm^3 aliquot of the prepared sample solution for HG–AAS technique was pipetted into a reaction vessel connected to the MHS-10 system. An aliquot of the $NaBH_4$ solution was added and the selenium hydride generated was swept with argon into the quartz cuvette. The selenium absorption signal was measured at 196.0 nm, with a 2 nm band pass.

RESULTS AND DISCUSSION

Polarographic determination

The components and preparation of the polarographic medium used in this study is essentially the same as that reported by Hang *et al.*[9] However, the following modification was applied: 0.50 cm^3 of perchloric acid (70%) was added before the addition of sodium sulphite and the solution allowed to stand for 20 min at room temperature before the addition of the buffer solution. It should be emphasized that the above modification is very important. In our study, tests demonstrated that there was no catalytic polarographic wave in the absence of perchloric acid. According to the processes reported in the following equations:[9]

$$H_2SeO_3 + 3H_2SO_3 \xrightarrow{\{H^+\}} H_2Se(O)SO_3 + 2H_2SO_4 + H_2O \quad (1)$$

$$Se(O)SO_3^{2-} \xrightarrow[(+2e,\ \text{electrode process})]{\{OH^-\}} Se^{2-} + SO_3^{2-} \quad (2)$$

$$3Se^{2+} 3SO_3^{2-} + IO_3^- + 3H_2O \xrightarrow[(\text{Parallel chemical reaction})]{\{OH^-\}} I^- + 6OH^- + 3Se(O)SO_3^{2-} \quad (3)$$

Only in strong acid solution can selenite and sulphite be transferred to selenious and sulphurous acid, the latter then react with each other to form $Se(O)SO_3^{2-}$ [equation (1)]. After about 20 min the pH was adjusted to about 10 by the addition of the buffer solution, and then

potassium iodate was added. Thereafter, reactions described by equations (2) and (3) would take place, and a very sensitive catalytic polarographic wave of selenium would be produced. The peak potential of the catalytic wave was at −0.79 V *vs* Ag/AgCl–KCl (satd.).

The catalytic polarographic wave of selenium was greatly influenced by the instrumental parameters. The DPP peak current increased with pulse amplitude, but the symmetry of the peak was also affected. From the consideration of the factors of peak current and peak symmetry, a pulse amplitude of 50 mV was selected. The peak current was found to be higher at lower scan-rate. However, a comprised scan-rate of 5 mV/sec was selected, since decreasing the scan-rate would lengthen the analysis time.

Microwave dissolution of sample

The microwave oven acid dissolution system used in our study is similar to that used by Mayer *et al.*[16] with slight modification. It is possible that biological samples produce more gaseous degradation products which increase the pressure in the system during heating. Therefore, prior to placement in the microwave oven, Mayer *et al.*[16] pre-digested their samples with digestion acid mixture at room temperature and in a water-bath at 90°C for about 2 hr. However, our study showed that pre-digestion was not required to decompose about 100 mg fish tissue samples with 5 cm^3 digestion acid mixture. Using the experimental condition described in the digestion procedure, a clear digestion solution was obtained. The procedure does not seem to digest the fat portion of the tissue. But, since the size of the sample is so small, 100 mg, that the small percentage of fat in the tissue is not recognizable on the surface.

Kingston and Jassie[14] reported an efficient decomposition of zoological and botanical samples in the microwave oven using only nitric acid. In our study, a clear digestion solution was also obtained with the nitric acid alone digestion procedure, but, the data determined showed that lower selenium contents was obtained for the determination of the NIES CRM No. 6 mussel [yielded a value of 0.88 ± 0.07 μg/(mean ± S.D. $n = 4$) compared with the reference value of 1.5 $\mu g/g$]. Similarly, lower recoveries and poor precision have been previously reported for the nitric acid only digestion with conventional wet digestion technique and subsequent determination by stripping voltammetric method.[11] However, the data obtained showed that the decomposition with nitric and sulphuric acids with hydrogen peroxide gave a comparable concentration for the determination of the NIES CRM No. 6 mussel [yielded a value of 1.43 ± 0.05 $\mu g/g$ (mean ± S.D. $n = 4$) compared with the reference value of 1.5 $\mu g/g$]. The reason may be due to the higher boiling temperature and stronger oxidizing power of the sulphuric acid, resulting in complete destruction of the organic matrix while maintaining the selenium in an oxidizing state to prevent its loss from volatilization. Also, hydrogen peroxide can promote the digestion temperature. Adeloju *et al.*[11] reported that complete destruction of the organic matrix with nitric acid alone may be somewhat limited by the boiling temperature of this acid. On the other hand, many selenium compounds are volatile and can be lost during the process of converting the organically bound selenium into its ionic forms. It is therefore necessary to maintain oxidizing conditions at all stages of the decomposition process.

Reduction of the selenate to selenite

After digestion, one must be sure that all the selenium in the sample be converted into its tetravalent state. This is the only form of selenium that reacts with sulphite in acidic medium and gives a $Se(O)SO_3^{2-}$–KIO_3 catalytic polarographic wave in NH_3–NH_4Cl medium.[22] The reduction is accomplished by reacting the digested sample with hydrochloric acid. In most cases, a large amount of hydrochloric acid is used. This is not a problem in the HG–AAS technique. However, in the DPP method, the presence of large amount of acid will pose a problem, that is, the buffer capacity of 5 cm^3 of NH_3–NH_4Cl is not enough for pH-adjustment to about 10. Therefore, the reduction should preferably be performed by a moderate amount of hydrochloric acid. In this study, it was found that 2 cm^3 of 4 mol/dm^3 hydrochloric acid placed in a boiling water-bath for 20 min was sufficient to reduce any selenate to selenite. The residual hydrochloric acid did not effect pH-adjustment by the buffer solution.

Calibration and analytical characteristics

In order to examine the effect of sample matrix, a 10 cm^3 aliquot of the blank matrix was pipetted into test standard solutions for the measurement of the calibration curve. A comparison between the calibration curve with the

Table 1. Selenium recoveries obtained in the analysis of fish samples

Sample	Selenium Content (μg/g dry mass) Present	Added	Found	Recovery (%)
Flower grouper				
	1.54	0.00	1.59	103
	1.54	1.00*	2.42	95
	1.54	2.00*	3.29	93
	1.54	1.00†	2.47	97
	1.54	2.00†	3.21	91
Tilapia				
	0.70	0.00	0.68	97
	0.70	0.50*	1.25	104
	0.70	1.00*	1.63	96
	0.70	0.50†	1.17	98
	0.70	1.00†	1.56	92

*Standard selenite solutions were used for standard addition.
†Standard selenate solutions were used for standard addition.

blank matrix and that without the blank matrix indicated that the slope of the former curve is lower than that of the latter. In other words, sample matrix does suppress the selenium signal. Therefore, in the real sample analysis, the calibration curve with the blank matrix or standard addition method should be used so as to eliminate the effect of sample matrix and give the same matrix concentration as for the sample solutions.

For the instrumental conditions used in real sample analyses, the calculated detection limit, defined as the selenium concentration corresponding to three times the standard deviation of the blank, according to IUPAC recommendations,[23] was 0.06 μg/dm^3. The calibration curve was linear up to 8 μg/dm^3.

The accuracy of the method was tested on the basis of recovery experiments, made by adding the amounts of selenite and selenate indicated in Table 1 before the treatment of the solid samples. The results are given in Table 1.

Sample analysis

The muscle of eight of the common fish species from local market was analyzed by DPP and HG–AAS techniques, respectively. The results are shown in Table 2. The differences between the results obtained by DPP and those by HG–AAS was analyzed by Student's t-test. From the significance test values included in Table 2, it can be seen that at the 95% confidence level, results obtained by DPP are in good agreement with those found by HG–AAS, except the sample Trevally.

CONCLUSIONS

The microwave dissolution method can be applied to the determination of selenium in fish by differential pulse polarography. The digestion procedure can be performed rapidly and effectively with a simple domestic microwave oven instead of an expensive commercial microwave digestion system. Only a few modifications

Table 2. Contents of selenium in fish as determined by DPP and HG–AAS

Sample	Selenium content (μg/g dry mass) DPP*	HG–AAS†	$t^{\ddagger}$ (cal.)
Black pomfret	2.76 ± 0.11	2.64 ± 0.07	1.921
Boon tong	1.68 ± 0.09	1.76 ± 0.13	1.162
Flower grouper	1.54 ± 0.09	1.50 ± 0.05	0.801
Red grouper	1.82 ± 0.12	1.69 ± 0.09	1.629
Sea bream	1.86 ± 0.08	1.91 ± 0.05	1.102
Tilapia	0.70 ± 0.04	0.74 ± 0.03	1.703
Trevally	3.09 ± 0.14	2.90 ± 0.08	2.437
Yellow tail	1.56 ± 0.06	1.64 ± 0.05	2.193

*Mean value $\pm$ S.D. $n = 6$.
†Mean value $\pm$ S.D. $n = 4$.
‡t (cal.) is the calculated significance test value.[24] Value of t is 2.306 at the 95% confidence level and eight degrees of freedom.

are required. The results obtained by differential pulse polarography are in accord with those found by hydride generation atomic absorption spectrometry. However, the instrumental expenditure is much cheaper using DPP method.

Acknowledgements—W. G. Lan wishes to thank the National University of Singapore for the award of a research studentship. This study was supported by a grant from the National University of Singapore (RP 920604).

REFERENCES

1. L. Schwaer and K. Suchy, *Collect. Czech. Chem. Commun.*, 1935, **7**, 25.
2. J. J. Lingane and L. W. Niedrach, *J. Am. Chem. Soc.*, 1949, **71**, 196.
3. G. D. Christian, E. C. Knoblock and W. C. Purdy, *Anal. Chem.*, 1963, **35**, 1128.
4. P. Lanza and S. Zappoli, *Anal. Chim. Acta*, 1986, **185**, 219.
5. G. E. Batley, *ibid.*, 1986, **187**, 109.
6. B. V. Trivedi and N. V. Thakkar, *Talanta*, 1989, **36**, 786.
7. T. Ferri, R. Morabito, B. M. Petronio and E. Pitti, *ibid.*, 1989, **36**, 1259.
8. X. Liu, Y. Tu, Y. Zhao, L. Zhu, H. Liu, H. Yu, Y. Ding and Y. Ren, *ibid.*, 1992, **39**, 207.
9. T. Hang, Z. Zhang, S. Dong and Y. Zhu, *ibid.*, 1992, **39**, 1277.
10. F. Alt and J. Messerschmidt, in *Quantitative Trace Analysis of Biological Materials*, H. A. McKenzie and L. E. Smythe (eds), p. 487. Elsevier, Amsterdam, 1988.
11. S. B. Adeloju, A. M. Bond and M. H. Briggs, *Anal. Chem.*, 1984, **56**, 2397.
12. A. Abu-Samra, J. S. Morris and S. R. Koirtyohann, *ibid.*, 1975, **47**, 1475.
13. R. T. White and G. E. Douthit, *J. Assoc. Off. Anal. Chem.*, 1985, **68**, 766.
14. H. M. Kingston and L. B. Jassie, *Anal. Chem.*, 1986, **58**, 2534.
15. S. Y. LamLeung, V. K. W. Cheng and Y. W. Lam, *Analyst*, 1991, **116**, 957.
16. D. Mayer, S. Haubenwallner, W. Kosmus and W. Beyer, *Anal. Chim. Acta*, 1992, **268**, 315.
17. D. C. Reamer and C. Veillon, *Anal. Chem.*, 1981, **53**, 1192.
18. V. W. Bunker and H. T. Delves, *Anal. Chim. Acta*, 1987, **201**, 331.
19. L. Hansson, J. Pettersson and A. Olin, *Analyst*, 1989, **114**, 527.
20. I. Kojima, A. Kato and C. Iida, *Anal. Chim. Acta*, 1992, **264**, 101.
21. Z. Yi, W. Shi, A. Li and H. Hou, *J. of Huazhong University of Science and Technology* (*Chinese*), 1985, **3**, 127.
22. X. Zhang, C. He and C. Xu, *Acta Scientiarum Naturalium Universitatis Pekinensis* (*Chinese*), 1985, **1**, 1.
23. IUPAC, *Pure & Appl. Chem.*, 1976, **45**, 99.
24. W. G. Lan, *J. of Xiamen Fisheries College* (*Chinese*), 1990, **12**, 21.