

**SULPHIDE AS AN INTERNAL STANDARD FOR SELENIUM DETERMINATION
BY CHRONOPOTENTIOMETRIC STRIPPING ANALYSIS**

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Abstract

Internal standard method for content calculation offers many advantages over other commonly used methods in electrochemical stripping techniques such as calibration curve method or standard addition method. The work describes the possibility of using sulphide as an internal standard for selenium determination on a mercury film electrode by chronopotentiometric stripping analysis. The constants of the standard were defined for different selenium contents on the basis of numerous analyses performed in model systems. The influence of the most prominent experimental parameters of the technique, such as electrolysis potential, dissolution current and electrolysis time, on the determination was investigated and defined. Defined method besides providing reliable, rapid, and simple selenium quantitation, enabled the improvement of the determination sensitivity in comparison to the method without sulphide addition.

Key words: *Selenium, Chronopotentiometric stripping analysis, Internal standard, Sulphide*

1. INTRODUCTION

Selenium is naturally occurring element, widely used in electronic, glass and pigment industry, as well as in metallurgy and agriculture. The element is essential for humans, animals, plants and microorganisms and its biochemical and metabolic role is very complex. Selenium participates in the formation of integral parts of many important body enzymes, such as glutathione-peroxidase (Rotruck et al. 1973), iodothyronine deiodinase, thioredoxin reductase (Berry et al. 1991), as well as many other essential molecules such as metalloproteins and selenoproteins P and W (Bansal et al. 1989, Read et al. 1990). In several intra- and extra-cellular glutathione peroxidases, iodothyronine 5 α -deiodinases, and in thioredoxin reductase, selenium is incorporated in enzyme's active center as selenocysteine. As selenomethionine selenium is incorporated non-specifically in many different proteins, as it competes with methionine in general protein synthesis (Švarc-Gajić 2009). Apart from being essential, elevated selenium levels are toxic. Early signs of chronic selenium poisoning include nausea, weakness and diarrhea. With continuation of selenium intake, deformed fingernails and hair loss may be demonstrated, as well as symptoms of adversely affected nervous system, such as coordination difficulties. Excessive long-term intake of selenium increases the potential risk of developing osteoporosis, enlarged prostate and dermatitis. The symptoms are frequently accompanied with reduced glucose tolerance and peripheral neuropathy which leads to numbness (Švarc-Gajić 2009).

Usually low selenium contents in biological samples demand highly sensitive analytical methods for their determination and numerous methods have been developed for the purpose. One of them involves the reaction between selenium(IV) and aromatic diamine to obtain a piarselenol, which is

determined by fluorimetry (Lu & Zheng 1991). In practice, however, for determination of total selenium content most frequently atomic absorption spectrometry is applied. In order to fulfill the requirement of highly sensitive determination, important when real samples are analysed, mostly electrothermal atomization (Theodorolea et al. 2005) or preconcentration in the form of hydride are performed (Gomez-Ariza et al. 2002). In order to perform the speciation of selenium compounds modern analytical approach combines chromatographic techniques with specific and selective detection. Both gas chromatography (Salenko et al. 1991) and high performance liquid chromatography (Gomez-Ariza et al. 2002) can be used to separate different selenium compounds. Separated compounds are subsequently determined by inductively coupled plasma atomic emission spectrometry (ICP AES) (Narasaki et al. 2000) or inductively coupled plasma mass spectrometry (ICP MS) (Darrouzes et al. 2005).

All these techniques require very sophisticated and expensive instrumentation and their application is complex, time consuming and expensive. Much simpler and less expensive techniques, but on the other hand, sufficiently sensitive and selective to selenium, are stripping techniques. High sensitivity of all stripping techniques is attributed to the preconcentration step that is performed prior analytical one. The aim of the preconcentration step is to concentrate the analyte in the electrode medium. The dissolution of the deposit can be performed in different ways providing the qualitative and quantitative data about the analyte. In voltammetric stripping techniques the dissolution is performed by the potential scan and in chronopotentiometric stripping techniques by the constant current. In cathodic stripping voltammetry selenium is usually accumulated at the mercury electrode surface by the electroreduction either in the form of mercury-selenide (Pottin-Gautier et al. 1995) or by the formation of intermetallic compounds such as copper-selenium (Van den Berg & Khan 1990, Matsson et al. 1995) or rhodium-selenium (Wang & Lu 1993). Selenium can also be preconcentrated by the adsorptive collection in the form of complex with 3,3'-diaminobenzidine on a hanging mercury drop electrode (Stara & Kopanica 1998). Anodic stripping voltammetry can be applied only if solid working electrodes are used, for example gold electrode (Tan & Kounaves 1998). Besides stripping voltammetry other stripping techniques that have been applied for selenium determination with detection limits down to $\mu\text{g}/\text{dm}^3$ levels are reductive potentiometric stripping analysis (PSA) (Christensen et al. 1980) and chronopotentiometric stripping analysis (CSA) (Suturović et al 2006).

Quantitative analysis in stripping techniques usually implies the definition of the calibration curve or a standard addition. Calibration curve method suffers from many drawbacks, especially in respect to accuracy of the determination and practical considerations. Calibration curve is usually defined in the matrix of blank which can never simulate adequately the behavior of the analyte in the sample matrix due to influence of many substances accompanying the analyte in the sample. Consequently, electrode processes, baseline and the measurement of the analytical signal are different in the blank and in the sample so the defined calibration curve does not represent the dependence adequately. Furthermore, since usually traces of selenium are to be determined, in order to achieve sufficient sensitivity relatively long electrolysis times are required, usually up to 600-900 s. In stripping techniques the application of the electrolysis time longer than 900 s is usually considered irrational and poorly justified. When defining calibration curve analysis for each selenium concentration should be repeated several times, as well as the analysis of the sample itself. In cases when mercury film electrodes are used durable analysis compromises the stability of the electrode and damages its surface reproducing the erroneous results. Standard addition method, on the other hand, demands less number of analyses to be performed, minimizes the influence of some interfering substances from the sample matrix and partially compensates them. However, this elegant method for content calculation can be applied only in the cases of linear dependences of the analytical signal on the concentration and for negligible intercept of such dependence on the "signal" axis. Internal standard method enables very simple and rapid concentration calculation avoiding many common procedural steps. The method is based on

defining the constants between the analyte and the standard in model systems. Once calculated, the constants enable calculation of the analyte concentration in the sample by performing a single analysis.

The method of the internal standard is very common in chromatographic techniques where it is required for the standard to fulfill certain criteria, such as the absence from the sample and good separation from other components of the mixture. It is recommended to use the standard which would represent the behavior of all mixture components and therefore its elution should be performed in retention window to approximate all components of the mixture. In other words, used internal standard should be approximately in the middle of the chromatogram or multiple internal standards must be applied. On opposite to chromatographic techniques where the method is somewhat common, the method of internal standard is rarely applied in stripping techniques for content calculation. Publications describing the application of internal standards for content calculation in stripping techniques are very rare and only few can be found, such as in the case of lead and cadmium determination (Liu et al. 1997, Fernando & Kratochvil 1991). Difficulty of finding the substance which would fulfill both criteria to be rare in the environment, i.e. absent from the sample, and which gives a well defined signal under the same experimental conditions as the analyte of interest, is the main cause of rare method application in stripping techniques. Even though stripping techniques offer the possibility of performing multielemental analysis, the number of potential internal standards is very limited because each analyte demands very strict experimental conditions in respect to the type of the working electrode, the medium, electrolysis potential etc.

The aim of this work was to eliminate the errors in calculation of selenium content arising when calibration curve is defined and to simplify selenium determination by stripping chronopotentiometry. Literature search demonstrated that selenium content has never previously been calculated on the basis of the internal standard in electrochemical methods. Furthermore, sulphide hasn't been used as the internal standard for the purposes of determination of any analyte. This work represents a novel approach to the concentration calculation of quite problematic analyte and describes successful application of sulphide for the determination of selenium content by chronopotentiometric stripping technique minimizing the total duration of the analysis, improving the accuracy and sensitivity of the determination and simplifying the overall analytical procedure.

2. MATERIALS AND METHODS

2.1 Instrumentation

Chronopotentiometric stripping analysis was performed by using the computerised system for electrochemical stripping analysis of our own construction. Mercury film electrode was used as a working electrode. Mercury was deposited at the glassy carbon support ($d = 3 \text{ mm}$) by a constant current ($50 \text{ }\mu\text{A}$) electrolysis from the separate solution containing 100 mg/dm^3 of mercury(II) and 0.02 mol/dm^3 of hydrochloric acid. Before each deposition of the mercury glassy carbon was cleaned with acetone and double distilled water. Platinum wire ($\varnothing = 0.7 \text{ mm}$, $l = 7 \text{ mm}$) served as a counter electrode and the reference was Ag/AgCl, KCl (3.5 mol/dm^3) electrode.

2.2 Chemicals and vessels

Acids used in this work were of ultra pure grade. For all dilutions and dissolutions triply distilled water was used. All vessels and cells were washed with nitric acid (1:1), distilled and triply distilled water. Nitrogen used for the deaeration of the analysed solutions was of extra purity.

2.3 Solutions

Selenium(IV) stock solution (2 g/dm^3) was prepared by dissolving sodium-selenite pent hydrate (p.a., Merck, min. 99% of $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$, iodometric) in 0.1 mol/dm^3 hydrochloric acid and kept in polyethylene bottle in the dark. Working solutions of selenium(IV) were prepared by diluting selenium stock solution with triply distilled water. Sulphide solution was prepared each day by dissolving $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (p.a., Merck, min. 99%) in deaerated triply distilled water. The stock solution of sulphide was kept under nitrogen atmosphere in a tightly closed flask.

3. RESULTS AND DISCUSSION

3.1 Influence of the electrolysis potential

Electrolysis potential is one of the most important experimental parameters in stripping techniques and when more than one element is to be deposited the choice of adequate electrolysis potential is more critical. By adequately choosing the potential value at which preconcentration is conducted, it is possible to perform multielemental analysis or to avoid the overlapping of the analytical signals of elements with close dissolution potentials. Selectivity can thus be improved and possible interferences from other elements may be avoided by optimal potential value. In systems containing both selenium and sulphide the role of electrolysis potential was central, because it should enable the formation of both mercury selenide (HgSe) and mercury sulphide (HgS). Potential values more positive than $+0.1 \text{ V}$ caused complete coverage of the electrode surface by HgS and impossibility of mercury selenide formation. Furthermore the risk of damaging the electrode due to mercury dissolution limited the potential window which was investigated. For this reason the potential values up to $+0.1 \text{ V}$ were examined. Experiments were performed in selenium solutions containing $20 \text{ } \mu\text{g/dm}^3$ of selenium (IV) and $500 \text{ } \mu\text{g/dm}^3$ of sulphide. These and all further analysis were performed in deaerated solutions. During deaeration, which lasted 600 s, previously formed mercury film electrode was held in triply distilled water in order to avoid its damage by nitrogen bubbles. Upon completion of the deaeration step the electrode was returned into solution and the electrolysis was performed for 120 s, after what the formed deposit was dissolved by applying the current of $-7.4 \text{ } \mu\text{A}$. The rest period between accumulation and dissolution step lasted 15 s. The results of investigation of the influence of the electrolysis potential (E) on selenium analytical signal (τ) are shown in Figure 1. Each point on the diagram represents the mean of five replicates. Reproducibility of the signal for each potential value is also presented on a diagram as interval around the value representing 2SD.

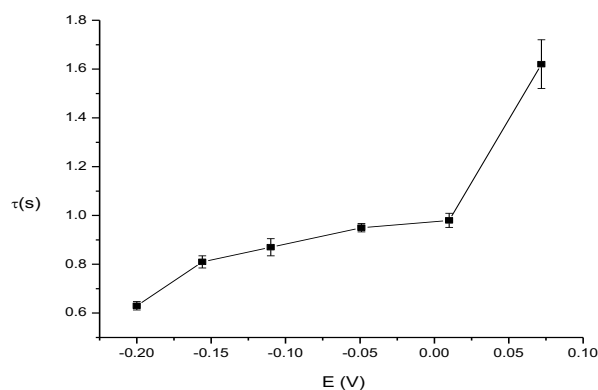


Figure 1. Dependence of selenium dissolution signal on the electrolysis potential.

Potential window which allowed both selenium and sulphide to accumulate in the electrode was very narrow and potentials more negative than -0.05 V caused only selenium accumulation and impossibility to use sulphide as an internal standard. Potential values more positive than 0.0 V caused poor reproducibility of selenium analytical signal. Electrolysis at -0.05 V provided sharp signals for both selenium and sulphide and was accepted in all further investigations, because it enabled at the same time well defined and reproducible selenium signal.

3.2 Influence of the dissolution current

Influence of the dissolution current was examined in solution containing $20 \mu\text{g}/\text{dm}^3$ of selenium (IV) and $500 \mu\text{g}/\text{dm}^3$ of sulphide under common experimental conditions as in the case of investigation of the electrolysis potential influence. Under adopted electrolysis potential of -0.05 V greater dissolution currents contributed to better reproducibility (2SD) but also to reduced sensitivity of the determination (Figure 2.).

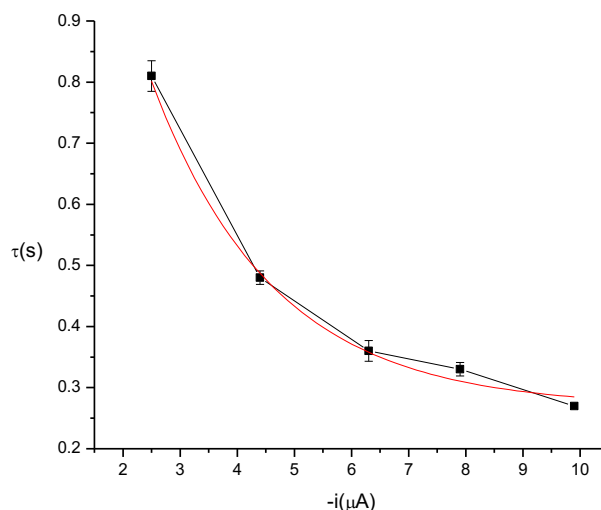


Figure 1. Dependence of selenium analytical signal on the dissolution current.

In conducted experiment obtained dependence of exponential decay was characteristic for stripping chronopotentiometry and demonstrated that the choice of adequate dissolution current balances between the sensitivity and reproducibility of determination.

3.3. Linearity

The linearity of selenium analytical signal was defined for two content ranges: 2-10 $\mu\text{g}/\text{dm}^3$ and 20-60 $\mu\text{g}/\text{dm}^3$ of selenium. Sulphide was added to all analysed solutions in the amount of 700 $\mu\text{g}/\text{dm}^3$. The dependence of selenium analytical signal on the content was defined on the basis of five measurements performed on a different mercury films. In general, the dependence showed better correlation with assumed linear dependence in solution containing sulphide (Table 1.). Surprisingly, the slope of the linear dependence was the same for both concentration ranges in systems containing sulphide and very close in systems without added sulphide.

Table 1. – The linearity in systems with and without added sulphide.

Range ($\mu\text{g}/\text{dm}^3$)		Electrolysis time (s)	Dissolution current (μA)	Intercept (s)	$\text{SD}_{\text{intercept}}$ (s)	Slope ($\text{s}\cdot\text{dm}^3/\mu\text{g}$)	SD_{slope} ($\text{s}\cdot\text{dm}^3/\mu\text{g}$)	Correlation coefficient
2 - 10	With sulphide	600	-5.6	0.17	0.031	0.019	0.0030	0.9989
	Without sulphide	600	-5.6	0.19	0.033	0.028	0.0035	0.9912
20 - 60	With sulphide	30	-7.2	-0.013	0.002	0.019	0.0021	0.9995
	Without sulphide	30	-7.2	-0.011	0.001	0.032	0.0023	0.9956

3.4 Reproducibility

Reproducibility of selenium analytical signal in systems containing sulphide was calculated both as analytical reproducibility, i.e. repeatability, and real reproducibility. Repeatability was calculated on the basis of analyses performed on a same mercury film by analysing the same solution in seven replicates, while real reproducibility was calculated on the basis of analysis of different solutions containing same selenium concentrations performed on a different mercury films. Reproducibility was calculated on the basis of five analysis. Calculated variation coefficients (CV) and experimental conditions are presented in Table 2. Added amount of sulphide was in all cases $500 \mu\text{g}/\text{dm}^3$.

Table 2 - Reproducibility and repeatability of selenium analytical signal.

Se content ($\mu\text{g}/\text{dm}^3$)	CV (%)		Experimental conditions	
	Reproducibility	Repeatability	Electrolysis time (s)	Dissolution current (μA)
5	6.3	4.7	300	-6.1
20	3.4	2.3	60	-8.6

3.5. Detection limit

Detection limit of chronopotentiometric stripping determination of selenium in systems containing sulphide was calculated on the basis of 3SD criterion, since the blank yielded only sulphide signal. Solutions containing $500 \mu\text{g}/\text{dm}^3$ of sulphide were electrolysed during 600 s and then left quiescent for 15 s. Formed deposits were stripped by applying the reduction current of $5.6 \mu\text{A}$. Standard deviation was calculated on the basis of five replicates yielding the detection and quantitation limit of $0.04 \mu\text{g}/\text{dm}^3$ and $0.13 \mu\text{g}/\text{dm}^3$ of Se (IV), respectively. Reproducibility of selenium signal at detection limit was relatively good and expressed as a coefficient of variation determined on the basis of analyses of separate solutions performed on a different mercury films was 6.1% ($n = 5$). In systems containing both selenium and sulphide selenium analytical signal was better defined and greater in comparison to systems without sulphide addition contributing to the improvement of the determination sensitivity

(Suturović et al., 2005).

3.6. Internal standard method

The application of internal standard method relies on previous calculation of factors (f) describing the signal formation and relative behaviour of the analyte and the internal standard in model systems. The possibility of using sulphide as an internal standard for selenium determination by stripping chronopotentiometry on a mercury film electrode arises from the fact that sulphides may not be present in real samples that have previously been digested. Digestion is usually performed by strong oxydising agents which oxidise any sulphide from the sample. Sulphides deposit on a mercury electrodes in the form of mercury-sulphides and can be dissolved in cathodic range similarly as selenium. Once calculated factors enable the calculation of the analyte content in the sample on the basis of signals ratio and known amount of the added standard:

$$C(Se^{4+}) = f \cdot C(S^{2-}) \cdot \frac{\tau(Se^{4+})}{\tau(S^{2-})}$$

Factor f comprises ratios defined on the bases of numerous analyses performed in standard solutions containing known amount of both selenium and sulphide:

$$f = \frac{\tau(S^{2-})}{C(S^{2-})} \cdot \frac{C(Se^{4+})}{\tau(Se^{4+})}$$

In order to define factors applicable for certain content range of selenium, solutions containing different selenium and sulphide contents were analysed in five separate probes. For each probe factor was calculated on the basis of a mean of five consecutive analyses of the same solution. Electrolysis time varied from 30 s to 600 s depending on the selenium content. After deposition of the elements the solution was left quiescent for 15 s and the deposits were dissolved by applying the current of $-5.6 \mu A$. The obtained dependence of factors on selenium content for $500 \mu g/dm^3$, $1000 \mu g/dm^3$ and $3000 \mu g/dm^3$ of sulphide is presented in Figure 3.

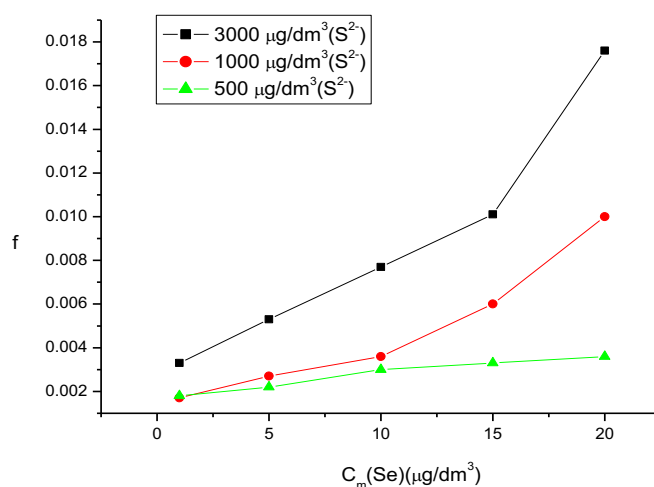


Figure 3. The dependence of factors on selenium content for different sulphide contents.

In order to have a flexible method which would allow calculation of the unknown selenium concentration it is necessary to clearly define the range in which certain factors may be used. As can be seen from Figure 3. sulphide content of $500 \mu\text{g}/\text{dm}^3$ produced the factors which could be used for calculation of selenium contents up to $20 \mu\text{g}/\text{dm}^3$. For selenium determination in real samples, where after sample preparation the selenium content is expected to fall in this range, it is recommended to add $500 \mu\text{g}/\text{dm}^3$ of sulphide because the factors do not change significantly with selenium concentration. Greater quantities of sulphide are not appropriate since the factor values change significantly depending on the selenium content. Under defined experimental parameters oriented primarily on selenium determination, sulphide deposition from solutions containing below $500 \mu\text{g}/\text{dm}^3$ of sulphide was poor yielding the absence of the sulphide signal or its poor definition.

3.7 Verification of the method

In order to verify the accuracy of content calculation by applying the defined factors, five spiked samples were analysed under the same conditions as in the case of factor definition. To all spiked samples $500 \mu\text{g}/\text{dm}^3$ of sulphide was added. Calculated selenium contents by applying the internal standard method are presented in Table 3.

Table 3 - Selenium contents calculated on the basis of internal standard.

Electrolysis time (s)	Selenium content in spiked samples ($\mu\text{g}/\text{dm}^3$)	Calculated selenium content ($\mu\text{g}/\text{dm}^3$)	f
600	1.5	1.5	0.003
600	3.5	3.9	0.003
600	5.6	6.0	0.003
30	15	16.3	0.018
30	19	22	0.018

For selenium contents greater than $10 \mu\text{g}/\text{dm}^3$ electrolysis time of 30 s was sufficient for obtaining well defined and sharp signal and for such solutions factors defined for the electrolysis time of 30 s and dissolution current of $-5.4 \mu\text{A}$ were used. In all other cases factors defined for electrolysis time of 600 s and dissolution current of $-5.4 \mu\text{A}$ were used. By choosing adequately experimental conditions and factors selenium content could be calculated by performing a single analysis. Selenium contents calculated by defined internal standard method were very close to selenium concentrations added to model systems confirming the accuracy of the defined method.

Addition of sulphide contributed to more accurate time measurement of selenium analytical signal due to existence of a previous plateau which besides being a referent signal also served to eliminate false time measurement arising from the extension in the first part of a diagram (Figure 4). In original chronopotentiogram first analytical signal occurring $\sim -211 \text{ mV}$ is corresponding to the dissolution of sulphide, while the second plateau corresponds to selenium analytical signal. Dissolution times measured by the analyser are given by the analyser in both seconds and internal units ($1 \text{ s} = 81.37 \text{ I.U.}$).

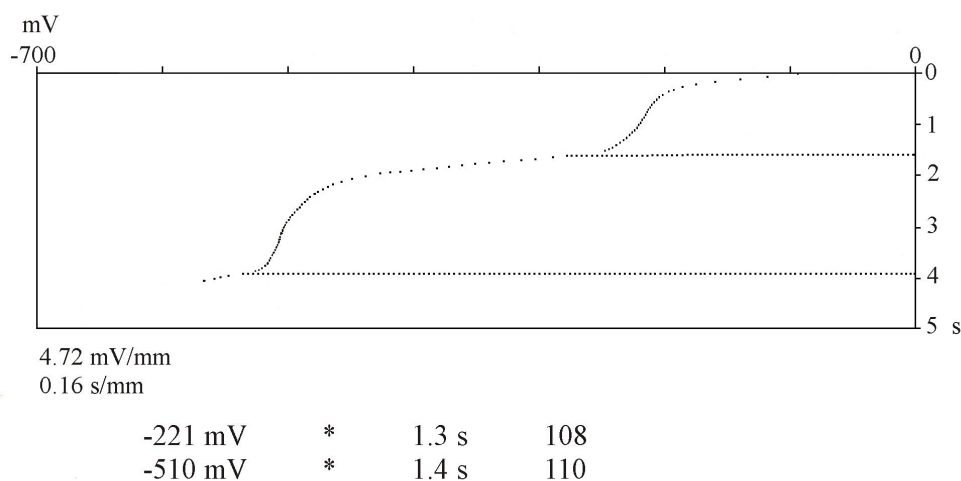


Figure 4. Obtained chronopotentiogram of $6 \mu\text{g}/\text{dm}^3$ of selenium and $500 \mu\text{g}/\text{dm}^3$ of sulphide. Electrolysis potential -0.05 V ; Electrolysis time 600 s ; Dissolution current $-5.4 \mu\text{A}$.

4. CONCLUSIONS

In solutions containing selenium and sulphide sulphide produces well resolved signal after its accumulation in a mercury film that can be used for the calculation of selenium content by chronopotentiometric stripping analysis. The method of using a sulphide as an internal standard relies on the fact that the real samples prepared for analysis by digestion don't contain sulphides. For concentration range in which selenium content from real samples is expected to fall in, sulphide content of $500 \mu\text{g}/\text{dm}^3$ is optimal because it produces factors which are not changing significantly with the selenium content and could be used for the calculation of unknown selenium concentrations. By analysing 5 spiked samples containing between $1.5 \mu\text{g}/\text{dm}^3$ and $20 \mu\text{g}/\text{dm}^3$ of selenium it was confirmed that sulphide can be used as an internal standard for accurate selenium quantitation by stripping chronopotentiometry. Calculated selenium contents showed good agreement with added selenium concentrations. Developed method significantly reduces analysis time and improves the accuracy of determination since it overcomes the disadvantages of commonly used calibration curve method. Sulphide addition contributes to sharper and better resolved selenium analytical signals, as well as influencing its height and contributing to the improvement of the determination sensitivity. In systems containing both selenium and sulphide determined detection limit for selenium ($0.04 \mu\text{g}/\text{dm}^3$) demonstrated 10 fold better sensitivity in comparison to the same method without sulphide addition.

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