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Selenium determination in biscuits and pasta: Development of chronopotentiometric stripping determination by using a sulphide as an internal standard

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ABSTRACT

Being common in chromatographic techniques internal standard method is rarely applied in electrochemical stripping determinations. One of the reasons for such rare use of this elegant quantification method is because optimal conditions of accumulation at the electrode for individual compounds producing a reproducible signal may vary significantly. These criteria are much stricter when selenium is in question due to very complex mechanism of its accumulation at mercury electrodes which implies simultaneous cathodic mercury dissolution and chemical reaction. Elements that are in the analytical step stripped cathodically from mercury electrodes are rare, further limiting the application of the internal standard method when electrochemical selenium determination is in question.

In this work the possibility of using sulphide for selenium quantification by chronopotentiometric stripping analysis was investigated. Optimal experimental parameters were defined in two-component systems. Dimensionless factors defining the ratio of proportionality constants of the two elements were calculated for different selenium concentration ranges at different sulphide contents. Sulphide content that was chosen as adequate for selenium concentrations reasonably to be expected in food samples was 500 μg/dm^3. Determined detection limit of chronopotentiometric stripping determination of selenium by using a sulphide as an internal standard was 0.04 μg/dm^3 (RSD=7.6%; n=5). Defined quantification method was confirmed by analysing spiked standard solutions and standard reference material. The method was used for selenium determination in biscuit and pasta samples. Calculated contents were statistically compared with those obtained by using graphite furnace atomic absorption spectrometry.

1. Introduction

Selenium is an essential element that exerts its effects through selenoenzymes involved in essential functions such as redox homoeostasis and thyroid hormone metabolism. The best know selenium containing enzymes are glutathione peroxidase and iodotironine-5-deiodinase. Required daily intake of selenium for healthy adults of average weight is 0.04–0.1 mg [1]. Selenium deficiency has been associated with several diseases such as heart failure and cancer [2], whereas the element is toxic at levels little above those required for its essential functions. Biochemical and physiological effects of selenium depend mostly on its amount and chemical form. Selenide, selenomethionine, selenocysteine and other organoselenium compounds are efficient in preventing certain types of diseases, while in case of some other diseases inorganic selenite is recommended [3].

A variety of analytical methods can be applied for the determination of trace amounts of selenium in different samples. Considering that selenium can be present in −2, 0, +4, and +6 oxidation states and that different forms exhibit different effects, applied instrumental method for selenium determination must be specific to particular species or the species must be separated prior determination. Routine analytical methods for detecting selenium quantitate only total selenium. The most commonly reported instrumental methods used for the determination of total selenium are fluorimetry [4,5], neutron activation analysis [6,7], atomic absorption spectroscopy [8,9], and inductively coupled plasma emission spectrometry [10]. More recent methods utilise inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry for total selenium determination, and in conjunction with high-performance liquid chromatography [11–13] or gas chromatography [12,14] for speciation.

Among electrochemical techniques that are selective towards Se^{4+}, differential pulse polarography [15,16] and differential pulse

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cathodic stripping voltammetry [17,18] can be used. In many applications, chronopotentiometric stripping analysis of selenium appeared to be well suited because of the inexpensive instrumentation, and the excellent sensitivity [3]. Speciation is possible since only tetravalent selenium is electrochemically active.

In stripping techniques quantitative analysis is usually performed by the calibration curve or standard addition method. Calibration approach suffers from many drawbacks, especially in respect to the accuracy of the determination and practical considerations. Dependence of the analytical signal on concentration is usually defined in matrix of blank which can never simulate adequately the behaviour of the analyte in the sample matrix due to influence of other substances accompanying the analyte in the sample. Consequently, electrode processes and baseline are different in the blank and in the sample, modulating analytical signals differently. Defined calibration curve, thus, does not represent the dependence adequately. Standard addition method minimises the influence of interfering substances from the sample matrix and partially compensates their influence. This simple method for content calculation can be applied only in the linear range and for the negligible intercept.

Since usually traces of selenium are determined in food samples, in order to achieve sufficient sensitivity relatively long electrolysis is required, usually up to 600–900 s. In stripping techniques electrolysis longer than 900 s is 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. Great number of analyses compromises the stability of the mercury film electrodes leading to erroneous results, especially under long electrolysis times. The internal standard method enables simple and rapid concentration calculation avoiding multiple steps and electrode damage. The method is based on comparing and expressing through numerical factors the behaviour of the analyte and the standard. Once calculated, the factors enable calculation of the analyte concentration in the sample by performing a single analysis.

The method of internal standard is very common in chromatographic techniques where it is required for the standard to fulfil 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 chromatographic behaviour of all mixture components, therefore, it should elute in retention window to approximate all components of the mixture. In another word, 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. Difficulty in finding a compound absent from the sample which would produce well defined analytical signal under experimental conditions optimal to the target analyte, make this quantification approach very challenging and rare in electroanalytical practice.

Publications describing the application of the internal standard for content calculation in stripping techniques are very few and refer mostly to lead and cadmium determination [19–21]. Difficulty of finding the substance which would fulfil criteria to be absent from the sample, producing well defined signal under same experimental conditions as the target analyte, is the main cause of method limitation in stripping techniques. Even though stripping techniques offer the possibility of performing multielement 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. Furthermore, chosen internal standard must not interfere with the target analyte in the electrochemical concentrate nor in the dissolution step.

The aim of this work was to eliminate the errors in selenium content calculation arising when calibration curve is used and to simplify selenium determination by stripping chronopotentiometry. Literature search demonstrated that in electroanalysis selenium content previously has never been calculated via internal standard. Furthermore, sulphide has never played the role of the internal standard for the purposes of quantification of any analyte. This work represents a novel approach to quantification of quite problematic analyte, selenium, and describes successful application of sulphide for the determination of selenium in biscuits and pasta by chronopotentiometric stripping technique minimising total duration of the analysis, improving the sensitivity of the determination and simplifying the overall analytical procedure.

2. Materials and methods

2.1. Instrumentation

Chronopotentiometric stripping analysis was performed using the computerised system for electrochemical stripping analysis of our own construction (M1 analyser). The instrument has a programme for automatic calibration of the current and voltage, with the parameter setting accuracies $\Delta E < 2$ mV and $\Delta i < 0.2$ μA. Accuracy of dissolution time measurement is 50 ms, and in all other cases 0.25 ms. Quantitative characteristic of the analyte, i.e. the transition time, was measured as a time between two inflection points. Inflection points are determined by programme derivation and are indicated at the chronopotentiogram as horizontal dotted lines [22]. During analytical step, the potential is measured with the frequency of 40 Hz between two inflection points. Derivative curve is internally registered, even though only standard potential vs. time curve is displayed to the user. The M1 analyser was connected to Epson LX-850 printer.

Mercury film electrode was used as a working electrode. Films of mercury were formed on the glassy carbon surface ($d=3$ mm) by a constant current (−50 μA) electrolysis from the separate solution containing 100 μg/dm^3 of Hg^{2+} and 0.02 mol/dm^3 of HCl for 240 s. The thickness of the formed mercury film was ~130 nm. Prior each mercury film deposition glassy carbon was cleaned mechanically by a filter paper wetted, first with acetone, and then with triply distilled water. Platinum wire ($\varphi=0.7$ mm, $l=7$ mm) served as a counter electrode and the reference was Ag/AgCl, KCl (3.5 mol/dm^3) electrode.

Atomic absorption measurements were made by using spectrometer with graphite furnace (Graphite furnace atomic absorption spectrometer—GFAAS) (Thermo Electron Corporation, S series GE711344v 1.26).

Samples were prepared in automated system for microwave digestion (Milestone Srl).

2.2. Chemicals and reagents

All chemicals used in this work were of analytical reagent grade (Merck, Darmstadt, Germany, pro-analysis) except acids which were of extra purity (Merck, Darmstadt, Germany, suprapur). Used chemicals included: $Na_2SeO_3 \cdot 5H_2O$, $Na_2S \cdot 9H_2O$, HCl, HNO_3, acetone, H_2O_2, acetic acid, ascorbic acid, $MgNO_3$, $Ni(NO_3)_2 \cdot 6H_2O$, and $Pd(NO_3)_2$. For all dilutions and dissolutions triply distilled water was used.

Selenium stock solution (2 g/dm^3 of Se^{4+}) was prepared by dissolving sodium–selenite pentahydrate in 0.1 mol/dm^3 hydrochloric acid and was kept in polyethylene bottle in the dark. Working solutions of selenium were prepared by diluting selenium stock solution with triply distilled water. Stock solution of sulphide was prepared daily by dissolving appropriate weight of

$Na_2S \cdot 9 \cdot H_2O$ in triply distilled water to obtain concentration of 1 g/dm^3 of S^{2-}. Solution of Hg^{2+} (1 g/dm^3) was prepared by dissolving elemental mercury in 3 cm^3 of nitric acid and subsequent dilution with triply distilled water. Nickel nitrate, magnesium nitrate, palladium nitrate, ascorbic acid and acetic acid stock solutions were prepared in concentration of 4 g/dm^3, by dissolving $Ni(NO_3)_2 \cdot 6H_2O$, $MgNO_3$, and $Pd(NO_3)_2$, acetic acid and ascorbic acid, respectively, in triply distilled water. Nitrogen used for the deaeration of the analysed solution, as well as argon for GFAAS determinations were of extra purity.

All vessels and cells were washed with nitric acid (1:1), distilled and triply distilled water.

2.3. Samples

To confirm method applicability 14 different samples of dry pasta and biscuits were analysed. Samples were collected in the local markets, applying probability sampling dependent on the element size [23]. Collected samples included the products of domestic and foreign producers (Table 1).

2.4. Standard reference material

The certified reference material of durum wheat flour, RM 8436 was supplied by the National Institute of Standards and Technology (NIST), USA. Certified reference material was used to confirm the accuracy of the developed method.

2.5. Sample preparation

Samples were digested by microwave-assisted mineralisation. Biscuit and pasta samples (0.5 g) were transferred to the reaction vessel and 7 cm^3 of nitric acid and 1 cm^3 of hydrogen peroxide were added. The samples were pre-heated 10 min at 200 °C under the magnetron power of 700 W. Digestion proceeded for next 15 min under the same conditions. Digested samples were diluted to 25 cm^3 with triply distilled water. Standard reference material was prepared in the same way as the analysed samples. A blank digest was carried out in the same way, as well.

2.6. Atomic absorption spectroscopy

For GFAAS determination, prepared samples or standard solutions (20 mm^3) with added modifier were placed into graphite cuvettes and a following temperature programme was applied as specified in Table 2. Used modifier was nickel-nitrate. Selenium absorbance was measured at characteristic wavelength of 196 nm, whereas background correction was made by deuterium lamp. The calibration curve method was used for quantitative analyses.

Table 1
Analysed samples.

		Producer
Biscuit samples		
1	Whole grain biscuit with oats	Serbia
2	Regular biscuit	Serbia
3	Biscuit with butter	Serbia
4	Biscuit with cinnamon	Serbia
5	Biscuit with low fat content	Serbia
6	Whole grain biscuit	Italy
7	Regular biscuit	Italy
Pasta samples		
8	Regular pasta	Serbia
9	Whole grain pasta	Serbia
10	Regular pasta	Bosnia and Herzegovina
11	Whole grain pasta	Italy
12	Regular pasta	Italy
13	Durum pasta	Italy
14	Durum pasta	Italy

Table 2
Temperature programme for GFAAS determination.

	Temperature (°C)	Time (s)	Heating speed (°C/s)	Gas flow (dm^3/min)
Drying	100	30	10	0.2
Ashing	1100	30	150	0.2
Atomisation	2200	3	0	off
Cleaning	2500	3	0	0.2

3. Results and discussion

3.1. Optimisation of the chronopotentiometric stripping determination of selenium

Chronopotentiometric stripping determination was performed after deaeration by nitrogen purging. This step lasted for 600 s and during this working electrode was held in triply distilled water in order to avoid mercury film damage by nitrogen bubbles. After each analysis the solutions were additionally deaerated for 90 s.

In electroanalytical determinations the role of supporting electrolyte is of extreme importance, to provide good electrical conductivity, adjust pH and minimise migration current. In chronopotentiometric stripping determination of selenium, 0.1 mol/dm^3 hydrochloric acid was used as a supporting electrolyte. At this electrolyte concentration selenium signal was sharp, well defined and reproducible.

Agitation during accumulation step in stripping techniques influences directly the amount of the formed deposit. In case of mercury film electrodes whose thickness is of the order of nanometre, agitation should be applied with precaution in order to avoid the electrode damage by a cavitation phenomenon that occurs under high stirring rates. In this work a stirring rate of 4000 rpm provided a good balance between the height of the analytical signal and its reproducibility.

3.1.1. Influence of the electrolysis potential

Selenium as well as sulphide are deposited according to similar mechanism at the mercury electrodes, chemically reacting with mercury:

$$Se^{4+} + Hg^0 + 4e^- \rightarrow HgSe$$

$$HS^- + Hg^0 \rightarrow HgS + H^+ + 2e^-$$

Electrolysis potential should provide the generation of well defined analyte signal. In case of several elements the choice of adequate electrolysis potential is much more complex. In case of systems containing both selenium and sulphide, applied electrolysis potential should have provided the formation of mercury(IV) selenide and mercury(II) sulphide. Electrochemical formation of each of these compounds is complex per se because electrochemically induced mercury dissolution is superseded by a chemical reaction.

In this study electrolysis potentials (E) in the range of −0.2 to +0.072 V were examined in model solutions of selenium containing 20 μg/dm^3 of Se^{4+} and 500 μg/dm^3 of sulphide. Solutions were subjected to electrolysis for 120 s and formed deposits were stripped by applying the current of −5.6 μA. Potentials more anodic than +0.072 V provoked very intense reactions with sulphides at the electrode, covering it completely with mercury(II) sulphide. Under such conditions selenium could not be deposited at the

working electrode. Sulphide analytical signal diminished at the potentials more negative than −0.049 V (Fig. 1).

Values represented in the Fig. 1 are the mean of three replicates, performed at the same mercury film, whereas intervals given around each value are signal reproducibilities calculated as 2SD. Analytical signal of selenium increased with the application of more anodic potentials and peaked at the +0.072 V with comparatively poor reproducibility (RSD=6.2%). Well defined signals of both elements appeared at potentials more positive than −0.049 V. As a matter of fact potential window which allowed reproducible deposition of both elements at the electrode was quite narrow (−0.049 V to +0.072 V). Applying electrolysis potential of −0.049 V analytical signal of selenium was well defined and the most reproducible (RSD=1.8%) and was adopted in all further investigations.

3.1.2. Influence of the electrolysis time

The change of selenium analytical signal (τ) with electrolysis time (t_{el}) was examined in model solution containing 20 μg/dm^3 of Se^{4+} and 500 μg/dm^3 of S^{2-}, applying dissolution current of −6.3 μA. Functional dependence of the signal and electrolysis time was defined on the basis of three series of measurements in the electrolysis time interval from 30 s to 480 s. Each series of measurements was defined at new mercury film. Averaged function correlated well with the linear dependence ($\tau=0.0013t_{el}+0.28$; $r=0.9967$; $n=3$).

3.1.3. Influence of the dissolution current

Among electrochemical stripping techniques the chronopotentiometric method is featured with flexibility in sense that by right choice of the dissolution current matrix interferences can be compensated and the sharpness, as well as the height of the signal, can be adjusted to some instance. Dissolution current is, thus, one of the most important experimental parameters in chronopotentiometric stripping determinations. In model solutions of Se^{4+} (20 μg/dm^3) electrolysis lasted 30 s while in the analytical step dissolution current varied from −2.5 μA to −9.9 μA. Similarly as in the case of the investigation of the electrolysis time influence, the trend of the selenium signal vs. current dependence was defined by averaging three series of measurements, each performed on a new mercury film (Fig. 2).

Typically for chronopotentiometric measurements [24–26], selenium analytical signal decreased with higher currents applied according to exponential function of the first order ($\tau=1.78\exp(I/2.15)+0.27$; $r=0.9925$; $n=3$). Reproducibility was better when

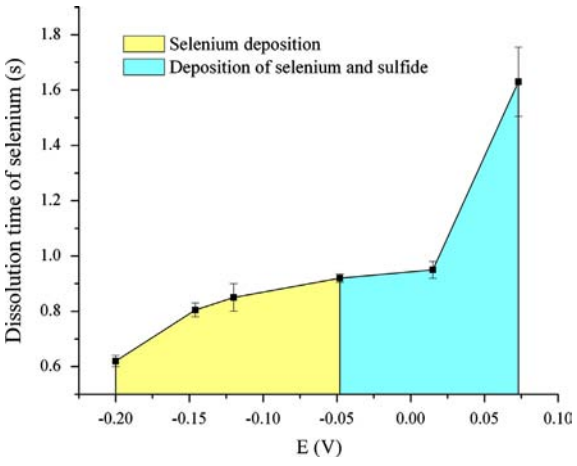

Fig. 1. The influence of the electrolysis potential on the electrochemical deposition of selenium and sulphide at the mercury electrode. C(Se^{4+})=20 μg/dm^3; C(S^{2-})=500 μg/dm^3; t_{el}=120 s; I=−5.6 μA.

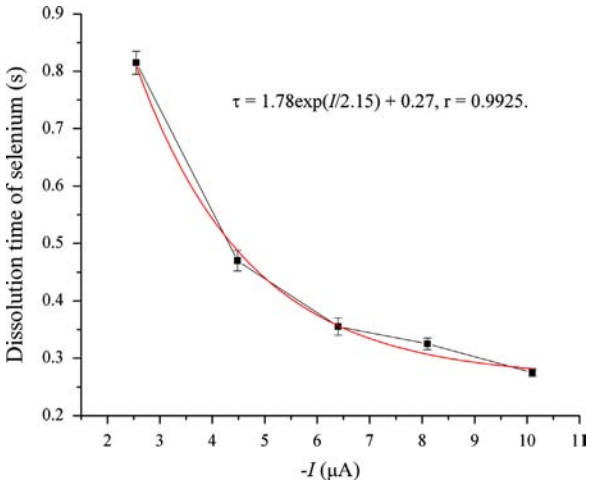

Fig. 2. Dependence of selenium analytical signal on the dissolution current. C(Se^{4+})=20 μg/dm^3; C(S^{2-})=1000 μg/dm^3; t_{el}=30 s; I=−5.6 μA.

higher currents were applied due to chronopotentiograms extension under small currents. In examined range of dissolution currents the reproducibility, expressed as RSD, varied from 0% to 4.8%. When selecting adequate dissolution current in chronopotentiometry it is important to make a compromise between the sensitivity and acceptable reproducibility.

3.1.4. Linearity

Under optimal conditions linearity was defined for two concentration ranges of Se^{4+}, 2–10 μg/dm^3 and 20–60 μg/dm^3, separately for systems with and without added sulphide. After electrolysis that lasted 600 s for lower concentration range and 30 s for higher concentration range, in systems with sulphide, dissolution current of −7.9 μA was applied for both concentration ranges. For both concentration ranges slopes of the defined linear dependences were in agreement independently on sulphide added (Table 3). Intercepts in systems with sulphide were lower due to existence of sulphide plateau, which, besides serving as internal standard, also enabled the avoidance of chronopotentiogram extension and more accurate measurement of selenium dissolution time.

3.1.5. Concentration calculation by internal standard method

In stripping techniques content calculation is often accompanied with errors arising from numerous factors, such as the influence of the interfering elements and substances from the sample. Interferences may have pronounced impact on both deposition and dissolution processes, as well as on the electroactivity of the electrode surface. Produced error is particularly pronounced when the calibration curve method is applied. Furthermore, the reproducibility of determination is compromised. The standard addition method compensates to some instance the error arising from differences in the composition of samples and standard solutions. In addition, the method saves the analysis time.

The internal standard method is based on defining the proportionality constants of the analyte and the standard. The constants are defined by analysing series of standard solutions containing both the target analyte and the internal standard. Once defined constants enable the calculation of the analyte concentration in the sample by performing a single analysis. In this respect this quantification method provides very rapid, simple and elegant approach. Electroanalytical techniques, however, suffer from difficulty of finding adequate internal standard. In order to assure the

Table 3
Linearity of selenium analytical signal in systems with and without sulphide.

Concentration range (μg/dm^3)	Systems without sulphide		Systems with sulphide	
	2–10	20–60	2–10	20–60
Slope (s dm^3/μg)	0.029 ± 0.002[a]	0.032 ± 0.002	0.019 ± 0.003	0.019 ± 0.003
Intercept (s)	0.19 ± 0.02	−0.13 ± 0.03	0.017 ± 0.003	−0.011 ± 0.02
Correlation coefficient	0.9945 ± 0.0015	0.9993 ± 0.0010	0.9955 ± 0.0012	0.9985 ± 0.0009

[a] mean ± 2SD, $n=3$.

absence of the internal standard from the sample synthetic derivatives can be used.

Reference element used as an internal standard ought to fulfil several important requirements. In electroanalytical techniques, substance used as an internal standard must be chemically related to analytes and must demonstrate similar electrochemical behaviour. The reference element must be absent from the sample or, alternatively, should be present in significantly lower concentration than the analyte, and in this case its content must be known. Another important term is a good resolution of the standard and the analyte and no interaction between the two in the electrochemical concentrate.

The application of the internal standard method consists of two phases. After choosing adequate internal standard, proportionality constants (K) are defined on the basis of repeated analyses performed in standard solutions with known concentrations of all analytes and the standard:

$$K_a = \frac{S_a}{C_a}$$

$$K_s = \frac{S_s}{C_s}$$

where K_a is the proportionality constant for the analyte, K_s is the proportionality constant for the internal standard, S_a is the analytical signal of the analyte, C_a is the concentration of the analyte, S_s is the analytical signal of the internal standard, and C_s is the internal standard concentration.

When internal standard is chosen adequately any unanticipated change in experimental parameters will lead to the same changes in proportionality constants of both internal standard and the analyte. This means that in reliable analysis the ratio of proportionality constants of the analyte and the internal standard must be constant. Dimensionless factors f can be defined for each analyte of interest as constants ratio:

$$f = \frac{K_a}{K_s}$$

By repeating analyses in standard systems calculated factors can be averaged becoming robust enough to compensate reproducibility limitation of the analysis. Well chosen internal standard will produce factors that will oscillate only around the value within the random experimental error.

In the second phase of the analysis internal standard is added to the sample. After the analytes have been identified according to their dissolution potentials, proportionality constant of the internal standard (K_s') should be calculated for those particular experimental conditions:

$$K_s' = \frac{S_s'}{C_s'}$$

where: S_s' is the analytical signal of the internal standard under experimental condition of analysis, C_s' is the added concentration of the internal standard.

Table 4
Experimental parameters in different selenium/sulphide systems.

Selenium content (μg/dm^3)	Electrolysis time (s)	Electrolysis potential (V)	Dissolution current (μA)
1	600	−0.049	−5.6
5	300	−0.049	−5.6
10	300	−0.049	−5.6
15	120	−0.049	−5.6
20	30	−0.049	−5.6

Proportionality constant of the analyte for particular experimental conditions (K_a') is calculated by multiplying the constant of the standard with factor calculated in series of previous experiments:

$$K_a' = fK_s'$$

Relying on the calculated constants and signal measured in the sample (S_a'), concentration of the analyte (C_a') can be calculated:

$$C_a' = \frac{S_a'}{K_a'}$$

In order to define adequate sulphide concentration that could be used in real samples in wide concentration range of selenium, systems containing 500 μg/dm^3, 1000 μg/dm^3 and 3000 μg/dm^3 of sulphide were analysed. The factor f values were calculated for five levels of selenium, namely 1 μg/dm^3, 5 μg/dm^3, 10 μg/dm^3, 15 μg/dm^3 and 20 μg/dm^3 on the basis of five replicates. Experimental parameters in different selenium/sulphide systems are shown in Table 4.

Obtained results of investigations are shown in Figs. 3 and 4. In systems containing 1000 μg/dm^3 and 3000 μg/dm^3 of sulphide factor values changed with selenium concentration exhibiting sharp increase at 15 μg/dm^3 of selenium. Dependences for 1000 μg/dm^3 and 3000 μg/dm^3 of sulphide could be approximated with exponential function. In solutions containing 500 μg/dm^3 of sulphide factor values oscillated around the mean value of 0.002 ± 0.0004 indicating that lower sulphide concentrations are more favourable in systems with selenium. This was obvious also from dependences of signal ratio vs. concentration ratio that are more commonly used for quantitation purposes when the internal standard method is used (Fig. 4). Only for sulphide content of 500 μg/dm^3 this dependence could have been approximated well with linear function. Thus, sulphide content of 500 μg/dm^3 could be recommended in the analysis of real samples, where the content of selenium is unknown.

After defining f values, five spiked samples were analysed in five replicates and the content was calculated by applying previously defined factors under adopted optimal experimental conditions. Obtained results are given in the Table 5. Each value represents the mean of five replicates. Calculated parameters of Student t-test demonstrated that there was good agreement (level of significance 0.05) between contents of spiked samples and contents of selenium calculated by the proposed method ($t=1.116<2.776$).

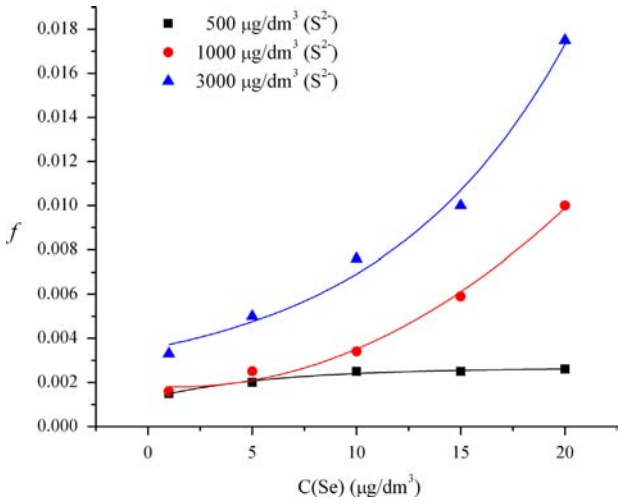

Fig. 3. Calculated factor values depending on selenium and sulphide concentrations.

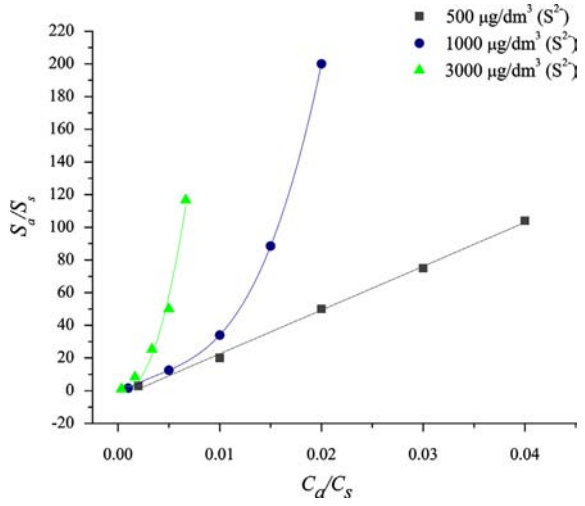

Fig. 4. Selenium/sulphide signal ratio vs. selenium/sulphide concentration ratio for different sulphide contents.

Quantification via sulphide was demonstrated to be a time and effort consuming method. Significant contribution is seen in the analyses of real samples, where the risk of impaired accuracy is much higher in comparison to model systems.

3.1.6. Reproducibility

Analytical reproducibility or repeatability, was calculated in standard solutions of selenium and sulphide applying optimal experimental conditions, which implied electrolysis time of 300 s under the potential of −0.049 V and dissolution current of −5.9 μA. Repeatability was calculated in selenium solutions containing 5 μg/dm^3 and 20 μg/dm^3 of the element. Original chronopotentiogram is presented in the Fig. 5. First observed plateau appearing at ~−230 mV corresponded to sulphide dissolution, whereas second signal is the signal of selenium dissolution. In the first column are shown dissolution potentials, the second column lists the measured dissolution times in seconds, whereas in the third the analyser expresses dissolution times in its internal units (1 s=81.37 IU). For both concentration levels calculated relative standard deviations (RSD) of signals and potentials of both elements were good (Table 6).

Table 5
Selenium content calculated by the internal standard method.

Sample	Selenium added (μg/dm^3)	Selenium found (μg/dm^3)
1	1.50[a]	1.47 ± 0.29[b]
2	4.00	4.14 ± 0.31
3	6.80	7.10 ± 0.33
4	11.20	10.10 ± 0.25
5	20.00	19.10 ± 0.22

[a] Measurement uncertainty of standard solution preparation was not calculated.
[b] mean ± 2SD, n=5.

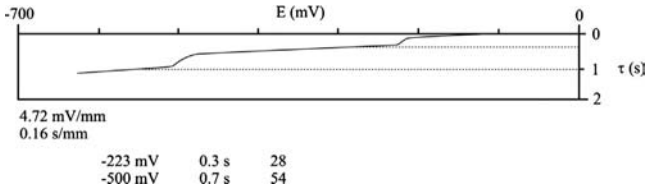

Fig. 5. Original chronopotentiogram obtained in the solution of selenium (5 μg/dm^3) and sulphide (500 μg/dm^3).

Table 6
Reproducibility in selenium/sulphide systems.

Selenium content (μg/dm^3)	**Selenium**		**Sulphide**	
	RSD [a] of dissolution time (%)	RSD [a] of dissolution potential (%)	RSD [a] of dissolution time (%)	RSD [a] of dissolution potential (%)
5	4.6	0.9	3.3	1.8
20	3.3	1.5	5.6	0.9

[a] Relative standard deviations calculated on the basis of five replicates.

Table 7
Results of analysis of the certified reference material.

Certified reference material	Certified (mg/kg)	Found[a] (mg/kg)	|Relative error|[b] (%)
RM 8436 (Durum Wheat flour)	1.23 ± 0.09	1.21 ± 0.06	1.63

[a] mean ± 2SD, n=3.
[b] |Relative error| (%)=|(Found−Certified)/Certified|100.

3.1.7. Limit of detection

There are several criteria that can be used for sensitivity calculation of the specific analytical method. In general, limit of detection (LOD) represents the lowest analyte concentration that can be reliably detected within statistically-justified range. A common criterion in calculating detection limit is the signal in blank enlarged by three standard deviations of that signal in order to ensure that determination is within reproducibility limits.

Taking into consideration that in blank selenium signal was not detected and that the accuracy of time measurement by used analyser was ± 50 ms, a selenium content that produced analytical signal of at least 0.2 s was chosen as a criterion for calculating limit of detection. Three standard deviations of the signals were interpolated into dependence of the signal vs. content for low concentration range in order to transform time units into content units.

Limit of detection was determined by applying electrolysis time of 600 s and dissolution current −5.6 μA. Calculated LOD was 0.04 μg/dm^3 of Se^{4+} with RSD of 7.6% (n=5). Limit of quantification

Table 8
Selenium content determined by GFAAS and CSA by the internal standard and standard addition method.

Sample	Content determined by GFAAS (μg/kg)	Content determined by CSA-internal standard method (μg/kg)	Content determined by CSA-standard addition method (μg/kg)
Biscuit sample			
Whole grain biscuit with oats	3.9 ± 0.2 (87%)[a]	4.6 ± 0.3 (103%)	4.1 ± 0.5 (97%)
Regular biscuit	26.9 ± 0.3 (89%)	28.2 ± 0.4 (94%)	26.1 ± 0.4 (93%)
Biscuit with butter	14.4 ± 0.2 (84.7%)	15.7 ± 0.3 (84%)	13.1 ± 0.5 (90%)
Biscuit with cinnamon	20.1 ± 0.3 (91%)	23.0 ± 0.4 (89%)	19.3 ± 0.5 (81%)
Biscuit with low fat content	21.9 ± 0.3 (92%)	24.1 ± 0.5 (91%)	20.6 ± 0.7 (83%)
Whole grain biscuit	17.6 ± 0.2 (85%)	15.9 ± 0.3 (74%)	18.3 ± 0.5 (91%)
Regular biscuit	23.1 ± 0.2 (93%)	25.1 ± 0.4 (87%)	25.4 ± 0.6% (86%)
Pasta sample			
Regular pasta	60.7 ± 0.4 (99%)	65.2 ± 0.5 (100.5%)	61.4 ± 0.6 (96%)
Whole grain pasta	41.3 ± 0.3 (98%)	40.4 ± 0.4 (96.8%)	44.1 ± 0.6 (87%)
Regular pasta	65.5 ± 0.4 (101%)	60.9 ± 0.7 (78%)	60.1 ± 0.7 (90%)
Whole grain pasta	45.9 ± 0.3 (100.6%)	47.2 ± 0.5 (99%)	43.3 ± 0.6 (101%)
Regular pasta	48.9 ± 0.3 (99.8%)	45.6 ± 0.3 (100.9%)	43.7 ± 0.4 (87%)
Durum pasta	52.6 ± 0.4 (102%)	55.2 ± 0.5 (102%)	56.2 ± 0.6 (99%)
Durum pasta	198.7 ± 0.5 (105%)	201.3 ± 0.6 (117%)	203.4 ± 0.4 (97%)

Value in parenthesis represents mean recoveries ($n=3$).

[a] mean ± 2SD, $n=5$.

(LOQ) was determined under same experimental parameters and was calculated by using 10SD criterion (LOQ = 0.13 μg/dm^3).

3.1.8. Accuracy

The reliability of the method was confirmed by using standard reference material (RM 8436). Standard reference material was prepared in the same way as the samples and was analysed under defined optimal experimental conditions. By comparing the certified and the found selenium contents obtained by the proposed method (Table 7), it is observed that the values did not differ more than 0.02 mg/kg, with acceptable relative error of 1.63%, confirming the accuracy of the defined chronopotentiometric stripping method.

3.2. Selenium determination in real samples

Prepared samples were analysed in five replicates by using GFAAS and chronopotentiometric stripping analysis (CSA) in which selenium quantification was performed with the commonly used standard addition method, and the developed method of internal standard. Quantification in CSA was performed by double standard addition and for internal standard method via sulphide and previously defined factors. In spectrometric determination calibration curve method was applied.

Determined contents are presented in Table 8 as mean values ± 2SD. In all analysed samples selenium was detected at levels ranging from 3.9 μg/kg to 198.7 μg/kg.

The agreement between the results obtained by the two methods was verified by the paired Student's *t*-distribution test that was applied for GFAAS and the developed method, as well as for GFAAS and the standard addition method. At confidence level of 0.05 there was no significant difference between the results of GFAAS and the developed method ($t=-1.147 < 2.160$). Good agreement with GFAAS also demonstrated CSA with the standard addition method ($t=0.21435 < 2.160$); however *t*-test indicated slightly better agreement with the developed method ($P_{GFAAS–CSAinternal\ standard}=0.18633 > P_{GFAAS–CSAstandard\ addition\ method}=0.05455$, $\alpha=0.05$). In addition, better reproducibility of results was noticed when applying internal standard method. Mean relative standard deviation for all analysed samples was 0.82% for the developed method, whereas for the standard addition method mean reproducibility was 1.37%.

Calculated recoveries for the samples were in the range 74–117% for all used methods confirming the correctness of the sample preparation, as well as the accuracy of the developed electroanalytical method.

4. Conclusions

The possibility of using a sulphide as an internal standard for chronopotentiometric stripping determination of selenium at mercury film electrode was investigated in this work. At 500 μg/dm^3 sulphide showed to be an excellent internal standard for rapid and simple selenium quantification in real samples because under optimised experimental conditions it formed a well defined, sharp stripping plateau. Furthermore, at this concentration level factors for sulphide and selenium were relatively stable and robust in selenium concentration range that can be realistically expected in real samples. Another analytically important effect of the existence of sulphide plateau at potentials more positive of those for selenium is increase of the accuracy of analytical signal measurement for selenium. The defined internal standard method for selenium quantification was confirmed by analysing standard reference material and spiked standard solutions. Reproducibility of determination was in the range 3.3–4.6% depending on selenium content. With the defined quantification method remarkable detection limit of 0.004 μg/dm^3 (RSD = 7.6%) of selenium could be reached.

The defined method of the internal standard was applied in the analyses of biscuit and pasta samples. Samples were analysed by GFAAS and CSA with the standard addition method as well. Statistical comparison of determined selenium contents by three analytical techniques showed good agreement.

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