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Chronopotentiometric Stripping Analysis of Selenium in Feed: Development of a Method

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Abstract: Chronopotentiometric stripping analysis is proposed for selenium determination in feed. Experimental conditions of the technique were investigated and optimised. As a working electrode served mercury film depositet at glassy carbon. Accuracy of the method was confirmed by analysing the standard reference material-wheat durum flour. Detection limit obtained for proposed technique was $0.5 \mu\text{g dm}^{-3}$, while limit of quantitation was $1 \mu\text{g dm}^{-3}$. Developed method was used for selenium determination in fish meal, meat-bouny meal, sows feed and yeast.

Key words: Chronopotentiometric stripping analysis, selenium, feed

Introduction

Selenium is naturally occuring element essential for humans, animals, plants and microorganisms. Many substances express both toxic and positive effect depending on its amount and chemical form, but for selenium is specific that the ratio of toxic to essential dose is very narrow.

Biochemical and metabolic role of selenium is rather complex. Selenium forms an integral part of the enzymes glutathione-peroxidase (Rotruck *et al.*, 1973), iodothyronine deiodinase, thyoredoxin reductase (Berry *et al.*, 1991), metalloproteines, fatty and binding proteins (Bansal *et al.*, 1989), selenoproteines P and W (Read *et al.*, 1990) and many other proteins.

The inhibition of the toxic effects of heavy metals such as arsenic, cadmium, mercury and tin is due to the formation of their stable selenides (Ganther, 1978).

Selenium deficiency causes a number of specific degenerative diseases in livestock associated with the growth and reproduction (Oldfield, 1997). In food animals and poultry, low selenium intake is associated with a wide range of practical and costly problems including both male and female infertility, low immune system of animals and disorders of thyroide hormone metabolism (Whitehair and Miller, 1985). In Table 1 are presented selenium-related problems in food animal production.

The distribution of selenium in the soil varies widely. For example, the contents of selenium in some soils of Ireland reaches 1250 mg kg^{-1} , while the soil of the certain areas of New Zeland contain less than 0.1 mg kg^{-1} of selenium. As a result the selenium content of plant-derived feed ingredients is difficult to predict. Research in selenium content of such samples requires measurement of widely different selenium concentrations in many types of feed with different origin. To be useful, a method for selenium determination needs to work equally well in any type of the sample and must be sensitive enough to measure the very lowest levels encountered. Knowing the exact selenium content in feed is necessary because of positive as well as toxic effects of the element. On the basis

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Table 1: Problems in food animal production related to selenium deficiency

Pigs ^a	Cattle and Sheep ^b	Poultry ^c
Dietary liver necrosis	Immune system effect	Exudative diathesis
Mulberry heart (acute circulatory failure)	Myodegeneration in adult cattle	Liver necrosis
Muscle degeneration (Miopathy)	White muscle disease	Pancreatic necrosis
Gastric ulcers	Retained placenta	Slow feather growth ^d
Mastitis/metritis/agalactia	Abortions, stillbirth	Drip loss increased ^e
Prolonged parturition	Neonatal weakness	Egg freshness affected
Infrequent milk letdown	Diarhea	
Neonatal weakness	Infertility	
Piglet diarrhea		
Reduced sperm motility in boars		

^aMarin-Guzman and Mahan (1989), ^bMaas and Koller (1985), ^cPatterson *et al.* (1957), ^dEdens (1998), ^eWakebe (1998)

of this data selenium supplementation is performed if necessary. Nowadays, feed supplementation with selenium has become common way of modern meat production, influencing not only animals health but, actually, aiming food products with higher selenium content and better quality. In Korea the production of pork meat enriched with selenium is well known. Similar programme is being performed with poultry meat. New Zeland, which is largely selenium deficient has a history now of over 35 years of selenium use in agriculture, with enormous benefits to animal production and human health.

The low content of selenium in biological samples demands highly sensitive analytical methods for its determination. A lot of methods have been developed for the determination of selenium. Some of them involve the reaction between selenium (IV) and aromatic diamine to obtain a piaszelenol, which is determined by fluorimetry (Mejuto-Marti *et al.*, 1987; Lu and Zheng, 1991). Modern methods in order to perform the speciation of selenium compounds combine some chromatographic technique with specific and selective detector. Separation techniques that can be used are gas chromatography (Measures and Burton, 1980; Al-Attar and Nickless, 1990; Salenko *et al.*, 1991) or high performance liquid chromatography (Gilon *et al.*, 1995; Gomez-Ariza *et al.*, 2002; Angeles *et al.*, 2000). Separated compounds are subsequently determined by inductively coupled plasma atomic emission (ICP AES) (Narasaki *et al.*, 2000) or mass spectrometry (ICP MS) (Darrouzes *et al.*, 2005; Feldmann *et al.*, 2000), atomic spectrometry after electrothermal atomisation (Gilon *et al.*, 1995) or after hydride generation (Sun *et al.*, 2002; Gomez-Ariza *et al.*, 2002). These techniques require very sophisticated and expensive instrumentation as well as exploitation. There application is complex and time consuming. Simpler and less expensive techniques that can be used for selenium determination are stripping techniques. 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 date about the analyte. In voltametric stripping techniques the dissolution is performed by the potential swep and in chronopotentiometric stripping techniques by the constant current. In cathodic stripping voltammety selenium is accumulated at the mercury electrode surface by the electroreduction either in the form of mercury-selenide (Batley, 1986; Companella *et al.*, 1989; Pottin-Gautier *et al.*, 1995) or by the formation of the intermetallic compounds such as copper-selenium (Van den Berg and Khan, 1990; Matsson *et al.*, 1995) and rhodium-selenium (Wang and 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 and Kopanica, 1998). Anodic

stripping voltammetry can be applied only if the solid working electrodes are used, for example gold electrode (Tan and Kounaves, 1998). Besides stripping voltammetry stripping techniques that have been applied for selenium determination with detection limits down to $\mu\text{g dm}^{-3}$ levels are reductive Potentiometric Stripping Analysis (PSA) (Christensen *et al.*, 1980) and Chronopotentiometric Stripping Analysis (CSA). Chronopotentiometric stripping analysis has been rarely applied for selenium determination. Method was used for selenium determination in urine and plasma samples (Gozzo *et al.*, 1999) and in flow system for the determination in muscle and bovine liver (Eskilsson and Haraldsson, 1987). In CSA minor charging currents and more accurate time measurement comparing to current measurement in voltametric techniques makes the CSA determinations more selective and accurate. Considering all advantages of the technique, the aim of this study was to define simple and inexpensive technique which can be used for sensitive determinations of selenium in natural samples. After the optimisation of the sample pretreatment developed method was applied for selenium determination in different types of feed, such as fish meal, meat-bony meal, sows feed and yeast.

Materials and Methods

Instrumentation

Investigation was performed in laboratory for Instrumental Methods of Analysis, Department for Applied and Engineering Chemistry at the Faculty of Technology. Chronopotentiometric stripping analysis was performed using the computerised system for electrochemical stripping analysis of our own construction (Suturović *et al.*, 1992; Suturović, 1996). Mercury film electrode was used as a working electrode. Mercury was deposited at the glassy carbon by a constant current ($50 \mu\text{A}$) 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 washed with acetone and double distilled water. As a counter electrode served platinum wire ($\varphi = 0.7 \text{ mm}$, $l = 7 \text{ mm}$) and the reference was Ag/AgCl, KCl (3.5 mol dm^{-3}) electrode.

Chemicals And Vessels

All chemicals used in this study were of ultra pure grade (Supra pure). 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.

Solutions

Selenium (IV) stock solution (2 g dm^{-3}) was prepared by dissolving sodium-selenite pentahydrate (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 dark. Working solutions of selenium (IV) were prepared by diluting selenium stock solution with triply distilled water. Nitrogen used for the deaeration of the analysed solution was of extra purity.

Reference Material

Wheat durum flour with the certified selenium content of $1.23 \pm 0.09 \text{ mg kg}^{-1}$ was used as a reference material (RM 8436) for the verification of the method accuracy. Prior analysis the material was dried at 85°C for 4 h (1 cm layer).

Sample Pretreatment

Sample (3 g) was transferred to a quartz long-necked flask and 10 cm³ of nitric and 6 cm³ of perchloric acid were added. After evolution of dark nitrogen-oxides fumes 6 cm³ (6×1 cm³) of nitric acid was added aiming the complete decomposition, i.e., to obtain clear and colourless solution. The obtained solution was completely evaporated. Perchloric acid fumes were collected with the glass tube connected to a vacuum-pump. After cooling, dry residue was treated with 1.5 cm³ of 5 mol dm⁻³ hydrochloric acid and heated for 20 min at 120°C in order to reduce Se(VI) to Se(IV), since only Se(IV) is electroactive. Finally, solution was filtered and transferred to a 25 cm³ glass flask with triply distilled water.

CSA Analysis

Prepared sample solution (20 cm³) was deaerated by passing nitrogen for 600 sec. During the deaeration time working electrode was held in triply distilled water in order to avoid the damage of the electrode surface with nitrogen bubbles. After the deaeration the electrode was returned into the solution and electrolysis was performed during 600 sec at a potential of -0.1 V. Stirring rate was 4000 rpm. After the rest period of 10 sec the stripping step was performed by the reduction current. Because of a different sample matrix, dissolution currents varied. For the sample of sows feed dissolution current was 10.2 μA, while currents of 13.3 μA; 17.1 μA and 18.6 μA were used for the samples of fish meal, meat-bony meal and yeast, respectively. All samples were analysed in five probes. Selenium content was calculated by the method of a calibration curve. In blank solutions selenium was not detected.

Accuracy Test

Samples of the reference material were analysed after the wet acid digestion and reduction of Se(VI) as described above. The electrolysis time (t) was 180 sec and the applied dissolution current was 1.8 μA.

Results and Discussion

Influence Of The Electrode Thickness

Mercury film thickness (d) influences directly at the amount of the mercury-selenide formed and therefore at the methods sensitivity. Influence of the mercury film thickness on selenium dissolution time (τ) was examined by varying the electrolysis time from 60 sec (~31 nm) to 420 sec (~226 nm). Solutions containing 60 μg dm⁻³ of selenium (IV) were used for the examination. Applied electrolysis potential was -0.15 V, electrolysis time 60 sec dissolution current 3 μA and stirring rate 4000 rpm. According to the reproducibility and height of the analytical signal (Table 2), electrolysis time of 300 sec was accepted as an adequate and corresponded to ~163 nm film thickness (presuming 100% electrolysis efficiency). In Table 2 are also shown the values of standard deviation (S) and coefficient of variation (CV) calculated on the basis of five analysis. Mercury film depositet under this conditions enabled 15-20 analysis.

Influence Of The Supporting Electrolyte Concentration

The role of supporting electrolyte is to provide the conductivity of the solution, to adjust pH value and to minimise the migration current. In this study as a supporting electrolyte was used hydrochloric acid. Influence of its concentration was investigated in the range 0.1-1 mol dm⁻³.

Table 2: Influence of the mercury film thickness on selenium analytical signal

t (s)	d (nm)	τ (s)	S (s)	CV (%)
20	31	0.29	0.08	27.6
120	65	0.50	0.05	10.6
180	98	2.19	0.06	5.06
300	163	1.09	0.03	2.48
360	195	1.06	0.12	11.6
420	226	0.88	0.03	3.40

Table 3: Influence of the dissolution current on selenium analytical signal

i (μ A)	τ (s)	CV (%)
1.5	0.74	5.2
1.8	0.62	3.3
2.2	0.56	2.8
2.6	0.50	2.1

Table 4: Determined selenium contents in feed

	Content (mg kg^{-1})	S (mg kg^{-1})	Recovery (%)
Fish meal	0.165	0.008	98.7
Yeast	0.08	0.005	95.3
Meat-bony meal	0.171	0.009	99.5
Sows feed	0.09	0.004	96.5

Although the concentration of 0.2 mol dm^{-3} did not provide highest sensitivity it was chosen as optimal, because it enabled sharp, well-defined signal and its good repeatability.

Influence Of The Electrolysis Potential On Selenium Analytical Signal

In order to obtain sharp and well-defined dissolution signal of mercury-selenide, determination of optimal electrolysis potential is of a great importance. Electrolysis potential examined was in the range from -0.05 to -0.3 V. Solutions containing $20 \mu\text{g dm}^{-3}$ of Se (IV) were implied in the investigation. Dissolution time for each potential value shown in Fig. 1 represents average value of five analyses on the same mercury film. Dissolution time decreased with more negative potential, probably because of less amount of Hg^{2+} ions available for the reaction with selenium, since mercury solubility is higher at more anodic potentials. Highest analytical signal of selenium was obtained applying electrolysis potential of -0.05 V, but with poor reproducibility. Best reproducibility was obtained applying electrolysis potentials of -0.1 V and -0.15 V. As an optimal electrolysis potential was chosen -0.1 V.

Influence of the Electrolysis Time on Selenium Analytical Signal

Influence of the electrolysis time on selenium analytical signal was investigated in standard solutions containing $10 \mu\text{g dm}^{-3}$ of selenium (IV). Electrolysis time varied from 60 sec to 300 sec. Dissolution current was $1.8 \mu\text{A}$. Longer electrolysis time contributed to the higher sensitivity, because it corresponded to higher selenium concentrations in the electrode medium.

Influence Of The Dissolution Current

Dissolution current is one of the most important experimental conditions of chronopotentiometric stripping analysis. Its influence was investigated in solutions containing $10 \mu\text{g dm}^{-3}$ of selenium (IV) and varied from 1.5 to $2.6 \mu\text{A}$ (Table 3). Each shown value of the analytical signal represents average value of five analyses on different mercury films. In case when analytical signal corresponding to certain current value represented average value of five analyses on the same mercury film, the dependence could not be defined, although the reproducibility was much more satisfactory. Electrolysis time was 90 sec.

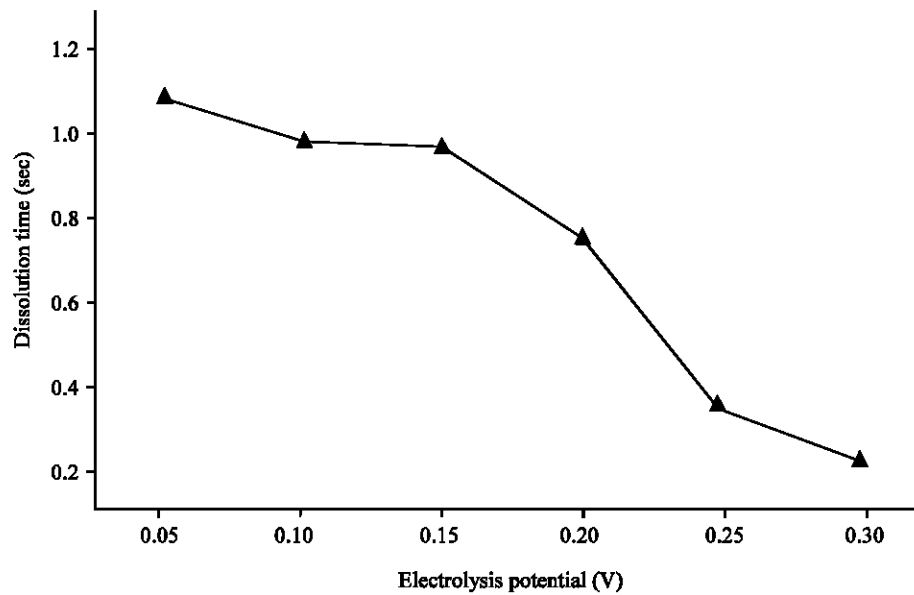


Fig. 1: Dependence of the selenium analytical signal on the electrolysis potential

With the decrease of the dissolution current selenium analytical signal increased approximately exponentially. Dissolution currents higher than $2.6 \mu\text{A}$ caused the significant decrease of the sensitivity, while currents lower than $1.5 \mu\text{A}$ were insufficient for the dissolution of the deposit and caused the extension of the chronopotentiogram and problems in the determination of the inflection point.

Linearity

The dependence of selenium analytical signal on the content (C_m) was defined for the range $20\text{-}60 \mu\text{g dm}^{-3}$. Electrolysis time was 90 sec and reducing current was $1.8 \mu\text{A}$. In the examined range experimental results were in correlation ($r = 0.9947$) with the assumed linear dependence ($\tau = 0.0301C_m + 0.84$; $n = 5$). Obtained results are in agreement with the results of the examination of the influence of the electrolysis time, because longer electrolysis time in stripping techniques is analogous with higher concentration of the analyte. Even though the dependence was linear method of the standard addition which is simpler and which compensate the matrix influence could not be applied due to the significant τ intercept. For the calculation of the concentration the method of calibration plot was used.

Repeatability (Analytical Reproducibility)

After the optimisation of the experimental conditions repeatability was determined by seven consequent analysis at the same mercury film. Solution containing $10 \mu\text{g dm}^{-3}$ of Se(IV) was analysed applying electrolysis time of 90 sec and dissolution current of $1.5 \mu\text{A}$. Qualitative analysis of selenium was based on the dissolution potential shown in the first column (Fig. 2).

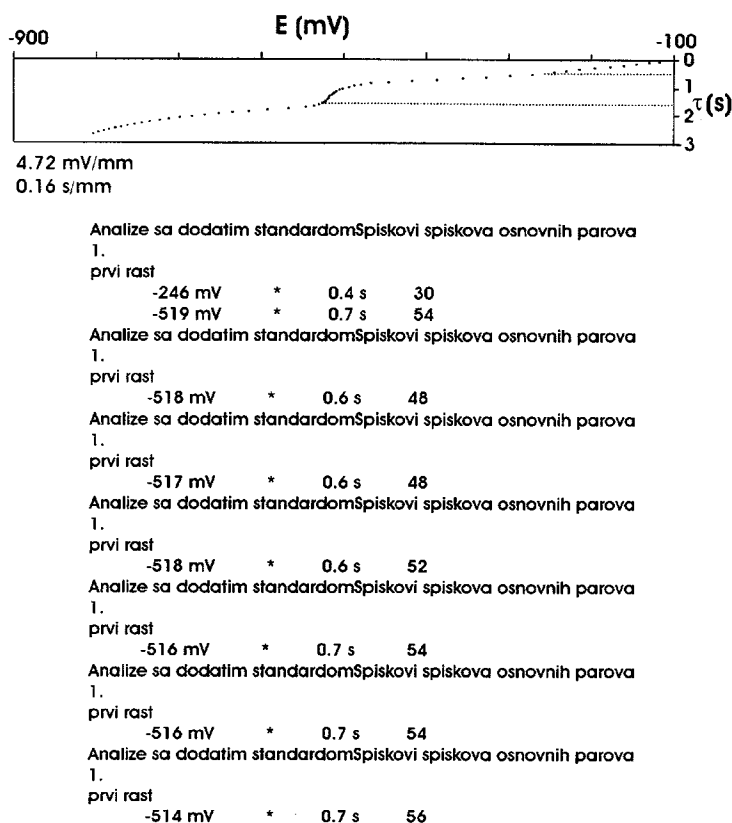


Fig. 2: Repeatability of the selenium determination

Reproducibility of the dissolution potential was very good and expressed as variation coefficient was 0.32%. Quantitation was performed according to the dissolution time shown in second and third columns in seconds and the intern units of the analyser (1 sec = 81.37 units), respectively. Repeatability expressed as a coefficient of variation was 6.02%.

Detection Limit And Limit Of Quantitation

After the optimisation of the CSA parameters the detection limit (LOD) of $0.5 \mu\text{g dm}^{-3}$ was obtained for the electrolysis time of 600 sec and the quantitation limit (LOQ) of $1 \mu\text{g dm}^{-3}$ for same electrolysis time. The method of calibration plot was used for the determination of LOD and LOQ (ACS Committee on Environmental Improvement, 1980).

Accuracy Test

The method accuracy was confirmed by analysing standard reference material in five probes. All determined contents were in the range $1.23 \pm 0.09 \text{ mg kg}^{-1}$, which is certified for the standard reference material RM 8436.

Selenium Determination In Feed

As can be seen in Table 4 selenium content in all samples of feed was below 0.2 mg kg^{-1} . According to the Serbian regulations (Regulation book, 2000) minimal selenium content recommended for sows feed is 0.1 mg kg^{-1} . Determined content in analysed sample was 10% lower. Taking into account that fish meal and meat-bony meal are added in the premixes in 5-7 %, while yeast is added in 2-3 %, it is obvious that these ingredients can not be considered as selenium source in feed, where selenium content should be depending on the type of the feed in the range from 0.1 to 0.45 mg kg^{-1} . On the basis of these results it can be concluded that selenium supplementation in feed produced from these ingredients is required.

Conclusions

Simple and inexpensive technique for chronopotentiometric stripping analysis of selenium was defined. The sensitivity of the defined method enables the determination of low levels of selenium present in biological samples. The correctness and the accuracy of the sample pretreatment procedure were confirmed with the good results of recovery assay. Methods accuracy was confirmed by analysing the standard reference material. Besides methods applicability for selenium determination in feed, it can be assumed that the method can also be applied with success for selenium determination in various premixes where expected contents of selenium are higher or for analysis of different biological samples.

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