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Determination of selenium in clinical plasma samples related to atopic dermatitis study by chronopotentiometric stripping method

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Galvanostatic stripping chronopotentiometry (GSC) was developed and applied for the determination of selenium in human plasma. In this work GSC based on composite carbon electrode coated by a gold layer was optimized concerning various electrochemical parameters (coating procedure, electrolysis potential, electrolysis time, dissolution current). Along with this, the sample preparation was optimized with respect to mineralization conditions (type and concentration of decomposition agent, temperature, time). The human plasma samples mineralized in an autoclave under the optimized conditions (160 °C, 100 min, 22 mol/l HNO_3) were appropriately diluted by background electrolyte solution (0.100 mol/l H_2SO_4 + 0.001 mol/l HCl) and directly analyzed by the optimized GSC method. The proposed method was characterized by excellent performance parameters, the limit of detection was 0.2 ng/ml, accuracy <5%, reproducibility <4%. The proposed method was applied for the investigation of the relationship between atopic dermatitis and selenium concentration in human plasma. Here, patients suffering from atopic dermatitis were monitored during their treatment with a pharmaceutical preparation containing inorganic selenium (Zinkosel®). After six months therapy increased levels of selenium in plasma were detected in 76% of the patients with an improvement of the clinical state in 65% of the patients.

1. Introduction

In humans, selenium is a trace element nutrient with many important biological functions (Bellisola et al. 1996). It was demonstrated in numerous studies that selenium deficiency correlated with the progression of various diseases such as cancer (Russo et al. 1997; Patterson and Levander 1997), HIV/AIDS (Baum et al. 1997; Campa et al. 1999; Baum and Shor-Posner 1998), and atopic dermatitis (Ranjbar and Pizzulli 2002).

Atopic dermatitis (a type of eczema) is an inflammatory, chronically relapsing, non-contagious and pruritic skin disease (De Benedetto et al. 2009). Since the beginning of the twentieth century, many mucosal inflammatory disorders have become dramatically more common; atopic eczema is a classic example of such a disease. It now affects 10–20% of children and 1–3% of adults in industrialized countries, and e.g., its prevalence in the United States alone has nearly tripled in the past thirty to forty years (Saito 2005).

Because of the wide range of biologically relevant selenium concentrations and chemical forms, the determination of selenium in biological samples has been problematic, leading to the development of many methods suitable for specific sample types. The concentration of selenium in most human materials is between 0.01 and 1 mg/g, and the most frequently used analytical techniques for determining nano- and microgram amounts of selenium are fluorimetry, atomic absorption spectrometry (hydride generation, graphite furnace), and inductively coupled plasma atomic emission spectrometry, as reviewed by Borella et al. (1998).

Electrochemical methods, such as stripping voltammetry and potentiometry are suitable alternatives for selenium determination in biological samples. Comparing to atomic absorption spectrometry and fluorimetry, electrochemical techniques enable a selective determination of the particular selenium species since only tetravalent selenium is electrochemically active. Moreover, electrochemical techniques are less time consuming and cheaper. Chronopotentiometric stripping analysis (CSA) compared to voltammetric stripping techniques has better selectivity due to lesser influence of the capacity current. Accuracy of time measurement (quantitative characteristic in CSA) is higher comparing to current measurement (quantitative characteristic in stripping voltammetry). Nevertheless the technique has been less frequently used for selenium determination (Eskilsson and Haraldson 1987; Gozzo et al. 1999; Suturovic et al. 2005). CSA was applied for biological samples like urine and plasma (Eskilsson and Haraldson 1987; Gozzo et al. 1999) and food samples (Suturovic et al. 2005).

The aim of this work is to develop a sensitive CSA method for the determination of total selenium content in plasma for a clinical study related to atopic dermatitis. Particular steps of the research were optimization of galvanostatic stripping chronopotentiometry (GSC) method, optimization of plasma sample preparation procedure, evaluation of performance parameters of the optimized method, application of the developed method to clinical plasma samples, investigation of the relationship between atopic dermatitis and selenium concentration in human plasma.

Table 1: Optimum working parameters of GSC method for selenium

Parameter	Value	Parameter	Value
Accumulation potential	−200 mV	Sample volume	25 ml
Initial potential	−200 mV	Sample accumulation time	60 s
Final potential	1000 mV	Background accumulation time	10 s
Dissolution current	10 μA	Standard sample accumulation time	60 s
Settling time	5 s	Stand by potential	650 mV
Measuring time	30 s	Standard addition volume	100 μl
Regeneration potential	1100 mV	Standard addition concentration	10 mg/l
Regeneration time	10 s		

2. Investigations, results and discussion

2.1. Method optimization

GSC based on composite carbon electrode coated by gold layer was optimized concerning various electrochemical parameters such as coating procedure, electrolysis potential, electrolysis time, dissolution current. Optimum parameters of the GSC method, enabling reliable, sensitive and selective measurements of selenium content in model samples (section 2.2.), are given in Table 1.

The sample preparation procedure was optimized with respect to mineralization conditions (type and concentration of decomposition agent, temperature, time). The optimization strategy is shown in Table 2. Optimum conditions were chosen with respect to the shortest decomposition time and lowest temperature needed to the complete mineralization of plasma. Concentrated acids, HNO_3 and H_2SO_4, were tested as decomposition agents for plasma samples. It was found out that 22 mol/l HNO_3 alone is sufficient for the total decomposition of plasma. The final mineralized sample was clear (without any turbidity) and matched all criteria for qualitative and quantitative GSC analysis. The pretreated human plasma samples were appropriately diluted by background electrolyte solution (section 3.3.) and directly analyzed by the optimized GSC method.

2.2. Performance parameters of the method

GSC profile of selenium obtained in the working background electrolyte solution was characterized by the single peak with the maximum at an 870 mV potential. For the quantitative determination of selenium content, the area of the selenium peak was evaluated. The GSC method was characterized by excellent performance parameters. The limit of detection (3σ, σ is baseline noise) for selenium was 0.2 ng/ml. Working range for the determination of selenium by the GSC method was 0.5–500 ng/ml.

Table 2: Mineralization strategy of plasma samples[a]

	Temperature (°C)			
Time (min)	120	140	160	180
60	-	-	-	-
80	-	-	(+)	(+)
100	-	-	+	+
120	-	(+)	+	+

[a] Mineralization was carried out with 22 mol/l HNO_3 as a decomposition agent
- Insufficient mineralization, the sample was turbid
(+) Insufficient mineralization, the sample was clear but does not match qualitative and quantitative criteria of GSC analysis
+ Sufficient mineralization, the sample was clear and matched qualitative and quantitative criteria of GSC analysis

Accuracy, repeatability and reproducibility were tested in the concentration range of selenium 10–100 ng/ml that was suitable for further application of the method. Recovery test as well as the analysis of the reference material confirmed the correctness of the defined methods. Accuracy, expressed via relative error (RE) of selenium concentration comparing peak area of sample with and without matrices, was lower than 5%. Precision was expressed via relative standard deviation (RSD) of concentration measurements of model samples. Here, RSD obtained from 10 consecutive measurements (repeatability) was 2.1%. RSD obtained from the 10 measurements, two on each day (reproducibility), was 3.9%. No interferences (from iron, chlorides or electroactive organic compounds) were registered in GSC profiles when analyzing model as well as real (mineralized plasma) samples that indicated suitable selectivity of the method. These parameters approved a suitability of the GSC method for its routine application.

2.3. Application of the method to clinical study

The proposed analytical procedure was applied for the investigation of the relationship between atopic dermatitis and selenium concentration in human plasma. In this study, patients suffering from atopic dermatitis were monitored during their treatment with the pharmaceutical preparation containing inorganic selenium (Zinkosel®) as described in section 3.3. Concentration levels of selenium in human plasma samples as obtained by the proposed method along with the statistical evaluation of the results are given in Table 3. At the beginning of the experiment, the concentration level of selenium in plasma of 100 dermatitic patients was in the interval 25.20–56.56 ng/ml with the average value 40.28 ng/ml. On the other hand, the concentration level of selenium in plasma of 100 healthy volunteers was in the interval 46.70–72.50 ng/ml with the average value 61.52 ng/ml. This was in a good agreement with the average selenium value (58.40 ng/ml) for the adult population in Slovakia (Mad'arič and Kadrabová 1997). The comparison of the initial selenium status of the ill and healthy participants indicated a significant difference in selenium concentrations between pathological and normal plasma samples with a potential relationship to the atopic dermatitis. An influence of the selenium therapy on the selenium concentration in the plasma of dermatitic patients is shown in the Fig. This dependency clearly indicates a continual increase of selenium levels in plasma during its constant supplementation. After six months therapy increased levels of selenium in plasma (35.40–67.09 ng/ml, average 51.32 ng/ml) were detected in 76% of the patients with an improvement of the clinical state in 65% of the patients (dermatitic symptoms were suppressed).

It is concluded that the proposed analytical procedure based on the oxidative decomposition of the biological matrices and direct GSC analysis of the mineralized sample is suitable

Table 3: Concentration levels of selenium in human plasma samples with statistical evaluation[a]

Parameter	Healthy volunteers	Patients with atopic dermatitis		
		Before Se therapy	After 3 months of Se therapy	After 6 months of Se therapy
Minimum	46,70 μg/l	25,20 μg/l	29,50 μg/l	35,40 μg/l
Maximum	72,50 μg/l	56,56 μg/l	61,24 μg/l	67,09 μg/l
$\bar{x}$	61,52 μg/l	40,28 μg/l	46,93 μg/l	51,32 μg/l
s	7,74	8,40	8,62	8,52
$L_{1,2}$	61,52 μg/l ± 2,19	40,28 μg/l ± 1,66	46,93 μg/l ± 1,61	51,32 μg/l ± 1,69

[a] For the therapy procedure and sample preparation procedure see section 3.3. and Table 2. For the GSC operating conditions see section 3.2 and Table 1.
$\bar{x}$= average; s = standard deviation of average, $s = (\Sigma \Delta i^2/n)^{1/2}$; $L_{1,2}$=confidence interval, $L_{1,2} = \bar{x} \pm s.t_\alpha/n^{1/2}$, t_α=1,984 for n = 100, n = number of samples, each sample was measured three times

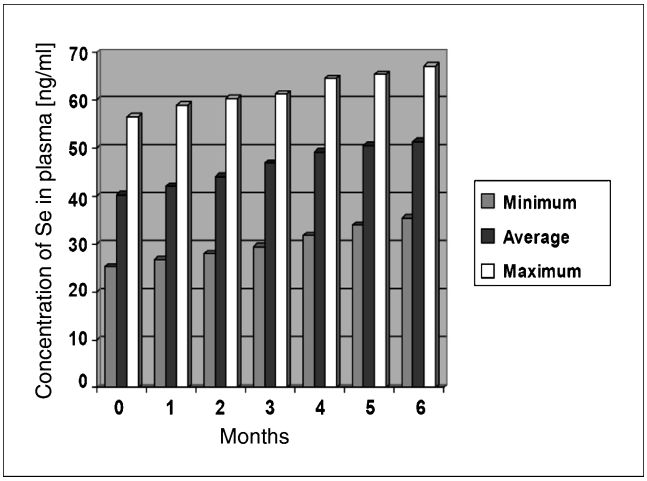

Fig.: The dependence of the selenium supplementation therapy on the selenium concentration in the plasma of dermatitic patients. For the therapy procedure and sample preparation procedure see section 3.3. and Table 2. For the GSC operating conditions see section 3.2. and Table 1

for the monitoring of total selenium concentration in clinical plasma samples. Great advantage of the proposed method is its extremely high sensitivity (sub ng/ml concentrations). It allows performing not only ultratrace but also microscale analyses that could be essential when limited amounts of sample are available. Simple analytical procedure and relatively short analysis time provide good conditions for a routine use of the proposed method. It can be a good alternative to fluorimetry, atomic absorption spectrometry, and inductively coupled plasma atomic emission spectrometry because of a low cost.

The results obtained in the presented clinical study indicated a relationship between selenium plasma concentrations and atopic dermatitis in humans. It was also demonstrated that a long term selenium supplementation increases the selenium concentration levels in plasma with suppressing symptoms of atopic dermatitis.

3. Experimental

3.1. Samples and reagents

Chemicals used for the preparations of the electrolyte solutions (H_2SO_4, HCl), electrode goldening solution ($HAuCl_4$), and sample mineralization solution (HNO_3) were obtained from Spolchim (Bratislava, Slovak Republic), Centralchem (Bratislava, Slovak Republic), Fluka (Buchs, Switzerland), Lachema (Brno, Czech Republic), respectively. All chemicals used were of analytical grade. The water was demineralised by a Rowapure-Ultrapure water purification system Premier (Phoenix, Arizona, U.S.A.).

The certified reference material of selenium with a declared content 1.000 g/l Se(IV) was obtained from Slovenský Metrologický Ústav (Bratislava, Slovak Republic). The dosage form Zinkosel® tablets containing 40 μg of inorganic Se(IV) per dose was obtained from a local pharmacy.

3.2. Instrumentation and electrochemical conditions

An ECA-SENSOR electrochemical analyser with composite carbon working electrode, argentochloride reference electrode and Pt auxiliary electrode (Istran, Bratislava, Slovak Republic) was used for galvanostatic stripping chronopotentiometry (GSC) measurements. Electrode reaction for selenium is Se(-II) ↔ Se(+IV). All basic principles and procedures are described by the manufacturer (EcaSensor 1989). For the determination of selenium the electrode was coated by gold layer. The gold blocking was carried out in a goldening solution (see section 3.3.) at the potential of the working electrode −500 mV, for the period of 15 min and without stirring of the solution during the process. Then, the potential was set at +650 mV and the sensor was washed by demineralized water. The coated electrode was conditioned before a set of experiments. The conditioning was carried out in the synthetic sample environment (section 3.3.) under the optimum electrochemical conditions (Table 1). A relative error of selenium content, obtained from the three paralel conditioning measurements, lower than 10% indicates suitable quality of the prepared electrode.

3.3. Sample and standard solution preparations

The stock solution of background electrolyte consisted of 1.0 mol/l H_2SO_4 and 0.01 mol/l HCl. It was diluted 10-times by demineralised water to prepare working background electrolyte solution. The goldening solution consisted of 0.001 mol/l $HAuCl_4$ and 0.02 mol/l HCl. Standard solution, prepared from the certified reference material of selenium by its diluting (100-times), was used in the standard addition method. The synthetic sample for the verification of electrode quality was prepared in the working background electrolyte solution with a 10 mg/l concentration of Se(IV).

One tablet of Zinkosel (equivalent to 40 μg of selenium) was administered as a daily dose to patients suffering from atopic dermatitis as well as to healthy volunteers. The plasma samples were taken in monthly intervals during the six-month continual selenium therapy. Each plasma sample was frozen (−18 °C) immediately after the sampling and kept in the freezer until the use. The sample was thawed just before the manipulation. Each sample was pretreated by mineralization procedure optimized in this work (Table 2). The mineralization was based on an acid decomposition under increased pressure and temperature in an autoclave Laboratorní autoklav ZA-1 (JZD Pokrok, Zahnašovice, Czech Republic) using a PTFE vessel. The optimized mineralization conditions were temperature 160 °C (controlled by thermostatic electrical oven Chirana STE 52/I, Stará Turá, Slovak Republic), decomposition time 100 min, decomposition agent 22 mol/l HNO_3 (2 ml plasma: 2 ml decomposition agent). After cooling down, the clear sample solution was quantitatively transferred into a 25 ml volumetric flask, 5 ml of the working background electrolyte solution was added to it and the flask was filled up to the mark by demineralised water. Such solution was directly analyzed by the optimized GSC method (Table 1).

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