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Determination of As(III) and As(V) in oilseeds by chronopotentiometric stripping analysis: Development of a method

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Chronopotentiometric stripping analysis (CSA) was used for selective determination of As(III) and As(V) in different oilseeds. After the optimization of experimental parameters an appropriate procedure for sample pretreatment was developed. A detection limit of 2 μg/dm^3 for As(III) was obtained with an electrolysis time of 600 s. This method was used for arsenic determination in sunflower, pumpkin, and flax seed, as well as for soy flakes and almond.

Keywords: Arsenic / Chronopotentiometric stripping analysis / Oilseeds

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1 Introduction

Arsenic naturally occurs in water, soil, ores, and atmosphere. It exists in four valence states: −3, 0, 3, and 5. This metalloid is emitted into the atmosphere by volcanic activity and burning of fossil fuels primarily in the form of As_2O_3 which adsorbs at solid particles that settle in soil. Arsine (AsH_3), the gas which is 2.5 times denser than air is the most toxic form of arsenic and can be released into the environment by microbial activity. By various oxidation processes it can be converted in nonvolatile forms. In addition to these natural processes, electronic industry processes, use of pesticides, and use of wood preservatives influence arsenic distribution into the environment as well. The average arsenic intake from consumption of food and drink by humans is 20–300 μg/day. Smokers inhale approximately 10 μg/day arsenic compared to 1 μg/day inhaled by nonsmokers. Arsenic has been recognized as a poison since ancient times. The major public health issue is the presence of elevated arsenic concentrations in ground water used for drinking in many parts of the world, especially in Bangladesh and West Bengal in India. The contamination of ground water by arsenic in Bangladesh represents the largest poisoning of a population in history [1]. Soluble inorganic compounds can cause cardiovascular, gastrointestinal, and nervous system disorders. Long-term arsenic exposure is correlated with increased risk of skin, lung, and kidney cancers. Chronic poisoning eventually leads to "black foot disease", a severe form of peripheral vascular system illness. Generally, organic arsenic (arsenocholine, arsenobetaine, and arsenosugars, typically present in sea food) is considered less toxic than inorganic forms of the metalloid. Trivalent arsenic is the most toxic form. Therefore, detection, speciation, and monitoring of arsenic content plays a crucial role in food, water, and soil analysis.

Currently, arsenic determination is made by various instrumental methods, including neutron activation analysis (NAA), atomic absorption spectrometry (AAS) [2, 3], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [4], and inductively coupled plasma-mass spectrometry (ICP-MS) [5]. Some of these techniques, coupled with chromatographic techniques, such as HPLC or gas chromatography, can be used for arsenic speciation. Among electrochemical methods for arsenic determination, most often used are voltammetric stripping techniques which possess the highest sensitivity after NAA. Anodic stripping voltammetry involves application of working electrodes made mostly from gold, platinum, and various modifications of the carbon [6–8]. An increase in sensitivity is observed when the gold electrode is heated [7] or when gold film electrodes are used [8]. Direct determination of As(V) without prior reduction to As(III) is possible if the applied current density during the preconcentration step is great enough [9, 10]. Heating of the microelectrodes has the effect of enhancing the electrode reaction, of increasing the convective mass transfer near the electrode surface due to

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Abbreviation: CSA, chronopotentiometric stripping analysis

the formation of a temperature gradient, and of increasing the diffusive coefficient in the stripping step due to higher applied temperature [9]. Since arsenic has low solubility in mercury, when cathodic stripping voltammetry is used in combination with mercury working electrodes, copper addition is neccessary and arsenic is concentrated in the intermetallic form [11, 12]. Speciation of inorganic arsenic in natural waters by square-wave cathodic stripping voltammetry has been performed by varying the composition of the supporting electrolyte. As(III) + As(V) was determined in the presence of reducing agent [13]. Chronopotentiometric stripping analysis (CSA) has previously been used for arsenic speciation chiefly by the use of gold-plated electrodes and anodic dissolution currents [10, 14, 15]. Selectivity between arsenic(III) and arsenic(V) was achieved either by application of selective potential [14] or by sample analysis with and without reduction of pentavalent arsenic [15]. Constant current cathodic stripping potentiometry of arsenic has also been performed with the implementation of mercury film electrodes which enabled overcoming interferences related to the usage of gold electrodes [16]. Compared to other electroanalytical techniques, simplicity, better selectivity, and accuracy are attributed to CSA. In the first step of CSA (electrolysis at constant potential) the element becomes concentrated at the electrode under conditions of reproductive solution stirring. After a rest period that enables deposits to homogenize, the analyte is stripped applying constant current. This step is conducted in conditions of diffusive mass transfer. The dissolution time is a quantitative characteristic of the analyte. Qualitative identification is based on the element dissolution potential [17–19]. In the present work, CSA was used for the determination of As(III) and As(V) in various oilseeds after optimization of experimental parameters. Sample pretreatment procedures were developed accordingly.

2 Materials and methods

CSA measurements were carried out on an automatic self-constructed system for potentiometric and chronopotentiometric stripping analysis [20]. A glassy carbon disc working electrode with a total surface area of 7.07 mm^2 was used as an inert support for a gold film. Glassy carbon was washed with acetone and double-distilled water prior to each deposition of gold. An Ag/AgCl, KCl (3.5 mol/dm^3) electrode was used as reference and a platinum wire (l = 7 mm, φ = 0.7 mm) as the counter electrode. A stock solution containing 10 g/dm^3 As(III) was prepared by dissolving 1.32 g As_2O_3 in 5 cm^3 10 mol/dm^3 NaOH and 25 cm^3 HCl and making the volume up to 100 cm^3 with double-distilled water. The solution was kept in a polyethylene bottle in the dark. Working solutions containing 2 and 60 mg/dm^3 As(III) were prepared daily and every week, respectively, by diluting with double-distilled water. A stock solution containing 1 g/dm^3 As(V) was prepared by dissolving $Na_2HAsO_4 \cdot 7H_2O$ (p.a., Merck, Darmstadt, Germany) in double-distilled water and was kept in a polyethylene bottle in the dark. A solution containing 1 g/dm^3 Au(III) was prepared by dissolving 0.1 g gold (99.99%) in 2 cm^3 aqua regia and making the volume up to 100 cm^3 with double-distilled water. All chemicals were of supra pure-grade (Merck). As reference material RM 8436 durum what flour (U.S. Department of Commerce National Institute of Standards and Technology, Gaithersburg, MD, USA) was used. Cationic resin Chelex-100 (50–100 mesh) with styrene-divinylbenzene matrix was used for copper elimination from the samples. The capacity of ion-exchange resin was 0.33 $mmol/cm^3$ to $[Cu(NH_3)_4]^{2+}$. Prior to column preparation the resin was suspended in double-distilled water overnight. Nitrogen of extra purity was used for deaeration. All containers and cells were washed with nitric acid (1 : 1) and double-distilled water. Samples of dehulled sunflower, pumpkin and flax seed, soy flakes, and almond were randomly collected from the Novi Sad market.

2.1 CSA of arsenic

The working electrode was formed by electrolysis at –0.4 V during 480 s from a solution containing 10 mg/dm^3 Au(III) and 0.03 mol/dm^3 HCl. Electrodes formed in this way could be used for 20 analyses each lasting 120 s. As a supporting electrolyte, 3 mol/dm^3 sulfuric acid was used. Prior to analysis, solutions were deaerated by passing through nitrogen gas for 300 s. During the deaeration step the working electrode was held in double-distilled water in order to avoid damage of the electrode surface by nitrogen bubbles. Arsenic deposition at the gold film electrode was performed with a stirring rate of 2000 rpm. After a rest period of 15 s, stripping was performed by applying a constant current.

2.2 Pretreatment of the samples

Various procedures for the sample pretreatment, such as dry ashing and wet acid digestion, in both closed and opened systems were examined. During dry ashing arsenic loss was observed due to formation of volatiles. Wet acid digestion in closed systems has the advantage of a shorter pretreatment time due to increased pressure in the vessel. Arsenic loss due to volatilization is avoided as well. Considering that in most real samples arsenic contents are very low and that vessels for this procedure can contain only a limited sample weight and volume, the procedure was unsuitable for arsenic determination. Wet acid digestion in quartz long-necked flasks was used for the destruction of the organic matter in the samples. In order to determine the total arsenic content it was necessary to reduce pentavalent to trivalent arsenic which is the only electroactive form of

arsenic. Application of various reducing agents, such as hydroxylamine-hydrochloride, hydrazine-sulfate + HCl, mixture of ascorbic acid and KI, Na_2SO_3 solution, 0.1 mol/dm^3 HCl, L-cysteine, and ascorbic acid, was investigated. Best results were accomplished using Na_2SO_3 solution.

3 Results and discussion

3.1 Influence of the CSA experimental parameters

Influence of the electrolysis potential (E) on the arsenic analytical signal (τ) was examined in the range of $-0.15 + (-0.35)$ V in a model solution containing 60 μg/dm^3 As(III). Electrolysis time was 60 s and dissolution current 4.8 μA. Over the examined voltage range experimental results agreed well with an assumed linear dependence (Fig. 1). The arsenic dissolution time (τ) increased proportionally with increased negative electrolytic potential. A potential of -0.27 V was chosen as optimal because it resulted in satisfactory sensitivity with very good reproducibility (0.25%, expressed as variation coefficient).

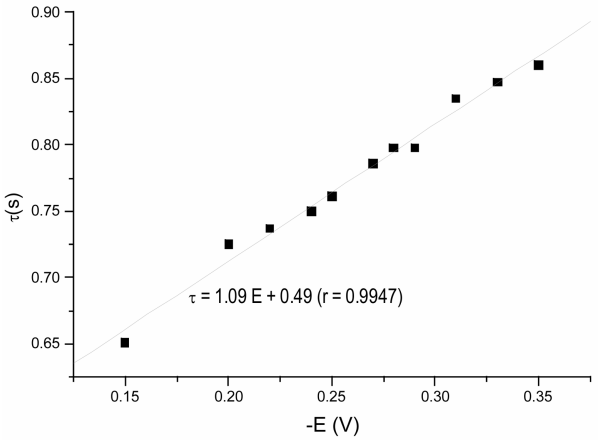

Figure 1. Influence of the electrolysis potential on the arsenic analytical signal. 60 μg/dm^3 As(III) in 3 mol/dm^3 sulfuric acid; electrolysis time, 60 s; stirring rate, 2000 rpm; dissolution current, 4.8 μA.

Dependence of the analytical signal on arsenic concentration (C_m) was examined for two concentration ranges: from 5 to 30 μg/dm^3 and from 80 to 180 μg/dm^3. For the lower concentration range, the electrolysis time was 240 s and the dissolution current 4.5 μA, while for the higher concentration range the electrolysis time was 120 s and the dissolution current 7.9 μA. Obtained dependences were linear in both ranges. For the range of 5 – 30 μg/dm^3 the zero voltage intercept was at 1.07 s and the slope 0.032 s · dm^3/μg (r = 0.9979, n = 6), while for the range of 80 – 180 μg/dm^3 the zero voltage intercept was 0.65 s and the slope 0.0038

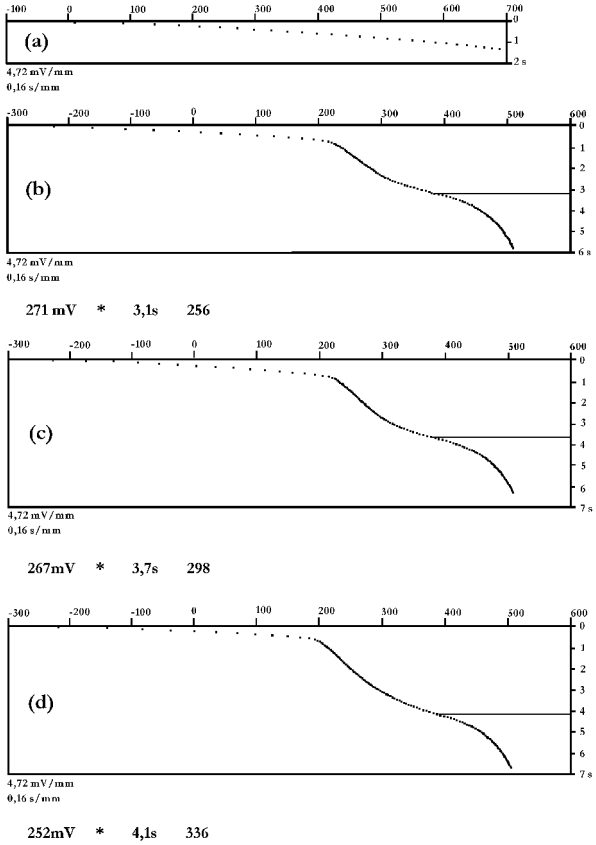

Figure 2. Calibration series: (a) blank, (b) 30 μg/dm^3 As(III), (c) 40 μg/dm^3 As(III), and (d) 50 μg/dm^3 As(III). Electrolysis potential, -0.27 V; electrolysis time, 120 s; stirring rate, 2000 rpm; dissolution current, 6.1 μA.

s · dm^3/μg (r = 0.9963, n = 6). Considering the results obtained for the determination of the arsenic concentration, the method of calibration plot was applied (Fig. 2). The resulting small slopes of the calibration curves and significant "τ" intercepts excluded the application of the standard addition method. The obtained inadequate enlargement of the analytical signal after the standard addition caused the enlarged results calculated by the analyzer.

In the first column of the original chronopotentiogram is presented the oxidation potential of arsenic and in the second and third column the dissolution time in seconds and internal units of the analyzer (1 s = 81.37 units), respectively. The horizontal line shown in the position of the inflection point corresponds to the dissolution time of arsenic.

Since dissolution current is one of the most important experimental parameters in CSA, its behavior was investigated in solutions containing 10 and 60 μg/dm^3 As(III). For low arsenic concentrations the electrolysis time (t_{el}) was 600 s and the current varied from 3.5 to 6.8 μA. For high arsenic

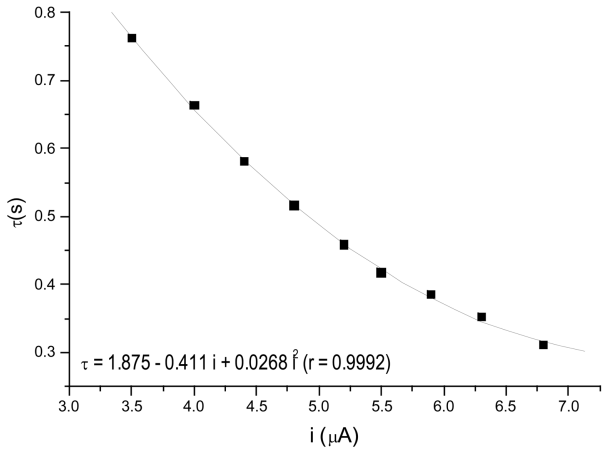

Figure 3. Influence of the oxidation current on the dissolution time of arsenic. C_m (As) = 10 μg/dm^3, t_{el} = 600 s.

concentrations the electrolysis time was 240 s and the investigated current range was from 4.8 to 8.4 μA. In both cases, the dependence of the arsenic analytical signal on dissolution current was in the form of a second-order polynomial (Fig. 3). The dependence obtained for 60 μg/dm^3 As(III) was $\tau = 18.668 - 4.5616 \times i + 0.3026\, i^2$ ($r = 0.9943$).

For an arsenic concentration of 10 μg/dm^3, currents lower than 3.5 μA caused the extension of the chronopotentiogram and caused problems in the detection of the inflection point (Fig. 4). Greater required dissolution currents for measurements at low arsenic concentrations resulted in significant decrease in measurement sensitivity. For an arsenic concentration of 60 μg/dm^3 a greater current resulted in a sensitivity decrease as well, but contributed to greater resolution of the analytical signal. Generally, a smaller dissolution current was used for lower arsenic concentrations.

Reproducibility of the CSA of arsenic was determined for solutions containing 10 and 60 μg/dm^3 As(III). Electrolysis times were 240 s and 180 s, respectively, while dissolution currents were 4.5 μA and 6.1 μA, respectively. Reproducibility of the dissolution time was very good, and expressed as variation coefficients, were 1.8% (n = 7) for the lower concentration and 1.3% (n = 7) for the higher concentration. Reproducibility of the oxidation potential was 2.04% expressed as variation coefficient.

After optimization of the experimental parameters the detection limit was determined applying the calibration plot method [21]. The detection limit of the technique was 2 μg/dm^3 As(III) for an electrolysis time of 600 s and dissolution current of 4.5 μA. Reproducibility for five consecutive analyses was 4.14% expressed as a variation coefficient. The detection limit for As(V) determined in model solutions of pentavalent arsenic was equivalent to the detection limit for trivalent arsenic because of the high efficiency of the reduction procedure described below.

3.2 Sample pretreatment

Destruction of organic matter in the samples was performed by aqueous acid digestion using a mixture of nitric and perchloric acids (1 : 1). The sample (3 g) was transferred to a quartz long-neck flask and 40 cm^3 of acid mixture was added. The mixture was heated to 110°C for 45 min until the evolution of dark nitrogen oxide fumes was completed. Heating was continued for 30 min at 140°C. In order to complete the destruction of the organic matter, 9 cm^3 of nitric acid was added. Perchloric acid was completely evaporated at 230°C under vacuum. The resulting dry residue was dissolved in 20 cm^3 double-distilled water. In order to eliminate copper from the samples, which can significantly influence the arsenic analytical signal, ion-exchange chromatography was performed. Firstly, copper was converted to $[Cu(NH_3)_4]^{2+}$ by adding 15 mol/dm^3 ammonia until pH 10. After passing the alkaline sample over the column,

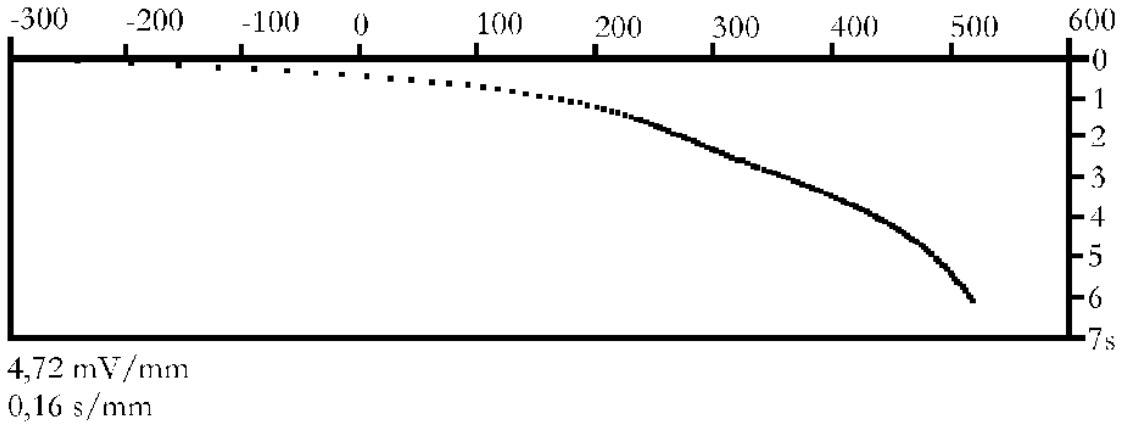

Figure 4. Extended chronopotentiogram (inflection point missing). Sample: 10 μg/dm^3 As(III). Electrolysis potential, –0.27 V; electrolysis time, 240 s; stirring rate, 2000 rpm; dissolution current, 3 μA.

the column was rinsed with double-distilled water until a 50 cm^3 eluate volume was collected containing the sample arsenic. In order to wash out bound copper and regenerate the column for subsequent samples, it was rinsed with 2 mol/dm^3 nitric acid followed by double-distilled water. Regeneration was performed by washing with 0.5 mol/dm^3 aqueous ammonia followed by 0.1 mol/dm^3 aqueous ammonia. In this way all copper was removed. It was established that there was no arsenic loss or arsenic contamination as a result of the column procedure.

Reduction of pentavalent arsenic was performed by heating the samples in polyethylene bottles in a water bath at 75°C for 45 min with 0.02 mol/dm^3 Na_2SO_3. Reduction efficiency was confirmed using model systems containing 30 $\mu g/dm^3$ As(V). For five replicates the efficiency of the reduction was 98.7%. Other reducing agents did not completely reduce pentavalent arsenic under these conditions and caused poor reproducibility, absence of the arsenic analytical signal, or shifting potential to more negative values.

3.3 Arsenic determination in oilseeds

Prepared samples were analyzed in five replicates. The electrolysis time was 120 s and the dissolution current 4.9 μA. The arsenic concentration was calculated by a calibration plot. Standard solutions were prepared in the matrix of blank solution. The pentavalent arsenic content was calculated as the difference between total arsenic content determined after reduction and the content of trivalent arsenic. A recovery assay was performed by adding 10 $\mu g/dm^3$ As(III) to the mixture prepared for the destruction of organic matter. The efficiency of the reduction of pentavalent arsenic had previously been confirmed. When the total arsenic content was determined, deaeration time was 600 s in order to completely eliminate SO_2 formed from excess reducing agent and supporting electrolyte. The results of arsenic determination in the samples of oilseeds are presented in Table 1.

The results presented in Table 1 show that sunflower seed has the highest content of both trivalent and pentavalent arsenic. The total arsenic content in all samples was below 1 mg/kg which is the highest permitted content in oilseeds as governened by Serbian regulatory laws [22]. The results of the recovery assay validated the procedure we developed for sample pretreatment as well as the arsenic quantitative technique, assuming that systematic errors were avoided. Also, since the recovery assay was performed assessing for only trivalent arsenic, it was further demonstrated that, during the destruction of organic matter, As(III) was not oxidized to As(V). This result was, however, not expected on the basis of As(III) → As(V) standard potential [23]. The method accuracy was confirmed by analyzing wheat durum flour as a reference material. Certified total arsenic in the reference material was 0.03 mg/kg. Results of five replicates were in the range 0.03 ± 0.002. This indicates that the developed method enables reliable determination of the arsenic content in oilseeds. Comparing the sensitivity to other instrumental techniques for arsenic determination, selectivity and cost of the instrumentation, as well as exploitation of the method, are advantages of the technique we developed and present here.

Table 1. Arsenic content in oilseeds

	As(III) (mg/kg)	As(V) (mg/kg)	Recovery (%)
Sunflower seed	0.28 ± 0.06[a)]	0.66 ± 0.02	95.9
Pumpkin seed	0.50 ± 0.03	0.09 ± 0.006	98.5
Flax seed	Not detected	Not detected	100
Almond	0.46 ± 0.05	0.09 ± 0.007	101.6
Soy flakes	0.26 ± 0.06	0.19 ± 0.04	103

a) Variability expressed as 2 SD (2 standard deviations)

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