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OPTIMIZATION OF METHOD FOR ZINC ANALYSIS IN SEVERAL BEE PRODUCTS ON RENEWABLE MERCURY FILM SILVER BASED ELECTRODE

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Abstract: Zinc is an interesting target for detection as it is one of the elements necessary for the proper functioning of the human body, its excess and deficiency can cause several symptoms. Several techniques including electrochemistry have been developed but require laboratory equipment, preparative steps and mercury or complex working electrodes. We here described the development of a robust, simple and commercially available electrochemical system. Differential pulse (DP) voltammetry was used for this purpose with the cyclic renewable mercury film silver based electrode (Hg(Ag)FE) and 0.05 M KNO_3 solution as a supporting electrolyte. The effect of various factors such as: preconcentration potential and time, pulse amplitude and width, step potential and supporting electrolyte composition are optimized. The limits of detection (LOD) and quantification (LOQ) were 1.62 ng/mL and 4.85 ng/mL, respectively. The repeatability of the method at a concentration level of the analyte as low as 3 ng/mL, expressed as RSD is 3.5% (n = 6). Recovery was determined using certified reference material: *Virginia Tobacco Leaves* (CTA-VTL-2). The recovery of zinc ranged from 96.6 to 106.5%. The proposed method was successfully applied for determination of zinc in bee products (honey, propolis and diet supplements) after digestion procedure.

Keywords: zinc, mercury film electrodes, stripping voltammetry, bee products

Bee products are common in majority of households. The most known of the bee products are honey, propolis, royal gelly, bee pollen and beeswax. They are widely used for nutritional and medical purposes (1). In medicine, honey and royal gelly are strictly administrated to digestive tract. However, propolis is administered internally and externally in tablet form or as ethanol extracts, rarely as water extract. Propolis has a multidirectional activity in wound healing. Its chemical composition is complex and varied, depending on the type of plant, from which it was collected by bees and seasonally changeable. Bee products contain organic compounds (phenolic acids, flavonoids, vitamins) and macro- and microminerals (including calcium, magnesium, manganese, zinc, copper, iron, silicon, aluminum) (2-4).

Zinc is one of the elements necessary for the proper functioning of the human body, its excess and deficiency can cause several symptoms. Zinc is a component of over 300 enzymes, fulfills the functions of the catalyst, is an element of the structure of nucleic acids. It stabilizes the structure of the cell membrane, ensures proper construction of the ribosome, is involved in catalysis, the intercellular signal transduction, regulates the oxidation and reduction processes (4, 5). Zinc impose a large impact on the majority of immune cells, plays an important role in viral infections – shortens the duration of the disease. It participates in the storage of insulin, it was shown that zinc supplement can reduce blood sugar levels (6). It has been reported that the supplementation could reduce the therapeutic doses of antidepressants (7).

Zinc is ubiquitous in the environment and laboratories, causing difficulties in a proper analysis and misinterpretation of obtained data (8). Analytical procedure for zinc determination in real (environmental and pharmaceutical) samples frequently requires matrix digestion. The most popular

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techniques for zinc analysis are atomic spectroscopy (9-13), ion chromatography (14), neutron activation analysis (15, 16) and stripping analysis (17-20). In the case of voltammetry, mercury drop electrodes are often used due to their high sensitivity, reproducibility and linearity. However, the toxicity of mercury limits the usage of the mercury electrodes in the analytical application.

In this work, the experimental conditions for the electrochemical detection of zinc(II) using Hg(Ag)FE electrode have been optimized. This electrodes are characterized by low analytical cost, low mercury consumption and simplicity of application procedure. The principle of working and first proposal of a construction of the Hg(Ag)FE was described in (21-23). The new procedure have been studied and successfully utilized for determination of zinc content of several bee products with various matrix.

MATERIALS AND METHODS

Measuring apparatus and software

A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland) were used for all voltammetric measurements. The classical three electrode quartz cell, volume 5 mL, consisting of a home-made cylindrical silver based mercury film electrode (Hg(Ag)FE), refreshed before each measurement and with a surface area of 1–12 mm^2, as the working electrode, a double junction reference electrode Ag/AgCl/ 3 M KCl with replaceable outer junction (2.5 M KNO_3) and a platinum wire as an auxiliary electrode. The certified reference material and bee products were digested with a Multiwave 3000, microwave digestion system (Anton Paar GmbH, Germany). Stirring was performed using a magnetic bar rotating at approximately 500 rpm. All experiments were carried out at room temperature.

Chemicals and glassware

All solutions were prepared with quadruply distilled water. All reagents used were of analytical grade. For digestion procedures HNO_3 conc. (Merck, Suprapur®) was used. Also Zn(II) standard stock solution (1000 mg/L, OUM, Łódź, Poland) was applied. Solutions with lower zinc concentrations were prepared just before the measurements. As a supporting electrolyte 0.05 M KNO_3 (Merck, Suprapur®) was used. Certified reference material: tobacco leaves "Oriental" CTA-OTL-1, was obtained from the Institute of Nuclear Chemistry and Technology, Poland. For amalgam's preparing, mercury GR for polarography (Merck) was used. Prior to use, glassware was cleaned by immersion in

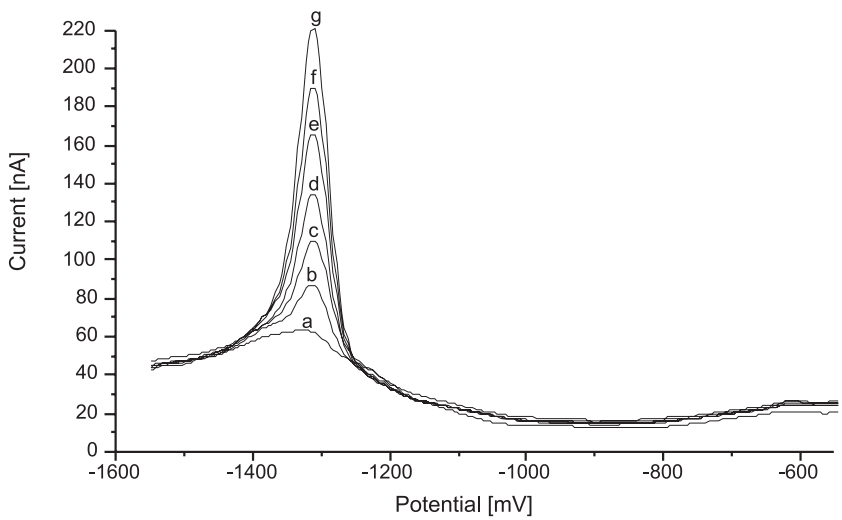

Figure. 1. Linearity range of voltammetric determination of Zn(II) on Hg(Ag)FE electrode: a) 0.05 M KNO_3; b)10 ng/mL; c) 20 ng/mL; d) 30 ng/mL e) 40 ng/mL; f) 50 ng/mL; g) 60 ng/mL.; DP mode: pulse amplitude, 40 mV; pulse width (waiting time + current sampling time) 40 ms; potential step, 4 mV. Preconcentration potential E_{acc} = -1300 mV and time t_{acc} = 20 s. Stirring rate 500 rpm (without background correction)

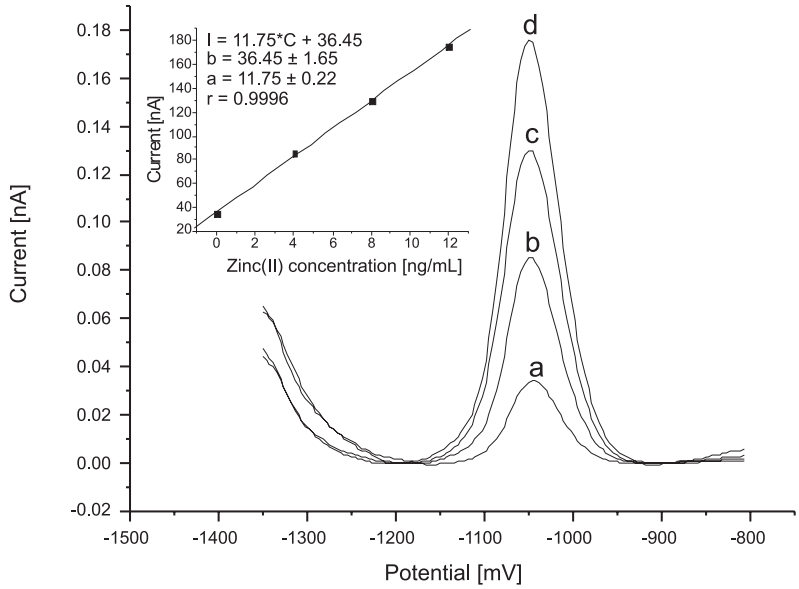

Figure 2. Typical DP ASV voltammograms of zinc(II) obtained for Propolis Plus® sample in 0.05 M KNO_3 (a) and standard addition of zinc(II): b) 4 ng/mL; c) 8 ng/mL; d) 12 ng/mL. DP mode: pulse amplitude, 40 mV; pulse width (waiting time + current sampling time), 40 ms; potential step, 4 mV. Preconcentration potential E_{acc} = -1300 mV and time t_{acc} = 20 s. Stirring rate 500 rpm (after background correction)

a 1 : 1 aqueous solution of HNO_3 (Merck, Suprapur®), followed by copious rinsing in quadruple distilled water.

Standard procedure of measurements

Quantitative measurements were performed using differential pulse anodic stripping voltammetry (DP ASV) by standard addition procedure (three additions). The procedure of refreshing the mercury film Hg(Ag)FE electrode was carried out before each measurement. The potential of the electrode was changed in the following sequence: conditioning (E_{cond}) = accumulation (E_{acc}) = starting potential (E_{start}) = -1300 mV for accumulation time (t_{acc}) 20 s. The differential pulse voltammogram was recorded in the anodic direction from -1300 to -800 mV. The other experimental parameters were as follows: step potential, 4 mV; pulse amplitude, 40 mV; pulse width, 20 ms (10 ms waiting time + 10 ms current sampling time). The measurements were carried out from deaerated solutions.

Analysis of zinc in bee products

Pharmaceuticals were purchased from drugstore and were from familiar manufacturer, other bee products were purchased from commercial sources. Propolis extract was prepared by weighing of 10 g of raw pure propolis (Apipol Farma, Poland) and adding 50 mL of 96% ethanol. Then, left for 48 h in the dark, stirring occasionally. Next, the extract solution was decanted from the solid residue and supernatant was used for further analysis. Dry samples were powdered in agate mortar and then dried at over 70°C for 4 h. Approximately 250 – 500 mg of sample material was weighed and inserted in a high pressure Teflon container and treated with 6 mL of nitric acid. The digestion of the sample was carried out according to the following on the ramp program: 30 min under microwave irradiation (600 W, 60 bar) 20 min under microwave irradiation (400 W, 60 bar) and 15 min cooling time. The propolis extract (5 mL) was prepared by ethanol evaporation and residue was quantitatively transferred to Teflon container using distilled water and digested with the same procedure. The digested sample was placed at the heated plate to let it evaporate and to remove the nitrate. The solutions were cooled to room temperature and transferred quantitatively into volumetric flasks (10 mL) and filled up to the mark with distilled water. All the procedures were repeated three times for each sample.

RESULTS AND DISCUSSION

Influence of DPV parameters on technique of zinc peak

In order to adapt the DP ASV method to nanomolar concentrations, Zn(II) five parameters were optimized: pulse amplitude (ΔE), step potential (E_s), pulse time ($t_p = t_w$ (waiting time) + t_s (current sampling time)) and accumulation potential (E_{acc}) and time (t_{acc}). Consequently, these parameters were investigated. To optimize the conditions for zinc ions measurements, the following instrumental parameters were systematically varied: E_s in the range 1-6 mV; DE in the range 10-100 mV (both positive and negative mode) and t_p from 10 to 60 ms. Changes of the step potential (in the given range) caused an increase of peak current but time caused growth of the background current. Therefore, the step potential of 4 mV was applied in further work. For a pulse amplitude 10 mV, the zinc peak current was equal to 0.05 μA and increased with increasing pulse amplitude. The best results were obtained for an amplitude of 50 mV. Higher pulse amplitude (> 50 mV) caused significant growth of the background current. The increase in pulse amplitude from 10 to 100 mV caused the peak potential to shift from -1036 to -1140 mV and was the same for negative pulse amplitude (not significant differences in peak currents). For further work, the pulse amplitude of 50 mV was applied. The waiting time and probing time were changed in the range from 10 to 50 ms. The change of pulse amplitude has no influence on potential peak and the peak at its half-height. The best result (precision, reproducibility and the signal-to-background current ratio) was obtained for $t_p = 20$ ms ($t_w = t_p = 10$ ms), and this was the value chosen for further work. The influence of the accumulation potential was studied in the range from -1550 to -1150 mV with supporting electrolyte spiked with 10 ng/mL Zn(II). The repeatability and the magnitude of the analytical signal were found to be independent of the accumulation potential in the potential range -1550 to -1300 mV. The accumulation potential -1300 mV was chosen. The accumulation time have been studied in the range of 0 - 90 s. The peak current for supporting electrolyte containing 10 ng/mL Zn(II) increased linearly with the accumulation times. For further study, the accumulation time of 20 s was chosen. The zinc peak potential is not dependent on either the accumulation time and potential.

Analytical performance

The DP ASV voltammograms were ploted in the concentration range 0 - 60 ng/mL and are presented in Figure 1. For a short time of analysis with short preconcentration step, the obtained LOD is 1.62 ng/mL and the linearity is up to 60 ng/mL (slope for regression line is 2.60 ± 0.09 [nA/ng/mL], intercept -3.41 ± 3.56 nA, correlation coefficient 0.9975). The value of LOD was determined as 3 times the standard deviation of the background in the potential region of the peak. For short-term reproducibility of the analytical response for the Hg(Ag)FE electrode is very good (for 10 ng/mL Zn(II) at $t_{acc} = 20$ s is 1 - 2%). The long-term stability (one week) of the Hg(Ag)FE is good. The recovery of zinc was determined using certified reference material: tobacco leaves "Oriental" (CTA-VTL-2) and was ranged from 96.6 to 106.5%. The repeatability of the method at a concentration level of the analyte as low as 3 ng/mL, expressed as RSD is 3.5% (n = 6). The presented results were obtained by subtracting the blank signal.

Bee products

Samples were analyzed according to the described procedure using the Hg(Ag)FE electrode and were performed using the standard addition method. Results for zinc content of selected bee products are presented in Table 1. The highest zinc concentration was found in raw propolis, 69.18 mg/kg, whereas the lowest was found in its ethanol extract 0.52 mg/L. Other products were similar considering the zinc level. The obtained results were comparable with the other study (14, 17) and

Table 1. Zinc content in analyzed bee products (n = 3)

Sample	Zn(II) [mgkg^{-1}] ± SD	RSD [%]
Royal Gelly®	6.40 ± 0.22	3.44
Propolis Plus®	6.23 ± 0.29	4.65
Honeydew	5.69 ± 0.20	3.51
Propolis	69.18 ± 2.2	3.18
Propolis extract	0.52* ± 0.02	3.85

* mgL^{-1}

were of the same order of magnitude. Typical voltammograms with calibration curve are shown in Figure 2. The investigated samples have been also studied using controlled growth mercury electrode (CGMDE) and results were similar in both cases.

CONCLUSIONS

The main goal of this work was the optimization of the DP ASV method for the determination of Zn(II) ions in bee products using a cylindrical silver based mercury film electrode Hg(Ag)FE, refreshed before each measurement. The optimal experimental variables as well as accumulation parameters were investigated as: potential increment, 4 mV; time of potential increment, 20 ms; scan pulse amplitude, 50 mV and an accumulation potential at -1300 mV (t_{acc} = 20 s) using a 0.05 M KNO_3 solution as a supporting electrolyte. The proposed method is characterized by low instrumental and analysis costs (ten times lower) and yielding comparable results with ICP-MS (1), AAS (24) and ICP-OES methods (23, 25). The reproducibility of the method is very good, i.e., when measured as RSD is 3.5% (with each measurement performed at a fresh surface of the working electrode). The limit of detection (LOD) was 1.62 ng/mL. Acceptable recovery shows that the proposed method can be used for the determination of Zn(II) with the same efficacy like voltammetric method (with hanging mercury drop electrode) but with lower mercury consumption. Moreover, the obtained results confirm that in the future the method may be incorporated into out-of laboratory sensor systems.

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