Development of an electroanalytical method for the determination of lead in Argentina raw propolis based on bismuth electrodes

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ABSTRACT

Propolis is a honeybee product which contains lead as one of the main contaminants. Taking into account that this product is used in the food industry, the Código Alimentario Argentino establishes maximum allowable level for raw propolis. Therefore, in this study an electroanalytical method to determine this metal in Argentina raw propolis samples was developed. A bismuth film electrode plated on a modified glassy carbon electrode was prepared. The use of these electrodes has been proposed as an alternative to mercury film electrodes in electroanalytical chemistry due to low toxicity of bismuth (environmentally friendly metal). The method presents a linear range between 1 and 50 μg L^{-1} of Pb(II). The limits of detection and quantification were 0.6 μg L^{-1} and 1.84 μg L^{-1}, respectively. The reproducibility and repeatability calculated as the relative standard deviations were 7.4% and 7.0%, respectively. The sample throughput was 21 sample h^{-1}. Argentina raw propolis samples were analyzed and some of them showed high levels of lead. The obtained results with this method are in good agreement with that obtained with the ICP-AES technique. Moreover, it is a good alternative to the quality control of raw propolis.

1. Introduction

Propolis (registration number chemical abstracts service (CAS) 9009-62-5) is an awfully complex resinous substance produced by bees (*Apis mellifera*) from plant exudates, beeswax, mechanical impurities and salivary secretions. The bees use propolis as a sealer for cracks and crevices of the hive, smooth out the inner walls and as a means of preventing decomposition of dead intruder animals [1]. The composition of raw propolis varies according to the local flora used by bees as resin sources, climatic conditions, season of the year and other constituents such as wax, pollen, essential oils, organic compounds and impurities. Propolis contains a great variety of chemical compounds especially flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones, sesquiterpene quinones, coumarins, steroids, amino acids and inorganic compounds. The color of propolis can be ocher, red, brown or green and presents a rubbery or elastic appearance [2,3].

This product of the hive is not used in Argentina and is generally discarded by the beekeeper. In other parts of the world (Brazil, Cuba, Japan, Romania, and Russia), it is highly appreciated and required for its extraordinary properties. Preliminary studies indicate that the Argentina propolis has good quality [4]. However, its use is limited, being marketed as raw material to Italy, USA, Belgium, Spain, Japan, Uruguay and Brazil [5,6].

Taking into account that raw propolis is used in medicine and food industry due to its biological and pharmacological effects [7], the level of contaminants must be low. This product can be contaminated through different sources such as air, water, plants and soil, therefore it is a bio-indicator of pollution [8,9]. One of the main pollutants of propolis is lead, a toxic heavy metal, which can come mainly from motor traffic. Usually, lead is not transported by plants [8]. In Argentina, since 2008 the Código Alimentario Argentino incorporates propolis in their regulation, including technical specification and physicochemical requirements and establishes a maximum lead concentration of 2.0 mg kg^{-1} for raw propolis [10].

In the literature there are studies that provide information related to lead contamination of propolis [6,8]. However, there are few researches about lead determination as contaminant in this sample. Sales et al. [6] carried out three propolis harvest method, in order to demonstrate that the lead content in propolis depends on the procedure performed. The authors employed electrothermal atomic absorption spectrometry and UV–vis spectrophotometry methods to evaluate the lead level. Cantarelli et al. performed chemical characterization of Argentina propolis from mineral contents by Neutron Activation Analysis. However, the authors did not report information about the lead concentration [11]. In the AOAC method to determine lead in food, ammonium citrate

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solution is added to the sample in a first step. Then, the lead is extracted with dithizone in chloroform forming a lead–dithizone complex that absorbs at 510 nm [12]. This method uses a liquid–liquid extraction and large amounts of hazardous organic solvents. Moreover, it has the disadvantage of being laborious, time consuming procedure and numerous mistakes can occur during this stage.

Considering the productive potential of raw propolis in Argentina and its therapeutic properties, it is necessary to verify the existence of contaminants. Electrochemical techniques are a good option to carry out the determination of pollutants, especially lead in propolis. The voltammetric technique presents the analytical advantages of high degree of accuracy, precision, sensitivity and selectivity. Moreover, measurements are carried out in a rapid analysis time and the instrumentation used is less expensive than spectrometric techniques [13,14]. In the last years, bismuth-based electrodes have increasingly become a very attractive system for the determination of trace metals through anodic stripping voltammetry (ASV) in a wide variety of samples of different origins. Nowadays, the use of bismuth-coated electrode is an alternative to mercury in electroanalysis due its low toxicity [15–17]. Several analytical methods are available to analyze the level of lead in water samples employing bismuth electrodes [18,19]. However, we have not found any report about their application to complex samples as propolis.

The aim of this work is to determine lead level in Argentina raw propolis to assure the safety and quality of this product for marketing in national and international markets. For this purpose an anodic stripping voltammetry and bismuth electrodes prepared in situ on modified glassy carbon electrodes were used. The electrode modification was carried out with Nafion (NA) and multi-wallet carbon nanotubes (MWCNT). Nafion improves the detection sensitivity under certain conditions and increases the electrode's resistance [20]. On the other hand, MWCNT can enhance the electrocatalytic activity and minimize the fouling on the surface of electrodes [21]. In this study, a MWCNT/NA coated glassy carbon electrode was optimized for anodic stripping voltammetry detection of lead. Effect of potential interferences, such as zinc and copper usually present in raw propolis could be eliminated through the variation of experimental conditions. The method was tested successfully on propolis samples obtained from different localities of the Province of Buenos Aires, Argentina and the results were compared with those of inductively coupled plasma atomic emission spectrometry (ICP-AES).

2. Materials and methods

2.1. Propolis origin

Propolis samples, produced by *A. mellifera* honeybees, were collected from September to May in several beekeeping situated at different localities in the Province of Buenos Aires, Argentina, i.e. Bahía Blanca, Tornquist, Hilario Ascasubi and Ciudad Autónoma de Buenos Aires. In order to harvest the bees' propolis, plastic netting meshes were placed on the top of the hive. To insulate the hive, the bees seal the slots in the mesh with propolis. Finally, the nets were removed from the hive and frozen to promote propolis removal.

2.2. Sample treatment

Propolis removed from the meshes must be conditioned for later analysis. The insect parts and other easily visible debris must be eliminated. Then, to determine metals in these samples, a mineralization treatment was carried out. For this purpose an appropriate amount of propolis was weighted and calcined in furnace at 400 °C to white ashes. Then, the ashes were taken up with 20% (v/v) nitric acid solution with heating in a hot plate, filtered and diluted with water to 25.0 mL [5].

2.3. Reagents and solutions

All reagents were of analytical grade and ultra pure water (18 MΩ) was used. 0.9216 g L^{-1} bismuth (III) standard stock solution was prepared by dissolving an appropriate amount of Bi(NO$_3$)$_3$·5H$_2$O (99.999% Sigma-Aldrich) in 5 mL of 20% (v/v) nitric acid and taken up to 25.0 mL with water. 0.0922 g L^{-1} bismuth (III) working solution was prepared by a suitable dilution of the standard stock solution. 2000 mg L^{-1} lead stock solution was prepared by dissolving an appropriate amount of Pb(NO$_3$)$_2$ (Merck) in 20% (v/v) nitric acid and taken up with water. 20 mg L^{-1} lead working solution was prepared by a proper dilution of the standard stock solution. An acetate buffer solution (0.1 mol L^{-1}, pH 4.5) was employed as supporting electrolyte. Nafion, a perfluorinated ion-exchange resin, (5%, w/v solution in a mixture of water and lower alcohols) was purchased from Aldrich. A 0.5% (v/v) ethanolic Nafion solution (NA) was prepared by diluting 100 μL of Nafion with ethanol (Merck p.a.). Multi-wallet carbon nanotubes (MWCNT) were purchased from Aldrich (inner diameter 110–170 nm and 5–9 μm of length). N-N-dimethylformamide (DMF) and potassium hexacyanoferrate(II) were provided from Aldrich.

2.4. Instrumentation

Voltammetric measurements were performed using an Epsilon potentiostat (BASi—Bioanalytical System, USA) and run with an electrochemical analysis software. A three-electrode configuration consisting of MWCNT/DMF/NA modified glassy carbon electrode, Ag/AgCl (3 M NaCl), and a platinum foil as working, reference, and counter electrodes, respectively, were used. A C3 cell stand (BASi-Bioanalytical System, USA) was used for all the stripping analysis. For ICP-AES measurements a Shimadzu 9000 was used.

2.5. Preparation of MWCNT/DMF/NA suspension

In order to prepare MWCNT/DMF/NA suspension in a first stage, 2 mg of MWCNT was mixed with 1 mL of DMF and sonicated in an ultrasonic bath for 30 min and stirred every 5 min on vortex for 10 s. Then, 50 μL of MWCNT/DMF suspension was added to 450 μL of NA and performed the same procedure of sonication. All dispersions were conducted in duplicate.

2.6. Preparation of the MWCNT/DMF/NA coated glassy carbon electrode (MWCNT/DMF/NA/GC)

The glassy carbon electrode surface was polished manually with a 1 μm, 0.3 μm and 0.05 μm alumina, respectively, and sonicated in water during 30 s and then dried with nitrogen. Then, the electrode surface was coated with 10 μL of MWCNT/DMF/NA suspension and allowed to evaporate at room temperature.

2.7. Procedure for square wave anodic stripping voltammetry measurements (SWASV)

In order to determine lead in propolis samples, a pre-treatment step was necessary to stabilize the surface of the modified electrode before each measurement. This step involved cyclic voltammograms from −1.40 V to 0.20 V with a scan rate of 0.100 V s^{-1} in pH 4.5 acetate buffer solution. SWASV measurements were carried out in pH 4.5 acetate buffer solution containing 1000 μg L^{-1} of Bi(III). The experiments were performed with an in situ deposited bismuth film on MWCNT/DMF/NA/GC (BiFE) with a deposition time of 120 s at −1.40 V, under conditions of forced convection (stirring). Then, the stirring was stopped and after 10 s the voltammogram was recorded in square-wave voltammetric mode between −1.40 and 0.20 V. Other experimental parameters were: step potential, $\Delta E_s = 2$ mV; frequency, $f = 25$ Hz and amplitude of the square wave, $\Delta E_{sw} = 40$ mV. After a series of measurements, the

bismuth film and the residual metals were removed by applying 0.20 V during 30 s under stirring conditions. All the measurements were carried out at room temperature in non-deaerated solutions.

3. Results and discussion

The SWASV analytical characteristics of BiFE in the absence and presence of lead by in situ plating bismuth film for trace metal determination are shown in Fig. 1.

The stripping voltammograms were checked in a solution containing 30 µg L^{-1} of Pb(II) and 1000 µg L^{-1} Bi(III) in pH 4.5 acetate buffer. Very well defined peaks at about -0.60 V and -0.12 V for lead and bismuth, respectively, were found. The influence of the concentration of Bi(III) on the stripping peak of Pb(II) was studied in the range from 200 to 2000 µg L^{-1}, each containing 30 µg L^{-1} of Pb(II) with a deposition time, $t_d = 120$ s. As shown in Fig. 2, the height of the Pb(II) stripping peaks displayed a clear dependence on the Bi film thickness as they increased with increasing concentrations of bismuth ions from 200 to 1000 µg L^{-1}.

However, for Bi(III) concentrations higher than 1000 µg L^{-1}, the stripping peak currents of Pb(II) decreased gradually and peaks became wider, which is probably due to the fact that thick bismuth film may hinder the mass transfer of metal ions during the stripping step [22]. Therefore, the optimized concentration of bismuth was chosen as 1000 µg L^{-1}.

3.1. Analytical parameters

Fig. 3a displays square wave anodic stripping voltammograms and Fig. 3b shows the corresponding correlation plot for increasing concentrations of Pb(II) obtained from a BiFE under optimal conditions.

As it is shown, the resulting calibration plot is linear over the 1–50 µg L^{-1} range for Pb(II). The slope of the Pb(II) curve on the BiFE correlation plot was 0.135 µA L/µg^{-1}. The calibration equation and the correlation coefficient for the linear fitting were $I_{p,n} = (-0.021 \pm 0.034) + (0.135 \pm 0.007)$ c^*_{Pb}, r = 0.9963. The detection and quantification limits were 0.6 µg L^{-1} and 1.84 µg L^{-1}, estimated as 3 and 10 times $S_{y/x}$/slope, respectively [23]. Limit of detection obtained is lower than those found in the literature, i.e. 18 µg L^{-1} on µ-NP/BiFE and 29 µg L^{-1} on BiFE [24], 1.3 µg/L^{-1} on CNT screen-printed electrodes [21], comparable to others i.e. 0.95 µg L^{-1} on OMC-NA coated GCE [25], and also slightly higher than others [26]. The reproducibility of BiFE was evaluated by running 6 calibration curves obtained from 6 dispersions prepared in a similar way on different days. The repeatability was calculated as the percentage variation of slopes of 6 calibration curves obtained from a given dispersion. Then, the percent relative standard deviations were 7.4% and 7.0%, respectively. The sample throughput was 21 h^{-1}. The results
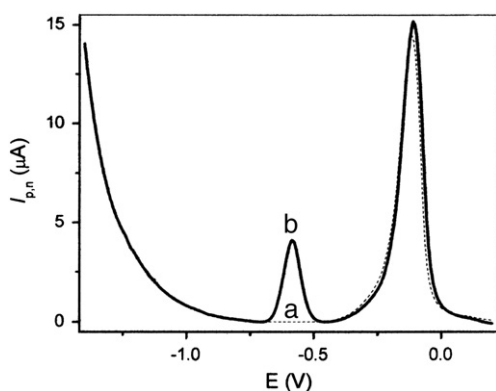

Fig. 2. Effect of the Bi(III) bulk concentration on the stripping peak of Pb(II) (30 µg L^{-1}). Conditions as in Fig. 1.

demonstrate that the BiFE shows a good performance. Statistical parameters are depicted in Table 1.

3.2. Interferences

Main existing interferences of inorganic species found in raw propolis, such as Zn(II) and Cu(II) were investigated to test the selectivity of BiFE. The cations to be tested were introduced into a solution of 30 µg L^{-1} Pb(II). Zn(II) yielded a peak at about -1.18 V as shown in Fig. 4. Zn(II) had no effect up to about 300 µg L^{-1}. Then, the signal of Pb(II) decreases smoothly. At a 900 µg L^{-1} level, a diminution on Pb(II) peak height of about 5% was found (see Fig. 4).

In literature, concentrations of Zn(II) up to 17 times larger than Pb(II) in propolis samples [20], so that the proposed method appears satisfactory under these conditions.

Fig. 1. SWASV voltammograms of 1000 µg L^{-1} Bi(III) in pH 4.5 acetate buffer in the absence (a) and presence (b) of 30 µg L^{-1} Pb(II). Experiments were performed on an in situ deposited bismuth film. Deposition time, $t_d = 120$ s at -1.40 V with stirring. $\Delta E_s = 2$ mV, f = 25 Hz, $\Delta E_{sw} = 40$ mV.

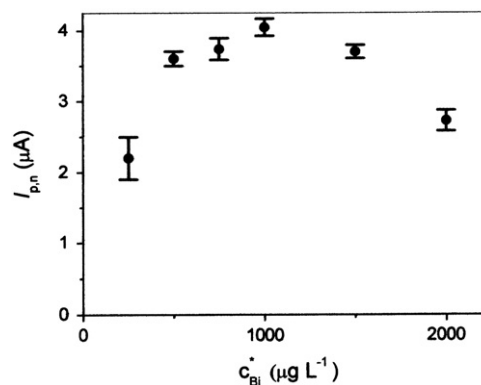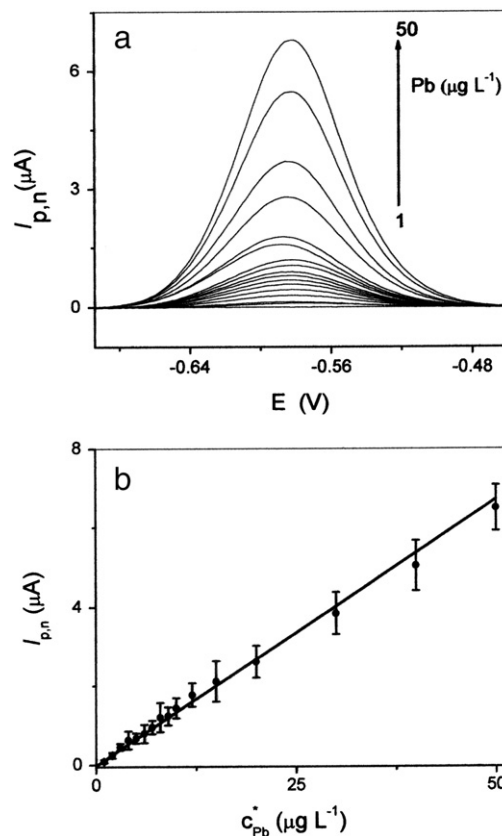**Fig. 3.** a) Square wave anodic stripping voltammograms for Pb(II) at BiFE in the 1–50 µg L^{-1} concentration range. b) Corresponding calibration curve. $c^*_{Bi(III)} = 1000$ µg L^{-1}, pH 4.5 acetate buffer solution. SWASV conditions same as in Fig. 1.

Table 1
Analytical parameters.

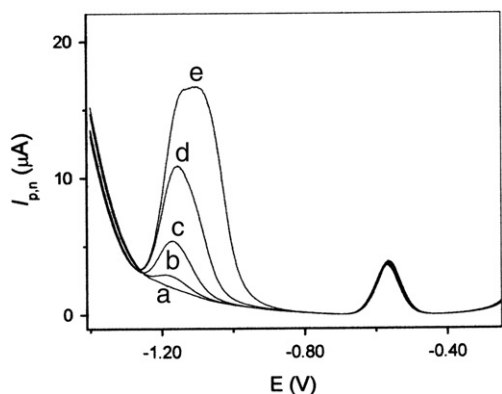| Linear range	LOD[a]	LOQ[b]	Reproducibility[c]	Repeatability[d]	Sensibility
(μg L^{-1})	(μg L^{-1})	(μg L^{-1})	(n = 6)	(n = 6)	(μA L μg^{-1})
1–50	0.60	1.84	7.4%	7.0%	0.135

[a] LOD: limit of detection.
[b] LOQ: limit of quantification.
[c] Calculated as the percentage variation of slopes of 6 calibration curves obtained from 6 dispersions prepared in a similar way.
[d] Calculated as the percentage variation of slopes of 6 calibration curves obtained from a given dispersion.

On the other hand, Cu(II) yielded a peak at 0.0 V (Fig. 5a). Cu(II) is commonly found in propolis and other environmental samples and usually acts as a major interference in the determination of lead. Hexacyanoferrate(II) has been proved to be a good masking agent to mask copper and minimize its effect as interference in the determination of lead [20]. Fig. 5a shows square wave anodic stripping voltammograms in the absence and in the presence of Cu(II) and hexacyanoferrate(II). The effect of increasing concentrations of Cu(II) in the amplitude of the current peak of Pb(II) is shown in Fig. 5b. As it can be observed, its interference effect is tremendous.
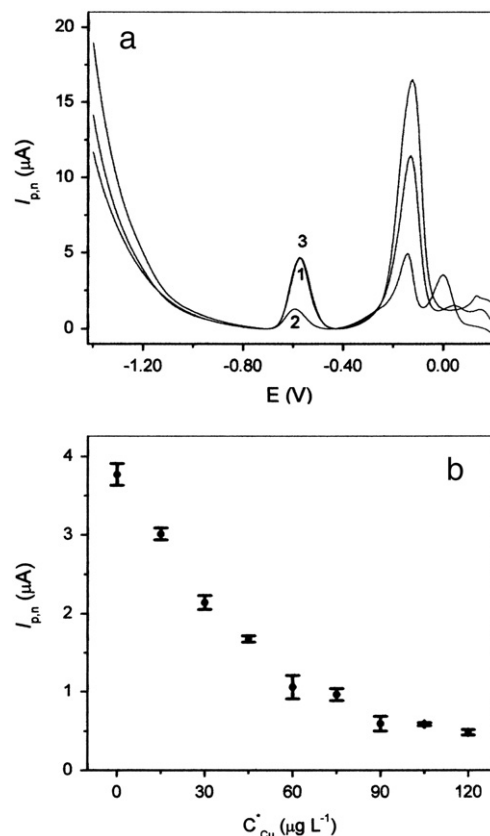
Hexacyanoferrate(II) was found to have no effect on the peak current of lead to concentrations in the order of 0.05 mmol L^{-1} (results not shown). However, as shown in Fig. 5a, small amounts of hexacyanoferrate(II) (about 1 mmol L^{-1}) are sufficient to mask completely the copper at the concentrations used in this work. We found that a reaction time of about 10 min is sufficient to mask the copper present in the samples of propolis. Thus, as it is shown in Fig. 5a, the original signal of 30 μg L^{-1} Pb(II) is recovery in the presence of 120 μg L^{-1} Cu(II) after addition of 1 mmol L^{-1} hexacyanoferrate(II).

3.3. Application to propolis samples

The BiFE prepared as previously described was used for the determination of Pb(II) in propolis samples. Four different raw propolis samples were analyzed with the proposed method. The values of Pb(II) concentration obtained are shown in Table 2. As can be seen, only M4 sample showed a Pb(II) concentration under the maximum permitted by the Código Alimentario Argentino (2.0 mg kg^{-1} for raw propolis) [10]. On the other hand, lead content in samples M1, M2 and M3 exceeds about 4, 5 and 0.5 times the maximum allowed. These results show differences between lead levels obtained from apiaries near and far industrial zones and vehicular traffic. M1 sample was collected from an apiary located in Bahía Blanca city, near the industrial zone. M2 and M3 samples were collected from apiaries in an area

Fig. 5. a) SWASV of Bi(III) in pH 4.5 acetate buffer containing 1000 μg L^{-1} Bi (III) + 30 μg L^{-1} of Pb(II) (curve 1); 1000 μg L^{-1} Bi(III) + 30 μg L^{-1} of Pb(II) + 120 μg L^{-1} Cu(II) (curve 2) and 1000 μg L^{-1} Bi(III) + 30 μg L^{-1} of Pb(II) + 120 μg L^{-1} Cu(II) + 1 mmol L^{-1} hexacyanoferrate(II) (reaction time: 10 min) (curve 3). b) Decrease in the peak current of Pb(II) with increasing Cu(II) concentration in the absence of hexacyanoferrate(II).

around Tornquist near national route 33 (RN33) and in an area around Hilario Ascasubi near route 3 (RN3) respectively. M4 sample was obtained from an apiary far away industrial zone and motor traffic, near Ciudad Autónoma de Buenos Aires.

The lack of certified reference materials of propolis with lead can be attributed to the difficulty of obtaining a stable and homogeneous matrix for its certification. Therefore, the obtained results were validated with inductively coupled plasma atomic emission spectrometry (ICP-AES). Then, the values of Pb(II) in raw propolis are compared with those obtained by ICP-AES as shown in Table 2. The results obtained by the proposed method were plotted versus the ICP-AES values. The identity line gives a linear correlation with an intercept of −0.02 μg L^{-1}, a slope of 1.035, and a correlation coefficient r = 0.995. Therefore, there is no significant difference between the results obtained by both methods. From these results, we can conclude

Fig. 4. Effect of the addition of Zn(II) on the electrochemical response of 30 μg L^{-1} Pb(II). c*$_{Zn(II)}$ (μg L^{-1}): a) 0; b) 30; c) 90; d) 300; and e) 900. Experimental conditions are the same as in Fig. 1.

Table 2
Analysis of real samples.

Samples	Pb(II) (mg kg^{-1})		Relative error %
	Proposed method[a]	ICP-AES[a]	
M1	7.81 ± 0.03	7.69 ± 0.02	+ 1.56
M2	10.13 ± 0.03	9.94 ± 0.03	+ 1.86
M3	3.18 ± 0.01	2.94 ± 0.01	+ 8.16
M4	1.70 ± 0.02	1.89 ± 0.02	− 10.05

Samples were taken from: M1—Bahía Blanca, M2—Tornquist, M3—Hilario Ascasubi, and M4—Ciudad Autónoma de Buenos Aires.
[a] Samples were analyzed in triplicate.

that the as-prepared electrode could provide a satisfactory method for the determination of lead ions in propolis samples.

4. Conclusions

An electroanalytical method for the determination of lead in Argentina raw propolis samples was developed. In order to achieve this goal, a bismuth electrode prepared in situ onto a modified glassy carbon electrode was developed. A suspension of multi-wallet carbon nanotubes, N-N-dimethylformamide and Nafion was used for this modification in order to improve the electrochemical sensitivity.

A linear response was observed from 1 to 50 $\mu g\,L^{-1}$. The proposed method is selective, and presents good reproducibility (7.4%) and repeatability (7.0%). The sample throughput was 21 h^{-1} and the detection limit was 0.60 $\mu g\,L^{-1}$. This methodology regarding the AOAC method is simple, low cost, low consumption of reagents and provides results in considerably less time. Moreover, ICP-AES technique requires high cost instrumentation and employs higher amounts of reagent and sample than the proposed electroanalytical method.

By using this method, it is possible to minimize the use and generation of hazardous substances, which produces less chemical waste than classical methods. The bismuth film is a good alternative as a suitable material to replace the often used mercury electrodes. Besides, bismuth can be considered a "green" element, with very low toxicity.

Four Argentina raw propolis samples were analyzed and the obtained results were validated using the ICP-AES technique, showing good agreement between them.

This method is a very good alternative to carry out the quality control of raw propolis and can be seen as a promising methodology for achieving green chemistry goals.

Acknowledgments

References

[1] M.I. Nieva Moreno, M.I. Isla, A.R. Sampietro, M.A. Vattuone, Comparison of the free radical-scavenging activity of propolis from several regions of Argentina, J. Ethnopharmacol. 71 (2000) 109–114.
[2] V. Francine de Castro Ishida, G. Negri, A. Salatino, M.F.C.L. Bandeira, A new type of Brazilian propolis: prenylated benzophenones in propolis from Amazon and effects against cariogenic bacteria, Food Chem. 125 (2011) 966–972.
[3] D. Valencia, E. Alday, R. Robles-Zepeda, A. Garibay-Escobar, J.C. Galvez-Ruiz, M. Salas-Reyes, M. Jiménez-Estrada, E. Velazquez-Contreras, J. Hernandez, C. Velazquez, Seasonal effect on chemical composition and biological activities of Sonoran própolis, Food Chem. 131 (2012) 645–651.
[4] L.L. Chaillou, M.A. Nazareno, Bioactivity of propolis from Santiago del Estero, Argentina, related to their chemical composition, LWT, J. Food Sci. Technol. 42 (2009) 1422–1427.
[5] E. Bedascarrasbure, L. Maldonado, W. Fierro Morales, A. Alvarez, Propóleos. Caracterización y normalización de propóleos Argentinos, 1th ed Magna, Argentina, 2006.
[6] A. Sales, A. Álvarez, M. Rodriguez Areal, L. Maldonado, P. Marchisio, M. Rodríguez, E. Bedascarrasbure, The effect of different propolis harvest methods on its lead contents determined by ET AAS and UV–vis, J. Hazard. Mater. 137 (2006) 1352–1356.
[7] R.A. Laskar, I. Sk, N. Roy, N. Ara Begum, Antioxidant activity of Indian propolis and its chemical constituents, Food Chem. 122 (2010) 233–237.
[8] S. Bogdanov, Contaminants of bee products, Apidologie 37 (2006) 1–18.
[9] M.E. Conti, F. Botrè, Honeybees and their products as potential bioindicators of heavy metals contamination, Environ. Monit. Assess. 69 (2001) 267–282.
[10] Código Alimentario Argentino, Chapter XIV, 3th ed., De La Canal y Asoc. SRL, Buenos Aires, Argentina, 2008.
[11] M.Á. Cantarelli, J.M. Camiña, E.M. Pettenati, E.J. Marchevsky, R.G. Pellerano, Trace mineral content of Argentinean raw propolis by neutron activation analysis (NAA): assessment of geographical provenance by chemometrics, LWT, J. Food Sci. Technol. 44 (2011) 256–260.
[12] AOAC – Association of Official Analytical Chemists, Official Methods of Analysis, 15th ed. AOAC International, Gaithersburg, 1993 Methods 934.07.
[13] C. Locatelli, G. Torsi, Voltammetric determination of ultra-trace total mercury and toxic metals in meals, Microchem. J. 78 (2004) 175–180.
[14] C. Locatelli, D. Melucci, Simultaneous square wave anodic stripping voltammetric determination of Cr, Pb, Sn, Sb, Cu, Zn in presence of reciprocal interference: application to meal matrices, Food Chem. 130 (2012) 460–466.
[15] D. Demetriades, A. Economou, A. Voulgaropoulos, A study of pencil-lead bismuth-film electrodes for the determination of trace metals by anodic stripping voltammetry, Anal. Chim. Acta 519 (2004) 167–172.
[16] I. Svancara, C. Prior, S.B. Hocevar, J. Wang, A decade with bismuth-based electrodes in electroanalysis, Electroanalysis 22 (2010) 1405–1420.
[17] F. Arduini, J.Q. Calvo, A. Amine, G. Palleschi, D. Moscone, Bismuth-modified electrodes for lead detection, TrAC, Trends Anal. Chem. 29 (2010) 1295–1304.
[18] D. Pan, L. Zhang, J. Zhuang, T. Yin, W. Lu, W. Qin, On-line determination of lead in tap waters at two-step prepared bismuth electrode, Int. J. Electrochem. Sci. 6 (2011) 2710–2717.
[19] L. Chen, Z. Su, X. He, Y. Liu, C. Qin, Y. Zhou, Z. Li, L. Wang, Q. Xie, S. Yao, Square wave anodic stripping voltammetric determination of Cd and Pb ions at a Bi/Nafion/thiolated polyaniline/glassy carbon electrode, Electrochem. Commun. 15 (2012) 34–37.
[20] K. Crowley, J. Cassidy, Trace analysis of lead at a Nafion-modified electrode using square-wave anodic stripping voltammetry, Electroanalysis 14 (2002) 1077–1082.
[21] G.H. Hwang, W.K. Han, J.S. Park, S.G. Kang, Determination of trace metals by anodic stripping voltammetry using a bismuth-modified carbon nanotube electrode, Talanta 76 (2008) 301–308.
[22] L.Y. Cao, J.B. Jia, Z.H. Wang, Sensitive determination of Cd and Pb by differential pulse stripping voltammetry with in situ bismuth-modified zeolite doped carbon paste electrodes, Electrochim. Acta 53 (2008) 2177–2182.
[23] J.C. Miller, J.N. Miller, Estadística para química analítica, 2th ed. E.U.A.: Addison-Wesley Iberoamericana, S.A., Wilmington, Delaware, 1993 Chapter 5.
[24] J. Saturno, D. Valera, H. Carrero, L. Fernandez, Electroanalytical detection of Pb, Cd and traces of Cr at micro/nano-structured bismuth film electrodes, Sens. Actuators B 159 (2011) 92–96.
[25] L.D. Zhu, C.Y. Tian, R.L. Yang, J.L. Zhai, Anodic stripping voltammetric determination of lead in tap water at an ordered mesoporous carbon/Nafion composite film electrode, Electroanalysis 20 (2008) 527–533.
[26] D. Li, J. Jia, J. Wang, Simultaneous determination of Cd(II) and Pb(II) by differential pulse anodic stripping voltammetry based on graphite nanofibers—Nafion composite modified bismuth film electrode, Talanta 83 (2010) 332–336.