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Determination of heavy metals in honey by potentiometric stripping analysis and using a continuous flow methodology

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Abstract

A methodology for the determination of Zn(II), Cd(II) and Pb(II) directly in dissolved honey samples by potentiometric stripping analysis with a flow cell is proposed. Heavy metals in honey are of interest not only for quality control, but can be used also as an environmental indicator. In this work honey samples were collected in different places of Burgos (Spain). Lead (II) and cadmium (II) can be directly determined. It is necessary to avoid the Zn–Cu interference for zinc (II) determination by adding Ga(III) in the adequate concentration, which has been calculated by using an experimental design methodology. The results were compared with inductively coupled mass plasma spectrometry as reference method.

Keywords: Heavy metals; Wall-jet cell; Potentiometric stripping analysis; Honey

1. Introduction

Honey, a viscous and aromatic product prepared by bees mainly from nectar or flowers or honey dew (Dustmann, 1993), is an excellent and widely used food that is popular all over the world. In addition, the biological role of honey intake may be important in cases of heavy metal contamination. Honey can characterise the level of soil, plant and air pollution (Jones, 1987) in an area of some square kilometres.

Considered as an analytical sample, honey is one of the most complex mixtures of carbohydrates produced in nature. In common honey, mono- and disaccharides constitute 80–85% (w/w), water is around 15–20% (w/w) and other organic compounds and inorganic ions being present to a minor extent (Sanna, Pilo, Piu, Tapparo, & Seeber, 2000). However, the minor components are often of great importance from many points of view. The evaluation of heavy metals content in honey has a twofold significance: the former one lies in the toxicity of theses metals, with the consequent necessity to develop adequate analytical procedures for their monitoring; the latter one is suggested by the possibility of using bees and their products as bioindicator of possible environmental pollution (Tong, Morse, Bache, & Lisk, 1975), taking advantage of both the large covering area where they live and of the concentration effect of the possibly present environmental pollutants into the "products" of bees (Bromenshenk, Carlson, Simpson, & Thomas, 1985; Bromenshenk, Gudatis, Cronn, & Olbu, 1988; Leita, Muhlbachova, Cesco, Barbattini, & Mondini, 1996; Rowarth, 1990). The mineral content of honey is recognised as an environmental indicator at least since 1984, when Crane published the first data on metals content in honey collected near or far from highways (Buldini, Gavalli, Mevoli, & Sharma, 2001).

Several techniques were proposed to determine metallic impurities in honey, but in most cases, a matrix mineralization is required. The largely preferred analytical

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approaches to the determination of transition metals in honey are spectroscopy techniques (Caroli, Forte, Iamiceli, & Galoppi, 1999; Chung & Tsai, 1992; Fodor & Molnar, 1993; Herrero & Peña, 1993; Jones, 1987; Rodriguez-Otero, Paseiro, Simal, Terradillos, & Cepada, 1992; Scheubeck, Nielsen, & Iwantscheff, 1979; Stein & Umland, 1986; Viñas, Lopez-Garcia, Lanzon, & Hernandez-Cordoba, 1997). For metal ion analysis, electrochemical techniques are potentially the cheapest and quickest method of carrying out a determination, that is, when they are compared with instrument techniques such as atomic absorption spectroscopy and inductively coupled plasma (Bersier, Howell, & Bruntlett, 1994). Recent papers show stripping techniques (Buldini et al., 2001; Li, Wahdat, & Neeb, 1995; Nanos & Karayannis, 1991; Sanna et al., 2000) to determinate heavy metals in honey. Depending upon the type of technique, they can be very specific and extremely sensitive.

The purpose of the present paper is to describe a method that allows for the direct determination of the heavy metals in honey (a matrix with high contents of sugars and low in metals), i.e., without digesting and pretreating the samples. A laboratory-made flow-cell and potentiometric stripping analysis (PSA) have been used as detection technique with a working electrode consisted of a mercury film electrode. Which was formed on the electrode simultaneously with the metals electrodeposition (Muñoz, Palmero, & García-García, 2002).

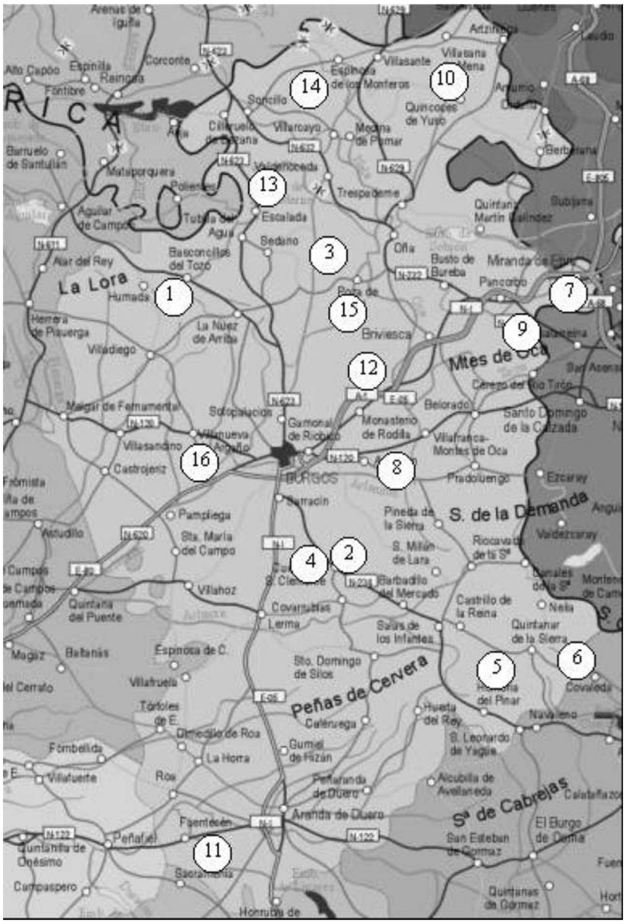

Fig. 1. Location map of sample sites.

2. Experimental

2.1. Reagents and solutions

All the solutions were prepared with high purity deionised water (Milli Q, Millipore instrument). The metal standard solutions were prepared using their nitrates salts (Zn(II), Cd(II) and Pb(II)) and Hg(II) solution by dissolving the nitrate in nitric acid. The reagents used were of "Suprapur" and P.A. (Merck) and "Chemika" (Fluka) quality.

High purity nitrogen was necessary for the prior deoxygenation of the sample in order to displace oxygen from it.

2.2. Sample preparation

The samples of honey were collected in different places of Burgos (Spain) (Fig. 1), and stored in plastic bottles in order to prevent contamination from metal or glass. The sample preparation consisted of dissolving honey (between 28 and 36 g L^{-1} depending of the sample density) directly in water and acidifying to a pH value of 1 with concentrated HCl. Solution also contained 1×10^{-4} mol L^{-1} Hg(II) because a mercury film was formed on the electrode simultaneously with the metals electrodeposition (Muñoz et al., 2002).

The samples honey for inductively coupled plasma mass spectroscopy, ICP-MS, (Agilent Technologies 7500i ICP-MS) were prepared in 1% HCl.

2.3. Instrumentation and procedure

The equipment comprised a computer-controlled potentiostat (PSTAT 10, Eco Chemie/Autolab, Utrecht, The Netherlands), a flow cell made in the laboratory (Muñoz et al., 2002; Palmero, Muñoz, & García-García, 2000) with a "wall-jet" configuration and a three-electrode system and a Miniplus 3 peristaltic pump (Gilson S.A., France) equipped with polyvinyl chloride tubing of appropriate diameter (0.2–4 mm). The glassy carbon disk working electrode is a commercial electrode (3 mm diameter); the reference electrode of Ag/AgCl and the counter electrode were made in the laboratory (Muñoz et al., 2002).

The potentiometric measurements were taken using the following procedure: once the solution has been deoxygenated, the electrodeposition began according to a time and potential determined for each experiment.

As solution contained 1×10^{-4} mol L^{-1} Hg(II), the mercury film and metal electrodeposition were carried out simultaneously over the working electrode.

$$Hg^{2+} + M^{n+} + (n+2)e^{-} \rightarrow Hg(M) \text{ (Electrochemical process)}$$

When the time had elapsed, the electric circuit is interrupted and a chemical oxidant such Hg(II), which remains in the dissolution, will establish the reoxidation.

$$Hg(M) - ne^{-} \rightarrow Hg + M^{n+} \text{ (Chemical process)}$$

The change in the electrode potential, E, over time, t, during the reoxidation process is monitored. A serie of plateau is obtained in function of reoxidated metal and their characteristic potentials (Fig. 2). A differential stripping potentiogram can be obtained by differentiating the analytical signal t with respect to the variable E and plotting dt/dE vs. E (Christensen, Kelding, Kryger, Rasmussen, & Skov, 1981) (Fig. 3). Integration of the dt/dE function between two plateau values of the potential yields to the total stripping period, A_p, in s/V for the metal investigated. Ion concentrations are linearly related to this area, which will be useful in the calibration step. Metal concentrations were calculated by applying the standard addition method over each sample.

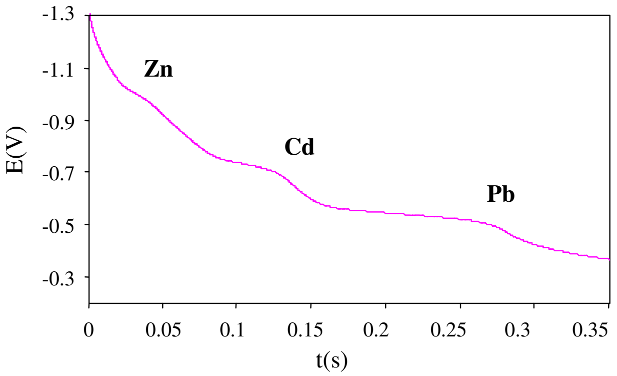

Fig. 2. Stripping potentiogram for a honey solution containing 1×10^{-4} mol L^{-1} of Hg(II) and 0.1 mol L^{-1} of HCl. Experimental conditions: $E_d = -1.3$ V, $t_d = 240$ s and $v_{flow} = 1.13$ mL min^{-1}.

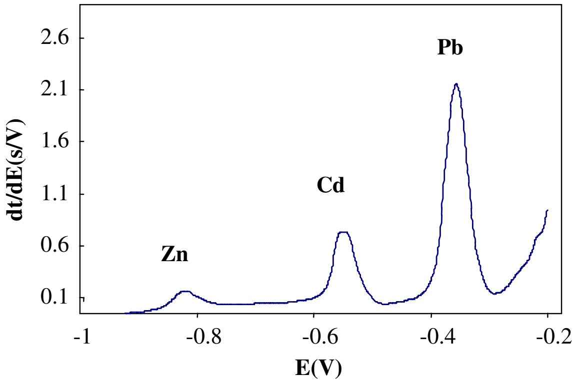

Fig. 3. Differential stripping potentiogram for a honey solution containing 1×10^{-4} mol L^{-1} of Hg(II) and 0.1 mol L^{-1} of HCl. Experimental conditions: $E_d = -1.3$ V, $t_d = 240$ s and $v_{flow} = 1.13$ mL min^{-1}.

The electrodeposition potential was obtained in previous work ($E_d = -1.3$ V) (Muñoz et al., 2002) and the electrodeposition time depends of metal concentration in honey samples.

Lead (II) and cadmium (II) were simultaneously determined and for zinc ions was necessary gallium (III) addition, because of Cu–Ga is more stable than Cu–Zn intermetallic compound, thus allowing zinc to be oxidized without interferences.

Copper concentration is not determined in this work because a signal appears overlapped with the peak corresponding to the copper ions oxidation. Interfere peak must be due to a very fast and strong adsorption of one or more compounds present in honey since it appears even if small times are used (Bousemart, Menarguy, & Benaim, 1993; Khoulif, Jambon, Chatelut, & Vitton, 1995). Determination of this metal will be object of a later work by using statistical analysis.

3. Results and discussion

A two part experiment was carried out. In the first step addition of gallium (III) nitrate leads to the determination of zinc concentration without interferences and, in the second step, cadmium and lead ions are directly measured after electrodeposition step.

3.1. Determination of Zn(II)

The zinc determination in honey samples was carried out in a solution containing 1×10^{-4} mol L^{-1} Hg(II), 0.1 mol L^{-1} HCl and 3×10^{-7} mol L^{-1} Ga(III).

The parameters that had more influence in the process of the zinc determination, calculated in a previous works (Muñoz et al., 2002), were the electrodeposition potential (E_d), the flow rate (v_{flow}) and the gallium (III) concentration ($c_{Gallium}$). Electrodeposition potential was fixed in -1.3 V and electrodeposition time, t_d, was chosen depending on the concentration of metal in each sample (between 240 and 400 s).

After previous experiments, the following experimental conditions were finally chosen

$$c_{Gallium} = 3.00 \times 10^{-7} \text{ mol L}^{-1}, \quad v_{flow} = 1.13 \text{ mL min}^{-1}.$$

Obviously, copper concentration can change in different honey samples. In this case, copper concentration (obtained by inductively coupled plasma mass spectroscopy) presents values between 0.8 and 2.4 mg kg^{-1} (less than an order) and the obtained results can be applied for all the samples.

The results have been compared with those obtained by ICP-MS (Table 1).

Table 1
Concentration of zinc ions for honey samples with the two methods ($N = 4$)

Zinc	$\bar{x}_{\text{ICP-MS}}$[a] (mg kg^{-1} honey)	$\bar{x}_{\text{flow system}}$[b] (mg kg^{-1} honey)	$S_{\text{flow system}}$[c] (mg kg^{-1} honey)	$S_{\text{ICP-MS}}$[d] (mg kg^{-1} honey)	t_{exp}[e]
Sample 1	0.284	0.282	0.009	0.017	0.233
Sample 2	0.333	0.325	0.012	0.010	1.222
Sample 3	0.419	0.340	0.019	0.021	1.965
Sample 4	0.160	0.150	0.004	0.002	5.680
Sample 5	0.386	0.379	0.006	0.014	1.227
Sample 6	2.720	2.620	0.462	0.294	0.3737
Sample 7	0.507	0.484	0.045	0.068	0.682
Sample 8	0.480	0.471	0.035	0.028	0.418
Sample 9	0.458	0.456	0.060	0.037	0.064
Sample 10	0.401	0.392	0.015	0.011	0.962
Sample 11	0.390	0.386	0.0109	0.029	0.420
Sample 12	0.934	0.936	0.007	0.032	0.311
Sample 13	–	–	–	–	–
Sample 14	0.097	0.116	0.028	0.001	1.273
Sample 15	2.197	2.212	0.082	0.152	0.229
Sample 16	0.247	0.250	0.008	0.009	0.663

[a] Mean of four measurements by ICP-MS.
[b] Mean of four measurements by flow system.
[c] Standard deviation of measurements by flow system.
[d] Standard deviation of measurements by ICP-MS.
[e] t_{exp} show the experimental student-t value. t value from the table for ($n_1 + n_2 - 2$) degrees of freedom at the 95% confidence level is 2.262.

For comparing the results of this flow system with those of an established procedure (ICP-MS), we applied the Student t test to compare the two sample means. It can be concluded from Table 1 that there is no significant difference between the results obtained by the two methods.

3.2. Determination of Cd(II) and Pb(II)

The study was performed in a solution which contained 1×10^{-4} mol L^{-1} Hg(II) and 0.1 mol L^{-1} HCl. Electrodeposition potential was fixed in −1.3 V (Muñoz et al., 2002), and a $v_{\text{flow}} = 1.13$ mL min^{-1} was used as above.

Under these conditions, by using an electrodeposition time between 280 and 400 s (depending of honey sample), metal concentrations was calculated by using the addition standard method.

Results can be seen in Tables 2 and 3, following the same statistical treatment as for the zinc. Statistical agreement was observed in the range of values obtained by the two methods.

Table 2
Concentration of cadmium ions for honey samples with the two methods ($N = 4$)

Cadmium	$\bar{x}_{\text{ICP-MS}}$[a] (μg kg^{-1} honey)	$\bar{x}_{\text{flow system}}$[b] (μg kg^{-1} honey)	$S_{\text{flow system}}$[c] (μg kg^{-1} honey)	$S_{\text{ICP-MS}}$[d] (μg kg^{-1} honey)	t_{exp}[e]
Sample 1	1.430	1.200	0.05	0.110	4.670
Sample 2	1.580	1.460	0.09	0.090	2.280
Sample 3	1.590	1.890	0.12	0.400	1.920
Sample 4	0.450	0.460	0.06	0.090	0.370
Sample 5	0.780	0.810	0.14	0.130	0.260
Sample 6	1.170	1.300	0.50	0.150	0.510
Sample 7	0.640	0.670	0.16	0.130	0.290
Sample 8	0.880	0.890	0.13	0.130	0.080
Sample 9	0.810	0.890	0.15	0.090	0.990
Sample 10	0.630	0.560	0.03	0.070	2.190
Sample 11	0.150	0.170	0.02	0.020	1.460
Sample 12	0.450	0.430	0.14	0.080	0.250
Sample 13	0.070	0.080	0.02	0.010	0.620
Sample 14	0.240	0.240	0.01	0.020	0.510
Sample 15	0.840	0.830	0.06	0.060	0.340
Sample 16	0.120	0.120	0.02	0.020	0.560

[a] Mean of four measurements by ICP-MS.
[b] Mean of four measurements by flow system.
[c] Standard deviation of measurements by flow system.
[d] Standard deviation of measurements by ICP-MS.
[e] t_{exp} show the experimental student-t value. t value from the table for ($n_1 + n_2 - 2$) degrees of freedom at the 95% confidence level is 2.262.

Table 3
Concentration of lead ions for honey samples with the two methods ($N = 4$)

Lead	$\bar{x}_{\mathrm{ICP\text{-}MS}}$[a] (mg kg^{-1} honey)	$\bar{x}_{\mathrm{flow\ system}}$[b] (mg kg^{-1} honey)	$S_{\mathrm{flow\ system}}$[c] (mg kg^{-1} honey)	$S_{\mathrm{ICP\text{-}MS}}$[d] (mg kg^{-1} honey)	t_{exp}[e]
Sample 1	0.023	0.023	0.001	0.001	0.826
Sample 2	0.048	0.051	0.002	0.003	1.746
Sample 3	0.045	0.044	0.001	0.001	0.960
Sample 4	0.013	0.037	0.001	0.0005	0.050
Sample 5	0.013	0.014	0.0001	0.001	0.001
Sample 6	0.013	0.021	0.001	0.002	0.421
Sample 7	0.011	0.012	0.0011	0.001	3.908
Sample 8	0.010	0.014	0.002	0.0011	2.220
Sample 9	0.010	0.013	0.0003	0.001	0.803
Sample 10	0.004	0.010	0.001	0.0004	0.031
Sample 11	0.004	0.004	0.001	0.0004	0.030
Sample 12	0.035	0.040	0.0011	0.001	0.403
Sample 13	0.005	0.005	0.0002	0.001	1.248
Sample 14	0.004	0.004	0.0001	0.001	0.852
Sample 15	0.025	0.030	0.003	0.004	0.105
Sample 16	0.121	0.127	0.002	0.007	2.131

[a] Mean of four measurements by ICP-MS.

[b] Mean of four measurements by flow system.

[c] Standard deviation of measurements by flow system.

[d] Standard deviation of measurements by ICP-MS.

[e] t_{exp} show the experimental student-t value. t value from the table for ($n_1 + n_2 - 2$) degrees of freedom at the 95% confidence level is 2.262.

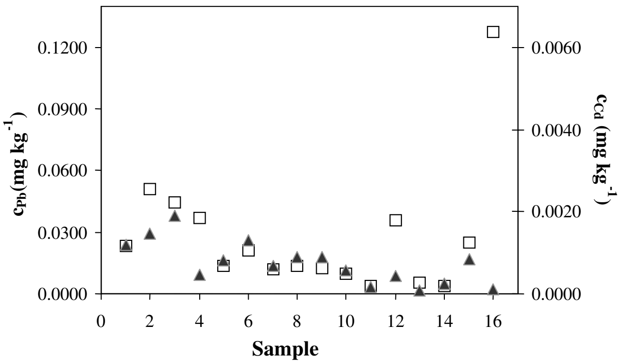

Fig. 4. Correlation data between Cd ▲ and Pb □.

All these samples present concentration values lower permitted limits, but ranged over two o three orders of magnitude.

Fig. 4 shows the correlation data between Cd(II) and Pb(II) concentrations for the most of the samples. However it can be observed that samples 4, 12 and 16 differ significatively from this behaviour. The high contents in lead ions in these samples are related with proximity of honeycombs to major roads.

4. Conclusion

The proposed method allows the fast determination of the concentration of heavy metals in free-digestion samples of honey. The results present concordance with those obtained by inductively coupled plasma mass spectroscopy.

This method can be applied to characterize the level of metal contamination in honey from different matrix like pollen, bees, plants, etc., with the adequate treatment.

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