On-Line Preconcentration/Determination of Lead Traces in Bee Honey by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Using a Conical Minicolumn Packed with Activated Carbon

Soledad Cerutti [a]; Ricardo Fernández Orsi [a]; José A. Gásquez [a]; Roberto A. Olsina [ab]; Luis D. Martinez [ab]
[a] Department of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, Chacabuco and Pedernera, National University of San Luis, San Luis, Argentina [b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad de Buenos Aires, Argentina

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INSTRUMENTATION, METHODOLOGY,
AND TECHNICAL DEVELOPMENTS

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Soledad Cerutti,[1] Ricardo Fernández Orsi,[1] José A. Gásquez,[1]
Roberto A. Olsina,[1,2] and Luis D. Martinez[1,2,*]

[1]Department of Analytical Chemistry, Faculty of Chemistry,
Biochemistry and Pharmacy, National University of San Luis,
Chacabuco and Pedernera, San Luis, Argentina
[2]Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET), Ciudad de Buenos Aires, Argentina

ABSTRACT

In this study, the preconcentration of Pb^{2+} using a conical minicolumn packed with activated carbon (AC) was studied for preconcentration of Pb^{2+}. The experiments showed that Pb^{2+} reacts with 8-hydroxyquinoline to form a complex at pH 10.0 and can be retained "on-line" on a conical minicolumn packed with activated carbon, then eluted with 20% (v/v) nitric acid. The conditions for the quantitative and reproducible preconcentration and subsequent ICP-OES determination with ultrasonic nebulization system (USN) were studied. A total enhancement factor (N) of 500 was obtained with respect to ICP-OES using pneumatic nebulization (10 for USN and 50 for enrichment factor) for a

*Correspondence: Luis D. Martinez, Department of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Chacabuco and Pedernera, P.O. Box 375, 5700 San Luis, Argentina; E-mail: ldm@unsl.edu.ar.

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sample volume of 25 mL. The detection limit (DL) was $0.04\,\mu g\,L^{-1}$. The relative standard deviation (RSD) were 3.0%. The optimized procedure was successfully applied to the determination of trace levels of Pb^{+2} in bee honey samples.

Key Words: Lead determination; On-line preconcentration; Activated carbon; Bee honey.

INTRODUCTION

Bee honey is an excellent and widely used food that is popular all over the world. It can be consumed as its, as a sugar substitute or in confectionery industries.

In addition, bee honey have been used as monitors of a variety of environmental contaminants, including trace metals,[1–3] low level radioactivity and pesticides.[3–7]

It is well known that lead is a very abundant heavy metal,[8] it represents a potential problem not only because of its widespread distribution throughout the environment, but also because of its toxicity.[9]

Lead contents in bee honey may vary within a broad range and it is possible to find very low concentration levels in the order of a few $ng\,g^{-1}$.[10]

Some authors, such as Caroli[10] found lead concentrations so low as $0.0032\,\mu g\,g^{-1}$. Therefore, in order to determinate Pb in bee honey at such low concentration levels, sensitive analytical techniques are required to obtain low detection limits.

In the past years, several methods have been developed for the determination of Pb at low concentrations, among them, inductively coupled plasma mass spectrometry (ICP-MS)[11], and atomization atomic absorption spectrometry[2,7].

Within the last decade, ICP-MS has proved ideally suited as an alternative approach for the determination of lead because of its high sensitivity, high selectivity and high sample throughput, however, the cost of instrumentation may be prohibitive to many laboratories.

Inductively coupled plasma optical emission spectrometry (ICP-OES) is widely recognized as a suitable technique for the determination of trace elements.[12,13]

On the other hand, if inductively coupled plasma optical emission spectrometry (ICP-OES) is used, the low level of lead concentration in bee honey is not compatible with the determination limit of this technique. In order to achieve accurate, reliable and sensitive results, preconcentration is needed when the concentrations of the analyte elements in the original material or the prepared solution are too low to be determinated directly by ICP-OES.

Since its introduction in analytical chemistry, activated carbon (AC) has been used as a collector, permitting the preconcentration and separation of low levels of analytes in several matrices, such as high-purity substances, water and rocks.

The mechanism of sorption on AC is still under investigation. The most common models used for heavy metal adsorption are the classic empirical adsorption (e.g. Langmuir and Freundlich equations). The major advantage of these models is their simplicity; however, the models fail to describe accurately the adsorption equilibrium under varying conditions, such as pH and ionic strength. The adsorption

equilibrium studies have revealed that pH is the dominant parameter controlling the adsorption.[14]

However, it was verified that in the presence of a chelating or precipitating agent, metal retention can be improved. Therefore, trace level of lead have been determined using pyrocatechol viole,[15,16] anthranilic acid,[17] O,O-diethyl-dithiophosphate,[18] potassium ethyl xanthate,[19] potassium xanthogenate,[20] 1,2-Ciclohexanediondioxima,[21] 8-hydroxyquinoline[22] as complexing reagents.

In this work, a simple method using 8-hydroxyquinoline as the complexing agent to preconcentrate lead ''on-line'' from bee honey samples at low concentration levels on activated carbon is proposed.

The determination was performed using USN-ICP-OES associated with flow injection (FI) methodology.

MATERIALS AND METHODS

Apparatus

The measurements were performed with a sequential inductively coupled plasma spectrometer [BAIRD (Bedford, MA, USA) ICP 2070]. The 1m-Czerny Turner monochromator had a holographic grating with 1800 grooves mm^{-1}. The ICP operating conditions are listed in Table 1. The measurements were performed with a Shimadzu AA-6800 atomic absorption spectrometer equipped with graphite furnace and a pyrolitic graphite tube with a L'vov platform. A lead hollow cathode lamp was used as the radiation source at a wavelength of 283.3 nm with a 5 mA lamp current and a 0.7 nm slit-width (low). A deuterium background corrector was also used. A Ultrasonic Nebulizer (U-5000 AT [CETAC Technologies (Omaha, NE, USA)]) with a desolvation system was used. A Minipuls 3 peristaltic pump [Gilson (Villiers, Le-Bell, France)] was used. Sample injection was achieved using a Rheodyne Model 50 4-way (Cotati, CA, USA). A conical minicolumn (40 mm length, 4.5 mm internal upper-diameter and 1.5 mm internal lower-diameter) was used as the activated carbon holder. Pump tubes—Tygon type (Ismatec, Cole-Parmer Instrument Company, Niles, IL, USA) were employed to propel the sample, reagent and eluent.

Table 1. ICP-OES instrumental parameters employed to lead determination.

Forward power	1.0 kW
RF generator	40.68 MHz
Nebulizer	Ultrasonic
Coolant gas flow rate	8.5 l min^{-1}
Auxiliary gas flow rate	1.0 l min^{-1}
Carrier gas flow rate	0.5 l min^{-1}
Solution uptake rate	1.5 mL min^{-1}
Observation height (above load coil)	15 mm

The 220.353 nm spectral line was used and measurements of the FI system were expressed as peak-height, which was corrected against the reagent blank.

Reagents

The activated carbon (Merck, Darmstadt, Germany, 50–70 mesh) was used after pretreatment with acid [activated carbon was heated with 10% (v/v) hydrochloric acid for 30 min and then with 10% (v/v) nitric acid for 20 min and finally washed with deionized water until neutral pH was reached].

Lead standard solutions was prepared by appropriate dilutions of a $1000\,mg\,L^{-1}$ stock solution (Merck, Darmstand, Germany) immediately before use.

8-hydroxyquinoline ($10^{-2}\,mol\,L^{-1}$) was prepared by dissolution of 8-hydroxyquinoline (Merck, Darmstadt, Germany) in ethanol. Lower concentrations were prepared by serial dilution. Buffer solution was prepared by diluting a $5.0\,mol\,L^{-1}$ ammonium hydroxide solution adjusted to pH 10.0 with acetic acid. Ultrapure water ($18\,M\Omega\,cm^{-1}$) was obtained from an EASY pure RF (Barnstedt, Dubuque, IA, USA). All other solvents and reagents used were of analytical-reagent grade or better, and the presence of lead was not detected in the working range.

Column Preparation

The conical minicolumn was prepared by replacing 100 mg of activated carbon into an empty column using the dry packing method. To avoid loss of activated carbon when the sample solution passes through the conical minicolumn, a small amount of quartz wool was placed at both sides of the conical minicolumn. The column was then connected to a peristaltic pump with PTFE tubing to form the preconcentration system.

Sample Pretreatment

Each bee honey pot was heated in a water bath to about $40^{\circ}C$ so that the bee honey flowed easily enough. To 2.5 g of bee honey, 10 mL of perclohoric acid concentrated and 20 mL of nitric acid were added and the sample was digested by heating the mixture until dryness. The residues were dissolved in 1 mL of nitric acid $2.5\,mol\,L^{-1}$ plus 2 mL of water. The solution was heated to boiling and diluted with water to 25 mL.
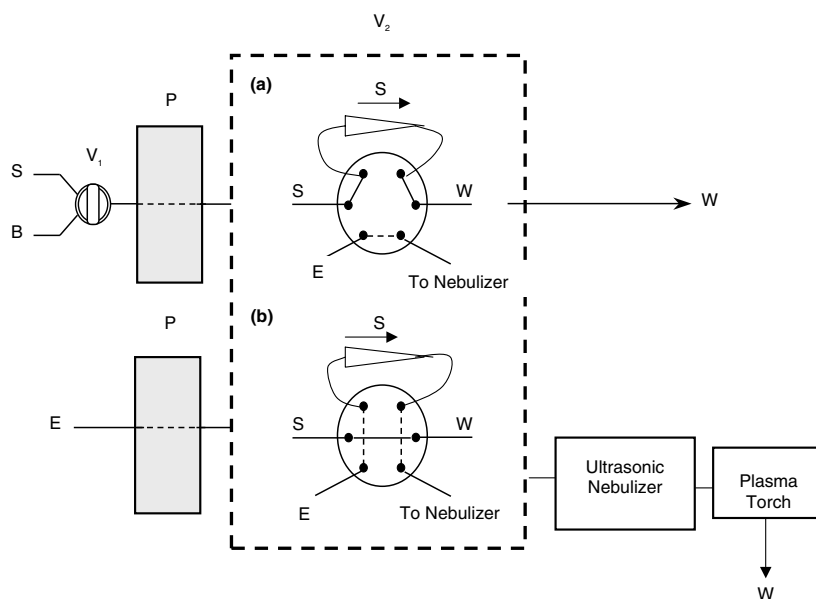
The sample pretreatment takes approximately 40 min. However, it must be taken into account that it is possible to simultaneously treat as many samples as can be placed in the water bath.

Preconcentration Procedure

Before loading, the column was conditioned for preconcentration at the correct pH with the buffer diluted (1:10) solution, valve V_1 in position B (Fig. 1).

Figure 1. Schematic diagram of the instrumental setup. S, sample (flow rate: $15\,mL\,min^{-1}$); E, eluent (flow rate: $1.5\,mL\,min^{-1}$); A, Ar (flow rate: $0.7\,l\,min^{-1}$); W, waste; P , peristaltic pump; C, minicolumn packed with activated carbon; V_2, injection valve. Valve positions: (a) sample loading; (b) injection.

The complex was then loaded on the activated carbon at a flow rate of $15\,mL\,min^{-1}$ with valve V_1 in position S and valve V_2 in load position (a).

Finally, peristaltic pump P was stopped and the injection valve V_2 was switched on to the injection position (b) and the retained metal complex was eluted with 20% (v/v) nitric acid directly in USN-ICP-OES. The operating system measurements were expressed as peak-height emission, which was corrected against the reagent blank.

RESULTS AND DISCUSSION

The preconcentration of lead on activated carbon from bee honey samples was necessary because its concentration can be too low to be compatible with ICP-OES detection limit. This preconcentration, performed prior to the ICP-OES measurement, permitted to obtain accurate and precise analytical results.

The ultrasonic nebulization system, coupled to the preconcentration system, allowed the lowering of the ICP-OES detection limits to values compatible with the lead content in the bee honey.

Effect of pH

In order to optimize the sorption conditions for the retention of the metal complex on activated carbon, the lead signal was monitored by measuring it with
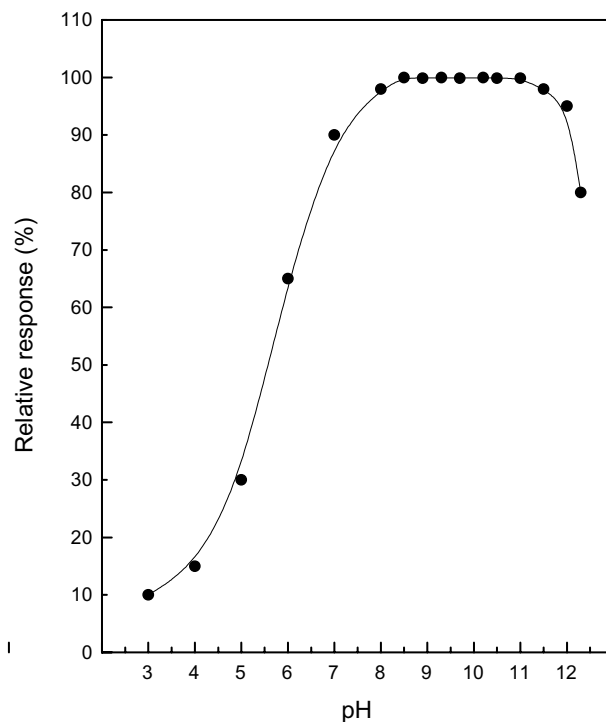
Figure 2. Dependence of Pb-8-hydroxyquinoline preconcentration on pH of loading solutions. Sample loading volume 25 mL; loaded flow rate was $15 \, \text{mL min}^{-1}$; the elution flow rate was $1.5 \, \text{mL min}^{-1}$; Pb concentration was $5.0 \, \mu\text{g L}^{-1}$; 8-hydroxyquinoline concentration was $4 \times 10^{-4} \, \text{mol L}^{-1}$.

ICP-OES while changing the pH of the solution that passes through the conical minicolumn packed with activated carbon. Figure 2 shows that the optimal pH values were in the range of 8.5–11.0. This phenomenon is understandable, since a better complexation occurs within this range. Accordingly, the selected pH was 10.0.

Effect of Sample Loading Rate

The flow rate sample through the column packed with AC is a very important parameter, since this is one of steps that controls the time of analysis.

We could verify that with flow rates up to $15 \, \text{mL min}^{-1}$ there is no effect on the analyte recovery, which in optimum conditions is 100%. Figure 3 shows that at higher flow rates the recovery decreases.

We must emphasize that the analyte retention factors for other reported "on-line" preconcentration systems using minicolumns packed with different sorbents, is lower than 90% for all cases.[23,24]
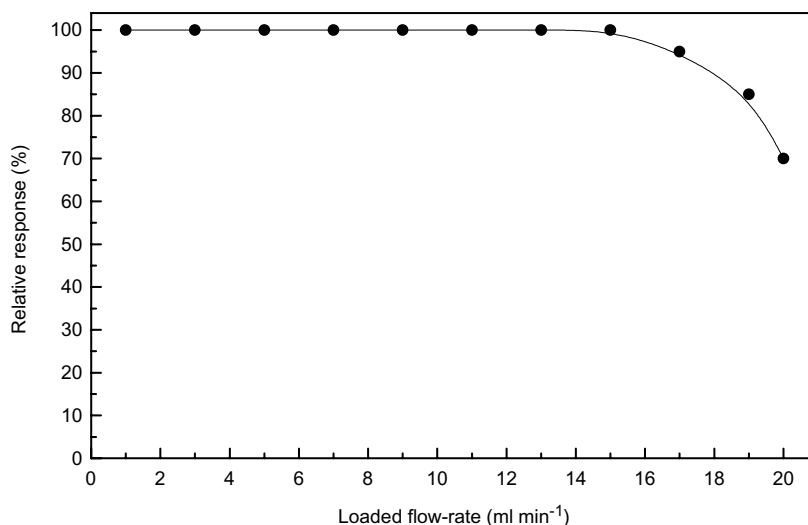
Figure 3. Dependence of recovery of metal complex on sample flow rate.

Effect of 8-Hydroxyquinoline Concentration

As regards the response variation with the molar concentration of the reagent 8-hydroxyquinoline, the signal remained constant between 10^{-5} and 10^{-3} mol L^{-1}. A 4×10^{-4} mol L^{-1} 8-hydroxyquinoline concentration was adopted for the following experiments.

Elution of Pb-8-Hydroxyquinoline Complex

In order to elute Pb-8-hydroxyquinoline complex adsorbed on the activated carbon, nitric acid was adopted as eluent. The complex retained was completely eluted with 20% (v/v) HNO$_3$. The optimun eluent flow rate was 1.5 mL min^{-1}.

Effect of Column Design

The column design strongly influences the performance of preconcentration systems[25]. The proposed method was applied to a classical column −3.0 mm internal diameter and a conical minicolumn −4.5 mm internal upper-diameter and 1.5 mm internal lower-diameter; both columns packed with the same activated carbon mass.

From our studies, we could verify that the conical minicolumn performance was much better than that from the classical column, the improvement being of 120%.

Effect of Size Particles

In the present work a size of activated carbon of 50–70 mesh was considered adequate for the preconcentration procedure in the conical minicolumn. Smaller activated carbon particles could have improve retention capacity, but this would have increased back-pressure of the minicolumn, and the flow rate ought to have been reduced, with the subsequent increase in preconcentration time.

Effect of Interferents

The effects of representative potential interfering species (at the concentrations levels at which the may occur in the sample studied) were also tested. Thus Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} could be tolerated up to at least $20\,\mu g\,g^{-1}$.

Commonly encountered matrix components as alkali and alkaline earth elements generally do not form stable complexes at the working conditions and are not retained on the activated carbon. A possible interfering effect of elements commonly found in honey samples, such as Ca and Mg, may be discarded in the here proposed preconcentration method, since at the selected pH value these elements do not form complexes with 8-hydroxyquinoline and thus are not retained on the AC. The recoveries were not influenced by these ions because they are not complexed with the 8-hydroxyquinoline reagent at the working pH value. Thus, they are not retained on the AC prior to the elution of the retained Pb. The value of the reagent blank signal was not modified by the presence of the potentially interfering ions assayed.

Evaluation of the FI On-Line Preconcentration System Performance

The overall time required for preconcentration of 25 mL of solution (1.66 min, at a flow rate of $15\,mL\,min^{-1}$); washing (0.30 min, at a flow rate of $1.2\,mL\,min^{-1}$); eluting (approx. 0.5 min, at a flow rate of $1.5\,mL\,min^{-1}$) and conditioning (0.4 min, at flow rate of $1.2\,mL\,min^{-1}$) was about 2.86 min, the throughput being about 21 samples per hour. A total enhancement factor of approximately 500-fold (10 for USN and 50 for enrichment factor) and an elevated throughput sample were obtained with the proposed preconcentration system.

The relative standard deviation (RSD) was 3% calculated using the obtained peaks heights. The calibration graph using the preconcentration system for lead was linear with a correlation coefficient of 0.9993 at levels near to the detection limits up to at least $100\,ng\,mL^{-1}$. The detection limit (DL) was calculated as the amount of lead required to yield a net peak equal to three-times the standard deviation of the background signal (3σ). The value of DL obtained for the preconcentration of 25 mL of aqueous solution was $0.04\,\mu g\,L^{-1}$.

Recovery and Validation Studies

In order to evaluate the lead recovery of this method, a 25 g bee honey sample was divided into 10 portions of 2.5 g each. The proposed method was applied to six

Table 2. Recovery study.

Aliquots	Base value ($\mu g\, g^{-1}$)	Quantity of Pb added ($\mu g\, g^{-1}$)	Quantity of Pb found ($\mu g\, g^{-1}$)	Recovery (%)[a]
1	—	0.0	0.52 ± 0.02	—
2	0.52	0.20	0.71	95.0
3	0.52	0.40	0.91	97.5
4	0.52	0.80	1.33	101.2
5	0.52	1.00	1.52	100.0

Table 3. Concentrations of lead in honey samples (95% confidence interval, $n = 6$). The honey samples were collected in the province of La Pampa, Argentina.

Sample	Pb concentration ($\mu g\, g^{-1}$) proposed method	Pb concentration ($\mu g\, g^{-1}$) ETAAS
A	0.52 ± 0.02	0.53 ± 0.02
B	0.70 ± 0.02	0.69 ± 0.02
C	0.18 ± 0.01	0.18 ± 0.01
D	0.34 ± 0.02	0.36 ± 0.02

portions and the average quantify of lead obtained was taken as base value. Then increasing quantities of lead were added to the other aliquots of sample and lead was determined by the same method. The results are shown in Table 2.

Additionally, the proposed method was validated by comparison with electrothermal atomic absorption spectrometry (ETAAS) technique (Table 3).

Determination using ETAAS were performed measuring direct aliquots with previous dissolution of the honey in water. This was possible due to the ETAAS low detection limits for lead.

Lead in Bee Honey Samples

Finally, the results of the method applied to total lead determination in 4 bee honey samples are shown in Table 3. The lead concentrations in bee honey found in this work were in the range 0.18–0.70 $\mu g\, g^{-1}$.

CONCLUSIONS

The connection of an on-line preconcentration system using activated carbon with a FI-ICP-OES coupled to ultrasonic nebulization system increased the speed of

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the preconcentration and analysis process and reduced sample consumption and contamination risks. The system of preconcentration allowed lead determination in bee honey samples in which the concentration was on the order of the $\mu g\,g^{-1}$. A 500-fold enhancement factor with respect to lead determination by ICP-OES without both preconcentration and USN was obtained. The determination showed excellent reproducibility and accuracy.

ABBREVIATIONS

AC	Activated carbon
DL	Detection limit
ETAAS	Electrothermal atomic absorption spectrometry
FI	Flow injection
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
RSD	Relative standard deviation
USN	Ultrasonic nebulization system

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