RESIDUES AND TRACE ELEMENTS

On-Line Preconcentration and Determination of Cadmium in Honey Using Knotted Reactor Coupled to Flow Injection-Flame Atomic Absorption Spectrometry

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An on-line cadmium preconcentration and determination system implemented with flame atomic absorption spectrometry (FAAS) associated with flow injection was studied. Cadmium was retained as Cd-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol Cd-(5-Br-PADAP) complex, pH 9.3. The Cd complex was removed from the knotted reactor (KR) with ethanol. A total enhancement factor of 140 was obtained with respect to FAAS (40 for KR and 3.5 due to the use of ethanol) with preconcentration time of 120 s. The detection limit value for preconcentration of 1 g sample was 0.5 ng/g. The repeatability for 10 replicate determinations at 5.0 ng/g Cd level was 3.5% relative standard deviation, calculated from peak heights obtained. The calibration graph using the preconcentration system for Cd was linear with a correlation coefficient of 0.9990 at levels near the detection limits to at least 2000 ng/g. The method was successfully applied to determination of total Cd in honey samples.

Honey is used worldwide as a basic foodstuff, either by direct ingestion or as a sweetener in a variety of foods. Honeybees cover great distances and contact innumerable surfaces during their foraging activities. In their search for food, bees can transport a variety of contaminants present on the surface of flowers (1). Previous studies have demonstrated that honey, through its elemental concentration, can be used as an indicator of environmental pollution (1, 2). Moreover, when beehives are located in areas of industrial air pollution, the mean content of mineral substances in honey has been calculated to be 0.17%, although this can vary within a wide range (3).

It is well known that cadmium and its compounds are highly toxic even at low concentration levels and may result in bioaccumulation (4). The kidney is a critical target organ for accumulation of Cd, which has a half-life in this tissue of about 30 years (5). Environmental pollution is the main cause of heavy metal presence in the food chain. Processing, packaging, and other technological processes used to bring foods to the consumer can significantly increase the total Cd concentration (6). Cadmium content in honey may vary within a broad range, and it is possible to find concentration levels as low as a few ng/g (2). To determine Cd in honey at these low concentration levels, sensitive analytical techniques are required.

Several methods have been developed for determination of Cd at low concentrations, among them, neutron activation analysis (NAA; 7), inductively coupled plasma-mass spectrometry (ICP–MS; 8), and electrothermal atomic absorption spectrometry (ETAAS; 9). The NAA method is time-consuming, and routine analysis of numerous samples is laborious. This method requires sophisticated instrumentation that may be not available in most analytical laboratories. Within the past decade, ICP–MS has proved ideally suited as an alternative approach for determination of Cd in various matrixes. ICP–MS is used to determine Cd because of its high sensitivity, selectivity, and sample throughput; however, the cost of instrumentation may be prohibitive to many laboratories.

However, flame atomic absorption spectrometry (FAAS), with its relatively low cost and excellent analytical performance, is probably the most widely used technique for determining a variety of metals in foods. However, the low level of Cd concentration in honey is not compatible with the determination limit of this technique. To achieve accurate, reliable, and sensitive results, preconcentrations and separations are needed when analyte concentrations in the original material or the prepared solution are too low to be determined directly by FAAS.

Preconcentration is effective for extending the detection limits of FAAS methods. Many flow injection (FI) separation and preconcentration methods were developed for this purpose, including on-line ion exchange (10), on-line solid extraction (11), and on-line coprecipitation-dissolution (12). The FI associated with FAAS (13, 14) has significantly improved the general drawbacks of batch preconcentration procedures, and on-line preconcentrations can now be achieved almost as efficiently as a simple FAAS determination. Reagent consumption is usually reduced to a few percent of those in-batch

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Table 1. FAAS instrumental parameters used in cadmium determination

Flame type	Air-C_2H_2
Burner height	7 mm
Wavelength	228.8 nm
Slit width	1.0 nm
Lamp current	8 mA
Measurement mode	Height
Air flow rate	8.0 L/min
Acetylene flow rate	1.8 L/min
Sample introduction flow rate	4.5 mL/min

procedures, and requirements of the laboratory environment for trace analysis are much less stringent. Until now, the most dramatic improvements in FI–FAAS have been in on-line preconcentration.

FI on-line preconcentration based on the sorption of metallic complexes on the inner walls of a PTFE knotted reactor (KR) has been used successfully. FAAS is an interesting alternative for coupling with KR for preconcentration and determination of metal traces, because it has the advantage of coupling to systems working on-line. An additional advantage of KR is its low hydrodynamic impedance, which permits high load sample rates and elevated sample throughput.

The most widely used reagents for metal preconcentration in KR have been diethyldithiocarbamate (DDTC; 15, 16) and ammonium pyrrolidine dithiocarbamate (APDC; 16). The reagent 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) has been used in the spectrophotometric determination of numerous metallic ions (17–19) and forms insoluble chelates in the absence of surfactant compounds or organic solvents. Therefore, 5-Br-PADAP could be suitable for preconcentration of cadmium on KR. Its application in preconcentration systems with KR has been published (20, 21).

In the present work, we propose a method for preconcentration and determination of total Cd in different types of honey samples at low concentration levels by using a KR. The Cd was retained in the form of Cd-(5-Br-PADAP) complex; the determination was performed using FAAS associated with an FI methodology.

Experimental

Reagents

(a) *5-Br-PADAP (10^{-2}M) solution.*—Prepared by dissolving 5-Br-PADAP (Aldrich, Milwaukee, WI) in ethanol. Lower concentrations were prepared by serial dilution.

(b) *Cadmium standard solutions.*—Prepared by appropriate dilutions of 1000 mg/L stock solution with 0.1M nitric acid as diluent.

(c) *Buffer solution.*—Prepared by diluting 5.0M ammonium hydroxide solution adjusted to pH 9.3 with HCl solution.

(d) *Ultrapure water (18 MΩ/cm).*—Obtained from EASYpure RF (Barnstedt, Dubuque, IA).

All other solvents and reagents were of analytical-reagent grade or better, and Cd was not detected in the working range.

Instrumentation

(a) *Atomic absorption spectrometer.*—The measurements were made with a Shimadzu AA-6800 spectrometer (Tokyo, Japan) equipped with Cd hollow-cathode lamp. The FAAS instrumental and operating conditions that provided the best sensitivity are listed in Table 1. The FI system used is shown in Figure 1. To validate the method, a Shimadzu AA-6800 atomic absorption spectrometer equipped with graphite furnace and a pyrolitic graphite tube with L'vov platform at 228.8 nm with spectral bandwidth of 1.0 nm was used. A deuterium background corrector was also used.

(b) *Peristaltic pump.*—Minipuls 3 (Gilson, Villiers-le-Bel, France).

(c) *Rotary valve.*—Rheodyne Model 50 4-way (Cotati, CA) was used for sample injection.

(d) *Knotted reactor.*—Composed of 2 m length of PTFE tubing, 0.5 mm id, with interlaced knots of ca 5 mm diameter loops.

(e) *Pump tubes.*—Tygon-type (Ismatec, Cole-Parmer Instrument Co., Niles, IL) were used to propel the sample, reagent, and eluent.

Sample Pretreatment

Each honey pot was heated in a water bath to ca 40°C so that the honey flowed easily. To 2.5 g honey, 10 mL concentrated perclohoric acid and 20 mL nitric acid were added, and the sample was digested by heating the mixture to dryness. The residues were dissolved in 1 mL nitric acid, 2.5M, plus

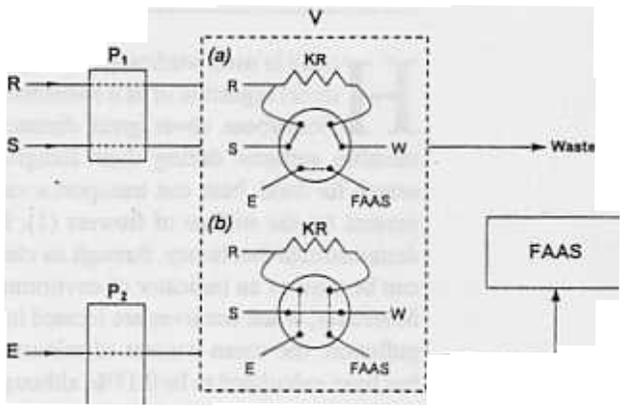

Figure 1. Schematic diagram of instrumental setup. R: 5×10^{-4}M 5-Br-PADAP solution (2.5 mL/min) in 20% (v/v) ethanol solution; S: sample (flow rate: 5.0 mL/min); E: eluent (flow rate: 4.5 mL/min); W: waste; P_1 and P_2: peristaltic pumps; KR: knotted reactor; V: injection valve. Valve positions: (a) sample loading; (b) injection.

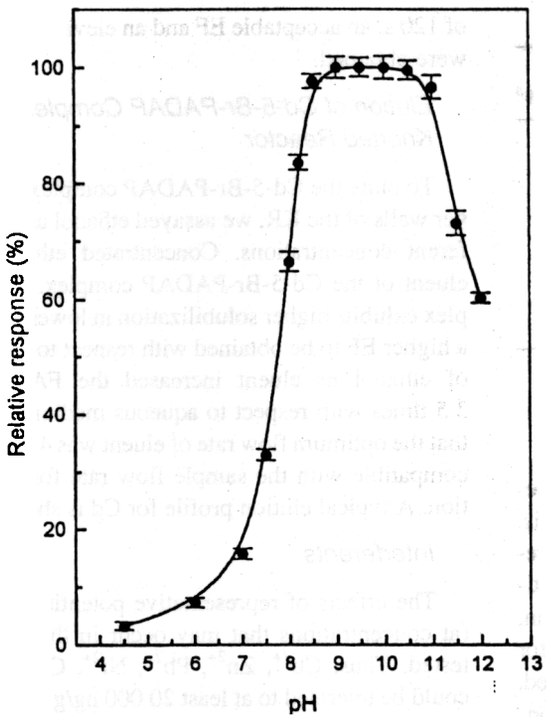

Figure 2. Dependence of Cd-5-Br-PADAP preconcentration on pH of loading solutions. Sample loading time was 120 s; loaded flow rate was 5 mL/min; elution flow rate was 4.5 mL/min; Cd concentration was 20 μg/L; 5-Br-PADAP concentration was 5×10^{-4}M.

2 mL water. The solution was heated to boiling and diluted to 25 mL with water.

The sample pretreatment takes ca 40 min. However, it is possible to simultaneously treat as many samples as can be placed in the water bath.

Preconcentration Procedure

The sample solution containing Cd, at a flow rate of 5.0 mL/min, and 5-Br-PADAP (5×10^{-4}M), at a flow rate of 2.5 mL/min, buffered to pH 9.3 with ammonia–ammonium chloride (1.0M), were mixed on-line to form the metal complex. This mixture was then loaded on the KR for 120 s, valve V in load position (a) (Figure 1). Finally, peristaltic pump P_1 was stopped, the injection valve V was switched on to the injection position (b), and the retained metal complex was eluted with ethanol at a flow rate of 4.5 mL/min, directly in FAAS. The operating conditions were established and the determination was performed. FI system measurements were expressed as peak-height absorption, which was corrected against the reagent blank.

Results and Discussion

The preconcentration of Cd from honey samples was necessary because its concentration can be too low to be compatible with the FAAS detection limit. This preconcentration, performed before the FAAS measurement, permitted us to obtain accurate and precise analytical results.

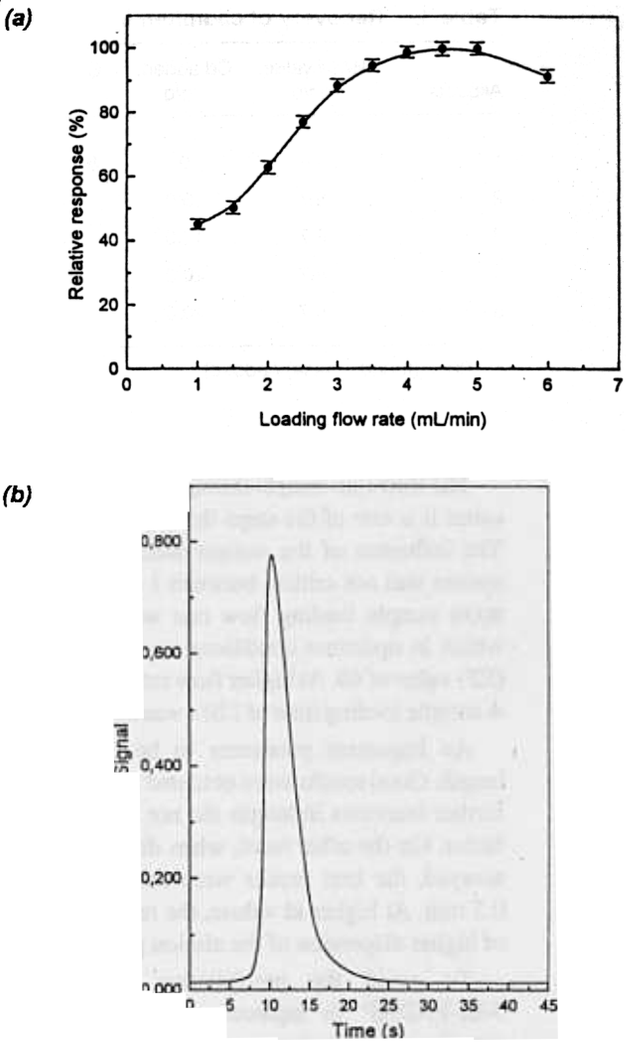

Figure 3. (a) Effect of elution flow rate on performance of reconcentration system. Loaded flow rate was 5 mL/min; Cd concentration was 20 μg/L; 5-Br-PADAP concentration was 5×10^{-4}M. A 2 m KR was used. (b) Peak profile obtained for preconcentration time of 120 s of Cd-5-Br-PADAP complex using system depicted in Figure 1. Loaded flow rate was 5 mL/min; elution flow rate was 4.5 mL/min; Cd concentration was 20 μg/L; 5-Br-PADAP concentration was 5×10^{-4}M in 20% (v/v) ethanol solution.

Influence of Loading Variables

To optimize the sorption conditions for retention of metal complexes, we monitored the Cd signal by measuring it with FAAS while changing the pH of the solution that passes through the KR. Figure 2 shows optimal pH values in the range of 8.5–10.0. This phenomenon is understandable, because a better complexation occurs within this range. The selected pH value was 9.3

Table 2. Recovery of cadmium study

Aliquots	Base value, ng/g	Cd added, ng/g	Cd found, ng/g	Recovery, %[a]
		0	8.7 ± 0.7	
	8.7	5.0	13.4	94
	8.7	10.0	18.6	99
	8.7	20.0	29.1	102
	8.7	40.0	48.8	100

[a] 100 × [(found – base) / added].

The flow rate sample through the KR is very important, because it is one of the steps that controls the time of analysis. The influence of the sample loading rate on analytical response was not critical between 1 and 10 mL/min. The optimum sample loading flow rate was achieved at 5 mL/min, which in optimum conditions reached an enrichment factor (EF) value of 40. At higher flow rates, the response decreased. A sample loading time of 120 s was used for preconcentration.

An important parameter to be optimized was the KR length. Good results were obtained for a length of 200 cm, and further increases in length did not improve the enhancement factor. On the other hand, when different KR id values were assayed, the best results were obtained with a diameter of 0.5 mm. At higher id values, the response decreased because of higher dispersion of the elution profiles.

To avoid the precipitation of reagent complexing 5-Br-PADAP in aqueous medium the solution of the complexing reagent was prepared in the presence of ethanol. With 10% (v/v) ethanol, 5-Br-PADAP remained in solution to concentrations of 10^{-4}M. Subsequently, the influence of ethanol concentration on the preconcentration of complex Cd-5-Br-PADAP was assayed. The preconcentration decreased when ethanol concentrations >35% (v/v) were used in the 5-Br-PADAP solution. This behavior was expected because increasing solvent concentrations leads to higher solubility of the Cd-5-Br-PADAP complex, which hinders its precipitation and adsorption on the KR walls. The optimum load rate of the 5-Br-PADAP solution was 2.5 mL/min. Regarding the response variation with the molar concentration of 5-Br-PADAP, the signal remained constant between 10^{-5}M and at least 10^{-3}M. A 5×10^{-4}M 5-Br-PADAP concentration was adopted for the following experiments.

The flexibility of adopting different sampling loading times to attain different EFs is one of the advantages of the proposed method. The value of EF increased in a relatively linear way throughout a long preconcentration time. This might be explained by assuming that the Cd-5-Br-PADAP precipitated complex is retained in layers on the KR walls after a certain preconcentration time has passed. However, we observed an increase of color intensity of the Cd-5-Br-PADAP complex retained in the first sections of the KR, which would also be indicative of the above observations. With a preconcentration time of 120 s, an acceptable EF and an elevated throughput sample were obtained.

Elution of Cd-5-Br-PADAP Complex from the Knotted Reactor

To elute the Cd-5-Br-PADAP complex retained on the inner walls of the KR, we assayed ethanol and nitric acid at different concentrations. Concentrated ethanol was the best eluent of the Cd-5-Br-PADAP complex. The retained complex exhibits higher solubilization in lower volumes, allowing a higher EF to be obtained with respect to nitric acid. The use of ethanol as eluent increased the FAAS sensibility by 3.5 times with respect to aqueous medium. Figure 3a shows that the optimum flow rate of eluent was 4.5 mL/min, which is compatible with the sample flow rate for FAAS determination. A typical elution profile for Cd is shown in Figure 3b.

Interferents

The effects of representative potential interfering species (at concentrations that may occur in the sample) were also tested. Thus, Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Fe^{3+} could be tolerated to at least 20 000 ng/g. Commonly encountered matrix components such as alkali and alkaline earth elements generally do not form stable complexes and are not retained on the KR. A possible interfering effect of elements, such as Ca and Mg, commonly found in honey, may be discarded in the proposed preconcentration method. At the selected pH value these elements do not form complexes with 5-Br-PADAP and are not retained in the KR walls. Recoveries were not influenced by these ions because they are not complexed with 5-Br-PADAP at pH 9.3. Thus, they were not retained on the KR before elution of the retained Cd. The reagent blank signal was not modified by the presence of the potentially interfering ions assayed.

Evaluation of the Flow Injection On-Line Preconcentration System Performance

The overall time required for preconcentration of 10 mL digestion solution (2.0 min, at a flow rate of 5.0 mL/min), elution (about 0.16 min, at a flow rate of 4.5 mL/min), and washing (0.10 min, at a flow rate of 4.5 mL/min) was about

Table 3. Concentrations of cadmium in honey samples (95% confidence interval, $n = 6$)[a]

Sample	Cd, ng/g	
	ETAAS	Proposed method
A	9.2 ± 0.10	8.7 ± 0.7
B	18.9 ± 0.12	20.2 ± 0.8
C	14.5 ± 0.11	13.4 ± 0.7
D	121.0 ± 0.15	122.1 ± 0.9

[a] Honey samples were collected in the province of La Pampa, Argentina.

2.26 min. Thus, 26 determinations can be realized per hour. An enrichment factor of 40 was obtained with the proposed preconcentration system.

The relative standard deviation for 10 replicates containing 5.0 ng/g Cd was 3.5%. The calibration graph was linear with a correlation coefficient of 0.9990 at levels near the detection limits (DL) to at least 2000 ng/g. The DL was calculated as the amount of Cd required to yield a net peak equal to 3 times the standard deviation of the background signal (3σ). The value of DL obtained for preconcentration of 1 g sample was 0.5 ng/g.

Recovery and Validation Studies

To evaluate the Cd recovery of this method, we divided a 25 g honey sample into 10 portions of 2.5 g each. The proposed method was applied to 6 portions, and the average quantity of Cd obtained was taken as a base value. Increasing quantities of Cd were then added to other sample aliquots, and Cd was determined by the same method (Table 2).

The proposed method was also validated by comparison with the ETAAS technique (Table 3). The results were compared with the *F*-test, and no significant differences at the 95% confidence level were observed. Data were compared through study of the regression between values obtained by ETAAS and those obtained by the proposed method. The regression analysis yielded the following statistics: slope = 1.01, r^2 (squared correlation coefficient) = 0.9996, s (standard deviation) = 1.21. Determinations using ETAAS were performed by measuring direct aliquots with previous dissolution of honey in water. This was possible because of the ETAAS low DLs for Cd.

Cadmium in Honey Bee Samples

The results of the method applied to total Cd determination in 4 honey samples are shown in Table 3. The Cd concentrations in honey found in this work were in the range of 8.7–122.1 ng/g. The results obtained are in good agreement with those found by Jones (22) and Morse and Lisk (1), who reported Cd concentration ranges of 0.3–300.0 and 102–267 ng/g, respectively.

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