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Determination of mercury in biological samples by cold vapor atomic absorption spectrometry following cloud point extraction with salt-induced phase separation

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Abstract

Method development for the pre-concentration of mercury in human hair, dogfish liver and dogfish muscle samples using cloud-point extraction and cold vapor atomic absorption spectrometry is demonstrated. Before the extraction, the samples were submitted to microwave-assisted digestion in a mixture of H_2O_2 and HNO_3. Cloud point extraction was carried out using 0.5% (m/v) ammonium *O*,*O*-diethyldithiophosphate (DDTP) as the chelating agent and 0.3% (m/v) Triton X-114 as the non-ionic surfactant. Phase separation was induced after the addition of Na_2SO_4 to a final concentration of 0.2 mol L^{-1}. Aliquots of the final extract were transferred to PTFE tubes and $NaBH_4$ and HCl were added. The mercury vapor was driven to a non-heated quartz tube for measuring the absorbance. The results obtained with salt-induced phase separation were in good agreement with the certified values at a 95% confidence level. An enrichment factor of 10 allowed a detection limit of 0.4 ng g^{-1} to be obtained, which demonstrates the high sensitivity of the proposed procedure for the determination of mercury at trace levels.

Keywords: Cloud point extraction; Cold vapor atomic absorption spectrometry; Mercury; Biological samples; *O*,*O*-diethyl dithiophosphate; Triton X-114

1. Introduction

Mercury is one of the elements with the highest environmental risk and it is considered a global pollutant, mostly due to its wide use, high toxicity and large distribution. Elemental Hg is used in several applications, such as electrical devices, lamps, batteries and dental amalgam [1,2]. The concentration of Hg in the environment is also due to the combustion of coal and fuel oil, resulting in the emission of considerable amounts of the metal to the atmosphere. The ability of Hg to accumulate in biological tissues might influence the entire food chain [3]. Mercury can also be readily absorbed by the human body when inhaled by mucous membranes, damaging mainly the central nervous system [1,3].

Due to the usually low concentration of Hg found in biological samples, a pre-concentration step is often required prior to its determination. One interesting alternative to conventional pre-concentration techniques is the use of cloud point extraction (CPE), which is based on the phase behavior exhibited by aqueous solutions of non-ionic surfactants, which become turbid after an increase in temperature or the addition of additives such as salting-out electrolytes. The generation of two distinct phases can be visualized: a water phase containing the surfactant near the critical micelle concentration, and a surfactant-rich phase, concentrating most of the added surfactant. Under optimized conditions, the analyte can be extracted to the surfactant-rich phase and determined using an adequate measuring technique. CPE has been successfully applied to the determination of polycyclic aromatic hydrocarbons [4–8] and pharmaceutical products [9,10], among many other applications.

Metallic elements that can bond to the micelles in an aqueous solution can also be extracted from the original solution

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and concentrated in the surfactant-rich phase as hydrophobic complexes formed between the analyte ion and an appropriate chelating agent. This has been demonstrated for several analytes in the most diverse samples [11–15]. Particularly, the combination of ammonium *O*,*O*-diethyldithiophosphate (DDTP) as the chelating agent and Triton X-114 as the non-ionic surfactant have proved to be of advantage, mostly due to the high stability of DDTP in acidic media, good hydrophobicity of the complexes formed and the relatively low cloud point of Triton X-114 [16]. However, the application of CPE for Hg determination is very limited and restricted to a few complex examples, mostly due to the peculiar characteristics of this element [17,18].

In this work, cloud point extraction with salt-induced phase separation using Triton X-114 and DDTP will be demonstrated for the determination of mercury in certified reference samples of human hair, dogfish liver and dogfish muscle using cold vapor atomic absorption spectrometry (CV AAS) after the addition of sodium borohydride as the reducing agent.

2. Experimental

2.1. Instrumentation

All measurements were carried out using a model Analyst 100 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA). Cold vapor was produced using a MHS-15 chemical vapor generation system (Perkin-Elmer), coupled to the AA spectrometer. A Hg hollow cathode lamp (Perkin-Elmer) operated at 6 mA was used. Measurements were carried out at 253.7 nm. Integrated absorbance was used exclusively. Argon 99.996% (White Martins, São Paulo, SP, Brazil) was used as the carrier gas. Microwave-assisted digestion was carried out in a MLS-1200 MEGA microwave digestion system (Milestone, Sorisole, Italy), using a five-step program: 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, and 5 min at 650 W, followed by 5 min of ventilation.

2.2. Reagents, standards and certified reference materials

All reagents used were at least of analytical grade. Water was de-ionized to a resistivity of 18.2 MΩ cm in a Milli-Q system (Millipore, Bedford, MA, USA). Hydrochloric acid (Merck, Darmstadt, Germany), methanol (Carlo Erba, Milan, Italy) and nitric acid (Merck) were doubly distilled in a quartz sub-boiling distillation apparatus (Kürner Analysentechnik, Rosenheim, Germany). Hydrogen peroxide 30% (Merck), octylphenoxy polyethoxyethanol (Triton X-114, Sigma, St. Louis, MO, USA), ammonium *O*,*O*-diethyldithiophosphate (DDTP, Aldrich, Milwaukee, WI, USA), sodium borohydride (Aldrich), sodium sulphate (Nuclear, Diadema, SP, Brazil) and *Antifoam-A* (Sigma) were used as supplied.

The following certified reference materials (CRM) were used: BCR 397 Human Hair (Institute for Reference Materials and Measurements, Brussels, Belgium), and DOLT-3 Dogfish Liver and DORM-2 Dogfish Muscle (National Research Council of Canada, Ottawa, OT, Canada).

2.3. Procedure

Aliquots containing 250–500 mg of each CRM were weighed directly into poly (tetrafluorethylene) (PTFE) flasks; 3.0 mL HNO_3 and 1.0 mL H_2O_2 were added for 250 mg aliquots or twice these volumes for 500 mg aliquots. The flasks were sealed and the mixture submitted to the above-described microwave heating program. Following digestion, samples were transferred to 50 mL polypropylene (PP) tubes and the volume was made-up with de-ionized water; the digested samples were stored under refrigeration until use.

For the CPE procedure, aliquots containing between 0.5 and 2.5 mL of the digested samples were transferred to 14 mL PP tubes and HCl, DDTP and Triton X-114 were added to the mixture at optimized concentrations (0.1 mol L^{-1}, 0.5% (m/v) and 0.3% (m/v), respectively). Phase separation was then induced by two different procedures. First, de-ionized water was added to a final volume of 14 mL and the samples were submersed in a water bath at 50 °C for 25 min. In a second approach, phase separation was induced by the addition of Na_2SO_4 to a final concentration of 0.2 mol L^{-1}, before making up the volume to 14 mL with de-ionized water. In both cases, phase separation was accelerated by centrifuging the tubes at 3500 rpm for 15 min. The PP flasks were then immersed in an ice bath, allowing the surfactant-rich phase to become viscous. Water was removed by simply inverting the tubes. Residues of the water phase were removed using a Pasteur pipette. Prior to the analysis, 500 μL of methanol acidified with 0.1 mol L^{-1} HNO_3 were added to the surfactant-rich phase to reduce the viscosity. A volume of 500 μL of this final solution was then transferred to the PTFE flasks of the MHS-15 system, and 2.5 mL of a 1.0 mol L^{-1} HCl solution were added, along with 40 μL of the antifoam agent. The system was sealed and 3% (m/v) $NaBH_4$ was added for 5 s to the PTFE flask, resulting in the injection of an approximate volume of 1.5 mL. A stream of argon carried the Hg vapor generated in the system to the quartz cell, where measurements were made. Calibration was performed using aqueous standards submitted to the same CPE extraction and CV AAS procedure described above. The initial Hg concentrations in the volume of 14 mL of the standard solutions were in the range 1–12 μg L^{-1}.

3. Results and discussion

3.1. Extraction parameters

The complexation and extraction parameters have all been optimized to obtain the highest signal. The influence of the concentration of DDTP on the analytical signal has been evaluated, and the results are shown in Fig. 1. From this Figure, it is possible to observe that the integrated absorbance for Hg decreases slightly for concentrations above 0.5% (m/v) in the DORM-2 sample, while for Hg in an aqueous solution submitted to CPE this decrease is more pronounced, resulting in a signal loss of about 50% for a DDTP concentration of 2.0% (m/v). One possible explanation for this different behavior could be that competition for complexation occurs in the biological material, which therefore requires a slightly higher concentration of

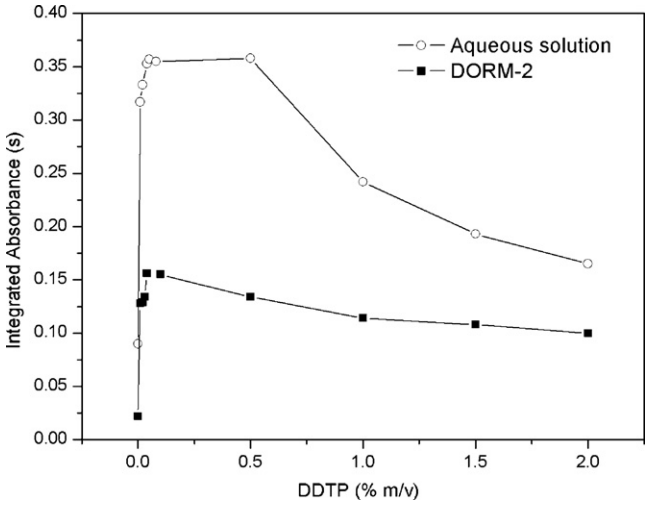

Fig. 1. Influence of DDTP concentration on the integrated absorbance obtained by CV AAS for Hg in the DORM-1 dogfish muscle CRM and in an aqueous solution, submitted to cloud point extraction. *Conditions*: 0.1 mol L^{-1} HCl; 0.3% (m/v) Triton X-114; 0.2 mol L^{-1} Na_2SO_4; 3% (m/v) $NaBH_4$.

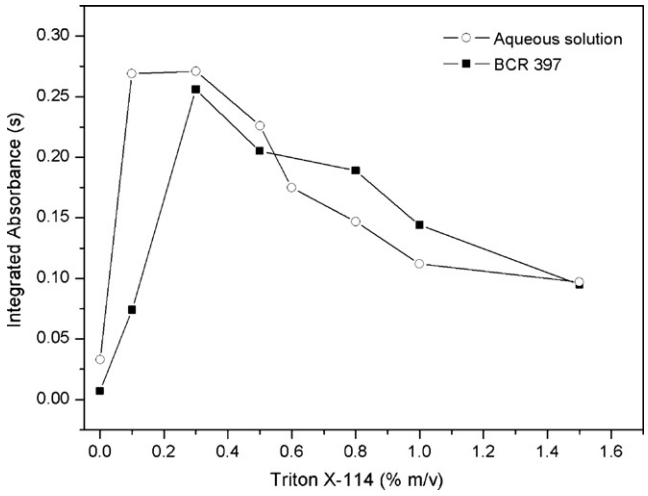

Fig. 3. Influence of Triton X-114 concentration on the integrated absorbance obtained by CV AAS for Hg in the BCR 397 human hair CRM and in an aqueous solution, submitted to cloud point extraction. *Conditions*: 0.5% (m/v) DDTP; 0.1 mol L^{-1} HCl; 0.2 mol L^{-1} Na_2SO_4; 3% (m/v) $NaBH_4$.

DDTP. For the aqueous solution, a large excess of DDTP might influence more severely the mechanism of vapor generation, affecting the reaction equilibrium. A DDTP concentration of 0.5% (m/v) was selected.

The concentration of HCl required for formation of the Hg complex with DDTP was evaluated in the range 0–1.0 mol L^{-1}, resulting in the curves shown in Fig. 2. Although complexation with DDTP occurs most likely in acidic media, the integrated absorbance signal for Hg in an aqueous solution submitted to CPE decreased almost linearly with the increase in HCl concentration from 0.1 to 1.0 mol L^{-1}, which might be partially explained by a possible reduction in the extraction efficiency due to formation of charged chloride complexes with Hg. This effect was not noticed for Hg in BCR 397 submitted to CPE, as in this case Hg may already be found as a complex, therefore

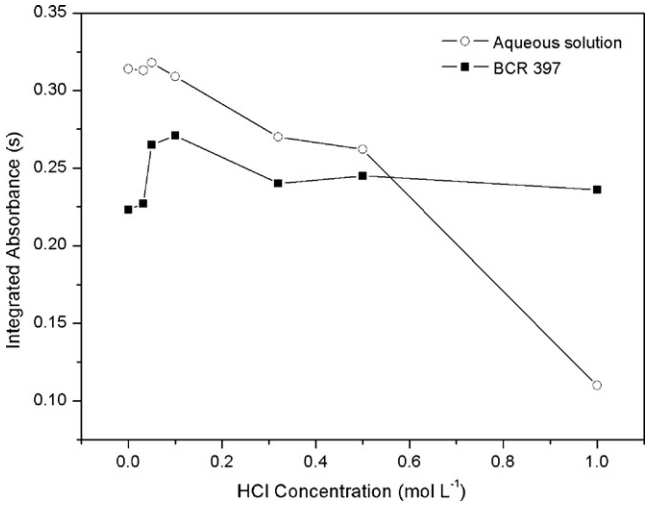

Fig. 2. Influence of HCl concentration on the integrated absorbance obtained by CV AAS for Hg in the BCR 397 human hair CRM and in an aqueous solution, submitted to cloud point extraction. *Conditions*: 0.5% (m/v) DDTP; 0.3% (m/v) Triton X-114; 0.2 mol L^{-1} Na_2SO_4; 3% (m/v) $NaBH_4$.

facilitating the reaction with DDTP. As a compromise, a final HCl concentration of 0.1 mol L^{-1} was used.

The concentration of Triton X-114 has also been optimized, and the results are shown in Fig. 3. For Hg in aqueous solution and BCR 397, the integrated absorbance for Hg increased up to a Triton X-114 concentration of 0.3% (m/v), and a further increase in the surfactant concentration resulted in a decrease in the analytical signal, which is typically due to the higher dilution of the analyte in the surfactant-rich phase, compensating for a possible increase in the extraction efficiency. A Triton X-114 concentration of 0.3% (m/v) was selected.

Phase separation in CPE is commonly induced by heating the mixture containing the surfactant up to a temperature above the cloud point. However, Hg is an element particularly sensitive to this kind of approach, due to its inherent volatility, and heating the solution might actually lead to analyte losses. In this sense, the *salting-out* effect of Na_2SO_4 was adopted as an alternative to induce phase separation in the aqueous solutions of Triton X-114. Sodium sulphate acts as a "drying" agent, causing dehydration of the polyoxyethylene chain in the surfactant molecules, an effect that arises from the increase in the association of water molecules with the salt, resulting in cleavage of the hydrogen bonding between water and surfactant molecules [19]. This obviously results in a significant reduction of the cloud point in a way that phase separation occurs already at room temperature. The added concentration of Na_2SO_4 should be sufficient to induce phase separation, but the increase in the ionic strength might also be of advantage.

The effect of the addition of Na_2SO_4 on the integrated absorbance for Hg has also been evaluated, and the results are shown in Fig. 4. The increasing signal that is obtained for Hg in an aqueous solution with the addition of increasing amounts of Na_2SO_4 can be explained by the higher extraction efficiency obtained with an increase in the ionic strength of the aqueous phase, resulting in higher sensitivity. This effect is much less pronounced in BCR 397 submitted to CPE, probably

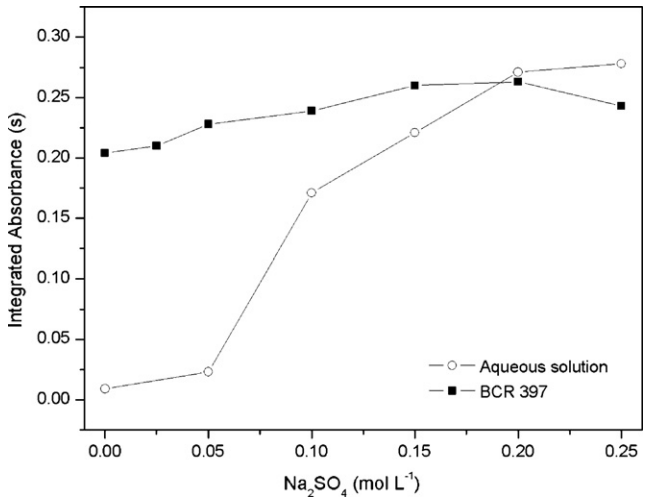

Fig. 4. Influence of Na_2SO_4 concentration on the integrated absorbance obtained by CV AAS for Hg in the BCR 397 sample and in an aqueous solution, submitted to cloud point extraction. *Conditions*: 0.5% (m/v) DDTP; 0.1 mol L^{-1} HCl; 0.3% (m/v) Triton X-114; 3% (m/v) $NaBH_4$.

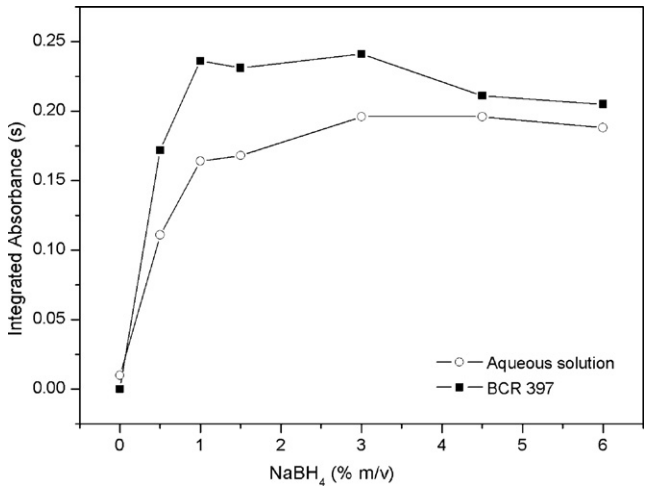

Fig. 5. Influence of $NaBH_4$ concentration in the reaction flask of the CVG system on the integrated absorbance obtained by CV AAS for Hg in the BCR 397 sample and in an aqueous solution, submitted to cloud point extraction. *Conditions*: 0.5% (m/v) DDTP; 0.1 mol L^{-1} HCl; 0.3% (m/v) Triton X-114; 0.2 mol L^{-1} Na_2SO_4.

because the ionic strength of the aqueous phase in this case is already high enough, due to the large amount of dissolved electrolytes. A Na_2SO_4 concentration of 0.2 mol L^{-1} was selected as optimum to induce phase separation and improve extraction efficiency. In order to evidence the need for a heatless procedure, a parallel experiment has been carried out using the 'traditional' CPE approach (i.e., phase separation by heating the surfactant-containing solution). The determined values for Hg in the biological samples were found to be lower than the certified values, which indicates that losses of volatile Hg compounds (such as methylmercury) might occur when heating is employed.

3.2. CV AAS Parameters

The chemical generation of Hg vapor consists in the addition of $NaBH_4$ to the sample aliquot in the PTFE reaction flask coupled to the chemical vapor generation system. The reaction between $NaBH_4$ and the analyte species in aqueous medium is typically catalyzed by an acid. Therefore, the concentration of HCl added to the reaction flask has been optimized for best sensitivity. The results showed that a HCl concentration of 0.5 mol L^{-1} is sufficient to produce the highest integrated absorbance signal. It is known that the addition of an excess of HCl might induce the formation of an excess of H_2, resulting in dilution of the analyte vapor with consequent reduction of the analytical signal, as could be in fact observed for Hg in an aqueous solution submitted to CPE. In this sense, to assure efficient generation of vapor, 1.0 mol L^{-1} HCl was used in all experiments using CV AAS.

The concentration of $NaBH_4$ has also been optimized, as shown in Fig. 5. The signal increases up to a $NaBH_4$ concentration of 1% (m/v), and remains approximately constant for higher concentrations. A concentration of 3.0% (m/v) was adopted. For higher concentrations, the reaction becomes more turbulent, resulting in lower precision due to kinetic effects resulting from a more difficult release of Hg from the condensed phase. In addition, the formation of water in the transfer line is reduced by the use of lower amounts of $NaBH_4$.

Table 1
Results (μg g^{-1}) obtained for the determination of Hg in biological samples by CV AAS following cloud point extraction ($n = 3$; 95% confidence level)

Sample	Certified	Found
DOLT-3	3.37 ± 0.14	3.27 ± 0.24
BCR-397	12.3 ± 0.5	11.8 ± 0.4
DORM-2	4.64 ± 0.26	4.33 ± 0.60

3.3. Results and Figures of Merit

The results of the analysis of three certified reference samples of biological origin are shown in Table 1. As can be seen, good agreement was obtained between certified and determined values at a 95% confidence level. Calibration was performed against aqueous standards submitted to the CPE procedure, resulting in a slope of 0.08 s L μg^{-1}, with a linear correlation coefficient of 0.9997. An enhancement factor of 10 was calculated as the ratio between the slopes of a calibration curve submitted to the CPE procedure and a calibration curve without pre-concentration. This resulted in a detection limit (3 s) of 0.4 ng g^{-1}, which is quite low and adequate to determine Hg at trace levels in biological samples. The procedure allowed an extraction efficiency of 90% to be obtained, as determined by mass recovery experiments for Hg in aqueous solutions submitted to CPE, with calibration against standards in Triton X-114 and methanol not submitted to the CPE procedure.

4. Conclusions

Cold vapor AAS in association with cloud point extraction has been shown to be a highly efficient procedure to determine Hg in biological samples submitted to microwave-assisted

acid digestion due to its simplicity, low cost and sensitivity. The possibility to analyze samples of very different nature and the low detection limit obtained due to the high efficiency of the pre-concentration procedure are valuable parameters, which facilitate considerably the difficult task of determining Hg in biological samples. Phase separation induced by the addition of Na_2SO_4 has also proved to be effective, avoiding possible losses of Hg due to heating, as is applied in most CPE procedures. This same procedure can certainly be extended to the determination of Hg and possibly to hydride-forming elements in other samples.

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