On-line Preconcentration and Determination of Mercury by Flow Injection Inductively Coupled Plasma Atomic Emission Spectrometry

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An inductively coupled plasma atomic emission spectrometry (ICP-AES) flow injection method for the determination of trace levels of mercury in biological materials is described based on complexation of the metal ion with 1,5-bis(di-2-pyridyl)methylene thiocarbonohydrazide (DPTH) and its subsequent on-line extraction into isobutyl methyl ketone (IBMK). The organic phase (containing the complex) is mixed on-line with SnCl₂ in dimethylformamide. The optimum experimental conditions are evaluated for the continuous extraction of Hg⁺. The direct generation of mercury vapour from the organic phase and the final determination of this element by ICP-AES. The detection limit for mercury is 0.002 μg ml⁻¹ and the calibration is linear from 0.01 to 5 μg ml⁻¹. The precision of the method (evaluated as the relative standard deviation obtained after analysing ten series of ten replicates) was ±3.3% at the 0.25 μg ml⁻¹ level of Hg⁺. The effect of various inorganic ions and some organic masking substances on the determination of mercury by the proposed method is examined under the optimum working conditions. The tolerance limits found show that mercury can be determined in the presence of a variety of ions including most of those that commonly occur with mercury in natural samples.

Keywords: Flow injection extraction; inductively coupled plasma atomic emission spectrometry; mercury; 1,5-bis(di-2-pyridyl)methylene thiocarbonohydrazide, biological samples

Today, the most commonly used method for determining mercury is cold vapour atomic absorption spectrometry (CVAAS), which permits the determination of mercury with good selectivity and sensitivity in a variety of samples. Although CVAAS has now become a widely accepted and utilized technique, there are several disadvantages associated with the use of AAS as the detection method. These result from the limited linear calibration range and spectral interference arising from non-specific background absorption of volatile organic species.

Notwithstanding an impressive number of analytical advantages, inductively coupled plasma atomic emission spectrometry (ICP-AES) has a significant drawback because its detection power is sometimes inadequate to comply with the requirements for the determination of trace elements in biological samples. Solvent extraction has proved to be an effective means of increasing sensitivity and a means of removing matrix interferences. However, the use of a common batch-type solvent extraction procedure was found to be time consuming and incompatible with the intrinsic speed of ICP-AES analysis. Recently, large advances have been made in developing systems for on-line solvent extraction with the aim of speeding up and simplifying the preconcentration step. Thus, to improve detection for ICP, Greenfield¹ examined some fundamental considerations using flow injection (FI) to preconcentrate samples by either extraction or ion exchange. This technique was applied by Kumamaru et al.,² who extracted cadmium from an aqueous sample into CCl₄ using diethyl-dithiocarbamate as the complex-forming agent. Yamamoto et al.,³ used a system for the determination of beryllium in alloys by extraction as the acetyl acetone into CCl₄; the system was constructed of a dual tube separator for the purpose of FI or suction-flow solvent extraction followed by introduction into an ICP. The well known lanthanum–alizarin complexone complex, used habitually for the spectrophotometric determination of fluoride, was applied by Monzooori and Miyazaki⁴ to the indirect determination of fluoride (by monitoring lanthanum) in water after extraction of the ternary complex into hexanol. In some recent work, Sanz-Medel and co-workers⁵ determined arsenic by ICP-AES in an organic phase by coupling continuous flow extraction with hydride generation in the so-called 'tandem on-line' separation.

In this work, an ICP-AES–FI method for the determination of trace amounts of mercury is described. In the FI system, a complex of the metal with 1,5-bis(di-2-pyridyl)methylene thiocarbonohydrazide (DPTH) was extracted on-line into isobutylmethylketone (IBMK) with the aid of a phase separator furnished with a poly(tetrafluoroethylene) (PTFE) membrane. The DPTH–IBMK system described has been used previously for the batch extraction of various transition elements in conjunction with atomic spectroscopic determination.⁶⁻⁹

Experimental

Reagents

Analytical-reagent grade chemicals were used throughout.

The ligand for the DPTH solution was synthesized as described elsewhere.¹⁰ The 0.05% m/v DPTH stock solution was prepared by dissolving 0.05 g of DPTH in 9 ml of N,N-dimethylformamide (DMF) and diluting to 100 ml with IBMK. The solution was found to remain stable for more than a week.

The tin(II) chloride solution was prepared by dissolving SnCl₂·2H₂O (Carlo Erba) in DMF. A stock solution of HgCl₂ was prepared from the nitrate (Merck) and standardized complexometrically; standards of working strength were made by appropriate dilution as required. A 0.1 mol ⁻¹ solution of NaClO₄ (Merck) was also used. A pH 3.6 buffer was prepared by mixing 5 ml of 0.2 mol ⁻¹ HCl and 50 ml of 0.2 mol ⁻¹ glycine in a 250 ml calibrated flask and making up to the mark with distilled water. The carrier solution was prepared by mixing IBMK and DMF to a volume ratio of 1:1.

Apparatus

The ICP-AES system used was a Perkin-Elmer 40 sequential emission spectrometer controlled by an IBM XT-286 personal computer. The spectrometer output was connected to a PE Nelson Model 1020 personal integrator. The plasma operating conditions used in this work are summarized in Table 1.

A schematic diagram of the FI system is shown in Fig. 1. The aqueous and organic solutions were pumped using a four channel peristaltic pump (Gilson Minipuls 3) fitted with vinyl and silicone pump tubes, respectively. The
Table 1 Optimum operating conditions for the FI-ICP determination of Hg

| ICP system   | Wavelength/nm | R.f. generator Frequency 40 MHz, incident power 1.1 kW | Photomultiplier voltage/V | Plasma gas flow rate/l min⁻¹ | Auxiliary gas flow rate/l min⁻¹ | Nebulizer gas flow rate/l min⁻¹ | Plasma viewing height 1.5 mm (above the induction coil) | Nebulizer | FI system   | Internal diameter of coils/mm | Extraction coil length/cm | Mixing coil length/cm | Aqueous phase flow rate/ml min⁻¹ | Organic phase flow rate/ml min⁻¹ | Injection volume/µl
<table>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>253.652</td>
<td></td>
<td>600</td>
<td>12</td>
<td>0.6</td>
<td>1.3</td>
<td>Cross-flow</td>
<td></td>
<td>Internal diameter of coils/mm</td>
<td>0.5</td>
<td>250</td>
<td>50</td>
<td>1.52 (sample, 1.15 + buffer, 0.37)</td>
<td>0.37</td>
<td>375</td>
<td></td>
</tr>
</tbody>
</table>

sample and the reductant were injected manually by means of two six-port rotary valves (Rheodyne Type 50) fitted in a device which allowed them to merge in a concreted point or a reproducible mode (merging zones). Tubings for the mixing and extraction coils were of Teflon with different internal diameters. A T-solvent segmentor (Omnifit Three-way connector) in which the aqueous phase, which flows straight through, and the organic phase, at right angles, was used for mixing organic and aqueous phases. Phase separation was effected in a laboratory-made phase separator (the phase separator was constructed following a previous design) furnished with a PTFE membrane (Millipore FHL2500, 0.5 µm pore size) permeable to IBMK. This porous membrane was wetted with methyl alcohol prior to use according to the manufacturer's instructions.

Description of the FI System

Operation of the FI system (Fig. 1) is as follows: first the carrier solution is pumped into the system to establish a baseline and to stabilize the plasma. Samples or standard carrier solution is pumped into the system to establish a sample and the reductant were injected manually by means of mixing and extraction coils were of Teflon with different internal diameters. A T-solvent segmentor (Omnifit Three-way connector) in which the aqueous phase, which flows straight through, and the organic phase, at right angles, was used for mixing organic and aqueous phases. Phase separation was effected in a laboratory-made phase separator (the phase separator was constructed following a previous design) furnished with a PTFE membrane (Millipore FHL2500, 0.5 µm pore size) permeable to IBMK. This porous membrane was wetted with methyl alcohol prior to use according to the manufacturer's instructions.

General Procedure

With the manifold described in Fig. 1 and under the optimum instrumental conditions shown in Table 1, a 1.15 ml min⁻¹ flow of sample (standard or blank) is mixed with a 0.37 ml min⁻¹ flow of 0.1 mol l⁻¹ NaClO₄ plus buffer solution. The resulting aqueous flow reaches the solvent segmentor, merging with a 0.37 ml min⁻¹ flow of 0.05% DPTH in IBMK. The mixture is passed through an extraction coil (length 250 cm, i.d. 0.5 mm) where the complex is formed and extracted. After extraction, the organic phase is separated from the aqueous phase and introduced into the sample loop (L₁). A stream of 0.1 mol l⁻¹ SnCl₂ in DMF is simultaneously pumped filling the injection loop L₂. The sample and reductant loops are 375 µl in volume. As shown in Fig. 1 and described above, the sample and the reductant are mixed into the mixing coil (length 50 cm, i.d. 0.5 mm) and transferred into the ICP by a continuous stream of the carrier solvent (3.3 ml min⁻¹).

Sample Preparation

The certified reference materials (CRMs) analysed to determine the accuracy of the proposed procedure were: National Research Council Canada (NRCC), CRM DORM-1 Dogfish Muscle; Community Bureau of Reference (BCR), CRMs 186 Pig Kidney and 397 Human Hair. These samples were first dried in accordance with the instructions of the respective analysis certificates. Human Hair was prepared by microwave digestion according to the following procedure. A 0.200 g sample was weighed directly into the digestion vessels of the bombs on a digital electronic balance and 4 ml of 65% nitric acid were added. After 30 min, 2 ml of 37% HCl were added, the digestion bomb was closed and placed in the microwave oven, where it was heated at 360 W for 4 min. Followed by 10 min at 180 W. The oven was allowed to cool for 14 min (same time period as the heating step). After digestion, the solutions were evaporated to a small volume, neutralized with sodium hydroxide and finally diluted with de-ionized water to 10 ml in a calibrated flask.

Pig Kidney and Dogfish Muscle were prepared by reflux mineralization with nitric acid and hydrogen peroxide. In a reaction flask were placed 0.500–2.000 g of weighed sample and 15 ml of 65% nitric acid and the mixture was heated under reflux until the nitrous fumes disappeared. Then, 3 ml of hydrogen peroxide were added and the mixture was concentrated (this step was repeated until the solution was completely colourless). Next, the mixture was neutralized with NaOH and made-up to 10 ml with de-ionized water.

Aliquots of these sample solutions were taken and the analysis of each sample, in triplicate, was completed as described under General Procedure, using the method of standard additions.
Results and Discussion

Optimization of Extraction in the FI System

Extraction of metal ions by an organic reagent is known to be dependent on several factors such as the type and amount of reagent, organic solvent, chemical form of metal ion and pH of solution. The extraction process was investigated in order to establish optimum conditions. The IBMK has significant solubility in water but was chosen as the organic solvent because of its high extraction efficiency for the Hg(II)-DPTH complex. The main chemical variables were examined by measuring the ICP signals for the extraction of 1 μg ml⁻¹ of Hg(II), using the flow system shown in Fig. 1 and an injection volume of 200 μl.

The influence of the pH was studied over the range 1–12. The optimum pH range for quantitative extraction of the complex was around 2.5–10.5. All subsequent studies were carried out at pH 3.6; this pH was adjusted using a glycine-HCl buffer solution. A plot of the peak height versus the ionic strength showed that the height of the FI peak increased with increasing ionic strength, hence 10 ml of 1 mol l⁻¹ NaClO₄ were added to 100 ml of buffer solution.

The extraction behaviour of Hg(II) was examined with varying concentrations of DPTH in the organic phase. The results obtained showed that the extracted fraction remains constant for reagent concentrations equal or greater than 2.3 × 10⁻⁴ mol l⁻¹ (0.01%). A concentration of 0.05% of DPTH was used in practice in order to prevent reagent depletion by other extractable ions potentially occurring in the aqueous medium.

With regard to the optimum concentration of SnCl₂ in DMF, 0.1 mol l⁻¹ was selected as a compromise between the efficiency required for mercury vapour generation and high plasma stability.

Selection of Flow Injection Variables

The effect of flow rates, reaction/extraction coil characteristics and sample injection volume were studied. In all studies the sample solution used contained 1 μg ml⁻¹ of Hg(II).

The influence of the sample flow rate arriving at the segmentor was studied using a constant flow rate of reagent of 0.37 ml min⁻¹. The results obtained are shown in Fig. 2. As can be seen, the peak area increases with increasing sample flow rate, because the mass of analyte reaching the segmentor increases. The precision, however, worsened at flow rates > 1.2 ml min⁻¹ owing to poor phase separation.

Hence, the sample flow rate is very important and a compromise (precision versus preconcentration factor) value of 1.15 ml min⁻¹ was selected. For this value the influence on the signal of the reagent flow rate was investigated. Fig. 3 shows the dependence of peak area on the aqueous:organic phase flow rate ratio. As expected, higher organic phase flow rates brought about a decrease in the ICP emission signal (the preconcentration factor decreases), however, this decrease is limited by the dissolution of IBMK in the aqueous phase. An organic phase flow rate of 0.37 ml min⁻¹ (aqueous:organic phase flow ratio = 3.1) was selected for subsequent experiments because at values ≤ 0.3 ml min⁻¹ the precision was poor as irregular organic phase segments were formed in the segmentor. The effect of the carrier flow rate on emission signals was also examined. The results obtained showed that when the flow rate increases the peak height increases and the peak width decreases, this can be attributed to a reduction in dispersion. At flow rates higher than 4.0 ml min⁻¹ the noise increased, probably as a result of plasma perturbation. As a compromise between analytical sensitivity, plasma stability and sampling rate, the latter being rather dependent on the flow rate, a carrier flow rate of 3.3 ml min⁻¹ was chosen. Finally, the NaClO₄-buffer flow rate was varied from 0.37 to 1.15 ml min⁻¹. The results obtained showed that the extracted fraction remains constant for flow rates equal or greater than 0.37 ml min⁻¹. A flow rate of 0.37 ml min⁻¹ was selected for this study.

The extraction coil length was varied from 100 to 500 cm while the flow ratio was maintained constant. Fig. 4 shows that the peak area increased with increasing extraction coil length up to 250 cm; at values > 250 cm the FI peak started to decrease slightly. Therefore, an extraction coil length of 250 cm was chosen. Preliminary tests showed that the i.d. of the extraction coil was not an important factor (0.3 to 0.8 mm i.d. coils gave similar results) when the volume of the extraction coil was kept constant. An inner diameter of 0.5 mm was selected.

The influence of mixing coil length was also examined to ensure a homogeneous mixture between the sample and
SnCl₂ solutions. Tygon tubing coils (0.5 mm i.d.) of different lengths (0–300 cm) were tested. The ICP emission signals were constant for coil lengths ≥50 cm so a 50 cm coil was chosen.

The effect of sample injection volume on peak shape was examined. The volume of sample injected was varied from 100 to 592 µl by changing the length of sample loop in the injection valve. The peak heights increased with increasing sample size and the peak widths also became broader. The peak height, however, was almost constant above a sample size of 375 µl. There was not a linear relationship between the sample size and the peak height, but there was good linearity between the sample size or the number of moles of mercury injected and the peak area. This means that the method involving the use of peak areas to construct a calibration graph was more sensitive than that using peak heights when large sample sizes are injected. An injection volume of 375 µl was chosen for all subsequent studies. The effect of reductant injection volume was also examined and an injection volume of 375 µl was selected for further investigations as a compromise between the efficiency required for mercury vapour generation and high plasma stability (when the volume of SnCl₂ solution injected was higher than 400 µl plasma perturbation was observed).

Parameter Selection for ICP-AES

The main objective of this study was to find a suitable wavelength for the determination of mercury. To that end [using the minimum background equivalent concentration, (BEC) as the optimization criterion], the following wavelengths were investigated: 194.227, 253.653, 296.728, 435.835, 265.204, 302.150 and 365.483 nm. From these wavelengths, measurement made at 435.835 nm produced the most intense emission but the presence of IBMK in the extractant produced a considerable background. Thus, the Hg 253.652 nm line was selected for this work.

Other operating variables such as type of nebulizer, nebulizer flow rate, photomultiplier voltage and plasma observation height were established to achieve the best signal-to-noise (S/N) ratios. The results are given in Table 1. The cross-flow nebulizer is generally not as efficient as the concentric nebulizer at creating the small droplets needed for ICP analysis, but was chosen because the larger diameter of the liquid capillary and the longer distance between liquid and gas injectors minimize clogging problems.

Calibration Graph and Precision

Under the optimum conditions, a linear calibration graph was obtained from 0.01 to 5 µg ml⁻¹ of Hg⁰ with a regression coefficient of 0.9998. At concentrations of Hg⁰≥5 µg ml⁻¹, a negative deviation from linearity was observed owing to insufficient complexing agent and/or incomplete extraction. Under the optimum conditions, the signal appeared 15 s after sample injection. Within 180 s after injection, the FI system was ready for the next injection, thus giving a sampling rate of about 20 per hour.

The relative standard deviations for ten replicate measurements were ±0.84% (10–900 ng ml⁻¹) and ±0.25% (1–5 µg ml⁻¹). For a series of ten samples, each containing 0.25 µg ml⁻¹ of mercury, a relative standard deviation of ±3.3% was obtained. The detection limit defined as the concentration of analyte giving a signal equivalent to three times the standard deviation of blank signal plus the net blank intensity was measured to be 0.002 µg ml⁻¹.

Interferences

The effect of various ions on the determination of mercury by the proposed method was examined under the optimum working conditions. For this study, different amounts of the ionic species tested were added to a 0.25 µg ml⁻¹ solution of Hg⁰. The starting point was an interferent:mercury ratio of 4000 m/m; if any interference occurred, the ratio was gradually lowered until the interference disappeared. The tolerance limits, defined as the concentration (µg ml⁻¹) causing a deviation of less than ±3% in the analytical response, are shown in Table 2. As can be seen, the tolerance limits found show that mercury can be determined in the presence of a variety of ions including most of those that commonly occur with mercury in biological samples. The ions that interfere most strongly are Co³⁺, Ni²⁺ and Fe³⁺ because they form complexes with DPT where DPT, although the interferences from these ions were minimized by the addition of glycine as a masking agent. For this reason the HCl–glycine buffer was chosen for the determination of mercury in biological samples.

Sample Analysis

In order to test the accuracy and applicability of the proposed method to the analysis of the real samples, three biological reference materials were analysed. The standard additions method was used in all instances and the results were obtained by extrapolation. These results, as the average of three separate determinations, are shown in Table 3. As can be seen, the mercury concentrations determined by the proposed method are in close agreement with the certified values, although samples of Pig Kidney and Human Hair show a slight decrease in the recovery, probably due to the incomplete digestion of the samples; this fact has been observed by Landi et al.¹² in the recovery of mercury in other diverse biological samples.

Table 2 Tolerance of foreign ions in the determination of 0.25 µg ml⁻¹ of mercury

<table>
<thead>
<tr>
<th>Species</th>
<th>Tolerance limit/µg ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺, Sr²⁺, Ca²⁺, Ba²⁺, Mg²⁺, Pb²⁺, Bi³⁺</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Al³⁺, Zn²⁺, fluoride</td>
<td>750</td>
</tr>
<tr>
<td>Mo⁵⁺, thiourea, oxalate, nitrite</td>
<td>500</td>
</tr>
<tr>
<td>Li⁺, Cd²⁺, Sn²⁺, Cr³⁺, Se⁴⁺, bromide</td>
<td>250</td>
</tr>
<tr>
<td>Cd⁴⁺, Ni²⁺, Fe³⁺</td>
<td>125</td>
</tr>
<tr>
<td>Ag⁺, Mn⁺, Cu⁺</td>
<td>31</td>
</tr>
</tbody>
</table>

* With addition of glycine.

Table 3 Results for the determination of mercury in real samples; n=3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Found value/µg g⁻¹</th>
<th>Certified value/µg g⁻¹</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Kidney</td>
<td>1.84±0.3</td>
<td>1.97±0.04</td>
<td>93.4</td>
</tr>
<tr>
<td>Dogfish Muscle</td>
<td>0.80±0.1</td>
<td>0.798±0.0074</td>
<td>100.2</td>
</tr>
<tr>
<td>Human Hair</td>
<td>11.8±0.2</td>
<td>12.3±0.5</td>
<td>95.9</td>
</tr>
</tbody>
</table>

Table 4 Results for the determination of mercury in spiked samples; n=3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added µg g⁻¹</th>
<th>Found µg g⁻¹</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Liver</td>
<td>0.300</td>
<td>0.29±0.05</td>
<td>97.0</td>
</tr>
<tr>
<td>Lobster</td>
<td>0.250</td>
<td>0.252±0.03</td>
<td>101.1</td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>0.250</td>
<td>0.259±0.006</td>
<td>103.8</td>
</tr>
<tr>
<td>Citrus Leaves</td>
<td>0.250</td>
<td>0.259±0.006</td>
<td>103.8</td>
</tr>
</tbody>
</table>
Materials (SRMs) spiked with mercury was investigated. For this purpose, standard solutions containing mercury were added to 0.2 g of Bovine Liver [National Institute of Standards and Technology (NIST) 1577a], Citrus Leaves (NIST 1572) and Lobster Hepatopancreas (NRCC TORT-1) and the resulting material was prepared by microwave digestion as described under Experimental. The results of these analyses are summarized in Table 4. The proposed method clearly gave satisfactory average recoveries.

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References