A Novel Approach to Cold Vapor Generation for the Determination of Mercury in Biological Samples

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A novel approach to the generation of mercury vapor at high pH by the simple addition of NaOH or other base to a solution containing mercury ions is described. Subsequent addition of another reducing agent, such as NaBH₄ or SnCl₂, is unnecessary. Following total dissolution of sample using HNO₃ and H₂O₂ in a closed microwave vessel, the pH of the sample solution is increased to 13 by the addition of NaOH. After standing for 90 min in a closed vessel, the Hg⁰ is directed to the plasma by a flow of argon. Emission from mercury is measured at 253.652 nm by an axial view inductively coupled plasma optical emission spectrometer (ICP OES). The procedure was applied to five certified biological samples, yielding a detection limit (3σ, n = 10) of 0.04 µg g⁻¹ for a nominal mass of 0.5 g in a final volume of 50 mL. Calibration was achieved using simple aqueous standard solutions containing 0.1 mol L⁻¹ NaOH. The procedure was efficient, with determined values lying in the range of 85–113 % of the certified values, showing good agreement at the 95% confidence level (t-test). The precision was fit for purpose, with relative standard deviations ranging from 7 to 9%. Organomercury species in solution were not detected; only Hg²⁺ in solution produces a signal. This new procedure provides for a simple approach to quantitation (and potentially speciation).

Keywords: mercury, alkaline pH, inductively coupled plasma optical emission spectrometry, cold vapor generation, biological samples

Introduction

The chemical transformation of an analyte species to a volatile form is known as chemical vapor generation (CVG). The main advantages of CVG are the separation of the analyte from the matrix and high sample introduction efficiency, resulting in enhanced sensitivity, selectivity and detection limits.¹ This is a widely utilized methodology in atomic spectrometry for the determination of trace and ultra-trace concentrations of elements such as As, Sb, Bi,
Ge, Pb, Hg, Se, Te and Sn. Currently, NaBH₄ remains the most popular reagent for CVG, forming volatile species of not only the aforementioned elements, but also of Ag, Au, Cd, Cu, In, Ni, Pt, Rh, Tl, and Zn with important analytical applications.¹⁻⁴ Several alternative means of CVG, not using NaBH₄, have also been proposed: electrochemical vapor generation;⁵ alklylation reactions, such as those using Grignard reagents (e.g., for tributyltin with pentylmagnesium bromide);⁶ and, more recently, photochemical vapor generation with UV irradiation. Examples of the latter include generation of volatile species of Se(IV) in formic, acetic and propionic acids (with formation of SeH₂, dimethylselenium and diethylselenium respectively)⁶ and of Ni by reaction with CO (yielding Ni(CO)₄).⁷ There is a particular interest in the determination of Hg because of its toxicity, mobility and ability to accumulate in various organisms. Cold vapor (CV) generation coupled to atomic absorption (AAS) or atomic fluorescence spectrometry (AFS) are the techniques most frequently used for detection.⁸⁻¹⁰ In addition, photochemical vapor generation¹¹⁻¹³ and ultrasound-assisted vapor generation¹⁴,¹⁵ have recently been proposed.

We have found that mercury ions in aqueous solution can be reduced to elemental mercury by simply increasing the pH of the solution through addition of NaOH or other base, without the need for any additional reducing agent. Taking into account this finding, an analytical method for the determination of mercury by CV-ICP OES is presented herein.

**Experimental**

**Instrumentation**

All measurements were made using a simultaneous axial view ICP OES spectrometer, model VISTA PRO (Varian, Mulgrave, Australia), coupled to an on-line continuous vapor generation system (Varian, model VGA-76P). Within the VGA-76P system, the argon gas supply is divided into two branches by a suitable “T” piece. One branch is controlled by a solenoid stop valve and argon gas to the inlet side of the reaction coil. The other branch supplies argon gas directly through the gas/liquid separator. With this system, only the sample line was employed, using 2.90 mm i.d medical grade PVC purple/black tubing (Kendall No. 1160549160) for the sample treated with NaOH. A scheme illustrating the vapor generation system coupled to the ICP OES spectrometer is shown in Figure 1. The experimental conditions are summarized in Table 1. Peak height emission intensities were measured at 253.652 nm. Argon (99.996% purity, White Martins, São Paulo, Brazil) was used. Samples were totally digested in a closed CEM microwave system (model MDS 2100, Matthews, NC, USA). A Shimadzu model AA-6601F single beam atomic absorption spectrometer (Nakagyo-Ku, Kyoto, Japan) equipped with deuterium lamp background correction and Hg hollow cathode lamp L233 from Hamamatsu Photonics K.K. (Shizuoka, Japan) was used to verify the generation of Hg⁹.

![Figure 1. Scheme of chemical vapor generation system (Varian model VGA-76P): 1. to the ICP OES; 2. peristaltic pump (50 rpm); 3. sample (flow rate of 8 mL min⁻¹); 4. carrier gas flow rate (120 mL min⁻¹); 5. flow controller; 6. drain (Nalgene tubing); 7. phase separator.](image)

| Table 1. Instrumental parameters for ICP OES and cold vapor generation |
|-----------------------|------------------|
| **ICP OES**           |                  |
| Radiofrequency        | 40 MHz           |
| Radiofrequency power  | 1.2 kW           |
| Plasma gas flow rate  | 15.0 L min⁻¹     |
| Auxiliary gas flow rate| 1.5 L min⁻¹      |
| Replicate read time   | 3 s              |
| Stabilization time    | 35 s             |
| Replicates            | 4                |
| Torch                 | Quartz for axial view |
| **Analytical line**   | 253.652 nm       |
| **Vapor generator**   |                  |
| Sample flow rate      | 8 mL min⁻¹       |
| Carrier gas flow rate | 120 mL min⁻¹     |

**Reagents and materials**

All chemicals were of analytical grade, unless otherwise specified. High purity water (resistivity of 18.2 MΩ cm) was de-ionized in a Milli-Q system (Bedford, MA, USA). The following reagents were used: Suprapur® 65% v/v HNO₃ (Merck, Darmstadt, Germany No. 1.00441.1000), sodium hydroxide (Merck, No. 1.06498.1000), 30% (v/v) hydrogen
peroxide (Merck No. 1.07210.1000), 1000 μg mL⁻¹ Hg standard solution (Merck No. 1.19795.0500). A Thimerosal stock solution, containing 100 μg L⁻¹ of Hg, was prepared by dissolving the salt (C₄H₉HgNaO₂S) (Merck No. 8.17043.0100) in water. A methylmercury chloride stock solution was prepared by dissolving CH₃HgCl (Fluka, 98% m/m purity) in ethanol (Merck No. 1.00983.1000).

The following certified reference materials were analyzed: DOLT-2 (Dogfish Liver), TORT-2 (Lobster Hepatopancreas) and DORM-1 (Dogfish Muscle) from the National Research Council Canada (NRCC, Ottawa, Ontario, Canada); BCR 186 (Lyophilized Pig Kidney), from the European Community Bureau of Reference (Brussels, Belgium); SRM 1566a (Oyster Tissue) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

 Procedures

Nominal 0.1-0.5 g samples of biological tissue were weighed into each microwave vessel (Advanced Composite Vessel, from CEM), followed by the addition of 5 mL concentrated HNO₃ and 2.5 mL of 30% (v/v) H₂O₂. After standing for 1 h, the samples were digested in the microwave system in accordance with EPA method SW 846-3051 (closed vessel). The resultant clear solutions were transferred to 50 mL volumetric flasks. Sufficient NaOH was added to each digested sample solution to achieve pH 13 (reached when the NaOH concentration was typically 0.1 mol L⁻¹); the volume was made up with high purity water and the flask was capped. After standing for 90 min in the closed flask under normal lab conditions, the sample (or standard calibration solutions which had been similarly mixed with the base) was introduced into the on-line generation system and emission from Hg was measured. Quantitation was performed against simple aqueous calibration solutions containing 1.0-20 μg L⁻¹ Hg²⁺. A reagent blank solution was run parallel to the determination and its result was taken into consideration. To study the effect of temperature, a solution containing 10 μg L⁻¹ of Hg²⁺ in 0.1 mol L⁻¹ NaOH was placed in a closed vessel and left to stand for 90 min under normal lab conditions. The solution was then transferred to a beaker, a thermometer inserted, and the solution then placed on a heating plate. When a predetermined temperature was reached, the solution was immediately aspirated into the on-line vapor generator. Experiments were also undertaken in the dark to assess the influence of ambient laboratory lighting. After the addition of the NaOH solution to a 10 μg L⁻¹ Hg²⁺ standard, the container was covered with aluminum foil and left to stand for 90 min in the dark before being introduced into the on-line vapor generator.

 Results and Discussion

This study initially utilized a tetramethylammonium hydroxide (TMAH) medium for vapor generation, resulting in the production of a signal for mercury after adjusting the solution pH to 13 with NH₄OH. Although it was then concluded that this reagent was reducing mercury in solution, it was subsequently determined that it was the pH change and not the TMAH that was responsible for production of mercury vapor. Using only TMAH, without adding another base to change the pH, a small intensity signal is obtained for mercury. However, if TMAH is added until pH = 13, the signal intensity is relatively high.

 Effect of pH

Figure 2. Effect of pH on signal intensity from an aqueous standard solution containing 10 μg L⁻¹ Hg²⁺ with pH adjusted with NaOH and/or HNO₃. Error bars represent standard deviation of three replicate measurements.

Aqueous solutions containing 10 μg L⁻¹ of Hg²⁺ were adjusted to different pHs by addition of NaOH or HNO₃. As shown in Figure 2, the signal increases with pH, most intensively in the range pH = 11 to 13. We do not yet know the mechanism of reduction arising by the addition of NaOH and therefore currently have no explanation for this observation.

 Effect of time

Figure 3 shows the signal intensity arising from a 10 μg L⁻¹ solution of Hg²⁺ containing 0.1 mol L⁻¹ NaOH (pH = 13) as a function of the reaction time. It is clear that an incubation period of 90 min is required to obtain a maximum signal. This time was adopted for the standard and for the sample solutions. The vessel was kept capped during the incubation time.
Effect of the carrier gas flow rate

Different carrier gas flow rates in the range 60-180 mL min⁻¹ were tested, again for a solution containing 10 μg L⁻¹ Hg²⁺ in 0.1 mol L⁻¹ NaOH. Measurements commenced 90 min after the addition of the NaOH. The results in Figure 4 show that response increases rapidly for flow rates up to 60 mL min⁻¹ and thereafter less intensively. A flow rate of 120 mL min⁻¹ was adopted for further study as this was optimal for efficient operation of the phase separator and the transfer of Hg⁰ from the solution. Without a carrier gas, the signal intensity is the same as for the blank, indicating that insufficient Hg⁰ is transferred from the solution to the plasma to produce a signal under this condition.

Effect of visible light

The signal intensity arising from a solution containing 10 μg L⁻¹ Hg²⁺ in 0.1 mol L⁻¹ NaOH, prepared and measured in the dark (flask covered with aluminum foil in the dark) was identical to that arising from a solution prepared under normal laboratory illumination conditions, demonstrating that the reduction of mercury is not influenced by light.

Effect of temperature

Figure 5 shows the signal intensity arising from a solution containing 10 μg L⁻¹ Hg²⁺ in 0.1 mol L⁻¹ NaOH as a function of the temperature of the reaction vessel. The signal intensity increases up to 50 °C, decreasing for higher temperature. Certainly, mercury is lost from the flask during heating. The loss is especially noticeable for temperatures higher than 50 °C. Increased temperature likely favors liberation of the vapor from the solution due to decreased solubility. Better precision was obtained at room temperature, which was adopted for all further experiments.

Figures of merit

Calibration curves, using Hg standard solutions spanning the concentration range 1.0-20 μg L⁻¹, were obtained using the selected conditions. The linear correlation coefficient was 0.997, and the limit of detection (LOD), defined as 3 times the standard deviation of 10 measurements of the blank divided by the slope of the calibration curve, was 0.04 μg g⁻¹ for 0.5 g of the solid sample in a final volume of 50 mL. This LOD was adequate for the analysis of the certified biological samples.
Generation of Hg\textsuperscript{0}

In order to verify the formation of Hg\textsuperscript{0} by the addition of NaOH, as opposed to other Hg species that would be decomposed in the plasma, measurements were also made based on cold vapor atomic absorption spectrometry using a quartz cell at room temperature and the same VGA-76P vapor generation equipment. A calibration curve was obtained, demonstrating that Hg\textsuperscript{0} is in fact formed.

Analytical application

The procedure was applied to the analysis of five biological certified reference materials. The results are summarized in Table 2. The recoveries of the certified values, between 85 and 113\%, were acceptable and all results were in agreement with the certified values in accordance with a \emph{t}-test at the 95\% level of confidence.\footnote{Precision was acceptable, with relative standard deviations (RSD) in the range 7-9\%.}

Additional studies

Possible vapor generation of Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Ni, P, Pb, Sb, Se, Si, Sn, Ti, U, V, Y and Zn species from aqueous solutions in 0.1 mol L\textsuperscript{-1} NaOH was also investigated, but only Hg produced an ICP OES signal. The presence of these elements in solution did not influence the Hg signal, even at added concentrations of 1000 \(\mu\)g L\textsuperscript{-1}.

The procedure was applied to the analysis of other environmental certified samples, including river and marine sediments as well as sewage sludge, but the recovery was too low, between 20\% and 70\%. This may be explained because the addition of NaOH resulted in the formation of hydrous oxides and hydroxides from concomitants in the sample solution which produced a gelatinous precipitate that occluded Hg\textsuperscript{0} and Hg\textsuperscript{2+}.

Several alternative strong bases were also examined for their efficiency of cold vapor generation, including 0.1 mol L\textsuperscript{-1} solutions of hydroxides of K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+} and NH\textsubscript{4}\textsuperscript{+}. Approximately the same signal intensity was obtained for all bases, except for NH\textsubscript{4}OH, for which the intensity was about half.

Aqueous solutions of thimerosal (\(\text{C}_9\text{H}_9\text{HgNaO}_2\text{S}\)) and ethanolic solutions of methylmercury (\(\text{CH}_3\text{HgCl}\)), containing 10 \(\mu\)g L\textsuperscript{-1} total Hg were mixed with different proportions of an aqueous standard solution of Hg\textsuperscript{2+}. The mixtures were (or not) submitted to acid digestion in a microwave oven. The results are shown in Table 3. It was found that, without digestion, only Hg\textsuperscript{2+} was detected, while after digestion both were detected. Only Hg\textsuperscript{2+} appears to lead to the formation of Hg\textsuperscript{0} by reduction with NaOH, while other Hg species do not respond.

Conclusions

Aqueous inorganic mercury can be reduced to Hg\textsuperscript{0} by the single addition of NaOH to an aqueous standard solution containing Hg\textsuperscript{2+} is about five-fold lower than that arising from the addition of NaBH\textsubscript{4} and HCl in the traditional online flow injection manifold using a strong reducing agent. Certainly, the presence of a reducing agent enhances the rate of reduction and its efficiency, providing a much higher intensity signal.

### Table 2. Analytical results for biological samples, \(\mu\)g g\textsuperscript{-1} (\(n = 3\))

<table>
<thead>
<tr>
<th>sample</th>
<th>certified</th>
<th>found</th>
<th>recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOLT-2</td>
<td>2.14 ± 0.28</td>
<td>2.35 ± 0.22</td>
<td>110</td>
<td>9</td>
</tr>
<tr>
<td>TORT-2</td>
<td>0.27 ± 0.06</td>
<td>0.23 ± 0.02</td>
<td>85</td>
<td>9</td>
</tr>
<tr>
<td>DORM-1</td>
<td>0.798 ± 0.074</td>
<td>0.745 ± 0.05</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>BCR 186</td>
<td>1.97 ± 0.04</td>
<td>2.24 ± 0.18</td>
<td>113</td>
<td>8</td>
</tr>
<tr>
<td>SRM 1566a</td>
<td>0.0642 ± 0.0067</td>
<td>0.069 ± 0.005</td>
<td>107</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table 3. Analytical results for spiked and digested thimerosal and methylmercury solutions, 10 \(\mu\)g L\textsuperscript{-1} (\(n = 3\))

<table>
<thead>
<tr>
<th>Spike Hg\textsuperscript{2+}/(\mu)g L\textsuperscript{-1})</th>
<th>MeHg/(\mu)g L\textsuperscript{-1})</th>
<th>Thimerosal/(\mu)g L\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (with digestion)</td>
<td>10.3 ± 0.7</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>5 (with digestion)</td>
<td>15.2 ± 0.2</td>
<td>14.5 ± 0.4</td>
</tr>
<tr>
<td>10 (with digestion)</td>
<td>20.5 ± 0.4</td>
<td>21.7 ± 0.4</td>
</tr>
<tr>
<td>0 (without digestion)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5 (without digestion)</td>
<td>5.2 ± 0.2</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>10 (without digestion)</td>
<td>9.8 ± 0.3</td>
<td>10.5 ± 0.1</td>
</tr>
</tbody>
</table>

ND: not detectable at level of 0.4 \(\mu\)g L\textsuperscript{-1}.
before being treated with NaOH. This observation opens the possibility for development of a rapid and easy speciation methodology for mercury. The procedure can likely be improved by designing a more efficient vapor generation apparatus, by accelerating reduction/phase separation steps by heating or sonication and/or by collecting the vapor before measurement (i.e., trapping on gold).

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References


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