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Determination of total and inorganic mercury in whole blood by cold vapor inductively coupled plasma mass spectrometry (CV ICP-MS) with alkaline sample preparation

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A simple method with a fast sample preparation procedure for total and inorganic mercury determinations in blood samples is proposed based on flow injection cold vapor inductively coupled plasma mass spectrometry (FI-CV ICP-MS). Aliquots of whole blood (500 μL) are diluted 1 + 1 v/v with 10.0% v/v tetramethylammonium hydroxide (TMAH) solution, incubated for 3 h at room temperature and then further diluted 1 + 4 v/v with 2.0% v/v HCl. The inorganic Hg was released by on-line addition of L-cysteine and then reduced to elemental Hg by $SnCl_2$. On the other hand, total mercury was determined by on-line addition of $KMnO_4$ and then reduced to elemental Hg by $NaBH_4$. Samples were calibrated against matrix-matching. The method detection limit was found to be 0.80 μg L^{-1} and 0.08 μg L^{-1} for inorganic and total mercury, respectively. Sample throughput is 20 samples h^{-1}. The method accuracy is traceable to Standard Reference Material (SRM) 966 Toxic Metals in Bovine Blood from the National Institute of Standards and Technology (NIST). For additional validation purposes, human whole blood samples were analyzed by the proposed method and by an established CV AAS method, with no statistical difference between the two techniques at 95% confidence level on applying the *t*-test.

Introduction

Mercury (Hg) is one of the most hazardous pollutants in the environment. Mercury exists basically in three forms: elemental mercury (Hg^0), known as metallic mercury; inorganic mercury compounds (Hg-i), primarily mercuric chloride; and organic mercury (Hg-o), primarily methylmercury (MeHg),[1] being organic forms more toxic than the inorganic ones.[1]

The main source of human exposure to organic mercury is the consumption of fish or seafood, mainly as MeHg.[1] On the other hand, the exposure to inorganic forms is more common by inhalation of Hg vapor released from dental amalgams or from gold mining activities.

Measurements of Hg in both blood and urine are used to determine whether adverse health effects are likely to occur. The total mercury concentration (Hg-t) in blood is often used as a proximate measure of MeHg exposure in individuals eating fish with the assumption that the Hg-i exposure, and thereby the Hg-i concentration in blood, is much lower. However, the proportion of MeHg in blood may vary among individuals, which makes essential to have analytical methods able to differentiate between chemical forms in blood to diagnose risks of toxicity.[2]

Until the last decade, cold vapor atomic absorption spectrometry (CV AAS) was the most commonly used technique for mercury determination in clinical samples.[3–6] The information on organic and inorganic forms of mercury could also be obtained with the same instrumentation by varying the reducing agents with different reducing powers.[7,8] However, more and more clinical laboratories are transitioning away from CV AAS methods toward those based on inductively coupled plasma mass spectrometry (ICP-MS).[9–11]

For fractionation of mercury in blood, a key step is the sample preparation procedure before CV AAS analysis. Usually on-line microwave-assisted sample digestion has been recommended.[12,13] However, these methods have several notable drawbacks. Some workers have reported difficulties with the flow injection (FI) instrumentation when blood specimens are analyzed, including blockages from precipitation of organic residues within the microwave reaction coil, and troublesome back pressure within the FI manifold that may be the result of exothermic reactions occurring within the microwave cavity.[14]

Moreover, clinical laboratories must cope with an increasing demand for trace element analysis in body fluids and tissues, in response to increasing concern for occupational and environmental exposure to mercury. Then, procedures for sample preparation with minimal handling and time consumption are much more desirable in routine analysis.

Tetramethylammonium hydroxide (TMAH) has been previously used for sample pre-treatment as an alternative to microwave-assisted acid digestion of biological materials for the determination of Hg and other elements.[15–21] Barbosa *et al.* (2004)[5] proposed a fast and simple sample preparation procedure

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for Hg determination in blood by CV AAS based on sample incubation at room temperature, resulting in a considerable improvement of the sample throughput.

The aim of this work was to evaluate a simple procedure for Hg-t and Hg-i determination (Hg-o by difference) in whole blood by flow injection cold vapor inductively coupled plasma mass spectrometry (FI-CV ICP-MS) with a fast and simple sample preparation procedure based on incubation of samples in alkaline medium at room temperature prior to analysis.

Experimental

Instruments and apparatus

For the CV ICP-MS method, all measurements were carried out with an ICP-MS (Elan DRC II PerkinElmer, Norwalk, CT, USA). A peristaltic pump of 8 channels (Ismatec) for propulsion of fluids was also used.

For the CV AAS comparative method, adapted from the work reported by Torres *et al.*,[21] a chemical vapor generation system (MHS-15 from PerkinElmer, Norwalk, CT, USA) operated in batch mode and coupled to an Analyst 100 atomic absorption spectrometer equipped with a mercury hollow cathode lamp (Perkin Elmer) and a deuterium background corrector was used. The following operating conditions were adopted: wavelength, 253.7 nm; spectral band pass, 0.7 nm; current, 6.0 mA. For all measurements, a quartz cell with a path-length of 165 mm and a diameter of 12 mm was used and kept at room temperature. For the CV AAS method, peak area was used for signal evaluation. In both methods, argon with a purity of 99.999% (White Martins, São Paulo, Brazil) was used.

Reagents

All reagents used were of analytical grade and the solutions were prepared using high-purity water with a resistivity of 18.2 MΩ cm, obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). 37% hydrochloric acid (Merck, Darmstadt, Germany) was doubly distilled in a quartz sub-boiling apparatus (Kürner Analysentechnik, Rosenheim, Germany).

A clean laboratory and laminar-flow hood capable of producing class 100 were used for preparing solutions and samples for the CV ICP-MS method. All solutions were stored in high-density polyethylene bottles. Materials were cleaned by soaking in 10% v/v HNO_3 for 24 h, rinsing five times with Milli-Q water and dried in a class 100 laminar flow hood before use. All operations for the CV ICP-MS method were performed on a clean bench.

A 10 mg L^{-1} standard solution of inorganic mercury was obtained from PerkinElmer (PerkinElmer, Norwalk, CT) whereas a 1000 mg L^{-1} standard solution of methylmercury chloride (MeHgCl) in water was obtained from Alfa Aesar (Ward Hill, MA, USA). Analytical calibration standard solutions of Hg-i were prepared daily over the range of 0.5–16.0 μg L^{-1} for the CV ICP-MS method. For the CV AAS method, a 1000 mg L^{-1} inorganic mercury stock solution (Merck) was used to prepare analytical calibration standard solutions of Hg-i, by suitable serial dilutions, in the range between 5.0 and 50.0 μg L^{-1}. The reductant solution was prepared by dissolving $NaBH_4$ powder (Sigma-Aldrich, Seelze, Germany) in NaOH (Carlo Erba, Milan, Italy). $SnCl_2$ (Sigma-Aldrich) solutions were also prepared by dissolving the salt in 10.0% v/v HCl. L-cysteine (Fluka, Buchs, Switzerland) and $KMnO_4$ (low in mercury, Sigma–Aldrich, St. Louis, MO, USA) were prepared in aqueous medium. Antifoam B (Fluka) was used for both methods. The antifoam B was diluted 10 times and the resulting diluted was added to the HCl carrier solution (1.0 mL for each 50.0 mL of HCl) for the CV ICP-MS method.

Solutions of 2-mercaptoethanol (Fluka), potassium dichromate (Fluka) and gold (Perkin Elmer) were diluted and used to minimize memory effects of mercury.

Sample preparation

CV AAS Method

For the determination of inorganic mercury, an aliquot of 250 μL of the sample was transferred to the reaction flask together with 1.0 mL of 1.0 mol L^{-1} HCl and 50 μL of antifoam B, following the addition of a $NaBH_4$ solution 1.5% m/v stabilized with 0.5% m/v NaOH. For total mercury determination, an aliquot of 100 μL of the sample was transferred to the reaction flask together with 1.0 mL of 1.0 mol L^{-1} HCl and 50 μL of antifoam B, following the addition of 150 μL of a $KMnO_4$ solution 2.0% m/v. After 2 minutes, 3.0% m/v $NaBH_4$ solution stabilized with 1.0% m/v NaOH was added to the reaction flask. The organic mercury concentration was defined as being the difference between the Hg-t and Hg-i concentrations.

CV ICP-MS method

An aliquot of blood (500 μL) was transferred to a conic flask of 15.0 mL and 500 μL of 2.5% m/v TMAH was added. The mixture was incubated at room temperature for 3 h for total and inorganic mercury determinations. After incubation, the volume was made up to 10.0 mL with a solution containing 2.0% v/v HCl. Matrix-matched calibration standard solutions were prepared daily by adding 500 μL of each intermediate Hg standard solution to five 15.0 mL Falcon tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA), 500 μL of a base blood material, and 500 μL of 10.0% v/v TMAH. The calibration standard solutions were incubated at room temperature for 3 h and were diluted to a final volume of 10.0 mL with 1.0% v/v HCl. The base blood was obtained from a caprine (goat) source, and Hg concentrations were below the method detection limit of 0.08 μg L^{-1}.

The flow injection configuration used for the determination of the total and inorganic mercury by CV ICP-MS is shown in Fig. 1. In the same way, the organic mercury concentration was calculated as being the difference between total and inorganic mercury concentrations.

Standard reference material and human blood specimens

Standard Reference Material (SRM) 966 Toxic Metals in Bovine Blood was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). For additional validation purposes, human blood specimens (n = 4) collected as part of another research study involving Hg exposure

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in the Brazilian Amazon region were available for method comparison purposes with identifiers removed. These specimens had been obtained with informed consent from humans subjects in accordance with procedures approved by our Institutional Review Board.

Flow injection cold vapor generation for ICP-MS quantification

Fig. 1a and 1b represent the schematic diagram used in the flow injection proposed system and the gas/liquid separator (GLS) adapted to interface, the cold vapor generator, and the ICP-MS, respectively. The peristaltic pump of the ICP-MS (P1) was used to pump the sample solution and the acid, and another pump (P2, (Ismatec, IP12N) was used for pumping the other reagents. A 0.8 mm id PTFE manifold tubing and peristaltic pump tubings were used with various diameters (Cole-Parmer, Vernon Hills, IL). Specifically, a 0.76 mm id pump tubing (black-black, at 1.0 mL min^{-1}) was used to propel the samples and standard solutions, a 1.14 mm id pump tubing (red-red, at 1.2 mL min^{-1}) to propel L-cysteine (Hg-i) or $KMnO_4$ (Hg-t), other 1.14 mm id pump tubing (red–red, at 1.2 mL min^{-1}) to propel $SnCl_2$ (Hg-i) or $NaBH_4$ (Hg-t) streams, and other 0.76 mm id pump tubing (black–black, at 1.0 mL min^{-1}) to propel the HCl solution (containing the antifoam B) and sample streams. A 3.18 mm id pump tubing (black–white, at 10.0 mL min^{-1}) was connected to the waste drain of the GLS (Fig. 1b). The argon gas line was disconnected from the nebulizer system and connected to the flow system to work as carrier gas (Fig. 1b). The operating conditions for the cold vapor generation coupled to the ICP-MS are summarized in Table 1. The volatile mercury vapor is introduced into the plasma following extraction into the argon carrier gas stream connected to the flow injection system by using a home made adapter (Fig. 1b). The ICP-MS measurement is started when the liquid sample enters the GLS. Sample data is acquired by using 70 sweeps/reading, 3 replicates, and a dwell time of 50 ms. Data were acquired in counts per second (cps) for Hg (*m*/*z* 202).

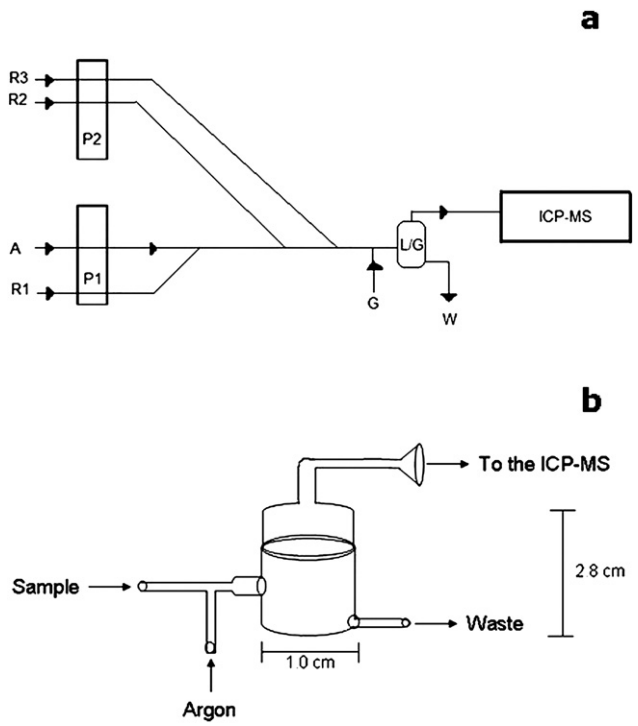

Fig. 1 (a) Schematic system for the flow injection cold vapor mercury generation coupled to ICP-MS and (b) gas/liquid separator adapted to interface the flow system with the ICP-MS. (A = Sample or standard; P1 and P2 = Peristaltic pumps; R_1 = HCl; R_2 = $KMnO_4$ or L-cysteine for Hg-t and Hg-i determination, respectively; R_3 = $NaBH_4$ or $SnCl_2$ for Hg-t and Hg-i determination, respectively; W = Waste; L/G = Chamber liquid–gas separation; G = Carrier gas (Argon).

Table 1 ICP-MS operating conditions for the fractionation of Hg in blood samples

Cold vapor flow injection parameter	
Hg-i determination	
HCl concentration (%, v/v)	4.0
L-cysteine (%, m/v)	1.1
$SnCl_2$ (%, m/v)	1.2
Carrier Argon flow rate/L min^{-1}	0.75
Hg-t determination	
HCl concentration (%, v/v)	4.0
$NaBH_4$ concentration (%, m/v)	0.4
$KMnO_4$ concentration (%, m/v)	0.4
Carrier Argon flow rate/L min^{-1}	0.75
ICP-MS experimental conditions	
Radiofrequency Power/W	1100
Scan Mode	Peak hopping
Resolution/amu	0.7
Replicate time/s	1
Dwell time/ms	50
Sweeps/reading	70
Replicates	3
Isotopes	^{202}Hg

For inorganic mercury determinations, a diluted sample was merged with the acid solution (4.0% v/v HCl) and further merged with L-cysteine (1.1% m/v) and $SnCl_2$ (1.2% m/v) solutions in this sequence in the chemifold. The mercury vapor formed was separated in the GLS and transferred by argon carrier into the ICP-MS for measurement (Fig. 1a and 1b).

For total mercury determinations, a diluted sample was merged with the acid solution (4.0% v/v HCl) and further merged with $KMnO_4$ 0.4% m/v and $NaBH_4$ 0.4% m/v solutions in this sequence in the chemifold. The mercury vapor formed is separated in the GLS and transferred by argon carrier into the ICP-MS for measurement as depicted in Fig. 1.

Memory effect experiments

The effectiveness of a mixture of 0.4% v/v 2-mercaptoethanol, 2.0% v/v HCl and 1 mg L^{-1} gold to reduce memory effects, by taking readings at 15 s intervals with a dwell time of 50 ms and 70 sweeps for the sampling sequences described as follows, was evaluated. To test equilibrium and washout, three blanks of solubilized base blood were measured first. The sample probe was then inserted into a solubilized blood spiked with 5.0 μg L^{-1} Hg-i and 5.0 μg L^{-1} MeHg. The measurement sequence was started as the liquid sample entered the GLS. Measurements at 15 s intervals were accumulated until a steady state signal was

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observed. The probe was then inserted back into the blank solution.

Results and discussion

Preliminary experiments

The alkaline sample dilution method adopted here is based in part on a previous work reported by Barbosa *et al.*[5] with little modifications. In that method, blood samples (500 μL) were first diluted 1 + 1 with 10.0% v/v TMAH, incubated for 2 h and then diluted by a further 1 + 4 with 2.0% v/v HCl. The selection of 2.0% v/v HCl was based on optimization studies showing the absence of protein precipitation. In the present work, this same sample preparation procedure was used, but with 3 h of sample incubation in alkaline medium instead of 2 h. All subsequent experiments were carried out with this dilution protocol.

Optimization of carrier gas flow rate

For the choice of the best carrier gas flow rate, a solubilized base blood was contaminated to contain 5.0 μg L^{-1} of Hg-i and analyzed by CV ICP-MS, according to the proposed method, with the use of $NaBH_4$ (0.4% m/v) as the reducing agent. The nebulizer gas line was disconnected from the nebulizer system and connected just before the GLS to work as carrier gas. To maintain the stability of the plasma, the argon flow rate was varied from 0.5 to 0.9 L min^{-1}. Higher sensitivity was obtained for a 0.75 L min^{-1} argon flow rate.

Memory effects of mercury

A significant and difficult problem to overcome when using cold vapor or direct ICP-MS mercury determination is the severe memory effect that is apparent for mercury in the instrument, which has been attributed to a combination of several factors.[22] The consequences of these effects include non-linear calibration graphs, long washout times, decreasing sensitivity with time, and signals dependent on the matrix.

In this study, potential mercury memory effects were examined for the proposed coupling of flow injection system to the cold vapor generator.

A solubilized and diluted blood sample (base blood) was contaminated to contain 5.0 μg L^{-1} of Hg-i and 5.0 μg L^{-1} of MeHg and analyzed according to the proposed sample introduction system with $NaBH_4$ (0.4% m/v). Another diluted base blood was also prepared without spiking (blank). A considerable memory effect was observed. To overcome this limitation, it was necessary to evaluate different reagents or a combination of them in the rinsed solution:[22–24] (i) a solution containing potassium dichromate, (ii) 2-mercaptoethanol and gold and (iii) a mixture of 2-mercaptoethanol 0.4% v/v, 2.0% v/v HCl and 1.0 mg L^{-1} gold. The solution containing 2-mercaptoethanol (0.4% v/v), HCl (2.0% v/v) and 1.0 mg L^{-1} gold was found to be superior to the other solutions with 1 minute of washing time (this time was sufficient for the signal of the base blood to decay to the original level). Then, after each running, this solution was passed through the flow system for one minute.

Evaluation of $NaBH_4$ concentration for Hg fractionation in blood samples by CV ICP-MS

Some recent publications demonstrated the feasibility of the separated determination of inorganic mercury and total mercury only with the use of different concentrations of sodium borohydride as reducing agent in CV AAS.[7,8] Inorganic mercury is selectively determined after reduction with 10^{-4}% m/v $NaBH_4$, while total mercury was determined after reduction with 0.75% m/v $NaBH_4$.[25] Based on these previous works, different concentrations of sodium borohydride were evaluated in the proposed CV ICP-MS system for a possible selective determination of total and inorganic mercury in blood. For this experiment, two aliquots of base blood were diluted 1 + 1 with 10.0% v/v TMAH and were incubated for 3 h as described previously, followed by a 1 + 4 dilution with 2.0% v/v HCl. One diluted blood was spiked with Hg to produce (i) 5.0 μg L^{-1} Hg-i and the other (ii) 5 μg L^{-1} MeHg. The concentration of $NaBH_4$ was varied from 0.0001% to 0.4% m/v. However, even at a very low $NaBH_4$ concentration (0.0001% m/v), a clear recovery of mercury (~15%) was found in the base blood spiked with methylmercury. Therefore, the specific determination of Hg-i based only in the variation of the reducing agent concentration is not possible. This fact probably occurred due to the formation of methylmercury hydride (H-MeHg) in the flow injection system, since the formation of this compound in $NaBH_4$ reducing conditions has been previously demonstrated.[26] On the other hand, it was also observed that quantitative recoveries of Hg-o in blood samples were not acquired with the use of only $NaBH_4$ and HCl. The best recovery (80%) of methylmercury was achieved with the use of 0.4% m/v $NaBH_4$. Higher concentrations of $NaBH_4$ were not evaluated, since that leads to plasma instability due to the higher formation of hydrogen. On the other hand, potassium permanganate is frequently used in flow injection CV AAS methods to oxidize those present organomercury species to inorganic Hg.[5] When this approach is combined with a subsequent on-line reaction using sodium borohydride, most organomercury species found in biological specimens are subsequently reduced and are easily determined by CV AAS, as reported by Barbosa *et al.*[5] for blood and by Tao *et al.*[12] for fish tissues. Then, further experiments were carried out to optimize the concentrations of $KMnO_4$ for total mercury determination in the solubilized blood samples.

Optimization of $KMnO_4$ concentration for total mercury determination

The effect of the $KMnO_4$ concentration for Hg-t mercury determination in blood with the proposed system was evaluated. The $KMnO_4$ solution was added on-line (see R2 on Fig. 1). Again, two aliquots of base blood were diluted 1 + 1 with 10.0% v/v TMAH, incubated for 3 h, as described previously, followed by a 1 + 4 dilution with 2.0% v/v HCl. A base blood was spiked with Hg to produce (i) 5.0 μg L^{-1} Hg-i and (ii) 8.0 μg L^{-1} MeHg. The concentration of $NaBH_4$ was fixed in 0.4% m/v.

The concentration of $KMnO_4$ was then varied from 0.05 to 0.4% m/v. Under these conditions, film residues of hydrated manganese(IV) oxide were not observed in the GLS. Fig. 2 shows that a better sensitivity, for both inorganic and organic mercury, was achieved with the on-line addition of a solution containing 0.4% m/v $KMnO_4$. Moreover, this concentration associated with

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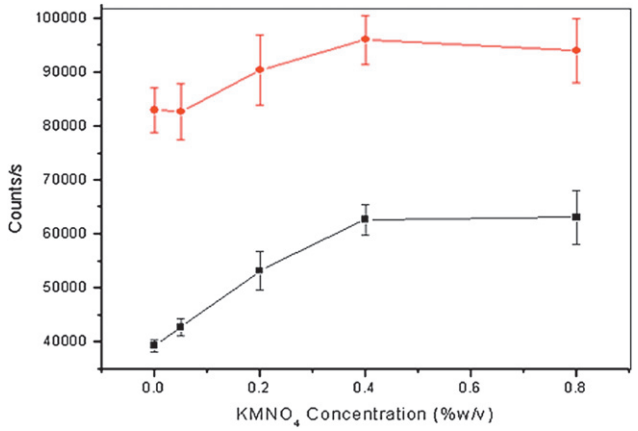

Fig. 2 Optimization of the $KMnO_4$ concentration for Hg-t determination (0.4% m/v $NaBH_4$; 4.0% v/v HCl; 0.75 L min^{-1} carrier gas flow rate). (—●—) Hg-o and (—■—) Hg-i. For more details of the experiment see the text.

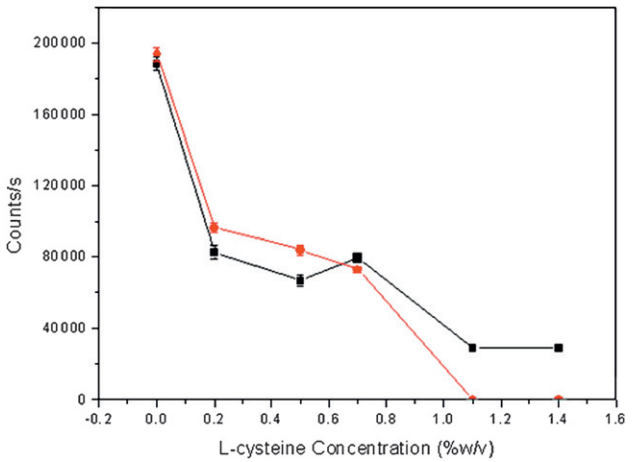

Fig. 3 Optimization of L-cysteine concentration for seletive inorganic mercury determination in blood samples (4.0% v/v HCl; 0.75 L min^{-1} carrier gas flow rate). (—●—) Hg-o and (—■—) Hg-i. For more details of the experiment see the text.

0.4% m/v $NaBH_4$ provided a recovery of total mercury in the spiked blood sample close to 100%.

No attempt was made to recover organomercury species other than MeHg, since this is considered one of the most difficult mercury species to reduce, and it is the predominant species found in human blood. However, we recognize the possibility that there may be some obscure organomercury species that require different operational conditions to be determined.

Optimization of $SnCl_2$ and L-cysteine concentrations for selectivity inorganic mercury determination in blood samples

Since the separated determination of inorganic mercury and total mercury with the use of different concentrations of sodium borohydride was not possible with the proposed system, we further evaluated the use of a different reducing agent, such as $SnCl_2$ or $SnCl_2$ in combination with L-cysteine. Then, optimization of the $SnCl_2$ concentration was performed as a compromise between mercury sensitivity and specificity for inorganic Hg determination in blood. Concentrations of $SnCl_2$ from 0.0 to 1.5% m/v were evaluated. Better sensitivity and specificity were achieved with the use of 1.2% m/v $SnCl_2$. Unfortunately, even at this concentration the use of only $SnCl_2$ was not able to effectively promote a separate determination of inorganic mercury in blood, since a recovery of Hg in a blood spiked with MeHg was observed. Since L-cysteine form stable complexes with Hg-o and may help with the specific reduction of Hg-i in presence of $SnCl_2$,[12] it was further evaluated the use of L-cysteine associated with $SnCl_2$ in the proposed system. Two aliquots of base blood were diluted 1 + 1 with 10.0% v/v TMAH and were incubated for 3 h, as previously described, followed by a 1 + 4 dilution with 2.0% v/v HCl. A diluted blood sample was spiked with Hg to produce (i) 5.0 μg L^{-1} Hg-i and (ii) 5.0 μg L^{-1} MeHg. The concentration of $SnCl_2$ was fixed in 1.2% m/v. Results for these experiments are shown in Fig. 3. The concentration of 1.1% m/v was chosen for L-cysteine, since under this condition only inorganic mercury was measured. Then, Hg-i determination in blood was carried out with the sequential on-line addition of 1.2% m/v $SnCl_2$ and 1.1% m/v L-cysteine (Fig. 3).

Recovery studies of Hg-t and Hg-i in whole blood samples

Initially, recovery studies were carried out with base blood samples contaminated to contain different concentrations of

Table 2 Recovery tests for Hg-t and Hg-i determination in blood samples by CV ICP-MS. Mean value (SD), n = 3

Sample	Hg-i add (μg L^{-1})	Hg-o add (μg L^{-1})	Hg-t obtained (μg L^{-1})	% recovery
1	0	7	7.5 (0.2)	107
2	0	6	6.2 (0.5)	103
3	6	0	5.8 (0.3)	96
4	6	6	12.4 (0.1)	103
5	2	0	2.1 (0.2)	106
Sample	Hg-i add (μg L^{-1})	Hg-o add (μg L^{-1})	Hg-i obtained (μg L^{-1})	% recovery
6	3.6	3.6	4 (0.3)	111
7	3.6	0	3.7 (0.1)	103
8	7	0	7.8 (0.2)	111
9	7	7	8.0 (0.3)	114
10	2	0	1.9 (0.1)	98

Table 3 Comparison of methods for Hg fractionation in ordinary blood samples and in NIST 966 SRM. Obtained concentration values, mean (SD), n = 3

Sample	CV ICP-MS		CV AAS	
Whole Blood	Hg-t (μg L^{-1})	Hg-i (μg L^{-1})	Hg-t (μg L^{-1})	Hg-i (μg L^{-1})
1	27.9 (1.8)	12.0 (1.3)	25.9 (2.3)	10.7 (1.7)
2	46.0 (4.5)	10.0 (2.5)	47.6 (3.1)	12.5 (1.6)
3	58.1 (3.3)	20.1 (2.0)	58.9 (4.4)	18.2 (1.2)
4	22.1 (0.9)	5.5 (0.2)	21.3 (0.9)	4.9 (0.9)
NIST 966[a]	30.2 (0.5)	15.4 (0.4)	—	—

[a] NIST SRM 966 Bovine Blood (Target value for total mercury = (31.4 ± 1.7) μg L^{-1} and for inorganic mercury = (14.87 ± 0.93) μg L^{-1}).

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Table 4 Analytical performance parameters for determination of total and inorganic Hg in whole blood: comparison between published methods and the proposed method with CV ICP-MS and alkaline sample preparation

Analytical parameter	Proposed method	Torres *et al.* (2009)[21]	Albalak *et al.* (2005)[10]	Chen *et al.* (1998)[9]	Bergdahl *et al.* (1995)[25]	Berglung *et al.* (2005)[27]
Method detection limit (μg L^{-1})	0.08 (Hg-t) 0.80 (Hg-i)	11.5 (Hg-t) 3.0 (Hg-i)	0.35 (Hg-i)	0.14 (Hg-t) 0.45 (Hg-i)	0.06 ng g^{-1} (Hg-t) 0.04 ng g^{-1} (Hg-i)	0.09 (Hg-t) 0.06 (Hg-i)
Sample volume (μL)	500	300 (Hg-t) 750 (Hg-i)	200	200	0.5 g	1000
Calibration method	Matrix matching	Aqueous standard	Matrix matching	Matrix matching	Matrix matching	Aqueous standard
Sample preparation procedure	Alkaline treatment at room temperature	Simple dilution	On-line digestion	On-line digestion	Off-line digestion	Stored overnight at room temperature
Sample throughput/h (duplicate)	20	6	17	—	—	4

Hg-i and Hg-o as methylmercury. The recovery results are presented in Table 2. For Hg-t, the obtained recovery values were from 96 to 107% and for Hg-i the values were from 98 and 114%, which is a preliminary indication of the good performance of the method.

Validation studies

Validation of the proposed method was accomplished using NIST SRM 966 Bovine Blood. For additional validation, 4 human blood specimens were analyzed using the proposed method and our data were compared to the results obtained using a reference method based on CV AAS.[18] Results for NIST SRM 966 are shown in Table 3. Values found using the proposed method are in good agreement with established target values for the SRM 966. Moreover, no statistical differences between the two techniques at 95% level on applying the *t*-test were observed for the analysis of the four human blood samples (Table 3).

The CV AAS method limit of detection (LOD) was 11.5 μg L^{-1} and 3.0 μg L^{-1} for total mercury and inorganic mercury, respectively. The LOD was defined as three times the standard deviation of ten measurements of the blank (base blood), divided by the slope of the calibration curve.

The CV ICP-MS proposed method detection limit (3 SD) was 0.08 μg L^{-1} and 0.80 μg L^{-1} for Hg-t and Hg-i, respectively, based on the analysis of 1 + 9 diluted base blood (n =10). Typical within-day precision was always lower than 7.0% (NIST 966), while between-day precision was < 12.0% RSD (NIST 966) for Hg-t and Hg-i.

Comparison of figures of merit between the proposed method and various published methods for Hg fractionation in whole blood by CV AAS are shown in Table 4. The proposed method compares well with most methods in detection capability, required sample volume and shows the best sample throughput in the Table.

Conclusion

The proposed non-chromatographic method by CV ICP-MS is attractive for the determination of total and inorganic mercury in blood samples due the simplicity of the sample preparation procedure. It can easily be used in routine fractionation analysis. The method is accurate as no statistical differences between results obtained by this proposed method and by another one using CV AAS were observed for the analysis of four human blood samples. Moreover, it can be an additional and alternative method for those clinical laboratories equipped with multi-elemental facilities, such as ICP-MS, eliminating the necessity of acquisition of additional and dedicated equipments for Hg fractionation in clinical samples.

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