

Post-Addition of Sulfuric Acid to Wet Digested Biological and Environmental Materials for Mercury Determination by Cold Vapor Atomic Absorption Spectrometry

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The charring of samples and loss of mercury by a nitric-sulfuric acid mixture has been overcome by prior digestion with nitric acid, followed by the addition of sulfuric acid into the final solution. This approach provides optimum decomposition and analytical conditions for a reliable cold vapor atomic absorption spectrometric determination of mercury in biological and environmental materials. In all cases, the presence of sulfuric acid improved the sensitivity of the mercury response significantly. Furthermore, the prior- and post-addition of sulfuric acid gave similar sensitivity and mercury concentrations for all samples. The adequacy of the prior digestion with nitric acid and post-addition sulfuric acid for reliable mercury determination was successfully demonstrated for blood, bone, bovine liver, mushroom, lake sediment, peat and prawn samples.

Keywords Cold vapor atomic absorption spectrometry, mercury, wet digestion, biological and environmental materials

Cold vapor atomic absorption spectrometry (CV-AAS) is the preferred technique for the determination of trace/ultra-trace concentrations of mercury in various samples because of its ease of operation and attainment of a much lower detection limit.^{1,2} However, like other techniques, the decomposition of the solid samples and, when necessary, the conversion of mercury to the divalent form, are required for an accurate determination by CV-AAS. Some of the approaches that have been employed for this purpose include the use of nitric acid³⁻⁵ and its mixture with sulfuric acid^{1,6,7}, hydrochloric acid⁸ and perchloric acid.^{6,9,10} The oxidizing power of the digestion acid or mixtures is often supplemented with the addition of either H₂O₂, KMnO₄, V₂O₅, K₂Cr₂O₇ or K₂S₂O₄.^{5,11-14}

A recent evaluation of some of these digestion methods in our laboratories revealed that the nitric-sulfuric acid mixtures was most effective for the CV-AAS determination of mercury in biological and environmental materials.¹⁵ However, it was noted that complete recovery of organomercury was not accomplished in some cases. Nevertheless, the recovery of organomercury obtained with the use of nitric-sulfuric acid mixture

(89.0–99.8%) was better than those (43.9–109.5) of the other acids or mixtures. It has also been reported in another study¹ that the nitric-sulfuric acid digestion mixture is effective for the decomposition of mercury sulfide prior to a CV-AAS measurement. The use of certified reference materials in our previous study¹⁵ also indicated that decomposition with the nitric-sulfuric acid digestion mixture gave reliable results. However, there is an underlying limitation in the use of a nitric-sulfuric acid digestion mixture, which is its tendency to char biological and environmental samples. If care is not taken, the charring of a sample can result in a loss of mercury during digestion and may give erroneous mercury results in solid samples.¹⁶

In this study, the prior digestion of solid biological and environmental materials with nitric acid, followed by post-addition of sulfuric acid, was explored as a mean of overcoming the charring problem and reducing losses of mercury caused by the use of nitric-sulfuric acid mixture. The post-addition of sulfuric acid to the nitric acid digests was considered as a means of obtaining an optimum CV-AAS measurement, as previously indicated.¹⁵ To ascertain the analytical benefit of this approach, a comparison was made between the prior- and post-addition of sulfuric acid to the digestion medium or sample digest, respectively. Also, the possibility of further improving the recovery efficiency by the addition of a strong oxidizing agent was considered. The prior digestion with nitric acid, followed by post-addition of

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sulfuric acid, was employed for accurate determination of mercury in bovine liver, prawn, river sediment, lake sediment, blood, bone, mushroom and peat samples.

Experimental

Reagents and standards

All reagents used in this study were of analytical grade and Milli-Q water was used for all solution preparations. A mercury stock solution (1 g/l) was purchased from PROLABO, Paris (France). A mercury standard (0.1 mg/l) was prepared daily by diluting the stock in 2%(v/v) hydrochloric acid. Tin(II) chloride solution (30%w/v) was prepared daily by dissolving an appropriate amount in 20%(v/v) hydrochloric acid and stabilized with the addition of a piece of tin. The concentrated acids used in this study were nitric acid (70%, Rhone-Poulem), hydrochloric acid (32.3%, BDH), sulfuric acid (98%, BDH) and perchloric acid (70%, BDH). Potassium permanganate (10%w/v, BDH) and 1.1 M oxalic acid (BDH) were prepared by dissolving appropriate amounts of their salts.

Biological and environmental materials

Prawn (AGAL-3), bovine liver (AGAL-20) and river sediment (AGAL-10) were obtained from Australian Government Analytical Laboratories, Pymble, NSW (Australia) and the lake sediment (SL-1) material was from International Atomic Energy Agency, Vienna.

Mushroom and peat samples were obtained from a local mushroom farm. Blood and bone samples were obtained from the Department of Biomedical Sciences at University of Sydney, Lidcombe, NSW (Australia).

Instrumentation and glassware

CV-AAS measurements were performed on a Varian Spectra 20 and GBC 902 atomic absorption spectrophotometers operated in double-beam mode. The conditions employed for the measurements were: wavelength, 253.7 nm; slit width, 0.5 nm; lamp current, 3.0 mA.

All glassware and plastic containers were soaked in nitric acid (2 M) for at least 24 h and rinsed 4–5 times with water prior to use.

Procedure

CV-AAS measurements. A 100 ml Erlenmyer flask containing 20 ml samples or the standard solution was connected to the mercury vapor generation system. Then, 1 ml tin(II) chloride solution was added and the contents of the flask were stirred with a magnetic stirrer (SYBRON Thermolyne Model S-1740) at maximum speed for 3 min. The resulting mercury vapor was displaced from the flask to the mercury cell by the water-displacement method¹⁷ at a rate of 10 ml/s. After recording the mercury response as the peak height, the cell was flushed out with instrumental-grade air.

Sample decomposition. A 0.5 g of prawn sample (or

0.5 g of peat at a peat:lime ratio 5:1 or 0.5 g bone, mushroom (freeze dried), lake sediment or 0.2 g bovine liver or 0.1 g of river sediment or 3 ml blood) was weighed into a pre-cleaned 100 ml (ground glass neck) Erlenmeyer flask and two procedures, prior-addition and post-addition, were then followed for the digestion of the sample. The prior-addition sample digestion procedure involved the addition of 3 ml of concentrated HNO₃ to the sample followed by heating at 90°C for 10 min. One milliliter of concentrated sulfuric acid (and/or 1.5 ml concentrated perchloric acid or 1.5 ml of potassium permanganate) was then added and the flask, partly covered with a pointed glass stopper, was then further heated for 80 min at 90°C. The post-addition sample digestion procedure involved the addition of 3 ml of concentrated HNO₃ to the sample followed by heating at 90°C for 90 min. The flask was cooled to room temperature, and 1 ml of concentrated sulfuric acid (and/or 1.5 ml concentrated perchloric acid or 1.5 ml of potassium permanganate) was then added. The excess potassium permanganate was neutralized by adding 1.0 ml oxalic acid.¹⁸ The digest was diluted to 25 ml (250 ml for river sediment) in a volumetric flask and 20 ml of this solution was used for the mercury measurements. Three separate digestions were performed on each sample. Mercury recovery was established by spiking samples with known concentrations of mercuric chloride solution.

Results and Discussion

Since the digestion of the prawn sample with HNO₃ only gave mercury concentrations very close to the certified value (Table 1), this suggests that the sample matrix is relatively easy to decompose. This sample therefore, provides an excellent matrix for investigating the effect of prior and post addition of acids and mixtures on the mercury response and concentrations.

Table 1 shows that the post-addition of sulfuric and perchloric acid mixture to a nitric acid digested sample slightly suppressed the sensitivity of the mercury response. This may have been due to the presence of a relatively higher concentration of perchloric acid compared with the prior-addition, where some of this acid would have been used up during digestion. The suppressive effect of perchloric acid is also evident in Table 1 for the nitric-perchloric acid mixture. However, it is interesting to note that both the prior- and post-addition strategies gave a similar sensitivity for mercury response with HNO₃+H₂SO₄ and HNO₃+H₂SO₄+KMnO₄. Also, identical mercury concentrations were obtained in the prawn sample by the prior- and post-addition strategies with these mixtures.

In contrast, prior and the post-addition of mixtures that contained perchloric acid gave significantly higher mercury concentrations for the prawn samples than with the other digestion mixtures. Nevertheless, the concentrations of mercury obtained with the mixtures were

Table 1 Influence of prior- and post-addition of acid components on sensitivities and mercury concentrations found in prawn sample

Digestion acid or mixture	Prior addition ^a		Post addition ^b	
	Mean Hg ^c /μg g ⁻¹	CV-AAS signal ^d	Mean Hg/μg g ⁻¹	CV-AAS signal ^d
HNO ₃	0.146±0.009	0.113±0.005	—	—
HNO ₃ +H ₂ SO ₄	0.159±0.002	0.126±0.002	0.159±0.004	0.126±0.003
HNO ₃ +H ₂ SO ₄ +KMnO ₄ ^e	0.143±0.002	0.128±0.002	0.144±0.003	0.129±0.001
HNO ₃ +HClO ₄	0.182±0.005	0.119±0.003	0.176±0.007	0.115±0.006
HNO ₃ +H ₂ SO ₄ +HClO ₄	0.171±0.002	0.125±0.002	0.163±0.003	0.116±0.001

a. Sample digested with the specified acid or mixture only. b. Sample digested with HNO₃, followed by addition of other component prior to CV-AAS measurement. c. $n=3$; mean±mean deviation (certified value: 0.15±0.03 μg/g). d. Signal for 60 ng of mercury. e. Measurements performed on GBC 902 atomic absorption spectrometer. All the above results were corrected for blank levels.

Table 2 Mercury concentration found in some biological and environmental reference materials with prior- and post-addition of sulfuric acid

Sample (Certified Hg value μg/g)	Digestion acid or mixture	Prior addition ^a		Post addition ^b	
		Mean Hg ^c /μg g ⁻¹	CV-AAS signal ^d	Mean Hg/μg g ⁻¹	CV-AAS signal ^d
Prawn (0.15±0.03)	HNO ₃	0.146±0.009	0.113±0.005	—	—
Prawn (0.15±0.03)	HNO ₃ +H ₂ SO ₄	0.159±0.002	0.126±0.002	0.159±0.004	0.126±0.006
Bovine liver (0.67±0.06)	HNO ₃	0.693±0.024	0.116±0.005	—	—
Bovine liver (0.67±0.06)	HNO ₃ +H ₂ SO ₄	0.690±0.012	0.120±0.002	0.689±0.018	0.119±0.004
River sediment ^e (11.79±1.10)	HNO ₃	10.92 ±0.24	0.129±0.001	—	—
River sediment (11.79±1.10)	HNO ₃ +H ₂ SO ₄	12.04 ±0.13	0.139±0.003	12.06 ±0.16	0.142±0.003

a. Sample digested with the specified acid or mixture only. b. Sample digested with HNO₃, followed by addition of H₂SO₄. c. $n=3$; mean±mean deviation. d. Signal for 60 ng of mercury. e. Measurements performed on GBC 902 atomic absorption spectrometer.

within the range of the certified value. As discussed above, the sensitivities obtained with nitric acid-sulfuric acid, and nitric acid-sulfuric acid-potassium permanganate were significantly higher and more stable than the other mixtures. Although potassium permanganate is preferred over potassium dichromate and hydrogen peroxide in oxidizing stable mercury compounds such as cinnabar (HgS), it lacks some important features of the other two substances.⁶ It can absorb mercury from air, and may result in higher blank concentrations if proper care is not taken. A slight excess of hydroxylammonium chloride used to neutralize the excess of permanganate can interfere with the mercury response.^{18,19} Moreover, potassium permanganate, even in an acid medium, is reduced by organic substances to solid hydrated manganese dioxide, which precipitate on the sample particles, thus hindering their complete oxidation.⁶ In a recent study it has been reported that the nitric acid and sulfuric acid mixture is capable of dissolving considerable amount of HgS.¹ These observations confirm that the addition of sulfuric acid alone is suitable for the determination of mercury in biological and environmental samples. Also, the data given in Table 1 indicate that the post-addition of sulfuric acid is useful for obtaining a more sensitive mercury

response. It is interesting to note that the addition of sulfuric acid to nitric acid and perchloric acid digestion mixture gave values closer to the certified values.

Table 2 shows that the prior and post-addition of sulfuric acid gave similar results for the digested biological and environmental reference materials. However, the initial digestion of the samples with nitric acid is advantageous in reducing sample charring, which often occurs with a nitric-sulfuric acid mixture. The post-addition of sulfuric acid to nitric acid digested samples was useful for improving the sensitivity and stability of the mercury response in real samples. The combination of nitric acid digestion with a post-addition of sulfuric acid therefore provides an excellent basis for accomplishing both the optimum decomposition and CV-AAS measurement conditions. The accomplishment of optimum decomposition with only nitric acid is clearly reflected in Table 2 by the closeness of the mercury concentrations in nitric acid digested samples to the certified values. It is important to note that the data in this table also indicate that the sensitivity of the mercury response was consistently higher for all samples in the presence of sulfuric acid.

Table 3 provides the attainable recovery efficiency for spiked inorganic mercury from various biological and

Table 3 Spiked recovery of mercury in nitric acid digested samples after post-addition of sulfuric acid

Sample	Hg ²⁺ added/ ng	Mean Hg recovered ^a /ng	% Recovery
Blood	25.0±0.1	23.2±1.0	92.8±3.9
Bone	50.0±0.1	51.3±0.7	102.6±1.5
Mushroom	50.0±0.1	52.0±3.0	104.0±6.0
Peat	25.0±0.1	20.5±0.6	82.0±1.9
Lake sediment	50.0±0.1	47.8±1.9	95.6±3.9

a. n=3; mean±mean deviation.

Table 4 Mercury concentrations obtained with post-addition of sulfuric acid to nitric acid digested samples

Sample	Number of samples	Mean Hg found ^a / µg g ⁻¹
Blood	2	0.867±0.033 ^b
Bone	1	0.112±0.001
Mushroom	3	0.051±0.003
Peat	2	0.022±0.004
Lake sediment	1	0.134±0.003

a. n=3; mean±mean deviation.

b. µg/l.

environmental samples. Apart from the peat sample, good percentage recoveries of mercury were obtained for blood, bone, mushroom and lake sediment samples (92.8±3.9 to 104.0±6.0%). The low recovery of mercury in the peat sample may have been due to some unknown interferants in the sample.

Table 4 shows the mercury concentrations obtained in biological and environmental samples with the post addition of sulfuric acid to nitric acid digested samples. The mean mercury concentration found in the blood sample (0.867±0.033 µg/l) was within the reference range.²⁰ The mercury concentration in mushroom samples varied from 0.042±0.002 to 0.060±0.002 µg/g. It is noted that the concentration of mercury in mushroom increased from the first harvest to the third harvest. This may have been due to the longer contact of the mushroom with the growing materials. The concentration of mercury in these mushroom samples was much lower than those reported for wild mushrooms.²¹ The amounts of mercury in the peat samples were similar.

In conclusion, the presence of sulfuric acid improved the sensitivity of mercury response obtained by CV-AAS for nitric acid digested samples considerably. The prior- and post-additions of acid components to diges-

tion mixtures or sample digests revealed that the inclusion of perchloric acid is undesirable for a reliable mercury determination owing to its interference which results in higher than expected concentrations. Most reliable mercury concentrations were obtained with the prior- and post-addition of sulfuric acid. It is therefore recommended that the prior digestion of a sample with nitric acid, followed by post-addition of sulfuric acid be used to accomplish optimum decomposition and analytical conditions for the CV-AAS determination of mercury in biological and environmental materials.

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