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Advanced oxidation processes for degradation of organomercurials: determination of inorganic and total mercury in urine by FI-CV-AAS

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A new methodology, based on advanced oxidation processes, for the determination of inorganic and total mercury in human urine is proposed. Ultrasound in conjunction with ozone (sonozone) was used to avoid urine dilution for inorganic mercury determination. A new oxidation method in urine based on $KMnO_4$–HCl–focused ultrasound is proposed for conversion of methylmercury into inorganic mercury and subsequent determination by flow injection-cold vapour atomic absorption spectrometry (FI-CV-AAS). The methodology was applied to the determination of inorganic and total mercury in spiked urine from different non-exposed volunteers.

Introduction

Mercury is a toxic element of widespread environmental and clinical significance. Inorganic mercury (Hg^{2+}) and methylmercury ($MeHg^{+}$) are the two major mercury species found in biological samples.[1] Table 1[2–7] summarizes the sample treatment and mercury levels found in urine taken from selected references in which mercury (total and organic) was determined by CV-AAS. Advanced oxidation processes (AOPs), which involve the *in situ* generation of highly potent chemical oxidants such as the hydroxyl radical (OH), have recently emerged as an important class of technologies by accelerating the oxidation and hence destruction of a wide range of organic contaminants in polluted water and air.[8] Based on previous works[7,9] a new oxidation method based on room temperature ultrasonic irradiation is proposed for the degradation of organic matter/methylmercury present in urine and subsequent total mercury determination by FI-CV-AAS. The use of sonozone (*i.e.*, ultrasounds and ozone) is proposed to release the inorganic mercury present in the urine, avoiding urine dilution.

Experimental

Apparatus

The flow injection system used for cold vapour generation consisted of a four channel Gilson (Villiers le Bel, France) Minipuls 2 peristaltic pump, a PerkinElmer (Überlingen, Germany) membrane gas–liquid separator, a four-way Rheodyne (Supelco, Bellefonte, PA, USA) injection valve with a 500-mL loop, and a Fisher and Porter (Warminster, PA, USA) flow meter (0–100% N_2). Tygon tubing of different internal diameters was used for carrying the reducing agent, carrier solution, carrier gas and waste solution. The initial conditions for cold vapour generation using $NaBH_4$ as a reducing agent were established in a previous work.[10] A FI system was used with: 0.3% m/v $NaBH_4$ solution stabilized in 1% m/v NaOH, 3 mL min^{-1}; 3% v/v HCl solution used as carrier, 10 mL min^{-1}; carrier gas (N_2), 200 mL min^{-1}. Ozone was generated from air with an Iberozono compressor (Cantabria, Spain), Model DNP/A 50 (ozone generation rate 200 mg h^{-1}). KI solutions (2% m/v) were used in order to control the ozone production of the compressor as follows: 5 mL of the KI solution was transferred into an ozone reactor[10] and ozone was bubbled during 3 min at a flow rate of 5 L min^{-1}. The absorbance of the solution was 0.901 measured at 270 nm and 1.368 at 350 nm. **Warning**: ozone is an irritant. All experiments with ozone must be carried out in a fume cupboard.

A Branson Sonifier 150 ultrasonic cell disruptor/homogeniser (63 W, 22.5 kHz, Branson Ultrasonics Corporation, USA) equipped with a 3-mm titanium microtip was used. Ultrasonic energy irradiation was fixed at any desired level using a power setting in the 40–70% range. The Sonifier 150 has a digital LCD display which provides a continuous read-out of the watts delivered to the end of the probe (range 5–12 W in this work). Mercury absorbance was measured with a Varian (Cambridge, UK) atomic absorption spectrometer Model SpectrAA 20 plus equipped with a home-made quartz tube. The quartz tube was kept at room temperature during operation. A mercury hollow-cathode lamp operated at 4 mA was used as a radiation source. The mercury line at 253.7 nm and a slit width of 0.5 nm were used for measurements.

Reagents

All chemicals were of analytical-reagent grade. Milli-Q water was used throughout. Sodium tetrahydroborate(III) (Merck) was prepared fresh daily by dissolving the solid in 0.25 mol L^{-1} sodium hydroxide solution (Merck). An inorganic mercury stock

Table 1 Selected references. Mercury determination in urine

Type of urine	Sample dilution	Reagents added	Additional devices/remarks	Mercury/ng ml^{-1}	Ref.
Non-exposed subjects	No dilution direct to FI	(a) $H_2S_2O_8$ + HCl; (b) HCl	On-line microwave digestion mercury speciation	1.65–6.50	2
Patients with Blackfoot disease	5–25 mL	HNO_3 + HCl (1 : 1)	Off-line microwave digestion	2.5–22.0	3
Workers exposed to mercury vapour	1–10 mL	(a) HNO_3; (b) H_2SO_4 + $KMnO_4$; (c) H_2SO_4 + $KMnO_4$ + heat	Mercury speciation	N.D.–21.0	4
Non-exposed workers	1–5 mL	HCl + KBr + $KBrO_3$	On line microwave digestion	3.04–7.03	5
Workers exposed to mercury vapour	1–10 mL	(a) HNO_3; (b) HNO3 + heat; (c) H_2SO_4 + $KMnO_4$ + heat	Total mercury	5–21	6
Control urine samples	No dilution	$KMnO_4$ + KBr + $KBrO_3$	Total mercury	7.8–119	7

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standard solution (Merck, 1 g L^{-1}) was used. A methylmercury stock standard solution (0.10 g L^{-1}) was prepared from methylmercury chloride (Riedel-de Häen, Seelze, Germany). All stock standard solutions were stored in a refrigerator at 4 °C and protected from light. Working standard solutions were prepared just before use by appropriate dilution of the stock standard solution. Nitric acid (Merck, Suprapur, 65% m/v), potassium permanganate (Merck), hydrochloric acid (Merck, 37% m/v pro analyse), were used when necessary. All solutions were prepared freshly every day.

Specimen collection

Exogenous contamination was avoided by cleaning all the plastic bottles used for specimen collection with HNO_3 10% v/v and then rinsed gently with ultrapure water and dried at room temperature. Urine specimens were collected on each day of analysis in clean plastic bottles and acidified with HCl (5 mL of HCl to *ca.* 250 mL of urine). Parameter optimisation was performed with 24 h urine. The urine was taken from a female volunteer, a healthy student (22 years old). When necessary, for comparative purposes, urine from other non-exposed students from the IST was also used.

Oxidation procedure

(i) *Inorganic mercury*. Ten millilitres of urine in HCl 1 M was transferred into a reactor specially designed for sonozone application. Ozone was generated from air with a flow rate of 5 L min^{-1} and the ultrasonic probe was set at 50% (output power 7 W). Whilst being sonicated, ozone passed through the solution for 30 min. The sonozone reactor was inserted in an ice bath so that the initial temperature (*i.e.*, before sonication) was 15 °C. The final temperature (*i.e.*, after sonication) was 35 °C. Three replicates were performed for each sample.

(ii) *Total mercury*. Ten millilitres of urine in HCl 1 M was transferred into a polyethylene tube (50 mL capacity) with 0.05 g of $KMnO_4$, and sonicated by means of a probe ultrasonic processor for 3 min at 50% sonication amplitude (7–8 W delivered as digital LCD display). The oxidation vessel was inserted in an ice bath so that the initial temperature (*i.e.*, before sonication) was 15 °C. The final temperature (*i.e.*, after sonication) was 22 °C.

Three replicates were performed for each sample. Oxidation was performed just before measurement by FI-CV-AAS.

Results and discussion

In this work the determination of inorganic and total mercury in spiked urines of non-exposed volunteers was analysed using different methodologies. The spiked Hg concentration (20×10^{-6} g L^{-1} Hg^{2+}) was found in people exposed to mercury vapour and in patients with blackfoot disease (Table 1). The parameters influencing the proposed processes were investigated so that the best conditions of analysis could be chosen. In absorbance measurements produced by cold Hg vapour generation the figure of merit was peak height, as this is more sensitive to experimental variables than peak area.[11]

The initial efforts were oriented to studying the inorganic mercury recovery in undiluted urine as a function of the reducing agent concentration (range concentration 0.05–1.5 % m/v) in the absence of any previous treatment. Absorbances produced by the cold vapour generation from urine samples spiked with Hg(II) were compared with that of an aqueous standard solution of the same concentration. $NaBH_4$ was used as the reducing system for the release of Hg in acidic medium (HCl 1 M), the best recovery (85 ± 7%, $n = 3$) being attained with 0.3% m/v, which was the concentration selected as optimum in subsequent experiments. $NaBH_4$ concentrations higher than 0.3% led to a decrease in the mercury recovery and in the sensitivities of both urine and Hg(II) aqueous solution. Finally, with the selected reducing agent concentration, the mercury recovery was studied in urine diluted by a factor of 1–6. In our experience, a dilution factor of 4 was the minimum necessary in order to obtain the total mercury recovery. Higher dilution factors may be necessary as a function of the organic matter content present in the urine. The dilution was necessary in order to cancel the interference of the organic matter present in urine, which decreases mercury recovery. Negligible recoveries of mercury in non-treated urine samples spiked with methylmercury were obtained.

Sonozone treatment in the determination of inorganic Hg

In order to avoid urine dilution and, as a consequence, an increment in the detection limit, different strategies were used in this work to release the inorganic mercury from the urine samples. All the approaches investigated were based on the following advanced oxidation processes: 10 min of ultrasonic irradiation by probe sonication (method A); 30 min of ozonation (method B); 10 min of ultrasonic irradiation, 10 min of ozone and 10 min of ultrasound and ozone, sonozone (method C); 30 min of sonozone (method D). Urine from two volunteers was used in this study. The results are shown in Table 2. As can be seen, when the urine is diluted 6 times the inorganic mercury is totally recovered from untreated samples. However, the recovery fell down below 80% when undiluted urine was used. The use of ultrasonic irradiation (method A) or ozonation (method B) did not improve the recovery to the desired level. Only methods C and D provided the total recovery of spiked mercury in undiluted urine. Since the method D met the requirement of simplicity when compared with method C, that is, with the sequential application of ultrasound and ozone, method D was chosen as the optimum.

Influence of the sample treatment and liquid medium composition on mercury determination in urine

Preliminary studies were performed to evaluate the influence of the medium composition and temperature on the mercury determination. Absorbances produced by the cold vapour generation from urine samples spiked with Hg(II) and methylmercury (20×10^{-6} g L^{-1} in Hg) were compared against aqueous standard

Table 2 Effect of different factors on sonozone treatment of urine and water samples spiked with both Hg(II) and methyl-Hg(I)

	Hg(II) recovery (%)		Methyl-Hg recovery (%)	
Experimental conditions[a]	Water	Urine	Water	Urine[c]
Sonozone + HNO_3 + ice bath	96 ± 5	93 ± 6	16 ± 3	N.D.
Sonozone + HCl + ice bath	97 ± 5	101 ± 7	99 ± 4	N.D.
Sonozone + HNO_3 + room temp.[b]	93 ± 6	80 ± 6	11 ± 2	N.D.
Sonozone + HCl + room temp.[b]	99 ± 3	73 ± 3	91 ± 4	N.D.
HNO_3 without sonozone treatment	—	81 ± 2	7 ± 2	N.D.
HCl without sonozone treatment	—	79 ± 5	9 ± 3	N.D.

[a] Urine samples spiked with 20×10^{-6} g L^{-1} of Hg as Hg(II) and as methylmercury; HNO_3 1 M; HCl 1 M; sonozone with 30 min sonication time and 5 L min^{-1} ozone flow rate; reducing agent $NaBH_4$ 0.3% m/v in 1% m/v NaOH. [b] Sporadic foaming in the gas–liquid separator and precipitate formation. [c] N.D. = not detected.

Table 3 Determination of inorganic and total mercury in spiked urine from non-exposed volunteers following sonozone irradiation or $KMnO_4$–HCl–focused ultrasound

	Hg found/g $L^{-1} \times 10^{-6}$					
	Sonozone treatment[a]			$KMnO_4$–ultrasonic iradiation[a]		
Urine	A	B	C	A	B	C
Volunteer 1	18 ± 2	N.D.	9 ± 1	19 ± 2	17 ± 2	21 ± 3
Volunteer 2	20 ± 1	N.D.	11 ± 1	19 ± 1	19 ± 2	20 ± 2
Volunteer 3	19 ± 2	N.D.	9 ± 1	18 ± 1	21 ± 1	20 ± 1

[a] Experimental conditions: sonozone treatment and $KMnO_4$–ultrasonic irradiation according to the Experimental section. Hg species added: (A) 20×10^{-6} g L^{-1} Hg(II); (B) 20×10^{-6} g L^{-1} MeHg(I); (C) 10×10^{-6} g L^{-1} Hg(II) + 10×10^{-6} g L^{-1}MeHg(I).

solution of the same concentration under the following conditions: in the presence of HNO_3 or HCl, with and without sonozone treatment; with the sonozone reactor immersed in an ice bath or at room temperature. The results shown in Table 2 indicated that under typical reaction conditions in the manifold used for cold vapour generation, the sensitivity for spiked Hg(II) in urine or aqueous solution is similar after sonozone treatment with either HNO_3 or HCl acidification. On the other hand, low mercury recoveries (<80%) and poor analytical performance of the sonozone treatment (*e.g.*, foaming, formation of a precipitate) were obtained when the treatment was developed under conditions of no cooling. This finding was expected since solution heating should be avoided owing to the decreased cavitation that occurs with increasing temperature and the enhanced volatilization risk of Hg species.

Table 2 also indicates that methylmercury is only fully decomposed by sonozone treatment in aqueous solution in the presence of HCl. This finding has been reported previously.[12] In addition, none of the different AOP approaches presented in Table 2 decomposed the methylmercury present in the urine.

Finally, a study of the influence of $NaBH_4$ concentration on the mercury recovery in treated urine spiked with inorganic mercury and methylmercury was performed. Results show that total recoveries are achieved only in the case of inorganic mercury in the $NaBH_4$ concentration range of 0.01–0.3% m/v. Higher borohydride concentrations led to lower recovery values. Moreover, no recovery of mercury from urine spiked with methylmercury was observed at any of the reducing agent concentrations used. $NaBH_4$, 0.05% m/v, was used as the reducing agent concentration when sonozone treatment in urine was applied.

Total mercury determination in urine after US/$KMnO_4$ treatment

Guo *et al.* verified that different organic mercury compounds can be decomposed in urine by the combined effect of $KMnO_4$–$NaBH_4$, previous addition of bromate–bromide reagent and Triton X-100 to the sample.[7] It might be expected that $KMnO_4$, in conjunction with ultrasonic energy irradiation, could also decompose methylmercury in urine. However, some problems associated with the use of $KMnO_4$ might occur, since its reduction may lead to the formation of manganese(IV) oxide instead of Mn(II), depending on the pH of the solution. MnO_2 has been cited as forming a film on the surfaces of sample vessels, tubing and other manifold components in which mercury is adsorbed.[13] Some authors have used hydroxylamine hydrochloride to dissolve the hydrated manganese oxide and to remove the excess of $KMnO_4$.[4] Initial experiments, under ultrasonic energy irradiation, showed that when urine (10 ml) in HCl 0.1 M was mixed with 0.5% m/v $KMnO_4$, a characteristic red-coloured precipitate was formed owing to the formation of MnO_2. However, by using the ultrasonic probe in conjunction with $KMnO_4$ in HCl 1 M, the characteristic purple colour of the $KMnO_4$ totally disappeared and no precipitate was formed. In addition, the urine became colourless after treatment. This finding was used to check the final point of the treatment. It should be stressed at this point that the reaction time between the urine (10 ml in HCl 1 M) and $KMnO_4$ (0.5% m/v) was less than 3 min when probe sonication was used, against 30 min in its absence. Moreover, the methylmercury added to the urine samples was totally recovered, that is, the proposed methodology was effective for the decomposition of methylmercury. The influence of $KMnO_4$ concentration on methylmercury decomposition in urine or aqueous solutions, both in HCl 1 M, was studied. Methylmercury in aqueous solution was totally decomposed by the couple ultrasound–$KMnO_4$ with a concentration as low as 0.01%. On the other hand, total methylmercury recovery in urine (10 ml in HCl 1 M) was only achieved if $KMnO_4$ concentrations higher than 0.4% m/v were used.

Determination of inorganic and total mercury in urine

The feasibility of sonozone and $KMnO_4$–ultrasonic irradiation couple in the determination of, respectively, inorganic and total mercury in spiked urine from three different non-exposed volunteers was presented in Table 3 (experimental conditions of sonozone and $KMnO_4$–HCl–focused ultrasound described in the Experimental section). The results confirmed that inorganic mercury can be determined by sonozone treatment, and the total content of methylmercury in urine can be estimated from the difference between the total mercury ($KMnO_4$–HCl–ultrasonic irradiation) and inorganic mercury content.

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References

1 C. Baird, *Environmental Chemistry*, W. H. Freeman and Co., New York, 1999, pp. 386–390.
2 M. Gallignani, H. Bahsas, M. R. Brunetto, M. Burgera, J. L. Burgera and Y. Petit de Peña, *Anal. Chim. Acta*, 1998, **369**, 57–67.
3 C. J. Horng and S. R. Lin, *Talanta*, 1997, **45**, 75–83.
4 O. Zenebon, A. M. Sakuma, F. D. de Maio and I. A. Okada, *Anal. Lett.*, 1999, **32**, 1339–1349.
5 M. F. M. Noth, T. A. Hamid and Z. Ismail, *At. Spectrosc.*, 1998, **19**, 95–99.
6 A. M. Sakuma, F. D. de Maio, R. Q. Utishiro and C. S. Kira, *At. Spectrosc.*, 1999, **20**, 186–190.
7 T. Guo and J. Baasner, *Anal. Chim. Acta*, 1993, **278**, 189–196.
8 J. L. Capelo-Martínez, P. Ximenez-Embun, Y. Madrid and C. Camara, *Trends Anal. Chem.*, 2004, in the press.
9 J. L. Capelo, I. Lavilla and C. Bendicho, *Anal. Chem.*, 2001, **73**, 3732–3736.
10 J. L. Capelo, H. A. Pedro and A. M. Mota, *Talanta*, 2003, **61**, 485–491.
11 C. Vargas-Razo and J. F. Tyson, *Fresenius' J. Anal. Chem.*, 2000, **366**, 182–190.
12 J. L. Capelo, I. Lavilla and C. Bendicho, *Anal. Chem.*, 2000, **72**, 4979–4984.
13 B. Welz, D. L. Tsalev and M. Sperling, *Anal. Chim. Acta*, 1992, **261**, 91–103.