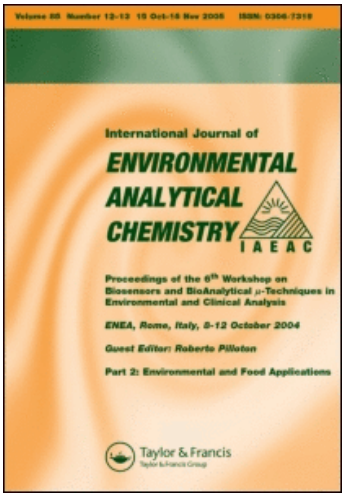

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SEPARATION OF METHYLMERCURY FROM HUMAN HAIR BY SOLVENT EXTRACTION

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An improved method of separation of methylmercury from inorganic mercury in hair has been developed. The method is based on alkaline dissolution of human hair and on extraction of methylmercury into benzene from an acidic solution of potassium iodide. Radiotracer experiments were used in the development and testing of the method. First a suitable method for preparation of spiked hair with radioactive or stable inorganic mercury and methylmercury was given. Human hair samples spiked with radioactively labelled inorganic mercury and methylmercury were then used for testing of the various possibilities of hair dissolution without changing the chemical form of the mercury and for finding optimal conditions for the solvent extraction separation of both mercury species.

KEY WORDS: Mercury, methylmercury, speciation, human hair, standard sample, separation, solvent extraction.

INTRODUCTION

Environmental or professional exposure of man to mercury is often analysed using measurements of mercury content in human hair[1]. It is well known that the toxicity of different forms of mercury differs considerably. Alkylmercury and especially methylmercury (MeHg) belong to the most toxic forms of mercury and therefore in advanced pollution studies they are distinguished from inorganic forms of mercury (Hg_{in}). Various methods for determination of methylmercury in environmental samples have been developed[2-4]. The methods are mostly based on separation of methylmercury from inorganic mercury using gas chromatography, ion exchange, solvent extraction, etc. At present the separation method most frequently used is solvent extraction[4].

However, limited knowledge still exists on the influence of the matrix on the efficiency of the separation and on possible changes in the species of mercury during the separation processes. This also applies for determination of mercury in hair. Therefore we studied the separation of methylmercury and inorganic mercury from hair using solvent extraction and the effect of dissolution of human hair on the species of mercury added to the hair.

Neutral halogenide complexes of the type HgX_2 or CH_3HgX can be extracted into

non-polar organic solvent such as benzene, toluene or xylene. The distribution constant K_D of individual complexes increases in the order: chloride < bromide < iodide[5,6]. At higher concentrations of ligands, inorganic mercury forms non-extractable anionic complexes, whereas methylmercury remains extractable as electroneutral complex. From the values of stability and extraction constants of methylmercury and inorganic mercury complexes[7], suitable conditions for their separation were calculated and experimentally verified.

The radiotracer method was used throughout this work. The application of radioactively labelled mercury species greatly facilitates the study of behaviour of the species in the separation. The labelled species can be easily traced during the separation by measurement of its (or their) activity, if isotope exchange between the labelled species and other mercury species is negligible or slow. The rate and extent of the isotope exchange between methylmercury and inorganic mercury is known from the paper by Stary and Prasilova[8]. Therefore, conditions could be selected to maintain the isotopic exchange negligible.

Basic problem in the use of the radiotracer method for the given purpose was to achieve equal behaviour between the labelled mercury species added to hair samples and the same mercury species naturally present in the hair. Problem of similar nature was also brought by the need to prepare standard hair sample with artificially elevated content of mercury or methylmercury for analytical purposes. The equal behaviour cannot easily be proved by model experiments and must be checked in practical analyses, for instance by comparison of results obtained using different separation methods. Therefore we carried out parallel determination of MeHg in nonlabelled human hair by the method described in this paper and by the acid leaching of hair[9]. The good agreement of the results provide the indirect evidence that our assumption about the equal behaviour made in this paper is correct.

EXPERIMENTAL

Apparatus

Radiometric Assembly NV 3102 TESLA with well-type NaI(Tl) crystal pH-meter PHM Radiometer Copenhagen

Chemicals

All reagents were of A.R. purity.

—^{203}Hg, specific activity 250 GBq/g (Du Pont de Nemours, Germany)

—Methylmercury labelled with ^{203}Hg, prepared by the isotope exchange method[5]: 2 ml of 10^{-3} M CH_3HgCl in 0.1 M NaOH were mixed with 0.1 ml 4 M HNO_3, 0.3 ml 0.1 M $AgNO_3$ and 0.2 ml 10^{-3} M $^{203}Hg(NO_3)_2$ in a ground stoppered test tube. Under these conditions a rather rapid isotopic exchange occurs. The mixture was left standing in the dark for 72 hours whereby, isotope exchange equilibrium was reached. Then 1 ml of conc. HCl was added and the labelled $CH_3{}^{203}HgCl$ was successively extracted with two 2 ml portions of benzene (2 min shaking), whereas inorganic mercury(II) was retained in the aqueous phase in anionic

form, $^{203}HgCl_4^{2-}$. The combined organic extracts were washed with 2 ml of 0.1 M NaCl and methylmercury was stripped from the organic phase into 2 ml 0.02 M NaOH as $CH_3{}^{203}HgOH$. The solution prepared in this way was diluted with 0.02 M NaOH to the required concentration.

—Hair sample: about 0.5 kg of human hair from different persons was cut to less than 5 mm long pieces by stainless scissors, washed with water and acetone according to the procedure recommended by IAEA and WHO[10] and homogenized by mixing.

Procedure for spiking of human hair samples

On the basis of preliminary tests, the following procedure for preparation of spiked hair samples was proposed: 20 ml of an aqueous solution containing 0.01 M acetate buffer (pH 4.7), 0.001M NaCl and 0.03–0.8 μg Hg/ml (as radioactively labelled Hg_{in} or MeHg) are stirred with 1 g cut hair for 1 h. The hair is separated by centrifugation, washed twice with 40 ml of distilled water, twice with 40 ml of acetone and dried on air.

The hair prepared in this way contains more than 90% of MeHg present in the loading solution. The uptake of inorganic mercury depends on the concentration of mercury. Under the conditions used, it is 75% for 0.03 μg/ml of Hg and 90% for 0.5 μg/ml of Hg. Grinding of hair in an agate mill accelerates and increases (by several percents) the uptake of both methylmercury and inorganic mercury.

The added mercury species are firmly fixed by hair. The measurement of radioactivity of prepared samples showed that the samples did not lose mercury to any significant extent when stored in open glass ampoules for as long as 125 days. Determination of activity of $Me^{203}Hg$ in the samples after 35 and 125 days of the storage using the extraction procedure described below proved that the transformation of methylmercury to inorganic forms of mercury and vice versa was negligible.

The proposed method can be also used for preparation of nonradioactive standard samples of hair containing artificially elevated concentration of methylmercury or inorganic mercury for standardization and intercomparison purposes.

All further experiments were carried out with hair containing 1–2 μg/g of Hg as labelled $^{203}Hg_{in}$ or $Me^{203}Hg$, respectively.

Dissolution of hair samples

Dissolution of hair samples was studied in various concentrations of H_2SO_4, HCl, NaOH and KOH at various temperatures: 100 mg of hair labelled with $Me^{203}Hg$ or $^{203}Hg_{in}$ were placed in a centrifuging tube and 1 ml of the dissolving medium was added. After determination of the initial activity (A_0) of the samples the tube was kept in thermostat at desired temperature for time required for total dissolution of the sample. From the activity of the dissolved sample (A_1) percentage of volatilized mercury was calculated.

Decomposition of MeHg during dissolution was studied by the extraction method. A hair sample labelled with $Me^{203}Hg$ was dissolved by the procedure studied. The pH value of the dissolved sample was adjusted to 0–1 by addition of H_2SO_4 or NaOH. Solid KI was added

to bring the concentration of iodide in the sample to 0.5 M, and CH_3HgI thus formed was extracted into benzene (efficiency of the extraction is higher than 97%, while Hg_{in} remains almost quantitatively in aqueous phase —see below). The extent of transformation of MeHg into Hg_{in} was determined from the comparison of the activity of the organic phase (A_2) with A_1.

Separation of MeHg from hair using solvent extraction

The values of the stability and extraction constants of halogenide and hydroxo complexes of MeHg and Hg_{in} were used for the calculation of distribution ratios of individual complexes between benzene and the aqueous phase under different experimental conditions. From the results it was concluded that the best mutual separation of MeHg and Hg_{in} by the solvent extraction should be achieved from aqueous solution of 0.5 M KBr at pH <10 or of 0.5 M KI at pH <11.

In the study on the extraction of both mercury species from dissolved hair samples in the pH range 0–14, the following experiments were carried out. After determination of the initial activity, 100 mg of radioactively labelled (with either $Me^{203}Hg$ or $^{203}Hg_{in}$) hair in centrifugation tube were dissolved in 0.2 ml 10 M NaOH at 90–95°C. Then 1.8 ml of distilled water were added and pH was adjusted to the desired value by adding concentrated H_2SO_4. The solution was cooled to room temperature and solid KBr or KI was added so that its final concentration was 0.5 M. After 10 min shaking with 2 ml of benzene, the aqueous and organic phases were separated by centrifugation and pH of the aqueous phase as well as the activity of organic phase were measured. The percentage of extraction (%E) of labelled mercury was calculated. For comparison, similar extraction experiments were carried out with solutions containing only labelled MeHg or Hg_{in} (without dissolved hair) and with labelled solutions of hair without addition of KBr or KI.

Reextraction of MeHg

The reextraction of MeHg into aqueous phase containing various concentrations of NaOH and/or 0.1 M cysteine was studied as follows: A hair sample labelled with $Me^{203}Hg$ was processed by the above mentioned procedure. 1.5 ml of the separated organic extract were shaken for 15 min with 1.5 ml of aqueous phase under study. After centrifugation pH of the aqueous phase as well as the activities of both phases were measured and percentage of reextraction (%RE) of labelled MeHg was calculated.

RESULTS AND DISCUSSION

Dissolution of hair samples

In the dissolution studies it was found that human hair can be dissolved within 1 h by heating at 90–95°C with ≥6 M HCl or ≥8 M H_2SO_4. Under these conditions, however, partial

decomposition of MeHg and volatilization of both MeHg and Hg_{in} occur. A 0.2–1 M solution of NaOH or KOH dissolves hair at 90–95°C in 1 h, in hot 10 M NaOH or KOH the dissolution is completed already after 15 min. The alkaline dissolution does not result in any significant volatilization of mercury species or decomposition of MeHg. The latter conclusion is based on the fact that >95% of the added $Me^{203}Hg$ can be extracted into the organic phase after dissolution.

Separation of MeHg from hair using solvent extraction

The extraction of labelled MeHg from hair solutions and from aqueous solutions without dissolved hair (both containing 0.5 M appropriate halogenide) is compared in Figures 1 and 2. Figure 3 presents the data on the extraction of $Me^{203}Hg$ from hair solutions without addition of halogenides. It can be seen that more than 87% of MeHg is extracted at pH 0.5–14 from 0.5 M solutions of KBr or KI without dissolved hair. The extraction from the hair solution in the presence of KBr decreases below pH 4 and above pH 7 (Figure 1). In the presence of KI such a decrease occurs in the alkaline range only (Figure 2). Rather high extraction of MeHg was observed also from hair solutions without addition of halogenides; the dependence on pH is then similar to that for hair solutions with added KBr.

The results obtained seem to indicate that the hair solution contains substances able to bind methylmercury, and the complexes formed in this way are probably extractable to benzene in weakly acidic or neutral solutions. [Methylmercury is not extracted to benzene from solutions containing only sulphuric acid or sodium sulphate.] The complexes obviously suppress the extraction (and formation) of neutral complexes of MeHg with bromide in the

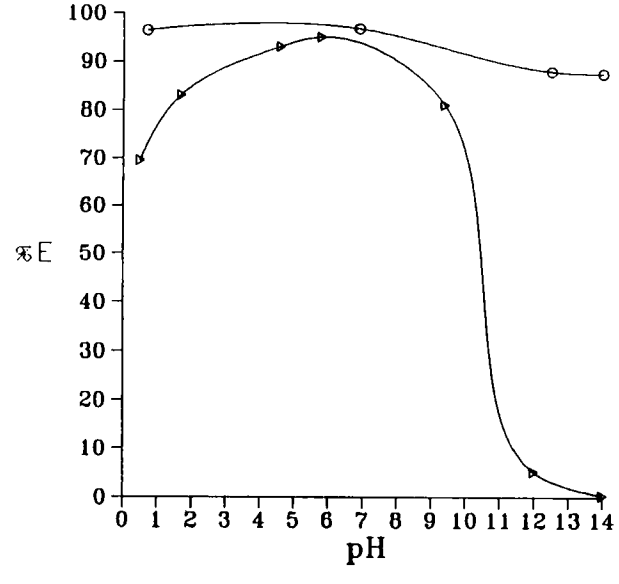

Figure 1 Extraction of MeHg from 0.5 M KBr. ⊖⊖⊖⊖⊖, without hair; ⊳⊳⊳⊳⊳⊳⊳, hair solution.

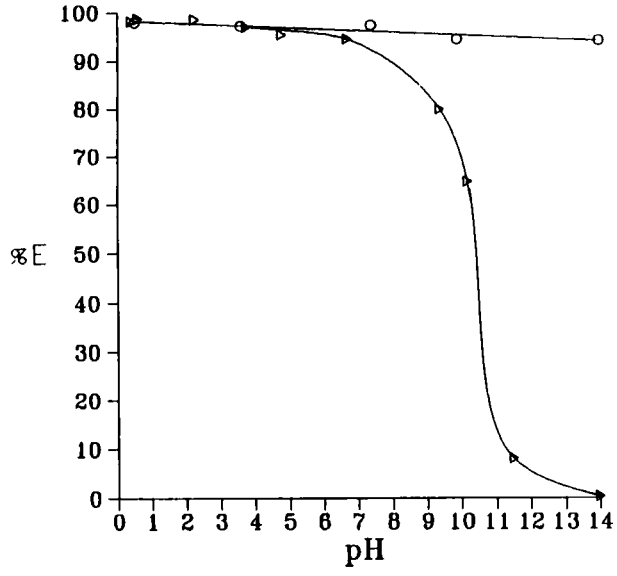

Figure 2 Extraction of MeHg from 0.5 M KI. ⊖⊖⊖⊖⊖, without hair; ⊳⊳⊳⊳⊳⊳, hair solution.

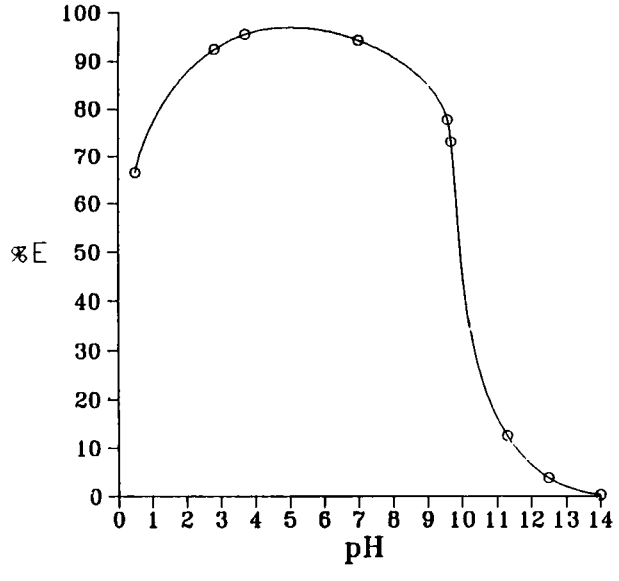

Figure 3 Extraction of MeHg from hair solution.

whole pH region studied and with iodide at pH >7. At pH 0.5–3 a substantial part of MeHg is extracted as iodide complex even from the hair solution.

No significant extraction of inorganic mercury (%E < 0.5) under all conditions studied was observed. Formation of small quantities of a solid phase was observed after the alkaline solutions containing dissolved hair were acidified to pH <5. The solid phase retained 70–90% of inorganic mercury present in the system. This phenomenon has no negative effect on the efficiency of the separation of MeHg from Hg_{in} by extraction, because the precipitate remains in the aqueous phase.

On the basis of the results obtained, the following procedure is proposed for the separation of MeHg from human hair: 0.2 ml of 10 M NaOH or KOH is added to 100–150 mg of a sample of hair in a centrifuge tube. The tube is kept in a thermostat at 90–95°C for 15 min. Then 1.8 ml of distilled water are added to the dissolved sample and its pH value is adjusted to 0.5–1.0 using concentrated H_2SO_4. The sample is cooled to room temperature, 150 mg of solid KI are added and MeHg is extracted into the organic phase by 10 min shaking with 2 ml of benzene.

The reproducibility of the recommended separation procedure was verified by processing eight hair samples containing labelled $Me^{203}Hg$ or $^{203}Hg_{in}$. The results are given in Table 1. It has been found that more than 97% of MeHg and less than 0.4% of Hg_{in} were extracted into organic phase. The separation factor α thus exceeds 8.10^3. In comparison with the earlier methods[4,11,12] based on the extraction of MeHg into toluene from a KBr solution, the proposed method requires neither repeated extraction nor a purification step and enables easy, quick and quantitative separation of methylmercury from human hair.

Reextraction of MeHg

With some analytical methods determination of methylmercury dissolved in benzene can be difficult or imprecise. This can be avoided by reextraction of MeHg into an aqueous solution. The results of the reextraction study of MeHg into solutions of cysteine or sodium hydroxide of variable pH are presented in Figure 4. More than 98% of MeHg present in the organic extract can be reextracted into 0.1 M cysteine at pH >10.5 or into a NaOH solution with a concentration higher than 0.5 M.

Table 1 Reproducibility of the separation procedure

$^{203}Hg_{in}$ *Hair weight [mg]*	*%E*	$Me^{203}Hg$ *Hair weight [mg]*	*%E*
98.4	0.40	102.0	99.9
99.0	0.41	104.0	99.5
100.8	0.39	109.2	102.2
108.0	0.37	115.4	97.1
120.7	0.37	127.0	98.4
130.3	0.28	131.9	96.7
133.6	0.24	135.0	96.8
147.9	0.22	154.8	100.3
Mean ± S.D.:	0.34 ± 0.07%	Mean ± S.D.:	98.9 ± 1.8%

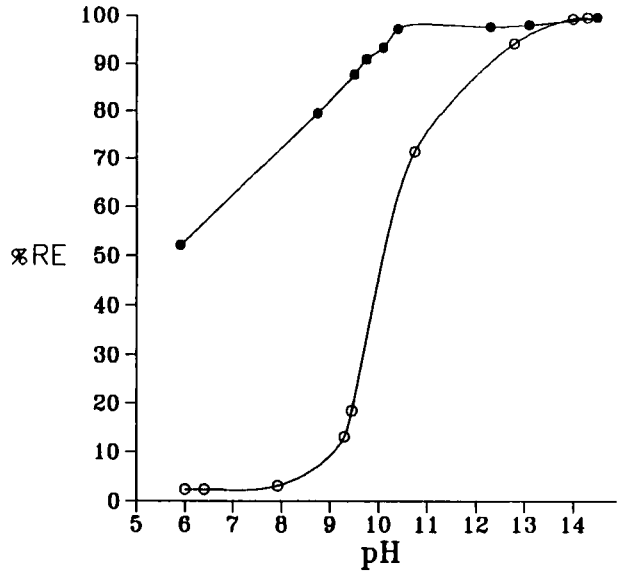

Figure 4 Reextraction of MeHg. ⊖⊖⊖⊖⊖, NaOH solution; ●●●●●, 0.1 M cysteine.

Acknowledgement

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