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Mercury(II) and methyl mercury speciation on *Streptococcus pyogenes* loaded Dowex Optipore SD-2

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ABSTRACT

A solid phase extraction procedure based on speciation of mercury(II) and methyl mercury on *Streptococcus pyogenes* immobilized on Dowex Optipore SD-2 has been established. Selective and sequential elution with 0.1 mol L^{-1} HCl for methyl mercury and 2 mol L^{-1} HCl for mercury(II) were performed at pH 8. The determination of mercury levels was performed by cold vapour atomic absorption spectrometry (CVAAS). Optimal analytical conditions including pH, amounts of biosorbent, sample volumes, etc., were investigated. The influences of the some alkaline and earth alkaline ions and some transition metals on the recoveries were also investigated. The capacity of biosorbent for mercury(II) and methyl mercury was 4.8 and 3.4 mg g^{-1}. The detection limit (3 sigma) of the reagent blank for mercury(II) and methyl mercury was 2.1 and 1.5 ng L^{-1}. Preconcentration factor was calculated as 25. The relative standard deviations of the procedure were below 7%. The validation of the presented procedure is performed by the analysis of standard reference material (NRCC-DORM 2 Dogfish Muscle). The procedure was successfully applied to the speciation of mercury(II) and methyl mercury in natural water and environmental samples.

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1. Introduction

Mercury is a toxic element. Mercury is generally found as mercury(II) and methyl mercury in natural waters. The toxicology of methyl mercury is higher than mercury(II). Their accurate and sensitive determination is very important due to these points. Mercury may enter a human body by inhalation or mercury vapour, drinking water and by the consumption of fish and fish products in the diet [1–3]. The World Health Organization (WHO) recommends a limit of 1 ng mL^{-1} of mercury in drinking water. Due to the very low concentration levels of mercury(II) and methyl mercury in water samples, a enrichment step should be included prior to the analysis in order to achieve a final concentration level matching the detection limits accessible with the technique selected [4]. Also other problem in mercury determination is the interference effects of matrix components of the sample. In order to solve these problems, preconcentration and separation techniques such as solid phase extraction, cloud point extraction, solvent extraction, ion-exchange, coprecipitation, etc., have been used [5–10].

Some analytical methods have been developed for the determination of mercury at low concentrations, but the most commonly used ones are cold vapour atomic fluorescence spectrometry (CV-AFS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) [11]. Owing to its simplicity, high sensitivity, relative freedom from interferences, low costs and speed, cold vapour atomic absorption spectrometry (CVAAS) have been widely used technique for mercury species in environmental samples [4,12–14].

The use of biological system for speciation and preconcentration has proven to be an interesting alternative to existing separation methods. Algae and yeast have been used as extractants. Nonetheless, dead or immobilized bacterial cells have been applied to the preconcentration of metals from environmental samples [15]. Biosorption is exclusively responsible for metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation [16]. The system is based on biosorption of the heavy metals and desorption of these metals from the organisms. An important part of the mercury studies on biosorption is based on the immobilization of the organisms on the natural or synthetic polymeric materials [17,18]. Microorganisms immobilized natural and synthetic adsorbents have been used for heavy metal separation and preconcentration from various media with successfully analytical results. Limited informations have been reported about biosorption of mercury speciation.

Streptococcus pyogenes is one of the most frequent pathogens of humans. It is estimated that between 5 and 15% of normal individuals harbor the bacterium, usually in the respiratory tract, without signs of disease. As normal flora, *S. pyogenes* can infect

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when defenses are compromised or when the organisms are able to penetrate the constitutive defenses. When the bacteria are introduced or transmitted to vulnerable tissues, a variety of types of suppurative infections can occur [19]. According to our literature survey, *S. pyogenes* is not used on the biosorption of mercury(II) and methyl mercury speciation prior to cold vapour atomic absorption determination of them. *S. pyogenes* immobilized on Dowex Optipore SD-2 as environment friendly, low cost and having high adsorption capacity biosorbent was used in this study.

In this work, a solid phase extraction procedure based on speciation of mercury(II) and methyl mercury on *S. pyogenes* immobilized on Dowex Optipore SD-2 was presented. The analytical conditions for the quantitative recoveries of the analytes including pH of solutions, sample volume, etc., were investigated.

2. Experimental

2.1. Instrument

A Perkin Elmer AAnalyst 700 atomic absorption spectrometer equipped with MHS-15 Mercury/Hydride System was used in this study. All measurements were carried out using high purity argon. A hallow cathode lamp operating at 6 mA was used and a spectral bandwidth of 0.7 nm was selected to isolate the 253.7 nm mercury line. Peak height was used for quantitation [20].

A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Digestion conditions for microwave system for the samples were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, ventilation: 8 min [21–23].

2.2. Reagents and solutions

All chemicals used were of analytical reagent grade and were used without further purification. Deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1}, resistivity) was used for all dilutions. Laboratory glassware was kept overnight in a 10% (v/v) HNO_3 solution and then rinsed with deionized double distilled water.

A 1000 mg L^{-1} stock solution of Hg(II) was prepared by $HgCl_2$. Similarly, a 1000 mg L^{-1} stock solution of CH_3Hg^+ was prepared by dissolving CH_3HgCl (E. Merck (Darmstadt, Germany)). Accurately diluted solutions of Hg(II) and CH_3Hg^+ were prepared daily using standard stock solutions. The calibration curve was established using the standard solutions prepared in 1 mol L^{-1} HNO_3 by dilution from stock solutions. The calibration curve solutions were prepared daily. The calibration standards were not submitted to the preconcentration procedure. Stock solutions of diverse elements were prepared from high purity compounds. $NaBH_4$ (1.5%) (w/v) in NaOH (0.5%) (w/v) was used as reducing agent.

Phosphate buffer solution ($H_2PO_4^-/H_3PO_4$) were prepared by mixing of appropriate volumes of 1 mol L^{-1} sodium dihydrogen phosphate and phosphoric acid solutions for pH 2. Acetate buffer solutions (CH_3COO^-/CH_3COOH) were prepared by mixing of appropriate volumes of 1 mol L^{-1} acetic acid and 1 mol L^{-1} sodium acetate solutions for pH 4. Phosphate buffer solutions ($H_2PO_4^-/HPO_4^{2-}$) were prepared by mixing of appropriate volumes of 1 mol L^{-1} sodium dihydrogen phosphate and 1 mol L^{-1} sodium hydrogen phosphate for pH 5 and 6. Ammonium buffer solutions were prepared by mixing of appropriate amounts of 1 mol L^{-1} ammonia and 1 mol L^{-1} ammonium chloride solutions for pH 8–10. Standard reference material (NRCC-DORM 2 Dogfish Muscle) was used in the experiments supplied from National Research Council of Canada (Ottawa).

Dowex Optipore SD-2 is a styrene-divinylbenzene copolymer. Its surface area, mesh size and pore size are 800 m^2 g^{-1}, 18–50 mesh and 50 Å, respectively. Dowex Optipore SD-2 was purchased from Sigma Chem. Co. St. Louis, USA. In order to remove organic and inorganic contaminants, the resin was washed successively with methanol, distilled water, 1 mol L^{-1} HNO_3 in acetone, distilled water, 1 mol L^{-1} NaOH and distilled water.

2.3. Preparation of biomass

A solid medium (nutrient agar) was prepared by mixing 3 g of beef extract, 5 g of peptone, 8 g of sodium chloride and 12 g of agar. This 28 g of powder was dissolved in 1L of deionized water. It was taken 28 g from this mixture and dissolved in the 100 mL. After this medium was cooled (40–50 °C), It was transferred to the sterilized Petri dishes. The bacterium, *S. pyogenes*, was inoculated on the solid medium and stored at 36 °C for 24 h in the incubator. It was inoculated into a 10 mL liquid medium. This medium was incubated at 36 °C for 24 h. Then, it was transferred into flasks (1 L) that contains 800 mL the sterilized liquid medium (nutrient broth). This culture was incubated at 36 °C for 48 h in the incubatory shaker. After reaching stationary phases, bacterial cells (*S. pyogenes*) were harvested and separated from the media using centrifugation at 6000 rpm for 20 min. The isolated biomass was washed three times with 0.1 mol L^{-1} HCl, and rinsed with distilled water and dried. *S. pyogenes* is highly pathogenic microorganism while it is living. It is not pathogenic while it is dead. We use dry and dead bacteria powder in a solid phase extraction method. While preparation of living biomass, the necessary precautions were taken.

150 mg of dry and dead bacteria powder was mixed with 0.5 g of Dowex Optipore SD-2. The mixture was wetted with 2 mL of doubly distilled water and thoroughly mixed. After mixing, the paste was heated in an oven at about 105 °C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *S. pyogenes* and Dowex Optipore SD-2 resin, thereby improving the immobilization efficiency. Then, the product obtained was ground to get original size (18–50 mesh) and used as an adsorbent.

2.4. Test studies

The *S. pyogenes* immobilized on Dowex Optipore SD-2 column was 10 cm long, and 0.5 cm in diameter. A small plug of glass wool was placed on the bottom of the column. The column contained about 500 mg resin. The *S. pyogenes* immobilized on Dowex Optipore SD-2 column was prepared by aspirating water slurry of the *S. pyogenes* immobilized on Dowex Optipore SD-2 into the glass column. It was conditioned by passing 10–15 mL of related buffer solution then it was used in the presented work.

The biosorption procedure on *S. pyogenes* immobilized on Dowex Optipore SD-2 for mercury(II) and methyl mercury speciation was tested with model solutions. For mercury(II) and methyl mercury determinations, to 40–50 mL of solution containing 0.10 μg mercury(II) and 0.10 μg methyl mercury ion was added 10 mL of buffer solution (to give the desired pH between 2–9). The *S. pyogenes* immobilized on Dowex Optipore SD-2 column was preconditioned by passing buffer solution. The solution was passed through the column at a flow rate of 5 mL min^{-1}. The sample solution was permitted to flow through the column under gravity. After passing of this solution, the column was rinsed twice with 5–8 mL of water. The adsorbed methyl mercury and mercury(II) on the biosorbent column was sequential eluted with 10 mL portion of 0.1 mol L^{-1} HCl and 10 mL of 2.0 mol L^{-1} HCl. The eluent was analyzed for the determination of mercury(II) and methyl mercury concentrations by cold vapour atomic absorption spectrometry

(CVAAS). The number of replicates for the test workings was three. Then for mercury quantification, 1 mL sample was pipetted into a 50 mL volumetric flask where 10 mL of 1.5% HCl was added as diluent. $NaBH_4$ (1.5%) (w/v) in NaOH (0.5%) (w/v) was used as reducing agent. The analytical measurement was based on peak height. Reading time and argon flow rate was selected as 10 s and 50 mL min^{-1}.

2.5. Applications to real samples

Natural water samples analyzed by the presented work were filtered through Millipore cellulose membrane filter (0.45 μm pore size). The pH of the samples was adjusted to 8.0 with buffer solution. Then the procedures given Section 2.4 were applied to the final solutions. The sample was passed through the column. The adsorbed methyl mercury and mercury(II) on *S. pyogenes* immobilized on Dowex Optipore SD-2 column were sequential eluted with 0.1 mol L^{-1} HCl and 2.0 mol L^{-1} HCl. Blank samples were also analyzed. The levels of analyte ions in the samples were determined by cold vapour atomic absorption spectrometry.

NRCC-DORM 2 Dogfish Muscle (250 mg), human hair (1.0 g), waste battery (1.0 g) and fish (1.0 g) were digested with 6 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 in microwave digestion system and diluted to 50 mL with deionized water. The blanks were prepared in the same way as the sample, but omitting the sample. Digestion of solid samples for mercury and methyl mercury determinations in literature were used similar methods [1,9]. The preconcentration procedure given above was applied to the samples.

3. Results and discussion

3.1. Influences of pH

The influences of pH of the sample solution on the recoveries of mercury(II) and methyl mercury ions were separately investigated in the pH range of 2.0–9.0 by adjusting pH of the model solutions with buffers given in Section 2. The results for this study were depicted in Fig. 1. While methyl mercury was quantitatively (≥95%) recovered at the pH range of 7–8, the recoveries of mercury(II) were generally below 10% in the pH range of 2–9 using 0.1 mol L^{-1} HCl as eluent. Mercury(II) were quantitatively (≥95%) recovered at the pH range of 7–8 using 2 mol L^{-1} HCl as eluent. Selective and sequential elution with 0.1 mol L^{-1} HCl for methyl mercury and 2 mol L^{-1} HCl for mercury(II) were performed at pH 8 by using ammonia/ammonium buffer solution.

The recovery values on the column filled with Dowex Optipore SD-2 without *S. pyogenes* at pH range of 7–8 were found below 50% for CH_3-Hg and 65% for Hg(II). The recoveries for the analytes on the column filled 150 mg of *S. pyogenes* without Dowex Optipore SD-2 at pH range of 7–8 were found below 60% for CH_3-Hg^+ and 70% for Hg(II). These points show that for the quantitative recoveries of analyte ions, it is necessary that the combination of *S. pyogenes* and Dowex Optipore SD-2.

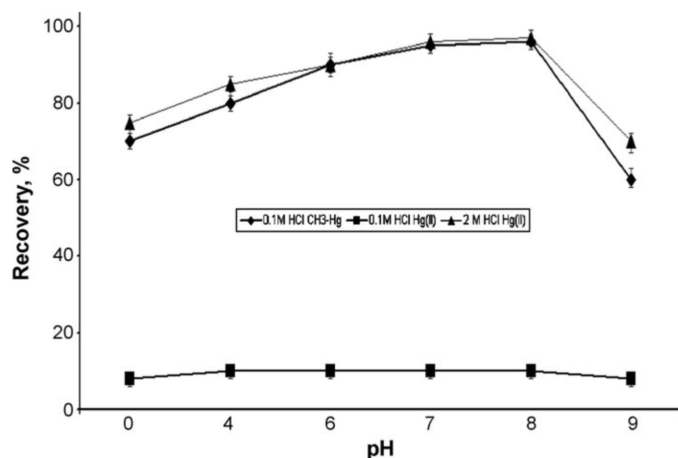

Fig. 1. Relation between pH and mercury species (N = 3).

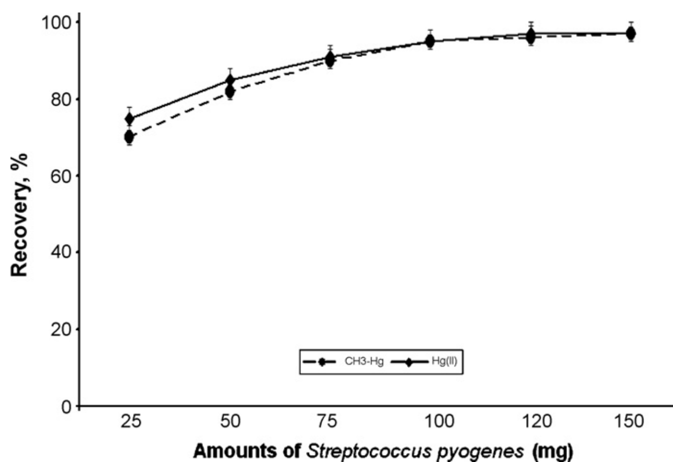

Fig. 2. The influences of amounts of *Streptococcus pyogenes* immobilized on Dowex Optipore SD-2 (N = 3).

It could be concluded that the cell surface becomes positively charged at low pH values which decrease the attraction between metal ions and the functional groups on the cell wall, whereas the cell surface becomes negatively charged at high pH values, increasing the attraction until a maximum is reached at around pH 7. For pH values higher than the optimum values, the retention decreases again due to the competition between the hydroxylated complexes of the metal and active sites of the cell [24–26].

3.2. Effect of amounts of S. pyogenes on the recoveries of analytes

In order to observe the influences of the amounts of *S. pyogenes* on the retentions of analyte ions, the procedure given in Section 2.4 was performed with different amounts of *S. pyogenes* and then the preconcentration procedure given in Section 2.5 was applied. The results were depicted in Fig. 2. The recoveries of analytes were increased with the increased amounts of *S. pyogenes* that immobilized on Dowex Optipore SD-2 resin. Quantitative recovery values for analytes were obtained after 100 mg of *S. pyogenes*. In all subsequent works, 150 mg of *S. pyogenes* was immobilized on 0.5 g of Dowex Optipore SD-2.

3.3. Eluent type and its volume

The effects of various eluent on the recoveries of the mercury(II) and methyl mercury from *S. pyogenes* immobilized on Dowex Opti-

Table 1
Effects of various eluents on the recoveries of mercury species from *Streptococcus pyogenes* immobilized on Dowex Optipore SD-2 (pH 8, N = 3).

	Recovery (%)	
	CH_3-Hg^+ [a]	Hg(II) [a]
0.03 mol L^{-1} HCl	80 ± 2 [b]	5 ± 1
0.1 mol L^{-1} HCl	96 ± 2	10 ± 2
0.5 mol L^{-1} HCl	98 ± 3	40 ± 1
1.0 mol L^{-1} HCl	98 ± 2	75 ± 2
1.5 mol L^{-1} HCl	98 ± 3	90 ± 3
2.0 mol L^{-1} HCl	98 ± 3	97 ± 2

[a] Eluent type.
[b] Mean ± standard deviation.

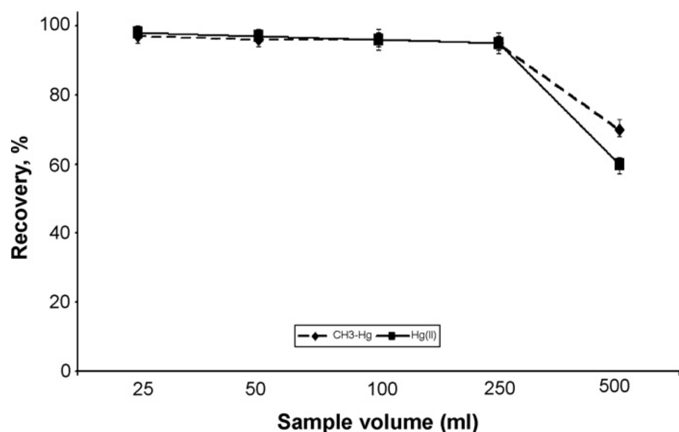

Fig. 3. Effects of sample volume on the retentions of mercury species on *Streptococcus pyogenes* immobilized on Dowex Optipore SD-2 (N = 3).

pore SD-2 column were given in Table 1. In order to check the influence of the HCl concentration on mercury(II) and methyl mercury recoveries from the biosorbent, different concentrations of the HCl solutions (5–10 mL) were tested for each case. For this study, solutions of 10 mL containing either mercury(II) and methyl mercury were passed through the column at the optima conditions and the elution was carried out. As it can be seen in Table 1, the use of 0.1 mol L^{-1} HCl allowed the selective elution of methyl mercury in the presence of mercury(II). The quantitative elution of mercury(II) was only possible with HCl concentrations higher than 2 mol L^{-1}. A 2 mol L^{-1} HCl solution was tested as a blank for the mercury determination by CVAAS. The signal obtained was negligible.

The volume of eluent is important for the high concentration factor. The recoveries of analytes were quantitative in the eluent volume range of 8–10 mL. Afterwards, the sequential elution of methyl mercury and mercury(II) was carried out by passing through the column 10 mL of 0.1 mol L^{-1} HCl and 10 mL of 2 mol L^{-1} HCl.

3.4. Flow rates of sample and eluent solutions

It is important to choose a flow rate that ensures an adequate mercury species uptake for the solid phase extraction works. So, the influences of the sample and eluent flow rates on the retentions and recoveries of mercury(II) and methyl mercury ions on *S. pyogenes* immobilized on Dowex Optipore SD-2 were also examined in the flow rate range of 2–10 mL min^{-1}. The analyte ions were quantitatively retained and recovered in the sample and eluent flow range of 2–6 mL min^{-1}. In the all-further works, 5 mL min^{-1} was selected as sample and eluent flow rate.

3.5. Effect of sample volume

To obtain high preconcentration factor, sample volume is one of the important analytical factor for solid phase extraction studies for metal ions. The influences of sample volume on the recoveries of analyte ions on *S. pyogenes* immobilized on Dowex Optipore SD-2 were investigated in the range of 25–500 mL range. The results are depicted in Fig. 3. Methyl mercury and mercury(II) ions were quantitatively (≥95%) recovered till 250 mL. After 250 mL of sample volume, recovery values not quantitative. The preconcentration factor was 25 when eluent volume is 10 mL.

Table 2
Influences of some foreign ions on the recoveries of analytes (N = 3).

Ion	Added as	Concentration (mg L^{-1})	Hg(II)	CH_3-Hg^+
Na^+	NaCl	10,000	95 ± 3[a]	96 ± 3
K^+	KCl	3,000	97 ± 2	95 ± 3
Ca^{2+}	$CaCl_2$	3,000	96 ± 3	96 ± 3
Mg^{2+}	$MgCl_2$	3,000	95 ± 2	96 ± 3
Cl^-	NaCl	20,000	96 ± 3	96 ± 2
F^-	NaF	1,000	97 ± 2	96 ± 2
NO_3^-	KNO_3	1,000	95 ± 2	96 ± 3
SO_4^{2-}	Na_2SO_4	1,000	96 ± 2	97 ± 2
PO_4^{3-}	Na_3PO_4	1,000	96 ± 4	95 ± 3
Al^{3+}	$Al_2(SO_4)_3$	10	95 ± 2	98 ± 2
Fe^{3+}	$FeCl_3$	10	95 ± 2	96 ± 3
Cu^{2+}	$CuSO_4$	10	96 ± 3	97 ± 3
Pb^{2+}	$Pb(NO_3)_2$	10	95 ± 1	95 ± 2
Zn^{2+}	$ZnSO_4$	10	95 ± 3	96 ± 3
Cr^{3+}	$Cr(NO_3)_3$	10	96 ± 2	97 ± 4
Cd^{2+}	$Cd(NO_3)_2$	10	95 ± 3	96 ± 3
Ni^{2+}	$NiSO_4$	10	97 ± 3	96 ± 3
Co^{2+}	$CoSO_4$	10	96 ± 2	96 ± 3
Mn^{2+}	$MnSO_4$	10	96 ± 3	97 ± 4
Zn^{2+}	$ZnSO_4$	10	96 ± 2	95 ± 3
Hg^{2+}	$Hg(NO_3)_2$	50	–	96 ± 3
CH_3Hg^+	CH_3HgCl	50	96 ± 2	–

[a] Mean ± standard deviation.

Table 3
Speciation of Hg(II) and CH_3-Hg^+ in spiked test solutions (volume: 50 mL, N = 4).

Added (μg L^{-1})		Found (μg L^{-1})		Recovery (%)	
Hg(II)	CH_3-Hg^+	Hg(II)	CH_3-Hg^+	Hg(II)	CH_3-Hg^+
0	20	–	19.6 ± 0.6[a]	–	96 ± 1
10	10	9.8 ± 0.3	9.7 ± 0.4	98 ± 1	97 ± 2
15	5	14.6 ± 0.7	4.8 ± 0.2	97 ± 2	96 ± 1
20	0	19.7 ± 0.8	–	99 ± 2	–

[a] Mean ± standard deviation.

Table 4
The results for NRCC-DORM 2 Dogfish Muscle reference standard materials (N = 4).

	Certified value (μg g^{-1})	Our value (μg g^{-1})
Hg(II)	4.64	4.50 ± 0.25[a]
CH_3-Hg^+	4.47	4.35 ± 0.30

[a] Mean ± standard deviation.

Table 5
Speciation of Hg(II) and CH_3-Hg^+ in some natural water samples (sample volume: 250 mL, final volume: 10 mL, N = 4).

Samples	Added (ng L^{-1})		Found (ng L^{-1})		Recovery (%)	
	Hg(II)	CH_3-Hg^+	Hg(II)	CH_3-Hg^+	Hg(II)	CH_3-Hg^+
Tap water	–	–	28 ± 2[a]	BDL	–	–
	50	50	75 ± 4	49 ± 3	96 ± 3	98 ± 3
	100	100	125 ± 8	97 ± 5	98 ± 4	97 ± 3
River water	–	–	45 ± 3	BDL	–	–
	50	50	93 ± 4	48 ± 3	98 ± 3	96 ± 2
	100	100	140 ± 5	97 ± 4	97 ± 3	97 ± 3
Sea water	–	–	22 ± 2	BDL	–	–
	50	50	70 ± 3	48 ± 3	97 ± 3	96 ± 2
	100	100	117 ± 5	95 ± 3	96 ± 3	95 ± 3
Spring water	–	–	32 ± 2	BDL	–	–
	50	50	78 ± 3	49 ± 3	95 ± 3	98 ± 3
	100	100	127 ± 6	96 ± 3	96 ± 3	96 ± 3

BDL: Below detection limit.
[a] Mean ± standard deviation.

Table 6
The application of presented method in real samples (N = 4).

Samples	Hg(II) (μg g^{-1})	CH_3-Hg^+ (μg g^{-1})
Human hair	0.30 ± 0.03[a]	0.23 ± 0.02
Waste battery	0.45 ± 0.03	0.28 ± 0.02
Fish	0.32 ± 0.02	0.19 ± 0.01

[a] Mean ± standard deviation.

Table 7
Comparative data from some recent studies on mercury speciation.

Analytes	Media	Detection System	PF	DL	RSD (%)	Ref.
Phenyl mercury	Living *Escherichia coli*	CVAAS	–	0.05 μg L^{-1}	2.2–5.3	[15]
Hg(II) and CH_3-Hg^+	*Saccharomyces cerevisiae* immobilized on silica gel	CVAAS	15	–	2	[17]
Hg(II) and CH_3-Hg^+	*Chlorella vulgaris* immobilized on silica gel	CVAAS	75	0.5–4.0 μg–L^{-1}	–	[18]
Hg(II) and CH_3-Hg^+	*Streptococcus pyogenes* immobilized on Dowex Optipore SD-2	CVAAS	25	1.5–2.1 ng L^{-1}	<7	This study

PF: Preconcentration factor, DL: Detection limit, RSD: Relative standard deviation.

3.6. Influences of matrix ions on the recoveries

The effects of matrix ions for cold vapour atomic absorption spectrometric determinations on the recoveries of methyl mercury and mercury(II) ions on *S. pyogenes* immobilized on Dowex Optipore SD-2 column were also investigated. The results are given in Table 2. A fixed amount of metal ions was taken with different amounts of foreign ions and recommended procedure was followed. The recoveries of the analytes from the *S. pyogenes* immobilized on Dowex Optipore SD-2 column was not affected from the solution containing the high concentrations of matrix ions. The tolerance limit of foreign ions was taken as that value which caused an error of not more than ±5% in the absorbance. The ions normally present in water do not interfere under the experimental conditions used. Also, some of the transition metals at mg L^{-1} did not interfere with the recoveries of the analyte ions on the *S. pyogenes* immobilized on Dowex Optipore SD-2 column. This results show that the proposed preconcentration/separation method could be applied for mercury species to the highly saline samples and the samples that contains some transition metals.

3.7. Adsorption capacity

In order to study the adsorptive capacity of *S. pyogenes* immobilized on Dowex Optipore SD-2 for mercury species, batch method was used. To 0.1 g resin was added 50 mL of solution containing 1.0 mg of metal ion at pH 8.0. After shaking for 1 h, the mixture was filtered. 10 mL of the supernatant solution was diluted to 100 mL. Mercury species were determined by cold vapour atomic absorption spectrometry. This procedure was repeated for each analyte ions separately. The capacity of sorbent for analytes were found as: Hg(II): 4.8 mg g^{-1}, CH_3-Hg: 3.4 mg g^{-1}.

3.8. Column reuse

In order to examine the long term stability of the biomass, it was subjected to successive adsorption and desorption cycles (5 runs in a day and the next 5 runs 1 day later, and so on, total 20 runs) by passing 100 mL of metal solutions through the column. The stability and potential recyclability of the column containing biomass were assessed by monitoring the change in the recoveries of the analytes. After 25 runs, the recoveries of all of the analytes slightly decreased to below 95%. Similar results were obtained in other reported studies [24,25–29].

3.9. Analytical performance

The analytical performance of the procedure for mercury species were calculated for the results from cold vapour atomic absorption spectrometric measurements. The reproducibility of the method was evaluated by passing 50 mL of solution containing analyte ions through the column and repeating this procedure ten times. The relative standard deviations (RSD) were below 7%. Relative error was found to be lower than 5%. It was found that the recovery of mercury(II) and methyl mercury was 97 ± 2 and 96 ± 2 at 95% confidence level. Speciation of Hg(II) and CH_3-Hg in spiked test solutions were performed. The results were given in Table 3.

The limit of detection (LOD) of the presented solid phase extraction study was calculated under optimal experimental conditions (pH: 8, sample volume: 250 mL, eluent volume: 10 mL) after application of the presented preconcentration procedure to blank solutions. The limit of detections, defined as the concentration equivalent to 3 times the standard deviation ($n = 20$) of the reagent blank were found as: mercury(II): 2.1 ng L^{-1} and methyl mercury: 1.5 ng L^{-1}.

3.10. Applications

The validation of the presented procedure was performed by the analysis of standard reference material (NRCC-DORM 2 Dogfish Muscle). The results are given in Table 4. The results are in agreement with certified values with a precision of less than 7%. The relative error was found to be lower than 5%.

The procedure was successfully applied to the speciation of mercury(II) and methyl mercury in natural water samples including tap water, river water, sea water and spring waters. The results are given in Table 5. This method is also applied to microwave digested environmental samples including human hair, waste battery and fish. The results are given in Table 6. Mercury(II) and methyl mercury concentrations in water, human hair and fish samples are very important for human health. It was found lower than toxic limits.

4. Conclusion

Dowex Optipore SD-2 and *S. pyogenes* combination is used in the presented study for speciation of mercury(II) and methyl mercury at first time. The proposed method has the following advantages: simple, rapid and low analysis cost. The method proposed here is rapid and has good reproducibility. The usefulness of the method is shown by the control analyses of standard reference material. The comparisons of the presented procedure with the some works in literature are given in Table 7. The detection limits of analytes are superior to those of preconcentration and speciation techniques for analyses. This study shows that *S. pyogenes* immobilized on Dowex Optipore SD-2 allows the retention of methyl mercury and mercury(II) from natural water and microwave digested environmental samples and their sequential elution and determination.

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