

## Cr(VI) and Cr(III) speciation on *Bacillus sphaericus* loaded diaion SP-850 resin

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### Abstract

A speciation procedure for chromium(III) and chromium(VI) in the environmental samples has been established in the presented work. The procedure presented based on quantitative biosorption of chromium(III) on *Bacillus sphaericus* loaded Diaion SP-850 at pH 5. The Cr(VI) recoveries at pH 5 were below 5% on the biosorbent. After reduction of Cr(VI) by concentrated H<sub>2</sub>SO<sub>4</sub> and ethanol, the system was applied to the total chromium. Cr(VI) was calculated as the difference between the total chromium content and the Cr(III) content. Optimal analytical conditions including pH, amounts of biosorbent, etc. for Cr(III) recoveries 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 chromium(III) was 6.95 mg g<sup>-1</sup>. The detection limit (3 sigma) of the reagent blank for chromium(III) was 0.50 µg L<sup>-1</sup>. The procedure was successfully applied to the speciation of chromium(III) and chromium(VI) in natural water samples (R.S.D. lower than 5%, recoveries greater than 95%).

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**Keywords:** *Bacillus sphaericus*; Diaion SP-850; Biosorption; Speciation; Chromium; Atomic absorption spectrometry

### 1. Introduction

The determination of metals in environmental samples continues to be a challenge for analytical chemists around the world due to the contribution of metal ions into the effluent by mining, tannery, cement, textile and iron industries [1–4]. Contaminant metals are potentially toxic and may cause health problems to humans via the food chain [5–7]. Metal speciation studies have shown that various oxidation states of metals are toxic to humans and also that they are associated with several types of diseases. The form or species the metal is in is very important, because of the effect it may have on the environment, as some forms of the same metal are more toxic than others. Chromium(VI) is carcinogenic and chromium(III) is found to be involved in some enzyme systems [8–10]. It is therefore important to be able to separate these two species in a water sample and to determine how much of each is present. There is an increasing need to know the concentrations of chromium(III)

and (VI) separately rather than only the total chromium content [11–14].

Traces heavy metals quantitatively adsorbed on the higher organisms including mosses, bacteria, algae [15,16]. Biosorption is responsible for metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation [15,16]. The biosorption systems for preconcentration-separation and speciation of chromium species are based on biosorption of chromium species on the organisms and desorption of adsorbed chromium from the organisms [17–21]. Bag et al. [22] have been proposed an enrichment and speciation procedure for Cr(III) and Cr(VI) ions on the biosorption on *Saccharomyces cerevisiae* loaded on sepiolite. The possibility of using moss (*Funaria hygrometrica*) immobilized in a polysilicate matrix as substrate for speciation and preconcentration of Cr(III) and Cr(VI) in various water samples has been studied by Krishna et al. [23]. *Saccharomyces cerevisiae* was used to selectively accumulate and preconcentrate Cr(III) from aqueous matrices. The substrate was used in batch and continuous operation immobilized in the latter case on alginate [24]. Biosorption of chromium(VI) was studied by using non-living free and immobilized biomass of *Rhizopus arrhizus*

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by Prakasham et al. [25]. The applicability of a fungal biomass *Aspergillus niger* immobilized on a polysilicate matrix as substrate for the separation and determination of Cr(III) and Cr(VI) in natural water samples has been investigated by Rao et al. [26].

*Bacillus sphaericus* is a naturally occurring bacteria— isolated, cultured, and labeled for mosquito control. *B. sphaericus* has been accepted as one of target specific biological pest control agent. *B. sphaericus* produces an endotoxin that is toxic to some species of mosquito larvae. *B. sphaericus* is found widely in soil substrate available nature and selected because of its effective control of mosquito larvae [27]. *B. sphaericus* has been used at first time by our working group for the preconcentration and separation of copper(II), lead(II), iron(III) and cobalt(II) from the environmental samples. According to our literature survey, it is first time used in the speciation of chromium in the presented work.

In the presented work, a biosorption procedure on *B. sphaericus* loaded Diaion SP-850 for speciation of chromium(III) and chromium(VI) in natural water samples has been established.

## 2. Experimental

### 2.1. Instrument

A Perkin-Elmer Analyst 700 atomic absorption spectrometer with deuterium background corrector, equipped with a chromium hollow cathode lamp operated at 25 mA, was used throughout this work. The wavelength and spectral band pass were set at 357.9 nm and 0.7 nm, respectively. A 10 cm long slot-burner head and an air-acetylene flame were used. The nebulizer uptake rate was adjusted to give the optimum response for conventional sample introduction, the resulting rate being 6.0 mL min<sup>-1</sup>. An acetylene flow rate of 2.5 L min<sup>-1</sup> was used with an air flow rate of 17.0 L min<sup>-1</sup>. A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase.

### 2.2. Reagents and solution

All chemicals used in this work, were of analytical reagent grade and were used without further purification. Deionised water (Milli-Q Millipore 18.2 MΩ cm<sup>-1</sup> resistivity) was used for all dilutions. All the plastic and glassware were cleaned by soaking in dilute HNO<sub>3</sub> (1 + 9) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L<sup>-1</sup> of the given element supplied by Sigma and Aldrich. Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

Sodium phosphate buffer (0.1 M) was prepared by adding an appropriate amount of phosphoric acid to sodium dihydrogen phosphate solution to result in a solution of pH 2 and 3. Ammonium acetate buffers (0.1 M) were prepared by adding an appropriate amount of acetic acid to ammonium acetate solutions to result in solutions of pH 4–6. Ammonium chloride

buffer solutions (0.1 M) were prepared by adding an appropriate amount of ammonia to ammonium chloride solutions to result in solutions of pH 8–9.

Diaion SP-850 is an aromatic type adsorbent is the standard grade and is based on crosslinked polystyrenic matrix. Its surface area is 1000 m<sup>2</sup> g<sup>-1</sup>. Diaion SP-850 was purchased from Sigma Chem. Co., St. Louis, USA. It (20–60 mesh) was washed successively with methanol, water, 1 mol L<sup>-1</sup> HNO<sub>3</sub> in acetone, water, 1 mol L<sup>-1</sup> NaOH, and water, sequentially.

### 2.3. Model studies

*B. sphaericus* Loaded Diaion SP-850 was prepared according procedure given by Tuzen et al. [28]. The *B. sphaericus* loaded Diaion SP-850 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 *B. sphaericus* loaded Diaion SP-850 column was prepared by aspirating water slurry of the *B. sphaericus* loaded Diaion SP-850 into the glass column. It was conditioned by passing 10–15 mL of buffer solution then it was used in the presented work.

The biosorption procedure on *B. sphaericus* loaded Diaion SP-850 for chromium speciation was tested with model solutions. For chromium(III) determinations, to 40–50 mL of solution containing 20 μg of chromium(III) ion was added 10 mL of buffer solution (to give the desired pH between 2 and 9). The *B. sphaericus* loaded Diaion SP-850 column was pre-conditioned by passing buffer solution. The solution was passed through the column at a flow rate of 5 mL min<sup>-1</sup>. 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 chromium on the biosorbent column was eluted with 10 mL portion of 1.0 mol L<sup>-1</sup> HCl. The eluent was analyzed for the determination of chromium(III) concentrations by an atomic absorption spectrometer.

Total chromium was determined as Cr(III) by the method described above after reducing Cr(VI) to Cr(III). The reduction of Cr(VI) to Cr(III) was performed by the addition of 0.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 0.5 mL of ethanol to the test solution [22]. The level of Cr(VI) is calculated by difference of total chromium and Cr(III) concentrations. The optimum conditions for separation of Cr(III) from Cr(VI) and for preconcentration of Cr(III) have been determined by using the test procedure given above.

### 2.4. Application to natural waters

Water samples analyzed were filtered through a cellulose membrane filter (Millipore) of 0.45 μm pore size. The pH of the samples was adjusted to 5.0 with buffer solution. The sample was passed through the *B. sphaericus* loaded Diaion SP-850 column. Chromium(III) adsorbed on column was eluted with 1 mol L<sup>-1</sup> HCl. The levels of chromium in the samples were determined by flame atomic absorption spectrometry.

Total chromium in natural water samples was determined as chromium(III) after reducing chromium(VI) to chromium(III)

in the natural waters. The reduction of Cr(VI) to Cr(III) was performed by the addition of 0.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 0.5 mL of ethanol to the water samples.

### 3. Results and discussion

#### 3.1. Optimization of the analytical variables

Due to pH of aqueous solutions is the one of the main variables on the biosorption procedures for heavy metals including chromium species, the influences of pH on the recoveries of Cr(III) and Cr(VI) on *B. sphaericus* loaded Diaion SP-850 was investigated in the pH range of 2–9. The results were given in Fig. 1. Chromium(III) was quantitatively (>95%) recovered in the pH range of 5–9. The recoveries of chromium(VI) were generally below 5% in the pH range of 2–9. These results show that separation of chromium(III) is possible from chromium(VI) by the presented biosorption procedure at the pH range of 5–9. All further experiments were performed at pH 5 by using acetate buffer solution. The recovery values of Cr(III) on the column filled with Diaion SP-850 without *B. sphaericus* at pH 5 were below 40%. The recoveries for analytes on the column filled 150 mg of *B. sphaericus* without Diaion SP-850 at pH 5 were below 40%. For the quantitative recoveries of Cr(III), it is necessary that the combination of *B. sphaericus* and Diaion SP-850.

The influence of the amounts of *B. sphaericus* on the recoveries of chromium(III) was investigated. The results were depicted in Fig. 2. The recoveries of chromium(III) were increased with the increased amounts of *B. sphaericus* that loaded on Diaion SP-850 resin. Quantitative recovery values for analytes were obtained after 75 mg of *B. sphaericus*. In all subsequent works, 100 mg of *B. sphaericus* was loaded on 500 mg of Diaion SP-850.

The possibility of quantitative desorption of biosorbed chromium(III) from the *B. sphaericus* loaded Diaion SP-850 column was also studied with various eluents. The results are given in Table 1. Quantitative recoveries for Cr(III) from the biosorbent were obtained only by using 1 mol L<sup>-1</sup> HCl. The effect of volume of 1 mol L<sup>-1</sup> HCl as eluent was also examined

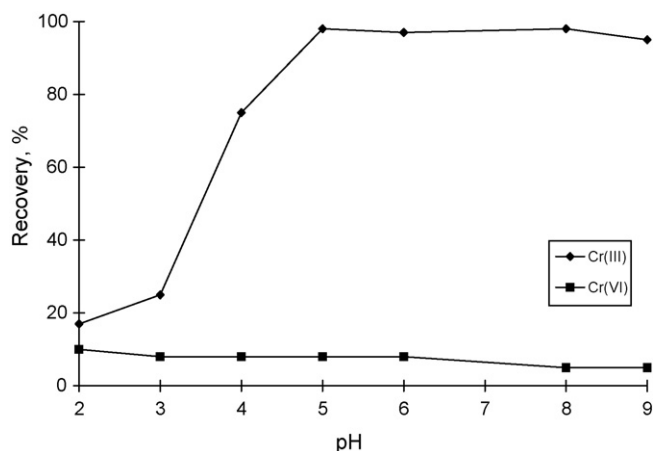


Fig. 1. Influences of pH on the recoveries of Cr(III) and Cr(VI) on *Bacillus sphaericus* loaded Diaion SP-850 ( $N=3$ ).

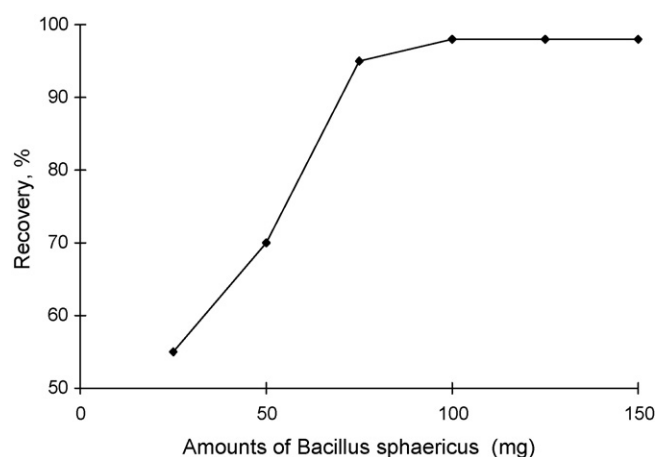


Fig. 2. Effect of amounts of *B. sphaericus* on the recoveries of chromium(III) (amounts of Diaion SP-850: 500 mg,  $N=3$ ).

Table 1  
Effects of various eluents on the desorption of Cr(III) from biosorbent ( $N=3$ )

Eluent type	Recovery (%)
0.5 mol L <sup>-1</sup> HCl	85 ± 3
1 mol L <sup>-1</sup> HCl	97 ± 2
0.5 mol L <sup>-1</sup> HNO <sub>3</sub>	82 ± 3
1 mol L <sup>-1</sup> HNO <sub>3</sub>	95 ± 3

on the recoveries. All Cr(III) was quantitatively recovered by using 8–10 mL of 1 mol L<sup>-1</sup> HCl.

The effect of sample volume on chromium extraction on the *B. sphaericus* loaded Diaion SP-850 column was studied by passing 20 µg of Cr(III) in increasing volumes, in a range from 25 mL to 750 mL. The results were depicted in Fig. 3. Quantitative recoveries were obtained with volumes up to 500 mL (corresponding to a preconcentration factor of 50, when 10 mL of eluent was utilized).

Sample and eluent flow rates on the solid phase extraction studies are important parameters to obtain quantitative retention and elution, respectively. The effects of the sample and eluent

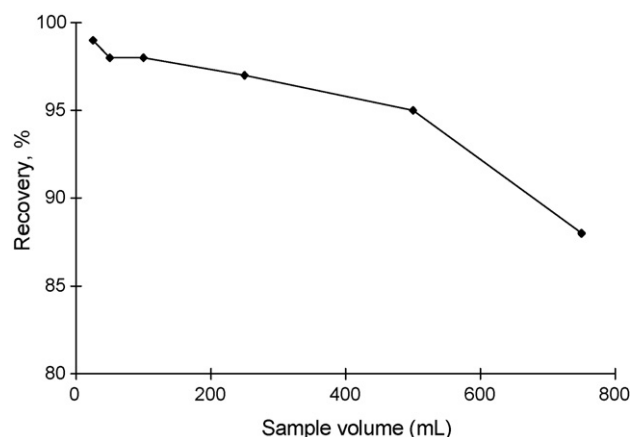


Fig. 3. Influences of sample volume on the recoveries of Cr(III) on *B. sphaericus* loaded Diaion SP-850 ( $N=3$ ).

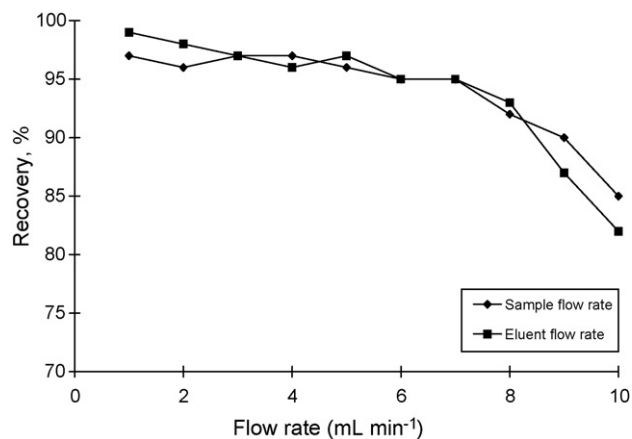


Fig. 4. Influences of flow rates on the recoveries of Cr(III) ( $N=3$ ).

flow rates on the retentions and recoveries of chromium(III) on *B. sphaericus* loaded Diaion SP-850 were also examined in the flow rate range of 2–10 mL min<sup>-1</sup>. The results were given in Fig. 4. All the analyte ions were quantitatively retained and recovered in the sample and eluent flow range of 1–7 mL min<sup>-1</sup>. In the all-further works, 5 mL min<sup>-1</sup> was selected as sample and eluent flow rate.

Various salts and metal ions were added individually to a solution containing of Cr(III) and the test procedure given above was applied. The tolerance limit was set, as the diverse ion amount require causing  $\pm 3\%$  error in the determination of Cr(III). The results obtained are given in Table 2. Under these optimized conditions, most of the probable concomitant cations and anions remained in the first phase. The tolerable levels of the some heavy metal ions are suitable for the separation and preconcentration of chromium(III) in the natural water samples examined present study, because of the levels of transition metals in these samples are lower than their interferic level.

To determination of total chromium, test solutions that contain different amounts Cr(VI) and Cr(III) were prepared. Then reduction of Cr(VI) ions to Cr(III) in the model solutions were performed by the procedure given by Bag et al. [22]. Then the procedure presented was applied to these solutions. The results are given in Table 3. The results show that the proposed preconcentration method could be applied for the determination of total chromium.

Table 2

Influences of some foreign ions on the recoveries of analytes ( $N=3$ )

Ion	Added as	Concentration (mg L <sup>-1</sup> )	Recoveries of Cr(III)
Na <sup>+</sup>	NaCl	20,000	95 $\pm$ 2 <sup>a</sup>
K <sup>+</sup>	KCl	5,000	97 $\pm$ 2
Ca <sup>2+</sup>	CaCl <sub>2</sub>	5,000	95 $\pm$ 2
Mg <sup>2+</sup>	MgCl <sub>2</sub>	5,000	95 $\pm$ 3
Cl <sup>-</sup>	NaCl	25,000	97 $\pm$ 3
F <sup>-</sup>	NaF	1,000	95 $\pm$ 2
NO <sub>3</sub> <sup>-</sup>	KNO <sub>3</sub>	3,000	95 $\pm$ 2
SO <sub>4</sub> <sup>2-</sup>	Na <sub>2</sub> SO <sub>4</sub>	3,000	96 $\pm$ 2
PO <sub>4</sub> <sup>3-</sup>	Na <sub>3</sub> PO <sub>4</sub>	3,000	96 $\pm$ 3
CH <sub>3</sub> COO <sup>-</sup>	CH <sub>3</sub> COONa	1,000	97 $\pm$ 2
HCO <sub>3</sub> <sup>-</sup>	NaHCO <sub>3</sub>	1,000	96 $\pm$ 3
Al <sup>3+</sup>	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	50	95 $\pm$ 3
Cd <sup>2+</sup>	CdSO <sub>4</sub>	50	97 $\pm$ 2
Mn <sup>2+</sup>	MnSO <sub>4</sub>	50	96 $\pm$ 3
Zn <sup>2+</sup>	ZnSO <sub>4</sub>	50	97 $\pm$ 3
Ni <sup>2+</sup>	NiSO <sub>4</sub>	50	96 $\pm$ 3
Fe <sup>3+</sup>	FeCl <sub>3</sub>	50	96 $\pm$ 2
Cu <sup>2+</sup>	CuSO <sub>4</sub>	50	97 $\pm$ 3
Pb <sup>2+</sup>	PbSO <sub>4</sub>	50	95 $\pm$ 2
Co <sup>2+</sup>	CoSO <sub>4</sub>	50	97 $\pm$ 2
Cr <sup>6+</sup>	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	250	95 $\pm$ 3

<sup>a</sup> Mean  $\pm$  standard deviation.

### 3.2. Figure of merits

In order to study the adsorptive capacity of *B. sphaericus* loaded Diaion SP-850, batch method was used. To 0.1 g resin was added 50 mL of solution containing 1.0 mg of metal ion at pH 5.0. After shaking, the mixture was filtered. Ten millilitres of the supernatant solution was diluted to 100 mL and determined by flame atomic absorption spectrometry. This procedure was repeated for each analyte ions separately. The capacity of sorbent for chromium(III) was 6.95 mg g<sup>-1</sup>.

The relative standard deviations for flame atomic absorption spectrometric determinations for analytes are between 1.0 and 5.0%. The detection limit, defined as the concentration equivalent to three times the standard deviation ( $N=11$ ) of the reagent blank for chromium(III) was 0.50  $\mu$ g L<sup>-1</sup>.

### 3.3. Application to water samples

We have explored the feasibility of presented speciation procedure for the determination of Cr(III), Cr(VI) and total chromium in natural waters including a tap water from Tokat

Table 3

Total chromium determinations in spiked test solutions (volume: 100 mL,  $N=4$ )

Added ( $\mu$ g)		Found ( $\mu$ g)		Total Cr	Recovery (%)		
Cr(III)	Cr(VI)	Cr(III)	Cr(VI)		Cr(III)	Cr(VI)	Total Cr
0	30	–	29.3 $\pm$ 0.8	29.3 $\pm$ 0.8	–	98 $\pm$ 2	98 $\pm$ 2
5	25	4.8 $\pm$ 0.2 <sup>a</sup>	24.6 $\pm$ 0.7	29.4 $\pm$ 0.9	96 $\pm$ 2	98 $\pm$ 2	98 $\pm$ 2
10	20	9.9 $\pm$ 0.4	19.6 $\pm$ 0.5	29.5 $\pm$ 0.7	99 $\pm$ 2	98 $\pm$ 2	98 $\pm$ 2
20	10	19.4 $\pm$ 0.6	9.9 $\pm$ 0.5	29.3 $\pm$ 0.8	97 $\pm$ 2	99 $\pm$ 2	98 $\pm$ 2
25	5	24.3 $\pm$ 0.5	4.9 $\pm$ 0.1	29.2 $\pm$ 0.5	97 $\pm$ 1	98 $\pm$ 1	97 $\pm$ 1
30	0	29.1 $\pm$ 0.8	–	29.1 $\pm$ 0.8	97 $\pm$ 2	–	97 $\pm$ 2

<sup>a</sup> ( $x \pm t.s.$ )/ $\sqrt{N}$ ,  $p: 0.95$ .

Table 4  
Speciation of Cr(III) and Cr(VI) and total chromium in some natural water samples (sample volume: 100 mL, N = 4)

Samples	Added ( $\mu\text{g}$ )		Found ( $\mu\text{g}$ )			Recovery (%)			Relative error (%)		R.S.D. (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total Cr	Cr(III)	Cr(VI)	Total Cr	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Tap water from Tokat city	–	–	$0.40 \pm 0.02^a$	$0.55 \pm 0.03$	$0.95 \pm 0.05$	–	–	–	–	–	5.0	5.5
	5.0	10.0	$5.35 \pm 0.30$	$10.40 \pm 0.45$	$15.75 \pm 0.75$	$99 \pm 1$	$99 \pm 1$	$99 \pm 1$	–0.9	–1.4	5.6	4.3
	10.0	5.0	$10.32 \pm 0.50$	$5.36 \pm 0.20$	$15.68 \pm 0.80$	$99 \pm 1$	$97 \pm 1$	$98 \pm 1$	–0.8	–3.5	4.8	3.7
Spring water from Sivas City	–	–	$0.30 \pm 0.01$	$0.44 \pm 0.02$	$0.74 \pm 0.04$	–	–	–	–	–	3.3	4.5
	5.0	10.0	$5.25 \pm 0.20$	$10.37 \pm 0.63$	$15.62 \pm 0.85$	$99 \pm 1$	$99 \pm 1$	$99 \pm 1$	–0.9	–0.7	3.8	6.1
	10.0	5.0	$10.10 \pm 0.65$	$5.30 \pm 0.25$	$15.40 \pm 0.70$	$98 \pm 2$	$97 \pm 1$	$98 \pm 2$	–1.9	–2.6	6.4	4.7
Sea water from Blacksea	–	–	$0.60 \pm 0.04$	$0.90 \pm 0.05$	$1.50 \pm 0.08$	–	–	–	–	–	6.7	5.6
	5.0	10.0	$5.45 \pm 0.25$	$10.75 \pm 0.70$	$16.20 \pm 0.70$	$97 \pm 2$	$99 \pm 1$	$98 \pm 2$	–2.8	–1.4	4.6	6.5
	10.0	5.0	$10.30 \pm 0.50$	$5.70 \pm 0.30$	$16.00 \pm 0.60$	$97 \pm 1$	$97 \pm 2$	$97 \pm 2$	–2.9	–3.5	4.9	5.3
Waste water	–	–	$7.65 \pm 0.32$	$4.40 \pm 0.15$	$12.05 \pm 0.50$	–	–	–	–	–	4.2	3.4
	5.0	10.0	$12.50 \pm 0.70$	$14.25 \pm 0.63$	$26.75 \pm 0.95$	$99 \pm 2$	$99 \pm 2$	$99 \pm 2$	–1.2	–1.1	5.6	4.4
	10.0	5.0	$17.45 \pm 0.65$	$9.32 \pm 0.45$	$26.77 \pm 0.82$	$99 \pm 2$	$99 \pm 2$	$99 \pm 2$	–1.1	–0.9	3.7	4.8
	10.0	10.0	$17.20 \pm 0.50$	$14.18 \pm 0.88$	$31.38 \pm 0.97$	$97 \pm 2$	$98 \pm 2$	$98 \pm 2$	–2.6	–1.6	2.9	6.2

<sup>a</sup>  $(x \pm \text{t.s.})/\sqrt{N}$ ,  $p:0.95$ .

Table 5  
Characteristic performance of some recent studies on chromium speciation

Technique	System	Media	Detection system	PF	DL ( $\mu\text{g L}^{-1}$ )	R.S.D. (%)	Reference
SPE	Cr(VI)—ADPC and oxidation of Cr(III)	Ambersorb 563	FAAS	125	2.70	<9	12
SPE	Sorption of Cr(III) and reduction of Cr(VI)	Saccharomyces cerevisiae immobilized on sepiolite	FAAS	75	94	<5	22
SPE	Cr(VI)—DPC and oxidation of Cr(III)	<i>Funaria hygrometrica</i> immobilized in a polysilicate matrix	ICP-MS, FAAS	20	0.15 for ICP-MS and 145 for FAAS	<10	23
Solvent extraction	Cr(VI)—DPC and oxidation of Cr(III)	MIBK	Spectrophotometry	20	2.22	<1	29
CPE	Cr(III)—1-phenyl-3-methyl-4-benzoylpyrazol-5-one and reduction of Cr(VI)	Triton X-100	ICP-AES	20	0.81	3.2	30
SPE	Cr(VI)—cetyltrimethyl ammonium bromide and oxidation of Cr(III)	C-18 bonded phase silica SPE disks	FAAS	45	15–20	<5	31
Coprecipitation	Cr(VI)—ethyl xanthate complex and oxidation of Cr(III)	–	FAAS	100	0.5	3.1	32
CPE	Cr(III)— <i>N,N'</i> -bis-(alpha-methyl salicylidene) propane-1, 3-diimine and reduction of Cr(VI)	Triton X-100	FAAS	–	0.1	2.3	33
SPE	At pH 6 Cr(III) was quantitatively separated from Cr(VI)	Chelex-100	ETAAS	–	46 ng/kg	<8.5	34
SPE	Cr(III) and Cr(VI)—APDC	LiChrospher 100 RP-8 and LiChrospher 60 RP-18	HPLC-UV detection	–	0.2	2	35
SPE	Cr(III) and reduction of Cr(VI)	<i>Bacillus sphaericus</i> loaded Diaion SP-850	FAAS	50	0.50	<5	This work

CPE: cloud point extraction, SPE: solid phase extraction, PF: preconcentration factor, and DL: detection limit.

City, a spring water from Sivas City, a waste water sample from Tokat City, Turkey and a sea water sample from Blacksea, Turkey. The preconcentration-speciation procedure was applied to the determination of chromium species by standard addition method. The reliability was checked by spiking experiments. The results were presented in Table 4. The recovery of spiked samples was satisfactory reasonable and was confirmed using addition method, which indicate the capability of the system in the speciation of chromium in natural water samples.

#### 4. Conclusion

A simple, economic and rapid speciation biosorption procedure for chromium species in natural water samples was presented. Six hundred milligrams of biosorbent could be used in all throughout the studies. The procedure was successfully applied to speciation of chromium species of various natural water samples. The comparative data from some recent studies on chromium speciation are given in Table 5. The optimal conditions are comparable for the procedure in the literature [22–27,28–35] with quantitative recovery values and lower detection limit for chromium speciation. The proposed method is superior to those reported chromium separation-preconcentration methods in term of selectivity, linear range, detection limit, applicable pH range, capacity, no need to consumption of organic solvents and enrichment factor.

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