Use of *Escherichia coli* Immobilized on Amberlite XAD-4 as a Solid-Phase Extractor for Metal Preconcentration and Determination by Atomic Absorption Spectrometry

A. Rehber TÜRKER*† and Sıtkı BAYTAK**

*Department of Chemistry, Gazi University, Faculty of Art and Science, 06500, Ankara, Turkey

**Department of Chemistry, Harran University, Faculty of Art and Science, 63100, Şanlıurfa, Turkey

A sensitive solid-phase extraction technique (SPE) for the enrichment of Fe(III), Co(II), Mn(II) and Cr(III) prior to atomic absorption spectrometric analysis is described. *Escherichia coli* immobilized on Amberlite XAD-4 was used as a solid-phase extractor. The effects of the pH, amount of solid-phase, eluent type and volume of the sample solution on the recovery of the metal ions were investigated. The effect of diverse ions was also investigated. The recoveries of Fe(III), Co(II), Mn(II) and Cr(III) under the optimum conditions were found to be 99 ± 2 , 99 ± 3 , 98 ± 2 , $98 \pm 3\%$, respectively, at the 95% confidence level. The detection limits of the metal ions were found as to be 2.4, 3.8, 1.3 and 1.7 ng ml⁻¹ for Fe(III), Co(II), Mn(II) and Cr(III) respectively, by applying a preconcentration factor of 25. The proposed enrichment method was applied to the determination of analytes in Atatürk Dam water samples, and alloy samples (RSD < 5%). The accuracy of the method was verified on certified alloy samples (NBS SRM 85b and NBS SRM 59a). The analytes were determined with a relative error lower than 5% in water and alloy samples.

(Received August 26, 2003; Accepted October 8, 2003)

Introduction

The increasing environmental pollution by toxic metals has required an accurate and sensitive quantitative metal determination in environmental samples. One of the most important targets of analytical chemist is metal quantification at low concentration levels below ng ml⁻¹ or ng g⁻¹. Despite recent advances in instrumental techniques and an increase in the selectivity and sensitivity of analytical techniques, a direct determination of trace amounts of elements occurring in samples is still difficult. There is still a need for the preconcentration and separation of trace elements before their analysis due to their low concentrations and potential interfering matrix constituents in numerous samples, such as water, biological and geological samples.

Various separation and preconcentration techniques have been used for trace metals, such as solvent extraction,¹⁻³ coprecipitation,⁴⁻⁶ electrodeposition,⁷ ion-exchange,⁸⁻¹⁰ cloud point extraction technique¹¹ and solid-phase extraction¹² (SPE), adsorption on activated carbon,¹³ on dithixone-anchored poly(EGDMA-HEMA) microbeads,¹⁴ on Amberlite resins¹⁵ and on sepiolite.¹⁶

The preconcentration procedures employing a solid phase and applied to atomic spectrometry present some advantageous characteristics, such as simplicity, column applicability and a higher preconcentration factor. There is current interest in the

development of a new solid-phase extractor of high sorption capacity, selectivity and preconcentration factor.

In recent years, preconcentration by microorganisms, such as yeast, fungi, bacteria and algae, based on solid-phase extraction has been widely used.^{17,18} The use of microorganisms as a biosorbent for metals has many advantages over the other preconcentration methods. Some of them are higher recovery, economical advantages, simplicity and environmental protection. In general, microorganisms selectively adsorb a specific element without preconcentrating the matrix.¹⁹ Microorganisms have been used as freely suspended cells or as loaded cells on a support. The use of immobilized cell systems on a support has many advantages over the use of freely suspended cells. These include better capability of re-using the biomass, better precision for recovery, easy separation of cells from the reaction mixture, high biomass loadings and minimal clogging in continuous flow systems. Immobilized cell systems can be used in both batch and column experiments.

Bağ *et al.*²⁰ studied *E. coli* immobilized on sepiolite for the preconcentration of Cu, Zn, Fe, Ni and Cd. The results indicated that the proposed solid phase is suitable for the preconcentration of analytes from alloys. Gonzalez *et al.*²¹ proposed the on-line preconcentration of Cd(II), Cr(III), Cu(II) and Pb(II) and the chemical speciation of Cr(III) and Cr(VI) using dealginated seaweed biomass. Carrillo *et al.*²² studied the biosorption of Al, Co, Cu and Fe by brown alga *Pilayella littoralis* in a flow-injection system. In other studies, algae cells²³ and bacteria, such as *E. coli* and *P. putida*,²⁴ have been used as biomass. Robles *et al.*²⁴ used two types of living and dead bacteria, *E. coli* and *P. putida*, to investigate the accumulation of gold. Mahan and Holcombe²⁵ studied algae-silica to preconcentrate Cu, Cd, Pb and Zn in simulated seawater and river-water solutions. Pardo *et al.*²⁶ investigated

A part of this manuscript was presented at the 3rd Aegen Analytical Chemistry Days, September 29 – October 3, 2002, Polihnitos, Lesvos, Greece.

† To whom correspondence should be addressed.

E-mail: aturker@gazi.edu.tr

the accumulation of Cd(II), Cu(II), Pb(II) and Zn(II) at mg l⁻¹ levels by inactive freeze-dried biomass of *Pseudomonas putida*.

In previous studies, various materials, such as controlled pore glass (CPG),²⁷ silica gel²⁸ and sepiolite²⁰ have been used as substrates for the immobilization of microorganisms.

In this study, Amberlite XAD-4 resin, which was previously used as an adsorbent, was used as a substrate for the immobilization of *E. coli*. The main advantage of Amberlite XAD-4 over pore glass, silica and sepiolite is the commercially availability of pure resin having a uniform pore distribution, high surface area, durable and chemically homogeneous structure. The resulting solid-phase extractor was examined for the enrichment of Fe(III), Co(II), Mn(II) and Cr(III) in a column procedure. The optimum enrichment conditions of Fe(III), Co(II), Mn(II) and Cr(III) by this solid-phase extractor were determined. Atomic absorption spectrometry was used as a detection technique. The proposed method was applied to the determination of these elements in water and alloy samples. Because Amberlite XAD-4 has been used as an adsorbent in previous studies, some experiments were also performed like using Amberlite XAD-4 alone as a solid phase for a comparison.

Experimental

Apparatus

A Philips PU 9285 Model atomic absorption spectrometer equipped with deuterium lamp background correction and an air-acetylene burner was used for the determination of iron, cobalt, manganese and chromium, under the following conditions: wavelength, 248.3 nm, 240.7 nm, 279.5 nm and 357.9 nm; spectral bandwidth, 1.0 nm, 1.0 nm, 1.0 nm and 1.0 nm; lamp current, 11.2 mA, 11.0 mA, 9.0 mA and 12.0 mA; acetylene flow rate, 0.9, 1.1, 1.0 and 1.4 l min⁻¹ for iron, cobalt, manganese and chromium, respectively. All pH measurements were performed with a JENWAY 3010 Model digital pH meter. Metal sorption studies on the solid-phase extractor were performed using a column of 1 cm in diameter and 20 cm in height.

Reagents

Doubly distilled water and analytical reagent-grade chemicals were used unless otherwise specified. Manganese, cobalt, iron and chromium stock solutions (1000 µg ml⁻¹) were prepared by dissolving appropriate amounts of MnSO₄·H₂O (Merck), Co(NO₃)₂·6H₂O (Merck), iron powder and Cr(NO₃)₃·9H₂O (Merck) by an appropriate solvent. Working solutions of the metal ions were prepared by a suitable dilution of a stock solution with doubly distilled water. Amberlite XAD-4 (Sigma Chem. 20–40 mesh, 780 m² g⁻¹) was used as a substrate for the immobilization of *Escherichia coli*.

Preparation of solid-phase extractor

The bacterium *Escherichia coli* was maintained, cultivated, grown and prepared as a dry bacterial powder according to a procedure given by Bağ *et al.*²⁰ Commercially available Amberlite XAD-4 was prepared as a substrate by washing successively with methanol, water, 1 mol l⁻¹ HCl and water, to remove organic and inorganic contaminants. Then, the immobilization of *Escherichia coli* on the substrate was performed as follows: 150 mg of dry and dead bacteria powder was mixed with 1 g of Amberlite XAD-4. 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

Table 1 Effect of the type and volume of elution solutions on the recoveries of Fe(III), Co(II), Mn(II) and Cr(III)

Element	Type of elution solution	Volume/ ml	Concentration/ mol l ⁻¹	Recovery, ^a %
Fe(III)	HCl	5	1	85
		10	1	99
	HNO ₃	5	1	75
		10	1	82
Co(II)	HCl	5	1	88
		10	1	98
	HNO ₃	5	1	74
		10	1	80
Mn(II)	HCl	5	1	87
		10	1	98
	HNO ₃	5	1	65
		10	1	72
Cr(III)	HCl	10	1	35
	HNO ₃	10	1	27
	HCl (in acetone)	10	1	98
	HNO ₃ (in acetone)	10	1	84

a. Mean of three determinations.

105°C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *Escherichia coli* and Amberlite XAD-4, thereby improving the immobilization efficiency.

Preparation of the column

First, 0.3 g of Amberlite XAD-4 loaded with *Escherichia coli* was packed in a glass column (10 mm i.d. and 200 mm length). Before use, a 1 mol l⁻¹ HCl solution and doubly distilled water were passed through the column in order to condition and clean it. Then, the column was conditioned to the studied pH by passing an aqueous solution of HCl or NH₃ having the same pH as that of sample solution through the column, prior to passage of the sample solution.

General enrichment procedure

An off-line column-SPE procedure was applied for preconcentration. An aliquot of the sample solution (100 ml) containing 50 µg of Fe(III), 30 µg of Co(II), 30 µg of Mn(II) and 20 µg of Cr(III) was taken and the pH was adjusted to the optimum value, determined experimentally with hydrochloric acid or ammonia. The resulting solution was passed through the column at a flow rate of 2 ml min⁻¹. The retained metal ions were then eluted from the solid phase with a suitable eluent, determined experimentally (Table 1). The concentration of the metal ions in the eluate was determined by atomic absorption spectrometry. *Escherichia coli* immobilized on Amberlite XAD-4 was used repeatedly after washing with a 1 mol l⁻¹ HCl solution and then distilled water.

The recovery (*R*) of the metal ions was calculated from the ratio of the concentration found by FAAS to that calculated theoretically.

Dissolution of alloy samples

The dissolution of ferrosilicon alloy (NBS SRM 59a) was performed according to the procedure adopted by Acar *et al.*²⁹ After 0.3 g of alloy was taken and the dissolution procedure was applied, the resulting solution was transferred into a 250 ml

Fig. 1 Effect of the pH on the recoveries of Fe(III), Co(II), Mn(II) and Cr(III). Fe(III), $0.5 \mu\text{g ml}^{-1}$; Co(II) and Mn(II), $0.3 \mu\text{g ml}^{-1}$; Cr(III), $0.2 \mu\text{g ml}^{-1}$; solid-phase, 0.3 g; sample volume, 100 ml; flow rate, 1 ml min^{-1} ; elution solution, 10 ml of 1 mol l^{-1} HCl (in acetone for Cr).

Fig. 2 Effect of the flow rate of the sample solutions on the recoveries of Fe(III), Co(II), Mn(II) and Cr(III). Fe(III), $0.5 \mu\text{g ml}^{-1}$; Co(II) and Mn(II), $0.3 \mu\text{g ml}^{-1}$; Cr(III), $0.2 \mu\text{g ml}^{-1}$; solid-phase, 0.3 g; sample volume, 100 ml; elution solution, 10 ml of 1 mol l^{-1} HCl (in acetone for Cr).

volumetric flask and diluted to the mark with doubly distilled water.

Aluminum alloy (NBS SRM 85b) was dissolved as follows: 0.2 g of aluminum alloy was dissolved in a 250 ml beaker by adding 5 ml of concentrated hydrochloric acid. The solution was heated on a water bath ($\sim 80^\circ\text{C}$) for 30 min to complete dissolution. The solution was cooled and transferred to a 100 ml volumetric flask and diluted to the mark with water. After 0.1 ml of each sample solution was diluted again to reduce the analyte concentration at the working range of the atomic absorption spectrometric method, the matrix concentration was reduced.

For analyzing aluminum foil, a 0.1 g of aluminum foil was dissolved in 10 ml of 2 mol l^{-1} hydrochloric acid and 5 ml of 2 mol l^{-1} nitric acid in a 50 ml conical flask. The flask was heated on a hot plate ($70 - 80^\circ\text{C}$) until the solution became clear. The solution was transferred to a 100 ml volumetric flask with small portions of 0.05 mol l^{-1} nitric acid and the volume was made up with 0.05 mol l^{-1} nitric acid. The resulting solution was spiked with an appropriate amount of analytes in order to check the accuracy. Spiked and unspiked sample solutions were subjected to the preconcentration and determination procedure.

Results and Discussion

Effect of the pH

The pH of the sample solution plays a crucial role in microbial biosorption. Thus, the retention of the analytes on the column containing *Escherichia coli* immobilized on Amberlite XAD-4 was studied as a function of the pH. For that purpose, a set of solutions (100 ml) each containing one of the four metal ions at a concentration given in the section of "General enrichment procedure" was taken. The pH value of the sample solutions was adjusted to a range of 2–10 with HCl or NH_3 . The obtained solutions were passed through the column at a flow rate at about 2 ml min^{-1} . The metal ions were then eluted by an appropriate eluent (Table 1) and determined by FAAS.

In all cases, metal retention by the adsorbent (biomass) increased with increasing pH and reached a maximum, after which the retention decreased. As can be seen in Fig. 1, the optimum pH of the sample solution was about 8 for Mn(II), Co(II) and Cr(III), and about 6 for Fe(III). These results show

that the adsorption could be attributed to ionic attraction between the metal ions and the biomass. The microbial cell products, such as metabolites, polysaccharides, and the cell-wall constituents, are effective in metal accumulating. There are many binding sites on the cell wall of microorganisms, and the binding sites of microorganisms have a diversity property. When the cell surface becomes more positively charged, the attraction between metal ions and the functional groups on cell wall at low pH values is reduced. When more negatively charged, the attraction at higher pH values is increased until a maximum is reached at around pH 7. For pH values higher than the optimum values, the retention decreases again because of the formation of hydroxylated complexes of the metal.²⁶

Effect of the amount of solid phase (bed height)

The retention of the analytes was examined in relation to the amount of solid phase, which was varied from 100 mg to 400 mg. It was found that the recoveries of Fe(III), Co(II), Cr(III) and Mn(II) were gradually increased up to 200, 200, 200 and 300 mg of the solid phase, respectively. Therefore, 200 mg of the solid phase was used for Fe(III), Co(II) and Cr(III), and 300 mg of the solid phase was used for Mn(II) in subsequent experiments.

Effect of the type and volume of elution solutions

Optimization of the elution conditions was performed in order to obtain the maximum recovery with the minimal concentration and volume of the elution solution. The different concentrations of nitric acid and hydrochloric acid in water and in acetone were tested to remove the bound metal ions from the biomass. As can be seen from Table 1, 10 ml of 1 mol l^{-1} HCl solution was found to be satisfactory (recovery > 95%) for Fe(III), Co(II) and Mn(II), and 10 mL of 1 mol l^{-1} HCl (in acetone) was for Cr(III).

Effect of the flow rates of sample solutions

The retention of an element on an adsorbent also depends on the flow rate of the sample solution. Thus, the effect of the flow rate of the sample solution on the recovery of the analytes was investigated under the optimum conditions (pH, eluent type, etc.). The sample solution was passed through the column with the flow rates adjusted in a range of $1 - 5 \text{ ml min}^{-1}$ by gravity. As can be seen in Fig. 2, at flow rates greater than 2 ml min^{-1}

Fig. 3 Effect of the volume of the sample solution on the recoveries of Fe(III), Co(II), Mn(II) and Cr(III). Fe(III), $0.5 \mu\text{g ml}^{-1}$; Co(II) and Mn(II), $0.3 \mu\text{g ml}^{-1}$; Cr(III), $0.2 \mu\text{g ml}^{-1}$; pH, 6 for Fe and 8 for Co and Mn; solid-phase, 0.2 g for Fe and Co, 0.3 g for Mn; elution solution, 10 ml of 1 mol l^{-1} HCl (in acetone for Cr).

there was a decrease in the recovery of all analytes. The reason for this decrease is probably insufficient contact of the metal ions and the adsorbent to reach equilibrium. Therefore, as the flow rate of the sample solution, 2 ml min^{-1} was adapted in subsequent experiments. The flow rate of the elution solution was 1 ml min^{-1} .

Effect of the volume of the sample solution

In order to determine the volume of an applicable sample solution (or minimum analyte concentration), the effect of changes in the volume of the sample solution passed through the column on the retention of the analytes was also investigated. First, 100, 250, 500, 750, and 1000 ml of sample solutions containing fixed amounts of analytes ($50 \mu\text{g}$ of Fe(III), $30 \mu\text{g}$ of Co(II), $30 \mu\text{g}$ of Mn(II) and $20 \mu\text{g}$ of Cr(III)) which corresponds to 0.5 , 0.2 , 0.1 , 0.066 , $0.05 \mu\text{g ml}^{-1}$ Fe(III), 0.30 , 0.12 , 0.06 , 0.04 , $0.03 \mu\text{g ml}^{-1}$ Mn(II) and Co(II), and 0.2 , 0.08 , 0.04 , 0.027 , $0.02 \mu\text{g ml}^{-1}$ Cr(III) were passed through the column under the optimum conditions (pH, eluent type, flow rate, *etc.*). It was then found that all analytes could be recovered quantitatively ($> 95\%$) up to 250 ml of the sample solution. At higher sample volumes, the recoveries gradually decreased with increasing volume of the sample (Fig. 3). In this study, because of the elution volume was 10 ml, a 25-fold preconcentration could be obtained for all of the analytes studied. It can be concluded that iron, cobalt, manganese and chromium can be determined at concentrations of $0.2 \mu\text{g ml}^{-1}$, $0.12 \mu\text{g ml}^{-1}$, $0.12 \mu\text{g ml}^{-1}$, and $0.08 \mu\text{g ml}^{-1}$, respectively. These concentrations can not be determined directly by atomic absorption spectrometry with sufficient accuracy.

Effect of interfering ions

In order to investigate the effect of the interfering ions, especially alkaline and alkaline earth elements, the main components of the alloys to be analyzed were added to a synthetic sample solution containing analytes. The recoveries of Fe(III), Co(II), Mn(II) and Cr(III) were also examined when they existed together in the same medium. The concentrations of iron, cobalt, manganese and chromium were fixed at $0.5 \mu\text{g ml}^{-1}$, $0.3 \mu\text{g ml}^{-1}$, $0.3 \mu\text{g ml}^{-1}$, $0.2 \mu\text{g ml}^{-1}$, respectively, and the concentration of interfering metal ions were adjusted over the range of $0.5 - 1000 \mu\text{g ml}^{-1}$. Other ions present in the solution

Table 2 Effect of other ions on the recoveries of Fe(III), Co(II), Mn(II) and Cr(III)^a

Interfering ion	Concentration/ $\mu\text{g ml}^{-1}$	Recovery, %			
		Fe(III)	Co(II)	Mn(II)	Cr(III)
Na ⁺	—	99	98	98	98
	25	99	98	98	98
	50	99	98	98	97
	100	99	—	98	97
	500	98	98	90	96
	1000	95	97	85	95
K ⁺	—	99	98	98	98
	20	99	—	98	97
	25	—	98	—	97
	30	—	—	98	97
	50	99	98	98	97
	100	98	98	98	96
Ca ²⁺	250	—	98	95	96
	500	95	—	85	95
	—	99	98	98	98
	10	—	96	98	97
	20	95	80	83	96
	50	85	65	—	89
Mg ²⁺	—	99	98	98	98
	0.5	99	98	98	98
	1	99	—	95	97
	2.5	95	98	80	96
	5	87	98	60	90
	10	—	82	—	—
Al ³⁺	—	—	98	98	98
	250	—	98	97	97
	500	—	96	95	97
Fe ³⁺	—	—	97	98	98
	15	—	96	97	—
	30	—	95	95	—

a. Concentrations of Fe(III), Co(II), Mn(II) and Cr(III) are 0.5 , 0.3 , 0.3 , $0.2 \mu\text{g ml}^{-1}$, respectively.

generally have a negative impact on the metal retention because of the competition between ions. The results were given in Table 2. As can be seen in the table, Na and K did not interfere in the determination of analytes up to $500 \mu\text{g ml}^{-1}$. However, Ca and Mg interfered in the determination when they existed above $10 \mu\text{g ml}^{-1}$ and $2.5 \mu\text{g ml}^{-1}$, respectively.

Effect of column reuse

To test the long-term stability of the adsorbent, the column was subjected to successive adsorption and desorption cycles by passing 100 ml of metal solutions through it. The procedure was carried out five times in a day and the next five runs were made one day later, and so on. The stability and potential recyclability of the column were assessed by monitoring the change in the recoveries of the analytes. For all of the analytes studied, after 15 runs a decrease appeared in the recoveries. The column seems to have been relatively stable up to 15 runs.

Capacity studies

To evaluate the amount of metal adsorbed onto the coordination sites of the immobilized *E. coli*, the breakthrough capacity was studied. The breakthrough capacity was evaluated from the breakthrough curve plot by a method given by Bağ *et al.*³⁰ The breakthrough capacities were found as to be 21 and $28 \mu\text{mol g}^{-1}$ for Mn(II) and Co(II) when using *E. coli*, and 15 and $20 \mu\text{mol g}^{-1}$ without using *E. coli*, respectively. As can be seen from the results, the capacity of *E. coli* immobilized on

Table 3 Recovery of the analytes and repeatability of the method

Element	With microorganism		Without microorganism	
	%R $\pm$ ts ^a / $\sqrt{N}$	RSD ^b , %	%R $\pm$ ts/ $\sqrt{N}$	RSD, %
Fe(III)	99 $\pm$ 2	1.6	35 $\pm$ 2	4.6
Co(II)	99 $\pm$ 3	2.4	40 $\pm$ 2	4.0
Mn(II)	98 $\pm$ 2	1.6	68 $\pm$ 3	2.4
Cr(III)	98 $\pm$ 3	2.0	35 $\pm$ 2	4.6

a. Uncertainty at 95% confidence limit ($N = 5$).

b. Relative standard deviation.

Table 4 Determinations of Fe(III), Co(II), Mn(II) and Cr(III) in alloys

Sample	Element	Certified, % ^a (m/m)	Found, ^b % (m/m) ($x \pm ts/\sqrt{N}$)	Error, %
NBS SRM 85b	Fe	0.24	0.23 $\pm$ 0.02	-4
	Mn	0.61	0.58 $\pm$ 0.03	-5
	Cr	0.211	0.20 $\pm$ 0.03	-5
	Co ^c	0.50	0.48 $\pm$ 0.02	-4
NBS SRM 59a	Mn	0.75	0.74 $\pm$ 0.05	-2
	Cr	0.080	0.076 $\pm$ 0.010	-5
	Co ^c	0.32	0.31 $\pm$ 0.02	-4

a. The composition of the aluminum based alloy (NBS SRM 85b) was Al 93.097, Mn 0.61, Si 0.18, Cu 3.99, Ni 0.084, Cr 0.211, V 0.006, Ti 0.022, Ga 0.019, Fe 0.24, Pb 0.021, Mg 1.49 and Zn 0.030% (m/m). The composition of the ferrosilicon based alloy (NBS SRM 59a) was Al 0.35, Mn 0.75, Si 48.10, Cu 0.052, Ni 0.033, Cr 0.080, Fe 50.05, C 0.046, S 0.002, P 0.016, Ca 0.042 and B 0.058% (m/m).

b. Mean of five determinations at 95% confidence level.

c. Co was added to the solutions of NBS SRM 59a and 85b.

Amberlite XAD-4 was higher than that Amberlite XAD-4 alone for both of the ions studied. The capacity of the column for Fe(III) and Cr(III) was not studied, because precipitation of Fe(III) and Cr(III) occurred at higher concentration levels over the studied pH.

Analytical features

To determine the precision of the method, successive retention and elution cycles were performed with 100 ml of a sample solution containing 50 μ g of Fe(III), 30 μ g of Mn(II) and Co(II), and 20 μ g of Cr(III) under the optimum conditions, mentioned above. As can be seen from Table 3, the recoveries of Fe(III), Co(II), Mn(II) and Cr(III) were quantitative (> 95%) and the precision of the method was very good (RSD < 3%) for *Escherichia coli* immobilized on Amberlite XAD-4. The results found by using Amberlite XAD-4 alone were also given for a comparison. As can be seen from the table, the recoveries of the analytes were very low when using Amberlite XAD-4 alone as an adsorbent. To increase the recovery of the elements by using Amberlite XAD-4, it is usually necessary to use a chelating agent prior to the column procedure.¹⁴ By using *Escherichia coli* immobilized on Amberlite XAD-4, there is no need to use a chelating agent.

The linear calibration ranges for measurements under the optimum conditions were 1.0 – 8.0 μ g ml⁻¹ for Fe(III), 0.5 – 5.0 μ g ml⁻¹ for Co(II), Mn(II) and Cr(III).

To determine the detection limit, 50 ml of a blank solution

Table 5 Determinations of Fe(III), Co(II), Mn(II) and Cr(III) in Atatürk Dam water (sample volume 250 ml)

Element	Added/ μ g l ⁻¹	Found/ μ g l ⁻¹ ($x \pm ts/\sqrt{N}$)	Error, %
Fe(III)	—	38.0 $\pm$ 0.7	—
	100	135 $\pm$ 2	-2
Co(II)	—	24.0 $\pm$ 0.9	—
	40	63 $\pm$ 1	-2
Mn(II)	—	15 $\pm$ 1	—
	40	52 $\pm$ 1	-5
Cr(III)	—	50 $\pm$ 2	—
	50	95 $\pm$ 3	-5

a. Mean of five determinations at 95% confidence level.

was passed through the column and retained metals were eluted by 50 ml of the elution solution mentioned above. The detection limits based on three-times the standard deviation of the blank solution were found to be 60, 95, 33 and 43 ng ml⁻¹ for Fe(III), Co(II), Mn(II) and Cr(III) ($N = 20$), respectively. It may be concluded that by applying a preconcentration factor³¹ of 25 for the all analytes, 2.4, 3.8, 1.3 and 1.7 ng ml⁻¹ detection limit for Fe(III), Co(II), Mn(II) and Cr(III), respectively, could be obtained.

To demonstrate the validity and accuracy of the proposed enrichment and determination method, standard reference materials of some alloys have been analyzed. The analytes in the diluted solution of alloys were preconcentrated and determined five times. The results are summarized in Table 4. As can be seen from Table 4, the accuracy of the method was very satisfactory; the percent relative error was lower than 5%.

Application

To evaluate the applicability of the proposed preconcentration and determination method, aluminum foil and water samples were analyzed as real samples. Co(II), Mn(II), Fe(III) and Cr(III) were determined in a water sample collected from Atatürk Dam and in aluminum foil used for food protection. A water sample was filtered through a MFS membrane filter (pore size 0.45 μ m) and analyzed as soon as possible after sampling. A 250 ml sample was adjusted to the optimum pH and subjected to the recommended column procedure for the preconcentration and determination of metal ions. The water sample was also spiked with the analytes to test the accuracy of the method. The results reported in Table 5, with a confidence interval for the 95% confidence level, show the applicability of the proposed method to water analysis.

Spiked and unspiked solutions of aluminum foil were subjected to the preconcentration and determination procedure described above. The results are given in Table 6. The accuracy of the results was quite satisfactory. The relative error was about 5%.

Conclusion

The proposed SPE enrichment procedure described in this paper provides a simple, sensitive, precise, reliable and accurate technique for the preconcentration of Fe(III), Co(II), Mn(II) and Cr(III). The recoveries of all four metal ions were nearly quantitative (> 95%). The relative standard deviations for five experiments were < 5%. It can also be concluded that the use of microorganism increased the retention of the analytes with

Table 6 Determinations of Fe(III), Co(II), Mn(II) and Cr(III) in aluminum foil

Element	Added/ $\mu\text{g g}^{-1}$	Found/ $\mu\text{g g}^{-1}$ ($\bar{x} \pm s^a$)	Error, %
Fe(III)	—	5100 ± 200	—
	10000	14600 ± 500	-3
Co(II)	—	—	—
	200	190 ± 4	-5
Mn(II)	—	54.6 ± 0.6	—
	100	150 ± 1	-3
Cr(III)	—	ND ^b	—
	100	95 ± 3	-5

a. Standard deviation ($N = 5$).

b. ND: Not detected.

respect to Amberlite XAD-4 alone. The analytes could be preconcentrated directly by using the proposed method without using any chelating or complexing agent. The preconcentration technique used in this work is also cheap. Only 0.3 g of adsorbent is needed, and repeated use is possible. The main disadvantage of the proposed method is the duration time of the preconcentration step. The duration time is about 2 h for a 250 ml sample solution.

This technique could be combined with other methods of analysis, such as ICP-AES, ICP-MS and electroanalytical methods, and used as an on-line preconcentration system.

Acknowledgements

The support of Harran University Research Fund is gratefully acknowledged.

References

1. B. Holynska, B. Ostachwicz, and D. Wegrzynek, *Spectrochim. Acta*, **1996**, *51B*, 769.
2. D. Kara and M. Alkan, *Talanta*, **2001**, *55*(2), 415.
3. I. Kojima and M. Katsuzaki, *Anal. Sci.*, **1997**, *13*, 1021.
4. S. J. J. Tsai, C. C. Jan, and L. L. Chang, *Spectrochim. Acta*, **1994**, *49B*, 773.
5. S. Kagaya, M. Saiki, Z. A. Malek, Y. Araki, and K. Hasegawa, *Fresenius J. Anal. Chem.*, **2001**, *371*, 391.
6. D. Atanassova, V. Stefanova, and E. Russeva, *Talanta*, **1998**, *47*, 1237.
7. A. Ritschel, P. Wobrauschek, E. Chinea, F. Grass, and Ch. Fabjan, *Spectrochim. Acta*, **1999**, *54B*, 1449.
8. G. H. Tao and Z. L. Fang, *Fresenius J. Anal. Chem.*, **1998**, *360*, 156.
9. R. M. Cespon-Romero, M. C. Yebra-Biurrun, and M. P. Bermejo-Barrera, *Anal. Chim. Acta*, **1996**, *327*, 37.
10. A. Cuesta, J. L. Todoli, and A. Canals, *Spectrochim. Acta*, **1996**, *51B*, 1791.
11. J. L. Manzoori and G. Karim-Nezhad, *Anal. Sci.*, **2003**, *19*, 579.
12. H. Bağ, A. R. Türker, R. Coşkun, M. Saçak, and M. Yiğitoğlu, *Spectrochim. Acta*, **2000**, *55B*, 1101.
13. L. Elçi and M. Doğan, *Fresenius Z. Anal. Chem.*, **1988**, *330*, 610.
14. B. Salih, R. Say, A. Denizli, Ö. Genç, and E. Pişkin, *Anal. Chim. Acta*, **1998**, *371*, 177.
15. L. Elçi, M. Soylak, and M. Doğan, *Fresenius J. Anal. Chem.*, **1992**, *342*, 175.
16. A. R. Türker, H. Bağ, and B. Erdoğan, *Fresenius J. Anal. Chem.*, **1997**, *357*, 351.
17. H. A. M. Elmahadi and G. M. Greenway, *J. Anal. At. Spectrom.*, **1991**, *6*, 643.
18. H. Bağ, A. R. Türker, M. Lale, and A. Tunçeli, *Talanta*, **2000**, *51*, 895.
19. R. H. Crist, K. Oberhaser, N. Shank, and M. Nguyen, *Environ. Sci. Technol.*, **1981**, *15*, 1212.
20. H. Bağ, A. R. Türker, and M. Lale, *Talanta*, **2000**, *51*, 1035.
21. M. E. Romero-Gonzalez, C. J. Williams, and P. H. D. Gardiner, *J. Anal. At. Spectrom.*, **2000**, *15*, 1009.
22. E. N. V. M. Carrilho, J. A. Nobrega, and T. R. Gilbert, *Talanta*, **2003**, *60*, 1131.
23. L. C. Robles and A. J. Aller, *Quim. Anal.*, **1996**, *15*, 21.
24. L. C. Robles, C. Garcia-Olalla, and A. J. Aller, *J. Anal. At. Spectrom.*, **1993**, *8*, 1015.
25. C. A. Mahan and J. A. Holcombe, *Spectrochim. Acta*, **1992**, *47B*, 1483.
26. R. Pardo, M. Herguedas, E. Barrado, and M. Vega, *Anal. Bioanal. Chem.*, **2003**, *376*, 32.
27. A. Maquieira, H. A. M. Elmahadi, and R. Puchades, *Anal. Chem.*, **1994**, *66*, 1462.
28. B. Godlewska-Zylkiewicz, *Spectrochim. Acta*, **2003**, *58B*, 1531.
29. O. Acar, A. R. Türker, and Z. Kılıç, *Fresenius J. Anal. Chem.*, **1997**, *357*, 656.
30. H. Bağ, M. Lale, and A. R. Türker, *Talanta*, **1998**, *47*, 689.
31. A. C. Sahayam, *Anal. Bioanal. Chem.*, **2002**, *372*, 840.