Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 65 (2005) 938–945
Talanta
www.elsevier.com/locate/talanta

The use of *Agrobacterium tumefacients* immobilized on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron(III), cobalt(II), manganese(II) and chromium(III)

Sıtkı Baytak[a], A. Rehber Türker[b,*]

[a] *Deparment of Chemistry, Science and Art Faculty, Harran University, TR-63100 Şanlıurfa, Turkey*
[b] *Deparment of Chemistry, Science and Art Faculty, Gazi University, TR-06500 Ankara, Turkey*

Received 26 May 2004; received in revised form 23 July 2004; accepted 16 August 2004
Available online 18 September 2004

Abstract

A microorganism *Agrobacterium tumefacients* as an immobilized cell on a solid support was presented as a new biosorbent for the enrichment of Fe(III), Co(II), Mn(II) and Cr(III) prior to flame atomic absorption spectrometric analysis. Amberlite XAD-4 was used as a support material for column preconcentration. Various parameters such as pH, amount of adsorbent, eluent type and volume, flow rate of sample solution, volume of sample solution and matrix interference effect on the retention of the metal ions have been studied. The optimum pH for the sorption of above mentioned metal ions were about 6, 8, 8 and 6, respectively. The loading capacity of adsorbent for Co(II) and Mn(II) were found to be 29 and 22 μmol g^{-1}, respectively. The recoveries of Fe(III), Co(II), Mn(II) and Cr(III), under the optimum conditions were found to be 99 ± 3, 99 ± 2, 98 ± 3 and 98 ± 3%, respectively, at the 95% confidence level. The limit of detection was 3.6, 3.0, 2.8 and 3.6 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 for metal ion determination from water samples, alloy samples, infant foods and certified samples such as whey powder (IAEA-155) and aluminum alloy (NBS SRM 85b). The analytes were determined with a relative error lower than 10% in all samples.

Keywords: Flame atomic absorption spectrometry; Enrichment; *Agrobacterium tumefacients*; Water; Alloy; Infant food

1. Introduction

The industrial use of metals leads to increase of metal concentrations in air, waters and soils. Unlike organic pollutants, metals are non-biodegradable and hence are accumulated by living organisms. Trace metals are widely spread in environment and may enter the food chain from the environment. Some trace metals are essential elements and play an important role in human metabolism. On the other hand, at higher concentrations all of the metals are recognized as potentially toxic. Therefore, accurate determination of trace metals in environment is very important.

Flame atomic absorption spectrometry (FAAS) is extensively employed for quantification of metallic species. This technique presents desirable characteristics such as operational facilities, good selectivity and low cost. However, in the presence of very high excess of diverse ions compared with the level of analyte, some limitations, mainly those related to the sensitivity are observed [1,2]. In spite of the inherent high sensitivities obtained for ETAAS, ICP–OES and ICP–MS, these techniques are relatively expensive and present some limitations related to the concomitants such as high dissolved solid contents of samples. Therefore, it is evident that despite recent advances in analytical instrumentation, the use of separation and/or preconcentration procedures is still necessary before the determination step.

Chemical precipitation and complexation and extraction with organic solvents are the available techniques for metal

* Corresponding author. Tel.: +90 312 2122900; fax: +90 312 2122279.
E-mail address: aturker@gazi.edu.tr (A.R. Türker).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.08.021

separation/preconcentration, but they become insufficient when they applied to metals present at trace concentrations. Adsorption is one of the few alternative methods available for such situation. For this purpose, some inorganic, organic and biological adsorbents have been proposed to accumulate and preconcentrate metal ions. Biological materials such as bacteria [3], algae [4] and yeast [5] are able to accumulate metals from aqueous solutions. This accumulation by biological substance is known under the general term biosorption [6]. Biosorption takes place on cellular membrane by two different processes: (1) with biological activity (2) without biological activity. The former occurs when live cells are used. The metallic species are firstly adsorbed on a cellular membrane and after passing through the membrane they are absorbed into this structure [7]. This process may only take place in the restricted range of conditions (pH, temperature, etc.) that will allow the cells to maintain their life functions. In the latter process, on the other hand, there is no biological activity and the main process may be considered as adsorptive that mainly takes place in dead microorganisms and occur in broader range of environmental conditions [8]. The efficiency of biosorption is usually higher in the latter process and it seems to occur via an ion-exchange process, in which metal ions compete with hydrogen ions for negatively charged binding sites on the cell wall [9]. There are evidences that some phenomena, such as adsorption, ion exchange, chelating, precipitation and crystallization take place in cellular membrane of microorganisms [10]. Biomoleculles (proteins, polysaccharides and cellulose), which contain sulfates, carboxylates and phosphates, etc. in their structure are responsible for binding metal ions. Previous experiments have indicated that carboxyl groups play an important role in metal binding by the biomass [11]. However, the actual mechanisms involved in the adsorption process are not fully understood.

Many researchers have found that nonliving biomaterials can be used to accumulate metal ions from environment [12–14]. In recent studies, mainly two processes are used in biosorption experiments: (1) the use of free cells; and (2) the use of immobilized cells on a solid support. The first process is difficult the use of on-line preconcentration system. In addition, use of free microorganisms cause relatively low precise results. Consequently, it is favorable to immobilize the microbial cells on appropriate supports. A variety of inert supports such as silica gel, sepiolite, glass beads and Amberlite XAD resins have been used to immobilize biomaterials either by adsorption or physical entrapment [15–18].

Recently, preconcentration and speciation methods by biological organisms and synthetic resins, like Chelex-100 for trace metals have been widely used [19,20]. Bağ et al. [15–17] reported a pretreatment by yeasts, bacteria and fungus immobilized on sepiolite for the preconcentration and determination of Ni, Cu, Zn, Fe and Cd in various samples by FAAS. Ohta et al. [21] presented a new preconcentration method with with yeast for copper. Zylkiewichz [22] used beaker yeast, *Saccharomyces cerevisia* and green algae, *Chtorella vuaris* either free or immobilized on silica gel to accumulate platinum and palladium from water samples in acidic medium. Carrilho et al. [23] used the brown alga, *Pilayell litorolis* as a new biosorbent in an on-line preconcentration of Al, Co, Cu and Fe in lake water.

In this paper, biosorption of Fe, Co, Mn and Cr by *Agrobacterium tumefacients* has been investigated. *A. tumefacients* was selected because it was not used before for this purpose and it is not a hazardous bacterium for human. *A. tumefacients* is a Gram-negative, non-sporing, motile, rod-shaped bacterium, closely related to *Rhizobium*, which forms nitrogen-fixing nodules on clover and other leguminous plants and isolated from soil and the stems and roots of plants. It causes oncogenic transformations (tumour formation) in a wide variety of higher plants after wounding and crown gall in plants and is commonly used as a transgene vector in gene transformation. *A. tumefacients* causes crown gall disease by transferring some of its DNA to the plant host [24,25].

The present work proposes the use of the *A. tumefacients* immobilized on Amberlite XAD-4 as a new biosorbent in trace metal determination. Amberlite XAD-4 resin, which was previously used alone as an adsorbent, was used as a support material for the immobilization of *A. tumefacients*. Commercial availability of pure resin, uniform pore distribution, high surface area, durability and chemically homogenous structure are the main advantageous of Amberlite-XAD-4 over other supports such as pore glass and sepiolite. The resulting biomass was examined for the enrichment of Fe(III), Co(II), Mn(II) and Cr(III) in a column procedure. Flame atomic absorption spectrometry was used as a detection technique. The proposed method was applied to the determination of these elements in water, food and alloy samples.

2. Experimental

2.1. Apparatus

A Philips PU 9285 Model flame atomic absorption spectrometer equipped with deuterium lamp background correction and an air–acetylene burner was used under the following conditions: wavelength, 248.3, 240.7, 279.5 and 357.9 nm; spectral bandwidth, 1.0, 1.0, 1.0 and 1.0 nm; lamp current, 11.2, 11.0, 9.0 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 biomass were performed using a glass column of diameter 1 cm and height 20 cm.

2.2. Reagents

Doubly distilled water and analytical reagent-grade chemicals were used unless otherwise specified. Mn(II),

Co(II), Fe(III) and Cr(III) stock solutions (1000 μg ml^{-1}) were prepared by dissolving appropriate amounts of $MnSO_4 \cdot H_2O$ (Merck), $Co(NO_3)_2 \cdot 6H_2O$ (Merck), iron powder and $Cr(NO_3)_2 \cdot 9H_2O$ (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 m^2 g^{-1}) was used as a support material for the immobilization of *A. tumefacients*.

2.3. Preparation of bacterial biomass

A solid medium (nutrient agar) was prepared by mixing 10 g of meat extract, 10 g of peptone, 5 g of sodium chloride and 150 g of agar. This nutrient agar (5 g) was dissolved with water and diluted to 200 ml. The mixture was sterilized in the previously sterilized Petri dish at 120 ± 1 °C and leaved to become solid. The bacterium, *A. tumefacients*, was inoculated on the solid medium and stored at 28 ± 2 °C in order to growth the bacterium. Liquid medium was prepared by mixing the substances mentioned above except agar and sterilized at 120 ± 1 °C for about 30 min. Firstly, in order to prepare the starter culture, *A. tumefacients* grown on the solid medium was inoculated to 100 ml of liquid medium. Then, it is incubated for 48 h at 28 ± 2 °C on a shaker (about 200 rpm). For preparing the experimental culture, 200 ml of liquid medium was prepared and inoculated with 10 ml of the starter culture and incubated on a shaker for 48 h at 28 ± 2 °C. Then, the bacteria grown in the experimental culture was separated from the media using centrifugation (5000 × *g* for 5 min) to isolate the biomass. In order to obtain the dead and dry bacteria, 10 ml of 0.1 mol l^{-1} HCl was added to the isolated biomass. After 10 min, the mixture was centrifuged and the acid solution was discarded. This procedure was repeated three times and then followed by rinsing the acid-washed biomass in distilled water. These rinsed bacteria were again centrifuged and the resulting biomass was lyophilized to yield a dry bacterial powder.

2.4. Immobilization of bacteria onto Amberlite XAD-4

Commercially available Amberlite XAD-4 was prepared as a substrate by washing successively with methanol, water, 1 mol l^{-1} HCl and water, respectively, to remove organic and inorganic contaminants. Then, the immobilization of *A. tumefacients* 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 105 °C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *A. tumefacients* and Amberlite XAD-4, thereby improving the immobilization efficiency. Then, the product obtained was ground to get original size (20–40 mesh) and used as an adsorbent.

2.5. Preparation of the column

First, 0.2–0.3 g of Amberlite XAD-4 loaded with *A. tumefacients* was packed in a glass column. 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 NH_3 having the same pH as that of sample solution through the column, prior to passage of the sample solution.

2.6. Preparations of the samples

Aluminum alloy (NBS SRM 85b) was dissolved as follow: 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 (∼80 °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. 0.1 ml of this sample solution was diluted again to 100 ml to reduce analyte concentration at the working range of the flame atomic absorption spectrometer. The matrix concentration was also reduced by this dilution procedure.

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 °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.

Whey powder and infant food were dissolved as follows: 0.1 g of sample was weighted exactly into a 250 ml beaker. Firstly, the sample was wetted with 5 ml of 0.5 mol l^{-1} HNO_3 and then 5 ml of concentrated HNO_3 was added to the beaker. The beaker was heated on a hot plate at about 80 °C for 2 h. The beaker and the contents were cooled to room temperature and the inner surface of the beaker was washed with 10 ml of 0.5 mol l^{-1} HNO_3. Then, 5 ml of concentrated $HClO_4$ (about 72%, m/m) was added to the mixture and heated on a hot plate until complete dissolution. The resulting solution was diluted to 100 ml with water.

Water samples collected from Atatürk Dam were acidified with 1.0 ml of concentrated hydrochloric acid per liter of sample and then filtered through a cellulose membrane filter of 0.45 μm pore size to remove the particulate matters.

2.7. General enrichment procedure

An off-line column 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 flow rates determined experimentally. The retained metal ions were then eluted from the solid phase with

a suitable eluent, determined experimentally. The concentration of the metal ions in the eluate was determined by flame atomic absorption spectrometry. *A. tumefacients* immobilized on Amberlite XAD-4 was used repeatedly (up to 10) after washing with a 1 mol l^{-1} HCl solution and distilled water, respectively.

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

3. Results and discussion

3.1. Effect of the pH

The pH of the sample solution plays a important role in microbial biosorption. Thus, the effect of the pH on the ability of the column containing *A. tumefacients* immobilized on Amberlite XAD-4 to preconcentrate the metal ions was studied. For that purpose, a set of solutions (100 ml) each containing one of the four metal ions at a concentration given in the Section 2.7, 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 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) and Co(II) and about 6 for Fe(III) and Cr(III). From these results, it could be concluded that the reason of retention of metal ions on the biomass may be attributed to ionic attraction between the metal ions and functional groups of the biomass. At low pH values, there is a competition between H^+ ions and metal ions. The cell surface becomes more positively charged at low pH values which decrease the attraction between metal ions and the functional groups on cell wall. At high pH values, the cell surface becomes more negatively charged, increasing the attraction until a maximum is reached at around pH 7. For pH values higher than the optimum values, the retention decreases again because of the competition between the formation of hydroxylated complexes of the metal and active sites of the cell [8].

Table 1
Effect of the type and volume of elution solutions on the recovery 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	82
		10	1	98
	HNO_3	5	1	70
		10	1	84
Co(II)	HCl	5	1	86
		10	1	98
	HNO_3	5	1	75
		10	1	82
Mn(II)	HCl	5	1	85
		10	1	97
	HNO_3	5	1	67
		10	1	75
Cr(III)	HCl	10	1	54
	HNO_3	10	1	40
	HCl (in acetone)	10	1	75
	HNO_3 (in acetone)	10	1	70
	HCl (in acetone)	10	2	98

[a] Mean of three determinations.

3.2. Effect of the amount of adsorbent (bed height)

The retention of the analytes was examined in relation to the amount of adsorbent, which was varied from 100 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, 300 and 300 mg of the adsorbent, respectively. Therefore, 200 mg of the adsorbent was used for Fe(III) and Co(II) and 300 mg of the adsorbent was used for Mn(II) and Cr(III) in subsequent experiments.

3.3. Effect of the type and volume of elution solutions

The other important factor that affects the preconcentration procedure is the type, volume and concentration of the eluent used for the remove of metal ions from the biomass. 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 2 mol l^{-1} HCl (in acetone) for Cr(III).

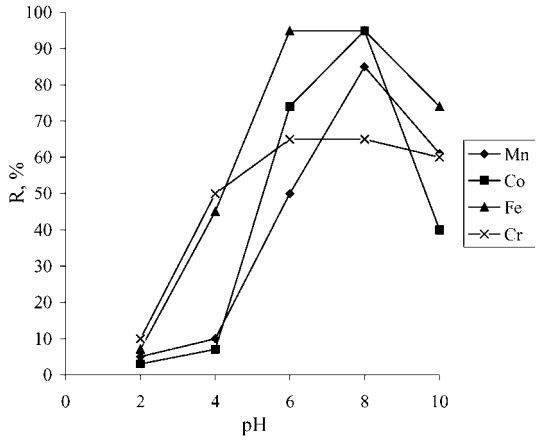

Fig. 1. The effect of pH on the recovery of Fe(III), Co(II), Mn(II) and Cr(III) [Fe(III), 0.5 μg ml^{-1}; Co(II) and Mn(II), 0.3 μg ml^{-1}; Cr(III), 0.2 μg ml^{-1}; sample volume, 100 ml].

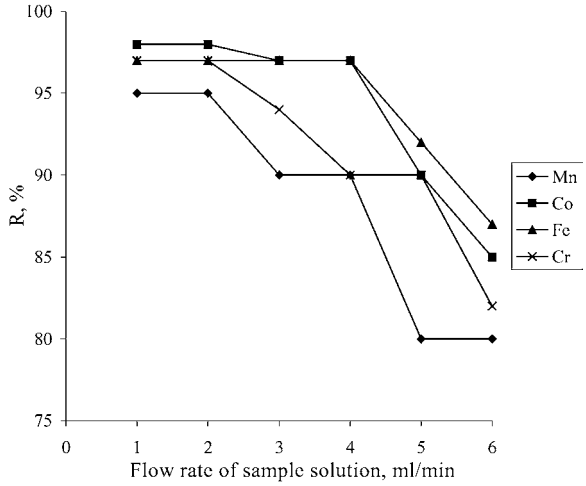

Fig. 2. Effect of the flow rate of the sample solutions on the recovery [Fe(III), 0.5 μg ml^{-1}; Co(II) and Mn(II), 0.3 μg ml^{-1}; Cr(III), 0.2 μg ml^{-1}; sample volume, 100 ml].

3.4. 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. Because, the mass transfer from the solution to the binding sites on the cell wall of microorganism is affected by the flow rate of sample solution. Therefore, 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 1–6 ml min^{-1} by gravity. As can be seen in Fig. 2, at flow rates greater than 2 ml min^{-1}, there was a decrease in the recovery of Cr(III) and Mn(II) and 4 ml min^{-1} for Fe(III) and Co(II). The reason for this decrease is probably insufficient contact of the metal ions and the adsorbent to reach equilibrium. Therefore, a flow rate of 2 ml min^{-1} was applied for Cr(III) and Mn(II) and 4 ml min^{-1} was applied for Fe(III) and Co(II) in subsequent experiments. The flow rate of the elution solution was 1 ml min^{-1}.

3.5. Effect of the volume of the sample solution

Real samples such as water, biological, etc. contain metal ions in very low concentrations. Thus, it is important to know the applicable volume of sample solution to be able to determine these trace concentrations. In order to determine the maximum 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 μg of Fe(III), 30 μg of Co(II), 30 μg of Mn(II) and 20 μg of Cr(III)) which corresponds 0.5, 0.2, 0.1, 0.066, 0.05 μg ml^{-1} Fe(III), 0.30, 0.12, 0.06, 0.04 and 0.03 μg ml^{-1} Mn(II) and Co(II) and 0.2, 0.08, 0.04, 0.027 and 0.02 μg ml^{-1}Cr(III)] were passed through the column

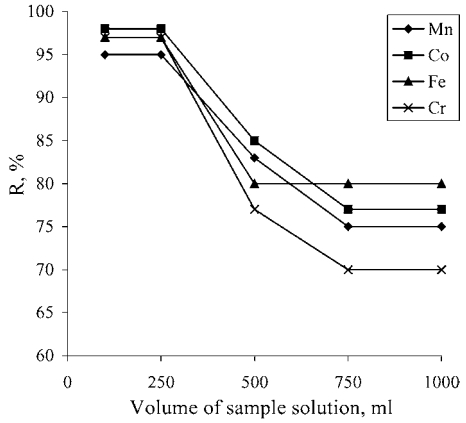

Fig. 3. Effect of the volume of the sample solution on the recovery [Fe(III), 0.5 μg ml^{-1}; Co(II) and Mn(II), 0.3 μg ml^{-1}; Cr(III), 0.2 μg ml^{-1}; pH 6 for Fe(III) and Cr(III), and 8 for Co(II) and Mn(II)].

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, 0.12, 0.12 and 0.08 μg ml^{-1}, respectively. These concentrations can not be determined directly by flame atomic absorption spectrometry with sufficient accuracy.

3.6. Effect of interfering ions

In order to investigate the effect of the interfering ions, especially alkaline and alkaline earth elements and 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 Fe(III), Co(II), Mn(II) and Cr(II) were fixed at 0.5, 0.3, 0.3 and 0.2 μg ml^{-1}, respectively, and the concentration of interfering metal ions were adjusted over the range of 0.5–1000 μg ml^{-1}. Other ions present in the solution have generally a negative impact on the metal retention because of the competition between analyte and diverse ions. The results were given in Table 2. As can be seen in the table, Na^+, K^+ and Al^{3+} did not interfere in the determination of analytes up to 500 μg ml^{-1}. However, Ca(II) and Mg(II) interfered in the determination when they existed in the range of 10–20 and 2.5–5.0 μg ml^{-1}, respectively.

3.7. Effect of 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 one day later, and

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

Interfering ion	Concentration ($\mu g\,ml^{-1}$)	Recovery (%)			
		Fe(III)	Co(II)	Mn(II)	Cr(III)
Na^+	–	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
	50	99	98	98	97
	100	98	98	98	96
	250	–	98	95	96
	500	95	–	85	95
Ca^{2+}	–	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
Al^{3+}	–	99	98	98	98
	250	98	98	97	97
	500	97	96	95	97
Fe^{3+}	–	–	99	98	98
	15	–	96	97	97
	30	–	95	95	96
Co^{2+}	–	99	–	98	98
	2	99	–	98	98
	4	98	–	97	97
	8	97	–	95	95
Mn^{2+}	–	98	99	–	98
	2	98	99	–	98
	4	97	98	–	96
	8	95	96	–	95
Cr^{3+}	–	99	99	98	–
	2	99	99	98	–
	4	97	98	97	–
	8	96	97	96	–

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

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 10 runs, the recoveries of all of the analytes slightly decreased to below 95%.

3.8. Loading capacity

The loading capacity of *A. tumefacients* immobilized on Amberlite XAD-4 was evaluated from the beakthrough curve plot by a method given by Bağ et al [26]. The capacities were found as to be 22 and 29 $\mu mol\,g^{-1}$ for Mn(II) and Co(II) when using *A. tumefacients* and 15 and 20 $\mu mol\,g^{-1}$ without using *A. tumefacients*, respectively. The capacity of *A. tumefacients* immobilized on Amberlite XAD-4 was higher than that Amberlite XAD-4 alone and that *Escherichia coli* immobilized on Amberlite XAD-4 [18] for both of the ions studied. The capacity of the adsorbent for Fe(III) and Cr(III) was not studied, because precipitation of Fe(III) and Cr(III) occurred at the studied pH, at higher concentration levels.

Table 3
Recovery of the analytes and repeatability of the method

Element	% $R \pm t^a s/\sqrt{N}$	R.S.D.[b] (%)
Fe(III)	99 ± 3	3
Co(II)	99 ± 2	2
Mn(II)	98 ± 3	3
Cr III)	98 ± 3	3

[a] Uncertainity at 95% confidence limit (N = 5).
[b] R.S.D.: relative standard deviation of the recovery.

3.9. Analytical features

The analytical features of the proposed method such as precision, linear range of calibration curve, limit of detection were also examined. Precision of the method were estimated by applying successive retention and elution cycles with 100 ml of a sample solution containing 50 μg of Fe(III), 30 μg of Mn(II) and Co(II) and 20 μg 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 (R.S.D. <3%) for *A. tumefacients* immobilized on Amberlite XAD-4. The recoveries and the precision found by using Amberlite XAD-4 alone were very low (below 70%) when using Amberlite XAD-4 alone as an adsorbent [18].

The linear calibration ranges for measurements under the optimum conditions were 1.0–8.0 $\mu g\,ml^{-1}$ for Fe(III), 0.5–5.0 $\mu g\,ml^{-1}$ for Co(II), Mn(II) and Cr(III).

The detection limits based on three-times the standard deviation of the blank solution were found to be 90, 75, 70 and 90 $ng\,ml^{-1}$ for Fe(III), Co(II), Mn(II) and Cr(III) (N = 20), respectively. It may be concluded that 3.6, 3.0, 2.8 and 3.6 $ng\,ml^{-1}$ detection limit for Fe(III), Co(II), Mn(II) and Cr(III) respectively, could be obtained by applying a preconcentration factor of 25 [27].

3.10. The validation of the method

In order to demonstrate the validity and accuracy of the proposed enrichment and determination method, the analytes were determined in standard reference materials of aluminum alloy (NBS SRM 85b) and whey powder (IAEA-155), and spiked aluminum foil, infant food and water samples. All the results found were in the range of 95% confidence levels except for aluminum foil. The results are summarized in Tables 4–8. As can be seen from the tables, the accuracy of the method was very satisfactory; the percent relative error

Table 4
Determination of Fe(III), Co(II), Mn(II) and Cr(III) in reference standard aluminum based alloy (NBS SRM 85b)

Sample	Element	Certified[a], % (m/m)	Found[b], % (m/m), $\bar{x} \pm ts/\sqrt{N}$	Error (%)
NBS SRM 85b	Fe	0.24	0.23 ± 0.02	−4
	Mn	0.61	0.59 ± 0.03	−3
	Cr	0.211	0.20 ± 0.02	−6
	Co[c]	0.50	0.49 ± 0.02	−2

[a] The composition of the aluminum based alloy (NBS SRM 85b): 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).
[b] Mean of five determinations at 95% confidence level.
[c] Co was added to the solutions of NBS SRM 85b.

Table 5
Determination of Fe(III), Co(II), Mn(II) and Cr(III) in reference standard whey powder (IAEA-155)

Sample	Element	Certified[a] (μg/g)	Found[b] (μg/g), $\bar{x} \pm ts/\sqrt{N}$	Error (%)
IAEA-155	Fe	62 ± 12	58 ± 3	−6
	Mn	9.3 ± 0.52	8.8 ± 0.03	−5
	Cr	0.59 ± 0.07	0.55 ± 0.05	−7
	Co	0.0427 ± 0.0134	ND[c]	–

[a] The composition of the whey powder (IAEA-155): Mg, 3.19 ± 0.12 mg/g; Mn, 9.3 ± 0.52 μg/g; Na, 15.82 ± 0.6 μg/g; P, 16.21 ± 0.79 mg/g; Br, 39.31 ± 2.9 μg/g; Cd, 16 ± 3.6 ng/g; Cl, 69.2 ± 3.2 mg/g; Co, 42.7 ± 13.4 ng/g; Cr, 0.59 ± 0.07 μg/g; Cs, 86 ± 16 ng/g; Hg, 2.6 ± 1.2 ng/g; Ni, 0.54 ± 0.1 μg/g; Pb, 104 ± 32 ng/g; Rb, 39.2 ± 2.8 μg/g; Sc, 28 ± 6 ng/g; Se, 64 ± 13 ng/g; Zn, 34.3 ± 1.4 μg/g; Fe, 62 ± 12 μg/g.
[b] Mean of five determinations at 95% confidence level.
[c] ND: not detected.

Table 6
Determination of Fe (III), Co(II), Mn(II) and Cr(III) in Atatürk Dam water (sample volume 250 ml)

Element	Added (μg/l)	Found[a] (μg/l), $\bar{x} \pm ts/\sqrt{N}$	Error (%)
Fe(III)	–	38 ± 2	–
	100	133 ± 2	−4
Co(II)	–	24 ± 1	–
	40	63 ± 2	−2
Mn(II)	–	15 ± 1	–
	40	52 ± 2	−5
Cr(III)	–	58 ± 2	–
	50	105 ± 3	−3

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

Table 7
Determination of Fe(III), Co(II), Mn(II) and Cr(III) in infant food

Element	Added (μg/g)	Found[a] (μg/g), $\bar{x} \pm ts/\sqrt{N}$	Error (%)
Fe(III)	–	73 ± 3	–
	50	118 ± 5	−4
Co(II)	–	ND[b]	–
	0.5	0.46 ± 0.02	−8
Mn(II)	–	0.30 ± 0.05	–
	0.5	0.75 ± 0.08	−6
Cr(III)	–	ND[b]	–
	0.5	0.45 ± 0.04	−10

[a] Mean of five determinations at 95% confidence level.
[b] ND: not detected.

Table 8
Determination of Fe(III), Co(II), Mn(II) and Cr(III) in aluminum foil

Element	Added (μg/g)	Found (μg/g), ($\bar{x} \pm s$[a])	Error (%)
Fe(III)	–	4900 ± 230	–
	5000	9600 ± 425	−3
Co(II)	–	–	–
	50	48 ± 2	−4
Mn(II)	–	60 ± 2	–
	50	105 ± 3	−5
Cr(III)	–	ND[b]	–
	50	47 ± 2	−6

[a] Standard deviation (N = 5).
[b] ND: not detected.

was lower than 10%. The results obtained for Atatürk Dam water and aluminum foil were also in agreement with our previous results [18].

3.11. Application

In order to check the applicability of the proposed method, aluminum foil, infant food 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, in infant food bought from the market and in aluminum foil used for food protection. An appropriate volume of sample solutions was adjusted to the optimum pH and subjected to the recommended column procedure for the preconcentration and determination of metal ions.

The results reported in Tables 6–8, with a confidence interval for the 95% confidence level, show the applicability of the proposed method to water analysis, alloy analysis and infant food analysis. The analytes were determined with a relative error lower than 10% in all samples.

4. Conclusion

The results presented in this paper demonstrate the usability of a new biosorbent, *A. tumefacients*, for the preconcentration of trace metal ions. The ability of *A. tumefacients* for selective biosorption of trace metals from aqueous solution was confirmed. The proposed procedure provides a simple, sensitive, precise, reliable and accurate technique for the preconcentration and determination of Fe(III), Co(II), Mn(II) and Cr(III). The recoveries of analytes studied were nearly quantitative (>95%). The analytes could be preconcentrated directly by using the proposed method without using any chelating or complexing agent which usually used to increase retention. The main disadvantage of the proposed method is the long duration time of the preconcentration step. The capacity of *A. tumefacients* immobilized on Amberlite XAD-4 was higher than that Amberlite XAD-4 alone and that *E. coli* immobilized on Amberlite XAD-4 [18] for both of the ions studied.

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

This work was supported financially by Harran University Scientific Research Fund.

References

[1] A.M. Nagmush, K. Pyrzynska, M. Trojanowicz, Talanta (1995) 42.
[2] H. Matusiewichz, R. Sturgeon, V. Luong, K. Moffatt, Fresenius J. Anal. Chem. 340 (1991) 35.
[3] L.C. Robles, A.J. Aller, Quim. Anal. 15 (1996) 21.
[4] L. Shunxin, Q. Shahua, H. Ganquan, H. Fei, Fresenius J. Anal. Chem. 365 (1999) 469.
[5] P. Smichowski, J. Marrero, A. Ladesma, G. Polla, D.A. Batisoni, J. Anal. At. Specrom. 15 (2000) 1493.
[6] P. Solari, A.I. Zouboulin, K.A. Matis, G.A. Stalidis, Sep. Sci. Technol. 31 (8) (1996) 1075–1092.
[7] E. Becerio Gonzales, A.T. Calzade, E. Alonso-Rodrigez, Trends in Anal. Chem. 10 (2000) 475.
[8] R. Pardo, M. Herguedas, E. Barrado, M. Vega, Anal. Bioanal. Chem. 376 (2003) 26.
[9] J. Dougney, J.B. Fein, N. Yee, Chem. Geol. 144 (1998) 161.
[10] M. Bhanoori, G. Venkateswerlu, Biochim. Biophys. Acta 1519 (2000) 22.
[11] K.J. Tiemann, J.L. Garden-Torresday, G. Gamez, K. Dokken, S. Sias, M.W. Renner, L.R. Furenlid, Environ. Sci. Technol. 33 (1999) 150.
[12] C. Nagendra, R. Rao, L. Iyengar, C. Venkobachar, J. Environ. Eng. 119 (1993) 260.
[13] J.R. Lujan, D.W. Darnall, P.C. Stark, G.D. Rayson, J.L. Gardea-Torresdey, Solvent Extr. Ion Exch. 12 (1994) 803.
[14] T. Viraraghaven, R. Saskatchewan, M.M. Dronamraju, J. Environ. Sci. Technol. 33 (1993) 150.
[15] H. Bağ, A.R. Türker, M. Lale, Talanta 51 (2000) 1035.
[16] H. Bağ, A.R. Türker, M. Lale, Anal. Sci. 15 (1999) 1251.
[17] H. Bağ, M. Lale, A.R. Türker, Fresenius J. Anal. Chem. 363 (1999) 224.
[18] A.R. Türker, S. Baytak, Anal. Sci. 20 (2004) 329.
[19] T.S. Lin, J.O. Nriagu, Anal. Chim. Acta 395 (1999) 301.
[20] S. Yalçın, R. Apak, Anal. Chim. Acta 505 (2004) 25.
[21] K. Ohta, H. Tanahasi, T. Suzuki, S. Kaneco, Talanta 53 (2001) 715.
[22] B. Godlewska-Zylkiewichz, Spectrochim. Acta 58B (2003) 1531.
[23] E.N.V.M. Carrilho, J.A. Nobrega, T.R. Gilbert, Talanta 60 (2003) 1131.
[24] T.D. Brock, M.T. Madigan, Biology of Microorganisms, fifth ed., Prentice Hall, New Jersey, 1988, pp. 645–648.
[25] S. Özcan, E. Gürel, M. Babaoğlu, Bitki Biyoteknolojisi, Genetik Mühendisliği ve Uygulamaları, S.Ü. Vakfı Yayınları, 2001, pp. 112–159.
[26] H. Bağ, M. Lale, A.R. Türker, Talanta 47 (1998) 689.
[27] A.C. Sahayam, Anal. Bioanal. Chem. 372 (2002) 840.