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Speciation of Cr(III) and Cr(VI) in waters using immobilized moss and determination by ICP-MS and FAAS

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Abstract

The possibility of using moss (*Funaria hygrometrica*), immobilized in a polysilicate matrix as substrate for speciation of Cr(III) and Cr(VI) in various water samples has been investigated. Experiments were performed to optimize conditions such as pH, amount of sorbent and flow rate, to achieve the quantitative separation of Cr(III) and Cr(VI). During all the steps of the separation process, Cr(III) was selectively sorbed on the column of immobilized moss in the pH range of 4–8 while, Cr(VI) was found to remain in solution. The retained Cr(III) was subsequently eluted with 10 ml of 2 mol l^{-1} HNO_3. A pre-concentration factor of about 20 was achieved for Cr(III) when, 200 ml of water was passed. The immobilized moss was packed in a home made mini-column and incorporated in flow injection system for obtaining calibration plots for both Cr(III) and Cr(VI) at low ppb levels that were compared with the plots obtained without column. After separation, the chromium (Cr) species were determined by inductively coupled plasma mass spectrometry (ICP-MS) and flame atomic absorption spectrometry (FAAS). The sorption capacity of the immobilized moss was found to be ~11.5 mg g^{-1} for Cr(III). The effect of various interfering ions has also been studied. The proposed method was applied successfully for the determination of Cr(III) and Cr(VI) in spiked and real wastewater samples and recoveries were found to be >95%.
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Keywords: Moss; Immobilization; Chromium; Speciation; ICP-MS; FAAS

1. Introduction

Toxicological studies have indicated that the degree of toxicity of metals including chromium (Cr), depends on the chemical form in which the element is present [1]. Chromium(III) is considered as an essential micronutrient for humans playing a role in the maintenance of normal glucose, cholesterol and fatty acid metabolism [2], whereas Cr(VI) is highly toxic than Cr(III). Its acute toxic effects include immediate cardiovascular shock, with later effects on kidney, liver and blood-forming organs [3,4]. The toxic nature of the Cr(VI) ions is attributed to their high oxidation potential and their relatively small size, which enables them to penetrate through biological cell membranes [5]. Moreover, in air, chromium particulates play a role in the oxidation of sulphur dioxide (SO_2) and formation of acidic aerosols involved in global acid rain [6]. Owing to the different toxicities of Cr(III) and Cr(VI), it is important to determine them separately, in addition to the total chromium content [7]. Such difference in toxicity is one of the main reasons for the enormous recent development of analytical methods for differentiating the various forms of Cr existing in the medium of interest. Water is probably the most studied environmental sample and in fact, the major part of speciation studies has been carried out in waters. Also information about the oxidation state of Cr is also very important for many industrial processes and waste purification methods.

The importance of Cr speciation originates from the extensive use of this metal in various industries such as metallurgical (steel, ferro- and non-ferrous alloys), refractories (chrome and chrome-magnesite) and chemical (pigments, electroplating, tanning and other); this has resulted in the release of aqueous Cr to the sub-surface at numerous sites [8]. Also Cr compounds have been used as corrosion inhibitors in water pipes that constitute a potential source of

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Cr(VI) in the drinking water distribution system [9]. The total chromium concentration in unpolluted natural waters is 1–10 $\mu g\,l^{-1}$ [10]. Reviews on chromium speciation methods [11,12], indicate that the conversion of chromium species may occur during storage and transport of natural waters. Therefore, the speciation study of Cr is very important in environmental, clinical and biological research and also in the control of wastewaters, natural water and drinking waters. Hence, total chromium measurements alone cannot be used to determine actual environmental impact. The sources of Cr(III) and Cr(VI) must therefore be monitored and this requires speciation techniques with sufficient selectivity and high sensitivity.

The determination of Cr at trace level usually requires previous separation and/or pre-concentration stages, in-spite-of, the increasingly sensitive analytical instrumentation. Various methods such as selective volatilization [13], precipitation, liquid–liquid extraction [14], ion-exchange [15], adsorption [16,17] and various liquid chromatographic separations [18] have been used widely for the pre-concentration and separation of chromium. But most of the methods for metal containing wastewaters are relatively complex for routine analysis and also reported to exhibit reduced efficiency at low concentrations [19]. Many methods also produce a sludge that results in further waste disposal problem [20].

It is therefore essential to investigate other alternatives which are reliable and effective sorbents for the separation of Cr(III) and Cr(VI), followed by their determination at trace levels. In recent years, biological substrates have been used for the economic removal, pre-concentration of suspended solids, dissolved nutrients, pathogens and metals from wastewaters [21] and to a lesser extent speciation purposes. Microorganisms such as yeast, bacteria, fungi and materials of plant origin have often been proposed for the pre-concentration and speciation of trace metals since, they are capable of accumulating metals present in liquid media. Bag et al. used *Saccharomyces cerevisiae* immobilized on sepolite for separation and speciation of Cr(III) and Cr(VI) [22]. Elmahadi and Greenway [23] used two types of algae for the pre-concentration of Cr(III) and Cr(VI) along with Cu(II) and Ag(I). Neidhart et al. [24] used human red erythrocytes under physiological conditions for the selective determination of chromate in the presence of Cr(III). The utilization of plant materials as biosorbents for Cr(III) adsorption has been well-documented [25]. The removal of trivalent and hexavalent chromium by a seaweed biosorbent was reported by Kratochvil et al. [26]. Milled peat was also used as biosorbent for Cr(III) and Cr(VI) from aqueous solutions [27]. An attempt was made with a plant biomass (*Garcinia cambogia*) for chromium removal and its speciation studies [28].

Among the plants, various species of mosses, which belong to the Bryophyte division, have been found to be suitable bio-monitors because, they obtain most of their nutrient supply directly from atmospheric deposition and have a great capacity to retain many elements [29]. Several works are available in literature in which moss was used as bio-monitor with particular concern to metals [30,31]. Other researchers also proposed the use of moss as a sorbent material for the removal of heavy metals from wastewaters [32,33]. But so far, moss has not been used for speciation purposes.

With all these considerations, we have evaluated the use of moss (after immobilization) ,as a sorbent material, for the first time, for it's potential applications for separation of Cr(III) from Cr(VI) and in the selective pre-concentration of Cr(III), prior to their determination by inductively coupled plasma mass spectrometry (ICP-MS) and flame atomic absorption spectrometry (FAAS). The optimum conditions such as pH, bed height and flow rate for speciation of chromium were evaluated. The breakthrough capacity of the sorbent for Cr(III) was also studied. Experiments were carried out using mini-column to investigate the efficacy of the immobilized moss for the speciation of chromium at low ppb levels.

2. Experimental

2.1. Instrumentation

The trace chromium determinations were made with VG Plasma Quad 3, ICP-QMS (VG Elemental, Winsford, Cheshire, UK) system. The data were collected by monitoring *m/z 52* and *m/z 53* using the peak jump mode. For the experiments with flow injection system, data were collected in time resolved mode. The optimized conditions are given in Table 1(a).

A GBC 932AA (Australia), FAAS with deuterium lamp background correction and air-acetylene burner was used to carry out absorption measurements of Cr in the aqueous solutions. Chromium hollow cathode lamp was used for determination of Cr. The operating parameters are listed in Table 1(b).

A double beam UV–vis spectrophotometer (U-3210, Hitachi Ltd., Tokyo, Japan) with 10 mm quartz cells was used for spectrophotmetric measurements of Cr(VI) at 540 nm. pH adjustments were done with dilute solutions of HCl and NaOH. All the pH measurements were made with a digital pH meter and a combined glass electrode.

Table 1a
Instrumental and operating parameters for VG plasma quad PQ3 ICP-MS

Instrumental parameters	Scanning parameters
Coolant gas: 13.4 l min^{-1}	Scanning mode: peak jump
Aux. gas: 0.66 l min^{-1}	Number of replicates: 3
Nebulizer gas: 0.85 l min^{-1}	Dwell time: 300 $\mu s\,channel^{-1}$
Sampler cone: 1.0 mm Ni	Sample delay: 30 s
Skimmer cone: 0.7 mm Ni	Stabilization delay: 20 s
Torch type: Fassel	Rinse time: 30 s
Plasma FW power: 1350 W	Sample pump rate: ~0.4 ml min^{-1}
Reflected power: <10 W	Isotopes used: *m/z* 52, 53

Table 1b
Instrumental and operating parameters employed to determine Cr by FAAS

Wavelength (nm)	357.9
Flame	Air-acetylene
Lamp current (mA)	10
Slit width (mm)	0.2
Air flow ($l\,min^{-1}$)	5
Acetylene flow ($l\,min^{-1}$)	1.2

2.2. Reagents

All the solutions were prepared using analytical reagent grade chemicals and Millipore water. 1,5-Diphenylcarbazide (DPC) solution ($0.01\,mol\,l^{-1}$) was prepared daily by dissolving appropriate amount of DPC (Merck) in acetone and then diluting with water. Stock standard solutions of Cr(III) and Cr(VI) were prepared from $CrCl_3\ 6H_2O$ (LDH, India) and $K_2Cr_2O_7$ (Merck), respectively. Working solutions of Cr(III) and Cr(VI) were prepared daily by appropriate dilutions of stock solutions. A range of solutions at different pH values was prepared by adjusting with HCl or NaOH. The cation solutions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}) used in the study of interferences were prepared by appropriate dilution of stock solutions. Millipore water was used for preparing synthetic sample solutions throughout this work.

2.3. Materials and methods

2.3.1. Sampling of moss

The moss *(Funaria hygrometrica)* was collected from the hill station at Kodaikanal, a southern state of India, because of cool climatic conditions. Kodaikanal is located at a high altitude (about 7000 ft) from sea level and has a good forest cover. Only the top portion of the fresh-green coloured-moss was collected, discarding the basal portion with adhered soil. After, the soil adhered to the material was removed, the samples were stored in a refrigerator until further processing.

2.3.2. Sorbent preparation

The moss sample was further cleaned of any adhering particles in the laboratory by suspending in water and agitating in an ultrasonic bath for about 30 s, followed by rinsing twice with fresh water. After drying the moss samples were kept in an oven for 24 h at 40 °C, they were ground to a fine powder in an agate planetary ball-mill (FRITSCH, Germany), and sieved to get 200–300 mesh particles.

2.4. Immobilization of moss on sodium silicate

This moss consists of very small particles (200–300 mesh) with low density, poor mechanical strength and little rigidity. When the plain moss was packed into a column, it clumps together and the flow rates are reduced significantly. It is suggested that immobilizing biomass in a granular or polymeric matrix improves biomass performance and facilitate faster separation of metal ions from solution [34]. Hence, when performing column experiments, it is preferable to immobilize the moss to avoid reduction in flows due to clumping.

A polysilicate matrix support material was used in this study to immobilize the moss. This combines the physical properties of polymer resin and the binding properties of the moss. The method adopted for immobilization of material within a polysilicate matrix was similar to that reported by Gardea et al. [35].

Seventy-five milliliters of 5% H_2SO_4 was mixed with sufficient sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. Five grams of powdered moss was added to the silica solution and stirred for 15 min. The pH was then raised slowly by the addition of 6% Na_2SiO_3 to reach pH 7.0. The polymer gel was washed with water enough times to remove all sulphates. This was further confirmed by the addition of few drops of barium chloride ($BaCl_2$) to water and there was no formation of white precipitate. The polymer gel with immobilized moss was dried overnight at 45–50 °C and ground by mortar and pestle and sieved to get 50–100 mesh sizes.

2.5. Column preparation

A polyacrylic column (25 cm × 1.0 cm, i.d.) with a Teflon stopcock was used for column studies. Accurately weighed amount of immobilized moss (1 g) was suspended in distilled water and then transferred to the column. Swelling of the matrix after packing in the column was negligible. The column was washed thoroughly with Millipore water and then conditioned to the respective pH before passing Cr containing solutions.

To investigate the efficacy of immobilized moss for the separation of chromium species at low ppb levels, a home made PTFE mini-column with end caps was used. One hundred milligrams of immobilized moss was filled into a PTFE mini-column (30 mm × 3.0 mm, i.d.) plugged with a small portion of glass wool at both ends. A minimum length of PTFE tubing was used for flow injection connections. Before use, Millipore water was passed through the column in order to condition it. Then the column was conditioned to the desired pH.

2.6. General procedure for species separation and determination

The pH of the metal containing solution plays a crucial role in passive biosorption. It has been shown that the affinity of cationic species for functional groups present in the cellular surface is strongly dependent on pH. The retention of Cr(III) and Cr(VI) by the column (25 cm × 1.0 cm, i.d.), as a function of pH was investigated individually, as well as together. For the optimization of column conditions, for the separation of individual species, 20 ml of sample solution spiked with 10 μg of Cr(III), Cr(VI) or mixture of both the species was used. Then the pH of the spiked sample solution was adjusted to desired value (in the range, 1.5–10), and

passed through the column loaded with immobilized moss (1 g). The column was conditioned by thoroughly washing with respective pH solution before passing sample solution. Then the respective sample solution was passed through the column at a flow rate of 2 ml min^{-1}. Chromium(VI) in the effluent was determined by both DPC-spectrophotometric method, whereas the total Cr content in the effluent was determined by ICP-MS and FAAS after reducing Cr(VI) to Cr(III). Then the concentration of Cr(III) was calculated by subtracting the concentration of Cr(VI) from total chromium concentrations.

Different portions of synthetic samples were prepared by spiking known concentration of different amounts of Cr(III), Cr(VI) or a mixture of both and pH of the sample solution was adjusted to desired value. The resulting solution was passed through the column at a flow rate of 2.0 ml min^{-1}. After passing the sample solution, the column was washed with Millipore water and the retained Cr(III) was eluted with 2 mol l^{-1} HNO_3. The total Cr in the eluate was determined by ICP-MS and FAAS.

2.7. Experiments with home made mini-column for the separation of Cr(III) and (VI) at low ppb levels

The analytical performance of mini-column loaded with immobilized moss using Flow Injection, ICP-MS (FI-ICP-MS) systems with 1 ml loop was studied for the separation of Cr(III), Cr(VI) at low ppb levels. Calibration plots of standards of Cr(III) and Cr(VI) separately, as well as, together were obtained by injecting a series of standard solutions (5, 10, 20 ng ml^{-1}; pH 4; flow rate 1 ml min^{-1}), through 1 ml loop with direct flow injection (without column). Similar calibration plots were also obtained by injecting same standard solutions through the same loop via mini-column.

3. Results and discussions

The effects of various parameters such as pH, flow rate, amount of sorbent and sample volume, affecting the speciation of Cr by immobilized moss were investigated.

3.1. Effect of pH on the sorption of Cr(III) and Cr(VI)

The influence of the pH on the retention of Cr(III) and Cr(VI) on the column has been studied by passing the Cr solutions containing one of the species. Fig. 1 shows the sorption behaviour of both Cr(III) and Cr(VI) on the column, as a function of pH. As seen in Fig. 1, the sorption of Cr(III) onto immobilzed moss increases (80–99%) when pH of the sample solution increases from 1.5 to 4 and the quantitative (>98%) sorption of Cr(III) occurred in the pH range of 4–8, whereas the sorption of Cr(VI) was rather low (<5%). This makes it possible to separate Cr(III) and Cr(VI). However, at pH >8, the sorption of Cr(III) was decreased to about 88%,

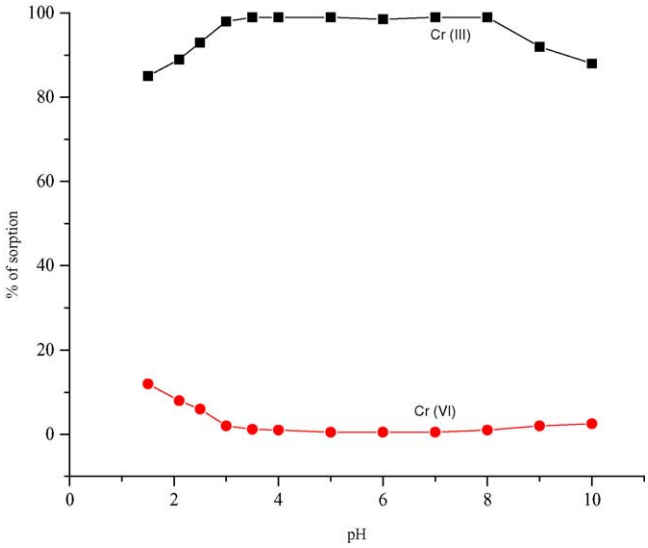

Fig. 1. Effect of pH on the separation of Cr(III) and Cr(VI).

possibly due to decrease in the cationic charge of chromium because of the formation of various hydroxide chromium species such as $Cr(OH)_4^-$ and $Cr(OH)_3$. It was confirmed that, Cr(III) was retained quantitatively on the column in the pH range of 4–8, which is the range of positively charged Cr(III) species formation. Therefore, pH ∼4 was selected for all subsequent experiments.

Perusal of literature on Cr speciation diagram shows that in the pH range of 3–8, the possible Cr species are Cr^{3+}, $Cr(OH)^{2+}$, $Cr(OH)_2^+$ etc. [36] The reason for the maximum retention of Cr(III) is possibly due to exchange of various cationic forms of Cr(III) with H^+ ions of carboxylic acid functional groups present in the moss, whereas, in the pH range of 3–8, Cr(VI) is present mainly in anionic forms of ($HCrO_4^{-1}$) and (CrO_4^{2-}) [11].

Studies with other metal ions by moss have also shown similar sorption behaviour [32,33]. In mosses, one of the main factors influencing the cation-exchange mechanism of various metal ions is the presence of polygalacturonic acids on the external part of the cellular wall and proteins in the plasmatic membrane [31]. Our earlier studies on the removal of Cs and Sr from actual low level radioactive waste solutions with esterified moss also confirmed that the sorption behaviour of both Cs and Sr might be an ion-exchange type mechanism involving carboxylate groups [32]. The present study further confirms that the uptake of metal ions by moss is mainly through cation-exchange mechanism.

3.2. Effect of amount of immobilized moss (bed height)

The retention of Cr(III) was examined in relation to the amount of immobilized moss loaded in the column. For this purpose, the amounts of sorbent were tested in the range of 0.2–1.2 g. About 20 ml of sample solutions spiked with 1 μg ml^{-1} of Cr(III), were passed through the column by keeping pH (∼4) of sample solution and flow rate

Table 2
Effect of volume and concentration of HNO_3 on the recovery of Cr(III) (10 μg of Cr(III) in 20 ml sample)

Volume (ml)	Concentration (mol l^{-1})	Recovery (%)[a]
5	1	71
10	1	78
15	1	86
5	2	82
10	2	97
15	2	98
5	3	90
10	3	98
15	3	98

[a] Mean of three determinations, analysed by both ICP-MS and FAAS.

(2 ml min^{-1}) constant. It was found that the retention of Cr(III) ions increased from 85 to 99%, with increasing the amount of sorbent upto 1.0 g. Above this, there was no significant change in the quantitative retention of Cr(III) and reached plateau at ~99%. Hence, about 1.0 g of immobilized moss was used in all the separation studies. Bed height was about 2.5 cm, when 1.0 g of immobilized moss was loaded in the column.

3.3. Elution of Cr(III)

The pH profile experiments suggested that the Cr(III) ion could be removed by increasing the strength of the acid. But non-destructive recovery is required for regeneration of the column for its re-use. Hence, strength of acid solution used for stripping of sorbed Cr must be as low as possible. Similarly, to obtain a higher pre-concentration factor, volume of the eluent solution must be also as less as possible. For that reason, eluent studies were performed in the range of 0.5–4 mol l^{-1} of HNO_3 to optimize the eluent concentration. The volume of the eluent solution was fixed at 5, 10 and 15 ml, whereas, flow rate was maintained at 1 ml min^{-1}. The results are presented in Table 2. As may be seen from Table 2, elution of Cr(III) was quantitative (>95%) with 10 ml of ≥2.0 mol l^{-1} HNO_3, whereas, lower concentrations of HNO_3 (<2.0 mol l^{-1}) gave only about 70–85%. To ensure quantitative elution of Cr(III), 10 ml of 2.0 mol l^{-1} HNO_3 was used as eluent in this work.

3.4. Effect of flow rate of loading solution

The flow rate of sample solution is also very important parameter for quantitative separation of Cr(III) from Cr(VI) on the adsorbent and duration of complete analysis. Therefore, the effect of the flow rate of sample solution was examined using general procedure under the optimum conditions such as pH, weight of immobilized moss (1 g). About 20 ml of sample solutions (pH ~4) spiked with mixture of Cr(III) and Cr(VI) of 1 μg ml^{-1} of each, were passed through the column at different flow rates. The flow rates varied in the range of 0.5–3 ml min^{-1}. This study indicates that maximum retention (98%) of Cr(III) occurred upto a flow rate of 2 ml min^{-1} while, >95% of Cr(VI) remained in effluent solution. At higher flow rates (>2 ml min^{-1}), retention of Cr(III) decreased gradually due to decrease in the adsorption kinetics at higher flow rates. Hence, a flow rate of 2 ml min^{-1} was used in all the subsequent experiments.

3.5. Effect of volume/concentration of sample solution

In order to estimate the achievable pre-concentration factor of very dilute analyte solutions, the maximum applicable volume of sample that can be passed through the column loaded with immobilized moss must be determined. To study this effect, a series of solutions with increasing sample volumes 50, 100, 150, 200, 250, 300 ml of sample solutions containing total content of 10 μg of Cr(III) were passed through the column under the optimum conditions such as pH, bed height, flow rate. The general procedure described previously was followed. After following the described procedure, the column was eluted with 10 ml of 2 mol l^{-1} HNO_3. Fig. 2 shows effect of sample volume on Cr(III) recovery, where it is evident that, for a given immobilized moss of 1.0 g, quantitative recovery could be achieved upto 200 ml sample solution. As shown in Fig. 2, above 200 ml of the sample solution, the recovery decreased gradually. As sample volume increases, the concentration of analyte decreases. It is generally observed that the behaviour of dilute solutions at ppb levels may not be identical to the behaviour at higher concentrations. This feature may be responsible for the low recovery, as sample volume increases. Similar observations were noticed by Tunceli and Turker [38], as well as, by Liang et al. [39]. The recovery was evaluated by comparing the signal with that of signal obtained with sample solution containing 10 μg of Cr(III) initially. A pre-concentration factor of 20, calculated as the ratio of initial to final volume was achieved.

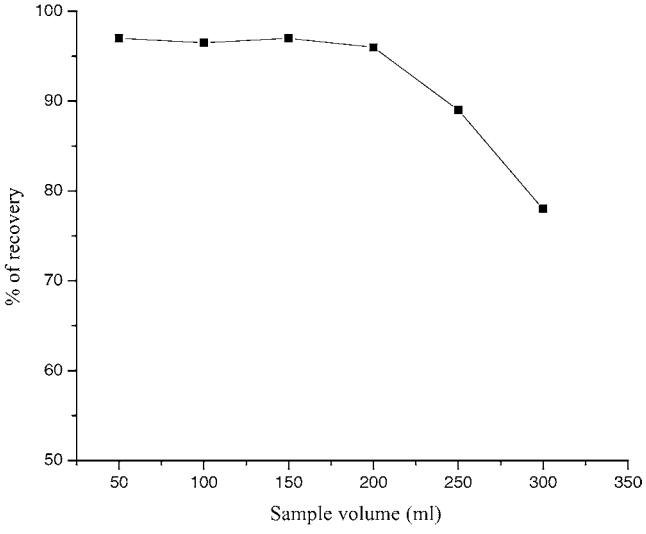

Fig. 2. Effect of sample volume on the recovery of Cr(III).

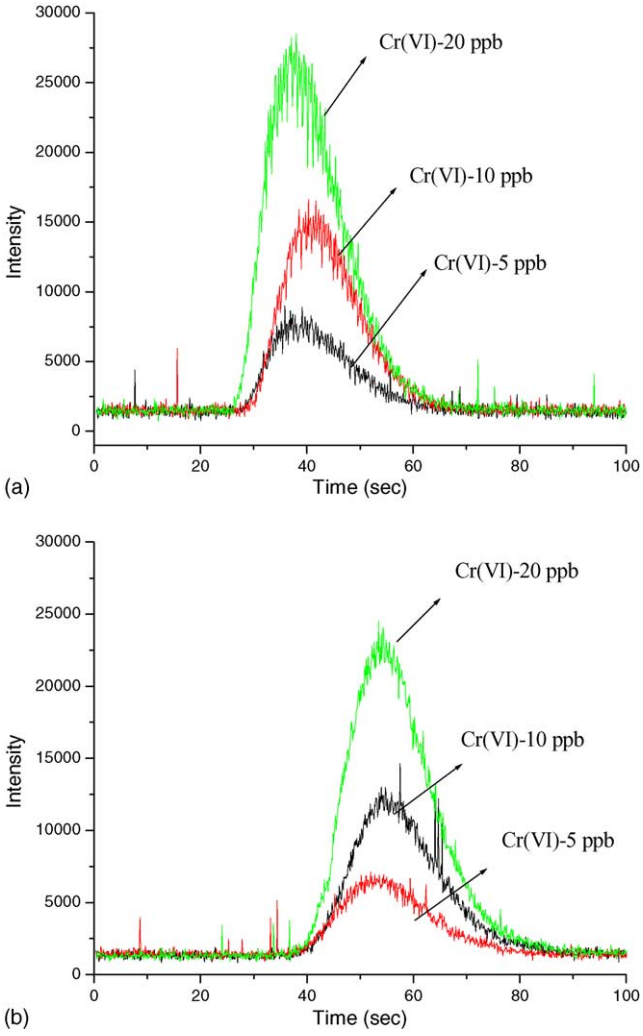

Fig. 3. (a) Calibration plots obtained for Cr(VI) before passing through the column by flow injection method. (b) Calibration plots obtained for Cr(VI) after passing through the column by flow injection method.

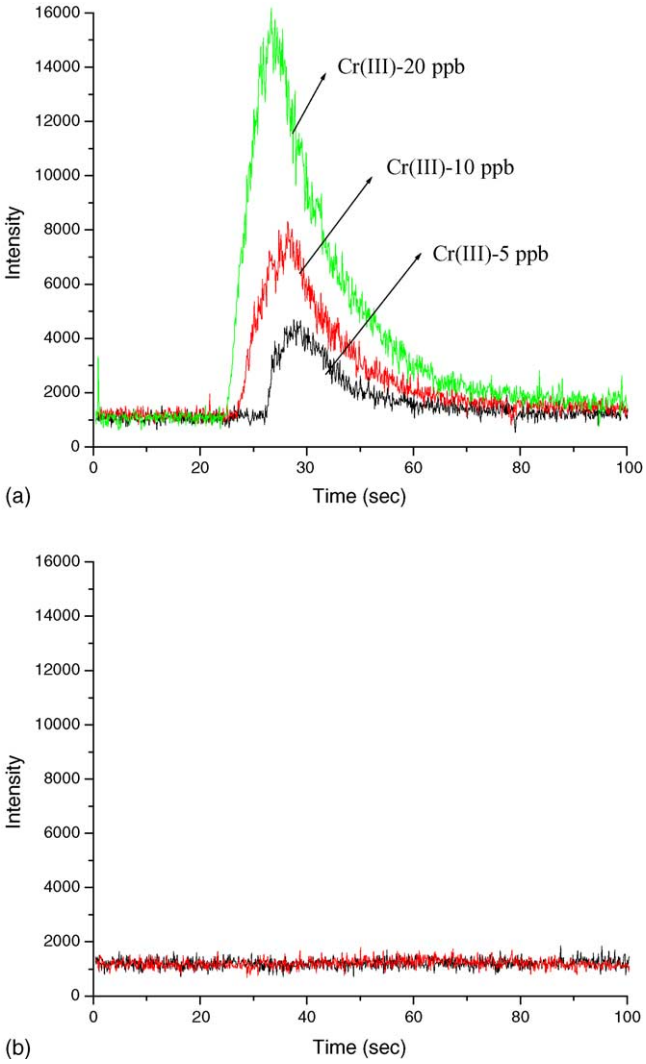

Fig. 4. (a) Calibration plot obtained for Cr(III) before passing through the column by flow injection (FI) method (without column). (b) Plot obtained for Cr(III) after passing through the column.

3.6. Speciation studies with mini-column using FI-ICP-MS method

From Fig. 3(a) and (b), it may be seen that the recovery of Cr(VI) on passing through the mini-column was well above >95%. The marginal decrease in the peak height from these figures resulted from the peak broadening due to the passage through the column. Thus, there is no significant sorption of Cr(VI) is seen to occur on the column. These figures also show that no conversion of Cr(VI) to Cr(III) occurs during passage of the column. As seen from Fig. 4(a) and (b), Cr(III) is completely sorbed on the micro-column, when different concentrations of standard solutions are passed. The calibration plots (Fig. 5(a) and (b)) obtained when mixture of different concentrations (5, 10, 20 ng ml^{-1} each) of Cr(III) and Cr(VI) passed through the mini-column shows that quantitative separation of Cr species occurs in all the sample solutions. The sorbed Cr(III) was stripped off from the mini-column with 1 ml of 2 mol l^{-1} HNO_3 and quantitative recovery (>90%) was achieved at these low levels.

3.7. Limit of detection (LOD) and precision of the method

The detection limit was evaluated as the concentration corresponding to three times the S.D. of the blank signal. Detection limit for Cr was found to be 0.15 ng ml^{-1} and 145 ng ml^{-1} for ICP-MS and FAAS, respectively. For DPC-spectrophotometric method, the limit of detection value achieved was 5 ng ml^{-1}.

The precision of the determination of Cr(III) was evaluated under the optimum conditions mentioned above. For this purpose, three successive retentions and elution cycles with 20 ml of sample solution containing 10 μg of mixture of Cr(III) and Cr(VI) were performed. These studies indicate that the recovery of Cr(III) from the column was 96 ± 1% while the recovery of Cr(VI) in the effluent was 98 ± 3%.

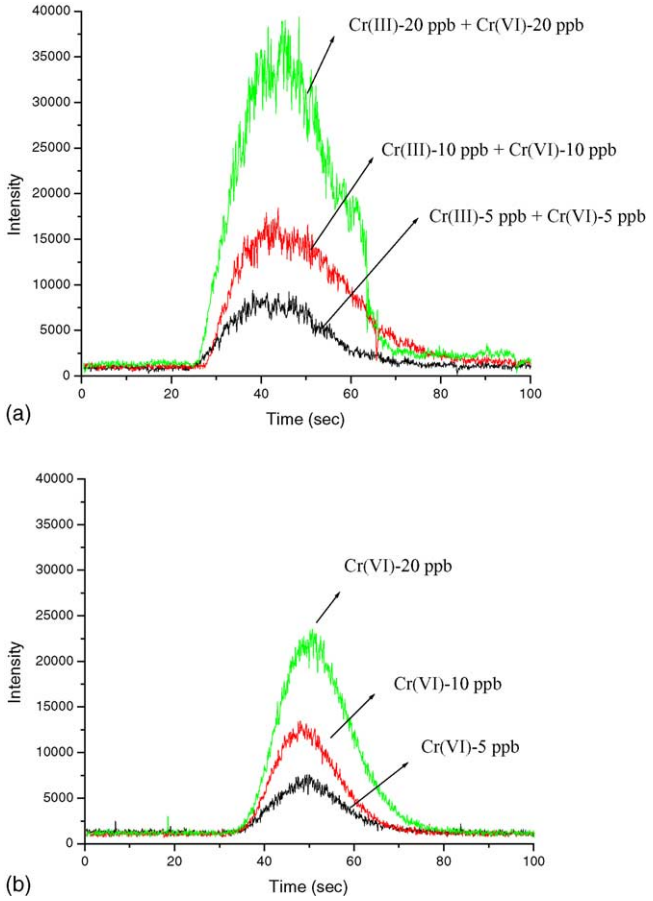

Fig. 5. (a) Calibration plot obtained for Cr(III) + Cr(VI) by flow injection method. (b) Plot obtained for mixture of Cr(III) + Cr(VI) after passing through the column.

3.8. Capacity of immobilized moss for Cr(III) using breakthrough curves

In this work, breakthrough capacity is used to assess the capability of immobilized moss for Cr(III). A feed solution containing 5 μg ml^{-1} of Cr(III) solution was passed through a column loaded with 1 g of immobilized moss at an optimized experimental conditions. The pH of the feed solution was maintained at ~4. Samples were collected from the column periodically and assayed for residual chromium content using ICP-MS. A breakthrough curve for Cr(III) was obtained by plotting percentage (%) breakthrough [$(C/C_0) \times 100$] against number of bed volumes, where, C_0 and C are the concentrations of chromium in the initial solution and the effluent, respectively. From the breakthrough curve (Fig. 6), the sorption capacity of immobilized moss for Cr(III) was found to be ~11.5 mg g^{-1}.

After attaining 100% breakthrough, the retained Cr(III) was eluted with 2 mol l^{-1} HNO_3. As seen from Fig. 7, 10 ml (3 bed volumes) of 2 mol l^{-1} HNO_3 solution was required for quantitative recovery (>95%) of Cr(III) from the column after sorption of Cr(III). Hence, 10 ml eluent solution of 2 mol l^{-1} HNO_3 was preferred to get a good pre-concentration factor.

3.9. Effect of interfering ions

Study of other cations' effect on the speciation of Cr is very important because natural and wastewaters rarely exist with single toxic metallic species. The presence of a multiplicity of metals often gives rise to interactive effects. Hence, the effect of the presence of common co-existing ions Na^+,

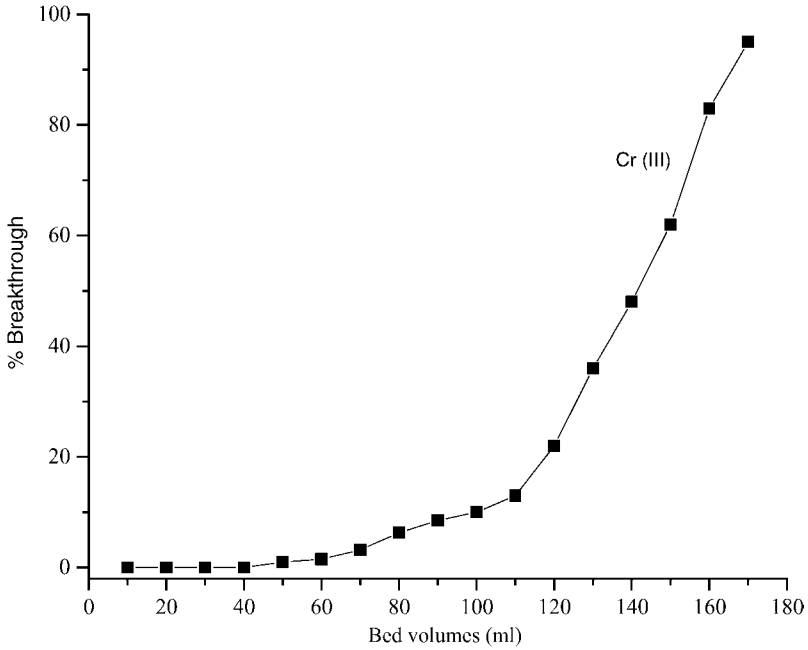

Fig. 6. Percentage (%) breakthrough in the effluent after being passed through a column of immobilized moss. 5 ppm solution of Cr(III) was passed at a flow rate of 2 ml min^{-1}. One bed volume equals to 3 ml.

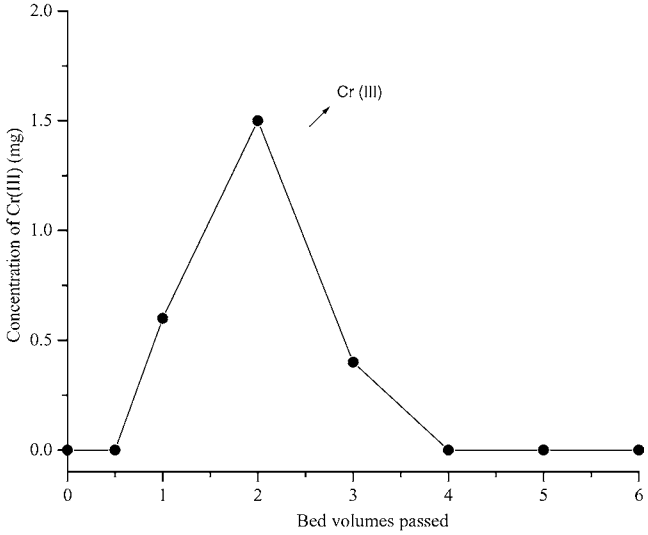

Fig. 7. Recovery of Cr(III) from the column of immobilized moss with 2 M HNO_3. Flow rate of 1 ml min^{-1} was used.

K^+, Ca^{2+}, Mg^{2+}, Fe^{3+} that are commonly encountered in waters, on the speciation of Cr was investigated. For this purpose, different concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+} (10–200 μg absolute), were added individually to a 20 ml solution containing 10 μg each of Cr(III) and Cr(VI). All the tests were performed under optimum operating conditions such as pH, weight of sorbent and flow rate, by following general procedure as described above. These studies indicated that quantitative separation of Cr(III) and Cr(VI) was achieved even when the sample solution containing the ratio of interfering ions to chromium was 20.

3.10. Application to different water samples

3.10.1. Analysis of synthetic samples

In order to demonstrate the reliability of this proposed speciation method, experiments were carried out by adding known amounts of Cr(III) and Cr(VI) to different kinds of synthetic and collected potable water samples and applying the general procedure previously described. As shown in Table 3, synthetic sample solutions were prepared by spiking different amounts of Cr(III) and Cr(VI). After adjusting pH of sample solutions to ∼4, a 50 ml portion of these samples was passed through the column of immobilized moss and the general procedure was applied. Similar experiments were carried out with sample solutions containing mixture of Cr(III) and Cr(VI) in different amounts after adjusting the pH to 4. The recoveries of both Cr(III) and Cr(VI) are presented in Table 3.

The water samples were collected in pre-cleaned polyethylene bottles and samples were analysed immediately after collection. For recovery studies, six different water samples of bottled lake and ground water were obtained from different sources in Hyderabad and after being

Table 3
Determination of Cr(III) and Cr(VI) in spiked sample solutions

Added (μg ml^{-1})[a]		Found (μg ml^{-1})		Recovery (%)	
Cr(III)	Cr(VI)	Cr(III)[b]	Cr(VI)[c]	Cr(III)	Cr(VI)
0.5	–	0.48	–	96 ± 2	–
–	0.5	–	0.48	–	96 ± 3
0.5	0.5	0.47	0.49	95 ± 2	98 ± 2
1.0	0.5	0.96	0.48	96 ± 1	96 ± 2
0.5	1.0	0.48	0.97	96 ± 3	97 ± 3
1.0	1.0	0.96	0.97	96 ± 2	97 ± 1
2.0	0.5	1.94	0.48	97 ± 1	96 ± 2

[a] Sample volume 20 ml, ± S.D. ($n = 3$)
[b] After eluting from the column.
[c] Measured in the effluent.

Table 4
Determination of Cr(III) and Cr(VI) in spiked natural water samples

Sample	Added (μg ml^{-1})[a]		Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)[b]	Cr(VI)[c]
Bottled water (mineral)	0.1	0.1	95 ± 1	96 ± 2
	0.5	0.5	96 ± 2	97 ± 1
Lake water	0.1	0.1	95 ± 2	96 ± 1
	0.5	0.5	97 ± 1	98 ± 2
Ground water	0.1	0.1	94 ± 1	95 ± 2
	0.5	0.5	95 ± 2	97 ± 1

[a] Sample volume 20 ml, ± S.D. ($n = 3$).
[b] After eluting from the column.
[c] Measured in the effluent.

confirmed that Cr level of these samples was under LOD of the methods employed, the samples were spiked with known concentrations of Cr(III), Cr(VI) or both the species. Then these water samples were passed through the column without adjustment of sample pH (the pH of sample solution as received was ∼6.5), because usual acidification would change the chemical species [37].

Quantitative sorption of Cr(III) occurred in all the samples while the sorption of Cr(VI) was low (<5%). After sorption, Cr(III) was eluted with 2 mol l^{-1} HNO_3 followed by measurement with ICP-MS and FAAS, whereas Cr(VI) in the effluent was measured by DPC method. The mean recoveries of Cr(III) and Cr(VI) in spiked potable water samples were presented in Table 4. In all the cases the recoveries of Cr(III) and Cr(VI) were found to be >95%.

3.10.2. Analysis of real wastewater samples

The two different real wastewater samples containing both Cr(III) and Cr(VI) was obtained from the plants of tannery and bulk-drug industry located in Hyderabad, as well as, from the ground waters collected near chromate mine areas located in the state of Orissa. All the samples were analysed as soon as possible, after sampling. Then these wastewater samples were passed through the column loaded with immobilized moss and the proposed method was applied under optimal experimental conditions as described above. The

Table 5
Speciation of Cr(III) and Cr(VI) in real wastewater samples ($\mu g\,ml^{-1}$)

Species	Tannery effluent (pH 7.5)	Bulk-drug industrial waste water (pH 8.24)	Ground water collected near chromate mines (pH 8.35)
Total Cr	4.8 ± 0.3	0.118 ± 0.007	0.195 ± 0.012
[a]Cr(III)	0.46 ± 0.05	0.044 ± 0.004	0.012 ± 0.003
[b]Cr(VI)	4.3 ± 0.2	0.072 ± 0.006	0.184 ± 0.009

Volume of the sample passed = 20 ml, ± S.D. ($n = 3$).
[a] After eluting from the column.
[b] Measured in the effluent.

total amount of Cr has been estimated by ICP-MS, whereas, Cr(VI) has been determined by DPC-spectrophotometric method. The obtained results are given in Table 5. As can be seen from Table 5, the measured total Cr and the sum calculated from Cr(III) and Cr(VI) were in good agreement. The results show that the immobilized moss is thus well suited for quantitative separation and determination of Cr(III) and Cr(VI) from real wastewaters.

4. Conclusion

Speciation of Cr(III) and Cr(VI) can be carried out very effectively using silica-immobilized moss. Chromium (III) was almost quantitatively retained in the pH range of 4–8, while Cr(VI) remained in the solution. Quantitative separation was achieved with mini-column even at low ppb levels. Another advantage in the use of moss is the capability of being used within a wide range of pH. The breakthrough capacity of the immobilized moss was found to be $\sim$11.5 mg g^{-1} for Cr(III). The proposed method was applied successfully for the determination of Cr(III) and Cr(VI) in spiked, natural and wastewater samples and recoveries in each phase were >95% in all the cases. Another advantage in the use of moss is, there is no chemical hazard, it is cheap and easy to obtain.

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