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A dual column system using agarose-based adsorbents for preconcentration and speciation of chromium in water

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Abstract

Three different agarose-based chelating adsorbents with, respectively, iminodiacetic acid (IDA), tris(2-aminoethyl)amine (TREN) and dipicolylamine (DPA) functional groups and an agarose-based anion exchanger (Q-Sepharose), were studied for the separation and preconcentration of Cr(III) and Cr(VI) species in water. Column recoveries of all the adsorbents were plotted against pH, and it was found that at pH 3.0 the IDA adsorbent selectively adsorbs Cr(III), with a 100 ± 1.0% recovery. The Q-Sepharose, on the other hand, accumulated only Cr(VI) at this pH, again with a recovery of 100 ± 1.0%. A dual column system was accordingly designed, using the two adsorbents in tandem, for the separation and preconcentration of the chromium species.

The effects of pH, sample flow rate, column length, eluent type, eluent volume, acid concentration and interfering ions on the recoveries of Cr(III) and Cr(VI) were carefully studied. It was shown that by passing test solutions, at pH 3.0; through the dual column system, the two chromium species could be individually collected on the columns, respectively, and eluted, one after the other. A portion of 2 mol l^{-1} hydrochloric acid was used for elution of each column before final measurement by flame AAS method. A preconcentration factor of 12, a detection limit of 7.7 ± 0.1 μg l^{-1} and a precision expressed as relative standard deviation of 0.4% (at 0.3 mg l^{-1}) were achieved for six replicates.

Application of the developed method to the determination of chromium species in spiked river and tap water and wastewater samples, from a dye production plant, resulted in excellent agreements with accepted concentrations.

Keywords: Chromium speciation; Agarose adsorbents; Iminodiacetic acid; Tris(2-aminoethyl)amine; Dipicolylamine; Q-Sepharose

1. Introduction

Speciation, separation and preconcentration of metal ions are of great interest in analytical, industrial and environmental fields of studies. Direct analysis of natural samples can give rise to unreliable results unless a separation step is used before final measurement. In addition, many of the separation and/or preconcentration techniques result in valuable speciation information. Solid phase extraction by polypropylene columns packed by chelating or ion exchange sorbents have been widely used for this purpose [1]. The usefulness of a solid phase extraction column depends on its functional groups, support material, particle size, etc. Agarose-based adsorbents, due to their hydrophilicity and chemical resistance in a wide pH range of 0–14, have been shown to be excellent support materials for column preconcentration and speciation experiments [2,3].

Chromium preconcentration and speciation is of great interest from an environmental point of view, because of different toxicities of its species [4] and low concentrations of this element in natural waters. Cr(III) is an important nutrient for humans; the insulin hormone does not function without it [5]. Cr(VI), on the other hand, is known as a poison that causes cancer. A number of different methods have been employed for chromium speciation and/or preconcentration. Among them, column techniques are of great interest [6–11]. In these methods, usually only one column is used to adsorb one of the chromium species [6–8]. Hence, the sample must be either oxidized or reduced [9,10] in order to determine total chromium. Mondal et al. [11] reported

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selective retention of Cr(III) and Cr(VI), respectively, by changing the pH of a 2-naphtol-3,6-disulphonic acid column from 6.5 to 1.5. Marques and co-workers [12], on the other hand, accumulated both chromium species on an activated alumina column, followed by selective elution by nitric acid and ammonium hydroxide, respectively. Use of a dual column system, in which each species was retained on one column with their respective elution, was first reported by Naghmush et al. [13]. In this method, however, the effluent pH of the first column must be readjusted before it enters the other column, which makes the procedure somewhat complicated.

In this work, four different types of agarose-based chelating adsorbents are studied in an attempt to design a dual column method in which the adsorption pH and other conditions for the two columns are the same, while each column accumulates only one species of chromium.

2. Experimental

2.1. Chemicals and apparatus

Agarose-based chelating adsorbents functionalized by iminodiacetic acid (IDA-Novarose), tris(2-aminoethyl)amine (TREN-Novarose) and dipicolil amine (DPA-Novarose) were generous gifts from Inovata AB (Stockholm, Sweden). Q-Sepharose, a ternary ammonium agarose-based anion exchanger was obtained from Pharmacia (Sweden).

All other chemicals were purchased from Merck or Fluka. The chemicals were analytical grade and used as they were received.

Measurements were performed by a flame AAS instrument (Shimadzu AA-670, Japan). Spectrophotometric determination of Cr(VI) was made by a Spectronic 20D spectrophotometer. For pH determinations, a Jeneway (USA) model 3020 with a combined glass electrode was used after calibration against standard Merck buffers. All the dilutions were made by double distilled water, prepared by a Fision (UK) double distiller.

Preconcentration columns were 5.7 mm i.d. polypropylene tubes with two frits at their bottoms and tops, packed with an adsorbent. A peristaltic pump (EYLA RP-1000, Japan) was used for pumping solutions through the columns.

2.2. Methods

Stock solutions of Cr(III) were prepared by dissolution of required amounts of $Cr(NO_3)_3$ salt in 0.1 M hydrochloric acid and its dilution to volume by the same acid. Cr(VI) stock solutions were prepared in the same way using $K_2Cr_2O_7$ salt. Test solutions and standard solutions for calibration curves were prepared by dilution of the same stocks.

Test solutions were prepared by dilution of required volumes of the stocks in an acetate buffer media. The final concentrations of chromium and acetate were usually 0.3 mg l^{-1} and 0.01 M, respectively. Droplets of 1 M hydrochloric acid or sodium hydroxide were used for adjustment of pH before dilution of the solutions to the volume.

For packing the columns, suspensions of the adsorbents were pipetted into an empty column with a frit at the bottom. A gentle vacuum was applied for faster settling of the particles before mounting the top frit. The column pretreatment was made by passing 10 ml double distilled water, 10 ml 2 M hydrochloric acid and 10 ml 0.1 M acetate buffer (pH 5.5) through it.

Preconcentration and recovery experiments were made, usually, by pumping 40 ml of a buffered (by 0.01 M acetate buffer) test solution through a pretreated column with a flow rate of 3.5–4.0 ml min^{-1}. The column was then washed with a few millilitres of double distilled water and eluted by 6 ml 2 M hydrochloric acid (unless otherwise stated). The eluate was collected in small capped vessels and analysed by flame AAS against matched standards. Column length was usually 8 mm for Q-Sepharose and 20 mm for the other adsorbents.

The Khoramabad's river and tap water samples were acidified to pH 2 on collection, filtered through a membrane filter (0.45 μ), spiked by Cr(III) and Cr(VI), and pH adjusted by the addition of drops of sodium hydroxide before column preconcentration. Waste water from a dye production plant was also acidified on collection and used as a real sample. This sample was filtered, pH adjusted and analysed as soon as possible after collection. About 40 ml of the field samples were enriched on selected columns and they were eluted by 6 ml HCl before measurement.

Spectrophotometric determination of Cr(VI) in real samples, for evaluation of the speciation method, was made by addition of diphenylcarbazide in acidic media to the samples, and absorbance determination at 540 nm in accordance with standard procedures [14].

Capacity measurements for the chelating adsorbents were performed in column mode as reported elsewhere [2]. A 100 ml solution of 100 mg l^{-1} copper at pH 5.5 was used for saturation of a column before its elution with 2 M hydrochloric acid. The capacity for copper was considered as a measure of total active sites of a chelating adsorbent.

The detection limit of the system used was calculated from the detection limit of AAS for direct determination of chromium, using ordinary methods, divided by the highest sample preconcentration factor (PF) achieved.

All the experiments were performed with at least two and usually three replicates.

3. Results and discussion

3.1. Comparison of the adsorbents

Column recoveries of Cr(III) and Cr(VI) were determined as a function of pH for the four different adsorbents in order

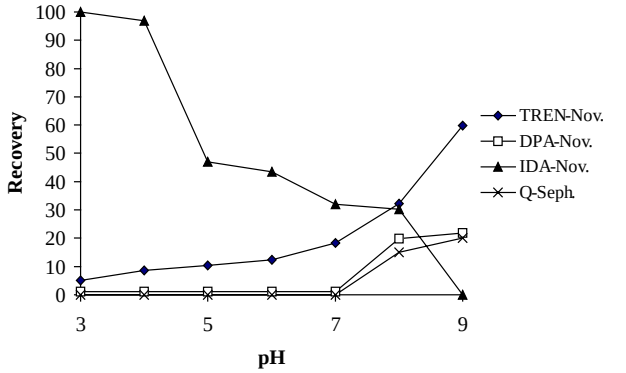

Fig. 1. Percent recovery of Cr(III) from different adsorbents as a function of pH. Sample volume 40 ml, 0.01 M acetate buffer, eluent 6.5 ml 2 M HCl, flow rate 4 ml min^{-1}, column length 2.0 cm.

to find the most suitable column packing for the purpose of speciation and preconcentration. TREN-Novarose, with a capacity of 120 ± 6 μmol ml^{-1} ($n = 3$) for copper, was the first adsorbent studied. Percentage recoveries of Cr(III) and Cr(VI) from this adsorbent can be followed as a function of pH in Figs. 1 and 2, respectively. As the graphs depict, Cr(III) is not quantitatively recovered in the whole pH range studied and Cr(VI) shows a quantitative recovery only at pH 9.

Figs. 1 and 2 also indicate percentage recoveries from DPA-Novarose, with a capacity of 18.3 ± 0.3 μmol ml^{-1} ($n = 3$), as a function of pH. As is obvious from the results, in no case were high recoveries obtained for Cr(III) or Cr(VI).

The third adsorbent studied was IDA-Novarose, with a capacity of 92.5 ± 7 μmol ml^{-1} for Cu(II). This adsorbent is negatively charged in the pH range studied and, as expected, does not significantly adsorb Cr(VI) anionic species. Cr(III) shows a quantitative recovery, beginning at pH 3.0 and decreasing gradually with increasing pH. Figs. 1 and 2 summarize the results.

Q-Sepharose is an anion exchanger with ternary ammonium functional groups. Its recovery for Cr(III) with positive charges is expected to be negligible, but it should efficiently

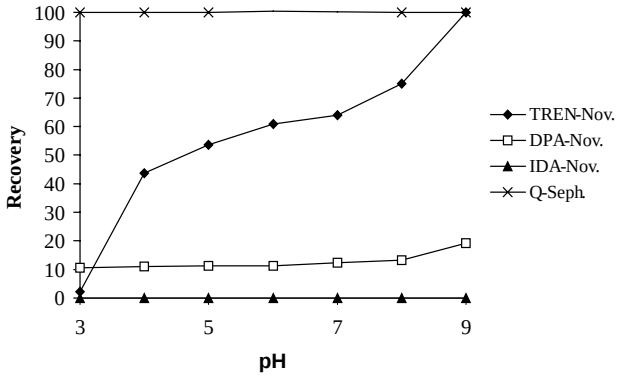

Fig. 2. Percent recovery of Cr(VI) from different adsorbents as a function of pH. Other conditions are as in Fig. 1.

scavenge negatively charged Cr(VI). The results (Figs. 1 and 2) confirm these expectations. As shown, the recovery of Cr(VI) on this adsorbent was quantitative in the whole pH range. Cr(III), on the other hand, is not significantly recovered in the pH range of 3–7. The capacity of this adsorbent for Cr(VI) was measured at up to 248 μmol ml^{-1} under optimized conditions. Compared to the chelating adsorbents studied this adsorbent contained more functional groups per millilitre. It was also observed that Q-Sepharose particles are softer than Novarose sorbents and therefore produced higher backpressures in column.

Taking into consideration the results obtained from the recovery experiments, it was decided to use the IDA-Novarose and Q-Sepharose adsorbents to design a dual column system for separation and preconcentration of Cr(III) and Cr(VI). At pH 3.0, the IDA-Novarose selectively adsorbs Cr(III) with no effect on Cr(VI). The Q-Sepharose, on the other hand, accumulates only Cr(VI) at the same pH. At pH 3.0, Cr(III) species are positively charged and are adsorbed or chelated efficiently by the negatively charged iminodiacetate functional groups of the IDA-Novarose. Cr(VI), on the other hand, is mainly in the anionic form of $HCrO4^-$ at the same pH and is hold only by the positively charged groups of the Q-Sepharose anion exchanger. Hence, the two columns, can be enriched using the same pH and the accumulated analytes may be then eluted one after the other from the columns.

3.2. Effect of different parameters

After selection of suitable adsorbents, the effect of different parameters on the accumulation and recovery of Cr(III) and Cr(VI) was studied in order to optimize conditions for achieving the best results. A one-at-a-time method was used for this purpose, changing one parameter each time while keeping the others constant.

The effect of sample flow rate was the first parameter studied. When the sample flow rate was increased from 3 to 7 ml min^{-1}, the recovery of Cr(III) decreased slightly for the IDA-Novarose, from 100 ± 1.0% to 98 ± 1.1%. The sample flow rate had no significant effect on the accumulation of Cr(VI) by Q-Sepharose. Higher flow rates were not tested due to high backpressures encountered and some limitations of the peristaltic pump used.

Four different types of eluents were tested for elution of the analytes. Six-millilitre portions of 2 M HCl or 2 M H_2SO_4 efficiently desorbed both the analytes from the columns. Using nitric acid (2 M) as an eluent for Cr(III) on the IDA adsorbent also resulted in quantitative recoveries. An average value of 82 ± 2% was obtained, however, for triplicate measurements of Cr(VI) recovery from the Q-Sepharose column. The use of an EDTA solution (0.1 M) as eluent resulted in low recoveries of 40 ± 4 and 63 ± 3 for Cr(III) and Cr(VI), respectively, from the adsorbents.

Fig. 3 depicts the effect of eluent (HCl) concentration on the recoveries of Cr(III) and Cr(VI) from IDA-Novarose

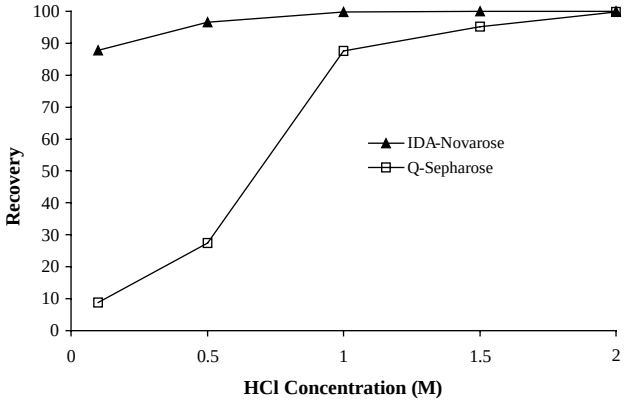

Fig. 3. Percent recoveries of Cr(III) from IDA-Novarose and Cr(VI) from Q-Sepharose as functions of HCl concentration. Other conditions are as in Fig. 1.

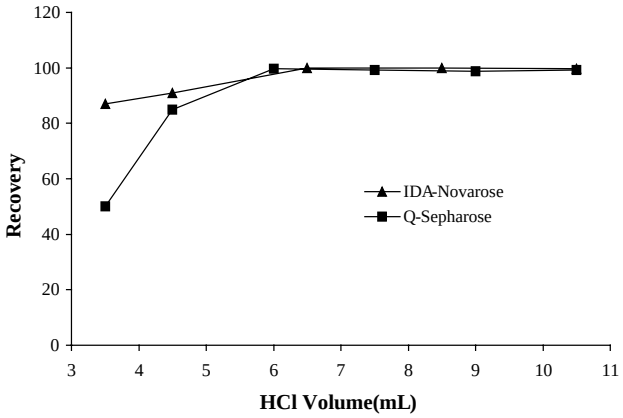

Fig. 4. Percent recoveries of Cr(III) from IDA-Novarose and Cr(VI) from Q-Sepharose as functions of eluent (2 M HCl) volume. Other conditions are as in Fig. 1.

and Q-Sepharose columns, respectively. The results indicate that an efficient elution of the chromium species requires minimum acid concentrations of 1 and 2 M, respectively.

The effect of eluent volume also was investigated using 2 M HCl for elution. As shown in Fig. 4, at least 6.0–6.5 ml of acid is required to achieve quantitative recoveries from both columns.

Different sample volumes of 40, 60 and 80 ml for enrichment of the columns were tested and no significant decrease in the recovery was observed. All the samples contained the same amount of an analyte, i.e. 0.012 mg Cr(III) or Cr(VI), despite of their different volumes. The recoveries in no case were less than 100 ± 1%.

Matrix ions can often affect recovery of an analyte or interfere with its final measurement. Na^+, K^+, Mg^{2+} and Ca^{2+} cations and NO_3^-, PO_4^{3-}, F^-, Cl^- and SO_4^{2-} anions are the most commonly occurring ions in natural waters. Effects of the matrix cations on the recovery of Cr(III) and the matrix anions on the recovery of Cr(VI) were carefully investigated. In each case, at least four different concentrations of the potentially interfering ion were added to the sample and the recovery was determined. The results, summarized in Table 1, depict good tolerance of the adsorbents for most of the matrix ions. The additions of 70 and 100 mg l^{-1} Ca^{2+}, however, resulted in Cr(III) recoveries averaging 86 ± 0.8% and 50 ± 2.8% for triplicate measurements, respectively. This implies that the method is not suitable for hard waters with high Ca^{2+} contents.

The optimum column length for IDA-Novarose and Q-Sepharose was found to be 2.1 and 0.8 cm, respectively. Longer columns increased backpressure, and a shorter column did not scavenge the analyte completely. A study of the effect of acetate buffer concentration resulted in recoveries of 77 ± 2% for Cr(III) and 63 ± 2% for Cr(VI) when an acetate concentration of 0.05 M was used. This means that a high acetate or ammonium ion concentration can partly elute the Q-Sepharose or IDA-Novarose columns, respectively, and may be explained by incorporation of an ion exchange mechanism in the adsorption of the analytes. Ammonium acetate concentrations higher than 0.01 M were, hence, avoided.

Table 1
Effect of interfering ions on the recovery of Cr(III) and Cr(VI)

Analyte	Interfering ion	Maximum tolerance (mg l^{-1})	Interfering ion/analyte mole ratio	Recovery (%)
Cr(III)	K^+	500	2222	99.6 (±0.8)
	Na^+	400	3014	99.9 (±0.8)
	Mg^{2+}	100[a]	713	99.7 (±1.0)
	Ca^{2+}	50	217	99.8 (±0.8)
Cr(VI)	NO_3^-	500	1398	99 (±1)
	PO_4^{3-}	1000[a]	1825	100 (±1)
	F^-	1000[a]	9123	100 (±1)
	Cl^-	500	2441	100 (±1)
	SO_4^{2-}	120	217	99 (±1)

Here, the recoveries are reported only when the maximum tolerable amount of an interfering ion is used. Other conditions are as in Fig. 1. The numbers in parentheses are standard deviations for triplicate measurements.

[a] The maximum concentration studied.

Table 2
The optimum conditions for the preconcentration of Cr(III) and Cr(VI) on the IDA-Novarose and Q-Sepharose columns, respectively

Parameter	Optimum condition
Sample pH	3.0
Flow rate	3 ml min^{-1}
Eluent type	HCl
Eluent concentration	2 M
Eluent volume	6.5 ml
Sample volume	80 ml or less
Buffer concentration	0.01 M or less
Column length (for IDA-Novarose)	2.1 cm
Column length (for Q-Sepharose)	0.8 cm

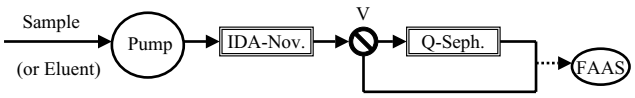

Fig. 5. Schematic diagram of the flow system. The peristaltic pump forces a sample, buffer or eluent through the columns. The valve (V) allows separate elution of the columns for their final measurement by the flame AAS method.

It was found that temperature also affects column recoveries by affecting backpressure and, accordingly, sample flow rates. On hot summer days some losses of analytes were observed. It was, therefore, decided to perform all the experiments at a temperature not higher than 25 °C.

3.3. Design of the dual column system

Decisions regarding the best experimental conditions for the preconcentration and speciation system were based on the results obtained from the parameters study. Table 2 lists the optimized settings for the IDA-Novarose and Q-Sepharose columns.

A tandem, dual column system was accordingly designed for the separation and preconcentration of the chromium species. A schematic representation of the system is depicted in Fig. 5. In this system, the sample's pH is set at 3.0 and then passed through the system. The first column, containing the IDA-Novarose adsorbent, accumulates Cr(III) with no effect on the Cr(VI) species. The Q-Sepharose column then adsorbs Cr(VI) with no need to readjust the pH. In the elution step, a rotating valve allows the columns to be eluted individually by a similar eluent. Then the eluates are collected in small vessels and analysed later in an offline mode or, alternatively, they can be monitored by a flame AAS instrument in an online flow system.

The efficiency of the system was tested first in an offline mode with synthetic standard solutions containing both Cr(III) and Cr(VI) at known concentrations (see Table 3). Quantitative recoveries were obtained for both the analytes. The detection limit for the chromium species was calculated to be $7.7 \pm 0.1\,\mu g\,l^{-1}$ for a preconcentration factor of 12. The detection limit of the method easily can be reduced further by using a more sensitive analytical technique, such as electrothermal AAS or ICP-MS. The precision of the method was estimated by six times analysis of a mixed Cr(III) and Cr(VI) sample by the speciation system. A precision expressed as relative standard deviation of 0.4% (at $0.3\,mg\,l^{-1}$) was obtained.

To see how the system works for real samples, a spiked (with $0.3\,mg\,l^{-1}$ chromium species) river water and a wastewater sample from a dye production plant were analysed. Certification of the results were performed by determination of Cr(VI) with a diphenyl carbazide spectrophotometric method and measurement of total chromium by the flame AAS instrument. The Cr(III) concentration then was calculated by difference. The system was also tested for a low concentration sample by preconcentration of a tap water sample spiked by only $50\,\mu g\,l^{-1}$ chromium species. The results, as shown in Table 3, indicate a good confirmation between reference and determined concentrations by the designed method with a maximum relative error of only 1.3%. The results are shown in Table 3. A *t*-test indicated no significant difference between the reference and measured values, with a 95% confidence level.

It can be concluded that the dual column system can efficiently separate and preconcentrate Cr(III) and Cr(VI) species in water samples. The system is simple and can be easily automated and used in online modes as well. Samples with high ionic strengths or high calcium contents, however, are not recommended to be used with this system due to a risk of some analytes losses.

Table 3
Results obtained for synthetic, natural water and wastewater samples by the designed dual column system in the optimized conditions mentioned in Table 2

Sample	Reference values ($mg\,l^{-1}$)		Measured values ($mg\,l^{-1}$)		Relative error (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Synthetic	0.300	0.300	0.299 (±1.3)	0.299 (±1.2)	−0.3	−0.3
Spiked Khoramabad's river water	0.300	0.300	0.299 (±1.4)	0.299 (±1.3)	−0.3	−0.3
Wastewater from a dye plant	0.415 (±1.2)	0.300 (±2.1)	0.410 (±1.6)	0.296 (±1.5)	−1.2	−1.3
Spiked Khoramabad's tap water[a]	0.0500	0.0500	0.0492 (±0.1)	0.0509 (±2.1)	−1.6	1.8

The figures in parentheses are relative standard deviations (%) for duplicate measurements.
[a] Chromium concentrations in this case were measured by an electrothermal AAS instrument (Model 6650 Shimadzu, Japan).

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