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ANALYTICAL METHODOLOGY

Determination of Cr(VI) and Cr(III) species in parenteral solutions using a nanostructured material packed-microcolumn and electrothermal atomic absorption spectrometry

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Abstract

A sequential on-line preconcentration and separation system for Cr(VI) and Cr(III) species determination was developed in this work. For this purpose, a microcolumn filled with nanostructured α-alumina was used for on-line retention of Cr species in a flow-injection system. The method involves the selective elution of Cr(VI) with concentrated ammonia and Cr(III) with 1 mol L^{-1} nitric acid for sequential injection into an electrothermal atomic absorption spectrometer (ETAAS).

Analytical parameters including pH, eluent type, flow rates of sample and eluent, interfering effects, etc., were optimized. The preconcentration factors for Cr(VI) and Cr(III) were 41 and 18, respectively. The limit of detection (LOD) was 1.9 ng L^{-1} for Cr(VI) and 6.1 ng L^{-1} for Cr(III). The calibration graph was linear with a correlation coefficient of 0.999. The relative standard deviation (RSD) was 8.6% for Cr(VI) and 6.1% for Cr(III) ($c = 10\,\mu g\,L^{-1}$, $n = 10$, sample volume = 25 mL). Verification of the accuracy was carried out by analysis of a standard reference material (NIST SRM 1643e "Trace elements in natural water") with a reported Cr content of $20.40 \pm 0.24\,\mu g\,L^{-1}$. Using the proposed methodology the total Cr content, computed as sum of Cr(III) and Cr(VI), in this SRM was $20.26 \pm 0.96\,\mu g\,L^{-1}$. The method was successfully applied to the determination of Cr(VI) and Cr(III) species in parenteral solutions. Concentration of Cr(III) species was found to be in the range of 0.29–3.62 $\mu g\,L^{-1}$, while Cr(VI) species was not detected in the samples under study.

Keywords: Chromium speciation; Nanostructured alumina; Preconcentration; Parenteral solutions

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Introduction

Chromium is a major pollutant for environment, usually as a result of some industrial pollution including tanning factories, steel works, industrial electroplating, wood preservation and artificial fertilizers [1]. Chromium species exist mainly in two different oxidation states in the environment, Cr(III) and Cr(VI), which have contrasting physiological effects [2]. Cr(III) is considered as an essential trace elemental species for the maintenance of an effective glucose, lipid, and protein metabolism in mammals [3,4]. On the other hand, Cr(VI) species can be toxic for biological systems, and water-soluble Cr(VI) is extremely irritating and toxic to human body tissues owing to its oxidizing potential and its easy permeation through biological membranes [5,6]. Thus, considerable emphasis has been given to the development of analytical methodologies for Cr species separation and determination [4].

Since one of the routes of incorporation of Cr into the human body could be parenteral nutrition (PN), its determination in this type of samples becomes very important [7,8]. Parenteral nutrition is the administration of all nutrients directly into the bloodstream. This nutrition is an important supportive for patients with gut failure or who cannot receive normal alimentation due to other pathologies. However, the quantitative requirements or the toxicity of trace elements in parenteral solutions are difficult to assess. Long-term total parenteral nutrition (TPN) patients can inadvertently receive excess or different redox state of trace elements as contaminants of other nutrients in TPN, e.g., Al in Ca/P supplements, Cr in amino acids [9]. Many of the solutions for PN support could have a chromium content that exceeds, in part considerably, the suggested threshold concentration of $5\,\mu g\,L^{-1}$ [10,11]. Therefore, powerful analytical techniques are required and only few of them show enough sensitivity. Among them, ETAAS and inductively coupled plasma mass spectrometry (ICP–MS) are the most commonly used for trace Cr determination [12], but the low level of Cr species is often not compatible with the detection limits of these techniques. Moreover, salt concentration normally occurring in parenteral solutions could be a serious drawback for ICP–MS analysis, due to nebulizer blocking and salt deposition on the cones.

The most widely used techniques for separation and preconcentration of analytes include, among others, coprecipitation [13], liquid-phase extraction [14], solid-phase extraction [15] and high-performance liquid chromatography [16]. In recent times, the solid-phase extraction (SPE) technique has increasingly become popular in comparison with the traditional liquid–liquid extraction methods because of its several major advantages like: high preconcentration factors; simple operation conditions; a rapid-phase separation; possibility of choosing active solid phases and ability of coupling SPE with different detection techniques [17,18]. When preconcentration and speciation techniques are applied in batch mode, the time of analysis is increased, turning these procedures unpractical for routine analysis [2]. However, this situation can be significantly improved employing on-line speciation and preconcentration systems.

Regarding the different solid phases that may be used, a particular interest in nanostructured materials such as: ZrO_2, TiO_2, CeO_2 or Al_2O_3, lies in the fact that their physicochemical properties, especially those affecting their affinity with different analytes, may be remarkably different from the observed behavior of conventional microstructured oxides [17]. These materials, which regularly show high specific area values, present an abundance of surface hydroxyl groups that can bind to analyte trace metals due to its strong chemical activity enabling a high retention capacity [19]. Solid phases with these characteristics make possible to build columns with small volume and high retention capacity, which enables in turn the determination of the analyte at trace levels, and using low volumes of eluent and wastes. Thus, micro- and mini-columns packed with nanometer-sized materials have been used for speciation of dissolved inorganic and organic Se species [20], simultaneous determination of trace metals (V, Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb) [17]. Moreover, nanostructured TiO_2 has been employed for speciation of Cr(III) and Cr(VI) in environmental samples [21,22]. Likewise, the advantage of nanostructured Al_2O_3 are their known properties as material for retention plus the high specific area values [18].

In this work, the application of a microcolumn packed with nanostructured α-Al_2O_3 was evaluated for the on-line separation and preconcentration of Cr(VI) and Cr(III). The nanostructured material was obtained by a fuel-rich nitrate–glycine gel-combustion method [23]. Finally, the nanostructured oxide was used as a solid-phase retention material in an on-line preconcentration system for Cr(III) and Cr(VI) species determination in parenteral solutions samples by ETAAS.

Experimental

Instrumentation

The measurements were performed with a Perkin Elmer (Uberlingen, Germany) Model 5100 ZL atomic absorption spectrometer, equipped with a transversely heated graphite atomizer, a Zeeman-effect background correction system and a Cr hollow cathode lamp. The ETAAS instrumental and operating conditions that

Table 1. Instrumental and experimental conditions for Cr species determination.

Instrumental conditions	
Wavelength	357.9 nm
Spectral band width	0.7 nm
Lamp current	25 mA
Injection mode	Automatic
Sample volume	20 μL
Matrix modifier	30 μg $Mg(NO_3)_2$

Graphite furnace temperature program

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying	110	1	30	250
Drying	130	15	30	250
Pirolysis	1500	10	20	250
Atomization	2300	0	3	–
Cleaning	2500	1	2	250

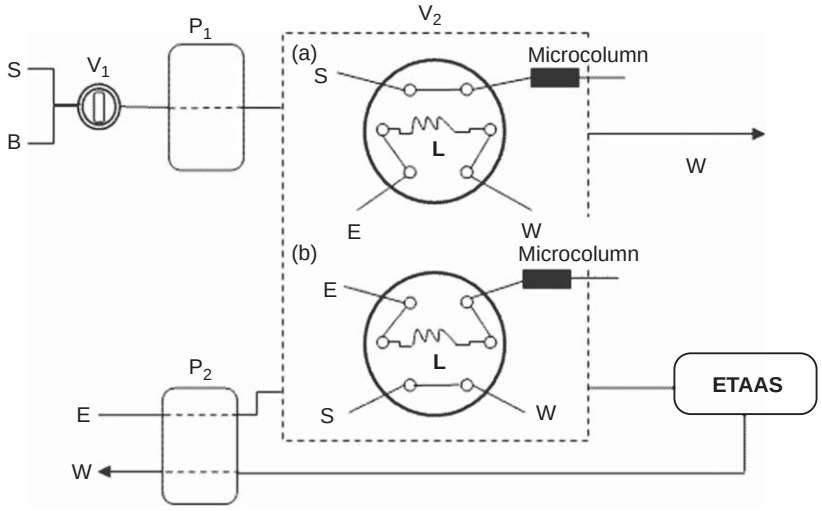

Fig. 1. Schematic diagram of the instrumental setup. S, sample, E, eluent; W, waste; P_1 and P_2, peristaltic pumps; V_1, six-way valve; and V_2, six-way valve. (a) Load position and (b) injection position.

provided the best sensitivity for Cr signal are listed in Table 1.

The flow-injection system is shown in Fig. 1. A Minipuls 3 peristaltic pump (Gilson, Villiers Le- Bell, France) was used. The sample injection was achieved using a six-way rotary valve (Upchurch Scientific, Oak Harbor, WA, USA).

Tygon-type pump tubes (Gilson Inc., Middleton, WI, USA) were employed to propel the sample, reagent and eluent.

Reagents and chemicals

Individual stock standard solutions of 1000 mg L^{-1} Cr were prepared from 7.6930 g chromium nitrate (99.99%) ($Cr(NO_3)_3 \cdot 9H_2O$) (Merck, Darmstadt, Germany) for Cr(III) and 2.8287 g potassium dichromate (99.5%) ($K_2Cr_20_7$) (Aldrich, Milwaukee, WI, USA) for Cr(VI). The Cr salts were dissolved in ultrapure water and diluted to 1000 mL with a final HNO_3 concentration of 0.05 mol L^{-1}. Working solutions were prepared by dilution of these stock standard solutions. Ammonia (Aldrich) was used for elution of Cr(VI) retained in the column. A 1 mol L^{-1} nitric acid solution was prepared from HNO_3 (65% w/w) (Merck) and used for elution of Cr(III). A 0.5 mol L^{-1} nitric acid solution was prepared from HNO_3 (65% w/w) (Merck) as a carrier for conditioning and regeneration of the column-filling material. Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France).

All reagents were of analytical reagent grade and the presence of Cr was not detected in the working range. All bottles used for storing samples and standard solutions and the glassware were washed in 10% (v/v) nitric acid for 24 h and later rinsed with ultrapure water.

Synthesis and characterization of nanostructured α-Al_2O_3

The nanostructured packing material was obtained by a nitrate–glycine gel-combustion method. The combustion route requires a minimum of laboratory equipment and infrastructure, yielding a very acceptable amount of ultrafine and compositionally homogeneous powders of the nanostructured oxide [24,25]. For this work, aluminum flakes (Merck) were dissolved in a slight excess of concentrated nitric acid (Merck). Once the metal was dissolved, this concentrated mixture was diluted (1:3) with distilled water. A little amount of insoluble residue was observed in the diluted mixture (due to preexisting surfacial aluminum oxide) and hence removed by filtration. The resulting solution of aluminum nitrate in nitric acid was added with a proper amount of glycine (Merck), in a ratio of aprox. 2 mol of glycine per mol of aluminum. Once the fuel was dissolved, the pH was increased by means of the addition of ammonium hydroxide (Merck) until pH 4.

The so-prepared, precursor solution remains homogeneous during the subsequent thermal concentration on a hot plate at 250 °C. In this concentration stage, as the water content of the solution became critical, the preparation turned into a fused gel, which soon evolved as a foam and, at last, ignited in a self-sustained combustion stage. When this brief (30 s approx.) combustion stage ceased, the resulting ashes were collected and calcined in an open air oven at 1200 °C for 2 h.

After calcination, the stabilized α-Al_2O_3 powders were characterized by X-ray diffractometry (XRD) (Philips PW 3710 diffractometer, Eindhoven, The Netherlands, Holland) and observed by scanning electron microscopy (SEM) (LEO 1450 VP, Zeiss, Germany), a BET surface area preliminary value of 25 $m^2 g^{-1}$ was also obtained for this material (Micromeritics Accusorb 2100E Micromeritics Instrument Corporation, Norcross, GA, USA). In Fig. 2(a) is shown a SEM micrography of the powder exhibiting the morphology obtained as result of the gel-combustion method. In the insert of Fig. 2(b) is plotted its XRD spectrum, which clearly is assigned to a corundum, α-Al_2O_3 phase [26]. From this spectrum, and employing the Scherrer equation [27], a crystallite size of 130 nm was evaluated.

Preparation of the nanometer-sized α-alumina-packed microcolumn

Before any use, the nanometer-sized α-Al_2O_3 was individually washed with 1 mol L^{-1} ammonia, 1 mol L^{-1} nitric acid, and finally water. A glass column (3 mm i.d. and 25 mm length) was used for preconcentration. An amount of 6.6 mg of α-Al_2O_3 was suitable to pack the column up to an effective bed length of 16 mm. The filling material was finally washed with ultrapure water followed by conditioning with 0.5 mol L^{-1} nitric acid solution.

Separation and preconcentration procedure

The procedure for speciation analysis was based on the retention of Cr(VI) and Cr(III), followed by sequential elution of each species. Initially, the column was conditioned for preconcentration at the correct pH with 0.5 mol L^{-1} nitric acid, valve V_1 in position B (Fig. 1). A sample volume of 25 mL was then loaded on the nanometer-sized α-Al_2O_3 microcolumn at a flow rate of 5 mL min^{-1}, with valve V_1 in S position and valve V_2 in load position (a). After the loading time, the sample still present in the lines and the column was removed, with valve V_1 again in position B. Finally, valve V_2 was switched to the injection position (b) and Cr species were sequentially eluted. The elution was performed with a 400 μL PTFE loop. In a first step, the microcolumn was loaded with ammonia and kept in contact with the retention material for 5 min. After this time Cr(VI) species was eluted at 5 mL min^{-1} directly into the autosampler cup of the ETAAS instrument for Cr determination. In a second step, the valve V_2 was switched-back to the load position (a) and the loop was this time filled with 1 mol L^{-1} nitric acid. Finally, the valve V_2 was switched to the injection position (b) and the eluent solution transferred into the microcolumn for 5 min. Then, Cr(III) species was eluted into the autosampler cup for Cr determination by ETAAS.

Samples collection and conditioning

Samples analyzed were collected from the Argentinean market. The samples under study were: Ringer physiological solution (100 mL: 0.86 g NaCl, 0.03 g KCl, 0.03 g $CaCl \cdot 2H_2O$, H_2O); $NaHCO_3$; parenteral solution (100 mL: 8.4 g $NaHCO_3$, H_2O) and dextrose 10% physiological solution (100 mL: 10 g D-glucose monohydrate, H_2O).

Results and discussion

Study of loading variables

The pH value plays an important role regarding the predominant species of Cr(VI) and Cr(III) in solution

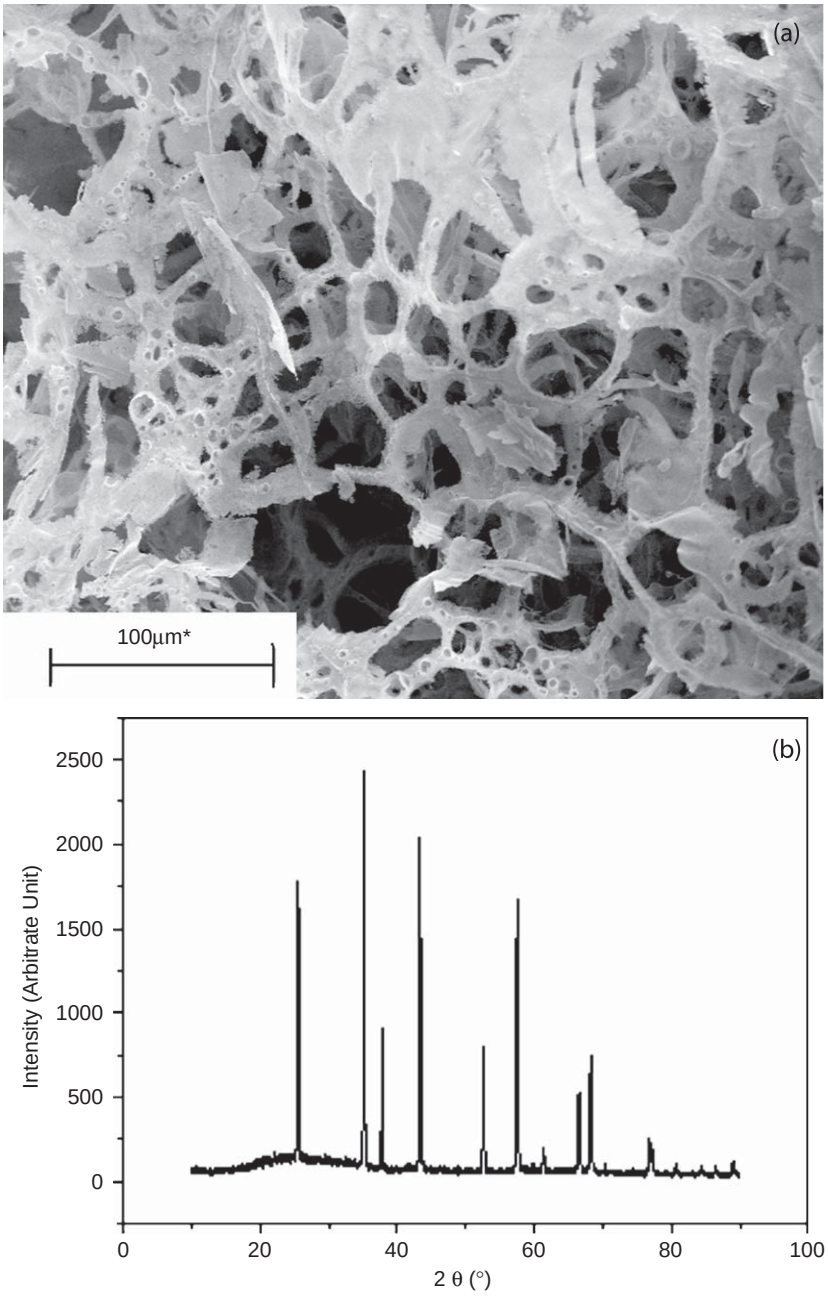

Fig. 2. (a) SEM micrography of α-Al_2O_3 nanostructured and (b) XRD plot of α-Al_2O_3 nanostructured.

[28]. In order to evaluate the effect of pH on the retention of Cr on nanostructured α-Al_2O_3, the pH of sample solutions was adjusted between 1.5 and 11.5 with nitric acid or ammonia. The results are shown in Fig. 3. Considering these results, the selected pH was 4.0. At this pH, the predominant species are: chromate ion (CrO_4^{2-}) for Cr(VI) and, Cr^{3+} and $CrOH^{2+}$ for Cr(III) [28].

The use of a buffer solution to keep a constant pH of 4 was also investigated. In this case, two different buffers solutions were tested: sodium acetate/acetic acid (in the concentration range of 6.7×10^{-3}–$0.13\,mol\,L^{-1}$) and potassium phthalate/phthalic acid ($5 \times 10^{-4}\,mol\,L^{-1}$). When these buffers systems were used, a drop in sensitivity was noted due to low analyte retention. The effect was more notorious for Cr(VI) than Cr(III). This phenomenon can be explained considering a possible competition between phthalate or acetate ions with Cr(VI), and H^+, Na^+ or K^+ ions with Cr(III), by the active sites of the nanostructured

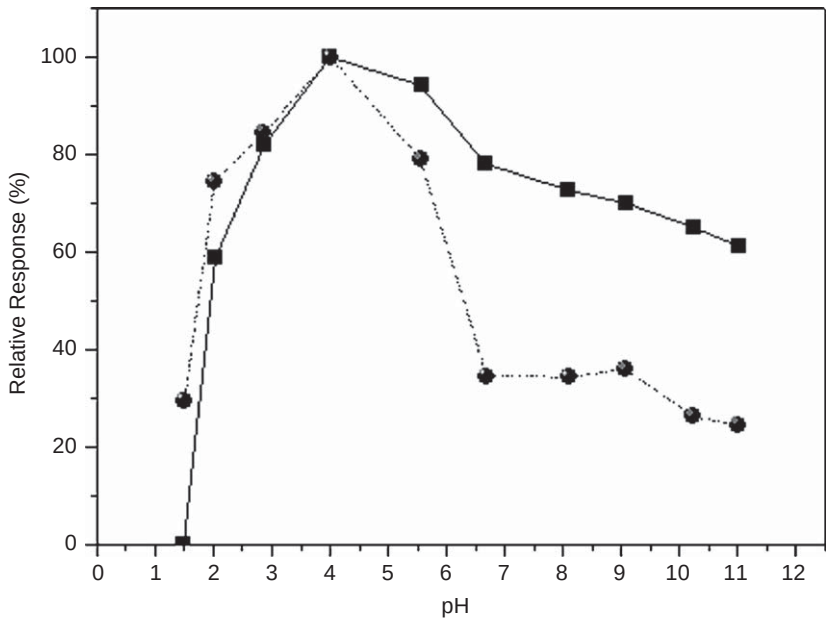

Fig. 3. Effect of pH of loading solutions vs. the retention of Cr on nanostructured α-Al_2O_3. (–■–) Cr(III) eluted with nitric acid (–●–) Cr(VI) eluted with ammonia. Preconcentration of 25 mL of 0.2 μg L^{-1} Cr(VI) and 2 μg L^{-1} Cr(III) at pH 4.0. Other conditions were as shown in Table 1.

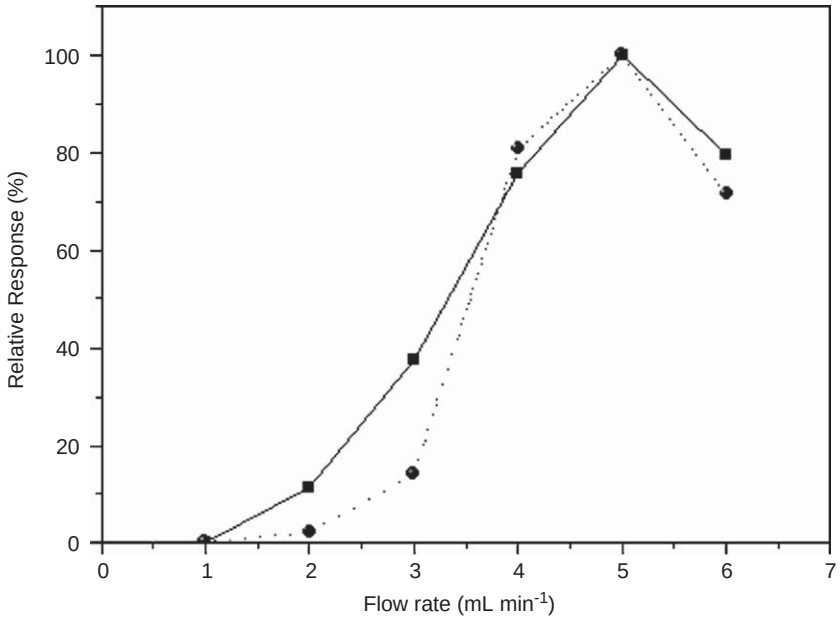

Fig. 4. Effect of sample loading flow rate on Cr retention. (–■–) Cr(III) eluted with nitric acid (–●–) Cr(VI) eluted with ammonia.

α-Al_2O_3. This is comparable with results obtained by Pannain et al. [29]. Therefore, pH was adjusted by the sole addition of proper amounts of acid or base.

A flow rate varying between 1 and 6 mL min^{-1} was found to be suitable for optimum loading on the nanometers α-Al_2O_3. The best result was obtained with a flow rate of 5 mL min^{-1} (Fig. 4).

Elution of Cr species from the nanostructured material

Different eluting agents, and at different concentration levels, were assayed to elute Cr species retained on the nanostructured α-Al_2O_3. For Cr(VI), ammonium hydroxide at concentrations ranging from 0.5 mol L^{-1} to

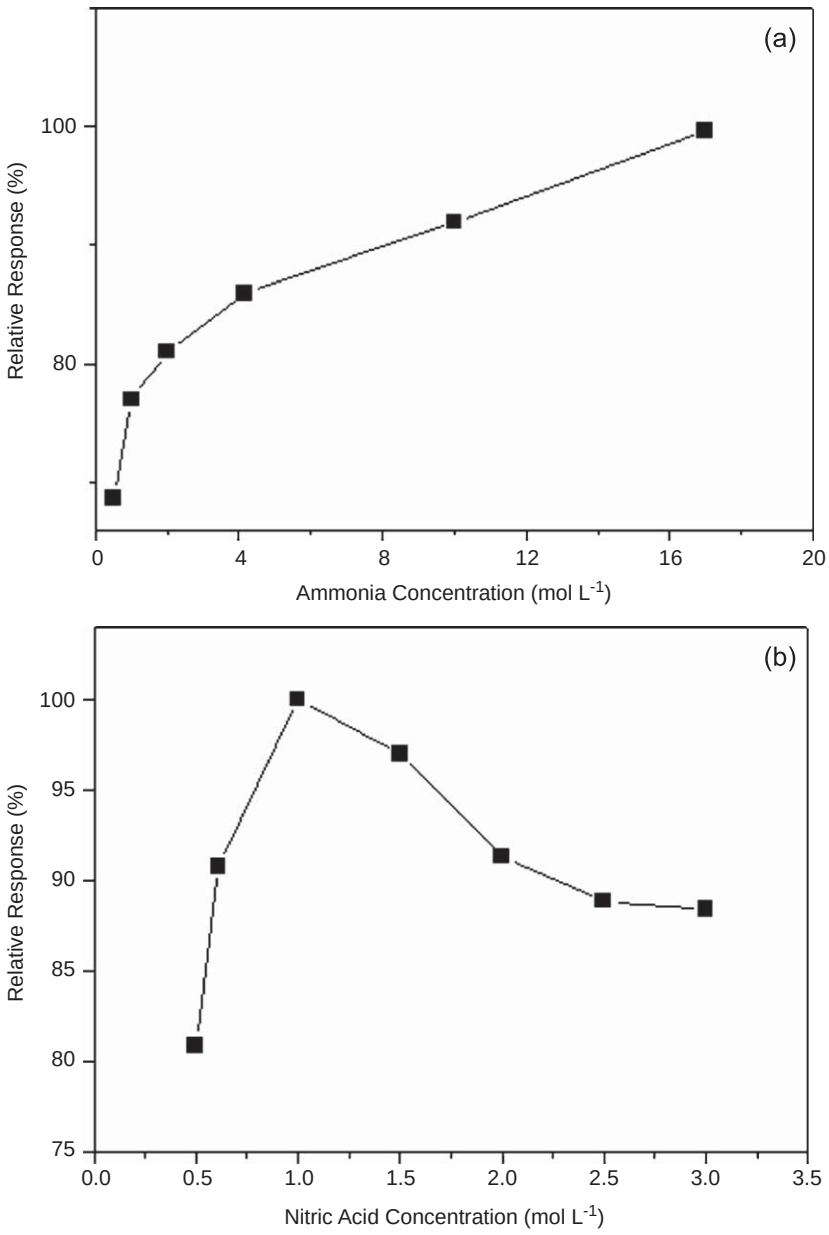

Fig. 5. Effect of eluent concentration on the analytical response. (a) Cr(VI) eluted with ammonia and (b) Cr(III) eluted with nitric acid.

concentrated ammonia was evaluated. On the other hand, elution of Cr(III) species was studied using nitric acid solution with concentrations ranging from 0.5 to 3 mol L^{-1}. As it is shown in Fig. 5, the highest efficiency for Cr elution from the column was achieved with concentrated ammonia for Cr(VI) (Fig. 5(a)) and 1 mol L^{-1} nitric acid for Cr(III) (Fig. 5(b)).

An important variable to be optimized was the contact time of the eluent with the nanostructured α-Al_2O_3 required for total removal of the Cr species from the microcolumn. Thus, it was observed that a minimal contact time was needed to achieve total elution of both Cr species. Elution was performed by a stopped-flow procedure filling the column with eluent and keeping it for 5 min. This was necessary due to the low rate of the ionic exchange process.

The volume of eluent required for complete analyte removal from the column was evaluated by loading 10 mL of a solution of 0.2 mg L^{-1} of Cr(VI) and 2 mg L^{-1} of Cr(III) and secondly performing the elution. Loops with volumes of 80, 200, 400 and 600 μL were used for these experiments. The best results were reached with a loop of 400 μL. No improvement of analyte elution was observed for a higher volume. On the other hand, incomplete analyte elution was observed when the eluent volume was lower than 400 μL.

Retention efficiency and preconcentration factor

Retention percentages of 95.8% and 73.8% for Cr(VI) and Cr(III), were achieved when the procedure was carried out under optimum experimental conditions (Table 1). Therefore, preconcentration factors of 41 and 18 were achieved for Cr(VI) and Cr(III), respectively, with a sample volume of 25 mL. For determination of retention capacity, a 50 mL portion of 60 mg L^{-1} of total Cr solution was adjusted to pH 4 with nitric acid and shaken with 50 mg of alumina in a glass flask for 30 min. Retention capacity of alumina material was found to be 57.5 mg Cr g^{-1} of dried material.

Analytical performance and determination of Cr species

In order of assess the possible interference between Cr species it was applied to several standard solutions with different concentration ratios of the two oxidation states. Table 2 indicates that Cr(III) and Cr(VI) species were completely separated and quantitatively recovered. The method was thus shown to have an acceptable selectivity under different conditions. Likewise, the effects of representative potential interfering species (at the concentration levels at which they may occur in the sample studied) were tested. The recovery of the analyte was not significantly influenced by anions such as CO_3^{2-}, Cl^-, SO_4^{2-} and PO_4^{3-}; and cations like Fe^{3+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+}. This probably is due to the high retention capacity of the nanostructured material that avoided fast and total saturation of the column.

After separation and preconcentration of Cr species by the proposed procedure, the calibration graphs for ETAAS determination were linear, achieving a relative standard deviation (RSD) of 8.6% for Cr(VI) and 6.1% for Cr(III) ($c = 10\,\mu g\,L^{-1}$, $n = 10$, sample volume = 25 mL). The calibration graph showed a correlation coefficient of 0.999 for both species. The limits of detection (LOD), calculated based on three times the standard deviation of the background signal (3σ), was 1.9 ng L^{-1} for Cr(VI) and 6.1 ng L^{-1}. The preconcentration factor was obtained as the ratio of the slopes of the calibration curves for Cr with and without the

Table 2. Evaluation of the separation of Cr(VI) and Cr (III) species (t-test; 95% confidence interval; $n = 6$).

Chromium (VI)			Chromium (III)		
Added ($\mu g\,L^{-1}$)	Found ($\mu g\,L^{-1}$)	Recovery (%)[a]	Added ($\mu g\,L^{-1}$)	Found ($\mu g\,L^{-1}$)	Recovery (%)[a]
30	29.80	99.3	0	0.00	0.0
20	19.98	99.9	2	1.95	97.5
10	9.89	98.9	10	9.87	98.7
2	1.93	96.5	20	19.89	99.4
0	0.00	0.0	30	29.96	99.9

[a] 100 × (found/added).

Table 3. Determination of Cr(VI) and Cr(III) species in parenteral solutions samples (*t*-test; 95% confidence interval; $n = 6$)*.

Sample	Chromium(III)			Chromium(VI)		
	Added ($\mu g\,L^{-1}$)	Found ($\mu g\,L^{-1}$)	Recovery (%)[a]	Added ($\mu g\,L^{-1}$)	Found ($\mu g\,L^{-1}$)	Recovery (%)[a]
1[b]	0	2.61 ± 0.41	–	0	nd	–
	2	4.59 ± 0.74	99.0	2	1.99 ± 0.24	99.5
2[c]	0	0.29 ± 0.04	–	0	nd	–
	2	2.29 ± 0.36	100.0	2	1.94 ± 0.23	97.0
3[d]	0	3.62 ± 0.57	–	0	nd	–
	2	5.60 ± 0.89	99.0	2	1.96 ± 0.23	98.0

nd: not detected.
*Uncertainties expressed as one standard deviation (1σ).
[a] 100 × ((found-sample)/added).
[b] Ringer physiological solution.
[c] Parenteral solution.
[d] Dextrose 10% physiological solution.

preconcentration step. The accuracy of the proposed method was evaluated by analyzing a standard reference material, NIST SRM 1643e ''Trace Elements in Water'', with a reported Cr content of $20.40 \pm 0.24\,\mu g\,L^{-1}$. Using the proposed methodology the Cr content determined in this SRM was $20.26 \pm 0.96\,\mu g\,L^{-1}$.

The results of the method applied to Cr(III) and Cr(VI) determination in parenteral solutions samples were in the range of 0.29–3.62 $\mu g\,L^{-1}$ for Cr(III); while Cr(VI) was not detected on any sample. The results are shown in Table 3. Concentration levels observed in this work were not significantly different to those reported by Gil et al. [7] and Pluhator-Murton et al. [10] for Cr(III) and Cr(VI) species.

Conclusion

The application of this system, using nanostructured α-Al_2O_3 as packing material, allowed the separation and determination of Cr(III) and Cr(VI) species in parenteral solutions in one step. The determination was possible at levels as low as $\mu g\,L^{-1}$ with good accuracy and good reproducibility. The manifold presented provided an enrichment factor of 41 for Cr(VI) and 18 for Cr(III) as consequence of good retention (95.8% and 73.8% for Cr(VI) and Cr(III), respectively) of Cr species derived from the high superficial area of the nanostructured α-Al_2O_3. Thus, this flow-injection system consisting of a microcolumn filled with this high superficial area material is presented for the on-line retention and separation of the species leading to fast preconcentration and analysis processes, low consumption of reagents and sample as well as faster speciation analysis. Finally, the applicability of the proposed method was demonstrated by performing Cr speciation analysis in several parenteral solutions.

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References

[1] Priego-Capote F, Luque de Castro MD. Speciation of chromium by in-capillary derivatization and electrophoretically mediated microanalysis. J Chromatogr A 2006; 1113:244–50.

[2] Gil RA, Cerutti S, Gásquez JA, Olsina RA, Martinez LD. Preconcentration and speciation of chromium in drinking water samples by coupling of on-line sorption on activated carbon to ETAAS determination. Talanta 2006;68:1065–70.

[3] Kot A, Namiesnèik J. The role of speciation in analytical chemistry. Trend Anal Chem 2000;19:69–79.

[4] Kotas J, Stasicka Z. Chromium occurrence in the environment and methods of its speciation. Environ Pollut 2000;107:263–83.

[5] Bag H, Turker AR, Lale M, Tunceli A. Separation and speciation of Cr(III) and Cr(VI) with *Saccharomyces cerevisiae* immobilized on sepiolite and determination of both species in water by FAAS. Talanta 2000;51:895–902.

[6] Wen B, Shan XQ, Lian J. Separation of Cr(III) and Cr(VI) in river and reservoir water with 8-hydroxyquinoline immobilized polyacrylonitrile fiber for determination by inductively coupled plasma mass spectrometry. Talanta 2002;56:681–7.

[7] Gil RA, Cerutti S, Gásquez JA, Olsina RA, Martinez LD. On-line preconcentration and determination of chromium in parenteral solutions by inductively coupled plasma optical emission spectrometry. Spectrochim Acta B 2005; 60:531–5.

[8] Wuilloud GM, Wuilloud RG, De Wuilloud JCA, Olsina RA, Martinez LD. On-line preconcentration and determination of chromium in parenteral solutions by flow injection-flame atomic absorption spectrometry. J Pharmaceut Biomed 2003;31:117–24.

[9] Shenkin A. Basics in clinical nutrition: Trace elements and vitamins in parenteral and enteral nutrition. e-SPEN 2008;3.

[10] Pluhator-Murton MM, Fedorak RN, Audette RJ, Marriage BJ, Yatscoff RW, Gramlich LM. Trace element contamination of total parenteral nutrition. 1. Contribution of component solutions. J Parenter Enteral Nutr 1999;23:222–7.

[11] Gunnar F Nordberg, Bruce A Fowler, Monica Nordberg, Friberg L. Handbook on the toxicology of metals. Atlanta, GA, USA: Elsevier; 2007.

[12] Gomez V, Callao MP. Chromium determination and speciation since 2000. TrAC Trends Anal Chem 2006;25:1006–15.

[13] Prasad K, Praveen RS, Prasada Rao T, Gopikrishna P, Naidu GRK. Sequential preconcentrative separation of Cr(VI) and Cr(III) species from tannery effluents and subsequent determination by flame MS. Atom Spectrosc 2005;26:173–81.

[14] Agrawal YK. Liquid–liquid extraction, separation recovery and transport of tantalum by crown-ether. Talanta 2002;58:875–82.

[15] Mohammadhosseini M, Tehrani MS, Ganjali MR. Preconcentration, determination and speciation of chromium(III) using solid phase extraction and flame atomic absorption spectrometry. J Chin Chem Soc—Taip 2006; 53:549–57.

[16] Hagendorfer H, Goessler W. Separation of chromium(III) and chromium(VI) by ion chromatography and an inductively coupled plasma mass spectrometer as element-selective detector. Talanta 2008;76:656–61.

[17] Yin J, Jiang Z, Chang G, Hu B. Simultaneous on-line preconcentration and determination of trace metals in environmental samples by flow injection combined with inductively coupled plasma mass spectrometry using a nanometer-sized alumina packed micro-column. Anal Chim Acta 2005;540:333–9.

[18] Pazos-Capeáns P, Barciela-Alonso MC, Bermejo-Barrera A, Bermejo-Barrera P, Fisher A, Hill SJ. On-line sequential determination of Cr(III) and Cr(VI) with selective elution of solid extracts using an alumina column. Atom Spectrosc 2006;27:107–16.

[19] Liu R, Liang P. Determination of trace lead in water samples by graphite furnace atomic absorption spectrometry after preconcentration with nanometer titanium dioxide immobilized on silica gel. J Hazard Mater 2008;152:166–71.

[20] Huang C, Hu B, He M, Duan J. Organic and inorganic selenium speciation in environmental and biological samples by nanometer-sized materials packed dual-column separation/preconcentration on-line coupled with ICP–MS. J Mass Spectrom 2008;43:336–45.

[21] Liang P, Qin Y, Hu B, Peng T, Jiang Z. Nanometer-size titanium dioxide microcolumn on-line preconcentration of trace metals and their determination by inductively coupled plasma atomic emission spectrometry in water. Anal Chim Acta 2001;440:207–13.

[22] Liang P, Shi T, Lu H, Jiang Z, Hu B. Speciation of Cr(III) and Cr(VI) by nanometer titanium dioxide micro-column and inductively coupled plasma atomic emission spectrometry. Spectrochim Acta B 2003;58:1709–14.

[23] Lascalea GE, Lamas DG, Djurado E, Cabanillas ED, Walsöe De Reca NE. Comparative study of nanocrystalline Zr0.85Ce 0.15O_2 powders synthesised by spray-pyrolysis and gel-combustion methods. Mater Res Bull 2005;40:2029–38.

[24] Juárez RE, Lamas DG, Lascalea GE, Walsöe De Reca NE. Synthesis and structural properties of zirconia-based nanocrystalline powders and fine-grained ceramics. Defect Diffus Forum 2000;177:1–26.

[25] Juárez RE, Lamas DG, Lascalea GE, Walsöe De Reca NE. Synthesis of nanocrystalline zirconia powders for TZP ceramics by a nitrate–citrate combustion route. J Eur Ceram Soc 2000;20:133–8.

[26] Lewis J, Schwarzenbach D, Flack HD. Electric field gradients and charge density in corundum, [alpha]-Al_2O_3. Acta Crystallogr A 1982;38:733–9.

[27] Klug HP, Alexander LE. X-ray diffraction procedures for polycrystalline and amorphous materials. J Appl Crystallogr 1975;8:573–4.

[28] Sperling M, Xu S, Welz B. Determination of chromium(III) and chromium(VI) in water using flow injection on-line preconcentration with selective adsorption on activated alumina and flame atomic absorption spectrometric detection. Anal Chem 1992;64:3101–8.

[29] Pannain MC, Santelli RE. On the spectrophotometric flow injection determination of chromium(VI) in natural waters after on-line preconcentration on activated alumina. Talanta 1995;42:1609–17.