



Novel on-line sequential preconcentration system of Cr(III) and Cr(VI) hyphenated with flame atomic absorption spectrometry exploiting sorbents based on chemically modified silica

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ABSTRACT

In the present study, a flow injection system using dual mini-columns, SiO₂/Al₂O₃/TiO₂ and silica gel functionalized with [3-(2-aminoethylamino)propyl] trimethoxysilane (SiO₂/AAPTMS) for the sequential preconcentration of Cr(III) and Cr(VI), respectively, from water samples with FAAS detection was proposed. A two-level full factorial design (2⁴) and desirability function were employed for the optimization of variables related to the system performance. The detection limits of 0.66 and 0.27 μg L⁻¹ for Cr(III) and Cr(VI), respectively, were obtained under the optimized preconcentration conditions (flow rate of 7.0 mL min⁻¹, pH 5.0, buffer concentration (acetate buffer) of 0.01 mol L⁻¹, and eluent (2.5 mol L⁻¹ HCl) flow rate of 5.0 mL min⁻¹). The other parameters including preconcentration factor (PF), consumptive index (CI), and concentration efficiency (CE) were found to be 17.62/32.98, 1.13/0.6 mL, and 6.2/11.54 min⁻¹ for Cr(III)/Cr(VI), respectively. The developed method was applied to the Cr(III) and Cr(VI) determination in water samples [tap, lake and mineral water, artificial saliva and parenteral solutions (physiological serum, water for injection, and glucose physiological solution)]. The method accuracy was checked by the analysis of standard reference materials (trace elements in water).

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1. Introduction

Cr(III) is considered to be an essential trace nutrient for humans, and it plays an important role in lipid, glucose and protein metabolism. Conversely, Cr(VI) is highly toxic, since it is a strong oxidant and also a potential carcinogen. The difference in toxicity between Cr(VI) and Cr(III) leads to a great interest in the speciation and determination of chromium species in environmental and biological samples [1]. Cr(III) is often used as a dietary supplement for some patients with diabetes, and absorption rates of this reduced form of chromium can be evaluated by monitoring its urinary levels. The normal chromium level in urine should be less

than 1.0 μg L⁻¹, whereas for occupationally exposed patients and complementary diets it may vary from 20 to 30 μg L⁻¹ [2]. The Brazilian National Council for the Environment (CONAMA) fixed the maximum amount of Cr(III) and Cr(VI) in effluents discharged into aquatic bodies at 0.1 and 1.0 mg L⁻¹, respectively [3]. The maximum contaminant levels established by the US Environmental Protection Agency (US EPA) for total chromium in drinking water and by the World Health Organization (WHO) for Cr(VI) are 0.1 and 0.05 mg L⁻¹, respectively [3,4]. Therefore, the development of sensitive and selective methods for the chromium speciation is of great importance, and some solid-phase separation/preconcentration systems coupled to spectroanalytical techniques (FAAS, GFAAS, and ICP-OES) have been employed to remove matrix interferences and improve the detectability.

In some studies, Cr(III) was selectively sorbed on a β-cyclodextrin cross-linked polymer as a 4-(2-pyridylazo)-resorcinol complex and then eluted with 1.0 mol L⁻¹ HCl [5]. Since Cr(VI) does not form complexes with 4-(2-pyridylazo)-resorcinol,

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it remains in the aqueous phase. Total chromium was determined by graphite furnace atomic absorption spectrometry (GFAAS) after the reduction of Cr(VI) to Cr(III) using hydroxylamine hydrochloride in 2.0 mol L⁻¹ HCl, and the Cr(VI) concentration was calculated by subtracting the value for Cr(III) from that for total chromium. Other researchers employed MCM-41 mesoporous silica functionalized with 3-aminopropyl groups for the selective retention of Cr(VI) in its anionic form at pH 2.0 [6], which was eluted with a mixture of 0.1 mol L⁻¹ hydroxylammonium chloride and 1 mol L⁻¹ hydrochloric acid and determined by GFAAS, whereas Cr(III), not retained by the sorbent, was quantified after collecting the solution at the micro-column end [6]. In other works, Cr(VI) and Cr(III) were selectively sorbed from aqueous solutions at pH 2 and 6, respectively, using Duolite C20 modified with active hydrazone [7]. Similar experiments were carried out with activated alumina for the Cr(III) and Cr(VI) retention at pH 7.0 and pH 2.0, respectively [8]. Besides, using ethanol as eluent, Cr(III) was sorbed as dithionated chelate on a Chromosorb 108 resin, and Cr(VI) was reduced by concentrated H₂SO₄ for the total chromium determination [9]. This approach was also applied for the Cr(VI) sorption on polytetrafluoroethylene (PTFE) as a metal complex with ammonium pyrrolidine dithiocarbamate (APDC) using isobutyl methyl ketone (IBMK) as eluent [10].

These methods have some unavoidable shortcomings regarding the chemical speciation, since they require sample pH changes or chemical treatments in order to determine total chromium amounts. To some degree, dual mini-columns can be used for solving such problems. Some authors employed silica gel modified with zirconium phosphate (SiO₂-ZrP) or cation-exchange iminodiacetate extraction disks and zirconium-oxide-modified silica (SiO₂-Zr) or anion-exchange iminodiacetate extraction disks, respectively, for the Cr(III) and Cr(VI) sequential sorption at different pH values [11,12]. For these methods, aggressive sample pretreatment procedures are not necessary, but only the sample pH readjustment is required for an effluent from the first sorbent, which makes the method performance somewhat complicated and results in the sample contamination. In other cases, it was possible to use dual mini-columns for the chromium speciation without readjusting the sample pH, but a reducing agent was still required in the preconcentration system [4,13]. Therefore, the development of sequential preconcentration methods using dual columns can be very desirable for the chemical speciation using minimal sample treatment, although such strategies have been poorly implemented. In some other works, Cr(III) and Cr(VI) were determined by ICP OES after their sequential retention on a ME-03 commercial resin and chitosan cross-linked with 4-hydroxyphthalic acid modified ethyleneglycoldiglycidylether (CCTS-HPA resin) at pH 3.5 [14]. Zou et al. [15] employed a biosorbent (*Chlorella vulgaris*) and 717 anion exchange resin to selectively retain Cr(III) and Cr(VI) at pH 6.0. Considering the aforementioned, in order to preconcentrate Cr(III) and Cr(VI) species at the same sample pH, the present research focuses on the synthesis of two sorbent materials based on the chemical modification of a silica matrix with Al₂O₃/TiO₂ and [3-(2-aminoethylamino) propyltrimethoxysilane (AAPTMS), respectively. Silica gel was chosen as a support material due to its intrinsic characteristics, such as large surface area, high adsorption capacity and absence of swelling effects at high flow rates, which are very important in on-line preconcentration systems. Apart from the fact that the chemical modification brings more selectivity to silica gel, our research group has recently reported that grafting with mixed oxides leads to a significant improvement in its chemical stability and adsorption capacity [16–18]. Finally, considering the nature of the on-line sequential preconcentration system developed herein for the Cr(III) and Cr(VI) determination,

chemometric tools were implemented based on a factorial design and multiple response optimization.

2. Experimental

2.1. Instrumentation

All absorbance measurements were carried out using a Shimadzu AA-6601 flame atomic absorption spectrometer (Tokyo, Japan) equipped with a hollow cathode lamp for chromium determination and deuterium lamp for background correction. The hollow cathode lamp was operated at 8.0 mA, and the wavelength was set at 357.8 nm. The flame composition was operated with an acetylene flow rate of 2.9 L min⁻¹ and air flow rate of 10.0 L min⁻¹. The on-line sequential preconcentration system was built up using an Ismatec IPC-08 peristaltic pump (Glattbrugg, Switzerland) equipped with Tygon[®] and polyethylene tubing (0.5 mm id). Two home-made minicolumns made of polyethylene (1.0 × 0.5 mm i.d.) for packing SiO₂/Al₂O₃/TiO₂ and (1.5 × 0.5 mm i.d.) for packing SiO₂/AAPTMS with end caps were used in the experiment. A home-made commutator injector was used for switching between the stages of preconcentration and elution.

Scanning electron micrographs (SEM) were acquired with a JEOL JSM 300 scanning electron microscope (Tokyo, Japan) operating at 30.0 kV. Samples were deposited onto a double-sided carbon tape fixed to a brass sample holder and subsequently coated with a 30-nm gold film. For energy dispersive spectroscopy (EDS) analyses, the same procedure was performed, excluding gold plating. The specific surface area (*S*₀) of the mixed oxide obtained was determined with a Quantachrome Nova 1200e equipment (Boynton Beach, Florida, US) and estimated by the multipoint BET (Brunauer–Emmett–Teller) method; the samples were preactivated at 300 °C for 2 h under vacuum. The average pore size and average pore volume were obtained through the BJH (Barrett–Joyner–Halenda) method. The Al₂O₃ and TiO₂ contents in the sample were measured with a Shimadzu EDX-800HS energy dispersive X-ray fluorescence (EDXRF) spectrometer (Tokyo, Japan). X-ray diffraction (XRD) data were collected at room temperature on a Rigaku Ultima IV instrument (The Woodlands, Texas, US) with CuK_α radiation (1.5418 Å) generated at 40 kV and 40 mA. Diffractograms were obtained in the range of 5° < 2θ < 80° with a step of 0.1 and a counting time of 1 s. Infrared spectra (range 4000–400 cm⁻¹) were recorded in the range of 4000–400 cm⁻¹ on a Shimadzu FTIR-8300 spectrometer (Tokyo, Japan) operating in transmission mode at 4 cm⁻¹ resolution and using KBr pellets (1% w/w). The sample pH was measured with a Metrohm 826 pH mobile digital pH meter (Herisau, Switzerland).

2.2. Reagents and solutions

All reagents used were of the highest available purity and prepared with ultrapure water (resistivity: 18.2 MΩ cm⁻¹) from a Milli-Q purification system (Millipore, Bedford, MA, US). To prevent any risk of metal contamination, all glassware was soaked in 10% (v/v) HNO₃ for 24 h and rinsed with high-purity water before use. 1000.0 mg L⁻¹ standard solutions of Cr(III) and Cr(VI) were prepared from CrCl₃·6H₂O (Vetec, Rio de Janeiro, Brazil) and K₂Cr₂O₇ (Merck, Darmstadt, Germany), respectively, dissolved in 5% HCl. The working solutions were obtained by stepwise dilution of the standard solutions in ultra-pure water. Solutions of HCl and HNO₃ were prepared by appropriate dilution of their concentrated solutions purchased from Merck (Darmstadt, Germany). Solutions of cationic [Al(III), Ba(II), Cd(II), Co(II), Pb(II), Ca(II), Mg(II), K(I), Cu(II), Fe(III), Mn(II), Ni(II), and

Zn(II)], used to study interference effects, were prepared from their respective nitrate and sodium salts, respectively (all of analytical grade). The solutions were stored in polyethylene bottles and stored in at 4 °C until analysis. The solution pH was adjusted with nitric acid to pH 1.0, sodium acetate/acetic acid to pH 3.0–5.0 and sodium phosphate to pH 7.0–8.0. Tetraethylorthosilicate (TEOS, 98%), titanium butoxide (97%), aluminum isopropoxide (98%) and trifluoroacetic acid were purchased from Sigma-Aldrich (Steinheim, Germany), and ethanol (PA) was acquired from Vetec (Rio de Janeiro, Brazil). Micrometer-sized silica (63–210 μm), with pore volume of 60 Å e surface area of 550 m² g⁻¹ and [3-(2-aminoethylamino)propyltrimethoxysilane (AAPTMS) were acquired from Sigma-Aldrich.

2.3. Al₂O₃/TiO₂ mixed oxide preparation and grafting on a silica matrix and silica functionalization with AAPTMS

The detailed protocol for the synthesis of Al₂O₃/TiO₂ mixed oxide grafted on silica and its chemical characterization can be found in reference [18]. The preparation of AAPTMS-functionalized silica gel was carried out according to reference [19]. Approximately 50 g of micrometer-sized silica gel was dried at 130 °C overnight, and 150 °C for 6 h under vacuum. Then, the gel was mixed with 30 mL of AAPTMS in 100 mL of toluene used as solvent. The reaction was maintained at 100 °C for 24 h under constant stirring in nitrogen atmosphere. After that, the mixture was filtered and washed with toluene and ethanol. The resulting material was washed with ethanol in a Soxhlet extractor for 6 h. Finally, the solid (SiO₂/AAPTMS) was dried at 60 °C for about 4 h under vacuum (at about 10⁻³ mmHg). The infrared spectra (data not shown) of the AAPTMS-functionalized silica gel showed a band at around 1580 cm⁻¹ attributed to the N–H bond, which was not observed for the non-modified silica material, thus indicating successful grafting of AAPTMS on the silica surface [20].

2.4. On-line sequential preconcentration system for the Cr(III) and Cr(VI) determination

In the on-line sequential preconcentration system coupled to FAAS (Fig. 1a and b), 20 mL of a solution containing Cr(III) and Cr(VI), buffered with 0.05 mol L⁻¹ acetate/acetic acid at pH 5.0, were loaded into the mini-columns, packed with SiO₂/Al₂O₃/TiO₂ (C₁, 200.0 mg) and SiO₂/AAPTMS (C₂, 200.0 mg) to retain Cr(III) and Cr(VI) species, respectively, at 7.0 mL min⁻¹. When Cr(III) was sorbed on SiO₂/Al₂O₃/TiO₂, a 2.5 mol L⁻¹ HCl solution was percolated simultaneously in countercurrent through the mini-column with SiO₂/AAPTMS at the same flow rate in order to strip off Cr(VI). Then, preconcentrated Cr(III) was stripped off with 2.5 mol L⁻¹ HCl by switching the injector to the elution position (Fig. 1b) and determined by FAAS. Therefore, in this procedure, Cr(III) and Cr(VI) were selectively preconcentrated on SiO₂/Al₂O₃/TiO₂ and SiO₂/AAPTMS, respectively, at the same pH (5.0) using the same eluent (2.5 mol L⁻¹ HCl).

2.5. Sample preparation

Water taken from Lake Igapó (Londrina, Brazil) was collected in polyethylene bottles and then directly filtered through 0.45 μm cellulose acetate membranes under vacuum. Mineral water (two different brands) was acquired from supermarkets, whereas tap water (1) was obtained from the Department of Chemistry of the State University of Londrina. Tap water samples (2 and 3) were taken at São José do Rio Preto City (São Paulo State, Brazil). Water for injection, acquired from the University Hospital of the State University of Londrina, and parenteral solutions, including physiological saline (NaCl 0.9% w/w, two different brands), glucose (5% w/w) and artificial saliva [21], were also examined. The sample pH was adjusted to pH 5.0 with a 0.01 mol L⁻¹

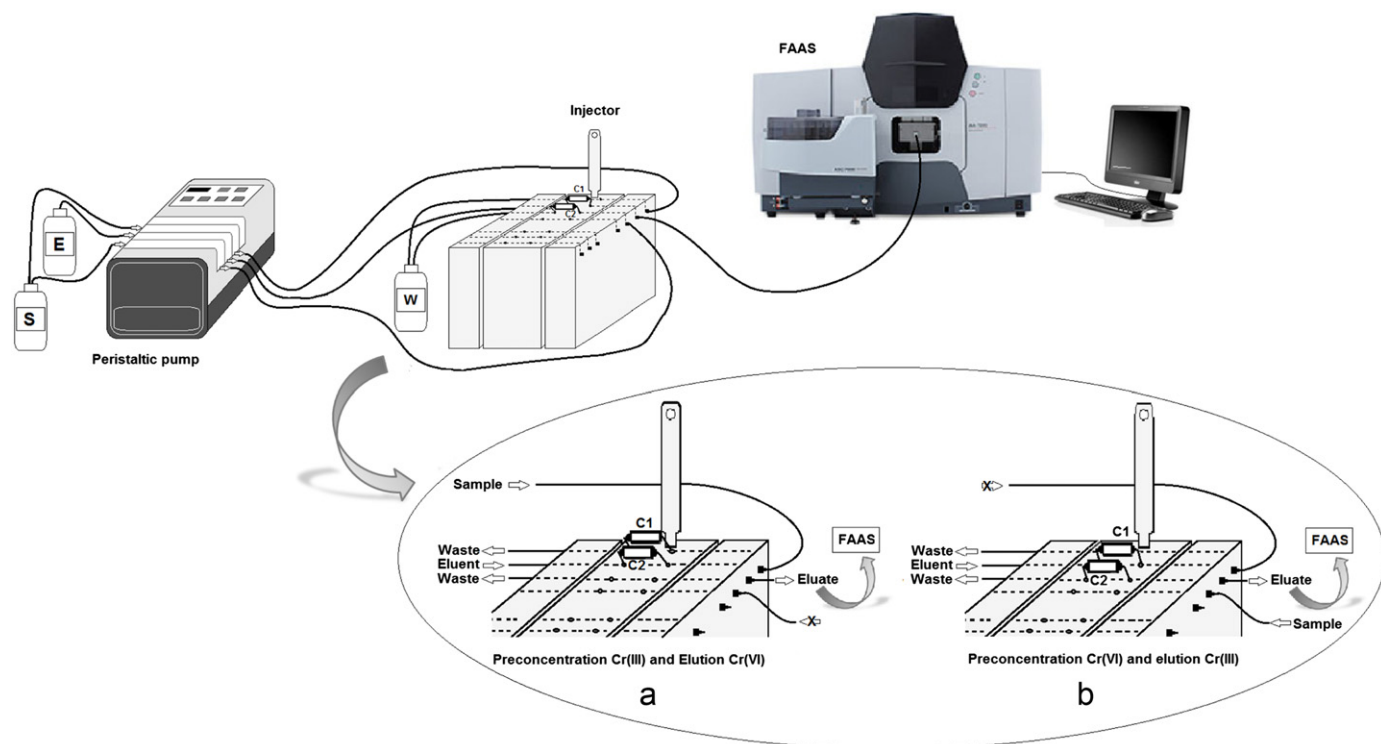


Fig. 1. Diagram of the flow system for preconcentration and sequential speciation of Cr(III) and Cr(VI) using mini-columns packed with SiO₂/Al₂O₃/TiO₂ and SiO₂/AAPTMS, respectively. (a) Preconcentration of Cr(III) and elution of Cr(VI), (b) Preconcentration of Cr(VI) and elution of Cr(III). PP=peristaltic pump, C1=mini-column packed SiO₂/Al₂O₃/TiO₂ (200 mg), C2=mini-column packed with SiO₂/AAPTMS (200 mg), W=waste, S=sample containing Cr(III) and Cr(VI), E=eluent (2.5 mol L⁻¹ HCl).

Table 1
Factors, levels, and experimental matrix of 2⁴ factorial design.

Factors						Levels		
						Low (-)	High (+)	
Buffer concentration (BC) (mol L ⁻¹)						0.01	0.10	
Eluent concentration (EC) (mol L ⁻¹)						0.8	2.5	
Flow preconcentration (FP) (mL min ⁻¹)						3.5	7.0	
Eluent type (ET)						HCl	HNO ₃	
Exp.	BC	EC	FP	ET	Absorbance			
					Cr(III)	Cr(VI)	Overall desirability	
1(9°)	-(0.01)	-(0.8)	-(3.5)	-(HCl)	0.1142	0.0916	0.5241	
2(16°)	+(0.10)	-(0.8)	-(3.5)	-(HCl)	0.0991	0.0634	0.2756	
3(11°)	-(0.01)	+(2.5)	-(3.5)	-(HCl)	0.1200	0.1734	0.8907	
4(15°)	+(0.10)	+(2.5)	-(3.5)	-(HCl)	0.1155	0.1895	0.8872	
5(4°)	-(0.01)	-(0.8)	+(7.5)	-(HCl)	0.0825	0.1035	0.1618	
6(5°)	+(0.10)	-(0.8)	+(7.5)	-(HCl)	0.0935	0.0573	0.2047	
7(1°)	-(0.01)	+(2.5)	+(7.5)	-(HCl)	0.1190	0.2050	0.9754	
8(6°)	+(0.10)	+(2.5)	+(7.5)	-(HCl)	0.1164	0.1884	0.8950	
9(10°)	-(0.01)	-(0.8)	-(3.5)	+(HNO ₃)	0.1033	0.0712	0.3447	
10(14°)	+(0.10)	-(0.8)	-(3.5)	+(HNO ₃)	0.0996	0.0360	4.400 × 10 ⁻⁹	
11(12°)	-(0.01)	+(2.5)	-(3.5)	+(HNO ₃)	0.1130	0.0922	0.5177	
12(13°)	+(0.10)	+(2.5)	-(3.5)	+(HNO ₃)	0.0928	0.0620	0.2203	
13(2°)	-(0.01)	-(0.8)	+(7.5)	+(HNO ₃)	0.1000	0.0670	0.2999	
14(7°)	+(0.10)	-(0.8)	+(7.5)	+(HNO ₃)	0.0798	0.0372	1.500 × 10 ⁻⁹	
15(3°)	-(0.01)	+(2.5)	+(7.5)	+(HNO ₃)	0.1210	0.1038	0.6334	
16(8°)	+(0.10)	+(2.5)	+(7.5)	+(HNO ₃)	0.0839	0.0585	0.1151	

Values in parenthesis express actual levels of studied factors.

sodium acetate/acetic acid buffer solution, and the samples were analyzed immediately.

2.6. Optimization strategy for the proposed system

Chemical and flow factors that influence the Cr(III) and Cr(VI) on-line sequential preconcentration system were investigated using a full 2⁴ factorial design. Experiments were carried out in duplicate. The advantages of this design for optimization of analytical methods have been well documented, since it reduces the number of tests and, consequently, analysis time and allows to evaluate interaction effects between factors studied [22]. Herein, multiple response optimization based on overall desirability functions was performed, considering that two responses were sequentially obtained [Cr(III) and Cr(VI) absorbances]. Table 1 presents low and high levels established for each factor studied and experimental matrix employed. All data were processed using the STATISTICA software package (StatSoft, Tulsa, US).

3. Results and discussion

3.1. pH effect

The sample pH plays an essential role in the Cr(III) and Cr(VI) sorption on SiO₂/Al₂O₃/TiO₂ and AAPTMS. A aliquot of 20 mL of Cr(III) or Cr(VI) solution at 100 µg L⁻¹ concentration was submitted to the preconcentration procedure at varied pH (1.0–9.0) using the mini-columns. As seen in Fig. 2a, Cr(III) was sorbed very well on SiO₂/Al₂O₃/TiO₂ in the pH range of 3.0–7.0, whereas the Cr(VI) sorption was negligible. On the other hand, Cr(III) was not sorbed on SiO₂/AAPTMS in the same pH range, whereas the Cr(VI) sorption was considerably improved by increasing the sample pH (Fig. 2b). It can be observed that Cr(III) and Cr(VI) were selectively

sorbed on SiO₂/Al₂O₃/TiO₂ and SiO₂/AAPTMS, respectively, at pH 5.0 without any interference.

The Cr(III) retention on SiO₂/Al₂O₃/TiO₂ could be explained by electrostatic interactions with the oxygen atoms coming from the Si–O–Al–O–H and Si–O–Ti–O–H bonds grafted to the silica matrix. The Cr(III) sorption was low at smaller pH values due to the protonation of the oxygen atoms, and it augmented when increasing the pH values until pH 7.0, since SiO₂/Al₂O₃/TiO₂ acquired negative charges leading to effective Cr(III) retention. The decrease in the Cr(III) sorption at pH > 7.0 could be due to the formation of unsorbed [Cr(OH)₄]⁻. On the other hand, the Cr(VI) sorption on SiO₂/Al₂O₃/TiO₂ was negligible due to the existence of CrO₄²⁻, HCrO₄⁻ and H₂CrO₄, whereas on the SiO₂/AAPTMS it was more effective, since the amino groups of AAPTMS, being weak bases and presenting positive charges, were able to retain anionic species and strip off cationic ones.

3.2. Multivariate optimization of the on-line preconcentration method

The use of desirability functions for multiple response optimization experiments was proposed by Derringer and Suich [23]. To obtain overall desirability, individual desirability of all responses (in this case, Cr(III) and Cr(VI) absorbances) should be determined. Thus, each response y_i ($i=1,2,\dots,m$) is transformed into a scale-free value, which is called an individual desirability function (d_i). Individual scales can vary from 0 (for an unacceptable response) to 1 (for a desirable response). The individual desirability function was calculated according to Eq. (1) (the larger-the-better (LTB) type), which was used to maximize the Cr(III) and Cr(VI) analytical responses.

$$d_i = \left(\frac{y_i - L}{H - L} \right) \quad (1)$$

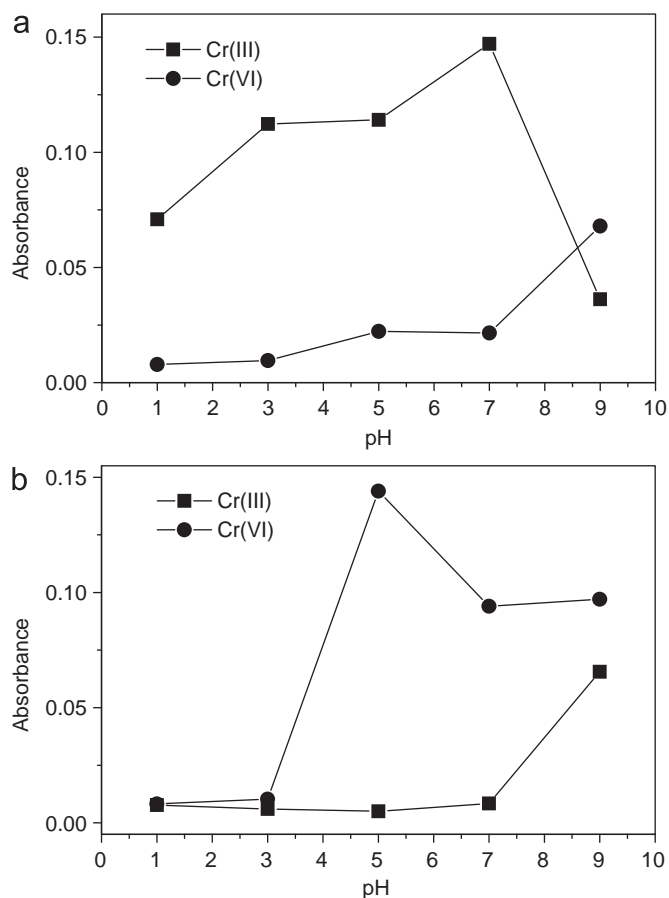


Fig. 2. Sample pH effect on the absorbance of Cr(III) and Cr(VI) ($100 \mu\text{g L}^{-1}$) (a) using the mini-column with $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$, and (b) using the mini-column with $\text{SiO}_2/\text{AAPTMS}$.

where, y_i is the Cr(III) or Cr(VI) absorbance; L and H are the lower and upper absorbances, respectively, observed in the experiments for these chromium forms. The overall desirability (D) was calculated by determining the geometric mean of the individual desirabilities (Eq. (2)).

$$D = \sqrt[m]{d_1^{p_1} d_2^{p_2} \dots d_n^{p_n}} \quad (2)$$

where, m is the number of responses (in this case, 2); P_n is the weight corresponding to each of these responses; the unitary weight of the Cr(III) and Cr(VI) responses was used herein, giving the same importance to both species. $D=1$ indicates a fully desired response, above which further improvements would have no importance.

The individual and overall desirability profiles for Cr(III) and Cr(VI) are shown in Fig. 3. It can be observed that the eluent concentration (EC), eluent type (ET) and buffer concentration (BC) play an important role in the system due to their interrelationship, which was confirmed by the analysis of variance (ANOVA) at the 95% confidence level. The BC was found to be inversely proportional to the overall desirability probably due to the high ionic strength (0.1 mol L^{-1}), thus complicating the Cr(III) and Cr(VI) retention at the material surface. Therefore, the 0.01 mol L^{-1} acetate/acetic acid buffer solution was chosen for further experiments. The high eluent concentration (2.5 mol L^{-1}) increased the overall desirability, demonstrating good efficiency of the Cr(III) and Cr(VI) release from $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$, respectively, without any memory effect. The eluent type (HCl or HNO_3) was

also studied; a significant decrease in responses was observed when HCl was changed for HNO_3 . Hence, the 2.5 mol L^{-1} HCl solution was chosen as the best eluent condition for the system. Fig. 3 reveals that the flow rate had no significant effect on the preconcentration system, indicating very fast kinetics of the Cr(III) and Cr(VI) sorption on $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$, respectively. Therefore, the efficient sample throughput of 21 h^{-1} was achieved with a sample loading volume of 20 mL at the flow rate at 7.0 mL min^{-1} . In order to confirm whether the best analytical signals were obtained under the chosen conditions, three experiments were performed, and the following absorbance values were obtained: 0.1152 ± 0.003 [for Cr(III)] and 0.2020 ± 0.0013 [for Cr(VI)].

3.3. Multivariate analysis of potentially interfering species

Considering that the same sample was percolated simultaneously at pH 5.0 through the mini-columns packed with $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$ in the proposed system, the effect of potentially interfering species on the Cr(III) and Cr(VI) preconcentration was investigated herein by means of factorial designs, and the concentration of both Cr(III) and Cr(VI) was fixed at $100 \mu\text{g L}^{-1}$. Therefore, the following factorial designs were used: 2^{5-1} —for Al(III), Ba(II), Cd(II), Co(II), and Pb(II), 2^3 —for Ca(II), Mg(II), and K(I), and 2^{5-1} —for Cu(II), Fe(III), Mn(II), Ni(II), and Zn(II). The potential interfering species levels are presented in Table 2. The significance of each species and their interactions were confirmed by probability plots, as shown in Fig. 4. It can be seen that all the effects and contrasts of these species, as well as their interactions, were closer to zero and thus insignificant. The only statistical interference at the 95% confidence interval was observed for Ca(II), Mg(II) and K(I) at their highest concentrations, 175, 45 and 12.5 mg L^{-1} , respectively. Despite this finding, the analytical signal recovery rates found within a $\pm 10\%$ error range in the presence of the first [Al(III), Ba(II), Cd(II), Co(II), and Pb(II)] and second [Ca(II), Mg(II), and K(I)], [Cu(II), Fe(III), Mn(II), Ni(II), and Zn(II)] cationic groups ranged in the following intervals: 90.5–104.5%, 94.6–109.5%, and 93.0–109.7%, respectively [for Cr(III)], and 96.6–103.8%, 99.1–104.2%, and 92.8–106.7%, respectively [for Cr(VI)]. According to these data, it could be stated that the Cr(III) and Cr(VI) sorption on $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$ was not affected by the interfering species.

3.4. Breakthrough curve

Breakthrough curves were determined to show the loading behavior of Cr(III) and Cr(VI) to be sorbed from aqueous media in a fixed bed mini-column. They make it possible to estimate the maximum sorption capacity under dynamic conditions, as well as the breakthrough volume that would not allow the column to quantitatively retain the analyte. Experiments were performed under the optimized conditions. 5.0 mL aliquots of 2.0 mg L^{-1} Cr(III) and Cr(VI) solutions were successively percolated through $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$, respectively. Initial and final concentrations were determined by FAAS. The Cr(III) and Cr(VI) amounts sorbed on $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$ from each aliquot were calculated according to Eq. (3).

$$Q(\text{mg/g}) = \frac{(C_0 - C) \times V(L)}{m} \quad (3)$$

where C_0 and C are the Cr(III) and Cr(VI) initial and final concentrations (in the column effluent), respectively (mg L^{-1}); V is the solution volume (mL); m is the $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$ weight (g) (in this case, 200 mg). Fig. 5 indicates that the Cr(III) concentration in the effluent increased rapidly as the solution continued to flow, giving

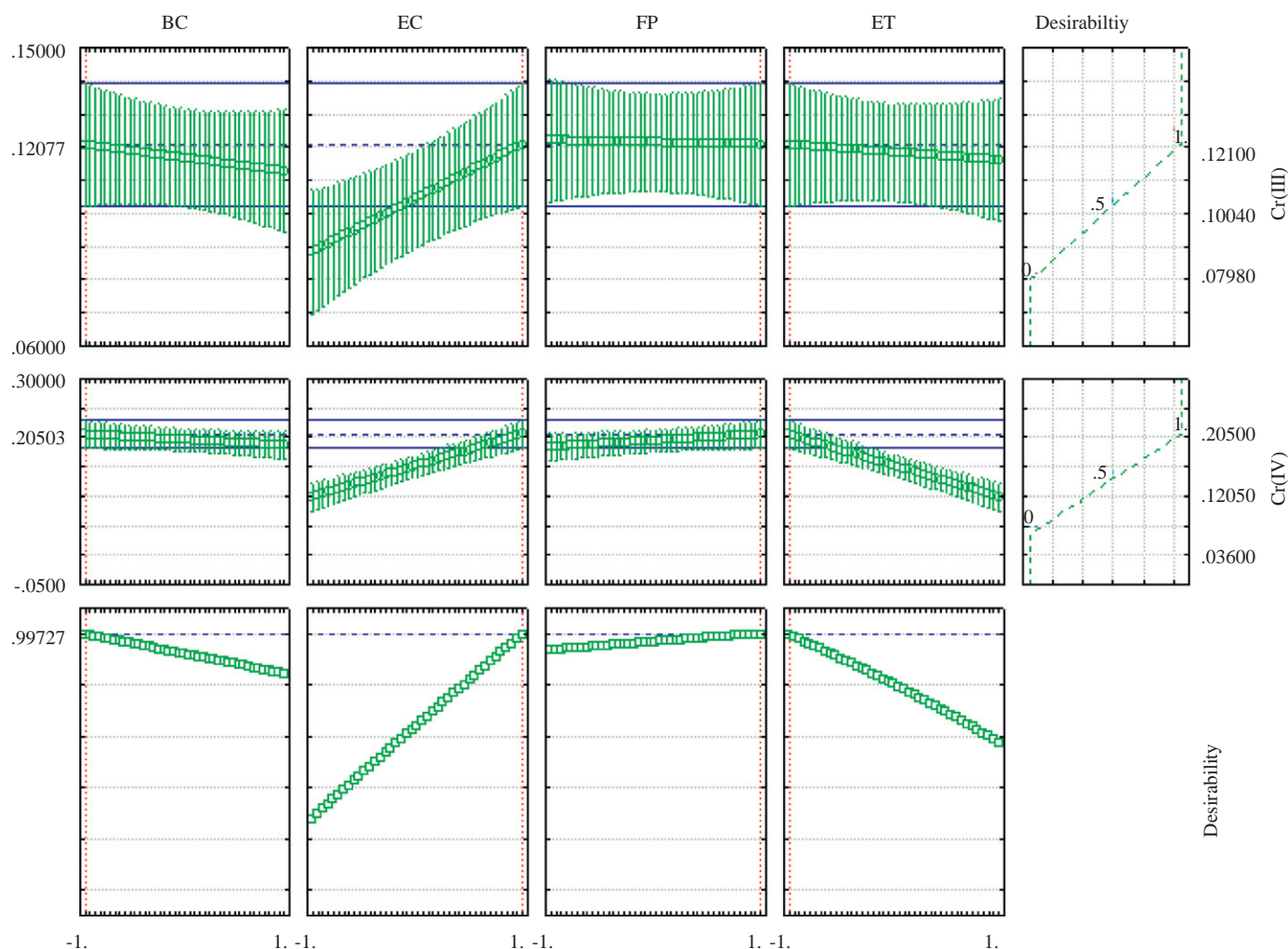


Fig. 3. Prediction and desirability profiles for simultaneous optimization of analytical signals from Cr(III) and Cr(VI) species. Dashed line indicates current values after optimization.

Table 2
Potential interfering species and their levels based on factorial design.

Levels	Concentration of potential interfering species ($\mu\text{g L}^{-1}$)				
	Al(III)	Ba(II)	Cd(II)	Co(II)	Pb(II)
-1	54	12.2	12	33	86
1	650	250	500	125	200
	Ca(II)		Mg(II)		K(I)
-1	6200		1600		680
1	175000		450000		125000
	Cu(II)	Fe(III)	Mn(II)	Ni(II)	Zn(II)
-1	181	100	33.7	67	93
1	1000	5000	200	300	350

rise to saturation of $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ with a sample loading volume of 175 mL. On the other hand, a higher sorption rate was observed for Cr(VI) at the beginning of the column operation, since the C/C_0 ratio was very low for the saturation using the sample loading volume of 215 mL. Thus, the maximum sorption capacities of $\text{SiO}_2/\text{AAPTMS}$ and $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ toward Cr(VI) and Cr(III) were found to be 1.44 mg g^{-1} and 0.44 , respectively.

3.5. Analytical features of the on-line sequential preconcentration system

The optimized conditions were used to determine the analytical performance of the developed method. The calibration graphs for both Cr(III) and Cr(VI) were constructed in the concentration ranges of $10.0\text{--}375 \mu\text{g L}^{-1}$, and the regressions were as follows: $\text{Abs} = 0.00189 + 0.00127[\text{Cr(III)} (\mu\text{g L}^{-1})]$ (linear correlation: 0.9981) and $\text{Abs} = 0.00817 + 0.00233[\text{Cr(VI)} (\mu\text{g L}^{-1})]$ (linear correlation: 0.9993). According to the IUPAC definition [24], the detection limits of the method, defined as three times the standard deviation of the blank signal intensity, were 0.66 and $0.27 \mu\text{g L}^{-1}$ for Cr(III) and Cr(VI), respectively, whereas the quantification limits, defined as ten times the standard deviation of the blank signal intensity, were 2.21 and $0.93 \mu\text{g L}^{-1}$ for Cr(III) and Cr(VI), respectively. The corresponding preconcentration factor (PF) values, calculated through the slope ratio of the calibration graphs obtained with and without the preconcentration step [25], were found to be 17.62 and 32.98 for Cr(III) and Cr(VI), respectively. It is worthy to emphasize that the sensitivity of the calibration graph for Cr(III) sorbed on $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ was improved 8.5-fold when compared to the sensitivity of the graph for Cr(III) sorbed on SiO_2 . This finding confirms the efficiency of the $\text{Al}_2\text{O}_3/\text{TiO}_2$ mixed oxide dispersed in the silica matrix for the Cr(III) extraction. The sample throughput obtained was 21 h^{-1} . The relative standard deviation for 10 replicate measurements (precision) estimated for Cr(III) and

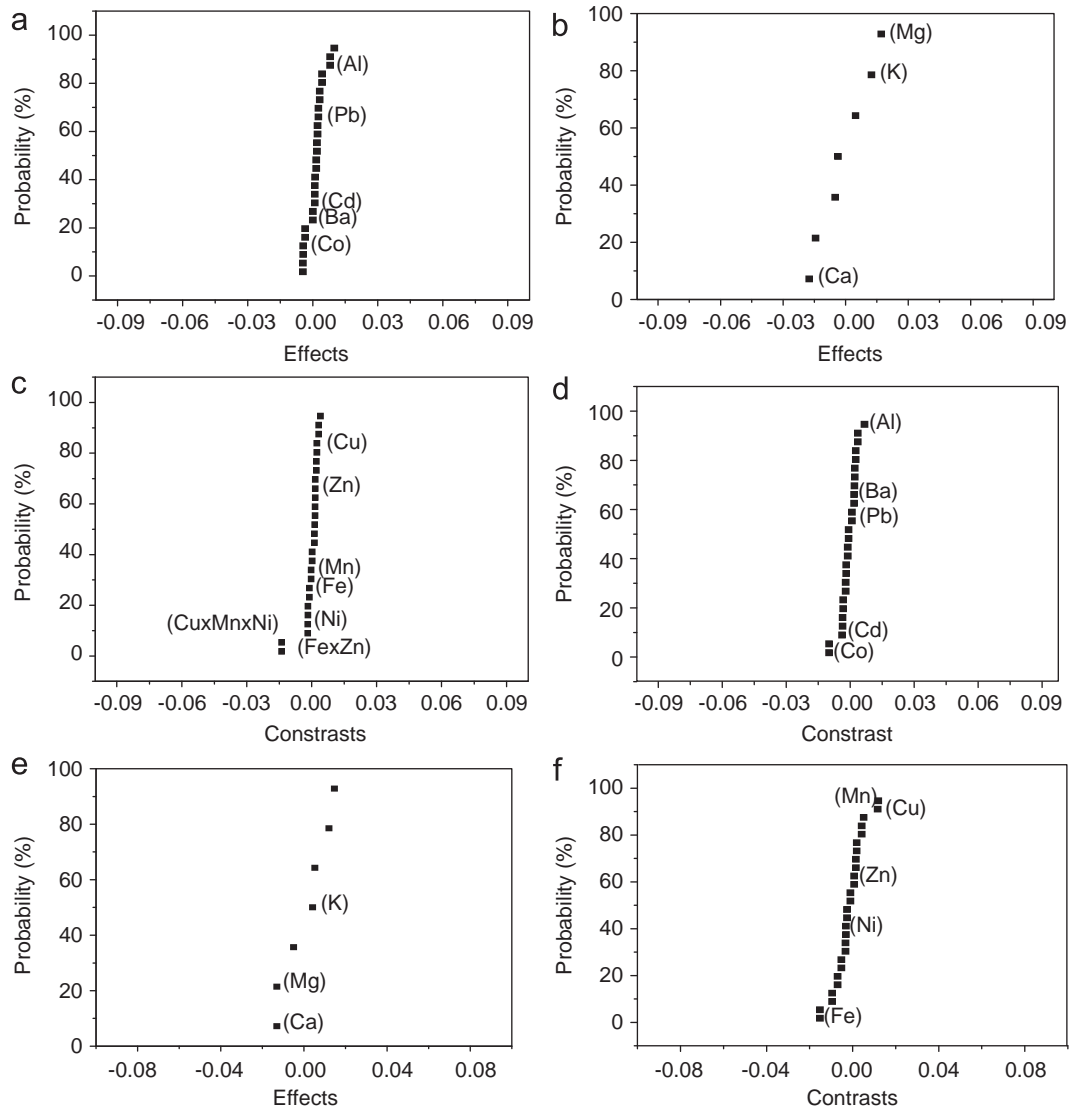


Fig. 4. Probability plots related to the experimental design used for assessing interferences for Cr(III) (a)–(c) and Cr(VI) (d)–(f).

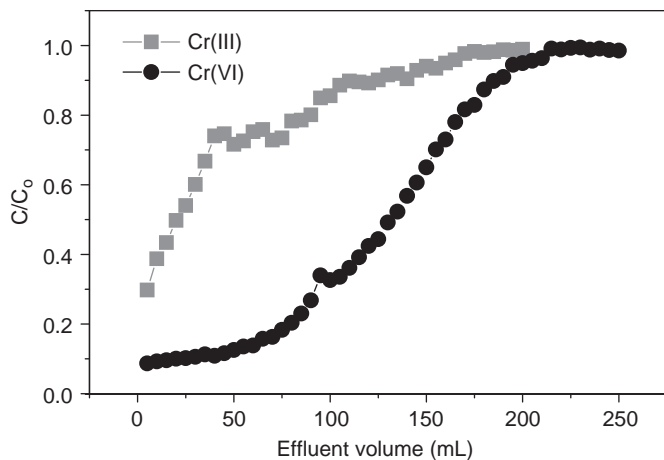


Fig. 5. Breakthrough curve for Cr(III) on $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and Cr(VI) on $\text{SiO}_2/\text{AAPTMS}$.

Cr(VI) at $20 \mu\text{g L}^{-1}$ was 3.52 and 4.08%, respectively, and for Cr(III) and Cr(VI) at $100 \mu\text{g L}^{-1}$, it was 3.58 and 3.61%, respectively. The efficient concentrations were found to be 6.2 and 11.4 min^{-1} , and

the consumptive index (mL) was 1.12 and 0.6 for Cr(III) and Cr(VI), respectively [25]. The reproducibility study results showed that the relative standard deviations for the columns preparation were 2.4 and 6.4% for $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPTMS}$ ($n=3$), respectively. The $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ mass effect (200, 350 and 400 mg) was also investigated, and no significant differences (deviation: 4.19%) were observed for the Cr(III) analytical signal. However, for $\text{SiO}_2/\text{AAPTMS}$, the analytical signal decreased when increasing the mass. Thus, the mass of 200 mg was chosen for both sorbents. A detailed comparison of the present speciation method with other previously reported ones is given in Table 3. The method described herein is characterized by low detection limits and sample consumption. Moreover, it appears to be of particular interest, since it does not require sample pH readjustments and chemical treatments to give the total chromium amount usually required for speciation.

3.6. Sample analysis

The developed method was applied to determine Cr(III) and Cr(VI) in various water and parenteral samples using the optimized conditions and external calibration curves. As Cr(III) and Cr(VI) were below the detection limits in some samples, recovery

Table 3
Comparison of different preconcentration analytical methods for Cr determination.

System	Detection	Preconcentration modality	Mode	DL ($\mu\text{g L}^{-1}$)	FP	Applications	References
Sorption of Cr(III) and reduction of Cr(VI)	FAAS	Sorption of Cr(III) as dithizonate chelate on Chromosorb 108	Off-line	Cr(III) 0.75	71	Water, tomato leaves, soil, tobacco and coffee	[9]
Sorption of Cr(III) and Cr(VI)	HPLC	Cloud point extraction using DDTC as chelating agent	Off-line	Cr(III) 3.4 Cr(VI) 5.2	Cr(III) 65 Cr(VI) 19	Water	[26]
Sorption of Cr(III) and oxidation of Cr(III) to Cr(VI)	FAAS	Sorption of Cr(III) on Amberlite XAD-1180 as diphenylcarbazone complex	Off-line	Cr(VI) 7.7	75	Water/stream sediment/pharmaceutical	[27]
Sorption of Cr(III) and reduction of Cr(VI)	ICP-AES	Titanium dioxide	Off-line	Cr(III) 0.32	50	Water	[28]
Sorption of Cr(III) and reduction of Cr(VI)	FAAS	Saccharomyces cerevisiae immobilized on sepiolite	Off-line	Cr(III) 94.0	75	Water	[29]
Sorption of Cr(VI)-cetyltrimethyl ammonium bromide and oxidation of Cr(III)	FAAS	C-18 bonded silica SPE disks	Off-line	Cr(III) 15.0 Cr(VI) 20.0	45	Water	[30]
Sorption of Cr(VI)-DPC and oxidation of Cr(III)	FAAS	Amberlite XAD-16	Off-line	Cr(VI) 45.0	25	Water	[31]
Sorption of Cr(VI)-ADPC and oxidation of Cr(III)	FAAS	Ambersorb 563	–	2.70	125	Water	[32]
Sorption of Cr(III) and Cr(VI)	UV-Visible	Sawdust	Off-line	Cr(III) 50.0, Cr(VI) 40.0	Cr(III) 100, Cr(VI) 80	Lake/river water	[33]
Sorption of Cr(III) and reduction of Cr(VI)	ICP-AES	Nanometer titanium dioxide immobilized on silica gel	Off-line	Cr(III) 0.22	Cr(III) 50	Water samples	[34]
Sorption of Cr(III) and reduction of Cr(VI)	FAAS	Nb ₂ O ₅ -SiO ₂	On-line	Cr(III) 0.34	Cr(III) 23	Water samples	[35]
Sorption of Cr(III) and reduction of Cr(VI)	FAAS	Moss (<i>Funaria hygrometrica</i>) immobilized in a polysilicate matrix	Off-line	Cr(III) 145	Cr(III) 20	Wastewater samples	[36]
Sorption of Cr(VI) and oxidation of Cr(III)	FAAS	Fiber of animal origin	On-line	Cr(III) and Cr(VI) 0.30	Cr(III) and Cr(VI) 32	Drinking water samples	[37]
Sorption of Cr(III) and Cr(VI)	FAAS	SiO ₂ /Al ₂ O ₃ /TiO ₂ , SiO ₂ /AAPTMS	On-line	Cr(III) 0.66, Cr(VI) 0.27	Cr(III) 17.62 Cr(VI) 32.98	Water/Physiological serum/Parenteral solution	This work

PF=preconcentration factor; DL=detection limit.

Table 4
Recovery rates for Cr species in water samples.

Samples	Cr added ($\mu\text{g L}^{-1}$)	Cr found ^a ($\mu\text{g L}^{-1}$)	Recovery rate(%)
Tap water 1	Cr(III) 0	ND	–
		3	3.03 ± 0.58
		40	38.38 ± 0.30
	Cr(VI) 0	ND	–
		3	2.87 ± 0.09
		40	43.55 ± 0.63
Lake Igapó water	Cr(III) 0	1.66 ± 0.13	–
		3	5.40 ± 0.17
		40	41.13 ± 0.78
	Cr(VI) 0	ND	–
		3	2.75 ± 0.26
		40	43.03 ± 1.0
Mineral water 1	Cr(III) 0	2.42 ± 0.25	–
		3	6.23 ± 0.36
		40	38.38 ± 0.30
	Cr(VI) 0	ND	–
		3	3.42 ± 0.15
		40	43.55 ± 0.63
Mineral water 2	Cr(III) 0	2.71 ± 0.33	–
		3	5.28 ± 0.28
		40	48.59 ± 0.67
	Cr(VI) 0	ND	–
		3	2.90 ± 0.60
		40	44.42 ± 1.40
Tap water 2	Cr(III) 0	4.41 ± 0.69	–
		5	10.29 ± 0.71
	Cr(VI) 0	13.36 ± 0.70	–
		15	31.68 ± 1.40
Tap water 3	Cr(III) 0	5.39 ± 0.69	–
		6	13.23 ± 2.07
	Cr(VI) 0	11.38 ± 0.70	–
		12	26.86 ± 1.22

ND=Not detected.

^a Results are expressed as mean value ± standard deviation (n=3).

Table 5
Recovery rates for Cr species in parenteral solutions.

Samples	Cr added ($\mu\text{g L}^{-1}$)	Cr found ^a ($\mu\text{g L}^{-1}$)	Recovery rate (%)
Physiological serum 1	Cr(III) 0	1.11 ± 0.24	–
		3	4.61 ± 0.32
	Cr(VI) 0	ND	–
		3	2.53 ± 0.11
Physiological serum 2	Cr(III) 0	ND	–
		3	3.03 ± 0.58
	Cr(VI) 0	ND	–
		3	2.87 ± 0.10
Water for injection	Cr(III) 0	ND	–
		3	2.95 ± 0.09
	Cr(VI) 0	ND	–
		3	2.77 ± 0.09
Artificial saliva	Cr(III) 0	ND	–
		3	3.29 ± 0.33
	Cr(VI) 0	ND	–
		3	2.71 ± 0.16
Physiological glucose (5%) solution	Cr(III) 0	ND	–
		3	3.23 ± 0.20
	Cr(VI) 0	ND	–
		3	2.83 ± 0.11

ND=Not detected.

^a Results are expressed as mean value ± standard deviation (n=3).

tests were performed by spiking their known amounts. Tables 4 and 5 present satisfactory recovery rates (84.3–116.6%), thus confirming the reliability of this speciation method for different kinds of samples even at concentrations lower than the allowable limit for drinking water. It is worth noting that chromium species were determined in the tap water samples (2 and 3), especially Cr(VI) at high concentrations. The presence of

Table 6

Analytical results for chromium determination in certified reference material using the on-line sequential preconcentration system.

Sample	Certified value ($\mu\text{g L}^{-1}$)	Cr(III) found ^a ($\mu\text{g L}^{-1}$)	Cr(VI) found ^a ($\mu\text{g L}^{-1}$)
Standard reference material NIST SRM 1643e ("Trace elements in natural waters")	20.40 ± 0.24	20.06 ± 1.40	ND

ND=not detected.

^a The results are expressed as mean value ± standard deviation based on three replicates ($n=3$).

chromium in these samples may be associated with the presence of leather processing industries near São José do Rio Preto City. Although chromium was not detected in some parenteral samples, the method can be successfully used to monitor this element, taking into account that some studies have reported its presence in the concentration range of 0.3–5.0 $\mu\text{g L}^{-1}$ [38,39]. The method accuracy for the chromium determination in water samples was evaluated by the analysis of the NIST 1643e standard reference material (trace elements in water) (Table 6). A good agreement between the found ($20.06 \pm 1.40 \mu\text{g L}^{-1}$) ($n=3$) and certified ($20.40 \pm 0.24 \mu\text{g L}^{-1}$) values was achieved by applying the *t*-test at the 95% confidence level.

4. Conclusions

In this work, the performance of the selective on-line Cr(III) and Cr(VI) preconcentration procedure using dual mini-columns packed with $\text{SiO}_2/\text{Al}_2\text{O}_3/\text{TiO}_2$ and $\text{SiO}_2/\text{AAPtMS}$ was described. The silica modification with mixed oxides and organosilanes allowed to determine Cr(III) and Cr(VI) in different kinds of water and parenteral samples at the same pH (pH 5.0), and not as a difference between the total chromium content and the amount of just one single species. Thus, common drawbacks of previous publications, such as sample pH adjustment, chemical pretreatments for the total chromium determination and the presence of auxiliary chelating agents that form complexes only with chromium species were overcome in the present research. Moreover, due to high preconcentration factor values and reduced sample consumption (20.0 mL), lower detection limits for Cr(III) and Cr(VI) were obtained in comparison with the previously published methods. The materials synthesized also showed high stability, since the same mini-columns were used throughout the study, without loss of sorption capacity. These features appear promising for further researches on chemical modifications of a silica matrix, aiming at the development of novel speciation methods that would have better analytical performance.

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