

Speciation of Chromium in Water Samples after Dispersive Liquid-Liquid Microextraction, and Detection by Means of High-Resolution Continuum Source Atomic Absorption Spectrometry

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A newly analytical method has been developed to determine total chromium and speciation of this element in water samples through dispersive liquid-liquid microextraction combined with a high-resolution continuum source flame atomic absorption spectrometry. The most significant variables affecting complexation and extraction were optimized by using response surface methodology and univariate optimization. The best conditions for both the complexation and extraction elements in this study were: complexing agent ammonium pyrrolidine dithiocarbamate (APDC 6.0 mmol L⁻¹); pH at 2.0 (Cr^{VI}) and at 7.0 (Cr total); NaCl (5% m/v); 1-undecanol (50 µL) and ethanol 300 (Cr^{IV}) and 275 µL (total Cr). Under optimal conditions, this method resulted in a 20-100 µg L⁻¹ linear range for Cr^{VI} and total chromium, detection limits of 0.35 (Cr^{VI}) and 6.7 µg L⁻¹ (total Cr), as well as enriching factor of 26 (Cr^{VI}) and 19 for total Cr. The method accuracy was carried out by using certified water reference material (NIST CRM 1643e), and the results achieved were in agreement with the certified value (*t*-test at a confidence interval of 95%). The method developed was applied in samples of mineral water, tap water (the recovery values ranged from 88 to 115%) and seawater.

Keywords: dispersive liquid-liquid microextraction, chromium, speciation, trace element, atomic absorption spectrometry

Introduction

Chromium is an element commonly found in rocks, animals, plants, soil, rivers and seawater, dusts and volcanic fumes, as well as in Earth's crust at a concentration of approximately 100 mg kg⁻¹.¹ Chromium in the environment can be of natural origins, such as rock and soil erosion, volcanic eruptions, or of anthropogenic origins, through the use of this chemical element in a range of industrial activities such as metallurgy (steel, aluminum and alloys), refractories (cement, glass and clay) and chemical industries (leather tanning, wood preservation and pigments).² The second source is significantly responsible for Cr^{III} and Cr^{VI} in the environment.

Trivalent chromium, Cr^{III}, is essentially found in the mechanisms for metabolism of carbohydrates, lipids and

proteins, with a lower level of toxicity and less mobility than hexavalent chromium in the environment, due to the fact that the element is present in mineral structures, in the form of precipitates with some elements as iron, aluminum and/or manganese, and complexed with organic matter.³ In contrast, hexavalent chromium, Cr^{VI}, is said to be carcinogenic as it is highly permeable in biological membranes, this being related to the fact that the chromate ion is a predominant specie of Cr^{VI} in physiological pH, whose tetrahedral structure is similar to other ions, such as sulfate and phosphate, leading it to make a path into cells by carrying ions.^{4,5} Since chromium's toxicological properties are dependent on the element's oxidation state, it is, therefore, important to carry out the correspondent chemical speciation for quantification purposes of these species.⁵ On the other hand, considering the low concentrations of chromium found, for example, in natural water samples as µg L⁻¹ it is important to follow a preconcentration procedure

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prior to the detection process.⁶ This procedure will allow the concentration and separation of the matrix analyte, aiming to further detectability and eliminate any expected effects of the matrix and foreign ions.⁷

The so-called dispersive liquid-liquid microextraction (DLLME) was created by Rezaee *et al.*⁸ in 2006 and consists of quickly inserting, with the aid of a microsyringe, a mixture of extractant and disperser solvents with an aqueous solution containing the analyte. The build-up of fine and disperse droplets in the extractant solvent allows for extraction the analyte. Among other advantages of DLLME, an important one is that can enhance the use of reduced volumes of organic solvents, at low cost, with quickness, good values of recovery and enrichment factors.⁹ DLLME has been widely used to determine organic and inorganic analytes,¹⁰ considering that for the second analyte a complexing agent is usually added, thus resulting in a hydrophobic complex for an extraction at a later stage.¹¹ Dithiocarbamates (DTCs) are mostly used as complexing agents, forming stable and neutral complexes with a wide range of metals.¹² Sodium diethyldithiocarbamate (DDTC) and ammonium pyrrolidine dithiocarbamate (APDC) are two of the most widely used in DLLME.¹³

Generally, the detection of metals after DLLME is carried out using absorption and atomic emission techniques. Some of the techniques most widely applied are flame atomic absorption spectrometry (FAAS) and graphite furnace absorption atomic spectrometry (GFAAS).¹³ Other detection methods have been combined with DLLME including inductively coupled plasma optical emission spectrometry (ICP OES),¹⁴ laser-induced breakdown spectrometry (LIBS)¹⁵⁻¹⁹ and tungsten coil atomic emission spectrometry (WCAES).²⁰ In this work, the authors used a high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) for chromium detection, which in contrast with the line source (LS FAAS) uses a radiation source of high-intensity emission in continuum 190-850 nm (Xe short-arc lamp), a high-resolution double Echelle monochromator (DEMO) and a charge coupled device (CCD) array detector, allowing a resolution of ca. 2 pm *per* pixel as well as an increased detection capacity.²¹⁻²⁵

In this context, this work aims at developing an analytical method for determination and speciation of Cr in water samples (tap water, mineral water and seawater) by using the DLLME procedure combined with HR-CS FAAS. To do so, the authors of this study assessed two complexing agents widely used, DDTC and APDC. Those variables encountered in complexation and microextraction were optimized by using response surface

methodology and univariate optimization, which makes it possible to look into the influences of such variances, as well as the interaction effects between them on the analytical response.

Experimental

Reagents and solutions

All the solutions used in this work were prepared with analytical-grade reagents and ultrapure water (Milli-Q Plus system, Millipore, Bedford, MA, EUA). The standard solutions of chromium were then prepared by diluting standard solutions of K_2CrO_4 10% (m/v) and Cr^{III} 1000 mg L⁻¹ (Specsol, Jacaré, SP, Brazil).

DDTC and APDC (Sigma-Aldrich, St. Louis, MO, USA) were two complexing reagents used. For adjusting the values of pH, solutions of sulfuric acid and ammonium hydroxide were used in different concentrations that resulted from diluting sulfuric acid at 95-97% (Merck, Darmstadt, Germany) and ammonium hydroxide at 28-30% (Sigma-Aldrich).

In the DLLME procedure, this was used as an extractant solvent, i.e., 1-undecanol (Sigma-Aldrich) and disperser solvent ethanol $\geq 99.5\%$ PA (Fluka Analytical). A NaCl solution 25% (m/v) obtained from Sigma-Aldrich reagent was used as an electrolyte.

To study the effect of foreign ions, solutions containing aluminum, barium, cadmium, calcium, lead, chlorite, cobalt, copper, iron, manganese, nickel, nitrate, potassium, sodium, sulfate and zinc were prepared by diluting the standard solutions with a concentration of 1000 $\mu\text{g mL}^{-1}$ (Ultra Scientific, USA).

To assess the method accuracy, a certified reference material (CRM) of water was used (NIST 1643e trace elements in water) from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Tap water (from our lab in the city of Santo André, SP, Brazil), mineral water (bought locally) and seawater (from city of Santos, SP, Brazil) were used as real samples. The tap and mineral water samples were used without any previous treatment and to remove particle material from the seawater the sample was filtered using a 0.45 μm pore size membrane filter.

DLLME procedure

For the complexation and extraction of chromium species by means of DLLME, different volumes of samples or reference solutions were transferred to a glass tube of 10 mL.

For determining total chromium

The pH value was adjusted with ammonium hydroxide (pH 7.0) then 600 μL APDC (90 mmol L^{-1}) and 1800 μL NaCl 25% (m/v) solutions were added. The resulting solution was heated in a water bath at 80 °C for 15 minutes²⁶ and cooled at room temperature, then the mixture 50 μL of 1-undecanol and 275 μL of ethanol was added using a glass syringe. The separation of phases was carried out in a centrifuge at 2000 rpm for 3 minutes. The organic phase was collected with a micropipette, then diluted in 200 μL of ethanol and then all volume introduced into HR-CS FAAS, with the aid of a pipette tip.

For the determination of Cr^{VI}

After acidification with sulfuric acid 1 mol L^{-1} (pH 2.0), 600 μL APDC (90 mmol L^{-1}) and 1800 μL NaCl 25% (m/v) were added. By using a glass syringe, the mixture 50 μL of 1-undecanol and 300 μL of ethanol were added. The other steps followed the same procedure used for total chromium.

For the determination of Cr^{III}

It was evaluated from the difference between the total Cr and Cr^{VI} concentrations found using the above mentioned procedures.

Instrumentation

A pH meter model 780 Metrohm (Herisau, Switzerland) equipped with a combined glass electrode was used to measure pH, a hotplate model IKA C-MAG HS 7 (Germany) was used to heat to 80 °C, and a Quimis centrifuge model Q222T (São Paulo, Brazil) for speeding up the separation of phases. The stirred extracting and dispersing solvents were added to the sample through a glass syringe with a capacity of 1000 μL (SGE Analytical Science, USA).

The detection of chromium by HR-CS FAAS was carried out through a ContrAA® 300 (Analytik Jena AG, Jena, Germany) equipped with a xenon short-arc lamp (XBO 301, 300 W, GLE, Berlin, Germany) operating in hotspot mode. The measurements were made at 357.9 nm, the main line of chromium. The other instrumentation conditions used were: air flow 490 L h^{-1} , acetylene flow 110 L h^{-1} , burner height 13 mm, sample flow rate at 6 mL min^{-1} and spectral resolution 0.2 nm.

Results and Discussion

Effect assessment of sample volume in analytical signal

In the proposed DLLME procedure, the volume

produced within the organic phase, containing the analytes extracted in 1-undecanol, was 45 μL . Even after dilution, the final volume with ethanol did not enable the continuous nebulization process in the detection. As result, it was then decided to carry out discrete nebulization. Discrete volumes (50 to 200 μL) of a solution containing $\text{Cr } 50 \mu\text{g L}^{-1}$ obtained from the DLLME procedure were manually added to a pipette tip (20 to 200 μL) connected to the nebulizer aspiration tube.²⁷ By keeping the instrumental parameters as above mentioned and a reading time of 10 s, transient signals were produced and then measured at peak area. Taking into account that the manual insertion of the sample can lead to a higher standard deviation of measurements, the sample volume selected was based on the peak area and corresponding relative standard deviations ($n = 3$). The intensity of peak area increased with the sample volume, and the relative standard deviations reached 15, 11, 7.0 and 7.7% for volumes of 50, 100, 150 and 200 μL , respectively. A sample volume insertion of 200 μL was selected for further experiments.

Oviedo *et al.*²⁸ achieved analytical calibration curves by performing continuous and discrete nebulization of Mo in an aqueous and organic medium. The ratio of angular coefficients of analytical calibration curves of samples in an organic medium obtained by discrete and continuous nebulization was around 10, displaying an increased sensitiveness level. That is, another advantage from discrete nebulization in addition to a reduced sample volume.

Optimization of flame composition in chromium detection

A study was carried out for flame composition in chromium analytical signal. Five different flame compositions were assessed with acetylene flow rate at 40, 100, 105, 110 and 120 L h^{-1} and by also keeping the air flow rate at 490 L h^{-1} (C:O 0.40; 0.97; 1.0; 1.1 and 1.2, respectively). Figure 1 shows that the stoichiometric flame with a C:O 0.40 ratio has the lowest sensitiveness level. On the other hand, in an air-acetylene flame formed by composition of 490 and 110 L h^{-1} air-acetylene, respectively (C:O 1.1), it was observed the highest sensitiveness level. A strategy widely used to increase sensitiveness in determining chromium consists of using a reducing flame;^{29,30} however, as noticed in Figure 1, in higher flow rates of acetylene at 120 L h^{-1} , for example, the reduction of sensitiveness can be the result of carbides formation which makes the atomization process difficult. Thus a flame composition of 490 and 110 L h^{-1} of air and acetylene, respectively, was selected for further experiments.

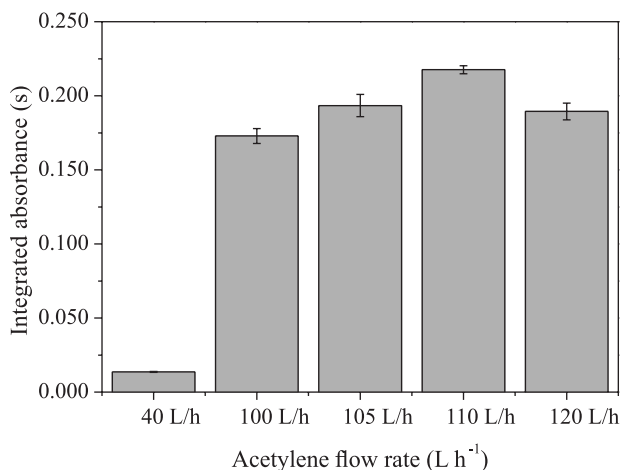


Figure 1. Flame composition study. Experimental conditions: 1000 $\mu\text{g L}^{-1}$ Cr^{III} , 6.0 mmol L^{-1} APDC, 0.02% (v/v) NH_4OH (pH = 7.0), NaCl 5% (m/v), 200 μL of 1-undecanol and 300 μL ethanol. Air flow rate: 490 L h^{-1} .

DLLME procedure optimization

The DLLME procedure was optimized by using multivariate designs in an Excel spreadsheet. The integrated absorbance signal achieved after microextraction was based on Origin®, version 9.0 (Copyright® 1991-2012, OriginLab Corporation) and Statistica®, version 10 (Copyright® 1984-2011, StatSoft). The optimization study was carried out independently for Cr^{III} and Cr^{VI} .

In order to perform the first screening of assessed variables, a fractional factorial design 2_{v}^{5-1} was carried out. Table 1 shows all the factors studied and their respective levels, Table S1 (APDC complexant) and Table S2 (DDTC complexant) (Supplementary Information section) complete matrix of factorial design and integrated absorbance values.

Table 1. Factors and levels assessed to fractional factorial design 2_{v}^{5-1}

Factor	Level		
	(-1)	(0)	(+1)
pH	2.0	4.5	7.0
Complexant concentration / (mmol L^{-1})	0.40	3.2	6.0
Extractant volume / μL	50	125	200
Disperser volume / μL	100	200	300
NaCl (m/v) / %	not added	2.5	5.0

Along with the results displayed in Tables S1 and S2, Pareto charts are presented in Figures 2a-2d, making it possible to assess the influence of variables and interaction between them on the analytical response. The horizontal bars represent the absolute values of estimated effects, while the vertical bar is known as a reference line. The effects that go beyond the reference line are significant

for the analytical response within the confidence interval of 95% ($p < 0.05$).³¹

Figures 2a and 2b show that the two chromium species have the most significant effects for variables, pH value, complexant concentration and interaction between them, thus enhancing the importance of both variables in the DLLME procedure. At low pH values, Cr^{III} is complexed with water molecules forming aqua complexes which are kinetically inert. By increasing pH value, the water molecules are then replaced with OH^- ions, forming complexes with higher lability thus increasing the interaction of Cr^{III} with complexant reagent molecules APDC and DDTC.^{26,32-37} The pH value is one of the variables allowing the speciation of chromium, as different from Cr^{III} , the hexavalent specie is immediately reduced to the trivalent form in the presence of the complexant agent, thus quickly forming the complexes.

Figure 2c indicates the same occurrence to variables, pH value, complexant concentration and interaction between them show significant. As for Cr^{VI} , Figure 2d, the only variable seen as significant was the disperser solvent (ethanol) volume.

Based on the results from the fractional factorial design and considering: (i) the need to keep the concentration of complexants, APDC and DDTC, in excess related to the chromium species in order to ensure the quantitative complexation in the presence of other metal ions; (ii) the value of pH is one of the variables allowing the speciation of chromium; (iii) the increase of extractant solvent volume (1-undecanol) results in a reduced analytical signal due to lower enrichment factor; and (iv) the increase of electrolyte concentration displayed a positive effect on complex Cr^{III} /APDC it was then decided to carry out a factorial design 3^2 with variables, pH value and disperser solvent in complexation and extraction of Cr^{III} with APDC and DDTC. The remaining variables such as 6.0 mmol L^{-1} of APDC and DDTC (highest level), 50 μL of 1-undecanol (lowest level), 5.0% (m/v) of NaCl (highest level) for Cr^{III} /APDC and 0.0% (no addition) of NaCl (lowest level) for Cr^{III} /DDTC were kept constant.

The design experimental matrix generated a total of 11 experiments, and the values of integrated absorbance are shown in Table S3 (Supplementary Information section). Table 2 shows statistical results calculated to 3^2 factorial design.

Taking into account the results presented in Table 2, it can be observed that just the average was considered significant within 95% confidence interval (p -value < 0.05). The factors investigated did not present significance within 95% interval because all p -values were higher than 0.05.

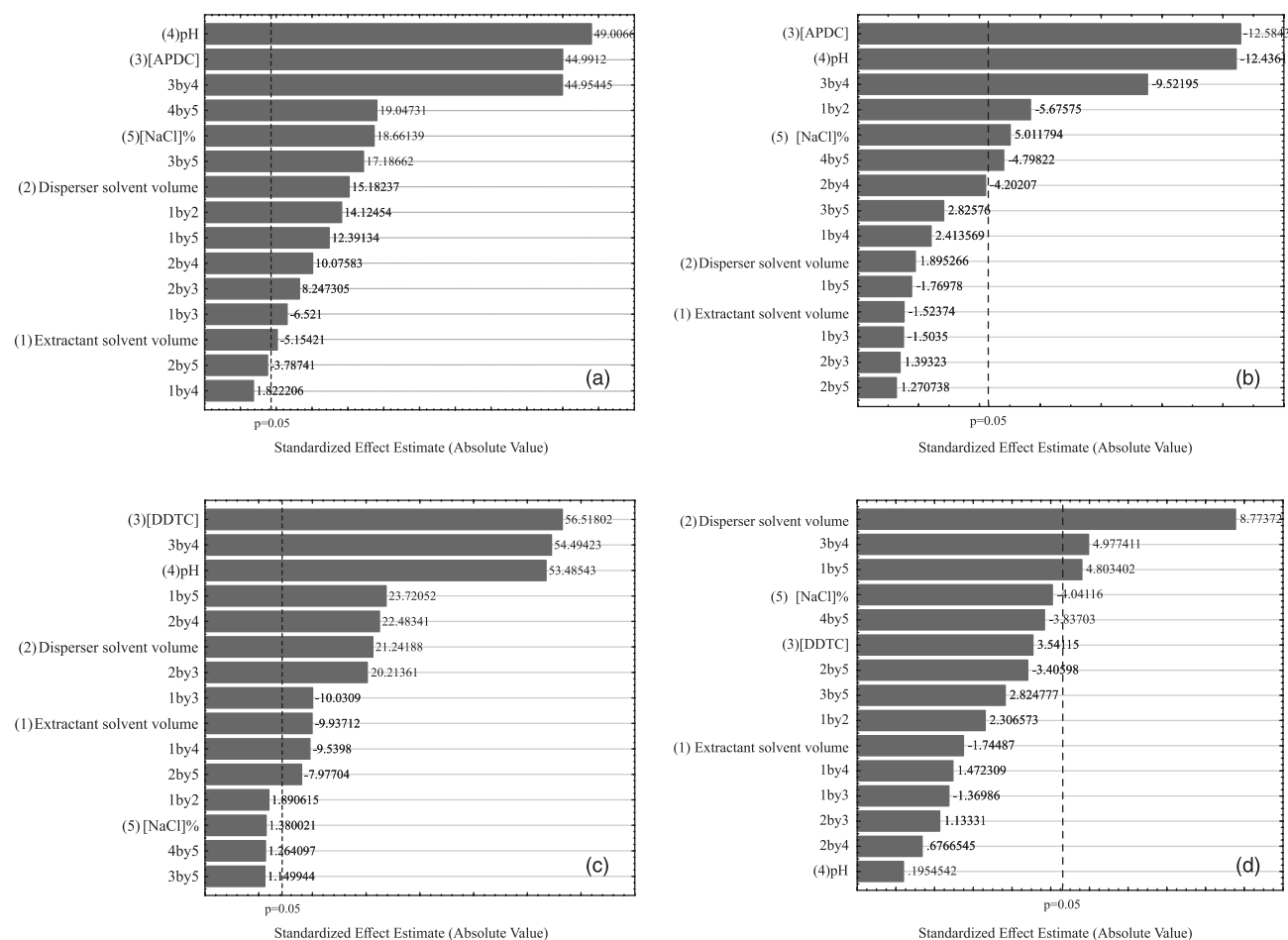


Figure 2. Pareto charts achieved in optimizing factors from fractional factorial design 2_{v-1}^{v-1} (a) Cr^{III} with APDC; (b) Cr^{VI} with APDC; (c) Cr^{III} with DDTC; (d) Cr^{VI} with DDTC.

Table 2. Statistical results for 3^2 factorial design

Factor	Cr ^{III} with APDC			Cr ^{III} with DDTC		
	Coefficient	Error	p-value	Coefficient	Error	p-value
Constant	0.067	0.011	0.024	0.827	0.146	0.030
X ₁	0.014	0.008	0.231	0.249	0.117	0.166
X ₂	0.002	0.008	0.866	0.030	0.117	0.821
X ₁ X ₁	0.005	0.013	0.755	-0.325	0.179	0.212
X ₂ X ₂	-0.042	0.013	0.084	-0.194	0.179	0.390
X ₁ X ₂	-2E-04	0.010	0.989	0.010	0.143	0.951

APDC: ammonium pyrrolidine dithiocarbamate; DDTC: sodium diethyldithiocarbamate.

The lack of fit test returned $F_{\text{calculated}} (MS_{\text{lack of fit}}/MS_{\text{pure error}})$ equal to 4.33 for Cr^{III}/APDC and 0.57 for Cr^{III}/DDTC, lower than $F_{\text{critical}0.05, v_1=3, v_2=2} = 19.16$, which indicates absence of lack of fit within 95% confidence interval (Tables S4 and S5, Supplementary Information section). The regression significance test returned the value of $F_{\text{calculated}} (MS_{\text{model}}/MS_{\text{residual}})$ equal to 0.91 for Cr^{III}/APDC and 2.84 for Cr^{III}/DDTC, lower than $F_{\text{critical}0.05, n_1=5, n_2=5} = 5.05$ which indicates a no significant regression within 95% confidence interval (Tables S4 and S5, Supplementary Information

section).³¹ However, taking into account that the percentage of variation explained by regression and maximum percentage of variation explained can be considered acceptable and the models residue for Cr^{III}/APDC and Cr^{III}/DDTC presented normal behavior through Shapiro-Wilk test for 95% confidence ($p\text{-value} > 0.05$), the response surface was built from the experiments.

Figures 3a and 3b, these graphs show that by using APDC, optimal conditions for DLLME were disperser solvent volume 275 μL and pH value 7.0, and also using

DDTC the best conditions for DLLME corresponded to the central point, that is, 275 μL of disperser solvent and pH value of 6.5.

For Cr^{VI} based on the results obtained from the fractional factorial design, Figures 2b and 2d, and considering: (i-iii) as previously mentioned for Cr^{III} ; (iv) that the increase of concentration of electrolyte displayed a positive effect on complex $\text{Cr}^{\text{VI}}/\text{APDC}$ and (v) that the only significant variable for $\text{Cr}^{\text{VI}}/\text{DDTC}$ was the disperser solvent volume, it was also decided to assess the disperser solvent (ethanol) volume influence on microextraction of Cr^{VI} with DDTC. The other variables were maintained fixed: 6.0 mmol L^{-1} of DDTC (highest level), pH value 2.0 (lowest level), 0.0% (no addition) of NaCl (lowest level) and 50 μL of 1-undecanol (lowest level).

Figure 4 shows an increased integrated absorbance up to 280 μL of disperser solvent. In DLLME, the disperser

solvent promotes the extractant solvent dispersion in aqueous solution, but an excess of disperser solvent should be avoided due to an increase in solubility of hydrophobic complex in aqueous phase, thus its reduced quantity in the organic phase.¹⁹ Even though the integrated absorbance displayed a slight increase at 310 and 320 μL of disperser solvent (Figure 4) the analytical signal was kept below the initial volumes, 250 and 260 μL . As the extraction efficiency was higher between 270 and 280 μL of disperser solvent, we decided then to keep the volume of 275 μL for remaining part of this study.

In summary, using DDTC the selected experimental conditions as follows: value of pH 2.0 for Cr^{VI} and pH 6.5 for total Cr, concentration of DDTC 6.0 mmol L^{-1} , with no addition of NaCl, 50 μL for 1-undecanol and 275 μL of ethanol. By using APDC, conditions as follows: value of pH 2.0 for Cr^{VI} and pH 7.0 for total Cr, concentration

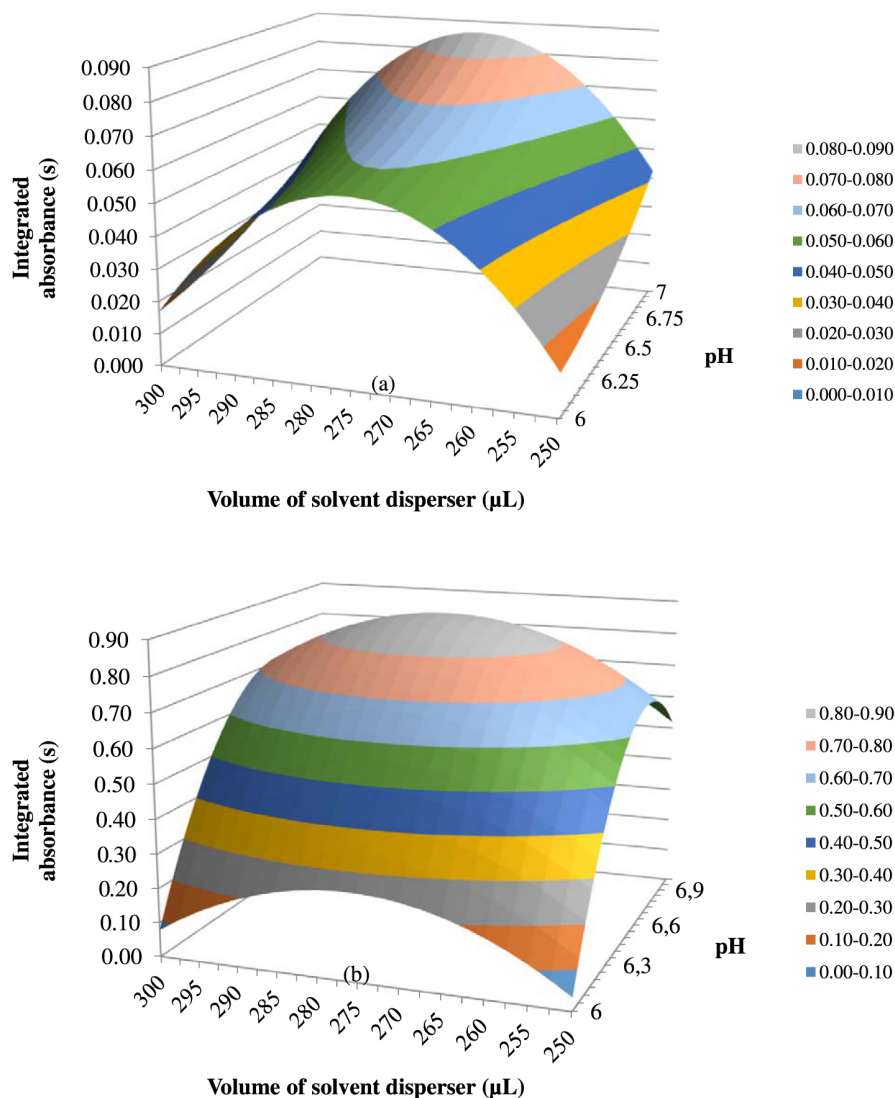


Figure 3. Response surface graphs for response of factorial design 3^2 . (a) Cr^{III} with APDC; (b) Cr^{III} with DDTC.

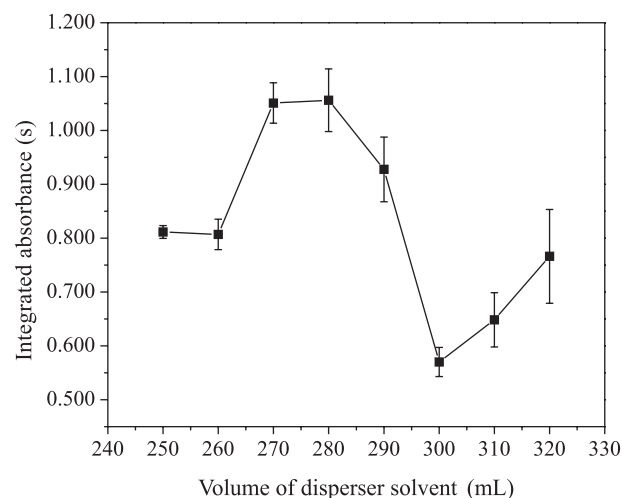


Figure 4. Effect of disperser solvent (ethanol) volume for Cr^{VI} using DDTC. Experimental conditions: $1000 \mu\text{g L}^{-1} \text{Cr}^{\text{VI}}$, H_2SO_4 (pH = 2.0), 6.0 mmol L^{-1} DDTC and $50 \mu\text{L}$ of 1-undecanol.

of APDC 6.0 mmol L^{-1} , 5.0% (m/v) NaCl, 50 and $275 \mu\text{L}$ of 1-undecanol and ethanol, respectively, for total Cr and $300 \mu\text{L}$ of ethanol for Cr^{VI} .

The reaction between Cr^{III} and APDC is then accelerated by heating the solution prior to the extraction procedure.^{26,33,35,36} To assess the influence of temperature and time on formation of the Cr^{III} complex with APDC after adjusting pH value (7.0) and addition of APDC (6.0 mmol L^{-1}) to an aqueous solution containing $50 \mu\text{g L}^{-1}$ of Cr^{III} , the resulting solution was heated to 80°C for 15 minutes²⁶ and after cooling at room temperature, microextraction took place. Integrated absorbance increased by 32 times in compared with the absorbance achieved without the heat step, thus showing the importance of the latter in higher sensitivity.

To select the type of complexant, the DLLME procedure was followed ($n = 3$), using experimental conditions previously selected and a heating step using APDC at pH 7.0, in aqueous solutions containing a mixture of Cr^{III} and Cr^{VI} $50 \mu\text{g L}^{-1}$. The selection of complexant was based on both the peak area and the relative standard deviations (RSD). At pH 2.0 (Cr^{VI}), integrated absorbance corresponded to 0.129 (RSD 21%) and 0.257 (RSD 3.2%) for DDTC and APDC, respectively. For total chromium-pH 6.5 (DDTC) and pH 7.0 (APDC)-the values of integrated absorbance were respectively 0.469 (RSD 9.3%) and 0.418 (RSD 0.98%). Apart from the lowest relative standard deviation using APDC for Cr^{VI} and total chromium, it was possible to notice that the analytical signal was twice as high for determining Cr^{VI} with APDC. It was then decided to use APDC as the complexant in the DLLME procedure proposed for determination and speciation of chromium. Table 3 shows optimal conditions for the DLLME procedure as well as for detection by HR-CS FAAS.

Analytical features of the proposed method

The analytical curves were achieved with and without the DLLME procedure (DLLME-HR-CS FAAS and HR-CS FAAS). Both curves were also obtained in triplicate and for the analytical curve without the DLLME procedure, the standards solutions of Cr were prepared in a medium of ethanol. Table 4 summarizes the analytical features for DLLME-HR-CS FAAS and HR-CS FAAS.

Table 3. Commitment conditions used for chromium speciation and detection

Variable	Condition
Complexant agent	APDC
Concentration / (mmol L^{-1})	6.0
pH	2.0 (Cr^{VI}) 7.0 (total Cr)
NaCl (m/v) / %	5.0
Heat temperature and time	total Cr 80°C for 15 min
Extractant solvent / μL	50 (1-undecanol)
Disperser solvent / μL	ethanol 300 (Cr^{VI}) 275 (total Cr)
Centrifugation condition / rpm	2000 (for 3 min)
Organic phase diluent / μL	200 (ethanol)
Instrumental parameter	
Wavelength / nm	357.9
Flame composition / (L h^{-1})	490 (air); 110 (acetylene)
Burner height / mm	13
Nebulization type	discrete
Aspiration rate / (mL min^{-1})	6
Analytical signal	peak area
Time of integration / s	10

APDC: ammonium pyrrolidine dithiocarbamate.

By comparing the angular coefficient of the analytical curves with and without the DLLME procedure, an increased level of sensitivity was obtained (26 times for Cr^{VI} and 19 times for total Cr). The combination of DLLME and HR-CS FAAS made it possible to reach quantification limits considerably below the acceptable levels of Cr established for drinking water by the World Health Organization (WHO),³⁸ Brazil's Ministry of Health³⁹ and European Drinking Water Directive of the European Union Council (EUC)⁴⁰ of $50 \mu\text{g L}^{-1}$ and the Environmental Protection Agency (EPA) of $100 \mu\text{g L}^{-1}$.⁴¹

Effect of foreign ions

The study of interfering elements is carried out, in general, in an univariate way, where only one ion is assessed at a time, although this type of study requires a

Table 4. Analytical features of DLLME-HR-CS FAAS and HR-CS FAAS methods for Cr^{VI} and Cr total

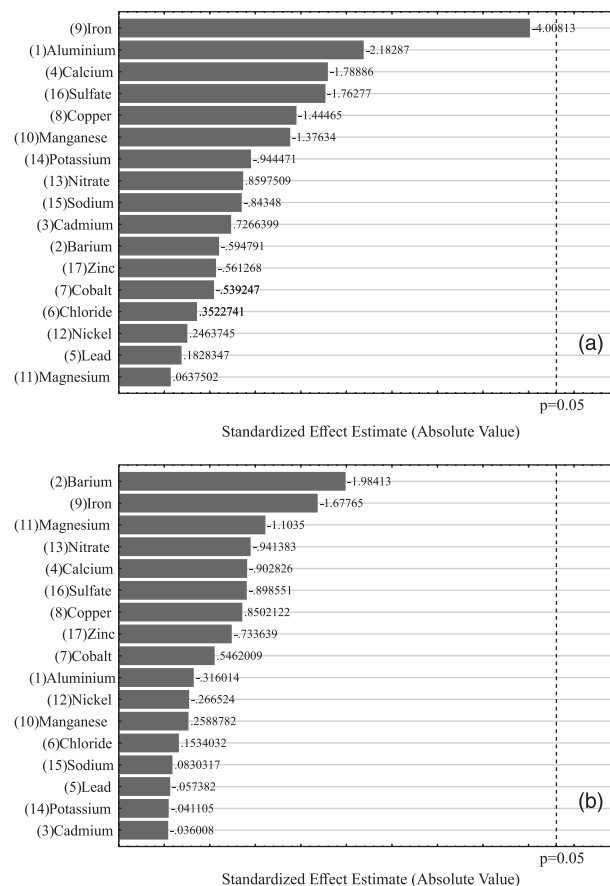
DLLME-HR-CS FAAS	Cr ^{VI}	Cr total
Analytical curve equation	$A = 5.1 (\pm 0.2) \times 10^{-3} C_{Cr^{VI}} + 1.3 (\pm 5.0) \times 10^{-3}$	$A = 3.7 (\pm 0.2) \times 10^{-3} C_{Cr^{total}} + 15 (\pm 6) \times 10^{-3}$
R ^{2a}	0.9987	0.9998
Linear range / ($\mu\text{g L}^{-1}$)	20-100	20-100
LOD / ($\mu\text{g L}^{-1}$)	0.35	6.7
LOQ / ($\mu\text{g L}^{-1}$)	1.1	22
Repeatability (RSD) ^b / %	7	18
Relative sensitivity ^c	26	19
HR-CS FAAS	Chromium	
Analytical curve equation	$A = 0.198 (\pm 0.006) C_{Cr^{total}} + 0.028 (\pm 0.006)$	
R ^{2a}	0.9994	
Linear range / (mg L^{-1})	0.5-3.0	
LOD / (mg L^{-1})	0.032	
LOQ / (mg L^{-1})	0.11	

^aNumber of calibration points, n = 8; ^brelative standard deviation, n = 10, Cr^{VI} 20 $\mu\text{g L}^{-1}$ and Cr total 80 $\mu\text{g L}^{-1}$; ^csensitivity DLLME-HR-CS FAAS/sensitivity HR-CS FAAS; DLLME-HR-CS FAAS: dispersive liquid-liquid microextraction high-resolution continuum source flame atomic absorption spectrometry; LOD: limit of detection; LOQ: limit of quantification.

long time frame, as the ions are simultaneously present in the sample. In this paper, the study of foreign ions was conducted in a multivariate way by means of a two-level Plackett-Burman design allowing the calculation of the main effects of an experimental set through a reduced number of experiments.^{42,43}

The foreign ions assessed were the following: aluminum, barium, cadmium, calcium, lead, chloride, cobalt, copper, iron, manganese, nickel, nitrate, potassium, sodium, sulfate and zinc. Except for chloride anions, nitrate and sulfate, the selection for the concentration of foreign ions was based on the value stated in the certified reference material NIST CRM 1643e (trace elements in water). The concentrations of foreign ions and levels assessed in the Plackett-Burman design are shown in Table S6 (Supplementary Information section).

Based on the design used, a matrix was designed with 27 experiments, out of which three correspond to the triplicate in the central point. For the DLLME procedure, the solution used was one containing 50 $\mu\text{g L}^{-1}$ Cr^{VI} and Cr^{III} in the presence of foreign ions as well as in the conditions previously set (Table 3). The entire matrix and integrated absorbance values are displayed in Tables S7 and S8 (Supplementary Information section). Such values were used to design Pareto charts, Figures 5a and 5b, where it can be noticed that in the conditions as selected for the DLLME procedure, all the foreign ions did not interfere in the complexation, microextraction and detection of chromium, due to the fact that the variances encountered did not deviate any further from the reference line. As a result, they were not seen as significant for the analytical response within the confidence interval of 95% ($p < 0.05$).

**Figure 5.** Pareto chart achieved from Plackett-Burman factorial design aiming at the effect of interfering ions in optimized conditions of extraction and detection at (a) pH 2.0; (b) pH 7.0.

Assessment of accuracy evaluation and applicability of the method to water samples

Table 5 shows the results obtained for the addition and

recovery trials of Cr^{III} and Cr^{VI} in different water samples and for determining Cr^{III} on certified reference material NIST CRM 1643e (trace elements in water). Recovery values ranged from 88 to 115%, suggesting that the proposed method is free from matrix effect. According to the *t*-test carried out (at a confidence interval of 95%) there were no significant differences between the values set and certified in the water reference material.

The proposed method was also applied to the detection of Cr in seawater, but both species of Cr were not detected. In order to compare the obtained results, total Cr was determined for mineral water 3 and seawater samples using the graphite furnace absorption atomic spectrometry (GFAAS) method. Table S9 (Supplementary Information section) presents information about the heating program using GFAAS, and some analytical features provided by the method. As obtained for the proposed method, Cr was not detected in both samples using GFAAS (LOD: 0.03 and LOQ: 0.10 µg L⁻¹).

Comparison of the proposed method with those previous reported

Table 6 shows a comparison of some experimental conditions and analytical features with those attained in procedures for Cr speciation and/or determination using liquid phase microextraction (LPME) procedures and FAAS detection.^{26,27,44-46} The proposed method leads

to detection limits higher than or comparable to the ones obtained by using different chelating reagents and extractant solvents. The proposed DLLME procedure uses a reduced volume of non-chloride solvents and low toxicity by combining 1-undecanol as an extractant solvent and ethanol as a disperser solvent. Moreover, the speciation of Cr was performed without adding any other reagent such as KMnO₄ for the oxidation of Cr^{III} to Cr^{VI} or Na₂SO₃ to reduce Cr^{VI} to Cr^{III}, and the organic phase was introduced without any previous evaporation of chloride solvent.

The results presented in the study of influence from other ions, Figures 5a and 5b, as well as those presented in Table 5 showed the good method selectivity without the need to add masking agent such as ethylenediamine tetraacetic acid (EDTA), that are at times necessary to the elimination of the interference of other ions, Co, Fe and Ni, for the detection of Cr by FAAS.^{26,35,36} The combination of DLLME with HR-CS-FAAS to detect Cr presents, in addition to the advantages previously showed, the possibility to a simultaneous correction of the background within the proximities of the analytical line, enabling to view the spectral region around the analytical line and automatic correction covering every continuum events.²⁴

Conclusions

The development of analytical methods that allow speciation and determination of potentially toxic chemical

Table 5. Determination of Cr^{III} and Cr^{VI} in water samples and certified reference materials using the proposed DLLME-HR-CS FAAS method

Sample	Added / (µg L ⁻¹)		Found concentration ^a / (µg L ⁻¹)			Recovery / %		
	Cr ^{III}	Cr ^{VI}	Cr ^{IIIb}	Cr ^{VI}	Cr total	Cr ^{III}	Cr ^{VI}	Cr total
Tap water	0.0	0.0	–	< LOQ	< LOQ	–	–	–
	20	20	19 ± 2	19.7 ± 0.1	39 ± 2	96	98	97
	30	30	32 ± 8	31 ± 4	63 ± 5	107	103	105
	40	40	36 ± 15	39 ± 5	75 ± 12	90	98	94
Mineral water 1	0.0	0.0	–	< LOQ	< LOQ	–	–	–
	20	20	18 ± 5	22 ± 1	40 ± 4	90	108	100
	30	30	31 ± 3	33 ± 1	64 ± 3	103	111	107
	40	40	36 ± 13	40 ± 1	76 ± 11	90	100	95
Mineral water 2	0.0	0.0	–	< LOQ	< LOQ	–	–	–
	20	20	20 ± 10	23 ± 2	43 ± 11	100	114	107
	30	30	26 ± 19	32 ± 3	58 ± 17	88	106	97
	40	40	38 ± 17	43 ± 2	81 ± 18	95	108	101
Mineral water 3	0.0	0.0	–	< LOQ	< LOQ	–	–	–
	20	2	23 ± 2	1.9 ± 0.5	25 ± 2	115	93	114
	50	20	56 ± 1	20 ± 2	76 ± 5	112	100	109
CRM NIST 1643e ^c	–	–	–	–	16.01 ± 1.93	–	–	–

^aValue ± standard deviation, n = 3; ^bCr^{III} concentration was evaluated from the difference between total Cr and Cr^{VI} concentration, (value ± standard deviation, n = 3); ^ccertified value 20.40 ± 0.24 µg L⁻¹ Cr; At 95% confidence limit (n = 3) *t*_{theoretical} = 4.303 and *t*_{experimental} = 3.939; LOQ: limit of quantification.

Table 6. Comparison of the proposed method with other extraction methods for Cr determination

Sample treatment/ spectrometric technique	Chelating reagent	Extractant solvent / μL	Disperser solvent	Sample volume / mL	LOD / ($\mu\text{g L}^{-1}$)		Reference
DLLME/FAAS	APDC	60 carbon tetrachloride	2 mL ethanol	25	0.07 Cr ^{VI}	0.08 total Cr	26
DLLME/MIS-FAAS	APDC	100 carbon tetrachloride	1 mL ethanol	40	0.037 Cr ^{VI}	–	27
RTILs-DLLME/FAAS	DDTC	40 [C ₈ MIM][PF ₆]	1 mL methanol	5	0.41 Cr ^{VI}	1.00 Cr ^{III}	44
M-IL-CIA-DLLME/FAAS	Schiff's base	90 mg [Hmim][PF ₆]	500 μL ethanol	30	–	0.7	45
SALLME-IL/FAAS	DPC	150 [C ₄ mim][BF ₄]	–	10	1.25 Cr ^{VI}	–	46
DLLME/HR-CS FAAS	APDC	50 1-undecanol	300 μL Cr ^{VI} 275 μL total Cr ethanol	9	0.35 Cr ^{VI}	6.7 total Cr	this work

LOD: limit of detection; DLLME: dispersive liquid-liquid microextraction; FAAS: flame atomic absorption spectrometry; MIS-FAAS: microsample injection system; RTILs: room temperature ionic liquids; APDC: ammonium pyrrolidine dithiocarbamate; DDTC: sodium diethyldithiocarbamate; [C₈MIM][PF₆]: 1-octyl-3-methylimidazolium hexafluorophosphate; M-IL-CIA: modified ionic liquid cold-induced aggregation; Schiff's base: bis(2-methoxybenzaldehyde) ethylene diimine; [Hmim][PF₆]: 1-hexyl-3-methylimidazolium hexafluorophosphate; SALLME-IL: salt-assisted liquid-liquid microextraction with ionic liquid; DPC: 1,5-diphenylcarbazide; [C₄mim][BF₄]: 1-butyl-3-methylimidazolium tetrafluoroborate.

elements has become of paramount importance, mainly when toxicological characteristics are dependent on the oxidation state, which can be seen for Cr^{III} and Cr^{VI}. The DLLME procedure proposed in this paper made it possible to carry out the determination and speciation of chromium in different water samples by using reduced volumes of non-chloride solvents, 1-undecanol and ethanol, and a consequent reduction in residue produced. The combination of DLLME and HR-CS FAAS also made it possible to reach quantification limits, 1.1 and 22 $\mu\text{g L}^{-1}$ for Cr^{VI} and total Cr, respectively, considerably below the acceptable levels of Cr established for drinking water.

Supplementary Information

Supplementary data (tables and figures) are available free of charge at <http://jbsc.s bq.org.br> as PDF file.

Acknowledgments

This research has been supported by National Council for Science and Technological Development (CNPq) and São Paulo Foundation Research (FAPESP Project No. 2011/19730-3). We thank Rodrigo Papai (PhD student) for assistance with GFAAS water sample analysis.

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Submitted: February 11, 2016

Published online: June 7, 2016

FAPESP has sponsored the publication of this article.