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A microextraction procedure based on an ionic liquid as an ion-pairing agent optimized using a design of experiments for chromium species separation and determination in water samples†

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Paula Berton,[ab] Luciana Vera-Candioti,[bc] Héctor C. Goicoechea[bc] and Rodolfo G. Wuilloud*[ab]

A microextraction methodology based on a room temperature ionic liquid (IL) as an ion-pairing reagent for determination of trace Cr(III) and Cr(VI) species is proposed. First, an ion-pair was formed between Cr(VI) species and the hydrophobic IL trihexyl(tetradecyl)phosphonium chloride (CYPHOS® IL 101). A simple and rapid microextraction procedure named ultrasound-assisted emulsification-microextraction (USAEME) was then developed for Cr species separation and preconcentration. Determination of Cr was performed by direct injection of the organic phase into an electrothermal atomic absorption spectrometer (ETAAS). Parameters that affect the efficiency of the microextraction step were investigated using a Plackett–Burman screening design. Then, the variables showing significant effects on the analytical response were considered within a further central composite design to optimize the experimental conditions. For 10 mL of water sample, the optimized USAEME procedure used 40 μL of tetrachloroethylene as extraction solvent, 5 min of extraction and 5 min of centrifugation at 1700 rpm. Selectivity among Cr species was obtained through pH selection. The concentration of Cr(III) species was calculated from the difference of total Cr and Cr(VI) concentrations. Under optimum conditions, the analyte extraction recovery was higher than 99% and yielded a preconcentration factor of 250. The limit of detection (LOD) obtained was 14.8 ng L^{-1} and the relative standard deviation for 10 replicate determinations at the 0.05 μg L^{-1} Cr(VI) level was 3.8%, calculated at peak areas. A correlation coefficient of 0.9983 was achieved. The method was successfully applied for Cr species determination in tap and river water samples.

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1 Introduction

Chromium (Cr) is one of the most abundant elements on earth and is widespread among the environmental compartments. The two oxidation states most commonly present in aqueous solution, *i.e.* Cr(III) and Cr(VI), differ drastically in their physiological and toxicological effects, as well as their chemical transformations, distribution and transport in the environment.[1] While Cr(III) is considered to be an essential trace element, Cr(VI) exerts toxic effects and it is known to be carcinogenic and mutagenic for biological systems.[2] Since the natural concentration of total Cr in surface waters is typically between 0.5 and 2 μg L^{-1}, it is obvious that Cr species will occur at levels of tenths or hundredths of μg L^{-1}.[3] Thus, considerable emphasis has been given to the development of sensitive analytical methodologies for Cr species separation and determination.[4] Even though atomic spectrometry-based detectors have been most widely employed for Cr species determination, the low concentrations of Cr usually found in water are not compatible with the detection limit achieved by these detectors.[5] Therefore, sample pre-treatment techniques are required in order to determine the individual Cr species.[3]

Conventional liquid–liquid extractions (LLE) can effectively decrease detection limits and eliminate matrix interference. However, several liquid phase microextraction (LPME) techniques have recently emerged as an attempt to miniaturize and to overcome some shortcomings originated from LLE, such as limited enrichment factors, slow and tedious procedures and the use of large volumes of organic solvents.[6,7] In 2006, Rezaee *et al.* reported for the first time a dispersive liquid–liquid microextraction (DLLME) technique.[8] In spite of its main advantages, this novel technique presents some disadvantages

[a]*Laboratory of Analytical Chemistry for Research and Development (QUIANID), Instituto de Ciencias Básicas, Universidad Nacional de Cuyo, Padre J. Contreras 1300, Parque Gral. San Martín, Mendoza, Argentina. E-mail: rwuilloud@mendoza-conicet.gob.ar; rodolfowuilloud@gmail.com; Tel: +54-261-4259738*

[b]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

[c]*Laboratory of Analytical Development and Chemometrics (LADAQ), Analytical Chemistry I, Faculty of Biochemistry and Biological Sciences, National University of the Litoral, University City, S3000ZAA Santa Fe, Argentina*

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such as the need for a third component (disperser solvent) that usually decreases the partition coefficient of analytes into extraction solvent. In order to overcome this drawback, ultrasound-assisted emulsification-microextraction (USAEME) was recently developed.[9] Ultrasound radiation causes acoustic cavitation, which enhances chemical reactions and mass transfer. It facilitates the dispersion of the extractant in the aqueous phase and the formation of organic vesicles, thus providing efficient extraction without the need for a third solvent.[10] On the other hand, ionic liquids (ILs) are a generation of fluids that, due to their specific properties such as negligible vapor pressure, have been proposed, among other applications, for separation processes.[11] Among them, the hydrophobic long-chain quaternary phosphonium IL trihexyl(tetradecyl)phosphonium chloride (CYPHOS® IL 101) has been employed as an extraction solvent in LLME for Co(II), Hg species, Se species and Pb(II) preconcentration.[12–15] Furthermore, the use of CYPHOS® IL 101 has been recently investigated as a potential novel ion-pair reagent dissolved in conventional organic solvents for metal extraction from aqueous phases.[16–18] Recently, this IL was proposed as an ion-pairing reagent for Tl–tetraiodide complex extraction into the IL $[C_6mim][PF_6]$.[19] In most of these methods, metal anionic chloro- or iodo-complexes are formed before ion-pair formation with CYPHOS® IL 101.

By their very nature, LLMEs require handling small volumes under strictly defined extraction and/or reaction conditions. Statistical analyses are available to evaluate which variables are significant in either mode of microextraction and to determine which combination of values (of the variables) produces the optimum results. The combination of microextraction and chemometrics significantly simplifies sample processing and also addresses problems related to improvement in detectability and method validation.[20]

In the present work, an IL is proposed for the first time as a direct ion-pair reagent for Cr speciation analysis, without the need for an extra ligand reagent. The speciation analysis was developed with initial ion-pair formation between Cr(VI) and CYPHOS® IL 101 followed by USAEME. An experimental design including both Plackett–Burman (P–B) and central composite design (CCD) allowed reduction in the number of optimization experiments. The multiple response criterion was successfully used to optimize the extraction of Cr(VI).[21] Selectivity of Cr species was achieved by choosing specific pH conditions based on the differences between Cr species formed at different pH values.[22,23] The total Cr was determined after oxidation of Cr(III) into Cr(VI) by hydrogen peroxide. The concentration of Cr(III) species was calculated from the difference of total Cr and Cr(VI) concentrations. The proposed method was successfully applied to the determination of Cr species at trace levels in natural and drinking water samples.

2 Materials and methods

2.1 Instrumentation

Experiments were performed using a Perkin-Elmer (Shelton, CT, USA) model 5100ZL atomic absorption spectrometer equipped with a pyrolytic graphite tube (Perkin-Elmer) and a transversely heated graphite atomizer Zeeman-effect background correction system. A Cr hollow cathode lamp (PerkinElmer) operated at a current of 25 mA and a wavelength of 357.9 nm with a spectral bandwidth of 0.7 nm was used. All measurements were performed using integrated absorbance with an integration time of 5 s. Temperature and time programs for the ETAAS instrument were as shown in Table 1.

A Horiba F-51 pH meter (Kyoto, Japan) was used for pH determination. A vortex model Bio Vortex V1 (Boeco, Hamburg, Germany) was used for mixing the phases. An US-bath (40 kHz and 600 W) with temperature control (Test Lab, Buenos Aires, Argentina) was employed in order to generate the dispersion. A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used to accelerate the phase separation process.

2.2 Reagents

A 1000 mg L^{-1} Cr(III) stock standard solution was prepared by dissolving 0.769 g of chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$, 99.99%) (Merck, Darmstadt, Germany) in 100 mL with a final HNO_3 concentration of 0.05 mol L^{-1}. A stock standard solution of 1000 mg L^{-1} Cr(VI) was prepared from 0.283 g potassium dichromate ($K_2Cr_2O_7$, 99.5%) (Aldrich, Milwaukee, WI, USA) dissolved and diluted to 100 mL with ultrapure water. Lower concentrations were prepared by diluting the stock solution with ultrapure water. A 0.75 mol L^{-1} phosphate buffer solution

Table 1 Instrumental and experimental conditions for Cr determination

Instrumental conditions				
Wavelength				357.9 nm
Spectral band width				0.7 nm
Lamp current				25 mA
Injection volume				40 μL
Modifier mass				17 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 1	110	1	30	250
Drying 2	130	15	40	250
Pyrolysis 1	600	30	15	250
Pyrolysis 2	800	15	30	250
Atomization	2300	0	5	—
Cleaning	2400	1	2	250
Extraction conditions				
Pre-treated sample volume				10 mL
CYPHOS® IL 101 concentration				1.2×10^{-4} mol L^{-1}
Working pH				7.0
Buffer concentration				9.0×10^{-3} mol L^{-1}
Solvent extraction volume				40 μL
Ion-pair formation time				1 min
Extraction time				5 min
Centrifugation time				5 min ($342 \times g$)

(Merck) adjusted to pH 7.00 was prepared by dissolution of potassium phosphate monobasic (Aldrich) and adjusted with hydrochloric acid (Merck). For a chemical modifier, a 0.87 g L^{-1} $Mg(NO_3)_2$ solution was prepared by dissolving 75.0 mg $Mg(NO_3)_2 \cdot 6H_2O$ (Merck) in 50 mL of 0.1% (v/v) HNO_3 solution. Organic solvents, such as chloroform, 1,2,4-trichlorobenzene, tetrachloroethylene, trichloroethylene and carbon tetrachloride, were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). The IL $[C_6mim][PF_6]$ was synthesized according to a method proposed by Huddleston and coworkers[24] and stored in contact with ultrapure water to equilibrate the water content in the IL phase.[25] Qualitative analysis of synthesized IL was performed by comparison of infrared spectra with commercially available $[C_6mim][PF_6]$ (Solvent Innovation GmbH, Köln, Germany). CYPHOS® IL 101 was kindly donated by Prof. Ullastiina Hakala (University of Helsinki, Finland) and supplied by CYTEC (Canada).

Ultrapure water (18 MΩ cm) was obtained from a Milli-Q Academic Water Purification System (Millipore, Billerica, MA, USA). All glassware was washed with a 0.1 mol L^{-1} HNO_3 solution at least for 24 h and thoroughly rinsed 5 times with ultrapure water before use.

2.3 Ion-pair formation and USAEME procedure

A volume of 10.0 mL of sample, or standard solution containing 0.1 μg L^{-1} of Cr(VI), was placed in a 10 mL graduated glass centrifuge tube with 120 μL of 0.75 mol L^{-1} phosphate buffer (pH 7.00) and 10 μL of 0.12 mol L^{-1} (6.3% (v/v)) CYPHOS® IL 101 solution (in methanol). The mixture was shaken for 15 s and kept still for 1 min. After ion-pairing, 40 μL of tetrachloroethylene were added. Immediately, the mixture was shaken for 15 s and then placed in an ultrasound bath at room temperature for 5 min. A cloudy solution was immediately formed, extracting the CYPHOS® IL 101–Cr(VI) ion-pair into the organic phase. Finally, centrifugation at 1700 rpm (342 × *g*) for 5 min allowed the formation of two well-defined phases. The upper aqueous phase solution was then manually removed with a syringe and the lower organic phase was directly injected into the graphite furnace of the ETAAS instrument for Cr determination (Table 1). Calibration was performed against aqueous standards and blank solutions applying the same procedure as described above.

2.4 Oxidation of Cr(III) species

Oxidation of Cr(III) to Cr(VI) species was performed following the procedure described by Monasterio *et al.*,[26] with a slight modification. Prior to oxidation, the pH of 70 mL of the standard solution (0.1 μg L^{-1} Cr(III)) was adjusted to 12.0 with NaOH. Then, 500 μL of 100 vol hydrogen peroxide were added to the solution. This solution was heated in a thermostatic water bath for 80 min at 80 °C and then boiled on a heating plate for 30 min in order to remove any excessive hydrogen peroxide. The resulting solution was then cooled to room temperature, neutralized and diluted to 100 mL with ultrapure water.

2.5 Sample collection and conditioning

For tap water sample collection, domestic water was allowed to run for 20 min and approximately a volume of 1000 mL was collected in a beaker. Tap water samples were analyzed immediately after sampling. River water samples were collected from Mendoza River (Mendoza, Argentina) in cleaned bottles rinsed three times with a water sample prior to collection. A sample volume of 1000 mL was collected at a depth of 5 cm below the surface. The river samples were filtered through 0.45 μm pore size membrane filters (Millipore Corporation, Bedford, MA, USA) immediately after sampling, stored in the dark at 4 °C in bottles (Nalgene; Nalge, Rochester, NY, USA) and analyzed within 48 h after collection.[27] The bottles used were previously washed with a 10% (v/v) HNO_3 water solution and then with ultrapure water.

2.6 Statistical methods for optimization

The main variables (factors) affecting the efficiency of USAEME, *i.e.* buffer concentration, CYPHOS® IL 101 concentration, ionic strength (salt concentration), sample volume, ion-pair formation time, solvent extraction volume, dispersion mode, extraction and centrifugation time and velocity, were evaluated by a P–B design with 12 experiments.[28]

Then, the variables showing significant effects such as CYPHOS® IL 101 concentration, ion-pair formation time, extraction time and solvent extraction volume were considered in a CCD consisting of 30 experiments in order to find optimum variable values for the response signal by optimizing an objective function. Finally, the multiple response criteria using the desirability function were successfully used to optimize the extraction of Cr(VI).[28] Experimental design, data analysis and desirability function calculations were performed by using the software Stat-Ease Design-Expert Version 8.0.7.1 (2011) (Stat-Ease Inc., Minneapolis).

3 Results and discussion

3.1 ETAAS conditions for Cr determination in IL-containing matrix

In this work, Cr was determined in the presence of the IL-organic matrix by direct injection of that phase into the graphite furnace of the ETAAS instrument. Since trace element detection by ETAAS in an organic-rich phase can carry some drawbacks when the matrix is present, there is a need for efficient matrix elimination. Therefore, pyrolysis and atomization temperatures were carefully optimized in order to obtain the highest absorbance-to-background signal ratio. Different amounts of $NH_4H_2PO_4$, $Mg(NO_3)_2$, $Pd(NO_3)_2$ and a mixture of them were tested as chemical modifiers to improve the Cr signal. The matrix modifier made a significant contribution to obtain high sensitivity, sharp and well defined absorption peaks and a reduced background. This was obtained by injecting 17 μg of $Mg(NO_3)_2$ into the graphite furnace. Therefore, further analyte measurements were performed with this chemical modifier. Optimal pyrolysis and atomization temperatures were 800 °C and 2300 °C, respectively (Table 1).

3.2 Effect of extraction solvent and pH

The extraction solvent to be used in USAEME should have higher density than that of water, a good extraction capability for the compounds of interest and low solubility in water.[29] Bearing in mind these considerations, chloroform, trichloroethylene, carbon tetrachloride, 1,2,4-trichlorobenzene, tetrachloroethylene and $[C_6mim][PF_6]$ were assayed as extraction solvents. The highest extraction recovery of Cr(VI) was obtained with tetrachloroethylene (Fig. 1). The highest affinity of the ion-pair towards this solvent could be due to its lowest polarity in comparison with the rest of the extraction solvents evaluated (Table 2). Furthermore, its lower water solubility, lower viscosity, and higher density make tetrachloroethylene a good extraction solvent for dispersive liquid phase microextraction techniques.

Selective microextraction of Cr(VI) was assayed with different pH values. The effect of pH on the extraction performance was studied within the range of 1–10 by adding appropriate volumes of HNO_3 or NaOH solution to the samples. As shown in Fig. 2, significant recoveries for both species [Cr(VI) and Cr(III)] were achieved at basic pHs (higher than 8.5). However, under acidic conditions, only Cr(VI) was recovered. At low concentrations of Cr(VI), acid chromate ($HCrO_4^-$) is the predominant species in the pH interval between 1 and 6, whereas dichromate ($Cr_2O_7^{2-}$) species becomes more important at higher pH.[22,23] On the other hand, while Cr(III) species at acidic pHs are cationic (*i.e.* $Cr(H_2O)_6^{3+}$), $Cr(OH)_4^-$ species became dominant at basic pHs.[23] The dissimilar extraction behavior of Cr(III) and Cr(VI) species towards the organic phase could be thus interpreted by the different charges observed for Cr(III) and Cr(VI) depending on pHs.

On the basis of the above description and the anion exchange properties of CYPHOS® IL 101, the following extraction reactions proposed by Alguacil *et al.*[17] must be considered when Cr(VI) forms an ion-pair with CYPHOS® IL 101.

$$[HCrO_4^- + R_4P^+ + Cl^-]_{aq} \leftrightarrow [R_4P^+HCrO_4^-]_{org} + [Cl^-]_{aq} \quad (1)$$

$$[Cr_2O_7^{2-} + 2R_4P^+ + 2Cl^-]_{aq} \leftrightarrow [(R_4P^+)_2Cr_2O_7^{2-}]_{org} + 2[Cl^-]_{aq} (2)$$

where the subscripts aq and org represent the aqueous and organic phases, respectively.

Consequently, complete separation of Cr species was achieved at pHs lower than 7.5. Therefore, a simple approach based on right selection of pH was pursued for selective extraction and determination of Cr(VI) species.

3.3 Screening phase

Systematic optimization procedures are carried out by selecting an objective function, finding the most important variables and investigating the relationship between responses and variables using the so-called response surface methodology (RSM).[28]

An experimental P–B design was built for the determination of the main variables affecting the extraction efficiency. The analyzed variables were buffer concentration ($7.5–15.0 \times 10^{-3}$ mol L^{-1}), CYPHOS® IL 101 concentration (2–10% (v/v)), ionic strength (0–0.51 g of KCl), sample volume (5–10 mL), ion-pair formation time (3–10 min), solvent extraction volume

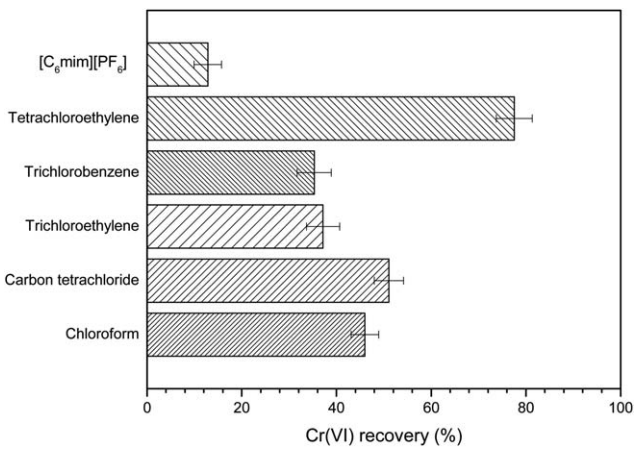

Fig. 1 Effect of extraction solvent on Cr(VI) species recovery (%). Other experimental conditions were as illustrated in Table 1.

Table 2 Physicochemical properties of different extraction solvents[6,37–40]

Solvent	Density (g cm^{-3})	Water solubility (g L^{-1})	Viscosity (mPa s)	Normalized polarity
Chloroform	1.48	8.50	0.54	0.259
Carbon tetrachloride	1.58	0.77	0.90	0.052
1,2,4-Trichlorobenzene	1.45	0.03	32.9	0.170
Trichloroethylene	1.45	1.10	0.53	0.160
Tetrachloroethylene	1.61	0.15	0.80	0.043
$[C_6mim][PF_6]$	1.29–1.37	7.50	560–586	0.657

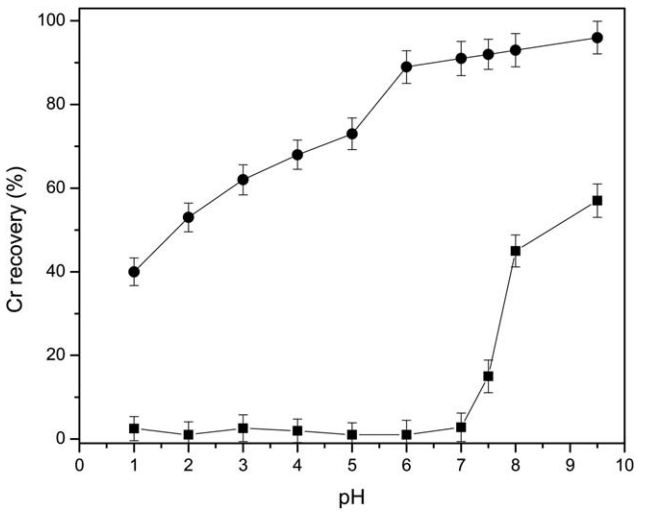

Fig. 2 Effect of pH on recovery (%) of Cr(III) (■) and Cr(VI) (●) species. Other experimental conditions were as illustrated in Table 1.

(40–90 μL), dispersion mode (vortex-ultrasonication), extraction time (1–5 min), centrifugation speed (1400–2000 rpm or 282–402 × *g*) and centrifugation time (5–10 min). The selected ranges for each variable were chosen according to previous experiments. The evaluation consisted of analyzing a sample spiked at constant mass of Cr(VI) and for each variable combination suggested by the P–B design, followed by determination of extraction recovery in each case.

A Pareto graph was used to choose significant effects. In this sort of graph, the bar height is proportional to the absolute value of the effect of each variable and can be used for comparing its significance (Fig. 3). There are two different *t* limits plotted in Fig. 3 (based on the Bonferroni corrected *t* and a standard *t*). These *t*-limits are only approximations at a significant level of 5%. Variables with effects above the Bonferroni limit are almost certainly significant, those with effects between the *t*-value limit and Bonferroni limit are likely significant and should be considered and at last those with effects below the *t*-value limit are not likely to be significant. According to this graph, the variables with significant effects were CYPHOS® IL 101 concentration, ionic strength, ion-pair formation time, solvent extraction volume and extraction time.

Furthermore, a Shapiro–Wilk normality test was performed for the normality of the unselected variables using the Pareto graph. The null hypothesis is that the data (the unselected variables) come from a normal distribution. A high *p*-value ($p = 0.108$) was obtained as a result of the Shapiro–Wilk test, indicating that the non-selected variables do not show deviation from normality, showing an agreement with the finding made with the previous test. In addition, the selected variables from the Pareto graph were examined by analysis of variance (ANOVA), reaching a similar result to that obtained by application of previous tests (data shown in Table S1 of the ESI†).

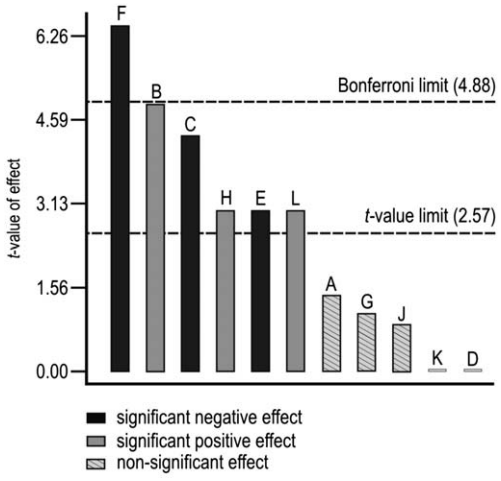

Fig. 3 Pareto graph used to determine significant effects. A: buffer concentration, B: CYPHOS® IL 101 concentration, C: ionic strength, D: sample volume, E: ion-pair formation time, F: solvent extraction volume, G: dispersion medium, H: extraction time, J: centrifugation velocity, K: centrifugation time, and L: dummy variable (unused variable).

The extraction recovery was statistically improved when the CYPHOS® IL 101 concentration and extraction time increased from low to high levels. The ion-pair formation is promoted when the concentration of CYPHOS® IL 101 increases, thus reducing the polarity of the Cr(VI) species. As a consequence, the extraction recovery is improved. Additionally, the extraction time interval is defined as the time elapsed between the extractant solvent addition and the end of the sonication stage. Analyte recovery increased for longer extraction times, since it plays an important role in the emulsification and mass-transfer phenomena (see Table S1 of the ESI†).

On the other hand, ionic strength, ion-pair formation time and solvent extraction volume showed negative effects (by decreasing the extraction efficiency) when levels changed from low to high values. Generally, the addition of salt in traditional LLE using conventional organic solvents increases the extraction performance due to the salting out effect. However, a negative effect on analyte extraction was observed when salt was added. Thus, salt addition was not adopted as it would significantly affect negatively either the ionic pair formation or the biphasic system generation.

Finally, the large adjusted *R*-square of 0.9045 indicates a good relationship between the experimental data and the fitted model.

3.4 Response surface method

A CCD was applied to find out the exact values of the four previously selected variables to obtain maximum recovery of Cr(VI). It consisted of 30 experiments based on combinations of the selected independent variables within the following ranges: (a) CYPHOS® IL 101 concentration: 2–15% (v/v) ($3.85–19.3 \times 10^{-5}$ mol L^{-1}); (b) ion-pair formation time: 0.5–10 min; (c) solvent extraction volume: 30–90 μL and (d) extraction time: 1–20 min (Table S2, ESI†). Other variables such as buffer concentration, ionic strength, sample volume, dispersion mode, centrifugation time and speed were set according to the results obtained in the screening phase (see above). Their values were 10.0 mL of sample volume, 9.0×10^{-3} mol L^{-1} of buffer concentration, pH 7.00, without salt addition, using ultrasound-assisted dispersion and 1700 rpm (342 × *g*) centrifugation speed for 5 min. The experiments were performed in two blocks (two consecutive days) in order to remove the expected variation caused by any change during the course of the experiment.[28]

Recovery of Cr(VI) for all the experiments was fitted to the modified cubic model once outliers were removed by analyzing the differences between fitted values test (DFFITS). This test measures the influence that each point has on the predicted value, computing a standardized value, which can be interpreted as the number of standard deviation units owed to experimental data which exert disproportionate influence on the model.[28] The model coefficients were calculated by backward multiple regression and validated by ANOVA. The model contains the intercept and the coefficients of the linear, the squared and the interaction terms between the variables:

$$\begin{aligned}\text{Recovery of Cr(VI) (\%)} = {} & 269.8 - 48.63A - 73.44B - 1.06C \\ & + 1.71D + 11.03AB + 2.58AC + 0.03AD \\ & - 1.30BC + 0.09BD + 0.04CD + 1.78A^2 \\ & + 5.95B^2 - 0.27C^2 - 0.27D^2 + 0.03ABC \\ & - 0.01ABD - 0.01ACD + 0.01BCD \\ & - 0.15A^2B - 0.12A^2C - 0.76AB^2\end{aligned}$$

where A is the CYPHOS® IL 101 concentration, B is the ion-pair formation time, C is the solvent extraction volume and D is the extraction time. The equation corresponds to real units.

An ANOVA test was employed to confirm that the fitted model explains significantly the experimental recovery (p-value < 0.0001). In addition, there was no evidence of lack of fit (F-value = 0.009 and p = 0.9911) at a significance level of 5% with a pure error mean square of 2.30. Further, the large adjusted R-square of 0.9917 indicates a good relationship between the experimental data and the fitted models. Finally, a variation coefficient of 1.40% is indicative of a low standard deviation, being a measure of the signal (response) to noise (deviation) ratio. In the present study, this ratio was equal to 36.33, which is indicative of an adequate signal (generally, ratio > 4 is desirable).

3.5 Optimization of Cr(VI) recovery

The aim of this optimization procedure was to find the USAEME conditions which provide the maximum extraction recovery of Cr(VI), employing the desirability function in the optimization process. Although the desirability function has been widely used to simultaneously optimize several responses,[30–33] in the present work the desirability function was employed to optimize not only the recovery of Cr(VI) (response) but also variables such as ion-pair formation time, solvent extraction volume and extraction time. Therefore, three variables and one response were simultaneously optimized by using the desirability function. Table 3 shows the criteria followed to adjust to a fixed value of 100% recovery of Cr(VI), the lower and upper limits and the optimal conditions. In order to obtain reproducible procedures, ion-pair formation time and extraction time were adjusted to fixed values of 1.0 and 5.0 min, respectively, while the solvent extraction volume was minimized.

Following the conditions and restrictions previously discussed, the optimization procedure was carried out and response surfaces obtained for global desirability functions are presented in Fig. 4. These plots were obtained for a given pair of variables, while maintaining the others fixed at their optimal values. This figure shows only two surfaces as examples: CYPHOS® IL 101 concentration *vs.* ion-pair formation time (Fig. 4a) and extraction time *vs.* extraction solvent volume (Fig. 4b).

Under the above-mentioned optimization criteria, the experimental conditions corresponding to one maximum in the desirability function (D = 1.00; recovery = 100%) were CYPHOS® IL 101 concentration 1.2×10^{-4} mol L^{-1}, ion-pair formation time 1.00 min, solvent extraction volume 40 μL and extraction time 5.0 min.

Table 3 Criteria for the optimization of individual responses in order to obtain the overall desirability response (D)

Variable/Response	Goal	Lower limit	Upper limit	Optimal conditions
CYPHOS® IL 101 concentration (% (v/v))	In range	2	15	6.3%
Ion-pair formation time (min)	Target = 1.0	0.5	10	1.0 min
Solvent extraction volume (μL)	Minimize	40	90	40 μL
Extraction time (min)	Target = 5.0	3	20	5.0 min
Extraction recovery (%)	Target = 100	62.07	104.4	100%

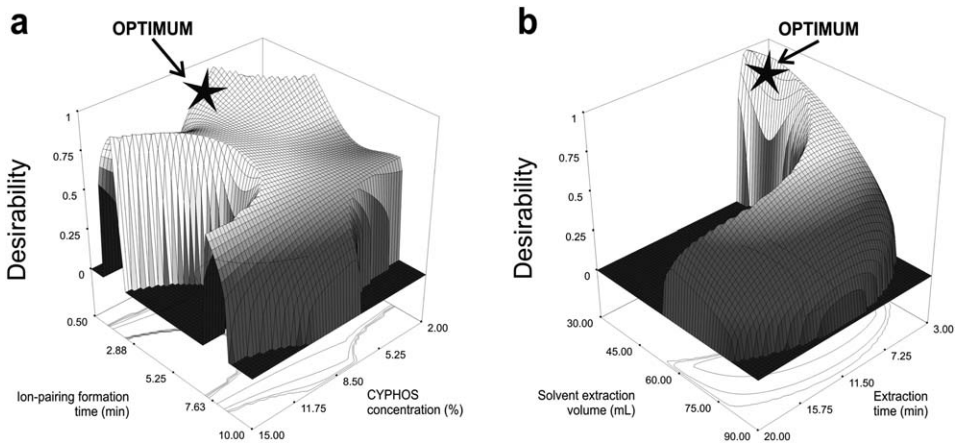

Fig. 4 Response surface plots corresponding to the desirability function when optimizing (a) CYPHOS® IL 101 concentration *vs.* ion-pair formation time and (b) extraction time *vs.* extraction solvent volume.

Table 4 Selectivity of the method for Cr(III) and Cr(VI) species determination

Cr(III)/Cr(VI) ratio	Cr(III)			Cr(VI)		
	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery (%)	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery (%)
0.20	0.050	0.049	99.1	0.250	0.254	101.6
1.00	0.150	0.150	100.3	0.150	0.147	97.8
5.00	0.250	0.245	98.2	0.050	0.050	100.9

The experimental values suggested after the optimization procedure were corroborated and the experimental recovery was compared with the theoretical one. As a result, it can be concluded that there is no significant difference between the predicted (99.99%) and experimental values (99.10%, CV = 3.8%).

3.6 Study on potential interfering species

To study potential interferences, several anions and cations at the concentration levels at which they may occur in the samples under study were added to a 0.1 $\mu g\ L^{-1}$ Cr(VI) solution and the procedure was followed as described earlier in this work. A concomitant ion was considered to interfere if it resulted in an analytical signal variation of ±5%. Analyte recovery was not influenced by Fe^{3+}, Cd^{2+}, Ca^{2+}, Mn^{2+}, Na^{+}, K^{+} and Mg^{2+} in concentrations up to at least 2000 $\mu g\ L^{-1}$. On the other hand, the number of anions usually present in the samples under study (such as Cl^{-}, NO_3^{-}, PO_4^{3-} and SO_4^{2-}) did not cause any adverse effects on the analytical signal. Elements such as As, Mn or V cannot be extracted directly using an IL as the ion-pairing reagent, since a complexing reagent is needed for extraction of these elements, such as molybdate heteropoly acid for As extraction.[34] Further, these elements are normally present at low concentrations in water samples. The value of the reagent blank signal was not modified by the presence of potentially interfering ions.

3.7 Analytical performance

Extraction recoveries higher than 99% were obtained when the procedure was performed in the sample matrices under optimum experimental conditions (Table 1). The enhancement factor (EF), obtained from the slope ratio of the calibration graph after and before application of the microextraction procedure, was also evaluated.[35] The obtained EF for a sample volume of 10 mL was 250. The relative standard deviation (RSD) resulting from the analysis of 10 replicates of 10 mL of standard solution containing 0.05 $\mu g\ L^{-1}$ Cr(VI) was 3.8%. The calibration graph was linear with a correlation coefficient of 0.9983 at levels near the detection limits and up to at least 0.4 $\mu g\ L^{-1}$ Cr(VI). The limit of detection (LOD), calculated based on the signal at the intercept and three times the standard deviation about regression of the calibration curve, was 14.8 ng L^{-1} Cr(VI) for the proposed methodology.

Since there is no certified reference material with certified concentrations of Cr species and Cr total which could be considered as a representative water sample, in order to evaluate the selectivity of the method on the Cr(III) and Cr(VI) species, the procedure was applied to various synthetic samples containing both species at different concentration ratios. It can be observed in Table 4 that both species were completely separated and quantitatively recovered. The method was thus shown to have an acceptable accuracy under different conditions, with recovery percentages between 98.2 and 100% for Cr(III) and between 97.8 and 102% for Cr(VI).

In comparison with other microextraction methods developed for Cr species determination, the proposed one presents a limit of detection comparable to, or better than, the others

Table 5 Performance data obtained by using the proposed method and other extraction methods based on ionic liquids reported for the determination of Cr species in water samples[a]

Method	Species	LOD (ng L^{-1})	RSD (%)	Calibration range ($\mu g\ L^{-1}$)	Microextraction procedure time	Enhancement factor	Ref.
HF-LPME-FAAS	Cr(III) and Cr(VI)	700 [Cr(VI)]	4.9 [Cr(VI)]	3–200	15 min	175	41
Ultrasonic probe-assisted IL-DLLME-ETAAS	Cr(VI)	70	9.2	0.50–8.00	16 min	300[b]	42
IL-DLLME-FAAS	Cr(III) and Cr(VI)	410 [Cr(VI)] 1000 [Cr(III)]	4.0 [Cr(VI)] 3.3 [Cr(III)]	3–800 [Cr(VI)] 5–200 [Cr(III)]	12 min	16.7[c]	43
IL-LLE-HPLC	Cr(III) and Cr(VI)	1000 [Cr(VI)] 1900 [Cr(III)]	0.3	25–200	13 min	[d]	44
TCME-ETAAS	Cr(III) and Cr(VI)	2.45 [Cr(VI)] 5.40 [Cr(III)]	4.24 [Cr(VI)] 3.05 [Cr(III)]	25–150 [Cr(VI)] 50–200 [Cr(III)]	25 min	43[c] [Cr(VI)] 42[c] [Cr(III)]	45
IL-DLLME-ETAAS	Cr(III) and Cr(VI)	2	8.1 [Cr(III)] 7.2 [Cr(VI)]	0.005–0.1	5 min	300[c]	46
SALLME-IL-FAAS	Cr(III) and Cr(VI)	1250 [Cr(VI)]	1.51 [Cr(VI)]	3–150 [Cr(VI)]	[d]	100[b]	47
USAEME-ETAAS	Cr(VI) and Cr(III)	14.8 [Cr(VI)]	3.8 [Cr(VI)]	0.03–0.40 [Cr(VI)]	11 min	250	Present work

[a] HF-LPME: hollow fiber liquid phase microextraction; FAAS: flame atomic absorption spectrometry; HPLC: high-performance liquid chromatography; TCME: temperature-controlled microextraction; SALLME-IL: salt-assisted liquid–liquid microextraction with ionic liquid. [b] Enrichment factor. [c] Preconcentration factor. [d] Not reported.

Table 6 Determination of Cr species in water samples (95% confidence interval; $n = 6$)

		Cr(III)			Cr(VI)		
	Sample	Added (ng L^{-1})	Found (ng L^{-1})	Recovery[a] (%)	Added (ng L^{-1})	Found (ng L^{-1})	Recovery[a] (%)
River water	1	—	234 ± 10	—	—	157 ± 6	—
		100	331 ± 13	96.6	100	257 ± 10	100
	2	—	193 ± 9	—	—	283 ± 11	—
		100	294 ± 11	101	100	381 ± 14	97.5
	3	—	305 ± 12	—	—	[b]	—
		100	404 ± 16	99.3	100	103 ± 8	103
Tap water	1	—	258 ± 11	—	—	54 ± 4	—
		100	356 ± 13	98.3	100	155 ± 9	101
	2	—	152 ± 7	—	—	[b]	—
		100	252 ± 11	100	100	98 ± 5	98.0
	3	—	248 ± 10	—	—	128 ± 7	—
		100	348 ± 14	99.8	100	227 ± 10	98.9

[a] [(Found-base)/added] × 100. [b] Not detected.

(Table 5). Likewise, most of the methodologies previously proposed for Cr species determination were more time-consuming than the present one.

3.8 Determination of Cr in water samples

Since one of the main routes of incorporation of Cr into the human body is water, its determination in these types of samples becomes very important. Therefore, the proposed method was applied to the determination of soluble Cr species present in several tap and river water samples. The proposed method was applied to six portions of different matrices and the average concentrations of Cr species obtained were taken as base values. The selectivity of the proposed method for Cr species determination was assayed adding 0.1 μg L^{-1} of Cr(III) and Cr(VI) species to samples and the same procedure was followed. The results obtained are summarized in Table 6. The concentrations in river water samples were in the range of 0.19–0.31 μg L^{-1} for Cr(III) and not detected (0.28 μg L^{-1}) for Cr(VI). The concentrations of Cr species in tap water were in the range of 0.15–0.26 μg L^{-1} for Cr(III) and not detected (0.13 μg L^{-1}) for Cr(VI). The results are in good agreement with a previous study, where similar Cr species concentrations in water samples were reported.[36]

4 Conclusions

The present work proposes a novel methodology based on the innovative application of CYPHOS® IL 101 as an ion-pairing reagent to achieve separation and preconcentration of Cr(III) and Cr(VI) species. For the first time, the addition of a third component to form a complex prior to the ion-pair formation was not necessary. Moreover, it has been demonstrated that selective extraction of Cr(VI) species can be obtained under specific pH conditions.

Through the multivariate optimization strategy, a successful determination of optimal USAEME conditions was achieved, thus obtaining a novel, simple, rapid and low-cost approach to determine Cr(III) and Cr(VI) species in several types of water samples.

The present work confirms the great potential that ILs have, not only for direct separation of elemental species but also as real derivatizing agents for highly efficient extraction (99%) and preconcentration. The low consumption of organic solvent and simplicity of the proposed USAEME associated with ETAAS detection turns this technique into a low cost and environmentally friendly tool for elemental speciation studies.

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