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Cloud point extraction for high-performance liquid chromatographic speciation of Cr(III) and Cr(VI) in aqueous solutions

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Abstract

Cloud point extraction (CPE) was applied as a preconcentration step for HPLC speciation of chromium in aqueous solutions. Simultanous preconcentration of Cr(III) and Cr(VI) in aqueous solutions was achieved by CPE with diethyldithiocarbamate (DDTC) as the chelating agent and Triton X-114 as the extractant. Baseline separation of the DDTC chelates of Cr(III) and Cr(VI) was realized on a RP-C_{18} column with the use of a mixture of methanol–water–acetonitrile (65:21:14, v/v) buffered with 0.05 M NaAc–HAc solution (pH 3.6) as the mobile phase at a flow rate of 1.0 ml min^{-1}. The precision (R.S.D.) for eight replicate injections of a mixture of 100 $\mu g\,l^{-1}$ of Cr(III) and Cr(VI) were 0.6 and 0.5% for the retention time, 4.1 and 4.6% for the peak area measurement, respectively. The concentration factor, which is defined as the concentration ratio of the analyte in the final diluted surfactant-rich extract ready for HPLC separation and in the initial solution, was 65 for Cr(III) and 19 for Cr(VI). The linear concentration range was from 50 to 1000 $\mu g\,l^{-1}$ for Cr(III) and 50–2000 $\mu g\,l^{-1}$ for Cr(VI). The detection limits of Cr(III) and Cr(VI) were 3.4 and 5.2 $\mu g\,l^{-1}$, respectively. The developed method was applied to the speciation of Cr(III) and Cr(VI) in snow water, river water, seawater and wastewater samples.

Keywords: Cloud point extraction; Extraction methods; Water analysis; Environmental analysis; Chromium; Metal cations

1. Introduction

Separation and preconcentration based on cloud point extraction (CPE) are becoming an important and practical application of the use of surfactants in analytical chemistry [1,2]. CPE as a preconcentration method offers many advantages, such as low cost, safety, and a high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and high concentration factors. Aqueous solutions of almost all non-ionic surfactants become turbid when heated to a temperature known as the cloud point. Above this temperature the isotropic micellar solution separates into two transparent liquid phases: a surfactant-rich phase of very small volume, composed mostly of the surfactant plus a small amount of water, and an aqueous phase, in equilibrium with the former, which contains a surfactant concentration close to its critical micellar concentration. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficiency, speedy and of lower toxicity to the environment than those extractions that use organic solvents. The first use of the CPE technique was pioneered by Goto et al. [3]. The CPE phenomenon has been used for the extraction and preconcentration of organic compounds [4,5] and metal cations [6,7] after the formation of sparingly water-soluble complexes. CPE has been shown to be an effective sample preconcentration technique for improving sensitivity and selectivity prior to atomic spectrometry [6,7], high-performance liquid chromatography [8,9], and flow injection analysis [10]. CPE as a preconcentration technique prior to HPLC was mostly used for organic compounds (polycyclic aromatic hydrocarbons, PAHs) [8,9].

Speciation of trace elements has become important in the past decade, due to its impact on environmental chemistry, ecotoxicology, clinical toxicology and food industry. The element Cr occurs in samples of natural origin in two relatively stable valence states, i. e. in the form of Cr(III) and Cr(VI), which exert quite different effects on biological

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systems. Cr(III) is an essential component having an important role in the glucose, lipid and protein metabolism, whereas Cr(VI) has a definitely adverse impact on living organisms. Cr(VI) can easily penetrate the cell wall and exert its noxious influence in the cell itself, being also a source of various cancer diseases [11]. Chromium is widely used in industry [12], and as corrosion inhibitors used in water pipes [13] that constitute a potential source of Cr(VI) and Cr(III) in the drinking water distribution system. The sources of Cr(VI) and Cr(III) must therefore be monitored and this requires speciation techniques with sufficient selectivity and high sensitivity [14]. The speciation methods of chromium, involved a separation step with ion chromatography [15] prior to detection or coprecipitation, solvent extraction using different reagents [16], solid sorbent extraction [17], electrodeposition of Cr(VI) in a graphite furnace [18], etc. Several electrochemical [19], photometric [20] methods employed suffered either from high detection limits and numerous interference or poor reproducibility. CPE as a preconcentration step for Cr speciation before detection by flame atomic absorption spectrometry [21] and spectrofluorimetry [22] was also reported. To date, inductively coupled plasma (ICP) MS is the most sensitive technique. However, such instruments are expensive. HPLC for Cr(III) and Cr(VI) speciation has been reported [23,24]. However, the use of CPE as a preconcentration step for HPLC speciation of trace Cr(III) and Cr(VI) has not been reported before.

The aim of the present work was to apply CPE as a preconcentration step for HPLC speciation of chromium. In the developed system, diethyldithiocarbamate (DDTC) was used as the chelating agent and Triton X-114 as the extractant. Potential factors affecting the CPE preconcentration and the subsequent HPLC separation of the DDTC chelates of Cr(III) and Cr(VI) were investigated in detail.

2. Experimental

2.1. Instrumentation

All separations were achieved on an analytical reversed-phase column (Maxsil ODS 5 μm, 25 cm × 4.6 mm i.d., Hertz Biotech., Zibo, China) at room temperature under isocratic conditions using a Waters model 600 HPLC system equipped with a Waters 600 Controller, Waters 600 Pumps, a Waters 2996 photodiode array detector, and injector (sample loop: 20 μl). The Empower software was used to acquire and process spectral and chromatographic data from the photodiode array detector 2996. The chromatograms were monitored at 254 nm for optimization experiments and peak area measurements.

A thermostated water bath maintained at the desired temperatures (Tianjin MinLi Science Instrument, Tianjin, China) was used for equilibration temperature experiments and the phase separation was assisted with a centrifuge (Shanghai Operation Apparatus, Shanghai, China).

2.2. Reagents

All chemicals were at least of the analytical grade. Doubly deionized water (DDW, 18 MΩ cm) obtained from a Water-Pro water system (Labconco Kansas City, MO, USA) was used throughout. Triton X-114 (Sigma) was used as the non-ionic surfactant. DDTC (Tianjin Chemicals, Tianjin, China) was used as the chelating agent to form the hydrophobic metal complexes. A 0.1% (m/v) of DDTC solution was prepared by dissolving suitable amount of DDTC in DDW. A mixture of methanol (Chromatographic Grade, Tianjin Kangkede Chemicals, Tianjing, China)–water–acetonitrile (Chromatographic Grade, Tianjin Kangkede Chemicals) (65:21:14, v/v) buffered with 0.05 M NaAc–HAc solution (pH 3.6) was employed as the mobile phase at a flow rate of 1.0 ml min^{-1}. The pH of the sample solution was adjusted to 7.0 with HAc. The mobile phase was filtered through a 0.45 μm filter, and was degassed in an ultrasonic bath for 20 min just prior to use. To ensure good day-to-day precision for the HPLC separation, methanol at 1.0 ml min^{-1} was used to wash the HPLC column for 30 min to remove residual surfactant adsorbed on the column after 1 day experiment.

Stock standard solutions of Cr(III) and Cr(VI) at a concentration of 1000 mg l^{-1} were prepared from chromium nitrate (Tianjin Chemicals) and potassium chromate (Tianjin Chemicals). Working standard solutions were prepared by stepwise diluting the stock solutions just before use.

2.3. Samples

Snow water and river water, seawater and wastewater samples were collected locally, filtered through 0.45 μm filter and analyzed immediately.

2.4. Procedures for cloud point extraction

For CPE preconcentration, aliquots of 10.0 ml of the solution (pH 7.0) containing the analytes, 0.005% (m/v) DDTC and 0.20% (v/v) Triton X-114 were heated in a thermostated water bath at 40 °C for 10 min. The mixture was centrifuged at 3500 rpm for 5 min for phase separation, and then cooled in an ice-bath for 10 min to increase the viscosity of the surfactant-rich phase. The supernatant aqueous phase was carefully removed with a pipette. Fifty microliters methanol was added to the surfactant-rich phase (ca. 100 μl) to reduce its viscosity just before HPLC separation.

3. Results and discussion

3.1. Factors affecting the CPE preconcentration

The CPE can be used for the preconcentration of metal ions after the formation of sparingly water-soluble complexes. The CPE efficiency depends on the hydrophobicity

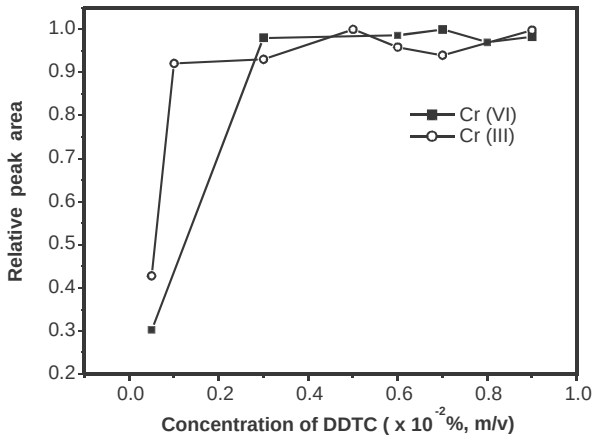

Fig. 1. Effect of the concentration of DDTC on the cloud point extraction of 100 μg l^{-1} Cr(III) and Cr(VI). The values for relative peak area were calculated relative to the highest peak area. Other conditions: 0.20% (v/v) Triton X-114, pH 7.0, equilibration temperature 40 °C. HPLC conditions: methanol–water–acetonitrile (65:21:14, v/v) buffered with 0.05 M NaAc–HAc buffer (pH 3.6). The flow rate was 1.0 ml min^{-1}. The monitoring wavelength was 254 nm.

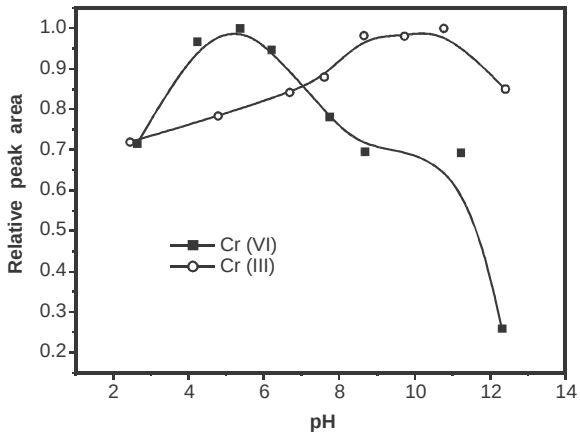

Fig. 2. Effect of pH on the cloud point extraction of 100 μg l^{-1} Cr(III) and Cr(VI). Other cloud point extraction conditions: 0.005% (m/v) DDTC, 0.20% (v/v) Triton X-114, equilibration temperature 40 °C. HPLC conditions as in Fig. 1.

of the ligand and the complex formed, the apparent equilibrium constants in the micellar medium, the kinetics of the complex formation, and the transference between the phases [1]. In this work, DDTC was used as the chelating agent due to the highly hydrophobic nature of its metal complexes. Fig. 1 shows the effect of DDTC concentration on the CPE of Cr(III) and Cr(VI). The concentration of DDTC tested ranged from 0.0005 to 0.009% (m/v). The CPE efficiency for Cr(VI) and Cr(III) increased rapidly as the concentration of DDTC increased from 0.0005 to 0.003% (m/v), and to 0.001%, respectively, then kept almost constant with further increase in the DDTC concentration up to 0.009% (m/v). Therefore, a DDTC concentration of 0.005% (m/v) was employed for further experiments.

Because pH plays a unique role in metal–chelate formation and subsequent extraction [1], the pH of the sample solution was the next critical factor evaluated for its effect on the CPE preconcentration of Cr(III) and Cr(VI). The examined pH ranged from 2.6 to12.3. As shown in Fig. 2, the maximum absorbance for Cr(VI) and Cr(III) was achieved in the range of 4.2–6.2, and 8.6–10.8, respectively. However, in order to extract Cr(VI) and Cr(III) simultaneously with the efficiency as high as possible, a pH value of 7.0 was used as a compromise.

The effect of Triton X-114 concentration was investigated between 0.05 and 0.25% (v/v). Fig. 3 shows variation of peak area of the analytes as a function of the surfactant concentration. The absorption of the analytes increased as the concentration of Triton X-114 increased from 0.05 to 0.15% (v/v), and remained constant between 0.15 and 0.25% (v/v) Triton X-114. So, a 0.20% (v/v) of Triton X-114 was employed.

To achieve easy phase separation and preconcentration as efficient as possible, optimal incubation time and equilibration temperature are necessary to complete reactions. The effect of the equilibration temperature was investigated from 20 to 80 °C. It was found that the CPE efficiency increased with increase in equilibration temperature from 20 to 35 °C, and reach maximum in the range of 35–45 °C. Over 40 °C, the CPE efficiency decreased probably due to the stability problems for chelates and chelating agents [1]. So, an equilibration temperature of 40 °C was used. Studies on the effect of the incubation time (Fig. 4) showed that the maximum extraction efficiency was observed from 8 to 12 min for Cr(III) and Cr(VI), and further increase in the incubation time resulted in a significant decrease of the efficiencies probably due to the thermal instability of the formed DDTC complexes. Further study is required to understand why the CPE efficiency for Cr species decreased as incubation time increased over 12 min. For the rest experiments, an incubation time of 10 min was used.

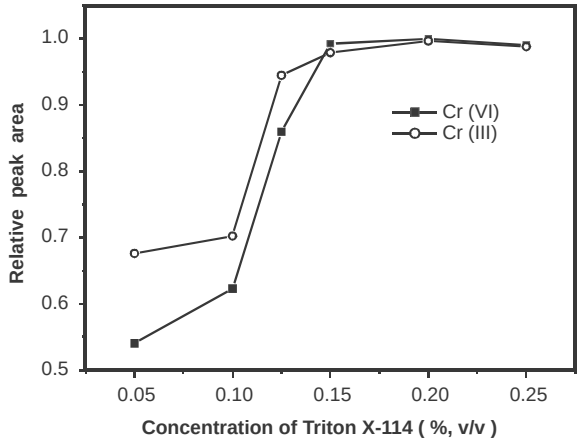

Fig. 3. Effect of the concentration of Triton X-114 on the cloud point extraction of 100 μg l^{-1} Cr(III) and Cr(VI). Other conditions: 0.005% (m/v) DDTC, pH 7.0, equilibration temperature 40 °C. HPLC conditions as in Fig. 1.

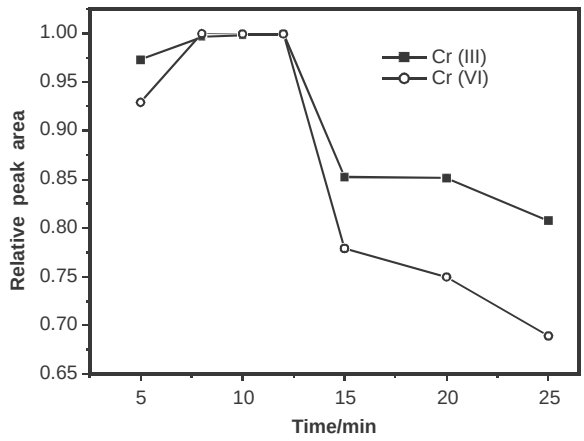

Fig. 4. Effect of the concentration of incubation time on the cloud point extraction of 100 μg l^{-1} Cr(III) and Cr(VI). Other conditions: 0.005% (m/v) DDTC, 0.20% (v/v) Triton X-114, pH 7.0, equilibration temperature 40 °C. HPLC conditions as in Fig. 1.

3.2. Consideration of mobile phase composition for HPLC speciation of chromium

A major disadvantage of using Triton X-series surfactants as CPE agents is that UV absorbance of these non-ionic surfactants could severely interfere with the detection of the analytes. Such potential interference should be avoided using an optimal composition of the mobile phase. For this purpose, the optimization of mobile phase composition first focused on the baseline separation of the DDTC, the DDTC complexes of Cr(III) and Cr(VI) and the Triton X-114 from each other. Because injection of viscous surfactant-rich phase into the HPLC column may impair the precision for Cr speciation due to deterioration of column efficiency caused by the adsorption of viscous surfactant onto the column, the composition of mobile phase should also facilitate the removal of the injected surfactant from HPLC column. Considering the above two important issues, a mixture of methanol, water and acetonitrile was used as the mobile phase and the composition of the mobile phase was optimized for baseline separation of the DDTC, the DDTC complexes of Cr(III) and Cr(VI) and the Triton X-114 with good precision for Cr speciation. After careful optimization, it was found that the use of a mixture of methanol–water–acetonitrile (65:21:14, v/v) buffered with 0.05 M NaAc–HAc solution (pH 3.6) as the mobile phase at a flow rate of 1.0 ml min^{-1} could serve well the above purposes.

Table 1
Analytical figures of merit for the developed CPE–HPLC methodology for the speciation of Cr(III) and Cr(VI)

	Cr(III)	Cr(VI)
Precision (R.S.D., $n = 8$) (%)		
Retention time	0.6	0.5
Peak area	4.1	4.6
Linear concentration range of the calibration graph[a] (μg l^{-1})	50–1000	50–2000
Detection limit (3σ)[b] (μg l^{-1})	3.4	5.2

[a] The concentration range in which the peak area of chromatographic peak increased linearly with the analyte concentration.
[b] Based on three times the standard deviation of the repeated peak area measurements of the reagent blank.

3.3. Interference studies

There are two kinds of potential interference in the present system. One is the interference due to the competition of other heavy metal ions for the chelating agent and their subsequent co-extraction with the Cr species. The other is the interference resulting from the co-extracted heavy metal complexes with strong UV absorption due to the overlap of the chromatographic peaks between the analyte complexes and other heavy metal complexes. The former gives negative interference effect, whereas the later presents positive interference effect. To evaluate the selectivity of the proposed method, the effect of typical potential interfering ions was investigated. The tolerable limit was taken as a relative error $\leq \pm 5\%$. The tolerable concentration ratio of foreign ions to 100 μg l^{-1} Cr(III) was found to be 500 for Mg(II)

Table 2
Analytical results for speciation of Cr(III) and Cr(VI) in aqueous solutions

Samples	Concentration (mean ± σ, $n = 3$) (μg l^{-1})			
	Determined by present method		Determined by a published method [25]	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Snow water	<3.4 (DL[a])	<5.2 (DL)	1.3 ± 0.06	1.5 ± 0.07
Spiked snow water	209.5 ± 1.8	190.8 ± 0.7	199.6 ± 4.6	202.5 ± 4.7
River water	<3.4 (DL)	<5.2 (DL)	2.3 ± 0.1	3.0 ± 0.1
Spiked river water	106.1 ± 3.5	100.4 ± 2.1	101.4 ± 2.5	101.6 ± 3.0
Seawater	<3.4 (DL)	<5.2 (DL)	2.0 ± 0.1	2.5 ± 0.1
Spiked seawater	97.1 ± 2.7	97.3 ± 0.2	103.6 ± 3.3	97.5 ± 3.2
Wastewater 1	60.3 ± 2.0	647.8 ± 9.9	61.7 ± 1.0	646.3 ± 16.0
Wastewater 2	126.2 ± 5.0	281.7 ± 6.5	124.7 ± 4.0	280.0 ± 4.3
Wastewater 3	117.2 ± 2.9	185.1 ± 3.9	118.7 ± 2.8	183.3 ± 4.3

[a] DL, detection limit.

and Ca(II); 20 for Cd(II); 10 for Hg(II), Cu(II), Mn(II) and Zn(II); 2 for Pb(II); 0.5 for Co(II) and Fe(III). To 100 μg l^{-1} Cr(VI) was 500 for Mg(II) and Ca(II); 10 for Hg(II), Cu(II) and Mn(II); 4 for Zn(II); 2 for Pb(II); 1 for Cd(II); 0.5 for Co(II) and Fe(III). The interference over the above concentration ratios of foreign ions to the Cr species represents a comprehensive effect from both the CPE and the chromatography. To date, most of the studies conducted have shown that ionic strength has no appreciable effect on the magnitude of CPE [1]. An increase in the ionic strength in the micelle mediated extraction systems does not seriously alter the efficiency of extraction of the chemical forms [1]. The quantitative recovery of the Cr species spiked in the seawater and wastewater in our study also demonstrated no interference from seawater and wastewater matrices.

3.4. Analytical figures of merit

Analytical characteristic data of the proposed CPE-HPLC for Cr(III) and Cr(VI) speciation were summarized in Table 1. The precisions (R.S.D.s) for eight replicate injections of a mixture of 100 μg l^{-1} of Cr(III) and Cr(VI) were 0.6 and 0.5% for the retention time, 4.1 and 4.6% for the peak area, respectively. The concentration factor, which is defined as the concentration ratio of analyte in the final diluted surfactant-rich extract ready for HPLC separation and in the initial solution, was 65 for Cr(III) and 19 for Cr(VI). The linear concentration ranges were from 50 to 1000 μg l^{-1} for Cr(III) and 50 to 2000 μg l^{-1} for Cr (VI). The detection limits (3σ) of Cr(III) and Cr(VI) based on the three times standard deviation of the peak area measurements of the reagent blank were 3.4 and 5.2 μg l^{-1}, respectively.

3.5. Application to chromium speciation in aqueous solutions

The proposed method was applied to the separation and determination of Cr(III) and Cr(VI) in snow water, river water, seawater and wastewater samples. The concentrations of Cr(III) and Cr(VI) in these samples were quantified using a simple external calibration method based on peak area measurement. In the original snow water, river water and seawater samples no Cr(III) and Cr(VI) were detected. The recoveries for 100 μg l^{-1}of Cr(III) and Cr(VI) spikes from these water samples ranged from 95 to 106%. The concentrations of Cr(III) and Cr(VI) in the spiked snow water, spiked river water, spiked seawater and original wastewater samples were also determined. As shown in Table 2, the concentrations of Cr(III) and Cr(VI) in these samples determined by the present method were in good agreement with those determined by an independent flow injection on-line separation coupled with flame atomic absorption spectrometry [25]. Fig. 5 compares the chromatograms of a standard solution of 100 μg l^{-1} Cr(III) and Cr(VI), wastewater 1, and wastewater 1 spiked with 200 μg l^{-1} of Cr(III) and Cr(VI) under the optimal conditions. As can be seen from Fig. 5, no difference of the retention times for Cr(III) and Cr(VI) was observed in the standard mixture, unspiked and spiked wastewater samples.

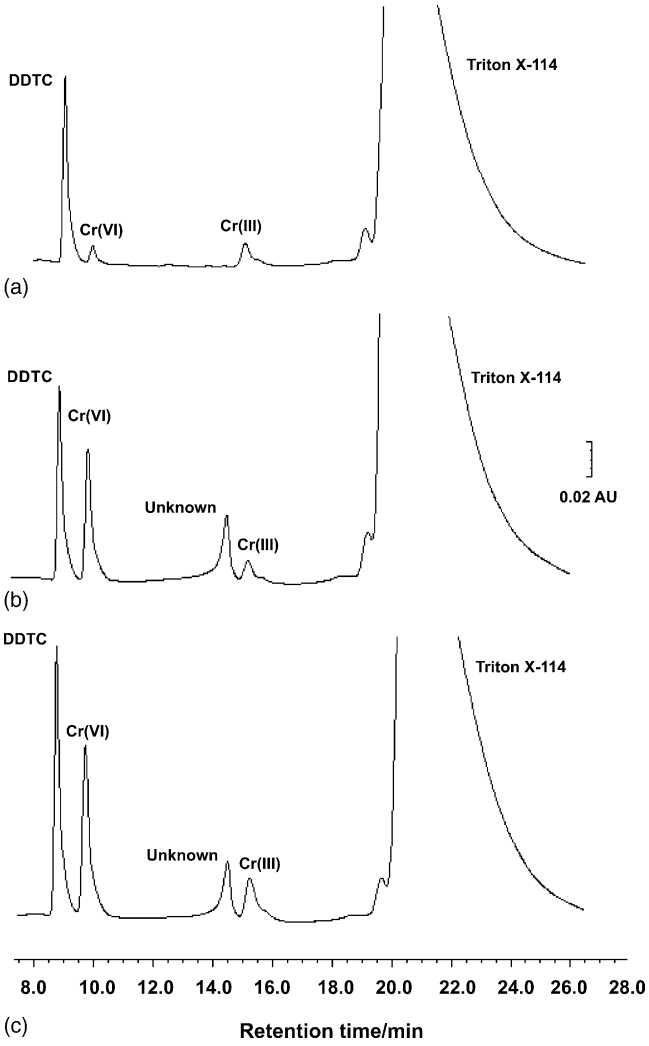

Fig. 5. Chromatograms of (a) a standard solution of 100 μg l^{-1} Cr(III) and Cr(VI), (b) wastewater 1, and (c) wastewater 1 spiked with 200 μg l^{-1} of Cr(III) and Cr(VI) under the optimal conditions. Cloud point extraction conditions: 0.005% (m/v) DDTC, 0.20% (v/v) Triton X-114, pH 7.0, equilibration temperature 40 °C. HPLC condition as in Fig. 1.

Acknowledgements

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