



*J. Serb. Chem. Soc.* 81 (6) 645–659 (2016)  
JSCS–4874

## Speciation of chromium through aqueous two-phase extraction of complexes of Cr(III) with 4-(2-pyridylazo)resorcinol and Cr(VI) with 1,5-diphenylcarbazide

TAMARA N. SIMONOVA<sup>1</sup>, VALENTINA A. DUBROVINA<sup>1</sup>  
and ANDRIY B. VISHNIKIN<sup>2\*</sup>

<sup>1</sup>Department of Analytical Chemistry, Faculty of Chemistry, Donetsk National University, Donetsk 83001, Ukraine and <sup>2</sup>Department of Analytical Chemistry, Faculty of Chemistry, Oles Honchar Dnipropetrovsk National University, Dnipropetrovsk 49010, Ukraine

(Received 30 June 2015, revised 3 January, accepted 11 February 2016)

**Abstract:** A green, sensitive and selective methods are described for the pre-concentration and speciation of chromium species in natural waters. The developed methods are based on the environmentally friendly aqueous two-phase extraction with a system consisted of ammonium sulfate and polyethylene glycol, or, alternatively, alcohol (ethanol, isopropanol). The extraction of chromium species from aqueous solution into the upper organic phase was performed with 4-(2-pyridylazo)resorcinol and 1,5-diphenylcarbazide as chelating agents for Cr(III) and Cr(VI), respectively. Some predominant factors affecting the pre-concentration and speciation of both Cr(III) and Cr(VI) species were evaluated and optimized. The concentration of chromium species in upper organic phase was determined by both spectrophotometric and electrothermal atomic absorption spectrometry (ET AAS) methods. Under the optimum conditions, the calibration graphs obtained with spectrophotometric method were linear over the concentration ranges from 5 to 500  $\mu\text{g L}^{-1}$  for Cr(III) and from 2.5 to 50  $\mu\text{g L}^{-1}$  for Cr(VI). Corresponding concentration ranges determined by using ET AAS method were from 1 to 10  $\mu\text{g L}^{-1}$  for both chromium species. The proposed methods were applied to the determination of chromium species in mine and waste waters with satisfactory results.

**Keywords:** chromium speciation; aqueous two-phase extraction; 1,5-diphenylcarbazide; 4-(2-pyridylazo)resorcinol; spectrophotometry; electrothermal atomic absorption spectrometry; waters.

### INTRODUCTION

Chromium is metal element with significant economic and environmental interest because it is extensively used in metal smelting, electroplating, tanning,

\* Corresponding author. E-mail: vishnikin@hotmail.com  
doi: 10.2298/JSC150630016S

metallurgy and dyestuff industry. It is especially significant to determine its forms because the toxicity of chromium compounds depends on the oxidation state. The Cr(III) is essential for plants and animals at trace concentration and occurs naturally in the environment. Cr(VI) is considered to be a more toxic form. Environmental contamination with Cr(VI) has been recognized as very dangerous source of pollution.

As the assessment of chromium toxicity requires speciation studies, often involving very low concentration, procedure is needed that will permit enrichment of the analyte while eliminating the matrix effect. The speciation methods of chromium, published hitherto, involved a separation as well as preconcentration step with solvent extraction using different reagents, solid sorbent extraction, high-performance liquid chromatography, ion chromatography, and so on.<sup>1-7</sup> For the speciation analysis, the separation techniques are combined with highly selective and sensitive detection systems such as flame atomic absorption spectrometry,<sup>7,8</sup> inductively coupled plasma atomic emission spectrometry,<sup>9</sup> inductively coupled plasma mass spectrometry,<sup>10</sup> electrothermal atomic absorption spectrometry (ET AAS),<sup>6,11,12</sup> spectrophotometry<sup>13</sup> and fluorometry.<sup>14</sup>

Extraction is one of the predominant method used for the chromium speciation.<sup>4</sup> Due to an increasingly stringent environmental regulations, there is a strong demand for less polluting safe analytical methods that are consistent with the principles of green chemistry.<sup>15,16</sup> Replacement of volatile organic compounds, the class of organic solvents commonly used in liquid-liquid extraction, is among the themes addressed. Use of toxic organic solvents, such as chloroform, 1,2-dichloroethane and others containing chlorine, for extraction of metal complexes is strictly forbidden by law. The toxic solvents such as ethyl acetate, isobutanol, methyl isobutyl ketone, and chloroform are used in the existing extractive procedures for the separation of chromium forms.<sup>17,18</sup> In addition, in certain cases, the extraction is incomplete, and the procedures are complex and time-consuming.

Aqueous two-phase systems (ATPS) are an excellent alternative for the selective extraction of metals because they are composed mainly of water and other components that are nontoxic, nonflammable and, in some cases, biodegradable and recyclable.<sup>19-22</sup> The possibility of linear scale-up, ease of use, low-cost and rapid phase separation without the formation of stable emulsions are advantages of the ATPS technique.<sup>19,20</sup> The separated organic phase always contains a lot of water and salt, resulting in a highly polar medium compared to the corresponding pure organic solvents. Thus, the separated organic phase can easily extract ion-pairs and highly charged species, which normally cannot be extracted using conventional solvents such as chloroform.<sup>23-25</sup> The modern versions of the aqueous two-phase extraction systems are represented by salt-assisted liquid-liquid microextraction and salting-out homogeneous liquid-liquid extraction.<sup>26-28</sup> Salting-out effect is used to initiate separation with cloud-point

extraction.<sup>29</sup> However, the application of ATPS to the extraction and separation of metals has not been explored extensively. A few extractants were used for the metal extraction, including thiocyanate and halide.<sup>19,20,22,30,31</sup> Less attention was given to the utilizing of chelate organic reagents.<sup>21,32–34</sup>

4-(2-Pyridylazo)resorcinol (PAR) is one of the best chelating reagents for the complexation of many metal ions, because of its sensitivity and solubility in water. The reagent has been used for separation and determination of over 45 metals, particularly of chromium(III) by extraction–spectrophotometric,<sup>17,35</sup> spectrophotometric methods,<sup>17</sup> and ion-pair high-pressure liquid chromatography.<sup>36</sup> The extraction-spectrophotometric method with 1,5-diphenylcarbazide (DPC) as the complexing agent is the most commonly used method for the separation, speciation and spectrophotometric determination of chromium(VI).<sup>17,37,38</sup>

The purpose of this work is the development of new types of extraction systems for chromium speciation based on the environmentally friendly aqueous two-phase extraction systems. The optimal conditions for the extraction of the colored chelate complexes in the systems Cr(III)–PAR and Cr(VI)–DPC were found. On this basis, the highly sensitive, selective, and green spectrophotometric and ET AAS methods for the determination of Cr(III,VI) have been developed.

## EXPERIMENTAL

### Instrumentation

All absorbance values were measured using a Genesys 10 S UV–Vis spectrophotometer (Thermo Electron Corp., USA) with 1 or 2 cm silica cells. The pH of the solution was measured with an ionometer I-160 MI (Measuring equipment, Russia). The chromium concentrations in the top phase of the ATPS were determined by atomic absorption spectroscopy (AAS) with a model Solaar MQZ atomic absorption spectrometer (Thermo-Electron Corp., USA) equipped with a Zeeman-effect background correction device and a transversely heated graphite atomizer. Pyrolytic graphite platforms inserted into pyrolytically coated tubes were obtained from the same manufacturer (part number B050-4033). The optimum operating parameters for ET AAS determination of chromium are given in Table I.

TABLE I. Operating conditions for the determination of chromium by ET AAS

Parameter	Value
Wavelength, nm	357.9
Slit width, nm	0.7
Lamp current, mA	10
Sample volume, $\mu\text{L}$	20
Chemical modifier	None
Background correction	Zeeman
Drying temperature, $^{\circ}\text{C}$	150 (Ramp 10 s, hold 20 s) 250 (Ramp 10 s, hold 10 s)
Ashing temperature, $^{\circ}\text{C}$	1200 (Ramp 10 s, hold 15 s)
Atomization temperature, $^{\circ}\text{C}$	2200 (Step, hold 5 s)
Cleaning temperature, $^{\circ}\text{C}$	2600 (Step, hold 5 s)

### *Standard solutions and reagents*

Analytical grade chemical and reagents were used. Double distilled water was used throughout the experiments. The chromium(III) and chromium(VI) stock standard solutions ( $1000 \text{ mg L}^{-1}$ ) were prepared from  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and  $\text{K}_2\text{Cr}_2\text{O}_7$ , respectively, and diluted daily to obtain appropriate working solutions. 4-(2-Pyridylazo)resorcinol (Panreac) was used without further purification. The solutions of 1,5-diphenylcarbazide and 1,5-diphenylcarbazone (DPCN) were prepared daily from reagent-grade reagents purified by recrystallization from acetone.

The inorganic salts and acids were of analytical purity grade. The pH of salt solutions was adjusted by sulfuric acid or sodium hydroxide aqueous solutions. Polyethylene glycols of average molecular masses 1500 (PEG-1500) and 2000 (PEG-2000) were purchased from EppliChem and LobaChemie, respectively, and used without further purification. Ethanol (EtOH) and 2-propanol (2-PrOH) of high pure grade (Reachim) were used without further purification.

### *Procedure for Cr(III) determination with PAR*

Exactly 100 mL of sample solution were measured and placed in a beaker. Then 10 mL of  $1 \text{ mol L}^{-1}$   $\text{KHCO}_3$  and 5 mL of 0.2 % PAR were added to it. The mixture was heated in a boiling water bath for 5 min. The obtained solution was cooled to room temperature and transferred to a 500 mL separatory funnel. Then 10 mL of  $0.1 \text{ mol L}^{-1}$  EDTA solution, 30 g of  $(\text{NH}_4)_2\text{SO}_4$ , and 20 mL of isopropanol (alternatively 10 mL of 20 % PEG-1500 can be used) were added to the mixture in the funnel. After shaking for 1 min, the mixture was allowed to stand until phase separation. The upper phase was separated, transferred to a 25 mL volumetric flask, and the volume was made up to the mark with distilled water. The absorbance was measured after 5 min at 535 nm against the blank. The concentration of chromium in the upper phase was independently determined by ET AAS in the conditions described in the section Instrumentation.

### *Procedure for Cr(VI) determination with DPC*

100 mL of sample solution were placed in a separatory funnel. 2.5 mL of  $4 \text{ mol L}^{-1}$   $\text{H}_2\text{SO}_4$  and 20 mL of the 0.2 % DPC solution in ethanol or isopropanol were then added. After the solution was left standing for 10 min, 30 g of  $(\text{NH}_4)_2\text{SO}_4$  was added to promote the phase separation. The mixture was gently shaken for about 1 min, and then it was allowed to stand until the upper phase became clear. The upper phase was separated and transferred to a 25 mL volumetric flask. Salt-rich phase was washed with 2 mL of ethanol or isopropanol, and the washing was combined with the first extract in a volumetric flask. After the addition of 1–2 drops of conc. orthophosphoric acid and then 1–2 drops of conc. sulfuric acid, the solution was diluted to the mark with alcohol. The absorbance was measured at 545 nm against the blank. The upper phase was also subjected to the determination of chromium by ET AAS. Noteworthy is that it is possible to reduce up to ten times the volumes or quantities of reagents used in both proposed procedures.

## RESULTS AND DISCUSSION

### *Optimization of phase separation conditions*

Aqueous biphasic systems are commonly obtained by adding the inorganic salt to the aqueous solution of water-soluble polymer. In a few papers, two-phase aqueous systems formed by salting-out the water-soluble organic solvents were

used to extract metal ions.<sup>20,21,39-42</sup> Both soluble polymer and water-organic solvent mixtures are nonflammable, nontoxic, durable, and contain components that are commercially available at a reasonable price.

Influence of nature of inorganic salt on the formation of aqueous two-phase system was studied (Table II). The phase separation occurred at the following concentrations of inorganic salts: 2.6–3.2 mol L<sup>-1</sup> for ammonium sulfate, 7.4–7.6 mol L<sup>-1</sup> for sodium chloride, 0.5–0.7 mol L<sup>-1</sup> for sodium phosphate, 1.3 mol L<sup>-1</sup> for sodium carbonate, and 0.7 mol L<sup>-1</sup> for sodium bicarbonate. Upper limit of the concentration range is determined by solubility of the salt while the lower one – by absence of phase separation. For all the salting-out agents used, the optimal ratio of aqueous and organic phases was near to 3:1. In such conditions, volume of upper phase was equivalent to the volume of the organic solvent used and separation took about 0.5–1 min.

TABLE II. Influence of the nature of salting-out agent, polymer or water-soluble organic solvent on the extraction percentage of complexes of Cr(III) with PAR or DPC by the ATPS top phase.  $c_{\text{PAR}} = 0.4 \text{ mmol L}^{-1}$ ,  $c_{\text{DPC}} = 1.4 \text{ mmol L}^{-1}$ ,  $c_{\text{Cr(III)}} = 15\text{--}20 \text{ }\mu\text{mol L}^{-1}$

Optimal conditions of the extraction			Extraction percentage, $E / \%$			
Salting-out agent	$c / \text{mol L}^{-1}$	pH	PEG-1500	PEG-2000	C <sub>2</sub> H <sub>5</sub> OH	2-PrOH
Cr(VI) – DPC						
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2.8	6	96	95	98	98
NaNO <sub>3</sub>	7.5		98	96	–	100
NaCl	2.7		–	–	–	100
Cr(III) – PAR						
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2.8	6	97	96	94	98
NaNO <sub>3</sub>	7.5		96	96	–	98
Na <sub>3</sub> PO <sub>4</sub>	0.6		97	97	–	96
KHCO <sub>3</sub> / K <sub>2</sub> CO <sub>3</sub>	0.7 / 1.3	8	97	96	–	94
NaCl	4		–	–	–	97

Sodium chloride can salt out only isopropanol. Phase separation by using sodium nitrate was achieved in the presence of rather high concentration of the salt. By using salts with water-structuring anions SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup> and CO<sub>3</sub><sup>2-</sup>, having high negative value of Gibbs free energy of hydration, formation of two-phase system occurred in the less concentrated solutions. Ethanol formed biphasic system solely with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> that was caused by its low hydrophobicity. Nevertheless, in this case an extraction percentage for Cr(III)–PAR complex was of 94% by single extraction and increased to 99.8 % by repeated extraction. In line with many preceding studies,<sup>19,20,32,43</sup> ammonium sulfate was chosen as the salting-out agent owing to its high solubility in water and high extraction efficiency with respect to chromium complexes. In all the studied ATPS systems, use of both PEG and isopropanol provided complete extraction of chromium(III,VI), quick separation, easy operation, clear phase boundary and no

emulsification. Cr(III) and Cr(VI) are scarcely distributed into organic phase in the absence of complexing agents.<sup>41</sup> Distribution coefficient was 0.06 for Cr(III) at pH 4.0 in 15 % PEG–14.4 % (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ATPS.<sup>20</sup>

*Investigation of the optimal conditions for the separation and determination of Cr(VI) and Cr(III)*

It is well known that the highly absorbing Cr(III)–DPCN complex is formed by oxidation of DPC with chromium(VI).<sup>20</sup> Owing to kinetic inertness, Cr(III) did not significantly react with DPCN. The absorption spectra of Cr(III)–DCN complex were measured in the upper organic phase for the ATPS systems consisting of ammonium sulfate and ethanol, isopropanol, PEG-1500 or PEG-2000 and were found very close to each other as well as to the spectrum of the complex formed in aqueous solution.<sup>38</sup> They all have the same absorption peak situated at about 545 nm and related to the formation of the complex Cr(III)–DPCN.<sup>38</sup>

Cr(VI) is extracted with DPC into upper organic phase of an aqueous two-phase system from bottom salt-rich phase over a wide acidity range from 4 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> to pH 4 (Fig. 1). The pH 1 was chosen to carry out further experiments. Practically complete extraction of Cr(VI) was observed at 0.8 mmol L<sup>-1</sup> and higher concentrations of DPC (Fig. 2).

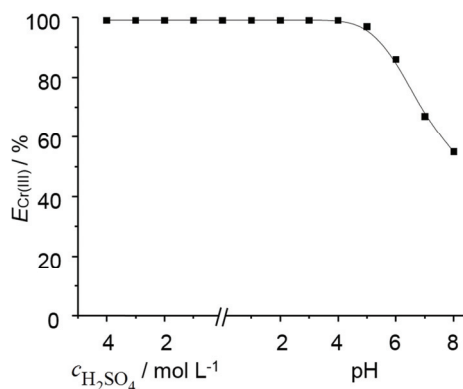


Fig. 1. The effect of the acidity of salt-rich phase on the extraction yield of Cr(III)–DPCN complex in APTS system. Extraction conditions:  $c_{\text{Cr(VI)}} = 0.02 \text{ mmol L}^{-1}$ ;  $c_{\text{DPC}} = 1.4 \text{ mmol L}^{-1}$ .

It was shown that, with the proposed ATPS systems in the optimal conditions, the percentage of Cr(VI)–DPCN and Cr(III)–PAR complexes extracted into upper organic phase was of 94–99 % (Table II). Without extractant, Cr(III) is not extracted ( $E = 0.2 \%$ ). This can be explained, on one hand, by inertness of chromium(III) aqua complexes. On the other hand, highly charged sulfate complexes of chromium(III)  $[\text{Cr}(\text{SO}_4)_x]^{3-2x}$ , which can be formed in the presence of  $10^5$ -fold excess of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, have poor extractability. The extractability of Cr(III) increases in the studied system if the nitrate solutions are used ( $E = 56 \%$ ).

Thus, extraction from sulfate solutions can be recommended for the effective separation and determination of Cr(VI) in the presence of large excess of Cr(III).

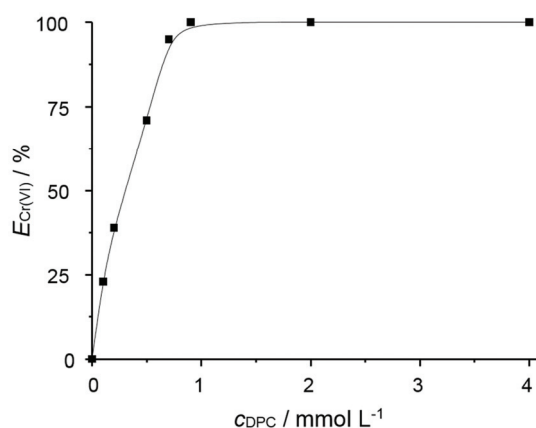


Fig. 2. Dependence of the chromium(VI) extraction on the concentration of DPC. Extraction conditions:  $c_{Cr(VI)} = 0.02 \text{ mol L}^{-1}$ ; pH 1.

Spectrophotometric determination of chromium(III) with chelate reagents in salt-rich phase after extractive separation of chromium(VI) is complicated in view of the high salt concentration. In addition, in such solutions very high concentration of photometric reagent has usually to be created to secure complete formation of the colored complex. Choice of suitable spectrophotometric reagent for the determination of Cr(III) after its extraction into upper phase is rather limited because in many cases significant quantities of the strongly colored reagent could be extracted into organic phase along with chelate complex.

Extraction of Cr(III) in ATPS system as chelate complex with PAR was proposed for its selective determination in the presence of high amounts of Cr(VI). It can be seen from Fig. 3 that complex of chromium(III) with PAR is best extracted with isopropanol or PEG in the wide pH range of 4 to 11. Decrease of the absorbance at pH > 11 is explained by Cr(III) hydrolysis while that at pH < 4 occurs owing to the protonation of PAR.

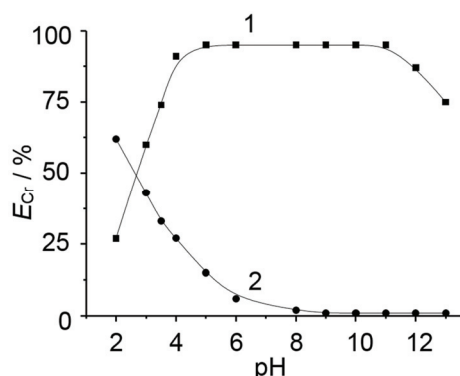


Fig. 3. Effect of pH on the extraction of  $0.015 \text{ mmol L}^{-1}$  Cr(III) (1) and  $0.015 \text{ mmol L}^{-1}$  Cr(VI) (2) in the presence of  $0.4 \text{ mmol L}^{-1}$  PAR.

0.27 mmol L<sup>-1</sup> or a minimum 18-fold excess of PAR to chromium(III) is necessary to attain the maximum absorbance and quantitative extraction (Fig. 4). Further increase in the reagent concentration did not cause any difficulty either in the separation of the phases or in the absorbance of the reagent blank. A 1 min equilibration time was sufficient in order to reach quantitative extraction.

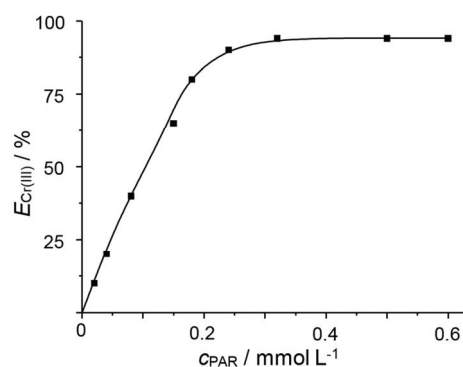


Fig. 4. Effect of PAR concentration on the extraction percentage ( $E$  in %) of 0.015 mmol L<sup>-1</sup> Cr(III) at pH 8.

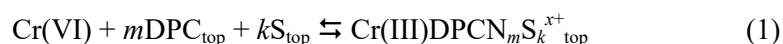
Investigation of Cr(VI) extraction in the optimal conditions of the procedure proposed for the determination of Cr(III) has shown that best separation of two forms of chromium was observed in the pH range from 6 to 11 (Fig. 3). Separation factor for Cr(III)/Cr(VI) was equal to 450.

Chromium(VI) interferes to the extraction-photometric determination of Cr(III) with PAR due to the oxidation of reagent which becomes appreciable due to long heating time needed for the formation of Cr(III) chelate complex. Therefore, in order to increase the selectivity of the determination of Cr(III) it was found reasonable to increase pH of the solution and to reduce the duration of heating.

It was established that the time during which the complex is formed by heating in the boiling water bath at pH 8.3 could be reduced to 5 min in the presence of bicarbonate ions (Fig. 5, curve 2). This time is six times smaller than that found in the absence of bicarbonate (Fig. 5, curve 1). Variation of the bicarbonate concentration between 0.07 and 0.62 mol L<sup>-1</sup> had no significant influence on the effect achieved. Catalytic action of bicarbonate ion was explained by increasing rate of the exchange of the coordinated water molecules by PAR due to the intermediate formation of soluble in water carbonate complex.

#### *Investigation of the mechanism of the extraction of chromium species in the studied ATPS*

The extraction of Cr(III) with PAR and Cr(VI) with DPC can be described as:





$$D_{\text{Cr(VI)}} = \frac{[\text{Cr(III)DPCN}_m\text{S}_k^{x+}]_{\text{top}}}{[\text{Cr(VI)}]} \quad (2)$$

$$K_{\text{ex}} = \frac{D}{[\text{DPC}]_{\text{top}}^m [\text{S}]_{\text{top}}^k} \quad (3)$$

where S – molecules of solvent.

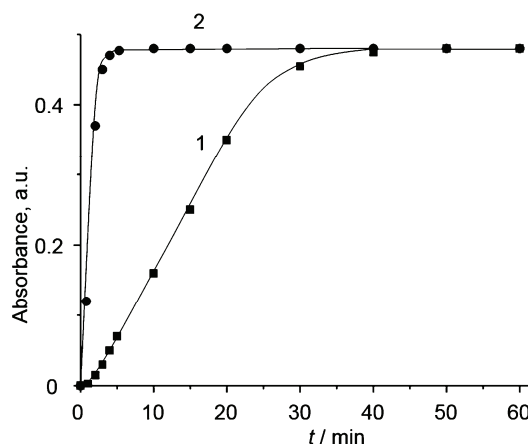


Fig. 5. Dependence of absorbance of chromium(III) complex with PAR on the heating time without (1) and in the presence of sodium bicarbonate (2). Extraction conditions:  $c_{\text{Cr(III)}} = 0.015 \text{ mmol L}^{-1}$ ,  $c_{\text{PAR}} = 0.4 \text{ mmol L}^{-1}$ ;  $c_{\text{bicarbonate}} = 0.09 \text{ mol L}^{-1}$ , pH 8.3.



$$D_{\text{Cr(III)}} = \frac{[\text{CrR}_m\text{S}_k^{(2m-3)-}]_{\text{top}}}{[\text{Cr}(\text{SO}_4)_x^{(2x-3)-}]} \quad (5)$$

$$K_{\text{ex}} = \frac{D[\text{H}^+]^{2m}[\text{SO}_4^{2-}]^x}{[\text{H}_2\text{R}]_{\text{top}}^m [\text{S}]_{\text{top}}^k} \quad (6)$$

where R – anion of PAR.

In this work, any chemical species in the organic-rich phase is denoted by the subscript “top”, while that in the salt-rich phase is given without any subscript. The real number of  $\text{H}^+$  produced or charge of chelate complex can differ depending on the reagent used and solution acidity. The chromium(III, VI) ions could be extracted as anionic or cationic complexes so that different cations and anions may distribute into the top phase as their counterions.

The chromium(III) can be found in both phases as complexed with  $\text{SO}_4^{2-}$  or PAR. The stability constant of chromium(III)–PAR complex is larger than that with  $\text{SO}_4^{2-}$ . Nevertheless, in the  $(\text{NH}_4)_2\text{SO}_4$ -rich phase of the present system, the

initial concentration of  $\text{SO}_4^{2-}$  is much higher than the concentration of the chelating agent. In addition, the chelating agent is distributed into the organic-rich phase with the high distribution ratio. This means that chromium(III) sulfate complexes are predominant species in the lower phase at a sufficiently low concentration of reagent. On the other hand, it is obvious that  $[\text{CrR}_m\text{S}_k^{y-}]_{\text{top}} \gg [\text{Cr}(\text{SO}_4)_x^{(2x-3)-}]_{\text{top}}$  since the chromium(III) ions are scarcely extracted into organic-rich phase in the absence of  $\text{H}_2\text{R}$ . Considering all that, the distribution ratio of a metal ion,  $D$ , defined as the ratio of the metal concentrations in the organic-rich phase to that in the  $(\text{NH}_4)_2\text{SO}_4$ -rich phase can be given by Eqs. (2) and (5).

By taking logarithms of both sides of the equations (3) and (6) we get:

$$\log D_{\text{Cr(VI)}} = \log K_{\text{ex}} + m \log [\text{DPC}]_{\text{top}} + k \log [\text{S}]_{\text{top}} \quad (7)$$

$$\log D_{\text{Cr(III)}} = \log K_{\text{ex}} + 2\text{mpH} - x \log [\text{SO}_4^{2-}] + m \log [\text{H}_2\text{R}]_{\text{top}} + k \log [\text{S}]_{\text{top}} \quad (8)$$

Equations (7) and (8) indicate that the slope of a log–log plot of  $D$  against  $[\text{H}_2\text{R}]$  or  $[\text{S}]$  will give, respectively, the number of reagent or organic solvent molecules per chromium atom related to the chelate complex. The log–log plot of distribution ratio *versus* PAR concentration made at fixed values of other parameters yielded a mole ratio of 1:2 with respect to PAR (Fig. 6a). PAR molecule acts as a tridentate ligand and forms complex in a 2:1 ratio with Cr(III) like the behavior in an aqueous system. The isopropanol solvation number for the extracted chelate complex was determined by the method based on a study of the dependence of distribution coefficient on the concentration of active solvent in an inert diluent. The log–log plot of  $D_{\text{Cr(III)}}$  *versus* isopropanol concentration in toluene was linear and showed that two alcohol molecules solvate the chromium complex (Fig. 6b). Hence, the extracted specie was thought to be a complex of probable composition  $\text{NH}_4[\text{Cr}(\text{PAR})_2] \cdot 2\text{C}_3\text{H}_7\text{OH}$  assuming that it contains the doubly charged anion of PAR.

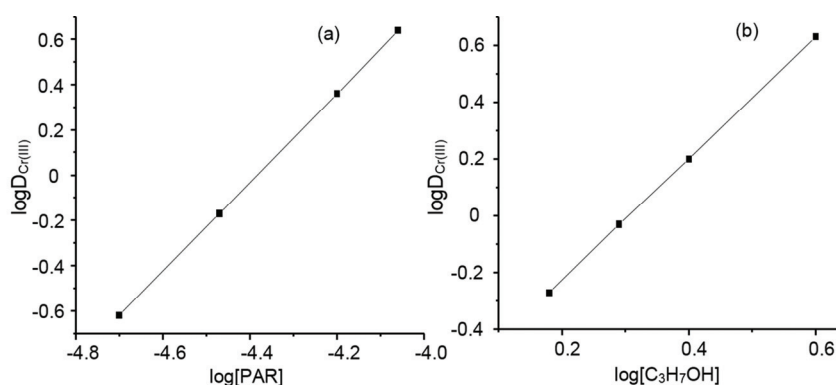


Fig. 6. The dependence between logarithm of distribution coefficient of Cr(III) and logarithms of equilibrium concentrations ( $\text{mol L}^{-1}$ ) of  $\text{H}_2\text{PAR}$  (a) or  $\text{C}_3\text{H}_7\text{OH}$  (b) in top phase.

Similar investigation was undertaken for the ATPS Cr(VI)-DPC. The slopes of a log–log plot of  $D$  against concentration of DPC or isopropanol indicated that chromium is extracted as a complex  $[\text{Cr(III)DPCN}]_A \cdot 4\text{C}_3\text{H}_7\text{OH}$  where  $A$  – counterions  $\text{SO}_4^{2-}$  and so on.

#### Calibration graphs, interference study and application

The optimal conditions found for the ATPS separation of chromium chelate complexes were used for the speciation analysis of chromium(III) and chromium(VI). The content of chromium(III,VI) in the upper phase of the proposed ATPS systems was determined both by spectrophotometry and by atomic absorption spectrometry with electrothermal atomization. Table III presents the concentration ranges and other characteristics of the obtained calibration curves. Limit of detection ( $LOD$ ) and limit of quantification ( $LOQ$ ) were calculated based on the signal to noise ratio ( $S/N$ ) of 3 and 10, respectively.

TABLE III. Figures of merit for chromium(III) and chromium(VI) determination by ATPS-spectrophotometric and ET AAS methods

Parameter	Cr(III)		Cr(VI)	
	ATPS-SP	ATPS- -ET AAS	ATPS-SP	ATPS- -ET AAS
pH	8.3		1.0	
Reagent concentration, mmol L <sup>-1</sup>	PAR – 0.4 HCO <sub>3</sub> <sup>-</sup> – 90		DPC – 1.4	
Time of heating, min	5		–	
Linear range, µg L <sup>-1</sup>	5–500	1–10	2.5–50	1–10
Calibration graph				
Intercept	0.021	0.026	0.006	0.026
Slope, L µg <sup>-1</sup>	0.017	0.057	0.016	0.057
Correlation coefficient	0.995	0.997	0.996	0.997
Detection limit, µg L <sup>-1</sup>	1.4	0.2	0.5	0.2
Quantification limit, µg L <sup>-1</sup>	5	1	2.5	1
RSD / % (n = 12)	2.4	3.5	1.8	3.7

#### Effect of foreign ions and application

It was established that selectivity of the extraction–spectrophotometric determination of chromium(III) with PAR with respect to Fe(III), Ni(II), Ce(III), Sc(III), U(VI), Cu(II), Al(III), and Mg(II) ions is strongly increased in comparison with that found for the aqueous solution. This can be explained by formation of poorly extractable highly charged sulfate complexes such as  $[\text{Fe}(\text{SO}_4)_3]^{3-}$ ,  $[\text{Sc}(\text{SO}_4)_3]^{3-}$ ,  $[\text{Ce}(\text{SO}_4)_3]^{3-}$ , and so forth. Tolerable ratio of interfering metal ions to Cr(III) was found to be 500:1 for Al(III) and Mg(II), 250:1 for Cu(II), 20:1 for Sc(III), Ce(III), and U(VI), 10:1 for Fe(III), 5:1 for Ni(II), and 1:1 for Co(II). The interfering effects of many metal ions including that of

Fe(III), Cu(II) and Ni(II) were eliminated by masking with EDTA. A 500-fold excess of these ions can be tolerated. The rate of the substitution reaction of PAR with EDTA is very low in Cr(III) complex due to its kinetic inertness. Chromium(VI) does not interfere to the determination of chromium(III) with PAR up to ratio 150:1.

The procedure for the spectrophotometric determination of chromium(VI) with DPC in aqueous solution is distinguished by high selectivity. Only high concentrations of Fe(III) ( $>1 \text{ mg L}^{-1}$ ), V(V) ( $>4 \text{ mg L}^{-1}$ ), Mo(VI), Hg(II) and Cu(II) ( $>200 \text{ mg L}^{-1}$ ) interfere to the determination. It was established that selectivity of the Cr(VI) reaction with DPC increases in ATPS system in relation to Mo(VI), V(V), Fe(III), Hg(II), and Cu(II) ions owing to the competitive influence of the formation of sulfate complexes. No interference was observed at the following concentrations of metal ions:  $20 \text{ mg L}^{-1}$  for V(V),  $400 \text{ mg L}^{-1}$  for Mo(VI) and  $500 \text{ mg L}^{-1}$  for Hg(II) and Cu(II). Influence of big quantities of iron(III) ( $200 \text{ mg L}^{-1}$ ) can be eliminated by masking with orthophosphoric acid. The determination of Cr(III) and Cr(VI) is possible in the presence of high excess of anions:  $5 \times 10^5$ -fold for  $\text{NO}_3^-$ ,  $2 \times 10^5$ -fold for  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$ ,  $9 \times 10^4$ -fold for  $\text{CO}_3^{2-}$ , and  $4 \times 10^4$ -fold for  $\text{PO}_4^{3-}$ .

The developed methods for the speciation analysis of chromium were applied to the determination of chromium forms in mine and waste waters of Donetsk region (Table IV). The water samples were analyzed at the day of sampling. They were not acidified in order to avoid reduction of Cr(VI). Before the analysis, the water samples were filtered through a 0.45 mm membrane filter to remove suspended particulate matters. Obtained results testify that proposed procedures are accurate and precise. Results of the speciation analysis of chromium by both spectrophotometric end ET AAS methods are in good agreement with each other and with the total chromium content determined by ET AAS method.

TABLE IV. Results obtained for the speciation analysis of chromium in mine and waste waters by using ATPS-spectrophotometric and ET AAS methods ( $c \pm$  confidence limits for  $n = 5$  and 95 % confidence level)

Sample	$c_{\text{Cr(III)}}$ found, $\mu\text{g L}^{-1}$ (reagent PAR)		$c_{\text{Cr(VI)}}$ found, $\mu\text{g L}^{-1}$ (reagent DPC)		$c_{\text{Cr}}$ total found $\mu\text{g L}^{-1}$
	ATPS-SP	ATPS-ET AAS	ATPS-SP	ATPS-ET AAS	ET AAS
Standard test solution 1 <sup>a</sup>	19.4±1.3	20.7±0.7	<2.5	<1	20.4±1.2
Standard test solution 2 <sup>b</sup>	20.2±0.8	21±2	4.9±0.3	5.04±0.17	25±2
Waste water 1	68±3	66±3	<2.5	0.32±0.02 <sup>c</sup>	67±4
Waste water 2	112±6	116±5	4.7±0.2	5.5±0.4	118±8
Mine water 1	9.3±1.2	8.6±0.3	<2.5	1.63±0.13	11.4±1.3
Mine water 2	11±2	10.4±0.6	<2.5	2.12±0.11	12.6±1.2

<sup>a</sup>20  $\mu\text{g L}^{-1}$  Cr(III), 100  $\mu\text{g L}^{-1}$  of Fe(III), Ni(II), Al(III); <sup>b</sup>20  $\mu\text{g L}^{-1}$  Cr(III), 5  $\mu\text{g L}^{-1}$  Cr(VI), 100  $\mu\text{g L}^{-1}$  of Fe(III), Ni(II), Al(III); <sup>c</sup> after ten-fold concentration by evaporation

## CONCLUSIONS

This work describes one of the first attempts to combine the green extraction method – aqueous two-phase extraction (ATPE) – with speciation analysis of metal forms. On this basis new, sufficiently sensitive and selective SP and ET AAS methods have been developed for the speciation of Cr(III) and Cr(VI) using the ATPE of chelate complexes formed in the reactions of Cr(III) with 4-(2-pyridylazo)resorcinol and Cr(VI) with 1,5-diphenylcarbazide, respectively.

It is important that content of chromium forms can be determined directly without subtracting.<sup>14</sup> The inertness of chromium(III) chelate complexes and masking action of sulfate ions is the reason for improvement in the selectivity of the determination of chromium forms with the proposed reagents with respect to most of other metal ions. 150-fold excess of Cr(VI) does not interfere with the spectrophotometric determination of Cr(III) with PAR, while determination of Cr(VI) with DPC is possible in the presence of 150-fold excess of Cr(III) ions. Ammonium sulfate offers an optimum combination of properties as salting-out agent. Not only polyethylene glycols but also such easily accessible and non-toxic organic solvents as ethanol and isopropanol can be used as ecofriendly extragents. The time of reaction between PAR and Cr(III) was reduced from 30 to 5 min by using catalytic action of bicarbonate ions.

The EPA recommends that the concentration of Cr(VI) in water destined for human consumption should not exceed  $100 \mu\text{g L}^{-1}$ .<sup>13</sup> California's drinking water standard established a maximum contaminant level of  $10 \mu\text{g L}^{-1}$  for Cr(VI) in drinking water.<sup>44</sup> A highly sensitive determination of chromium species was done not only by ET AAS method but also with spectrophotometric method taking into account the use of strongly colored chelate systems. Detection limit for both the proposed SP and ET AAS methods are at sub  $\mu\text{g L}^{-1}$  level completely satisfying these requirements. The methods are adequately selective for the direct and accurate determination of the analytes in a variety of real samples, including waste and mine waters. The advantage inherent to the proposed ATPS systems is that they allow to carry out the speciation analysis of Cr(III) and Cr(VI) in highly salty solutions.

## ИЗВОД

СПЕЦИЈАЦИЈА ХРОМА ДВОФАЗНОМ ЕКСТРАКЦИЈОМ КОМПЛЕКСА Cr(III) СА 4-(2-ПИРИДИЛАЗО)РЕЗОРЦИНОЛОМ И Cr(VI) СА 1,5-ДИФЕНИЛКАРБАЗИДОМ

TAMARA N. SIMONOVA<sup>1</sup>, VALENTINA A. DUBROVINA<sup>1</sup> и ANDRIY B. VISHNIKIN<sup>2</sup>

<sup>1</sup>Department of Analytical Chemistry, Faculty of Chemistry, Donetsk National University, Donetsk 83001, Ukraine and <sup>2</sup>Department of Analytical Chemistry, Faculty of Chemistry, Oles Honchar Dnipropetrovsk National University, Dnipropetrovsk 49010, Ukraine

У раду је описана “зелена”, осетљива и селективна метода за преконцентровање и специјацију хемијских врста хрома у природним водама. Развијена метода је базирана на пријатељској према околини, воденој двофазној екстракцији системом, који садржи

амонијум-сулфат и полиетиленгликол или алтернативно, алкохол (етанол или изопропанол). Екстракција хемијских врста хрома из воденог раствора у горњу органску фазу је изведена са 4-(2-пиридилазо)резорцинолом и 1,5-дифенилкарбазидом као хелирајућим агенсом за Cr(III) и Cr(VI), редом. Евалуирани су и оптимизовани неки битни фактори који утичу на преконцентровање Cr(III) и Cr(VI). Концентрација врста хрома у горњој органској фази је одређена спектрофотометријски и електротермалном атомском апсорпционом спектрометријом (ЕТ ААС). Добијене су калибрационе праве под оптималним условима спектрофотометријске методе, са опсегом линеарности 5–500  $\mu\text{g L}^{-1}$  за Cr(III) и 2,5–50  $\mu\text{g L}^{-1}$  за Cr(VI). Кореспондентни концентрациони опсег у ЕТ ААС методи износио је 1–10  $\mu\text{g L}^{-1}$  за обе хемијске врсте хрома. Предложена метода је примењена за одређивање хрома у подземним и отпадним водама са задовољавајућим резултатима.

(Примљено 30. јуна 2015, ревидирано 3. јануара, прихваћено 11. фебруара 2016)

#### REFERENCES

1. N. Unceta, F. Séby, J. Malherbe, O.F.X. Donard, *Anal. Bioanal. Chem.* **397** (2010) 1097
2. V. Gómez, M. P. Callao, *Trends Anal. Chem.* **25** (2006) 1006
3. J. Kotaš, Z. Stasicka, *Environ. Pollut. (Oxford, U.K.)* **107** (2000) 263
4. M. J. Marqués, A. Salvador, A. Morales-Rubio, M. de la Guardia, *Fresenius, J. Anal. Chem.* **367** (2000) 601
5. L.-L. Wang, J.-Q. Wang, Z.-X. Zheng, P. Xiao, *J. Hazard. Mater.* **177** (2010) 114
6. P. Liang, H. Sang, *J. Hazard. Mater.* **154** (2008) 1115
7. I. Narin, A. Kars, M. Soylak, *J. Hazard. Mater.* **150** (2008) 453
8. K. Kiran, K. S. Kumar, B. Prasad, K. Suvardhan, L. R. Babu, K. Janardhanam, *J. Hazard. Mater.* **150** (2008) 582
9. P. Liang, Q. Ding, Y. Liu, *J. Sep. Sci.* **29** (2006) 242
10. G. M. M. Rahman, H. M. S. Kingston, T. G. Towns, R. J. Vitale, K. R. Clay, *Anal. Bioanal. Chem.* **382** (2005) 1111
11. X. Zhu, B. Hu, Z. Jiang, M. Li, *Water Res.* **39** (2005) 589
12. I. López-García, M. Briceño, Y. Vicente-Martínez, M. Hernández-Córdoba, *Talanta* **115** (2013) 166
13. D. G. Themelis, F. S. Kika, A. Economou, *Talanta* **69** (2006) 615
14. M. S. Hosseini, F. Belador, *J. Hazard. Mater.* **165** (2009) 1062
15. S. Armenta, S. Garrigues, M. de la Guardia, *Trends Anal. Chem.* **27** (2008) 497
16. D. L. Rocha, A. D. Batista, F. R. P. Rocha, G. L. Donati, J. A. Nóbrega, *Trends Anal. Chem.* **45** (2013) 79
17. Z. Marczenko, M. Balcerzak, *Separation, Preconcentration and Spectrophotometry in Inorganic Analysis*, Elsevier, Amsterdam, 2000, pp. 159–166
18. V. M. Rao, M. N. Sastri, *Talanta* **27** (1980) 771
19. R. D. Rogers, A. H. Bond, C. B. Bauer, *Sep. Sci. Technol.* **28** (1993) 1091
20. T. I. Zvarova, V. M. Shkinev, G. A. Vorob'eva, B. Ya. Spivakov, Yu. A. Zolotov, *Microchim. Acta* **84** (1984) 449
21. C. E. Matkovich, G. D. Christian, *Anal. Chem.* **45** (1973) 1915
22. M. Shibukawa, N. Nakayama, T. Hayashi, D. Shibuya, Y. Endo, S. Kawamura, *Anal. Chim. Acta* **427** (2001) 293
23. N. H. Chung, J. Nishimoto, O. Kato, M. Tabata, *Anal. Chim. Acta* **477** (2003) 243
24. M. Tabata, M. Kumamoto, J. Nishimoto, *Anal. Sci.* **10** (1994) 383
25. M. Tabata, M. Kumamoto, J. Nishimoto, *Anal. Chem.* **68** (1996) 758

26. M. Gupta, A. Jain, K.K. Verma, *Talanta* **80** (2009) 526
27. M. A. Farajzadeh, S. Sheykhizadeh, P. Khorram, *J. Sep.Sci.* **36** (2013) 939
28. M. A. Farajzadeh, B. Feriduni, M. R. A. Mogaddam, *Anal. Chim. Acta* **885** (2015) 122
29. N. Sato, M. Mori, H. Itabashi, *Talanta* **117** (2013) 376
30. L. Bulgariu, D. Bulgariu, *J. Serb. Chem. Soc.* **73** (2008) 341
31. L. Bulgariu, D. Bulgariu, *J. Serb. Chem. Soc.* **72** (2007) 289
32. N. Yoshikuni, T. Baba, N. Tsunoda, K. Oguma, *Talanta* **66** (2005) 40
33. G. D. Rodrigues, L. R. de Lemos, L. H. M. da Silva, M. C. H. da Silva, *J. Chromatogr. A* **1279** (2013) 13
34. G. D. Rodrigues, L. R. de Lemos, L. H. M. da Silva, M. C. H. da Silva, *Anal. Sci.* **28** (2012) 1213
35. T. Yotsuyanagi, Y. Takeda, R. Yamashita, K. Aomura, *Anal. Chim. Acta* **67** (1973) 297
36. X.-S. Zhang, X.-P. Zhu, C.-S. Lin, *Talanta* **33** (1986) 838
37. C. K. Pires, B. F. Reis, A. Morales-Rubio, M. de la Guardia, *Talanta* **72** (2007) 1370
38. H. Marchart, *Anal. Chim. Acta* **30** (1964) 11
39. N. H. Chung, M. Tabata, *Talanta* **58** (2002) 927
40. Z.-H. Wang, M. Song, Q.-L. Ma, H.-M. Ma, S.-C. Liang, *Microchim. Acta* **134** (2000) 95
41. S. S. Samaratunga, J. Nishimoto, M. Tabata, *Env. Sci. Pollut. Res.* **15** (2008) 27
42. I. A. Shevchuk, T. N. Simonova, E. S. Gontar, *Ukr. Khim. Zh.* **72** (2006) (9) 29
43. Y. Akama, A. Sali, *Talanta* **57** (2002) 681.
44. California Environmental Protection Agency, *State Water Resources Control Board. Chromium-6 Drinking Water MCL* ([http://www.waterboards.ca.gov/drinking\\_water/cert-lic/drinkingwater/Chromium6.shtml](http://www.waterboards.ca.gov/drinking_water/cert-lic/drinkingwater/Chromium6.shtml)).