1	Speciation of chromium through aqueous two-phase extraction of
2	complexes of Cr(III) with 4-(2-pyridylazo)resorcinol and Cr(VI) with
3	1,5-diphenylcarbazide
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13	Abstract: A green, simple, rapid, sensitive and selective methods are described for the
14	preconcentration and speciation of chromium species in natural waters. The developed
15	methods are based on the environmentally friendly aqueous two-phase extraction with a
16	system consisted of ammonium sulfate and polyethylene glycol, or, alternatively, alcohol
17	(ethanol, isopropanol). The extraction of chromium species from aqueous solution into the
18	upper organic phase was performed with 4-(2-pyridylazo)resorcinol and 1,5-
19	diphenylcarbazide as chelating agents for Cr(III) and Cr(VI), respectively. Some predominant
20	factors affecting the preconcentration and speciation of both Cr(III) and Cr(VI) species were
21	evaluated and optimized. The concentration of chromium species in upper organic phase was
22	determined by both spectrophotometric and electrothermal atomic-absorption spectrometry
23	(ET AAS) methods. Under the optimum conditions, the calibration graphs obtained with
24	spectrophotometric method were linear over the concentration ranges from 5 to 500 $\mu g \ L^{\text{-1}}$ for
25	Cr(III) and from 2.5 to 50 μ g L ⁻¹ for Cr(VI). Corresponding concentration ranges determined
26	by using ET AAS method were from 1 to 10 μ g L ⁻¹ for both chromium species. The proposed
27	methods were applied to the determination of chromium species in mine and waste waters
28	with satisfactory results.

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

32

INTRODUCTION

Chromium is metal element with significant economic and environmental interest because it is extensively used in metal smelting, electroplating, tanning, 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 41 very low concentration, procedure is needed that will permit enrichment of the analyte while 42 eliminating the matrix effect. The speciation methods of chromium, published hitherto, 43 involved a separation as well as preconcentration step with solvent extraction using different 44 45 reagents, solid sorbent extraction, high-performance liquid chromatography, ion chromatography, and so on.¹⁻⁷ For the speciation analysis, the separation techniques are 46 combined with highly selective and sensitive detection systems such as flame atomic 47 absorption spectrometry,^{7,8} inductively coupled plasma atomic emission spectrometry,⁹ 48 inductively coupled plasma mass spectrometry,¹⁰ electrothermal atomic absorption 49 spectrometry (ET AAS),^{6,11,12} spectrophotometry,¹³ and fluorometry.¹⁴ 50

Extraction is one of the predominant method used for the chromium speciation.⁴ Due to 51 an increasingly stringent environmental regulations, there is a strong demand for less 52 polluting safe analytical methods that are consistent with the principles of Green 53 Chemistry.^{15,16} Replacement of volatile organic compounds, the class of organic solvents 54 commonly used in liquid-liquid extraction, is among the themes addressed. Use of toxic 55 organic solvents, such as chloroform, dichlorethane and others containing chlorine, for 56 extraction of metal-complexes is strictly forbidden by law. The toxic solvents such as ethyl 57 acetate, isobutanol, methyl isobutyl ketone, and chloroform are used in the existing extractive 58 procedures for the separation of chromium forms.^{17,18} In addition, in certain cases, the 59 60 extraction is incomplete, and the procedures are complex and time-consuming.

Aqueous two-phase systems (ATPS) is 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.^{19–22} The possibility of linear scale up, ease of use, low-cost and rapid phase separation without the

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formation of stable emulsions are advantages of the ATPS technique.^{19,20} 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.^{19,20,22–24} Less attention was given to the utilizing of chelate organic reagents.^{21,25,26}

69 4-(2-Pyridylazo)resorcinol (PAR) is one of the best chelating reagents for the 70 complexation of many metal ions, because of its sensitivity and solubility in water. The 71 reagent has been used for separation and determination of over 45 metals, particularly of 72 chromium(III) by extraction-spectrophotometric,^{17,27} spectrophotometric methods,¹⁷ and ion-73 pair high-pressure liquid chromatography.²⁸ The extraction-spectrophotometric method with 74 1,5-diphenylcarbazide (DPC) as the complexing agent is the most commonly used method for 75 the separation, speciation and spectrophotometric determination of chromium(VI).^{17,29,30}

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 simple, highly sensitive, selective, and green spectrophotometric and ET AAS methods for the determination of Cr(III, VI) have been developed.

82

EXPERIMENTAL

83 Instrumentation

All absorbance values were measured using a Genesys 10 S UV-Vis spectrophotometer 84 (Thermo Electron Corp., USA) with 1 or 2 cm silica cells. The pH of the solution was 85 measured with an ionometer I-160 MI (Measuring equipment, Russia). The chromium 86 concentrations in the top phase of the ATPS were determined by atomic absorption 87 spectroscopy (AAS) with a model Solaar MQZ atomic absorption spectrometer (Thermo-88 Electron Corp., USA) equipped with a Zeeman-effect background correction device and a 89 transversely heated graphite atomizer. Pyrolytic graphite platforms inserted into pyrolytically 90 coated tubes were obtained from the same manufacturer (part number B050-4033). The 91 optimum operating parameters for ETAAS determination of chromium are given in Table I. 92

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- 95
- 96

Parameters	Value
Wavelength, nm	357.9
Slit width, nm	0.7
Lamp current, mA	10
Sample volume, µL	20
Chemical modifier	None
Background correction	Zeeman
Drying temperature, °C	150 (Ramp 10 s, hold 20 s)
	250 (Ramp 10 s, hold 10 s)
Ashing temperature, °C	1200 (Ramp 10 s, hold 15 s)
Atomization temperature, °C	2200 (Step, hold 5 s)
Cleaning temperature, °C	2600 (Step, hold 5 s)

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

98 Standard solutions and reagents

Analytical grade chemical and reagents were used. Double distilled water was used 99 throughout the experiments. The chromium (III) and (VI) stock standard solutions 100 (1000 mg L⁻¹) were prepared from Cr(NO₃)₃·9H₂O and K₂Cr₂O₇, respectively, and diluted 101 daily to obtain appropriate working solutions. 4-(2-Pyridylazo)resorcinol (Panreac) was used 102 The solutions of 1,5-diphenylcarbazide 103 without further purification. and 1.5diphenylcarbazone (DPCN) were prepared daily from reagent-grade reagents purified by 104 recrystallization from acetone. 105

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.

112 Procedure for Cr(III) determination with PAR

Exactly 100 mL of sample solution were measured and placed in a heat-resistant beaker. Then 10 mL of 1 mol L^{-1} KHCO₃ 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 mol L^{-1} EDTA 117 solution, 30 g of (NH₄)₂SO₄, and 20 mL of isopropanol (alternatively 10 mL of 20% PEG-118 1500 can be used) were added to the mixture in the funnel. After shaking for 1 min, the 119 mixture was allowed to stand until phase separation. The upper phase was separated, 120 transferred to a 25 mL volumetric flask, and the volume was made up to the mark with 121 distilled water. The absorbance was measured after 5 min at 535 nm against the blank. The 122 concentration of chromium in the upper phasewas independently determined by GF-AAS.

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

100 mL of sample solution were placed in a separatory funnel. 2.5 mL of 4 mol L⁻¹ 124 H₂SO₄ and 20 mL of the 0.2% DPC solution in ethanol or isopropanol were then added. After 125 the solution was left standing for 10 min, 30 g of (NH₄)₂SO₄ was added to promote the phase 126 separation. The mixture was gently shaken for about 1 min, and then it was allowed to stand 127 until the upper phase became clean. The upper phase was separated and transferred to a 25 128 mL volumetric flask. Salt-rich phase was washed with 2 mL of ethanol or isopropanol, and 129 the washing was combined with the first extract in a volumetric flask. After the addition of 1-130 2 drops of conc. orthophosphoric acid and then 1-2 drops of conc. sulfuric acid, the solution 131 was diluted to the mark with alcohol. The absorbance was measured at 545 nm against the 132 blank. The upper phase was also subjected to the determination of chromium by GF-AAS in 133 the conditions described in the section Instrumentation above. 134

135

RESULTS AND DISCUSSION

136 *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.^{20,21,31–34} Both soluble polymer and water-organic solvent mixtures are nonflammable, nontoxic, durable, and contain components that are commercially available at a reasonable price.

143 Influence of nature of inorganic salt on the formation of aqueous two-phase system was 144 studied (Table II). The phase separation was occurred at the following concentrations of 145 inorganic salts: 2.6-3.2 mol L⁻¹ for ammonium sulfate, 7.4-7.6 mol L⁻¹ for sodium chloride, 146 $0.5-0.7 \text{ mol } \text{L}^{-1}$ for sodium phosphate, 1.3 mol L⁻¹ for sodium carbonate, and 0.7 mol L⁻¹ for 147 sodium bicarbonate. Upper limit of the concentration range is determined by solubility of the 148 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

150 of upper phase was equivalent to the volume of the organic solvent used and separation took

about 0.5-1 min.

- 152 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
- 154 top phase. $c_{PAR} = 0.4 \text{ mmol } L^{-1}$, $c_{DCD} = 1.4 \text{ mmol } L^{-1}$, $c_{Cr(III)} = 15-20 \text{ } \mu \text{mol } L^{-1}$

Optimal conditions of the extraction			Extraction percentage, %E			
Salting-out agent	c, mol L ⁻¹	pН	PEG-1500	PEG-2000	C2H5OH	С3Н7ОН
	Cr(VI) – DPC					
(NH4)2SO4	2.8		96	95	98	98
NaNO ₃	7.5	6	98	96	-	100
NaCl	2.7		_	_	_	100
Cr(III) – PAR						
(NH4)2SO4	2.8		97	96	94	98
NaNO ₃	7.5	6	96	96	-	98
Na ₃ PO ₄	0.6		97	97	_	96
KHCO3 / K2CO3	0.7 / 1.3	8	97	96	_	94
NaCl	4		_	_	_	97

155 Sodium chloride can salt out only isopropanol. Phase separation for sodium nitrate was achieved in the presence of rather high concentration of the salt. By using salts with water-156 structuring anions SO_4^{2-} , PO_4^{3-} , and CO_3^{2-} , having high negative value of Gibbs free energy of 157 hydration, formation of two-phase system occurred in the less concentrated solutions. Ethanol 158 formed biphasic system solely with (NH₄)₂SO₄ that was caused by its low hydrophobicity. 159 Nevertheless, in this case an extraction percentage for Cr(III)-PAR complex was of 94% by 160 single extraction and increased to 99.8% by repeated extraction. In line with many preceding 161 studies,^{19,20,32,35} ammonium sulfate was chosen as the salting-out agent owing to its high 162 solubility in water and high extraction efficiency with respect to chromium complexes. In all 163 the studied ATPS systems, use of both PEG and isopropanol provided complete extraction of 164

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.³³ Distribution coefficient was 0.06 for Cr(III) at pH 4.0 in 15% PEG –
 14.4% (NH₄)₂SO₄ ATPS.²⁰

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

It is well known that the highly absorbing Cr(III)-DPCN complex is formed by 171 oxidation of DPC with chromium(VI).²⁰ Owing to kinetic inertness, Cr(III) did not 172 significantly react with DPCN. The absorption spectra of Cr(III)-DCN complex were 173 measured in the upper organic phase for the ATPS systems consisting of ammonium sulfate 174 and ethanol, isopropanol, PEG-1500, or PEG-2000 and were found very close to each other as 175 well as to the spectrum of the complex formed in aqueous solution.³⁰ They all have the same 176 absorption peak situated at about 545 nm and related to the formation of the complex Cr(III)-177 DPCN.³⁰ 178

179 Cr(VI) is extracted with DPC into upper organic phase of an aqueous two-phase 180 system from bottom salt-rich phase over a wide acidity range from 4 mol L^{-1} H₂SO₄ to pH 4 181 (Fig. 1). The pH 1 was chosen to carry out further experiments. Practically complete 182 extraction of Cr(VI) was observed at 0.8 mmol L^{-1} and higher concentrations of DPC (Fig. 2).



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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: $c_{Cr(VI)} = 0.02$ mmol L⁻¹; $c_{DPC} = 1.4$ mmol L⁻¹.



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Fig. 2. Dependence of the chromium(VI) extraction on the concentration of DPC. 188 Extraction conditions: $c_{Cr(VI)} = 0.02 \text{ mol } L^{-1}$; pH = 1. 189

It was shown that, with the proposed ATPS systems in the optimal conditions, the 190 percentage of Cr(VI)-DPCN and Cr(III)-PAR complexes extracted into upper organic phase 191 was of 94-99% (Table II). Without extragent, Cr(III) is not extracted (E = 0.2 %). This can be 192 explained, on one hand, by inertness of chromium(III) aqua complexes. On the other hand, 193 highly charged sulfate complexes of chromium(III) $[Cr(SO_4)_x]^{3-2x}$, which can be formed in the 194 presence of 10⁵ fold excess of (NH₄)₂SO₄, have poor extractability. The extractability of 195 Cr(III) increases in the studied system if the nitrate solutions are used (E = 56 %). Thus, 196 extraction from sulfate solutions can be recommended for the effective separation and 197 198 determination of Cr(VI) in the presence of large excess of Cr(III).

Spectrophotometric determination of chromium(III) with chelate reagents in salt-rich 199 200 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 201 202 has usually to be created to secure complete formation of the colored complex. Choice of 203 suitable spectrophotometric reagent for the determination of Cr(III) after its extraction into 204 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. 205

206 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 207 Fig. 3 that complex of chromium(III) with PAR is best extracted with isopropanol or PEG in 208

- 209 the wide pH range of 4 to 11. Decrease of the absorbance at pH > 11 is explained by Cr(III)
- 210 hydrolysis while that at pH < 4 occurs owing to the protonation of PAR.



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

 $0.27 \text{ mmol } \text{L}^{-1}$ 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.



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Fig. 4. Effect of PAR concentration on the extraction percentage (E%) of 0.015 mmol L⁻¹
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^{-1} 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.



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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: $c_{Cr(III)} = 0.015 \text{ mmol } \text{L}^{-1}, c_{PAR} = 0.4 \text{ mmol } \text{L}^{-1}; c_{\text{bicarbonate}} = 0.09 \text{ mol } \text{L}^{-1}, \text{pH} = 8.3.$

241 Investigation of the mechanism of the extraction of chromium species in the studied ATPS

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

243
$$Cr_2O7^{2-} + 2mH_4R'_{top} + 2kS_{top} \stackrel{\leftarrow}{\to} 2CrR_mS_k^{x+}_{top} + yH^+ + 7H_2O$$
(1)

244
$$D_{Cr(VI)} = \frac{[CrR_m S_k^{x+}]_{top}^2}{[Cr_2 O_7^{2-}]}$$
(2)

245
$$K_{ex} = \frac{D[H^+]^{y}}{[H_4 R]_{top}^{2m} [S]_{top}^{2k}}$$
(3)

246 where R', R – anions of DPC and DPCN, and S –molecules of solvent

247
$$Cr(SO_4)_x^{(2x-3)-} + mH_2R_{top} + kS_{top} = CrR_mS_k^{y-}_{top} + zH^+ + xSO_4^{2-}$$
(4)

248
$$D_{Cr(III)} = \frac{[CrR_m S_k^{y-}]_{top}}{[Cr(SO_4^{2-})_x^{(2x-3)-}]}$$
(5)

249
$$K_{ex} = \frac{D[H^+]^z [SO_4^{2-}]^x}{[H_2 R]_{top}^m [S]_{top}^k}$$
(6)

where R – anion of PAR.

In this work, any chemical specie 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 H⁺ ions 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 SO₄²⁻ or PAR. The 257 stability constant of chromium(III)-PAR complex is larger than that with SO₄²⁻. Nevertheless, 258 in the (NH₄)₂SO₄-rich phase of the present system, the initial concentration of SO₄²⁻ is much 259 higher than the concentration of the chelating agent. In addition, the chelating agent is 260 261 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 262 263 low concentration of reagent. On the other hand, it is obvious that $[CrR_mS_k^{y}]_{top} \gg [Cr(SO_4)_x^{(2x-3)}]_{top}$ since the chromium(III) ions are scarcely extracted into 264 organic-rich phase in the absence of H₂R. Considering all that, the distribution ratio of a metal 265 ion, D, defined as the ratio of the metal concentrations in the organic-rich phase to that in the 266 (NH₄)₂SO₄-rich phase can be given by eqs. (2) and (5). 267

- 268
 - By taking logarithms of both sides of the equations (3) and (6)we get:
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$$logD_{Cr(VI)} = logK_{ex} + ypH + 2mlog[H_4R']_{top} + 2klog[S]_{top} \qquad j \qquad (7)$$

$$logD_{Cr(III)} = logK_{ex} + zpH - xlog[SO42-] + mlog[H_2R]_{top} + klog[S]_{top}$$
(8)

Eqs. (7) and (8) indicate that the slope of a log-log plot of D against [H₂R] or [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 molar 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 loglog plot of $D_{Cr(III)}$ 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 NH4[Cr(PAR)₂]·2C₃H₇OH 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 of H₂PAR (a) or C₃H₇OH (b) in top phase.

Similar investigation was undertaken for the ATPS Cr(VI)-DPC. The slopes of a loglog plots of D against DPC or isopropanol indicated that chromium is extracted as a complex $[Cr(III)DPCN]A\cdot 4C_3H_7OH$ where A – counterions SO4^{2–} and so on.

289 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.

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- 298
- 299

	Cr(III)		Cr(VI)	
Parameter	ATPS –	ATPS –	ATPS –	ATPS –
	SP	ET AAS	SP	ET AAS
рН	8.3		1.0	
Reagent concentration,	PAR - 0.4		DPC – 1.4	
mmol L ⁻¹	$HCO_{3}^{-} - 90$			
Time of heating, min	5		_	
Linear range, µg L ⁻¹	5 - 500	1 – 10	2.5 - 50	1 – 10
Calibration graph				
Intercept	0.021	0.026	0.006	0.026
Slope, L µg ⁻¹	0.017	0.057	0.016	0.057
Correlation coefficient	0.995	0.997	0.996	0.997
Detection limit, µg L ⁻¹	1.4	0.2	0.5	0.2
Quantification limit, $\mu g L^{-1}$	5	1	2.5	1
RSD, % (n = 12)	2.4	3.5	1.8	3.7

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

302 *Effect of foreign ions and application*

It was established that selectivity of the extraction-spectrophotometric determination of 303 304 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. 305 This can be explained by formation of poorly extractable highly charged sulfate complexes 306 such as $[Fe(SO_4)_3]^{3-}$, $[Sc(SO_4)_3]^{3-}$, $[Ce(SO_4)_3]^{3-}$ and so forth. Tolerable ratio of interfering 307 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 308 309 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 310 masking with an EDTA. A 500-fold excess of these ions can be tolerated. The rate of the 311 substitution reaction of PAR with EDTA is very low in Cr(III) complex due to its kinetic 312 inertness. Chromium(VI) does not interfere to the determination of chromium(III) with PAR 313 up to ratio 150:1. 314

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) $(>1 \text{ mg } \text{L}^{-1})$, V(V) (>4 mg L⁻¹), Mo(VI), Hg(II) and Cu(II) (>200 mg L⁻¹) interfere to the determination.¹⁷ 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 mg L⁻¹ for V(V), 400 mg L⁻¹ for Mo(VI), and 500 mg L⁻¹ for Hg(II) and Cu(II). Influence of big quantities of iron(III) (200 mg L⁻¹) can be eliminated by masking with orthophosphoric acid.

The developed methods for the speciation analysis of chromium were applied to the 324 determination of chromium forms in mine and waste waters of Donetsk region (Table IV). 325 The water samples were analyzed at the day of sampling. They were not acidified in order to 326 avoid reduction of Cr(VI). Before the analysis, the water samples were filtered through a 0.45 327 328 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 329 330 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. 331

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)

Samples	<i>c</i> _{Cr(III)} found, μg L ⁻¹ (reagent PAR)		<i>c</i> _{Cr(VI)} found, μg L ⁻¹ (reagent DPC)		c _{Cr} total found, μg L ⁻¹
	ATPS SP	ATPS ET AAS	ATPS SP	ATPS ET AAS	ET AAS
Standard test solution 1 ^a	19.4±1.3				20.4±1.2
Standard test solution 2 ^b	20.2±0.8		4.9±0.3		25±2
Waste water 1	68±3			0.32±0.02	67±4
Waste water 2	112±6		4.7±0.2		118±8
Mine water 1		8.6±0.3		1.6±0.1	11.4±1.3
Mine water 2	1	10.4±0.6		2.1±0.1	12.6±1.2

335 ${}^{a}20 \ \mu g \ L^{-1} \ Cr(III), \ 100 \ \mu g \ L^{-1} \ of \ Fe(III), \ Ni(II), \ Al(III)$

336 ^b 20 μ gL⁻¹ Cr(III), 5 μ gL⁻¹ Cr(VI), 100 μ g L⁻¹ of Fe(III), Ni(II), Al(III)

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, simple, rapid, 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.

344 Optimal conditions have been specified for the separation of chromium forms by aqueous two-phase systems. 150-fold excess of Cr(VI) does not interfere with the spectrophotometric 345 346 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. It is important that content of chromium forms can be 347 determined directly without subtracting.¹⁴ The inertness of chromium(III) chelate complexes and 348 masking action of sulfate ions is the reason for improvement in the selectivity of the determination 349 350 of chromium forms with the proposed reagents with respect to most of other metal ions. Ammonium sulfate offers an optimum combination of properties as salting-out agent. Not only 351 polyethylene glycols but also such easily accessible and non-toxic organic solvents as ethanol and 352 isopropanol can be used as ecofriendly extragents. The time of reaction between PAR and Cr(III) 353 was reduced from 30 to 5 min by using catalytic action of bicarbonate ions. 354

The EPA recommends that the concentration of Cr(VI) in water destined for human consumption should not exceed 100 μ g L⁻¹.¹³ In some countries, the maximum permitted concentration is 20 mg L⁻¹ [12]. 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 μ g L⁻¹ level completely satisfying these requirements.

The new methods were validated by statistical evaluation which indicated that the sum of concentrations of chromium species is the same as the total concentration of total chromium determined by ET AAS at 95% level of confidence. The method is adequately selective for the direct and accurate determination of the analytes in a variety of real samples, including waste and mine waters.

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