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Ion chromatographic speciation of chromium with diphenylcarbazide-based spectrophotometric detection[1]

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Abstract

Two different ion chromatographic procedures were developed for the speciation of different oxidation states of chromium with use of anion-exchange column Hamilton PRP-X100, phthalate mobile phase and spectrophotometric detection based on post-column reaction of Cr(VI) with diphenylcarbazide. Due to element-specific detection such a determination can be carried out with retention of Cr(VI) and elution of Cr(III) in the void peak. In the second procedure developed, Cr(III) is complexed with DCTA, and the obtained complex is also retained on an anion-exchange column. For 200 μl sample volume the estimated limits of detection were for the first procedure 2.5 and 1.8 ng/ml for Cr(III) and Cr(VI), respectively, whereas for the second one 4.5 and 1.5 ng/ml for Cr(III) and Cr(VI), respectively. In the method with complexation of Cr(III) a better resolution of analytes was obtained and this method was applied for chromium speciation in spiked river waters. The obtained results show also a potential of the developed method for more detailed speciation of chromium, including complexes of various stability.

Keywords: Derivatisation, LC; Complexation; Environmental analysis; Chromium; Diphenylcarbazide; Metals

1. Introduction

It is commonly known, that toxicity and the biochemical role of chromium depend essentially on the oxidation state of this element present in a given environment [1]. Hence there are numerous different methods of chromium speciation reported in the literature, employing mostly atomic and molecular spectroscopy and voltammetry [2,3]. They are continuously being improved in terms of detectability levels, simpler operations and required instrumentation and also regarding the elimination of the effect of various interferences occurring in natural matrices. Among various methods used for speciation of chromium compounds at different oxidation states much attention in recent years is focused on various kinds of high-performance liquid chromatography (HPLC) methods with different mechanisms of separation and different detection methods. Several papers have been published on the use for this purpose of reversed-phase chromatography after derivatisation of inorganic chromium species to lipophilic compounds [4–7]. Especially widely employed for this purpose is interactive ion-pair chromatography [8–19]. This wide interest can be attributed to a possibility of the use of a large variety of chemical conditions for separation, a common availability, and the use of reversed-phase columns.

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A smaller number of applications can be found, so far, for HPLC using ion-exchange columns. Among these, most often anion-exchange columns were used, on which Cr(VI) species were retained only, whereas Cr(III) was determined in a void peak [20–23], or Cr(III) species were converted into negatively charged complexes, which can also be retained on anion-exchange columns [12,24]. Retention of two oxidation states of chromium was achieved also in the system with both anion- and cation-exchange columns connected in parallel [25,26] or using an anion-exchange column containing a small proportion of cation-exchange groups [27]. Separation of Cr(III) and Cr(VI) using a cation-exchange column, where Cr(III) species were retained was reported in one paper only [28].

Ion chromatographic speciation of chromium was carried out most often with the use of atomic spectroscopy detections such as atomic absorption spectrometry (AAS) [20,21], inductively coupled plasma atomic emission spectrometry (ICP-AES) [12,22], and inductively coupled plasma mass spectrometry (ICP-MS) [23]. In several papers chemiluminescence detection was reported, which was based on sensitive post-column reaction of Cr(III) with luminol, producing chemiluminescence [25,27,28]. Sporadically, simpler instrumentally detection methods were reported such as UV spectrophotometry, where measurements of Cr(VI) and Cr(III)–EDTA complex had to be made at different wavelengths [24], or conductivity detection [26].

A general task of this approach was to develop a chromatographic method for chromium speciation, which can be used for environmental purposes without hyphenation of the HPLC setup with expensive atomic spectroscopy instrumentation and with less interferences than the very sensitive chemiluminescence detection. The aim of this study was to develop the ion chromatography method for the determination of chromium species using an anion-exchange column and spectrophotometric detection in the visible range based on post-column reaction of Cr(VI) with diphenylcarbazide (DPC). Such a detection requires the post-column oxidation of separated Cr(III) species to Cr(VI) and it was already successfully employed to chromium speciation using ion-pair chromatography [13]. In the routine use of that procedure some interferences were found in the presence of excess of chloride and sulphate; therefore, it seemed to be interesting to investigate whether these also occur when using anion-exchange chromatography instead of ion-pair chromatography. Due to high specificity of the reaction of Cr(VI) with DPC, this detection may be considered as element-specific detection and can then be used to quantify unretained Cr(III) species. Studies on the possibilities of retention of Cr(III) as negatively charged complexes were also carried out in order to improve the selectivity of Cr(III) determination.

2. Experimental

2.1. Apparatus

The HPLC instrumentation consisted of a high-performance liquid chromatographic pump Series 100 from Perkin-Elmer (Norwalk, CT, USA) with a 200-μl injection loop, an UV–Vis detector from Knauer (Bad Homburg, Germany) and strip chart recorder TZ 4620 from Laboratorni Pristroje (Prague, Czech Republic). The separation column used was a Hamilton PRP-X100 anion column (100×4.1 mm I.D.) with trimethylammonium polystyrene copolymer as stationary phase of high pH stability. Reagents for post-column reactions were delivered using a multi-channel peristaltic pump Ismatec MS-4 REGLO/8-100 (Zürich, Switzerland). A schematic diagram of the setup used is shown in Fig. 1.

2.2. Reagents and solutions

All chemicals used for preparations of mobile phases and solutions for post-column reactions were of analytical reagent grade and were obtained from POCh (Gliwice, Poland), except 3- and 4-hydroxybenzoic acids, which were purchased from Aldrich. Deionised water was obtained by passing distilled water through a Waters Milli-Q water-purification system. For studies of Cr(III) complexation, oxalic acid, EDTA and Tiron (1,2-di-hydroxybenzene-3,5-sulphonic acid sodium salt) were from POCh, and DCTA (disodium salt of 1,2-diaminecyclohexane-N,N,N′,N′-tetraacetic acid) from Fluka.

Stock solutions of Cr(III) in concentrations of 1

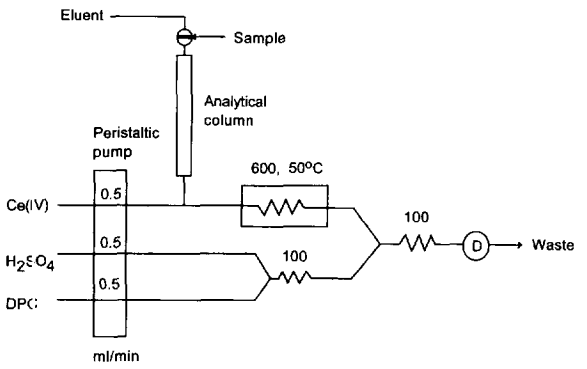

Fig. 1. Schematic diagram of the ion chromatographic setup with post-column reaction spectrophotometric detection used in this study. Length of mixing and reaction coils shown in cm. D= spectrophotometric detector.

mg/ml were prepared from Titrisol 1000 mg/l standard chromium(III) chloride solution (Merck) or from potassium chromium(III) sulphate and chromium(III) nitrate from POCh. Stock solution of Cr(VI) was prepared from sodium chromate (POCh). Standard solutions used for evaluation of the method were obtained by appropriate dilution of stock solution with eluent. For the post-column reaction detection 0.5 g/l Ce(IV) sulphate in 0.8 *M* sulphuric acid, 0.05% aqueous solution of diphenylcarbazide (DPC) and 0.8 *M* sulphuric acid were used. All mobile phases were freshly prepared prior to the measurements, filtered through a 0.45-μm membrane filter and 20 min ultrasonicated.

2.3. Procedures

In measurements without retention of Cr(III), as eluent 4 m*M* potassium hydrogenphthalate of pH 3.5 was used which contained 50 m*M* sodium chloride and 2 m*M* sodium sulphate. Measurements were carried out at a flow-rate of 2.0 ml/min and a sample volume of 200 μl. Mixtures of potassium chromium(III) sulphate and potassium chromate in the eluent were used for the calibration.

In measurements with retention of Cr(III)–DCTA complex, as eluent 2 m*M* potassium hydrogenphthalate solution of pH 3.5 was used containing sodium sulphate. Measurements were carried out at a flow-rate of 2.0 ml/min and a sample volume of 200 μl. To 50 ml sample or standard solutions containing Cr(III) and Cr(VI) 1 ml 0.1 *M* solution of disodium salt of DCTA was added. The obtained mixture was heated for 15 min at 80°C, then cooled to room temperature and after dilution to 100 ml was used for the chromatographic measurements.

3. Results and discussion

3.1. Separation without retention of Cr(III)

The conditions of the spectrophotometric detection at 540 nm with post-column oxidation of Cr(III) to Cr(VI) and the reaction of Cr(VI) with diphenylcarbazide were adapted from earlier work with ion-pair chromatography [13]. Four different eluents were examined, namely 3- and 4-hydroxybenzoate, orthophosphate and phthalate. The most favourable response for both chromium species was obtained using potassium hydrogen phthalate as eluent, and therefore the effect of its concentration up to 6 m*M* and pH in the range from 3.0 to 4.6 was examined. The effects of these two parameters are shown in Fig. 2 and Fig. 3. An increase of phthalate concentration causes clear shortening of the retention time for Cr(VI), but simultaneously results in some increase of the retention time for Cr(III). The latter can be attributed to complexation of Cr(III) at increasing concentration of phthalate. As optimum mobile phase was taken 4 m*M* phthalate solution of pH 3.5. The obtained peak shape is to a certain extent also affected by the pH of the injected sample solution, and as most favourable was found pH 3.0. In such conditions linear dependencies of peak height versus concentration in injected samples were obtained up to 5 and 2 mg/l for Cr(III) and Cr(VI), respectively.

In the numerous papers cited above on liquid chromatographic speciation of chromium at different oxidation states a large variety of different standard Cr(III) solutions were used. However, only few authors have suggested that the counterion in the used solution may be very essential for whole chromatographic determination. It is associated with complex chemistry of aqueous Cr(III) solutions, the formation of complex compounds with different ligands and polymerisation processes [29]. The inert-

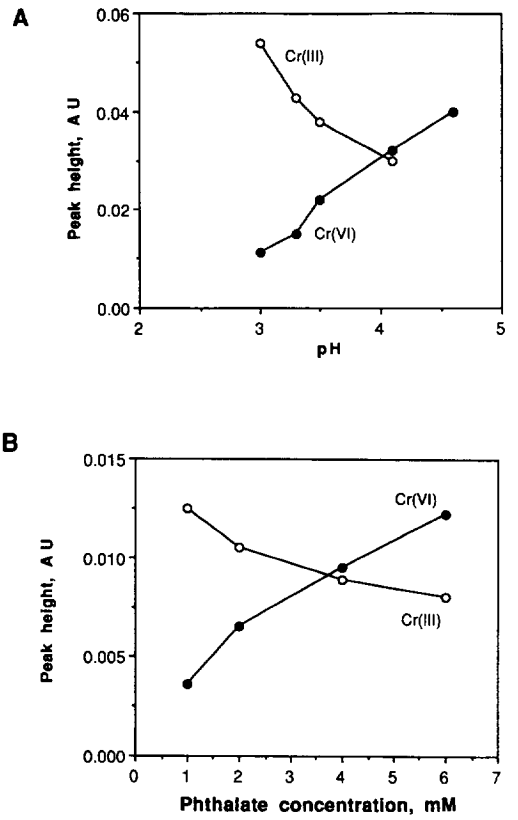

Fig. 2. Dependence of the signal magnitude for Cr(III) and Cr(VI) on (A) pH of 2 m*M* phthalate eluent and (B) concentration of phthalate eluent of pH 3.5 obtained for injections of 200 μl solution containing 5 mg/l Cr(III) and 1 mg/l Cr(VI). Flow-rate: 2.0 ml/min.

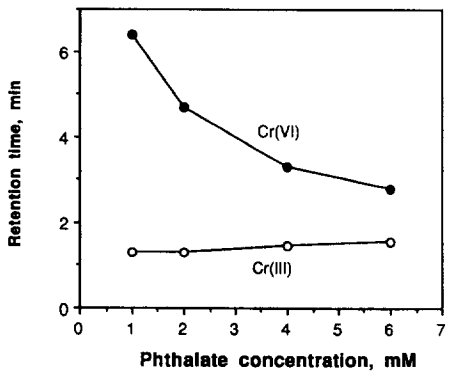

Fig. 3. Effect of the phthalate concentration in the mobile phase of pH 3.5 on the retention times of Cr(III) and Cr(VI) at a flow-rate of 2.0 ml/min.

ness of the majority of Cr(III) complexes can seriously complicate the chromatography of Cr(III).

The above-reported optimisation of phthalate eluent was carried out using standard solution of chromium chloride, which was used also by other authors [16,22,28]. For this purpose also other chromium(III) salts were used such as nitrate [10,15,20,27], sulphate [14,24], acetate [11] and potassium chromium sulphate [30]. The authors of only two papers indicate some problems associated with standardisation of Cr(III) response. In ion-pair chromatography, Syty et al. [11] obtained for chromium acetate standard solution two peaks and one of them disappeared with aging of solution. Using mobile phase of pH 7.3 for chromium chloride and perchlorate standard solutions, a Cr(III) peak was obtained only in the presence of acetate, which prevents formation of chromium(III) hydroxide. In another work with an anion-exchange column it was found that a single chromium(III) peak is recorded for freshly prepared chromium nitrate solution; however, after 12 h a second peak with a shorter retention time is observed [12].

In this study, besides chromium chloride as standard solutions were investigated chromium nitrate and potassium chromium sulphate. In the measuring system without the separation column all chromium(III) solutions showed the same signal magnitude for Cr(III). For the same standard solutions injected in the system with an anion-exchange column very different signals for Cr(III) were obtained. The largest signal magnitude was observed for potassium chromium sulphate; however, it has to be admitted that for freshly prepared solution the Cr(III) signal was much smaller and a stable value was observed after two days aging of the solution. This behaviour may be attributed to the slow processes of hydration and polymerisation of Cr(III) cations in non-complexing aqueous solutions. For chromium chloride the Cr(III) peak was smaller, broader and with some shift of the retention time in comparison to sulphate solution. These properties may be interpreted by the formation of Cr(III) chloride anionic complexes in the presence of some excess of chloride. They cause the broadening of the chromium(III) peak and an increase of the retention. Surprisingly low, but reproducible Cr(III) signals were observed for freshly prepared chromium nitrate

solution. This observation is quite controversial in ms of the common use of chromium nitrate solution as Cr(III) standard in the numerous, above-mentioned chromatographic works. As conclusion from the comparison of several chromium(III) standard solution , in further studies as standard solution the potassium chromium sulphate solution in mobile phase was employed, which was always left overnight for aging.

In the earlier works on ion chromatographic speciation of oxidation states of chromium several interferences in such determinations were reported, especially in the systems with chemiluminescence detection and post-column reaction with luminol. In this work the effect of Fe(III) presence up to 5 mg/l, Mg up to 20 mg/l, Ca up to 50 mg/l, nitrate up to 1 m*M*, sulphate up to 2 m*M* and chloride up to 100 m*M* was investigated. The measurements were carried out at a 0.1 mg/l level of Cr(III) and Cr(VI) using the optimised mobile phase of 4 m*M* phthalate at pH 3.5. In the flow-injection system (chromatographic setup after column was disconnected) no signal changes exceeding ±3% for both the Cr(III) and Cr(VI) signal were observed, which would be outside of repeatability of measurements.

Evident interferences, however, were found in several cases in a chromatographic determination (Table 1). In the investigated range of concentrations practically no effect on the peak shape or the signal magnitude was found for nitrate and Fe(III). As negligible interferences can be considered those which were observed in the presence of magnesium and calcium. At the highest concentrations used, 20 mg/l Mg and 50 mg/l Ca, besides the Cr(III) peak another one appeared, with a longer retention time (Fig. 4a,b), which can be assigned to another form of Cr(III) complex formed by nitrate counterions added with Mg and Ca.

Very substantial interference in Cr(VI) determination was observed in the presence of 0.5 m*M* and higher concentrations of sulphate, and in Cr(III) determination in the presence of chloride already at the 10 m*M* level. As such an effect was not observed in flow-injection measurements with the column, these interfering anions seem to affect the process of desorption of analytes from the stationary phase in the column. In the presence of 2 m*M* sulphate in the injected sample besides the evident increase of the

Table 1
Interference from ions present commonly in natural waters on ion chromatographic determination of 0.1 mg/l Cr(III) and 0.1 mg/l Cr(VI)

Ion added	Concentration	Recovery (%)	
		Cr(III)	Cr(VI)
Chloride	10 m*M*	124	103
	20 m*M*	132	100
	50 m*M*	153	100
	100 m*M*	260	103
Nitrate	0.1 m*M*	100	100
	1.0 m*M*	103	104
Sulphate	0.5 m*M*	97	107
	2.0 m*M*	103	128
Ca^{2+}	10 mg/l	100	100
	50 mg/l	94	100
Mg^{2+}	5 mg/l	100	100
	20 mg/l	97	100
Fe^{3+}	0.5 mg/l	97	100
	5.0 mg/l	95	96

As eluent 4 m*M* phthalate solution of pH 3.5 was used with flow-rate 2.0 ml/min.

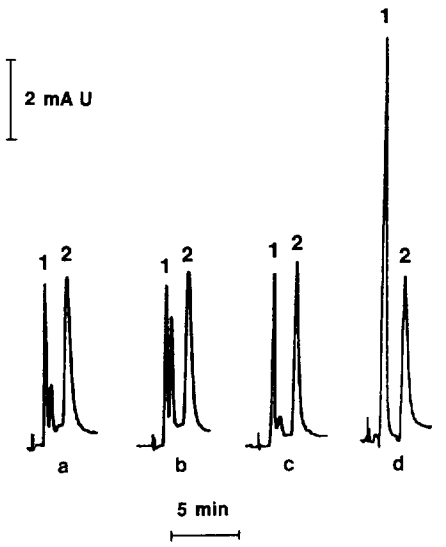

Fig. 4. Chromatograms recorded for a mixture of 0.1 mg/l Cr(III) and Cr(VI) prepared in the eluent solution with (a) 20 mg/l Mg as nitrate, (b) 50 mg/l Ca as nitrate, (c) 2.0 m*M* sulphate and (d) 100 m*M* chloride. Eluent: 4 m*M* phthalate pH 3.5; flow-rate: 2.0 ml/min. Peaks: 1, Cr(III); 2, Cr(VI).

signal magnitude for Cr(VI) (Table 1) also some additional peak appeared on the chromatogram (Fig. 4c). The signal increase for Cr(VI) can be eliminated by addition of 2 m*M* sulphate to the mobile phase, which deteriorates to some extent the resolution but eliminates a positive error of determination.

In the case of the presence of chloride in the injected sample a very substantial increase of the peak height was observed for Cr(III) (Fig. 4d). A multiple sequential injection of the same solution with chloride results in signals with decreasing positive errors down to the signal magnitude corresponding to standard chromium solution. In another experiment, after several injections of the standard mixture of Cr(III) and Cr(VI) multiple injections of 100 m*M* chloride solution (without chromium) were made, which initially gave a very large peak but rapidly descending in following injections, with retention time corresponding to Cr(III). This behaviour can be interpreted as the elution by chloride of a part of Cr(III) from the stationary phase, which is not completely desorbed with phthalate mobile phase. The injections of chloride solutions on a column which was earlier rinsed with EDTA do not give measurable signals. Also, in this case, it was efficient to add 50 m*M* chloride to the mobile phase in order to eliminate this effect.

The introduction of sulphate and chloride to the phthalate eluent eliminates the effect of excess of sulphate and chloride in the sample. In calibrations in the concentration range from 10 to 100 ng/ml for both chromium species, linear relationships between peak height and concentration in injected samples were obtained with correlation coefficients of 0.995 and 0.999 for Cr(III) and Cr(VI), respectively. For a signal-to-noise ratio (S/N) of 3, limits of detection were estimated as 2.5 and 1.8 ng/ml for Cr(III) and Cr(VI), respectively.

3.2. Separation with retention of Cr(III) complexes

The formation of neutral chelates by Cr(III) is utilised in reversed-phase chromatography [4–7]. Chromium(III) forms also a variety of anionic complexes, which can be employed for the chromatography of chromium at different oxidation states on an anion-exchange column. Very common are complexes of the type $[CrX_6]^{3-}$, where X may be fluoride, chloride, cyanide or thiocyanate, but they may have also lower charges if neutral ligands are present in the ion [31]. Complexes of bi- and polydentate anions are also known, one example being $[Cr(C_2O_4)_3]^-$ [32]. The most important aspect of the analytical application of these complexes is their usually low rate of formation. One example of this can be oxalate. Fig. 5A shows chromatograms obtained for a mixture of 1 mg/l Cr(III) and Cr(VI) in the presence of large excess of oxalate without

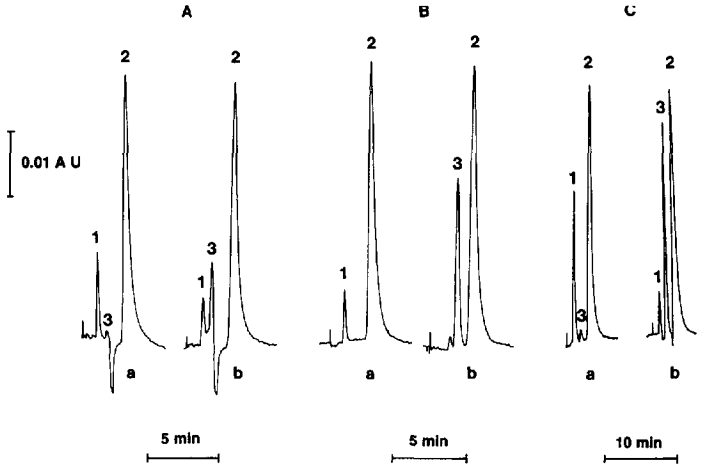

Fig. 5. Chromatograms of mixtures of 1 mg/l Cr(III) and Cr(VI) recorded in the presence of (A) 100 mg/l oxalate, (B) 2 m*M* Tiron, and (C) 0.2 m*M* EDTA in injected solutions (a) without heating and (b) with 15 min heating at 80°C prior to injection. Eluent: 4 m*M* phthalate pH 3.5; flow-rate: 2.0 ml/min. Peaks: 1, Cr(III); 2, Cr(VI); 3, complex of Cr(III) with the ligand used.

and with 15 min heating at 80°C. Only for the solution which was heated there is evident formation of Cr(III) complex, eluted from the column between not complexed Cr(III) and Cr(VI); however, under such a conditions still Cr(III) partially remains in the form eluted in peak 1. It is not obvious which exact form is eluted in peak 1, as for not heated solution the height of this peak is half of that obtained for the standard solution without oxalate. This system would be rather difficult to use for analytical purposes. The obtained data show, however, that the present system can be used for more detailed study of chromium speciation and not only to quantitate the total amount of Cr(III) and Cr(VI).

Another ligand forming strong Cr(III) complexes which was investigated is the common spectrophotometric reagent Tiron. Introduction of 2 m*M* of this reagent to the same standard solution containing Cr(III) and Cr(VI) causes significant decrease of the Cr(III) peak with retention time 1.1 min without change of its retention time and without formation of a new peak (Fig. 5B). After 15 min heating at 80°C the peak at 1.1 min completely disappears and instead two new peaks are observed, a small one at 1.5 min and a very large one at 2.0 min, which should be assigned to Cr(III) complexes. The irreproducibility of the height of this peak at 2.0 min (ca. 30% R.S.D.) in consecutive injections also does not allow the use of Tiron complexation of Cr(III) for analytical chromatography of chromium.

The formation of the negatively charged 1:1 complex Cr(III)–EDTA was already applied in chromatographic speciation of chromium either in ion-pair chromatography [15] or in ion chromatography on an anion-exchange column [12]. The chromatograms shown in Fig. 5C demonstrate also in this case very slow formation of the EDTA chelate, which gives a peak with a retention time of 2.0 min. Similarly to oxalate and Tiron, the introduction of 0.2 m*M* EDTA to the standard solution containing 1 mg/l of both chromium species does not decrease the peak for Cr(VI) even after heating. This means that it does not affect the disproportionation equilibrium Cr(VI)–Cr(III). Another aspect of such a system is the influence of the presence of other cations in the sample solution which can be complexed by EDTA, which may affect Cr(III) complexation by EDTA. Such interferences were not observed in ion-pair chromatography [15], and there is no information whether they were investigated by chromatography on an anion-exchange column [12]. The chromatogram shown in Fig. 5C indicates that, even after heating, in the presence of 0.2 m*M* EDTA some part of Cr(III) remains not complexed by EDTA. Introduction to the sample solution of 20 mg/l Mg or 50 mg/l Ca does not practically affects the height and retention time of all peaks recorded. The system is also insensitive to the presence up to 1 mg/l Fe(III), which forms more stable complexes with EDTA than Mg and Ca, especially in the weakly acidic medium used in the reported measurements. Introduction of 5 mg/l Fe(III) causes, however, significant disturbances. An about 50% smaller peak is observed for Cr(III)–EDTA and a double increase of the peak height was found for Cr(III). It was also associated with an about 15% decrease of the Cr(VI) peak.

The ligand which enables the most efficient retention of Cr(III) in the ion chromatography on an anion-exchange column and avoids the drawbacks of other examined ligands is another complexone, DCTA. It also binds Cr(III) in stable, negatively charged 1:1 chelate [33]. The complexation of Cr(III) with DCTA was very recently utilised in the ion-pair chromatography for speciation of chromium at different oxidation states, where DCTA was used as additive to the mobile phase [19].

The effectiveness of Cr(III) complexation by DCTA was investigated at different pH with 4 m*M* phthalate eluent (Fig. 6). Using 2 m*M* concentration of DCTA and heating the sample solution for 15 min at 80°C eliminates completely the chromatographic peak for not complexed Cr(III), and a peak for Cr(III)–DCTA is obtained with a retention time two times longer than that for Cr(VI). A longer time of heating does not affect the results. An increase of pH in the range from 3.5 to 4.3 causes an increase of height for both peaks but decreases the separation of the Cr(III)–DCTA peak from the large, negative signal of DCTA. The degree of complexation of Cr(III) depends significantly on the concentration of DCTA added to the mixture of Cr(III) and Cr(VI) (Fig. 7). Only at 2 m*M* DCTA, however, complete disappearance of the not-complexed-Cr(III) peak was obtained. An increase of the DCTA concentration from 0.01 to 2 m*M* is associated also with

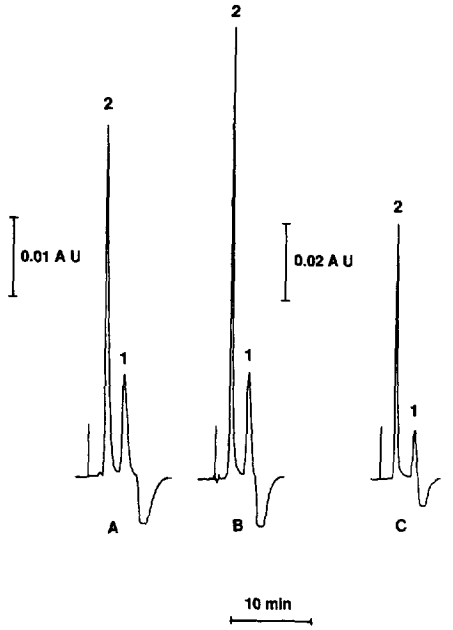

Fig. 6. Chromatograms of mixtures of 1 mg/l Cr(III) and Cr(VI) with 2 m*M* DCTA obtained after 15 min heating of the injected solutions at 80°C using as the mobile phase 4 m*M* phthalate solutions adjusted to pH (A) 3.5, (B) 3.85 and (C) 4.3. Flow-rate: 2.0 ml/min. Peaks: 1, Cr(III)–DCTA; 2, Cr(VI).

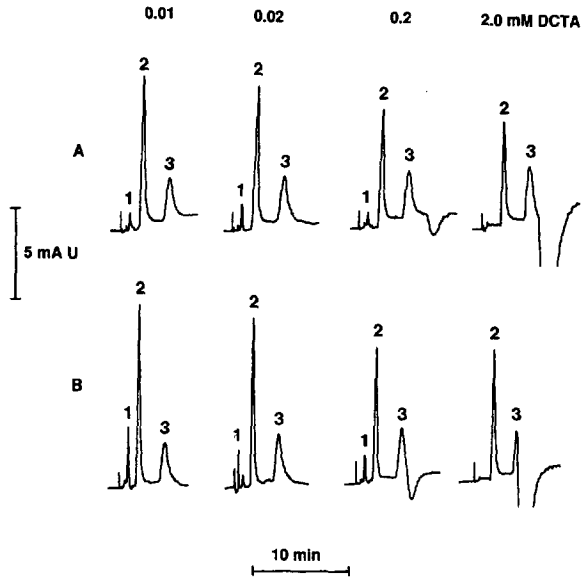

Fig. 7. Effect of pH of 4 m*M* phthalate used as the mobile phase and the concentration of DCTA added to the injected solutions containing 0.2 mg/l Cr(III) and 0.1 mg/l Cr(VI) on chromatograms recorded at a flow-rate of 2.0 ml/min. Peaks: 1, non-complexed Cr(III); 2, Cr(VI); 3, Cr(III)–DCTA. The pH values of the mobile phase: (A) 3.85, (B) 4.3.

some decrease of the Cr(VI) signal (about 30% at 2 m*M* DCTA) due to partial reduction of Cr(VI) to Cr(III). This effect is the same regardless of heating of the sample, and it should not affect the analytical determination providing that calibration is carried out with standard solutions containing the same concentration of DCTA.

Similarly to above-reported experiments with EDTA, also for DCTA the effect of the presence of 50 mg/l Ca, 20 mg/l Mg and 5 mg/l Fe(III) on the peaks of Cr(III) and Cr(VI) was examined. The observed peak heights did not show any systematic error caused by the presence of these cations.

For investigation of the possibility of the use of the chromatographic procedure with addition of DCTA to the speciation of chromium at much lower concentration levels, as the mobile phase phthalate solutions of pH 3.5 containing additionally 10 m*M* sulphate and 20 m*M* chloride were examined. The role of these anionic additives was discussed above as elimination of interferences in chromatographic determinations in the absence of Cr(III) complexing ligands. The increase of phthalate concentration in the range from 1 to 4 m*M* decreases the retention of both solutes; however, the most satisfactory value of the limit of detection for both oxidation states was found for the mobile phase containing 2 m*M* phthalate (Table 2).

The calibration plots obtained for 2 m*M* phthalate eluent in the range 10 to 100 ng/ml Cr(III) and 5 to 50 ng/ml Cr(VI) were linear with correlation coefficients of 0.999 and 1.000 for Cr(III) and Cr(VI), respectively. Example chromatograms recorded under the optimised conditions with addition of 2 m*M* DCTA and heating the sample solution prior to the injections are shown in Fig. 8.

The determinations carried out for two river water samples did not show Cr(III) or Cr(VI) contents above the detection limits of the developed procedure. The results of recovery tests for the samples spiked with chromium species indicate only differences, which are below the limit of detection (Table 3). The reproducibility of measurements estimated

Table 2
Comparison of the chromatographic data obtained for the mixture of 20 ng/ml Cr(III), 10 ng/ml Cr(VI) and 2 m*M* DCTA using a mobile phase of pH 3.5 containing different concentrations of phthalate, and 10 m*M* sulphate

Phthalate concentration in mobile phase (m*M*)	Cr(III)			Cr(VI)		
	t_R (min)	Peak height (mAU)	Detection limit (ng/ml)	t_R (min)	Peak height (mAU)	Detection limit (ng/ml)
1.0	8.7	0.20	8.0	2.7	0.64	1.7
2.0	6.7	0.30	4.5	2.5	0.72	1.5
4.0	4.5	0.36	6.0	2.0	0.68	1.6

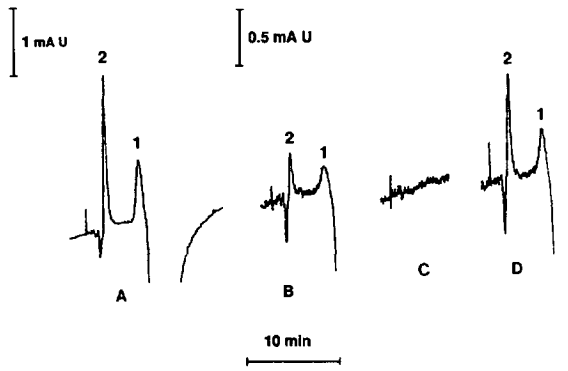

Fig. 8. Chromatograms recorded using a mobile phase of 2 m*M* phthalate (pH 3.5) containing 10 m*M* sulphate and 20 m*M* chloride. Solutions injected after addition of 2 m*M* DCTA and 15 min heating at 80°C. (A) Mixture of 60 ng/ml Cr(III) and 30 ng/ml Cr(VI), (B) 10 ng/ml Cr(III) and 5 ng/ml Cr(VI), (C) sample of river water, and (D) the same river water sample as (C) spiked with 20 ng/ml Cr(III) and 10 ng/ml Cr(VI). Flow-rate: 2.0 ml/min.

with standard solutions at 20 ng/ml of both species was 1.9 and 1.2% R.S.D. ($n=10$) for Cr(III) and Cr(VI), respectively.

Table 3
Results of the recovery test for the determination of Cr(III) and Cr(VI) in river water samples using 2 m*M* DCTA added to the injected solutions with mobile phase of pH 3.5 containing 2 m*M* phthalate, and 10 m*M* sulphate

Sample	Added amount (ng/ml)		Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
River water I	20	10	97	99
River water II	20	10	104	104

4. Conclusions

Both developed ion chromatographic procedures, without and with retention of Cr(III), show their own advantages and drawbacks, and therefore both are presented. Each of them can find different applications.

The advantage of the method without retention of Cr(III), which is eluted with the void peak, is no need of sample pretreatment. This procedure has, however, its drawbacks, such as larger sensitivity to interferences and lack of the baseline resolution of both analytes of interest. In the procedure with Cr(III) complexation by DCTA it is necessary to heat and then to cool the sample solution prior to the injection; however, the chromatographic resolution of both chromatographic signals is better.

The detection limits for both procedures are very similar and they are quite satisfactory for environmental purposes. Only chromatographic methods developed with chemiluminescence [25,27,28], ICP–AES [12,22] and ICP–MS [18] detection provide better detectability (Table 4). They require, however, much more sophisticated instrumentation. The detection limits of the developed procedures can certainly be lowered by at least a few times by decreasing the observed noise level. This can be achieved for instance by the use of more advanced signal recording devices or by digital filtering of the experimental data before their processing.

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Table 4
Comparison of the detection limits reported for ion chromatography speciation of chromium

Principle of separation	Detection[a]	Limits of detection (mg/l)		Ref.
		Cr(III)	Cr(VI)	
Anion-exchange (Cr(III) not retained)	AAS	200	100	[20]
	DCP-AES	100	100	[21]
	ICP-AES	0.25	0.27	[22]
	ICP-MS	0.06	0.18	[23]
	Spectrophotometry	2.5	1.8	This work
Anion-exchange (Cr(III) retained as negatively charged complex)	UV	2000	2000	[24]
	ICP-AES[b]	10	30	[12]
	Spectrophotometry	4.5	1.5	This work
Anion-exchange (column with cation exchange groups)	Chemiluminescence	0.05	0.1	[27]
Cation-exchange	Chemiluminescence	0.5	0.5	[28]
Dual column system with anion- and cation-exchange	Conductivity	1000	500	[26]
	Chemiluminescence	0.1	0.3	[25]

[a]AAS: atomic absorption spectrometry, DCP-AES: direct current plasma atomic emission spectrometry, ICP-AES: inductively coupled plasma atomic emission spectrometry, ICP-MS: inductively coupled plasma mass spectrometry, UV: ultraviolet spectrophotometry.
[b]Thermospray enhanced.

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