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On-line preconcentration and determination of chromium(VI) in waters by high-performance liquid chromatography using pre-column complexation with 1,5-diphenylcarbazide

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Abstract

A method for on-line chromatographic preconcentration and determination of chromium(VI) traces has been developed. Chromate was preconcentrated on a C_{18} column (50×6 mm I.D.) after complexation with diphenylcarbazide (DPC). Following the preconcentration step, analysis of the sample was performed using a C_{18} column (100×6 mm I.D.) with an eluent containing $6 \cdot 10^{-3}$ mol/l sulphuric acid and 20% (v/v) acetonitrile. Direct spectrophotometric detection at 546 nm was used. Experimental parameters such as mobile phase pH, DPC concentration, preconcentration flow-rate, sample volume were optimized for preconcentration and detection of Cr(VI)–DPC complex. Under the optimum conditions, most metal ions [Cr(III), Fe(III), Cu(II), Hg(II), Mo(VI), V(V)] and inorganic anions did not interfere. A detection limit of 0.02 ng/ml Cr(VI) can be attained when a sample volume of 100 ml is used. The technique has been applied successfully to the determination of Cr(VI) traces in drinking, surface and groundwater samples and the recoveries of added chromium were in the range 94–104%.

Keywords: Preconcentration; Water analysis; Chromium; 1,5-Diphenylcarbazide; Metal cations

1. Introduction

Chromium (Cr) exists in the environment predominantly in two oxidation states, Cr(III) and Cr(VI). Because chromates are used extensively in water treatment and Cr(VI) has a much higher toxicity than Cr(III) [1] most interest in chromium speciation has centred on the determination of Cr(VI).

A number of different analytical techniques are available for the determination of chromium species, as shown in review articles [2,3]. Most methods for separating the two chromium oxidation states involve ion-exchange, although coprecipitation and liquid–liquid extraction have also been used [4,5].

During the last ten years high-performance liquid chromatography (HPLC) in combination with various detection techniques has been extensively applied to the speciation of a number of metal ions. Cr(VI) or both chromium species were determined either by ion chromatography (IC) [6–13], or by reversed-phase HPLC after derivatization of the chromium species to a hydrophobic molecule [14–16] or by formation of an ion-pair with hydrophobic

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counter-ions [17–21]. Speciation of Cr using HPLC with conductometric detection, however, provides low sensitivity (~100 ng/ml) and poor selectivity because higher amounts of common inorganic anions interfere in the determination of chromate [8,19]. Significantly higher sensitivity and better selectivity are achieved using HPLC with spectrophotometric detection [10,12,21]. The best detection limit thus far [0.3 ng/ml for Cr(VI)] was reported by Arar and Pfaff [10] who used IC separation with post-column reaction of chromate with diphenylcarbazide and detection at 530 nm. This procedure was successfully applied to the determination of Cr(VI) in industrial wastewater. In several papers chemiluminescence detection was reported, which was based on the post column catalytic oxidation of luminol [9,11]. The detection limits were estimated to be 0.1 ng/ml and 0.3 ng/ml for Cr(III) and Cr(VI), respectively. However, this technique was not applied to the analysis of real environmental samples.

Several research groups have developed hyphenated HPLC methods for speciation of chromium [7,13,17,18,20]. Krull et al. [17] developed a method with on-line coupling of the HPLC system to a direct-current plasma or an inductively-coupled plasma atomic emission spectrometry (ICP-AES) system [17]. The detection limits obtained for both Cr species were in the range of 5 to 10 ng/ml. However, spiked Cr(VI) in environmental samples was not recovered. Syty et al. [18] determined Cr(III) and Cr(VI) in spiked natural water with on-line flame atomic absorption spectrometry (FAAS) but the detection limits [40 ng/ml and 80 ng/ml for Cr(III) and Cr(VI), respectively] were inadequate for the analysis of natural water samples. Several research groups obtained very low detection limits (0.3–0.5 ng/ml) for chromium species using HPLC in combination with ICP mass spectrometry (MS) [13,20]. However, ICP-MS is prone to some interferences when analyzing environmental samples. Probably the most serious interference is the formation of polyatomic ions, especially below atomic mass number 80.

The main problem with all these HPLC methods is that the normal concentration range of chromium in natural water samples is so low that it can not be determined without a preconcentration procedure. Although several on-line HPLC preconcentration methods combined with FAAS [22], flame AES [23] or ICP-MS [24] are reported for Cr(VI), the use of hyphenated techniques is to complicate for routine analysis.

The aim of this work was to develop a simple HPLC technique for the on-line preconcentration and determination of Cr(VI) traces in natural waters using selective pre-column complexation with 1,5-diphenylcarbazide.

2. Experimental

2.1. Instrumentation

Two Dionex 2000i/SP series high-pressure chemically inert plastic pumps (Dionex, Sunnyvale, CA, USA) were used for delivery of the mobile phase and sample. Two non-metallic injection valves (Dionex) were connected in consecutive order. On the first valve a 100-μl loop was used for direct injection. The loop on the second valve (preconcentration valve) was replaced by a concentrator column. All connections were achieved with PTFE tubing. Both concentrator (50×6 mm I.D.) and analytical (100×6 mm I.D.) titanium columns were packed with 10-μm Silasorb C_{18} (Tessek, Prague, Czech Republic). The detector was UV–Vis photometer LCD 2563 (Laboratorni Pristroje, Prague, Czech Republic) set to absorb at 546 nm. The results and data were collected and plotted on a plotter/integrator SP 4290 (Spectrophysics, San Jose, CA, USA).

2.2. Reagents and solutions

All chemicals used were of analytical-reagent grade. Deionized water was obtained by passing distilled water through a Waters Milli-Q water-purification system (Millipore, Eshborn, Germany).

Stock standard solution (100 mg/l) of Cr(VI) was prepared from potassium dichromate (Merck, Darmstadt, Germany). Solutions of lower concentrations were prepared daily by appropriate dilution. Diphenylcarbazide was obtained from Merck. Diphenylcarbazide stock solution ($2 \cdot 10^{-3}$ mol/l) was prepared daily in 10% (v/v) acetonitrile solution with appropriate amount of sulphuric acid. The mobile phase was a mixture of acetonitrile, sulphuric

acid and water. All mobile phases were filtered through 0.2-μm cellulose nitrate filter and degassed by ultrasonication.

2.3. Preconcentration procedure

The concentrator column was pre-equilibrated with the mobile phase before the first injection and no further pretreatment was done before subsequent injections. The measured volume of sample was passed by the sampling pump into the concentrator column. After a preconcentration period of normally 5–20 min the connecting tubing was washed with 5 ml of water. In the elution step, the position of preconcentration valve was changed, so the eluent flowed through the concentrator column in the same direction as the sample loading, and analyte was eluted from this column to the analytical column. Cr(VI) standards were injected directly onto the concentrator and analytical columns in equivalent amounts to those preconcentrated in order to calculate recoveries.

3. Results and discussion

3.1. Chromatographic separation

The reaction between Cr(VI) and 1,5-diphenylcarbazide (DPC) has been widely used for the spectrophotometric determination of chromium since 1900 [25]. According to the literature [26] the following reactions take place. First, chromate is reduced by DPC to Cr(III). At that time an oxidation of DPC to a diphenylcarbazone occurs. Then Cr(III) reacts with diphenylcarbazone by forming a highly absorbing violet complex (1:1; $\lambda_{max}=543$ nm; $\varepsilon=4.2\cdot10^4$ l/mol cm). Cr(III) does not react with both diphenylcarbazide and diphenylcarbazone or reacts only very slowly [27].

The cationic nature and relatively large molecule of the formed chelate offer a possibility to separate and determine this chelate by both reversed-phase HPLC and by ion pair HPLC methods. But on-line preconcentration and determination procedures require a rapid equilibration between stationary and mobile phases, therefore the reversed-phase HPLC method was selected for further investigations. In addition, by using a reversed-phase HPLC procedure a washing step with acetonitrile or methanol between consecutive injections is not required to restore the starting conditions.

Preliminary investigations of the elution efficiency were made using acetonitrile–water mixtures (at pH 3.0 adjusted with H_2SO_4) ranging from 5 to 50% (v/v) and carrying out each test by stepwise 5% increases in acetonitrile concentration. It was found that an elution of Cr(VI) in less than 10 min can be attained if 20% of acetonitrile in the mixture is exceeded.

The complex forming reaction between Cr(VI) and DPC is pH dependent. It has been recommended [28] that the DPC solution should be acidified with sulphuric acid before the reaction with the chromium sample solution. In order to optimise the detection sensitivity the effect of H_2SO_4 concentration in the mobile phase and sample solutions on the peak area of Cr(VI)–DPC chelate was studied over the range 10^{-3}–10^{-2} mol/l. The results showed that the maximum signal response was achieved at H_2SO_4 concentrations higher than $4\cdot10^{-3}$ mol/l. The decrease in response at lower H_2SO_4 concentrations may be attributed to slower complex forming reaction, which is consistent with earlier experiments [28]. However, a pH of the mobile phase lower than 2 can cause degradation of C_{18} column. Thus $6\cdot10^{-3}$ mol/l H_2SO_4 was selected as the working concentration.

The addition of DPC up to the concentration of $5\cdot10^{-4}$ mol/l to the mobile phase only negligibly decreases the migration time of Cr–DPC chelate without significant improvement in the signal response and the peak shape. An optimal separation resulted from tests made with mobile phase containing 20% (v/v) acetonitrile and $6\cdot10^{-3}$ mol/l H_2SO_4. Under these operational conditions, 6 min is sufficient for the elution of the analyte. Minimum detectable concentration ($S/N=3$) using direct injection (100 μl) and photometric detection at 546 nm is approximately 6 ng/ml Cr(VI).

3.2. Optimisation of preconcentration procedure

For a further improvement of the sensitivity the injection loop of the injection valve was replaced by

a 10-μm Silasorb C_{18} (50×6 mm I.D.) concentrator column. Several parameters for the preconcentration system were optimised in order to determine the optimal conditions. In all experiments the sample loading step was performed in 10 min after derivatization procedure.

A significant factor affecting the performance of the preconcentration procedure is the sample loading rate. In adsorption experiments, a slow sample flow-rate is preferred but time-consuming. Therefore, the maximum flow-rate for sample loading onto concentrator column was investigated. Sample solutions containing 0.5–2.5 ng/ml Cr(VI), $2.5 \cdot 10^{-4}$ mol/l DPC and $6 \cdot 10^{-3}$ mol/l H_2SO_4 were preconcentrated with flows ranging from 1 to 8 ml/min. The total volume used to load the sample was kept constant at 50 ml. With each of the standard solutions, estimates of the recoveries at various flow-rates were obtained by comparing peak areas from the preconcentration runs to those obtained using direct injection. The recoveries as a function of the preconcentration flow-rate is shown in Fig. 1. Flow-rates higher than 5 ml/min resulted in a peak broadening at identical peak area. At flow-rates>6 ml/min, the analytical signal became smaller probably owing to the decrease in the contact time between the chelate and sorbent. Therefore a flow of 5 ml/min was considered optimal.

If lower detection limits are required, larger sample volumes must be used for the preconcentration. Different sample volumes up to 150 ml containing the same total amount of Cr(VI) were studied. Volumes larger than 100 ml cause a decrease of signal area, because Cr(VI)–DPC chelate is gradually washed out of the column by the carrier containing an excess of hydrophobic DPC ($2.5 \cdot 10^{-4}$ mol/l), which competes on the adsorption sites with analyte. These results suggested that the critical volume for the concentrator column was approximately 100 ml under the conditions used.

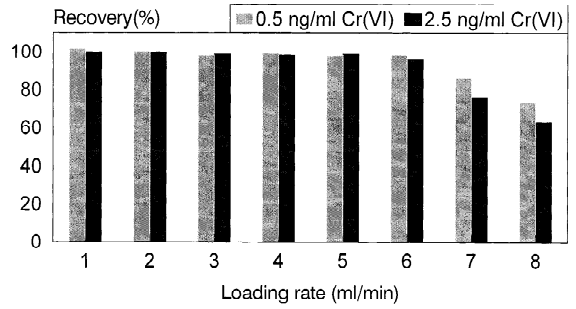

Fig. 1. Effect of sample loading rate on Cr(VI) preconcentration. Chromium standards were prepared in $2 \cdot 10^{-4}$ mol/l DPC and $6 \cdot 10^{-3}$ mol/l H_2SO_4. A Silasorb C_{18} concentrator column (50×6 mm I.D.) was used with 20% (v/v) acetonitrile and $6 \cdot 10^{-3}$ mol/l H_2SO_4 as the mobile phase. Mobile phase flow-rate 1 ml/min. Detection at 546 nm.

The effect of the DPC concentration in the sample was also studied over the range 10^{-5}–$5 \cdot 10^{-4}$ mol/l at different Cr(VI) concentrations (0.25 ng/ml; 1.0 ng/ml and 5.0 ng/ml) and no significant changes in the peak areas of Cr–DPC were found. Thus a $2 \cdot 10^{-4}$ mol/l DPC concentration was chosen for subsequent experiments.

One of the most common sources of error with preconcentration of metal traces using precomplexation procedure is the contamination of complexing and other reagents generally used in an excess. To test effect of contamination, different volumes of deionized water (25, 50 and 100 ml) containing $2 \cdot 10^{-4}$ mol/l DPC and $6 \cdot 10^{-3}$ mol/l H_2SO_4 were preconcentrated. Analytical blanks averaged 0.04 ng/ml (8% R.S.D.; $n=9$). Thus, the $2 \cdot 10^{-4}$ mol/l DPC and $6 \cdot 10^{-3}$ mol/l H_2SO_4 solution preconcentrated prior to sample could be used as the blank.

The recoveries of the preconcentration procedure were evaluated by comparing the peak areas of a direct 100-μl injection of 0.1–1.0 μg/ml Cr(VI) samples complexed with DPC with the peak areas from the preconcentration of 100 ml samples containing 0.1–1.0 ng/ml Cr(VI). The results obtained are shown in Table 1. It can be clearly seen that preconcentration and elution of chelated Cr(VI) is quantitative; recoveries are generally >96% with R.S.D.s of 1.5–4.5%.

Table 1
Preconcentration recoveries (%) of Cr(VI) standards from 100-ml samples ($n=5$)

Cr(VI) concentration (ng/ml)	Recovery (%)	R.S.D. (%)
0.10	97.4	4.5
0.25	96.1	2.9
0.50	101	1.5
1.00	98.7	1.8

Conditions as in Fig. 2.

3.3. Interferences

Iron(III), copper(II), mercury(II), molybdenium(VI) and vanadium(V) react with DPC and hence can interfere in the determination of Cr(VI). The effect of increasing concentrations of these ions and Cr(III) on the determination of Cr(VI) was therefore examined. A total of 50 ml of Cr(VI) standards spiked with foreign ion at a given concentration were complexed with DPC and preconcentrated. Recoveries were deduced from the comparison of the peak areas with those obtained by direct injection of standard solutions that were free of the foreign ion. Table 2 lists the tolerated ratio of foreign ions to analyte. As can be seen, the DPC provides a very high selectivity. The interferences of Hg(II), Mo(VI) and V(V) at higher concentrations arise probably from their competition with Cr for reagent because all these ions showed negative interferences.

Common inorganic anions (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) did not interfere in the determination of Cr with the tolerated ratios at least 10^6.

3.4. Quantification

Several parameters important for quantitative analysis, including linearity, minimum detectable concentration and reproducibility, were examined under the above optimised conditions. All the signals of preconcentrated solutes were corrected for the blank.

A linear relationship between peak area and concentration was obtained in the 0.05–2.5 ng/ml Cr(VI) range for preconcentration of 100-ml samples with correlation coefficient 0.998. A detection limit ($S/N=3$) for 100 ml samples was 0.02 ng/ml Cr(VI). Detection limits given in the literature for IC based on post-column reaction of Cr(VI) with DPC [10] and for HPLC with ICP-MS detection [13] were 0.3 and 0.5 ng/ml, respectively. Thus the presented method is 15–25-times more sensitive.

Table 3
Recoveries of Cr(VI) in spiked water samples ($n=5$)

Sample	Added (ng/ml) Cr(VI)	Recovery (%)
Drinking water	0.25	100
	0.50	104
	1.50	97.3
Groundwater	0.25	96.0
	0.50	94.0
	1.50	101
River water	0.25	96.0
	0.50	102
	1.50	96.6

The reproducibility was studied by making five consecutive runs with Cr(VI) concentrations of 0.25 ng/ml, 0.75 ng/ml and 1.5 ng/ml. The R.S.D.s of peak areas were less than 4.0%.

3.5. Analysis of water samples

In order to evaluate the quantitative performance of the method, several samples of drinking water, groundwater and river water taken from the Vilnius region were analysed. After collection the samples were filtered through a 0.20-μm membrane filter to eliminate suspended matter. Then the samples were treated with DPC and H_2SO_4 solution and after ~10 min were analysed.

Different amounts of Cr(VI) were added to water

Table 2
Tolerated ratios of foreign ions to analyte in the determination of 10 ng/ml Cr(VI) at various DPC concentrations

Foreign ion	Tolerated ratio		
	c(DPC)=$1\cdot10^{-5}$ mol/l	c(DPC)=$5\cdot10^{-5}$ mol/l	c(DPC)=$2\cdot10^{-4}$ mol/l
Cr(III)	>5000	>5000	>5000
Cu(II)	>5000	>5000	>5000
Fe(III)	>5000	>5000	>5000
Hg(II)	30	75	200
Mo(VI)	50	120	300
V(V)	50	200	500

Conditions as in Fig. 2.

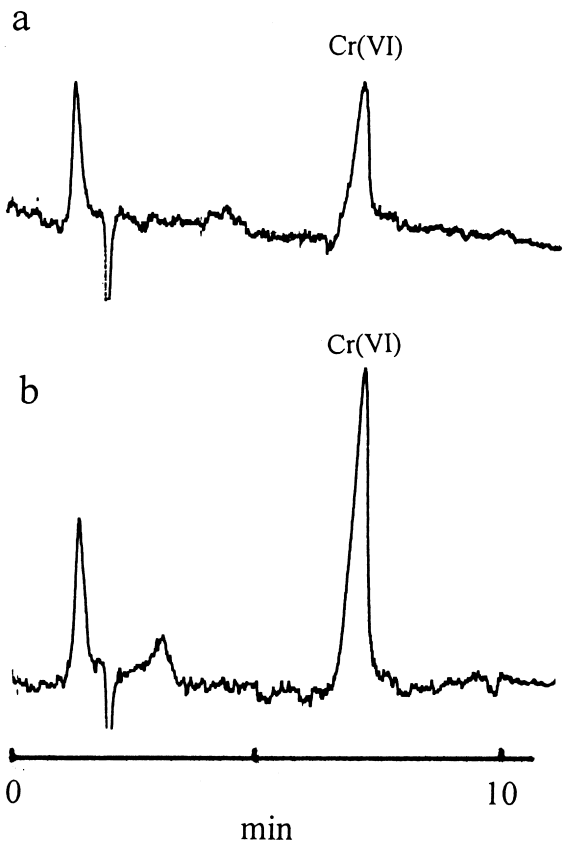

Fig. 2. Chromatograms of (a) groundwater sample and (b) groundwater sample spiked with 0.25 ng/ml Cr(VI). Sample loading rate 5 ml/min. Other conditions as in Fig. 1.

samples to study the matrix effect during the preconcentration. The results obtained are presented in Table 3. The recoveries are in the range 93–102% showing that the matrices of these samples had no remarkable effect on the preconcentration. The concentrator column could be used for a minimum of five months without any significant deterioration. It should be noted, that for more complicated river water samples the elimination of organic matter by passing the sample prior to complexation through a disposable solid-phase extraction cartridge that contains a hydrophobic stationary phase (e.g., C_{18} or polymeric) in tandem with a disposable 0.20-μm membrane filter in a single operation significantly prolongs the life of the concentrator column. The chromatograms for a groundwater sample and a groundwater sample spiked with 0.25 ng/ml of Cr(VI) are shown in Fig. 2.

The most challenging problem in the determination of Cr(VI) traces is the preservation of its oxidation state. Cr(VI) exists predominantly as $HCrO_4^-$ in acidic solution and is a strong oxidiser. It can be reduced to Cr(III) in the presence of organic matter. At pH>6.5 Cr(VI) exists predominantly as CrO_4^{2-}, which is less reactive than $HCrO_4^-$. For this reason, several water samples were analysed on the day of collection and the five days after. These samples were stored at 4°C without pH adjustment. The results of the analysis are summarised in Table 4. It can be seen that the reduction of Cr(VI) was not observable within five days in drinking water and groundwater samples but in the stored river water sample the Cr(VI) concentration was lower than the concentration originally present in the sample. The stability tests showed the importance of analyzing the river water samples as soon as possible after collection. These results are consistent with observations recently reported by Arar and Pfaff [10] and by Boussemart et al. [29].

The proposed method appears to be acceptable for the speciation of Cr(VI) traces in common water samples. Equipped with standard HPLC accessories (e.g., autosampler) this procedure can be fully automated.

Table 4
Results of the determination of Cr(VI) in water samples (n=5)

Sample	Cr(VI) (ng/ml)			
	6 h after collection	R.S.D. (%)	5 days after collection	R.S.D. (%)
Drinking water	0.12	5.2	0.11	5.0
Groundwater	0.16	4.6	0.16	4.2
River Neris at Vilnius	0.58	2.5	0.42	2.8

4. Conclusions

The described method allows the on-line preconcentration and determination of Cr(VI) selectively complexed with diphenylcarbazide from samples up to 100 ml in reversed-phase HPLC. Most of the common cations and anions had no effect on Cr(VI) enrichment. This on-line preconcentration technique was applied successfully to the determination of Cr(VI) in a water samples and the recoveries of added chromium were in the range 94–104%.

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