Talanta 99 (2012) 83–90

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Preparation of Cr(VI) and Cr(III) isotopic spike solutions from ^{50}Cr and ^{53}Cr enriched oxides without the use of oxidizing and/or reducing agents

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ARTICLE INFO

Article history:
Received 16 March 2012
Received in revised form
1 May 2012
Accepted 3 May 2012
Available online 19 May 2012

Keywords:
^{50}Cr and ^{53}Cr enriched oxides
Isotopic spike solutions of $^{50}Cr(VI)$ and $^{53}Cr(III)$
Alkaline melting
Microwave assisted digestion
Anion-exchange fast protein liquid chromatography
Inductively coupled plasma mass Spectrometry

ABSTRACT

The use of enriched stable isotopes as tracers in speciation procedures by ion-exchange chromatography coupled to ICP-MS enables to follow the oxidation–reduction processes of Cr. The most commonly available Cr stable isotopes are ^{50}Cr and ^{53}Cr enriched oxides or metallic Cr. For application of Cr enriched stable isotopes, adequate preparation of isotopic spike solutions is necessary. To ensure that Cr species present in the sample investigated are not compromised, no excess of the reducing neither oxidizing agents should remain in the isotopic spike solutions. Cr(VI) isotopic solutions are mostly prepared by dissolving of Cr oxide in $HClO_4$, followed by the addition of ammonia and H_2O_2 to quantitatively oxidize Cr, while the excess of H_2O_2 is removed by boiling or UV irradiation. If traces of H_2O_2 still remains, such isotopic spike solution may cause artefacts in Cr speciation in the sample investigated. In the present work, new procedure based on alkaline melting of ^{50}Cr enriched oxide for preparation of pure $^{50}Cr(VI)$ spike solution was developed. Cr(III) was quantitatively oxidized to Cr(VI) with air oxygen without use of other oxidizing agents. Moreover, the microwave assisted digestion procedure of ^{53}Cr enriched oxide was applied for preparation of $^{53}Cr(III)$ spike solution without use of reducing agents. The purity of $^{50}Cr(VI)$ and $^{53}Cr(III)$ isotopic spike solutions was verified by the speciation analysis applying hyphenation of anion-exchange FPLC to ICP-MS. Speciation analysis demonstrated suitability of the proposed procedures for preparation of Cr isotopic spike solutions. In addition, the artefacts in Cr speciation, which may be initiated by traces of oxidizing and/or reducing agents present in Cr spike solutions, were demonstrated. The outcomes of our investigation highlighted the importance of the adequate preparation of spike solutions of Cr isotopes that may be used as reliable tracers in the investigations of the oxidation–reduction processes of Cr in wide range of environmentally relevant pH values.

1. Introduction

Precise isotope ratio measurement by ICP-MS enables quantification of trace elements in environmental and biological samples. In combination with chromatographic procedures, species transformation may be followed in different environmental compartments and also during the analytical procedures [1,2]. Chromium (Cr) and Cr chemicals are widely used in different industrial applications, so Cr is frequently present as a pollutant in the terrestrial and aquatic environments. In the environment the most stable and the most abundant are trivalent Cr compounds, while the hexavalent Cr compounds are mainly present as a consequence of industrial activities [3]. Cr has been identified both as an essential micronutrient and as a toxic element. The essentiality and toxicity of Cr depend primarily on its chemical forms [4]. Cr(III) compounds are necessary for glucose metabolism and helps in maintaining normal cholesterol and fat levels. Cr(VI) is carcinogenic, mutagenic and inducer of skin dermatitis. Therefore, the accurate determination of Cr(III) and in particular highly toxic Cr(VI) in different environmental samples is of crucial importance for environmental protection. Enriched stable isotopes used as tracers in speciation analysis by hyphenating ion-exchange chromatography to ICP-MS make possible to follow the oxidation–reduction processes of Cr in the environment, the efficiency of remediation procedures and to control species conversion during the analytical procedures [5–13]. Cr has four stable isotopes: ^{50}Cr (4.35%), ^{52}Cr (83.8%), ^{53}Cr (9.50%) and ^{54}Cr (2.37%) [14]. In speciation analysis enriched ^{50}Cr and ^{53}Cr isotopes are frequently used as tracers. These two Cr enriched isotopes are the most commonly available as oxides and also as metallic Cr. When Cr enriched stable isotopes are used, it is necessary to prepare

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http://dx.doi.org/10.1016/j.talanta.2012.05.022

appropriate isotopic spike solutions. In order to assure that Cr speciation in the sample investigated is not disturbed, no excess of the oxidizing or reducing agents should remain in the isotopic spike solutions. Several procedures have been proposed for the preparation of Cr isotopic spike solutions. Nusko and Heumann [15] prepared spike solutions of Cr(III) and Cr(VI) by dissolving enriched metallic ^{53}Cr in HCl. Part of the ^{53}Cr(III) solution obtained by this dissolution process was oxidized in a NH_3/H_2O_2 mixture to obtain ^{53}Cr(VI). An excess of H_2O_2 was then catalytically decomposed by action of Pt wire and boiling. These spike solutions were used for speciation of Cr(III) and Cr(VI) in aerosol particles after alkaline leaching of samples collected on filters, followed by extractive separation of Cr(III) and Cr(VI) and thermal ionization isotope dilution mass spectrometry determination of the separated Cr species. United States Environmental Protection Agency (US EPA) issued method 6800 in which preparation of Cr enriched isotopic spike solutions is prescribed [16]. For preparation of ^{53}Cr(VI) isotopic standard solution ^{53}Cr enriched oxide is dissolved in hot $HClO_4$, followed by the addition of HN_4OH and 50 μL of H_2O_2 to quantitatively oxidize Cr, while the excess of H_2O_2 is removed by boiling for at least 15 min. When enriched metallic ^{53}Cr is available, it is recommended to dissolve Cr metal in 6 M HCl, followed by addition of HN_4OH and H_2O_2 as described previously. For preparation of ^{50}Cr(III) isotopic standard solution ^{50}Cr enriched metal is digested with 6 M HCl, evaporated to approximately 1 mL and diluted with 1% HNO_3. These Cr enriched isotopic spike solutions were applied in quantification of Cr species by speciated isotope dilution inductively coupled plasma mass spectrometry (SIDICP-MS) in a variety of sample matrices. The preparation of enriched spike Cr solutions based on the US EPA method 6800 [16] was used with slight modifications in different applications [17–19]. Kingston et al. [17] demonstrated the ability of SIDMS in compensating for species transformation during sampling, storage, sample preparation and speciated measurement of Cr(VI) and Cr(III) in various water samples. Yang et al. [18] applied double-spike isotope dilution for the accurate determination of Cr(III), Cr(VI) and total Cr in yeast. Ma and Tanner [19] reported speciated isotope dilution analysis of Cr(III) and Cr(VI) in water samples by ICP-DRC-MS. Authors followed the US EPA procedure for the preparation of isotopic standard solution of ^{50}Cr(III) from ^{50}Cr enriched metal and ^{53}Cr(VI) isotopic standard solution from ^{53}Cr enriched oxide, but they added much higher quantity (42.9 mL) of H_2O_2. This excessive H_2O_2 was then removed by boiling for 30 min. Tirez et al. [20] determined hexavalent Cr by species specific isotope dilution mass spectrometry in alkaline digests of packing materials. For preparation of ^{53}Cr(VI) isotopic spike solution Cr enriched oxide was digested with hot $HClO_4$. The same procedure of digestion in $HClO_4$ was applied in preparation of ^{50}Cr(III). After cooling the formed ^{50}Cr(VI) was quantitatively reduced to ^{50}Cr(III) by the addition of 2 mL of H_2O_2. When the latter spike was added to the sample of alkaline digest, significant oxidation of ^{50}Cr(III) was observed, since under alkaline conditions, the oxidation took place by the remaining H_2O_2. To overcome this problem, the excessive H_2O_2 was decomposed by UV irradiation.

By the use of the above reported procedures, there is a risk that in the isotopic spike solutions the excess of H_2O_2 still remains. Such spike solutions may cause artefacts in Cr speciation in the sample investigated. These artefacts may be pronounced especially in acidic samples like extracts of acid soils [21] or under alkaline conditions as for instance airborne chromium in alkaline extracts [22], alkaline extracts of packing materials [20], cement extracts [23], alkaline extracts of chromium corrosion protection coatings [24] and many other samples. Therefore, the aim of the present work was to develop reliable procedure for quantitative preparation of ^{50}Cr(VI) and ^{53}Cr(III) spike solutions without use of oxidizing and/or reducing agents that could remain in spiking media. For this purpose alkaline melting of ^{50}Cr enriched oxide was performed to prepare ^{50}Cr(VI) and microwave assisted digestion of ^{53}Cr enriched oxide was used to prepare ^{53}Cr(III) spike solutions. The purity of ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions was checked by the speciation analysis using anion-exchange FPLC coupled to ICP-MS. Investigation was also performed on artefacts in Cr speciation, which may be initiated by the traces of oxidizing and/or reducing agents present in Cr spike solutions.

2. Experimental

2.1. Instruments

HPLC separations were performed by using an Agilent (Tokyo, Japan) series 1200 quaternary pump equipped with a sample injection valve, Rheodyne, model 7725i (Cotati, Ca, USA) fitted with 0.5 mL injection loop. For separation of Cr species a strong anion-exchange FPLC column of Mono Q HR 5/5 (Pharmacia, Uppsala, Sweden) (column dimensions 5 × 50 mm, matrix polystyrene/divenyl benzene, pH stability 2–12, particle size 10 μm) was used. Detection of Cr species after chromatographic separation was performed using an inductively coupled plasma mass spectrometer, model 7700 ×, from Agilent Technologies (Tokyo, Japan). The outlet of the chromatographic column was directly connected to the Miramist nebulizer and a Scott-type spray chamber of ICP-MS instrument. A nickel sampler and skimmer with 1.0 and 0.4 mm cone orifices, respectively, were used. To control the stability of the ICP-MS the eluent was spiked (post column addition) with internal standards of 100 ng mL^{-1} Ge and Sc. Treatment of data was performed with the Agilent MassHunter software. Data processing was based on the peak area. Experimental working conditions for ICP-MS (summarized in Table 1) were optimized for plasma robustness and adequate sensitivity using High Matrix Introduction (HMI) system. HMI allows introducing low amounts of salts that were used in the separation procedure.

A CEM Corporation (Matthews, NC, USA) CEM MARS 5 Microwave Acceleration Reaction System was used for digestion of enriched $^{53}Cr_2O_3$.

A WTW (Weilheim, Germany) 330 pH meter was employed to determine the pH.

Analytical balance, Mettler AE 163 (Zürich, Switzerland), was used for all weighting.

Table 1
ICP-MS operating parameters.

Parameter	Value
Plasma conditions	
Forward power	1550 W
Plasma gas flow	15.0 L min^{-1}
Carrier gas flow	0.25 L min^{-1}
Dilution gas flow	0.92 L min^{-1}
He gas flow	10 mL min^{-1}
QP bias	−97 V
Oct bias	−100 V
Cell entrance	−130 V
Cell exit	−150 V
Deflect	−80 V
Plate bias	−150 V
Nebulizer type	Miramist
Sample uptake rate	1.5 mL min^{-1}
Data acquisition parameters	
m/*z* isotopes monitored in Cr speciation	^{50}Cr, ^{52}Cr,^{53}Cr
m/*z* isotopes of internal standards	^{45}Sc, ^{72}Ge
Total acquisition time	599 s

2.2. Reagents and materials

Merck (Darmstadt, Germany) suprapur nitric acid and Milli-Q water (Direct-Q 5 Ultrapure water system, Millipore Watertown, MA, USA) were used for the preparation of samples. Merck ultrapure hydrochloric acid was used for sample preparation and to adjust the pH of samples investigated. For pH adjustment in neutral and alkaline pH range, Merck suprapur sodium hydroxide monohydrate and suprapur sodium carbonate were applied. Enriched ^{50}Cr and ^{53}Cr isotopes as Cr_2O_3 were obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA) and were used for the preparation of $^{50}Cr(VI)$ and $^{53}Cr(III)$ isotopic spike solutions. The declared composition of enriched ^{50}Cr isotope was $96.82 \pm 0.05\%$ for isotope 50, $2.95 \pm 0.02\%$ for isotope 52, $0.18 \pm 0.01\%$ for isotope 53 and $0.05 \pm 0.01\%$ for isotope 54. The declared composition of enriched ^{53}Cr isotope was $0.03 \pm 0.005\%$ for isotope 50, $2.65 \pm 0.02\%$ for isotope 52, $97.20 \pm 0.02\%$ for isotope 53 and $0.12 \pm 0.005\%$ for isotope 54, respectively. To control the stability of the ICP-MS, Ge (1000 mg/L in water) and Sc (1000 mg/L in 5% nitric acid), both purchased from Merck were used. Sodium chloride of suprapur quality used in FPLC separations was also obtained from Merck.

2.3. Cleaning procedures

For the experiments Teflon laboratory ware was used. All laboratory ware and tubes for chromatographic separations and ICP determinations were treated with 10% nitric acid for 48 h, rinsed well with MilliQ water and dried at room temperature. Cleaning of the FPLC Mono Q column was performed after each set of experiments when pH of the samples investigated was changed. For cleaning 0.5 mL of 1 mol L^{-1} sodium hydroxide was injected onto the column resin and the chromatographic procedure applied. The cleaning procedure was repeated twice. Before each new series of the experiments two blank samples were first injected. To avoid blank arising from the stainless steel needle, reverse injection of

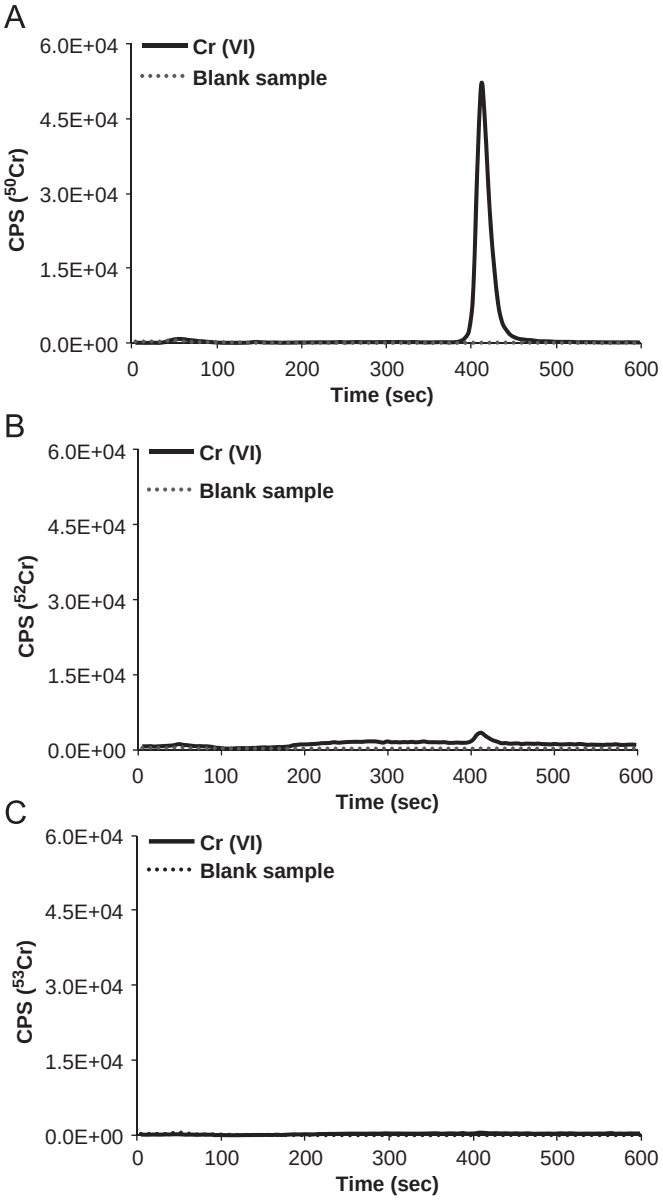

Fig. 1. Separation of $^{50}Cr(VI)$ isotopic spike solutions at pH 4 applying FPLC-ICP-MS procedure (10 ng mL^{-1} $^{50}Cr(VI)$). Chromatograms were recorded at m/z (A) 50, (B) 52 and (C) 53.

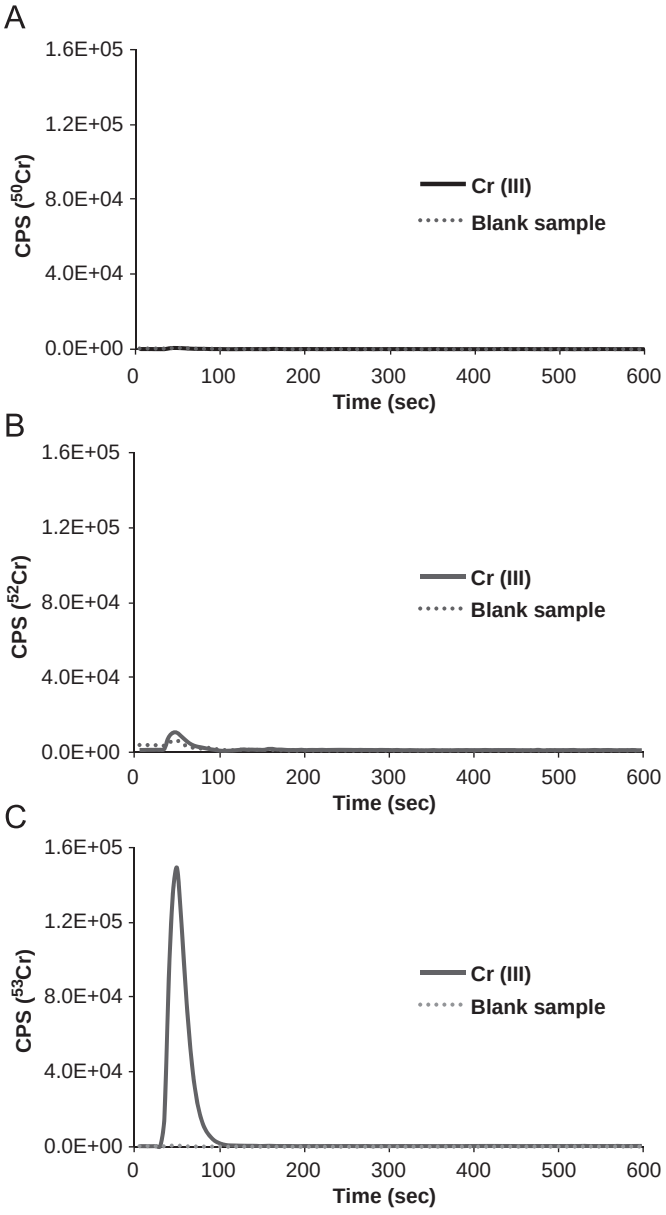

Fig. 2. Separation of $^{53}Cr(III)$ isotopic spike solutions at pH 4 applying FPLC-ICP-MS procedure (20 ng mL^{-1} $^{53}Cr(III)$). Chromatograms were recorded at m/z (A) 50, (B) 52 and (C) 53.

samples was applied (sample was pumped in opposite direction through the plastic tube of the waste exit from the injector).

2.4. FPLC mono Q procedure

In the present study the speciation procedure that was previously developed and validated in our group was applied [22–25]. The stability and robustness of the chromatographic column in the pH range 2–12 enabled analysis of acidic (pH 4), neutral (pH 7) and highly alkaline (pH 12) [25] samples with reproducible and quantitative elution of Cr(VI). 0.5 mL of sample was injected onto the column. Linear gradient elution from 100% water to 100% 0.7 mol L^{-1} NaCl was applied for 10 min at a flow rate of 1.5 mL min^{-1}. The eluate from the column was connected on-line to ICP-MS. After separation the column was regenerated with 2 mol L^{-1} NaCl for 3 min and in next 7 min equilibrated with water. The eluents from the regeneration and equilibration of the column were directed to waste. By the use of this procedure Cr(VI) was eluted from 400 to 460 s. The behavior of Cr(III) on the column was investigated in our previous work [22–25]. The elution of Cr(III) species depended significantly on the pH of sample. It was experimentally proven that at pH below 4, Cr^{3+} was quantitatively eluted with the solvent front. At pH 7.0 to 8.0 Cr that corresponded to hydroxo-Cr(III) species was strongly adsorbed on the column resin and did not disturb the following separations. At pH higher than 12 Cr(III) is partially eluted as negatively charged species from 200 to 250 s due to formation of $Cr(OH)_4^-$. This peak is well separated from Cr(VI). If not stated otherwise, all the analyses were done in two replicates.

2.5. Preparation of $^{50}Cr(VI)$ isotopic spike solution from enriched Cr_2O_3

0.0029 g of Cr_2O_3 (^{50}Cr enriched isotope) was transferred into a platinum beaker. After that 0.4 g of $NaKCO_3$ and 0.1 g of NaOH were added and the contents melted by the use of Bunsen burner until the yellow-orange melt was obtained. During the melting procedure quantitative oxidation of Cr by air oxygen was achieved only in alkaline media, so the addition of NaOH was mandatory. The melt was cooled to a room temperature and 1 mL of concentrated HCl was carefully added to dissolve the deposit. The clear solution was transferred into Teflon tube and diluted to 10 mL with MilliQ water. The concentration of Cr in stock isotopic spike solution was determined with reverse IDICP-MS and was found to be 197.2 ± 0.8 μg mL^{-1}.

2.6. Preparation of $^{53}Cr(III)$ isotopic spike solution from enriched Cr_2O_3

0.0029 g of Cr_2O_3 (^{53}Cr enriched isotope) was transferred into a Teflon vessel and 4 mL of concentrated HNO_3 was added. The Teflon vessel was subjected to closed vessel microwave assisted digestion performed at maximal power of 1200 W: ramp to temperature 20 min, 190 °C, pressure 10 bar, holding 20 min, cooling 20 min. Clear solution was quantitatively transferred into a platinum beaker and the contents carefully evaporated to approximately 0.2 mL. Then 1 mL of concentrated HCl was added, solution transferred into Teflon tube and diluted to 10 mL with MilliQ water. The concentration of Cr in stock isotopic spike solution was determined with reverse IDICP-MS and was found to be 200.3 ± 0.9 μg mL^{-1}.

2.7. Preparation of working solutions of isotopic standards

Fresh working standard solutions were prepared daily within the pH range from 4 to 12 by dilution of stock isotopic spike solutions. First appropriate dilution with water was applied to obtain $^{50}Cr(VI)$ and $^{53}Cr(III)$ standards with concentration 100 ng Cr mL^{-1}. From these solutions working standards in concentration 10 ng $^{50}Cr(VI)$ mL^{-1} and 20 ng $^{53}Cr(III)$ mL^{-1} were prepared at pH 4, 7 and 12. For this purpose 1 or 2 mL of isotopic spike standard (100 ng Cr mL^{-1}) was added to 10 mL flask and diluted to mark with 0.0006 mol L^{-1} HCl to obtain pH 4, mixture of 0.0016% NaOH + 0.0024% Na_2CO_3 to obtain pH 7 and mixture of 0.2% NaOH + 0.3% Na_2CO_3 to obtain pH 12, respectively.

In order to study the influence of residual concentrations of the reducing and/or oxidizing agents in spike solutions on Cr speciation at pH 4, 7 and 12, 1 mL of 5 μg mL^{-1} of ascorbic acid or 0.1 mL of 0.044 mol L^{-1} of H_2O_2 were added to isotopic standard solutions before final dilution to 10 mL. The concentration of ascorbic acid that represented the excessive amount of the reducing agent was 1000 times lower than that normally used for reduction of Cr(VI) [26]. The concentration of H_2O_2 represented the remaining H_2O_2 that would endure in solution if only 0.01% of added H_2O_2 [19] would not be removed after oxidation procedure in preparation of Cr(VI) enriched spike.

3. Results and discussion

3.1. Purity and stability of $^{50}Cr(VI)$ and $^{53}Cr(III)$ isotopic spike solutions

The purity of $^{50}Cr(VI)$ and $^{53}Cr(III)$ isotopic spike solutions, prepared as described in the Experimental section was verified by the speciation analysis applying anion-exchange FPLC-ICP-MS procedure at pH 4. The chromatograms along with the corresponding blank samples are presented in Figs. 1 and 2. It is evident from Fig. 1A

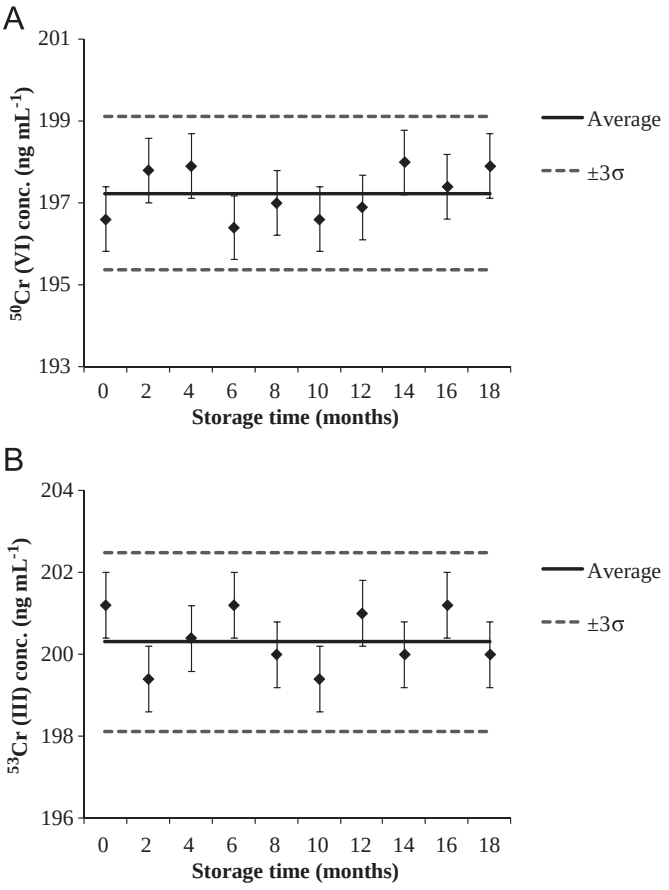

Fig. 3. Stability of A: $^{50}Cr(VI)$ and B: $^{53}Cr(III)$ isotopic spike solutions prepared from enriched Cr_2O_3 without the use of oxidizing and/or reducing agents in a time span of 18 months.

that ^{50}Cr(VI) is quantitatively eluted from 400 to 460 s. Since ^{50}Cr(III) is not detected in chromatogram at m/z 50, this indicates that no reduction of ^{50}Cr(VI) appeared at pH 4, confirming the adequate preparation of ^{50}Cr(VI) isotopic spike solution. Chromatogram at m/z 52 (Fig. 1B) indicates the presence of trace amounts of ^{52}Cr(VI) arising from enriched oxide (purchased ^{50}Cr enriched oxide contained $2.95 \pm 0.02\%$ of isotope 52), while no detectable amounts of Cr are observed in chromatogram at m/z 53 (Fig. 1C). Elution profiles for ^{53}Cr(III) are presented in Fig. 2. In aqueous solutions at pH 4 Cr(III) exists as $[Cr(H_2O)_6]^{3+}$ species which is not retained by the anion-exchange resin. Data from Fig. 2C indicates that ^{53}Cr(III) is quantitatively eluted with a solvent front. No detectable amounts of ^{53}Cr(VI) are observed confirming the adequate preparation of ^{53}Cr(III) isotopic spike solution. Chromatogram at m/z 52 (Fig. 2B) indicates the presence of trace amounts of ^{52}Cr(III) arising from enriched oxide (purchased ^{53}Cr enriched oxide contained $2.65 \pm 0.02\%$ of isotope 52), while no detectable amounts of Cr are observed in chromatogram at m/z 50 (Fig. 2C). Stability of prepared stock ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions was followed by speciation analysis in a time span of 18 months. The concentration of Cr in stock isotopic spike solutions were determined with reverse IDICP-MS. Stability control charts are presented in Fig. 3. As evident from Fig. 3, ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions were stable during the course of the experiment. Experimental data also verified that when the mixture of ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions was prepared under the proposed protocol, at pH 4, the only eluting species at m/z 50 was ^{50}Cr(VI) and at m/z 53 ^{53}Cr(III).

3.2. The influence of trace amounts of reducing and/or oxidizing agents on speciation of Cr in acidic, neutral and alkaline pH

Reported procedures for preparation of Cr(VI) isotopic spike solutions propose the use of H_2O_2 in alkaline media for complete oxidation of Cr(III) [15–19] followed by the removal of the excess of H_2O_2 by boiling [15–19]. These Cr isotopic solutions were used as spikes in samples of neutral pH. Tirez et al. [20] prepared Cr(III) enriched spike by reduction of Cr(VI) with H_2O_2 in strongly acid media. When this spike was added to the sample of alkaline digest, significant oxidation of enriched Cr(III) was observed, since under alkaline conditions, the oxidation took place by the remaining H_2O_2. To overcome this problem, the excessive H_2O_2 was decomposed by UV light. The observations of Tirez et al. [20] demonstrated that residual concentrations of oxidizing agent in enriched spike solution may cause artefacts in Cr speciation in the sample investigated.

Therefore, in the present study detail investigation of impacts of trace amounts of residual reducing and/or oxidizing agents in enriched spike solutions on Cr speciation was performed in wide pH range, typical for environmental samples. For this purpose ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions with addition of ascorbic acid or H_2O_2 were prepared at pH 4, 7 and 12 and the FPLC-ICP-MS speciation procedure applied. The results of these investigations are presented in Figs. 4 and 5. Data of Fig. 4A show quantitative elution of ^{50}Cr(VI) at pH 4, 7 and 12. At pH 12 sharper chromatographic peak is observed due to higher ionic strength of

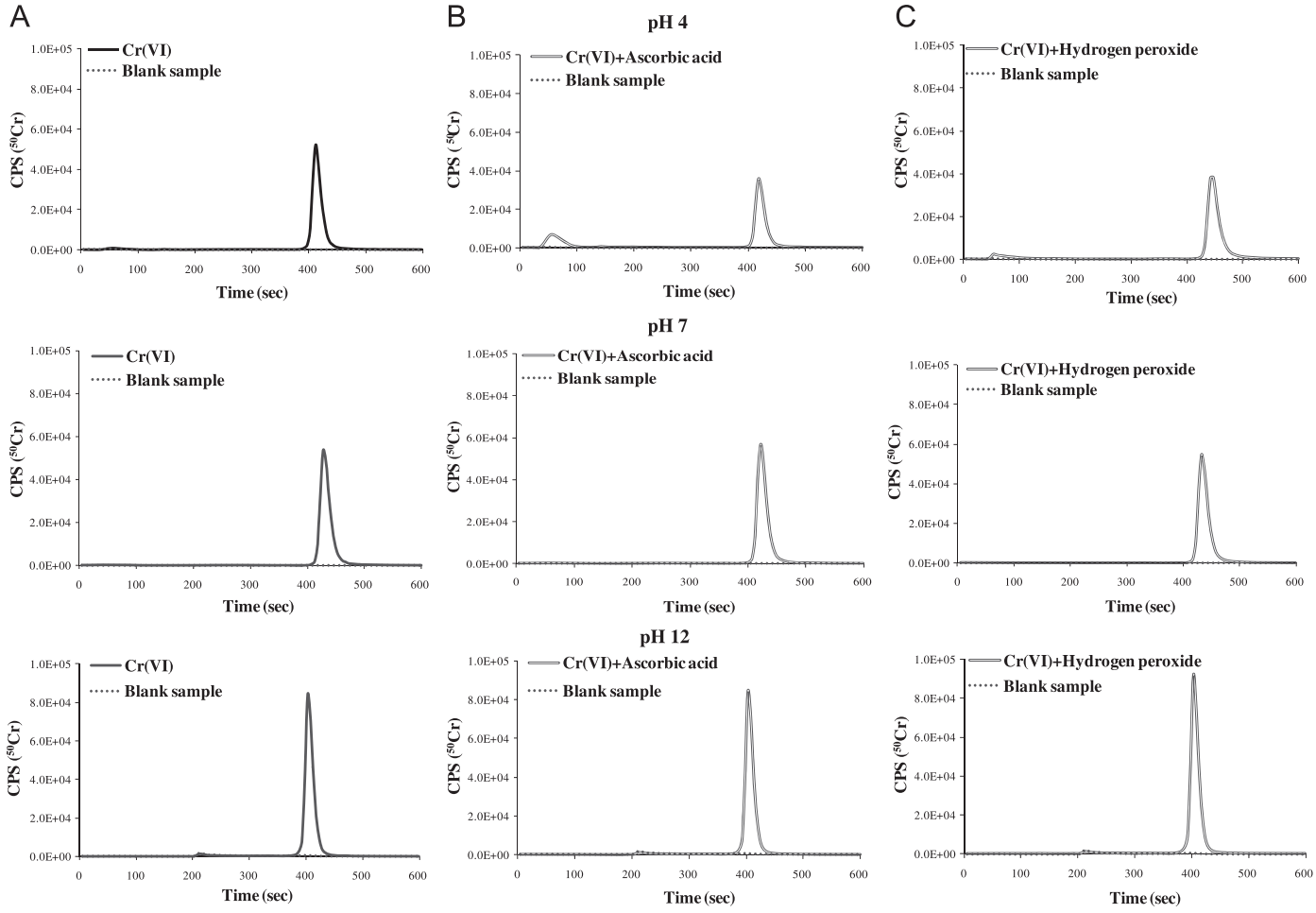

Fig. 4. Behavior of ^{50}Cr(VI) isotopic spike solution (A) (10 ng mL^{-1} ^{50}Cr(VI)), (B) (10 ng mL^{-1} ^{50}Cr(VI)+ascorbic acid) and (C) (10 ng mL^{-1} ^{50}Cr(VI)+hydrogen peroxide) at pH 4, pH 7 and pH 12. Chromatograms were recorded at m/z 50 applying FPLC-ICP-MS procedure.

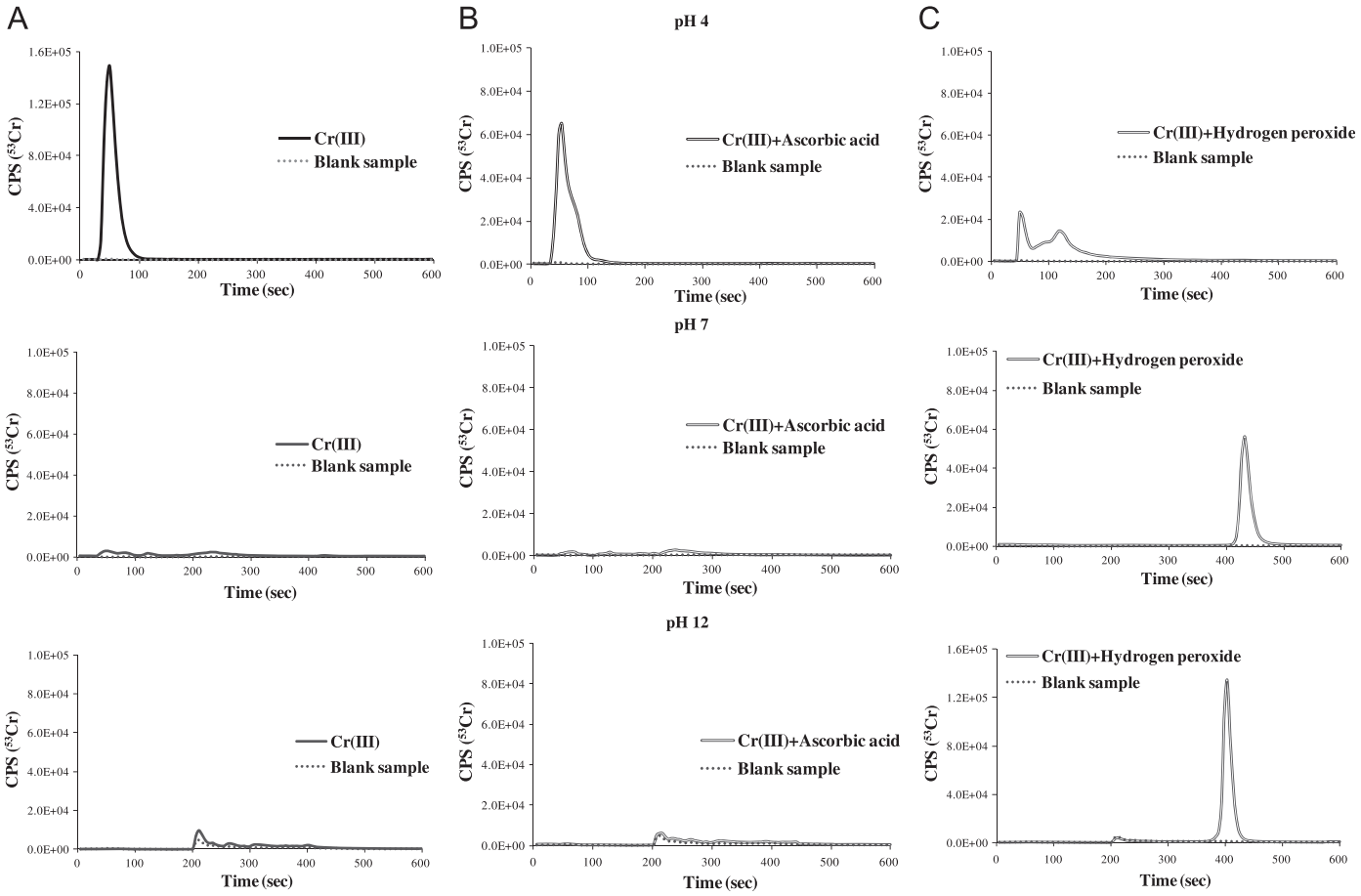

Fig. 5. Behavior of ^{53}Cr(III) isotopic spike solution (A) (20 ng mL^{-1} ^{53}Cr(III)), (B) (20 ng mL^{-1} ^{53}Cr(III)+ascorbic acid) and (C) (20 ng mL^{-1} ^{53}Cr(III)+hydrogen peroxide) at pH 4, pH 7 and pH 12. Chromatograms were recorded at *m*/*z* 53 applying FPLC-ICP-MS.

sample (^{50}Cr(VI) spike solution was prepared in 0.2% NaOH+0.3% Na_2CO_3 buffer). When ascorbic acid was added (Fig. 4B) about 15% of ^{50}Cr(VI) was reduced at pH 4 and the formed ^{50}Cr(III) was eluted with a solvent front, while at pH 7 and 12 addition of ascorbic acid did not influence ^{50}Cr(VI) speciation. Addition of H_2O_2 (Fig. 4C) resulted at pH 4 in slight reduction of ^{50}Cr(VI) for about 3%, whereas at pH 7 and 12 addition of H_2O_2 did not influence ^{50}Cr(VI) speciation. The percentage of ^{50}Cr(VI) reduction was calculated based on the ratio between the determined concentration of ^{50}Cr(VI) after the addition of ascorbic acid or H_2O_2, and added ^{50}Cr(VI) concentration. The above experiments confirmed that if spike solutions contain traces of reducing agents these may influence Cr(VI) speciation in the sample investigated at acidic pH values. Therefore, the use of reducing agents should be avoided in preparation of Cr enriched isotopic spike solutions that are used as tracers in samples with acidic pH.

The behavior of ^{53}Cr(III) alone or in the presence of ascorbic acid or H_2O_2 in pH range from 4 to 12 on FPLC column is shown in Fig. 5. Data of Fig. 5A show that at pH 4 ^{53}Cr(III) is quantitatively eluted with a solvent front, while at pH 7 Cr(III), being present mainly as $Cr(OH)_3$ precipitate is strongly adsorbed on the column resin. At pH 12 $Cr(OH)_3$ is partially transformed into the readily soluble $Cr(OH)_4^-$ species [27]. A small peak that corresponds to $^{53}Cr(OH)_4^-$ is eluted from 200 to 250 s, whereas the remaining ^{53}Cr(III) (present as $Cr(OH)_3$) is adsorbed. The addition of ascorbic acid (Fig. 5B) caused broadening of ^{53}Cr(III) peak at pH 4, and does not influence Cr(III) speciation at pH 7 and 12. When H_2O_2 is added to ^{53}Cr(III) solution (Fig. 5C), wide broadening of ^{53}Cr(III) peak is observed at pH 4. At pH 7 about 50% of ^{53}Cr(III) is oxidized, resulting in formation of ^{53}Cr(VI). At pH 12 almost 80% of ^{53}Cr(VI) is formed from ^{53}Cr(III). The percentage of ^{53}Cr(III) oxidation was calculated based on the ratio between the determined concentration of ^{53}Cr(VI) that was formed after the addition of H_2O_2, and added ^{53}Cr(III) concentration. These experiments clearly demonstrated that if spike solutions contain even trace amounts of H_2O_2, this oxidizing agent evidently provokes oxidation of Cr(III) present in the sample investigated in neutral and particularly in alkaline pH ranges. Thus, the use of H_2O_2 should be avoided in preparation of Cr enriched isotopic spike solutions that are used as tracers. Although the procedures reported in the literature that use H_2O_2 for complete oxidation of Cr(III) in preparation of Cr(VI) enriched spike solutions, or the use of H_2O_2 as reducent in acidic pH for preparation of Cr(III) enriched spike solutions, recommend its removal by boiling or UV irradiation [15–20], the risk still exists that in the isotopic spike solutions traces of residual H_2O_2 remains. Such spike solutions may cause artefacts in Cr speciation in the sample investigated.

3.3. The applicability of ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions in investigations of species interconversion during the extraction procedure

^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions prepared without use of oxidizing and/or reducing agents were applied in the development of the analytical procedure for the determination of Cr(VI) in various corrosion protection coatings (work under investigation). For this purpose ultrasonic alkaline extraction procedure was optimized. ^{50}Cr(VI) and ^{53}Cr(III) isotopic spikes

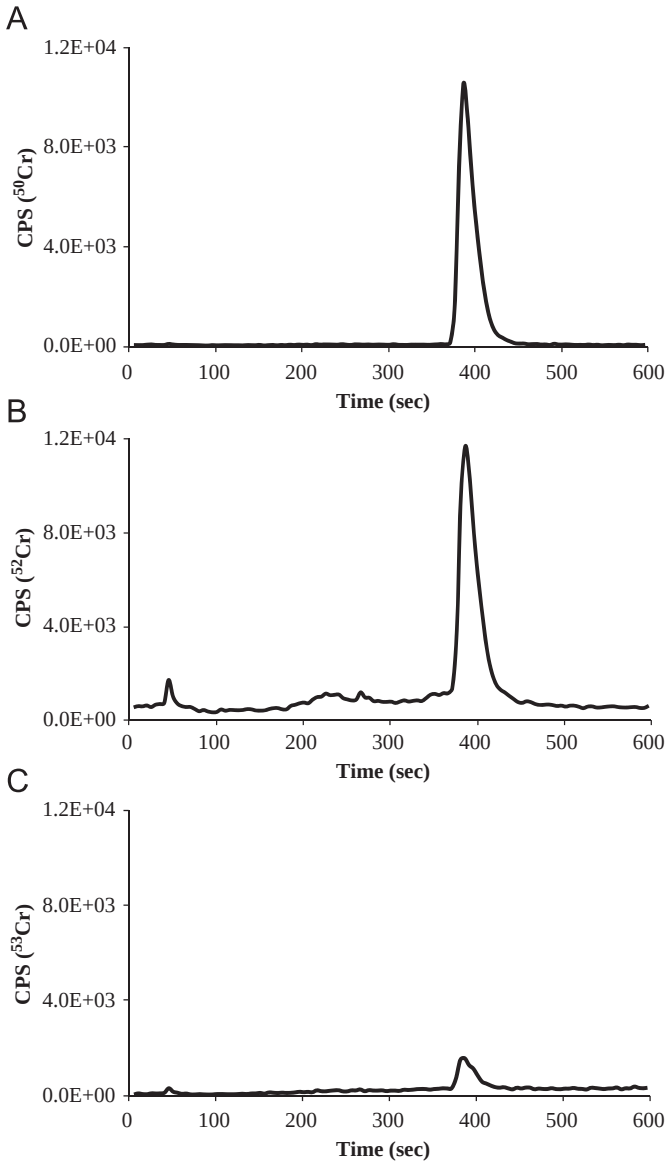

Fig. 6. Ultrasonic extraction (480 W, 70 °C, 30 min) of Cr(VI) from 10 μm hard chrome coating on copper electroplated metallic plate, using 2% NaOH+3% Na_2CO_3+0.1 mol L^{-1} $MgCl_2$ as extracting solution. Chromatograms of a doubly spiked sample (20 ng mL^{-1} ^{50}Cr(VI) and 20 ng mL^{-1} ^{53}Cr(III)) recorded at *m*/*z* (A) 50, (B) 52 and (C) 53.

were added into the extracting solution along with the sample (10 μm hard chrome coating on copper electroplated metallic plate) to follow species interconversion during the extraction. An example of chromatograms of a doubly spiked sample (20 ng mL^{-1} ^{50}Cr(VI) and 20 ng mL^{-1} ^{53}Cr(III)) obtained after ultrasonic extraction (480 W, 70 °C, 30 min), using 2% NaOH+3% Na_2CO_3+0.1 mol L^{-1} $MgCl_2$ as extracting solution, is presented in Fig. 6. The concentration of ^{50}Cr(VI) added and determined after the extraction remained the same (Fig. 6A). In calculation of the ^{50}Cr(VI) concentration after the extraction, the contribution of natural abundance ^{50}Cr(VI) content extracted from corrosion protection coating was considered. These findings confirmed that ^{50}Cr(VI) was not reduced during the extraction. It is further evident that small peak of Cr(VI) appeared at *m*/*z* 53 (Fig. 6C). Based on calculation of ^{53}Cr(VI) concentration it was proved that ^{53}Cr(VI) signal corresponds solely to natural abundance ^{53}Cr(VI) extracted from coating. These results confirmed that no ^{53}Cr(III) oxidation occurred during the extraction procedure. The ^{52}Cr(VI) signal (Fig. 6B) corresponds to ^{52}Cr(VI) extracted from coating and the contribution arising from ^{50}Cr(VI) spike solution that contains 2.95% of ^{52}Cr(VI). Data from Fig. 6 revealed that under the analytical procedure applied no species interconversions occurred during the extraction of Cr(VI) from corrosion protection coating examined. Such investigations are possible only if isotopic spike solutions are used that do not contain traces of residual oxidizing and/or reducing agents. By the use of adequate Cr(VI) and Cr(III) enriched spike solutions, which do not compromise Cr speciation in the sample investigated, artefacts that lead to erroneous interpretation of data are avoided.

4. Conclusions

New procedures were developed for preparation of ^{50}Cr(VI) and ^{53}Cr(III) enriched isotopic solutions that do not influence Cr speciation in the sample investigated. The alkaline melting of enriched ^{50}Cr oxide and dissolution of melt in HCl enabled preparation of pure ^{50}Cr(VI) spike solution. Applying this procedure ^{50}Cr(III) is quantitatively oxidized by air oxygen at high temperature and highly alkaline pH, without use of other oxidizing agents. For preparation of ^{53}Cr(III) isotopic spike solutions from enriched ^{53}Cr oxide, we proposed microwave assisted digestion by the use of HNO_3. After digestion HNO_3 is carefully evaporated to approximately 200 μL and enriched Cr(III) spiking solution stabilized by HCl.

The purity of ^{50}Cr(VI) and ^{53}Cr(III) isotopic spike solutions and influence of oxidizing and/or reducing agents on Cr speciation was established by the speciation analysis using anion-exchange FPLC coupled to ICP-MS. In the present investigation we clearly demonstrated that traces of remaining H_2O_2 may influence Cr(III) speciation in samples investigated at neutral and alkaline pH values, and that residual amounts of reducing agents may influence Cr(VI) speciation under acidic pH.

The advantages of the developed procedures over commonly applied are the following: (a) simplicity and speed of preparation; (b) the use harmful $HClO_4$ for digestion of Cr oxide is avoided; (c) the use of H_2O_2 to completely oxidize Cr(VI) in alkaline media or to reduce Cr(VI) under acidic conditions is omitted; (d) the artefacts that may be initiated by the residual amounts of reducing and/or oxidizing agents in Cr enriched spiking solutions are prevented.

The results of our investigation emphasized the significance of the adequate preparation of Cr isotopic spike solutions that may be used as reliable tracers in speciation of Cr in a wide range of environmentally relevant pH values.

Acknowledgments

This work was supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia (Program group P1-0143) and Project J1-2326.

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