



## Carrier element-free coprecipitation (CEFC) method for the separation, preconcentration and speciation of chromium using an isatin derivative

Volkan Numan Bulut<sup>a</sup>, Duygu Ozdes<sup>a</sup>, Olcay Bekircan<sup>a</sup>, Ali Gundogdu<sup>a</sup>, Celal Duran<sup>a</sup>, Mustafa Soylak<sup>b,\*</sup>

<sup>a</sup> Department of Chemistry, Karadeniz Technical University, Faculty of Arts & Sciences, 61080 Trabzon, Turkey

<sup>b</sup> Department of Chemistry, Erciyes University, Faculty of Arts & Sciences, 38039 Kayseri, Turkey

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### ABSTRACT

A new, simple, rapid and sensitive separation, preconcentration and speciation procedure for chromium in environmental liquid and solid samples has been established. The present speciation procedure for Cr(III) and Cr(VI) is based on combination of carrier element-free coprecipitation (CEFC) and flame atomic absorption spectrometric (FAAS) determinations. In this method a newly synthesized organic coprecipitant, 5-chloro-3-[4-(trifluoromethoxy) phenylimino]indolin-2-one (CFMEPI), was used without adding any carrier element for coprecipitation of chromium(III). After reduction of chromium(VI) by concentrated H<sub>2</sub>SO<sub>4</sub> and ethanol, the procedure was applied for the determination of total chromium. Chromium(VI) was calculated as the difference between the amount of total chromium and chromium(III). The optimum conditions for coprecipitation and speciation processes were investigated on several commonly tested experimental parameters, such as pH of the solution, amount of coprecipitant, sample volume, etc. No considerable interference was observed from the other investigated anions and cations, which may be found in natural water samples. The preconcentration factor was found to be 40. The detection limit for chromium(III) corresponding to three times the standard deviation of the blank ( $N=10$ ) was found 0.7  $\mu\text{g L}^{-1}$ . The present procedure was successfully applied for speciation of chromium in several liquid and solid environmental samples. In order to support the accuracy of the method, the certified reference materials (CRM-TMDW-500 Drinking Water and CRM-SA-C Sandy Soil C) were analyzed, and standard APDC-MIBK liquid-liquid extraction method was performed. The results obtained were in good agreement with the certified values.

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### 1. Introduction

The contamination of environmental samples by toxic heavy metals has become a serious environmental problem that has to be controlled. The importance of heavy metal pollution control has increased significantly in recent decades [1].

Chromium is one of the major pollutants for environment. The most stable and common forms of chromium in the environment are trivalent and hexavalent species. Chromium(III) is considered to be an essential trace element for living organisms. In contrast Cr(VI) is toxic and carcinogenic for humans due to its high oxidation potential [2]. As a result, the change of the oxidation state affects the toxicity of heavy metals, so the accurate and sensitive determination of the chromium species is very important in analytical chemistry.

The direct determination of chromium species in real samples may not be possible by using spectrometric techniques including atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS). By using these techniques only total chromium could be determined. Thus, the chromium speciation procedures are generally based on a separation and preconcentration of one of the chromium species (Cr(III) or Cr(VI)). The total chromium is determined after reduction of Cr(VI) to Cr(III) or by the oxidation of Cr(III) to Cr(VI) [3]. Various preconcentration methods for separating the two chromium species such as liquid-liquid extraction [4], solid phase extraction [5,6], ion-exchange [7], cloud point extraction [8], coprecipitation [9] etc. have been developed and widely used.

Coprecipitation methods are particularly recommended for preconcentration of trace metal ions from interfering species [10] and may overcome some limitations of solvent and solid-phase extractions, the most widely used techniques, due to the different mechanism of extraction [11]. Coprecipitation is characterised by

\* Corresponding author. Tel.: +90 352 4374933; fax: +90 352 4374933.

E-mail addresses: [soylak@erciyes.edu.tr](mailto:soylak@erciyes.edu.tr), [msoylak@gmail.com](mailto:msoylak@gmail.com) (M. Soylak).

the formation of insoluble compounds, and adopted when direct precipitation cannot separate the desired metallic species due to their low concentrations in solution. Coprecipitation occurs with retention of metals at trace levels onto the precipitate surface or precipitate structures via various mechanisms [12]. The combination of coprecipitation with filtration and/or centrifugation offers a simple and rapid preconcentration technique. This is especially useful for recovery of trace metals and determination of sufficient separation factors for alkali and alkaline earth elements. The main requirement is use of an efficient collector being able to separate the trace metal ions from the matrix solution [10]. Organic or inorganic coprecipitants can be used as efficient collectors for preconcentration of trace metals by coprecipitation.

Various metal hydroxides, such as iron(III), indium, gallium, cerium(IV), lanthanum, magnesium, hafnium, scandium, and zirconium, have been widely used as inorganic coprecipitants for the preconcentration of trace metal ions from aqueous media. However, many drawbacks have arisen during the applications [10,12]: magnesium and indium themselves cause serious high-background absorption during the analysis because of their large quantities in the medium. The method using Iron(III) hydroxide cannot avoid coprecipitation of large amounts of alkaline earth metals [10].

The organic coprecipitants such as 8-hydroxyquinoline [13], methylpiperidinedithiocarbamate [14] and rubeanic acid [15], usually chosen, that are able to form neutral chelates with metallic species [12]. However large amounts of a carrier element (Cu, Bi, Ni, Co etc.) together with organic coprecipitants are necessary to be added to the medium in order to form a precipitate. Large amounts of a carrier element may cause interferences during the analysis. Until now most of the coprecipitation procedures including the use of organic or inorganic coprecipitants have been performed by using a carrier element. Some researchers have not used a carrier element in coprecipitation studies, but they have collected the trace metals on a collector after complexation with a suitable chelating agent in aqueous solution [16,17]. Only one study, similar to the CEFC method, has been encountered in literature survey. The current study, a new coprecipitation method was developed using a triazole compound to separate and preconcentrate  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Pb}^{2+}$  ions [18].

Studies of chromium speciation by coprecipitation using various reagents have been suggested by many researchers. Ueda et al. [19] have proposed a speciation method based on coprecipitation for determination of chromium(III) and chromium(VI) by graphite-furnace atomic absorption spectrometry (GF-AAS) by using hafnium hydroxide as an inorganic coprecipitant. Wang et al. [20] have also proposed a speciation method for chromium(III) and chromium(VI) in aqueous samples by coprecipitation/slurry sampling fluorination assisted GF-AAS by using ammonium pyrrolidinedithiocarbamate as an organic coprecipitant and  $\text{Pb}^{2+}$  ions as a carrier element.

We have developed a new coprecipitation method in which no carrier element has been used. The method, thus, has been called carrier element-free coprecipitation (CEFC). We have utilized a water insoluble organic compound, used as coprecipitating agent, and the trace metal ions are adsorbed on the precipitate. CEFC method has great advantages for separation, preconcentration, and speciation of heavy metals as such that there is no contamination and background adsorption risks described above for metal ions from a carrier element or a chelating agent. The present procedure based on CEFC method is also a new way for metal speciation analysis.

We have used an isatin derivative 5-chloro-3-[4-(trifluoromethoxy)phenylimino]indolin-2-one (CFMEPI) as an organic coprecipitant. Isatin (indolin-2,3-dione) derivatives are reported to show a variety of biological activities including antibacterial, antifungal,

anti-HIV [21], anticonvulsant, antidepressant [22], and antiinflammatory activities [23]. Furthermore, isatin derivatives have been used in spectrophotometric [24] and electrochemical [25] applications. Isatin derivatives have been rarely used for metal analysis. Wang et al. [26] have determined the level of trace Ni in oil samples by a kinetic spectrophotometric method using isatin. Mahmoud et al. [27] have developed a new solid phase extraction system using an isatin derivative for speciation, selective extraction and preconcentration of chromium ions.

In this study, we applied the CEFC method for the speciation of chromium. Cr(III) was coprecipitated with CFMEPI and thus separated from Cr(VI). The total chromium level was determined after converting all the Cr(VI) to Cr(III). The application is a simple and rapid method for the chromium speciation. The influences of the analytical parameters, such as the effects of pH, quantity of CFMEPI, standing time, centrifugation rate and time, etc. were investigated. The proposed method was applied to the speciation of chromium in several environmental samples.

## 2. Experimental

### 2.1. Apparatus

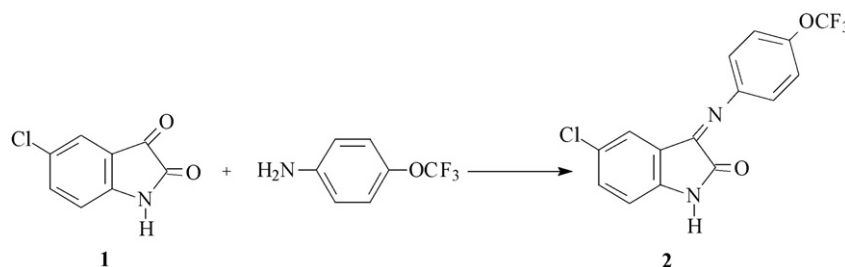
A Unicam model AA-929 Flame Atomic Absorption Spectrometer (FAAS) was used for chromium determination in solutions. All measurements were carried out in a  $\text{N}_2\text{O}$ /acetylene flame with 5 cm long burner head, and chromium hollow cathode lamps were used. The instrumental parameters were those recommended by the manufacturer. The wavelength (nm) selected for determination of chromium was 357.9 nm. MSE Mistral 2000 model centrifuge was used to centrifuge solutions. A pH meter, Hanna pH-211 digital glass electrode was employed for measuring pH values in the aqueous solution. pH meter was calibrated with standard buffer solutions. Distilled/deionized water was obtained from Sartorius Milli-Q system (arium® 611UV). Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was operated for digestion of the solid samples.

The instruments listed below were used to determine molecular features of CFMEPI: melting point was determined on Büchi oil-heated melting point apparatus and is uncorrected. IR spectra were obtained with a PerkinElmer 1600 FTIR spectrometer in KBr pellets.  $^1\text{H}$  NMR spectra was recorded on a Varian-Mercury 200 MHz spectrometer using tetramethylsilane as internal standard. The mass spectra were recorded on a MicroMass Quattro LC-MS/MS spectrometer. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. UV-vis analyses were made on Dr Lange Cadas 200 spectrophotometer.

### 2.2. Reagents and solutions

Almost all of the chemicals (some of the reagents used were ultra pure reagent grade, as  $\text{HNO}_3$ ,  $\text{HCl}$  and  $\text{HF}$ ) used in this work were analytical grade and were used without further purification. Distilled/deionized water was used for all dilutions. All glassware and plastic materials used were soaked in 10% (v/v) nitric acid solution for 1 day before use then cleaned repeatedly with distilled/deionized water. For storage of water samples prior to analysis, polypropylene bottles (5 L) were used. Working solutions of studied metals were prepared by diluting a stock solution of  $1000 \text{ mg L}^{-1}$  of the given elements supplied by Sigma and Aldrich.  $\text{HCl}$  (Merck) and  $\text{NaOH}$  (Merck) were used for pH adjustments.

Trace metal in drinking water standard (CRM-TMDW-500) and sandy soil standard (CRM-SA-C) were obtained from High-Purity Standard Inc.



**Scheme 1.** Synthesis of Schiff Bases of 5-chloroisatin (reflux time: 4 h in room temperature, solvent: ethanol, catalyst: glacial acetic acid).

### 2.3. Preparation of CFMEPI

4-(Trifluoromethoxy)-aniline was condensed with 5-chloroisatin **1** in ethanol containing a catalytic amount of acetic acid, to give the corresponding CFMEPI **2** (Scheme 1). Equimolar quantities (0.01 mol) of isatin and 4-(trifluoromethoxy)-aniline were dissolved in warm ethanol containing 1 mL of glacial acetic acid. The reaction mixture was refluxed for 4 h and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol. Yield 95%; m.p. 224 °C, IR (KBr,  $\text{cm}^{-1}$ ):  $\nu = 3255$  (NH), 1736 (C=O), 1611 (C=N), 1290 (C-F), 1205 (C-O), 1163 (C-Cl);  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ , ppm):  $\delta = 6.62$  (d, 1H, isatin-H), 6.89 (d, 2H, phenyl-H), 7.05 (d, 2H, phenyl-H), 7.31–7.35 (m, isatin-H), 12.34 (s, 1H, isatin-NH);  $\lambda_{\text{max}}$ : 420 nm (in ethanol),  $\epsilon$ :  $1255.3 \text{ L mol}^{-1} \text{ cm}^{-1}$ ; anal. calcd. for  $\text{C}_{15}\text{H}_8\text{ClF}_3\text{N}_2\text{O}_2$  (molecular weight:  $340.69 \text{ g mol}^{-1}$ ): C: 52.88, H: 2.37, N: 8.22, Found: C: 52.47, H: 2.48, N: 8.65; MS,  $m/z$  (I,%): 340.87 (100)  $[\text{M}]^+$ .

The IR spectrum of compound **2** showed absorption bands of 3255, 1736 and  $1611 \text{ cm}^{-1}$  regions resulting from the NH, C=O and C=N functions, respectively. The signal due to proton of NH was seen at  $\delta$  12.34 ppm. Protons of phenyl group appeared as two doubles at  $\delta$  6.89 and  $\delta$  7.05 ppm. Isatin protons were observed at  $\delta$  6.62 (d, 1H, isatin-H), and  $\delta$  7.31–7.35 (m, isatin-H). The mass spectrum of compound **2** showed a molecular ion peak at  $m/z$  340.87, and the molecular ion ( $\text{M}^+$ ) peak was found to be the base peak.

### 2.4. Determination of chromium(III)

The coprecipitation method was tested firstly by using model solutions, prior to application to the speciation procedure for chromium(III) and chromium(VI) based on CEFC method to environmental real samples. For that purpose, 50 mL of an aqueous solution containing  $12.5 \mu\text{g}$  of chromium(III) was placed in a centrifuge tube. The pH of the solution was adjusted to 8.0. Then 1.5 mg of CFMEPI, coprecipitating agent solution prepared in ethyl alcohol (0.5% w/v) was added into the tube. After standing 5 min, the solution was centrifuged at 3000 rpm for 20 min. The supernatant was removed. The precipitate remained adhering to the tube was dissolved with 1.0 mL of concentrated  $\text{HNO}_3$ . Final volume was completed to 5.0 mL with distilled/deionized water, and then the level of chromium(III) was determined by FAAS.

### 2.5. Determination of total chromium

After reducing chromium(VI) to chromium(III) by the addition of 0.5 mL of concentrated  $\text{H}_2\text{SO}_4$  and 0.5 mL of ethanol, total chromium was determined as chromium(III) by the method described in Section 2.4.

The given amounts of concentrated  $\text{H}_2\text{SO}_4$  and ethanol were added into a 50 mL of aqueous solution containing  $12.5 \mu\text{g}$  of chromium(III) and  $12.5 \mu\text{g}$  of chromium(VI). After cooling to room temperature, the pH of the solution was adjusted to 8.0. Then

the procedure described in Section 2.4 was applied to this solution for determination of total chromium. The concentration of chromium(VI) was calculated by subtracting the amount of chromium(III) from the total chromium content.

### 2.6. Application to real samples

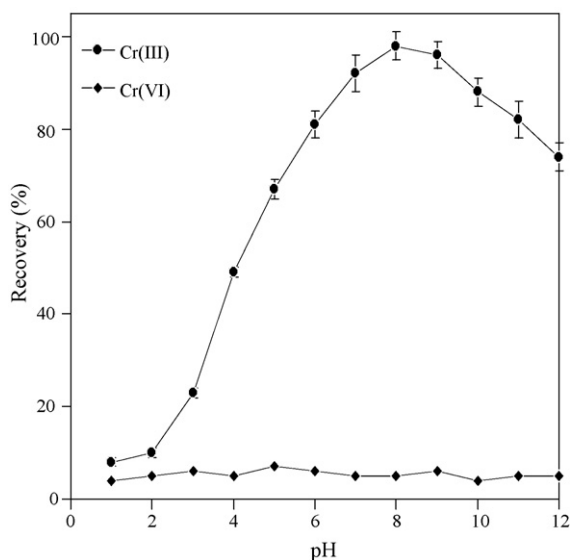
The present speciation procedure for chromium(III) and chromium(VI) based on CEFC method was applied to various environmental liquid and solid real samples. The stream water, sea water, and tannery waste-water were used as liquid real samples, and tobacco and anodic slime were used as solid real samples. The method was also applied to standard reference materials. For that purpose, CRM-TMDW-500 Drinking Water and CRM-SA-C Sandy Soil C were used. Various amounts of chromium species were also spiked to liquid real samples.

Twenty five milliliters of CRM-TMDW-500 Drinking Water were taken to determine the level of chromium(III). From the other real water samples, 200 mL of seawater from Rize, 200 mL of stream water from Of/Trabzon, and tannery waste-water samples from Bor Organized Industrial Region-Nigde (Turkey) were collected in polyethylene bottles prewashed with detergent, distilled water, diluted  $\text{HNO}_3$ , and doubly distilled water, respectively. Before the analysis, the pHs of the liquid samples were adjusted to 8.0. The appropriate amount of CFMEPI was added, and the procedure given above was applied. The final volumes of the solutions were diluted to 5 mL with distilled deionized water. The levels of chromium(III) in the samples were determined by FAAS. After reduction of chromium(VI) to chromium(III) by the addition of concentrated  $\text{H}_2\text{SO}_4$  and ethanol, total chromium in these liquid samples was determined as chromium(III).

The solid samples were digested with a closed microwave digestion system prior to the application of the present method. For that purpose, 1.0 g of tobacco samples, 0.5 g of anodic slime, and 0.1 g of CRM-SA-C Sandy Soil C were weighed with a sensitivity of 0.1 mg into Teflon vessels separately. 6 mL of  $\text{HNO}_3$ , and 2 mL of  $\text{H}_2\text{O}_2$  for tobacco sample, 4.5 mL of HCl, 1.5 mL of  $\text{HNO}_3$ , and 2 mL of HF for anodic slime, and CRM-SA-C Sandy Soil C standard were added into the vessels. Digestion conditions for the microwave system for the samples were applied as (45 bar) 6 min for 250 W, 6 min for 400 W, 6 min for 650 W, 6 min for 250 W, vent: 3 min. After microwave digestion, the volume of the sample was made up to 50 mL with distilled water. Blanks were prepared in the same way as the sample, but omitting the sample. The preconcentration procedure given above was applied to the samples. The final volume was 5 mL.

## 3. Results and discussion

CEFC method was optimized with some analytical parameters before applying it to the environmental samples. Each experiment was repeated three times and the results were presented with mean  $\pm$  standard deviation in 95% confidence intervals.



**Fig. 1.** Effect of pH on Cr(III) and Cr(VI) recoveries ( $N=3$ , sample volume: 50 mL, quantity of CFMEPI: 0.3 mL (0.5%, w/v), standing time: 5 min, centrifugation rate: 3000 rpm, centrifugation time: 20 min).

### 3.1. Effect of pH

pH plays a unique role for the quantitative recoveries of the analyte ions. Hence, the influences of pH on the retention of both chromium(III) and chromium(VI) were investigated in the pH range 1.0–12.0 by using 50 mL of a model solution containing 12.5  $\mu\text{g}$  of each chromium species (Cr(III) and Cr(VI)). Solution pHs were adjusted by using diluted NaOH and diluted HCl, and the present method was applied to these solutions. The results are given in Fig. 1. The highest recoveries for chromium(III) were obtained in the pH range 7.0–9.0. The recovery values for chromium(VI) were below 4% at all the investigated pHs. This makes it possible to separate chromium(III) from chromium(VI) at the pH range of 7.0–9.0. For the separation and speciation of chromium(III) and chromium(VI), pH 8.0 was selected in all subsequent works.

### 3.2. Effect of CFMEPI amount

The effect of CFMEPI amount on chromium(III) (12.5  $\mu\text{g}$  in a 50 mL solution) recoveries was investigated in the range of 0–2.5 mg (0–0.5 mL, 0.5%, w/v) under optimal conditions. The results are depicted in Table 1. The recoveries of chromium(III) were not quantitative (<95%) without adding CFMEPI. The recovery of chromium(III) increased with increasing the amount of CFMEPI. When 1 mg or more of CFMEPI was used, quantitative recoveries of chromium(III) were obtained, so all subsequent works were carried out with 1.5 mg of CFMEPI (0.3 mL 0.5%, w/v).

This agent provided effective and quantitative collection of Cr(III) on itself without adding any carrier element or collector for

**Table 1**

Effect of CFMEPI amount on Cr(III) recovery (mean  $\pm$  standard deviation, Cr(III) quantity: 12.5  $\mu\text{g}$   $N=3$ , sample pH: 8.0, sample volume: 50 mL, standing time: 5 min, centrifugation rate: 3000 rpm, centrifugation time: 20 min).

Quantity of CFMEPI (mg)	Cr(III) recovery (%)
0.0	78 $\pm$ 3
0.5	86 $\pm$ 2
1.0	94 $\pm$ 4
1.5	98 $\pm$ 3
2.0	96 $\pm$ 3
2.5	97 $\pm$ 2

the formation of insoluble complexes or salts, which may cause contamination and interference risk for analytes.

### 3.3. Effect of standing time and centrifugation rate and time

The standing time, centrifugation rate, and centrifugation time affect the quality of the precipitate that forms in the aqueous medium. Hence, these factors were also examined. For that purpose 1.5 mg of CFMEPI (0.3 mL, 0.5%, w/v in ethyl alcohol) were added into a series of 50 mL of solutions, containing 12.5  $\mu\text{g}$  of chromium(III). Then the standing time was investigated in the range of 0–20 min. After 5 min, quantitative recoveries were obtained, so optimum standing time was determined as 5 min for all subsequent works. This period was long enough for the coprecipitant formation and adsorption of chromium(III) on the coprecipitant.

The effect of the centrifugation rate was examined in the range of 2000–3500 rpm, under optimal conditions. The optimum centrifugation rate was determined as 3000 rpm. And the effect of centrifugation time was investigated on the recoveries of chromium(III) in the range of 10–25 min at 3000 rpm. After 20 min, quantitative recoveries were obtained, so all the other works were performed at 3000 rpm for 20 min.

### 3.4. Effect of sample volume

Sample volume is an important parameter to get high preconcentration factor in the analysis of real samples. The influence of a sample volume on the recoveries of chromium(III) was investigated in the sample volume range of 50–500 mL containing 12.5  $\mu\text{g}$  of chromium(III) by using model solutions. For the sample volumes above 50 mL, the precipitates formed in a polyethylene tube and solutions were separated from each other with centrifugation. For the sample volumes above 50 mL, the precipitates were filtered through 0.45  $\mu\text{m}$  cellulose nitrate membrane. The result showed that the recovery of chromium (III) was quantitative up to 200 mL of sample volume (Table 2). Above 200 mL, the recovery decreased with increasing the sample volume. Also any differences were not observed in terms of recoveries after the analysis performed with both centrifugation and filtration system for 50 mL sample volume. The preconcentration factor is calculated by the ratio of the highest sample volume and the lowest final volume, and it was found as 40 for chromium(III) when the final volume was 5.0 mL.

### 3.5. Effect of foreign ions

The effect of foreign ions on chromium(III) (12.5  $\mu\text{g}$  of Cr(III) in 50 mL aqueous solution) was investigated under the optimal conditions because it is an important factor to evaluate the possible analytical applications of recommended procedure. For this studies different amounts of salts and heavy metal ions were added in model solution containing fixed amount of chromium(III) and then the present procedure was applied. The recoveries of chromium(III) were higher than 95%. The results are presented in Table 3. The

**Table 2**

Effect of sample volume on the recoveries of Cr(III) (mean  $\pm$  standard deviation,  $N=3$ , sample pH: 8.0, quantity of CFMEPI: 0.3 mL (0.5%, w/v), standing time: 5 min, centrifugation rate: 3000 rpm, centrifugation time: 20 min).

Sample volume (mL)	Cr(III) recovery
50	98 $\pm$ 2
100	97 $\pm$ 1
150	98 $\pm$ 4
200	96 $\pm$ 3
250	91 $\pm$ 3
500	74 $\pm$ 2

**Table 3**

Influences of some foreign ions on the recoveries of Cr(III) (mean  $\pm$  standard deviation, Cr(III) quantity: 12.5  $\mu\text{g}$   $N=3$ , sample pH: 8.0, sample volume: 50 mL, quantity of CFMEPI: 0.3 mL (0.5%, w/v), standing time: 5 min, centrifugation rate: 3000 rpm, centrifugation time: 20 min).

Ions	Concentration (mg L <sup>-1</sup> )	Cr(III) recovery (%)
Na <sup>+</sup>	10000	96 $\pm$ 4
K <sup>+</sup>	750	95 $\pm$ 5
Ca <sup>2+</sup>	1000	96 $\pm$ 6
Mg <sup>2+</sup>	1000	94 $\pm$ 5
Cl <sup>-</sup>	15000	96 $\pm$ 5
PO <sub>4</sub> <sup>3-</sup>	500	96 $\pm$ 4
SO <sub>4</sub> <sup>2-</sup>	500	95 $\pm$ 3
NO <sub>3</sub> <sup>-</sup>	5000	94 $\pm$ 5
Al <sup>3+</sup> , Cd <sup>2+</sup> , Cu <sup>2+</sup> , Fe <sup>3+</sup> , Mn <sup>2+</sup> , Zn <sup>2+</sup> , Pb <sup>2+</sup>	25	101 $\pm$ 7
Mixed <sup>a</sup>		93 $\pm$ 5

<sup>a</sup> 10000 mg L<sup>-1</sup> Na<sup>+</sup>, 15000 mg L<sup>-1</sup> Cl<sup>-</sup>, 250 mg L<sup>-1</sup> Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, SO<sub>4</sub><sup>2-</sup> and PO<sub>4</sub><sup>3-</sup>, 1500 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> and 10 mg L<sup>-1</sup> Al<sup>3+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>.

results show that large number of anions and cations used have no considerable effect on the determination of chromium(III). Also some transition metals at mg L<sup>-1</sup> levels did not interfere with chromium(III) coprecipitation. As a result the proposed separation/preconcentration method could be applied to the highly saline samples and the samples that contain some transition metals at tolerable levels (Table 3).

### 3.6. Determination of total chromium

In order to determine the total chromium by the presented method, firstly model solutions that contain different amounts of chromium(III) and chromium(VI) were prepared. Different reducing agents including H<sub>2</sub>SO<sub>4</sub>-ethanol, HCl-K<sub>2</sub>SO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>-KI, ascorbic acid, and hydroxylamine can be used for reducing Cr(VI) to Cr(III). The agents mentioned above were tested for their reducing performances, but no significant differences were observed, and all the reagents reduced Cr(VI) to Cr(III) quantita-

tively (>95%). H<sub>2</sub>SO<sub>4</sub>-ethanol was preferred because it is available easily. Then chromium(VI) in the model solutions were reduced to chromium(III) by using 0.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 0.5 mL of ethanol. Because quantitative recoveries for chromium(III) were obtained at pH 8.0, the pH of the aqueous solution was adjusted to 8.0 by using diluted HCl and diluted NaOH. Then the presented procedure was applied to these solutions. The results are given in Table 4. Quantitative recovery values were obtained for these solutions and a good agreement was found between the added chromium(III) and measured chromium(III) amount. The recoveries of chromium(III) were higher than 96%. The results show that the proposed method can be applied for the determination of total chromium.

### 3.7. Analytical performance of the method

The analytical performance of the procedure can be calculated for the results from FAAS measurements. The precision of the method was evaluated as the relative standard deviations (RSD). In order to evaluate the precision of the determination of chromium(III) (12.5  $\mu\text{g}$  of Cr(III) in 50 mL aqueous solution), the procedure was repeated 10 times under optimum conditions mentioned above. RSD and the recovery of chromium(III), were found 5% and 98  $\pm$  5 at 95% confidence level, respectively.

The detection limit, defined as the concentration equivalent to three times the standard deviation of 10 replicate measurements of the blank samples, for chromium(III) was 0.7  $\mu\text{g L}^{-1}$  for 50 mL of sample volume and 5.0 mL of final volume. The limit of detection was calculated by dividing the instrumental detection limit by the preconcentration factor (40).

The sensitivity of the method was calculated as 0.6  $\mu\text{g L}^{-1}$  for chromium after preconcentration step.

### 3.8. Application to real samples and method accuracy

We have investigated the possibility of present speciation procedure for the determination of chromium(III), chromium(VI), and

**Table 4**

Total chromium determinations in spiked test solutions (mean  $\pm$  standard deviation,  $N=3$ , sample pH: 8.0, sample volume: 50 mL, quantity of CFMEPI: 0.3 mL (0.5%, w/v)).

Added ( $\mu\text{g}$ )			Found ( $\mu\text{g}$ )			
Cr(III)	Cr(VI)	Total chromium	Cr(III)	Cr(VI)	Total chromium	Recovery for total chromium (%)
0	25	25	ND	25.4 $\pm$ 1.4	25.4 $\pm$ 1.4	102
5	20	25	4.8 $\pm$ 0.2	19.3 $\pm$ 0.8	24.1 $\pm$ 0.8	96
10	15	25	10.0 $\pm$ 0.4	14.6 $\pm$ 0.9	24.6 $\pm$ 1.0	98
15	10	25	14.4 $\pm$ 1.2	10.1 $\pm$ 0.5	24.5 $\pm$ 1.3	98
20	5	25	20.6 $\pm$ 1.5	4.7 $\pm$ 0.3	25.3 $\pm$ 1.5	101
25	0	25	25.1 $\pm$ 1.1	ND	25.1 $\pm$ 1.1	100

**Table 5**

Speciation of Cr(III), Cr(VI) and total chromium in stream water and sea water (mean  $\pm$  standard deviation,  $N=3$ , V: 200 mL, CFMEPI amount: 0.5%, w/v in ethanol, 0.4 mL).

Samples	Added ( $\mu\text{g}$ )		Found ( $\mu\text{g}$ )			Recovery (%)		
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total chromium	Cr(III)	Cr(VI)	Total chromium
Stream Water	–	–	2.2 $\pm$ 0.1	BDL <sup>*</sup>	2.2 $\pm$ 0.1	–	–	–
	5	5	6.9 $\pm$ 0.4	5.1 $\pm$ 0.2	12.0 $\pm$ 0.5	96	102	98
	10	10	11.9 $\pm$ 0.5	9.7 $\pm$ 0.4	21.6 $\pm$ 0.6	98	97	97
Sea Water	–	–	2.8 $\pm$ 0.1	BDL	2.8 $\pm$ 0.1	–	–	–
	5	5	7.6 $\pm$ 0.4	4.7 $\pm$ 0.3	12.3 $\pm$ 0.5	97	94	96
	10	10	12.5 $\pm$ 0.7	10.2 $\pm$ 0.6	22.7 $\pm$ 0.9	98	102	99
**Tannery waste water	–	–	226.0 $\pm$ 8.1	33.6 $\pm$ 1.2	259.6 $\pm$ 8.2	–	–	–
	10	10	234.8 $\pm$ 9.1	42.9 $\pm$ 1.9	277.7 $\pm$ 9.3	99	98	99
	20	20	242.1 $\pm$ 11.3	52.0 $\pm$ 2.0	294.1 $\pm$ 11.5	98	97	98

<sup>\*</sup> Below the detection limit.

\*\* CFMEPI amount: 0.5%, w/v in ethanol, 4.0 mL.

**Table 6**  
Application of the present method to CRMs and real water samples and comparison of the method with the results from APDC–MIBK liquid–liquid extraction method (mean  $\pm$  standard deviation,  $N=3$ , pH: 8.0, standing time: 5 min, centrifugation rate: 3000 rpm, centrifugation time: 20 min (except for APDC–MIBK liquid–liquid extraction method)).

Samples	Total chromium			Cr(III)	Cr(VI)	Total chromium
	Obtained value	Certified value	Error (%)			
Analysis of CRMs with present method						
CRM-TMDW-500 ( $\mu\text{g L}^{-1}$ ) <sup>a</sup>	18.9 $\pm$ 0.9	20.0 $\pm$ 0.1	–5			
Sandy Soil-C ( $\mu\text{g g}^{-1}$ ) <sup>b</sup>	52.6 $\pm$ 3.1	54.1 $\pm$ 4.2	–3			
Analysis of real samples with present method ( $\mu\text{g L}^{-1}$ )						
Stream water <sup>c</sup>				11.0 $\pm$ 0.5	BDL	11.0 $\pm$ 0.5
Sea water <sup>c</sup>				14.0 $\pm$ 0.5	BDL	14.0 $\pm$ 0.5
Tannery waste water <sup>c</sup>				1130 $\pm$ 39	168 $\pm$ 6	1298 $\pm$ 39
APDC–MIBK liquid–liquid extraction method ( $\mu\text{g L}^{-1}$ ) <sup>d</sup>						
Stream water				10.3 $\pm$ 0.4	BDL	10.3 $\pm$ 0.4
Sea water				13.6 $\pm$ 0.3	BDL	13.6 $\pm$ 0.3
Tannery waste water				1123 $\pm$ 42	164 $\pm$ 7	1287 $\pm$ 43

<sup>a</sup> Sample volume: 25 mL, quantity of CFMEPI: 0.3 mL (0.5%, w/v).

<sup>b</sup> Sample quantity: 0.1 g, quantity of CFMEPI: 0.3 mL (0.5%, w/v).

<sup>c</sup> Sample volume: 200 mL, final volume: 5.0 mL, quantity of CFMEPI: 0.4 mL (0.5%, w/v) for present method.

<sup>d</sup> Sample volume: 200 mL, final volume: 5.0 mL,  $N=3$ .

**Table 7**  
Statistical evaluation of the result obtained in the accuracy study (Table 6) using student's  $t$ -test.

Samples	Element (Cr)					
	$s$	$X_R$	$\bar{X}$	$X_R - \bar{X}$	$^*t_s/\sqrt{N}$	Comparison
CRM TMDW-500 Drinking Water	0.9	20.0	18.9	1.1	2.2	1.1 < 2.2 (same)
Sandy Soil-C	3.1	54.1	52.6	1.5	7.7	1.5 < 7.7 (same)
Steram water	0.5	10.3 <sup>a</sup>	11.0	0.7	1.2	0.7 < 1.2 (same)
Sea water	0.5	13.6 <sup>a</sup>	14.0	0.4	1.2	0.4 < 1.2 (same)
Tannery waste water	39	1287 <sup>a</sup>	1298	11	97	11 < 97 (same)

<sup>\*</sup>  $t=4.30$  (95% confidence intervals),  $N=3$ ,  $s$ : standard deviation,  $X_R$ : Value of standard material,  $\bar{X}$  mean value.

<sup>a</sup> These values were obtained from standard APDC–MIBK liquid–liquid extraction method (Table 6).

**Table 8**  
Comparison of the method with some recent studies on chromium speciation reported in literature.

Method	System	Medium pH	LOD	RSD (%)	PF	Detection	Ref.
Ion-exchange	Amberlite CG-50	5.5	$8.00 \times 10^{-8}$ M for Cr(III)	2.56 for Cr(III)		FAAS	[30]
Liquid–liquid extraction	H <sub>2</sub> O <sub>2</sub> /ethyl acetate	1.7	200 ng L <sup>-1</sup> for Cr(VI)	<3 for Cr(VI)	–	GFAAS	[4]
Liquid–liquid extraction	DPC/ <i>n</i> -pentanol	4.3 mol L <sup>-1</sup> HClO <sub>4</sub>	7.5 $\mu\text{g L}^{-1}$ for Cr(VI)	–	–	CCD-Spectrometry	[31]
Solid phase extraction	Dowex M 4195 chelating resin	2.0	1.94 $\mu\text{g L}^{-1}$ for Cr(VI)	<10 for Cr(VI)	31	FAAS	[5]
Solid phase extraction	Diaion HP-2MG/APDC	3.5	0.03 $\mu\text{g L}^{-1}$ for Cr(VI)	9 for Cr(VI)	–	GFAAS	[6]
Solid phase extraction	Amberlite XAD-1180/DPC	0.5 mol L <sup>-1</sup> H <sub>2</sub> SO <sub>4</sub>	7.7 $\mu\text{g L}^{-1}$ for Cr(VI)	5.1–5.7	75	FAAS	[32]
Cloud point extraction	2-HNAT/Triton X-100	8.0	0.18 $\mu\text{g L}^{-1}$ for Cr(III)	2.13	58	FAAS	[8]
Cloud point extraction	PMBP/Triton X-114	5.0	21 ng L <sup>-1</sup>	3.5	42	GFAAS	[33]
Cloud point extraction	DDTC/Triton X-114	7.0	3.4 $\mu\text{g L}^{-1}$ for Cr(III)	4.1–4.6	65 for Cr(III) 19 for Cr(VI)	HPLC	[34]
Cloud point extraction	Br-PF/Triton X-100	4.5	0.01 mg L <sup>-1</sup> (LOQ)	2.6	50	GFAAS	[35]
Cloud point extraction	Acetylacetone/Triton X-100	2.0	0.32 ng mL <sup>-1</sup> for Cr(III)	3.4 for Cr(III)	35	FAAS	[36]
Coprecipitation	Thulium hydroxide	12.0	0.87 $\mu\text{g L}^{-1}$ for Cr(III) 1.18 $\mu\text{g L}^{-1}$ for Cr(VI)	<10	200	FAAS	[9]
Coprecipitation	Ethyl xanthate complex onto naphthalene	2.5	0.5 $\mu\text{g L}^{-1}$ for Cr(VI)	3.1	100	FAAS	[16]
Coprecipitation	Hafnium hydroxide	5.7 for Cr(VI) 11 for Cr(III)	0.02 $\mu\text{g L}^{-1}$ for Cr(III) and Cr(VI)	3.8 for Cr(III) 5.1 for Cr(VI) 0.4 for Cr(III)	–	GFAAS	[19]
Coprecipitation	Pb(PDC) <sub>2</sub>	4.0 for Cr(VI) 9.0 for Cr(III)	0.02 $\mu\text{g L}^{-1}$ for Cr(VI) and Cr(III)	3.2 for Cr(VI) 3.9 for Cr(III)	15	GFAAS	[20]
Coprecipitation	CFMEPI	8.0	0.7 $\mu\text{g L}^{-1}$ for Cr(III)	5.0	40	FAAS	This work

PDC: pyrrolidinedithiocarbamate; HPLC: high-performance liquid chromatography; APDC: ammonium pyrrolidinedithiocarbamate; DDTC: diethyldithiocarbamate; PMBP: 1-phenyl-3-methyl-4-benzoylpyrazol-5-one; Br-PF: dibromophenylfluorone; DPC: 1,5-diphenylcarbazide; 2-HNAT: Bis-[2-hydroxy-1-naphthaldehyde] thiourea; CCD: charge coupled device; CFMEPI: 5-chloro-3-[4-(trifluoromethoxy)phenylimino]indolin-2-one.

total chromium in water samples including stream water from Of, Trabzon-Turkey, seawater from Black Sea, Rize-Turkey, and tannery waste-water samples from Bor Organized Industrial Region-Nigde (Turkey).

Accuracy tests of methods can be performed with the technique of standard additions, which can also be used to determine recovery of spiked analyte. In order to determine the chromium(III), chromium(VI), and total chromium, different amounts of chromium species were also spiked to these samples. The results are given in Table 5. A good agreement was obtained between the added and measured analyte amounts, so the proposed method could be successfully applied for separation, preconcentration, and speciation of trace amounts of chromium in stream water, sea water, and tannery waste-water samples. The accuracy of the results was satisfactory. Relative error was lower than 7% both for chromium(III), chromium(VI), and total chromium.

The validation of the proposed method was performed by determination of total chromium using certified reference materials, CRM-TMDW-500 Drinking Water and CRM-SA-C-Sandy Soil C (Table 6). The accuracy of the developed procedure for water samples was also checked with standard MIBK-APDC liquid-liquid extraction method [28]. The results are given in Table 6. In order to decide whether the difference between  $\bar{X}$  (mean value) and  $X_R$  (value of standard material) is significant, statistical evaluation was applied to the results obtained from the accuracy study (Table 6) using student's *t*-test (Table 7) [29]. The results revealed good agreement between the observed values and certified values. The proposed separation/preconcentration method was also applied to the determination of total chromium in solid environmental samples, tobacco, and anodic slime. The concentrations of tobacco and anodic slime were found to be  $6.8 \pm 0.4$  and  $27.5 \pm 1.4 \mu\text{g g}^{-1}$ , respectively.

### 3.9. Comparison of the method with others

The data from the present method have been compared with those of recently reported methods on speciation of chromium (Table 8). Some parameters obtained are comparable to those presented by other methods. The present work has relatively low LOD and RSD when compared to other methods using FAAS, HPLC, and CCD-Spectrometric detections [30–36]. Preconcentration factor (PF) is relatively high when compared to others. In addition, the cited methods are more complicated and use more reagents than this method, namely application of this method is simpler and takes less time. For example, because the optimum pH value (8.0) of the work is close to neutral pH (7.0), low quantity of reagent is necessary for adjustment of the pH especially in water analysis.

## 4. Conclusion

A simple and sensitive separation, preconcentration and speciation procedure utilizing CEFC method combined with FAAS was established in the present work. We have used a new organic compound, CFMEPI, as the sole coprecipitating agent without using any carrier element for separation, preconcentration and speciation of chromium, unlike the coprecipitation methods reported in the literature until now. Hence, there is no contamination risk for chromium from a carrier element. No matrix interferences from

the various foreign ions and chemical reagents were observed. This method is also fairly rapid, low cost, and uses less chemicals than those of similar works in the literature (see Section 3.9). And also because CFMEPI is an organic compound, it is decomposed easily by flame and  $\text{HNO}_3$ , so no interferences have observed during the FAAS analysis from this compound. The proposed method was successfully applied for the determination of chromium species in various solid/liquid samples with a low detection limit and high accuracy and precision.

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