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A novel strategy for chromium speciation at ultra-trace level by microsample injection flame atomic absorption spectrophotometry

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Simple, robust and novel analytical procedures were developed for the speciation of chromium by carrier element co-precipitation (CECP) and dispersive liquid-liquid microextraction (DLLME) coupled with microsample injection system-flame atomic absorption spectrophotometry (MIS-FAAS). Ammonium pyrrolidine dithiocarbamate (APDC), carbon tetrachloride and ethanol were used as chelating agent, extraction solvent and disperser solvent, respectively for the determination of $Cr(v_1)$ by DLLME. For total chromium, Cr(III) was oxidized by Ce(SO₄)₂ in acidic media (0.07 mol L^{-1} H_2SO_4) and the resulting solution was co-precipitated with APDC. The concentration of Cr(III) was estimated by determining the difference between the concentration of total chromium and that of Cr(vi). The maximum recovery of Cr(vi) was obtained with DLLME at optimal conditions of pH 3.0, 0.25% APDC, 100 μ L CCl₄, 1.00 mL of CH₃CH₂OH and 0.01 mg L⁻¹ Cr(v1). Whereas, the optimal conditions for CECP were 40 mL initial volume of water samples, 0.25% APDC, 0.02% Ce(SO₄)₂ and $0.10 \text{ mg } \text{L}^{-1} \text{Cr}(\text{vI})$ concentration. The limits of detection and enrichment factor of DLLME and CECP were [0.037 and 2.13] and [400 and 100] μ g L⁻¹, respectively with 40 mL initial volumes. The relative standard deviations (RSD, n = 6) were <4%. The proposed method was successfully applied to the chromium speciation at ultra-trace levels in natural drinking water, industrial effluents waste water and an exchangeable fraction¹ of garden soil from Denizli. Moreover, the proposed methods compared well with the literature reported method.

Introduction

The scientific community and local populations throughout the world are worried about the complexity of nature and its weak balance in global ecology due to human activities and natural climatic changes. Chromium environmental pollution is of great concern because of its synergistic health consequences.^{2,3} The chromium species released from industries including tanning factories, steel works, industrial electroplating, wood preservation, *etc.* not only contaminate the aquatic system but also pollute soil and biotic life.⁴ Among the species of chromium with different oxidation states, Cr(III) is one of the most essential trace nutrients involved in the mechanism of the action of the pancreatic hormone insulin and/or glucose metabolism.^{5–7} Although, Cr(VI) is toxic for biological systems, due to its high water solubility and oxidizing potential, Cr(VI) can efficiently penetrated through biological membranes.⁷ Thus, Cr(VI)

compounds are approximately 100 times more toxic than Cr(III) salts.⁸⁻¹⁰ Th synergistic consequences of Cr(vI) are chronic ulcers, dermatitis, corrosive reaction in the nasal septum and local effects in the lungs.^{7,11} Chemical speciation is required for the proper assessment of the physiological and toxicological effects of chromium and its chemical transformations/distribution in water, soil and the atmosphere.

Several analytical techniques have been used for the quantitative determination of chromium species in environmental samples these include flame atomic absorption spectrometry (FAAS),^{7,12} inductively coupled plasma atomic emission spectrometry (ICP-AES),¹³ inductively coupled plasma optical emission spectrometry (ICP-OES),^{14,15} inductively coupled plasma-mass spectrometry (ICP-MS),¹⁶ electrothermal atomicabsorption spectrometry (ET-AAS),¹⁷ electrochemical,¹⁸ fluorimetry^{19,20} and chemiluminescence.²¹ Although, the determination of chromium species at trace levels in natural waters is difficult due to various factors, particularly their low concentrations and matrix effects. Pre-concentration and separation can solve these problems and can lead to a higher confidence level and easy determination of the chromium species.^{12,13}

Pre-concentration methods like solvent extraction, ion exchange, adsorption and co-precipitation have been frequently

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used for the trace level analysis of metals and metalloids.²²⁻²⁵ Liquid-liquid extraction, based on the transfer of analyte from the aqueous sample to a water-immiscible solvent, is widely used for sample preparation.²⁶ But, these methods have some drawbacks such as the length of time required, the use of significant amounts of chemical additives, solvent losses, large quantities of secondary wastes produced and requirement for complex equipment. Miniaturization of this extraction technique can be achieved by a drastic reduction of the extractant phase volume. Based on this assertion, three new methodologies have arisen, i.e., single-drop microextraction, hollow fibre liquid-phase microextraction and dispersive liquid-liquid microextraction (DLLME).²⁷ Among them, DLLME is effectively useful for the separation and pre-concentration of organic and inorganic contaminants in environmental samples in a single step.^{26,28,29} The speciation procedures for chromium have usually been based on the preconcentration-separation of single chromium specie [Cr(III) or Cr(VI)]. In these procedures, total chromium was determined after the reduction of Cr(vI) or by the oxidation of Cr(III).

On the basis of these facts, a new approach was designed for the selective extraction and preconcentration of chromium species in environmental samples based on DLLME and coprecipitation methods. Ammonium pyrrolidine dithiocarbamate " $C_5H_8NS_2NH_4$ " has been used as a selective chelating agent for Cr(vI). Therefore, it was selected for the pre-concentration of Cr(vi) by means of DLLME prior to analysis by flame atomic absorption spectrometry. Whereas, the determination of total chromium [Cr(T)] based on the oxidation of Cr(III) to Cr(VI) by Ce(IV) [Ce(SO₄)₂]³⁰ and co-precipitation with APDC in acidic media in the presence of Ce(III) as the carrier element is a novel carrier element co-precipitation (CECP) method and has not been reported elsewhere as per our knowledge. The Cr(III) concentration was estimated by the difference between the concentrations of Cr(vi) and Cr(T). The influences of the different analytical parameters for both methods were investigated in detail. The proposed method was applied to the speciation of chromium in water samples of different origins and an exchangeable fraction of soil samples.

Experimental

Reagents

All solutions were prepared using ultrapure water (resistivity 18.2 M Ω cm⁻¹) obtained with a reverse osmosis system (Human Corporation, Seoul, Korea). Stock solutions of Cr(vI) and Cr(III) were prepared by dilution of 1000 mg L⁻¹ certified solutions, Fluka (Buchs, Switzerland). Working solutions were freshly prepared by appropriate dilution of the stock standard solution. Ammonium pyrrolidine dithiocarbamate "C₅H₈NS₂NH₄" (APDC, Fluka) was used as the chelating agent/co-precipitant to form the hydrophobic metal complexes. A 5.0% (w/v) of APDC solution was prepared by dissolving a suitable amount of APDC in ultrapure water. Hydrochloric acid (HCl)/potassium chloride (KCl) buffer³¹ (pH 1–2) was prepared by adding an appropriate amount of HCl, Merck (Darmstadt, Germany) to KCl solution and sodium acetate (NaCH₃COO)/acetic acid (HCH₃COO) buffer³¹ was prepared by adding an appropriate amount of

HCH₃COO (Merck) to NaCH₃COO solutions at pH 3–6. The 1.0 mol L^{-1} solutions of CaCl₂, MgCl₂, NH₄CH₃COO, NaCH₃COO and NH₄NO₃ (Merck) were prepared by dissolving appropriate amounts of each in ultrapure water. All glassware used in the experiments were cleaned with pure water, soaked in 6.00 mol L^{-1} HNO₃ and washed with ultrapure water. The stream sediment standard reference material (SRM) (GBW-07309) was purchased from the National Research Centre for Certified Reference Materials (NRCCRM), China.

Apparatus

An atomic absorption spectrometer (PerkinElmer, AAnalyst 200), equipped with a chromium hollow cathode lamp, an airacetylene flame atomizer and a handmade microinjection system was used for the determinations. The wavelength, lamp current and spectral bandwidth used were 358.0 nm, 25.0 mA and 0.70 nm, respectively. The pH measurements were carried out with a pH meter (WTW-pH-meter-720). A centrifuge Nuve (Ankara, Turkey) was used to accelerate the phase separation process. The ultrasonic extractions were carried out with an ultrasonic bath capacity 4.0 L (Ultrasound Bendelin Electronic, Berlin), programmable for temperatures ranging from 0.0 °C to 80 °C with an intensification frequency of 35 kHz. A microinjector (Hamilton, Switzerland) was used for sediment phase separation. A MD domestic programmable microwave oven Arcelik (Istanbul-Turkey) was operated at a power of 900 W for the digestion and heating of the final solution obtained by the CECP method.

Preparation of handmade microsample injection system (MIS)

In routine practice, a sample volume of 2.0–4.0 mL is required for a single element determination by FAAS. However, a small volume was obtained by each pre-concentration method (<0.5 mL). As a result, there is need of high dilution. To resolve this problem, our group have made a microsample injection system (MIS), coupled to the nebulizer needle using a PTFE



Fig. 1 Microsample injection system-flame atomic absorption spectrophotometry (MIS-FAAS).

capillary tube (length of 12 cm) attached with a yellow micropipette tip (capacity 20–200 μ L) as shown in Fig. 1. MIS needed only a 150 μ L sample volume for each measurement for maximum absorbance signals. The absorbance signals as peak height/peak area were analyzed within 5.0 to 10.0 second. This innovative MIS enables a small volume of sample to be analysed by FAAS with high accuracy, reproducibility and an intense absorbance signal.

General procedure

Dispersive liquid-liquid micro-extraction. Cr(vi) was extracted by DLLME following complexation with APDC, the resulting complex was enriched into the extraction solvent through the disperser solvent. Four replicates of sub-samples (40 mL) of solution containing 10 μ g L⁻¹ Cr(vi) were taken into PTFE tubes (50 mL in capacity). The pH was adjusted to 1.00-2.00 and 3.00-6.00 with HCl/KCl and NaCH₃COO/HCH₃COO buffer solutions, respectively. Then, 0.05-2.00% (w/v) APDC was added and 1.0-4.0 mL CH₂CH₂OH containing 100-400 µL CCl₄ was rapidly injected into the aqueous solution with a 2.50/5.00 mL syringe. As a result, a turbid phase was immediately formed. The acquired solution was centrifuged at 3000 rpm (12 074g) for 5.0 min. The CCl₄ sediment $(85 \pm 10 \ \mu\text{L})$ was separated by microinjection into a 2.0 mL glass vial. The residual phase was vaporized to dryness and further dissolved in 0.40 mL of 0.10 mol L⁻¹ HNO₃. The final solution was determined by MIS-FAAS.

Carrier element co-precipitation method. Total chromium was determined by a carrier element co-precipitation (CECP) method using $Ce(SO_4)_2$ as the carrier. In this procedure, Ce(IV) in acidic media (0.07 mol L^{-1} , H₂SO₄), oxidized Cr(III) to Cr(VI) and the resulting solution was co-precipitated with a complexing reagent (APDC). Four replicate sub-samples (40 mL) of solution containing 100 μ g L⁻¹ Cr(III) were taken into PTFE tubes (50 mL in capacity). The pH was set with an appropriate buffer in the range of (1.00–6.00). Then $Ce(SO_4)_2$ and APDC were added in the range of 0.01 to 0.10% (w/v) and 0.05 to 2.00% (w/v), respectively. The co-precipitants were separated by centrifugation at 3000 rpm (12 074g) for 5 min. The aqueous phase was discarded by pipette and the residue was treated with 0.50 mL HNO₃ (conc.) in a microwave oven at 60% of total power (900 W) for 30 seconds. For minimizing the interferences of organic matrices, the residual phase was evaporated to dryness in a microwave oven and further dissolved in 0.4 mL of 0.10 mol L⁻¹ HNO₃ solution. The final solution was determined by MIS-FAAS.

Sampling. Six samples of each type of drinking water (tap and groundwater samples) were collected in November 2011 from different sampling sites of Denizli, Turkey (situated between the coordinates $37^{\circ}46'48''$ N and $29^{\circ}04'48''$ E in the Aegean region). Industrial effluent (n = 3) was sampled from a well organized industrial zone of Denizli with the assistance of Denizli environment quality laboratory (Denizli Cevre Kalite Laboratuari "DENCEV"). The garden soil samples (top soil from 1.0–2.0 cm depth) were collected from different gardens in central and southern parts of Denizli. Garden soil samples were air-dried for 72 h, followed by oven-drying at 70.0 °C for complete dryness.^{32,33} The dried garden soil samples were homogenized by

pulverizing in a mortar and pestle and were then sieved through a steel sieve (0.105 mm mesh) and stored at room temperature in labeled polypropylene containers.^{34,35}

Determination of chromium speciation in water and industrial effluents. Four 40 mL replicate sub-samples of tap water, ground water and industrial effluent were filtered with ordinary filter papers to remove insoluble matrices and taken into PTFE tubes (50 mL in capacity). Then, treated with the DLLME and CECP methods described above for the determination of $Cr(v_1)$ and Cr(T), respectively. The concentration of $Cr(v_1)$ was calculated by determining the difference between the concentrations of Cr(T) and $Cr(v_1)$.

Chromium speciation in exchangeable fraction of soil. 8.0 g of garden soil samples were weighed into 100 mL polyethylene bottles separately, 80.0 mL each of 1 mol L^{-1} CaCl₂, MgCl₂, NaCH₃COO, NH₄CH₃COO and NH₄NO₃ solutions were added followed by extraction in an ultrasonic water bath at room temperature for 30 min. The mixture was centrifuged to separate the extract from the residue for 5 min at 4000 rpm. Each of the extracted solutions was divided into two portions. Each portion was used for the determination of Cr(v1) by DLLME procedure and Cr(T) by CECP procedure, separately. The concentration of Cr(III) was calculated by determining the difference between the concentrations of Cr(v1) from Cr(T). Blanks were measured with each batch of analysis using only extracting reagents.

Procedure for total chromium in soil. Garden soil samples were digested by microwave acid digestion, in order to achieve a shorter digestion time. Four triplicate 0.2 g samples of air dried garden soil and standard reference material of stream sediment (GBW 07309) were weighed into flasks and then treated twice with 5.0 mL of HNO₃ and 2.0 mL of H₂O₂. The acid treated samples were kept at room temperature for 5 h. Followed by heating in a one-stage digestion programmed at 80% of total power (900 W) for 2.0–3.0 min, to completely oxidize the organic matter in the of soil samples. After cooling, the extra acid was evaporated to a semidried mass and 10 mL of 1.0 mol L⁻¹ HNO₃ was added. Each digested sample was filtered through a filter paper and the volume was made up to 40 mL with ultrapure water in a flask (capacity 50 mL). Then, CECP procedure detailed in the experimental sub-section procedure for total Cr was applied to the final solutions. The same procedure was also applied to the blank solution.

Results and discussion

To attain a higher enrichment factor and recovery of Cr(VI) and Cr(T) by DLLME and CECP, different analytical parameters were investigated by using MIS–FAAS. Each experiment was repeated four times and the results were presented with mean \pm standard deviation at 95% confidence intervals.

Investigation of the injection volume on the analytical signal obtained by MIS-FAAS

The injection volume effect was evaluated by MIS-FAAS according to the recommended manufacturer conditions given in

the instrumental manual using pre-concentrated solutions containing 1.0 mg L⁻¹ Cr in the final solution and obtained with both of the developed sample preparation methods. The intensity of the signal height was found to increase as the injection volumes were increased up to about 150 μ L and then remained constant. A volume of 150 μ L of the extracted solution was selected for further analysis by MIS–FAAS.

Investigation of analytical parameters of DLLME

Influence of the extraction solvent type and volume. The selection of an appropriate extractant phase is of major importance in microextraction schemes especially for DLLME. A low extractant phase-to-sample ratio and a high distribution ratio "K" (ratio of the concentrations of analyte in the extractant and aqueous phase at equilibrium) are needed to achieve high enrichment factors and extraction efficiencies.²⁶ This factor depends to a large extent on the type of extractant phase, considered as a key variable and the choice of which is therefore important. The final choice was made after comparing the different physical properties of various solvents in order to achieve good sensitivity, precision and selectivity (Table 1).

CHCl₃ and CCl₄ were selected for investigating the preconcentration of Cr(v1) on the basis of their high density and vapour pressure as well as low water solubility (Table 1). Samples were tested using 1.0 mL of CH₃CH₂OH (disperser solvent) containing 100 μ L of extraction solvent (Table 1) which was rapidly injected into 40 mL of the aqueous sample resulting from the complex formation reaction. In this experiment, CCl₄ and CHCl₃ resulted in enrichment factors of 400 and 380, respectively. CCl₄ established the maximum enrichment factor as compared to CHCl₃ because of its low solubility, high viscosity and other physical properties as shown in Table 1. The formed sediment phase could easily be separated by a micro-injector. As a result, CCl₄ was chosen as the best extraction solvent.

To evaluate the effect of the extraction solvent volume, solutions containing different volumes of CCl₄ (100 to 400 μ L) were subjected to the same DLLME procedures as shown in Fig. 2. It can be seen that the enrichment factor decreased with an increase in the volume of CCl₄ from 100 to 400 μ L. In the subsequent studies, 100 μ L CCl₄ was used as the optimum volume of the extraction solvent.

Influence of the disperser solvent type and volume. In DLLME, the disperser solvent must be miscible in both the aqueous phase and the extraction solvent. Thus, different solvents (CH_3CHO ,



Fig. 2 Influence of volume of the extraction solvent (CCl₄) and disperser solvent (CH₃CH₂OH) on the enrichment factor of Cr(v1) in 40 mL initial volume of water samples, 0.25% APDC, pH 3.00 and 0.01 mg L⁻¹ Cr(v1).

CH₃CN, CH₃CH₂OH and mCH₃OH) were selected on the basis of their physical properties as depicted in Table 1. 1.00 mL of each disperser solvent containing 100 µL CCl₄ (extraction solvent) was tested. The enrichment factors obtained show no significant differences between the disperser solvents. However, less toxicity and the lowest standard deviation of CH₃CH₂OH makes it a better choice. The effect of the volume of CH₃CH₂OH on the extraction recovery was also studied. The experimental conditions were fixed and included the use of different volumes of CH₃CH₂OH 1.00, 1.50, 2.00, 3.50 and 4.00 mL containing 100 µL of CCl₄, respectively. Under these conditions, the volume of the sediment phase was constant (85.0 \pm 10.0 μ L). The results showed that there was considerable variation in the enrichment factor using 1.00-4.00 mL of CH₃CH₂OH (Fig. 2). The enrichment factor decreased, when the CH₃CH₂OH volume increased. Thus, 1.00 mL of CH₃CH₂OH was selected for further study.

Salt effect. The solubility of $Cr(v_1)$ and organic solvent in aqueous phase usually decreases with an increase in ionic strength, which is favorable for obtaining high recoveries. Therefore, the influence of salt concentration was evaluated at 0.5–40.0% (w/v) NaCl levels while other parameters were kept constant. The results showed that salt addition up to 10.0% did not affect the enrichment factor considerably. By increasing the ionic strength (from 10.0% to 40.0%), the solubility of the

Table 1 Physical properties of understudied solvents for DLLME²⁶

Extractant	Boiling point/ °C	Vapour pressure/ Torr	Water solubility/ mg L^{-1}	Density/ g cm ⁻³	Viscosity/ cP	Surface tension/dyn cm ⁻¹	Dipole moment (D)	Dielectric constant
Methanol	64.5	127.0	8	0.79	0.55	22.30	2.87	32.66
Ethanol	78.3	59.0	00	0.79	1.08	22.32 (20 °C)	1.66	24.55
Acetone	56.1	231.1	00	0.78	0.30	22.68	2.69	20.56
Acetonitrile	81.6	88.81	00	0.78	0.34	28.25	3.53	35.94
Chloroform	61.2	194.8	8500	1.48	0.54	26.53	1.15	4.81 (20 °C)
Tetra chloromethane	76.6	115.2	770	1.58	0.90	26.13	0.00	2.23

extraction solvent in the aqueous phase diminishes. As a result, the volume of the sediment phase increases from almost 110 to 130 μ L, which decreases the enrichment factor. The enrichment factor drops from 362 to 308 on going from 10.0% to 40.0% (w/v) of NaCl in water. The developed method can be applied to the separation of chromium from saline solutions up to 10.0%.

Influence of metal-chelate formation parameters (sample pH and concentration of APDC). pH plays an important role in the extraction of inorganic compounds especially chromium in environmental samples as it affects the reaction with complexing reagents. APDC forms a complex with different metals and metalloids in acidic media. In acidic conditions Cr(vI) in the form of chromate is converted to dichromate $(Cr_2O_7^{2-})$ and forms a Cr(PDC)₂(OPDC) complex, where OPDC represents an oxygen atom inserted between the metal ion and one of the sulfur atoms and a small fraction of Cr(PDC)₃.^{36,37} On the other hand, Cr(III) does not react with APDC under the conditions (viz. ligand concentration, reaction temperature and time) used for the chelation of Cr(VI).³⁸ The inertness of Cr(III) towards APDC is due to the difficulty in displacing the coordinated water molecules of [Cr(H₂O)₆]³⁺complex.³⁹ The possible reaction of Cr(vi) with APDC is predicted as:

$$Cr(VI)_{(aq.)} + C_5H_8NS_2NH_{4(aq.)} \xrightarrow{CH_3CH_2OH+CCl_4} Cr(VI)$$
$$-C_5H_8NS_2NH_{4(CCl_4)}$$

In this experiment, the effect of pH on the extraction performance within the range of 1–6 was investigated (Fig. 3). It can be observed that the recovery percentages of $Cr(v_I)$ were found to be high with an increase of pH value (2.0 to 4.0). However, the recovery percentage of $Cr(v_I)$ decreased abruptly when the pH was increased to 4.0. The reason is that $Cr(v_I)$ is not stable at high pH. Therefore, pH 3.0 was selected for maximum extraction of $Cr(v_I)$. 0.25% (v/v) APDC was chosen as the optimal amount for the determination of $Cr(v_I)$ to prevent any interference (Fig. 3).



Fig. 3 Influence of sample pH on DLLME for a 40 mL initial volume of water samples, 0.25% APDC, 100μ L CCl₄, 1.00μ L of CH₃CH₂OH and 0.01 mg L⁻¹ Cr(v1) and CECP for 40 mL initial volume of water samples, 0.25% APDC, 0.02% Ce(SO₄)₂ and 0.10μ g L⁻¹ Cr(v1).

Optimization of analytical parameters of CECP

Influence of pH. At more acidic conditions, $HCr_2O_7^{-}$ and $Cr_2O_7^{-2-}$ dimers become the dominant Cr(vI) form and the pK_{a1} and pK_{a2} values of these ions are 0.74 and 6.49, respectively.⁴⁰ It becomes apparent that APDC mostly forms complexes with $HCr_2O_7^{--}$ ions. In aqueous solutions having a lower pH, the dichromate will be present primarily in its protonated form, *i.e.*, $HCr_2O_7^{-.40}$ The quantitative/selective oxidation of Cr(III) to Cr(vI) is possible at pH 2.0 by Ce(IV) as reported in the literature.³⁰ After oxidation, Cr(vI) co-precipitates in the presence of a mixture of Ce(III) and unreacted Ce(IV).

$$Cr(III)_{(aq.)} + Ce(SO_4)_{2 (aq.)} \longrightarrow Cr(VI)_{(aq.)} + C_5H_8NS_2NH_4 (aq.)$$

$$Ce((III)/Ce(IV)$$

$$Cr_{(VI)}-C_5H_8NS_2NH_4-Ce(III)/Ce(IV)_{(S)}$$

Thus, the effect of pH was studied in the range of 1.0–6.0 for CECP and the pH was adjusted with appropriate buffers. The recovery percentage decreased with an increase in pH because of the decrease in hydrogen ion concentration which also causes a decrease in the rate of association of Cr(vI) with APDC in the presence of carrier [Ce(III)/Ce(IV)]. The maximum co-precipitation was achieved at pH 2 (Fig. 3). Hence, all subsequent works were performed at pH 2.0 for Cr(T).

Effects of amounts of cerium (IV) and co-precipitating organic ligand (APDC). The effects of the amount of Ce(IV) on the oxidation of Cr(III) to Cr(VI) and Cr(T) in the presented coprecipitation system were examined in the range of 0.01-0.1% (w/ v). The recovery of Cr(T) was below 90% at the Ce(IV) concentration 0.01 to 0.02% (Fig. 4). The recovery values increased with an increase in the amounts of Ce(IV). At concentrations greater than 0.02% Ce(IV), the recovery values were quantitative (>90%). Further studies were carried out with 0.025% of Ce(IV). In order to optimize the concentration of organic co-precipitant (APDC) required to achieve the maximum extraction yield of Cr(T) the effect of adding 0.1 to 2% (w/v) was studied as shown in Fig. 4. A recovery of 70% of Cr(T) was obtained at a concentration of



Fig. 4 Influence of APDC and Ce(ν) concentration on the co-precipitation method for Cr(T) for 40 mL initial volume of water samples, pH 2.0 and 0.10 mg L⁻¹ Cr.

0.1% APDC but increased sharply when its concentration was raised to 0.15%. At higher concentrations, there was no significant difference in recovery percentages (p > 0.05). So, an optimal concentration of 0.25% was selected for subsequent experiments.

Effects of duration time for co-precipitation. The influence of duration time of co-precipitation on the recoveries of analytes has been studied by different scientists.^{10,41,42} The developed co-precipitation method has advantages over other methods because of the use of the organic co-precipitant (APDC). Maximum recovery percentages were obtained within 20–30 second. So, there is no effect of time duration on recovery percentage of Cr(T).

Sample volume. The sample volume is an important parameter for co-precipitation methods as it has an effect on the value of the pre-concentration factor.^{43,44} So, the influence of sample volume on the recovery percentages of Cr(T) was investigated in the range of 10–40 mL under the optimal conditions. The final volume of the co-precipitation work was 0.4 mL and a pre-concentration factor of 100 could be achieved with an initial sample volume of 40 mL.

Effect of the co-existing ions. APDC as the complexing agent reacts with several transition metals and metalloids in acidic media, but it does not react with alkali and alkaline earth metals.³⁰ In order to assess the feasibility of the recommended separation-preconcentration procedures (DLLME and CECP) for analytical applications, the effect of some foreign ions which interfere with Cr(vi) ion or/and often accompany analyte ions in various real environmental samples was investigated under the optimized conditions. It is a general rule for analytical chemistry that any matrix was considered to interfere if the resulting FAAS signal of understudied analyte was varied $\pm 5.0\%$. The recoveries of Cr(vi) and Cr(T) were almost quantitative in the presence of an excessive amount of the possible interfering cations and anions. The tolerance limits of [Na⁺ and Cl⁻], [Li⁺, K⁺, Ca²⁺ and Mg²⁺], (SO₄²⁻ and NO_3^{-}) and [Cu(II), Fe(II), Se(II), As(III), Co(II), Hg(II), Mn(II),Cr(III), Ni(II), Al(III), Zn(II)] were found to be 500 000, 250 000, 100 000 and 1000 μ g L⁻¹, respectively for Cr(VI) and Cr(T).

Analytical figure of merits. The analytical characteristics of the proposed procedures were calculated under the optimized conditions. The calibration and standard addition graphs were obtained for Cr(VI) and Cr(T), determined by MIS-FAAS. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as $3 \times S_{\rm b}/m$ and $10 \times S_{\rm b}/m$, respectively. Where $S_{\rm b}$ is the standard deviation of the blank (n = 10) and m is the slope of the linear section of the calibration graph. The LOD of Cr(vi) and Cr(T) were found to be 0.037 and 2.13 μ g L⁻¹, whereas, the LOQ were 0.124 and 7.12 μ g L⁻¹ with a linear range of the calibration graph from quantification limit to 100 μ g L⁻¹, respectively. The calibration curve of Cr(vi) and Cr(T) for this interval were determined to be $A = 2.10 \times 10^{-2} \times [Cr(vI)] + 6.00$ $\times 10^{-5}$ and $Y = 3.70 \times 10^{-2} \times [Cr(T)] + 9.00 \times 10^{-5}$, respectively. Where, A is the analytical signal, measured as absorbance and [] is the concentration of chromium in the solution ($\mu g L^{-1}$). The correlation coefficients (r²) were 0.999 and 0.984 for Cr(vI) and Cr(T), respectively.

The precision of the measurements was assessed as the relative standard deviation (RSD) for each value of concentration, calculated as $(SD/X) \times 100$, where SD is the standard deviation for seven measurements and X is the mean value of these measures. The %RSD of both methods was evaluated with 40.00 mL of solution containing 0.01 μ g L⁻¹ Cr(vI)/Cr(T) and was found to be <5% (n = 4). The reliability of the simplest calibration against aqueous standard solutions for DLLME was verified by means of spiking experiments at three concentration levels of Cr(vi) in the range of 10 to 50 μ g L⁻¹ with recoveries >98.5% (Table 2). The accuracy of Cr(T) determination by CECP after microwave acid digestion was checked by using certified reference materials (GBW-07309; Stream Sediment) as shown in Table 2. The paired *t*-test showed that the difference is not significant (p > 0.05) between the found values and the certified values (Table 2). The enrichment factor (EF) was calculated by the ratio of $Cr(v_1)/Cr(T)$ concentration in the sediment phase and the initial concentration in the original solution. Finally, enrichment factors of 400 and 100 were obtained by DLLME and CECP, respectively.

Comparison of the proposed methods with other literature reported methods. A comparison of the proposed methods with other approaches reported in the literature for the speciation of chromium is represented in Table 3. The results show that extraction times in DLLME and CECP are short (1.0 to 2.0 min). The enrichment factors, LODs and %RSDs of DLLME and CECP are comparable with those of the methods reported in the literature.^{8,10,12,25,45–55} The proposed methods (DLLME and CECP) coupled with MIS–FAAS are simple, most sensitive and selective. Therefore, the developed new methods could successfully be applied to the monitoring of trace amounts of chromium species in environmental, biological and soil samples.

Application to real samples

Chromium speciation in tap, groundwater, industrial untreated and treated industrial waste water, soil exchangeable and acid digested samples of garden soil samples are important in determining mobilization and toxicity. Thus, DLLME and CECP methods were applied in the analysis of real samples and the resulting data is presented in Table 3. Cr(vi), Cr(iii) and Cr(T) contents in tap and groundwater varied in the ranges [0.00 to 3.20 and 0.00 to 6.00], [0.00 to 6.00 and 0.00 to 10.3] and [0.00 to 10.0 and 0.00 to 20.0] μ g L⁻¹, respectively. The Cr(T) contents were within the US EPA maximum contaminant level (100 μ g L⁻¹). The Cr(III) contents were found to be higher than the Cr(VI) contents, because the reduction of Cr(vI) by iron containing silicates, oxides or sulfides was faster than the oxidation of Cr(III).⁵⁶ The level of Cr(VI) was found to be high in groundwater as compared to tap water. This may cause disorders of the skin, gastrointestinal tract, kidney, liver, circulatory and nerve tissues and carcinoma of the lung.57 But, Cr(VI) in ground water of Denizli was within WHO regulated values (50.0 μ g L⁻¹) of Cr(v1) for drinking water. Thus, both the studied waters (tap and ground water) have safe levels of chromium species for the local community as natural drinking water sources.

The chromium species in the tannery effluent samples collected from the organized industrial zone of Denizli-Turkey were also

Table 2	The results for tests of addition/recover	y for Cr(vi) by	DLLME in industrial	waste water samples $(n = 6)$
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	Amount added/µg L^{-1}	Found values Mean \pm SD/µg L ⁻¹	%Recovery ^a	%RSD
Cr(vi)	10 25 50	$\begin{array}{c} 12.5 \pm 2.4 \\ 24.7 \pm 0.3 \\ 40.7 \pm 0.7 \\ 61.4 \pm 3.4 \end{array}$	$\frac{-}{110.0 \pm 1.5} \\ 108.0 \pm 1.8 \\ 98.5 \pm 3.3$	1.4 1.7 3.4
Validation of Cr(T) by C	CECP			
Certified reference material	Certified value mean \pm SD/µg g ⁻¹	Found values mean \pm SD/µg g ⁻¹	%Recovery (% RSD)	$t_{\text{Experiment}}^{t} (\text{RSE} = \text{SD}\sqrt{n})$
GBW 07309 stream sediment	85.0 ± 7.0	83.3 ± 2.3	97.7 ± 1.5(2.74)	0.715 (<1.00)
^{<i>a</i>} %Recovery = $\frac{C_{\text{after}}}{C_{\text{initial}}}$	$\frac{rspiked}{+C_{spiked}} \times 100.$ ^b At $t_{certical} = 2.$.57 at 95% confidence limit, $(n = 6)$).	

determined by the developed preconcentration methods as shown in Table 4. Cr(vi) was observed in excess amounts in industrial water (Table 4), because of human activities especially through the production of waste water in electroplating, tanning, textile and dyestuff industries present in the industrial zone. The Cr(T) values in garden soil samples collected from central and southern areas of Denizli-Turkey were found to be in the ranges of 43.7–48.1 and 28.3–34.3 μ g g⁻¹, respectively (Table 4). The Cr(T) contents were found to be higher in garden soil of central Denizli examined this may be due to considerable traffic pollution in the central city, the type of soil and the use of contaminated organic animal manure. The toxicity of heavy metals as well as chromium in contaminated soils depends on their chemical state and bioavailability.⁵⁸ For current study, two types of salts; acetate (NH₄CH₃OO, NaCH₃OO) and non-buffer salts (MgCl₂, CaCl₂, NH₄NO₃) were selected for bioavailable

Table 3	Comparative da	ta of analytical charact	ristics of literature	e reported methods ar	nd proposed metho	ds for chromium speciation ^a
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Analyte(s)	Analytical technique	Extraction phase	Extraction time/ min	Enrichment factor	$\begin{array}{c} \text{LOD/} \\ \mu g \ L^{-1} \end{array}$	RSD (%)	Ref.
Cr(vi) Cr(iii)	GFAAS	Pb(PDC) ₂	15	15	0.020	3.9 3.2	25
Cr(vi)	FAAS	Ethyl xanthate complex onto naphthalene	15	100	0.500	3.1	10
Cr(vi)	CCD-spectrometry	DPC/n-pentanol			7.500		45
Cr(vi)	GFAAS	H ₂ O ₂ /ethyl acetate			0.200	<3.0	46
Cr(vi)	Direct-SDME-SIA-ETAAS	Toluene	5	20	0.020	7	47
Cr(vi)	FAAS	Dowex M 4195 chelating resin	1.0-5.0	31	1.490	<10	48
Cr(vi) Total	FAAS	Amberlite XAD-1180 resin	_	75	7.700 8.600	_	8
chromium Cr(III)	FAAS	bis-[2-Hydroxynaphthaldehyde] thiourea/triton X-100	20	58	0.180	2.13	12
Cr(III)	FAAS	CFMEPI	20	40.0	0.070	5.0	49
Cr(III) Cr(VI)	CE-FAAS	Dy ₂ O ₃ aqueous solution	20	100	$0.650 \\ 0.780$		50
Cr(vi)	UPAILDLLME-ETAAS	APDC/[Hmim][PF6]	5–6	300	0.07	9.2	51
Cr(III)	LLUSAEME-ICP-OES	Tetrachloroethylene	7	16	0.20	2.5	52
Cr(vi)	DLLME–UV-Vis spectrophotometry	DIC/toluene	_	—	30.0	<5.0	53
Cr(vi)	DLLME-ETAAS	APDC/carbon tetrachloride		171	0.00596	3.02	54
Cr(vi) Cr(T)	DLLME-FAAS	APDC + EDTA/carbon tetrachloride	0.08	275 262	0.07 0.08	2.0 2.6	55
Cr(vi) Cr(T)	DLLME–MIS–FAAS CECP–MIS–FAAS	APDC/carbon tetrachloride Ce(III)/Ce(IV)-APDC	1–2	400 100	0.037 2.130	<4.0	Current study

^{*a*} Ammonium pyrrolidine dithiocarbamate (APDC), charge coupled device (CCD), co-precipitation flame atomic absorption spectroscopy (CE-FAAS), methylindocarbocyanine (DIC), 1,5-diphenylcarbazide (DPC), 1-hexyl-3-methylimidazolium hexafluorophosphate ([Hmim][PF6]), electro thermal atomic absorption spectroscopy (ETAAS), graphite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma-optical emission spectrometry (ICP-OES), ligandless-ultrasound-assisted emulsification microextraction (LLUSAEME), pyrrolidine dithiocarbamate (PDC), 5chloro-3-[4-(trifluoromethoxy) phenylimino]indolin-2-one (CFMEPI), single drop micro extraction (SDME), sequential injection analysis (SIA), ultrasonic probe-assisted ionic liquid dispersive liquid–liquid microextraction (UPAILDLLME).

Table 4 Analytical results of chromium speciation in water, extractable and acid digestible soil samples of different origins (Denizli-Turkey)

Origin of water (no. of samples)TGround water $(n = 3)$ 1Tap water $(n = 4)$ 7Industrial effluent $(n = 4)$ 4			l Cr/μg L ⁻¹	$Cr(vi)/\mu g L^{-1}$		$Cr(III)/\mu g L^{-1}$
			± 1.8 = 1.3 ± 8.0	$\begin{array}{c} 3.7 \pm 1.8 \\ 2.6 \pm 0.5 \\ 23.3 \pm 5.3 \end{array}$		$\begin{array}{c} 6.9 \pm 2.5 \\ 4.9 \pm 1.4 \\ 16.8 \pm 9.6 \end{array}$
		Centr	ral Denizli		Southern Denizli	
Exchangeable reagents	Total Cr Mean \pm SD/µg g ⁻¹	Cr(vi)	Cr(III)	Total Cr	$Cr(v_1)$ Mean \pm SD/µg g ⁻¹	Cr(III)
NH ₄ -Ac Na-Ac MgCl ₂ CaCl ₂ NH ₄ NO ₃	$\begin{array}{c} 0.58 \pm 0.02 \\ 0.51 \pm 0.02 \\ 0.51 \pm 0.07 \\ 0.53 \pm 0.06 \\ 0.36 \pm 0.01 \end{array}$	$\begin{array}{c} 0.29 \pm 0.02 \\ 0.31 \pm 0.01 \\ 0.24 \pm 0.02 \\ 0.29 \pm 0.01 \\ 0.21 \pm 0.03 \end{array}$	$\begin{array}{c} 0.26 \pm 0.04 \\ 0.21 \pm 0.03 \\ 0.20 \pm 0.12 \\ 0.26 \pm 0.06 \\ 0.14 \pm 0.04 \end{array}$	$\begin{array}{c} 0.52 \pm 0.02 \\ 0.44 \pm 0.02 \\ 0.45 \pm 0.02 \\ 0.52 \pm 0.02 \\ 0.37 \pm 0.03 \end{array}$	$\begin{array}{c} 0.29 \pm 0.03 \\ 0.25 \pm 0.02 \\ 0.26 \pm 0.02 \\ 0.25 \pm 0.03 \\ 0.24 \pm 0.02 \end{array}$	$\begin{array}{c} 0.23 \pm 0.04 \\ 0.19 \pm 0.03 \\ 0.19 \pm 0.03 \\ 0.27 \pm 0.02 \\ 0.13 \pm 0.03 \end{array}$
			Cr(T) in acid digestee	d soil samples by CECP		
			Mean \pm SD/µg g ⁻¹		Mea	$n \pm SD/\mu g g^{-1}$
HNO ₃ + HCl (1 : 3)			45.8 ± 1.5		31.3	± 1.5

fractions of chromium species by the developed methods. The acetate and chloride salts enhanced the extraction of chromium species as compared to nitrate salt as a result of their complexing action (Table 4). The amount of Cr(vI) and Cr(III) in the exchangeable fraction of garden soil were found to be 50–70% and 30–50%, respectively.

Conclusion

The DLLME and CECP methods coupled with MIS–FAAS were novel, fast, simple, inexpensive and reproducible for ultratrace level chromium speciation with high enrichment factor. The applicability and the accuracy of DLLME was estimated by the analysis of Cr(v1) in industrial effluent using the standard addition method (recoveries > 96%). The accuracy of Cr(T) by CECP after microwave acid digestion was checked by using CRM (GBW-07309) and there was an insignificant (p > 0.05) difference between the found and certified values. The developed methods have more tolerance to the interference caused by the other coexisting metal ions because Cr–APDC complex formation and co-precipitation was achieved at low pH (<3.0). Analytical characteristics and applications in water and soil samples made these methods a good alternative for the determination of chromium species at ultra-trace level for routine analysis.

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