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Dispersive Liquid-Liquid Microextraction of Cr(VI) in Water and Hair Samples by Electrothermal Atomic Absorption Spectrometry

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In this study, a dispersive liquid-liquid microextraction technique was combined with electrothermal atomic absorption spectrometry for preconcentration and determination of Cr(VI) in water and hair samples. Ammonium pyrrolidine dithiocarbamate, carbon tetrachloride and methanol were used as chelating agent, extraction and disperser solvent, respectively. Some variables influencing the microextraction and complex formation, including extraction and disperser type and volume, pH of the sample solution, extraction time, salt effect and amount of chelating agent were investigated. Under optimized conditions, the enrichment factor of 171 with a sample volume 5 mL was achieved. The detection limit for Cr(VI) was 5.96 ng L^{-1} and the relative standard deviation was 3.02 % (n = 10, c = 20 ng L^{-1}). The method was successfully applied to the extraction and determination of Cr(VI) in several natural water and hair samples.

Key Words: Dispersive liquid-liquid microextraction, Ammonium pyrrolidine dithio carbamate, Cr(VI), Electrothermal atomic absorption spectrometry, Hair and water samples.

INTRODUCTION

Chromium is released into the environment as a result of industrial activities, such as steel work, electroplating and leather tanning industries, wood preservation and pigment, timber processing[1].

Two stable chromium species are known in the environment: Cr(III) and Cr(VI). Their biological activities, chemical behaviours and influences on the environment are different. Cr(III) is necessary for the synthesis of fat from glucose and also for the oxidation of fat to carbon dioxide. On the contrary the hexavalent chromium present toxic and carcinogenic effects, due to its strong oxidation potential[2,3].

Transformation of Cr(III)-Cr(VI) is rapid enough in presence of even mild oxidants as the potential necessity to oxidize Cr(III)-Cr(VI) decreases with the increase of pH of the media[4].

For a selective determination of Cr(VI) a variety of procedure were described up to this time. Sperling presented a reviewed introduction of the method for the determination of Cr(VI) and total chromium by graphite furnace atomic absorption spectrometry (GFAAS) which included different preconcentration methods used for the separation of Cr(VI)[5]. Chromium concentration in natural waters is normally at the low ng mL^{-1} level, so to achieve accurate and sensitive determination, a preliminary separation and preconcentration is required[6].

Various separation and preconcentration methods including liquid liquid extraction (LLE)[7], coprecipitation[8], solid phase extraction (SPE)[9] and cloud point extraction (CPE)[10,11] have been proposed for chromium determination. Although these methods have been widely employed for preconcentration of chromium, there are some limitations associated with them. Liquid-liquid extraction is time consuming and requires large amounts of toxic and expensive organic solvents. Cloud point extraction exhibits several limitations such as the viscose surfactant-rich phase that prevents an accurate injection to conventional analytical instruments[12].

Miniaturization of extraction procedure is one of the recent trends in sample preparation techniques. Miniaturization of the extraction technique can be achieved by a drastic reduction of the extractant phase volume. Based on this premise, three new methodologies have been introduced. Single-drop microextraction (SDME)[13,14], hollow fiber liquid-phase microextraction (HF-LPME)[12,15] and dispersive liquid liquid microextraction (DLLME).

Dispersive liquid liquid microextraction introduced by Assadi in 2006. In this method, the appropriate mixture of extraction solvent (with high density such as carbon tetrachloride) and dispersive solvent with high miscibility in both extractant and aqueous phase such as methanol is injected into an aqueous sample rapidly by syringe and as a result, a cloudy solution is formed.

The analyte in the sample is extracted into the fine droplets of extraction solvent. After extraction, phase separation is performed by centrifugation and the sedimented phase is removed for further analysis[16,17]. The advantages of DLLME method are its simplicity of operation, rapidity, low cost, high recovery and enrichment factors. Dispersive liquid liquid microextraction has been mainly employed in combination with electrothermal atomic absorption spectrometry (ETAAS) for quantitation. For instance, Au[18], Cd[19], Ni[20] and Pb[21,22] have been determined in different water and biological samples. In addition this method has been applied for the extraction and determination of polycyclic aromatic hydrocarbons (PAHs)[23] and organophosphorous pesticides (OPPs)[24].

The aim of the present work is to develop a new method for the determination of trace amounts of Cr(VI) in water and hair samples by ETAAS based on formation of complex. Various reagents such as diphenylcarbazide, crystal violet, benzyltributylammonium, O,O'-dibutyl dithiophosphate, chromotropic acid[25] and ammonium pyrrolidine dithiocarbamate (APDC)[26] have been used for the determination of Cr(VI). Ammonium pyrrolidine dithiocarbamate (APDC) is a kind of sensitive organic reagent used for the spectrometric determination of chromium, so it was selected as chelating reagent in this work.

EXPERIMENTAL

The measurements were performed with a Varian (Spectr AA 200) atomic absorption spectrometer with a heated graphite tube atomizer (GTA 100). A chromium hollow cathode lamp (Spectr AA, Australia by Varian) was used as radiation source, operated at a current of 7 mA and wavelength was set at 357.9 nm. The optimum operating parameters for ETAAS are given in Table-1. All measurements were based on peak height.

TABLE-1
GRAPHITE FURNACE TEMPERATURE PROGRAM
FOR CHROMIUM DETERMINATION

Step	Temperature (ºC)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying	120	20	10	200
Pyrolysis	600	10	20	200
Atomization	2300	0	3	0
Cleaning	2400	1	3	100

A volume of 20 µL for all sample and standard solution was injected manually into the graphite furnace 10 s after starting the drying step in all experiments. Argon (99.999 %) was employed as sheath gas. The pH values were measured with a Metrohm pH-meter (model 744). A (Koksun model H-11) centrifuge was used for centrifugation. A magnetic stirrer, hot plate (Heydolph MR 3001) was employed to stir and heat the sample solution. Reagents and solutions.

Stock standard solutions (1000 mg L^{-1}) was prepared by dissolving appropriate amount of $K_2Cr_2O_7$ (analytical grade, Merck) in high purity deionized water. All working standard solutions of chromium were prepared by stepwise dilutions of stock solution with ultra pure water prior to analysis. Ammonium pyrrolidine dithiocarbamate (Fluka) was used as chelating agent.

Other chemicals used were: carbon tetrachloride (proanalysis), chloroform (for synthesis), 1,2-dichlorobenzene (for synthesis) and carbon disulfide (for spectroscopy) as extraction solvents, methanol (extra pure), ethanol (for spectroscopy), acetone (extra pure), acetonitrile (HPLC grade) and ethyl acetate (extra pure) as disperser solvents. The mentioned chemicals were obtained from Merck.

All laboratory glass wares used for the trace analysis were kept in a 10 % (v/v) nitric acid for at least 24 h and rinsed tree times with high purity deionized water prior to use.

Dispersive liquid-liquid microextraction procedure: A 5 mL of sample solution containing Cr(VI) (pH = 2.2) was transferred to a 10 mL conic bottom screw cap glass test tube. Exactly 500 µL of methanol (as disperser solvent), containing 35 µL of carbon tetrachloride (as extraction solvent) and 0.0001 g of ammonium pyrrolidine dithiocarbamate (as chelating agent) were injected rapidly into each sample solution using 500 µL syringe. A cloudy solution was formed in the test tube. In this step Cr(VI) was extracted into the fine droplets of CCl_4. Then, the separation of the phases was obtained by centrifugation at 4000 rpm for 3 min. After this step, the dispersed fine droplets of carbon tetrachloride were sedimented at the bottom of the conical test tube (about 25 µL). Twenty microlitres of this sedimented phase was removed using a sampler (Brand, Germany) and manually injected into the graphite tube under the temperature program listed in Table-1.

Sample preparations

Water samples: Water samples were collected from Northern Iran. Sea water (Caspian sea), underground water (Soustan), spring water (Plour) and tap water was collected from our laboratory, after allowing the water to flow for 5 min. All water samples were collected in PTFE containers and the pH of the samples were adjusted to 3 with appropriate amount of HNO_3 and stored in dark at 4 ºC and analyzed afterward.

Hair samples: Human hair samples were collected from the habitants of Tehran (Tehran, Iran) and finely cut from the nap of the neck near the scalp section and washed with non-ionic detergent solution. The hair samples were rinsed twice with high purity deionized water and then with acetone and then air-dried prior to analysis. A 0.1 g portion of each sample was transferred into PTFE beakers, 10 mL of concentrated HNO_3 and 5 mL of H_2O_2 were added and heated on a hot plate until the solution become clear and heating continued to near dryness. The residue was dissolved in 0.1 M HNO_3 and the solution made up to 50 mL with deionized water in a volumetric flask.

RESULTS AND DISCUSSION

In order to reach the optimized experimental conditions for high-enrichment factor, the effect of different parameters influencing the complex formation and the extraction efficiency, were investigated. These parameters were: the nature and volume of both extraction and disperser solvents, pH, the chelating agent concentration and the extraction time. Finally these optimal conditions were applied to extract and detect Cr(VI) in water and hair samples.

The enrichment factor was defined as the ratio between the chromium concentration in the sedimented phase (C_{sed}) after preconcentration and the initial concentration (C_0).

C_{sed} was calculated from the calibration graph obtained by the conventional LLE-ETAAS (extraction condition: 5 mL of standard water sample in the concentration range of 0.1-15.0 µg $L^{-1}$of chromium, 5 mL of CCl_4, 0.005 g of APDC and pH = 2.2).

Effect of nature and volume of extraction solvent: The selection of extraction solvent used in DLLME is an essential consideration for efficient extraction. The extraction solvent should have a higher density than water, an extraction capability of the interested compounds and a low solubility in water.The organic solvent usually recommended for conventional LLE extractions of Cr (VI) with APDC is methyl iso butyl ketone (MIBK), but it has high solubility in water[13], so other organic solvents were tested in this experiment. Chloroform, carbon tetrachloride, carbon disulfide and 1,2-dichloro benzene were employed and compared for chromium extraction.

A series of sample solutions were tested using 500 µL methanol containing 0.0001 g of APDC and different volume of extraction solvents to achieve a 25 µL volume of sedimented phase. Because the solubility of the extraction solvents in water was different, 70, 55, 35 and 40 µL of chloroform, carbon disulfide, carbon tetrachloride and 1,2-dichloro benzene were used, respectively. The obtained enrichment factors were found to be 132.5, 115.0, 171.0 and 140.0 for $CHCl_3$, CS_2, CCl_4 and $C_6H_4Cl_2$, respectively. Among the above result, carbon tetrachloride demonstrated the maximum enrichment factor. In addition the sediment phase of CCl_4 can easily be removed by a microsampler. On the other hand the sediment of CS_2 and $C_6H_4Cl_2$ was difficult to be removed by a microsampler. Consequently, CCl_4 was the best extraction solvent and was chosen as the extraction solvent.

To evaluate the effect of the extraction solvent volume, different volume of CCl_4 (35, 45, 55, 65, 75 and 85 µL) were used as extraction solvent to the same DLLME procedure. Fig. 1 shows the curve of enrichment factor *versus* volume of the extraction solvent (carbon tetrachloride). As it is seen in Fig. 1, enrichment factor decreases with increasing the volume of the carbon tetrachloride, because the volume of the sediment phase increased from 25-70 µL. Thereby, 35 µL CCl_4 was used as extraction volume in the subsequent experiments.

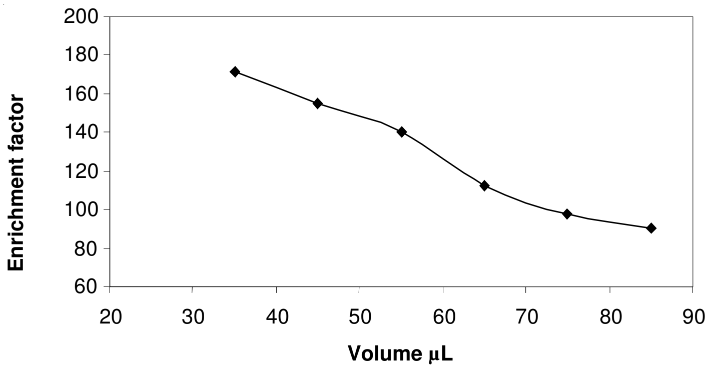

Fig. 1. Effect of the volume of extraction solvent (CCl_4) on the enrichment factor of Cr(VI) obtained from DLLME. Extraction conditions: 20 ng L^{-1} Cr (VI), sample volume; 5 mL, disperser solvent (methanol) volume; 0.5 mL, extraction solvent (CCl_4) volume; 35 µL; APDC amount 0.00010 g

Effect of nature and volume of disperser solvent: The miscibility of disperser solvent in extraction solvent and sample solution is the main criterion for the selection of the disperser solvent. Thereby, methanol, ethanol, acetone, acetonitrile and ethyl acetate were tested as disperser solvent.

The effect of these solvents on the extraction efficiency of DLLME was investigated using 500 µL of each solvent containing 35 µL of carbon tetrachloride (extraction solvent) and 0.0001 g of APDC (chelating agent). The sediment of ethyl acetate was very low, therefore ethyl acetate is not suitable as a disperser solvent in this experiment. The resulting data illustrated no statistical significant differences between disperser solvents, thus methanol was selected because the solubility of APDC in methanol makes it a better choice.

The influence of the volume of methanol on the extraction recovery was also studied. To obtain the optimized volume of methanol, a number of experiments were performed with different methanol volumes (0.25, 0.50, 0.75, 1.00, 1.50 mL), containing 0.0001 g of APDC and 33, 34, 35, 36 and 37 μL of carbon tetrachloride, respectively. The results indicated that, there was no considerable variation on the enrichment factor using 0.25 and 0.5 mL of methanol as disperser solvent. At higher methanol volumes, the decrease of enrichment factor is due to the increasing solubility of complex in water. Thus, 0.5 mL of methanol was chosen as the optimum volume.

Effect of extraction time: By definition, extraction time in DLLME is the interval time between the injection of the mixture of disperser solvent (methanol) and extraction solvent (CCl_4) before starting centrifuge.

The influence of extraction time was evaluated in the range of 0-10 min under constant experimental conditions. The results revealed that the proposed extraction method is very fast, so the extraction time has no significant effect on the extraction efficiency. In this method, the most time-consuming step was the centrifuging of sample solution in the extraction procedure. The process took about 3 min.

Effect of pH: The pH of the sample solution is an important factor in the metal-chelate formation and the subsequent extraction. The influence of pH on the complex formation and extraction of Cr(VI) from water and hair samples was studied in the pH range of 1-6. As can be seen in Fig. 2, the highest signal intensity of chromium obtained at pH 2.2. Therefore, pH 2.2 was selected for further studies. Moreover, to adjust pH 2.2, nitric acid was used.

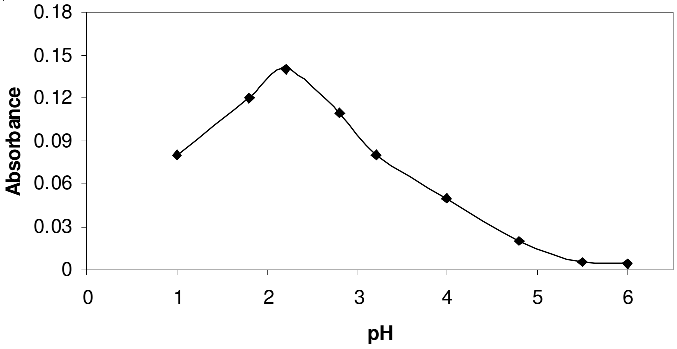

Fig. 2. Effect of pH on the absorbance of Cr (VI) after DLLME. Extraction conditions were the same as those of Fig. 1

Effect of ammonium pyrrolidine dithiocarbamate(APDC) concentration: The influence of the APDC amount on the DLLME was evaluated in the range of 10^{-5}-10^{-2} g. The results showed that the absorption was increased by increasing

APDC amount. Slight reduction of extraction at high concentration of APDC was occurred. In addition, at high amount of APDC the background absorbance was increased. Thus, the amount of 10^{-4} g of APDC was selected as the optimum amount for chromium determination to prevent any interferences.

Effect of salt: To investigate the effect of ionic strength on DLLME performance, a number of experiments were performed by adding different amount of NaCl 0-5 % (w/v), while other experimental conditions were kept constant. The results confirmed that salt addition has no significant effect on enrichment factor. Thus the proposed method can be employed for chromium separation from saline solution with a concentration up to 5 % (w/v).

Effect of the co-existing ions: The potential interferences in the present method were investigated. The effect of alkali and alkaline earth metals (Na^+, Li^+, Ca^{2+}) and transition metals (Cu^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Fe^{3+}, Ni^{2+}, Pb^{2+}) and some anions (Cl^-, NO_3^-, SO_4^{2-}) which co-existing in natural water and hair samples, on the measurement of 20 ng L^{-1} of Cr(VI) was studied. In this experiment, 5 mL of solutions containing 20 ng L^{-1} of Cr(VI) and different amount of interfering ions were treated according to the recommended procedure. The tolerance limits of the co-existing ions, defined as the maximum amount making the recovery of Cr(VI) less than 90 %. The results are summarized in Table-2.

Analytical figures of merit: The calibration graph was linear in the range of 0.1-15.0 µg L^{-1} with a correlation coefficient (r^2) of 0.9996. The limit of detection (LOD) calculated based on 3 S_b/m (where, Sb and m are the standard deviation of the blank and slop ratio of the calibration graph respectively) was 5.96 ng L^{-1} and the relative standard deviation (RSD) for 20 ng L^{-1} of Cr(VI) was 3.02 % (n = 10). The enrichment factor of 171 was obtained for only 5 mL of sample solution.

TABLE-2
EFFECT OF INTERFERING IONS ON THE RECOVERY OF 20 ng L^{-1} Cr(VI) IN WATER AND HAIR SAMPLES USING DLLME-ETAAS METHOD

Interferent	Concentration (µL^{-1})	Interferent/Cr(VI) ratio	Recovery (%)
Na^+	40,00,000	200,000,000	95.3
Li^+	200	10,000	101.2
Ca^{2+}	200	10,000	103.2
Cd^{2+}	200	10,000	93.8
Ni^{2+}	200	10,000	92.3
Cu^{2+}	200	10,000	96.5
Zn^{2+}	200	10,000	75.6
	20	1,000	95.0
Fe^{3+}	200	10,000	99.2
Al^{3+}	200	10,000	98.7
Pb^{2+}	200	10,000	79.8
	20	1,000	100.4
NO_3^-	200	10,000	103.0
Cl^-	60,00,000	300,000,000	94.3
SO_4^{2-}	2000	10,000	100.0

Analysis of water and hair samples: In order to assess the applicability of recommended procedure to real sample, the method was applied to the determination of Cr(VI) in several natural water and hair samples. Tap water, underground water, spring water and sea water were collected from the Northern Iran and hair samples were collected from habitants of Tehran and were analyzed by DLLME-ETAAS for deter-mination of Cr(VI) (Table-3). The accuracy of the method was verified by the analysis of the samples spiked with known chromium amounts. These result showed that the matrices of the tap water, underground water, spring water and sea water and hair samples had little effect on the DLLME method for determination of chromium.

TABLE-3
DETERMINATION OF Cr(VI) IN WATER AND HAIR SAMPLES AND RELATIVE RECOVERIES OF SPIKED Cr(VI) IN WATER AND HAIR SAMPLES

Samples	Cr(VI) Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$), mean ± SD[a]	Relative recovery (%)
Tap water[b]	0	n.d.[c]	–
	5	4.800 ± 0.34	97.6
	10	10.210 ± 0.21	102.1
Sea water[d]	0	1.860 ± 0.33	–
	5	6.750 ± 0.18	98.3
	10	11.710 ± 0.34	98.7
Spring water[e]	0	1.080 ± 0.14	–
	5	5.900 ± 0.35	97.0
	10	10.830 ± 0.23	97.7
Underground water[f]	0	2.130 ± 0.17	–
	5	6.880 ± 0.38	96.4
	10	11.920 ± 0.26	98.2
Human hair[g]	0	1.8300 ± 0.11	–
	5	6.590 ± 0.25	96.5
	10	11.640 ± 0.18	98.4
Human hair[h]	0	0.5350 ± 0.33	–
	5	5.270 ± 0.30	95.3
	10	10.110 ± 0.22	96.0

[a] Standard deviation (n = 3). [b] From drinking water system of Tehran, Iran. [c] Not detected. [d] Caspian sea, Iran. [e] Plour spring, Iran. [f] Soustan, Lahijan, Iran. [g] Female hair from habitant of Tehran, Iran. [h] Male hair from habitant of Tehran, Iran.

Comparison of DLLME with other methods: A comparison of the presented method with other reported preconcentration method for the Cr(VI) extraction from water samples is given in Table-4. In comparison with other reported methods, DLLME has lower LOD (5.96 ng L^{-1}), higher enrichment factor (171), shorter extraction procedure (about 3 min) and lower sample consumption (5 mL).

Conclusion

A dispersive liquid-liquid microextraction method was used for preconcentration and the determination of ultratrace amount of Cr(VI) from water and hair samples. The method is simple, rapid, inexpensive and has a lower limit of detection and

TABLE-4
COMPARISON OF THE PUBLISHED METHODS WITH THE PRESENT WORK

Mehtod	RSD (%)	EF	Do L (µg L^{-1})	Reference
SPE	–	25	5.000	[9]
CPE	3.50	42	0.021	[21]
CPE	2.13	58	0.180	[23]
Co-precipitation	–	100	0.500	[8]
SDME	7.00	70	0.020	[13]
DLLME	3.02	171	5.96×10^{-3}	Present method

higher enrichment factor than other reported methods in the literature. In this method, the sample preparation time as well as consumption of toxic organic solvents was minimized without affecting the sensitivity of the method.

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