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Research Article

SEPARATION AND PRECONCENTRATION OF ULTRA TRACE AMOUNTS OF Cr (III) IN ENVIROMENTAL WATER SAMPLES BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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ABSTRACT

Recent years have seen an upsurge of interest in developing a low-cost, easy-to-operate, reliable preconcentration technique for precise determination of ultra-trace concentrations of metal ions in aqueous matrices. Dispersive liquid-liquid microextraction (DLLME) is a novel sample-preparation technique offering high enrichment factors from low volumes of water samples.

In this microextraction technique, a mixture of 500 µL acetone (disperser solvent) containing the aqueous solution of 1% (v/v) ligand **A** (chelating agent) and 35 µL carbon tetrachloride (extraction solvent), was rapidly injected by syringe into the 5.00 mL water sample, spiked with chromium. In this process, the chromium ions reacted with the chelating agent and were extracted into the fine droplets of CCl_4. After centrifugation (2.5 min at 5000 rpm), the fine CCl_4 droplets were sedimented at the bottom of the conical test tube (50 ± 1 µL). Then, 20 µL from the sedimented phase, containing the enriched analyte,was determined by ET AAS. The next step was the optimization of various experimental conditions, affecting DLLME, such as the type and the volume of the extraction solvent, the type and the volume of the disperser solvent, pH, the extraction time, the salt effect, the sample volume and the ligand **A** concentration (the chelating agent amount). Moreover, the effect of the interfering ions on the analytes recovery was also investigated. Under the optimum conditions, the enrichment factor of 146 was obtained from only a 5.00 mL water sample. The calibration graph was linear in the range of 0.1–5 µg L^{-1} with the detection limit of 0.02 µg L^{-1}. The relative standard deviation (R.S.D.) for six replicate measurements of 0.50 µg L^{-1} of chromium was 1.8%. The relative chromium recoveries in mineral, tap, well and sea water samples at the spiking level of 0.50 µg L^{-1} varied from 96 - 102.

Keywords: *Dispersive Liquid–liquid Microextraction; Sample Preparation; Chromium; Graphite Furnace Atomic Absorption Spectrometry; Preconcentration*

INTRODUCTION

Traditional solvent extraction has been used as a basic and powerful method of concentrating for a long time. However, it requires extensive amounts of organic solvents. A special attention is nowadays focused on techniques, which are characterized by a considerable reduction or complete elimination of organic solvents. Such techniques protect the environment against additional quantities of solvents and reduce the cost of analysis. The solvent-free techniques such as gas phase extraction, membrane extraction and solid phase extraction are limited to volatile and relatively volatile organic compounds, volatile nonpolar compounds and relatively low volatile compounds, respectively. In 1996, Liu and Dasgupta used 1.3 µL chloroform as extraction reagent, thus decreased the environmental pollution greatly throughout the analytical procedure. Meanwhile, Jeannot and Cantwell introduced the single-drop microextraction (SDME) technique and these authors investigated a convective–diffusive mass transfer model to interpret the kinetic procedure in the novel SDME technique. Pedersen-Bjergaard and Rasmussen reported a microextraction method with a polypropylene hollow fiber as support for the extraction of methamphetamine from urine and plasma. The acceptor solution (0.1 mol L^{-1} HCl) inside the hollow fiber was analyzed by capillary electrophoresis (CE). The enrichment factor (EF) reached 75 times. Lee and co-workers combined the hollow fiber membrane–liquid phase microextraction (HFM–LPME) with GC (6) or HPLC to separate and determine aromatic compounds in various samples. Wu and co-workers developed a method that combined SDME with ETV-ICP-OES/MS for the determination of trace La, Be, Co, Pb and Cd and for speciation of Al. Li and co-workers determined trace Cd and Pb in environmental and biological samples by ETV-ICP-MS after single-drop microextraction.

Another extraction procedure, namely homogeneous liquid–liquid extraction, which extracts the target analyte in the homogeneous aqueous solution into a water-immiscible phase, was used by each kind of phase separation phenomenon. The methods, which used phase separation principle, depending on the temperature of the water–propylene carbonate system or the salt effect in the water–acetonitrile system have already been reported. Igarashi and co-workers reported a pH-dependent phase separation and used it in determination of copper and palladium as 5, 10, 15, 20-tetrakis (4*n*-pyridyl) porphine chelate by HPLC and spectrophotometric techniques. This method was mainly studied as a highly efficient preconcentration method for the separation of the target analyte before instrumental analysis.

Another microextraction technique, which uses extraction solvent at µL volume, is dispersive liquid–liquid microextraction (DLLME). It is simple, rapid, efficient and has a higher EF and recovery (R). It was first reported in 2006 by Assadi and co-workers. This method uses an extracting solvent dissolved in a dispersive solvent, which is miscible with both extraction solvent and water. Methanol, acetone, acetonitrile, etc., have been used as dispersive solvents. Recently we reported a DLLME method to preconcentrate some polymer additives followed by their determination by HPLC-DAD. This technique is based on dispersion of tiny droplets of the extraction solvent within the aqueous solution by the aim of a water miscible solvent which is called disperser. This method is a promising alternative to the classic liquid-liquid extraction (LLE) that uses microliter volume of the extraction solvent. DLLME is simple, rapid and inexpensive and provides high enrichment factors (EF). This technique has been applied for the extraction of several bio chemicals and organic and inorganic compounds.

In this work a (E)-2-(((4-methylpyridin-2-yl) imino) methyl) phenol is applied to the DLLME of Cr in aqueous solutions. This ligand forms selective and stable complexes with Cr.

No applications of (E)-2-(((4-methylpyridin-2-yl) imino) methyl) phenol ligands for the DLLME of Cr have been reported so far.

MATERIALS AND METHODES

Apparatus and Analysis

A graphite furnace atomic absorption spectrometry (PG 990, UK) with a deuterium lamp background Correction was used for the determination of chromium. Condensed PG graphite tubes of the wall atomization type were used for the chromium atomization. A PG hollow-cathode lamp was employed as the radiation source under the manufacturer's recommended conditions. All measurements were performed using the peak height.

For pH determinations, a Metrohm model 827 pH meters with a combined glass electrode was used after calibration against standard Merck buffers. A Centrifuge (Hermle model: Z200A, Germany) was used for centrifugation. Screw cap 15 mL falcon test tubes with conical bottom were used as extraction and centrifugation vessels.

Chemicals and Reagents

Stock standard solution (1000 g mL^{-1}) of $Cr(NO_3)_3$ was obtained from the National Institute of Standards Merck, Germany). Working standard solutions were obtained by appropriate dilution of the stock standard solution. All other reagents used were of the highest available purity and of at least analytical reagent grade (Merck, Germany). Doubly distilled water, prepared by a totally glass Fisons (UK) double distiller, was used throughout. Pipettes and vessels in the experiments were kept in 10% nitric acid for at least 24 h and subsequently washed four times with double distilled water.

Synthesize of the (E)-2-(((4-methylpyridin-2-yl) imino) methyl) phenol

Place 1.08 g (10 mmol) 2-amin-4-methyl- pyridine, 1.22 g (10 mmol) salicylaldehyde and 15 mL ethanol in a 50 mL round bottomed flask attached to a reflux condenser. The mixture was heated under reflux condition for 4 hours and the bright yellow precipitate was collected by filtration and washed thoroughly with ethanol; the yield is 46%. Figure 1 show structure of the (E)-2-(((4-methylpyridin-2-yl) imino) methyl) phenol (ligand A).

Dispersive Liquid-liquid Micro Extraction Procedure

The extraction of Cr(III) from an aqueous sample into a micro volume of the organic solvent (carbon-tetrachloride) was made by complexation with the ligand **A**. Five milliliters of ultra pure water were placed in the test tube with a conical bottom and were spiked at the chrumium concentration of 0.5µg L^{-1}.

A mixture of 35µL of carbon tetrachloride (extraction solvent) and 500 µL of acetone (disperser solvent) containing the aqueous solution of 1% (v/v) ligand **A** (chelating agent), were injected rapidly into a sample solution with a 0.50 mL syringe. A cloudy solution (water, acetone and carbon tetrachloride) was formed in the test tube. In this step, the chrumium ions reacted with chelating agent and were extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged for 2.5 min at 5000 rpm. After this process, the dispersed fine droplets of carbon tetrachloride were sedimented at the bottom of the conical test tube (50 ± 1 µL). Exactly fine droplets were injected into the GFAAS instrument for analysis.

RESULTS AND DISCUSSION

In order to obtain a high-enrichment factor, the effect of different parameters, influencing the complex formation and the extraction conditions, were optimized. These parameters were the type and the volume of the extraction solvent, the type of the disperser solvent, the disperser solvent volume**,** pH, the extraction time, the salt, the sample volume, the ligand **A** concentration (chelating agent concentration) and the interference studies. Finally, these optimal conditions were applied to extract and detect chrumium in the real water samples.

Eq. (1) was used for the calculation of the enrichment factor. $EF = C_{sed}/C_0$ (1)

where EF, C_{sed} and C_0 are the enrichment factor, the analyte concentration in the sedimented phase and the initial analyte concentration in the aqueous sample, respectively. C_{sed} was calculated from the calibration graph obtained by the conventional.

Effect of Type and Volume of the Extraction Solvent

Extraction solvent should have special characteristics; it should have higher density rather than water, high efficiency in the extraction of the interested compounds and low solubility in water.

Chloroform, carbon tetrachloride were examined in the extraction of chromium. A series of sample solution were studied by using 0.50mL from the aqueous solution of 1% (v/v) ligand **A** and different volumes of extraction solvent to achieve 25 µL volume of the sedimented phase. Thereby, 35, 50 and 75 µL of chloroform and carbon tetrachloride were used, respectively.

Chloroform and carbon tetrachloride as extraction solvents resulted enrichment factors of 73 and 70, respectively. The results are not statistically significantly different. In comparison between these two extraction solvents, carbon tetrachloride forms a well stable cloudy solution; sedimented phase can easily be removed by sampler to be introduced into the graphite furnace and has less consumption volume to obtain the 25 µL sedimented phase. Therefore carbon tetrachloride was the best to be used.

To examine the effect of the extraction solvent volume, solutions containing different volumes of carbon tetrachloride were subjected to the same DLLME procedures. The experimental conditions were fixed and included the use of 0.50mL from the aqueous solution of 1% (v/v) ligand **A** and different volume of carbon tetrachloride (35, 45, 55, 65, 75 and 85 µL). By increasing the volume of carbon tetrachloride from 35 to 85 µL, the volume of sedimented phase increases from 25 to 67 µL.

Figure 1 shows the curve of enrichment factor versus volume of the extraction solvent (carbon tetrachloride). According to Figure 1, enrichment factor decreases with increasing the volume of carbon tetrachloride, because the volume of the sedimented phase increases. Subsequently, at low volume of the extraction solvent high enrichment factor was obtained. Thereby, as shown in Figure 2 the gain in sensitivity was achieved by using 35 µL of carbon tetrachloride.

Selection of the Disperser Solvent

The main criterion for the selection of the disperser solvent is its miscibility in the organic phase (extraction solvent) and the sample. Acetone, acetonitrile, methanol and ethanol were tested for this purpose. A series of sample solutions were studied using 0.50mL from each disperser solvent, containing 35 µL of carbon tetrachloride (extraction solvent) and the use of 0.50mL from the aqueous solution of 1% (v/v) ligand **A**. The enrichment factors obtained for acetonitrile, acetone, methanol and ethanol were 145.5±2.1, 146.3±3.4, 151.1±4.2 and 134.8±3.6, respectively. The resulting data illustrated no significant statistical differences among the disperser solvents, apart from ethanol that presented the lowest enrichment factor. Thus, acetone was selected because of its low toxicity and cost.

Effect of the Disperser Solvent Volume

After choosing acetone as the disperser solvent, it was necessary to optimize its volume. For defining the optimal acetone volume, various experiments were performed with different acetone volumes (0.25, 0.50, 0.75, 1.0, 1.5 and 2 mL), containing 34.0, 35.0, 36.0, 40.0, 45.0 and 50.0µL of carbon tetrachloride, respectively. It was required to change the CCl_4 volume by changing the acetone volume in order to obtain a constant volume of the sedimented phase (25±1µL) in all experiments. Under these conditions, the volume of the sedimented phase remained constant (25±1µL). The results showed that there was no considerable variation on the enrichment factor using 0.25 and 0.50mL of acetone as disperser solvent. The enrichment factor slightly decreased, when the acetone volume exceeded 0.50 mL. It was clear that with the increase of the acetone volume, the complex solubility in water increased. Therefore, the extraction recovery diminished.

Accordingly, the acetone volume of 0.50mL was chosen as the optimum one for the achievement of a better and more stable cloudy solution.

pH Effect

The extraction of chromium ions by DLLME involves prior formation of a complex with sufficient hydrophobicity to be extracted into small volume of extraction phase, thus obtaining the desired pre-concentration. Extraction yield depends on the pH at which complex formation is carried out. The effect of pH on the complex formation and extraction of chromium from water samples was studied within the range of pH 1–9 by using HCl, CH_3COOH, H_3PO_4, H_3BO_3 and NaOH. The results illustrated in Figure 2 show that the recovery percentage is nearly constant in the range pH of 1–5 and pH 2.5 was chosen in all experiments. Because pH plays a unique role in the metal-chelate formation and the subsequent extraction, the pH of the sample solution was the next critical factor evaluated for its effect on the DLLME preconcentration of chromium. Figure 3 summarize the results.

Effect of the Extraction Time

The influence of the extraction time was examined in the range of 0–60 min with the experimental conditions remaining constant. The results displayed that the extraction time had no notable effect on the enrichment factor. It was revealed that after the formation of the cloudy solution, the surface area among the extraction solvent and the aqueous phase was essentially large. As a consequence, the chromium complex formation and its transfer from the aqueous phase to the extraction solvent were fast. This fact was one of the advantages of the DLLME technique.

Salt Effect

For investigating the influence of the ionic strength on the DLLME performance, several experiments were performed by adding varying NaCl amount from 0% to 5% (w/v). The rest of the experimental conditions were kept constant. The resulting data disclosed that the salt addition from 0% to 5% had no significant impact on the enrichment factor. These observations offered the possibility of utilizing this method for the chromium separation from saline solutions up to 5%.

Sample Volume

Different volumes of analyte solution (1–50 mL) were used to study the effect of sample size with a constant volume of extracting solvent (35 µL) and dispersive solvent (0.5 mL). The results (Figure 3) show that with a large sample size (50 mL) no sedimented organic phase was obtained. For this purpose, the acetone volume was selected 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 4 and 5mL when the volume of sample was 1, 2, 3, 4, 5, 10, 20, 40 and 50 mL, respectively. We used 0.5 mL acetone and a sample size of 5 mL in the further studies due to higher recovery and reasonable EF and sedimented phase volume. Figure 4 summarize the results.

Effect of the Ligand A Concentration

The next influential factor, whose effect on the factor was studied, was the ligand **A** amount. This study was conducted in the ligand **A** concentration range of 0.1 – 5% (v/v).

In this case, the enrichment factor increased with the increase of the ligand **A** amount up to 0.5% (v/v), reaching a plateau. A concentration of 1% (v/v) in 5.00mL of the aqueous solution was chosen as the optimum amount for the chromium determination to prevent any interference.

Interference Studies

Most common matrix constituents of real samples such as alkali and alkaline earth elements do not react with the (E)-2-(((4-methylpyridin-2-yl) imino) methyl) phenol (ligand **A**) because of its selectivity. However, large amounts of metal ions which react with ligand **A** appreciably reduce the efficiency of chromium extraction. The effects of common coexisting ions in natural water samples on the extraction recovery of chromium were studied. In these experiments, 5.00 mL of solutions contains 0.5μgL^{-1} of chromium and various amounts of interfering ions were treated according to the recommended procedure. A given spices was considered to interfere if it resulted in a ±5% variation of the GF AAS signal. The results obtained are given in Table 1.

Analytical Figures of Merit

Table 2 summarizes the analytical characteristics of the optimized method, including the linear range, the limit of detection, the reproducibility and the enrichment factor. The calibration graph was linear in the chromium concentration range of 0.1–5 μgL^{-1}. The detection limit (calculated as three times the standard deviation of 6 blank measurements, divided by the slope of the calibration curve) for the chromium ions was found to be 0.02 μgL^{-1}. Concerning the reproducibility of the method, it was evaluated with 5.00 mL from the solution, containing the analyte ions in the Cr (III) concentration of 0.5 μgL^{-1}. The relative standard deviation (R.S.D.) for 6 replicate measurements was 1.8%. Finally, the high-enrichment factor of 146 was obtained for only a 5.00mL water sample.

Figure 1: Structure of the (E)-2-(((4-methylpyridin-2-yl) imino) methyl) phenol (ligand A)

Table 1: Effect of interferents on the recovery of 0.50 μg L−1 Cr(III) in water sample using DLLME-ET AAS

Interferent	**Concentration (μg L^{-1})**	**Interferent/Cr(III) ratio**	**Recovery (%)**
Na^+	1,000,000	2,000,000	101.0
Li^+	5,000	10,000	101.4
K^+	5,000	10,000	105.4
aC(II)	5,000	10,000	100.3
Mg(II)	5,000	10,000	95.2
Ba(II)	5,000	10,000	102.0
Bi(III)	500	1,000	94.6
Mn(II)	500	1,000	97.1
Co(II)	500	1,000	101.4
Al(III)	500	1,000	100.0
Fe(II)	500	1,000	100.0
Ni(II)	500	1,000	94.0
Sn(IV)	500	1,000	96.3
Zn(II)	500	1,000	100.0
Cr(III)	500	1,000	96.7
Ag(I)	500	1,000	96.9
Cd(II)	500	1,000	94.6
Cu(II)	500	1,000	96.6
Si(II)	500	1,000	97.8
Se(II)	500	1,000	97.9
Hg(II)	500	1,000	104.4
Cl^-	1,000,000	2,000,000	94.2
$NO3^-$	5,000	10,000	105.8
$CH3COO^-$	50,000	100,000	94.8
SCN^-	5,000	10,000	94.5
$SO4^{--}$	5,0000	10,0000	100.0

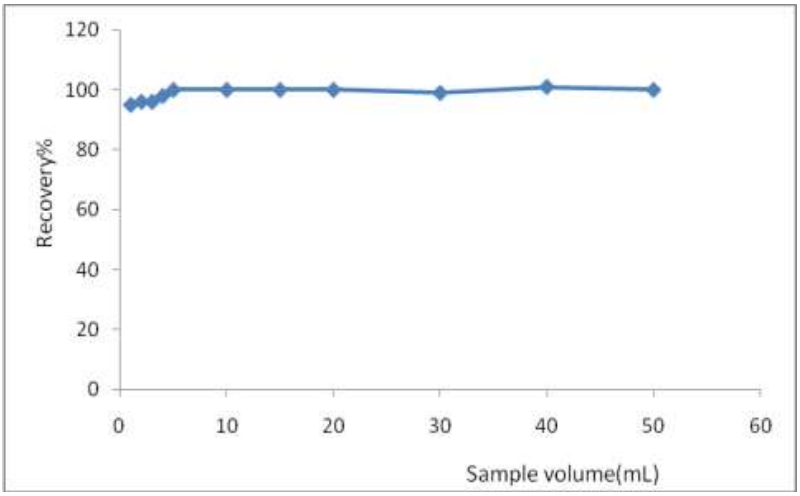

Figure 2: Effect of the volume of extraction solvent (CCl_4) on the enrichment factor of chromium obtained from DLLME

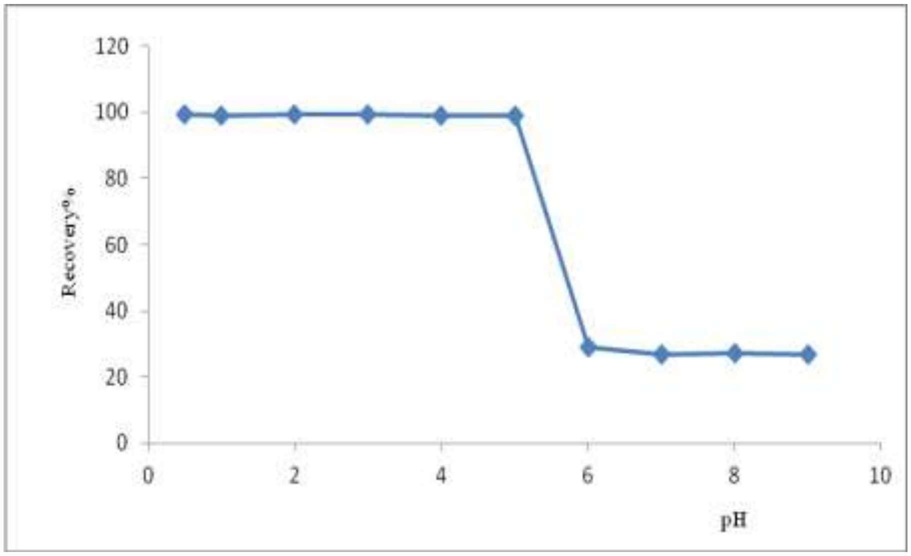

Figure 3: Effect of pH on the recovery percentage of chromium obtained from DLLME. Extraction conditions, as with Figure 2; extraction solvent (CCl_4) volume, 35 µL

Table 2: Analytical characteristics of DLLME-ET AAS for determination of Cr(III)

Parameter	Analytical feature
Linear range (µgL^{-1})	0.1-5
r^2	0.9996
Repeatability (R.S.D.^{a},%) (n=6)	1.8
Limit of detection (µgL^{-1}) (3σ,n=6)	0.02
Enrichment factor	146
Sample volume (mL)	5.00
Sample preparation time (sec)	150

[a] *Chromium concentration was 0.5 µgL^{-1} for which R.S.D. was obtained.*

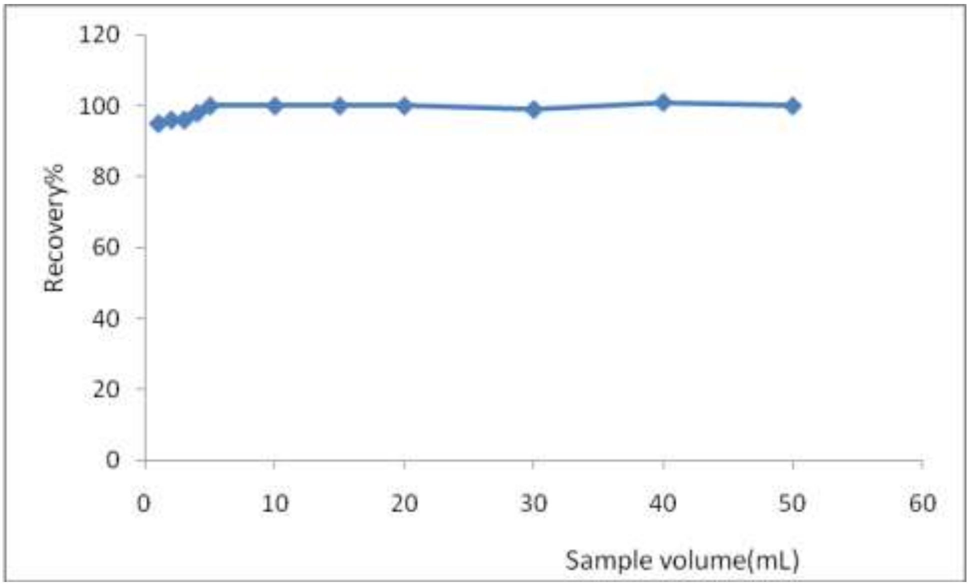

Figure 4: Effect of sample size on the Recovery in the extraction of Cr(III) ions by DLLME. Other conditions are the same as Figure 3

Table 3: Determination of Cr(III) in tap, sea and river water samples and relative recovery of spiked chromium in tap, sea and river water samples

Sample	**Concentration of Cr(III), μ g L^{-1}**	**Added Cr(III) , μ g L^{-1}**	**Found, μ g L^{-1}**	**Relative recovery/%**
Tap water [a]	n.d.[d]	0.50	0.50	100
River water [b]	n.d.[d]	0.50	0.51	102
Sea water [c]	n.d[d]	0.50	0.48	96

[a] *From the drinking water system of Mahshahr, Iran.*

[b] *Jarahie river water, South of Iran.*

[c] *Persian golf*

[d] *Not detected*

Application to Real Samples

The described DLLME–GF AAS technique was applied for the determination of chromium in several water samples. Tap, river and sea water were collected from the South of Iran and were analyzed by DLLME combined with GF AAS for determination of chromium. In order to study the effects of real sample matrix on the recovery of spiked samples, in further investigation, it was found that the addition of EDTA at the level of 200 mg L^{-1} as a masking agent in pH 2.5 can reduce interferences of metal ions. The concentration of chromium was calculated as the difference. The concentration of chromium in the tap, sea and river water samples was lower than the limit of detection of our technique (not detected, as shown in Table 3). Tap, river and sea water samples were spiked with chromium standards to assess matrix effects in the presence of EDTA. The relative recoveries for above species in tap, river and sea water at spiking level of 0.50 μg L^{-1} are listed in Table 3.

Conclusion

DLLME combined with the electrothermal atomic absorption spectrometry (ET AAS) was evaluated for the separation, the preconcentration and the determination of the ultra trace amounts of chromium (at sub-μg L^{-1} level) from water samples. DLLME proved to be a fast simple, inexpensive and reproducible technique for the determination of trace metals with the use of low sample volumes. The high-preconcentration factor and the low sample volume requirements were the major advantages of the technique. An enrichment factor of about 146 times was attained with only a 5.00 mL from the sample. In this method, the sample preparation time (less than 3 min) as well as the consumption of the toxic organic solvents (at micro liter level) was minimized without affecting the method sensitivity. Although the obtained results of this research were related to the chromium determination, the system could be readily applied to the determination of other metals with the help of various chelating agents, extractable by other organic solvents.

Extraction conditions: water sample volume 5.00 mL; disperser solvent (acetone) volume, 500 μL; pH 2.5; concentration of chromium 0. 5 μgL^{-1}.

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