Talanta 83 (2011) 885–890

Contents lists available at ScienceDirect

Talanta

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

Optimization of dispersive liquid–liquid microextraction coupled with inductively coupled plasma-optical emission spectrometry with the aid of experimental design for simultaneous determination of heavy metals in natural waters

Hassan Sereshti*, Vahid Khojeh, Soheila Samadi

School of Chemistry, University College of Science, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:
Received 13 August 2010
Received in revised form 22 October 2010
Accepted 28 October 2010
Available online 4 November 2010

Keywords:
Heavy metals
Dispersive liquid–liquid microextraction
Inductively coupled plasma-optical emission spectrometry
Sodium diethyldithiocarbamate
Experimental design

ABSTRACT

In this study, dispersive liquid–liquid microextraction (DLLME) combined with inductively coupled plasma optical emission spectrometry (ICP-OES) was developed for simultaneous preconcentration and trace determination of chromium, copper, nickel and zinc in water samples. Sodium diethyldithiocarbamate (Na-DDTC), carbon tetrachloride and methanol were used as chelating agent, extraction solvent and disperser solvent, respectively. The effective parameters of DLLME such as volume of extraction and disperser solvents, pH, concentration of salt and concentration of the chelating agent were studied by a (2^{f-1}) fractional factorial design to identify the most important parameters and their interactions. The results showed that concentration of salt and volume of disperser solvent had no effect on the extraction efficiency. In the next step, central composite design was used to obtain optimum levels of effective parameters. The optimal conditions were: volume of extraction solvent, 113 μL; concentration of the chelating agent, 540 mg L^{-1}; and pH, 6.70. The linear dynamic range for Cu, Ni and Zn was 1–1000 μg L^{-1} and for Cr was 1–750 μg L^{-1}. The correlation coefficient (R^2) was higher than 0.993. The limits of detection were 0.23–0.55 μg L^{-1}. The relative standard deviations (RSDs, $C = 200$ μg L^{-1}, $n = 7$) were in the range of 2.1–3.8%. The method was successfully applied to determination of Cr, Cu, Ni and Zn in the real water samples and satisfactory relative recoveries (90–99%) were achieved.

1. Introduction

In recent years, pollution of the environment by toxic elements has been dramatically increased; therefore, the determination of toxic metals such as chromium, copper, nickel and zinc in environmental samples is a very important task. The toxicity of chromium depends critically on its oxidation state. Chromium (III) is able to coordinate with several amino acids in the human body. It is also frequently supplied as a dietary complemented. On the other hand, chromium (VI) is known to be toxic to humans, capable of permeating cell membranes, being a powerful mutagen and potential carcinogen. Copper is an essential trace element, a component of crustacean respiratory pigments like hemocyanin, and is commonly present in the coastal marine environment, where it may reach toxic levels to both animals and plants. Increase in copper concentration in coastal areas have resulted from industrial and domestic waste discharge, disposal of mining washings, refineries and the use of the heavy metal as a base compound for antifouling paints. Thus, the monitoring of extremely low levels of copper in natural waters has received special attention as a way to assess the early impact of human sources and detect potential health hazards. Interest in zinc concentrations in environmental waters from its dual role as a required nanonutrient and potential toxicant due to its widespread industrial (viz. metallurgy, galvanizing and alloy manufacturing) and marine usage is recently shown. In the case of surface seawater the major Zn inputs include atmospheric deposition (both natural and anthropogenic origin), fluvial, run off and upwelled waters. Interest in the determination of nickel has been increased over the last few years because of its influence on human body. Adverse effects of water soluble inorganic nickel species occur when contact with the skin. After inhalation, it causes nickel dermatitis, respiratory tract irritation and asthma [1].

The trace elements level in natural water samples are usually lower than the detection limit of flame atomic absorption spectroscopy (FAAS), inductively coupled plasma-optical emission spectrometry (ICP-OES) and graphite furnace-atomic absorption spectrometry (GF-AAS) [2–5], therefore, due to analyte low con-

---

* Corresponding author. Tel.: +98 21 61113632; fax: +98 2166495294.
E-mail address: sereshti@khayam.ut.ac.ir (H. Sereshti).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.10.052

Table 1
Instrumental parameters of ICP-OES and emission lines of the elements.

Parameter	Value
RF generator power (kW)	1.3
Plasma gas flow rate (L min^{-1})	15
Auxiliary gas flow rate (L min^{-1})	1.5
Nebulizer pressure (kPa)	150
Torch mode	Axial
Analytical lines (nm)	Cr (267.716), Cu (324.754) Ni (231.604), Zn (213.857)

centration and matrix effects they often require a suitable sample pretreatment step (preconcentration and/or separation).

Several preconcentration and separation techniques such as solid phase extraction (SPE), co-precipitation and liquid–liquid extraction (LLE) of the metals from environmental samples have been developed [6–8]. These methods have some disadvantages such as high time consumption, significant chemical additives, solvent losses, large secondary wastes along procedure, and complex equipment. Solvent microextraction techniques such as dispersive liquid–liquid microextraction (DLLME) effectively overcomes these problems by reducing the amount of organic solvent as well as allowing sample extraction and preconcentration to be done in a single step.

DLLME that was introduced by Assadi and co-workers is a modified solvent extraction method and its acceptor-to-donor phase ratio greatly reduced comparing with other extraction methods. Simplicity of the operation, rapidity, low sample volume, low cost and high enrichment factor are the main advantages of this method [9]. DLLME has been widely used for extraction of organic compounds such as polycyclic aromatic hydrocarbons (PAHs) organophosphorus pesticides, chlorobenzenes [9–11] and metal ions such as cobalt, lead and palladium in water samples [12–14].

In this work, DLLME coupled with ICP-OES was applied for preconcentration and determination of trace amounts of Cu, Cr, Ni and Zn. Sodium diethyldithiocarbamate (Na-DDTC) which has numerous applications in separation and preconcentration of trace elements, was selected as the chelating agent [15,16]. The effect of various parameters affecting the extraction efficiency such as volume of extraction and disperser solvents, pH, concentration of salt and concentration of the chelating agent were studied simultaneously and optimized by experimental design to achieve high recovery.

2. Experimental

2.1. Instrumentation

An inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista-MPX, Australia) equipped with a slurry nebulizer and a charge coupled device (CCD) detector was used for determination of metal ions in this study. The instrument parameters and emission lines of each element are shown in Table 1. The values of pH were measured with a Metrohm pH-meter (Model: 691, Switzerland) with a combined glass electrode. Centrifuges were performed by a HERMEL-Z 200 A (Wehingen-Germany).

2.2. Reagents and solutions

All chemicals, such as chloroform, carbon disulfide, carbon tetrachloride, methanol, ethanol, acetone, sodium chloride, with the purity higher than 99%, HNO_3 (65%, suprapure), NH_4OH (25%, suprapure) were purchased from Merck (Darmstadt, Germany). The solutions of the chelating agent were prepared daily by dissolving appropriate amounts of Na-DDTC (Hopkin & Williams) in double distilled water. Stock solutions (1000 mg L^{-1}) of Cr, Cu, Ni and Zn were purchased from Merck (Darmstadt, Germany). Dilute solutions were prepared by appropriate dilution of stock solutions in double distilled water. The laboratory glassware was kept overnight in 1 mol L^{-1} HNO_3 solution and subsequently washed with double distilled water before applications. River, sea and tap water samples, used for development of the method were collected in PTFE tubes from the north region of Iran, and their pH were adjusted to 1 using HNO_3. The following certified reference materials were used: CASS.4 seawater and river water reference material SLRS-4 (National Research Council of Canada, Ottawa, Ontario) and CRM-TMDW-500 drinking water (High-Purity Standards Inc).

2.3. Preconcentration procedure

At first, 10 mL of sample solution containing Cr, Cu, Ni and Zn adjusted to pH 6.70 using aqueous solution of ammonium (2.5% v/v), was placed in a 12 mL screw cap glass test tube with conic bottom. Then, 1 mL of Na-DDTC solution containing 540 mg L^{-1} of Na-DDTC was added into the sample solution. In this step, the metal ions reacted with Na-DDTC to form the related complexes. 1.00 mL of methanol (disperser solvent) containing 113 μL carbon tetrachloride (extraction solvent), was injected rapidly into the sample solution using a 2.0 mL syringe. A cloudy solution was formed in the test tube. In this step, metal ion complexes in the water sample were extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged for 5 min at 3500 rpm. The dispersed fine droplets of carbon tetrachloride were sedimented at the bottom of the test tube. This sediment phase was quantitatively transferred to another test tube and dried by an oven in 90 °C. Finally, the residue was dissolved in 1 mL (1 mol L^{-1}) HNO_3 and the concentration of the metal ions were determined by ICP-OES.

3. Results and discussion

3.1. Selection of extraction and disperser solvents

The extraction solvent was selected based on higher density than water, extraction capability for the compounds of interest, and low solubility in water [17]. Several solvents such as chloroform (density: 1.48 g mL^{-1}), carbon disulfide (density: 1.26 g mL^{-1}), carbon tetrachloride (density: 1.59 g mL^{-1}), were tested to choose a suitable extraction solvent. On the other hand, the most important point for selection of disperser solvent is the miscibility of it in organic phase (extraction solvent) and aqueous phase (sample solution). Thereby, methanol, ethanol and acetone were selected for this purpose. The results indicated that the maximum recovery was achieved by using methanol-carbon tetrachloride as disperser–extractor solvents pair (Fig. 1). The recovery of the analytes was 90% for Cr, 86% for Cu, 79% for Ni and 90% for Zn.

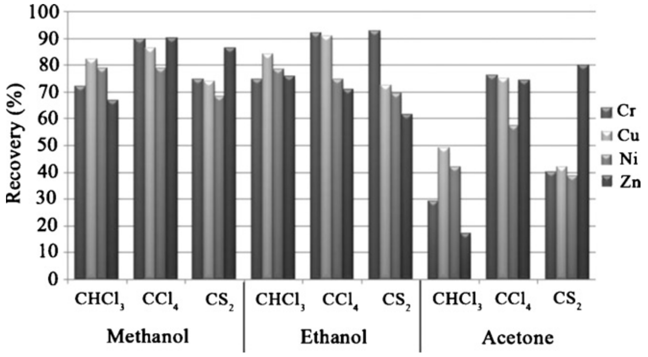

Fig. 1.

Table 2
Independent variables, their symbols and levels for a fractional factorial (2^{5-1}) design.

Variable	Effect symbol	Variable levels	
		−1	1
Volume of extraction solvent (CCl_4) (μL)	E	75	150
Volume of disperser solvent (methanol) (mL)	D	0.75	1.5
Salt concentration (w/v %)	S	0.0	5.0
pH	P	3	7
Concentration of chelating agent (μg L^{-1})	L	500	1500

3.2. *Optimization of the main microextraction parameters*

3.2.1. *Factorial design*

Factorial designs at two levels are mainly used for screening, which is to determine the influence of a number of effects on a response, and to eliminate those that are not significant [18]. In order to reduce the number of required experiments, a half-fractional factorial design (2^{5-1}) with 16 experiments was executed. The main factors, their symbols and levels are shown in Table 2. The levels of the factors were chosen based on the previous experiments. The experiments were divided into two blocks with eight experiments in each block and were run in a random manner in order to minimize the effect of uncontrolled variables [19]. The design matrix and the results are shown in Table 3. To perceive the most important effects and interactions analysis of variance (ANOVA) was used and the following information was obtained. The model *F*-value of 34.83 implies that the model is significant and there is only a 2.82% chance that a “model *F*-value” this large could occur due to noise. The three main effects E, P and L, and two two-factor interaction effects, PL and EP, were the most significant model terms. Therefore, the three factors E, P and L were to be used in the next step of the design. Considering the effect of each factor individually, it was concluded that decreasing volume of the extraction solvent, increasing pH level and increasing concentration of the chelating agent resulted into rise in the extraction efficiency. However, the effect of volume of disperser solvent and concentration of salt on the response was not considerable.

Table 3
Design matrix and the responses for a fractional factorial (2^{5-1}) design.

Block	Run No.	E	D	S	P	L	Recovery (%)
1	1	1	−1	1	1	−1	51.74
1	2	1	1	1	−1	1	53.32
1	3	−1	−1	−1	−1	−1	52.92
1	4	1	1	−1	−1	−1	35.81
1	5	−1	−1	1	−1	1	55.83
1	6	1	−1	−1	1	1	65.04
1	7	−1	1	1	1	−1	58.38
1	8	−1	1	−1	1	1	65.36
2	9	1	1	1	1	1	59.69
2	10	1	−1	−1	−1	1	69.74
2	11	−1	1	1	−1	−1	59.04
2	12	−1	−1	1	1	1	72.33
2	13	1	−1	1	−1	−1	29.38
2	14	−1	−1	−1	1	−1	75.29
2	15	1	1	−1	1	−1	80.75
2	16	−1	1	−1	−1	1	57.23

Table 4
Factors, their symbols and levels for the central composite design.

Variable	Symbol	Levels				
		−*a*	−1	0	1	+*a*
Volume of extracting solvent (μL)	E	58	75	100	125	140
Concentration of chelating agent (μg L^{-1})	L	160	500	1000	1500	1840
pH	P	1.6	3	5	7	8.34

3.2.2. *Central composite design*

In the next step, a rotatable, orthogonal central composite design (CCD) was employed to determine the optimal conditions for the critical factors. This design is a combination of a two-level full factorial design, a star design, and the points at the center of the experimental region. Center points (N_0) are usually repeated to get a good estimate of experimental error (pure error) [19]. A CCD is made orthogonal and rotatable by the choice of a suitable axial point, “*a*”, for the star design. The value of “*a*” needed to be ensure orthogonality and rotatability can be calculated from Eq. (1).

$$a = \sqrt[4]{N_f} \qquad (1)$$

where $N_f = 2^f$ is the number of factorial points. Using Eq. (1) the axial spacing was ±1.682. Then, N_0 was obtained using Eq. (2) equal to 9.

$$a = \sqrt{\frac{\sqrt{(N_f + N_a + N_0)N_f} - N_f}{2}} \qquad (2)$$

where N_a (=2*f*) is the number of axial points. The total number of experimental runs (*N*) is obtained by Eq. (3) equal to 23, where *f* is the number of variables.

$$N = 2^f + 2f + N_0 \qquad (3)$$

The experiments were divided into three blocks. The main factors, their symbols and levels are shown in Table 4 and the design matrix with the responses is shown in Table 5.

To evaluate the model and the significance of the effects, analysis of variance (ANOVA) was considered (Table 6). The *F*-value of 8.12 implies the model is significant. There is only a 0.50% chance that a “Model *F*-value” this large could occur due to noise. The second order polynomial with the most reasonable statistics, that is, higher *F*- and *R*-values and low standard error were considered as the satisfactory response surface model to fit the experimental data. This model is shown in Eq. (4), consisted of three main effects, three two-factor interaction effects and three curvature effects, where b_0

Table 5
Design matrix and responses for central composite design.

Run	Block	E	L	P	Recovery (%)
1	1	100	1000	5	77
2	1	100	1000	5	54
3	1	125	1500	3	66
4	1	100	1000	5	72
5	1	75	500	3	25
6	1	75	1500	7	58
7	1	125	500	7	82
8	2	100	1000	5	75
9	2	75	1500	3	59
10	2	125	1500	7	59
11	2	100	1000	5	73
12	2	125	500	3	20
13	2	75	500	7	56
14	2	100	1000	5	73
15	3	100	1000	5	68
16	3	100	1000	8.34	63
17	3	58	1000	5	50
18	3	100	1000	5	59
19	3	100	160	5	62
20	3	100	1000	1.6	20
21	3	140	1000	5	78
22	3	100	1840	5	45
23	3	100	1000	5	75

Table 6
Analysis of variance (ANOVA) for central composite design.

Source	Sum of squares	df [a]	Mean square	*F* value [b]	*p*-value, prob > *F* [c]	
Block	74.1184	2	37.0592	0.6108	0.5695	Significant
Model	6404.6306	13	492.6639	8.1206	0.0050	
E	379.2086	1	379.2086	6.2505	0.0410	
L	155.9797	1	155.9797	2.5710	0.1529	
P	901.6790	1	901.6790	14.8623	0.0063	
EL	21.0850	1	21.0850	0.3475	0.5740	
EP	75.1051	1	75.1050	1.2380	0.3026	
LP	1262.1243	1	1262.1243	20.8036	0.0026	
E^2	50.54821	1	50.5482	0.8332	0.3917	
L^2	461.9100	1	461.90100	7.6137	0.0281	
P^2	1502.7748	1	1502.7748	24.7702	0.0016	
ELP	95.5740	1	195.5739	3.2236	0.1157	
E^2L	518.0077	1	518.0077	8.5383	0.0223	
E^2P	13.6145	1	13.6145	0.2244	0.6501	
EL^2	72.1102	1	72.1102	1.1886	0.3117	
Residual	424.6801	7	60.6686			
Lack of Fit	7.9639	1	7.9639	0.1147	0.7464	Not significant
Pure error	416.7163	6	69.4527			
Cor. total [d]	6903.4291	22				

[a] Degrees of freedom.
[b] Test for comparing model variance with residual (error) variance.
[c] Probability of seeing the observed "*F* value" if the null hypothesis is true.
[d] Totals of all information corrected for the mean.

is the intercept and the *b* terms represent those parameters of the model which are optimized iteratively to fit, or model the data.

$$\begin{aligned} Y &= b_0 + b_1\mathrm{E} + b_2\mathrm{L} + b_3\mathrm{P} + b_4\mathrm{EL} + b_5\mathrm{EP} + b_6\mathrm{LP} + b_7\mathrm{E}^2 \\ &\quad + b_8\mathrm{L}^2 + b_9\mathrm{P}^2 \\ b_0 &= 69.92;\quad b_1 = 8.19;\quad b_2 = -5.25;\quad b_3 = 12.63; \\ b_4 &= -1.62;\quad b_5 = 3.06; \\ b_6 &= -12.56;\quad b_7 = -1.78;\quad b_8 = -5.39;\quad b_9 = -9.37 \end{aligned} \tag{4}$$

The "Lack of Fit (LOF) *F*-value" of 0.04 implies that it is not significant relative to the pure error. The quality of the polynomial model was expressed by the coefficient of determination (R^2, adjusted-R^2 and adequate precision). R^2 is a measure of the amount of variations around the mean explained by the model and it is equal to 0.9378. The adjusted-R^2 is adjusted for the number of terms in the model and it decreases as the number of terms in the model increases, if those additional terms do not add value to the model. It is equal to 0.8223. Adequate precision is a signal-to-noise ratio. It compares the range of the predicted values at the design points to the average prediction error (Eq. (5)). Ratios greater than 4 indicate adequate model discrimination. Here it is equal to 9.630.

$$\left[\frac{\max(\widehat{Y}) - \min(\widehat{Y})}{\sqrt{\bar{V}(\widehat{Y})}}\right] > 4, \quad \bar{V}(\widehat{Y}) = \frac{1}{n}\sum_{i=1}^{n}\bar{V}(\widehat{Y}) = \frac{p\sigma^2}{n} \tag{5}$$

$\hat{Y}$ is the predicted value, *p* is the number of model parameters (including intercept (b_0) and any block coefficients), σ^2 = residual MS from ANOVA table, and *n* is the number of experiments.

For the graphical interpretation of the interactions, the use of three-dimensional plots (3D) of the model is highly recommended. Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models were chosen for the axes of the response surface plots to account for curvature of the surfaces. This is useful to visualize the relationship between the responses and the experimental levels of each factor. The response model is mapped against two experimental factors while the third is held constant at its central level. Fig. 2 shows that, in low concentrations of the chelating agent, by increasing pH levels the recovery increases, this is because with increasing the pH, more complex is formed. In higher pH values, by increasing concentration of the chelating agent, the recovery decreases. This behaviour is related, in high concentration of Na-DDTC, to the extraction of the ligand itself, which can saturate the small volume of the extraction solvent. Here, the maximum recovery will be obtained when pH is 7 and concentration of the chelating agent is 500 mg L^{-1}. Finally, using optimization option of the software package Design-Expert 7.1.6. the optimal conditions were obtained as follows: volume of extraction solvent (CCl_4), 113 μL; volume of disperser solvent (methanol), 1.00 mL; concentration of the chelating agent, 540 mg L^{-1}; and pH 6.70.

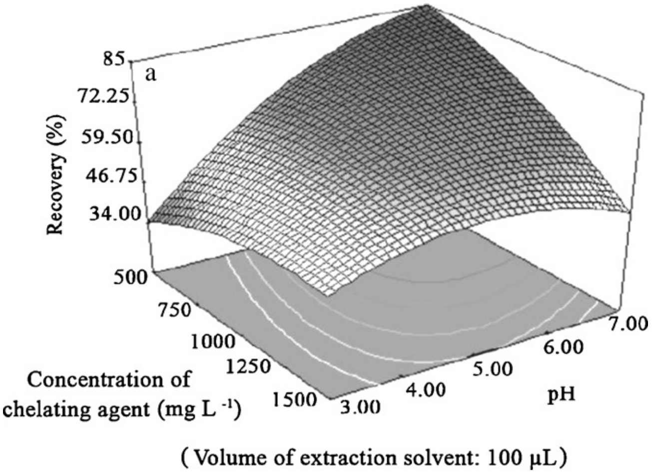

Fig. 2.

Table 7
Analytical figures of merit of DLLME-ICP-OES.

Metal ions	LDR [a]	R^2	LOD [b]	LOQ [c]	PF [d]	RSD [e]
Cr	1–750	0.994	0.27	0.90	8	3.0
Cu	1–1000	0.994	0.23	0.77	9	2.5
Ni	1–1000	0.995	0.40	1.34	8	3.8
Zn	1–1000	0.993	0.55	1.83	9	2.1

[a] Linear dynamic range (μg L^{-1}).
[b] Limit of detection (μg L^{-1}).
[c] Limit of quantification (μg L^{-1}).
[d] Preconcentration factor.
[e] Relative standard deviation, *n* = 7,(%).

Table 8
Comparison of the proposed method with CPE and DLLME–SFO of the metal ions in water samples.

Metal ions	System	RSD (%)	LDR [a]	LOD [b]	Detection	Ref.
Cr, Cu, Ni, Zn	DLLME	2.1–3.8	1–1000	0.23–0.55	ICP-OES	This work
Co, Cr, Cu, Mn	DLLME–SFO	3.4–7.5	1.25–250	0.10–0.30	ICP-OES	[20]
Cd, Cu, Ni, Zn	CPE	1.3–2.6	50–2500	1.00–6.00	ICP-OES	[21]
Co, Ni, Zn	CPE	1.1–4.3	2–250	n.r[c]	UV–vis	[22]

[a] Linear dynamic range ($\mu g\,L^{-1}$).
[b] Limit of detection ($\mu g\,L^{-1}$).
[c] Not reported.

Table 9
Effect of interfering ions on preconcentration and determination of Cr, Cu, Ni and Zn.

Interference	Ratio of interfering ions to the analytes (w/w) [a]	Recovery (%) ± S.D [b]			
		Cr	Cu	Ni	Zn
Na^+	6000	94 ± 2	96 ± 3	104 ± 1	99 ± 1
K^+	6000	102 ± 1	98 ± 3	95 ± 4	94 ± 3
Ca^{2+}	4000	101 ± 2	99 ± 4	97 ± 5	94 ± 3
Mg^{2+}	2000	99 ± 4	97 ± 3	95 ± 4	96 ± 2
Mn^{2+}	1000	103 ± 2	91 ± 5	96 ± 3	92 ± 4
Cl^-	6000	97 ± 4	94 ± 4	104 ± 1	100 ± 1
$SO_4{}^{2-}$	400	101 ± 2	99 ± 1	95 ± 2	97 ± 3
$CO_3{}^{2-}$	400	93 ± 5	90 ± 5	94 ± 5	99 ± 2
$CrO_4{}^{2-}$	600	–	94 ± 4	90 ± 3	97 ± 3

[a] Concentration of each analyte is 200 $\mu g\,L^{-1}$.
[b] Standard deviation($n = 5$).

Table 11
Statistical evaluation of the result obtained in the accuracy study of CRM-TMDW-500 drinking water.

Element	CRM-TMDW-500 drinking water					
	S	X_R	$\bar{X}$	$X_R - X$	[a] $ts/\sqrt{N}$	Comparison
Cr	0.6	20	19.6	0.4	0.75	0.4 < 0.75 (same)
Cu	0.7	20	19.5	0.5	0.87	0.5 < 0.87 (same)
Ni	1.1	60	58.7	1.3	1.37	1.3 < 1.37 (same)
Zn	0.9	70	69	1.0	1.12	1.0 < 1.12 (same)

[a] $t = 2.78$ (95% confidence intervals); N, 5; s, standard deviation; X_R, value of standard material; $\bar{X}$, mean value;.

Table 12
Trace determination of Cr, Cu, Ni and Zn in different water samples.

Sample	Cr	Cu	Ni	Zn
Tap water [a] mean ± SD [b] ($\mu g\,L^{-1}$)	n.d. [e]	6.2 ± 1.2	n.d.	60 ± 3.1
Added ($\mu g\,L^{-1}$)	100	100	100	100
Found, ±SD ($\mu g\,L^{-1}$)	90 ± 6.1	89 ± 4.4	93 ± 4.3	155 ± 5.4
Relative Rec. (%)	90	93	93	95
Sea water [c], ±SD ($\mu g\,L^{-1}$)	n.d.	4.3 ± 0.8	n.d.	73 ± 4.1
Added ($\mu g\,L^{-1}$)	100	100	100	100
Found ±SD ($\mu g\,L^{-1}$)	93 ± 5.4	96 ± 3.3	90.5 ± 7.3	165 ± 6.9
Relative Rec. (%)	93	91.7	90.5	93
River water [d]	n.d.	1.9 ± 0.5	n.d.	30 ± 3
Added ($\mu g\,L^{-1}$)	100	100	100	100
Found ±SD ($\mu g\,L^{-1}$)	99 ± 1.9	96 ± 5.4	94 ± 6.1	122 ± 5.4
Relative Rec. (%)	99	94.1	94	92

[a] The sample was collected from Caspian sea (Mazandaran, Iran).
[b] Standard deviation ($n = 3$).
[c] The sample was collected from Gorgan river (Golestan, Iran).
[d] The water was taken from university of Tehran (Tehran, Iran).
[e] Not detected.

3.3. *Analytical figures of merit*

The analytical figures of merit of the proposed method are summarized in Table 7. The limits of detection (LOD), which is defined as $C_{LOD} = 3S_d/m$ (where C_{LOD}, S_d and m are the limit of detection, standard deviation of the blank, and slope of a calibration graph, respectively) The limit of quantification (LOQ) was calculated based on standard deviation of the blank to the slope of calibration graph (S_d/m) which is equal to 10. Preconcentration factor (PF) was defined as the ratio of concentration of analyte in the final solution to its concentration in the aqueous sample [17], were obtained between 0.23 and 0.55 $\mu g\,L^{-1}$. The percent relative standard deviations (RSDs, $C = 200\,\mu g\,L^{-1}$) for seven replicated measurements in optimal conditions were 6.2–9.0%. Linear dynamic range (LDR) from simultaneous preconcentraion of 10 mL of 10 different standard sample solutions under the optimum conditions were 1–750 $\mu g\,L^{-1}$ for Cr, and 1–1000 $\mu g\,L^{-1}$ for Cu, Ni, and Zn. The correlation coefficient of the calibration curves was in the range of 0.993–0.995. A comparison of the results of the represented method with cloud point extraction (CPE) and DLLME–SFO (solidification of floating organic drop) methods to extract and determine the similar metal ions is shown in Table 8.

Table 10
Analysis of the three certified reference materials for the determination of Cr, Cu, Ni and Zn with DLLME-ICP-OES method.

	Cr	Cu	Ni	Zn
CRM-TMDW-500				
[a] Certified value ($\mu g\,L^{-1}$)	20 ± 0.1	20 ± 0.1	60 ± 0.3	70 ± 0.4
[b] Amount fund ($\mu g\,L^{-1}$)	19.6 ± 0.6	19.5 ± 0.7	58.7 ± 1.1	69 ± 0.9
Recovery (%)	98	97.5	97.8	98.5
CASS – 4 (Nearshore Seawater)				
Certified value ($\mu g\,L^{-1}$)	0.144 ± 0.029	0.592 ± 0.055	0.314 ± 0.030	0.381 ± 0.057
Added	5.00	5.00	5.00	5.00
Amount fund ($\mu g\,L^{-1}$)	5.08 ± 0.061	5.70 ± 0.095	5.21 ± 0.090	5.45 ± 0.083
Recovery (%)	98.72	102.16	97.92	101.38
SLRS – 4 (Riverine Water)				
Certified value ($\mu g\,L^{-1}$)	0.33 ± 0.02	1.81 ± 0.08	0.67 ± 0.08	0.93 ± 0.10
Amount fund ($\mu g\,L^{-1}$)	0.32 ± 0.03	1.89 ± 0.12	0.64 ± 0.05	0.97 ± 0.04
Recovery (%)	96.97	104.42	95.52	104.30

[a] Certified reference material trace metals in drinking water.
[b] The confidence interval was calculated at $P = 0.95$, ($N = 5$).

3.4. *Effect of coexisting ions*

The effect of common coexisting cations on the recovery of Cr, Cu, Ni, and Zn were also investigated. 10.0 mL of solution containing 200 μg L^{-1} of Cr, Cu, Ni and Zn with various amounts of interfering ions were treated according to the procedure mentioned in Section 2.3, and the results are given in Table 9.

3.5. *Accuracy of the method*

In order to verify the accuracy and applicability of the proposed procedure, the determination of Cr, Cu, Ni and Zn in three certified reference material (CRM-TMDW-500 drinking water, SLRS-4 riverine water and CASS4 nearshore seawater) were investigated (Table 10). Statistical evaluation (Table 11) was applied to the results obtained from the accuracy study of CRM-TMDW-500 drinking water (Table 10) using *t*-test. The result of this test show that the difference between X and XR is significant [23]. The results showed a good agreement between the analyzed and certified values.

3.6. *Analysis of real samples*

To evaluate the efficiency of the proposed DLLME-ICP-OES method, three types of water samples including tap water, seawater and river water were studied (Table 12). The results show that the relative recoveries for the spiked samples are acceptable (90–99%).

4. Conclusion

Hyphenation of DLLME with ICP-OES for simultaneous preconcentration and determination of Cr, Cu, Ni and Zn in water samples by Na-DDTC was introduced and applied for the first time in this study. The proposed procedure is simple, low cost, low consuming of organic solvents and specifically fast. The short extraction time is related to the infinitely large surface area between extraction solvent and aqueous phase after formation of cloudy solution. Thereby, complex formation/transfer from aqueous phase to extraction solvent is fast. Subsequently, equilibrium state is achieved quickly therefore, the extraction time is very short. To determine the optimum operating conditions of preconcentration stage (DLLME) of the method to yield the maximum efficiency, experimental designs (factorial design and central composite design) were applied. Factorial design gave important main and interaction effects. Using central composite design, quadratic and interaction terms revealed and the location of the optimum set of experimental conditions were determined, whereas, by classical methods we were not able to see the detailed effect of factors on each other and also on the efficiency to explain the behaviour of a system.

References

[1] M. Miro, J.M. Estela, V. Cerda, Talanta 63 (2004) 201.
[2] A.A. Shaltout, M.A. Ibrahim, Can. J. Anal. Sci. Spectros. 52 (2007) 276.
[3] S. Fragueiro, I. Lavilla, C. Bendicho, Talanta 68 (2006) 1096.
[4] J.N. Silveira, P.C.P. Lara, M.B. Dias, J.M.G. Matos, J.C.J. da Silva, C.C. Nascentes, V.S.T. Ciminelli, J.B.B. da Silva, At. Spectrosc. 28 (2007) 1.
[5] M.N.M. Reyes, R.C. Campos, Talanta 70 (2006) 929.
[6] F.Z. Xie, X.C. Lin, X.P. Wu, Z.H. Xie, Talanta 74 (2008) 836.
[7] V. Umashankar, R. Radhamani, K. Ramadoss, D.S.R. Murty, Talanta 57 (2002) 1029.
[8] Y. Boukraa, D. Barkat, T. Benabdellah, A. Tayeb, M. Kameche, Phys. Chem. Liq. 44 (2006) 693.
[9] M. Rezaee, Y. Assadi, M.R.M. Hosseinia, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1.
[10] S. Berijani, Y. Assadi, M. Anbia, M.R.M. Hosseini, E. Aghaee, J. Chromatogr. A 1123 (2006) 1.
[11] R.R. Kozani, Y. Assadi, F. Shemirani, M.R.M. Hosseini, M.R. Jamali, Talanta 72 (2007) 387.
[12] M.T. Naseri, M.R.M. Hosseini, Y. Assadi, A. Kiani, Talanta 75 (2008) 56.
[13] T.A. Kokya, K. Farhadi, J. Hazard. Mater. 169 (2009) 726.
[14] M. Gharehbaghi, F. Shemirani, M. Baghdadi, Int. J. Environ. Anal. Chem. 88 (2008) 513.
[15] P. Liang, E. Zhao, F. Li, Talanta 77 (2009) 1854.
[16] D. Atanassova, V. Stefanova, E. Russeva, Talanta 47 (1998) 1237.
[17] E.Z. Jahromi, A. Bidari, Y. Assadi, M.R.M. Hosseini, M.R. Jamali, Anal. Chim. Acta 585 (2007) 305.
[18] R.G. Brereton, Chemometrics: Data Analysis for the Laboratory and Chemical Plant, London, John Wiley & Sons Ltd, West Sussex, England, 2003.
[19] H. Sereshti, M. Karimi, S. Samadi, J. Chromatogr. A 1216 (2009) 198.
[20] Y. Yamini, M. Rezaee, A. Khanchi, M. Faraji, A. Saleh, J. Chromatogr. A 1217 (2009) 2358.
[21] E.L. Silva, P.S. Roldanb, M.F. Ginéa, J. Hazard. Mater. 171 (2009) 1133.
[22] A. Afkhami, M. Bahram, Microchim. Acta 155 (2006) 403.
[23] J.C. Miller, J.N. Miller, Statistics and Chemometrics for Analytical Chemistry, 5th ed., Prentice Hall, Upper Saddle River, NJ, 2005.