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Cold-Induced Aggregation Microextraction Technique Based on Ionic Liquid for Preconcentration and Determination of Zinc in Environmental Samples

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Abstract: The determination of zinc in food and environmental samples using cold induced aggregation microextraction combined spectrophotometry is described. The extraction of zinc was performed in the presence of 2-amino-6-(1,3-thiazol-2-yldiazenyl)phenol (ATDP) as the complexing agent. In this method, sodium hexafluorophosphate ($NaPF_6$) was added to the sample solution containing small amounts of 1-hexyl-3-methylimidazolium hexafluorophosphate [Hmim][PF_6] as extraction solvent. Parameters governing the extraction efficiency such as pH, amount of ionic liquid, ionic strength and temperature were optimized. The applicability of the technique was evaluated by the determination of trace zinc in different real samples. Under the optimum conditions, the calibration curve was linear in the concentration range 5.0-120 ng/mL with correlation coefficient (r^2) of 0.9994. The limit of detection (LOD) of 1.47 ng/mL and enhancement factor of 200 were obtained for zinc. The relative standard deviation (RSD) was 1.73% for 60 ng/mL zinc (*n* = 10).

Keywords: Ionic liquid; Cold induced aggregation microextraction; Zinc determination; Spectrophotometry; Environmental analysis.

1. INTRODUCTION

Zinc is an essential trace element for humans, animals, plants and microorganism. Zinc plays many fundamental roles in all replications, gene expression and in the metabolism of nucleic acids and different proteins [1]. A variety of preconcentration and determination procedures for trace determination of zinc are reported [2-4]. Also, the typical methods for determination zinc are atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and stripping voltammetry. Although these methods have good sensitivity, they require expensive instruments, well controlled experimental conditions, and profound sample-making. The development of selective and sensitive method for the quantitative analysis of zinc has become extremely important for environmental and biological applications.

Several procedures have been developed for the separation and preconcentration of contaminants from environmental matrices such as: liquid-liquid extraction [5], co-precipitation [6], and solid phase extraction [7]. However, liquid-liquid extraction (LLE) is time-consuming and requires large amounts of organic solvents that are potentially toxic. Solid phase extraction (SPE) uses much less solvent than LLE but can be relatively expensive. Additionally, evaporation of the final organic extract into a small volume is necessary to achieve high enrichment of the analytes. Batch-to-batch reproducibility continues to be the major concern for analysts in selecting SPE devices. Elution of sorbed solute must be performed after sample loading. Solvent evaporation and redissolution are often required [8]. A novel microextration technique as a high performance and powerful preconcentration method termed dispersive liquid-liquid microextration (DLLME) was demonstrated by Y. Assadi and coworkers [9].

Modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation, as well as the minimization of the used organic solvent. A new mode of liquid-phase microextraction (LPME) named dispersive liquid-liquid microextraction (DLLME) which is based on ternary component solvent system, such as cloud point extraction (CPE) and homogeneous liquid-liquid extraction (HLLE), as high performance, rapid and inexpensive microextraction method has been proposed [10]. DLLME has been successfully applied to the preconcentration of several families of organic [11-16] and inorganic species [17-19], prior to analysis with gas chromatography (GC), GC-mass spectrometry (GC MS), high-performance liquid chromatography (HPLC), UV-Vis spectrophotometry and atomic absorption spectrophotometry (AAS).

Room temperature ionic liquids (RTILs) with negligible vapor pressure and good selective solubility have aroused increasing interests for their promising role as alternative solvents in synthesis, separation and electrochemistry [20]. They are considered to be green solvents due to their negligible vapor pressure, good thermal stability, very wide liquid phase range and good dissolving and extracting abilities [21-23]. They have been recently used with success as replacement solvents to separate organic compounds [27], biologically important molecules [24,25] and metal ions [26,27]. Several efforts have been made based on extraction with an ionic liquid, such as ionic liquid-based headspace liquid phase microextraction [28], ionic liquid-based single-drop microextraction [29-31] and temperature-controlled ionic liquid dispersive liquid phase microextraction [32,33]. These methods present outstanding figures of low consumption of volatile organic solvents and high enrichment factors. Another useful method, which is termed cold-induced aggregation microextraction (CIAME) and is based on the use of ILs for homogeneous liquid-liquid microextraction (HLLME), was developed by Baghdadi and Shemirani in 2008 [34], and further modified in 2010 [35]. The mechanism of this method is very similar to that of DLLME, but the dispersal is accomplished by temperature-dependent dissolution instead of injection. In this method, a suitable temperature is used to fully dissolve the room temperature IL in the sample solution without the use of disperser solvent [36].

Both CIAME and DLLME methods have their merits. In comparison with CIAME, IL-based DLLME is more efficient and convenient for dispersing the room temperature ILs throughout the sample, thus significantly reducing the extraction time and improving the recovery. However, because the IL solubility in water can change considerably with changes in temperature, the IL-CIAME extraction process (which involves cooling) can require more IL than the DLLME extraction process, not involving cooling when the initial volume of IL is the same.

In this study, the CIAME method was applied for simple, rapid, sensitive, and reasonably selective determination of zinc(II) in environmental samples using spectrophotometry technique. The effects of various experimental conditions on the extraction were also investigated.

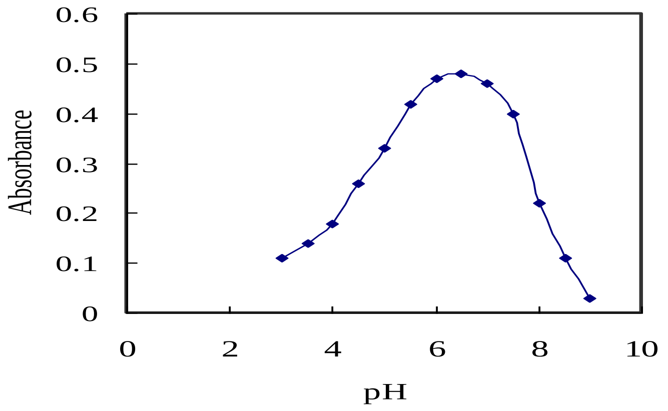

Fig. 1. Effect of on the absorbance of Zn-ATDP complex. Utilizing optimum conditions for 60 ng/mL

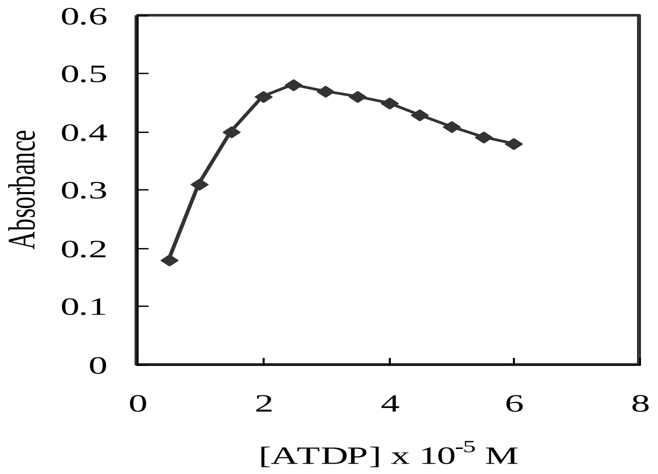

Fig. 2. Effect of ATDP concentration on 60 ng/mL zinc ion at the optimum conditions

2. EXPERIMENTAL

2.1 Apparatus

A Perkin Elmer Lambda 12 UV-Visible spectrophotometer with a 10 mm quartz cell was used for all spectral measurements. A Perkin Elmer atomic absorption spectrometry model A Analyst 300 was used for all GFAAS measurements. a laboratory centrifuge (Heraeus, Labofuge 400 model, Germany) was used to accelerate the phase separation. An Orion research model 601 A/digital ionalyzer pH meter was used for checking the pH of solutions.

2.2 Reagents

All chemicals, reagents and solvents such as $NiNO_3$, ethanol, acetone, sodium hydroxide, sodium nitrate, nitric acid, hydrochloric acid, Triton X-114 (octylphenoxy polyethoxyethanol) and sodium hexafluorophosphate ($NaPF_6$) were highly purified reagents produced in Merck Co. (Darmstadt, Germany). 1-Hexyl-3-methylimidazolium hexafluorophosphate [Hmim][PF_6] ionic liquid was produced by Aldrich (England). Deionized water (1-10 MΩ cm) obtained from a Labconco system (Labconco Co., Kansas City, USA) was used throughout this work. A 1.2×10^{-3} M zinc stock solution was prepared by dissolving 0.0785 g of $Zn(NO_3)2•6H_2O$ in exactly 200 mL of water and standardizing with EDTA [37]. Standard solutions were prepared by adequate dilution of the stock solution. A buffer solution (0.1 M) was prepared by dissolving the appropriate amount of sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) (Merck) in deionized water and adjusted to pH 6.5 by adding the diluted HCl and HNO_3 solutions [38].

The reagent, 2-amino-6-(1,3-thiazol-2yldiazenyl) phenol (ATDP) was prepared according to the procedure reported previously [39]. Stock solution 5 × 10−3 M of ATDP was prepared by dissolving appropriated weighed amounts of solid reagent in least amount of DMF and competed to the mark in a 100 mL measuring flask.

A solution of 100 mg/mL NaPF6 was prepared by dissolving appropriate amount of $NaPF_6$ in deionized water. The viscosity of IL was high and their handling was difficult, so working solution ([Hmim][PF_6], 0.8 mg/L) was prepared in acetone. In the experiments, vessels were kept in 10% nitric acid for at least 24 h and subsequently washed with the deionized water.

2.3 General analytical procedure

A volume of 10 mL of sample or standard solution containing Zn(II), adjusted in pH 6.5 using phosphate buffer, was transferred to a 20 mL conical-bottom glass centrifuge tube. Then, 0.5 % m/v $NaNO_3$, 50 µL of a mixture (5 × 10−4 M ATDP, 0.10% v/v Triton X-114, 50 mg of IL ([Hmim][PF_6] as extraction solvent) and 40 µL of the solution containing sodium hexafluorophosphate using a Hamilton syringe were rapidly injected into the above mentioned aqueous sample. After shaking, it was kept in a thermostated bath at 55 ^{o}C for 5.0 min. Then, the tube was cooled in an ice bath for a certain time and a cloudy solution was formed. Subsequently, the cloudy solution was centrifuged for 5.0 min at a centrifugation rate of 3800 rpm. As a result, the fine droplets of IL settled at the bottom of the tube. The upper aqueous phase was removed with a microsyringe, and the IL phase was dissolved in 100 µL of ethanol and transferred to quartz microcell. The absorbance of the complex was measured at 543 nm.

2.4 Preparation of hair sample

Hair sample was washed with acetone and water to remove the surface contamination. Then 1.0 g of clean hair sample was accurately weighed and dry-ashed in furnace at 450 ^{o}C. The residue was dissolved in nitric acid. The pH of the solution was adjusted to about 6.5 with sodium hydroxide and the solution diluted to 25 mL with water [40].

2.5 Preparation of powdered milk sample

Two grams of the sample was treated with 1.0 g of sodium hydroxide dissolved in a few mL of water and evaporated to dryness in a platinum dish. The residue was placed in a furnace at 500 ^{o}C for 20 min. Then it was removed, cooled and 15 mL of water and 2.0 mL of concentrated nitric acid were added to dissolve the residue [41]. The pH of the solution was adjusted to about 6.5 with sodium hydroxide and the solution diluted to 25 mL with water.

2.6 Preparation of water samples

Water samples including underground water, tap water and spring water were collected from local sources and prepared as follows [42]. Samples were filtered through a Millipore 0.45 µm pore-size membrane filter into cleaned polyethylene bottles, the pH of solutions were adjusted to ~6.5 and were treated according to the given procedure.

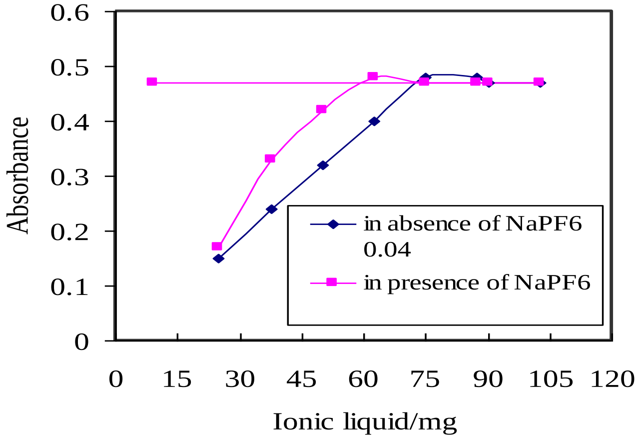

Fig. 3. Effect of IL amount and common ion on the absorbance of Zn-ATDP complex. Utilizing optimum conditions:

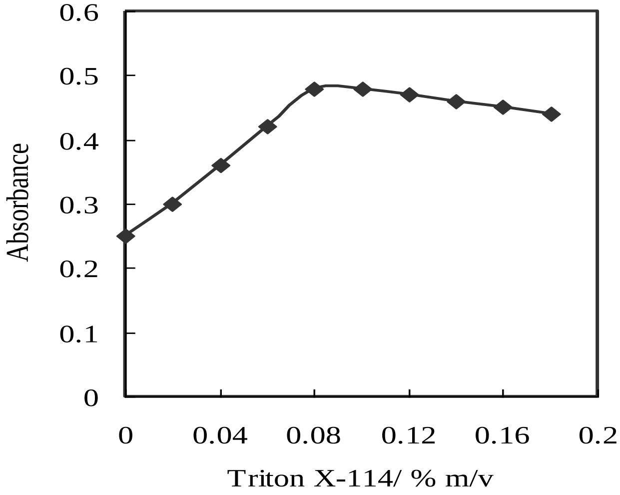

Fig. 4. Effect of Triton X-114 on the absorbance of the 60 ng/mL Zn(II) complex at the optimum conditions.

2.7 Preparation of Vegetables (Parsley, Basil)

Two types of vegetables were chosen for analysis. These were parsley and basil which were collected from the local supermarket. Parsley and basil samples were air-dried followed by drying at 105 ^{o}C for 2.0 h and then powdered. 0.5 g of basil sample and 0.3 g of parsley sample were taken in Teflon flasks, and 5.0 mL of concentrated nitric acid and 5.0 mL of 30% (v/v) hydrofluoric acid were added. After standing for 8.0 h, the samples were digested in a microwave system for 10 min at 106 ^{o}C. The clear solutions obtained were transferred to a 400 mL beaker; and diluted to 300 mL, then adjusted to the pH of 6.5.

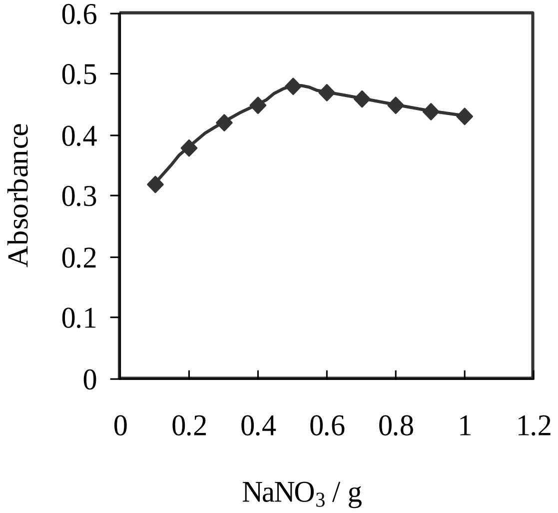

Fig. 5. Effect of $NaNO_3$ on the absorbance of 60 ng/mL complex. Utilizing the optimum conditions

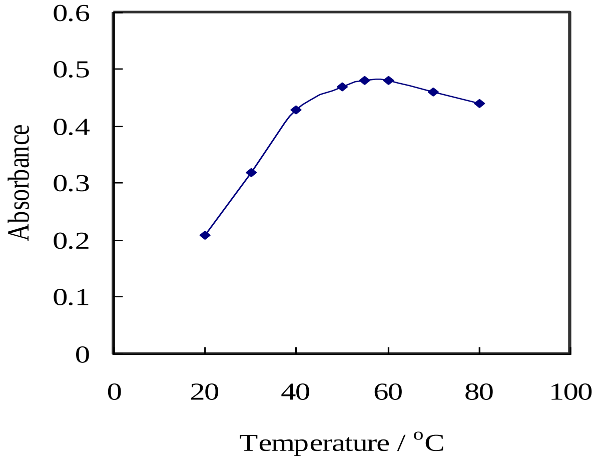

Fig. 6. Effect o temperature on the absorbance of 60 ng/mL Zn complex. Utilizing the optimum conditions

2.8 Preparation of Red meat

The meat samples were brought to the laboratory in plastic bags, cleaned and washed with distilled water, before cooking, they were cut in pieces with a stainless steel knife, and dried in 170 an oven at 65 °C for 48 h discontinuously until constant weight was obtained. The dried samples were then powdered. Accurately, 1.0 g of dried samples were weighted and put in polyethylene vials, and digested with a mixture of concentrated H_2SO_4 and HNO_3 (1:3 v/v). A small amount of 30% H_2O_2 was added to eliminate nitrogen dioxides. The digestion process was continued until the solution became clear. The samples were transferred into 250 mL beaker and diluted to 200 mL with distilled water.

Table 1. Tolerance limits of interfering ions in the determination of 60 ng/mL Zn.

Ions	Tolerance limits	Absorbance change (%)	Ions	Tolerance limits	Absorbance change (%)
Ba^{2+}	10000	−1.57	PO_4^{3-}	12000	+2.25
Sr^{2+}	10000	−3.20	HPO_4^{2-}	12000	+2.50
Ca^{2+}	8500	−1.97	$H_2PO_4^-$	11000	+2.75
Mg^{2+}	8500	−3.32	CH_3COO^-	8000	+3.80
Na^+	8000	+1.20	SO_4^{2-}	4000	−1.73
Li^+	8000	−0.84	F^-	2000	−2.75
K^+	7500	−0.60	Cl^-	1500	−1.23
Co^{2+}	7000	−2.12	NO_3^-	1250	−1.30
Al^{3+}	6800	+1.40	CO_3^{2-}	1000	+3.77
Cr^{3+}	6500	−3.20	I^-	800	+4.23
Pb^{2+}	5000	−2.82	HCO_3^-	750	−3.75
Fe^{3+}	3250	−3.79	$S_2O_8^{2-}$	600	−4.20
Ni^{2+}	2500	+3.70	Br^-	500	−4.00
Cd^{2+}	2250	+3.82	benzoate	500	−3.60
Cu^{2+}	1750	+4.10	Succinate	500	−4.40
Ag^+	250	+4.63	Citrate	400	−4.75
Pd^{2+}	250	+4.75	Phathalate	400	−4.80

2.9 Optimization of the CIAME sample preparation method

In the present study, for higher sensitivity, selectivity and precision of nickel determination, the

CIAME method combined with spectrophotometer technique, after selecting the maximum wavelength of nickel complex, the effect of the main parameters (like the amount of ionic liquid, pH, amount of chelating agent, sample ionic strength and extraction time), was studied and optimized.

The precision of the method was calculated as the relative standard deviation (RSD) of 10 independent measurements, carried out using 60 ng/L zinc. In order to define the linear dynamic range (LDR) of method, eight sample solutions of Zn(II) with different concentrations were extracted under the optimized conditions. After extraction, the IL phase was dissolved in 100 µL of ethanol and absorbance were measured at λmax. The limit of detection is defined as 3Sb/m (where Sb is standard deviation of the blank and m is the slope of the calibration graph). The enhancement factor (EF) is calculated as the ratio of the slope of preconcentrated samples using CIAME to that obtained without preconcentration.

2.10 Maximum wavelength of complex

To obtain the maximum wavelength of zinc complex, a solution of (10 mL, 0.01 µg/mL, pH 6.5) of Zn(II) was taken for extraction with ATDP reagent in DMF (1.5 mL, 5 × 10−4 M). Absorption spectra corresponding to the complex in DMF in the range 300−800 nm were taken.

2.11 Sample acidity and ATDP concentration

Among chemical variables, sample acidity, which was defined by the pH of the sample solution, played a significant role in the overall performance of the solvent extraction and affects the complex formation and the extraction efficiency. The effect of the pH on the absorbance was studied in the range of 2.0−12 by adjusting it in Zn(II) solution with phosphate buffer. In addition, the influence of ATDP concentration on the extraction in the range of 10−3−10−5 M in DMF was studied.

Table 2. Determination of zinc ion in vegetables (Parsley, Basil), and red meat.

Sample	Zn(II) added (µg/kg)	Zn(II) found[a] (µg/kg)		Recovery (%)	
		Proposed	GF-AAS	Proposed	GF-AAS
Parsley	0.0	n.d[b]	n.d[b]	---	
	20	20.15±0.33	20.25±0.60	100.75	101.25
	40	40.25±0.25	40.30±0.45	100.63	100.75
	80	80.40±0.40	79.55±0.70	100.50	99.44
Basil	0.0	---			
	30	30.45±0.15	30.50±0.80	101.50	101.67
	60	60.60±0.45	61.00±0.65	101.00	101.67
	90	90.65±0.27	90.85±0.77	100.72	100.94
Red meat	0.0	---			
	15	15.10±0.43	14.80±0.66	100.67	98.67
	30	29.65±0.19	30.35±0.70	98.83	101.17
	60	59.75±0.33	60.70±0.85	99.58	101.17

[a]: Mean ± SD (*n* = 6).
[b]: Not detected.

2.12 Amount of ionic liquid

To study the effect of extraction ([Hmim][PF$_6$]) solvent amount, solutions containing different amounts of [Hmim][PF$_6$] were tested. The experimental conditions were fixed and included the different amounts of [Hmim][PF$_6$] in the range of 30-80 mg. In addition, to evaluate the effect of common ion, similar experiments were done in the presence of common ion. A common ion is any ion in the solution that is common to the ionic liquid being dissolved. In this study, NaPF$_6$ was used as a common ion source and the effect of this agent on the analytical responses was investigated.

2.13 Amount of anti-sticking agent

The effect of Triton X-114, as an anti-sticking agent, was investigated in order to overcome the adherence of the IL-phase on the wall of the centrifuge tube in the range of 0.00−0.25% (v/v).

2.14 Amount of salt

In order to investigate the influence of the ionic strength on the CIAME performance, several experiments were performed with different NaCl concentrations. Due to high solubility of NaNO$_3$, salt effect was studied up to 30% (m/v).

2.15 Investigation of temperature

Temperature affects the partition coefficient of analytes and the solubility of ILs in water. As the temperature increases, the solubility of ILs in water also increases. Therefore, the selection of a suitable extraction temperature is necessary. To investigate this issue, nickel samples in water bath with different temperature in the range of 20−60 °C were extracted while other conditions were kept constant.

Table 3. Determination of zinc in hair and powdered milk samples

Sample	Found[a] (%)		t-test[b]	F-value[c]
	DLLME	ICP-AES		
Hair (male)	163.9 ± 0.05	162.4 ± 0.03	1.68	3.38
Hair (female)	120.78 ± 0.03	121.07 ± 0.05	1.89	3.94
Powdered milk 1	32.25± 0.01	32.10 ± 0.06	1.65	3.48
Powdered milk 2	43.94 ± 0.04	43.02 ± 0.04	1.99	4.14

[a]: Mean ± SD (*n* = 6).

[b] Tabulated t-value for five degrees of freedom at P (0.95) is 2.57;

[c] Tabulated F-value at P (0.95) is 5.05;

2.16 Centrifuge conditions

Centrifugation is a necessary step to obtain two distinguishable phases in the extraction tubes. The effect of centrifugation time on the extraction efficiency was evaluated in the range of 500−4500 rpm.

3. RESULTS AND DISCUSSION

3.1. Spectrophotometric study

The complex had an absorbance maximum at 543 nm while further experiments showed that ATDP reagent in DMF did not have any absorption at this wavelength, so this wavelength was chosen for the subsequent experiments.

3.2. Effect of sample pH and reagent concentration

As shown in Figure 1, the extraction recovery of zinc ion reaches a maximum in the pH ranges of 6.0−7.0. A decrease in metal extraction efficiency at pH < 6.0 is due to the low complex formation. At pH > 8.0, the analyte is mainly present as hydroxide species, which do not form complex with the studied reagent. Therefore, a buffer media of pH 6.5 was chosen for the subsequent experiments.

The metal extraction efficiency as a function of the ATDP concentration is shown in Figure 2. The results revealed that the extraction efficiency increased by increasing ATDP concentration up to 2.5×10^{-5} M and remained nearly constant at higher concentrations. Therefore, this concentration was selected as optimum value.

3.3. Effect of ionic liquid amount

Figure 3 shows the effect of ionic liquid amount in the presence and absence of $NaPF_6$ as a common ion source. As can be seen, in the absence of $NaPF_6$, little amount of ionic liquid used to obtain maximum absorption was 75 mg, whereas, in the presence of constant $NaPF_6$ (40 µL), this amount reduced to 50 mg. Naturally, in the presence of excess content of salt, the solubility of the ionic liquid increases. But, according to common ion effect, solubility decreases in the presence of IL with common ion and lower amount of ionic liquid is consumed. So, 50 mg of IL and 40 µL of $NaPF_6$ were chosen for the next steps.

3.4. Effect of anti-sticking agent

According to Figure 4, in the presence of Triton X-114, the absorbance increased up to 0.08% (v/v), and then remains approximately constant. Hence, 0.10% (v/v) was selected for the rest of the work.

Table 4. Determination of Zn(II) in spiked different water and biological samples.

Sample	Zn added ng/mL	Proposed method		GFAAS method		t-test[b]	F-test[c]
		Zn Found [a] ng/mL	Recovery %	Zn Found [a] ng/mL	Recovery %		
River water [d]	0.0	65.1	--	64.5	---		
	20	85.2 ± 0.33	100.12	84.2 ± 1.66	99.65	1.87	3.18
	40	104.8 ± 0.65	99.71	105 ± 0.67	100.48	1.78	3.09
	60	123.0 ± 0.41	98.32	124.9±0.52	100.32	1.84	3.14
waste Water [d]	0.0	97.5	---	98.0	---		
	7.5	104.5±0.67	99.52	105.4	99.91	1.21	3.15
	15	112.4 ± 0.31	99.91	113.7±1.67	100.62	1.75	3.01
	30	125.3 ± 0.65	98.27	127.3±0.45	99.45	2.27	4.07
Tap water [d]	0.0	52.5	---	52.2	---		
	25	77.2 ± 0.31	99.61	76.5 ± 1.97	99.09	1.60	3.16
	50	103.0 ± 0.57	100.49	101.7±1.42	99.51	1.82	3.11
	70	120.8 ± 0.40	98.61	123.4±1.48	100.98	1.72	2.98
Sea water [d]	0.0	85.3	---	84.7	---		
	10	95.7 ± 0.36	100.42	95.0 ± 1.84	100.32	1.87	3.22
	20	106.0 ± 0.42	100.66	104.2±1.43	99.52	1.76	3.11
	30	114.8 ± 0.21	99.57	113.8±1.29	99.22	1.95	3.33
Well water	0.00	47.5	---	48.2	---		
	25	72.9 ± 0.57	100.55	72.7 ± 1.66	99.32	1.85	3.16
	50	97.1 ± 0.31	99.59	99.0 ± 1.48	100.81	1.96	3.29
	70	116.8 ± 0.38	99.40	117.5±1.74	99.41	1.81	3.13
Spring water	0.00	15.0	---	14.5	---		
	30	44.7 ± 0.57	99.33	44.0 ± 1.57	98.88	1.95	3.36
	60	75.8 ± 0.33	101.07	75.2 ± 1.73	100.94	1.30	2.86
	90	104.6 ± 0.46	99.62	105.3±1.48	100.77	2.11	3.79

[a] Mean ± Relative Standard Deviation (n = 5);
[b] Tabulated t-value for five degrees of freedom at P (0.95) is 2.57;
[c] Tabulated F-value at P (0.95) is 5.05;

3.5. Effect of salt content

In the presence of high medium salt content, the solubility of ILs increases and the phase separation does not occur. According to the common ion effect, solubility decreases in the presence of NaPF$_6$. NaNO$_3$ was chosen in order to study the salt effect. It was shown in the presence of NaPF$_6$ the successfully occurred phase separation. Figure 5 shows the effect of salt in the range of 0.0-1.0%. Absorbance was slightly increased as a result of salting out effect. A concentration of 0.5% NaNO3 was selected for subsequent experiments to increase the recovery.

3.5. Effect of temperature

The experimental data showed (Figure 6) that in the range of 20-40 °C the absorbance increased due to increasing of analyte partition coefficient. At temperatures higher than 55 °C, absorbance slightly decreased because of ionic liquid solubility. In the range of 50-60 °C, absorbance was constant, hence, a temperature of 55 °C was selected for the rest of the work.

3.6. Effect of centrifuge conditions

It was found that over 3500 rpm, IL-phase completely settled, so the rate of 3800 rpm was selected as the optimum point. At the optimum rate, the absorbance was investigated as a function of centrifugation time. The maximum absorption was achieved after 5.0 min, and no significant variation was observed when the time exceeded 5.0 min. Therefore, a time of 5.0 min was selected as optimum centrifugation time for subsequent experiments.

3.7. Interferences

After selecting the optimum conditions, effects of common coexisting ions on the recovery of zinc were studied by spiking appropriate amounts of the relative ions to the solutions containing 60 ng/mL zinc, which treated according to the recommended procedure. Zn(II) recovery was almost quantitative in the presence of other species with tolerance limits indicated in Table 1. The tolerance limit was defined as the concentration of added ion causing less than ± 5.0 % relative error. As can be seen, some cations, such as palladium(II) and silver(I), may interfere with the determination of zinc(II) at 250:1 ratio. It was shown that the presence of most metals and anions, such as chloride, bromide, iodide, acetate and sulfate, did not have any adverse effects on the extraction efficiency.

3.8. Analytical performance

The performance of this method was investigated under optimized conditions. Excellent linearity was observed over the concentration range of 5.0-120 ng/mL with favorable coefficient of correlation (r^2) 0.9994. EF of zinc was as high as 200. The repeatability study was carried out by extracting spiked water samples at a concentration level of 60 ng/mL, and RSD was 1.73% (n = 10). The limit of detection (LOD), was 1.47 ng/mL. These results confirm that the method is sensitive and stable, and may facilitate the analysis of zinc at trace levels.

3.9. Real samples analysis

The proposed methodology was applied for the analysis of zinc in different real samples such as hair, powdered milk, water, vegetables (Parsley, Basil), red meat, by CIAME as a prior step to its determination by GF-AAS. No concentration of vegetables and red meat samples was detected. The results, along with the recovery for the spiked samples to assess matrix effects, are given in Table 2.

The recoveries for the addition of Zn(II) to all the samples were in the range of 98.83-101.67%. These results demonstrated that matrices of these samples, in our present context, had little effect on CIAME of zinc. To more verity the accuracy of the proposed procedure, Zn(II) ion was also determined in two hair, powdered milk, samples. The results for this test (Table 3), using t-test at 95% confidence interval, showed that there is not any significant statistical difference between the obtained results. These results demonstrated that the proposed method could be satisfactorily used for the analysis of real food samples.

The accuracy of the proposed method was tested by determination of Zn(II) ions in river, waste, tap, sea, well, and spring waters samples. In order to validate the method, analytes were determined in spiked real samples. The results obtained are shown in Tables 4. As is evident, the Zn(II) ions added were quantitatively recovered from water matrices. As seen, there is good agreement between the results obtained by proposed and GFAAS methods.

Table 5. Comparation of the proposed method with other preconcentration -FAAS methods.

Preconcentration technique	Linear range (µg/L)	LOD µg/L	EFa	Sample Consumption (mL)	RSD (%)	Analysis frequency (h^{-1})	Ref.
Solid-phase extraction based on MWCNT[b]	20–150	0.867	20	200	3.9	n.r.[c]	[43]
Batch preconcentration with CA–PAN[d] modified polymeric membranes	Up to 30	0.7	100	50	1.8	n.r.c	[44]
On-line preconcentration system based on oxine–chitosan chelating resin	2.5–75	0.8	17.6	-	2.7	18	[45]
On-line preconcentration system modified with niobium (V) oxide	10–100	0.77	77	-	1.5	27	[46]
Cloud point extraction	10–80	8	10	10	1.4	n.r.c	[47]
Cloud point extraction	10–200	1.1	52	15	2.1	n.r.c	[48]
Ionic liquid-based DLLME	0.8–33	0.22	71	30	1.9	40	[49]
IL-CIAME with Spectrophotometry	5.0–120	1.47	200	10	1.73	10	This work

[a] Enhancement or enrichment factor. [b] Multi-walled carbon nano tube. [c] Not reported. [d] Cellulose acetate-1-(2-pyridylazo)-2-naphthol

The performance of the proposed method was assessed by calculation of the t- value (for accuracy) and F-test (for precision) compared with GFAAS method. The mean values were obtained in a Student's t- and F-tests at 95% confidence limits for five degrees of freedom [50]. The results showed that the calculated values (Table 4) did not exceed the theoretical values. A wider range of determination, higher accuracy, more stability and less time consuming, shows the advantage of the proposed method over other method.

3.10. Comparison of CIAME with other methods

The determination of zinc in the water samples by cold-induced aggregation microextraction and spectrophotometric detection was compared with other methods and the results are shown in Table 5. As can be seen, the LOD value of the CIAME spectrophotometry using only 10 mL of the sample is better than that of other methods and this method is robust against high medium salt content. All these results indicate that CIAME is a reproducible, simple and low cost technique that can be used for the preconcentration of metal ions like zinc from water and food samples.

4. CONCLUSION

In this study, IL-CIAME method in combination with spectrophotometry was used for the determination of zinc in environmental samples. The proposed method has a high enhancement factor, sensitive, selective, acceptable recovery, good repeatability and a wide linear range for the determination of zinc. When compared to other methods, this extraction method reduces the danger of exposure to toxic solvents, used for extraction in conventional extraction procedures. It also requires a shorter extraction time. The limit of detection using only 10 mL of sample is better than that of other methods. The proposed method is robust against high medium salt content.

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