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Preconcentration based dispersive liquid-liquid microextraction for spectrophotometric determination of zinc in natural water and human blood after multivariate optimization based on Box-Behnken design

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A new simple and rapid dispersive liquid-liquid microextraction has been applied to preconcentrate trace levels of zinc as a prior step to its determination by spectrophotometric method. In the proposed method, 4-(2-pyridylazo) resorcinol (PAR) is used as a chelating agent, and chloroform and ethanol are selected as extraction and dispersive solvent. The optimization strategy is carried out by using two level full factorial designs. Results of the two level full factorial design (2^4) based on an analysis of variance demonstrated that the pH, concentration of PAR, amount of dispersive and extraction solvents are statistically significant. Optimal condition for three variables: pH, concentration of PAR, amount of dispersive and extraction solvents are obtained by using Box-Behnken design. Under the optimum conditions, the calibration graphs are linear in the range of 30-220 ng mL^{-1} with detection limit of 11.2 ng mL^{-1} ($3\delta_B/m$) and the enrichment factor of this method for zinc reached at 130. The relative standard deviation (RSD) is 1.4% (n=7) at 50 ng mL^{-1} level. The method is successfully applied to the determination of trace amount of zinc in water and human blood samples.

Keywords: Zinc; Dispersive liquid-liquid microextraction; Experimental design; Spectrophotometry; Water sample; Human blood sample.

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 cowrkers [9].

In this method, an appropriate mixture of the extraction solvent and the dispersive solvent is injected into aqueous sample by a syringe and forms a cloudy solution. The cloudy state results from the formation of fine droplets of the extraction solvent which disperse in the sample solution. The cloudy solution shall be centrifuged and the fine droplets sediment at the bottom of the conical test tube. Determination of analytes in the remained phase can be performed by instrumental techniques. Recently, several papers have been published about dispersive liquid-liquid microextration in preconcentration and determination of metals [10-18]. Although, satisfactory results can be obtained with the above mentioned separation/preconcentration techniques but they have some drawbacks such as large consumption of reagent, low enrichment factor, multistage operation and lengthy separation.

Spectrophotometric methods are the most commonly used techniques and continue to enjoy wide popularity. The common availability of the instrumentation, the simplicity of procedures, speed, precision and accuracy of the technique still make spectrophotometric methods attractive. In the present work, the DLLME is combined with UV-Visible Spectrophotometry by using micro-sample introduction system for the determination of zinc was proposed. In this method, 4-(2-pyridylazo) resorcinol (PAR), which forms complexes with zinc, is selected as chelating reagent. PAR contains azo and hydroxyl groups, which are good electron donors. PAR is widely used in extraction spectrophotometry because it can form stable complexes with many metal ions under proper conditions. The factors influencing the efficiency of DLLME and determination are systematically studied and optimized by Box-Behnken design [19]. Box-Behnken is a second-order multivariate design technique based on three-level incomplete factorial designs that received widespread application for evaluation of critical experimental conditions, that is, maximum or minimum of response functions. The number of experiments (N) required for the development of this design is defined as $N=2k(k-1)+C_0$, where k is the factor number and C_0 is the replicate number of the central point [20]. The applicability of this approach is validated for the determination of zinc in real samples.

2. EXPERIMENTAL

2.1. Apparatus

A Hewlett-Packard 8453 diode array spectrometer controlled by a Hewllet-Packard computer and equipped with a 100 μL quartz cell was used for recording the spectra. A centrifuge (Behdad Universal Centrifuge) was used to accelerate the phase separation process. The pH was determined with a model 780 Metrohm pH-meter with combined glass-calomel electrode. Box-Behnken design was accomplished with Minitab Version 16.

2.2. Reagents and materials

All reagents were of analytical reagent grade. The water utilized in all studies was double-distilled and deionized. Stock solution of Zn^{2+} (1000 µg mL^{-1}) was prepared by dissolving appropriate amounts of their corresponding salts in deionized water. Standards of working solution were made by appropriate dilution daily as required. The PAR, zephiramine and all solvents, such as methanol, ethanol, chloroform, carbon tetrachloride, acetonitrile and acetone were obtained from Merck. Universal buffer solutions were prepared from boric acid, citric acid and phosphoric acid (0.04 mol L^{-1}). The final pH was adjusted by the addition of 0.2 mol L^{-1} sodium hydroxide.

2.3. Dispersive liquid-liquid microextration procedures

A 10 mL of sample of standard solution containing 30-220 ng mL^{-1} of zinc and potassium nitrate (1%) was poured in a test tube with a conical bottom and 1 mL of PAR (5.0×10^{-4} mol L^{-1}) as chelating agent and 1 mL of zephiramine (5.0×10^{-4} mol L^{-1}) as auxiliary ligand was added into the solution. After a few minutes an orange complex of zinc was formed and the solution pH was adjusted to 8.0 by universal buffer. Then a binary solution containing 850 μL of ethanol (disperser solvent) and 150 μL of chloroform (extraction solvent) was injected rapidly into the sample. Then, Zn-PAR complex was extracted into fine droplets of chloroform. After that the mixture was centrifuged for 10 min at 4000 rpm. After this process the fine droplets of chloroform were joined together and sedimented at the bottom of the conical test tube. After removing the whole aqueous solution, it was transferred to a 70 μL cell and the absorbance was measured at 505 nm. The determined volume of the sedimented phase was about 150 μL.

2.4. Sample preparation before DLLME

Tap, mineral and waste water samples were collected from Arak (Arak, Iran). Prior to the preconcentration procedure, all the water samples were filtered through a 0.45 μm pore size membrane filter to remove suspended particulate matter and then stored at 4 °C in the dark. Blood serum samples were acidified using 2 mL of $HClO_4$:HNO_3 (1:3) mixture, boiled to near dryness, dissolved in doubly distilled water and pH was adjusted to 8.0 and diluted to 50 mL in a volummteric flask.

3. RESULTS AND DISCUSSION

Fig. 1 shows the absorption spectra after DLLME of PAR and Zn-PAR-Zephiramine complex at optimum conditions.

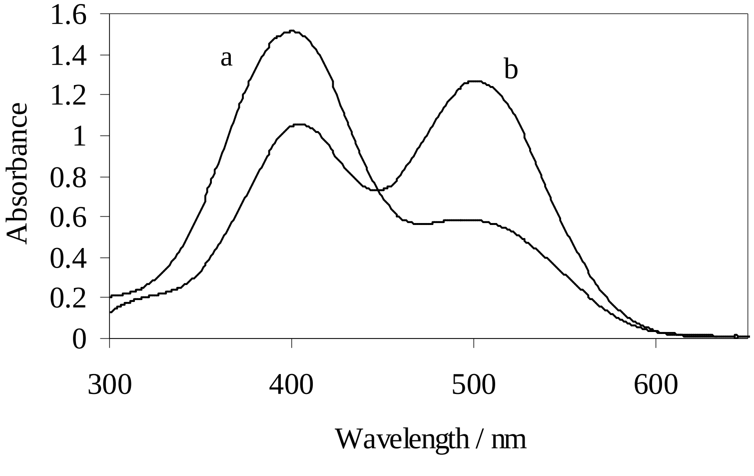

Fig. 1. Absorption spectra of (a) PAR and (b) Zn-PAR-Zephiramine complex (200 ng mL^{-1} of zinc) in under optimum conditions.

There are different factors that affect the extraction process. Some of them are selected as suitable pH, concentration of PAR and Zephiramine, extraction solvent, disperser solvent, volume of extraction solvent, volume of disperser solvent, ionic strength of aqueous phase, and extraction time. It is very important to optimize them in order to obtain good recovery and strategy forms.

3.1. Factorial Design

A two level factorial 2^4 design with two replicates of center point is performed in order to determine the influence of these factors on their interactions. The factorial design is evaluated using analytical response (Absorbance in 505 nm) of Zn-PAR-Zephiramine. The experimental matrix and the A for each trial are shown in Table 1. An analysis of the variance (ANOVA) demonstrated that, within the experimental range, pH, concentration of PAR, volume of extraction solvent and disperser solvent were statistically significant and Pareto chart (Fig. 2) interpretation it.

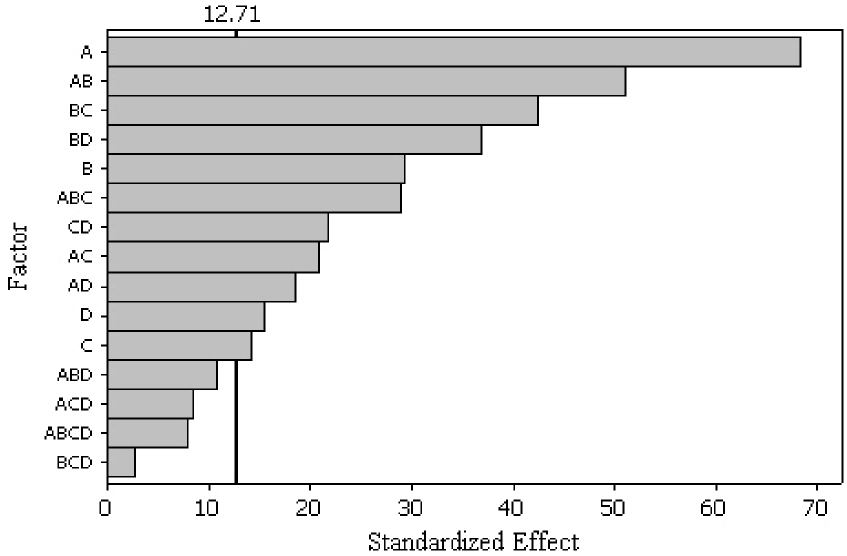

Fig. 2. Pareto chart of main effects obtained from 2^4 full factorial designs. The vertical line defines the 95% confidence interval (A = pH, B = Concentration of PAR, C = Volume of disperser solvent and D = Volume of extraction solvent).

3.2. Box-Behnken design

The significant variables like pH; concentration of PAR, volume of extraction solvent and disperser solvent are chosen as the critical variables and designated as pH, C_{Ligand}, $V_{disperser}$ and $V_{extration}$, respectively. The low, middle and high levels of each variable were designated as -, 0 and + respectively, are given in Table 2. The actual design of experiments is given in Table 3. In a system involving four significant independent variables, the mathematical relationship of the response on these variables can be approximated by the quadratic polynomial equation:

$$Y = \beta_0 + \beta_1 \, pH + \beta_2 \, C_{Ligand} + \beta_3 \, V_{disperser} + \beta_4 \, V_{extraction} + \beta_{12} \, pH \times C_{Ligand} + \beta_{13} \, pH \times V_{disperser} + \beta_{14} \, pH \times V_{extration} + \beta_{23} \, C_{Ligand} \times C_{Ligand} + \beta_{24} \, C_{Ligand} \times V_{extration} + \beta_{34} \, V_{disperser} \times V_{extration} + \beta_{11} \, pH^2 + \beta_{22} \, C_{Ligand}^2 + \beta_{33} \, V_{disperser}^2 + \beta_{44} \, V_{extration}^2 \qquad \text{Equation 1}$$

Table 1
Experimental design and the results obtained in A.

Number	pH	C_{Ligand} (mol L^{-1})	$V_{disperser}$ (μL)	$V_{extraction}$ (μL)	A

1	7	6.0×10^{-5}	900	200	1.6428
2	9	5.0×10^{-5}	900	100	0.7666
3	7	5.0×10^{-5}	800	100	0.8283
4	7	5.0×10^{-5}	800	200	1.3685
5	9	6.0×10^{-5}	800	100	0.5499
6	9	5.0×10^{-5}	900	200	0.8404
7	7	6.0×10^{-5}	800	200	1.1131
8	9	6.0×10^{-5}	900	100	0.8232
9	8	5.5×10^{-5}	850	150	1.5029
10	7	6.0×10^{-5}	800	100	1.5804
11	8	5.5×10^{-5}	850	150	1.4814
12	9	6.0×10^{-5}	800	200	0.2128
13	9	6.0×10^{-5}	900	200	0.8504
14	7	5.0×10^{-5}	900	200	1.2523
15	7	5.0×10^{-5}	900	100	0.1747
16	7	6.0×10^{-5}	900	100	1.7276
17	9	5.0×10^{-5}	800	200	0.7598
18	9	5.0×10^{-5}	800	100	0.7289

A multiple regression analysis is done to obtain the coefficients and the equation can be used to predict the response. The degree of experiments chosen for this study was Box-Behnken, a fractional factorial design for four independent variables. It is applicable as the critical variables have been identified. In the model given in equation (1), interactions higher than second order have been neglected.

Table 2

The levels of variables chosen for the trials.

Variable	Low level (-)	Middle level (0)	High level (+)
pH	7.0	8.0	9.0
C_{Ligand} (mol L^{-1})	5.00×10^{-5}	5.25×10^{-5}	5.50×10^{-5}
$V_{disperser}$ (μL)	800	850	900
$V_{extraction}$ (μL)	100	150	200

A total of 27 experiments were necessary to estimate of the full model. The results from this experimental design provided a statistical process, which was used to identify high yield trends for the DLLME process. Table 3 shows the matrix with coded and the responses for absorbance of Zn-PAR-Zephiramine complex at 505 nm. The equation below illustrates the relationship among the four variables.

$$A = 07642 + 0.0699\,(pH) + 00293\,(C_{Ligand}) - 0.0449\,(V_{extraction}) + 0.0176\,(V_{disperser}) + 0.1269\,(pH)^2 - 0.0323\,(C_{Ligand})^2 - 0.0276\,(V_{extraction})^2 + 00471\,(V_{disperser})^2 - 0.0943\,(pH)(C_{Ligand}) + 0.0322\,(pH)(V_{extraction}) + 0.0499\,(pH)(V_{disperser}) + 0.0724\,(C_{Ligand})(V_{extraction}) + 0.0219\,(C_{Ligand})(V_{disperser}) - 0.0961\,(V_{extraction})(V_{disperser}) \qquad \text{Equation 2}$$

The critical points in the surface response are found by solving the derivation of equation 2 for the condition of $\delta(A)/\delta(pH) = 0$, $\delta(A)/\delta(C_{Ligand}) = 0$, $\delta(A)/\delta(V_{disperser}) = 0$ and $\delta(A)/\delta(V_{extraction}) = 0$. The calculated values for the critical point are: pH = 8.0, $C_{Ligand} = 5.0\times10^{-5}$ mol L^{-1}, $V_{disperser}$ = 850 μL and $V_{extracion}$ = 150 μL.

Table 3

The Box-Behnken design for the optimization of the DLLME parameters.

Number	pH	C_{Ligand} (mol L^{-1})	$V_{disperser}$ (μL)	$V_{extraction}$ (μL)	A

1	9	5.5×10^{-5}	900	150	0.9901
2	8	6.0×10^{-5}	850	100	0.8120
3	8	5.5×10^{-5}	850	150	0.8129
4	8	5.5×10^{-5}	900	100	0.8020
5	8	5.5×10^{-5}	850	150	0.7899
6	7	5.0×10^{-5}	850	150	0.6659
7	8	5.0×10^{-5}	850	100	0.8319
8	8	5.0×10^{-5}	800	150	0.7292
9	8	5.5×10^{-5}	850	150	0.6899
10	8	5.0×10^{-5}	850	200	0.4401
11	8	5.5×10^{-5}	900	200	0.5908
12	7	5.5×10^{-5}	800	150	0.7745
13	8	5.5×10^{-5}	800	100	0.8192
14	8	6.0×10^{-5}	850	200	0.7098
15	9	5.5×10^{-5}	800	150	0.9718
16	9	5.5×10^{-5}	850	100	0.9301
17	9	5.0×10^{-5}	850	150	0.9601
18	8	5.5×10^{-5}	800	200	0.9923
19	7	6.0×10^{-5}	850	150	0.9807
20	8	5.0×10^{-5}	900	150	0.8819
21	7	5.5×10^{-5}	850	100	0.7777
22	8	6.0×10^{-5}	900	150	0.8503
23	8	6.0×10^{-5}	800	150	0.6098
24	7	5.5×10^{-5}	900	150	0.9923
25	9	6.0×10^{-5}	850	150	0.8976
26	9	5.5×10^{-5}	850	200	0.9912
27	7	5.5×10^{-5}	850	200	0.7102

3.3. Effect of type of the extraction and disperser solvents

Selecting the extraction solvent by paying attention to its characteristic properties is very important. It must have a higher density than water, be capable of extracting the compounds of interest, and also low solubility in water. Chloroform, carbon dichloride and carbon tetrachloride were compared in this extraction and the obtained recoveries were highest for chloroform. The main criterion for the selection of the disperser solvent is its miscibility in the extraction solvent and aqueous solution. In addition, the type of disperser directly influences the viscosity of the binary solvent. Thus, this solvent can control droplet production and extraction efficiency. To study this effect, four different solvents such as acetone, acetonitrile, ethanol and methanol were tested and according to the recoveries the most suitable solvent was ethanol.

3.4. Effect of extraction time and centrifuging rate

In DLLME, extraction time is defined as the time between the injection of the binary solvent and starting to centrifuge. The effect of extraction time was examined in the range of 1-10 min with constant experimental conditions. The surface area between extraction solvent and aqueous phase is infinitely large. Thereby, transfer of complex from aqueous phase to extraction solvent is fast. Subsequently, equilibrium state is achieved quickly; therefore, the extraction time is very short, which is the advantage of DLLME technique. In this method, the most time-consuming step is the centrifuging of sample solution, which is about 1 min.

A series of solutions with same other conditions were tested at various rates of centrifugation. The rate of centrifugation was adjusted between 1000 to 6000 rpm for 10 minutes. Fig. 3 shows the influence of rate of centrifugation in DLLME and also on R.S.D. The absorbance increased slowly with the increase of rate to 4000 rpm and after that, it approximately stayed constant. The rate of 4000 rpm was selected as the optimum rate for centrifuging.

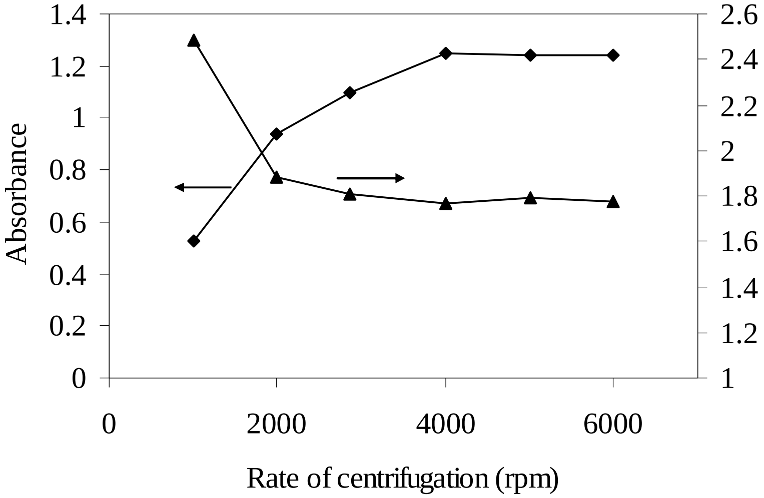

Fig. 3. Effect of rate of centrifugation on DLLME and also on R.S.D. under other optimum parameters.

3.5. Effect of salt

For studying the influence of ionic strength on the performance pf DLLME, we investigated NaCl concentration in the range of 0-15 % (w/v) while other experimental conditions were kept constant. By increasing NaCl concentration, extraction efficiency approximately stayed constant. These studies showed the applicability of DLLME for separation of zinc from saline solution to 15% (w/v).

3.6. Interferences study

The potential interference in the present method is investigated. The interference was due to the competition of other heavy metal ions for the chelating agent and their subsequent co-extraction with zinc. Although PAR is nonselective ligand, the selectivity may be improved by using masking agents and choosing the condition of solvent extraction because the extractability of the chelates varies to a great with metal ion, pH, and the nature of the auxiliary ligand. EDTA is useful masking agent in PAR photometry. In these experiments, solutions containing 50 ng mL^{-1} of zinc in presence of EDTA (0.001 mol L^{-1}) and the interfering ions were treated according to the recommended procedure. The tolerance limits of the co-existing ions, defined as the largest amount making the recovery of zinc less than 95%, are given in Table 4.

Table 4
Tolerance limits of co-existing ions in DLLME of zinc.

Co-existing ions	Tolerance limits (ng mL^{-1})

Co_3^{2-}, No_3^-, Br^-, $S_2O_3^{2-}$, SO_3^{2-}, I^-, CH_3COO^-, Cl^-, SCN^-, BrO_3^-, $C_2O_4^{2-}$, PO_4^{3-}	1000
Li^+, Rb^+, K^+, Na^+, NH_4^+, Cr^{3+}	1000
Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, As^{3+}, Mn^{2+}, Cs^+, Cd^{2+}, Zr^{4+}, Ga^{2+}, Pd^{2+}, Al^{3+}, In^{3+}, Tl^+, Sn^{2+}, Hg^{2+}	500
Ni^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}	100

3.7. Analytical performance

Table 5 summarizes the analytical characteristics of the optimized method, including linear range, limit of detection, reproducibility ad enhancement factor. The calibration graph was linear in the range of 30-220 ng mL^{-1} of zinc. The limit of detection, defined as $C_{DL} = 3\ S_B/m$ (where C_{DL}, S_B and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), was 11.2 ng mL^{-1}.

Table 5
Analytical characteristics of DLLME for determination of zinc.

Parameter	Analytical feature
Calibration curve before extraction	$A = 2.4150\ C_{Zn} + 0.0412$ ($R^2 = 0.9902$)
Calibration curve after extraction	$A = 0.0185\ C_{Zn} + 0.1036$ ($R^2 = 0.9923$)
Linear range (ng mL^{-1})	30 - 220
Limit of detection (ng mL^{-1})	11.2
Repeatability (R.S.D., %) (n = 10)	1.4
Enhancement factor (mL)	130

The relative standard deviation (R.S.D.) for ten replicate measurements of 50 ng mL^{-1} Zn(II) was 1.4%. The enhancement factor was obtained from the slope ratio of the calibration graph after and before extraction, which was about 130. The equations of calibration graphs after and before extraction are summarized in Table 5.

3.8. Analytical applications

The proposed DLLME method was validated by extraction and determination of zinc from spiked tap, mineral and waste water and real sample such as human blood samples and the results are summarized in Table 6. For this purpose, 10 mL of the water samples were spiked with 50 and 100 ng mL^{-1} zinc standard solution and the recommended procedure was followed to assess the matrix effects. The method was also applied to the determination of zinc content, as well as recovery of some added zinc to, a fish sample and the results are also included in Table 6. As it is obvious from Table 6, in all cases, the extraction efficiency of zinc was excellent, and showed no serious matrix effects.

Table 6
Determination of zinc in different samples.

Sample	Zinc spiked (ng mL^{-1})	Zinc found [a] (ng mL^{-1})	Recovery (%)
Tap water [b]	0	N.D. [c]	-
	50	58.6 ± 0.3	117.2
Mineral water [b]	0	N.D.	-
	50	53.7 ± 0.4	107.4
Waste water [b]	0	109.5 ± 0.4	-
	100	211.3 ± 0.6	101.8
Human blood	0	38.6 ± 0.4	-
	50	89.9 ± 0.4	102.6
Human Blood	0	42.6 ± 0.3	-
	100	144.7 ± 0.5	102.1

[a] Mean ± S.D., n = 3.
[b] Water samples were collected from Arak, Iran
[c] Not detected

4. CONCLUSION

A new method of DLLME combined with spectrophotometric has been proposed for the determination of zinc in tap, mineral, waste water and human blood samples. The proposed DLLME method takes advantages from low cost, use of minimized toxic organic solvents, simplicity of operation, rapidity, high enhancement factor and high sensitivity and selectivity. The limit of detection of proposed method (11.2 ng mL^{-1}) is significantly improved over that of the best of previously reported spectrophotometric methods for zinc determination. Also, the application of a Box-Behnken matrix became possible, rapid, economical and efficient way of an optimization strategy of the proposed procedure.

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