Research Article

Efficient and Clean Pre-concentration of Toxic Metal Ions from Real Samples Via a Brand-new Descendant of Eco-friendly Deep Eutectic Solvent Based Tandem Air-Agitated Liquid-Liquid Microextraction Followed by Inductively Coupled Plasma-Optical Emission Spectrometry

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PAPER INFO	ABSTRACT
	In this work, we present a novel extracting media named as deep eutectic solvent containing choline chloride and cinnamyl alcohol (ChCl: Cinnamyl alcohol), that has unique properties such as simple and cheap synthesize, eco-friendly, and bio-degradability. 2-amino-5-mercapto-1,3,4-thiadiazole was used as the chelating agent. Then, it was efficiently employed at the technique of Tandem air-agitated liquid-liquid microextraction (TAALLME) of metal ions (Pb^{2+} and Cd^{2+}) in biological and aqueous samples pursued by inductively coupled plasma-optical emission spectrometry (ICP-OES). This secure, impressive, and speedy pre-concentration procedure based on the modern DES was quickly practical with no extra requirement the emulsifier intermediates. Effective statistical optimization of basic variables results by the Central Composite Design (CCD) demonstrated that pH of acceptor phase 7.00, pH of donor phase 2.00, 1175µL of acceptor solvent, and 325µL of extraction solvent be consequented on topmost extraction recoveries. In optimum conditions, the reliable analytical features were found to be: large range of linearity (LDRs) 0.65-250 and 0.065-250 ng mL^{-1}, low limits of detection (LODs) of 0.2 and 0.02 ng mL^{-1}, and rational precision (%RSDs, n=5) of 2.98 and 2.93% for Pb^{2+} and Cd^{2+}, respectively. Also, recoveries of extraction and enrichment factors were 97-98% and 41.84, respectively. These sufficiently good results confirmed the powerful efficacy of the suggested microextraction technique for achievement to pure and proper enrichment of the aforementioned compounds in highly complex real samples.

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1. Introduction

Recently, growing amount of toxic metal ions in the environment, food, and cosmetic samples have indicated an earnest alarm to human health and eco-system. Elements at low concentrations levels have important positive and negative roles in biochemical functions in alive organisms. Some of them such as copper, zinc, and manganese are indispensable for health life, but high dosage amounts can cause solemn toxicity and unusual metabolism. While, some elements such as lead and cadmium are unnecessary for our life, and because of their high agglomeration propensity in alive weaves, they can reason harmful effects on environment and human health [1-3]. The attendance of toxic metals even in trace levels can causes to serious disorders such as heart disease, cancer, breathing problems, kidney disease, and problems of nervous system; hence, it is essential to apply efficacious and speedy techniques for determination of toxic metals in the environment [4].

Atomic spectrometric methods containing inductively coupled plasma optical emission spectrometry (ICP-OES), flame atomic absorption spectrometry (FAAS), electro-thermal atomic absorption spectrometry (ET-AAS), inductively-coupled plasma mass spectrometry (ICP-MS), are commonly used to determine metal ions in different real samples [5]. Due to its prominent figures of merit, ICP-OES is widely utilized in food sciences: (i) well precision and accuracy; (ii) low LOD; (iii) wide linear range; and (iv) Ability to measure multiple elements simultaneously. Despite the considerable progresses made in the analytical instruments, due to the low analytes concentration in real samples and the complexity of sample matrices, most analytical instruments cannot determinate complex matrices straightly, and thus need to pre-treatment steps [6].

Sample preparation is a key step prior to the analysis of most samples, affecting the accuracy and precision of the conclusive outcomes [7,8]. Recently, due the promising goals of the green chemistry, the usual methods utilized for the separation and extraction of

the analytes such as LLE (liquid-liquid extraction) and SPE (solid phase extraction) is already replaced by the bio-compatible and miniaturized extraction techniques such as LPME (liquid-phase microextraction) and SPME (solid-phase microextraction) [9]. because of easiness, cost-less, and high efficiency in the microextraction methods, LPME is rather more applicatory than SPME. Dispersive liquid–liquid microextraction, as a favorite technique, supplies an easy function, efficient recovery, and good enrichment factor at a high speed [10,11]. However, a little sample clean-up is the basic disadvantages of this technique [12]. Although, the method of dispersive liquid–liquid microextraction can be combined with another extraction method such as SPE [13], electro membrane extraction (EME) [12], molecular imprinted polymer extraction (MIPE) [14], and SFE [15] to improvement purification capability, but some are complex, grueling, and time consuming. In the recent years, our group has resolved the difficulty of low sample clean-up via an efficient technique named as tandem dispersive liquid–liquid microextraction [16]. In fact, this method improves the sample clean-up by using an easy back-extraction step [17]. As know, the use of chlorinated organic solvents is severe opposition with green chemistry principles. To prevail, some helpful suggestions are provided. solvents with lower toxicity, such as long-chained hydrocarbons and alcohols, can be introduced as suitable choices, but finite capability for extraction of polar analytes and/or requirement the special extraction instruments have surrounded their usage. Also, for improvement of extraction performance along with eco-compatibility, an extraction medium with special attributes (such as partial vapor pressure, changeable viscosity, and high thermal stability) named as ionic liquids (ILs) have been suggested [18-22]. Recently, some of ionic liquids based on imidazolium species, because of problems such as effortful and expensive synthesis process and low safety, were substituted with inexpensive and bio-compatible ones [23]. Deep eutectic solvent (DES) are modern and more bio-

degradable successors for ILs. They are quickly formed by combination of affordable and green crude materials, named as the quaternary ammonium salt (generally choline chloride; ChCl) and the hydrogen bond donor (HBD); containing amines, carboxylic acids, alcohol, and carbohydrates).

In these solvents, the dominant bonds can be efficaciously instated by powerful hydrogen bonding among hydrogen atoms and halide anions which are highly accountable for the significant decrement in the melting/freezing point of DESs [24,25]. For addition, one of the most important of their advantages is the great potency to determination of vast range of analytes with various polarities, which can make them excellent options for enrichment and pre-concentration of analytes [26-30]. Because of the high solubility in two phases, some of them such as the ones made of glycerol, ethylene glycol or urea are unsuitable in the separation of phases method in aqueous solutions with high volume, and are used in oily media. Although, using of materials such as phenol nearly offset the above problem, but the high toxicity of these substances is considered as an important point [31-33].

In this study, we employed an entirely eco-friendly extraction solvent based on ChCl and Cinnamyl alcohol for effective and secure tandem extraction of metal ions in food samples for the first time. ChCl is accepted as green material, choline which specified as a Water-soluble vitamin (B vitamin) and also widely used in Livestock food industry. Cinnamyl alcohol is an organic compound that are naturally present in Cinnamon leaves. this compound has a specific odor qualified as "sweetish, balsam, jacinth, green, spicy, cinnamic" and is applied in Perfume industry. Furthermore, easy and cheap synthesis without requirement the emulsifier intermediates is the other prominent facet.

2. Experimental

2.1. Chemicals

Stock solutions of Pb^{2+} and Cd^{2+} ions were prepared (1000 mg L^{-1}) via solving proper amounts of $Pb(NO_3)_2 \cdot 6H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$ in distilled water,

and stored at 4°C. The working solutions of the demanded concentrations were obtained daily by adequate stepwise dilutions of the stock solutions with distilled water. The other chemicals used to include Choline chloride (ChCl, 99%), phenol (Ph, 99%), H_3PO_4 (85%), HNO_3 (65%), HCl (37%), and NaOH (≥97%) were supplied from Merck (Darmstadt, Germany). Cinnamyl alcohol (%98) was obtained from Sigma–Aldrich (St. Louis, MO, USA). All the other reagents used were at least of the analytical reagent grade.

2.2. Instrumentation

Model GENESIS FEE ICP-OES (Spectro Genesis, Germany) was employed for the determination of the metal ions. Determination of Pb^{2+} and Cd^{2+} by ICP-OES was performed for comparison purposes after sample digestion. Proton Nuclear Magnetic Resonance (1H-NMR 500 MHz, DMSO-d6) spectra were obtained using a Bruker spectrometer. Chemical shifts were reported as 5 (ppm). Fouriertransform-infrared (FT-IR) spectrometer (Shimadzu, 8400 s, Japan) was used to identify functional groups of DESs using KBr pel-let technique. Phase's separation was performed using Hettich centrifuge model EBA20 (Tuttlingen, Germany). A PHS-3BW model pH-meter (Bell, Italy) with a combined Ag/AgCl glass electrode was applied for the pH measurements of the sample solutions. A SW3-type ultrasonic water bath (SONO SWISS, Switzerland) at 50/60 kHz (80 W) was used for a create good dispersion into the aqueous medium and attainment to efficient extraction in a short-time. For weighing the solid materials, an electronic analytical balance (Shimadzu LIBROR AEU-210) was utilized.

2.3. Preparation of extraction solvent

In this work, the reported method in literature [27] was applied for the preparation of the suggested extraction solvent. It included of a mixture of environmentally friendly ingredients named as ChCl and Cinnamyl alcohol (molar ratios of 1:4). firstly, the related ingredients were combined at room temperature until transparent fluids were formed. Hence, it was applied

for the microextraction method without requirement to any emulsifier agent and complicated instruments.

2.4. Procedure

TAALLME was carried-out via two consecutive stages. firstly, 50.00 mL of the sample solution (pH 2.00) was placed into a 50.00-mL screw-cap glass test tube with conic bottom. PAN (10^{-3} M), as the complexing agent, were added. Then, 325 μL of DES based on ChCl: Cinnamyl alcohol as a bio-compatible and effectual extraction solvent was added, and next the mixture was frequently sucked and dispensed into tube with a 10 mL glass syringe (ten times). Then, the resulting emulsion was centrifuged (4 min at $1132 \times g$), and the tiny droplets of organic phase enriched with the target analytes placed at the settle down of the centrifuge tube. At this stage, due to the simultaneous preconcentration of target analytes and interference ions, the sample clean-up is greatly reduced. The organic solution was transferred to a smaller glass tube with taper bottom, and 1175 μL of the aqueous solution with pH 7.00 was added to it. Afterwards, the second stepped-up dispersion microextraction was used via 15 air-agitation cycles using a glass syringe (100-μL), and then was centrifuged for 1 min at $1132 \times g$. This step provided a plain and effectual back extraction (in less than 4 min), and also more clean-up of sample. Finally, 1.0 mL of the supernatant phase injected into the ICP system for analysis. The performance of the suggested extraction process was evaluated by computation of ER% and EF (the extraction recovery percentage, and the enrichment factor, respectively) as follows:

$EF = C_{final}/C_{initial}$

$ER = n_{final}/n_{initial} \times 100 = EF \times VeVs \times 100$

Where $C_{initial}$ and C_{final} are the concentrations relevant to the initial and final concentrations of target analytes in donor and acceptor phase, respectively; $n_{initial}$ and n_{final} are the initial and final mole numbers of the analytes available in the sample solution and extracting phase and, respectively; Vs is the sample solution volume; and Ve is the extracting phase volume.

2.5. Preparation of sample

human bio-monitoring has become a precious implement for the probe of toxic elements in human body. therefore, urine samples are the most commonly used as matrices to recognize to toxic metals in human body. Other biological samples such as hair, saliva and nails have suitable advantages over the usual ones in the assessment of the dosage of toxic metals in human body since they contain simple availability, nice stability of matrices, easy collection and transportation, lack of the need for specific storage conditions, and repeated determinations over time.

a) Human urine

The urine sample was prepared from a healthful volunteer and collected in pre-washed laboratory containers. Then the urine sample was filtered by Whatman filter paper with a diameter of 125 mm and a hole size of 40. In order to prevent the growth of bacteria, the glass containers used were washed with hydrochloric acid and distilled water. Then for stabilization purposes, 5.00 ml of the urine sample was taken and made up to 50.00 ml using distilled water, and adjusted to pH 2.00 before use [34].

b) Human saliva

The saliva sample was collected within 10 min with the mouth closed, introduced into a polypropylene tube, and centrifuged at 10000 rpm for 5 min in order to eliminate any sediment cellular debris. Next,1.50 ml of the saliva sample was brought to a volume of 50.00 ml using distilled water. After adjusting the pH of the sample solution to a constant value of 2.00, the proposed method was used for extraction [35].

c) Human hair and nails

In order to eliminate the surface impurity and grease, the hair and nail samples were first placed in a mix solution with a ratio of 3:2:5 (v/v) of diethyl ether, acetone, and deionized water, respectively, for 1 h using an ultrasonic bath. Then the samples were washed with deionized water and dried in an oven at about 110 ∘C. A 50 mL of concentrated HNO_3 was added to accurately weighed samples (2.00 g). After evaporating to near dryness (150 ∘C), 5.00 mL of concentrated H_2O_2 (or $HClO_4$) was added to destroy the

organic matter in the samples, and the evaporation process was repeated. If digestion did not occur, more acid was added before evaporation. The mixture was then made up to 50.00 mL with distilled water, and finally filtered. The pH value for the filtrate was adjusted to 2.00 and the microextraction method was then performed [36].

d) Tap water

The tap water samples were taken from piped water of Semnan city. For this purpose, 50.00 ml of water sample was collected in a polyethylene container. In order to remove the existing impurities, the sample was smoothed by Whatman filter paper and kept at a temperature of 4 degrees Celsius. Next, the sample was set at pH 2.00 to perform the proposed method and submitted to the analytical device for final analysis.

2.6. Experimental design methodology

For statistical optimization of the current emulsification microextraction, a 5-levels CCD comprised 1-block and 4-factors was utilized. The involving experimental factors consisted of pH of donor phase (1.00-5.00), pH of acceptor phase (4.00-8.00), volume of extraction solvent (100–400 µL), and volume of acceptor phase (900-2000 µL). The operational domains of relevant factors as well as design matrix and the experimental responses

(extraction percentage) are displayed in Table 1. The software used for design of experiments was Design Expert 7.0.0.

3. Results and discussion

3.1. Characterization of DES

The crucial functional groups of the suggested Deep eutectic solvents can be specified by FT-IR spectrum. as seen as in Fig. 1(a-c), The FT-IR spectra related to structure of pure cinnamyl alcohol, pure choline chloride, and DES are showed. The Vibrations shown in areas of 3286 cm^{-1} and 1091 cm^{-1} are relevant to the O–H and C–N bonding of pure choline chloride, respectively. Also, stretching vibrations related to C–C, C–O, and O–H bonds of cinnamyl alcohol can be determined by the peaks shown in the areas of (1450 and 1550 cm^{-1}), (1210 cm^{-1}), and (3300 cm^{-1}), respectively. The dominant interaction between ChCl and cinnamyl alcohol in the DES is mostly hydrogen bonding, which easy shown via a broad peak at the range of 3300 cm^{-1}. The wider the peak and its little shift to lesser wavenumbers vs. to single components [27] is due to the subscription hydrogen atoms between electronegative atoms (Cl$^-$ and O) of ChCl and cinnamyl alcohol, that leading to the foundation of the related eutectic medium. These outcomes demonstrates that this product is made with confidence.
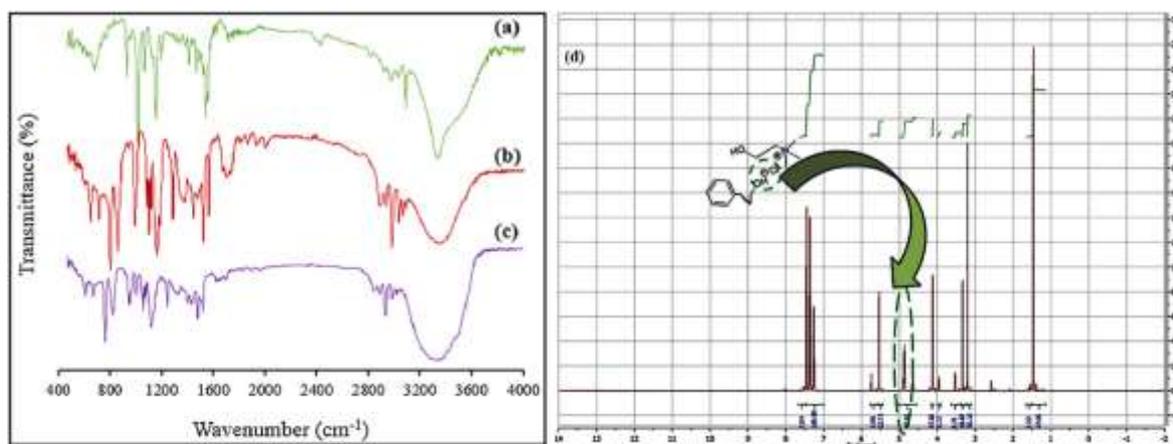
Fig. 1. (a-d) FT-IR and 1HNMR spectra of DES (ChCl: Cinnamyl alcohol).

The HNMR spectra of mentioned DES is showed in Fig. 1(d). firstly, it was diluted with helping of deuterated DMSO-d6. As seen, severe overlapping among the methylene groups of Cinnamyl alcohol and

ChCl causes some signals to merge (around 3–4.5 ppm), and resulting in uncertainty. As well as, multi-signals appeared in the proximity of 7.5 ppm confirm the hydrogen atoms connected to aromatic ring. In

addition, the observed signal is about 5 ppm for the bridged hydrogen atom among oxygen and chloride species, that implying on formation of hydrogen bonding between the involved components in the

related DES. Therefore, mentioned results show the successful synthesis of the proposed deep eutectic solvents included of ChCl: Cinnamyl alcohol.

Table 1. A five-level CCD based on 1-block and four main factors for TAALLME-DES of metal ions.

Independent variables			Ranges and levels				
			-α	-1	0	+1	+α
(A) pH of acceptor phase			4	5	6	7	8
(B) pH of donor phase			1	2	3	4	5
(C) Volume of organic solvent (µL)			100	175	250	325	400
(D) Volume of acceptor phase (µL)			900	1175	1450	1725	2000
	Factor				Recovery%		
[a]Run	A	B	C	D	R_1		R_2
1	7	2	175	1725	77.10		76.60
2	6	3	250	1450	85.10		85.90
3	7	2	325	1175	97.10		98.34
4	5	4	175	1725	79.52		79.30
5	6	3	100	1450	84.24		84.30
6	7	2	325	1725	82.36		81.90
7	6	3	250	1450	85.10		85.30
8	4	3	250	1450	70.65		70.30
9	6	1	250	1450	86.50		87.10
10	7	4	175	1175	83.78		84.10
11	6	3	400	1450	86.32		86.00
12	5	2	175	1725	76.62		76.40
13	6	3	250	1450	85.01		85.20
14	7	4	175	1725	86.10		86.40
15	6	3	250	1450	85.10		84.90
16	7	2	175	1175	92.31		93.40
17	5	4	175	1175	81.20		81.50
18	6	5	250	1450	83.70		84.10
19	6	3	250	1450	85.10		85.00
20	5	2	325	1175	84.55		84.50
21	8	3	250	1450	82.00		82.30
22	5	2	325	1725	75.10		74.91
23	6	3	250	2000	79.32		79.60
24	5	2	175	1175	86.00		85.40
25	6	3	250	1450	85.36		85.10
26	6	3	250	900	91.38		91.60
27	7	4	325	1175	83.40		83.60
28	5	4	325	1175	80.54		79.90
29	7	4	325	1725	86.23		86.10
30	5	4	325	1725	78.86		78.80

[a]The tested concentration was 200 ng mL^{-1}.

[b]R is the response analyzed (Extraction recovery); Indexes of 1 and 2 refer to Pb^{2+} and Cd^{2+}, respectively.

The HNMR spectra of mentioned DES is showed in Fig. 1(d). firstly, it was diluted with helping of deuterated DMSO-d6. As seen, severe overlapping among the methylene groups of Cinnamyl alcohol and ChCl causes some signals to merge (around 3–4.5 ppm), and resulting in uncertainty. As well as, multi-signals appeared in the proximity of 7.5 ppm confirm the hydrogen atoms connected to aromatic ring. In addition, the observed signal is about 5 ppm for the

bridged hydrogen atom among oxygen and chloride species, that implying on formation of hydrogen bonding between the involved components in the related DES. Therefore, mentioned results show the successful synthesis of the proposed deep eutectic solvents included of ChCl: Cinnamyl alcohol.

3.2. Optimization of the TAALLME-DES-ICP method

3.2.1 Extraction solvent effect

Choice of an extracting solvent is impressive value in the LPME techniques, but however this selection is conditional on several specifications: low water-solubility, lower density than water, high extraction efficiency, and good stableness. In this study, two types of deep eutectic solvents (ChCl: Cinnamyl alcohol and ChCl:Ph) were examined for TALLME-DES of target analytes. According to Fig. 2, ChCl: Cinnamyl alcohol was obtained the excellent extraction recovery for the Pb^{2+} and Cd^{2+}. Hence, this solvent was chosen as the extraction solvent for future steps.

3.2.2. Statistical assessment of main numeric factors

Table 2 shows the significant competency of fitted models, qualified by analysis of variance (ANOVA) based on Fisher's test (F-test) and rejection of null hypothesis. According to Table 3, the p-values for all models and Lack of Fit are less and more than 0.05, respectively, indicating the high significance of the fitted models at the 95% confidence level. According to above criteria, the resultant quadratic equations in terms of actual factors within TAALLME-DES of two metal ions can be expressed as follows:

For Pb^{2+}:

$$Y = -37.80 + 19.08A - 8.02B - 2.14D + 1.69AB + 0.46AD - 0.02BC - 0.36BD - 1.28A2 - 0.06D2$$

For Cd^{2+}:

$$Y = -16.10 + 17.75A - 9.02B - 0.10C - 0.97D + 1.69AB + 0.42AD - 0.53BD - 1.26A2 - 0.25B2 - 0.05D2$$

The values of R-squared and adj R-squared for both quadratic models are higher than 0.90 and close to each other. It means that he differences between the experimental and the predicted values is negligible and relevant quadratic models possess the satisfactory accuracy and reliability [37].

3.2.3. Interaction effects of process variables

Fig. 3(a–d) demonstrates the main 3D and 2D surface plots relevant to important Mutual effects within TAALLME-DES of two metal ions. These dual interaction effects and the excellent functional

conditions can be expressed with the help of the mentioned fitted models, as follows:
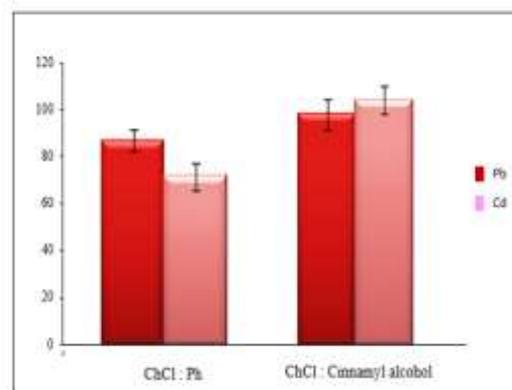

Fig 2. Effect of extracting solvent type onto TAALLME-DES of Pb^{2+} Cd^{2+}; (Conditions: pH of donor phase 2.00, pH of acceptor phase 7.00, 325 µL of extraction solvent, 1175 µL of acceptor phase, C = 200 ng mL^{-1}, n = 3).

As shown in this figure, by reducing and increasing the pH values for the donor and acceptor phases, respectively, the extraction efficiencies of the metal ions improved. This means that the acidic media in the first DLLME affected the simple and quick transfer of the metal ions from the aqueous phase to the organic solvent. Then, in the latter DLLME, the value of pH for the acceptor phase should be neutral to efficiently retake the metal ions from the organic solvent.

other great interaction effect (X1X2) for the extraction of Cd^{2+} is shown in Fig. 3(d). As shown in this figure, the efficiency of extraction for this ion metal was improved by the increase of the organic phase volume and the pH value of the acceptor phase. However, after a specific volume of the organic phase, the amount of extraction of the metal ions is reduced, which can be related to the dilution effects.

3.3. Investigation of coexisting ions

Investigation of the effect of common interfering ions, on the analytical signals of the target analytes is a significant factor to evaluate the applicability of the analytical technique. In these experiments, the method was carried-out in the attendance of different concentrations of other possible interfering ions under optimum conditions.

Table 2. ANOVA in CCD for TAALLME-DES of metal ions.

Source of variation	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Pb(II)					
Model	775.51	13	59.65	1281.65	< 0.0001
Residual	0.74	16	0.047		
Lack of fit	0.67	11	0.061	4.32	0.0592
Pure error	0.071	5	0.014		
R² = 0.98	Adj-R²= 0.9966	Pred-R²=0.9931			
Cd(II)					
Model	1550.21	13	118.54	1211.12	< 0.0001
Residual	0.96	16	0.061		
Lack of fit	0.87	11	0.079	4.68	0.0559
Pure error	0.082	5	0.016		
R² = 0.98	Adj-R²= 0.9866	Pred-R²=0.9531			

If an ion leaded to a ±5% variation in the absorbance signal, was considered an interfering ion. In Table 3, the allowable concentration ratios of the considered ions to the analytes are shown, and as seen as that the considered ions do not interfere with the measurement of analytes at high concentrations. Based on these

experiential outcomes, the suggested technique had an excellent selectivity, and could be applied to the real samples containing the target analytes with a complicated matrix since it was not affected by high concentrations of the interfering ions.
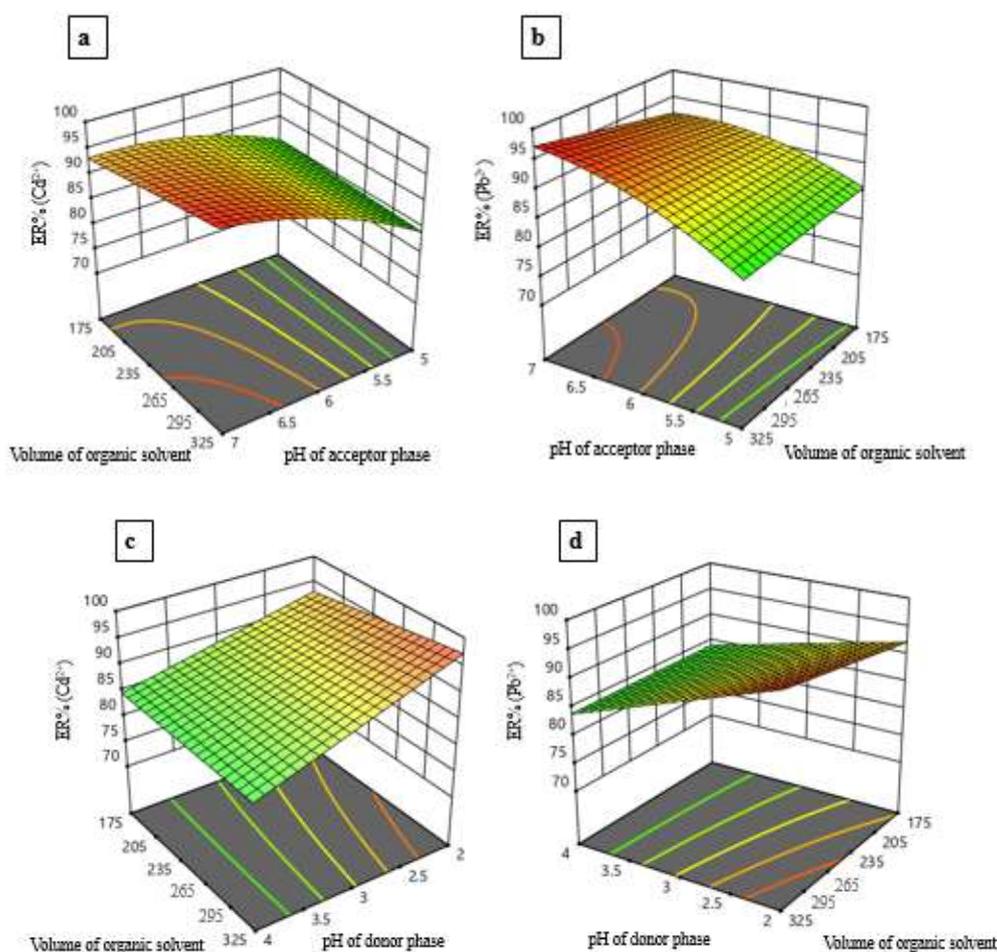

Fig. 3. Response surfaces for Pb^{2+} and Cd^{2+} ions as a representative analyte in TAALLME-DES method: (a) ,(b) pH of acceptor phase vs. Volume of organic solvent; (c),(d) pH of donor phase vs. volume of organic solvent.

Table 3. Influence of interfering species on recoveries of Pb^{2+} and Cd^{2+} and ions in optimum conditions.

Ion	Interference/Ion ratio	
	C_{Ions}/C_{pb}^{2+}	C_{Ions}/C_{Cd}^{2+}
Mg^{2+}	750	750
Cu^{2+}	250	100
Zn^{2+}	100	100
Ba^{2+}	250	750
Ca^{2+}	250	1000
Mn^{2+}	250	250
Na^+	750	1000
K^+	1000	1000
Li^+	1000	1000
Ag^+	250	500
Fe^{3+}	250	250
Al^{3+}	1000	250
F^-	1000	750
Cl^-	750	750
Br^-	1000	1000
NO_3^-	1000	750
CH_3COO^-	500	750
SO_4^{2-}	100	50
CO_3^{2-}	500	500

3.4. Analytical performance

In the aforesaid optimal experiential states, the analytical proficiency of the suggested method containing the linearity (LDR) of the calibration graphs, detection limit (LOD), relative standard deviation (RSD), and determination coefficient (R^2) were evaluated in the complex matrices such as biological and water samples (Table 4). As seen as,

good LDR in the range of 0.65-250 and 0.065-250 ng mL^{-1} with correlation coefficients (R^2) above 0.98 were obtained for Pb^{2+} and Cd^{2+}, respectively. Detection limits of in the range of 0.02-0.2 ng mL^{-1} was obtained. RSDs were sufficiently good, ranging from 2.98% to 2.93% for the target analytes, showing good repeatability of the method.

Table 4. Analytical results for Pb^{2+} and Cd^{2+} ions in real samples with TAALLME-DES method.

Sample	Pb^{2+}			Cd^{2+}		
	Added (ng mL^{-1})	Found (ng mL^{-1})	[a]RR (%)	Added (ng mL^{-1})	Found (ng mL^{-1})	RR (%)
Urine	0	19.80±[c]0.50	-	0	10.76±1.40	-
	20	39.20±0.51	97.00	20	30.03±0.69	96.50
	50	69.10±0.22	98.60	50	60.80±0.65	99.80
Saliva	0	7.60±1.31	-	0	[b]N.D	-
	20	27.20±0.36	98.00	20	19.60±1.37	98.00
	50	56.20±0.20	97.20	50	48.20±0.31	96.40
Hair	0	10.60±0.95	-	0	2.30±0.20	-
	20	29.90±0.33	96.50	20	21.70±1.40	94.00
	50	60.80±0.66	100.40	50	52.60±0.66	100.60
Nail	0	41.30±0.37	-	0	1.11±0.90	-
	20	61.00±0.43	98.50	20	19.53±0.70	93.00
	50	90.90±0.67	99.20	50	51.43±0.85	100.04
Tap water	0	33.50±0.45	-	0	N.D	-
	20	53.20±0.28	98.50	20	19.20±0.52	97.50
	50	83.40±0.30	99.80	50	49.53±0.616	99.60

[a] Relative Recovery.
[b] Not detection.
[c] Standard deviation.

Table 5. Comparison of TAALLME-DES method with other published methods.

Method	Sample	Analyte	LOD	LDR	PF(EF)	Ref
[a]UA-M-D-µSPE FAAS	Urine Plasma Nail Hair	Pb(II) Cd(II)	2.0 0.6	7-725 2-70	40 40	38
[b]DLLME-ICP-MS	Water	Pb(II) Cd(II)	0.015 0.006	0.5-250 0.1-250	20 19.7	39
[c]MSPE-ICP-MS	Biological	Pb(II) Cd(II)	0.157 0.038	1–150	-	40
DLLME-ICP-MS	Seawater	Pb(II) Cd(II)	0.02–0.11	0.2-200 0.1-100	-	41
[d]Reverse DLLME-ETAAS	water	Cd(II)	0.6	2 – 40	-	35
[e]SPE-ICP-MS	Well water	Pb(II) Cd(II)	0.2 0.012	1-160 0.5-160	150	42
[f]DMSPE-EDXRF	Water	Pb(II)	1.4	5-100	3.41	43
[g]TAALLME-DES-ICP	Urine Nail Hair Water	Pb(II) Cd(II)	0.12 0.02	0.3-250 0.065-250	41.84	This work

[a]Ultrasound assisted magnetic dispersive micro solid phase extraction flame atomic absorption spectrometry.

[b]Dispersive liquid liquid microextarction inductively coupled plasma mass spectroscopy.

[c]Micro solid phase extraction inductively coupled plasma mass spectroscopy.

[d]Reverse dispersive liquid liquid microextarction electrothermal atomic absorption spectroscopy.

[e]Solid phase extraction inductively coupled plasma mass spestroscopy.

[f]Dispersive micro solid phase extraction Energy-dispersive X-ray fluorescence spectrometry.

[g]Tandem air-agitated liquid-liquid microextraction deep eutectic solvent inductively coupled plasma.

3.5. Application of real samples

For that to demonstrate the feasibility and reliability of the presented approach, it was Confidently applied for the analysis of the Pb^{2+} and Cd^{2+} ions in some complex real samples. The real samples contained lipstick and brightening cream samples were treated (section 2-5), and subsequently, the proposed method was applied at spiked concentration in three levels of 20 and 50 ng mL^{-1}. In Table 4, The outcomes acquired in blank biological and water samples spiked at the similar concentrations in comparison with those gained the samples are listed as relative recoveries (RR% values). Referred to the outcomes, the matrices of

the different biological fluids and water sample have no considerable result on the performance of the suggested technique, and confirming the high ability of the synthesized DES based on ChCl: Cinnamyl alcohol serving for the extraction and preconcentration of Pb^{2+} and Cd^{2+} ions in these complex real samples.

3.6. Comparison with literature

the suggested method was compared with other studies based on liquid phase microextraction for the extraction of Pb^{2+} and Cd^{2+} ions to demonstrate the efficiency of the process. As seen as in table 6, the tandem dispersive liquid–liquid microextraction (TDLLME) technique compared with the other

extraction procedures, has some benefits containing: The high speed of this method is very significant compared to the other methods, also, excellent clean-up, to be cost-effective, and very simple

operation are among the very suitable features of this method. In addition, the suggested procedure enjoys a high enrichment factor with sufficiently good LODs.

Table 6. Validation for TAALLME-DES of metal ions.

Analyte	[a]LOD	[b]LOQ	[c]LDR	[d]R^2	[e]RSD%(n = 3) Intra-day (Interday)	[f]ER%	[g]PF
Pb (II)	0.2	0.66	0.65-250	0.99	4.52(2.98)	97.10	41.84
Cd (II)	0.02	0.066	0.065-250	0.99	3.98(2.93)	98.34	

a Limit of detection (ng mL−1).
b Limit of quantification (ng mL−1).
c Linear dynamic range (ng mL−1).
d Coefficient of determination.
e Relative standard deviation (100 ng mL−1), (n = 3).
f Extraction recovery.
g Preconcentration factor.

4. Conclusion

Briefly, we were developed a modern, entirely bio-compatible, highly efficient, and speedy microextraction technique named as TAALLME-DES (ChCl: Cinnamyl alcohol)-ICP) for analyses of trace amounts of Pb^{2+} and Cd^{2+} in biological fluid and water samples. For the first time, deep eutectic solvent based on ChCl: Cinnamyl alcohol was prepared in the Laboratory media via attainable and exactly safe components accompanied with easy and low-cost equipment, and in addition used with no requirement any effortful and heavy purification stages. The proposed solvent with facile function in the dispersion step (with no need to any emulsifier agents) is appropriate choice for effectual extraction of aforementioned metal ions at trace levels. Low

detection limits, proper enrichment factor, and finally precise and repeatable analyses confirmed the high competence of the suggested procedure.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, the authors have entirely observed the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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