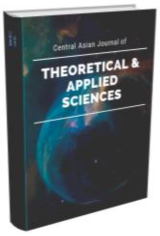

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Article

Deep Eutectic Solvents-Based Ultrasonic Assisted Dispersive Liquid-Liquid Microextraction with Using Nanoparticles for Determination of Cadmium and Lead in Real Samples

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Abstract: A simple, rapid, sensitive, and eco-friendly ultrasonic-assisted deep eutectic solvent-dispersive liquid-liquid microextraction method (USA-DES-DLLME) with modified nanoparticles (CuO NPs) for the separation/pre-concentration of cadmium and lead in water samples using a flame atomic absorption spectrometer. In this method, CuO NPs were synthesized and characterized using various techniques (FT-IR, XRD, FE-SEM, EDX, and AFM). The optimal conditions for the quantitative recovery of the analytes, including the effect of pH, type and volume of DES, volume of CuO NPs, extraction time, tetrahydrofuran (THF), speed and time of centrifugation, and ultrasonic time, were determined. Under optimized experimental conditions, the relative standard deviation (RSD%) at a concentration of 0.05 μg/mL was found to be 2.4% and 2.7%, with limits of detection (LOD) of 0.012 $\mu g.mL^{-1}$ and 0.017$\mu g.mL^{-1}$, and limits of quantification (LOQ) of 0.038$\mu g.mL^{-1}$ and 0.051 $\mu g.mL^{-1}$ for cadmium and lead, respectively. The enrichment factors (EF) were found to be 86.6 and 68.85, and the pre-concentration factor was 15 for cadmium and lead, respectively. The intra-day and inter-day precision of the method were calculated at 0.3 and 0.5 μg/mL, with intra-day precision (2.2%, 2.5%) for lead, and (2.6%, 3.2%) for cadmium, and inter-day precision (3.4%, 3.8%) for lead, and (3.0%, 4.8%) for cadmium, respectively. The method was successfully applied to the determination of cadmium and lead in real samples.

Keywords: Deep Eutectic Solvents (DESs), CuO NPs, Green Synthesis, Pre-Concentration, DLLME.

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1. Introduction

Lead and cadmium are widely recognized as toxic substances, with children being more sensitive to these metals than adults. The U.S. Environmental Protection Agency (EPA) has classified lead and cadmium as Group B2 possible human carcinogens. These elements are not essential within the human body. In recent years, concern has grown over the concentrations of lead and cadmium in soil, drinking water, and natural waters. The World Health Organization (WHO) recommends maximum allowable concentration limits of 10 and 3 ng/mL in drinking water, and 300 and 3 μg/g in soil for lead and cadmium, respectively. This necessitates a much greater sensitivity in measurement than what is offered by flame atomic absorption spectrometry, the most appealing analytical technique for metal ion determination. Therefore, an initial preconcentration of lead and cadmium is often an important step. [1]. In recent years, there has been an urgent need to expand modern and environmentally friendly technologies for treating infected water. Among those technology, nanomaterials synthesized from plant leaf extracts have emerged as promising answers. These nanomaterials efficiently eliminate pollution, as they're prepared the use of environmentally secure strategies, taking gain of the natural houses of plant compounds to reduce chemical substances to the nanoscale. This process will increase the lively floor place and enhances their capacity to soak up and engage with pollutants. Copper oxide nanoparticles have experienced substantial growth because of their unique properties and diverse applications, including use in solar cells, gas sensors, hydrogen storage materials, and medical fields. As a result, there is a need to develop biological methods for producing eco-friendly nanoparticles. [2].In this studies, the extract of Aeschynomeneindica leaves changed into used[3]. Additionally, deep eutectic solvents (DESs) have recently represent an eco-friendly alternative to dangerous traditional solvents. DESs are mixtures of two or extra compounds that, via interactions like hydrogen bonding and van der Waals forces, create a eutectic aggregate[4, 5]. Interactions among their additives, which include hydrogen bonding and van der Waals forces, form a liquid eutectic mixture at temperatures lower than the melting points of the individual compounds. [6]. Maximum of them are non-toxic and environmentally pleasant extraction solvents[7, 8]. DESs are usually made up of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), commonly in molar ratios of 1:1 or 1:2 [9, 10]. Recently, there has been a growing interest in using of DESs in microextraction processes, partly due to their structural flexibility and wide range of applications[11].These solvents are non-toxic and biodegradable, making them suitable for use in modern extraction techniques. DESs are utilized in dispersive liquid-liquid microextraction (DLLME) strategies, which rely upon forming a dispersed natural segment within the aqueous sample, thereby growing the effectiveness of pollutant extraction, along with industrial Cadmium and Lead elements[12]. By combining nanomaterials synthesized from plant extracts with deep eutectic solvents in extraction techniques, full-size enhancements can be done in the performance of pollutant elimination from water. This integration enhances the capability of these technology to shield the environment and make sure clean and safe water resources [13]. This study investigated an alternative technique to evaluate the feasibility of using Deep Eutectic Solvents (DESs) for extracting lead and cadmium from aqueous samples. By combining the benefits of DESs and copper oxide nanoparticles (CuO NPs), the researchers developed a novel, rapid, and straightforward method for the extraction of these metals using DESs. This approach facilitates the separation and preconcentration of lead and cadmium through a ligandless ultrasound-assisted dispersive liquid-liquid microextraction (DES-USA-DLLME) method. In this technique, DESs and CuO NPs are directly added to the sample solution, allowing the metal-containing DESs to be captured by the sorbent and efficiently separated. The primary goal of the study was to develop a new DES-based ultrasound-assisted dispersive liquid-liquid microextraction method, followed by the determination of the elements using atomic absorption spectroscopy. DESs and copper oxide nanoparticles (CuO NPs) were successfully synthesized, and optimal conditions for applying the USA-DLLME method for extracting elements from water samples were identified. Research Importance: This research is of significant importance as it contributes to the development of a new and environmentally friendly technique for extracting lead and cadmium from water samples using DESs and copper oxide nanoparticles (CuO NPs). Given the environmental challenges posed by water pollution with toxic chemicals, this research provides an innovative solution that enhances extraction efficiency while reducing the use of harmful solvents. Additionally, the use of nanoparticles synthesized through green methods increases interaction efficiency and improves the system's ability to effectively remove pollutants. This research opens new avenues for future applications in water treatment and environmental conservation.

2. Materials and Methods

2.1. *Materials*

All reagents and materials used in this work were in the purity of analytical reagent grade. they were purchased from Sigma Aldrich, BDH and Merck. Chemicals were used with no any purification process. It should be mentioned that deionized water was used for the dilution of the standard reagents and samples. All chemicals and reagents were of analytical grade and used without any further modification. The leaves of *AeschynomeneIndica* were collected from AL- Muthana Governorate.

2.2. *Instrumentation*

The Flame Atomic Absorption Spectrometry (AA-500 AFG) device from Shimadzu (Japan) was utilized for the analysis at the University of Thi-Qar / College of Science, where acetylene-air was used as the fuel. The temperature used was 2300°C. IR spectra were obtained using the FT-IR Affinity Spectrophotometer (Shimadzu, Japan) with KBr discs. Both instruments are available in the Department of Chemistry, College of Science, University of Thi-Qar, Iraq. For microscopic examinations, a Scanning Electron Microscope (FESEM) of the TESCAN Mira3 type, manufactured by TESCAN in the Czech Republic, was used. An EDAX device was employed as an attached detector with the FESEM. Additionally, an X-ray Diffraction (XRD) device, model PW1730, manufactured by Philips in the USA, and a CoreAFM 2023 Atomic Force Microscope from Nanosurf, Switzerland, were used in the laboratories of Tehran University.

2.3. *Synthesis of Aqueous Extract from AeschynomeneIndica Leaves*

Fresh leaves of Aeschynomene indica (Figure 1) were collected and washed first with tap water and then with deionized water to remove dirt and dust. The leaves were air-dried at room temperature for two weeks and then ground into a fine powder using a mortar and pestle. To prepare the aqueous extract, 10 grams of the powdered leaves were added to 90 mL of deionized water in a 250 mL Erlenmeyer flask. The mixture was stirred at 70°C for 3 hours using a magnetic stirrer with heating. Afterward, the solution was filtered using Whatman No. 1 filter paper to collect the plant extract. Finally, the filtered solution was stored in a refrigerator at 4°C until it was used to synthesize CuO nanoparticles.[14, 15].

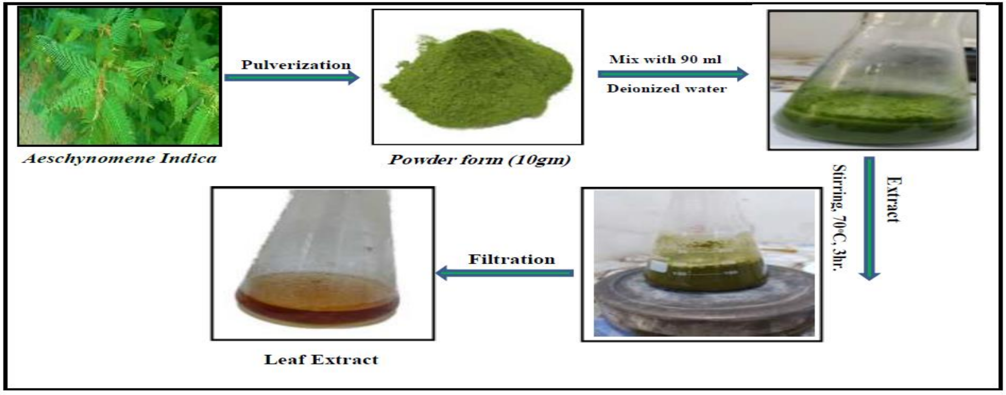


Figure 1 Synthesis of Aqueous Extract from *AeschynomeneIndica* Leaves

2.4. *Green synthesis of CuO nanoparticle*s

Copper acetate dihydrate ($Cu(CH_3COO)_2 \cdot 2H_2O$) was used as the source of Copper ions in the present study. A 0.2 M solution of Copper acetate dihydrate was prepared in deionized water. To synthesize CuO nanoparticles, 90 mL of the 0.2 M Copper acetate dihydrate solution was mixed with 10 mL of plant extract from *Aeschynomeneindica* in a 250 mL flask.. Use a magnetic stirrer to ensure homogeneous mixing of the solution. Heat the solution until the temperature reaches 70°C. Continue stirring and heating for three hours even as preserving the temperature at 70°C. After this, go away the solution blanketed for 3 days at room temperature. Separate the ensuing precipitate, then wash the precipitate with deionized water several times to purify the nanoparticles. Finally, dry the precipitate in an oven at a temperature of 100-110 °C [14].

2.5. Preparation of Deep Eutectic Solvents (DESs)

Ten distinctive varieties of choline chloride-primarily based DESs have been decided on for the extraction of cadmium and lead. These DESs had been prepared by way of combining choline chloride with numerous hydrogen bond donors (HBDs) primarily based on their useful organizations. For example, DES1 became created via mixing choline chloride with phenol in a 1:2 molar ratio, while DES2 involved choline chloride and thiourea, additionally in a 1:2 molar ratio. Similarly, DES3 turned into shaped by means of combining choline chloride with malonic acid in a 1:2 ratio, and DES4 became prepared by using mixing choline chloride with malic acid in a 1:1 ratio. Other combos protected DES5 with citric acid in a 1:1 ratio, and DES6 with urea in a 1:2 ratio. DES7 involved the combination of choline chloride with oxalic acid in a 1:1 ratio, DES8 with glucose in a 1:1 ratio, DES9 with ethylene glycol in a 1:1 ratio, and subsequently, DES10 mixed choline chloride with glycerol in a 1:2 ratio (Image2). All DESs were treated under the same conditions, with magnetic stirring at 60 °C for 10 minutes, resulting in homogeneous mixtures that were further utilized in optimization studies[16].

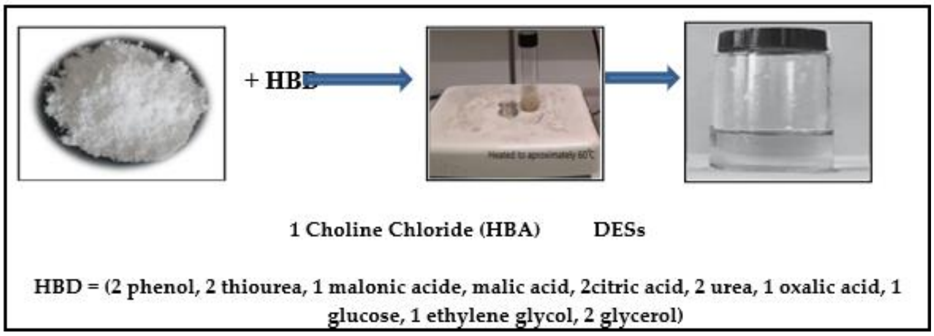


Figure 2: Preparation of Deep Eutectic Solvents (DESs)

2.6. DES-USA-DLLMEprosedure

In a centrifuge tube, 10 mL aliquots of a standard solution with known concentrations of lead and cadmium were adjusted to pH 6 by adding 0.1 M HCl or NaOH. Then, 0.6 mL of the prepared biosorbent (CuO nanoparticles) and 0.5 mL of DESs were added to the solution. The mixture was manually shaken for 5–6 seconds to achieve a homogeneous solution. Next, 0.5 mL of THF, used as an emulsifier solvent, was added and the solution was sonicated for 120 seconds. Ultrasonic vibration enhanced the dispersion of the extractant, causing the DESs in the aqueous solution to form fine droplets and create a cloudy appearance. The mixture was then centrifuged at 4,000 rpm for 10 minutes to separate the aqueous phase from the DES-rich phase. The DES phase containing the elements settled at the bottom of the tube. The aqueous phase was removed using a syringe, and the DES-rich phase was diluted to 1 mL with 0.5 M HNO_3 in ethanol. The final solution was measured using a flame atomic absorption spectrometer at the maximum wavelengths for lead and cadmium..

2.7. Application of real samples

The present ***DLLME*** method was applied for determination of lead and cadmium ions in river water. River water was collected from soil and oil from different areas in Thi-Qar Governorate.

2.7.1. River water Preparation

To eliminate suspended particulate matter, the water samples were filtered using Whatman No. 42 filter paper and stored in glass bottles at 4°C until analysis. A 15 mL aliquot of each sample was then treated according to the standard DLLME procedure. The concentrations of analyte ions were measured using flame atomic absorption spectrometry.

2.7.2. Soil digestion

The wet digestion method for soil aims to convert the elements in the soil into water-soluble forms to facilitate their analysis using techniques such as flame atomic absorption. In this study, soil samples from Thi Qar Governorate were analyzed for cadmium and lead. Two grams of soil were weighed for each sample, and 16 mL of aqua regia (a mixture of hydrochloric acid and nitric acid in a 3:1 ratio) was added. The samples were then heated at 110°C for 2-3 hours. After cooling, 25 mL of deionized water was added, and the mixture was filtered. The resulting solution was then analyzed using a flame atomic absorption spectrometer[17].

2.7.3. Oil digestion

Weighed triplicate of crude oil samples (0.5 g) in pre-weighed conical flasks (50 mL in capacity). Added 5 mL of concentrated HNO_3 and H_2SO_4 mixture at (2:1 ratio) to the content of flasks. Covered the flasks and heated on an temperature control electric hot plate at 100°C till semi dried mass was obtained (2 to 3h). Then added 10 mL of 0.2 mol/L of HNO_3 to the contents of the flasks[18]

3. Results and Discussion

3.1. UV-Visible Spectrum of CuONPs

The UV-Visible spectrum (UV-Vis) of copper oxide nanoparticles (CuO-NPs) can be analyzed to determine the optical and structural properties of the nanomaterial. This study investigated the UV-Visible spectra at wavelengths ranging from 200 to 800 nm. The peaks at 302 nm and 336 nm (Figure 3) indicate electron transitions in the direct energy band of the copper oxide nanoparticles. These peaks could be associated with electronic transitions from the valence band to the conduction band in the nanomaterial [16,17].

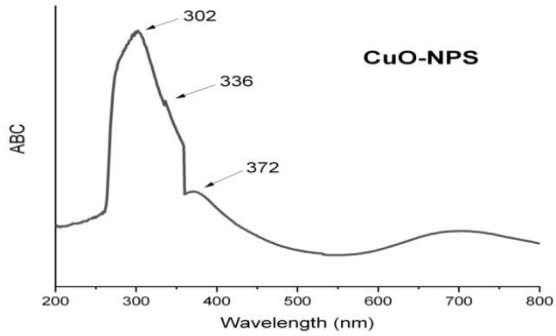


Figure 3. UV–Vis absorption spectra of CuO NPs

3.2. FT-IR Spectrum of Cu*O NPs*

To understand the nature of the capping agents and the presence of different functional groups responsible for the synthesis of monodispersed CuO nanoparticles, FT-IR measurements were carried out. The FT-IR spectrum of the CuO nanoparticles showed characteristic absorption bands at 3337 cm⁻¹, 1622 cm⁻¹, and 1050 cm⁻¹, corresponding to H-O-H stretching, hydrogen-bonded alcohols and phenols, and C-H alkyl stretching, respectively. These findings confirmed the presence of phytoconstituents such as polyphenols, alkaloids, and steroids that may act as capping agents on the surface of the nanoparticles. The peaks at 624 cm^{-1} (Cu-O symmetric stretching) and 690 cm^{-1} (Cu-O wagging) indicated the presence of metal-oxide groups in the sample. [19, 20].

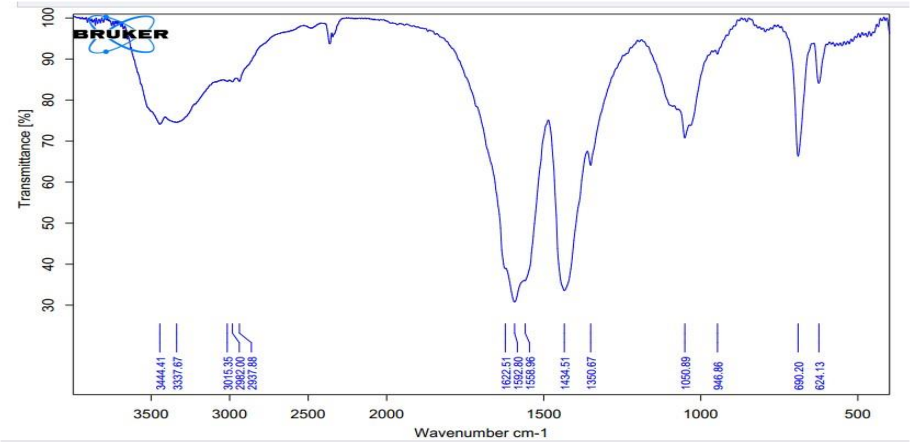


Figure 4. FT-IR Spectrum of CuO NPs

3.3. X-ray Diffraction (XRD) Spectrum of CuO NPs

X-ray diffraction (XRD) analysis is a technique used to determine the crystalline structure of materials. XRD can be employed to identify the type of crystal lattice and crystal size. The crystalline nature of copper oxide nanoparticles (CuO NPs) was confirmed through XRD analysis. The figure shows the XRD pattern of CuO NPs synthesized using a plant extract. The XRD diffractogram for CuO-NPs displays clear and strong peaks corresponding to 2θ values of 32.10, 35.40, 38.20, 41.84, 48.40, 53.50, 58.10, 61.20, 65.50, and 67.30 for the indices (110), (002), (111), (131), (202), (020), (123), (113), (022), and (113), respectively. The XRD results demonstrate that the CuO nanoparticles prepared from the plant extract are highly crystalline. The XRD results are consistent with the monoclinic structure of CuO. They also indicate that the nanoparticles have a small crystalline size. These findings clearly show the formation of a highly crystalline material. The X-ray diffraction pattern reflects the high crystalline nature of the CuO nanoparticles, confirming the quality of the material's crystalline structure. The average crystalline size was calculated using the Scherrer equation and found to be 36 nm[21].

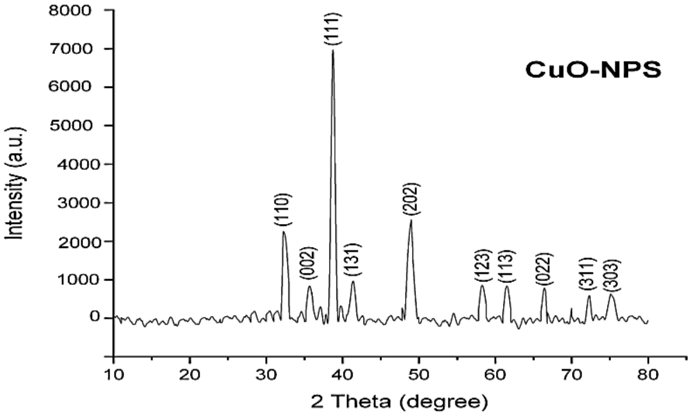


Figure 5. X-ray diffraction spectrum of CuO NPs

3.4. FESEM analysis of CuO NPs

Figure 5 shows various measurements of nanoparticle sizes, ranging between 36 nm to52 nm. The nanoparticles appear to be irregular in shape and form clusters. The distribution is uneven, indicating a variation in particle sizes. The size distribution ranges from 36 nm to 52 nm, indicating a wide range of nanoparticle sizes. The aggregation could be due to van der Waals forces or electrostatic interactions between the particles. Aggregation can affect certain applications, such as catalysis, where there can be a positive or negative impact. The uneven surface of the particles increases the specific surface area, making them useful in applications that require large surface reactions, such as chemical catalysis or environmental applications[16,19].

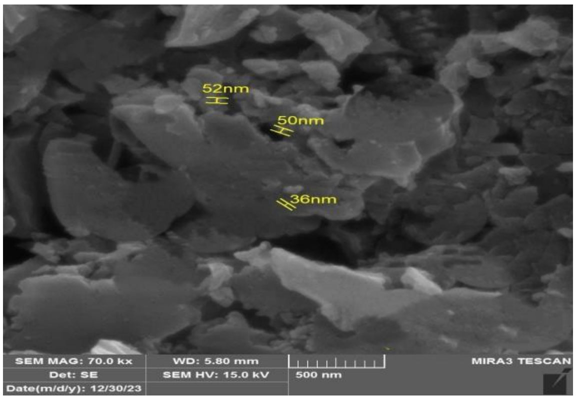


Figure 6. FE-SEM images of CuONPs

3.5 EDX analysis of CuO NPs

EDX revealed a strong signal for both Copper and Oxygen, confirming the presence of Copper in its oxide form. The EDX data provided the elemental composition of the sample, showing prominent peaks at 64.81% for Copper and 35.19% for Oxygen. These weight percentage values are consistent with previously reported data for the synthesis of CuO nanoparticles. Specifically, two significant peaks were observed for Copper at 1 eV, 8 eVand 8.6 eV, while Oxygen showed a distinct signal at 0.5 eV[21]. These values are specific to Copper and Oxygen, confirming the elemental composition of the synthesized compound. In addition to Copper and Oxygen, traces of other elements, such as Sodium, were also detected, as shown in Image7.

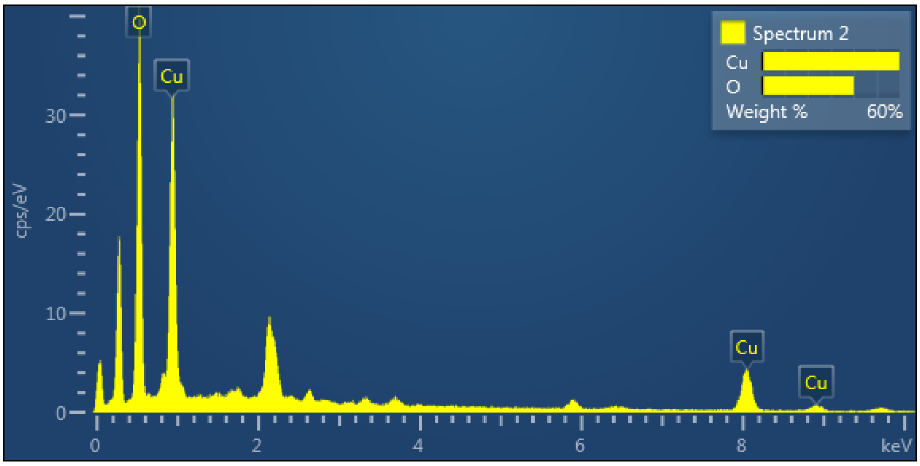
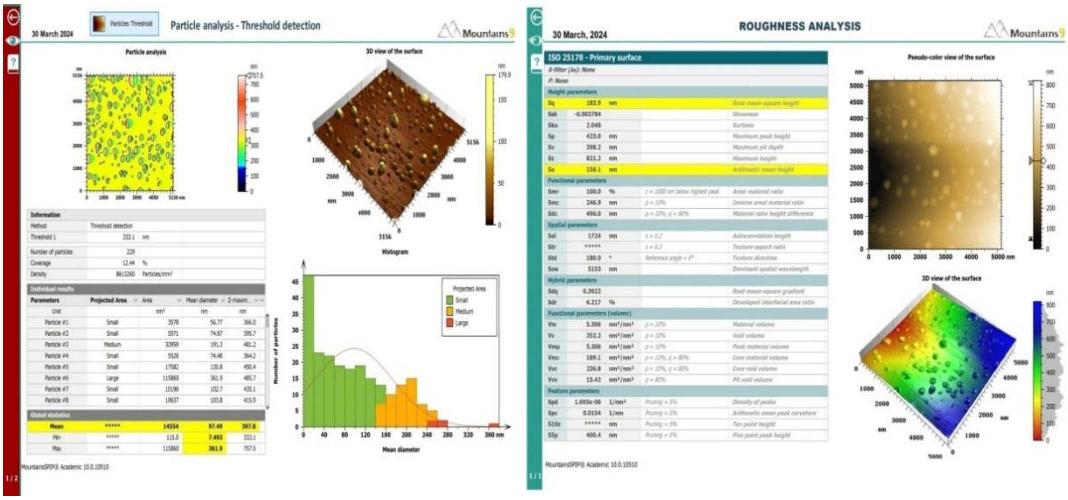


Figure 7 .EDX spectrum of CuO NPs

3.5.A FM analysis of CuO NPs

Atomic force microscopy (AFM) analysis reveals the size and roughness of CuO nanoparticles. The sample contains 229 particles, covering 12.44% of the surface area, with a high particle density of 8,613,260 particles/mm². The figure shows that the mean particle size was 97.49 nm. Particle heights range from 333.1 nm to 757.5 nm. The root mean square height (Sq) is 183.9 nm and the average arithmetic height (Sa) is 156.1 nm, indicating significant surface roughness. The high particle density and rough surface suggest the material is suitable for applications requiring extensive surface interaction[20].



Information		
Method	Threshold detection	
Threshold 1	333.1	nm
Number of particles	229	
Coverage	12.44	%
Density	8613260	Particles/mm²

Parameters	Projected Area	Area	Mean diameter	Z-maxim...
Unit		nm²	nm	nm
Particle #1	Small	3578	56.77	366.0
Particle #2	Small	5571	74.67	395.7
Particle #3	Medium	32959	191.3	481.2
Particle #4	Small	5526	74.48	364.2
Particle #5	Small	17582	135.8	450.4
Particle #6	Large	115860	361.9	485.7
Particle #7	Small	10196	102.7	435.1
Particle #8	Small	10637	103.8	415.9
Global statistics				
Mean	*****	14554	97.49	397.8
Min	*****	115.0	7.493	333.1
Max	*****	115860	361.9	757.5

ISO 25178 - Primary surface				
S-filter (λs): None				
F: None				
Height parameters				
Sq	183.9	nm		Root-mean-square height
Ssk	-0.003784			Skewness
Sku	2.048			Kurtosis
Sp	423.0	nm		Maximum peak height
Sv	398.2	nm		Maximum pit depth
Sz	821.2	nm		Maximum height
Sa	156.1	nm		Arithmetic mean height
Functional parameters				
Smr	100.0	%	c = 1000 nm below highest peak	Areal material ratio
Smc	246.9	nm	p = 10%	Inverse areal material ratio
Sdc	496.0	nm	p = 10%, q = 80%	Material ratio height difference
Spatial parameters				
Sal	1724	nm	s = 0.2	Autocorrelation length
Str	*****		s = 0.2	Texture-aspect ratio
Std	180.0	°	Reference angle = 0°	Texture direction
Ssw	5153	nm		Dominant spatial wavelength
Hybrid parameters				
Sdq	0.3922			Root-mean-square gradient
Sdr	6.217	%		Developed interfacial area ratio
Functional parameters (volume)				
Vm	5.306	nm³/nm²	p = 10%	Material volume
Vv	252.2	nm³/nm²	p = 10%	Void volume
Vmp	5.306	nm³/nm²	p = 10%	Peak material volume
Vmc	189.1	nm³/nm²	p = 10%, q = 80%	Core material volume
Vvc	236.8	nm³/nm²	p = 10%, q = 80%	Core void volume
Vvv	15.42	nm³/nm²	p = 80%	Pit void volume
Feature parameters				
Spd	1.693e-06	1/nm²	Pruning = 5%	Density of peaks
Spc	0.0154	1/nm	Pruning = 5%	Arithmetic mean peak curvature
S10z	*****	nm	Pruning = 5%	Ten point height
S5p	400.4	nm	Pruning = 5%	Five point peak height

Figure 8. AFM spectrum of CuO NPs

3.6.Optimization of Ligandless DES-USA-DLLME parameters

3.6.1. Effect of pH solution

The pH of the solution influences the charge and interaction of the analyte with the deep eutectic solvent (DES), impacting extraction efficiency. Adjusting the pH can change the ion state. To study this effect, six buffer solutions with pH ranging from 2 to 12 were prepared. The highest extraction efficiency for cadmium and lead was observed at pH 4. This moderate optimum pH makes the method particularly advantageous, as it reduces the need for significant amounts of strong acids or bases for pH adjustment. High pH levels can cause undesirable reactions, leading to cloudy solutions, phase separation difficulties, and nanoparticle aggregation or disintegration, which reduces surface area and extraction efficiency. High pH levels can negatively affect extraction efficiency. Adjusting pH is crucial for high extraction efficiency using DES. Optimal pH varies between analytes, requiring careful study. Nanoparticles enhance extraction efficiency, with moderate pH making the process more feasible. Understanding the impact of pH and other factors improves chemical extraction processes. All experiments were conducted at room temperature (25°C) [22]

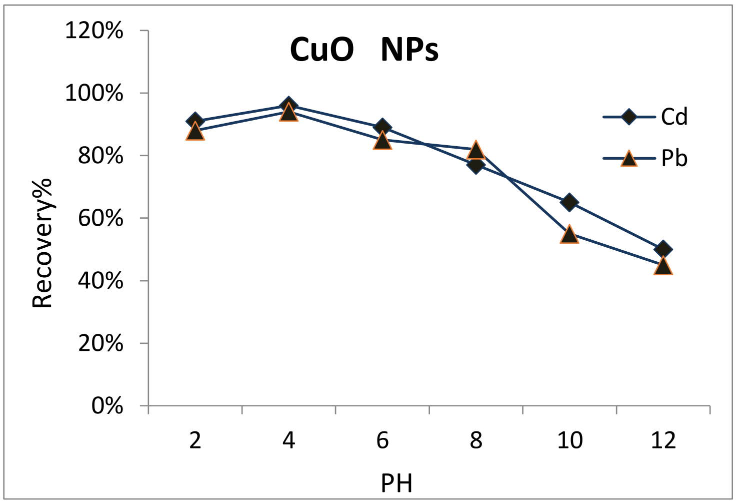


Figure 9. Effect of Ph on DLLME

3.6.2. Effect of Type and volume of DESs

Based on the criteria for selecting a suitable solvent in the Dispersive Liquid-Liquid Microextraction (DLLME) technique, several factors are considered, such as cost-effectiveness, availability, high selectivity, and extraction efficiency during the extraction process of analytes. In this study, a novel class of natural solvents known as "deep eutectic solvents" (DESs) was chosen for this technique. Ten different types of DESs were evaluated for the extraction of lead and cadmium, each with its optimal molar ratio and pH. The recovery rates for the various DESs under their optimal pH conditions are presented in Fig.10a. When using **copper oxide nanoparticles** for the elements, the deep eutectic solvent based on **choline chloride and urea** (1:2) showed the highest recovery rate (96%) for the extraction of **lead and cadmium**, therefore, **Ch:Cl:Urea**was selected for the final work [21,22].

The effect of different volumes of Deep Eutectic Solvents (DES) on the extraction efficiency was studied . The volume of DES was optimized within the range of 0.1 mL to 0.6 mL. Within this range, a gradual increase in the % recovery of **lead and cadmium**was observed. However, increasing the DES quantity past this point led to a dilution of the analyte, resulting in a slight lower in % recovery. The outcomes showed that the extraction efficiency reached its most whilst 0.3 mL of DES become used (Fig. 10b). This shows that this volume of DES presents the most advantageous stability between the quantity of solvent and the goal analytes for extraction. When a smaller amount of DES become used,the extraction performance reduced. This is because of a lower enrichment aspect, where the solvent quantity is insufficient to efficiently extract all of the goal analytes. When a bigger amount of DES turned into used, the extraction efficiency additionally reduced because of the problem in collecting the DES. This means that increasing the solvent amount leads to more dispersion of the solvent and an incapability to successfully separate the layers[16, 23].

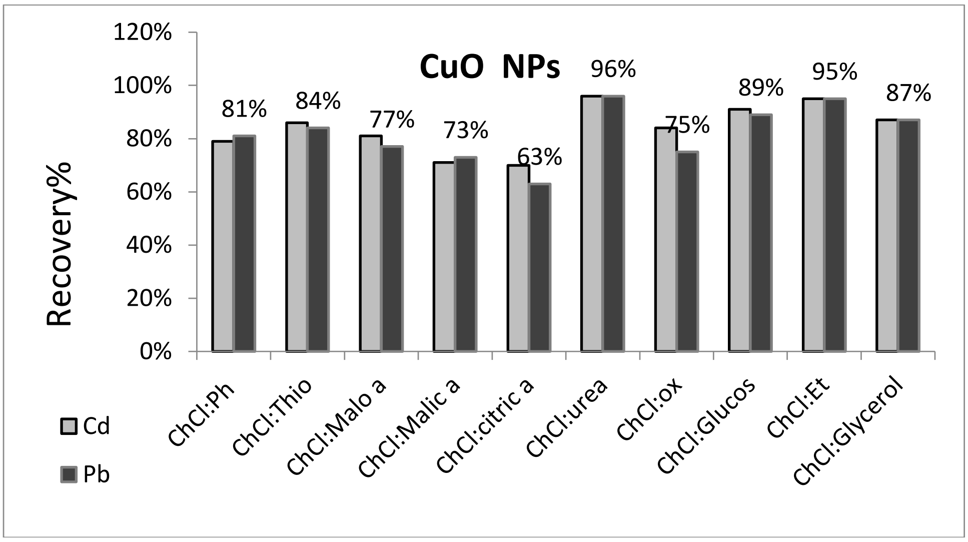


Figure 10a. Effect of type of DES on the DLLME

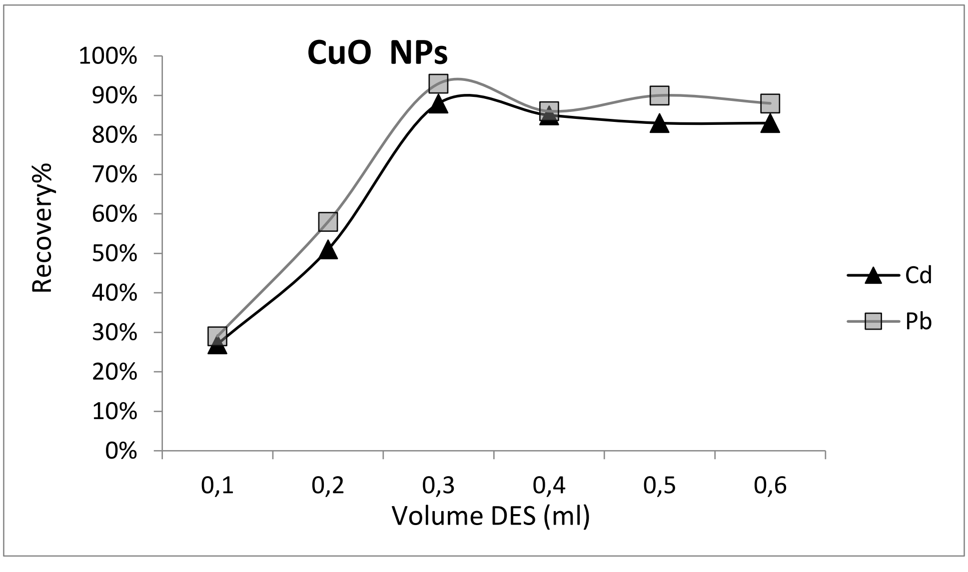


Figure 10b. Effect of volume of DES on the DLLME

3.6.3. *Effect of THF Volume*

In this methodology, the aprotic solvent THF was utilized for its ability to separate DES from the aqueous phase. Adding THF as an emulsifying agent causes DES droplets to become insoluble in the water/THF mixture. Under these conditions, DES molecules separate from water molecules, aggregate, and form an immiscible liquid that distinctly separates. The effect of THF volume on the extraction efficiency of DES-DLLME was studied using various volumes of THF ranging from 0.1 to 0.6 mL. It was observed that the extraction efficiency generally increased with increasing THF volume up to 0.6 mL, after which it decreased. Increasing the THF volume leads to an increase in the size of DES droplets, which reduces their surface area. The reduced surface area diminishes the interaction between DES and the organic compound, thereby reducing the extraction efficiency. Additionally, increasing the THF volume increases the volume of the organic phase, which dilutes the concentration of the target compound, further reducing the extraction efficiency. Therefore, a THF volume of 0.6 mL was chosen for the remaining study.[22, 23]**.**

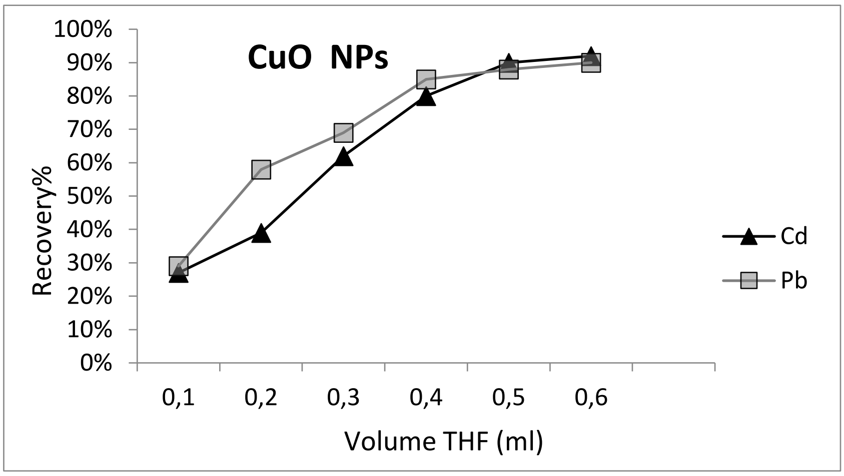


Figure 11. Effect of THF Volume

Figure 11. Effect of THF Volume

3.6.4. *Effect of CuO NPs Volume*

Experiments have proven that the quantity of nanomaterial immediately affects the extraction performance of the goal compound using DLLME with DES solvent. Extended surface area: As the quantity of the nanomaterial increases, its available floor location for interaction with the goal compound also increases. Enhanced Adsorption: This leads to more adsorption of the goal compound on the nanomaterial's surface. Improved Extraction: More adsorption results in higher extraction efficiency by way of DES and the dispersive solvent, facilitating the transition from the aqueous section to the natural section . Different volumes of nanomaterial ranging from 0.1 to 0.6 mL were used, and the extraction efficiency for each volume was measured. Results indicated that the highest extraction efficiency was achieved with 0.4 mL of nanomaterials[24].

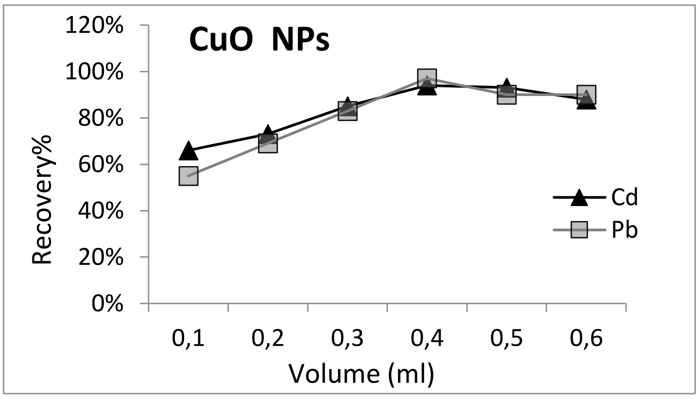


Figure 12. Effect of CuONPs Volume

3.6.5. Effect of extractiontime

Extraction time is critical in the efficiency of DES-DLLME. Prolonged extraction enhances interaction between the solvent and target substances, improving transfer to the DES phase. However, once equilibrium is reached, further time increase does not enhance efficiency. The study found that maximum dye extraction using CuO nanoparticles occurred at 5 min with efficiency remaining stable between 5-10 min..Therefore, 4 min was chosen as the extraction time for futhur experiments [25]..

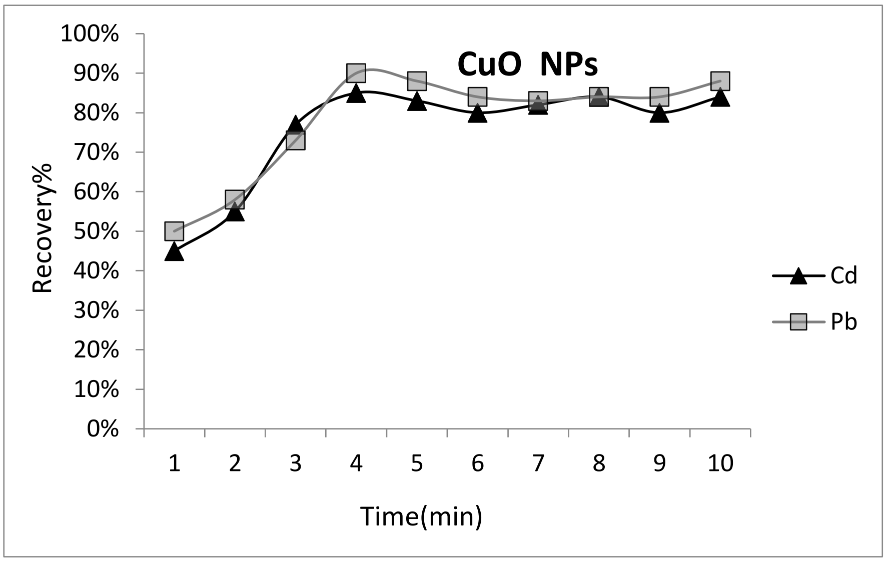


Figure 13. Effect of extraction time

3.6.6. Effect of Centrifugation conditions

The influence of centrifugation rate on extraction efficiency was investigated within the range of 1000 to 6000 rpm and a duration of 5 to 25 minutes. It was found that increasing the rate to 4000 rpm (Fig. 14a) provided the optimal extraction efficiency. This rate was determined to be the best for ensuring complete phase separation.. Centrifugation separates the organic phase (DES) from the aqueous phase, requiring sufficient time for complete separation. Short centrifugation times may lead to incomplete separation, reducing extraction efficiency by causing organic compounds to remain in the aqueous phase. Increase centrifugation times can increase the organic phase volume by reaggregating small DES droplets, diluting the compound, and decreasing extraction efficiency. The quantitative recovery was resulted at 10 min.(Fig. 14b) at 4000 rpm. When the centrifuging period exceeded 10 min., the recovery decreased due to generation study found that a 10 min centrifugation time at 4000 rpm maximized dye extraction efficiency. Decrease times resulted in incomplete separation, while longer times led to decreased efficiency due to phase instability and dilution[26, 27].In a previous study conducted in 2021, the effect of centrifugation speed on the efficiency of separating the DES-water system was investigated. The results showed that the highest extraction efficiency was achieved at a speed of 4000 rpm, where the signal response of the analytes stabilized at this speed [28].In a 2018 study, the centrifugation time 10 minutes was investigated[29].

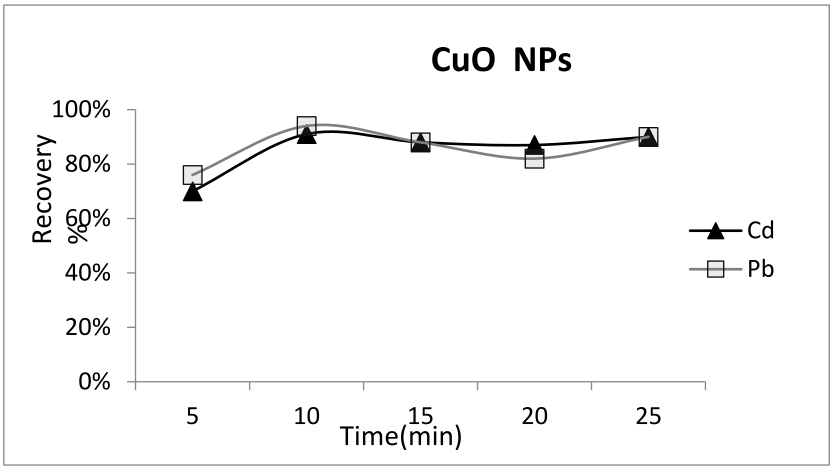


Figure 14a. Effect of Centrifuge time

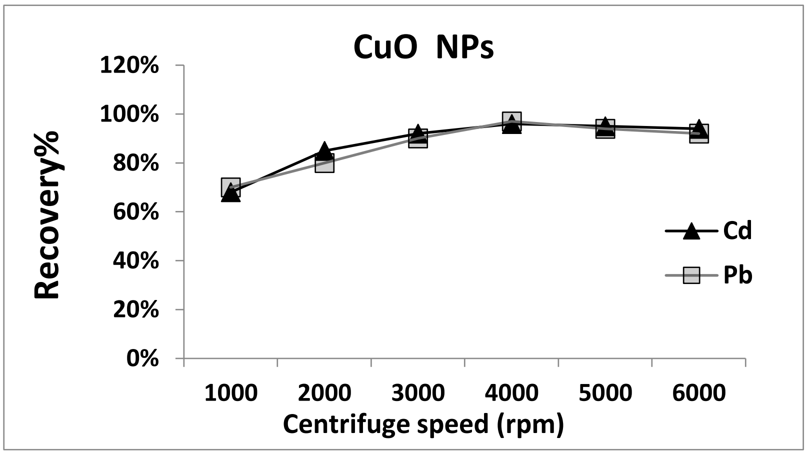


Figure 14b. Effect of Centrifuge speed

3.6.7. Effect of Ultrasonic Time

Ultrasonication time significantly influences droplet formation during the extraction process. In this study, DES aggregates in the aqueous phase were created by mixing with THF, and the application of ultrasonic radiation further broke these aggregates into smaller droplets. This process reduced the extraction time until the solution became cloudy and rapidly reached equilibrium, resulting in enhanced extraction efficiency. The effect of ultrasonication time was examined over a range of 1 to 5 minutes while keeping other variables constant, as shown in Fig. 17. The percentage recovery of the analyte stabilized when the ultrasonication time exceeded 3 minutes, with no significant improvement beyond that point. Shorter times led to incomplete droplet formation, reducing extraction efficiency. Conversely, extended ultrasonication times may cause sample heating or damage to sensitive components.[22]. In a previous study conducted in 2018, the effect of ultrasonication time on extraction efficiency was investigated, and the results showed that the extraction efficiency stabilized after 3 minutes of ultrasonication treatment [22].

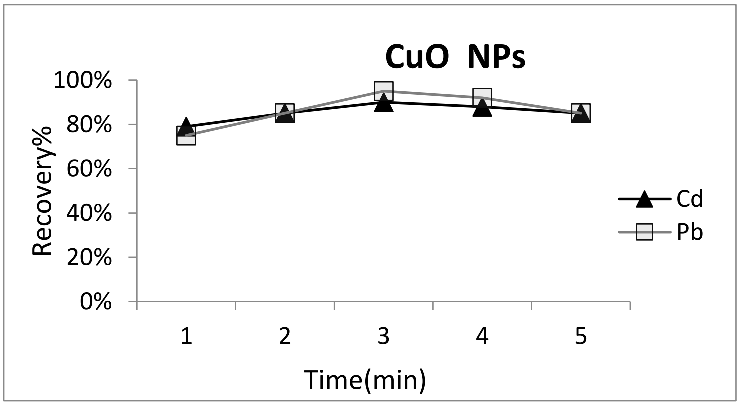


Figure 15. Effect of Ultrasonication time

3.7. Analytical features and validationof the USA-DES-DLLME

Under optimal experimental parameters, the analytical characteristic data of the recommended DLLME method were obtained to establish the calibration curve and determine the method's linear range. Standard aqueous solutions of Cd and Pb were prepared at a concentration of 100 μg/mL.. Various dilutions of these solutions were then made within the range of 0-15 μg.mL^{-1} of Pb and 0-12 Cd before extraction, and a range of 0-4 and 0-3 μg.mL^{-1} Cd and Pb after extraction using the optimized conditions. The results showed that the calibration curve for Cd had a good linear was obtained with a correlation coefficient (R^2) of 0.996 before extraction and 0.995 after extraction, while the results for Pb showed a good linear was obtained with a correlation coefficient (R^2) of 0.998 before extraction and 0.997 after extraction, and the regression equation was $y = 0.005x + 0.001$ andy = $0.433x + 0.012$ respectively, before and after extraction for Pb, the regression equation was $y = 0.014x - 0.002$ and $y = 0.964x - 0.057$ respectively, before and after extraction for Cd.

The method's relative standard deviation (RSD%) was determined by performing five consecutive experiments using a standard solution of Pb and Cd at a concentration of 0.5 μg/mL under optimal conditions, resulting in RSD values of 2.7% for Pb and 2.4% for Cd. The limits of detection (LOD), calculated as 3SD/m (where SD is the standard deviation of the blank sample with n=8 and m is the slope of the calibration curve), were found to be 0.012 μg/mL for Pb and 0.017 μg/mL for Cd. The limits of quantification (LOQ), defined as 10SD/m (with SD as the standard deviation of the blank sample and m as the slope of the calibration curve [16, 23]), were 0.038 μg/mL for Pb and 0.051 μg/mL for Cd. The enrichment factors, calculated as the ratio of the slopes of the calibration curves with and without preconcentration, were determined to be 86.6 for Pb and 68.85 for Cd. The preconcentration factor for both Pb and Cd was found to be 15.

The precision of the proposed DLLME method was investigated in terms of within-day precision (repeatability) and between-day precision (intra-laboratory reproducibility). Within-day precision was studied by extracting samples of Pb and Cd spiked at two different concentration levels (0.5 and 0.3 mg/L). Each concentration level was extracted in duplicates and each extract was also detected in duplicates on the same day (morning and evening). Under the same experimental conditions, between-day precision was also evaluated at the same concentration levels used for the within-day precision study over five consecutive during the same time period. The relative standard deviation (RSD) for within-day precision (repeatability, n = 8) and between-day precision (intra-laboratory reproducibility, n = 10) of the proposed method are shown in the table 1.

Table 1: Precision of DLLME method of the Pb and Cd

Analyte	**Intra-day**		**Inter-day**	
Pb(μg/mL)	**0.3**	**0.5**	**0.3**	**0.5**
%RSD	**2.2%**	**2.7%**	**3.4%**	**3.8%**

Analyte	**Intra-day**		**Inter-day**	
Cd (μg/mL)	**0.3**	**0.5**	**0.3**	**0.5**
%RSD	**2.6%**	**3.2%**	**3.7%**	**4.8%**

Comparison with other methods

As demonstrated in Table 2, this technique has been successfully compared with other methods. Its low cost, straightforward experimental procedures, and the use of a non-toxic extraction solvent (ChCl) make it a promising avenue for analytical applications.

Table 2. Comparison of present technique with reported methods

Sample types	Sorbent	Analyte	LOD (μg L^{-1})	RSD (%)	LDR (μg L^{-1})	PF	Ref.
Tea and water samples	Polyurethane foam/ Me-BTANC	Pb	2.0	3.1	100-5000	37	[30]
		Cd	0.8	4.4	10-1000	37	
Marine samples	Sulfur-nanoparticle-loaded Alumina	Pb	0.6	4.8	1-60	83	[31]
		Cd	0.3	2.4	0.8-30	83	
Soil and water samples	**Surfactant loaded Fe3O4 nano-particle**	Pb	0.7	3.83	100-1000	25	[32]
		Cd	0.1	3.15	1000-10000	25	
Soil and water samples	**DES mediated nano particles**	Pb	0.012 μgmL^{-1}	2.7	0-15000	86.6	This work
		Cd	0.017μgmL^{-1}	2.4	0-10000	68.85	

3.8. Determination of Pb and Cd in Water samples

To evaluate the effectiveness of the developed method for determining **lead and cadmium** in water samples, the results were applied as shown in Table 2 and Table 3. Good recoveries (101.303-106.667) were achieved for all analyzed samples by adding a standard solution of **lead and cadmium** to the sample solution. **Flame atomic absorption spectrometry** was used for the quantitative analysis of these elements. The results of this method for each type of selected sample and the analytical recovery rates are shown in the table. As indicated, the determination of **lead and cadmium** in real samples is remarkably accurate using the proposed method..

Table 3. Recovery percentage of the Pb in real samples

Sample	Pb add	Found	%Recovery	%Error
Internal water of the textile factory	0	0.078	-	-
	0.3	0.389	102.910	2.910
	0.5	0.597	103.287	3.287
External water of the textile factory	0	0.072	-	-
	0.3	0.385	103.494	3.494
	0.5	0.58	101.398	1.398
Euphrates River water near the power station	0	0.077	-	-
	0.3	0.39	103.448	3.448
	0.5	0.589	102.079	2.079
Euphrates River water near the College of Science	0	0.052	-	-
	0.3	0.369	104.829	4.829
	0.5	0.567	102.717	2.717
Euphrates River Water near the al-Hadarat Bridge	0	0.07	-	-
	0.3	0.389	105.135	5.135
	0.5	0.587	102.982	2.982
Euphrates River Water Near the Al-Zdinawea Area	0	0.092	-	-
	0.3	0.401	102.295	2.295
	0.5	0.617	104.223	4.223
Textile factory soil	0	5.61	-	-

	0.3	6.07	102.707	2.707
	0.5	6.311	103.289	3.289
The soil of the power station	0	5.4	-	-
	0.3	5.851	102.649	2.649
	0.5	6.094	103.288	3.288
The soil of the College of Science	0	2.782	-	-
	0.3	3.2	103.828	3.828
	0.5	3.371	102.711	2.711
The soil of the al-Hadarat Bridge	0	4.9	-	-
	0.3	5.405	103.942	3.942
	0.5	5.593	103.574	3.574
The soil of the Zinaweh area	0	6.5	-	-
	0.3	7.098	104.382	4.382
	0.5	7.241	103.442	3.442
The soil of the oil refinery	0	9.087	-	-
	0.3	9.581	102.066	2.066
	0.5	9.949	103.775	3.775
Heavy crude oil residues	0	1.818	-	-
	0.3	2.205	104.107	4.107
	0.5	2.401	103.580	3.580
Light crude oil residues	0	0.671	-	-
	0.3	1.023	105.355	5.355
	0.5	1.243	106.148	6.148

Table 4. Recovery percentage of the Cd in real samples

Sample	Cd add	Found	**%Recovery**	%Error
Internal water of the textile factory	0	0.042	-	-
	0.3	0.353	103.216	3.216
	0.5	0.558	102.952	2.952
External water of the textile factory	0	0.051	-	-
	0.3	0.359	102.279	2.279
	0.5	0.563	102.177	2.177
Euphrates River water near the power station	0	0.081	-	-
	0.3	0.401	105.249	5.249
	0.5	0.599	103.098	3.098
Euphrates River water near the College of Science	0	0.037	-	-
	0.3	0.342	101.483	1.483
	0.5	0.551	102.607	2.607
Euphrates River Water near the al-Hadarat Bridge	0	0.056	-	-
	0.3	0.362	101.685	1.685
	0.5	0.57	102.518	2.518
Euphrates River Water Near the Al-Zdinawea Area	0	0.081	-	-
	0.3	0.393	103.149	3.149
	0.5	0.598	102.926	2.926
Textile factory soil	0	0.309	-	-
	0.3	0.623	102.298	2.298
	0.5	0.831	102.719	2.719
The soil of the power station	0	0.481	-	-
	0.3	0.803	102.819	2.819
	0.5	1.017	103.669	3.669

The soil of the College of Science	0	0.394	-	-
	0.3	0.718	103.458	3.458
	0.5	0.927	103.691	3.691
The soil of the al-Hadarat Bridge	0	0.517	-	-
	0.3	0.829	101.468	1.468
	0.5	1.028	101.081	1.081
The soil of the Zdinaweh area	0	0.583	-	-
	0.3	0.904	102.378	2.378
	0.5	1.108	102.308	2.308
The soil of the oil refinery	0	0.639	-	-
	0.3	0.961	102.343	2.343
	0.5	1.182	103.775	3.775
Heavy crude oil residues	0	0.292	-	-
	0.3	0.607	102.534	2.534
	0.5	0.818	103.283	3.283
Light crude oil residues	0	0.182	-	-
	0.3	0.501	103.942	3.942
	0.5	0.697	102.199	2.199

The results showed that the concentration of lead ion in the river water was above the maximum permissible limit (0.01 μg/mL) for the protection of aquatic life and drinking water, as specified by the World Health Organization (WHO) [33]. The results also showed that the concentration of cadmium ion (Cd^{2+}) in the river water was slightly above the maximum permissible limit (0.003 μg/mL) for the protection of aquatic life and drinking water, as specified by the World Health Organization (WHO) [33]. This is due to the large amounts of wastewater being discharged into the Euphrates River in these areas. As for the water from the textile factory, the presence of these elements may be attributed to the use of dyes containing these substances. The concentrations of the same elements in the soil at the study locations were found to be within the permissible limits set by the World Health Organization, which are (3 mg/kg) for cadmium and (100 mg/kg) for lead in soil. However, the concentrations in heavy crude oil samples were above the permissible limits for both elements, with the WHO limits for heavy crude oil being (1 mg/L) for lead and (0.2 mg/L) for cadmium. Light crude oil was also contaminated with both elements, exceeding the permissible limits of (0.1 mg/L) for lead and (0.05 mg/L) for cadmium.

4. Conclusion

In this study, a relatively new, efficient, and environmentally friendly analytical method called USA-DES-DLLME, modified with copper oxide nanoparticles (CuO NPs), was developed for the separation and pre-concentration of lead and cadmium in water samples. A deep eutectic solvent (DES) based on choline chloride and urea demonstrated the highest recovery rates for both elements. These green solvents were used as eco-friendly alternatives to traditional toxic organic solvents. To achieve optimal extraction conditions, the separation and pre-concentration process was systematically optimized through single-factor experiments. The developed method was validated by assessing parameters such as linear range, limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD%), enrichment factor (EF), pre-concentration factor (PF), precision, and recovery rates. The proposed procedure was successfully applied for the pre-concentration and analysis of lead and cadmium in water samples.

REFERENCES

[1] M. Karimi, A. M. H. Shabani, and S. Dadfarnia, "Deep eutectic solvent-mediated extraction for ligand-less preconcentration of lead and cadmium from environmental samples using magnetic nanoparticles," *Microchimica Acta,* vol. 183, pp. 563-571, 2016.

[2] Y.-N. Chang, M. Zhang, L. Xia, J. Zhang, and G. Xing, "The toxic effects and mechanisms of CuO and ZnO nanoparticles," *Materials,* vol. 5, no. 12, pp. 2850-2871, 2012.

[3] M. Bandeira, M. Giovanela, M. Roesch-Ely, D. M. Devine, and J. da Silva Crespo, "Green synthesis of zinc oxide nanoparticles: A review of the synthesis methodology and mechanism of formation," *Sustainable Chemistry and Pharmacy,* vol. 15, p. 100223, 2020.

[4] H. Musarurwa and N. T. Tavengwa, "Deep eutectic solvent-based dispersive liquid-liquid micro-extraction of pesticides in food samples," *Food Chemistry,* vol. 342, p. 127943, 2021.

[5] U. Alshana and M. Soylak, "Deep eutectic solvents in microextraction," in *Analytical sample preparation with nano- and other high-performance materials*: Elsevier, 2021, pp. 471-512.

[6] D. J. Van Osch *et al.*, "A search for natural hydrophobic deep eutectic solvents based on natural components," *ACS Sustainable Chemistry & Engineering,* vol. 7, no. 3, pp. 2933-2942, 2019.

[7] A. Shishov, A. Bulatov, M. Locatelli, S. Carradori, and V. Andruch, "Application of deep eutectic solvents in analytical chemistry. A review," *Microchemical journal,* vol. 135, pp. 33-38, 2017.

[8] T. Khezeli, M. Ghaedi, S. Bahrani, A. Daneshfar, and M. Soylak, "Deep eutectic solvent in separation and preconcentration of organic and inorganic species," in *New Generation Green Solvents for Separation and Preconcentration of Organic and Inorganic Species*: Elsevier, 2020, pp. 381-423.

[9] D. C. de Andrade, S. A. Monteiro, and J. Merib, "A review on recent applications of deep eutectic solvents in microextraction techniques for the analysis of biological matrices," *Advances in Sample Preparation,* vol. 1, p. 100007, 2022.

[10] Y. H. Choi *et al.*, "Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology?," *Plant physiology,* vol. 156, no. 4, pp. 1701-1705, 2011.

[11] D. J. Ramón and G. Guillena, "Deep eutectic solvents: synthesis, properties, and applications," 2020.

[12] F. Oyoun *et al.*, "Deep Eutectic Solvents: An Eco-friendly Design for Drug Engineering," *ChemSusChem,* vol. 16, no. 20, p. e202300669, 2023.

[13] M. A. Farajzadeh, M. R. A. Mogaddam, and M. Aghanassab, "Deep eutectic solvent-based dispersive liquid–liquid microextraction," *Analytical Methods,* vol. 8, no. 12, pp. 2576-2583, 2016.

[14] S. Alamdari *et al.*, "Preparation and characterization of zinc oxide nanoparticles using leaf extract of Sambucus ebulus," *Applied Sciences,* vol. 10, no. 10, p. 3620, 2020.

[15] S. Abel, J. L. Tesfaye, N. Nagaprasad, R. Shanmugam, L. P. Dwarampudi, and R. Krishnaraj, "Synthesis and characterization of zinc oxide nanoparticles using moringa leaf extract," *Journal of Nanomaterials,* vol. 2021, no. 1, p. 4525770, 2021.

[16] S. Ullah *et al.*, "Ultrasound-assisted dispersive liquid-liquid microextraction using deep eutectic solvents (DESs) for neutral red dye spectrophotometric determination," *Molecules,* vol. 27, no. 18, p. 6112, 2022.

[17] A. Al-Bedhany, "Geochemical evaluation pollution for some heavy metals in basra soil," *University of Thi-Qar Journal of Science,* vol. 5, no. 2, pp. 34-42, 2015.

[18] A. Lashari, T. G. Kazi, H. I. Afridi, J. A. Baig, M. B. Arain, and A. A. Lashari, "Estimation of metal and metalloid in crude oil of newly developed oil field after acid digestion/extraction methods using different devices," *Journal of Trace Elements and Minerals,* vol. 4, p. 100064, 2023.

[19] L. Destiarti, B. N. Huda, R. Riyanto, R. Roto, and M. Mudasir, "Challenges of Using Natural Extracts as Green Reducing Agents in the Synthesis of rGO: A Brief Review," *Results in Chemistry,* p. 101270, 2023.

[20] S. Saran, G. Sharma, M. Kumar, and M. Ali, "Biosynthesis of copper oxide nanoparticles using cyanobacteria spirulina platensis and its antibacterial activity," *Int J Pharm Sci Res,* vol. 8, no. 9, pp. 3887-3892, 2017.

[21] Y. Kumar, V. Singh, A. Pandey, M. Genwa, and P. Meena, "Synthesis, characterization and antibacterial activity of ZnO nanoparticles," in *AIP Conference Proceedings*, 2020, vol. 2265, no. 1: AIP Publishing.

[22] R. A. Zounr, M. Tuzen, N. Deligonul, and M. Y. Khuhawar, "A highly selective and sensitive ultrasonic assisted dispersive liquid phase microextraction based on deep eutectic solvent for determination of cadmium in food and water samples prior to electrothermal atomic absorption spectrometry," *Food chemistry,* vol. 253, pp. 277-283, 2018.

[23] T. A. Jouybari, H. A. Jouybari, F. Hosseini, M. Nesari, and N. Fattahi, "Evaluation of blood lead levels in opium addicts and healthy control group using novel deep eutectic solvent based dispersive liquid–liquid microextraction followed by GFAAS," *Environmental Science and Pollution Research,* vol. 30, no. 9, pp. 24553-24561, 2023.

[24] E. Prasedya *et al.*, "Effect of particle size on phytochemical composition and antioxidant properties of Sargassum cristaefolium ethanol extract," *Scientific Reports,* vol. 11, no. 1, p. 17876, 2021.

[25] N. Liang, X. Hou, P. Huang, C. Jiang, L. Chen, and L. Zhao, "Ionic liquid-based dispersive liquid-liquid microextraction combined with functionalized magnetic nanoparticle solid-phase extraction for determination of industrial dyes in water," *Scientific Reports,* vol. 7, no. 1, p. 13844, 2017.

[26] E. Ragheb, M. Shamsipur, F. Jalali, M. Sadeghi, N. Babajani, and N. Mafakheri, "Magnetic solid-phase extraction using metal–organic framework-based biosorbent followed by ligandless deep-eutectic solvent-ultrasounds-assisted dispersive liquid–liquid microextraction (DES-USA-DLLME) for preconcentration of mercury (II)," *Microchemical Journal,* vol. 166, p. 106209, 2021.

[27] A. M. Hameed, "An eco-friendly ultrasound-assisted deep eutectic solvent-based liquid–phase microextraction method for enrichment and quantification of nickel in environmental samples," *Journal of Umm Al-Qura University for Applied Sciences,* vol. 8, no. 1, pp. 57-68, 2022.

[28] Z. Gholami, M. H. Marhamatizadeh, S. Yousefinejad, M. Rashedinia, and S. M. Mazloomi, "Vortex-assisted dispersive liquid-liquid microextraction based on hydrophobic deep eutectic solvent for the simultaneous identification of eight synthetic dyes in jellies and drinks using HPLC-PBA," *Microchemical Journal,* vol. 170, p. 106671, 2021.

[29] S. Sadeghi and Z. Nasehi, "Simultaneous determination of Brilliant Green and Crystal Violet dyes in fish and water samples with dispersive liquid-liquid micro-extraction using ionic liquid followed by zero crossing first derivative spectrophotometric analysis method," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* vol. 201, pp. 134-142, 2018.

[30] E. M. Gama, A. da Silva Lima, and V. A. Lemos, "Preconcentration system for cadmium and lead determination in environmental samples using polyurethane foam/Me-BTANC," *Journal of Hazardous Materials,* vol. 136, no. 3, pp. 757-762, 2006.

[31] K. Ghanemi, Y. Nikpour, O. Omidvar, and A. Maryamabadi, "Sulfur-nanoparticle-based method for separation and preconcentration of some heavy metals in marine samples prior to flame atomic absorption spectrometry determination," *Talanta,* vol. 85, no. 1, pp. 763-769, 2011.

[32] N. Jalbani and M. Soylak, "Ligandless surfactant mediated solid phase extraction combined with Fe3O4 nano-particle for the preconcentration and determination of cadmium and lead in water and soil samples followed by flame atomic absorption spectrometry: multivariate strategy," *Ecotoxicology and Environmental Safety,* vol. 102, pp. 174-178, 2014.

[33] T. Chiroma, R. Ebewele, and F. Hymore, "Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano," *International refereed journal of engineering and science,* vol. 3, no. 2, pp. 01-09, 2014.