

## EFFERVESCENT-ASSISTED DEEP EUTECTIC SOLVENT-BASED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION OF Cr(VI) IN AQUEOUS SAMPLES

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**ABSTRACT.** Effervescent-assisted deep eutectic solvent-based dispersive liquid-liquid microextraction (EA-DES-DLLME) was developed for the selective extraction and preconcentration of Cr(VI) in water samples before its spectrophotometric determination. Before using the method, Cr(IV) ions present in the samples were complexed with 1,5-diphenylcarbazine (DPC) in an acidic medium. Experimental parameters that influenced the extraction efficiency of the method including sample pH, volume of DPC, type and volume of DES, type and concentration of effervescent agents, extraction time, stability of the complex, and selectivity were thoroughly investigated. Under optimal conditions, the method showed excellent linearity ranging from 50 to 3200  $\mu\text{g L}^{-1}$ , with a coefficient of determination ( $R^2$ ) of 0.9991. The limits of detection and quantification (LOD and LOQ) of the method were 3 and 10  $\mu\text{g L}^{-1}$ , respectively. Intra- and inter-day precision studies conducted at three concentration levels yielded relative standard deviation (RSD) values of  $\leq 3.3$  and 7.2, respectively. The method's robustness, assessed at wavelengths of 535 to 545 nm had RSD  $< 4.3$ . The method provided an enrichment factor of 13.52 and recoveries between 81.1 and 111.2%. Overall, the developed method is applicable and selective for the determination of Cr(VI) in aqueous samples.

**KEY WORDS:** Cr(VI), Deep eutectic solvent, Dispersive liquid-liquid microextraction, 1,5-Diphenylcarbazine, Effervescence-assisted extraction, Water samples

### INTRODUCTION

Chromium (Cr) contamination is a serious problem that pollutes various environmental compartments [1]. Cr is widely used in industries such as stainless steel production, electroplating, textiles, leather, and paint. However, inadequate waste management has led to the release of Cr into the environment, posing significant health risks due to its toxicity to humans. As a result, monitoring the levels of Cr in water and other environmental matrices is crucial [1, 2]. Cr commonly exists in two stable oxidation states in water: Cr(III) and Cr(VI). Cr(III) plays a crucial role in the metabolism of fats and carbohydrates and is essential for plants and animals, exhibiting less mobility and toxicity. However, Cr(VI) is toxic and carcinogenic to humans [3, 4].

Cr(VI) has a high oxidation potential, small size, and the ability to easily penetrate cell membranes and attach to DNA, proteins, and membrane lipids. It is highly soluble in water and easily contaminates water bodies, causing serious health risks to humans and animals through the food chain [5]. Exposure to Cr(VI) can lead to health problems such as liver and kidney damage, skin rashes, dental abnormalities, and lung cancer [2, 5, 6]. The United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO) have set an acceptable limit for Cr(VI) in drinking water at 0.05  $\text{mg L}^{-1}$  [4, 7]. Therefore, it is important to use simple and sensitive techniques to determine Cr(VI) in various environmental sample matrices.

Various analytical techniques have been extensively used to determine the concentration levels of Cr and its species, such as Cr(III) and Cr(VI), in water and other environmental samples. These analytical techniques include X-ray fluorescence (XRF) spectroscopy [3], ultraviolet-visible (UV-Vis) spectrophotometry [5], high-performance liquid chromatography with UV

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detection (HPLC-UV) [8], flame atomic absorption spectrometry (FAAS) [9], and dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS) [5]. Among these techniques, UV-Vis spectrophotometry is the most accessible and cost-effective, making it the most commonly used analytical instrument for determining Cr(VI). However, due to its low concentrations in real samples and the complexity of the sample matrix, sample preparation is required before instrumental determinations can be conducted.

Sample preparation involves selective extraction and preconcentration of the target analyte before instrumental analysis [10]. Various sample preparation techniques have been reported for the extraction and preconcentration of Cr(VI) from various matrices. These techniques include salting-out assisted liquid-liquid extraction [11], solid-phase extraction (SPE) [9, 10, 12], electromembrane extraction (EME) combined with micro-EME ( $\mu$ -EME) [13], homogeneous liquid-liquid extraction (HLLE) [14], and dispersive liquid-liquid microextraction (DLLME) [1, 2, 4, 5, 15-21]. Among these techniques, DLLME is the most commonly used method for the extraction and preconcentration of organic and inorganic analytes. This is due to its ease of use, high extraction efficiency, versatility, minimal sample volume, reduced organic solvent consumption, high recovery, and enrichment factor [15, 21]. DLLME involves a ternary solvent system consisting of water-immiscible organic solvents, water-miscible disperser solvents, and an aqueous solution containing the analyte [22-25].

Recently, deep eutectic solvents (DESs) have been widely used in the DLLME method for extracting and preconcentrating both organic and inorganic chemicals [25, 26]. These solvents are formed from a eutectic mixture of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs) through hydrogen-bonding interactions, resulting in products with lower melting points than those of individual components [26, 27]. DESs are environmentally friendly, easily prepared, non-toxic, inexpensive, and biodegradable [2]. They also have tunable physicochemical properties: the ability to modify their viscosities, densities, conductivities, melting points, and solubilities through careful selection of their HBD and HBA constituents and their molar ratios. This allows them to be tailored for specific uses based on their required characteristics [23].

On the other hand, various mechanical approaches, such as air purging, mechanical shaking, ultrasonication, and vortex mixing, have been used to enhance the dispersion of extraction solvents in the DLLME method. These approaches also help to reduce extraction time and improve the simplicity, selectivity, and efficiency of the method [8, 28-30]. Recently, effervescence-assisted (EA) DLLME (EA-DLLME), has been reported and gained significant attention as an alternative method to the existing DLLME approaches [7, 28, 31-38]. The EA-DLLME method involves dispersing the extraction solvent using CO<sub>2</sub> bubbles. These bubbles are generated *in situ* by the effervescence reaction between effervescence agents, a CO<sub>2</sub> source, and a proton donor.

The EA-DLLME method has been used for the extraction and preconcentration of copper ions from aqueous solutions [28], Cr(VI) from water, milk, orange juice, and tannery effluent samples [7], organochlorine pesticides (OCPs) from water samples [31], polybrominated diphenyl ethers from water samples [32], fungicides from vinegar and apple juice samples [33, 34], benzoyl urea from water samples [35], triazine herbicides from water samples [36], alkyl phenols in drinks [37], and Cr(VI) and Cu(II) in edible vegetable oils [38]. Most of these methods used toxic organic solvents as extraction solvents. Therefore, the use of DES as an extraction solvent with CO<sub>2</sub> bubbles as a dispersing agent will have a significant advantage in advancing the DLLME method.

In this study, an EA-menthol-based DES-DLLME (EA-DES-DLLME) method was developed to extract Cr(VI) from water samples before determining it using UV-Vis spectrophotometry. To the best of our knowledge, EA-menthol-based DES DLLME has not been previously reported for extracting and preconcentrating Cr(VI) from any matrix. Various experimental parameters that affect the extraction efficiency of the method were optimized, and the optimal conditions were established. Under these optimal conditions, the analytical

performance characteristics of the method were validated and was applied to analyze Cr(VI) in different water samples.

## EXPERIMENTAL

### *Chemicals and reagents*

All chemicals and reagents utilized were analytical grades. DL-Menthol ( $C_{10}H_{20}O$ ) and glacial acetic acid ( $CH_3COOH$ ), sodium acetate ( $CH_3COONa$ ), and 1,5-diphenylcarbazide (DPC) were obtained from Merck (Darmstadt, Germany). Formic acid ( $HCOOH$ ), and acetone ( $C_3H_6O$ ) were purchased from Loba Chemie Pvt. Ltd (Mumbai, India). Nitric acid ( $HNO_3$ ), sulfuric acid ( $H_2SO_4$ ), sodium carbonate ( $Na_2CO_3$ ), and sodium bicarbonate ( $NaHCO_3$ ) were purchased from Fisher Scientific Limited (UK). Calcium carbonate ( $CaCO_3$ ), and potassium dichromate ( $K_2Cr_2O_7$ ) were purchased from BDH Chemicals Ltd (Poole, England). Cobalt nitrate ( $Co(NO_3)_2$ ), lead nitrate ( $Pb(NO_3)_2$ ), copper sulfate ( $CuSO_4 \cdot 5H_2O$ ), and vanadium pentoxide ( $V_2O_5$ ) were purchased from Finekem Pvt. Ltd (Aurangabad, India).

A stock solution,  $1000 \text{ mg L}^{-1}$  of Cr(VI) was prepared by dissolving 2.83 g of  $K_2Cr_2O_7$  in 0.01 mol  $L^{-1}$  of  $HNO_3$ . The working solutions were then daily prepared by diluting the stock solution with ultrapure water. Similarly, a stock solution of DPC,  $1000 \text{ mg L}^{-1}$ , was prepared in acetone. The prepared stock solutions were kept in the refrigerator, below  $4 \text{ }^\circ\text{C}$ . An acetate buffer was prepared using  $CH_3COOH$  and  $CH_3COONa$ .

### *Instrument and apparatus*

Double beam UV-Vis spectroscopy (SPECORDR200 PLUS, Analytik Jena, Germany) was used for the quantitative analysis of Cr(VI). A portable pH meter from Hanna Instruments (Póvoa de Varzim, Portugal) was used to determine pH values. A 10-mL medical syringe with a B. Braun Sterican needle  $21 \text{ G} \times 4\frac{3}{4}$  ( $0.80 \times 122 \text{ mm BL/LB}$ ) obtained from B. Braun Melsungen AG (Melsungen, Germany) was used during sample preparation. A centrifuge, model 800 from Jiangsu Zhenji Instruments Co. Ltd. (China, Jiangsu), and a 15-mL Falcon centrifuge tube were also used during sample preparation.

### *Sample collection*

The applicability of the proposed method was investigated by analyzing the level of Cr(VI) in river, spring, and tap water samples. A river water sample was collected from the Hawetu River, which flows through Jimma City in the southwest of the Oromia Region, Ethiopia. The spring water sample was collated from the Kochi area of Jimma City, and tap water was collected from the Analytical Chemistry Laboratory at Jimma University. Before the sample preparation procedure, the collected samples were filtered using Whatman filter paper to remove suspended particles.

### *Synthesis of deep eutectic solvents*

For the synthesis of DESs, menthol was used as the HBA and mixed separately with formic acid and acetic acid as HBDS in a 1:1 molar ratio to form DES-I and DES-II, respectively. The mixtures were then heated to  $70 \text{ }^\circ\text{C}$  with continuous mechanical stirring until they turned into a colorless liquid [21].

### *EA-DES-based DLLME procedure*

First, a 10 mL sample was added to a 15-mL Falcon centrifuge tube. Then, 0.5 mL of  $H_2SO_4$  ( $0.05 \text{ mol L}^{-1}$ ) and 300  $\mu\text{L}$  of DPC ( $1000 \text{ mg L}^{-1}$ ) were added and left for 5 min to complete the

complexation reaction. After adjusting the pH to 4 using an acetate buffer, 500  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  (2 mol  $\text{L}^{-1}$ ) and 600  $\mu\text{L}$  of DES were added to the sample solution. Subsequently, 1000  $\mu\text{L}$  of  $\text{CH}_3\text{COOH}$  (2 mol  $\text{L}^{-1}$ ) was added to the solution to rapidly generate  $\text{CO}_2$  bubbles, which thoroughly dispersed the DES droplets into the sample solution and enhanced the extraction of the metal-ligand complex into the DES droplets. Once the effervescence reaction was completed, the content was centrifuged at 4000 rpm for 2 min. Finally, the lower aqueous phase was carefully withdrawn and discarded. The remaining organic phase was transferred into a quartz cuvette, to measure its absorbance at 540 nm using a UV-Vis spectrophotometer. Figure 1 shows the schematic diagram of the proposed EA-DES-based DLLME method.

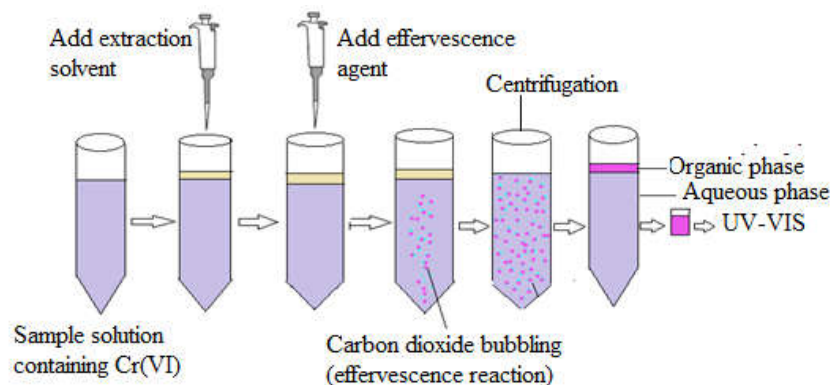


Figure 1. Schematic diagram of the developed EA-DES-based-DLLME method.

## RESULTS AND DISCUSSION

### *Spectral characteristics of Cr(VI)-DPC complex*

To extract and analyze the concentration of Cr(IV) using the proposed method, Cr(IV) should first form a complex by interacting with DPC in acidic media. The complex formed between Cr(VI)-DPC has a red-violet color [11]. The Cr(VI)-DPC complex is hydrophobic and can be easily extracted from aqueous samples into hydrophobic solvents such as hydrophobic DESs. The concentration of the extracted analyte was then determined by measuring its absorbance at 540 nm. Figure 2 shows the absorption spectra of the 0.75  $\text{ng mL}^{-1}$  Cr, DPC, and Cr(VI)-DPC.

### *Optimization of EA-DES-based DLLME procedure*

#### *Effect of pH and volume of DPC*

The extraction efficiency of the method and the formation of metal-ligand complexes are significantly influenced by the pH [5]. The influence of pH on the system was determined by adjusting the pH of the sample solution from 3 to 6 using acetate buffer at 0.5 unit intervals. The extraction efficiency of the method improved up to pH 4, but declined as the pH increased further. Therefore, pH 4 was selected for further experiments.

The volume of the complexing agent, DPC, is a crucial parameter as it needs to be sufficient to completely bind all available Cr(IV) in a sample. Various volumes of 1000  $\text{mg L}^{-1}$  DPC, ranging from 100 - 500  $\mu\text{L}$  were evaluated in a sample solution containing 2  $\text{mg L}^{-1}$  of Cr(VI). The findings

indicated that the method's extraction efficiency increased as the volume of DPC increased up to 300  $\mu\text{L}$ , with minimal changes observed with larger volumes. Therefore, 300  $\mu\text{L}$  of DPC was chosen for subsequent experiments.

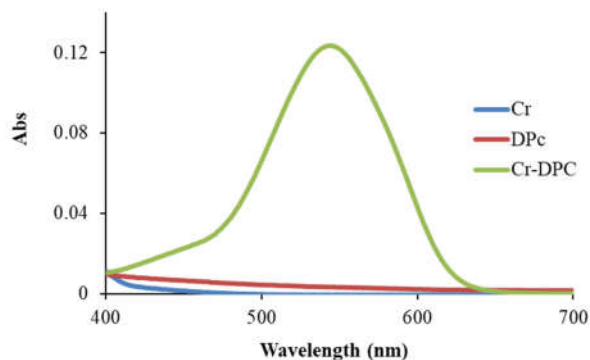


Figure 2. The UV-VIS spectra of Cr, DPC, and Cr(VI)-DPC complex.

#### *Type and volume of DESs*

The extraction efficiency of the EA-DES-based DLLME method is significantly influenced by the types of DESs, which vary based on their HBA and HBD precursors [20]. Therefore, an appropriate DES should be chosen to effectively extract the Cr(IV)-DPC complex from the aqueous sample [29]. In this study, two DESs were prepared separately by combining  $\text{CH}_3\text{COOH}$  and  $\text{CH}_3\text{COOH}$  (as HBDs) with DL-menthol (as the HBA). These were then labeled as DES-I and DES-II, respectively. As shown in Figure 3a, DES-I demonstrated superior extraction efficiency compared to DES-II. This can be attributed to the physicochemical properties of the DESs, such as density, viscosity, polarity, and miscibility. Generally, an extractant with low viscosity facilitates enhanced mass transfer and faster phase equilibration, while a significant density difference from the matrix aids in subsequent phase separation [38]. Therefore, DES-I was chosen for the subsequent experiments.

The volume of the extraction solvent is another crucial factor that affects the performance of the extraction method. To determine the optimum volume, various volumes of DES-I ranging from 100-800  $\mu\text{L}$  were tested. The highest extraction efficiency was achieved with 600  $\mu\text{L}$  (Figure 3b). When lower volumes were used, there was no clear or sufficient phase separation observed for subsequent spectrophotometric analysis. Conversely, when higher volumes were used, a dilution effect was observed [19]. Therefore, 600  $\mu\text{L}$  of DES-I was selected for further analysis.

#### *Selection of effervescence agents*

In an EA-DES-based DLLME, the selection of appropriate effervescent agents, including both a  $\text{CO}_2$  source and a proton donor is crucial. Three salts, including  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{CaCO}_3$  were evaluated as the  $\text{CO}_2$  source using  $\text{HCOOH}$  as a proton donor, and the results are displayed in Figure 4 (a). The highest extraction efficiency was obtained when  $\text{Na}_2\text{CO}_3$  was used, which is consistent with the literature [1].  $\text{NaHCO}_3$  exhibited lower extraction efficiency due to its rapid reaction, leading to incomplete dispersion of the extraction solvent into the aqueous phase. On the other hand,  $\text{CaCO}_3$  undergoes a slow effervescent reaction and produces less energetic  $\text{CO}_2$  bubbles leading to lower extraction efficiency. As indicated in the literature fast or slow effervescence reactions cause insufficient dispersion of the extraction solvent, resulting in decreased extraction efficiency of the method [38]. Thus,  $\text{Na}_2\text{CO}_3$  was chosen as the  $\text{CO}_2$  source.

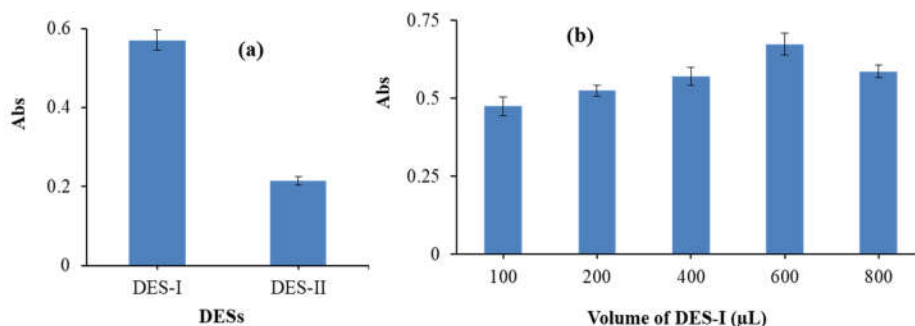


Figure 3. Effect of (a) the type of DESs and (b) volumes of DES-I. Experimental conditions for Figure 3 (a): sample pH, 4; reaction time, 3 min, DES and DPC used were 500  $\mu\text{L}$  and 300  $\mu\text{L}$ , respectively;  $\text{NaHCO}_3$  and  $\text{CH}_3\text{COOH}$  were used as effervescence precursors. For Figure 3 (b), similar experimental conditions to Figure 3 (a) were used, but DES-I was the extraction solvent with varying volumes.

As proton donors,  $\text{HCOOH}$  and  $\text{CH}_3\text{COOH}$  were investigated using  $\text{Na}_2\text{CO}_3$  as the  $\text{CO}_2$  source, at their stoichiometric ratio. The findings are shown in Figure 4 (b). Although both acids exhibited comparable extraction efficiencies, relatively better extraction efficiency was observed when  $\text{CH}_3\text{COOH}$  was used, so it was selected for further experiments.

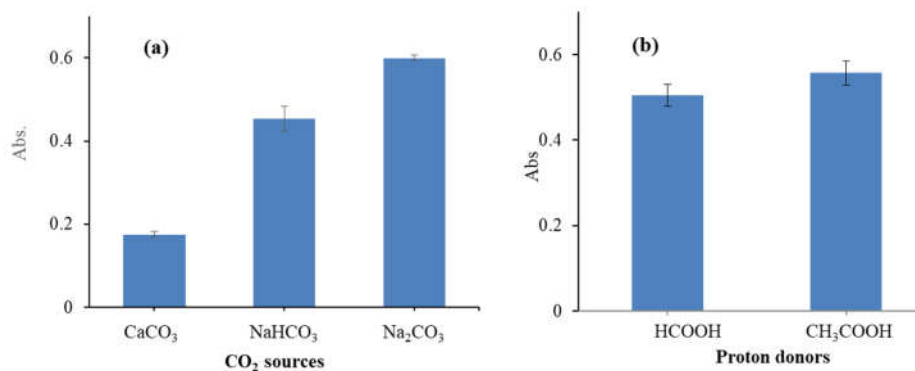


Figure 4. Effect of effervescence agents: The experimental conditions for Figure 4(a) were similar to those in Figure 3, but 600  $\mu\text{L}$  of DES-I was used for the extraction. In Figure 4 (b) similar experimental conditions to Figure 3 were used, but  $\text{Na}_2\text{CO}_3$  was used as the  $\text{CO}_2$  source.

#### *Effect of concentrations of $\text{Na}_2\text{CO}_3$ and $\text{CH}_3\text{COOH}$*

The amount of  $\text{CO}_2$  bubbles produced during the effervescence reaction depends on the concentrations and molar ratios of the two components: a  $\text{CO}_2$  source and a proton donor. It is crucial to add appropriate concentrations of these components to the samples to generate  $\text{CO}_2$  bubbles. This will aid in dispersing the extraction solvent within a reasonable timeframe [28, 39]. The concentrations of  $\text{CH}_3\text{COOH}$  and  $\text{Na}_2\text{CO}_3$  were investigated in the range of 1 - 5  $\text{mol L}^{-1}$  at a 2:1 molar ratio of the two components, Figure 5 (a). It was observed that the extraction

performance of the method increased as their concentrations increased up to 2 mol L<sup>-1</sup> and then slightly decreased when the concentrations of effervescent agents were further increased. This might be due to an increase in the solution's viscosity and ionic strength of the sample, which could hinder the mass transfer of the analyte from the sample phase to the extraction solvent [28]. Therefore, 2 mol L<sup>-1</sup> was selected as the optimum concentration for both components for further studies.

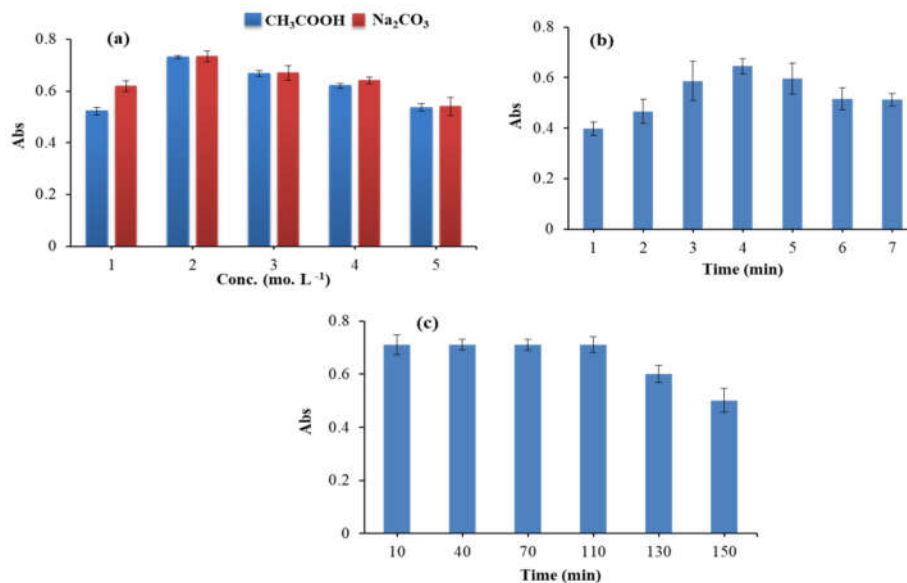


Figure 5. Effects of (a) effervescent agent concentrations, (b) extraction time, and (c) stability of the complex, Cr(VI)-DPC. The experimental conditions for Figure (a) were similar to those in Figure 4 (b), but CH<sub>3</sub>COOH was used as the proton donor. In Figure 5 (b) similar experimental conditions to Figure 5 (b) were used, but 500  $\mu$ L and 1000  $\mu$ L of 2 mol L<sup>-1</sup> of Na<sub>2</sub>CO<sub>3</sub> and CH<sub>3</sub>COOH, respectively, were used as effervescent agents for varied reaction times. In Figure 5 (c) the reaction time was 4 min, with other conditions similar to Figure 5 (b).

#### *Effect of the extraction time and stability of Cr-DPC complex*

Extraction time is the duration required for the transfer of the analyte from the aqueous sample to the extraction phase [2]. The impact of time was examined from 1-7 min. Figure 5 (b) shows that the extraction performance of the method increased up to 4 min and then remained constant with further increasing time. Therefore, 4 min was chosen for further experiments.

To evaluate the stability of the extracted analyte-containing complex (Cr(VI)-DPC), it was kept in the dark for 10-150 min. The absorbance of the complex remained consistent up to 110 min but decreased after 110 min (Figure 5 (c)). This aligns with literature indicating that when the complex is stored for an extended period, the metal-ligand complex breaks down [5, 11].

#### *Effect of interfering ions*

The selectivity of the proposed method may be influenced by the presence of potentially interfering ions such as Pb(II), Co(II), Cu(II), and V(V) in the samples [5, 11]. The impact of

these ions was examined by spiking known concentrations of each metal ion, ranging from 50 - 500 mg L<sup>-1</sup> into an aqueous sample. The interference of each ion is considered negligible or tolerable if it causes an absorbance fluctuation of less than 5% [5]. It was observed that all the studied metal ions exhibited negligible interference up to 500 mg L<sup>-1</sup>, demonstrating the selectivity of the method for the analysis of Cr(VI) in the presence of these ions in water samples (Table 1).

Table 1. Effect of interfering ions for determination of 2 mg L<sup>-1</sup> Cr(VI) with DPC.

Concentration (mg L <sup>-1</sup> )	%R±RSD when interfering ions are present			
	Pb(II)	Co(II)	Cu(II)	V(V)
0.0	100.0±1.5	100.0±1.7	100.0±2.4	100.0±2.6
50.0	98.4±2.1	98.8±2.8	103.6±1.7	101.4±2.3
100.0	97.0±1.9	97.3±0.9	102.5±2.3	100.5±0.8
200.0	99.0±3.2	97.6±3.0	99.4±0.8	97.8±4.1
500.0	95.6±2.7	100.6±0.6	99.8±1.2	101.9±3.5

#### Method validation

A calibration curve was constructed using seven concentration levels ranging from 50 to 3200 µg L<sup>-1</sup>. Each level was extracted in duplicates and the absorbance of each extract was measured in triplicates. The calibration curve, constructed by plotting analyte concentration as a function of absorbance, yielded a linear equation of  $y = 0.2534x + 0.0907$  with an R<sup>2</sup> of 0.9991. The limits of detection and quantification (LOD and LOQ) of the method were determined by multiplying the ratio of the standard deviation of the blank (n = 6) and the slope of the calibration curve by 3 and 10, resulting in values of 3 and 10 µg L<sup>-1</sup>, respectively. The enrichment factor (EF) of the developed method, as determined by Equation 1, was 13.52 [23].

$$EF = \frac{C_{DES}}{C_o} \quad (1)$$

where C<sub>o</sub> is the concentration of the analyte in the sample before extraction, and C<sub>DES</sub> is the concentration of the analyte in the extraction phase (extracted into DES).

#### Robustness/ruggedness of the proposed method

Robustness refers to the ability of a method to produce unbiased findings despite slight variations in experimental parameters [5]. The robustness of the method was investigated by measuring the absorbance of three different concentration levels at varying wavelengths ranging from 535 to 545 nm. The RSD values for the three concentration levels were below 4.2%, indicating the robustness of the proposed method.

#### Study of precision

The precision of the method was evaluated in terms of intra-day precision (repeatability) and inter-day precision (within-lab reproducibility) at three concentration levels: 100, 400, and 1,600 µg L<sup>-1</sup>. Intra-day precision was investigated by extracting three samples for each concentration level twice a day (in the morning and afternoon) and measuring the absorbance of each extract in triplicate. Inter-day precision was studied for three consecutive days, with three samples for each concentration level extracted per day and analyzed in triplicate. As shown in Table 2, the RSD values for intra- and inter-day precision values are ≤ 3.3% and 7.2%, respectively.



*Recovery studies and real sample analysis*

Percent recovery (%R) studies were conducted by spiking tap, river, and spring water samples with three concentration levels had been previously used for precision studies. For each concentration level, duplicate samples were extracted, and then each extract was analyzed in triplicate. The recoveries (%R±RSD) ranged from 81.1±5.3 to 111.2±5.9 (Table 2), falling within an acceptable range. The applicability of the proposed method was evaluated by analyzing various water samples, including tap, river, and spring waters. The river water contained 100.0±2.1 µg L<sup>-1</sup> of Cr(IV), however, it was not detected in the other two water samples.

Table 2. The precision and recovery of the proposed method.

Spiked concentration (µg L <sup>-1</sup> )	Precision		%R ± RSD		
	Intra-day (n = 18)	Inter-day (n = 27)	Tap water	Spring water	River water
100.0	3.3	6.4	81.1±5.3	93.9±4.3	90.8±4.7
400.0	2.7	6.3	94.1±3.0	105.8±2.9	109.1±2.5
1,600.0	1.7	7.2	104.6±0.4	103.9±1.2	111.2±5.9

*Comparison of the proposed method with other reported methods*

The developed EA-DES-DLLME method was compared with previously reported methods for the extraction and preconcentration of Cr(VI)-DPC before its spectrophotometric determination from water and other matrices. The EA-DES-DLLME method exhibited comparable LOD and LOQ compared to methods such as SA-LLE, SA-DLLME, LLE, and DLLME used for the extraction and preconcentration of Cr(VI)-DPC (Table 3). The method also showed comparable precision and recoveries with reported methods. Furthermore, the method was compared to the EA-DES-DLLME method, which was used for the extraction and preconcentration of Cu(II)-DPC before its determination by flame atomic absorption spectrometry (FAAS). The proposed method demonstrated comparable analytical performances in terms of LOD, LOQ, precision, and percent recoveries.

Table 3. Comparison of the proposed method with other reported methods.

Method	Sample (mL)	Analyte	Sample	LOD (µg L <sup>-1</sup> )	LOQ (µg L <sup>-1</sup> )	RSD (%)	%R	Ref.
EA-DLLME	10	Cr(VI)	Water tannery, milk, juice	1.0	5.0	<6.2	83.0-117.6	[5]
SA-LLE	5	Cr(VI)	Water	4.0	15.0	< 9.0	88.0-97.3	[11]
SA-DLLME	10	Cr(VI)	Water	0.9	-	< 6.0	80.0-104.0	[4]
LLE	10	Cr(VI)	Water	7.5	-	< 5.0	94.0-108.0	[40]
DLLME	10	Cr(VI)	Water	30.0	70.0	< 6.2	95.2-104.8	[2]
EA-DES-DLLME*	10	Cu(II)	Water	2.9	9.7	< 2.2	97.0-104.0	[28]
EA-DES-DLLME	10	Cr(VI)	Water	3.0	10.0	≤ 7.2	81.1-111.2	This work

\*The extract was determined by FAAS.

## CONCLUSION

In this study, EA-DES-DLLME has been proposed for the extraction and preconcentration of Cr(VI) from water samples before its determination using UV-Vis spectrophotometry. The method uses environmentally friendly extraction solvent (DES) and easily accessible effervescent precursors, Na<sub>2</sub>CO<sub>3</sub> and CH<sub>3</sub>COOH, for *in-situ* generation of CO<sub>2</sub> bubbles to disperse the extraction solvents in the sample. Several parameters affecting the extraction performance of the proposed method were evaluated, and optimal conditions were established. Under optimized conditions, the method exhibited satisfactory analytical performances, including a wide linear dynamic range, low LOD, and LOQ, as well as satisfactory precision and %R studied at three concentration levels. Overall, the developed method is simple, selective, and robust, making it an attractive alternative for monitoring Cr(VI) in various water samples and related matrices.

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### *Conflicts of interest*

The authors have no conflicts of interest.

## REFERENCES

1. Vakh, C.; Evadokimova, E.; Pochivalov, A.; Moskvina, L.; Bulatov, A. Effervescence assisted dispersive liquid-liquid microextraction followed by microvolume UV-Vis spectrophotometric determination of surfactants in water. *Toxicol. Environ. Chem.* **2017**, *99*, 613-623.
2. Pourmohammad, M.; Faraji, M.; Jafarnejad, S. extraction of Cr(VI) in water samples by dispersive liquid-liquid microextraction based on deep eutectic solvent and determination by UV-Vis spectrophotometry. *Int. J. Environ. Anal. Chem.* **2019**, *100*, 1146-1159.
3. Astatkie, H.; Ambelu, A.; Beyene, E.M. Sources and level of heavy metal contamination in the water of Awetu watershed streams, southwestern Ethiopia. *Heliyon* **2021**, *24*, e06385.
4. Marguí, E.; Torrent, L. Simple method for Cr(VI) determination by liquid-liquid microextraction combined with total reflection X-ray fluorescence spectrometry: application to water samples and industrial extracts. *Spectrochim. Acta Part. B At. Spectrosc.* **2023**, *204*, 106682.
5. Kebede, A.; Tilachew, G.; Chirfa, G.; Gure, A. Effervescence assisted dispersive liquid-liquid microextraction for spectrophotometric determination of Cr(VI) in water, tannery effluent, milk, and orange juice samples. *S. Afr. J. Chem.* **2022**, *76*, 127-133.
6. Khan, I.; Ali Shah, A.U.H.; Bilal, S. Effective adsorption of hexavalent chromium and divalent nickel ions from water through polyaniline, iron oxide, and their composites. *Appl. Sci.* **2020**, *10*, 2882.
7. Tumolo, M.; Ancona, V.; De Paola, D.; Losacco, D.; Campanale, C.; Massarelli, C.; Uricchio, V.F. Chromium pollution in European water, sources, health risk, and remediation strategies: an overview. *Int. J. Environ. Res. Public Health* **2020**, *28*, 17, 5438.
8. Zahedi, M.M.; Rezaei, A. Optimization of emulsification-based liquid phase microextraction of chromium in seawater of chabahar bay for its speciation by high-performance liquid chromatography. *J. Chromatogr. Sci.* **2016**, *54*, 1851-1857.

9. Moghimi, A. Solid phase extraction and determination of chromium species using multiwalled carbon nanotubes by solid phase extraction. *African J. Pure. Appl. Chem.* **2013**, *7*, 146-156.
10. Aranda, P.R.; Moyano, S.; Martinez, L.D.; De Vito, I.E. Determination of trace Cr(VI) in drinking water using X-ray fluorescence spectrometry after solid-phase extraction. *Anal Bioanal. Chem.* **2010**, *398*, 1043-1048.
11. Dame, J.; Wedajo, F.; Molole, G.J.; Gure, A. Salting-out assisted liquid-liquid extraction of Cr(VI) from water samples before its determination by UV-Vis spectrophotometry. *J. Chem.* **2023**, 8857010.
12. Rezvani, M.; Asgharinezhad, A.A.; Ebrahimzadeh, H.; Shekari, N. Polyaniline-magnetic nanocomposite as an anion exchange sorbent for solid-phase extraction of Cr(VI) ions. *Microchim. Acta* **2014**, *181*, 1887-1895.
13. Nojavan, S.; Rahmani, T.; Mansouri, S. Selective determination of Cr(VI) in industrial wastewater samples by micro-electromembrane extraction combined with electrothermal atomic absorption spectrometry. *Water. Air. Soil. Pollut.* **2018**, *229*, 89.
14. Abkenar, S.D.; Hosseini, M.; Dahaghin, Z.; Salavati-Niasari, M.; Jamali, M.R. Speciation of chromium in water samples with homogeneous liquid-liquid extraction and determination by flame atomic absorption spectrometry. *Bull. Korean. Chem. Soc.* **2010**, *31*, 2813-2818.
15. Alexovi, M.; Balogh, I.S.; Skrlkova, J.; Andruch, V. Dispersive liquid-liquid microextraction procedure for UV-Vis spectrophotometric determination of Cr(VI) in water samples. *Anal. Methods* **2014**, *4*, 1410-1414.
16. Fiorentini, E.F.; Oviedo, M.N.; Wuilloud, R.G. Ultra-trace Cr preconcentration in honey samples by magnetic ionic liquid dispersive liquid-liquid microextraction and electrothermal atomic absorption spectrometry. *Int. J. Environ. Anal. Chem.* **2018**, *75*, 6307-6314.
17. de Andrade, J.K.; de Andrade, C.K.; Felsner, M.L.; Dos Anjos, V.E. Ultrasound-assisted emulsification microextraction combined with graphite furnace atomic absorption spectrometry for the chromium speciation in water samples. *Spectrochim. Acta Part B* **2020**, *169*, 105879.
18. Hemmatkhan, P.; Bidari, A.; Jafarvand, S.; Hosseini, M.R.M.; Assadi, Y. Speciation of chromium in water samples using dispersive liquid-liquid microextraction and flame atomic absorption spectrometry. *Microchim. Acta* **2009**, *166*, 69-75.
19. Lasarte-Aragonés, G.; Lucena, R.; Cárdenas, S.; Valcárcel, M. Effervescence assisted dispersive liquid-liquid microextraction with extractant removal by magnetic nanoparticles. *Anal. Chim. Acta* **2014**, *807*, 61-66.
20. Fasihi, M.; Rajabi, M.; Barfi, B.; Sajjadi, S.M. Efficacious and environmentally friendly deep eutectic solvent-based liquid-phase microextraction for speciation of Cr(III) and Cr(VI) ions in food and water samples. *Int. J. Environ. Anal. Chem.* **2020**, *102*, 4331-4343.
21. Panhwar, A.H.; Tuzen, M. Ultrasonic assisted deep eutectic solvent liquid-liquid microextraction using azadipyromethene dye as complexing agent for assessment of chromium species in environmental samples by electrothermal atomic absorption spectrometry. *Appl. Organomet. Chem.* **2018**, *32*, e4319.
22. Rezaee, M.; Assadi, Y.; Hosseini, M.M. Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J. Chromatogr. A* **2006**, *1116*, 1-9.
23. Tesfaye, B.; Gure, A.; Asere, T.G.; Molole, G.J. Deep eutectic solvent-based dispersive liquid-liquid microextraction for determination of organochlorine pesticides in water and apple juice samples. *Microchem. J.* **2023**, *195*, 109428.
24. Lu, W.; Liu, S.; Wu, Z. Recent application of deep eutectic solvents as green solvent in dispersive liquid-liquid microextraction of trace level chemical contaminants in food and water. *Crit. Rev. Anal. Chem.* **2020**, *52*, 504-518.
25. Ripoll, L.; Rayos, J.; Aguirre, M.Á.; Vidal, L.; Canals, A. Natural deep eutectic solvent-based microextraction for mercury speciation in water samples. *Anal. Bioanal. Chem.* **2023**, *415*, 4435-4444.

26. Shishov, A.Y.; Savinov, S.; Volodina, N.; Gurev, I.; Bulatov, A. Deep eutectic solvent-based extraction of metals from oil samples for elemental analysis by ICP-OES. *Microchem. J.* **2022**, *179*, 107456.
27. Abbott, A.P.; Capper, G.; Davies, D.L.; Rasheed, R.K.; Tambyrajah, V. Novel solvent properties of choline chloride/urea mixtures. *R. Soc. Chem.* **2003**, 70-71.
28. Regueiro, J.; Llompарт, M.; Garcia-Jares, C.; Garcia-Monteagudo, J.C.; Cela, R. Ultrasound-assisted emulsification-microextraction of emergent contaminants and pesticides in environmental waters. *J. Chromatogr. A* **2008**, *1190*, 27-38.
29. Ezoddin, M.; Abdi, K.; Esmacili, N. Ultrasound enhanced air-assisted surfactant liquid-liquid microextraction based on the solidification of an organic droplet for the determination of chromium in water, air and biological samples. *Microchem. J.* **2016**, *129*, 200-204.
30. Maya, F.; Horstkotte, B.; Estela, J.M.; Cerda, V. Lab in a syringe: Fully automated dispersive liquid-liquid microextraction with integrated spectrophotometric detection. *Anal. Bioanal. Chem.* **2012**, *404*, 909-917.
31. Tesfaye, B.; Gure, A.; Asere, T.G.; Molole, G.J. Effervescence-assisted dispersive liquid-liquid microextraction for extraction and preconcentration of organochlorine pesticides in water samples. *Bull. Chem. Soc. Ethiop.* **2023**, *37*, 1109-1122.
32. Wang, Y.; Zhang, Q.; Chen, S.; Cheng, L.; Jing, X.; Wang, X.; Guan, S.; Song, W.; Rao, Q. Determination of polybrominated diphenyl ethers in water samples using effervescent-assisted dispersive liquid-liquid microextraction with solidification of the aqueous phase. *Mol.* **2021**, *26*, 1376.
33. Jiang, W.; Chen, X.; Liu, F.; You, X.; Xue, J. Effervescence-assisted dispersive liquid-liquid microextraction using a solid effervescent agent as a novel dispersion technique for the analysis of fungicides in apple juice. *J. Sep. Sci.* **2014**, *37*, 3157-3163.
34. Jing, X.; Zhang, J.; Zhu, J.; Chen, Z.; Yi, L.; Wang, X. Effervescent-assisted dispersive liquid-liquid microextraction based on the solidification of floating organic droplets for the determination of fungicides in vinegar and juice. *Food. Addit. Contam. Part A Chem. Anal. Control. Expo. Risk. Assess* **2018**, *35*, 2128-2134.
35. Hu, L.; Qian, H.; Yang, X.; Li, S.; Zhang, S.; Lu, R.; Zhou, W.; Gao, H. Effervescence-assisted dispersive liquid-liquid microextraction based on the solidification of a floating ionic liquid with a special collection method for the rapid determination of benzoylurea insecticides in water samples. *RSC. Adv.* **2016**, *6*, 95283-95291.
36. Jing, X.; Cheng, X.; Zhao, W.; Wang, H.; Wang, X. Magnetic effervescence tablet-assisted switchable hydrophilicity solvent-based liquid phase microextraction of triazine herbicides in water samples. *J. Mol. Liq.* **2020**, *306*, 112934.
37. Jing, X.; Xue, H. Y.; Huang, X.; Li, H. Y.; Wang, X. W.; Jia, L. Y. An effervescence-assisted dispersive liquid-phase microextraction based on solidification of switchable hydrophilicity solvents for detection of alkylphenols in drinks. *Chinese J. Anal. Chem.* **2021**, *49*, e21065-e21071.
38. Wu, J.; Lan, S.; Sun, J.; She, H.; Wang, G.; Wen, X.; Zhou, S.; Yoing, B.; Wnag, X.; Wang, H. Trace-level detection of Cr(VI) and Cu(II) in edible vegetable oils using dicationic ionic liquids and MFe<sub>2</sub>O<sub>4</sub>-based effervescence-enhanced emulsification microextraction followed by ICP-MS analysis. *Food. Anal. Methods* **2023**, *16*, 1655-1672.
39. Lasarte-Aragonés, G.; Lucena, R.; Cárdenas, S. Effervescence-assisted microextraction-one decade of developments. *Mol.* **2020**, *21*, 6053.
40. Chen, W.; Zhong, G.; Zhou, Z.; Wu, P.; Hou, X. Automation of liquid-liquid extraction-spectrophotometry using prolonged pseudo-liquid drops and handheld CCD for speciation of Cr(VI) and Cr(III) in water samples. *Anal. Sci.* **2005**, *21*, 1189-1193.