Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print) 2521-3520 (electronic)

ORIGINAL ARTICLE *OPEN ACCESS*

A Simple Dispersive Liquid-Liquid Microextraction for the Determination of Copper II in the Water Samples of the Halfaya Oil Field in Maysan

Anwer S. Salim [a], Mohammed Z. Thani [a], Abdull Jabar KH. Atia [a], and Hassan A. Fattah [b]

[a] *Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq*
[b] *Mining and Petroleum Engineering Department, Faculty of Engineering, Al Azhar University, Cairo, Egypt*

CORRESPONDENCE
Anwer S. Salim
anwaralasadi82@gmail.com

ARTICLE INFO
Received: September 03, 2023
Revised: December 27, 2023
Accepted: January 02, 2024
Published:September 30, 2024

ABSTRACT: ***Background:*** *Copper, an important trace element for life on Earth, is crucial to many metabolic processes. This includes its role in the production and breakdown of nucleic acids and the metabolism of proteins, lipids, and carbohydrates.* ***Objective:*** *We aim to integrate DLLME with a spectrophotometric method to establish a novel approach for quantifying trace amounts of copper in water samples.* ***Methods:*** *In this study, a new ligand known as a 2-(4 aminophenol) benzimidazole azo derivative was successfully synthesized. This ligand was created by converting 2-(4-aminophenyl) benzimidazole into a diazonium salt and coupling it with 8-hydroxyquinoline in an alkaline environment. The resulting product was a brown azo dye, which served as the ligand for detecting copper (II) in aqueous samples.* ***Results:*** *To analyze copper (II) in both pure and aqueous samples, we developed and validated micro-extraction techniques combined with UV-Vis measurement. Specifically, we used the DLLME (dispersive liquid-liquid microextraction) method to separate, enrich, and assess copper (II) in both its pure form and in aqueous samples. UV-Vis spectroscopy at a wavelength of 503 nm was used for this purpose. We considered several variables during the experiment, including the type and volume of the dispersive solvent, the extraction solvents, the temperature, the reaction duration, and the centrifuging time. By optimizing these conditions, we achieved linear procedures within the concentration range of (1.0–25.0) and (2.0–25.0) g/mL for spectroscopy and DLLME methods respectively. For the spectroscopic and DLLME approaches, the coefficient of determination (R2) was found to be 0.9963 and 0.9979 for the spectrophotometric and DLLME methods respectively. The limit of detection (LOD) for copper (II) was found to be 0.23 and 0.04 µg/mL respectively. Moreover, the recovery of the target analyte in aqueous samples was found to be between 95.6% and 101% for the spectroscopy method and between 102.4% and 108.2% for the DLLME method.* ***Conclusions:*** *These results demonstrate the effectiveness and accuracy of both methods in analyzing copper (II) in aqueous samples.*

KEYWORDS: *Copper; Microextraction; DLLME; Azo compound; Green chemistry*

INTRODUCTION

Copper, an important trace element for life on Earth, is crucial to many metabolic processes. These include its function in the production and breakdown of nucleic acids, as well as the metabolism of proteins, lipids, and carbohydrates [1], [2]. Diseases including biochemical abnormalities and physiological functioning disorders might result from a copper deficit. In contrast, excessive use can be detrimental and result in symptoms including nausea, vomiting, diarrhea, throat and nose irritation, liver disease, and even the formation of dangerous free hydroxyl radicals that can harm DNA and perhaps cause cancer. Copper is a widespread trace element found in water, soil, and

DOI: https://doi.org/10.23851/mjs.v35i3.1475

plants. Additionally, environmental copper pollution can occur due to its extensive use in various industries. Humans and aquatic life in general may be toxically affected by this pollution. Due to the importance of determining trace copper levels in understanding how it affects ecosystems and human health, biological and environmental analysis is essential for these reasons[3]–[6]. Various sensitive analytical techniques can be utilized to determine copper at low levels. These techniques include chemiluminescence (CL)[7], fluorescence [7], [8], voltammetry [9], [10], GFAAS [11]–[13], HPLC [14], ICP-MS [15], [16], and ICP-AES [17], [18]. In this particular case, spectrophotometric determination and DLLME were chosen as the methods of analysis due to their efficiency, high sensitivity, and cost-effectiveness [19], [20]. Solid-phase extraction [21], [22], cloud point extraction [23], [24], and liquid-liquid extraction (LLE) [25], [26] are the most commonly used methods for separating and preconcentrating trace copper. However, these techniques have certain drawbacks, including the use of large toxic organic solvents, unacceptable enrichment factors, long analysis time, and the production of secondary wastes. To address these issues, liquid-phase microextraction techniques such as dispersive liquid-liquid microextraction [12], [26], single-drop microextraction [27], [28], solidified floating organic drop microextraction [29], [30], and supramolecular solvent-based microextraction [31] have gained significant attention. The analyte is extracted from the aqueous sample solution using DLLME, a recently developed liquid-phase microextraction technology, which employs a binary solvent system made up of a high-density water-immiscible solvent (extractant) and a water-miscible solvent (disperser) [32], [33]. Due to the method's broad contact area between the sample's tiny droplets and the extraction solvent, the extraction equilibrium state is quickly reached and mass transfer kinetics are accelerated. For the extraction and preconcentration of heavy metals, DLLME has been frequently used [32]. Its key benefits include low cost, simplicity, quickness, high recovery, and high enrichment factors, as well as minimum organic solvent usage. In this study, we aim to integrate DLLME with a spectrophotometric method to establish a novel approach for quantifying trace amounts of copper in water samples.

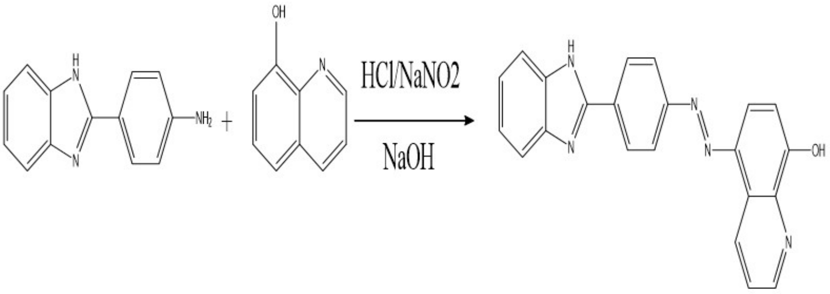
MATERIALS AND METHODS

The absorbance measurements were conducted at 510 nm using a single-beam UV-visible spectrophotometer (analyticgena, Germany) equipped with a 1 cm quartz cell. The evaluation of copper was achieved using an atomic absorption spectrophotometer (AAS) (Shimadzu AA-670, Japan). To expedite the phase extraction process, a centrifuge (Beckman model TJ-6, Germany) was employed.

Reagents and Solutions

Analytical grade copper chloride purchased from Riedel-de Haën and SDS from Fluka were used in the study. By blending 0.0672 g of CuCl2(Mwt. = 134.45 g/mol/) with 100 mL of deionized water, a stock solution of copper (II) with a concentration of (100 mg/L) was produced. A novel azo compound was additionally produced with a concentration of 100 mg/L, by dissolving 0.01 g in 5 mL of ETOH and then diluting it with deionized water to the appropriate volume (100 mL). Daily working solutions have been produced by properly diluting the stock solutions.

Synthesis of (E)-5-((4-(1H-benzo[d]imidazol-2-yl)phenyl)diazenyl)quinoline-8-ol [5BIPQ] [34]



To prepare the diazonium salt, 2-(4-aminophenyl) benzimidazole (3×10-3 mol) was combined with crushed ice, concentrated HCl (0.9 mL), and a solution of NaNO2 (3.3×10-3 mol) in distilled water

(10 mL). The resulting mixture was stirred at a temperature range of 0–10 ℃ for 12 minutes. In a separate solution, 8-hydroxyquinoline (3×10-3 mol) was dissolved in water (15 mL), followed by the addition of 10% aqueous NaOH (3 mL). The solution of the diazonium salt was then added to this mixture at a temperature range of 0–10 ℃. The resulting product was filtered, washed with small amounts of water, and left to dry in the air, resulting in the formation of an orange compound. The compound has the following properties as shown in Table 1.

Table 1. Synthesis, Characterization, and Thermal Analysis of a New Acidic azo Ligand's Metal Complexes

Name	**Properties**
Formula	C22H15N5O
Molecular weight	365.39 g/mol
Yield	91%
Melting point	220–224℃
FTIR (cm^{-1})	OH (2450-3561), CHor (3058), NH(3227), C=N(1628), C=C(1563), N=N(1531)
Mass spectra (m/z)	365, 337, 228, 209, 194, 181, 160, 145, 131, 117, 104, 91, 77, 63, 52

General procedure of spectroscopy method

In a test tube (10 mL), a range of standard solutions containing 1.0-25.0 µg/mL of copper (II) in an aqueous solution were combined with 2.5 mL of reagent 5BIPQ (100 g/mL). Then, 1 mL of ammonium hydroxide solution and 1 mL of SDS were added to the mixture. To complete the process, the mixture was diluted with deionized water up to the mark and heated to 50°C for 10 minutes. At 503 nm, the absorbance has been measured in comparison to a blank for the same reagent. A calibration curve established simultaneously was utilized to ascertain the amount of copper present in an unidentified sample.

General procedure of DLLME

In a 10 mL test tube, a copper standard solution ranging from 2.0 to 25.0 µg/mL was added along with 2.5 mL of 5BIPQ (100 µg/mL), 1.0 mL of ammonium hydroxide solution, and (1.0 mL) of SDS. The volume was subsequently adjusted by deionized water. to 10 mL. The mixture was heated at 50°C for 10 minutes after shaking it for 10 minutes. To create a cloudy solution, 600 µL of chloroform was rapidly injected as an extraction solvent, combined with 600 µL of ethanol as a dispersive solvent, using a microsyringe. The mixture was then centrifuged at 5000 rpm for 7.0 minutes. A microsyringe was used to obtain the copper complex, and its absorbance at 503 nm has been measured in comparison to a reference.

RESULTS AND DISCUSSION

In this study, we created a novel azo compound by combining 2-(4-aminophenyl) benzimidazole with 8-hydroxyquinoline using concentrated HCl and NaNO2. FTIR spectra of this compound give the following stretching bonds (OH (2450-3561), CHor (3058), NH(3227), C=N(1628), C=C(1563), N=N(1531) cm-1 and Mass spectra m/z(365, 337, 228, 209, 194, 181, 160, 145, 131, 117, 104, 91, 77, 63, 52), as shown in Figure 1. This newly synthesized compound was then employed as a ligand to detect copper ions (Cu+2) in aqueous solution. The primary focus of the study was the produce a complex between 5BIPQ and copper ions, which resulted in an orange color with a maximum absorption wavelength of 503 nm. Figure 2 illustrates the absorption spectra of the complex compared to the blank solution.

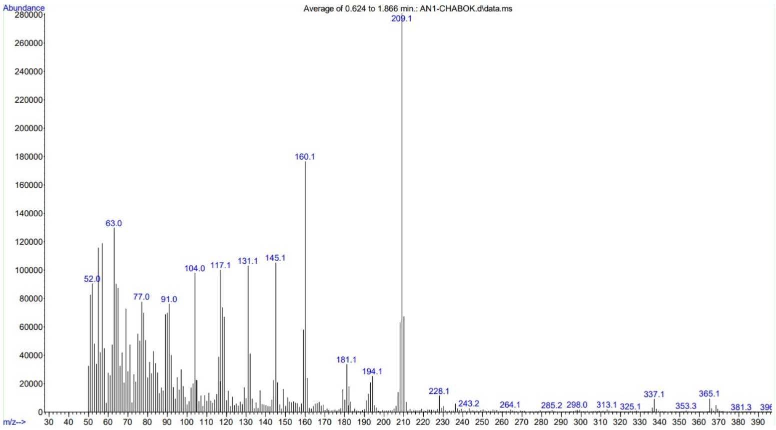


Figure 1. Mass spectra of the synthesized compound

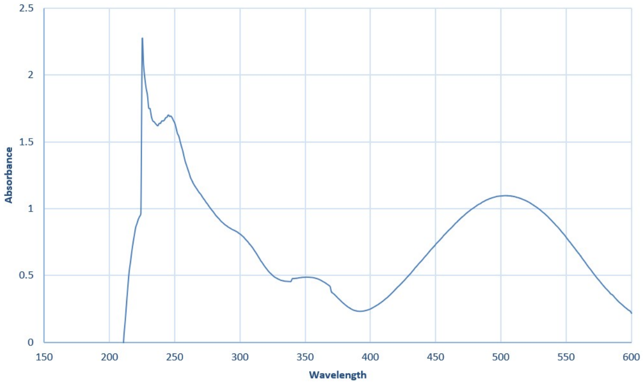


Figure 2. Absorption spectrum of cadmium complex

Spectrophotometric Preliminary Investigation for Determination of Copper Ion

1 Optimization of the Spectrophotometric Experimental Conditions

The investigation focused on the formation of the copper complex using different bases, including NaOH, KOH, NH4OH, and Na_2CO_3. The results, depicted in Figure 3, illustrate the outcomes of this study. The impact of varying base volumes (ranging from 0.1 to 2.0) mL on the developed copper complex solution at a fixed concentration was also examined. Figure 4 indicates that a volume of 1.0 mL of NH_4OH yielded the most favorable results.

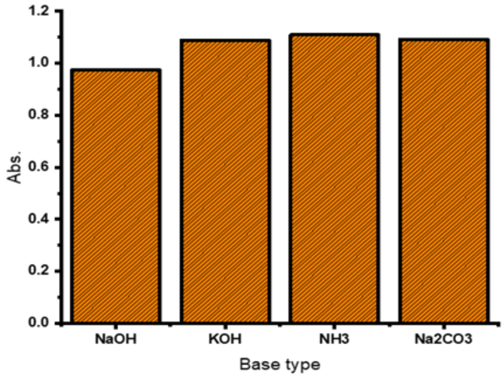


Figure 3. Effect of the base type

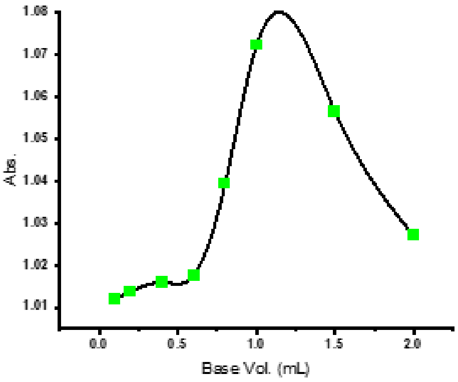


Figure 4. Effect of the base volume

The measurement of absorbance was conducted using different surfactants, including SDS, CTAB, and Tween-20. The results revealed that SDS yielded the most favorable outcomes, as depicted in Figure 5. Considering that the maximum absorbance can be influenced by the volume of the reagent (5BIPQ), different volumes of the 5BIPQ solution ranging between 0.25 mL and 3.0 mL were added. Figure 6 demonstrates that an addition of 2.5 mL of the 5BIPQ solution resulted in the highest absorbance signal, which was subsequently utilized to carry out the subsequent steps.

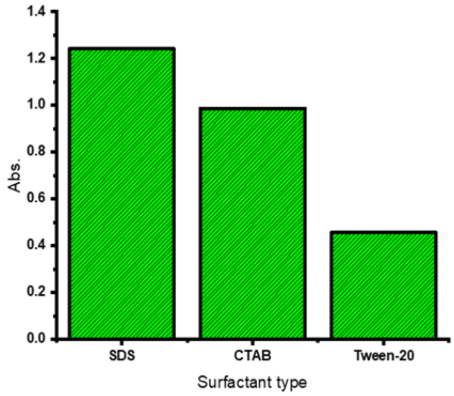


Figure 5. Effect of the base type

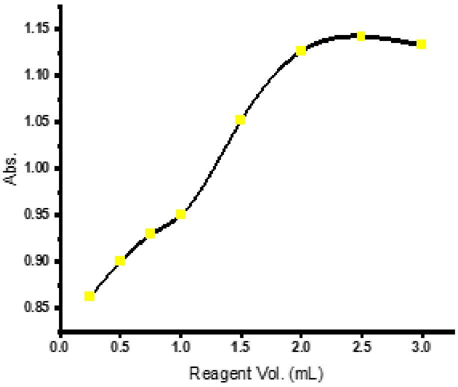


Figure 6. Effect of the base volume

The impact of the temperature of the reaction was examined within the range of 40–70 °C. The findings indicated that the best absorbance was obtained at 50 °C, which was chosen for the next steps. With the reaction temperature set at 50 °C, the influence of reaction time on the formation of the complex was examined within the range of 5–25 minutes. It was shown that a reaction time of 10 minutes was adequate to achieve complex formation. Consequently, a reaction time of 10 minutes was utilized in the subsequent experiments, as shown in Figures 7 and 8.

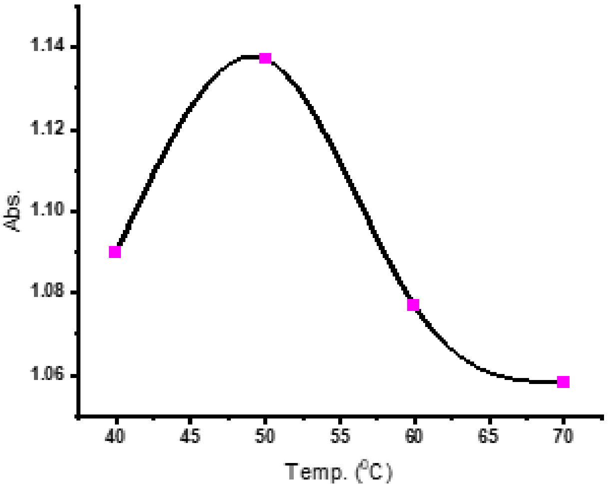


Figure 7. Effect of the base type

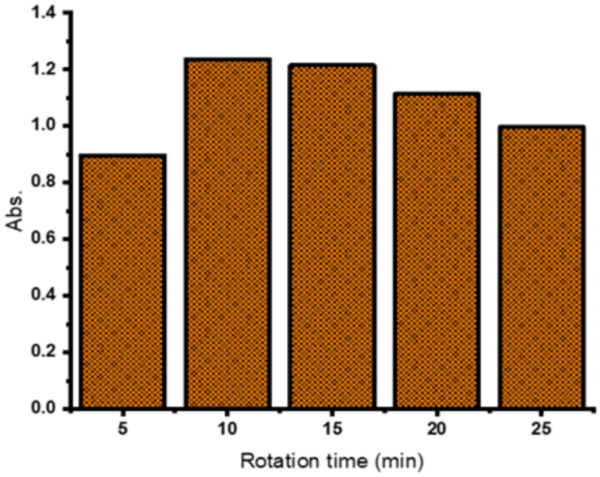


Figure 8. Effect of the base volume

A study was conducted to ascertain the makeup of the complex produced between the Cu (II) ion and the 5BIPQ reagent. Job's method [35] was utilized to determine the stoichiometry of the complex and the corresponding data is presented in Figure 10. It was observed that Cu (II) forms a stable brown-colored complex with a 1:2 ratio of metal to ligand, as depicted in Figures 9 and 10.

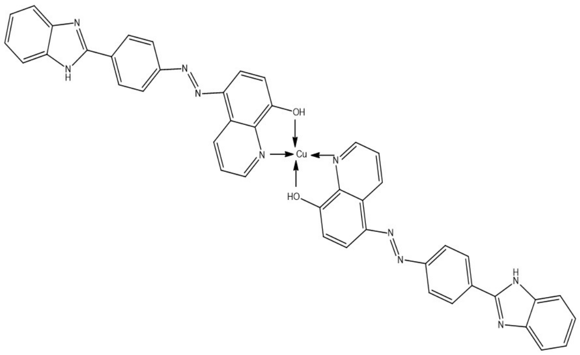


Figure 9. Effect of the base type

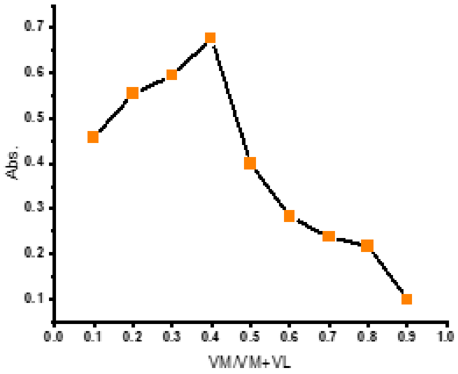


Figure 10. Effect of the base volume

The spectrophotometric method exhibits a high level of selectivity, although certain species may potentially interfere with its accuracy. In the conducted experiments, the influences of commonly coexisting foreign ions in natural aqueous samples on the recovery of copper were investigated. The summarized results can be found in Table 2. The obtained results demonstrate that the method is highly selective for accurately measuring trace levels of copper.

Table 2. Effect of the interference on the Cu-complex formation in the spectrophotometric method

Cation	**Reco. %**
Pb^{+2}	101.8
Cd^{+2}	91.7
Ni^{+2}	85.3
Cr^{+2}	99.3
Al^{+3}	92.1
Mn^{+2}	93.5

2 Calibration Graph, Accuracy and Precision

In the ideal conditions described earlier, a linear calibration curve was generated for Cu in the range of concentration 1.0–25.0 mg/L. The regression coefficients (R2) consistently exceeded 0.9963, as shown in Figure 11. LOQ and LOD for Cu were determined to be 0.69 mg/L and 0.23 mg/L respectively. These values indicate the lowest concentrations at which reliable quantification and detection of Cu can be achieved. The method's performance was assessed using the relative standard deviation (RSD %) at three replicates (10.0, 15.0, and 20.0 mg/L). The RSD % for Cu ranged from 1.6% to 4.72%. To evaluate the accuracy, the percentage of the final ion concentration compared with the beginning concentration in the sample solution was used to calculate the recovery. The recovery rate for Cu ranged from 95.6% to 101%.

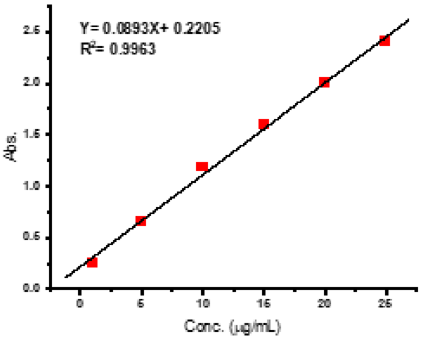


Figure 11. Effect of the base volume

DLLME-Spectrophotometric Determination of Determination of copper Ion.

3 Optimization of the DLLME Experimental Conditions

In the dispersive liquid-liquid microextraction technique, various factors significantly influence the system's performance and the entrapment of the analyte species. The kind and volume of the extraction solvent, the type and volume of the dispenser solvent, the number and duration of rotations, and other variables all affect the extraction of metal ions by DLLME. Our investigation focused on determining the optimal conditions for the DLLME procedure. The choice of solvent for extraction plays a crucial role in DLLME-based systems. The extraction solvent needs to possess specific properties to ensure efficient extraction of analytes, including lower density than water, low solubility in aqueous solutions, and high efficiency in analyte extraction. Based on these characteristics, CHCl3 was chosen as the solvent of extraction, as indicated in Table 3. In Figure 12, a range of extraction solvent volumes (300 - 900 µL) was investigated, and it was observed that 600 µL resulted in better absorbance, thus it was utilized in the subsequent steps.

Table 3. Effect of the extraction of solvent

Type of extraction solvent	Abs.
$CHCl_3$	0.6574
CH_2Cl_2	0.3072
CCl_4	0.5261

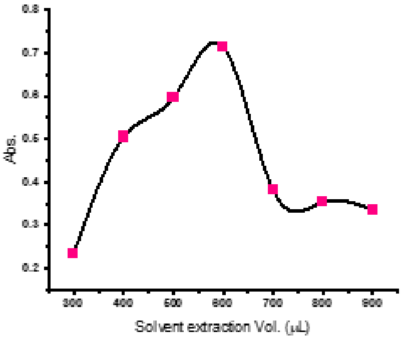


Figure 12. Effect of the base volume

The disperser solvent utilized in DLLME should have the ability to mix well with both the solvent of extraction and the aqueous solution. To meet this requirement, various solvents such as acetone, acetonitrile, MeOH, and ETOH were examined. Based on the results obtained from Table 4, ethanol proved to be an effective disperser solvent and was chosen for further experimentation. Ethanol demonstrated good solubility in chloroform and miscibility with water. The data indicated that ethanol facilitated the dispersion of chloroform as small particles in the aqueous phase, resulting in a cloudy solution and higher extraction efficiency. To estimate the impact of the volume of the disperser on analyte extraction, ethanol volumes ranging from 500 to 1500 µL were used in the experiments, and the corresponding signals were recorded in Figure 13. Lower volumes did not fully achieve the cloudy state in the solution, leading to inadequate analyte extraction. Conversely, volumes exceeding 600 µL caused the interested analytes to dissolve in the aqueous phase, resulting in decreased extraction efficiencies. Therefore, 600 µL of ethanol was determined to be the optimal disperser solvent volume.

Table 4. Effect type of dispenser solvent

Type of dispenser solvent	**Abs.**
ETOH	0.6584
MeOH	0.5582
Acetonitrile	0.5187
Acetone	0.2164

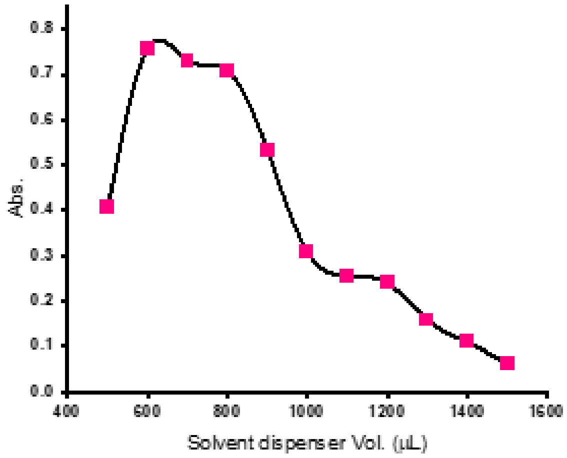


Figure 13. Effect of volume of dispenser solvent

A significant factor to take into consideration is how the number and duration of rotations affect the centrifuge's ability to extract Cu-complex. Based on the data presented in Figures 14 and 15, the highest absorbance is observed when the centrifuge is operated at 5000 rpm for a duration 7 minutes.

To assess the impact of a 20-fold molar excess of different cation species on the assessment of copper (II) utilizing the 5BIPQ ligand, experiments were conducted. The results, presented in Table 5, indicate that these cations did not cause interference in the determination of copper (II) with the 5BIPQ ligand.

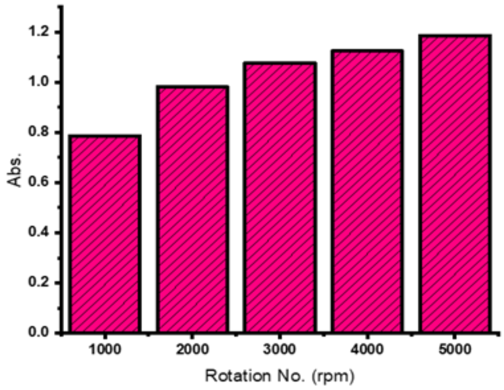


Figure 14. Effect of the number of rotations

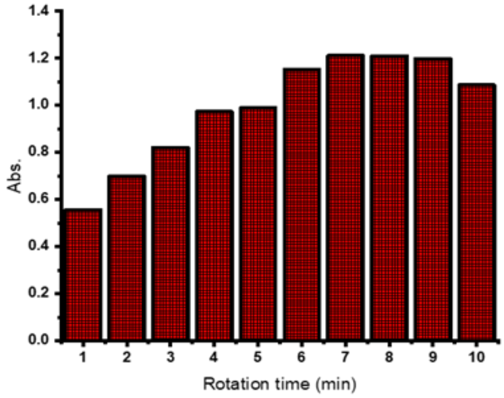


Figure 15. Effect of the time of rotation

Table 5. Effect of interference on the Cu-complex formation in the DLLME method

Cation	Reco.%
Pb^{+2}	93.4
Cd^{+2}	97.5
Ni^{+2}	101.2
Cr^{+2}	95.8
Al^{+3}	97.3
Mn^{+2}	95.6

4 Calibration Graph, Accuracy, and Precision

In the ideal conditions described earlier, a linear calibration curve was generated for Cu in the concentration range of (2.0–25.0) mg/L. The regression coefficients (R2) consistently exceeded 0.9979, as shown in Figure 16. LOQ and LOD for Cu were determined to be 0.45 mg/L and 0.15 mg/L, respectively. These values indicate the lowest concentrations at which reliable quantification and detection of Cu can be achieved. The method's performance was assessed using the relative standard deviation (RSD %) at three replicates (5.0, 10.0, and 15.0 mg/L). The RSD % for Cu ranged from 0.5 % to 4.6%. To evaluate the accuracy, the percentage of the final ion concentration compared with the beginning concentration in the sample solution was used to calculate the recovery. The recovery rate for Cu ranged from 102.4% to 108.2%.

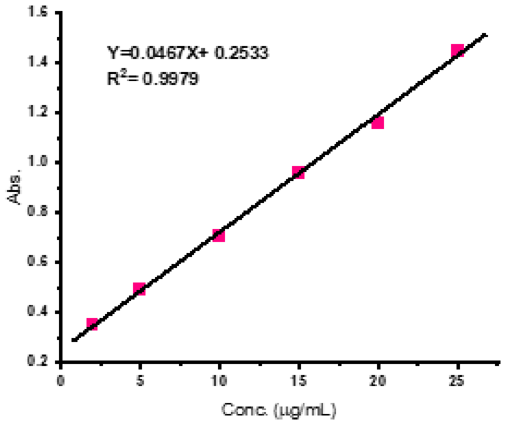


Figure 16. Calibration curve Cu-complex in the DLLME

Validation of the Method

The investigation focused on the validity of two procedures for analyzing copper ions in both their pure state and aqueous samples. Table 6 also includes the results of the copper ion analysis, which exhibited reproducibility with low RSD% values. The obtained results for pure ions are presented in Table 7. Table 8 shows the comparison between the recommended approach and previously published DLLME techniques. To assess the precision and accuracy of the procedures, three replicates of the copper were analyzed. The methods demonstrated excellent precision and reproducibility, as indicated by the low values of relative standard deviations (RSD%).

Table 6. Analytical and statistical parameters of Spectroscopy and DLLME method

Parameter	**Spectrophotometric method**	**DLLME**
λmax	503 (nm)	
color	Brown	
linearity range, mg/L	1.0-25.0	2.0-25.0
ϵ, (L.mol^{-1} cm^{-1})	5.65×10^3	2.96×10^3
Sandell’s sensitivity (µg/cm^2)	0.011	0.021
Correlation coefficient (R^2)	0.9963	0.9979
Regression equation	Y= 0.0893X+0.2205	Y= 0.0476X+0.2533
Slope	0.0893	0.0476
Intercept	0.2205	0.2533
LOD (mg /L)	0.23	0.15
LOQ (mg/L)	0.69	0.45
*C.L. for the X1 mg/L (95%)	10.10±0.23	5.37±0.21
*C.L. for the X2 mg/L (95%)	14.35±0.55	10.82±0.72
*C.L. for the X3 mg/L(95%)	19.43±0.34	15.36±0.31
T-testcal. (T-testtable)	-0.23(2.77)	-4.42(3.18)
F-testcal. (F-testtable)	1.32(19)	1.04(19)

Table 7. Application of the suggested methods (Spectroscopy, DLLME& AAS) for the evaluation of copper ions

Station	**Conc. of copper μg/mL^{-1}**			**RSD (%) n=3**
	Add (μg/mL)	**Spectrophotometric method**	**AAS**	
Station 1	-	4.72	5.14	0.81
	5	9.55		0.99
	10	14.01		0.72
Station 2	-	4.41	4.33	3.28
	5	8.88		2.77
	10	14.31		1.93
Station 3	-	6.56	6.72	0.24
	5	11.14		0.83
	10	15.97		1.44
		DLLME method		
Station 1	-	5.00	5.14	4.6
	5	10.88		3.8
	10	15.36		1.5
Station 2	-	4.04	4.33	1.6
	5	9.73		0.8
	10	14.04		1.01
Station 3	-	6.73	6.72	3.2
	5	11.67		1.9
	10	15.86		0.5

Table 8. The comparison between the recommended approach and previously published DLLME techniques

Reagent	**Technique**	**Solvent of Extraction**	**Solvent of Disperser**	**Sample Vol.**	**LOD (ng_mL^{-1})**	**Ref.**
HCDTC	SP	C_2Cl_4	Acetone	10 mL	0.3	[36]
BPDC	FO-LADS	$CHCl_3$	ETOH	10 mL	0.34	[37]
PAN	FAAS	1-Decanol	ETOH	10 mL	6.6	[38]
HBDAP	FAAS	CCl_4	Acetone	10 mL	0.75	[39]
Salophen	FAAS	$CHCl_3$	Acetone	10 mL	0.6	[14]
5-Br-PADAP	FAAS	$CHCl_3$	Acetone	15 mL	1.4	[40]
1N2N	FAAS	$CHCl_3$	ETOH	10.5 mL	0.95	[41]
p-SA	FAAS	Toluene	CH_3OH	10 mL	0.12	[42]
Curcumin	FAAS	[bmim] [PF6]	Ultrasonic bath	25 mL	0.19	[43]
-	ETAAS	Nitric acid	CH_3OH	5 g	0.52 ng/g	[44]
5BIPQ	UV-Vis	$CHCl_3$	ETOH	10 mL	40	Present work

CONCLUSION

In this research, we created a novel azo compound by combining 2-(4-aminophenyl)benzimidazole with 8-hydroxyquinoline using concentrated HCl and $NaNO_2$. This newly synthesized compound was then employed as a ligand to detect copper ions (Cu+2) in aqueous solution. The primary focus of the study was the produce a complex between 5BIPQ and copper ions, which resulted in an orange color with a maximum absorption wavelength of 503 nm. The extraction of Cu(II) using the 5BIPQ reagent as an auxiliary ligand occurs rapidly, taking only 20 minutes. As a result, a simple, quick, sensitive, and synergistic analytical technique has been established for the spectrophotometric determination of Cu(II). By utilizing a highly sensitive chromogenic reagent in a single step, trace amounts of Cu(II) can be accurately estimated with a minimal reagent concentration. The stability of the copper-5BIPQ complex was observed to last for more than 8 hours, with a likely stoichiometry of 1:2 (M:L) for the extracted species. The evaluation of copper in the produced aqueous samples from the Halfaya oil field yielded satisfactory results.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Anwer S. Salim experimented, Abdul Jabar Atia, Mohammed Z. Thani, and Hassan A. Fattah conducted the calculations, and analysis and wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

FUNDING

None.

DATA AVAILABILITY STATEMENT

None.

ACKNOWLEDGMENTS

The authors extend their heartfelt appreciation to Mustansiriyah University, College of Science, Department of Chemistry and its laboratories for their indispensable assistance in supplying the necessary equipment and chemical materials.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] I.-C. Lee, J.-W. Ko, S.-H. Park, J.-O. Lim, I.-S. Shin, C. Moon, S.-H. Kim, J.-D. Heo, and J.-C. Kim, “Comparative toxicity and biodistribution of copper nanoparticles and cupric ions in rats,” *International journal of nanomedicine*, pp. 2883–2900, 2016. doi: `10.2147/IJN.S106346`.

[2] M. Kucharzewski, J. Braziewicz, U. Majewska, and S. Góźdź, “Copper, zinc, and selenium in whole blood and thyroid tissue of people with various thyroid diseases,” *Biological Trace Element Research*, vol. 93, pp. 9–18, 2003. doi: `10.1385/BTER:93:1-3:9`.

[3] D. Mendil, Ö. D. Uluözlü, M. Tüzen, and M. Soylak, “Investigation of the levels of some element in edible oil samples produced in turkey by atomic absorption spectrometry,” *Journal of Hazardous Materials*, vol. 165, no. 1-3, pp. 724–728, 2009. doi: `10.1016/j.jhazmat.2008.10.046`.

[4] T. Theophanides and J. Anastassopoulou, “Copper and carcinogenesis,” *Critical reviews in oncology/hematology*, vol. 42, no. 1, pp. 57–64, 2002. doi: `10.1016/S1040-8428(02)00007-0`.

[5] A. K. Ibraheem, M. Z. Thani, and M. T. Mohammed, “Determination of vitamins, trace elements, and phytochemical compounds in ginkgo biloba leaves extracts,” *Egyptian Journal of Chemistry*, vol. 66, no. 4, pp. 159–166, 2023.

[6] M. Sultan, M. Thani, H. Khalaf, and A. Salim, “Determination of some heavy metals in solid waste from heavy water treatment station in baghdad,” *The Iraqi Journal of Agricultural Science*, vol. 49, no. 3, pp. 500–505, 2018. doi: `10.36103/ijas.v49i3.122`.

[7] Z. Saeedi, A. Lotfi, J. Hassanzadeh, and N. Bagheri, "Highly sensitive determination of copper (ii) ions using fluorescence and chemiluminescence emissions of modified cds quantum dots after preconcentration by dispersive liquid-liquid microextraction," *Canadian Journal of Chemistry*, vol. 95, no. 6, pp. 704–709, 2017. doi: `10.1139/cjc-2016-0558`.

[8] H. Vojoudi, B. Bastan, J. B. Ghasemi, and A. Badiei, "An ultrasensitive fluorescence sensor for determination of trace levels of copper in blood samples," *Analytical and bioanalytical chemistry*, pp. 5593–5603, 2019. doi: `10.1007/s00216-019-01940-w`.

[9] L. P. Silva, N. D. S. Campos, T. P. Lisboa, L. V. de Faria, M. A. C. Matos, R. C. Matos, and R. A. de Sousa, "Simultaneous determination of cadmium, lead and copper in chocolate samples by square wave anodic stripping voltammetry," *Food Additives & Contaminants: Part A*, vol. 38, no. 3, pp. 418–426, 2021. doi: `10.1080/19440049.2020.1857443`.

[10] N. Zaytsev, K. Stancheva, and V. Trifonova, "Application of anodic stripping voltammetry at a mercury film electrode for simultaneous determination of zinc, cadmium, lead and copper in water and soil samples.," *Oxidation Communications*, vol. 43, no. 3, 2020.

[11] J. R. Bacon, K. L. Linge, and L. Van Vaeck, "Atomic spectrometry update. atomic mass spectrometry," *Journal of Analytical Atomic Spectrometry*, vol. 18, no. 8, pp. 955–997, 2003. doi: `10.1039/b306545f`.

[12] S. M. Sorouraddin, "Simultaneous separation and preconcentration of ultra-trace of cu (ii), pb (ii) and zn (ii) in water samples by air-assisted liquid-liquid microextraction prior to determination by graphite furnace atomic absorption spectrometry," *Journal of the Iranian Chemical Society*, vol. 13, no. 12, pp. 2219–2227, 2016. doi: `10.1007/s13738-016-0940-9`.

[13] Z. Khayyat-Sarkar, S. H. Hashemi, A. Jamali Keikha, and M. Kaykhaii, "Application of polyethylene glycol-coated iron oxide nanoparticles for magnetic solid phase extraction of copper from seawater samples and its determination by graphite furnace atomic absorption spectrometry using response surface methodology for optimization," *Spectroscopy Letters*, vol. 54, no. 4, pp. 254–265, 2021. doi: `10.1080/00387010.2021.1926285`.

[14] G. Alpdoğan and Ş. D. Zor, "A new dispersive liquid-liquid microextraction method for preconcentration and determination of aluminum, iron, copper, and lead in real water samples by hplc," *Journal of AOAC International*, vol. 100, no. 5, pp. 1524–1530, 2017. doi: `10.5740/jaoacint.16-0376`.

[15] U. S. Erdemir, Y. Sahan, and S. Gucer, "Fractionation and bioaccessibility of manganese, copper, zinc, cadmium, and lead in commercial vegetable and rice baby foods using inductively coupled plasma-mass spectrometry (icp-ms) with central composite design (ccd)," *Analytical Letters*, vol. 52, no. 17, pp. 2840–2851, 2019. doi: `10.1080/00032719.2019.1636056`.

[16] I. Wysocka and E. Vassileva, "Determination of cadmium, copper, mercury, lead and zinc mass fractions in marine sediment by isotope dilution inductively coupled plasma mass spectrometry applied as a reference method," *Microchemical Journal*, vol. 128, pp. 198–207, 2016. doi: `10.1016/j.microc.2016.05.002`.

[17] Y. Çağlar and E. T. Saka, "Ionic liquid based dispersive liquid-liquid microextraction procedure for the spectrophotometric determination of copper using 3-dimethylamino rhodanine as a chelating agent in natural waters," *Karbala International Journal of Modern Science*, vol. 3, no. 4, pp. 185–190, 2017. doi: `10.1016/j.kijoms.2017.09.002`.

[18] H. Shoaee, M. Roshdi, N. Khanlarzadeh, and A. Beiraghi, "Simultaneous preconcentration of copper and mercury in water samples by cloud point extraction and their determination by inductively coupled plasma atomic emission spectrometry," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 98, pp. 70–75, 2012, issn: 1386-1425. doi: `10.1016/j.saa.2012.08.027`.

[19] N. K. Kareem, M. Z. Thani, and K. F. Al-Rawi, "New approach for determination of phenylephrine hcl in pure and pharmaceutical formulation using various microextraction methods," *Research Journal of Pharmacy and Technology*, vol. 15, no. 4, pp. 1648–1652, 2022. doi: `10.52711/0974-360X.2022.00275`.

[20] N. K. Kareem, M. Z. Thani, and K. F. Al-Rawi, "New microextraction methods for the evaluation of bromhexine hcl in pure and pharmacological formulations," *Egyptian Journal of Chemistry*, vol. 65, no. 4, 2022. doi: `10.21608/ejchem.2021.92380.4377`.

[21] L. Wang, J.-B. Zhou, X. Wang, Z.-H. Wang, and R.-S. Zhao, "Simultaneous determination of copper, cobalt, and mercury ions in water samples by solid-phase extraction using carbon nanotube sponges as adsorbent after chelating with sodium diethyldithiocarbamate prior to high performance liquid chromatography," *Analytical and Bioanalytical Chemistry*, pp. 4445–4453, 2016. doi: `10.1007/s00216-016-9542-8`.

[22] S. Shegefti, A. Mehdinia, and F. Shemirani, "Preconcentration of cobalt (ii) using polythionine-coated fe3o4 nanocomposite prior to its determination by aas," *Microchimica Acta*, vol. 183, pp. 1963–1970, 2016. doi: `10.1007/s00604-016-1837-0`.

[23] Q. Han, Y. Huo, N. Yang, X. Yang, and T. Hao, “Determination of cobalt in water by thermal lens spectrometry with cloud point extraction,” *Analytical Letters*, vol. 48, no. 13, pp. 2096–2106, 2015. doi: `10.1080/00032719.2015.1017762`.

[24] A. Torabi, M. Shirani, A. Semnani, and A. Akbari, “Deep eutectic solvent-based ligandless ultrasound-assisted liquid-phase microextraction for extraction of cobalt ions from food samples prior to spectrophotometric determination,” *Journal of the Iranian Chemical Society*, vol. 18, pp. 893–902, 2021. doi: `10.1007/s13738-020-02079-7`.

[25] M. R. Jamali, Y. Assadi, and F. Shemirani, “Homogeneous liquid-liquid extraction and determination of cobalt, copper, and nickel in water samples by flame atomic absorption spectrometry,” *Separation Science and Technology*, vol. 42, no. 15, pp. 3503–3515, 2007. doi: `10.1080/01496390701508784`.

[26] V. V. Divarova, K. T. Stojnova, P. V. Racheva, V. D. Lekova, and A. N. Dimitrov, “Liquid-liquid extraction of ion-association complexes of cobalt (ii)-4-(2-pyridylazo) resorcinol with ditetrazolium salts,” *Journal of the Serbian Chemical Society*, vol. 80, no. 2, pp. 179–186, 2015. doi: `10.2298/JSC140514102V`.

[27] T. S. Neri, D. P. Rocha, R. A. Munoz, N. M. Coelho, and A. D. Batista, “Highly sensitive procedure for determination of cu (ii) by gf aas using single-drop microextraction,” *Microchemical Journal*, vol. 147, pp. 894–898, 2019. doi: `10.1016/j.microc.2019.04.014`.

[28] X. Wen, Q. Deng, and J. Guo, “Ionic liquid-based single drop microextraction of ultra-trace copper in food and water samples before spectrophotometric determination,” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 79, no. 5, pp. 1941–1945, 2011. doi: `10.1016/j.saa.2011.05.095`.

[29] F. Islami-Bonab, S. Sajedi-Amin, S. M. Sorouraddin, S. Maleknia, and A. Naseri, “Multiple objective optimization of air assisted liquid-liquid microextraction combined with solidified floating organic drop microextraction for simultaneous determination of trace copper and nickel,” *Turkish Journal of Chemistry*, vol. 45, no. 4, pp. 1030–1044, 2021. doi: `10.3906/kim-2010-24`.

[30] L. K. Silva, J. H. Rangel, N. M. Brito, E. R. Sousa, É. M. Sousa, D. L. Lima, V. I. Esteves, A. S. Freitas, and G. S. Silva, “Solidified floating organic drop microextraction (sfodme) for the simultaneous analysis of three non-steroidal anti-inflammatory drugs in aqueous samples by hplc,” *Analytical and Bioanalytical Chemistry*, vol. 413, pp. 1851–1859, 2021. doi: `10.1007/s00216-021-03153-6`.

[31] V. Jalili, R. Zendehdel, and A. Barkhordari, “Supramolecular solvent-based microextraction techniques for sampling and preconcentration of heavy metals: A review,” *Reviews in Analytical Chemistry*, vol. 40, no. 1, pp. 93–107, 2021. doi: `10.1515/revac-2021-0130`.

[32] M. Saraji and M. K. Boroujeni, “Recent developments in dispersive liquid-liquid microextraction,” *Analytical and bioanalytical chemistry*, vol. 406, pp. 2027–2066, 2014. doi: `10.1007/s00216-013-7467-z`.

[33] S. Dmitrienko, V. Apyari, V. Tolmacheva, and M. Gorbunova, “Dispersive liquid-liquid microextraction of organic compounds: An overview of reviews,” *Journal of Analytical Chemistry*, vol. 75, pp. 1237–1251, 2020. doi: `10.1134/S1061934820100056`.

[34] A. M. Abdullah, M. M. Hasson, and M. Z. Thani, “Synthesis, thermal analysis, spectroscopy and magnetic properties of some transition metals complexes derived from new azo compound,” *Materials Today: Proceedings*, vol. 20, pp. 595–598, 2020. doi: `10.1016/j.matpr.2019.09.195`.

[35] S. S. Patil, “The estimation of small metal traces of cd (ii) from the alloy and water effluents using fast and accurate extractive spectrophotometric method with newly developed dba reagent,” *Current World Environment*, vol. 17, no. 3, p. 815, 2022. doi: `10.12944/CWE.17.3.26`.

[36] M. Faraji, M. Pourmohammad, F. Aryanasab, and M. Shabanian, “(4-hydroxy-2-oxo-2h-chromen-3-yl) methyl pyrrolidine-1-carbodithioate as a novel, highly selective and sensitive ligand for determination of copper in water and food samples by dispersive liquid-liquid microextraction coupled with microvolume uv-vis spectrophotometry,” *Journal of the Iranian Chemical Society*, vol. 16, no. 8, pp. 1579–1589, 2019. doi: `10.1007/s13738-019-01616-3`.

[37] M. Ezoddin, F. Shemirani, and M. R. Jamali, “Fiber optic-linear array detection spectrophotometry in combination with dispersive liquid-liquid microextraction for preconcentration and determination of copper,” *Journal of Analytical Chemistry*, vol. 65, pp. 153–158, 2010. doi: `10.1134/S1061934810020097`.

[38] M. Soylak and E. Kiranartligiller, “A simple vortex-assisted dispersive liquid–liquid microextraction system for copper(ii) to preconcentration and separation from natural water and table salt samples,” *Arabian Journal for Science and Engineering*, vol. 42, no. 1, pp. 175–181, 2017. doi: `10.1007/s13369-016-2208-1`.

[39] C. Karadaş, “A new dispersive liquid-liquid microextraction method for preconcentration of copper from waters and cereal flours and determination by flame atomic absorption spectrometry,” *Water Air and Soil Pollution*, vol. 225, pp. 1–9, 2014. doi: `10.1007/s11270-014-2150-3`.

[40] Z. A. Alothman, M. Habila, E. Yilmaz, and M. Soylak, "A dispersive liquid-liquid microextraction methodology for copper(ii) in environmental samples prior to determination using microsample injection flame atomic absorption spectrometry," *Journal of AOAC International*, vol. 96, no. 6, pp. 1425–1429, 2013. doi: `10.5740/jaoacint.12-422`.

[41] D. Citak and M. Tuzen, "Separation and determination of copper in bottled water samples by combination of dispersive liquid-liquid microextraction and microsample introduction flame atomic absorption spectrometry," *Journal of AOAC International*, vol. 96, no. 6, pp. 1435–1439, 2013. doi: `10.5740/jaoacint.12-084`.

[42] M. Farajvand, V. Kiarostami, M. Davallo, A. Ghaedi, and F. Fatahi, "Rapid extraction of copper ions in water, tea, milk and apple juice by solvent-terminated dispersive liquid-liquid microextraction using p-sulfonatocalix (4) arene: Optimization by artificial neural networks coupled bat inspired algorithm and response surface methodology," *Journal of food science and technology*, vol. 56, pp. 4224–4232, 2019. doi: `10.1007/s13197-019-03892-6`.

[43] Z. Al Othman, Y. E. Unsal, M. Habila, A. Shabaka, M. Tuzen, and M. Soylak, "Determination of copper in food and water by dispersive liquid-liquid microextraction and flame atomic absorption spectrometry," *Analytical Letters*, vol. 48, no. 11, pp. 1738–1750, 2015. doi: `10.1080/00032719.2014.999276`.

[44] N. Altunay, A. Elik, and R. Gürkan, "Monitoring of some trace metals in honeys by flame atomic absorption spectrometry after ultrasound assisted-dispersive liquid liquid microextraction using natural deep eutectic solvent," *Microchemical Journal*, vol. 147, pp. 49–59, 2019. doi: `10.1016/j.microc.2019.03.003`.