Dispersive Liquid-Liquid Semi-Microextraction of Cu(II) with 6,7-dihydroxy-2,4-diphenylbenzopyrylium Chloride for its Spectrophotometric Determination

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Abstract

A novel dispersive liquid-liquid semi-microextraction (DLLsME) procedure for copper(II) preconcentration is proposed. The system containing copper(II) and 6,7-dihydroxy-2,4-diphenylbenzopyrylium chloride (DHDPb), after addition a mixture of chloroform and methanol becomes cloudy and the formation of the organic phase was observed immediately. The optimal conditions of DLLsME were found to be: pH 5, absorption band maximum was 570 nm, 1 cm^3 of $1 \times 10^{-3} \text{ mol/dm}^3$ of DHDPb, and mixed extractant containing 1 cm^3 of chloroform and 1 cm^3 of methanol. Under optimal conditions, the calibration plot was linear in the range of copper(II) concentration $4.32\text{--}65 \text{ }\mu\text{g/dm}^3$ and the limit of detection was $1.29 \text{ }\mu\text{g/dm}^3$. The rocks and tap water samples were successfully analyzed according to the suggested procedure with RSD no more than 4.9%.

Keywords: Dispersive liquid-liquid semi-microextraction; spectrophotometry; copper(II); rocks analysis; water analysis.

1. Introduction

Copper is an essential element that plays a significant role in various biological processes that are necessary to sustain life. Wherein, excessive intake of copper(II) compounds in the human body can lead to irritation of the nose and throat, nausea, vomiting, and diarrhea.¹ Copper is usually in trace level in environmental samples and food products. Moreover, well-known analytical methods for the copper(II) determination, such as flame atomic absorption spectroscopy, atomic absorption spectroscopy with a graphite furnace, ion chromatography and UV/Vis spectrophotometry, are often coupled with analyte preconcentration stage.

Many various techniques for copper(II) preconcentration have been proposed. For example, cloud point extraction,^{2–4} solid-phase extraction,^{5–7} liquid extraction^{8–10} etc., while most often copper(II) is pre-bound into ion pairs or complexes (chelates in particular) using reagents such as sodium diethyldithiocarbamate,^{11–13} 1-(2-pyridylazo)-2-naphthol,^{4,14} and dimethyl-1,10-phenanthroline (neocuproine).^{15,16} It is important to note that interest in

the development and modernization of various cloud point extraction and liquid extraction techniques does not disappear even today.^{17–21}

Liquid-liquid extraction has well-known advantages, namely, such as the possibility of increasing the concentration of the analyte, simplicity, high speed, low cost, and efficiency. Such modifications of liquid-liquid extraction as vortex-assisted LLE and dispersive LLE have been actively developed recently. The last one is based on the transfer of the analyte from the aqueous phase to another immiscible liquid phase, which is an extractant. A certain amount of a mixture of extraction and dispersive solvents is rapidly introduced into the aqueous phase of the sample containing the analyte, forming a cloudy solution. The cloudy state is caused by the formation of small droplets of water-immiscible extracting solvent that is dispersed in the sample solution. However, this is not often effective enough and is also being modified. For example, it was proposed to additionally irradiate the solution with ultrasound,²² introduce an auxiliary solvent,²³ or use the vortex technique.²⁴

Since preconcentration of copper(II) initially implies its conversion into some intensely colored hydrophobic

complexes, UV/Vis spectrophotometry seems to be the most attractive detection method. At the same time, the wide availability of equipment, speed, accuracy, ease of operation, and low operating costs make UV/Vis spectrophotometry still attractive to use.

In this work, dispersive liquid-liquid semi-microextraction coupled with UV/Vis spectrophotometry (DLLsME-UV/Vis) was used for the preconcentration and quantification of Cu(II). On the one hand, the use of semi-microtechniques notably reduces the environmental load and does not require special equipment like microextraction and is not fraught with errors related to dosing microliter volumes. On the other hand, the proposed DLLsME-UV/Vis procedure for copper(II) determination does not need extra time-consuming steps like, for example, heating and cooling of solution,³ so the method is quite rapid. The 6,7-dihydroxy-2,4-diphenylbenzopyrylium chloride (DHDPbB) was chosen as the chelating ligand whose synthesis, physio-chemical, spectroscopic, and complexing properties were described in detail in our previous works.^{25,26}

2. Experimental

2.1. Chemicals

The Cu(II) 1×10^{-2} mol/dm³ stock solution was prepared by dissolving CuCl₂ · 2H₂O in distilled water and standardized by iodometric titration.

The DHDPbB was synthesized (Fig. 1) by condensation of 1,2,4-triacetoxybenzene (TOR, Ukraine) with 1,3-diphenyl-1,3-propanedione (Acros, Belgium) in glacial acetic acid, while sparging dry hydrogen chloride, and recrystallized from ethanol.²⁵

Fig. 1. Synthesis of DHDPbB reagent.

The chemical structure of DHDPbB was confirmed by ¹H and ¹³C NMR:

¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm): 8.327 (s, *J* = 7.14 Hz, 1H, Ar), 8.18–8.16 (m, 3H), 7.988 (d, *J* = 8.23 Hz, 1H, Ar), 7.37–7.64 (m, 4H, Ar), 7.58–7.55 (m, 4H, Ar), 7.34 (s, 1H, Hetaryl).

¹³C NMR (500 MHz, DMSO-*d*₆) δ (ppm): 185.24 (C=O); 136–127 (Ar, Hetaryl), 102.74 (C-OH), 93.17 (C-OH).

A 1×10^{-3} mol/dm³ DHDPbB solution was prepared by dissolving its suitable weight in ethanol. The pH of the mixture was adjusted by the acetate buffer solution (concentrations of acetic acid and sodium acetate were 0.1 mol/dm³ and 0.0468 mol/dm³, respectively). Some organic solvents, such as methanol, acetonitrile, acetone, ethanol, chloroform, benzene, butyl acetate, isoamyl alcohol, and their mixtures were used for dispersive extraction. All organic solvents and chemicals used in the present study were analytically pure grade.

2.2. Instrumentations

An SF-56 spectrophotometer (OKB “Spectr”, Russia), equipped with 10 mm semi-micro quartz cells, was used for absorbance measurements. The pH measurements were carried out on an I-160 potentiometer (ZIP, Belarus) equipped with a combined glass electrode. A centrifuge model MPW-340 with conical 50 cm³ tubes was used for phase separation acceleration.

2.3. General Procedure

Appropriate amounts (0.1–3.0 cm³) of 1×10^{-5} mol/dm³ Cu(II) solution, 1.0 cm³ of 1×10^{-3} mol/dm³ ethanolic solution of DHDPbB, 8 cm³ of acetate buffer with pH 5 were placed into 50 cm³ centrifuge test tubes and diluted up to 30 cm³ with distilled water. Then a mixture of 1 cm³ of chloroform and 1 cm³ of methanol was injected using a syringe for dispersive extraction and the solution immediately became cloudy. The tubes were centrifuged for 5 min at 3,000 rpm to accelerate phase separation. The heavier organic phase, which contained the extracted complex, was at the bottom, and the upper aqueous layer was carefully removed from the test tube.

2.4. Sampling and Sample Pretreatment

The certified reference materials (CRM) of geological samples, such as carbonate-silicate loose sediments SGHM-1 (CRM №3483-86) and rock ST-1a Trap (CRM №519-74) were used to test the proposed method. A 0.1–0.3 g of CRM sample was transferred to a platinum crucible and 2 g of potassium persulfate was added and melted at 600 °C in a muffle for 25–30 min. After that, the melt

was cooled and dissolved in water and diluted to 100 cm³. The obtained solutions were used for analysis by the proposed method.

Tap water samples were collected in our laboratory and directly analyzed according to the proposed procedure without any special treatment.

3. Results and Discussion

3. 1. Effect of Variables

Several factors were studied such as pH, type and volume of extracting solvent, type and volume of dispersive solvent, the concentration of DHDPhB, interfering ions that affect the efficiency of copper(II) determination after its DLLsME preconcentration.

3. 1. 1. Effect of pH

To study the effect of pH on the procedure, many solutions were prepared, which required pH level that was reached by the addition of standard acetate buffer with a pH of 3–8, and then 1 cm³ of chloroform was added for extraction.

Fig. 2. Effect of pH on the extraction: 3.33×10^{-5} mol/dm³ of DHDPhB, 5×10^{-7} mol/dm³ of Cu(II), $V_{\text{Chloroform}} = 1$ cm³, $\lambda = 570$ nm.

As seen in Fig. 2, the optical absorbance increases to pH 5 and then decreases. Probably, in a strongly acidic medium the reagent is in a protonated form, which prevents the effective binding of copper(II). In a strongly alkaline medium, destructive hydrolysis of the DHDPhB takes place; therefore, the influence of the acidity of the medium was studied in the pH range 3–7. Henceforward, whole analysis is carried out at a pH of 5.

3. 1. 2. Effect of Type and Volume of Extracting Solvent

The solvent which is used for extraction has some mandatory requirements: insolubility or poor solubility in

water, a large difference in density with water, an affinity for the extracted substance. According to these requirements, organic solvents such as benzene, chloroform, isoamyl alcohol, and butyl acetate were tested. It was shown that chloroform removes a complex of copper(II) with DHDPhB from an aqueous solution best of all.

Fig. 3. Effect of volume of extracting solvent (chloroform) on extraction: 3.33×10^{-5} mol/dm³ of DHDPhB, 6.67×10^{-7} mol/dm³ of Cu(II), $\lambda = 570$ nm.

The effect of the volume of extractant on the recovery of the copper(II) complex with DHDPhB was studied (Fig. 3). As seen, 1 cm³ of chloroform is sufficient to extract the complex.

3. 1. 3. Effect of Type and Volume of Dispersive Solvent

Dispersive solvents are often used to increase the rate and efficiency of LLE. Such a solvent must dissolve both in the selected organic solvent and in water. Among the solvents considered, such as acetonitrile, acetone, ethanol, and methanol, methanol proved to be the most effective (Fig. 4a).

To determine the optimal volume of methanol for extraction, 1 cm³ of chloroform and 0.25–3.5 cm³ of methanol were added to several solutions. As shown in Fig. 4b, as the amount of dispersing solvent increases, the degree of complex recovery also increases. Thus, the ratio of the extracting and dispersive solvents in the mixture was 1:1, since a further increase in the volume of the dispersing solvent does not lead to an increase in optical absorbance. It is interesting to note that the complex has a high affinity for chloroform, and the use of the vortex technique does not significantly affect the extraction efficiency.

3. 1. 4. Effect of the Concentration of DHDPhB

The effect of the concentration of the chelating ligand on the efficiency of copper(II) extraction was studied.

Fig. 4. Effect of type (a) and volume (b) of dispersive solvent on extraction: $3.33 \times 10^{-5} \text{ mol/dm}^3$ of DHDPhB, $6.67 \times 10^{-7} \text{ mol/dm}^3$ of Cu(II), $V_{\text{Chloroform}} = 1 \text{ cm}^3$, $\lambda = 570 \text{ nm}$.

Fig. 5. The effect of the concentration of DHDPhB on the extraction of $5 \times 10^{-7} \text{ mol/dm}^3$ of Cu(II), $V_{\text{Chloroform}} = 1 \text{ cm}^3$, $V_{\text{Methanol}} = 1 \text{ cm}^3$, $\lambda = 570 \text{ nm}$.

As seen in Fig. 5, it is necessary to introduce a 200-fold excess of the reagent to maximize the binding of copper(II) to the complex and its extraction.

3. 2. Analytical Figures of Merit and Interferences Study

Analytical figures of merit for the developed DLLsME – UV/Vis procedure obtained under optimal conditions are shown in Table 1. The precision and accuracy of the proposed technique were checked by performing 5 measurements at a concentration level of Cu(II) $30 \text{ } \mu\text{g/dm}^3$ over two consecutive days.

Table 1. Analytical figures of merit for Cu(II) determination by the developed DLLsME – UV/Vis spectrophotometry method

Regression equation	$A = 0.0154 C + 0.1273$
R^2	0.999
Linear range, $\mu\text{g/dm}^3$	4.32–65
LOD, $\mu\text{g/dm}^3$	1.29
LOQ, $\mu\text{g/dm}^3$	4.32
RSD ($n = 5$, $P = 0.95$, $C_{\text{Cu(II)}} = 30 \text{ } \mu\text{g/dm}^3$)	4.8%
Recovery	97–104%
Preconcentration factor	39

To investigate an interfering effect, the following ions were studied (Table 2):

Table 2. Study of interfering ions.

Interfering ions	Tolerable concentration (analyte:interfering ion) ^a
Fe^{2+} , Fe^{3+}	1:1
Al^{3+}	1:5
I^-	1:25
Zn^{2+} , Ni^{2+} , Co^{2+} , Cd^{2+} , Mn^{2+} , HPO_4^{2-} , CO_3^{2-} , SO_4^{2-}	1:100
Na^+	1:250
K^+ , Br^- , Cl^-	1:500
Ca^{2+} , Mg^{2+} , NO_3^-	1:5000

^a At this ratio no interfering effect was observed.

As seen, Fe^{2+} and Fe^{3+} ions interfere most of all and 2.5% solution of NaF was used to mask them. Besides, Al^{3+} ions can be masked by 0.1 mol/dm^3 solution of malonic acid. A 1–3 cm^3 of masking reagents solutions were used, because their required amount depends on the analyzed sample weight.

3. 3. Analysis of CRMs and Comparison with Literature Studies

The DLLsME–UV/Vis procedure was successfully applied to the preconcentration and determination of Cu(II) in CRM rocks samples and tap water sample (Table 3). As can be seen from Table 3, a good agreement was found between the proposed method data and certified values. Thus, the developed technique is suitable for the determination of copper in rocks and tap water samples.

Table 3. Application of suggested procedure to the preconcentration and spectrophotometric determination of Cu(II) in CRM and tap water samples (n = 5; P = 0.95)

CRMs	Certified values, $\mu\text{g/g}$	Spiked, $\mu\text{g/L}$	Found*	Recovery, %	RSD, %	**Student's <i>t</i> -test
SGHM-1	220 $\pm$ 20	–	215.7 $\pm$ 13	98.1	4.9	0.91
№3483-86		50	263.3 $\pm$ 15	97.0	4.5	–
ST-1a Trap	48 $\pm$ 5	–	46.4 $\pm$ 3	96.7	4.7	1.64
№519-74		50	97.2 $\pm$ 6	98.3	4.6	–
Tap water	–	–	19.9 $\pm$ 1	–	4.4	–
		10	30.1	99.0	4.8	–

* Concentration is given in $\mu\text{g/g}$ for rocks samples and in $\mu\text{g/dm}^3$ for tap water sample.

** The experimental *t*-values were calculated according to equation $\mu = x_{\text{average}} \pm tS/n^{1/2}$; The critical *t*-value for five replicate measurements at a confidence level of 0.95 was 2.78.

Table 4. Comparison between the analytical performances of the described method for Cu(II) quantification with some other studies previously reported in the literature

Method	Reagent	SV ^a , cm^3	LOD, $\mu\text{g/dm}^3$	PF ^b	Detection	Ref.
CPE ^c	–	10	1.5	14	FAAS ^f	27
CPE	Monocarboxylic acid	100	10	10	FAAS	28
HLLME ^d	8-Hydroxy quinoline	5	1.74	25	FAAS	29
DLLME ^e	Salophen	10	1.9	70.9	HPLC-UV ^g	30
CPE	Isoleucine	25	5	22	UV/Vis ^h	31
CPE	DHMPb ⁱ	15	6	–	UV/Vis	2
DLLsME	DHDPhB	30	1.29	39	UV/Vis	This study

^aSample volume, ^bPreconcentration factor, ^cCloud point extraction, ^dHomogeneous liquid–liquid microextraction,

^eDispersive liquid–liquid microextraction, ^fFlame atomic absorption spectroscopy, ^gHigh-performance liquid chromatography–ultraviolet detection, ^hUltraviolet–visible spectrophotometry, ⁱ6,7-dihydroxy-4-methyl-2-phenylbenzopyrilium perchlorate

A comparison of the suggested DLLsME – UV/Vis technique for preconcentration and quantification of Cu(II) with some studies described in the literature are summarized in Table 4. The suggested method has a wider or comparable linear range and a better limit of detection. Also, the developed DLLsME procedure does not require special equipment, large sample amounts and also does not require significant quantities of toxic organic solvents. Moreover, suggested DLLsME–UV/Vis method is cheap, sensitive and easy to perform.

4. Conclusions

A cheap, simple, sensitive and environmentally friendly dispersive liquid–liquid semi-microextraction method for preconcentration and quantification of Cu(II) which is based on the complex formation with 6,7-dihydroxy-2,4-diphenylbenzopyrilium chloride was described. In optimal conditions, the calibration graph was linear in the range of Cu(II) concentrations 4.32–65 $\mu\text{g/dm}^3$. The proposed DLLsME–UV/Vis method has been successfully applied to the quantification of Cu(II) traces in rocks and tap water samples.

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Povzetek

Predlagamo postopek nove disperzivne semi-mikroekstrakcije tekoče-tekoče (DLLsME) za predkoncentracijo bakra(II). Sistem, ki je vseboval baker(II) in 6,7-dihidroksi-2,4-difenilbenzopirilijev klorid (DHDPhB), je po dodatku zmesi kloroforma in metanola postal moten in takoj je bilo opaziti tvorbo organske faze. Optimalni pogoji DLLsME so bili: pH 5, absorpcijski maksimum 570 nm, 1 cm^3 raztopine $1 \times 10^{-3}\text{ mol/dm}^3$ DHDPhB in ekstrakcijska zmes, ki je vsebovala 1 cm^3 kloroforma in 1 cm^3 metanola. Pri optimalnih pogojih je bila kalibracijska krivulja linearna v območju koncentracij bakra(II) 4,32–65 $\mu\text{g/dm}^3$, meja zaznave pa je bila 1,29 $\mu\text{g/dm}^3$. S predlaganim postopkom smo uspešno analizirali vzorce kamnin in vodovodne vode, pri tem je bil RSD pod 4,9 %.

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