



Determination of Zinc, Copper, and Mercury in Water Samples by Using Novel Micro Cloud Point Extraction and UV-Vis Spectrophotometry

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ABSTRACT

A novel micro cloud point extraction (MCPE) was developed as a fast, simple, and economical preconcentration method for spectrophotometric determination of Zn, Cu, and Hg in water samples. In contrary to traditional cloud point extraction, this method does not need heating. To achieve the cloud point in room temperature, the MCPE procedure was carried out in brine. Triton X-114 was employed as a non-ionic surfactant and the analytes were chelated by 4-(2-pyridylazo) resorcinol prior to extraction. The important factors influencing the extraction efficiency were investigated and optimized. Under the optimized condition, calibration curve was found to be linear in the concentration range of 0.15-0.60, 0.02-0.10, and 0.30-0.80 mg.L⁻¹ for Zn, Cu, and Hg respectively with a limit of detection of 51.7, 9.8, and 13.1 µg.L⁻¹.

Keywords: copper, mercury, zinc, micro cloud point extraction, UV-Vis spectrophotometry

INTRODUCTION

Trace determination of heavy metals is one of the most important issues of chemical analysis [1]. Over the years, UV-Vis spectrophotometry has been widely used for the determination of metals [2-6], because it is an available and inexpensive technique which can be found in most laboratories. However, due to low concentration of heavy metals, insufficient sensitivity of the instrument, and matrix interferences, there are some difficulties for determination of traces of such analytes using this technique. Therefore, developing and application of a sample preparation/ preconcentration step prior to instrumental analysis is ineluctable [7]. A sample preparation step must be fast, economical, environmentally friendly, easy to perform, and useful for broad spectrum of analytes in various matrices [8]. In recent years, miniaturization of sample preparation has become an important factor for new sample preparation techniques. Some of these techniques include single drop microextraction (SDME) [9, 10], dispersive liquid-liquid microextraction (DLLME) [11-13], solid phase extraction (SPE) [14-16], solid

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phase microextraction (SPME) [17, 18], and cloud point extraction (CPE) [19-22]. Meanwhile, the concern for environmental pollution and human health has led to the development of green analytical methods. Green chemistry concentrates on eliminating or reducing the consumption of organic solvents, and/or replacing the organic solvent with non-toxic and environmental friendly solvents [23]. One of favorite approach in green chemistry is replacing organic solvent with non-toxic and non-flammable solvents such as ionic liquids [24-29]; however it must be noted that they are expensive solvents, many of them are not commercialized and they can also decompose in contact with moisture, giving harmful volatiles [30]. In 1978, Watanab introduced cloud point extraction (CPE) as a new extraction method for determination of Zn with 1-(2-Pyridylazo)-2-Naphthol ligand [31]. Since then, CPE was applied widely for determination of various analytes [32-33], mainly for the extraction of metal ions [34-38]. The most important feature of CPE is application of a surfactant, mostly non-ionic, as the extractant phase [39] which means that CPE avoids the consumption of large amounts of expensive, toxic and flammable organic solvents. In CPE, in a temperature above a well-defined point, known as cloud point temperature (CPT), non-ionic surfactant molecules form structures known as micelles which extract analytes. This step which takes place in water bath needs high temperature sometimes up to 60 °C [40] and it's usually time consuming [41]. CPE can be coupled with many analytical instruments including spectrophotometry. In spectrophotometric determination of analytes, it is common to use macrocells (3.5 mL). That means the consumption of diluting agent which is necessary to dissolve the micelles before introducing the sample to any instrument, can reach up to 2 mL [42].

In our previous work, we introduced a new variant of CPE, named new micro cloud point extraction (MCPE), for the determination of two metals (uranium and vanadium) [43] and some organic dyes [44, 45] in water samples in water samples. MCPE is a green and fast extraction method based on miniaturization of CPE. In MCPE, the consumption of organic solvents is reduced to 40-50 μ L which makes this method even more environmental friendly. Besides, since MCPE is performed in brine, the cloud point temperature (CPT) can be reached at room temperature, therefore, heating step is entirely eliminated. MCPE is a very flexible extraction method which can be potentially coupled with all analytical instruments, especially with spectrophotometer. Here we employed MCPE for the extraction of Zn, Cu, and Hg from aqueous media. Triton X-114 was utilized as extractant phase; and as a well-known chelating agent, 4-(2-pirydilazo) resorcinol (PAR) was used as chelating agent.

EXPERIMENTAL

Instrument

A Shimadzu UV/VIS spectrophotometer, UV-160 (Kyoto, Japan) equipped with two 10 μ L microcells (Starna, UK) was used for measuring the absorbance and recording the spectra.

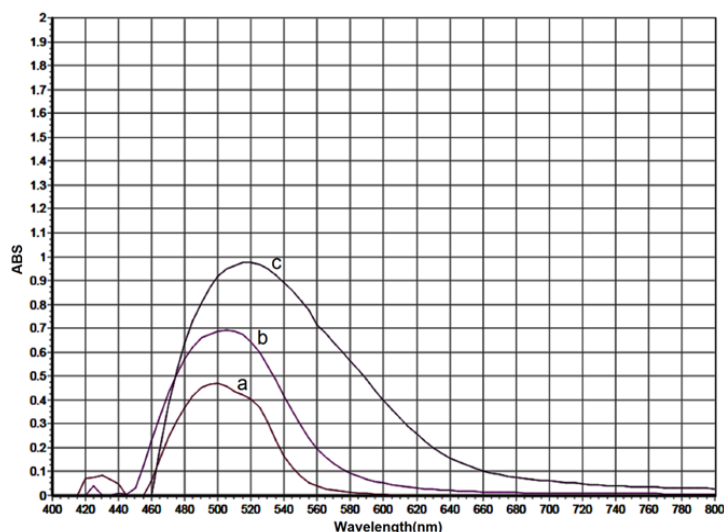


Figure 1. Absorption spectra of 0.2 mg.L^{-1} Zn (a), 0.04 mg.L^{-1} Cu (b), and 0.70 mg.L^{-1} Hg (c) after MCPE extraction

Reagents and chemicals

All reagents and solvents were of analytical grade and were purchased from Merck KGaA (Germany) and were used as received. Zinc and mercury standard solutions (1000 mg.L^{-1}) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were obtained from the same company. 4-(2-pyridylazo) resorcinol (0.01 M), Na_2SO_4 ($5\% \text{ w/v}$) and Triton X-114 ($2\% \text{ v/v}$) solutions were prepared by dissolving appropriate amounts of each compound in doubly distilled water. The pH of the sample solution was adjusted using a suitable phosphate buffer. Doubly distilled water was used throughout all the procedures.

Micro Cloud Point Extraction procedure

An aliquot of the sample solution containing appropriate amounts of the analyte was transferred into a centrifuge test tube with conical bottom containing PAR solution and $2\% \text{ v/v}$ Triton X-114. The pH was set with addition of 0.5 mL phosphate buffer solution. To reach cloud point and formation of a cloudy solution, 0.5 mL of Na_2SO_4 solution ($5\% \text{ w/v}$) was added to the mixture. Then the mixture was diluted to 10 mL with double distilled water. The obtained cloudy solution was centrifuged for 2 minutes for Zn and 5 minutes for Cu and Hg at 3500 rpm . During the centrifugation, the surfactant settled down at the bottom of the test tube in form of a very high density liquid phase. $20 \mu\text{L}$ of this sediment was transferred into a vial and dissolved in $50 \mu\text{L}$ of methanol. Finally $10 \mu\text{L}$ of this mixture was transferred to a microcell for spectrophotometric determination. The blank was prepared in the same way but without the analytes.

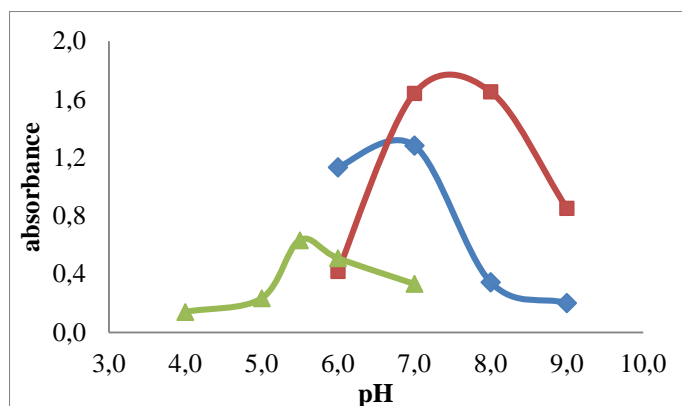


Figure 2. Effect of pH of aqueous solution on MCPE of 0.60 mg.L^{-1} Zn (◆), 0.10 mg.L^{-1} Cu (■), and 0.50 mg.L^{-1} Hg (▲)

RESULTS AND DISCUSSION

Absorption spectra of complex

In order to find the wavelength of maximum signals of the metal complexes, i.e. Zn-PAR, Cu-PAR, and Hg-PAR, the absorption spectra of each was determined in the wavelength range of 400 to 800 nm against the reagent blank. As can be seen in **Figure 1**, maximum absorption wavelength for Zn-PAR, Cu-PAR, and Hg-PAR complexes were found to be 517, 515, and 530 nm respectively. Therefore, these wavelengths were selected as the absorption wavelengths for further determinations. During all of the following experiments, the blank absorbance of all reagents was corrected.

Optimization of MCPE

To obtain the maximal extraction efficiency, important experimental parameters which can potentially influence enrichment factor of extraction, such as pH of sample solution, effect of ionic strength of the sample solution, amount of surfactant and ligand concentration, type of diluting solvents, and centrifugation speed have been investigated in detail for proposed MCPE method. The univariant method was used to simplify the optimization procedure. A series of experiments were designed for this goal as discussed below. Number of replicates of analysis was at least three for each experiment.

Effect of pH

To obtain the maximal extraction efficiency, important experimental parameters which can potentially influence enrichment factor of extraction, such as pH of sample solution, effect of ionic strength of the sample solution, amount of surfactant and ligand concentration, type of diluting solvents, and centrifugation speed have been investigated in detail for proposed MCPE method. The univariant method was used to simplify the optimization procedure. A series of experiments were designed for this goal as discussed below. Number of replicates of analysis was at least three for each experiment.

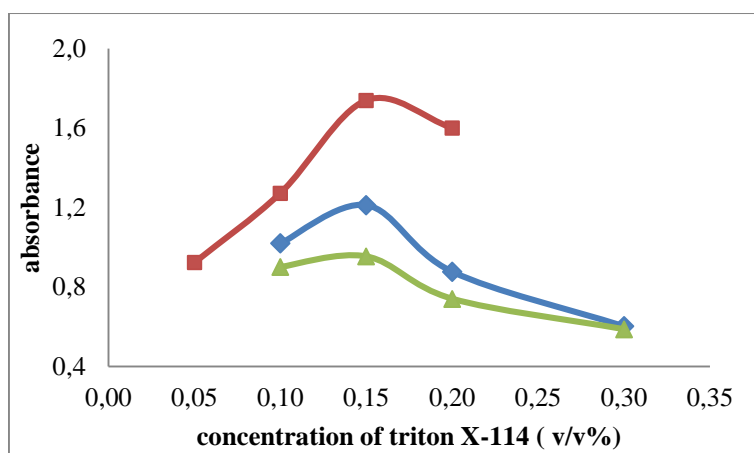


Figure 3. Effect of concentration of Triton X-114 on MCPE of 0.60 mg.L^{-1} Zn (◆), 0.10 mg.L^{-1} Cu (◆), and 0.50 mg.L^{-1} Hg (▲)

Effect of pH

pH plays an important role in complex formation of metals. Therefore, the effect of pH on the absorbance of Zn-PAR, Cu-PAR, and PAR has been examined with great concern. PAR acts as a tridentate chelating agent and bonds with metal ions through the *o*-hydroxyl group. This species is dominant in $\text{pH} > 5$ [46]. Therefore, pH of the sample solution was studied in the range of 4 to 9 (Figure 2). The maximum absorbance of Zn-PAR, Cu-PAR, and Hg-PAR were obtained at $\text{pH} = 7.0$, $\text{pH} = 8.0$, and $\text{pH} = 5.5$, respectively. In the next experiments, 0.5 mL of appropriate phosphate buffer was added to the sample solution for adjustment of the pH of sample solution to the desired value.

Effect of salt concentration

Addition of salt in MCPE is for increasing the ionic strength of the sample solution and formation of micelles. It is probably due to salting out effect which reduces the solubility of surfactant molecules in aqueous media. Therefore the concentration of salt has an important effect in reaching cloud point at ambient temperature. The chosen salt for this purpose was Na_2SO_4 because it implies more ionic strength to the sample solution in comparison with other tested salts (NaCl, KCl, and NaHSO_4).

The effect of Na_2SO_4 concentration on the absorbance was studied in the range of 0.12-0.75% w/v and it was found that the absorbance of all analytes reaches to a maximum value at 0.25% w/v of Na_2SO_4 and the cloudy solution was formed immediately. Therefore, there was no need for heating the sample solution to reach cloud point. Consequently, this concentration of sodium sulphate was used in all subsequent experiments.

Table 1. Analytical figures of merit for MCPE extraction of Zn, Cu, and Hg

Parameter	Zinc	Copper	Mercury
Equation of calibration curve	$A = 2.3476C_{Zn} - 0.0023$	$A = 18.005C_{Cu} - 0.0432$	$A = 1.9455C_{Hg} - 0.46$
Dynamic range (mg.L ⁻¹)	0.15-0.60	0.02-0.10	0.30-0.80
R ² (determination coefficient)	R ² = 0.9666	R ² = 0.9676	R ² = 0.9799
Repeatability ^a (RSD%, n = 5)	2.44	3.71	6.06
Limit of detection ^b (µg.L ⁻¹)	51.7	9.8	13.1
Enrichment factor (fold)	2.58	3.71	6.06

^aRSD, relative standard deviation, for 5 replicate measurements

^bLOD, was based on 3S_b/m criterion for 10 blank measurements

Effect of Triton X-114 concentration

The concentration of Triton X-114 as the extractant medium has great effect on the extraction efficiency. In order to find the best amount of it, different concentrations of Triton X-114 in the range of 0.05-0.3% v/v were subjected to the same procedure. **Figure 3** shows instrument responses for target analytes. According to these data, Triton X-114 concentration of 0.15% was chosen as the best concentration for the further experiments. In lower concentrations of Triton X-114, very little micelle formation was observed which consequently reduced the efficiency of extraction.

Selection of diluting solvent

In coupling CPE methods with UV-Vis spectrophotometer, it is necessary to dissolve the formed micelles. This is achieved by using diluting agents which usually are organic solvents. Diluting agent must be capable of dissolving the sedimented phase completely and rapidly. For this purpose, four solvents, acetone, ethanol, methanol, and acetonitrile, were considered. The results lead us to select acetonitrile for Zn and methanol for Cu and Hg as diluting solvent.

Effect of PAR concentration

The effect of concentration of PAR as a chelating agent on the absorbance of analytes was also investigated. The maximum absorbance was obtained at 2×10⁻⁴ M PAR for all three complexes and remained almost constant with increasing concentration up to 4×10⁻⁴ M.

Effect of time of centrifugation

Since separating of enriched micellar and aqueous phase is difficult, application of centrifugation is necessary. Keeping rotation constant, time of centrifugation was investigated in the interval of 1 to 7 min. according to the obtained data, the best time was found to be 2 min at 3500 rpm for Zn and 5 min at 3500 rpm for Cu and Hg.

Table 2. Comparison of MCPE with other preconcentration methods for determination of Zn, Cu, and Hg

Analyte	Microextraction Method	Extractant	Sample Volume (mL)	Volume of diluting solvent (mL)	LOD ($\mu\text{g}\cdot\text{L}^{-1}$)	EF ^a (fold)	RSD %	Recovery%	Approximated Total analysis time (min)	Ref
Zinc	DLLME/AAS ^b	CCl ₄	8	0.07	0.3	10.3	1.5	98.5-103.5	5	47
	CPE/UV-Vis	Triton X-114	25	2.5	1.2	≈ 10	2.1	95-104	22	48
	MCPE/U V-Vis	Triton X-114	10	0.05	51.7	2.58	2.44	93.42-112.66	2	This work
Copper	DLLME/AAS	CHCl ₃	10	-	7.92	6	3.22	94.94-103.21	5	49
	DLLME-SFO ^c /AAS	1-undecanol	20	0.5	3.4	10	0.7	91.1-92.9	25	50
	CPE/UV-Vis	Triton X-100	25	1	5	22	2.8	96-101	15	51
	MCPE/U V-Vis	Triton X-114	10	0.05	9.8	13.15	3.71	93.00-112.66	5	This work
Mercury	DLLME/UV-Vis	[Hmim][Tf2N]	10	0.35	3.9	18.8	1.7	92.2-103.2	6	52
	CPE/UV-Vis	Triton X-114	50	1.5	1.65, 14.35	33.3	2.75, 2.65	97.8-103.5	20	53
	MCPE/U V-Vis	Triton X-114	10	0.05	13.1	17	6.06	115.00-107.50	5	This work

^a Enrichment factor^b Atomic absorption spectrometry^c Dispersive liquid-liquid microextraction-solidification of floating organic drop

Linear range, limit of detection and precision

Analytical figures of merit for the proposed method obtained under optimal conditions are shown in **Table 1**. Detection limits (LODs) were obtained based on a signal-to-noise ratio of 3. The repeatability of the method, expressed as relative standard deviation (RSD), was calculated for five replicates of the standard at an intermediate concentration of the calibration curve. The enrichment factor (EF) that was calculated as the ratio of the analyte concentration after MCPE (C_{MCPE}) and the initial concentration of the analyte (C_0) within the sample (Eq. 1), was found to be 2.6, 13.2, and 17.0 fold for Zn, Cu, and Hg, respectively.

$$EF = C_{MCPE} / C_0 \quad (1)$$

Table 3. Analytical results for the determination of analytes in tap water (n=3)

	Added (mg.L ⁻¹)	Recovery (%)	RSD (%)
Zinc	0.15	112.6	1.95
	0.40	93.42	1.84
	0.60	109.60	4.73
Copper	0.02	93.00	5.93
	0.05	95.00	3.08
	0.10	98.33	2.56
Mercury	0.30	115.00	6.38
	0.50	108.00	5.44
	0.80	107.50	3.50

A comparison between data obtained with MCPE with those recently obtained with other microextraction methods coupled with spectrophotometer for determination of Zn, Cu, and Hg is summarized in [Table 2](#).

Analysis of real samples

The MCPE procedure was applied on tap water. Since no detectable analyte was observed, the samples were spiked with 3 different concentrations of each cation to investigate the matrix effect on their determination individually. The results are shown in [Table 3](#). As can be seen, very good recoveries between 93.00 and 115.00% were achieved with reproducibilities better than 6.38%. The total analysis time for any of the target analytes was less than 5 min.

CONCLUSION

As a novel, fast, economical, effective and easy to operate method, MCPE, was developed for preconcentration and determination of traces of three heavy metals (zinc, copper, and mercury) in aqueous samples. Triton X-114 was used as a non-ionic and green extractant solvent. In comparison to the similar methods of extraction like CPE, DLLME, and SPME, MCPE is much faster and simpler. Since the time consuming step of water bath (to reach cloud point) is eliminated for MCPE in brine, the total analysis time including microextraction was about 5 min; and consumption of solvents is minimized to 50 μ L. Spectrophotometric instrumentations also own merits of simplicity, cheapness, portability and so on. Enrichment factor and reproducibility of MCPE was found to be in the same order of complicated and time consuming extraction techniques such as DLLME-SFO/AAS, while better LODs were achieved. In this paper we coupled MCPE with spectrophotometry equipped with microcells, as a fast and available instrument; but MCPE can be potentially coupled with any analytical instrument. Therefore, the proposed MCPE method is applicable in ordinary laboratories with any instrument available.

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