Homogeneous Liquid-liquid Extraction for the Preconcentration of Copper Using a New Schiff Base and Determination in Water Samples by Flame Atomic Absorption Spectrometry

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Abstract
In this study, a simple and effective homogeneous liquid–liquid extraction method was applied for extraction and determination of Cu$^{2+}$ after formation of complex with a new Schiff’s base N,N-bis(diacetylmonoxim)-naphthylene-1,8-diimine using (water/tetrabutylammonium ion (TBA)/chloroform) as a ternary component system and the later was determined by flame atomic absorption spectrometry (FAAS). The phase separation phenomenon occurred by an ion-pair formation of TBA$^+$ and perchlorate ion. Then sedimented phase was separated using a 100 μl micro-syringe and diluted to 1.0 ml with ethanol. The sample was introduced into the flame by conventional aspiration. After the optimization of complexation and extraction conditions such as pH=8.0, [ligand]=1.0 × 10^{-4} mol L^{-1}, [TBA]= 2.0 × 10^{-2} mol L^{-1}, [ClO$_4^-$] = 2.0 × 10^{-2} mol L^{-1} and 100.0 μL of CHCl$_3$, a preconcentration factor (Va/Vs) of 100 was obtained for only 10 ml of the sample. The limit of detection was sufficiently low and lie at ppb level. The proposed method was applied for the extraction and determination of Cu$^{2+}$ in natural water samples with satisfactory results. The relative standard deviation was 2.5 % (n=10). Accuracy and application of the method was estimated by using test samples of natural and synthetic water spiked with different amounts of copper (II) ion. The method is very simple and inexpensive.

Keywords: Homogeneous liquid-liquid extraction; Schiff’s base, Copper; Preconcentration

Introduction
Copper is considered as an essential micronutrient. This element is needed by plants only at very low levels but it is toxic at high levels. Copper can bind to the cell membrane and
hinder the transport process through the cell wall. Copper at nearly 40 ng.l$^{-1}$ is required for normal metabolism of many living organisms [1,2]. On the other hand, copper is an important element in many industries. Thus the development of new methods for selective separation, preconcentration and determination of copper at sub-micro levels in different industrial, medicinal and environmental samples is of continuing interest. This determination is usually carried out by flame and graphite furnace atomic absorption spectrometry (AAS) [3, 4], spectrophotometric methods [5-7] and ICP-AES methods [8]. However due to the presence of copper in medicinal and environmental samples at low levels, its separation from other elements present in the sample and also the use of a preconcentration step prior to its determination is usually necessary. Different methods, especially liquid–liquid extraction of copper in the presence of various classical [9] and macrocyclic [10,11] co-extracting ligands, have attracted considerable attention. However, these methods which are typically time-consuming and labor-intensive have multi-step procedures prone to the loss of analytes and need high volumes of samples [12]. Therefore, simple, rapid, and efficient techniques that can be used easily are required.

Homogeneous liquid-liquid extraction is an excellent method that extracts desired solute existing in the homogeneous solution into the water–immiscible phase by each kind of phase separation phenomenon. In homogeneous liquid-liquid extraction, the initial condition (before phase separation) is homogeneous solution; namely, there is no interface between the water phase and organic solvent phase. In other words, the surface area of the interface is infinitely large initially. Accordingly, no vigorous mechanical shaking is necessary. The procedure is simple, rapid, and requires only the addition of the reagents [13, 14].

Homogeneous liquid-liquid extraction method using a ternary solvent system has been developed and applied as a simple and high-powered preconcentration for the instrumental analysis [15-18]. In order to obtain homogenous system, various systems exist such as: ion pair systems [19], surfactant systems [20] and pH dependent system [15].

In addition, the methods based on the pH dependent phase separation, which is used a water / acetic acid / chloroform ternary solvent system [15] and water / pyridine / ethyl chloroacetate system[21] were reported. However, in these methods, the widespread application for target analytes has been limited by the inconvenient pH condition in extraction.

Recently, a pH independent phase separation phenomenon using the ion-pair formation of tetrabutylammonium ion (TBA$^+$) and perchlorate (ClO$_4^-$) in homogeneous solution (water / TBA$^+$/ chloroform) has been reported. In this method, chloroform is solvated by TBA$^+$ and
dissolves in water. When NaClO$_4$ is added to the homogeneous solution that consists of a water / TBA$^+$/ chloroform, the solvation effect of TBA$^+$ is excluded by ion-pair formation of TBA$^+$ and ClO$_4^-$ . Therefore, the chloroform in homogeneous aqueous solution is postulated to form water-immiscible chloroform in aqueous solution by the phase separation[22,23].

The Schiff bases have been extensively studied because of their biological and structural importance, which lies mainly in their specific and selective reactions with metal ions[24,25]. Almost all of metals form 1:1 metal complexes with Schiffs bases. Schiffs bases with donor atoms are well known to form strong complexes with transition metal ions and have been proposed as spectrophotometric reagents for several metal ions [26, 27] and as ionophore in sensors for determining cations[28-32]. These compounds also have sufficient hydrophobocity to use as a complexing agent in homogeneous liquid-liquid extraction.

The aim of this work was the development of a homogeneous liquid-liquid extraction and preconcentration method for Cu by the use of a new Schiffs base as a complexing agent prior to flame atomic absorption spectrometric determination.

**Experimental**

**Apparatus**

A Shimadzu model 680 atomic absorption spectrometer equipped with deuterium background correction and Cu$^{2+}$ hollow cathode lamp, as the radiation source was the manufactures recommendations.

Phase separation was assisted using a centrifuge (Centurion Scientific Ltd. model: 1020D). The pH of the solutions was controlled with Metrohm pH-meter model 713.

**Reagents and solutions**

All reagents used were of analytical grade. Stock solution of copper (1000 mg L$^{-1}$) was prepared by dissolving appropriate amounts of Cu (NO$_3$)$_2$.3H$_2$O in aqua regia. Working solutions were prepared from the stock solution by serial dilutions with double distilled water.

Other reagents used were; tetrabutylammonium bromid (Merck), nitric and hydrochloric acid (Merck), sodium hydroxide and sodium perchlorate (Merck). Chloroform and ethanol were of analytical – grade from Merck. The chelating agent solution was prepared by dissolving 0.3243g, Schiffs base in 100 ml of chloroform 99.5%. The Schiff base N,N-bis(diacetylmonoxim)-naphthylene-1,8-diiine was synthesized and purified as described in Synthesis of ligand section. (Scheme 1).
A stock standard buffer solution, 0.1 molL\(^{-1}\) was prepared by dissolving appropriate amount of sodium carbonate and hydrochloric acid.

The pipettes and vessels used for trace analysis were kept in 10 % nitric acid for at least 24 h and subsequently washed four times with deionized water before use.

**Synthesis of ligand**

To an ethanolic solution (50 ml) of diacetylmonoxim (2.56 g, 25.28 mmol) was added an ethanolic solution (20 ml) of 1,8-diaminonaphthalene (2 g, 12.64 mmol) drop by drop with stirring. A yellow compound was obtained during mixing at room temperature. The mixture was heated under reflux on a water bath for 30 min and was cooled to room temperature. The yellow precipitates separated were suction filtered, washed with ethanol and dried under vacuum.

F.W. (formula weight) = 324.381 g/mol, Yield 64\%. Selective IR bands (cm\(^{-1}\)), KBr pellets, (O-H, C=N, C-O), 3352, 1597 (s), 1250. Anal. calcd for C\(_{18}\)H\(_{20}\)N\(_4\)O\(_2\): C, 66.65; H, 6.21; N, 17.27. Found: C, 66.53; H, 6.16; N, 17.34.

**Recommended procedure**

A 10 mL homogeneous solution (water / TBA\(^+\) / chloroform) containing analyte (7-2000 ng mL\(^{-1}\)), chloroform (100.0 µL), Schiff’s base (1.0 × 10\(^{-4}\) mol L\(^{-1}\)), TBA\(^+\) (2.0 × 10\(^{-2}\) mol L\(^{-1}\)) and 5.0 × 10\(^{-3}\) mol L\(^{-1}\) carbonate buffer (pH = 8.0) were kept in a thermostated bath at 25\(^\circ\)C for 2min. Then, the mixture was gently shaken (accordingly, no vigorous mechanical shaking was
necessary). Under these conditions, homogeneous solution was obtained. By adding NaClO₄ aqueous solution (2.0×10⁻² mol L⁻¹) and shaking, a cloudy mixture was formed in the test tube. The mixture was then centrifuged for 5 min at 3000 rpm. The sedimented phase (90 μL) in the bottom of the conical test tube was separated using a 100 μL micro-syringe, and diluted to 1 mL with ethanol. The sample was introduced into the flame by conventional aspiration[23]. Experimental scheme of the procedure is shown in scheme 2.

\[ \text{Scheme 2. Experimental scheme of preconcentration of metal cations from water sample by HLLE method.} \]

**Results and Discussion**

*Effect of pH*

The separation of metal ions by homogeneous liquid-liquid extraction involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of sedimented phase; thus obtaining the desired preconcentration. pH plays a unique role on metal–chelate formation and subsequent extraction. The effect of pH on the extraction of 1.0 μg of copper from 10 mL of the sample solution was studied in the pH ranges of 1-12. Figure 1 shows the influence of pH on the extraction recovery of copper.

As can be seen, at pH = 8.0 maximum extraction was obtained. Hence, pH = 8.0 was chosen as the working pH and subsequent extraction was performed in carbonate buffer solution.
Effect of pH on the absorbance of complex.

Conditions: Cu (II) 100.0 ng mL\(^{-1}\); ligand 1.0 × 10\(^{-4}\) mol L\(^{-1}\); TBA\(^+\) 2.0 × 10\(^{-2}\) mol L\(^{-1}\); CHCl\(_3\) 100.0 µL and NaClO\(_4\) 2.0 × 10\(^{-2}\) mol L\(^{-1}\).

Effect of ligand concentration

The effect of the concentrations of ligand on the extraction recovery of copper is shown in Figure 2. The results showed that the extraction increased up to a known concentration of ligand (1.0 × 10\(^{-4}\) mol L\(^{-1}\)), reaching plateau, which was considered as complete extraction. A concentration of 1.0 × 10\(^{-4}\) mol L\(^{-1}\) was chosen to account for other extractable species.

Effect of TBA\(^+\) concentration

In order to investigate the optimum amount of TBA\(^+\) on the quantitative homogeneous liquid-liquid extraction of copper ions, the extraction of 1.0 µg of copper from 10 mL of the sample
solutions under the optimal experimental conditions was conducted by varying the concentration of TBA⁺ (Figure 3).
As can be seen, the extraction of copper is quantitative above $2.0 \times 10^{-2}$ mol L$^{-1}$ of TBA⁺. Hence, subsequent homogeneous liquid-liquid extraction experiments were carried out with $2.0 \times 10^{-2}$ mol L$^{-1}$ of TBA⁺. At lower concentration, the system remains two phases and the extraction is not quantitative.

![Figure 3](image_url)

**Figure 3.** Effect of pH on the absorbance of complex. Conditions: Cu (II) 100.0 ng mL$^{-1}$; ligand $1.0 \times 10^{-4}$ mol L$^{-1}$; TBA⁺ $2.0 \times 10^{-2}$ mol L$^{-1}$; CHCl$_3$ 100.0 µL and NaClO$_4$ $2.0 \times 10^{-2}$ mol L$^{-1}$.

**Effect of ClO$_4^-$ concentration**

In order to determine the concentration of ClO$_4^-$ for quantitative recoveries, the extraction of 1.0 µg of copper from 10 mL of the sample solutions under the optimal experimental condition was conducted by varying the concentration of ClO$_4^-$ (Figure 4).

![Figure 4](image_url)

**Figure 4.** Effect of ClO$_4^-$ concentration on the extraction of copper. Conditions: Cu (II) 100.0 ng mL$^{-1}$; ligand $1.0 \times 10^{-4}$ mol L$^{-1}$; CHCl$_3$ 100.0 µL; TBA⁺ $2.0 \times 10^{-2}$ mol L$^{-1}$ and pH = 8.0.
As seen, the extraction of copper is quantitative above $2.0 \times 10^{-2}$ mol L$^{-1}$ of ClO$_4^-$ . The composition of ion-pair becomes approximately 1:1 in the neighborhood of $[\text{TBA}^+]=[\text{ClO}_4^-]$ . Hence, subsequent homogeneous liquid-liquid extraction experiments were carried out with $2.0 \times 10^{-2}$ mol L$^{-1}$ of ClO$_4^-$.

**Effect of buffer concentration**

The influence of buffer quantity was investigated, while other experimental variables remained constant. The results showed that above $4.0 \times 10^{-3}$ mol L$^{-1}$ buffer solutions, obvious variation did not take place in the extraction yield. Thus, $5.0 \times 10^{-3}$ mol L$^{-1}$ of buffer solution was chosen as the optimal to achieve higher buffering capacity. At lower concentration than $4.0 \times 10^{-3}$ mol L$^{-1}$ of buffer solution, the buffering capacity is not sufficient for adjusting pH.

**Effect of the volume of chloroform**

In this extraction method, the concentration factor strongly is dependent on the volume of the sedimented chloroform phase. Figure 5 shows the influence of the volume of added chloroform on the extraction efficiency of copper.

As can be seen, the extraction of copper is quantitative above 100.0 µL of CHCl$_3$. Hence, in order to achieve maximum concentration factor, 100.0 µL of added CHCl$_3$ was selected.

![Figure 5](Figure5. Effect of added volume of chloroform on the extraction of copper. Conditions: Cu (II) 100.0 ng mL$^{-1}$; ligand $1.0 \times 10^{-4}$ molL$^{-1}$; TBA$^+$ $2.0 \times 10^{-2}$ mol L$^{-1}$; NaClO$_4$ $2.0 \times 10^{-2}$ mol L$^{-1}$ and pH = 8.0.)

**Selectivity of the method for copper**

For detection interferences, the influence of several ions was tested. The effect of interfering
ions at different concentrations on the absorbance of a solution containing 100.0 µg L⁻¹ of copper was studied. An ion was considered to interfere when its presence produced a variation in the absorbance of the sample of more than 5 %.

Among the interfering ions tested; Cl⁻, I⁻, SO₄²⁻, NO₃⁻, ClO₄⁻, Li⁺, K⁺, Na⁺, Ba²⁺, Ca²⁺, Mg²⁺, (Ion / Cu (II) (w/w) = 1000) and Ce³⁺, Co²⁺, Pb²⁺, Cd²⁺, Al³⁺, Ni²⁺, Sr²⁺, Fe²⁺, Ag⁺, Fe³⁺, Cr³⁺, Sn²⁺, Cd²⁺, Zn²⁺ (Ion / Cu (II) (w/w) = 200) did not interfere in copper determination. Results are shown in Table 1.

<table>
<thead>
<tr>
<th>Ion</th>
<th>(Ion/Cu)(w/w)</th>
<th>(%) Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻, Cl⁻</td>
<td>1000</td>
<td>100.2</td>
</tr>
<tr>
<td>I⁻</td>
<td>1000</td>
<td>97.8</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>1000</td>
<td>98.5</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>1000</td>
<td>99.2</td>
</tr>
<tr>
<td>Na⁺, Li⁺</td>
<td>1000</td>
<td>99.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>1000</td>
<td>102.9</td>
</tr>
<tr>
<td>Ca²⁺, Ba²⁺</td>
<td>1000</td>
<td>97.8</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>1000</td>
<td>102.2</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1000</td>
<td>98.4</td>
</tr>
<tr>
<td>Fe²⁺, Pb²⁺</td>
<td>200</td>
<td>98.0</td>
</tr>
<tr>
<td>Cr³⁺, Ce³⁺</td>
<td>200</td>
<td>96.7</td>
</tr>
<tr>
<td>Co²⁺, Sr²⁺</td>
<td>200</td>
<td>99.2</td>
</tr>
<tr>
<td>Ni²⁺, Cd²⁺</td>
<td>200</td>
<td>100.0</td>
</tr>
<tr>
<td>Zn²⁺, Fe³⁺</td>
<td>200</td>
<td>97.8</td>
</tr>
<tr>
<td>Sn²⁺</td>
<td>200</td>
<td>98.7</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>200</td>
<td>101.2</td>
</tr>
</tbody>
</table>

**Figures of merit**

Table 2 summarizes the analytical characteristics of the optimized method, including linear range, limit of detection, reproducibility, and concentration factor. The limit of detection, defined as \( C_L = 3 S_B / m \) (where \( C_L \), \( S_B \) and \( m \) are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), was 7.0 ng/ml⁻¹. The relative standard deviation (RSD) for ten replicate measurements of 100.0 ng/ml⁻¹ Cu (II) was 2.5 %.

The concentration factor (the volume ratio (Va / Vs) of the aqueous phase (Va= 10 ml) and final volume of sedimented phase (Vs = 1.0 ml) after phase separation) was 10. Certainly a preconcentration factor of 100 (100 µl of chloroform phase was obtained from 10 ml of
the homogenous aqueous solution) in combination of this method with other techniques that required low volume of sample such as flow injection analysis (FIA) or graphite furnace atomic absorption spectrometry (GF AAS) can be obtained. Preconcentration factor of 100 will make the method very promising for use at ng/ml level.

### Table 2. Analytical characteristics of proposed method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analytical feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (ng mL⁻¹)</td>
<td>7.0-2000</td>
</tr>
<tr>
<td>Limit of detection (ng mL⁻¹)(n =10)</td>
<td>7.0</td>
</tr>
<tr>
<td>Repeatability (RSD a, %) ( n =10 )</td>
<td>2.5</td>
</tr>
<tr>
<td>Concentration factor b</td>
<td>10</td>
</tr>
</tbody>
</table>

⁢Cu (II) concentration was 100.0 ng mL⁻¹ for which RSD was obtained.

b Concentration factor is the volume ratio (Va/Vs) of the aqueous phase (Va) and final volume of sedimented phase (Vs).

### Application to samples

To access the capability of the method for real samples with different matrices containing varying amounts of diverse ions, the method was applied to separation, preconcentration and determination of copper from 10 mL of water samples. According to the results, the concentration of copper in analyzed water samples was below the LOD of the method. The suitability of the proposed method for the analysis of natural water samples was checked by spiking samples with 50 and 100 µg L⁻¹ of copper. Good recoveries (99-103%) were achieved for all analyzed samples. The data obtained with the proposed method are presented in Table 3. The results indicate that the proposed method can be reliably used for the determination of copper in various matrices.

### Table 3. Determination of Cu (II) in different water samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu²⁺ spiked (ng ml⁻¹)</th>
<th>Cu²⁺ detected (ng ml⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>0.0</td>
<td>29.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>81.7 (2.6b)</td>
<td>102.4</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>130.8 (2.5)</td>
<td>100.8</td>
</tr>
<tr>
<td>River water</td>
<td>0.0</td>
<td>53.2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>102.8 (2.4)</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>152.9 (2.8)</td>
<td>99.8</td>
</tr>
</tbody>
</table>

⁢From drinking water system of Tehran

bRSD of three replicate experiments

cRecovery = \( \frac{C_{\text{detected}}}{(C_{\text{sample}} + C_{\text{spiked}})} \)
Conclusion

In this work, we used a homogenous liquid–liquid extraction method for separation and determination of copper that utilizes an ion-pair formation of TBA+ and ClO4- for phase separation phenomenon (a pH independent phase separation) from the homogeneous solution. The method is simple, rapid, highly selective and sensitive. High preconcentration factor can be obtained easily by this method. The proposed method can be applied to environmental and/or other sample having Cu levels higher than the detection limit of the method.

References