Online solid-phase extraction coupled to flame atomic absorption spectrometry for determination of trace amounts of copper (II) ion in water samples

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A solid phase extraction-flow injection procedure for online determination of Cu(II) by flame atomic absorption spectrometry (FI-AAS) has been reported. The metal ions are retained as Cu(I)-neocuproine complex in a mini-column filled with octadecyl bonded silica (C_{18}) as sorbent material. Quantitative extraction has been obtained at pH 5.0. The retained metal ions are then eluted efficiently with ethanol into the nebulizer of flame atomic absorption spectrometer for on-line determination. Different variables affecting the preconcentration efficiency, including pH, neocuproine concentration, eluent type, sample, eluent flow rates and sample loading time, are optimized. Two minute preconcentration at flow rate of 6 mL min^{-1} provides the enhancement factor of 100 for Cu(II). The detection limit (3σ) is found to be 0.15 µg L^{-1} for Cu(II), and the relative standard deviation at 5.0 µg L^{-1} of target metal ions is 1.2% (n=7). The accuracy has been assessed by analysis of certified reference materials CASS-4 and CRM1643d, and the obtained results are found in good agreement with certified amounts of Cu(II). The procedure has been applied successfully to Cu(II) ion determination in water samples.

Keywords: Copper, Flame atomic absorption spectrometry, Flow injection, Neocuproine

Despite the sensitivity and selectivity of analytical techniques such as flame atomic absorption spectrometry (FAAS), there is a great necessity for the preconcentration of trace metal ions prior to their determination, basically due to its relatively low power of detection for some elements\(^{1,2}\). One of the main reasons for this is the low efficiency of the pneumatic nebulizer, because only 5–10% of the primarily produced aerosol reaches the flame\(^3,4\). Another reason for the poor sensitivity is the short residence time of the atoms in the flame. To improve the sensitivity and selectivity, preconcentration procedures such as liquid–liquid extraction, precipitation, cloud point extraction, co-precipitation and solid-phase extraction (SPE) are generally used before the detection. The above procedures, operated in the batch mode, are time consuming and labor-intensive, require large sample/reagent volumes, and suffer from risks of contamination and analyte loss. In recent years, flow injection (FI) on-line sorption technique has shown great promise and become one of the most active research fields in automated analysis, improving the performance characteristics of the methods\(^5,6\). When FAAS is coupled with a preconcentration procedure such as solid-phase extraction, it can reach a very low detection limit comparable to that obtained with graphite furnace atomic absorption spectrometry (GF-AAS)\(^7,8\). The nature and the properties of the sorbent materials are of prime importance for effective retention of metals\(^9\). The main requirements of a sorbent material are fast and quantitative adsorption/elution, regeneration ability, high capacity, accessibility, and the chemical and mechanical resistibility to endure harsh conditions. Various packing materials for on-line column preconcentration have been used for the determination of copper and other heavy metals in various types of samples such as multiwalled carbon nanotubes\(^10\), microcrystalline naphthalene\(^11\), polyurethane foam\(^12\), silica-gel\(^13\), styrene-divinylbenzene\(^14,15\), chitosan\(^16\), Styrene-divinylbenzene commercially available as Amberlite XAD resin series, and other sorbents\(^17\). Flow injection (FI) separation and preconcentration techniques have proved to be effective for enhancement of sensitivity of atomic spectrometry\(^18\). The on-line preconcentration systems using flow injection have several advantages in comparison to the off-line systems, such as higher analytical throughput, low

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sample and reagents consumption and less risks of analyte loss or sample contamination. In addition, these systems can be easily coupled to conventional analytical techniques, such as FAAS, ICP-MS and ICP-OES\textsuperscript{19,20}. Online solid phase extraction preconcentration and separation coupled with flame atomic absorption spectrometry have shown to be powerful for determination of trace elements in a variety of matrices\textsuperscript{21-23}. The present work includes the application of SPE online incorporated to atomic absorption spectrometry for the selective extraction, concentration and determination of trace amounts of copper.

**Experimental Procedure**

**Chemicals and reagents**

High purity methanol, ethanol, hydrochloric acid, acetic acid and ammonia solution, all from Merck (Darmstadt, Germany), were used. Reagent grade neocuproine (Merck) was used as received. Analytical grade cupric sulfate and hydroxylamine hydrochloride (as reducing agent) to convert Cu(II) to Cu(I) and nitrate salts of other metal ions (for interference study) with highest purity available were obtained from Merck. Doubly distilled deionized water was used throughout. The standard stock solution of Cu(II) was prepared by dissolving an appropriate amount of CuSO$_4$·5H$_2$O in deionized water. Working solutions were prepared by appropriate dilution of the stock solution. Octadecyl bonded silica was obtained from Applied Separations (Allentown, PA, USA).

**Apparatus**

All absorbance measurements of metal ions were performed using a Varian model Specter AA220 (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer equipped with a deuterium background corrector. A copper hollow cathode lamp operating at 324.7 nm was used as radiation source. The burner height and the gas flow rates were adjusted to obtain the maximum sensitivity. A Metrohm 692 pH/Ion meter equipped with a combined glass-calomel electrode was used to measure the pH of test solutions. A peristaltic pump (040D model) was used to propel the solutions.

**Column preparation**

The mini-column was prepared by firmly packing 100 mg C$_{18}$ in a polyethylene syringe tube (40 mm length and 2 mm inner diameter) plugged with glass wool at both ends. The sorbent was washed thoroughly by 1.0 mol L$^{-1}$ HNO$_3$ followed by ethanol and finally with distilled water. The performance of the mini-column was stable at least over 20 preconcentration cycles.

**Operating procedure**

The online manifold of the developed method is shown in Fig. 1. Details for the operational sequence of the solid phase preconcentration system are given in Table 1. In the preconcentration step [Fig 1(a)], the injection valve (V) turned to the load position and pump (P$_1$) was activated. In sample solution, Cu(II),

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**Table 1**—Operating sequence of FI on-line solid-phase preconcentration system for FI-FAAS determination of Cu(II)

<table>
<thead>
<tr>
<th>Step</th>
<th>Valve position</th>
<th>Pumps</th>
<th>Delivered medium</th>
<th>Flow rate, mL min$^{-1}$</th>
<th>Time, s</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Load</td>
<td>On</td>
<td>Off</td>
<td>Sample</td>
<td>6</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>Injection</td>
<td>Off</td>
<td>On</td>
<td>Ethanol</td>
<td>7.5</td>
<td>60</td>
</tr>
</tbody>
</table>

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Fig. 1.—FI-manifold and operation sequence for on-line copper FAAS determination (a) preconcentration step and (b) elution step [Sample—copper solution containing neocuproine and buffer, FAAS—flame atomic absorption spectrometer, P$_1$, P$_2$—peristaltic pumps, V—injection valve, C—column packed with octadecylsilica, and W—waste]
neocuproine, hydroxylamine hydrochloride and pH 5 buffer were merged together, and the formed Cu(I)-neocuproine complex was retained by the sorbent. During the elution step [Fig 1(b)], the injection valve (V) was turned to the injection position and P2 was activated, then the retained complex was eluted at the rate of 7.5 mL min$^{-1}$ and transported directly into the nebulizer of the spectrometer. For minimum dispersion, the elution was performed in the reverse direction relative to that of the loading. The peak height of the transient signal was proportional to Cu(II) concentration in the sample, and was used for all measurements. Three replicate measurements per sample were made in all instances.

**Results and Discussion**

Some preliminary experiments were carried out to investigate quantitative retention of Cu(II) ions by the C18 packed mini-column in the absence and presence of neocuproine. It is found that while the mini-column does not show any tendency for Cu(II) ions, it is capable of retaining copper-neocuproine complex in the sample solutions quantitatively. The test solution used contained 15 µg Cu(II) and 5 mg neocuproine in 50 and 250 mL water.

**Effect of pH**

The influence of pH on the SPE of Cu(II) ions was studied in the pH range 2-10, adjusted by using HCl (0.5 M) or NH$_3$ (0.5 M). The results reveal that the absorbance depends on the pH of the solution, which is found to be nearly constant in the pH range 4-6. At pH values higher than 6, however the absorbance decreases, probably due to the competition of NH$_3$ with neocuproine and formation of a non-retained Cu(NH$_3$)$_4^{2+}$ complex. At pH values lower than 4 the protons seem to compete with metal ions for the ligand, and hence the extraction yield is decreased. Thus, pH 5 is selected for subsequent extraction experiments.

**Effect of amount of neocuproine and hydroxylamine-hydrochloride**

In order to optimize the amount of neocuproine for quantitative extraction of copper by the mini-column, the extraction was conducted by varying the amount of ligand from 0 mg to 14 mg in presence of 0.3 g reducing agent. According to the obtained results the extraction of copper is found to be quantitative using above 6 mg of the ligand. Hence, subsequent extraction experiments were carried out with 8 mg of the ligand. In these experiments, Cu(II) ions are reduced to Cu(I) ions by using hydroxylamine hydrochloride, and form a very stable complex with neocuproine. Experiments show that for quantitative extraction of copper ions more than 0.2 g of reducing agent is needed. Thus, 0.5 g of reducing agent is used in further experiments.

**Effect of sample flow rate**

In on-line preconcentration systems with time-based sampling mode, the sample flow rate determines the amount of sample to be processed in a given time. This rate is limited by flow resistance of the column and the reaction rate. The effect of the sample flow rate during the preconcentration step was studied in the range 1.5-9 mL min$^{-1}$. It is observed that the absorbance is independent of sample flow rate up to 9 mL min$^{-1}$, implying that the kinetic of the complexation and extraction is very fast and the contact time for complete sorption is sufficient. This is a significant advantage over other on-line preconcentration systems using column or knotted reactors. At 120 s preconcentration time, a flow rate of 6 mL min$^{-1}$ is selected for high sensitivity.

**Effect of sample loading time**

The influence of sample loading time was investigated in the range 30-180 s. The absorbance increases almost linearly within the studied range, indicating that no partial leaching of the complex is observed as it is occurred in the systems that employ the tubing mini-column sorbents. A 120 s loading time is therefore chosen as a compromise between sensitivity and sampling frequency.

**Effect of eluent type and flow rate**

Organic solvents, like ethanol and methanol have been extensively used as effective eluents in FI on-line solid phase extraction preconcentration systems. However, ethanol is considered to be more convenient for FAAS, producing higher and sharper signals. Thus, ethanol is adopted as eluent and the elution time is fixed at 60 s for complete elution. The effect of ethanol flow rate was studied within the range 1.5-9 mL min$^{-1}$. Maximum absorbance is achieved within the range 6-9 mL min$^{-1}$, according to the obtained results. At flow rates lower than 6 mL min$^{-1}$, the absorbance decreases, mainly due to the incompatibility between elution and nebulizer uptake flow rates. Thus, a flow rate of 7.5 mL min$^{-1}$ is selected, to compromise between the concentration factor and analytical time.
Interference studies

Although it is well known that neocuproine is a selective chelating agent for Cu(I) due to its great ability to form stable complexes in acidic medium, the effect of potential interferents occurring in environmental samples on the on-line determination of Cu(II) was tested using the optimized on-line preconcentration system. The recovery of 5 µg L\(^{-1}\) Cu(II) solution was tested with individual interferents added. Taking as criterion for interference the deviation of the recovery more than ±3%, the obtained results show that Al(III), Cd(II), Co(II), Cr(III), Fe(III), Mn(II), Pb(II), Ag(I) and Zn(II) are tolerated up to 50 mg L\(^{-1}\), while Hg(II) and Ni(II) are tolerated up to 10 and 5 mg L\(^{-1}\) respectively. Moreover, the potential interferences from some common matrix cations such as Na(I), Ca(II), Mg(II) and Be(II) were also investigated. They are tolerated at concentrations at least up to 1000 mg L\(^{-1}\).

Analytical performance

The analytical performance characteristics of the proposed method are listed in Table 2. For 120 s preconcentration time, the enhancement factor is 100 (comparing the slope of the proposed method with that of direct aspiration of aqueous standard solutions into FAAS). The calibration graph is found to be linear over the linear range 0.1-20 µg L\(^{-1}\). The detection limit is calculated by 3σ criterion and found to be 0.15 µg L\(^{-1}\). The precision, as relative standard deviation (RSD), for 7 replicate measurements at the 5.0 µg L\(^{-1}\) level of Cu(II) is 1.2%.

Applications

In order to evaluate the accuracy of the proposed method for copper determination certified reference materials, CASS-4 (near shore seawater) and CRM1643d (Trace elements in water) were analyzed. As it is shown in Table 3, the observed values are in good agreement with the certified ones and the calculated recoveries (98–100%) are satisfactory. The proposed method has been applied also to the analysis of water samples (tap, mineral and waste water) and is validated by spiking the samples with known amounts of Cu(II). The recoveries from spiked samples are varied in the range 98–102% and the relative standard deviation for copper determination in the examined samples varied in the range 3.1–4.9%. The Comparative data with some other selected previous on-line solid phase extraction reports are given in Table 4. As can be seen, the relative standard deviation and preconcentration factor of the present method are superior to those of reported methods\(^{24-28}\).

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**Table 2**—Analytical performance of FI on-line solid-phase extraction FAAS method for copper determination using mini-column packed with C\(_{18}\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Column packed with C(_{18})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconcentration time, s</td>
<td>120</td>
</tr>
<tr>
<td>Enhancement factor</td>
<td>100</td>
</tr>
<tr>
<td>Sample flow rate, mL min(^{-1})</td>
<td>6</td>
</tr>
<tr>
<td>Elution time, s</td>
<td>60</td>
</tr>
<tr>
<td>Linear range, µg L(^{-1})</td>
<td>0.1-20.0</td>
</tr>
<tr>
<td>Detection limit (3σ), µg L(^{-1})</td>
<td>0.15</td>
</tr>
<tr>
<td>Precision (RSD, n = 7), %</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Table 3**—Analytical results of copper determination in the certified reference materials and water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Certified value</th>
<th>Found</th>
<th>Spiked</th>
<th>Found *</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>-</td>
<td>2.00</td>
<td>3.05±0.09</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Mineral water</td>
<td>-</td>
<td>2.00</td>
<td>2.50±0.08</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Waste water</td>
<td>-</td>
<td>2.00</td>
<td>4.70±0.05</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>CASS-4</td>
<td>0.59±0.05</td>
<td>0.58±0.02</td>
<td>98.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRM 1643d</td>
<td>20.5±3.8</td>
<td>20.5±1.0</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean value ± S.D. based on three replicate determinations.

**Table 4**—Comparison of the characteristic data with recently published on-line solid phase extraction methods

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Adsorbent</th>
<th>Reagent</th>
<th>E F</th>
<th>LOD µg L(^{-1})</th>
<th>RSD, %</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu, Pb</td>
<td>Polychlorotrifluoroethylene</td>
<td>Diethyldithiophosphate</td>
<td>250</td>
<td>2.0</td>
<td>1.8</td>
<td>24</td>
</tr>
<tr>
<td>Cu</td>
<td>Silica gel-modified 3(1-imidazolyl) propyl</td>
<td>3(1-imidazolyl) propyl</td>
<td>-</td>
<td>0.4</td>
<td>1.2</td>
<td>25</td>
</tr>
<tr>
<td>Cu</td>
<td>Amberlite XAD-2</td>
<td>-</td>
<td>32</td>
<td>0.15</td>
<td>4.5</td>
<td>26</td>
</tr>
<tr>
<td>Cu</td>
<td>Cotton</td>
<td>CTAB</td>
<td>-</td>
<td>0.4</td>
<td>1.3</td>
<td>27</td>
</tr>
<tr>
<td>Cu</td>
<td>Amberlite XAD-2</td>
<td>Calmagite</td>
<td>-</td>
<td>0.15</td>
<td>2.4</td>
<td>28</td>
</tr>
<tr>
<td>Cu</td>
<td>Silicoctadecyl (C(_{18}))</td>
<td>Neocuproine</td>
<td>100</td>
<td>0.15</td>
<td>1.2</td>
<td>This work</td>
</tr>
</tbody>
</table>

E F—Enhancement factor, LOD—Limit of detection, RSD—Relative standard deviation.
Conclusion
The newly developed method is simple, fast, selective and precise for separation, preconcentration and determination of ultra trace amounts of Cu(II). A mini-column packed with commercially available octadecyl bonded silica stationary phase has been used for the on-line concentration of Cu(II) ions. The method is highly accurate and reliable, permitting its application to the real samples (with complex matrices).

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References