In-Tube Solid-Phase Microextraction/Electrothermal Atomic Absorption Spectrophotometry of Copper

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A chemically modified silica capillary tube (50 cm × 0.22 mm i.d.) was used as the sorbent for on line in-tube solid-phase microextraction/electrothermal atomic absorption spectrometry (ITSPME/ETAAS). The inner wall of the silica capillary was modified with octadecyl siloxane by the repeated reaction with octadecyltrichlorosilane and the oligomeric phase was constructed on the surface of the inner wall. Then, ITSPME/ETAAS was carried out by connecting the modified silica capillary to a sample nozzle of an autosampler. A sample solution containing up to 0.5 ng Cu ml⁻¹ of the copper(I)-bathocuproine (Cu-BC) complex was sucked through the modified capillary at a flow rate of about 0.22 ml min⁻¹ for 6 min. The extracted Cu-BC complex was eluted by 60 µl of methanol and the eluate was directly injected into ETAAS. The linear relationship between absorbance divided by the sample volume and the concentration of copper in the sample solution (0 - 0.5 ng ml⁻¹) was obtained. The relative standard deviations were 2.1-8.4% for the repeated runs of 0.5 ng ml⁻¹ of copper. ITSPME/ETAAS is suitable for an enrichment of a desired trace element in a small sample.

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Preparation of a chemically modified silica capillary tube

A chemically modified silica capillary was prepared as follows. At first, the inner surface of a PEEKsil Tubing (aluminum coated fused silica capillary tube having 500 mm in long, 1.59 mm in external diameter and 0.22 mm in internal diameter) obtained from GL Sciences (Tokyo) was treated with 0.3 M sodium hydroxide solution for 48 hr. After the surface treatment, the capillary tube was washed by running each 25 ml of water, ethanol, acetone, and dry toluene in that order with a model UF 5004PS delivery pump (Tokyo Rika, Tokyo) at 1.6 ml min⁻¹ flow rate. Silanilization was carried out by circulating 1% n-octadecyltrichlorosilane solution through the capillary tube placed in a oven (Yamato, model DX-58) at 100 °C for 24 hr with the delivery pump. After silanilization, the capillary tube was washed with each 25 ml of dry toluene, acetone, ethanol, and water in that order. This sequence produces a monomeric bonded phase with additional silanol sites for further reaction. An oligomeric bonded phase was synthesized by building up one silane unit at a time by repeating the process between the initial and second wash steps. After the process was repeated ten times, the residual silanol group was endcapped with chlorotrimethylsilane and the resulting C₁₈ bonded capillary tube was washed as stated above.

Apparatus

The measurement of atomic absorption of copper was carried out by a model AA-6800 atomic absorption spectrophotometer equipped with a model GFA-EX7 graphite furnace atomizer (Shimadzu Corporation, Kyoto). A pyrolytic graphite tube was used for the atomization of copper. A hollow cathode lamp was obtained from Hitachi (Tokyo). The operating condition of ETAAS is shown in Table 1. A model ASC-6100 autosampler (Shimadzu Corporation, Kyoto) was modified for ITSPME as follows. (1) A six-port selection valve (Rheodyne, California, USA) connecting the chemically modified silica capillary was inserted between the sample nozzle and the microsyringe of the autosampler. The sample nozzle (14.5 cm × 0.2 mm i.d. of polytetrafluoroethylene (PTFE) tube) was connected to the capillary tube. (2) The 250 µl of microsyringe of the autosampler was replaced by a 500 µl one (Hamilton, Nevada, USA) to ensure that the eluent could be delivered to the graphite tube through the PTFE piping from the six-port selection valve to the silica capillary tube. (3) A model A-3S aspirator with a model VG-1 vacuum gauge (Tokyo Rika) was connected to the six-port selection valve for the suction of sample solution under controlled pressure. The schematic diagram of ITSPME/ETAAS system is shown in Fig. 1. The atomic absorption spectrometer and the autosampler, except for the six-port selection valve, were controlled by a software program "WizAArd" on a personal computer.

General Procedure

Table 2 shows the sequence of the operation of ITSPME/ETAAS. Two sample cup was set on the turntable of the autosampler. One contained water. To another cup, a 1-2 ml portion of test solution containing 0.5 ng Cu ml⁻¹ of the Cu-BC complex, 0.04 µM BC, 1.43 M hydroxylammonium chloride, and 1% methanol was poured. ITSPME was carried out as followed. At first, the chemically modified capillary tube was thoroughly washed with eight portion of 460 µl of methanol to elute any residual Cu-BC complex. Position of the six-port selection valve was set at VP 1 when methanol was sucked from the methanol reservoir and that was VP 2 when methanol was passed through the capillary tube (Figure 1). The switching of the valve position was done manually. Next, at VP 2, the capillary tube was conditioned with about 360 µl of water by repeating 'Nozzle Rinse' command of 'WizAArd' three times. At an extraction step, the sample nozzle was moved to the sample cup. The special command for the maintenance mode in the AAS software program "WizAArd" was used for the sample nozzle operation. After the vacuum pressure was reached to 0.04 MPa, then, the valve position was switched to VP 1 and the timer was started. The test solution was sucked from the sample cup about 0.24 ml min⁻¹ and taken in a plastic cup after passed through the capillary tube. After 360 s, the valve position was switched to VP 2 and the sample nozzle was moved to the water cup on the turntable. The volume of test solution in the plastic cup was measured by a micropipette. The valve was switched to VP 1 to wash the capillary tube by sucking water at 0.01 MPa for 30 s. The sample nozzle was then moved to the empty cup to drain water. Finally, the 'Start' icon of 'WizAArd' was clicked to initiate ETAAS measurement. In this step, 130 µl of methanol was sucked into the microsyringe from the reservoir. The volume of methanol depends on the length of PTFE tube used for the piping, i.e., the sum of the volume of the PTFE piping
from the valve, the capillary column, the sample nozzle, and the injecting volume (60 µl) was the volume required. After the suction of methanol was completed, the valve position was switched to VP 2, the Cu-BC complex adsorbed on the C18 phase was eluted at a flow rate of 6 µl s⁻¹, and the solvent front of 60 µl was directly injected to the graphite tube. The atomic absorption was measured under the operating conditions shown in Table 1. The integral absorbance obtained was divided by the sample volume passed through the capillary tube because it was difficult to take definite volume of test solution by suction with the aspirator. The measurements were repeated more than three times and the average was calculated.

Extraction following in situ complex formation
The conditions of the complex formation reaction of copper(II) with BC were described earlier and applied after the minor modifications as follows. The pH of the water sample containing up to 2.0 ng ml⁻¹ copper(II) was adjusted to pH 2 by adding 6 M hydrochloric acid and 2.0 ml of the aliquot was taken in a sample cup of ETAAS. To the test solution, 20 µl of 1.43 M hydroxylammonium chloride solution, 20 µl of 0.2 mM BC methanol solution, and 30 µl of 3 M sodium acetate solution were added. The whole was mixed well and allowed to stand for 10 min. Then, the test solution was subjected to ITSPME/ETAAS as described in the general procedure.

Results and Discussion
ITSPME/ETAAS system
Figure 1 and Table 2 show the schematic diagram and its operational sequence in ITSPME/ETAAS. One of the characteristics of this system is that a test solution is sucked from the open end of sample nozzle and the elution is performed with the reversed flow direction. This may result in the highly efficient extraction and elution. Furthermore, the undesirable adsorption of a hydrophobic complex of a desired element on the inner wall of the piping could be avoided. On the other hand, it is a disadvantage to prepare a test solution manually.

Effect of the eluent
The effect of the elution volume. As the eluent for ITSPME, methanol was used since it was expected that a polar solute such as the Cu-BC complex could be successively eluted. In the preliminary experimental to elucidate the effect of methanol on the ETAAS of copper, the effect of the injection volume into graphite tube was investigated by injecting 10-100 µl of the methanol solution of the Cu-BC complex containing the 0.2 ng of copper. The results showed that the maximum and constant absorbance was obtained up to 20 µl of the injection, however, the absorbance decreased with increasing volume of injection in the range 30-100 µl (Fig. 2a).

The effect of the elution rate. The effect of the elution rate was studied by varying the injection rate of the microsyringe of the autosampler in the range 6-40 µl s⁻¹. As expected, the absorbance increased with decreasing rate of the elution. Then, the elution was carried out at 6 µl s⁻¹ for further experiments.

Effect of the addition of BC
The equilibria involved in ITSPME of the Cu-BC complex are the complex formation or dissociation in the solution

$$\text{Cu}^+ + 2\text{BC} \rightleftharpoons [\text{Cu(BC)}_2]^+$$

and the adsorption or partition of the complex to the C₁₈ phase

$$[\text{Cu(BC)}_2]^+ + X^- \rightleftharpoons [\text{Cu(BC)}_2X]^{\text{in C}}_{\text{18}}$$

where X⁻ is a counter anion such as nitrate, chloride, or acetate. Then, the effect of the addition of BC was examined according to a general procedure varying the concentration of BC added in the range 0-0.12 µM. These correspond to zero to fifteen-fold concentration of the Cu-BC complex. It was found that the absorbance increased with the increased concentration of BC in the range 0-0.016 µM, and reached to the maximum and constant at 0.04 µM.

Table 2: Operational Sequence of ITSPME/ETAAS

<table>
<thead>
<tr>
<th>Step</th>
<th>Purpose</th>
<th>Pune</th>
<th>Sample nozzle position</th>
<th>Suction (vacuum pressure)</th>
<th>Command of ‘WizAArd’</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>washing by methanol</td>
<td>VP 1 ← VP 2</td>
<td>home</td>
<td>OFF</td>
<td>‘Move to the upper limit’ and ‘Move to the lower limit’ of the microsyringe position, four times, respectively</td>
</tr>
<tr>
<td>2</td>
<td>conditioning by water</td>
<td>VP 2</td>
<td>home</td>
<td>OFF</td>
<td>‘Nozzle Rinse’, three times</td>
</tr>
<tr>
<td>3</td>
<td>extraction (360 s)</td>
<td>VP 1</td>
<td>sample cup</td>
<td>ON (0.04 MPa)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>washing by water (30 s)</td>
<td>VP 1</td>
<td>water cup</td>
<td>ON (0.01 MPa)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>drain</td>
<td>VP 1</td>
<td>empty cup</td>
<td>ON (0.04 MPa)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>elution and injection (60 µl)</td>
<td>VP 1 → VP 2</td>
<td>graphite tube</td>
<td>OFF</td>
<td>‘Start’</td>
</tr>
</tbody>
</table>

Fig. 2 (a) Effect of the injection volume on the absorbance of copper. The methanol solutions containing 0.2 ng of copper was injected. (b) Effect of the elution volume used in ITSPME/ETAAS. Sampling time of 1 ng Cu ml⁻¹ Cu-BC complex solution, 120 s; BC solution and hydroxylammonium chloride solution were not added in the test solution. The blank was subtracted.
The effect of the addition of sodium dodecyl sulfate was also studied. However, no significant effect was observed.

**Effect of the sampling time**

The effect of a sampling time was studied by varying the sampling time in the range 2-6 min. The results were shown in Fig. 3a. A linear relationship between the sampling time and the suction time was observed. The flow rates were 0.22-0.23 ml min\(^{-1}\) and the relative standard deviations were 6.6-7.5%. It was found that it did not depend on the flow rate, as shown in Fig. 3b. This indicates that the sampling at a rapid flow rate is more advantageous and based on the slopes of the regression equation, it can be concluded that 8.6-fold enrichment was achieved by ITSPME/ETAAS, when 1.0 ml portion of sample solution was used. Furthermore, the yield of the enrichment was calculated to be 52%.

On the other hand, the regression equation of the calibration graph prepared as described in "Extraction following in situ complex formation" was \( y = 0.142x + 0.107 \) \((r = 0.997)\). It is apparent that the optimization of ITSPME was needed because the slope was lowered to one fourth. The rather high blank value may be attributed to the migration of copper from the silica wall in the relatively high ionic strength.

In conclusion, ITSPME/ETAAS with a modified silica capillary tube has a possibility to become a useful on-line SPE system for an enrichment and determination of a desired trace element in a small sample. We were able to perform over two hundred times extraction with the same capillary tube without appreciable changes in the extraction performance. Although, the attempts to lower the blank and to establish the optimum conditions for a real sample are required.

**Calibration graph**

The calibration graph was prepared by varying the concentration of the Cu-BC complex in the range 0-0.5 ng Cu ml\(^{-1}\) as described in general procedure. The regression equation was \( y = 0.588x + 0.059 \) \((r = 0.998)\), where \( y \) is the integrated absorbance divided by the sample volume in milliliter and \( x \) is the concentration of the Cu-BC complex in ng Cu ml\(^{-1}\). The other calibration graph was prepared by injecting 60 µl of 0-5 ng Cu ml\(^{-1}\) of the Cu-BC complex methanol solution. The regression equation was \( y = 0.0680x + 0.0016 \) \((r = 0.999)\), where \( y \) is the integrated absorbance. Comparing two regression equations, it can be concluded that 8.6-fold enrichment was achieved by ITSPME/ETAAS, when 1.0 ml portion of sample solution was used. Furthermore, the yield of the enrichment was calculated to be 52%.

**References**