Determination of Nanogram Amounts of Copper(II) by the Catalytic-Photometric Method

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The effect of several buffers was examined for the catalysis of copper(II) in the reaction of N,N-dimethyl-p-phenylenediamine with N,N-dimethylaniline to form Bindschedler's Green (λ_{max}=725 nm) in the presence of hydrogen peroxide. The buffer agents which were examined included imidazole, 2,4,6-trimethylpyridine (TMP), piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 3-(N-morpholino)-propanesulfonic acid (MOPS), and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES). Among them TMP was found to be the most favorable agent in this reaction system. The established catalytic method is given for the 0.05 - 1.0 ng cm^{-3} range of copper(II), and has an effective sensitivity index of 1.1X10^{-3} ng cm^{-2}.

Keywords Catalytic method, copper(II), N,N-dimethyl-p-phenylenediamine, N,N-dimethylaniline, natural water

The application of metal-catalyzed reactions to the determination of traces of metal ions, kinetic-catalytic methods, is now well established. The advantages of these methods include extremely high sensitivity, simple instrumentation and simplicity of operation. Numerous catalytic methods for determining of nanogram and/ or subnanogram levels of metal ions have been reported, which are based on their catalytic effects on a large number of indicator reactions. Trace amounts of copper have also been catalytically determined by using various redox reactions. Gutierrez et al. have developed a sensitive fluorometric method for copper(II) based on the hydrogen peroxide oxidation of 1,1,3-tricyano-2-amino-1-propene, and have successfully applied it to the determination of copper in blood serum. The oxidation of 3-hydroxybenzaldehyde azine by peroxidisulfate was employed for the photometric-catalytic determination of copper(II). This method had a detection limit of 0.17 ng cm^{-3}, and was applied to the determination of copper in food and industrial samples. Photometric-catalytic methods by using the oxidative coupling reactions of p-anisidine, N-phenyl-p-phenylenediamine and 3-methyl-2-benzothiazolione hydrazone with N,N-dimethylaniline have also been proposed. The dynamic range of these methods was from 0.1 ng cm^{-3} to 10 ng cm^{-3}; most of them suffered from interference by several ions, which severely limited their applications. Hence, the development of a sensitive and selective catalytic method for the determination of copper(II) is still expected.

A short communication by the present authors has been presented regarding the analytical application of the catalytic effect of copper(II) on the reaction of N,N-dimethyl-p-phenylenediamine (DPD) with N,N-dimethylaniline (DMA) to form Bindschedler's Green, N,N-dimethyl-N'-p-dimethylanilino-1,4-benzenediiminonium ion, in the presence of hydrogen peroxide. However, problems still remained; an increase in the concentration of phosphate used as a buffer decreased the rate of the catalyzed reaction and the working curves showed curvature. Lunar et al. improved this catalytic method by the addition of surfactants using the initial rate procedure; the detection limit of the improved method was 0.5 ng ml^{-1} at 50°C. In this work, we re-examined several conditions for the catalytic determination of copper(II) using this coloration. Especially, the effect of several buffer agents on color development was examined in detailed. As a result, the sensitivity and reproducibility of the method could be improved by using 2,4,6-trimethylpyridine as a buffer. Copper concentrations as low as 10^{-9} mol dm^{-3} can be determined from the increase in the absorbance by the fixed-time procedure at 25°C. The method is highly sensitive with an effective sensitivity index of 1.1X10^{-3} ng cm^{-2} and allows the direct determination of copper in tap, river, lake and sea-water samples at nanogram amounts.

Experimental

Reagents
A stock solution of copper(II) (1.0 mg cm^{-3}), prepared from copper(II) sulfate pentahydrate in 0.1 mol dm^{-3}
hydrochloric acid, was standardized by EDTA and was suitably diluted as required. A 1.2×10⁻³ mol dm⁻³ DPD solution was prepared from the dihydrochloride (Wako Junyaku Co.) in 0.1 mol dm⁻³ hydrochloric acid. A 4.0×10⁻² mol dm⁻³ DMA solution was prepared from a compound purified by distillation under reduced pressure in 0.1 mol dm⁻³ hydrochloric acid. A 30% hydrogen peroxide solution (Mitsubishi Gasu Kagaku Co.) was titrated by a standard permanganate solution and a 3.0 mol dm⁻³ aqueous solution was prepared. 2,4,6-Trimethylpyridine (TMP) was purified by distillation under reduced pressure and a 0.15 mol dm⁻³ TMP solution was prepared in water. Ammonia (for the determination of toxic metals from Wako Junyaku Co.) was used without further purification and a 1.0 mol dm⁻³ solution was prepared.

All solutions used were prepared with water from a Millipore Milli-Q purification system (Millipore Co.). All other chemicals used were of analytical grade.

**Apparatus**

Hitachi Model 100-10 and 200-10 spectrophotometers with 10-mm glass cells were used for absorbance and absorption spectra measurements, respectively. A Toa Model HM-6A pH meter was employed for pH measurements. All of the reactions were carried out in a Toyo Model EPS-45 circulating thermostatted bath. A Hitachi Model 170-70 Zeeman atomic absorption spectrophotometer was used to evaluate the proposed method.

**Recommended procedure**

To an aliquot sample solution containing up to 25 ng of copper in a beaker, add 2.5 cm³ of TMP, 1.5 cm³ of ammonia, 1.0 cm³ of DMA and 1.0 cm³ of DPD solutions. Adjust the pH of the solution to 7.1–7.6 with 1.0 mol dm⁻³ hydrochloric acid. Transfer the solution to a 25-cm³ volumetric flask and dilute to ca. 24 cm³ with water. After keeping the solution at 25±0.1°C in a thermostat for 10 min to attain thermal equilibrium, start the reaction by mixing 0.5 cm³ of a hydrogen peroxide solution and making up to the mark with 25°C water. Keep the mixed solution in the thermostat during the reaction. Exactly 10 min after the initiation of the reaction, measure the absorbance at 725 nm in a 10-mm glass cell against a water reference. Run a reagent blank concurrently. Obtain the net absorbance by subtracting the blank absorbance.

**Results and Discussion**

Bindschedler’s Green leuco base (BGL), N,N-di- methyl-(p-dimethylaminophenyl)-p-phenylenediamine, which is produced by the reaction between DPD and DMA in the presence of hydrogen peroxide, is oxidized by copper(II) to a green compound, N,N-diethyl-N’-(p-dimethylaminophenyl)-1,4-benzoquinonediiminium ion (λmax=725 nm). The reduced copper(I) is oxidized again to copper(II) by hydrogen peroxide. As a result of the regeneration of copper(II), the oxidation of BGL proceeds catalytically.

The absorption spectra of the reaction mixture with and without copper(II) showed an absorption maximum at 725 nm (described previously). The reaction was monitored at this wavelength.

**Selection of a suitable buffer**

In the previous work, the maximum rate of the copper(II)-catalyzed reaction was observed in the pH range 7.0–7.4. The pH of the solution was adjusted by using phosphate, ammonia and hydrochloric acid. However, the rate of the catalyzed reaction was retarded with increasing concentration of phosphate used as a buffer. However, the reaction proceeded faster at higher concentrations of ammonia, due to its activating effect for the catalytic action of copper(II) on the coloration. The suitability of several buffers was thus examined in this study. The buffers examined were as follows: TMP, imidazole, piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), N,N'-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 3-(N-morpholino)propanesulfonic acid (MOPS) and N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES). It was observed that the presence of these buffers in this system slightly changed the optimum reaction pH region. The effect of these buffer concentrations was examined in each optimal pH range. As shown in Fig. 1, these buffers (except for TMP and BES) retarded the reaction rate in a manner similar to that observed for phosphate. However, it is still not understood why the rate should decrease depending on the buffer used. On the other hand, TMP and BES were found to be suitable buffers since there was no effect on the rate of the catalyzed reaction. Figure 2 shows the effect of the pH on the color development in the presence of both buffers.
can be seen, TMP is found to be a suitable buffer in this reaction system, because the maximum and constant absorbance range for TMP is wider than that for BES. A 1.5×10^{-2} \text{ mol dm}^{-3} \text{ TMP concentration was used and a pH between 7.1 - 7.6 was selected for the procedure. As described above, ammonia acted as an activator on the catalysis of copper(II); the absorbance for the catalyzed reaction became higher upon increasing the concentration of ammonia. While the blank absorbance was kept low over the concentration examined. The total concentration of ammonia was fixed at 6.0×10^{-2} \text{ mol dm}^{-3} for the procedure.}

**Effect of the temperature and reaction time**

The effect of the temperature on the uncatalyzed and catalyzed reactions was examined, at 20°, 25° and 30°C in the presence of TMP. As expected, the reaction proceeded faster with an increase in temperature. However, an increase in temperature and/or the reaction time also increased the blank absorbance. Reaction times of 8, 10 and 14 min were recommended at 30°, 25° and 20°C, respectively; a reaction temperature of 25°C and a reaction time of 10 min were selected for the sake of low blank and reproducibility.

**Effect of reagent concentration**

The effect of DPD concentration on the color development is shown in Fig. 3. As can be seen, the absorbance of the reaction mixture with copper(II) remained constant at DPD concentrations higher than 4.0×10^{-5} \text{ mol dm}^{-3}. The blank absorbance slightly increased with increasing DPD concentration. Its concentration was selected as 4.8×10^{-5} \text{ mol dm}^{-3} for the procedure. The color development increased upon increasing the DMA concentration up to 1.5×10^{-3} \text{ mol dm}^{-3}; a slight increase in the absorbance was then observed at higher DMA concentrations. The blank absorbance was kept low over the DMA concentration examined. A 1.6×10^{-3} \text{ mol dm}^{-3} DMA concentration was selected for the procedure. The rate of the catalyzed reaction also increased with increasing the concentration of hydrogen peroxide, as shown in Fig. 4. The hydrogen peroxide concentration was fixed at 6.0×10^{-2} \text{ mol dm}^{-3} in order to realize high sensitivity and reproducibility.

**Calibration curves**

Typical working curves for the recommended procedure showed that Beer's law was obeyed over the copper(II) concentration range 0.05 - 1.0 ng cm^{-3} of the working solution. An effective sensitivity index calculated from the working curve is 1.1×10^{-3} \text{ ng cm}^{-2}; the sensitivity of the present method is higher than that reported by the others.\textsuperscript{4-12} The reproducibility of the method is satisfactory with relative standard deviations of 4.5, 3.2 and 2.0% for seven determinations of 0.2, 0.4 and 1.0 ng cm^{-3} of copper(II), respectively.

**Effect of diverse ions**

The effect of diverse ions on the determination of 15 ng
of copper(II) per 25 cm³ of the working solution was examined. The following ions and compounds caused no interference at least up to the specified amounts: 5 mg of Ba²⁺, Ca²⁺, Mg²⁺, Sr²⁺, F⁻, Br⁻, Cl⁻, sodium chloride, potassium chloride, potassium nitrate, sodium dihydrogen phosphate and sodium borate; 50 µg of As⁵⁺, As³⁺, Bi³⁺, Mn³⁺, S²⁻, and NO₂⁻. The results for the other ions are summarized in Table 1. Most of the ions listed in the Table (even at least 33-fold by weight than copper(II)) do not affect the determination of copper(II). It should be emphasized that coexisting iron(III) at amounts below 0.5 µg does not interfere with the determination of copper(II), even when no masking reagent is present.

### Table 1 Effect of diverse ions on the determination of 15 ng of copper(II) per 25 cm³ by the proposed method

<table>
<thead>
<tr>
<th>Ion added</th>
<th>Amount added/µg</th>
<th>Cu found/ng</th>
<th>Ion added</th>
<th>Amount added/µg</th>
<th>Cu found/ng</th>
<th>Ion added</th>
<th>Amount added/µg</th>
<th>Cu found/ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag⁺</td>
<td>5</td>
<td>15</td>
<td>Co²⁺</td>
<td>5</td>
<td>18</td>
<td>Sn⁴⁺</td>
<td>5</td>
<td>6</td>
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<tr>
<td>Al³⁺</td>
<td>5</td>
<td>16</td>
<td>Fe³⁺</td>
<td>5</td>
<td>15</td>
<td>Pb⁴⁺</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Ca⁴⁺</td>
<td>5</td>
<td>7</td>
<td>Mn⁵⁺</td>
<td>5</td>
<td>15</td>
<td>Ti⁴⁺</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.5</td>
<td>15</td>
<td>Mo⁴⁺</td>
<td>0.5</td>
<td>15</td>
<td>Te⁴⁺</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Sr²⁺</td>
<td>0.25</td>
<td>15</td>
<td>S²⁻</td>
<td>0.5</td>
<td>15</td>
<td>Zn²⁺</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>F⁻</td>
<td>5</td>
<td>15</td>
<td>Cl⁻</td>
<td>0.5</td>
<td>15</td>
<td>W⁶⁺</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Br⁻</td>
<td>5</td>
<td>15</td>
<td>Br⁻</td>
<td>0.5</td>
<td>15</td>
<td>I⁻</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>5</td>
<td>15</td>
<td>NO₂⁻</td>
<td>0.5</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Application to water analysis

The present method was applied to the analysis of copper in various types of water samples without any separation or preconcentration procedures. After using a pretreatment (described elsewhere), copper in tap, river, lake and sea-water samples was directly analyzed by the standard addition method in order to minimize the matrix effect. At the same time, the samples (except for seawater) were digested with a mixture of nitric and perchloric acids and were analyzed by the proposed method, since the fractions of organically-bound copper species in natural water did not exhibit any catalytic effect. The results obtained by the present method (Table 2) were compared with those obtained by graphite-furnace atomic absorption spectrometry (GFAAS) coupled with an extraction procedure. The values for river- and lake-water samples obtained by the present method are smaller than those obtained by GFAAS. On the other hand, the copper contents for the digested samples were almost the same as those obtained by GFAAS. From this result, the present method seems to be applicable to the determination of certain species of copper in natural water.

### Table 2 Determination of copper in tap, river, lake and seawater samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Copper in sample/µg cm⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present method</td>
</tr>
<tr>
<td></td>
<td>Standard addition method</td>
</tr>
<tr>
<td>Tap water</td>
<td>2.1 ±0.1</td>
</tr>
<tr>
<td>River water</td>
<td>2.1 ±0.1</td>
</tr>
<tr>
<td>Kyufukuro-gawa</td>
<td>0.2±0.006</td>
</tr>
<tr>
<td>Sendai-gawa</td>
<td>0.7±0.008</td>
</tr>
<tr>
<td>Lake water</td>
<td></td>
</tr>
<tr>
<td>Tanega-ike</td>
<td>0.2±0.008</td>
</tr>
<tr>
<td>Koyama-ike</td>
<td>0.3±0.005</td>
</tr>
<tr>
<td>Togo-ike</td>
<td>0.5±0.005</td>
</tr>
<tr>
<td>Seawater</td>
<td></td>
</tr>
<tr>
<td>Kuro</td>
<td>0.4±0.005</td>
</tr>
<tr>
<td>Sakonotsu</td>
<td>0.2±0.002</td>
</tr>
<tr>
<td>Hawaii</td>
<td>0.2±0.005</td>
</tr>
</tbody>
</table>

a. Collected in Tottori Prefecture, Japan.
b. Average of three determinations.
c. 5–15 cm³ of samples were used.
d. 300 cm³ of each sample was used.

### References


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