Kinetic-catalytic methods based on catalytic reactions have been recognized as effective techniques for trace analyses. In catalytic methods based on metal-catalyzed reactions, the addition of a ligand as an activator to the reaction system improves their sensitivity and/or selectivity. Another possibility for improving the sensitivity is to use a surfactant which is able to form organized assemblies. Perez-Bendito et al. found that a copper-catalyzed reaction of N,N-dimethyl-p-phenylenediamine with N,N-dimethylaniline in the presence of hydrogen peroxide and pyridine as an activator. A higher sensitivity and sampling rate were achieved by adding benzyltrimethylammonium chloride (Zephiramine) as a surfactant to this reaction system.

Numerous batchwise methods for the catalytic determination of copper have recently been reported based on its catalytic effect for various indicator reactions with spectrophotometric and fluorometric detections. The catalysis of copper was activated in the presence of suitable ligands, such as ammonia, pyridine and 2,2'-bipyridine. Although these methods allow as little as sub-nanogram and/or nanogram per cm³ levels of copper(II), their analytical conditions must be strictly controlled; also, some of them are time-consuming. On the other hand, the adaptation of a flow injection analysis (FIA) to the catalytic methods leads to many advantages, i.e., the reagent addition and the reaction time can be easily controlled and a large number of samples can be measured automatically with high precision.

A highly sensitive flow-injection spectrophotometric method has been developed for the determination of copper(II). It is based on the catalytic effect of copper(II) on the oxidative coupling reaction of 3-methyl-2-benzothiazolinone hydrazone with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline to form a red compound (λmax=525 nm) in the presence of hydrogen peroxide and pyridine as an activator. A higher sensitivity and sampling rate were achieved by adding benzyltrimethylammonium chloride (Zephiramine) as a surfactant to this reaction system. The calibration graph for copper(II) was linear over the range 0.005 – 0.75 ng cm⁻³ at a rate of 40 samples h⁻¹. The relative standard deviations for ten determinations of 0.5 and 0.05 ng cm⁻³ were 0.21 and 2.0%, respectively. The proposed method was successfully applied to the determination of copper in pepperbush.

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Many indicator reactions have been applied to the FIA method for copper determination. However, few papers on the use of a surfactant for a catalytic flow-injection determination of copper have been published. An oxidative coupling of N-phenyl-p-phenylenediamine with m-phenylenediamine in the presence of hydrogen peroxide was employed for the determination of copper(II) over the range 0.1 – 2.0 ng cm⁻³ in which polyoxyethylene(20) sorbitan monooleate (Tween 80) as a surfactant was added to the reaction system in order to obtain a higher sampling frequency and to prevent the adsorption of dye produced on the inner wall of the reaction coil. In previous work we developed a catalytic spectrophotometric method for the determination of 0.002 – 0.1 ng cm⁻³ of copper(II) using the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with N-ethyl-N-(2-hydroxy-3-sul-
Zephiramine was prepared by dissolving 4.0 dm–3 with water, respectively. A stock solution of 0.1 was prepared by diluting a commercial 30% solution and pyridine. The proposed method was successfully applied to the determination of copper in pepperbush (National Institute for Environmental Studies (NIES, No. 1)).

Experimental

Reagents

All reagents used were of analytical grade and used without further purification. The water used to prepare the reagent and buffer solutions was obtained from a Milli-Q water purification system (Millipore).

A stock solution of 1.0 µg cm–3 copper(II) was prepared from a commercially available copper solution for atomic absorption spectrometry (1000 µg cm–3) (Kanto Kagaku). Working standard solutions of lower concentrations were freshly prepared daily by serial dilutions of the stock solution with 1·10–2 mol dm–3 hydrochloric acid. A stock solution of 1·10–2 mol dm–3 MBTH was prepared by dissolving 0.43 g of 3-methyl-2-benzothiazolinone hydrazine hydrochloride (Tokyo Kasei) in 200 cm3 of water. A stock solution of 1·10–2 mol dm–3 DAOS was prepared by dissolving 0.68 g of N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-di-methoxyaniline sodium salt (Dojindo Lab.) in 200 cm3 of water and stored in a refrigerator. A 0.2 mol dm–3 MOPSO buffer was prepared by dissolving 22.5 g of 2-hydroxy-3-morpholinopropanesulfonic acid (Dojindo Lab.) in 500 cm3 of water. Solutions of hydrogen peroxide (3 mol dm–3) and pyridine (3 mol dm–3) were prepared by diluting a commercial 30% solution and pyridine with water, respectively. A stock solution of 0.1 mol dm–3 Zephiramine was prepared by dissolving 4.0 g of benzyl dimethyltetradecyl ammonium chloride (Dojindo Lab.) in 100 cm3 of water. Working solutions of these reagents were prepared by suitable dilution with water.

Apparatus

A schematic diagram of the flow system is shown in Fig. 1. Two double-plunger micropumps (Sanuki Kogyo, SVM-6M2) were used for propelling each solution at a flow rate of 0.5 cm3 min–1. Sample solutions were injected by a six-way injection valve (Sanuki Kogyo, SVM-6M2) into the carrier stream. The flow lines were made from Teflon tubing (0.5 mm i.d.) and connectors. The absorbance was measured at 525 nm with a Soma Kogaku S-3250 spectrophotometer equipped with a 8-µl flow cell (optical path length, 10 mm) and recorded on a Chino EB-22005 recorder. A Taiyo Model H-100 circulating thermostated bath was used to control the reaction temperature at 60±0.1°C. A Corning Model 120 pH/mV meter was used for pH measurements.

Procedure

In the flow system (Fig. 1), a 1·10–2 mol dm–3 hydrochloric acid carrier solution and a 0.8 mol dm–3 hydrogen peroxide solution in reservoirs C and R1 were pumped, respectively. A solution of 2·10–3 mol dm–3 DAOS and a mixed solution (pH 5.9) of 3·10–3 mol dm–3 MBTH, 0.1 mol dm–3 MOPSO, 0.5 mol dm–3 pyridine and 5·10–4 mol dm–3 Zephiramine in reservoirs R2 and R3 were also pumped at the same flow rate, respectively. An aliquot (130 µl) of a sample solution was injected into the carrier stream. The catalytic reaction of copper(II) on the color development proceeded in a reaction coil of 5 m length at 60°C. The increase in the absorbance was recorded continuously at 525 nm.

Results and Discussion

In the presence of hydrogen peroxide, MBTH reacts with DAOS to produce a red dye, which has an absorption maximum at 525 nm. Although the rate of the dye-forming reaction is very slow in the absence of copper(II), it is accelerated by trace amounts of copper(II) at pH range 5–6. The copper(I) produced during the redox reaction is oxidized again to copper(II) by hydrogen peroxide. As a result of the regeneration of copper(II), the rate of the reaction increases catalytically with increasing copper concentration. In the present...
Choice of surfactant

It was expected that micelles modified the rate of the copper-catalyzed reaction through a large electrostatic potential at the micellar surface or through hydrophobic forces. Thus, the effects of surfactants on the color development were examined over the range 0.02 – 1 w/v% by injecting a 0.5 ng cm$^{-3}$ copper(II) solution in the presence of pyridine. The surfactants examined were as follows: cationic {dodecyltrimethylammonium chloride (DTAC), cetyltrimethylammonium bromide (CTAB) and Zephiramine}, nonionic {polyoxyethylene-23-lauryl ether (Brij 35), polyoxyethylene(20) sorbitan monolaurate (Tween 20) and Tween 80} and anionic {SDS and sodium dodecylbenzenesulfonate (SDBS)}. The results are shown in Fig. 2. The catalytic effect of copper was suppressed in the presence of anionic surfactants, such as SDS and SDBS. In the case of nonionic surfactants, the peak heights for copper(II) increased slightly. On the other hand, significantly higher absorbances were obtained in the presence of cationic surfactants. A maximum and almost constant peak height was obtained in the range of Zephiramine (CMC=3.7×10$^{-4}$ mol dm$^{-3}$) and CTAB (CMC=1.0×10$^{-3}$ mol dm$^{-3}$). In the present copper-catalyzed coloration system, 0.02%(w/v) (5×10$^{-4}$ mol dm$^{-3}$) of Zephiramine was used. Figure 3 shows the peak profiles in the absence and presence of Zephiramine. The presence of Zephiramine decreased the peak tailing, which led to a higher sampling frequency as well as sensitivity.

Effect of variables

The effect of the temperature on the peak height was examined over the range 25 – 80˚C. The catalytic reaction proceeded faster with increasing temperature. However, the baseline became unstable at temperatures above 65˚C. A reaction temperature of 60˚C was selected for the sake of high sensitivity and baseline stability. The effect of the pH on the peak height was examined over the range 5.1 – 6.4. As shown in Fig. 4, the optimum pH range for the catalyzed reaction was 5.8 – 6.2. The reaction was carried out in the pH range 5.8 – 6.2. The effect of the MBTH concentration was examined over the range 3×10$^{-4}$ – 5×10$^{-3}$ mol dm$^{-3}$. The peak height increased with increasing MBTH concentration up to 2×10$^{-3}$ mol dm$^{-3}$, and remained constant over the range 2×10$^{-3}$ – 5×10$^{-3}$ mol dm$^{-3}$. A 3×10$^{-3}$ mol dm$^{-3}$ MBTH concentration was selected. The effect of the DAOS concentration was examined over the range 1×10$^{-3}$ – 5×10$^{-3}$ mol dm$^{-3}$. The maximum and constant peak height was obtained over the concentration range 1.5×10$^{-3}$ – 5×10$^{-3}$ mol dm$^{-3}$. A 2×10$^{-3}$ mol dm$^{-3}$ DAOS concentration was used for the procedure. The effect of the hydrogen peroxide concentration was examined over the range 0.03 – 1.4 mol.
The peak height increased with increasing the concentration. However, the baseline became unstable at concentrations above 1 mol dm$^{-3}$. A 0.8 mol dm$^{-3}$ hydrogen peroxide concentration was selected for the sake of high sensitivity and baseline stability. The effect of the pyridine concentration was examined over the range 0.01–0.9 mol dm$^{-3}$ (Fig. 5). The peak height increased with increasing the concentration, and the peak height showed a maximum at 0.5 mol dm$^{-3}$, gradually decreasing beyond this concentration. A 0.5 mol dm$^{-3}$ pyridine concentration was used for the procedure.

**Calibration graph**

The calibration graphs for copper(II) were prepared by the recommended procedure using the flow system shown in Fig. 1. The dynamic range for the copper(II) determination was from 0.005 to 0.75 ng cm$^{-3}$; $A=0.798C$ with a correlation coefficient of 0.999, where $A$ is the absorbance and $C$ is the concentration of copper(II) in ng cm$^{-3}$. The relative standard deviations ($n=10$) for 0.5 and 0.05 ng cm$^{-3}$ of copper(II) were 0.21 and 2.0%, respectively. The sampling rate was about 40 h$^{-1}$.

**Interferences**

The effect of foreign ions on the determination of 0.5 ng cm$^{-3}$ copper(II) was examined; an error of ±5% is considered to be tolerable. The results are summarized in Table 1. Most of the ions examined did not interfere up to at least 100-fold excess. Although positive interference was observed for chromium(VI), this ion was allowed to coexist up to 20-fold excess.

**Application**

The proposed method was applied to the determination of copper in pepperbush (NIES certified reference material No. 1). A pressure decomposition in a Teflon bomb for the wet digestion of the sample (232.0 mg) was carried out by the recommended procedure. The decomposed sample was dissolved in 100 cm$^{-3}$ of water; the sample solution was diluted 50 times with 0.01 mol dm$^{-3}$ hydrochloric acid before measurement. The result obtained by the proposed method (12.0±0.1 µg g$^{-1}$) was in good agreement with the certified value (12.0±1.0 µg g$^{-1}$).

**References**


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