

Flow Injection Determination of Copper Based on Its Quenching Effect on Fluorescence of Anisidine Blue

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Abstract

A novel method has been developed for the determination of copper by use of fluorescence quenching of Anisidine Blue by flow injection analysis. Copper (II) quenches Anisidine Blue's fluorescence and can then be determined indirectly by measurement of the decrease in the fluorescence of Anisidine Blue. The present method has been successfully applied to the determination of copper in copper alloy removing interfering metals by on-line cation exchange in tartrate medium. The detection limit is found to be 0.027 µg/ml and the sample throughput is 30 per hour.

Keywords: Copper, Anisidine Blue, fluorescence quenching, flow injection analysis, copper alloy

1. Introduction

Copper is one of very popular elements and extensively examined in environmental studies, industrial, biological, medical applications, etc. Many analytical techniques are actually available to determine copper concentration in samples with different matrices. Flow injection analysis (FIA) has frequently been applied to the determination of copper because of its advantages such as reproducibility, sample throughput, and cost performance.

Most of recent FIA investigations for determination of copper have focused on its sensitive determination in water and biological samples using spectrophotometry[1-5], chemiluminescence[6], flame atomic absorption spectrometry[7-11], and ICP-MS[12,13] as detection techniques coupled. In those investigations, on-line preconcentration has been often employed. On the other hand, information of FIA methods for copper in industrial samples is few and fluorimetric detection has hardly been applied to the

determination of copper. Dias et al. reported sequential injection determination of copper in brass based on the catalytic effect of copper (II) on the oxidation rates of resorcinol by hydrogen peroxide [14].

Anisidine Blue in ultraviolet light shows an intense blue fluorescence at pH 4-10 which is quenched by certain metal ions. The applications of this compound as a metallofluorescent indicator were first reported by Kirkbright and Stephen[15]. In their work, copper (II) and lead (II) were determined by titration with EDTA in the presence of Anisidine Blue, the blue fluorescence of which was restored at the end-point.

In this work, fluorescence quenching effect of copper (II) on Anisidine Blue [15] has been exploited to determine copper in brass coupled with on-line cation exchange separation of interfering metals. The present method is simpler than that proposed by Dias et al. [14] so that it is convenient from the practical point of view.

2. Experimental

2.1 Reagents

All the reagents used were of analytical

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reagent grade, and distilled and deionized water was further purified by a Milli-Q system (Japan Millipore, Tokyo) to be used throughout the work.

The solution of Anisidine Blue (3,3'-dimethoxybenzidine-*N,N,N',N'*-tetraacetic acid, tetrasodium salt, Dojindo Laboratories) was buffered at pH 6.3 with acetate.

Copper (ID stock solution was prepared by dissolving 1.1005 g copper plate in 20 ml of 7 M nitric acid and diluting to 1000 ml with water, the accurate copper concentration (1.10 mg/ml) being standardized by chelatometric titration with EDTA

The stock solutions (10.0 mg/ml) of aluminum (III), calcium (II), cobalt (II), iron (III), lead (II), magnesium (II), nickel (II), and zinc (II) were prepared as 0.1 M hydrochloric acid solutions from chloride of each metal.

Sodium nitrate, sodium sulfate, sodium chloride, disodium hydrogenphosphate, and sodium bromide were prepared as 0.1 M hydrochloric acid solutions and used for study on the effect of anions.

Tartaric acid solution was prepared by dissolving its L-form in water and its pH was adjusted with sodium hydroxide solution.

A strongly acid cation exchange resin Bio-Rad AG 50W-X8, 200-400 mesh (Bio-Rad Laboratories, U.S.A.) was used for on-line separation of copper. A mini column was prepared by packing the ion exchange resin in a 100 mm × 2 mm i.d. polytetrafluoroethylene (PTFE) column plugged at the ends with cotton.

2.2 Apparatus

A block diagram of the flow-injection analysis system used in this work is illustrated in Fig.1. The flow system was assembled from 1-mm bore PTFE tubing and connectors.

A Soma Kogaku spectrofluorometric detector S-3350 was used to monitor the fluorescence of Anisidine Blue (excitation wavelength, 321.0 nm; fluorescence wavelength, 403.0 nm). The carrier and reagent solutions were delivered by a GL Science PUD-16 type pump and a Sanuki Kogyo DMX-2000 pump. Sample solutions were charged into an injection valve by a Tokyo Rikakikai Micro Tube Pump MP-3N. The pH of solutions was measured by a Toadenpa Kogyo HM-7E pH meter.

2.3 Recommended FIA procedure

An aliquot (100 μ l) of sample solution is injected into the carrier solution (0.1 M tartrate, pH 4.0, 1.75 ml/min), passed through the cation exchange resin column (100 mm × 2 mm i.d.) and then mixed with 7.0×10^{-3} M Anisidine Blue solution (pH 6.3, 1.75 ml/min), followed by monitoring the fluorescence of Anisidine Blue.

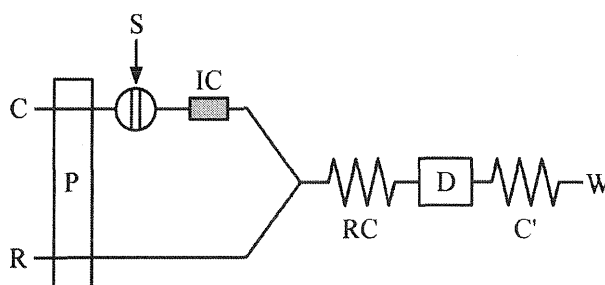


Fig.1 Block diagram for flow injection system used for determination of copper in brass.

C, carrier (0.1 M tartrate, pH 4.0); R, 7.0×10^{-5} M Anisidine Blue solution (pH 6.3); P, pump; S, sample (100 l) ; IC, ion exchange column; RC, reaction coil; D, fluorimetric detector; C', back pressure coil; W, waste.

2.4 Analysis of copper alloy

About 16 mg of naval brass was taken in a small beaker and dissolved in 3 ml of 7 M nitric acid and 1 ml of 6 M hydrochloric acid by gentle heating. The sample solution was evaporated nearly to dryness. One milliliter of 2 M hydrochloric acid was added to it and evaporated nearly to dryness. This treatment was repeated more two times. The sample was then diluted to 100 ml in a volumetric flask with 0.1 M hydrochloric acid. A one milliliter aliquot of the sample solution was taken in a 50-ml volumetric flask and diluted to the mark with 0.1 M tartaric acid of which pH value had been adjusted to 4.0 with sodium hydroxide solution. About 100 μ l of the sample solution prepared as above was injected into the FIA system.

3. Results and Discussion

3.1 Study of flow injection analysis parameters

Basic flow injection analysis parameters were first studied using a flow injection system without a cation exchange column as follows.

(1) Effect of concentration of Anisidine Blue

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Anisidine Blue was examined over the range of 3.0×10^{-5} to 8.0×10^{-5} M. The Anisidine Blue solution of higher concentration gave more intensive fluorescence and wider linearity in calibration graphs. However, the fluorescence intensity of 8.0×10^{-5} M Anisidine Blue solution was too strong to be detected, so that 7.0×10^{-5} M Anisidine Blue solution was used hereinafter.

(2) Effect of flow rates of carrier and reagent solutions

The effect of the total flow rate on the fluorescence intensity and the stability of the base line was examined over the range of 1.6 to 4.0 ml/min keeping the flow rates of the carrier and reagent at the same value each other. The increasing total flow rate resulted in the increase in the stability of the base line. However, the pressure in the flow system became so high at the flow rate of 4.0 ml/min that the solutions leaked at the connectors. Accordingly, subsequent experiments were carried out at the total flow rate of 3.5 ml/min.

3.2 Effect of foreign ions

The effect of eight metal ions was studied keeping the concentration of copper (II) at 2.0 $\mu\text{g/ml}$. The results obtained are summarized in Table 1. Magnesium (II), calcium (II), and aluminum (III) did not interfere even at 200 $\mu\text{g/ml}$, while the other (heavy) metals tested gave serious interferences at this concentration level. Iron (III) and lead (II) at 2 $\mu\text{g/ml}$ were tolerable, but cobalt (II), nickel (II), and zinc (II) at 2 $\mu\text{g/ml}$ gave the positive error of more than 5 % in the measurement of copper (II). These errors might be attributable to complex formation of the corresponding heavy metal ions with Anisidine Blue. Therefore, on-line cation exchange separation in tartrate medium was employed in the flow injection system to avoid the interference from other heavy metals described below.

The effect of common anions was examined over the concentration range of 0.01 to 0.1 M and the results obtained were listed in Table 2. Sulfate and bromide did not interfere, but nitrate gave positive errors and phosphate negative ones. Nitrate adsorbed the excitation light (321.0 nm) which should excite Anisidine Blue, resulting in the positive errors. On the other hand, phosphate might react with copper (II) to form stable complexes, leading to the negative errors.

Table 1 Effect of foreign metal ions

Metal ions	Added/ $\mu\text{g/ml}$	Cu recovery, %
Al(III)	2000	134.9
	200	104.5
	100	103.0
Ca(II)	2000	150.9
	200	105.2
	100	105.0
Co(II)	200	326.3
	20	183.7
	2	110.8
	0.2	102.4
Fe(III)	200	197.2
	20	127.8
	2	106.2
Mg(II)	2000	106.8
	200	102.4
	100	99.8
Ni(II)	200	348.8
	20	301.0
	2	142.2
	0.2	102.7
Pb(II)	200	199.5
	20	116.7
	2	102.2
Zn(II)	200	194.9
	20	149.3
	2	107.3
	0.2	101.2

2 μg Cu(II)/ml added.

Table 2 Effect of foreign anions

Anions	Added /M	Cu recovery, %
NO_3^-	0.10	143.7
	0.05	131.4
	0.01	108.5
SO_4^{2-}	0.10	99.9
	0.05	101.9
	0.01	104.6
Cl ⁻	0.10	101.9
	0.05	103.9
	0.01	103.0
PO_4^{3-}	0.10	68.5
	0.05	88.1
	0.01	100.2
Br ⁻	0.10	103.1
	0.05	103.6
	0.01	103.2

2 μg Cu(II)/ml added.

3.3 Removal of interfering heavy metals by on-line cation exchange separation

Yamane et al. reported high-performance liquid chromatographic separation of cobalt, nickel, zinc and copper using the cation exchange system in a tartrate medium [16]. In their chromatographic system, the metal ions studied eluted in the following order, copper (II), nickel (II), zinc (II), and cobalt (II) in 0.1 M sodium tartrate-hydrochloric acid (pH 4.0). Accordingly, a 100 mm × 2 mm i.d. cation exchange resin column was introduced in the flow injection system and 0.1 M tartrate solution (pH 4.0) was used as a carrier solution instead of 0.1 M hydrochloric acid.

The effect of cobalt (II), lead (II), and zinc (II) on the measurement of 2 µg/ml of copper (II) was examined again using the flow injection system containing the cation exchange column. The results are shown in Table 3. Cobalt (II) and zinc (II) of about 100 µg/ml and lead (II) of at least 20 µg/ml did not interfere (relative error, less than ca. 5 %). Based on these observations, we tried to determine copper in naval brass.

Table 3 Recovery of copper* by the modified FIA system with a cation exchange column

Metal ions	Added/µg/ml	Cu(II) recovery, %
Co(II)	100	96.4
	20	96.4
	2	98.8
Pb(II)	20	101.4
Zn(II)	100	105.6
	20	100.7

* 2 µg Cu(II)/ml added.

3.4 Determination of copper in naval brass

A mock solution of naval brass was prepared by reference to the certificate of Japan Copper and Brass Association (see Table 4) and analyzed for copper by the proposed method. Recovery of copper (II) obtained was 100.7 %. Consequently, the present method was applied to the determination of copper in naval brass distributed by Japan Copper and Brass Association. The analytical result of 5 runs, $62.4 \pm 0.52\%$, is in good agreement with the certified value, 62.16%.

The detection limit calculated as 3 times of standard deviation of 5 measurements of 0.05 µg/ml copper (II) solution was found to be

0.027µg/ml. The sample throughput was 30 per hour.

Table 4 Composition of Naval Brass*

Element	Content, %
Cu	62.16 ₃
Sn	1.00 ₇
Pb	0.0066
Fe	0.010 ₁
Zn	36.82

* Provided by Japan Copper and Brass Association.

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(Received October 10, 2004)

(Accepted November 16, 2004)