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Talanta 68 (2006) 527–534

Talanta

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Sequential injection lab-on-valve simultaneous spectrophotometric determination of trace amounts of copper and iron

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Received 15 February 2005; received in revised form 19 April 2005; accepted 19 April 2005
Available online 22 June 2005

Abstract

A sequential injection (SI) method in a lab-on-valve (LOV) format for simultaneous spectrophotometric determination of copper and iron has been devised. The detection chemistry is based on the complex formation of 2-(5-bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]aniline (5-Br-PSAA) with copper(II) and/or iron(II) at pH 4.6. Copper(II) reacts with 5-Br-PSAA to form the complex which has an absorption maximum at 580 nm but iron(III) does not react. In the presence of a reducing agent only iron(II)–5-Br-PSAA complex is formed and detected at 558 nm. Under the optimum experimental conditions, the determinable ranges are 0.1–2 mg l^{-1} for copper and 0.1–5 mg l^{-1} for iron, respectively, with a sampling rate of 18 h^{-1}. The limits of detection are 50 μg l^{-1} for copper and 25 μg l^{-1} for iron. The relative standard deviations ($n = 15$) are 2% for 0.5 mg l^{-1} copper and 1.8% for 0.5 mg l^{-1} iron when determined in standard solutions. The recoveries range between 96 and 105% when determining 0.25–2 mg l^{-1} of copper and 0.2–5 mg l^{-1} of iron in artificial mixtures at copper/iron ratios of 1:10 to 5:1. The proposed SI-LOV method is successfully applied to the simultaneous determination of copper and iron in multi-element standard solution and in industrial wastewater samples.

Keywords: Sequential injection; Simultaneous trace determination; Copper; Iron

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1. Introduction

Copper and iron are essential elements for living organisms including human being. Lack of these elements in the daily diet may result in the development of serious diseases. However, excess uptake of copper and iron through water pollution results in acute and/or chronic poisoning. As early as the late 1800s, water pollution occurred as a consequence of industrial waste dumping at the Ashio copper mine in Japan [1]. For sick infants and children, even tap water can cause their chronic copper poisoning [2]. It is well known that sheep are the domestic animals who are most prone to the copper poisoning [3]. And also, large amount of iron discharged from factories leads to effluvial and reddish water. Recent industrialization in Thailand has exerted considerable stress on the marine environments and provoked habitat degradation [4]. Therefore, the determination of copper and iron is required for quality assessment of wastewater.

The guideline values in wastewater set by Japanese government are 3 mg l^{-1} for copper and 10 mg l^{-1} for iron, respectively [5]. Although diethyldithiocarbamate and 1,10-phenanthroline spectrophotometric methods are utilized for individual determination of copper and iron [6], inductively coupled plasma atomic emission spectrometry (ICP-AES) is usually exploited for simultaneous determination of multi-elements including copper and iron. However, the running cost and operator skill requirement for ICP-AES are high, so a cost-effective and affordable technique for copper and iron determination is desired.

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doi:10.1016/j.talanta.2005.04.073

Flow injection analysis (FIA) conceived by Ruzicka and Hansen in 1975 [7] is one of flow-based techniques and has been applied to many fields such as atmospheric gas analysis [8], water analysis [9], bioassay [10,11], pharmaceutical analysis [12–14] and so on for the last few decades. Recently, Sakai and co-workers [15] reported a simultaneous spcetrophotometric determination for copper and iron in sera using 2-(5-bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]aniline [16] (5-Br-PSAA) by FIA equipped with twin flow cell. The limits of detection (LOD) were 0.39 μg l^{-1} for copper and 0.20 μg l^{-1} for iron. This high sensitivity allowed the volume of serum sample to be minimized. However, analysis of wastewater for copper and iron does not need such highly sensitive method because relatively large volumes of wastewater samples are usually available compared to biological materials. In addition, due to its continuous flow character, FIA essentially tends to consume reagent(s) even when an analyte is not measured.

Sequential injection analysis (SIA) was developed as the second generation of flow injection techniques in 1990 [17,18]. In a SIA system, microlitre volumes of sample and reagents are aspirated at programmed time intervals by a syringe pump that provides for bi-directional discontinuous flow. Therefore, the consumption of reagents and sample in SIA is usually smaller than that in FIA. Moreover, the same SIA setup can be used for different assay protocols without changing configuration of the system. As highlighted in a review by Lenehan et al. [19], over 200 papers on SIA have been published in the recent decade.

Many researchers utilized sequential injection (SI) technique with UV–vis detection for the assay of copper [20,21] or iron [22–29]. The SI technique was employed as a means of the sample introduction into flame atomic absorption spectrometric detector for the determination of zinc, manganese, iron and copper in wine [30]. FI and SI systems with anodic stripping voltammetric detection were exploited for the simultaneous determination of cadmium, copper, lead and zinc in wastewater samples [31]. SI method with UV–vis detection has also been utilized for the determination of binary species in water sample: iron(III)–iron(II) using Tiron [29], chromium(VI)–chromium(III) using 1,5-diphenylcarbazide with wetting film extraction technique [32] and cobalt(II)–nickel(II) with differential kinetics [33]. However, there is no report on simultaneous determination of copper and iron by SI system with UV–vis detection.

Most recently, a mini-conduit module with integrated flow cell and fiber optics was mounted atop a multi-port valve in SI manifold to form a ‘lab-on-valve’ (LOV) [17]. In the SI-LOV system, the volume of the sample path from injector to detector can be minimized. The volume of the generated waste solution is smaller than in a common SI system. This SI-LOV technique recently employed for spcetrophotometric chloride ion assay [34].

This paper describes a SI-LOV system using 5-Br-PSAA as a chromogenic reagent for the simultaneous determination of copper and iron. The high reagent blank was successfully suppressed by using spacers including 5-Br-PSAA. The proposed method can be applied to industrial wastewater analysis.

2. Experimental

2.1. Reagents

All of the chemicals were of analytical-reagent grade and they were used without further purification. All solutions were prepared with deionized water purified with an Advantec Aquarius GSH-210 system.

Commercially available 1000 mg l^{-1} copper(II) and iron(III) standard solutions for atomic absorption spectrophotometry (Wako, Osaka) were used. Working solutions of copper and iron were prepared by appropriate dilution of the standard solutions with 0.01 mol l^{-1} hydrochloric acid.

A 2×10^{-3} mol l^{-1} 5-Br-PSAA stock solution was prepared by dissolving 0.096 g of 2-(5-bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]aniline, sodium salt (Dojindo Laboratories, Kumamoto) in 100 ml of water. 4×10^{-4} (for copper determination) and 7×10^{-4} mol l^{-1} (for iron determination) 5-Br-PSAA solutions were prepared by dilution of the stock 5-Br-PSAA solution with water and a 5×10^{-5} mol l^{-1} 5-Br-PSAA solution as a carrier and a spacer as well was prepared by dilution of the same stock solution with 0.01 mol l^{-1} hydrochloric acid.

A 1×10^{-2} mol l^{-1} ascorbic acid solution was daily prepared by dissolving 0.035 g of L-ascorbic acid (Wako) in 10 ml of water. A 5×10^{-4} mol l^{-1} L-ascorbic acid solution was prepared by dilution of the stock solution with water.

A 0.2 mol l^{-1} acetic acid solution was prepared by diluting 0.6 ml of acetic acid (Sigma–Aldrich Japan, Tokyo) to 50 ml with water. A 0.2 mol l^{-1} sodium acetate solution was prepared by dissolving 1.35 g of sodium acetate trihydrate (Nakalai Tesque, Kyoto) in 50 ml of water. These solutions were mixed to prepare a buffer solution (pH 4.6).

2.2. Apparatus

The scheme of manifold of the computer-controlled SI-LOV system (FIAlab 3000, FIAlab Instruments, USA) used is shown in Fig. 1. The system consists of a 2.5 ml syringe pump, a six-port selector valve with integrated flow cell and a single speed unidirectional peristaltic pump. A UV–vis spectrophotometer (USB2000, Ocean Optics, USA) was used to measure absorbance of the formed complexes at 558 and 580 nm. A temperature control unit, TC (R-5000C, Sanuki Kogyo, Tokyo) was used to promote the complex formation of iron(II) with 5-Br-PSAA. All flow lines were made from Teflon tubing (0.5 or 1 mm i.d., see Fig. 1). FIAlab for Windows 2000 software was used to control the SIA-LOV system and to process the experimental data.

A Horiba Model F-22 pH/mV meter equipped with silver/silver chloride electrode (6378-10D, Horiba, Kyoto) was

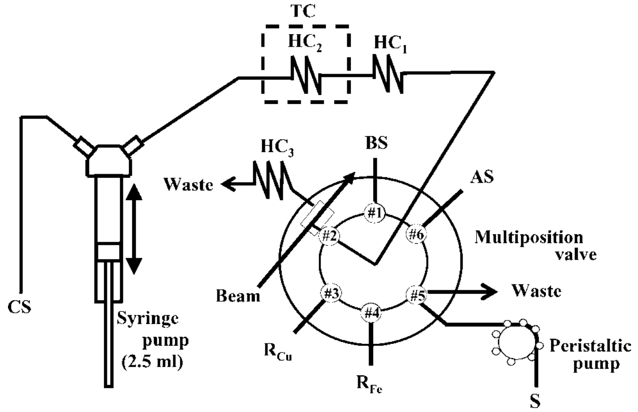

Fig. 1. Manifold of the SIA system in the proposed method. HC_1, holding coil$_1$ (0.5 mm i.d., 60 cm); HC_2, holding coil$_2$ (0.5 mm i.d., 55 cm); HC_3, holding coil$_3$ (1 mm i.d., 50 cm); TC, temperature controller (45 °C); CS, 5×10^{-5} mol l^{-1} 5-Br-PSAA in 0.01 mol l^{-1} HCl; R_{Cu}, 4×10^{-4} mol l^{-1} 5-Br-PSAA; R_{Fe}, 7×10^{-4} mol l^{-1} 5-Br-PSAA; S, sample containing copper and/or iron; AS, 5×10^{-4} mol l^{-1} ascorbic acid; BS, 0.2 mol l^{-1} acetate buffer at pH 4.6.

used for pH adjustment. Phthalate, phosphate and tetraborate pH standard solutions were used for calibration.

2.3. Procedure

The central inlet of the six-port valve shown in Fig. 1 was connected to a couple of holding coils, HC_1 (0.5 mm i.d., 60 cm) for copper and HC_2 (0.5 mm i.d., 55 cm) for iron. The HC_2 was heated by the above-mentioned TC at 45 °C. The flow cell (5 mm path length) was set to the port #2. The auxiliary peristaltic pump was used to transport sample solutions (S) to the sampling line. The sample and reagents were aspirated with a high precision syringe pump of the FIAlab 3000 system. A 0.2 mol l^{-1} acetate buffer solution at pH 4.6 (BS), 4×10^{-4} mol l^{-1} 5-Br-PSAA solution for copper determination (R_{Cu}), 7×10^{-4} mol l^{-1} 5-Br-PSAA solution for iron determination (R_{Fe}), 5×10^{-4} mol l^{-1} ascorbic acid solution (AS) were aspirated from ports #1, #3, #4 and #6, respectively. Sample was aspirated from port #5. The third holding coil, HC_3 (1 mm i.d., 50 cm), connected to the port #2 was used to keep a spacer solution (SS_{Cu}, SS_{Fe} in Fig. 2) of 5×10^{-5} mol l^{-1} 5-Br-PSAA in 0.01 mol l^{-1} hydrochloric acid. The spacer solution in the HC_3 was aspirated from port #2 twice as described below.

Diagram of sequence of reactant zones corresponding to one cycle of simultaneous determination of copper and iron is shown in Fig. 2. The SI-LOV protocol to carry out the sequence is shown in Table 1. At first, HC_1, HC_2 and HC_3 are filled with a carrier solution (CS, 5×10^{-5} mol l^{-1} 5-Br-PSAA in 0.01 mol l^{-1} hydrochloric acid), which is also used as SS_{Cu} and SS_{Fe}. The sample, reagents and SS_{Fe} for the determination of iron are aspirated from the respective ports (in the order: #1, #4, #6, #5, #6, #1, #2) into the HC_2. The sample, reagent and SS_{Cu} are then aspirated from corresponding ports port (in the order: #1, #5, #3, #2) into the HC_1. Thereafter, the reaction products of Cu(II) and Fe(II) with 5-Br-PSAA are dispensed to the flow cell through the port #2 by reversing the direction of the flow. The role of the SS_{Cu} and SS_{Fe} is to promote efficient mixing of the sample and reagents in each zone before reaching the flow cell and also to completely separate the zones of Cu(II) and Fe(II) complexes that results in well separated detection peaks.

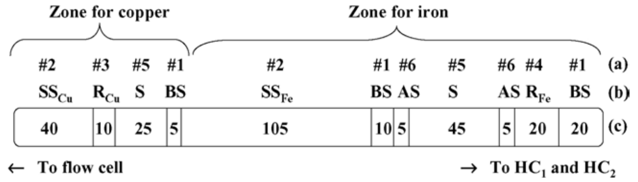

Fig. 2. Diagram of sequence by one cycle for the simultaneous determination of copper and iron: (a) port numbers on lab-on-valve; (b) reagents aspirated (abbreviations are shown in Fig. 1), (c) aspiration volume in microlitres.

Table 1
SIA protocol for the simultaneous determination of copper and iron

SIA protocol	Note
Loop start (#) 3	
Syringe pump valve in	Cleaning the whole flow line
Syringe pump flowrate (μl s^{-}) 185	
Syringe pump fill	
Syringe pump delay until done	
Delay (s) 1	
Multiposition valve flow cell	
Syringe pump valve out	
Syringe pump flowrate (μl s^{-}) 185	
Syringe pump dispense (μl) 300	
Syringe pump delay until done	
Delay (s) 1	
Loop end	
Delay (s) 2	
Multiposition valve acetate buffer	Aspiration of 0.2 mol l^{-1} acetate buffer
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 20	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve 5-Br-PSAA	Aspiration of 7×10^{-4} mol l^{-1} 5-Br-PSAA
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 20	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve ascorbic acid	Aspiration of 5×10^{-4} mol l^{-1} ascorbic acid
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 5	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve sample (Fe)	Aspiration of iron solution at a suitable concentration
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 45	
Syringe pump delay until done	
Delay (s) 2	

Table 1 (*Continued*)

SIA protocol	Note
Multiposition valve ascorbic acid	Aspiration of 5 × 10^{-4} mol l^{-1} ascorbic acid
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 5	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve acetate buffer	Aspiration of 0.2 mol l^{-1} acetate buffer
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 10	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve flow cell	Aspiration of carrier solution
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 105	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve acetate buffer	Aspiration of 0.2 mol l^{-1} acetate buffer
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 5	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve sample (Cu)	Aspiration of copper solution at a suitable concentration
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 25	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve 5-Br-PSAA	Aspiration of 4 × 10^{-4} mol l^{-1} 5-Br-PSAA
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 10	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve flow cell	Aspiration of carrier solution
Syringe pump flowrate (μl s^{-}) 15	
Syringe pump aspirate (μl) 40	
Syringe pump delay until done	
Delay (s) 2	
Multiposition valve flow cell	
Syringe pump flowrate (μl s^{-}) 20	
Spectrometer reference scan	
Spectrometer absorbance scanning	Absorbance monitoring starts
Syringe pump dispense (μl) 500	
Syringe pump delay until done	
Spectrometer stop scanning	Absorbance monitoring stops
Loop end	
Stop program	

3. Results and discussion

3.1. Peak profiles

The molar absorptivities for the copper(II)-5-Br-PSAA complex are 55,000 l mol^{-1} cm^{-1} at 558 nm and 65,000 l mol^{-1} cm^{-1} at 580 nm [35] and those for the Fe(II) complex are 87,000 l mol^{-1} cm^{-1} at 558 nm and 42,000 l mol^{-1} cm^{-1} at 713 nm [13]. In this work, we chose the monitoring wavelengths of 580 nm for copper and 558 nm for iron, respectively, to ensure maximum sensitivity. Fig. 3 shows the peak profiles for Cu(II)–5-Br-PSAA and Fe(II)–5-Br-PSAA complexes obtained by the proposed method. As shown in Fig. 2, there are two determination zones per one sequence; one zone containing SS_{Cu}, R_{Cu}, S and BS from the HC_1 gives a first peak for copper, and another zone containing SS_{Fe}, BS, AS, S and R_{Fe} from HC_2 gives a second peak for iron. When the sample solutions containing only copper were introduced into the SI-LOV system, only first peak heights depended on the concentration of copper (Fig. 3 (a–c)). On the other hand, the second peaks (Fig. 3 (a′–c′)) were proportional to the concentration of iron. Hence, copper and iron can be determined simultaneously.

3.2. Effects of 5-Br-PSAA concentrations

Fig. 4 shows the effect of 5-Br-PSAA concentration in R_{Cu} and R_{Fe} on color development with 1 mg l^{-1} copper and 1 mg l^{-1} iron. The concentrations were varied from 1 × 10^{-4} to 5 × 10^{-4} mol l^{-1} for copper and from 2 × 10^{-4} to 1 × 10^{-3} mol l^{-1} for iron. The net absorbance of the copper complex was almost constant over the examined range and that of iron complex was monotonically increased with an increase in 5-Br-PSAA concentration. Both of the blank peaks for copper and iron also increase with an increase in 5-Br-PSAA concentration. We chose therefore 5-Br-PSAA concentrations of 4 × 10^{-4} mol l^{-1} for copper and 7 × 10^{-4} mol l^{-1} for iron.

3.3. Effect of 5-Br-PSAA concentration in carrier solution

Fig. 5 shows the effects of 5-Br-PSAA concentration in carrier solution, which was used as SS_{Cu} and SS_{Fe} to suppress the high reagent blank. The 5-Br-PSAA concentration was varied from 0 to 1 × 10^{-4} mol l^{-1}. The blank peaks for copper and iron favorably decreased with an increase in the concentration of 5-Br-PSAA, because the 5-Br-PSAA concentration gap between R_{Cu} or R_{Fe} and the carrier solution was narrowed. At the same time, the net absorbance for copper increased with increasing the reagent concentration up to 2 × 10^{-5} mol l^{-1} and that for iron also increased over the range examined. These results indicated that 5-Br-PSAA in SS_{Cu} and SS_{Fe} suppressed each blank and moreover promoted the complex formations. Thus, a 5 × 10^{-5} mol l^{-1} of 5-Br-PSAA in 0.01 mol l^{-1} hydrochloric acid was chosen as SS_{Cu} and SS_{Fe}.

3.4. Effect of pH

The effects of the reaction pH with acetate buffer on the copper and iron determinations were studied in the pH range 3.8–5.0. As can be seen in Fig. 6, no colored complex of iron was produced at the lowest examined pH 3.9. The peak heights for copper and iron were maximum and essentially constant over the pH range 4.6–5.0. Thus, acetate buffer solution of pH 4.6 was used for the simultaneous determination of copper and iron.

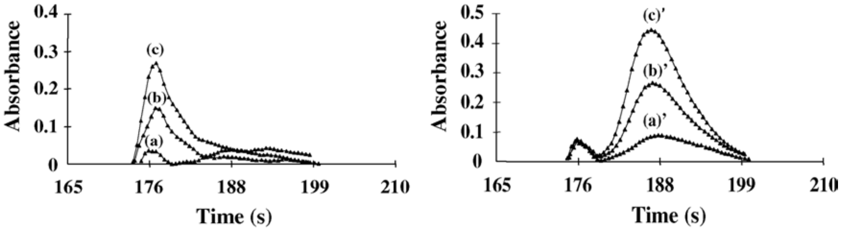

Fig. 3. Peak profiles obtained by the proposed method for copper at 580 nm and iron at 558 nm: (a) blank for copper; (b) 0.5 mg l^{-1} copper; (c) 1 mg l^{-1} copper; (a′) blank for iron; (b′) 0.5 mg l^{-1} iron; (c′) 1 mg l^{-1} iron. Other conditions are the same as in Fig. 1.

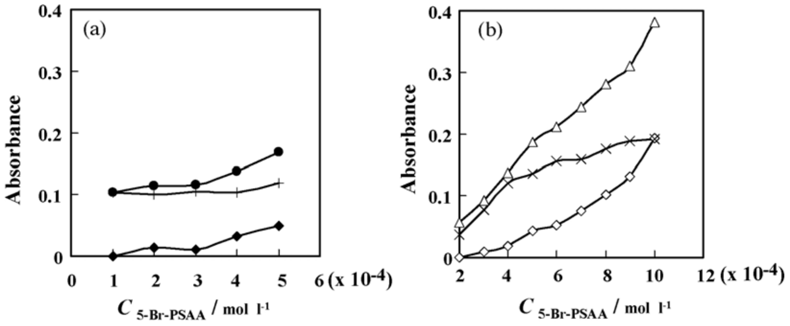

Fig. 4. Effects of 5-Br-PSAA concentration on the (a) copper and (b) iron determination. (♦) Blank for copper; (●) 1 mg l^{-1} copper; (+) net; (◊) blank for iron; (△) 1 mg l^{-1} iron; (×) net. Other conditions are the same as in Fig. 1.

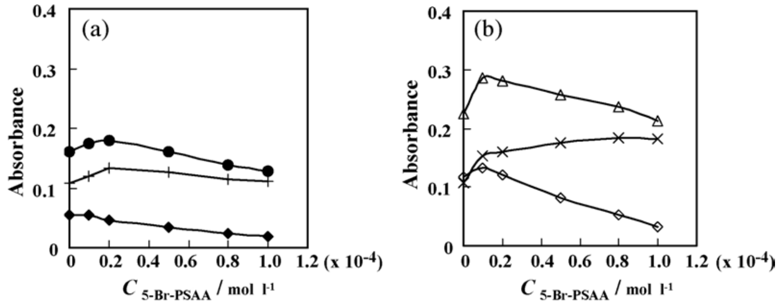

Fig. 5. Effects of 5-Br-PSAA concentration in carrier used as spacer on the (a) copper and (b) iron determination. (♦) Blank for copper; (●) 1 mg l^{-1} copper; (+) net; (◊) blank for iron; (△) 1 mg l^{-1} iron; (×) net. Other conditions are the same as in Fig. 1.

3.5. Effect of ascorbic acid concentration on iron determination

Ascorbic acid was used to reduce iron(III) to iron(II) and also to protect the peak for iron from the interference of copper(II) since the copper(II)–5-Br-PSAA complex can be decomposed by this reductant [36]. Therefore, sample in the iron determination zone was sandwiched between two zones of ascorbic acid solutions as shown in Fig. 2. Ascorbic acid concentration was varied from 0 to 1×10^{-3} mol l^{-1}. In the

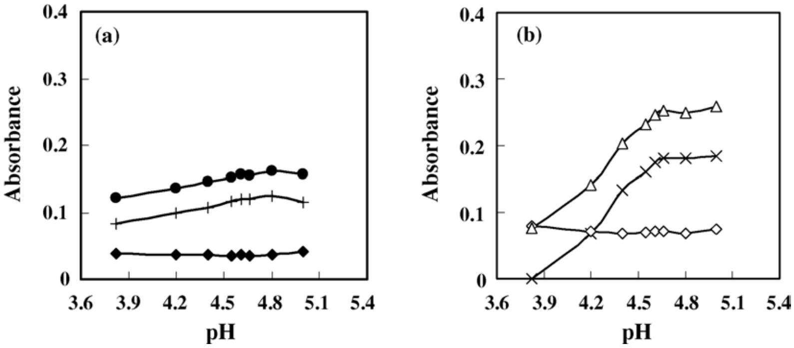

Fig. 6. Effects of pH on the (a) copper and (b) iron determination. (♦) Blank for copper; (●) 1 mg l^{-1} copper; (+) net; (◊) blank for iron; (△) 1 mg l^{-1} iron; (×) net. Other conditions are the same as in Fig. 1.

absence of ascorbic acid, an undesirable peak was obtained, i.e. copper(II) reacted with 5-Br-PSAA in the SS_{Fe}. At ascorbic acid concentrations higher than 1×10^{-4} mol l^{-1}, the interference from copper(II) was removed, and the peak for iron was maximum and constant. A 5×10^{-4} mol l^{-1} ascorbic acid was thus selected as reductant for the iron determination.

3.6. *Effect of reaction temperature on iron determination*

The effect of the temperature on the complex-formation reaction of iron with 5-Br-PSAA was studied in the range from 25 to 50 °C. The reaction was accelerated by increasing the temperature up to 40 °C and the absorbance was maximum and constant over the range from 40 to 50 °C. Thus, the optimum reaction temperature for the determination of iron was 45 °C.

3.7. *Effects of aspiration volumes*

The effects of aspiration volumes of spacers, sample and reagents were studied with test solutions containing 1 mg l^{-1} copper and 1 mg l^{-1} iron. In these optimizations the volume of SS_{Cu} or SS_{Fe} was simultaneously varied together with the other parameter optimized to maintain constant overall volume of the sequence of zones corresponding to the formation of the peak of the other analyte (so that the distance between the colored product zone and the flow cell is not changed). For instance, when the volume of R_{Cu} was increased by 10 μl during the R_{Cu} optimization, the volume of SS_{Fe} was reduced by 10 μl. Otherwise, the travel path of the iron zone to the flow cell would increase, in other words, the dispersion of the iron zone would increase. In this case, the compensation kept the peak height for iron constant even if the volume of R_{Cu} varied. When applying this kind of volume compensation, in all cases the changes of volume in the copper determination zone did not also affect the peak heights of iron and vice versa. In the following discussion, we focus on the effects of aspiration volumes on either copper or iron determination.

3.7.1. *Effects of sample volumes*

The sample volume in the copper zone was varied from 15 to 35 μl. The net absorbance at 580 nm linearly increased with increasing the sample volume. The sample volume in the iron zone was varied from 35 to 50 μl. At a sample volume higher than 45 μl, the net absorbance at 558 nm was almost constant. The blank values for copper and iron were constant. Thus, the optimum sample volumes were selected to be 25 μl for copper and 45 μl for iron.

3.7.2. *Effects of reagent volumes*

5-Br-PSAA is a sensitive chromogenic reagent for the determination of iron(II) [16], cobalt(III) [16], copper(II) [16,35], nickel(II) [16,37] and palladium(II) [38]. Nevertheless, this reagent is expensive and therefore reasonable reduction of its volume consumed is desirable. The effects of 5-Br-PSAA volumes were investigated from 5 to 20 μl for R_{Cu} and from 10 to 35 μl for R_{Fe}. Although the peak heights for copper and iron increased with an increase in the volumes of R_{Cu} and R_{Fe}, the blank values increased simultaneously. Taking into account the sensitivity and the blank value, the optimum volumes of R_{Cu} and R_{Fe} were selected to be 10 and 20 μl, respectively.

The volumes of two AS zones were individually varied from 5 to 15 μl; optimum values were 5 μl of each.

Optimizations for the BS volumes in both copper and iron zones were also carried out, and the optimum volumes are shown in Fig. 2.

3.7.3. *Effects of spacer solutions volumes*

The effect of SS_{Cu} volume on the determination of 1 mg l^{-1} copper was studied over the range from 20 to 50 μl. The net absorbance was constant in the examined range but too small SS_{Cu} resulted in high blank because the R_{Cu} got much closer to the flow cell. We chose therefore the SS_{Cu} of 40 μl. When varying the SS_{Fe} volume from 0 to 140 μl, the net absorbance of iron was also constant. Similarly, too small SS_{Fe} resulted in high blank and poor peak separation; thus, SS_{Fe} of 105 μl was chosen.

3.8. *Calibration graphs*

Calibration graphs for copper and iron were measured under the optimum conditions. The calibration graphs were linear for 0.1–2 mg l^{-1} copper ($r^2 = 0.997$) and 0.1–5 mg l^{-1} iron ($r^2 = 0.999$), respectively:

$$\text{Net absorbance for copper} = (1.22 \pm 0.02) \times 10^{-1}[\text{copper, mg l}^{-1}] - (9 \pm 2) \times 10^{-3} \quad (1)$$

$$\text{Net absorbance for iron} = (1.73 \pm 0.02) \times 10^{-1}[\text{iron, mg l}^{-1}] + (6 \pm 10) \times 10^{-3} \quad (2)$$

With replicate sample injections the relative standard deviations (R.S.D.) ($n = 15$) of peak heights were 2% and 1.9% (for 0.5 and 1 mg l^{-1} copper, respectively) and the R.S.D.'s for 0.5 and 1 mg l^{-1} iron were 1.8 and 1.2%. The detection limits (3σ) were 50 μg l^{-1} for copper and 25 μg l^{-1} for iron. The sampling rate was 18 samples h^{-1} for simultaneous analysis.

Copper and iron were determined simultaneously in artificial mixtures at known various concentration ranges. The concentration ranges were 0.25–2 mg l^{-1} for copper and 0.25–5 mg l^{-1} for iron (the Cu/Fe ratios were 1:10 to 5:1). The results are represented in Table 2. The recoveries of both analytes were 96–105%.

3.9. *Interference study*

The interferences of foreign ions on the determination of a mixture of 0.5 mg l^{-1} copper(II) and 0.5 mg l^{-1} iron(III) were

Table 2
Simultaneous determination of copper and iron in artificial mixtures

Added (mg l^{-1})		Found[a] (mg l^{-1})		Recovery (%)	
Cu	Fe	Cu	Fe	Cu	Fe
0.25	0.25	0.24 ± 0.01	0.24 ± 0.00	97	97
0.5	0.5	0.48 ± 0.01	0.51 ± 0.01	96	102
1	1	1.02 ± 0.01	1.01 ± 0.01	102	101
0.5	0.25	0.50 ± 0.02	0.24 ± 0.01	100	97
0.5	1	0.49 ± 0.02	1.02 ± 0.01	97	102
0.5	2	0.51 ± 0.01	1.99 ± 0.01	102	100
0.5	5	0.51 ± 0.02	5.04 ± 0.04	102	101
1	0.2	1.01 ± 0.01	0.21 ± 0.00	101	105
1	0.5	1.01 ± 0.02	0.52 ± 0.01	101	104
2	0.5	2.05 ± 0.01	0.50 ± 0.01	103	99

[a] Average value for three determinations.

studied. The results are summarized in Table 3. An error of less than 5% was considered to be tolerable on each peak height. Most of the metal ions and inorganic anions did not interfere at levels from 5 to 5000 mg l^{-1}. But the tolerance limits of some metals such as cobalt(II), palladium(II) and nickel(II) were low due to the formations of corresponding complexes with 5-Br-PSAA.

3.10. Applications

The proposed SI-LOV method was applied to the simultaneous determination of copper and iron in multi-element solution with certified content of the analytes (Table 4). The analytical values obtained by the proposed method were in good agreement with the certified values. In addition, the proposed method was applied to industrial wastewater sample analysis. Table 5 summarizes the results of copper and iron in industrial wastewater. The experimental *t*-values obtained by the proposed SI-LOV method were 0.284 for copper and 0.156 for iron. These *t*-values were smaller than *t*-value (2.776) for four degrees of freedom at the 95% confidence level. This indicates that there is no significant difference between the results obtained by the proposed SIA-LOV method and those of ICP-AES.

Table 3
Interference of foreign ions on the simultaneous determination of 0.5 mg l^{-1} copper and 0.5 mg l^{-1} iron

Tolerance limit (mg l^{-1})	Ion added[a]	
	For copper determination	For iron determination
5000	Na, K, Zn(II), Pb(II), Cd(II), Cl$^-$, NO$_3^-$, Br$^-$, SO$_4^{2-}$	Na, Cl$^-$, NO$_3^-$, Br$^-$, SO$_4^{2-}$
2000	Mn(II), Ca(II)	K, Pb(II)
1000	Al(III)	Mn(II), Ca(II)
500	Mg(II)	Mg(II), Zn(II), Cd(II)
200		Al(III)
50	Mo(IV), Sn(II), Se(IV)	Se(IV),V(V)
20		Mo(IV)
10	V(V)	
5		Sn(II)
2	Ni(II)	Pd(II)
0.2	Pd(II)	Ni(II)
0.1	Co(II)	Co(II)

[a] An error of ±5% is considered to be tolerable.

Table 4
Simultaneous determination of copper and iron in multi-element standard for ICP spectroscopy

Found (mg l^{-1})		Certified value (mg l^{-1})	
Cu	Fe	Cu	Fe
10 ± 0.4[a]	31 ± 0.3[a]	10	30
10 ± 0.3[b]	30 ± 0.4[b]		

[a] After neutralizing 6 ml of original solution, the neutralized solution was diluted to 100 ml with 0.01 mol l^{-1} HCl.

[b] After neutralizing 5 ml of original solution, the neutralized solution was diluted to 100 ml with 0.01 mol l^{-1} HCl.

Table 5
Simultaneous determination of copper and iron in industrial wastewater[a]

Sample	SIA method (mg l^{-1})		ICP-AES (mg l^{-1})	
	Cu	Fe	Cu	Fe
1[b]	1.12 ± 0.05	2.86 ± 0.06	1.1	2.9
2[c]	1.98 ± 0.02	7.00 ± 0.04	1.9	6.9
3[b]	0.79 ± 0.02	0.46 ± 0.02	0.81	0.46
4[c]	1.90 ± 0.04	0.98 ± 0.05	1.8	0.98
5[b]	0.74 ± 0.02	0.44 ± 0.02	0.83	0.48

[a] t_{exp} for Cu: 0.284; t_{exp} for Fe: 0.156; t (95%): 2.776.

[b] After 5 ml of each sample solution was neutralized, it was placed in a 10 ml. Volumetric flask with 0.01 mol l^{-1} HCl.

[c] After 2.5 ml of each sample solution was neutralized, it was placed in a 10 ml. Volumetric flask with 0.01 mol l^{-1} HCl.

4. Conclusions

We have described here an affordable and precise SI-LOV system for the simultaneous spectrophotometric determination of copper and iron in wastewater using 5-Br-PSAA. The use of spacer containing the chromogenic reagent resulted in the suppression of blank, the promotion of the complex formation reaction and the successful separation of peaks for copper and iron. The proposed SI-LOV method has wide determinable ranges from 0.1 to 2 mg l^{-1} for copper and from 0.1 to 5 mg l^{-1} for iron, respectively, with low R.S.D. values and low reagent consumption. The computer-controlled SI-LOV system is suitable for performing routine automated assays of copper and iron in wastewater.

Acknowledgements

The present work was partly supported by a grant of the Frontier Research Project “Materials for the 21st

Century—Materials Development for Environment, Energy and Information" (for 2002–2006 fiscal years) from Ministry of Education, Culture, Sports, Science and Technology.

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