Design of High Performance Flow-through Cells for Trace Analysis of Metals and Organic Compounds

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Abstract

New flow-through cells were designed and their functions were investigated. A temperature-controlled flow cell was effective for the selective determination of quaternary ammonium compounds with tetrabromophenolphthalein ethyl ester (TBPE) was used as an ion association reagent. Multi-channel flow cells were useful for simultaneous determination of metal ions. A flow cell with long path length was excellent for the detection of trace metals at ppb levels. 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) aniline (5-Br-PSAA) and 2-(5-Nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]phenol(Nitro-PAPS) were also utilized as a chelating reagent for the sensitive determination of several metals.

Keywords : Temperature-controlled flow cell, multi-channel flow cells, simultaneous assay of trace metals

1. Introduction

"Flow Injection Analysis. Part 1. A new concept of fast continuous flow analysis" was reported in Anal. Chim. Acta, Vol. 78, 145-157 (1975) by J.Ruzicka and E.H.Hansen. At that time, high performance liquid chromatography (HPLC) and a segmented flow analysis with an auto analyzer system were noticed as a rapid analytical method using the continuous flow. Especially, various kinds of separation modes and excellent columns for various analytes have been developed remarkably, and the techniques have contributed to practical analyses in such fields as pharmaceutical, food and industrial sciences. AutoAnalyzer was also applied to multi-element analyses in the clinical chemistry. In the beginning, the concept of flow injection analysis (FIA) was not necessarily acceptable; however, the advantages on rapidity, simplicity, reproducibility, reduction of reagent amounts consumed and small sample volume have been gradually recognized. Recently, the development of FIA techniques becomes more and more, and the methods are applied to in steel products, food sciences, clinical and environmental chemistry. However, the flow cell used in spectrophotometry has only a simple function. The author and his coworkers newly designed temperature-controlled flow cells for enhancement of selectivity and multi-channel flow cells for simultaneous assay of metals.

2. Development of temperature-controlled micro flow cell on-line extraction system

2.1. Flow injection analysis for thermo-spectrophotometric determination of acetylcholine and choline [1]

Acetylcholine(Ach) is an important neuro-transmitter in a central nervous system of man, and choline (Ch) is its metabolite. Recently, the relationship between Ach and senile dementia has been noted in clinical chemistry research, and a trace analysis methods for Ach and Ch are needed. For such measurements, liquid chromatography utilizing an immobilized enzyme electrode [3] and chemiluminescence detection with immobilized enzymes [4] have been reported. In addition, several amperometric detection systems with enzymes have been developed [5,6]. On the other hand, spectrophotometric methods have received less attention because of the lack of coloration reagents for Ach and Ch. Fortunately, it was found that Ach and Ch, having a quaternary ammonium structure, reacted with TBPE to form a blue ion association complex. Also, a method utilizing both a flow injection (FI) system and thermochromism phenomenon of ion associates [7] could be employed to enhance the sensitivity and selectivity for Ach and/or Ch determination. In addition, to

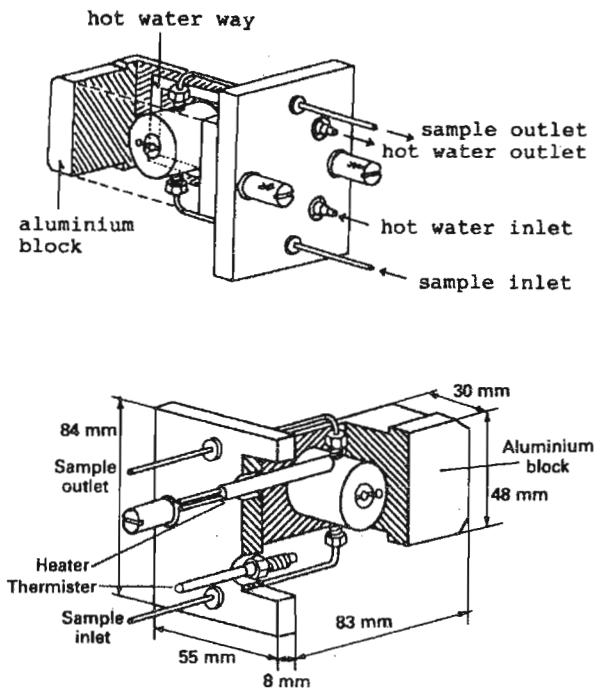

Fig.1 Thermo-controlled micro flow cells [1,2].

eliminate the interfering amine associate, thermo-controlled micro flow cells were developed.

2.1.1. Procedure for flow injection method

Absorbances were measured at 610 nm by using a Soma Optics S-3250 double-beam detector (data output 0-10 mV) with 10-mm laboratory-made flow cells (8 μl) as shown in Fig.1 and recorded as peak-shaped signals using a Toa Electronics FBR-251A recorder.

A double-plunger micro pump (Sanuki Kogyo, DM2U-1026) was used to propel a carrier solution buffered at pH 11 and a TBPEH /1,2-dichloroethane (DCE) solution. Samples (140 μl) were injected into the carrier stream by a six-way injection valve. A T-shaped connector was used as a segmentor. A phase separator with a sloped groove (depth 2 mm, width 2 mm) and a porous polytetrafluoroethylene (PTFE) membrane (0.8 μm pore size) as designed by Motomizu et al. [8] was used for smooth and efficient phase separation. All flow lines were made of PTFE tubing (0.5mm i.d.). The temperature in the flow cell was maintained at 25 or 45 ℃ by circulating the water. A diagram of the flow system is shown in Fig.2.

2.1.2. Spectral characteristics

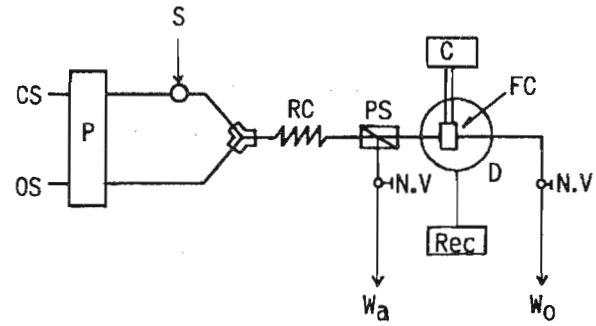

Fig.2 A flow diagram for Ach and Ch determination using thermo-controlled flow cell.

The yellow TBPEH/DCE solution can be back-extracted into an alkaline aqueous medium, and TBPE anion released in the aqueous phase can react with cationic compounds to form the ion association complexes as follow:

$$\underset{\text{yellow}}{TBPE{\cdot}H_o} \rightleftharpoons \underset{\text{blue}}{TBPE^-} + H^+$$

$$\underset{\text{blue}}{TBPE^-} + Ach^+ \rightleftharpoons \underset{\text{blue}}{(TBPE^-)(Ach^+)_o}$$

$$\underset{\text{blue}}{TBPE^-} + HNR_3^+ \rightleftharpoons \underset{\text{red-violet}}{(TBPE{\cdot}H{\cdot}NR_3)_o}$$

where the subscripts o refers to the organic phases. Ach and Ch, reacted with TBPE anion to form blue ion association complexes, which were successfully extracted into DCE. The blue association complex has an absorption maximum at 610 nm. The molar absorptivity obtained for Ach was 24000 l mol^{-1} cm^{-1} at pH 11 and that for Ch was 6000 l mol^{-1} cm^{-1} at pH 12. On the other hand, protonated secondary and tertiary amines formed red charge transfer complexes and the absorption maxima of the complexes were at 555-585 nm.

2.1.3.Thermochromism of charge transfer complexes of TBPE with amines

In previous papers [7,9], it was reported that charge transfer complexes formed between amines and TBPE showed reversible thermochromism with temperature changes and the absorbance decreased greatly with an increase in the temperature in the cell. It was assumed that thermochromism of the charge transfer complexes occurred as follow:

$$\underset{\text{red-violet}}{TBPE{\cdot}H{\cdot}NR_{3,o}} \overset{\text{heat}}{\underset{\text{cool}}{\rightleftharpoons}} \underset{\text{yellow}}{TBPE{\cdot}H_o} + \underset{\text{colorless}}{NR_{3,o}}$$

Table 1 Effect of diverse amines in the determination of facetylcholine and choline at 25 and 45℃ in the flow injection method[a]

Amine	Mole ratio[b]	Recovery (%)			
		Acetylcholine		Choline	
		25℃	45℃	25℃	45℃
Procaine	1	114	102	123	102
Diphenhydramine	1	111	101	122	103
Chlorpheniramine	0.1	113	101	110	102
Methylephedrine	1	110	102	118	100
Ephedrine	10	122	100		
	5			120	103
Lidocaine	10	110	100	98	

a. 5×10^{-6} mol/l acetylcholine or 1×10^{-5} mol/l choline was taken. 1×10^{-5} mol/l TBPE·H; pH 11; wavelength, 610 nm.
b. Mole ratio: [amine]/[acetylcholine] or [amine]/[choline].

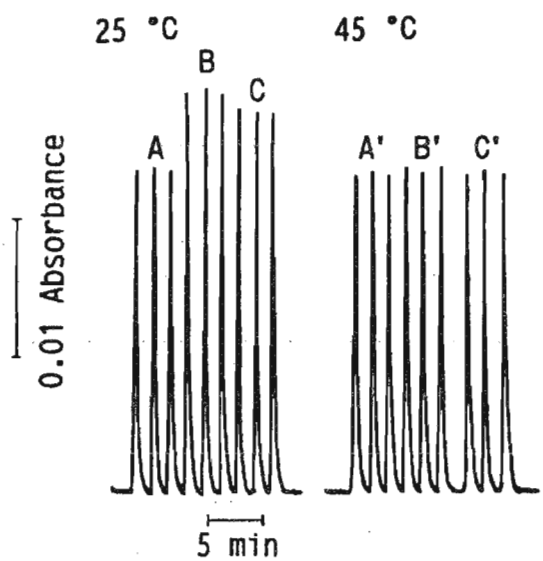

Fig.3 Flow signals of ion association complexes with TBPE·H at 25 and 45℃

On the other hand, the absorbance of the blue ion association complexes was not influenced by temperature changes. Consequently, the absorbance of the blue ion association complex can be measured without interference from amines when absorbance measurements are made at 45-60℃, but not at room temperature. This is because the above equilibrium shifts completely to the right on heating and the absorbance of the red species apparently disappears.

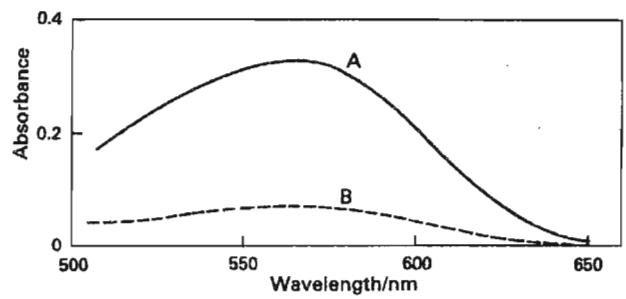

Fig.4 Absorbance changes of red ion associate with thermochromism.

2.1.4. Effect of amines on the determination of Ach and Ch

To a standard 1×10^{-5}mol/l Ch solution, various amines were added, and their interference with the determination of Ch using the FI system asstudied at both 25 and 45℃. Signals for the Ch-TBPE and other ion associates obtained at 25 and 45℃ are shown in Fig.3. Signals A are for the Ch-TBPE ion associate with 1×10^{-5} mol/l Ch, signals B and C are for the ion associates with 1×10^{-5} mol/lprocaine and/or methylephedrine. Procaine and methylephedrine gave positive errors with the Ch determination at 25℃. However, signals B' and C' in Fig.3 show almost the same peak heights as those for the Ch ion associates alone. Consequently, interference from procaine and methylephedrine could be eliminated effectively on the signal measurement of Ch

Table 2 Determination of cetylpyridinium and benzalkonium in commercial samples by the FI method, (n = 3)

Sample	Nominal / mg	25℃		45℃	
		Found / mg	Recovery (%)	Found / mg	Recovery (%)
1[a]	1.00	1.25±0.02	124	0.97±0.03	97.3
2[b]	4.00	4.37±0.13	109	3.97±0.12	99.2
3[c]	1.00	1.46±0.02	145	0.98±0.02	97.9
4[d]	0.1	0.101±0.01	101	—	—

a. Main content: cetylpyridinium chloride; chlorpheniramine maleate (2 mg); naphazorine hydrochloride (1 mg); and iproheptine hydrochloride (3 mg).
b. Main content: cetylpyridinium chloride; and dextromethorphan (90 mg).
c. Main content: cetylpyridinium chloride; chlorpheniramine maleate (5 mg); and procaine hydrochloride (5 mg).
d. Main content: benzalkonium chloride.

ion associates at 45℃.

The effect of co-existing amines on the determination of Ach and Ch by the proposed FI system is summarized in Table 1.The recoveries at 25℃ were not good, whereas those at 45℃ were satisfactory.

2.2. Thermo-spectrometry for the cetylpyridinium and benzalkonium determination [10]

2.2.1. Thermochromism of amine asssociation complexes with TBPE

In the previous section, it was reported that TBPEH-amine association complexes exhibit changes in absorbance with temperature (thermochromism). As can be seen in Fig.4, absorbance on the TBPEH-butylamine ion association complex (565 nm) approaches zero when the temperature is elevated from 20 to 45℃; that is, the measurement at 45℃ eliminates the interference from amines at 25℃ apparently and selectivity in the determination of cetylpyridinium and benzalkonium is enhanced.

2.2.2. Calibration graphs

At 25 and 45℃, good liner relationships were found over the range 5×10^{-7} – 2×10^{-6} mol/l cetylpyridinium when 140 μl of the standard solutions were injected. The relative standard deviations (n = 5) were 2.0% for 1×10^{-6} mol/l cetylpyridinium at 25℃ and 2.1% at 45℃. The limit of detection (S/N = 3) was 6.3×10^{-8} mol/l at 25℃ and 6.1×10^{-8} mol/l at 45℃. Peak height at 45℃ was about 10% shorter than that at 25℃. Similar results were obtained for benzalkonium. The relative standard deviations (n = 8) were 1.8% for 1×10^{-6} mol/l at 25℃ and 2.1% at 45℃. Sample throughputs were 60/hr for cetylpyridinium and 50/hr for benzalkonium.

2.2.3. Application

Practical use of the proposed method was assessed by applying it to the determination of cetylpyridinium and benzalkonium in pharmaceutical preparations. Sample solutions were prepared after filtration and suitable dilution. Table 2 shows the results obtained at 25℃ and 45℃ with the proposed FI system. Strong interference from co-existing amines were observed at 25℃, whereas the interference could be satisfactorily eliminated in the determination of cetylpyridium at 45℃.

3. Development of simultaneous determination method for metals using FI technique[11,12]

Although many researches on FIA have brought its great development in recent years, its potential has not been adequately realized and the commercialization is still limited. This is partially because it is difficult to determine several elements simultaneously with a simple detection system. Faizullah and Townshend [13] determined Fe(II) and total iron in a flow system by passing some parts of samples through a Jones reductor mini-column before spectrophotometric detection with 1,10-phenanthroline. Kuroda et al. [14] reported a simple approach to the determination of Fe(III) and total iron by the formation of a colored complex between Fe(III) and Tiron, in which the oxidation of Fe(II)

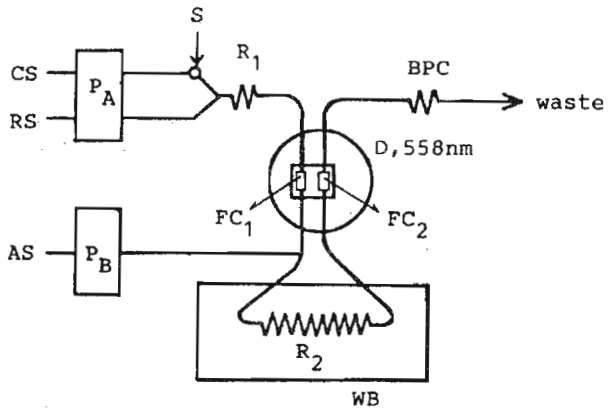

Fig. 5 Schematic flow diagram for the simultaneous determination of Cu(II) and Fe(II)[11]. CS, 0.1 mol/l HCl containing 1×10^{-4} mol/l KIO_4; RS, 1×10^{-4}mol/l 5-Br-PSAA solution buffered at pH 4.5; AS, 1×10^{-2} mol/l sodium ascorbate solution (pH 4.5); R_1, 15-cm reaction coil; R_2, 700-cm reaction coil; FC_1, FC_2, flow cells; BPC, 200-cm back-pressure coil (0.25 mm i.d.); WB, water-bath (60℃; D, double-beam spectrophotometer; P_A, P_B, Pumps.

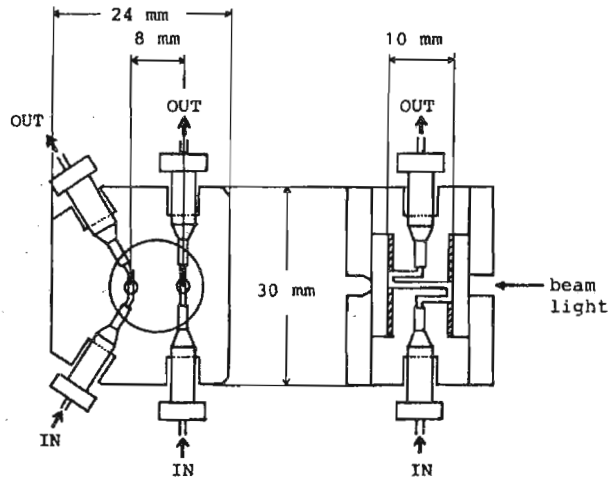

Fig.6 Configuration of double flow cell in double beam spectrophotometer[11].

was accelerated by irradiation with ultraviolet radiation. Flow injection procedures for the simultaneous spectrophotometric determination of Fe(II)/ Fe(III) and Co(II)/Ni(II) based on the difference in kinetic catalysis behavior in the redox reaction with and without an activator have been reported [15-17]. Most detection systems applied to the spectrophotometric determination of Fe(II), Fe(III), total iron and Ti(IV) used a single optical detector with two flow cells aligned with the same optical path to yield two positive peaks [14,18].

This section describes the development of simultaneous detection system for copper and iron using 5-Br-PSAA and the function of serial and double flow cells designed newly for the rapid determination of the metals.

The reagent solution containing 5-Br-PSAA (1×10^{-4} mol/l), sodium ascorbate solution (1×10^{-2} mol/l), sodium (potassium) metaperiodate solution(1×10^{-4} mol/l) were prepared. Super purified HCl and super pure water were used for diluting the solutions. The manifold of the flow injection system used is shown in Fig.5. Two double plunger micro pumps were used to pump the solutions. A carrier solution containing 1×10^{-4} mol/l potassium(sodium) metaperiodate and 0.1 mol/l HCl and the chromogenic reagent solution (1×10^{-4} mol/l 5-Br-PSAA buffered at pH 4.5) were propelled at a flow-rate of 0.9 ml/min by pump A and the samples (100 μl) containing up to 200 ppb each of Cu(II) and Fe(II) were injected into the carrier stream by a six-way injection valve. The sample and reagent were mixed in the 15-cm reaction coil (R_1). A double-beam spectrophotometer, fitted with laboratory-made double micro flow cells designed as shown in Fig.6 (8 μl, 10mm path length) was used for absorbance measurements. After the color development of Cu(II) with 5-Br-PSAA, absorbances of Cu(II) complexes were detected at 558 nm at the first flow cell, FC_1, and recorded as peak-shaped signals. The sodium ascorbate solution (1×10^{-2} mol/l) buffered at pH 4.5 was pumped at 0.25 ml/min by pump B and mixed in the 700-cm reaction coil (R_2) in a water-bath (60℃). Only reduced Fe(II) reacted with 5-Br-PSAA to form a colored product. Absorbance at the second flow cell, FC_2, was measured at the same wavelength as mentioned above. The first peak, corresponding to Cu(II), was positive and the second, corresponding to Fe(II), was negative.

3.1. Application of serum assay [11]

Horiguchi et al. [19] reported the synthesis of water-soluble 5-Br-PSAA and noted that the reagent reacted with Cu(II), Fe(II), Co(II), and Ni(II) to form red complexes. In the flow system used in this work, iron(II) is oxidized to Fe(III) with potassium metaperiodate (KIO_4). Iron(III) dose not give a color complex with 5-Br-PSAA, whereas all copper ions are present in the form of Cu(II) in the presence of KIO_4 and can form a chelate with 5-Br-PSAA. Hence, only the absorbance of the Cu(II) chelate can be measured in the first flow cell, FC_1, at the double-beam spectrophotometer. Subsequently, the solution

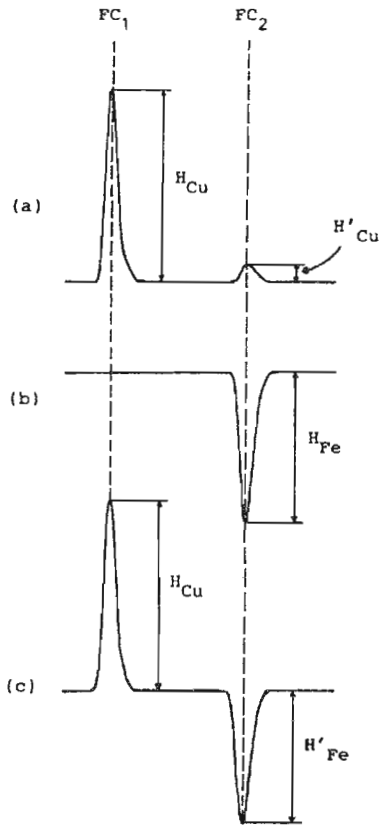

Fig.7 Typical flow signals with double flow cell [11].

is merged with a reducing agent (sodium ascorbate) to reduce Cu(II) and Fe(III) to Cu(I) and Fe(II), respectively. In this step, the Cu(II) chelate is decomposed and the Fe(II) chelate is formed. Thus only the Fe(II) chelate is detected in the second flow cell, FC_2, and an inverted peak is obtained. In this work, the coil R_1 was shortened and the coil R_2 was long enough to reduce Fe(II) and Cu(II), two peaks for Cu(II) and Fe(II) were obtained individually. The molar aborptivities for Cu(II) and Fe(II) at 558nm were 55000 and 87000 l mol^{-1} cm^{-1}, respectively, and those at 578nm were 65000 and 41000 l mol^{-1} cm^{-1}, respectively. There is a large shoulder which has sufficient sensitivity between 550 and 560 nm in the absorption spectra of Cu(II) complexes, and 558nm was chosen for simultaneous determinations of Cu(II) and Fe(II) in this manifold.

3.1.1. Effect of coil lengths R_1 and R_2

The effect of the lengths of the reaction coils R_1 and R_2 (Fig.5) was examined. The coil length of R_1 was varied in the range 10-50 cm. Cu(II) reacted with the reagent quickly to form a colored chelate in R_1. The largest peak height was obtained in the range 10-25 cm (0.5mm i.d.). When the coil length of R_1 was more than 50cm, the peak height decreased and the reproducibility was poor. A 15-cm coil was recommended. The effect of the coil length of R_2 was studied over the range 200-700 cm. The peak heights increased with increasing the length of R_2 because the rate of the reaction between Fe(II) and the reagent was slow. Further, the separation of the first peak for Cu(II) and the second peak for Fe(II) was not satisfactory when the R_2 length was less than 400 cm. A 700-cm reaction coil length was used for R_2 to separate completely the first and the second peaks and to achieve sufficient Fe(II) chelate formation.

3.1.2. Characteristics of peak signals

The signal profiles obtained by the manifold equipped with a parallel double-flow cell in the double-beam spectrophotometer are shown in Fig.7. The profile (a) was obtained when Cu(II) was injected into the carrier stream containing potassium metaperiodate. The Cu(II)-5-Br-PSAA chelate in FC_1 was detected as a first peak. When sodium ascorbate was added to the slugs passing through FC_1, Cu(II) was reduced to Cu(I) and the Cu(II) chelate was decomposed. However, a small second peak was observed in FC_2 and it was found that the peak heights were proportional to copper concentration. It was assumed that the second peak in FC_2 is caused by catalytic decomposition of the 5-Br-PSAA to decrease a background absorbance. The profile (b) was obtained when Fe(II) was injected. The reagent 5-Br-PSAA reacts with Fe(II), whereas it does not react with Fe(III). Accordingly, no signal was found in FC_1 and an inverted signal of Fe(II) chelate was found in FC_2 owing to the reduction of Fe(III) to Fe(II) with sodium ascorbate. The profile (c) was obtained when a mixed solution of Cu(II) and Fe(II) was injected. The height, H_{Cu}, of the first peak, for the Cu(II) chelate, agreed with that for the first peak in profile (a). On the other hand, for the second peak, the inverted peak with Fe(II) overlapped the positive second peak (H'_{Cu}) due to reduction of Cu(II). Consequently, the actual peak height (H_{Fe}) for Fe(II) should represent the sum of the apparent peak height, H'_{Fe}, and the peak height H'_{Cu} as shown in the profile (a). As mentioned above, the first peak height for copper is proportional to the second peak height caused by copper. Hence the following equation is obtained:

$$H'_{Cu} / H_{Cu} = a \qquad (1)$$

where a is a constant. The actual peak height of Fe, H_{Fe}, is represented by the equation

$$H_{Fe} = H'_{Fe} + H'_{Cu} = H'_{Fe} + aH_{Cu} \qquad (2)$$

In Eqn.(2), the proportionality factor, a, can be obtained experimentally; in the FI system in Fig.5, a=0.0938.

3.1.3. Calibration graphs and limit of detection

Calibration graphs were linear over 50-200 ppb of Cu(II) at both the first and second peaks. The limits of detection (LOD) were 2.4 ppb (S/N = 2) at the first peak and 10 ppb at the second peak. The relative standard deviations (R.S.D.) were 1.2% (first peak) and 5% (second peak) for ten runs of 100 ppb of Cu(II). Calibration graph was also linear over 50-200 ppb of Fe(II) at the inverted peak. The LOD and RSD were 2.4 ppb (S/N = 2) and 1.2%, respectively, for ten runs of 100 ppb of Fe(II). Sample throughput was 30/h in the proposed manifold. Common anions and most metal ions at levels from 2.5 to 50 ppm did not interfere with the determination of Cu and Fe. In particular, Zn(II), which is present in serum, gave no interference even when it is present at level of 25 ppm.

3.2. Ground water assay [11]

This section describes the function of a double and serial flow cell designed newly and the simultaneous determination system of trace amounts of copper and iron.

The reagents in the previous section were used. The manifold of the FI system is shown in Fig.8. For the simultaneous determination of Cu(II) and Fe(II), measuring wavelengths were investigated. The molar absorptivity of the 5-Br-PSAA-Cu(II) complex at 578 nm was large, and that of the Fe(II) complex at 558 was larger than that at 578 nm. However, 558 nm was chosen in the proposed manifold because there is a large and a wide shoulder, which has sufficient sensitivity, between 550 and 560 nm in the absorption spectra of the Cu(II) complex.

3.2.1. Design of two channel flow cells

Fig. 9 shows the configuration of a serial (A) and a double (B) flow cell designed newly by the author. The path length of the serial flow cell was 5 mm and the volume of the cell was 4 μl. The size of the cell

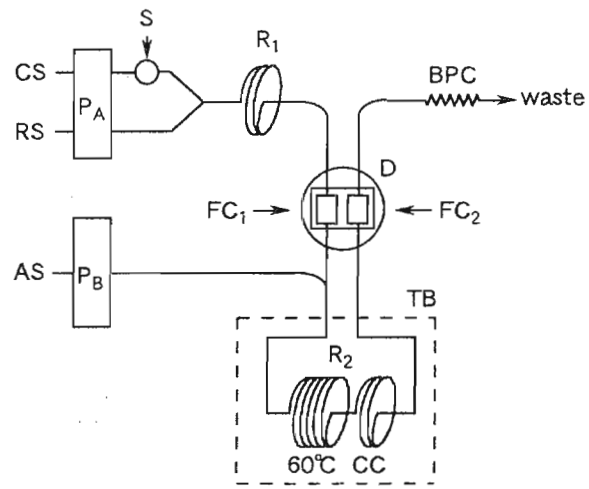

Fig. 8 Flow diagram for simultaneous determination of copper and iron.

holder was 55×84×90 mm and compact.

The peak signals for Cu(II) and Fe(II) using the serial flow cell are shown in Fig.10. The volume in the 10mm path length cell was 8 μl, that of the 20 mm length cell, 30 μl. For copper and iron below 10 ppb, the flow cell with 20 mm path length was useful

3.2.2. Calibration graphs and limit of detection

Calibration graphs for both Cu(II) and Fe(II) were made using 5-Br-PSAA and double flow cells with 10 mm and 20 mm path lengths. The calibration graphs were linear over 10-80 ppb. As a matter of course, when the cell with 20 mm path length was used, the slope of the calibration graph was 2-fold. The RSDs(n=5) with the cell of 10 mm path length were 0.8% for 30 ppb Cu(II) and 0.9% for 30 ppb Fe(II). The RSDs with the cell of 20 path length were 0.6% for 30 ppb Cu(II) and 0.99% for Fe(II). When the cell of 20 mm path length and the sensitivity range of AU(absorbance unit full scale) 0.05 of the detector were used, the graph was linear even in the range 3-10 ppb and R.S.D for 10 ppb Cu(II) was 0.5%, for 10 ppb Fe(II), 0.6%. The sampling rate was 12/h.

4. New design of a multi-channel micro cell for simultaneous analysis in the flow injection method

Many studies have undergone great developments concerning sensitivity, pretreatment and application in FIA. However, most of these investigations have been performed on a single-analyte detection. In rou-

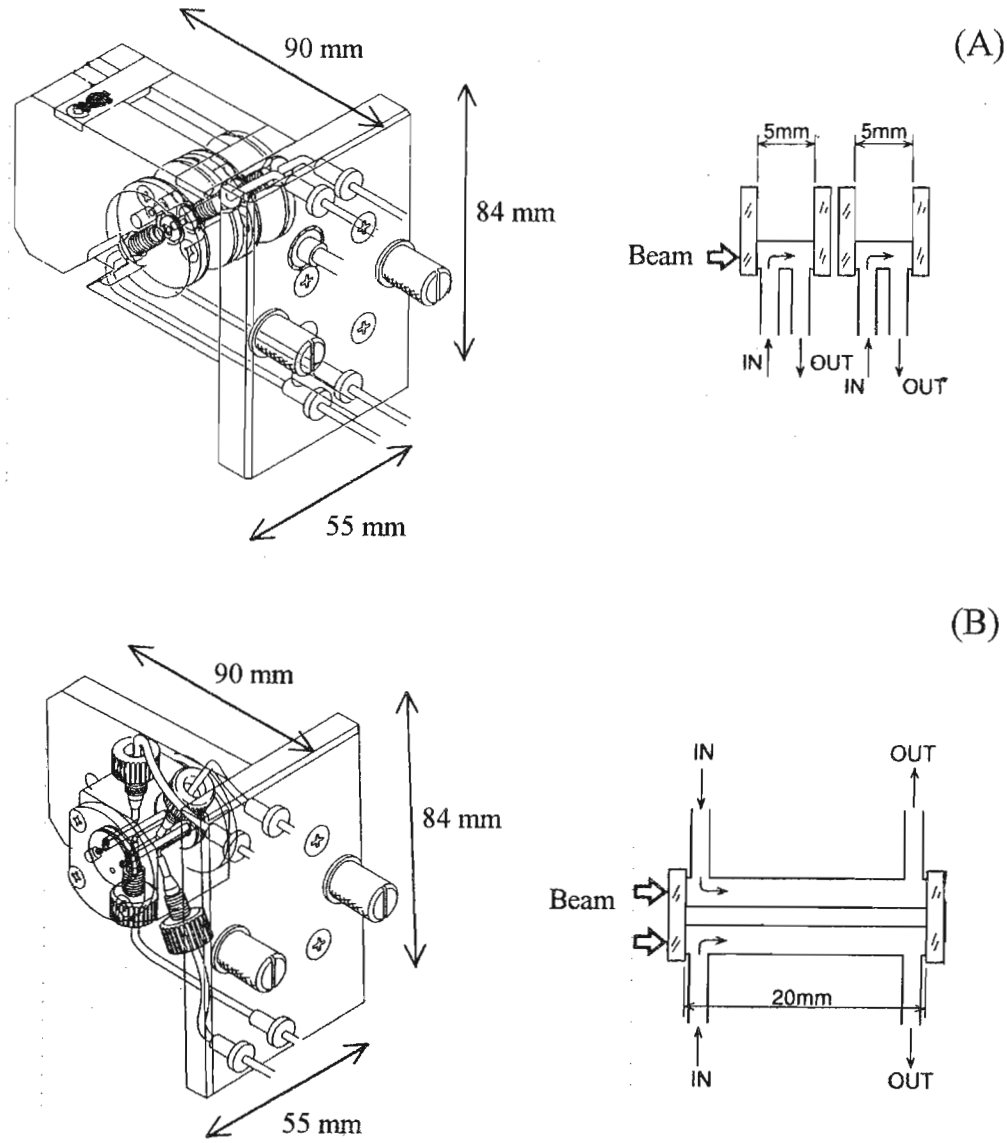

Fig. 9 Configration of a serial (A) and a double (B) flow cells [12.]

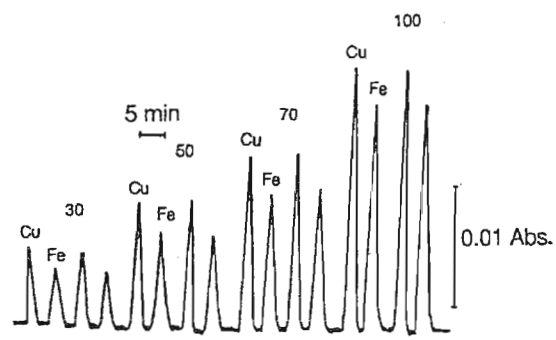

Fig. 10 Flow signals using serial flow cell [12].

tine FIA, it is difficult to analyze simultaneously several analytes by a single shot, when FIA does not involve separating system like a column. In detection system with a single beam, two flow cells were aligned in the same optical path. Yamane et al. [15] proposed the simultaneous intercalation of a reagent plug and two small sample plugs into the same carrier stream by a 16-way valve for the determination of Fe(III) and Fe(II). In the detection system, the difference in the rate of a reaction containing an oxidation-reduction reaction, coupled with a delayed coil and a single flow cell, were often used. A twin-compartment flow-through cell has been proposed for the first time by Muller et al [16],

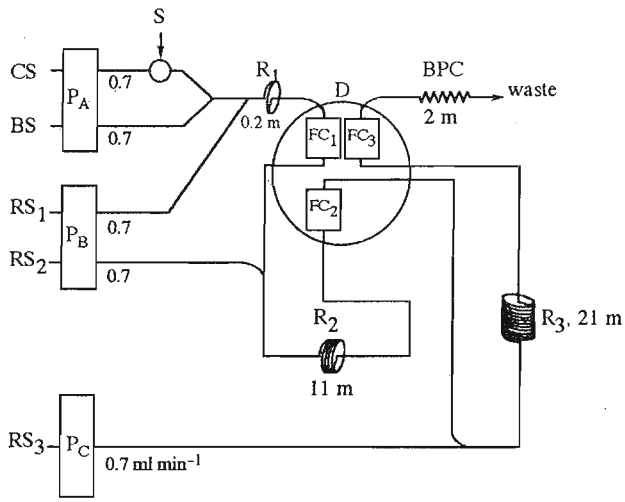

Fig. 11 Flow diagram for copper, iron and zinc analysis

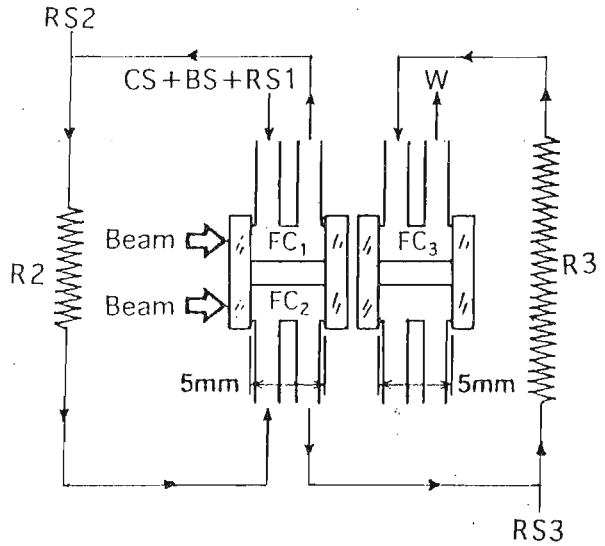

Fig. 12 Configuration of quadruple flow cell [20].

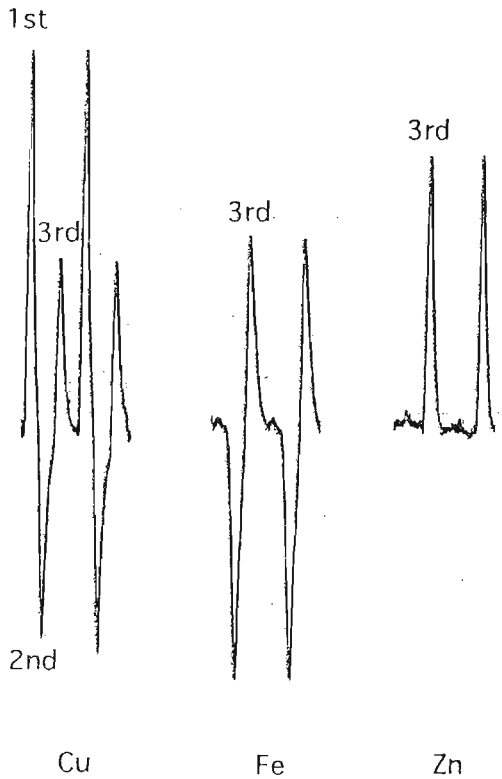

Fig. 13 Flow signals for copper, iron and zinc at FC_1, FC_2 and FC_3 [20].

which was used to determine Fe(II) and Fe(III); the definite configuration of the cell was not shown. The author and his colleagues [12] have designed three types of new compact double micro flow cells, which were applied to the selective and reproducible determination of trace amounts of Cu(II) and Fe(II) using a single- or double-beam spectrophotometer. However, simultaneous determination methods for three or more components have not been proposed in the spectrophotometry; also, there are no multi-channel flow cells, except for the double-flow cells, shown in a previous paper [12].

4.1. Simultaneous determination of copper, iron and zinc using quadruple flow cell [20]

The determination of three metal ions was examined with 2-(5-Nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl) amino]phenol (Nitro-PAPS). Nitro-PAPS reacts with copper(I, II), iron(II) and zinc. The chelate compounds were formed at pH 3 - 9 for copper and iron(II), and at pH 8 - 9 for zinc. Iron(III) did not react in any pH ranges. The characteristics were introduced for the simultaneous determination of copper, Fe(II) and zinc. A five-line manifold was assembled as shown in Fig.11. The 0.01mol/l HCl solution and acetate buffer (pH 4) were delivered at 0.7 ml/min by pump 1, and they were merged, going downstream. Then, the stream was mixed with 2×10^{-5} mol/l Nitro-PAPS (RS_1) delivered by pump 2; then, the copper chelate was detected at the FC_1. Fig.12 shows the configuration of the quadruple flow cell. A 5×10^{-2} mol/l ascorbate solution including 6×10^{-5} mol/l Nitro-PAPS (RS_2) was added to the solution coming out from FC_1, and a mixture Cu(II) and Fe(II) was detected at the FC_2. Then, 6×10^{-5} mol/l Nitro-PAPS buffered at pH 11 (RS_3) was mixed in the stream from FC_2, and the

total absorbance of Cu(II), Fe(II) and zinc was measured at the third. Duplicate flow signals obtained using the proposed flow cell are shown in Fig.13. The Nitro-PAPS-copper chelate was found at all compartments, and the peak height measured at each compartment was proportional to the copper concentrations. In the case of iron, the signals were obtained at the 2nd and 3rd cells. At pH 3 - 4, Nitro-PAPS-zinc chelate was not formed. As a result, no flow signals were found at the 1st and 2nd flow cells, and only the 3rd signal was obtained. The sample throughput for three analytes was 12/h. The relative standard deviations (n=5) were 1.0% for 0.2 ppm copper at each flow cell, 1.7% at the 2nd cell and 1.3% at 3rd cell for 0.2 ppm iron and 1.1% for 0.2 ppm zinc. The newly designed flow cell is compact, simple, easy to perform and useful for flow-injection analysis to detect multi-analytes.

5. Conclusion

FIA has many advantages on rapidity, simplicity, reproducibility and repeatability. However, its function is not sufficient on the multi-elements analysis because of simple detection system. In this study, to advance the FIA function, new flow cells were designed for selective determination of quaternary ammonium compounds and simultaneous determination of metal ions such as copper, iron and zinc. The selectivity was enhanced on the ion associate extraction-FIA using the thermo-controlled flow fell. And also, two channel flow cells and four compartment flow cell were favorable for the determination of two or three elements. Furthermore, the proposed flow cells are also available for many kinds of kinetic investigation.

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References

[1] T. Sakai, Y-H. Gao, N. Ohno and N. Ura, *Anal. Chim. Acta*, **255**, 135 (1991).
[2] T.Sakai and N.Ohno, *Anal.Sci.*, **7**, 297(1991).
[3] T. Yao, N. Sato and T. Wasa, *Nippon Kagaku Kaishi*, **1985**, 1501.
[4] K. Honda, K. Miyaguchi, N. Nishio, H. Tanaka, T. Yao and K. Imai, *Anal. Biochem.*, **153**, 50 (1986).
[5] T. Yao and M. Sato, *Anal. Chim. Acta*, **172**, 371 (1985).
[6] J.-L. Morrty, K. Sode and I. Karube, *Anal. Chim. Acta*, **228**, 49 (1989).
[7] T. Sakai and N. Ohno, *Analyst*, **107**, 634 (1982).
[8] S. Motomizu and M. Oshima, *Analyst*, **112**, 295 (1987).
[9] T. Sakai and N. Ohno, *Talanta*, **33**, 415 (1986).
[10] T. Sakai, *Analyst*, **117**, 211 (1991).
[11] S. W. Kang, T. Sakai, N. Ohno and K. Ida, *Anal. Chim. Acta*, **308**, 329 (1995).
[12] T. Sakai, Y. Maeda and N. Ura, *Talanta*, **49**, 989 (1999).
[13] A. T. Faizullach and A. Townshend, *Anal. Chim. Acta*, **167**, 225 (1985).
[14] R. Kuroda, T. Nara and K. Oguma, *Analyst*, **113**, 1557 (1988).
[15] T. Yamane and E. Goto, *Anal. Sci.*, **5**, 783 (1989).
[16] H. Muller, U. Muller and E. H. Hansen, *Anal. Chim. Acta*, **230**, 113 (1990).
[17] A.Fernandez, M.D.L.de Castro and M.Valcarcel, *Anal.Chem.*, **56**, 1146(1984).
[18] S.Kozuka, K.Saito, k.Oguma and R.Kuroda, *Analyst*, **115**, 431(1990).
[19] D. Horiguchi, M. Saito, T. Imamura and K. Kina, *Anal. Chim. Acta*, **151**, 457 (1983).
[20] T. Sakai, N. Sakashita, N. Teshima and N. Ura, *Anal. Sci.*, **16**, 251 (2000).

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