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Flow-Injection and Sequential-Injection Determinations of Paracetamol in Pharmaceutical Preparations Using Nitrosation Reaction

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A simple FI and two different SI systems have been investigated for the determination of paracetamol by employing a simple reagent for a nitrosation reaction. It is based on the on-line nitrosation of paracetamol with sodium nitrite in an acidic medium. The formed nitroso derivative species reacts further with sodium hydroxide to convert it to a more stable compound. The yellow product is continuously monitored at 430 nm. The FI system is very simple and cost effective for fast manual operation (60 injections/h; $y = 0.268x + 44.314$, $r^2 = 0.9910$ for 400 - 1000 mg/l and $y = 0.1687x + 145.72$, $r^2 = 0.9970$ for 1000 - 2500 mg/l). The two SI systems with different components and configurations are automated and optimized for the conditions for which no extra dilution is to be required for sample handling: one with a syringe pump and two selection valves (60 samples/h; $y = 0.1488x - 4.7297$, $r^2 = 0.9946$ for 400 - 1000 mg/l and $y = 0.0858x + 63.933$, $r^2 = 0.9849$ for 1000 - 2500 mg/l); the other is simpler and more cost-effective, with an autoburette and only one selection valve (15 samples/h; $y = 0.0072x + 1.1467$, $r^2 = 0.9977$ for 200 - 1000 mg/l and $y = 0.0028x + 5.4699$, $r^2 = 0.9879$ for 1000 - 2500 mg/l). They have all been applied to assay paracetamol in pharmaceutical preparations. The obtained results agree with those by the US Pharmacopeia method.

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Introduction

Paracetamol is an extensive analgesic and antipyretic drug. Several batch methods have been reported for the determination of paracetamol in pharmaceutical preparations, such as spectrophotometry,[1-6] reflectance near-infrared spectroscopy,[7] chemiluminescence[8] and liquid chromatography.[2] A number of flow-injection (FI) methods have also been reported for the determination of paracetamol, such as FI-spectrophotometry, using different on-line derivatization reactions. However, the control of such reactions and/or manifolds is still complicated.[9-12] Some methods, such as FI-FTIR[13] and FI with a boron-doped diamond thin film electrode,[14] involve relatively higher cost instruments.

A further generation of FIA, SIA is a great potential technique for chemical analysis due to much less reagent consumption, its simplicity and convenience with which manipulation can be automated. So far, an SI procedure for paracetamol determination has been reported. It is based on a reaction with hexacyanoferrate(III), followed by a reaction with phenol at elevated temperature in aqueous ammonia.[15]

Simple and inexpensive spectrophotometric flow-injection and sequential-injection systems for the determination of paracetamol using simple reagents based on preliminary concepts introduced at the ICFIA 2001 Conference,[16] are presented. The procedures employ the reaction of paracetamol with nitrous acid at room temperature, producing a derivative.

Experimental

Chemicals and reagents

All of the reagents used were of analytical reagent grade. Deionized water was used throughout the experiments. A stock solution (5000 mg/l) of 4-acetaminophenol standard (paracetamol, Fluka, Switzerland), which was assayed using the USP method,[17] was prepared by dissolving 0.5146 g of the standard in water and diluting to the mark in a 100 ml volumetric flask. Working standards were freshly prepared by diluting the stock solution with water to obtain appropriate concentrations.

Sample preparation

Some locally commercial pharmaceutical preparations were taken as samples to be assayed. For tablet samples, 20 tablets were accurately weighed and finely powdered. An amount of the powder equivalent to a tablet was dissolved in water. After stirring for 15 min at a temperature of 60 - 70°C, the volume was made to 250 ml. The solution was then filtered before analysis. Further appropriate dilutions for FIA procedures were made using water. For a syrup sample, an aliquot corresponding to 200 mg of paracetamol was diluted to 100 ml

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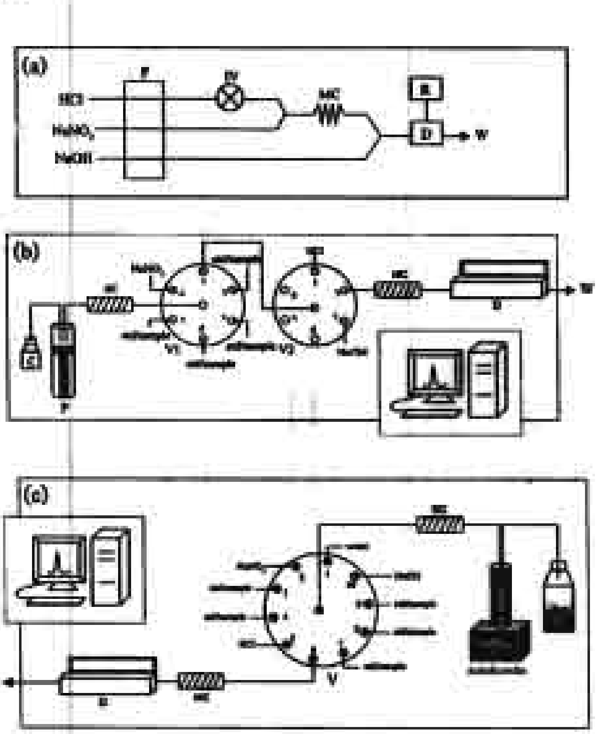

Fig. 1 Schematic diagrams of FI/SI systems for determination of paracetamol: (a) FIA, (b) SIA-I and (c) SIA-II. P, pump; C, carrier; HC, holding coil; MC, mixing coil; IV, injection valve; V, selection valve; D, detector; R, recorder; W, waste.

and then filtered.

FI system

The FI system, similarly to the previous reported one,[16] which is more simple than that described by Knochen *et al.*,[19] consisted of a peristaltic pump (Ismatec, Switzerland), a six-port injection valve (V-451, Upchurch), a reaction coil, a colorimeter (Cole Parmer, USA) with a flow through cell (Hellma, Germany) of 1 cm light path and a recorder (Philips PM 8251, Holland), as shown in Fig. 1(a). Through an injection valve, a solution containing paracetamol was injected into a HCl carrier and merged with NaNO$_2$ to form the nitroso derivative, which was subsequently stabilized with NaOH. The absorbance of the formed product was continuously recorded at a wavelength of 430 nm, and the peak height was used for an evaluation.

SI systems

Two SI systems were investigated. Both systems were aimed for operation with minimum steps in sample handling, such as no requirement for further dilution of the sample stock solution. The first one (SIA-I) was a SI Analyser (Laboratory made, Turku Center for Biotechnology, University of Turku and Åbo Akademi University, Finland) consisting of a 2500 µl syringe pump (Cavro), two six-port selection valves (Cavro), a holding coil (200 cm × 0.6 mm i.d.), a reaction coil (100 cm × 0.6 mm i.d.) and using a Spectronic 21 (Bausch & Lomb, USA) as a detector with a flow-through cell (Hellma, Germany) of 1 cm light path, as shown in Fig. 1(b). A personal computer was used for instrumental control, data acquisition and evaluation *via* a Lab PC+ interface card (National Instruments) and an AnalySIA program (The Biosense Team).

The second SI system (SIA-II) used is schematically depicted in Fig. 1(c). It consisted of an autoburette Dosimat 765 (Metrohm, Switzerland) equipped with a 10 ml exchange unit, for a pumping system, and connected to a personal computer *via* an RS232C interface, a 10-position selection valve VICI with a microelectric actuator (Valco Instruments, USA) and a Spectronic21 (Bausch & Lomb, USA) detector with a flow-through cell (Hellma, Germany) of 1 cm light path. The autoburette was connected to the center of the selection valve by means of a holding coil (250 cm × 1.27 mm i.d. Tygon tubing). A mixing coil (30 cm × 0.79 mm i.d. PTFE tubing) was placed between the selection valve and the detector. Both instrumental control and data acquisition were manipulated *via* software using LabVIEW, developed in-house and using a CYDAS ULV interfacing board (CyberResearch, USA). This software provided control of the volume to be dispensed or aspirated by the autoburette, flow rate, selection of the different valve positions and performed data acquisition. The data processing was computed by using MATLAB.

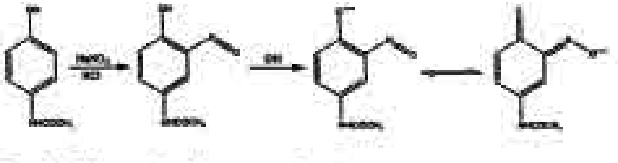

Fig. 2 Schematic diagram of the nitrosation reaction of paracetamol.[1]

The SI operation steps were as follows: firstly, the manifold lines were washed with water, and all of the reagents were filled into the ports of the selection valves. Then, suitable volumes of the reagents were sequentially aspirated and stacked as zones in a holding coil. Finally, these zones were propelled through a reaction coil. A zone penetration occurred. The absorbance of a product zone was continuously monitored at a wavelength of 430 nm.

Results and Discussion

The FIA

The flow system and chemical variables were investigated. Optimization studies were carried out for each individual variable and optimum values were selected. The effect of the mixing coil length, which influenced both the sensitivity (*i.e.* slope of a plot of concentration (mg/l) of paracetamol *vs.* peak height) of the method and the sampling frequency, was studied. The lengths of mixing coils between 50 - 280 cm were examined. A longer mixing coil resulted in increasing the sensitivity as a result of promoting better mixing of the sample and reagent. A mixing coil of 200 cm was selected for further experiments due to the linearity and sensitivity.

By fixing the flow-system variables (mixing coil length, 200 cm; flow rate, 2.0 ml/min and sample volume, 70 µl), the influence of the chemical variables was studied by varying the concentrations: NaOH (0.03 - 0.10 mol/l), HCl (0.03 - 0.15 mol/l) and NaNO$_2$ (0.01 - 0.14 mol/l). The conditions were selected to be 0.10, 0.07 and 0.07 mol/l for NaOH, HCl and NaNO$_2$, respectively.

The SIA-I

By using the SIA-I system, the sequence order of aspiration was firstly optimized to ensure good mixing of the sample zone with all of the reagents involved. Several sequence orders were examined and a suitable one that provided a good peak shape and high sensitivity (better response) was selected. The selected

Table 1 Selected conditions of the SIA-I system for the determination of paracetamol

Sequence	Valve no.	Position	Volume/µl	Description
1	2	1	200	Aspirate 0.05 mol/l HCl into HC
2	1	6	75	Aspirate 0.1 mol/l $NaNO_2$ into HC
3	1	2 - 5	10	Aspirate sample into HC
4	2	1	100	Aspirate 0.15 mol/l NaOH into HC
5	2	3	250	Aspirate 0.05 mol/l HCl into HC
6	1, 2	1, 2	2500	Pump to detector with flow rate of 9 ml/min

sequence of the sample and the reagent zones was created by first aspirating HCl into a holding coil, then, $NaNO_2$ and the standard/sample were inserted to the acid zone to increase mixing of the sample and the reagents. In this step, a nitroso derivative compound was formed. NaOH was finally aspirated to stabilize the product before detection of the signal.

Although some reported that the reaction product was a nitro derivative,[17,18] there has been a report mentioning the product being a nitroso derivative.[1] A study on the formation of metal chelates of such a reaction product indicated that the formation of a nitroso derivative of acetaminophen is more probable than the nitro derivative.[1]

According to the nitrosation reactions[1] of paracetamol, as shown in Fig. 2, the nitroso derivatization of paracetamol and $NaNO_2$ should take place under an acidic condition. Therefore, the $NaNO_2$ zone should overlap with the sample and the HCl zone to ensure an acidic medium for efficient forming of the nitroso compound before being stabilized by the NaOH zone to convert it into another species, to be monitored for absorbance. It was found that the best sequence was to sandwich the sample and the $NaNO_2$ zones with two HCl zones.

The concentrations of the three reagents ($NaNO_2$, HCl and NaOH) involved in the reactions were optimized. A series of concentrations of a single reagent was varied, while the others were kept constant. The reagent concentrations giving high sensitivity and good linearity were selected by considering a regression equation (*i.e.* slope and r^2-value) for a plot of the concentration of the paracetamol *vs.* peak area. The trends of the reagent concentrations were quite similar to those obtained from the FI system. However, it is not convenient to work with high concentrations of NaOH, since the Schlieren effect would have a great influence.

The effects of the reagent and sample volumes were evaluated by varying the volumes of NaOH (100 - 300 µl), HCl (100 - 350 µl), $NaNO_2$ (50 - 200 µl) and the sample (5 - 50 µl). An increase in the reagent and sample volumes resulted in increased sensitivity. However, a poor sensitivity was obtained by using a very high volume, probably due to less zone overlap.

In addition, the flow rate was optimized to achieve a good analytical signal and sensitivity. The selected conditions are summarized in Table 1.

SIA-II

Although the SIA-I system could be successfully applied to the determination of paracetamol, another SIA set-up with more cost effective considerations was investigated. Instead of a syringe pump, an autoburette was used, and only one 10-port selection valve was employed. Optimization was studied. The optimum conditions are described in Table 2.

Table 2 Selected conditions of the SIA-II system for the determination of paracetamol

Sequence	Valve position	Mode	Volume/µl	Description
1	5	PIP[a]	300	Aspirate 0.05 mol/l HCl into HC
2	6	DIS C[b]	110	Dispense HCl
3	2	PIP	110	Aspirate 0.10 mol/l $NaNO_2$ into HC
4	3 - 4, 7 - 9	PIP	20	Aspirate sample into HC
5	6	PIP	110	Re-aspirate HCl (sequence 2)
6	10	PIP	250	Aspirate 0.20 mol/l NaOH into HC
7	6	DIS C	5000	Dispense to detector with flow rate of 30 ml/min

a. Pipetting.
b. Cumulative dispensing.

Table 3 Analytical characteristics of the proposed methods

Parameter	FIA	SIA-I	SIA-II
Linear range (mg/l), r^2	400 - 1000, 0.9991 1000 - 2500, 0.9970	400 - 1000, 0.9946 1000 - 2500, 0.9849	200 - 1000, 0.9977 1000 - 2500, 0.3879
%RSD (n = 11)	2[a]	3[a,b]	4[a,b]
Detection limit (mg/l)	45	70	65
Sampling frequency (h^{-1})	60	60	15

a. At 2000 mg/l.
b. Without extra dilution for sample handling.

Analytical characteristics

An evaluation of the analytical characteristics of all the proposed procedures was carried out by studying the linear range, precision, detection limit and sample frequency, as summarized in Table 3.

The precision of each proposed procedure was studied. When using a standard (2000 mg/l), the RSDs for SIA procedures with no dilution for sample handling were less than 4%. RSDs of less than 1% for a particular case could be obtained by altering some conditions, which should be useful for the routine quality control of a particular formula of pharmaceutical preparation. The precision of such a procedure was tested daily for 5 days. It was found that the RSDs were also within 4%.

By using the FI method, the effect of foreign compounds that can be found in phamaceutical preparations containing paracetamol was also studied. Chlorphenramine maleate with a concentration of up to 80 mg/l was tested for the determination of paracetamol (2000 mg/l). The results showed that the presence of chlorphenramine did not interfere with the determination of paracetamol.

The accuracy of the proposed methods was evaluated by analyzing real samples. The results agreed with those obtained

Table 4 Determination of paracetamol in some pharmaceutical preparations

Sample	Type of preparation	%Label			
		FIA	SIA-I	SIA-II	Standard method[18]
1	Tablet	99	97	97	92
2	Tablet	98	98	98	92
3	Tablet	102	101	101	94
4	Tablet	95	100	100	93
5	Tablet	100	105	105	96
6	Tablet	107	90	90	98
7	Tablet	104	98	98	91
8	Tablet	107	99	99	94
9	Tablet	106	98	103	92
10	Suspension	108	102	95	92
11	Syrup	105	102	101	92

by the standard method (Table 4).[18]

Application to samples

The three proposed systems (FI, SIA-I and SIA-II) were applied to the determination of paracetamol in pharmaceutical preparation samples. Each sample was prepared to obtain solutions having concentrations of *ca.* 700 and 2000 mg/l of paracetamol. For SIA procedures, no sample dilution was made. The obtained results are summarized in Table 4. The evaluation by a t-test at the 95% confidence level indicates that there is no significant difference in the results obtained by the proposed and standard methods.

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ORIGINAL PAPER

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Simple and selective method for determination of iodide in pharmaceutical products by flow injection analysis using the iodine–starch reaction

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Abstract This work exploited the well-known iodine–starch reaction for development of a simple flow-injection (FI) method for determination of iodide in pharmaceutical samples. Iodide in an injected zone was oxidized to iodine. A gas diffusion unit enables selective permeation of iodine through a hydrophobic membrane. Detection was made very selective for elemental iodine by employing formation of the I_3^-–starch complex. The detection limit (3*S/N*) of the system was 1 mg I L^{-1}. For a liquid patent medicine used for asthma treatment we suggested modification of the system. Direct injection of this sample, which contains a particularly high concentration level of iodide (ca. 9000 mg I L^{-1}), can be achieved by coupling a dialysis unit to the FI system. This has increased the working range to 6000–10,000 mg I L^{-1} without employing complicated nanoliter injection.

Keywords Flow injection · Gas diffusion · Dialysis · Iodide · Iodine–starch

Introduction

Iodine compounds are often used in pharmaceutical products. Iodine in the form of tri-iodide is an antiseptic and disinfectant and potassium iodide is thought to act as an expectorant. In patients with hyperthyroidism iodide rapidly inhibits the synthesis of thyroid hormones. In some countries potassium iodide tablets are sold in drug stores for thyroid protection in the event of nuclear emergency. However, iodide should be used with extreme caution when patients are markedly sensitive to the element [1]. Therefore it is essential to have an accurate and precise method available to determine the iodine content of pharmaceutical preparations.

Several methods are available for determination of iodide at different levels. For determination of microgram and nanogram amounts of iodide, the most frequently used method is chromatography [2, 3, 4, 5]. Iodide can be determined by spectrometric [6], catalytic spectrometric [7, 8], and potentiometric [9, 10] methods. However, most available methods are suitable for trace levels of iodide [2, 3, 4, 5, 6, 7, 8].

Mixt. Stramonium Co. is a liquid patent medicine sometimes used in patients suffering from asthma [11]. The level of iodide in this drug is quite high, ca. 9000 mg I L^{-1}. Apart from potassium iodide 12 g, other ingredients are present in one liter: hyoscyamus tincture 134 mL, stramonium tincture 20 mL, and liquorice liquid extract 34 mL. The liquid medicine has a very dark brown color. It would be quite difficult to measure the iodide content using a common colorimetric method.

Usually a gas diffusion (GD) unit, as applied to flow injection analysis, is a form of an on-line clean-up system. Volatile analytes can be separated from interference in the sample by permeation across a hydrophobic membrane. This process is fairly selective, because few species are turned into gaseous form at room temperature [12]. Employment of a GD unit enables colored samples, or samples which differ in refractive index from the reagent, to be analyzed without optical interference. Motomizu and his group reported a number of GD applications in flow injection (FI) analysis [12, 13, 14, 15, 16, 17] including application to iodide and other halogens [15]. In 1997, Hakedal and Egeberg [18] proposed an FI system for determination of iodide in brine. This system employed a

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GD unit fitted with a PTFE membrane. Iodine (I_2), from oxidation of the iodide analyte permeated through the membrane into a stream of iodide. Absorbance of tri-iodide (I_3^-) was measured in the UV region for calibration.

In this work we incorporated a GD unit into a colorimetric FI system. Detection is by simple measurement of the absorbance of the I_3^-–starch complex, recently presented by our group [19]. The system was primarily employed to analyze pharmaceutical products containing iodide, for example KI tablets. These samples were dissolved in water and the iodide content of filtered samples could be determined directly by use of our system. However, for Mixt. Stramonium Co. the sample much be diluted before analysis. Staden et al. recently adopted dialysis for simultaneous dilution of a sample [20]. For our work on Mixt. Stramonium Co. a dialysis unit was coupled to the GD unit and was used as an on-line dilutor. The use of a GD unit with detection based on I_3^-–starch has made the system very selective and simple. The sensitivity in the visible range is twice that of the spectrometric detection of I_3^- in the UV region [18].

Experimental

Reagents and solutions

Flow injection

All chemicals were analytical reagent grade. Deionized–distilled water was used for chemical preparation. A stock solution of standard iodide (ca. 20,000 mg I L^{-1}) was prepared by dissolving approximately 26.16 g (accurate weight) potassium iodide crystals (Merck, Germany) in 1 L water. Working solutions of iodide were obtained by appropriate dilution with water.

The oxidant was prepared by dissolving 3 g potassium dichromate (Univar, Australia) in 1 L of 10% (v/v) sulfuric acid (Labscan, Ireland).

The acceptor stream was a solution of 0.016 mol L^{-1} potassium iodide and 0.1% (*w/v*) starch. This mixture was first made by mixing 1 g starch (Merck) with a few milliliters of water to form a slurry. The slurry was added to 1 L boiling water. This resulted in a starch solution of 0.1% (*w/v*). Potassium iodide (2.6 g) was then dissolved in this starch solution. This mixed solution was always prepared daily, because of deterioration of starch molecule.

Thiosulfate solution (0.1 mol L^{-1}) was prepared by dissolving 2.5 g sodium thiosulfate pentahydrate (Merck) in 100 mL water. This solution was diluted with 0.1% (*w/v*) starch to give 2×10^{-3} mol L^{-1} of thiosulfate. The solution was used to remove the starch complex or iodine residues deposited on tube walls.

Potentiometric analysis

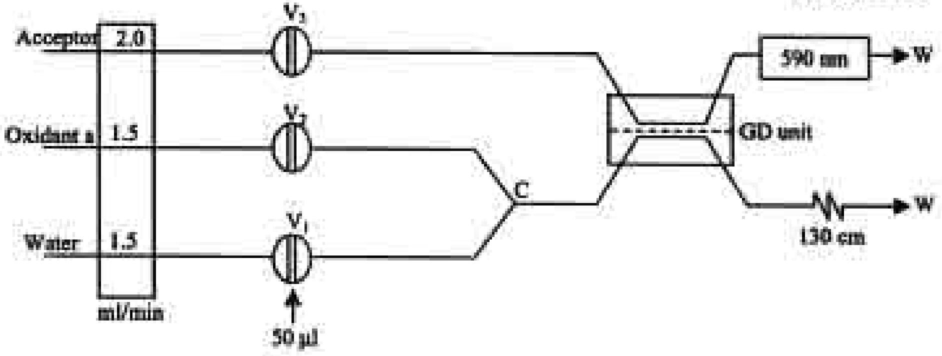
Potassium iodide standards for calibration were prepared from the same stock iodide solution prepared for FI analysis (20,000 mg I L^{-1}). Sodium nitrate (5 mol L^{-1}), the ionic strength adjustor, was prepared by dissolving 42.5 g crystalline sodium nitrate (Fluka, Switzerland) in 100 mL water.

Samples

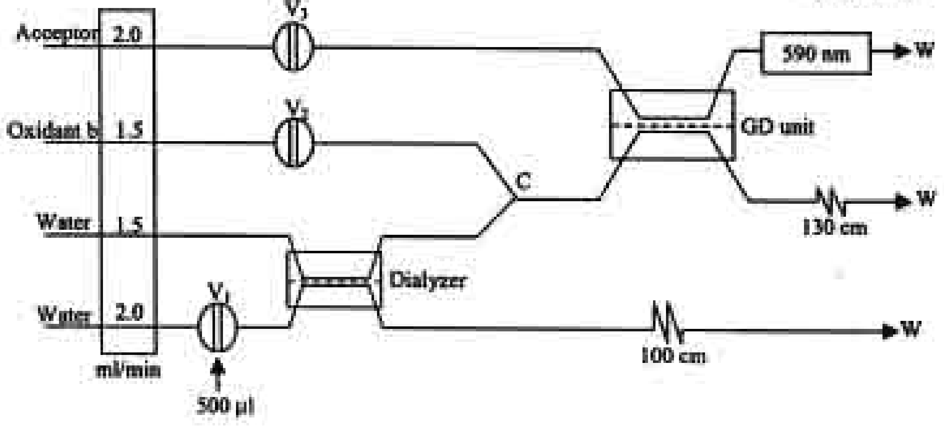
Commercial potassium iodide tablets were used for method validation. NO-RAD (65 mg KI per tablet) is a product of Body Gold, USA. IOSAT (130 mg KI per tablet) is distributed by Anbex (NY, USA). These sample tablets were dissolved in deionized–distilled

Fig. 1 The flow-injection systems used in this work for determination of iodide. Acceptor, 0.016 mol L^{-1} KI in 0.1% (*w/v*) starch; oxidant a, 0.01 mol L^{-1} $K_2Cr_2O_7$ in H_2SO_4 of different concentrations (2, 4, 6, 8, and 10%, *v/v*); oxidant b, 0.01 mol L^{-1} $K_2Cr_2O_7$ in 10% (*w/v*) H_2SO_4. *C*, a confluence point; V_1, sample injection port (under the optimized conditions), V_2 and V_3, 250 µL injection of cleaning solution (2×10^{-3} mol L^{-1} $Na_2S_2O_3$ in 0.1% (*w/v*) starch); *W*, waste

(a) System A



(b) System B



water. Suspension was eliminated by filtration through Whatman filter paper No. 1 before injection into the FI systems.

The medicinal sample Mixt. Stramonium Co. is produced by the Government Pharmaceutical Organization, Bangkok, Thailand. It is a dark-brown liquid.

FI apparatus

Figure 1 depicts two FI systems, A and B, which were used for method development. Each manifold employed an Ismatec peristaltic pump (model IS 7610, Switzerland) for propelling reagents. Three Rheodyne injection valves (model 5020, USA) were used. A Metrohm gas diffusion unit (model 754, Switzerland), fitted inside with a circular PTFE membrane (47 mm i.d. with pore size 0.45 µm; Sartorius, Germany), was employed. The unit consists of two Perspex blocks, each with a concentric spiral groove (2 mm×300 mm×0.2 mm, width×length×depth). System A, shown in Fig. 1a, was used in the preliminary studies. The system was later modified as shown in Fig. 1b (System B), by incorporating a dialysis unit for on-line dilution of samples containing high levels of iodide.

The homemade dialysis unit used in System B (Fig. 1b) consisted of two half-Perspex blocks. Each block had a straight groove (1.5 mm×120 mm×1 mm, width×length×depth). A cellulose acetate membrane (10 mm wide with molecular weight cut-off from 12,000 to 14,000 Dalton; Thomas Science, USA) was placed inside the dialysis unit.

A Jenway spectrophotometer (model 6405, UK), fitted with a Philips flow cell of 0.01 mL volume, was used for monitoring the absorbance at 590 nm. PTFE tubing of i.d. 0.5 mm was used for construction of the two FI systems. An Alltech chart recorder (model LR 93025, USA) was employed for recording the signal.

Table 1 is the summary of the operating steps of the FI systems shown in Fig. 1.

Potentiometric method

Sample solution (30.0 mL, accurately measured) was transferred into a 50-mL beaker. To this sample sodium nitrate solution (5 mol L^{-1}, 0.6 mL) was added for control of the ionic strength. The solution was analyzed by measuring the potential developed across the Orion (USA) Iodide-ISE (model 9453) and an Orion saturated calomel electrode. A digital Orion Ionanalyzer (model 601A) was used for this measurement. The operation of this technique was performed in accordance with the instruction manual [21]. Calibration was carried out with standard solutions (1 to 1000 mg I L^{-1}) prepared from potassium iodide stock solution (20,000 mg I L^{-1}). According to the specification, the linearity of the iodide electrode is from 10^{-1} to 10^4 mg I L^{-1}.

Table 1 Summary of the operating steps for the FI manifolds shown in Figs. 1a and 1b (Injections: V_1, sample; V_2 and V_3, cleaning solution)

Step	Valve position			Duration (s)	
	V_1	V_2	V_3	Fig. 1a[a]	Fig. 1b[b]
1	Inject	Load	Load	45	110
2	Load	Inject	Inject	75	75

[a] Analysis time per injection run is 45+75 s,=2 min
[b] Analysis time per injection run is 110+75 s,=3 min

Results and discussion

Manifold design

GD-FI system

First, the manifold of the gas-diffusion flow injection (GD-FI) system, shown in Fig. 1a, was examined. Introduction of a liquid sample was carried out via injection through V_1. In this manifold, iodide in sample plugs is oxidized after mixing with the stream of oxidant, acidic potassium dichromate. Liberated iodine (I_2), which diffuses through the PTFE membrane, forms the tri-iodide–starch complex in the acceptor stream. Detection of the blue complex zone and calibration are similar to previous work with iodized salt [19].

System cleaning

When measuring the tri-iodide–starch complex, a problem often arises from deposition of the complex on tube walls and the flow-cell. Injection of pure thiosulfate solution to remove the adsorbed starch complex [19] was found effective only for the acceptor stream (Fig. 1a). The species in the donor stream expected to be adsorbed on the manifold walls are $I_{2(aq)}$ and/or $I_3^-{}_{(aq)}$. However, the solution found suitable for removing the complex in this stream is a mixture of thiosulfate and starch (2×10^{-3} mol L^{-1} $Na_2S_2O_3$ in 0.1%(*w/v*) starch). Addition of starch into the previously employed cleaning solution [19] is necessary. It was observed that reduction of iodine by $S_2O_3^{2-}$ was easier (or more rapid) for the iodine complex than for the non-complex form. However, this was true for deposits on tube walls and is different from what is normally used and observed in titration [22].

The cleaning solution was injected at two positions (V_2 and V_3) as shown in Fig. 1a. The cleaning steps and sample injections suitable for this manifold are summarized in Table 1.

Appropriate concentration of sulfuric acid in the oxidant

For oxidation of iodide in a homogeneous system, H^+ must be present at sufficient concentration [18]. The system employed is based on non-homogeneous mixing and spectrometric detection was not at reaction equilibrium. For such a system concentrations of chemicals must be optimized. For the system in Fig. 1a different concentrations of sulfuric acid in the oxidant (2 to 10%, *v/v*) were investigated for the concentration of potassium dichromate selected, 0.01 mol L^{-1}.

To optimize the acid concentration a standard iodide solution of 300 mg I L^{-1} was chosen. By repeated injection of this iodide solution it was shown that absorbance increased dramatically with increasing concentration of sulfuric acid from 2 to 6% (*v/v*) before approaching a plateau at

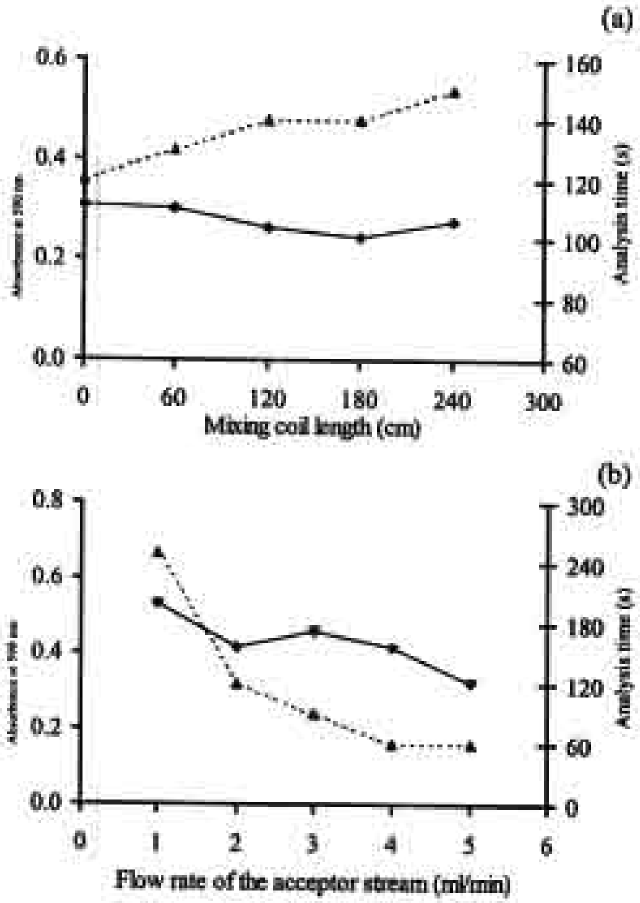


Fig. 2 Effect on analytical signal (*diamonds*) and analysis time (*triangles*) of variations in (a) coil length (between C_1 and GD unit) in System A (Fig. 1a) and (b) acceptor flow rate

8% (*v/v*). The highest concentration of sulfuric acid (10%, *v/v*) was chosen as the optimum.

System A

Optimization

Preliminary optimization was carried out using repeated injection (n=5) of 200 mg I L^{-1} standard iodide solution, to study two aspects of the manifold in Fig. 1a. The objective was to get a compromise between analytical signal and analysis time. "Analysis time" is defined as the time taken from injection of sample until the cycle of system operation is complete (for a run of 1 injection).

Mixing coil of different lengths, from 0 to 240 cm, were inserted between the confluence point (C) and the GD unit (Fig. 1a). According to the results in Fig. 2a a slight difference in the signals was observed with variation of coil length. The shortest analysis time was achieved when no mixing coil was used. Thus, this condition was chosen.

The flow rate of the acceptor stream (Fig. 1a) was varied from 1 to 5 mL min^{-1}. As expected, the absorbance decreased as the flow rate increased (Fig. 2b). This was because of the decrease in the yield of the reaction between tri-iodide and starch. At an acceptor flow rate of 1 mL min^{-1} it was observed that the system was too sensitive for samples containing high iodine concentrations (>300 mg I L^{-1}). However, too rapid a flow rate would lead to high consumption of reagent. We chose to set the flow rate at 2 mL min^{-1}. The analysis time of 2 min per injection was obtained at this flow rate.

Analytical features and applications of the GD-FI manifold (The System A)

To conclude, the optimized conditions for System A employ 10%(*v/v*) H_2SO_4 as the oxidant and an acceptor stream flow rate of 2 mL min^{-1}. System characteristics are summarized in Table 2.

Three samples were analyzed for iodide content. The results were compared with values obtained by using an ion-selective electrode (Table 3). The iodide content as determined by the two methods agreed significantly with each other and with the nominal values. A paired *t*-test was employed to compare the results in terms of the concentration (mg I L^{-1}) of the injected liquid samples. No significant difference was found between the two analytical methods (P=0.01). This good agreement demonstrates that the (GD-FI manifold in Fig. 1a) is suitable for these samples. No evidence was found that these pharmaceutical products cause interference.

System B

The GD-FI system with on-line dilution by dialysis

The level of iodide present in the liquid Mixt. Stramonium Co. medicine is particularly high (ca. 9,000 mg I L^{-1}) compared with KI tablets. Dissolution of a tablet in 500 mL of

Table 2 Analytical performance of GD-FI system

Analytical feature	GD-FI (System A, Fig. 1a)	GD-FI with on-line dilution (System B, Fig 1b)
1. Working range (mg I L^{-1})	50 to 300	6000 to 10,000
2. Standard equation	PH=3.4×10^{-3}[I$^-$]+9.1×10^{-2}; r^2=0.999	PH=8.3×10^{-5}[I$^-$]–2.3×10^{-1}; r^2=0.999
3. Precision (*RSD*)	1.27% (for 100 mg I L^{-1}, n=10)	1.44% (for 9000 mg I L^{-1}, n=25)
4. Sample throughput (injections h^{-1})	30	20
5. Limit of detection (mg I L^{-1}; 3*S/N*)	1	200

PH: peak height (absorbance)

Table 3 Determination of iodide in pharmaceutical products by this method and the ISE method, compared with the nominal content. The means and standard error were from a set of three samples of the same product

Trade name	Sample type	Concentration unit	Iodide content		
			Labeled	GD-FI (Fig. 1a)	ISE
1. NORAD	KI tablet	mg I per tablet	49.7	51.6±7.3	57.9±7.3
2. IOSAT	KI tablet	mg I per tablet	99.5	101.2±3.5	102.8±5.6
3. Mixt. Stramonium Co.	Liquid patent medicine	mg I L^{-1}	9,181	8,926±170	9,312±335

Table 4 Results obtained by use of different injection volumes when working on the GD-FI system coupled with a dialysis unit (System B, Fig. 1b). Calibration ranged from 6000 to 10,000 mg I L^{-1}

Injection volume (µL)	Signal range (a.u.)	Standard equation[a]	r^2
250	0.106–0.279	$PH=4.3\times10^{-5}[I^-]-1.5\times10^{-1}$	0.999
350	0.169–0.390	$PH=5.5\times10^{-5}[I^-]-1.6\times10^{-1}$	0.999
500	0.249–0.603	$PH=8.9\times10^{-5}[I^-]-2.9\times10^{-1}$	0.995
750	0.361–0.725	$PH=9.1\times10^{-5}[I^-]-1.9\times10^{-1}$	0.997

PH: peak height (absorbance)

[a] Iodide standards were 6,000, 8,000 and 10,000 mg I L^{-1}.

water will bring the concentration to approximately 100 or 200 mg I L^{-1}, much lower than in Mixt. Stramonium Co. For this medicine prior dilution with water (1:100) was necessary before the analysis using System A.

On-line dilution in flow-injection analysis is usually achieved by merging and mixing the sample zone with a suitable carrier. This technique is not always applicable when high dilution is required, unless a special mechanism for injecting very small samples is used.

When dialysis [23, 24] is applied to flow injection for pretreatment, analyte concentration is often quite dilute after diffusion through the membrane. Although in our work the sample (Mixt. Stramonium Co.) did not require removal of interferences by use of a dialysis membrane, we employed dialysis for the purpose of dilution by using System B (Fig. 1b).

To obtain the optimized conditions, summarized in Fig. 1b, the effect of injection volume was first studied. A suitable volume should give a signal reading between 0.2 and 0.8 a.u. for the desired calibration range of 6000 to 10,000 mg I L^{-1}. Replicate injections of iodide standards were performed using the manifold shown in System B. Results in Table 4 show that absorbance was not appropriate when the injection volume was below 500 µL. The sensitivity and signal range given for injection volumes of 500 and 750 µL were both applicable. However, because small volumes are simpler to handle, a volume of 500 µL was selected for further study.

Performance and application of the FI system with on-line dilution

Figure 1b depicts the manifold developed for final application in analysis of Mixt. Stramonium Co. We can inject the liquid medicine directly into the system without a need for off-line dilution. A summary of the analytical features is shown in Table 2. On-line dilution via dialysis has made the system capable of working at a much higher range than previously (up to 6000 to 10,000 mg I L^{-1}).

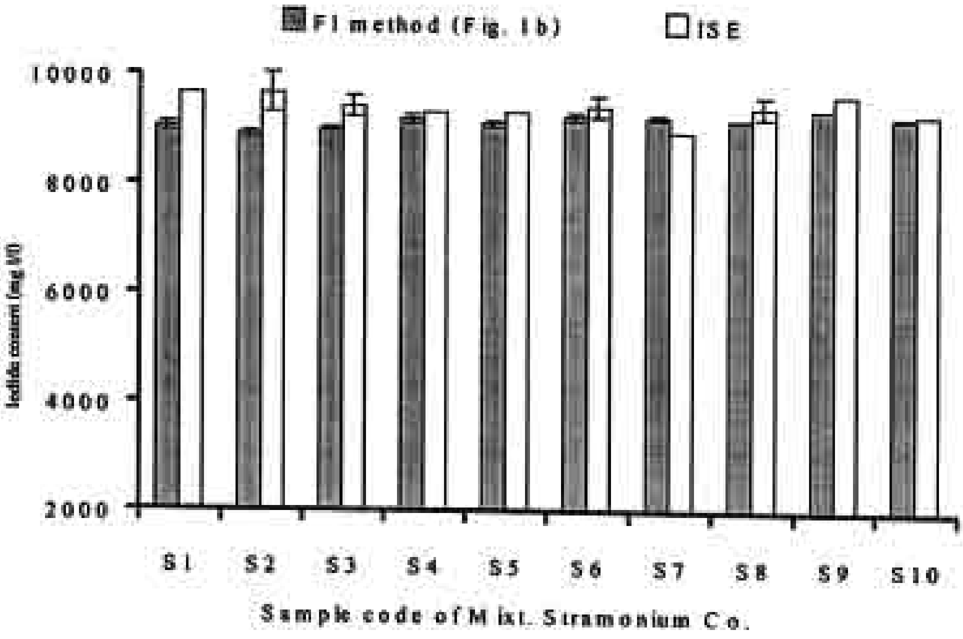


Fig. 3 Comparison of the iodide content determined by the FI method using System B (Fig. 1b) (n=3) and by the potentiometric method (n=3)

The on-line dilution manifold was used to determine iodide in Mixt. Stramonium Co. using direct injection without prior dilution. The results were compared with those determined by use of the iodide-ISE (Fig. 3). A paired t-test showed there was no significant disagreement at 99% confidence ($t_{observed}=2.82$; $t_{critical}=3.25$).

Recovery

Recovery was studied for all types of sample, for both FI manifolds. The results showed recovery was satisfactory – from 93.8 to 104%. This implies the samples contain no analytical interferences under the conditions employed for both systems.

Advantage of the proposed methods

For the ISE, which is operated batchwise, it can take longer than 1 min to obtain a stable reading. Electrode washing to ensure no cross-contamination between samples requires 5 to 6 min at least. One should also allow for the time required for transfer of sample. Thus the dominant advantage of our method is probably the throughput of samples. The ISE method enables analysis of approximately eight samples per hour whereas our methods enable throughput of 30 and 20 samples per hour for manifolds A and B, respectively.

For liquid samples containing 6500 to 9500 mg I L^{-1} System B has a good potential for integration into the process for manufacture of the pharmaceutical preparation. Maintenance should be simpler than using an iodide-selective electrode.

Coupling of the dialyzer for analysis of the Mixt. Stramonium Co. sample is useful for two reasons. Dialysis enables on-line dilution of the analyte, which is convenient and enables direct injection of the medicine. Sample matrix is also diluted through the dialyzer membrane. Mixt. Stramonium Co. contains 10% (*v/v*) ethanol. Dilution of this alcohol will help maintain proper function of the hydrophobic membrane (PTFE) in the GD unit.

Conclusions

We have developed two flow-injection systems, both employing a gas-diffusion unit, for selective detection of iodide. The principle of detection is very simple, based on formation of the I_3^-–starch complex. However, this complex is sparingly soluble in water and one must be careful not to exceed the appropriate working concentration range of iodide, otherwise clogging of the inside of the tube or deviation from Beer's law will occur. The first system (System A) is recommended for determination of KI tablets. The sample, after dissolution and normal filtration, can be analyzed directly. Use of this FI system for more concentrated samples requires dilution. For our liquid medicine that contains ca. 9000 mg I L^{-1}, System B is more suitable. After direct injection, the sample is sequentially diluted and analyzed for iodide content. In fact, the latter system can be adapted for monitoring iodide content in the manufacturing process of the medicine.

Acknowledgements This work was supported by grants from the Thailand Research Fund and the Postgraduate Education and Research Program in Chemistry. The authors would like to thank Associate Professor Prapin Wilairat for editing this paper.

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ผลงานวิจัย ก21

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Editorial

The 11th International Conference on Flow Injection Analysis (ICFIA 2001) was held in Chiang Mai, Thailand, December 16–20, 2001. This conference started informally in the USA in 1987, organized by Gary Christian, Gil Pacey and Jarda Ruzicka. The Conference grew and attracted numerous international participants and was changed to the ICFIA in 1995. At the same time, the Japanese Association for Flow Injection Analysis (JAFIA) was invited to join the Conference and they have held their semi-annual meeting jointly with ICFIA since. ICFIA 2001 was held jointly with the 38th Semi-annual meeting of JAFIA. An international steering committee is chaired by Gary Christian and includes Jose Luis Burguera (Venezuela), Kate Grudpan (Thailand), Bernard Lendl (Austria), Ian McKelvie (Australia), Shoji Motomizu (Japan, JAFIA), Jarda Ruzicka (USA), Tadao Sakai (Japan, J. Flow Injection Anal.), Rolf Sara (Finland), and Koos van Staden (South Africa), with Sue Christian as Advisor.

ICFIA 2001 was hosted by Chiang Mai University and organized by a team in the Department of Chemistry. The Conference was sponsored and supported by Chiang Mai University, Thailand, the JAFIA, the British Council, the Chemical Society of Thailand, the Post-graduate Education and Research Program in Chemistry (PERCH), the Science Society of Thailand (Chemistry and Northern Divisions), the Thai Ministry of University Affairs, the Thailand Research Fund (TRF), Constellation Technology, Corp., FIAlab Instruments, Inc., Foss Tecator, Metrohm Siam Co., Ltd, Perkin Elmer, Inc., and Thai Unique Co., Ltd, (Lachat).

There were 120 participants from 21 countries. Opening the conference was announced by the traditional striking of a Gong, by the President of Chiang Mai University, and was followed by traditional dancing and a drum show. Social programs included a wonderful Thai culture evening with a Khan Toke dinner, Traditional Northern Thai Music and hill tribe dancing; and an afternoon touring and sightseeing with all the participants invited to an elephant camp, and the Queen Sirikit Botanic Garden, followed by an evening with refreshments at the Chiang Mai University, Science Faculty Observatory.

The Conference was devoted exclusively to flow injection analysis and related techniques. Thirty-six papers and 72 posters were presented over three and a half days. Collaborations among the researchers and users of such techniques exit in—and across—various geographical parts of the globe. Some of the collaborations were initiated from the previous ICFIA conferences. Strengthening of the collaborative activities has been observed.

This special issue of Talanta includes manuscripts from the conference, representing state-of-the-art research and application from academic, government and industrial laboratories.

The Steering Committee has selected the next venue for the 12th ICFIA (ICFIA 2003) to be Los Andes University in Merida, Venezuela. Pioneering FIA colleagues Joes Luis Burguera and Marcella Burguera have graciously agreed to organize and host the conference. Professors J.F.

PII: S0039-9140(02)00447-2

(Koos) van Staden and Raluca-Ioana Stefan from the University of Pretoria will be co-organizers, in charge of the technical program. The scheduled date is 7–11 December, 2003, a very pleasant time of the year in this beautiful sub-tropical country. Information is posted at www.up.ac.za/academic/chem/analytical/first.pdf, and may be accessed at www.flowinjection.com. Direct contacts are burguera@ciens.ula.ve and koos.vanstaden@chem.up.za.

Those who shared in the contributions to the success of ICFIA 2001 are here thanked.

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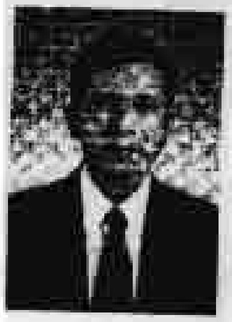
Editors' note: The Conference was proclaimed in honor of Kate Grudpan, in recognition of his being named the Outstanding Scientist of the Year in Thailand for 2001, the first time in the 19-year history of the award that it was given to a scientist from outside Bangkok.—GDC and J-MK.

ผลงานวิจัย ก22

Kate Grudpan

Associate Professor and Senior Research Scholar of the Thailand Research Fund



1953: born, 1974: B.S., Chiang Mai University, 1981: Ph.D., Liverpool John Moores University, UK, 1983-4: Post doctorate at the University of Karlsruhe/Karlsruhe Research Center, Germany, 1991-2: Alaxander von Humboldt Research Fellow 1999: Outstanding Researcher Award of Thailand, 2000-present: Member, Editorial Advisory Board, Talanta, 2001: Senior Research Scholar Award of the Thailand Research Fund, 2001: Outstanding Scientist Award of Thailand, 2002–present: member, International Advisory Board, JFIA

Development of Cost-Performance Flow-Based Chemical Analysis Systems

Various alternatives for instrumentation with parts/components are summarized. Some recent developments are briefed. They include uses of cost-effective reagents, FI, BI SI, LOV, LAV as well as a new concept for flow-based analysis using a stopped FI analyzer.

1.Instrumentation/parts/components of a beginner and/or a person with budget constrain. Various alternatives for cost performance have been proposed for FIA and relative techniques such as pumping system [1-4], injection devices [1-4], mixing devices [2,4,17], detection systems [1-17], and some on-line sample separation/treatment devices [4-7,10,16,18,19].

2.Hyphenated techniques/detection systems. FIA and related techniques have been hyphenated and/or connected to other techniques and devices such as a simple colorimeter [3,9-11,18,30-35], radiometry [1,2], chemiluminescence [11], NIR [36], Raman spectroscopy [37], conductometry [9,10], amperometry [38], voltammetry [15], IC [13] and DSTD[18, 19].

3.Cost-effective reagents. Unstable reagents [7,11,22] can be employed,e.g. permanganate, murexide.

4.Some recent developments. Size-based speciation using FFF has been reported [17]. Bead injection (BI) was for the first time coupled to FI [14,19], SI systems have been proposed: with modified autoburet for reversal flow [12], titration [11],: SI with standard addition-voltammetry. Lab-on-Valve (LOV) systems including micro-titration has been proposed. Novel SI with "Lab-at-Valve (LAV)" systems are being investigated. New concept for flow-based analysis using a stopped-FI analyzer for various analytes without changing any hardware parameters is introduced.

5.Applications have been aimed for various fields including environmental analysis [5,14,18,21], clinical/medical analysis [19-21,25], pharmaceutical [7,11,26],and agro-industry/agriculture [8, 11].

6.Networking. Some of the developments have been under active collaboration in various geographical areas [27].

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ผลงานวิจัย ก23

Title: *Simple Flow Injection System for Colorimetric Determination of Iodate in Iodized Salt*

Journal: Talanta, 58, 1195-1201 (2002).

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Summary

Complex formation of iodine-starch has long been used to indicate the end-point for titration in iodine analysis. The complex is formed by intercalation of tri-iodide (I_3^-) inside starch molecules. Since the complex absorbs visible light (λmax = 590 nm), we propose a simple colorimetric detection of this blue complex as applied to flow injection (FI).

This work demonstrates use of iodine-starch in flow injection for analysis of iodine. The application was focussed on determination of iodate in iodized salt. In Thailand, for a longer shelf life, salts are supplemented with potassium iodate, not potassium iodide. The recommended level of iodine in iodized salt is at 50 mg I/kg.

There have been some methods for quantitation of iodine in iodized salts but mostly for the supplementation with potassium iodide [1-4]. A flow injection (FI) method was presented for salts, which are iodized with potassium iodate. The FI method employed amperometric detection and provided a throughput of sample at 35 injections/h [5]. In this work, we present a different concept of detection that is the colorimetric analysis, which is much simpler than the former amperometric method.

We have utilized the Dushman reaction [6] by formation of tri-iodide (I_3^-) when there is a limited amount of iodate and excess amounts of iodide and hydronium ion. In a condition in which starch is present, the blue complex of I_3^--starch then forms. Linear calibration, based on the Beer's law, can be obtained from a plot of the relationship between the absorbance of the complex and the iodate concentration.

In our FI method, a sample plug of salt solution (made to approximately 6% (w/v)) is injected directly into a carrier stream which consists of 2.5×10^{-2} M KI in 6% (w/v) NaCl. The zone in the carrier line is then merged and mixed with a continuous flowing stream that contains starch and acid. Detection of the blue product in the FI manifold employs a colorimeter. In fact, a yellow LED can be an alternative light source, which will make the method even more, cost effective.

In the FI manifold, there are two injection ports. One is for injecting the liquid salt samples and standards. We use another injection valve for injecting a cleaning solution into the manifold. We observed that there was accumulation of tri-iodide starch complex on walls of tubing and the flow cell. This used to cause shift of baseline. We therefore present a very simple idea of cleaning off this deposit on the walls by injecting a small plug of thiosulfate solution. The purpose is to decolorize the blue complex deposit, by reducing tri-iodide (which intercalates inside the starch molecults) to iodide ion. We recommend an injection of thiosulfate solution after every sample injection. Injection of sample is usually carried out at approximately at 30 s intervals.

At the optimized condition, the calibration is perfectly linear in the range of 5.0×10^{-6} to 4.0×10^{-5} M: absorbance = $25{,}520[IO_3^-] - 0.052$, $r^2 = 0.999$. It is suspected that negative intercept may be caused by limitation in the reaction kinetics at low concentrations or it could be due to restriction of the dynamic range of the detector.

The proposed method although is simple but very efficient and precise (RSD = 0.66%). With this principle of detection, the method provides more rapid analysis than the former amperometric FI method. Our sample thoughput is 65 injections/h. A detection limit as low as 2 mg I/kg is achieved. Successful validation of our method was accomplished using other two analytical methods, which are the conventional titration method and the potentiometric method that uses an iodide-selective elective electrode.

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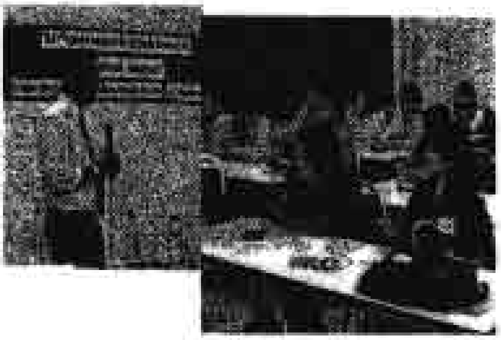
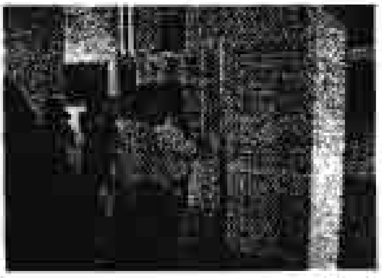
ผลงานวิจัย ก24

Report on the 2nd Annual Symposium on TRF Senior Research Scholar on Flow-based Analysis, Chiang Mai, Thailand, 6 September 2003

A-one-day symposium was organized with objectives: (1) to report research progress in the past years, especially the last year of the grant on the Thailand Research Fund (TRF) Senior Research Scholar (Kate Grudpan) on "Development of Flow-based Analysis" and (2) to open opportunities for discussion/exchange ideas and information in the research field among those who are in-and outside the group of the TRF Senior Research Scholar Grant.



The symposium invited 2 plenary lectures: "Flow injection analysis for determination of trace air pollutants" by Prof. Dr. Tadao Sakai, Editor-in-chief, JAFIA, Aichi Institute of Technology and "Ultratrace and trace determination with flow-based techniques" by Prof. Dr. Shiji Motomizu, President of JAFIA, Okayama University. There were 70 participants including faculty members, researchers and students from 10 universities with 30 contribution papers which were presented in poster format. Some of the authors were invited to present orally for brief concepts. This was aimed for more discussion and interaction among those who are in the same interests to meet maximum benefit at posters.



Some of the presentations were given by graduate students (master and doctoral). Some recent works on clinical analysis were reported, for examples, flow based ion exchange micro-column system for screening of thalassemia and hemoglobinopathies, a flow-based system to assay specific proteoglycans. Some developments on instrumentation were discussed. They included automated systems for paracetamol; bead injection combining with flow injection system ; an economical alternative for determination of some trace metals, boron doped -diamond thin film electrode in FI system; pervaporation for high speed GC for volatile organic compounds, and dynamic surface tension detector for flow analysis. Various applications were involved such as release of metal ions from contaminated soil in mining area to environment by humic acid colloids; phosphate contents in fertilizer and soil by stopped FI-Analyzer, iodide/iodine contents in various types of samples.

The enjoying and easy environment with good academic atmosphere encouraged the interaction among students, newer and older generations leading to build up relationship for the young and more senior researchers.

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Flow-injection in-line complexation for ion-pair reversed phase high performance liquid chromatography of some metal-4-(2-pyridylazo) resorcinol chelates

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Abstract

Flow injection (FI) was coupled to ion-pair reversed phase high performance liquid chromatography (IP-RPHPLC) for the simultaneous analysis of some metal-4-(2-pyridylazo) resorcinol (PAR) chelates. The reverse flow injection (rFI) was used as an in-line complexation of metal-PAR chelates prior to their separation by ion pair reversed phase HPLC. The lab made rFI was coupled to HPLC via switching valve and the performance of the system was fully manually operated. The rFI conditions were injection volume of PAR 85 µL, flow rate of metal stream 4.5 $\mathrm{mLmin^{-1}}$, concentration of PAR 1.8×10^{-4} $\mathrm{molL^{-1}}$ and the mixing coil length of 150 cm. IP-RPHPLC was carried out using a C_{18} µBondapak column with the mobile phase containing 37% acetonitrile, 3.0 $\mathrm{mmolL^{-1}}$ acetate buffer pH 6.0 and 6.2 $\mathrm{mmolL^{-1}}$ tetrabutylammonium bromide (TBABr) at a flow rate of 1.0 $\mathrm{mLmin^{-1}}$ and visible detection at 530 and 440 nm. The analysis cycle including in-line complexation and separation by IP-RPHPLC was 16 minutes which able to separate Cr(VI) and the PAR chelates of Co(II), Ni(II) and Cu(II).

Keywords: Flow injection; In-line complexation; Ion pair reversed phase high performance liquid chromatography; Metal-PAR chelates

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1. Introduction

Liquid chromatography has been widely recognized as one of the methods for multi-element and sensitive analysis of trace metals. Various modes of liquid chromatography have been used, including normal phase [1-3], reversed phase and ion exchange chromatography [4-10]. Since the introduction of ion-pair reversed phase high performance liquid chromatography (IP-RPHPLC) [11-12] for the separation of charged solutes. IP-RPHPLC has gained wide acceptance as an alternative method to ion exchange chromatography (IEC) for charged analytes, including metal ions. IP-RPHPLC offers multi-element detection capacity, selectivity and sensitivity of analysis. Moreover, the reversed-phase stationary phase has the benefit of lower cost compared to the IEC stationary phase.

Most of the reports on IP-RPHPLC for metal analysis [13-16] are based on the separation as their chelate ions. Pre-complexation of metal ions with appropriate ligands has many advantages such as increasing selectivity between metal ions, the ability to determine speciation, and, for chelates with high absorptivity, increasing sensitivity. Among the many ligands successfully used for IP-RPHPLC separation of metal ions, the azo dye, 4-(2-pyridylazo) resorcinol (PAR) is one of the most widely used ligands for the spectrometric determination of over 40 different metals [17]. PAR forms ionic complexes with large absorptivity (~ 10^4 L.cm^{-1}.mol^{-1}) [18] at about 500 nm. It has been shown to be an effective reagent for the determination of metals using HPLC with either pre-column [19] or post column complexation techniques [20-21].

Typically, complexation of metals is performed by batch or external to the chromatographic system before injection. External complexation is time consuming and the large amounts of chemicals used mean more waste to discharge. It is prone to contamination, especially for trace level determinations. Nowadays, the main consideration include automation of the method, low operating costs, less waste as well as high sample throughput.

Flow injection (FI) has been known with features of a simple operational basis, using inexpensive hardware, straightforward thus leading to convenient operation, high sample throughput, cost effective performance and versatility. FI has been widely used as an analytical tool and also as a complement to the other analytical techniques.

Flow injection (FI) coupled with HPLC systems, reviewed by Luque de Castro M.D. and Valcacel M.[22], is usually intended to improve general features of the analytical process such as sensitivity, precision, rapidity, cost, etc. FI coupled with HPLC is used in two different modes i.e., pre- or post-column arrangements. For the pre-column arrangement, as in the present study, the FI system is placed before the HPLC. The specific objectives of pre-column coupling are automation of sample clean-up and/or preconcentration steps, automatic implementation of derivatization reactions and saving reagents. Two methods have been used to couple FI as precolumn of HPLC. The first method, the sample plug is injected through the FI valve and then passed through HPLC loop. In the second method, the sample from the FI system is retained in a precolumn placed in HPLC loop.

Previous work in this laboratory has involved metal analysis by IP-RPHPLC via batch complexation with PAR [23]. In the work described here, a simple FI system was developed as the in-line precolumn for complexation of some metal-PAR chelates before being analysed by IP-RPHPLC. The FI part is operated in the "reverse mode" that is a metal solution is the flowing stream and the PAR reagent solution is injected into it. A portion of the PAR-metal mixture zone is then sampled with the HPLC injection valve for subsequent separation and further detection.

2. Experimental

2.1. Chemicals and Reagents

All the reagents used were of analytical reagent (AR) grade, 4-(2-pyridylazo) resorcinol and tetrabutylammonium bromide (TBABr) were purchased from Fluka (Switzerland). 2-diethylaminoethanol was obtained from Merck (Germany). Methanol and acetonitrile were of HPLC grade and were obtained from Lab-Scan (Thailand). The atomic absorption standard solutions (1000 mgL^{-1}) of Cu(II), Cd(II), Co(II), Hg(II), Zn(II), Fe(III) and Pb(II) were obtained from Ajax Finechem (Australia) whereas Ni(II) was purchased from BDH (England). Cr (VI) oxide was obtained from Merck (Germany). Aqueous solutions were prepared with deionized water obtained from RiOs^{TM} type I simplicity 185 (Millipore Waters, USA) throughout the experiment.

2.2. Instruments

A schematic representation of the reverse flow coupled with the high performance liquid chromatographic system is shown in Figure 1.

The configuration of the injection for the reverse flow injection system, using a 505s 505LA peristaltic pump (Watson Marlow, England). PFA Teflon tubes (1.5 mm i.d.) were employed for the reaction coils and were connected to a six-port low pressure injection valve, four way switching valve (Upchurch, USA) was used to allow the metal-chelates flow to HPLC system. A stop watch (Casio, Japan) was used for time control.

The chromatographic set-up consisted of a Waters 6000A Dual Pump, a Rheodyne injector with 20 µL sample loop and a Waters 484 Tunable Absorbance Detector (Waters, USA) equipped with Waters 740 Data Module Integrator (Waters, USA), A 996 photodiode array (Waters, USA) and the Millinium 32 Software data aquisition system was also used for the study of interferences. A C_{18}-µBondapak (3.9 x 300 mm i.d.) coupled to a guard column (Waters, USA) was used as the stationary phase.

The spectra of the metal chelates in batchwise experiments were obtained with a Agilent 8453 (USA) UV-visible spectrophotometer equipped with a 1 cm quartz cell. The pH measurements were performed on a SP-701 pH meter (Suntex, Taiwan). Micropipette Nichiyo 500 DG (Nichiyo, Japan) and Disposable syrige (1 mL) (Nipro, Thailand) were used.

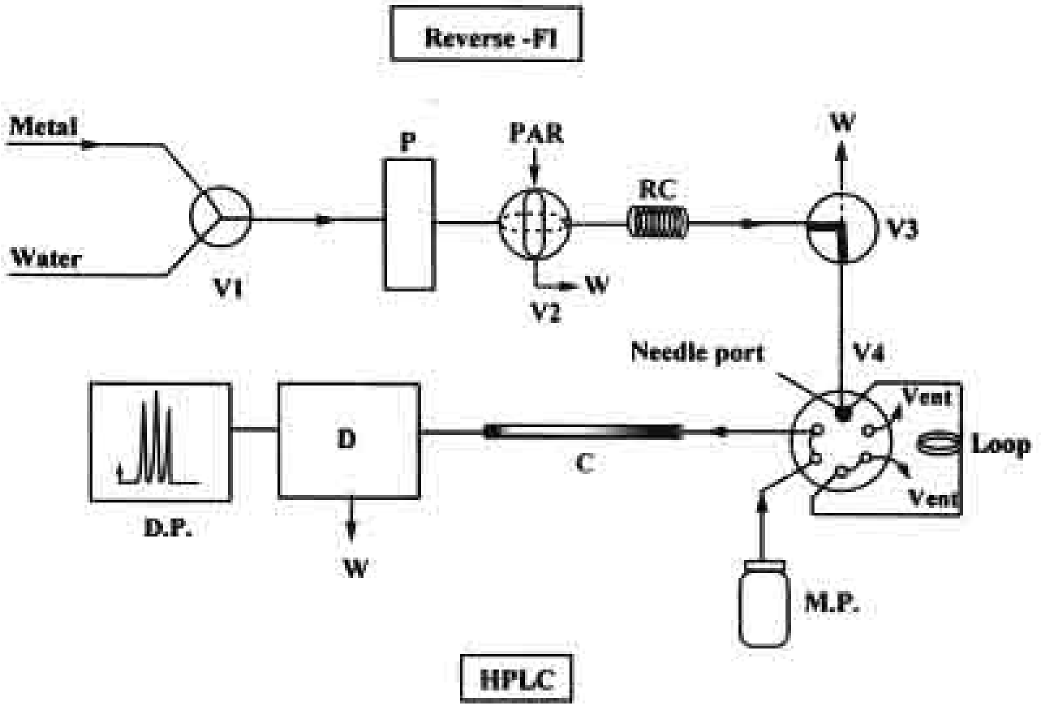


Figure 1 Flow diagram of FI-HPLC in-line derivatization system : P = peristaltic pump ; RC = reaction coil ; V1 = three way valve ; V2 = low pressure injection valve ; V3 = switching valve ; V4 = high pressure injection valve; C = analytical column ; M.P = mobile phase ; D = UV-visible detector/photodiode array detector ; D.P. = data processor ; W = waste

2.3 Procedure

2.3.1. Preparation of standard metal solution

Standard solutions of metal ions were prepared daily by stepwise dilution of 1000 mgL^{-1} stock solutions by deionized water.

Standard solution of Cr(VI) 1000 mgL^{-1} was prepared from chromium oxide.

2.3.2. Preparation of PAR solution

Stock standard solution (0.001 $molL^{-1}$) of PAR was prepared by dissolving an accurately weighed amount of 4-(2-pyridylazo)resorcinol in deionized water and stored in dark bottle. Working solution was prepared daily with deionized water and appropriate volume of 2-diethylaminoethanol was added to make the concentration of 2.5×10^{-4} $molL^{-1}$ in PAR solution.

2.3.3. Optimization of the reverse flow injection (rFI)

Factorial design was used to investigate the influence of the reverse flow injection (rFI) system on the peak height (absorbance). The four variables studied were flow rate of metal ions stream, injection volume of PAR, length of the mixing coils and concentration of PAR. A factorial design for four variables at two levels (2^4 resolution, 16 experiments) was performed. According to the results obtained from the factorial design, the chosen parameters to be optimized were the concentration of PAR, injection volume of PAR solution and flow rate of metal stream. The variable size simplex was then employed for optimization. The mixing coil length of 150 cm was used throughout the experiment.

3. Results and Discussion

3.1. Coupling of rFI to IP-RPHPLC

In-line complexation of metal-PAR chelates was performed using the rFI which the optimum conditions were mixing coil length of 150 cm, injection volume of PAR 85 μL, flow rate of metal stream 4.5 mLmin^{-1} and concentration of PAR 1.8 x10^{-4} molL^{-1}. The PAR chelates were then separated via IP-RPHPLC. The rFI was chosen instead of normal FI because of its low background noise for HPLC baseline as well as lower reagent consumption for the expensive ligand, PAR. The rFI was coupled to the HPLC by switching valve (V3) shown in the diagram in Figure 1.

Synchronization of the FI manifold and the HPLC is very important to achieve good performance of the coupling system. The time intervals and valve positions of the rFI-HPLC were investigated using the results obtained from the study of the optimization of the reverse flow injection . Manual operation of the rFI-HPLC system was sufficient for good precision. Each step was performed using a stopwatch as the timer control.

Once the baseline of the HPLC was steady, a complete cycle (4 steps) of the rFI-HPLC manifold was started. The 4 steps include prefill, complexation, separation and washing. In the first (prefill) step, the aqueous solution containing metal ions was pumped through the rFI manifold for 30 s, this period was long enough to fill the transmission line with metal solution. The HPLC was in the load mode throughout this step to maintain a steady baseline of solvent.

During the prefill step, an aliquot of PAR was filled into the loop connected to V2 (at LOAD position).

Step 2, the complexation step, was started by switching V2 to INJECT position. The metal ions merged with PAR and complexation occurred during their passage through the reaction coil (RC). To avoid a dilution edges of the zone and to allow only the middle zone of the PAR chelates to pass into the HPLC-loop, the valve V3 was switched after 8 seconds of injection of PAR. The subsequent time period of 2 s, was enough to rinse and fill the HPLC-loop (20 μL).

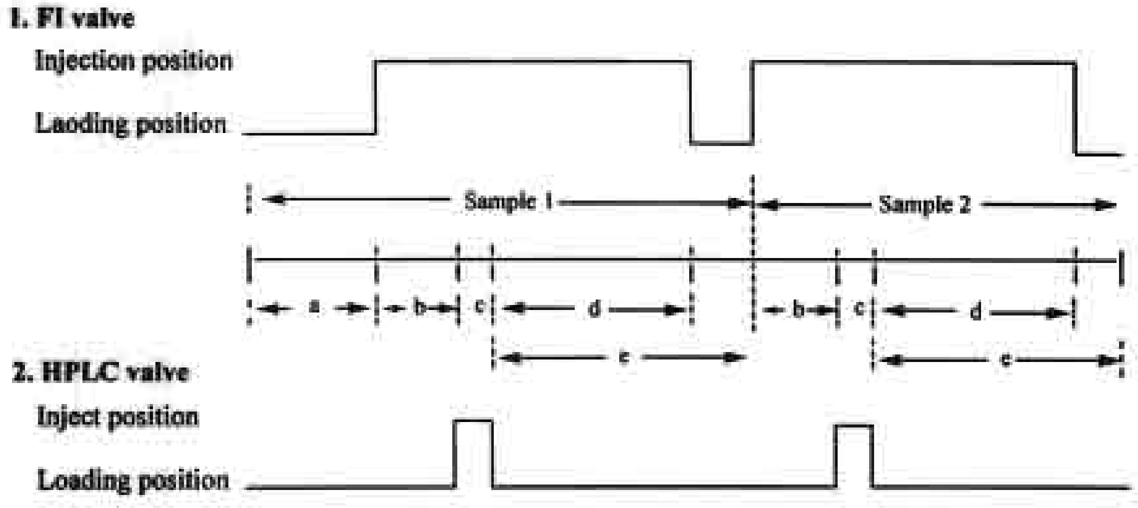
Then, step 3 (separation step), was initiated via HPLC-valve. The PAR-chelates were introduced and then separated in the HPLC system.

Finally, step 4 (washing step), was to wash the rFI and HPLC-loop for the next analysis. While separation was taking place on the HPLC column.

The complete cycle of rFI coupling to IP-RPHPLC was 120 seconds, where as the analysis time of the HPLC was 14 minutes. Operating periods and valve position for rFI –IPRPHPLC are summarized in Table 1 and Figure 2.

Table 1 Valves positions and operating times for rFIA -IPRPHPLC

Step	Time (s)	Valve position				In-line complexation operation	
		V1	V2	V3	V4	Medium pump	Stage of operation
Sample 1							
1	30	To V2	Load	To waste	Load	Metals	Prefill
2	38	To V2	Inject	To V4	Load	Metals	Complexation
3	40	To V2	Inject	To V4	Inject	Metals	Separation
4	120	To V2	Load	To V4	Load	Water	Washing
Sample 2							



a = prefill step (30 s)
b = complexation step (8 s)
c = transportation to HPLC valve (2 s)
d = washing step (80 s)
e = separation time (14 min)

Figure 2 Schematic diagram of timing control for operation of valves in the FI-HPLC system.

3.2. IP-RPHPLC of metal-PAR chelates

In IP-RPHPLC, the metal chelates which are successfully separated have to be stable and kinetically inert [24]. Typically, PAR forms anionic chelates with metals at the metal to ligand ratio of 1:2 [25]. It is known that the retention behavior of chelates in IP-RPHPLC depends strongly on complex composition (metal:ligand) which is governed by the nature of the central metal ion. The mobile phase composition is also govern the separation. The principal parameters of interest in the mobile phase are pH, buffer (type and concentration), organic modifier and ion pairing agent (long-chain alkyl ions with a charge opposite that of analytes). There are several mechanisms [26-28] explaining the retention behavior of IP-RPHPLC, such as the ion-exchange mechanism, the solvophobic theory and dynamic equilibrium.

The optimum mobile phase obtained by slightly adjusting the one obtained in our previous work [23]. The mobile phase composition was 37% acetonitrile, 6.2 mmolL^{-1} TBABr and 3.0 mmolL^{-1} acetate buffer pH 6.0.

Baseline separation of four metal-PAR chelates was achieved within 14 minutes, with the elution order of Co(II)-PAR, Ni(II)-PAR and Cu(II)-PAR. The excess PAR was detected at the retention time of 9.6 minutes. The chromatogram is shown in Figure 3.

Surprisingly that under the condition used and the detection at 440 nm, Cr(VI) was retained shortly (4.9 min) after unretained peak (at 3.3 min), as shown in Figure 4. The spectrochromatogram corresponding to Figure 4 is shown in Figure 5. Although, Cr(VI) does not form complex with PAR but it oxyanion ($HCrO_4^-$) could interacted with ion pairing agent in the same manner to the anionic chelates. The peak at 4.9 minutes which was identified as Cr(VI) gives the absorption spectrum (as shown in Figure 6.) identical to the spectrum of Cr(VI) detected by UV-vis spectrometer. The resolutions between pairs were as follow: 1.4 for Cr(VI) and Co(II)-PAR, 1.0 for Co(II)-PAR and Ni(II)-PAR, 2.4 for Ni(II)-PAR and excess PAR, and 1.3 for excess PAR and Cu(II)-PAR.

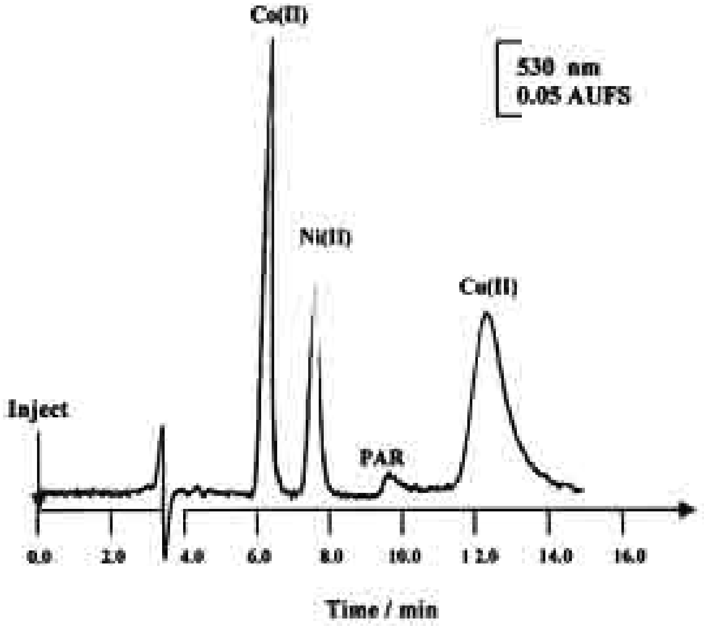


Fig. 3. Chromatogram of metal-PAR chelates : condition ; C_{18} column, mobile phase 37 % acetonitrile, 6.2 $mmolL^{-1}$ TBABr and 3.0 $mmolL^{-1}$ acetate buffer pH 6.0, flow rate of mobile phase 1.0 $mLmin^{-1}$ visible detection at 530 nm: peak ; 0.10 μgmL^{-1} Co(II), 0.20 μgmL^{-1} Ni(II), excess PAR and 0.80 μgmL^{-1} Cu(II)

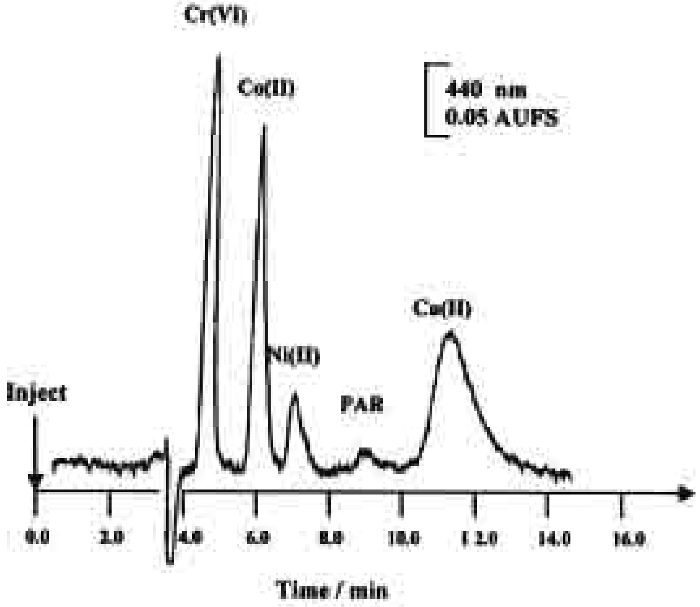


Figure 4. Chromatogram for Cr(VI) and metal-PAR chelates : peak ; 5.0 μgmL^{-1} Cr(VI), 0.10 μgmL^{-1} Co(II), 0.10 μgmL^{-1} Ni(II), excess PAR and 0.40 μgmL^{-1} Cu(II) (condition as described in Figure 3, except visible detection at 440 nm)

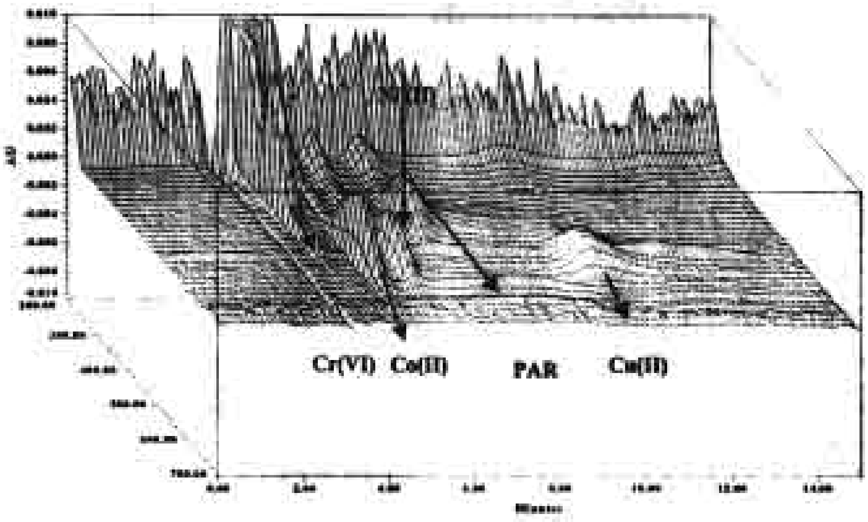


Figure 5. 3D plot of Cr(VI) and metal-PAR chelates of chromatogram in Figure 4.

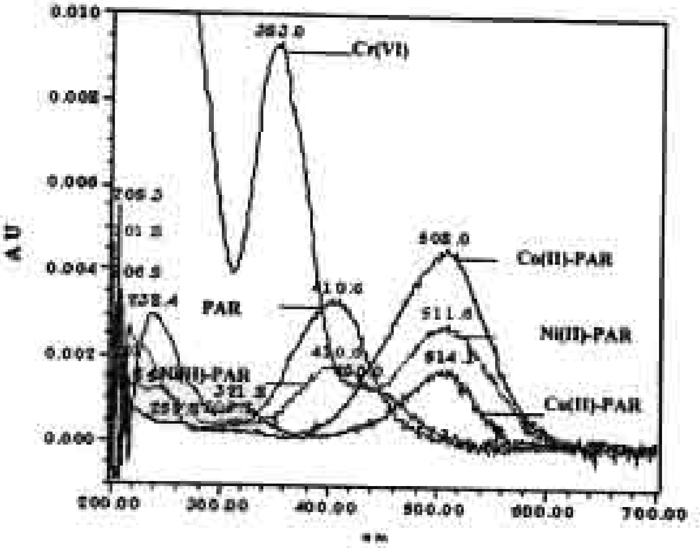


Figure 6. The absorption spectra of chromatogram in Figure 4.

3.3. Performance of rFI-IP-RPHPLC

Quantitative features including linearity and reproducibility for retention time and peak area were studied using the optimum condition. Calibration graphs were prepared by plotting the concentration of each metal ion (μgmL^{-1}) against the peak area. The limit of detection (LOD) was deduced based on 3 times of baseline signal. The calibration equation, coefficient of correlation (r^2), recovery, reproducibility and LOD are summarized in Table 2.

Table 2

The quantitative features of rFIA-HPLC

Chelates	Concentration (μg mL^{-1})	Linear equations Y = AX + C	Correlation Coefficient (r^2)	R.S.D* (%) (n =11)		LOD (3σ) (μg mL^{-1})	% Recovery
				t_R	Area		
Cr(VI)	1.00 - 6.00	473.51X – 0.17	0.99614	2.01	0.94	1.0	113
Ni(II) –PAR	0.010 – 0.20	341.66X – 4.73	0.99835	2.07	0.91	0.015	84
Co(II) –PAR	0.010 – 0.40	547.21X – 0.61	0.99516	0.56	0.97	0.030	96
Cu(II) –PAR	0.05 – 0.60	293.80X+ 5.49	0.99864	2.43	0.71	0.150	102

* concentration of each metal was described in Figure 4

3.4. Interferences

The effects of interferences on the chromatography of metal-PAR chelates was investigated. The chosen ions are the ions able to form chelate with PAR including Cd(II), Cr(III), Hg(II), Mn(II), Fe(III), Pb(II) and Zn(II). These metal ions were individually injected into the rFI-HPLC. All of the studied ions could not formed chelates with PAR under the condition used. Only peak which was identified as PAR (9.6 min) was observed.

The study on tolerance level of the metal ions which could not form chelates with PAR was studied by individually spiking the metal ions at difference amounts (ranging from 0.5-10.0 μgmL^{-1}) into the mixture of 0.10 μgmL^{-1} Co(II), 0.20 μgmL^{-1} Ni(II) and 0.40 μgmL^{-1}Cu(II). It was found that the presence of the foreign ions did not affect the retention time of the PAR chelates of Co(II), Ni(II) and Cu(II). However, the quantitative signals (both peak height and peak area) were affected by the addition of the foreign ions. Cu(II)-PAR was strongly influenced when the concentrations of the foreign ion increase to 2.5 times resulted in either decreased or increased of peak height and peak area. The effect on Ni(II)-PAR was observed when the foreign ion increase to 5 times greater than Ni(II), resulted in the decreasing of peak height and peak area. This effect was also observed for Co(II) when the concentration of the foreign ion was 10 times to Co(II). The peak area of Cr(VI) was not affected by the addition of the foreign ion. However, the obtained spectra and the 3-D plots (results not shown) revealed that neither PAR chelates of the foreign ions nor the ternary complexes was formed. According to the obtained results indicating that PAR was enough for all of metal ions. Furthermore, to ensure the excess amount of PAR, ten times higher concentration of PAR i.e. 1.0×10^{-3} $molL^{-1}$ was used. Similar results were obtained and large peak of excess PAR overlapped the analyte peaks. The effect of interference on the present method was obviously seen when compared to the previous work on precomplexation of metal-PAR chelates by batch method prior to the analysis by IP-RPHPLC. This may attribute to the nature of the flow system which a short time that stream of reagents are reacted. Neither physical equilibrium nor chemical equilibrium (i.e. the completeness of reaction) has been attained by the time it was detected.

3.5. Analysis of real sample

According to the study it is possible to analyse Cr(VI) simultaneously with Ni(II). The present method was applied to the analysis of chrome plating waste water. The samples were collected from chrome plating plant in Khon Kaen and were analysed after dilution, pH adjustment and filtration through 0.45 μm membrane. Data obtained are list in Table 3 which were in good agreement with atomic spectrometry.

Table 3

Result of analysis of chrome plating waste water

Metal	Concentration ($\mu g\ mL^{-1}$)	
	Sample 1	Sample 2
Cr(VI)	984.5 ± 6.48[b]	69.3 ± 10.74[b]
Co(II)	N.D.[a]	N.D.[a]
Ni(II)	1574.6 ± 10.25[b]	121.4 ± 7.42[b]
Cu(II)	N.D.[a]	N.D.[a]

[a] Not detected, [b] S.D. (n = 3)

5. Conclusion

In the present study, a simple combination of reverse flow injection and HPLC resulted in a powerful technique for simultaneous analysis of metal ion as their PAR chelates. The reversed flow injection (rFI) was coupled to HPLC and the performance of the system was fully manually operated. Using the developed rFI for in-line complexation gives benefit of less PAR consumption, less analysis time and less waste disposed comparison to the batch derivatization. The analysis cycle was 16 minutes including in-line complexation (ca. 2 minutes) and separation by IP-RPHPLC (14 minutes). The method was successfully applied for the separation of Co(II), Ni(II) and Cu(II) as their PAR chelates.

Acknowledgements

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ผลงานวิจัย ก26

Determination of Linear Alkylbenzene Sulfonates in Water Samples by Liquid Chromatography-UV Detection and Confirmation by Liquid Chromatography-Mass Spectrometry

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Abstract

A high performance liquid chromatography (HPLC) method was developed for the separation and determination of individual ($C_{10} - C_{13}$) linear alkylbenzene sulfonates (LAS). A new set of conditions has been established for routine analysis of individual chemical forms of four LAS surfactants, i.e. C_{10} - C_{13} LAS. The mobile phase containing 1.5 mM ammonium acetate in methanol/water mixture of 78 to 22 (v/v) was used. Under the optimum condition, detection limits obtained were in the range 1.43 pg μl^{-1} (for C_{10} LAS) to 11.35 pg μl^{-1} (for C_{13} LAS). This method offers the advantages of significant improvement in resolution, short separation time and using less amount of common salt under isocratic condition. In addition, the use of simple mobile phase containing a simple low amount of salt cannot deposit at the entrance of mass spectrometric detector. The method is applicable to the simultaneous determination of LAS surfactants in various water samples. LAS surfactants presented in these samples were also successfully confirmed by using electrospray mass spectrometry.

Keywords: anionic surfactant, linear alkylbenzene sulfonates, HPLC, mass spectrometry, water

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1. Introduction

Linear alkylbenzene sulfonates (LAS), synthetic anionic surfactants, have been used in household laundry and dishwashing detergents [1]. The commercial product is a mixture of homologues containing carbon atoms between 10 and 13 atoms. Each of these homologues consists of positional isomers resulting from the attachment of the phenyl ring to the carbon atoms of the linear alkyl chain (Fig. 1) [2, 3]. They are rapidly biodegraded under aerobic conditions. LAS-containing detergents are used in large quantities and are therefore released in the environment. LAS homologues with alkyl chain lengths from C_{10} to C_{13} have been found in municipal wastewaters and sediments at the ppm levels [4-6]. It has been reported that LAS and their degradation products can affect membrane permeability, enzyme and lysosomal activity [7, 8]. The toxicity of the LAS containing 13 carbon atoms to the microalgae namely *Chaetoceros gracitis* was found to be greater than that of the C_{11} LAS [9]. For these reasons, the identification and quantification of individual LAS species are invaluable for estimating the environmental impact and potential health effects of LAS species.

The standard methylene blue method has long been used for determining total amounts of sulfonate and sulfate based anionic surfactants in wastewater [10]. The method cannot differentiate individual anionic surfactant. The method is sensitive. It is time consuming and is often interfered by sample matrix, i. e. organic sulfonates, sulfates, carboxylates and phenol. This method also requires a large quantity of the toxic solvent for extraction such as chloroform.

A number of methods have been developed for identifying and quantifying individual chemical forms of anionic surfactants. Chromatographic techniques like gas chromatography (GC) [4, 11-15], electrophoresis [1] and high performance liquid chromatography (HPLC) [16-18] are efficient separation methods for the analysis of LAS mixture. Due to their low volatility and anionic form, derivatization of these compounds is necessary when GC-based method is used [19].

HPLC is currently a suitable method for the determination of LAS. Reversed-phase HPLC provides a good separation of LAS mixture when using various chromatographic detectors, e. g. ultraviolet (UV) [16-18], fluorescence [2, 20, 21] and mass spectrometry (MS) [22-24]. However, most existing methods and procedures are still far from being considered suitable for the routine determination of individual chemical forms of LAS. Most HPLC methods with UV detector require the mobile phase containing either sodium perchlorate [22] or additive mixture such as triethylamine and acetic acid [23, 24], trifluoroacetic acid (TFA) and tetrabutylammonium dihydrogenphosphate (TBA-H_2PO_4) [25] or cetyltrimethylammonium ($CTMA^+$) ions [26] in order to resolve LAS homologues under gradient conditions. In practical application of the mobile phase containing high amount of those compounds, particularly sodium perchlorate (10 g l^{-1}) can shorten the column life and can also clog the capillary when mass spectrometric detector is used. In addition, the complicated mass spectra of LAS homologues would be obtained. This makes the identification of individual LAS in environmental samples so difficult.

As reported earlier by other workers, LAS compounds containing 10 to 13 carbon atoms are used in large amounts and are therefore released in the environment [1, 4-6]. The toxicity of surfactant to aquatic organisms increases with increasing of carbon atom [9].

The main purpose of this study was to develop HPLC method that would allow for routine analysis of LAS mixture, particularly in respect to reducing analysis time, improving separation efficiency for all the four LAS surfactants, precision and accuracy under isocratic condition. As mentioned earlier, several publications have been reported to use complicated mixtures as mobile phase under gradient conditions for separating some LAS surfactants. These approaches can also cause either capillary blockage or additional spectral interferences when a mass spectrometric detector is used for confirmation results. Therefore, common salts, i. e. sodium chloride, sodium acetate and ammonium acetate added into mobile phase were chosen because of their suitability of identifying LAS in water samples using mass spectrometric detection.

2. Experimental

2.1 Chemicals

Linear alkylbenzene sulfonates in the forms of sodium salts were obtained from Henkel (Germany). HPLC grade methanol was purchased from BDH (Poole, England). Sodium chloride, sodium acetate and ammonium acetate were AnalaR grade and purchased from Carlo Erba (Barcelona, Spain). Milli-Q water was used in this study.

2.2 Instrumentation

A HP 1100 high performance liquid chromatograph (Agilent Corp., Wilmington, USA) consisting of an Agilent 1100 quaternary pump and an Agilent 1100 UV detector (224 nm) was employed. An inlet frit with 2 µm pore size was placed between the injector and