Table 4
Iodine contents in table salts obtained from the colorimetric FI method and the potentiometric method (ISE)

Sample	Iodine content (mg I kg^{-1})±S.D.	
	Colorimetric FI (n = 3)	Potentiometric method (n = 3)
S14	15.7±0.1	18.7±1.9
S15	15.8±0.1	19.3±2.1
S16	16.6±0.3	18.8±2.1
S17	17.3±0.4	21.2±2.0
S18	18.8±0.3	25.4±2.2
S19	29.4±0.1	28.3±2.0
S20	33.3±0.1	30.3±2.1
S21	33.5±0.4	29.8±1.9
S22	35.2±0.3	30.7±2.0
S23	38.0±0.3	33.7±2.2
S24	55.4±0.4	48.8±2.2
S25	56.5±0.3	47.5±2.1

recently with use of amperometric detection (35 injections per h) [6]. The method is much faster and more convenient than the conventional titration method. The FI method also gave higher sample throughput than the potentiometric method, which has the throughput of only seven measurements per h. As the iodide-required approximately 4–5 min for stable reading.

The detection limit of the FI method (3 S/N, $n = 10$) was 2 mg I kg^{-1}. The method has high precision with the R.S.D. of 0.5% (ten injections of the 2.0×10^{-5} M standard).

4. Conclusions

A FI system was developed and is suitable for determination of iodate in table salts. The system requires spectrometric detection in the visible region of the blue I_3^-–starch complex formed in sample zone. The FI detection, which exploited this complex formation, has not been reported elsewhere. There are a few systems that utilized similar chemistry for the detection [9–11] but their monitorings are based upon decoloration of the complex. Initially detection of the I_3^-–starch complex gave some problems (e.g. continuous shift of baseline, irreproducible signal after some injections). This was due to the adsorption of I_3^-–starch mainly on tube walls. The present use of an injection of thiosulfate solution, prior to injection of sample, eliminated this contamination. Air–oxygen oxidation of iodide, which tends to occur in batch operation, is easily prevented in this technique. The present method was successfully validated against the titration and the potentiometric methods.

Features of the system meet all requirements in terms of simplicity, rapidity, precision, linear working range, detection limit and economy. As the chemistry takes place in a close system, no blank signal was observed.

Acknowledgements

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Stopped-flow injection simultaneous determination of phosphate and silicate using molybdenum blue☆

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Abstract

Kinetic information for the phosphate–molybdate–ascorbic acid reaction can be obtained by making use of a very simple manually operated stopped-flow injection (FI) system. Various parameters (concentrations of reagents, flow rate, mixing coils, and volume of flow cell) were investigated for determination of phosphate. A stopped-FI system should be arranged for low degree of mixing (of reactants) and low dispersion so that good signals of rate changes will be observed. Simultaneous determination of phosphate and silicate by the stopped-FI technique is proposed, using a laboratory-made semi-automatic stopped-FI Analyzer with LED-based photometer. It is based on kinetic separation of phosphate and silicate using molybdenum blue. The proposed procedure has been demonstrated for the application to water samples. The results obtained agree with that of a standard method.

Keywords: Stopped-FI; Simultaneous determination; Phosphate; Silicate; Molybdenum blue; Kinetics

1. Introduction

Since the first application by Ruzicka and Hansen [1] for the determination of phosphate using molybdenum blue, a number of flow injection (FI) techniques have been reported for phosphate and silicate. They involve a continuous mode, such as on-line microwave digestion [2], determination of phosphate in silicate rocks by adding tartaric acid [3], on-line column separation [4–7]. Sequential injection analysis (SIA) has been applied to determine phosphate and silicate simultaneously by forming vanadomolybdo complexes [8], or using the molybdenum blue [9]. SIA with lab-on-valve using a stopped flow mode for determination of phosphate has been proposed [10,11].

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Kanaya and Hiromi [12] used stopped-flow to record the progress curve for the formation of the colored complex of 12-molybdophosphate with malachite green. Linares et al. [13] described the fluorometric differential kinetic determination of silicate and phosphate using two measurements at different times. Yoza et al. [14] determined ortho-, di-, and triphosphates using stopped-flow, based on substitution reactions with colored metal complexes. Lacy et al. [15] used sorbent extraction on a hydrophobic sorbent and optosensing measurement to determine mixtures of phosphate and silicate, based on differences in the reduction rates of the heteropoly complexes, using partial least squares analysis.

In this work, we employ stopped-FIA with simple instrumentation for kinetic information on phosphate and silicate using the molybdate and ascorbic acid reactions. Simultaneous determination of phosphate and silicate based on kinetic separation, using a simple semi-automatic stopped-FI Analyzer is proposed.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were analytical grade unless otherwise stated. Deionized water was used throughout. A stock phosphate standard solution (1000 μg ml^{-1}) was prepared by dissolving potassium dihydrogen phosphate (Merck, 0.4390 g) in a portion of water before making up to a volume of 100.00 ml. A commercial silicate standard solution of 1000 μg ml^{-1} (Merck) was used. Working phosphate and silicate standard solutions were obtained freshly by appropriate dilutions of the stock solutions.

A sodium molybdate solution (0.6% w/v) was prepared by dissolving sodium molybdate (Merck, 1.512 g) in 100 ml water, and 3.25 ml of conc. HNO_3 before making to a volume of 250.0 ml with water.

An ascorbic acid solution (0.5% w/v) was prepared freshly from 2.5 g of L-ascorbic acid (Fluka) which was dissolved in 500-ml water.

2.2. FI manifolds with manual operation

Two FI manifolds for manual operation were designed, as depicted in Fig. 1. In manifold I, a standard/sample solution (S) was injected via an injection valve (FIA-lab, USA, with a sample loop of 100 μl) into a molybdate line with a mixing coil (MC1) before merging with the ascorbic acid stream, passing through another mixing coil (MC2) before entering into a flow cell in a spectrometer (Spectronic 21, Spectronic Instruments, USA), connected to a chart recorder (R100 A, Perkin-Elmer, USA). Manifold II represents a different arrangement, in which a standard/sample solution (S) was injected into a merged stream of molybdate and ascorbic acid via an injection valve placed between mixing coils (MC3 and MC4).

2.3. Semi-automatic FI Analyzer

The laboratory-made system (Fig. 2) consists of a controller via a microprocessor to control a peristaltic pump (S-mini, Alitea) to propel re-

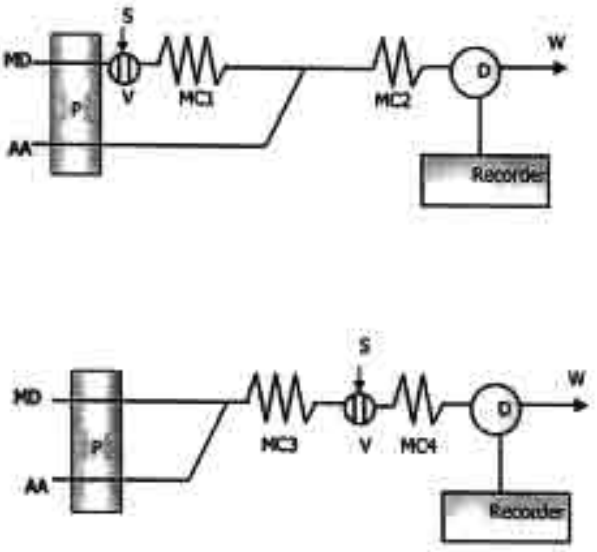

Fig. 1. FI manifolds with manual operation for stopped-FIA for phosphate, *top*: manifold I, S injected into MD before merging with AA; *bottom*: manifold II, S injected into a stream of mixed MD and AA; MD = molybdate solution, AA = ascorbic acid solution, P = peristaltic pump, MC = mixing coil, D = spectrometer, V = injection valve, W = waste.

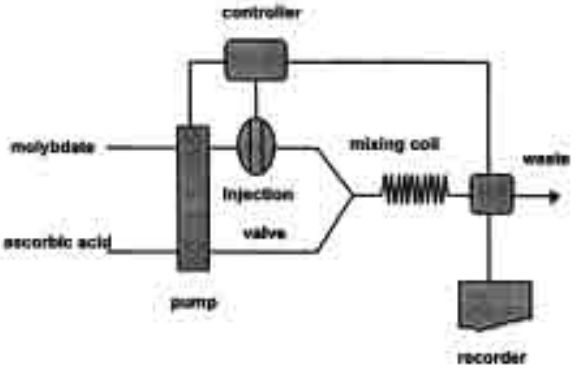

Fig. 2. Semi-automatic FI Analyzer.

agents, to switch an injection valve (V-451, Upchurch), and to set a photometer for data acquisition and to evaluate the signals. The photometer is a laboratory-design, using a LED red light source, with a peak height holding feature, similar to the ones previously reported [16–18]. The output signals can be via built-in digital read-out and/or a chart recorder (Philips, PM 8251, Holland). Signal profiles are illustrated in Fig. 3. The controller can be used to preset the traveling time (T), the period of flow between the point of injection to the point at which the flow is stopped for monitoring the reaction development at the flow-cell; the stopping time (S), the period during the flow stopped; and the washing time (W), the period when the stream is re-started to flow (after stopping) until this operation cycle ends and is ready for the next cycle. I and D, the signals at the first and last stopped points, respectively, will be given as digital read-outs on the photometer. Fig. 3(b–d) demonstrate stopped-FI recordings for phosphate, silicate, and mixtures of the standard solutions.

3. Results and discussion

3.1. Continuous FIA for phosphate

In preliminary investigation, it was found that the reactions to form the molybdenum blue product are reversible and will be in equilibrium. For continuous FI determination of phosphate, manifold II should be employed. Manifold II resulted in higher FI responses compared to the peaks obtained by using manifold I. Calibrations (a plot of peak height vs concentration) were: $y_1 = 65.08x_1 + 9.76$, $r^2 = 0.998$ and $y_2 = 91.93x_2 + 15.39$, $r^2 = 0.991$ for the manifolds I and II, respectively (0.5–3 μg P ml^{-1}). The reactions for molybdenum blue may involve [19]:

$$\text{heptomolybdate} + \text{phosphate} \overset{\text{fast}}{\rightleftharpoons} \text{yellow heteropoly complex} \quad (1)$$

$$\underset{\text{(Mo (VI))}}{\text{yellow heteropoly complex}} + \text{ascorbic acid} \overset{\text{slow}}{\rightleftharpoons} \underset{\text{(Mo(V)–Mo(III))}}{\text{molybdenum blue}}. \quad (2)$$

Using manifold II with a premixed stream of molybdate and ascorbic acid, the reactive intermediate of the reduced form of molybdate will be readily available and reactive to the injected phosphate, to yield the molybdenum blue very fast (on the order of seconds), whereas in the manifold I, phosphate injected into molybdate stream will form an observed yellow product of phosphomolybdate which is not as reactive, with slow reduction to yield molybdenum blue (on the order of minutes). This was found in the batch molybdenum blue method for determination of phosphate. It should be noted that for batch analysis, if molybdate was premixed with ascorbic acid before adding phosphate, no molybdenum blue was observed at all.

Using manifold I with MC1 and MC2 being 25 and 50 cm, respectively, and ascorbic acid solution being 0.5% w/v, molybdate concentrations were varied (0.1–1.2% w/v). It was observed (Fig. 4(a)) that the same steady state of the system should be established in the molybdate concentrations in the range of 0.5–1% w/v. Similar observation was found for the stopped-FIA studies (Fig. 4(b)). A lower molybdate concentration would cause slower approach to equilibrium. Disproportionation of Mo(VI) and Mo(III) yielding Mo(V) may occur if a higher concentration of molybdate is used. This shifts the equilibrium in Eq. (2), resulting in less phosphomolybdenum(V) blue.

Similarly, a study on the effect of ascorbic acid concentrations (Fig. 5(a and b)) indicated that the

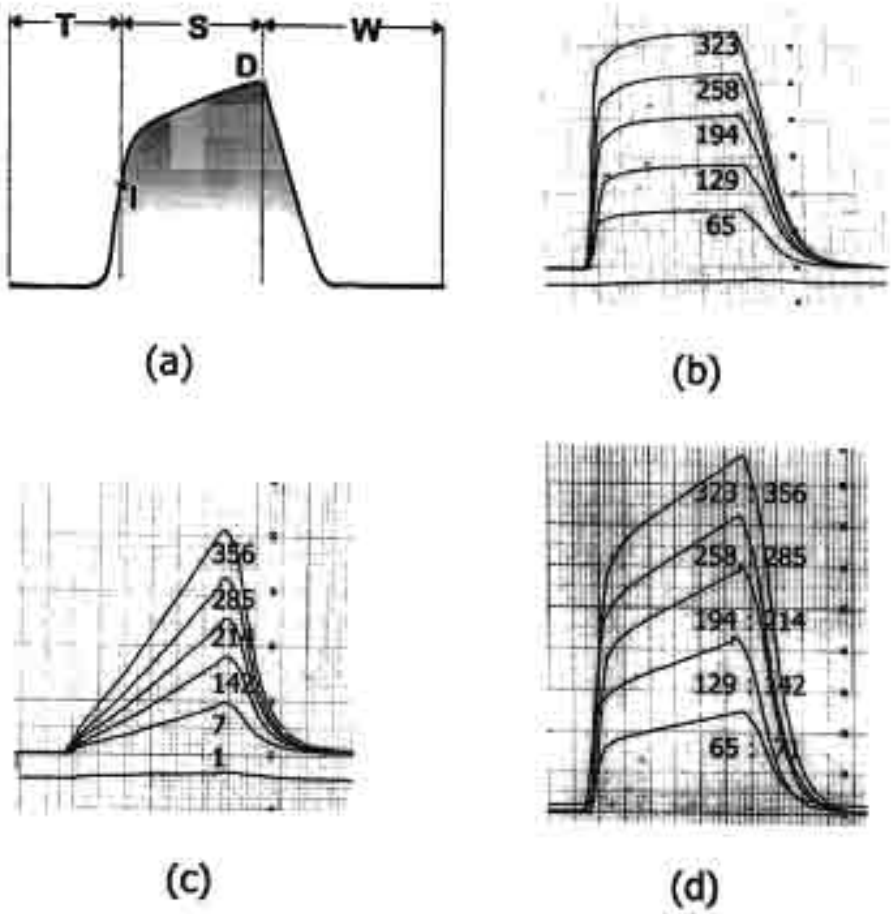

Fig. 3. Stopped-FI profiles obtained (a) in general, (b) phosphate, (c) silicate, (d) mixture of phosphate and silicate: T = traveling time, S = stop time, W = washing time, I = signal at the first stopped point, D = signal at the last stopped point before resuming flow. The numbers refer to concentrations [μM].

system with 0.3–0.8% w/v ascorbic acid resulted in reaction rates at steady state for the molybdate used (0.8% w/v). With lower concentrations of ascorbic acid, different rates were observed.

3.2. Stopped-FI determination of phosphate

At the flow-cell, in the stream, a bolus of the mixture (phosphate, molybdate and ascorbic acid) is stopped, and an increase in signal is observed for a period before gradually becoming constant (Fig. 3(b)). The signal represents the rate of the reactions, which come into equilibrium as discussed earlier.

A manifold used for stopped-FIA should provide both a low degree of axial mixing of reactants and low dispersion, to provide a suitable bolus of reaction mixture stopped at the flow cell for monitoring the reaction progress.

It was found that the manifold I (Fig. 1) without mixing coil and using a 8-μl flow cell is more suitable, compared to manifold II for stopped-FI determination of phosphate (Fig. 6). A dispersion study using a dye (bromothymol blue) [20] for manifold I is summarized in Table 1. As expected, apart from the effect of mixing coil length on dispersion, a higher volume flow cell creates more dispersion. It should also be noted that a signifi-

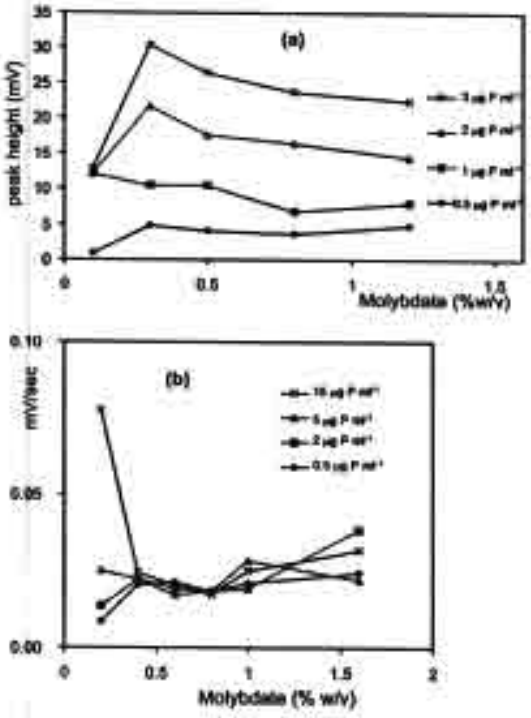

Fig. 4. Effect of molybdate: (a) continuous-FI; (b) stopped-FI.

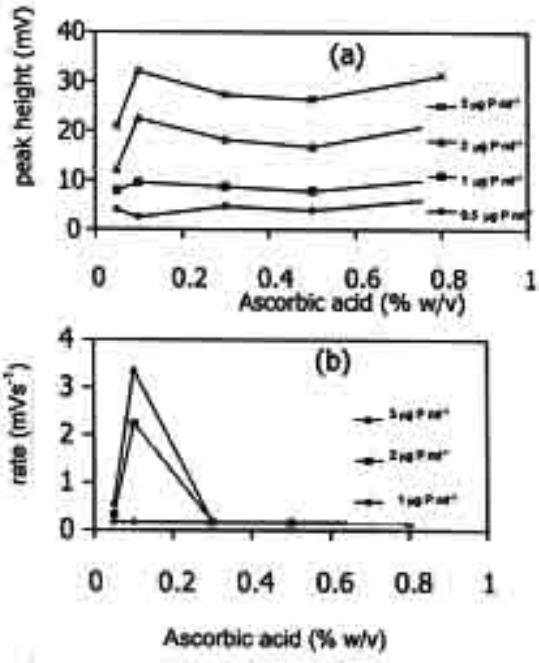

Fig. 5. Effect of ascorbic acid, (a) continuous-FI; (b) stopped-FI.

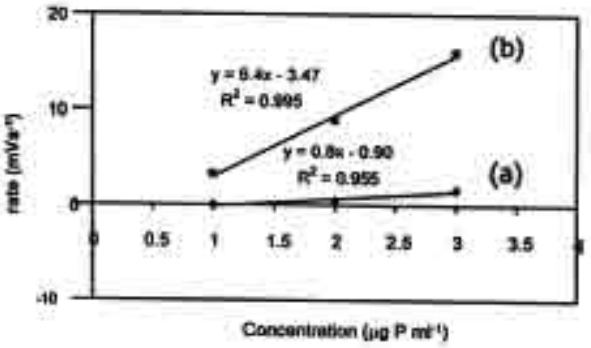

Fig. 6. Plot of reaction rates and phosphate (as P) concentrations using manifold I: (a) with and (b) without mixing coil.

cant effect of flow rate on dispersion was not observed.

3.3. Kinetic information obtained by the stopped-FIA

For a general reaction:

$$A + B \rightarrow C \quad (3)$$

with a rate expression: $r = d[C]/dt = k[A]^n$, where r = rate, k = rate constant, n = order of reaction, or $\ln r = \ln(k[A]^n)$, then:

$$\ln r = \ln k + n\ln[A] \quad (4)$$

From Eq. (4), a plot of $\ln r$ vs $\ln[A]$, the order of the reaction can then be obtained from the slope (n), and the rate constant (k) can be evaluated from the y-intercept.

From the stopped-FI experiments for phosphate (Table 2), the rate (mV s^{-1}) was obtained from each stopped-FI recording for each phosphorous (phosphate) concentration (i.e., slope of the signal). By assuming that the reagents (molybdate and ascorbic acid) were in large excess, then the rate of product formation should be proportional to the phosphorous (phosphate) concentration. A plot of ln(rate) and ln(phosphorous concentration) was found to be linear: $y = 1.3x - 3.99$, $r^2 = 0.992$ (Fig. 7). The slope of 1.3 reflects the order of the reaction under the experimental conditions to be close to unity, in agreement with other reports [21,22]. The rate constant obtained was 1.9×10^{-2} µM s^{-1}.

Table 1
Studies on dispersion in the manifold I

Total flow rate (ml min^{-1})	Dispersion (C_o/C_{max})			
	With mixing coils[a]		Without mixing coil[b]	
	8-μl flow-cell	80-μl flow-cell	8-μl flow-cell	80-μl flow-cell
2	3.1	4.9	1.2	2.8
3	2.9	4.7	1.3	2.8
4	2.7	4.1	1.1	2.8

[a] MC1 = 25 cm, MC2 = 50cm.
[b] No MC1 and MC2.

Table 2
Experiments for kinetic information on the reactions of phosphate with molybdate and ascorbic acid

Phosphate (P) concentration		ln P	Rate of reaction[a]	Ln (rate)
(μg P ml^{-1})	(μM)		(mV s^{-1})	
0.5	16.4	2.80	8.0×10^{-1}	-2.2×10^{-1}
1	32.8	3.49	1.6	4.70×10^{-1}
2	65.6	4.18	4.4	1.48
3	98.4	4.59	8.0	2.08

[a] d[Product]/dt.

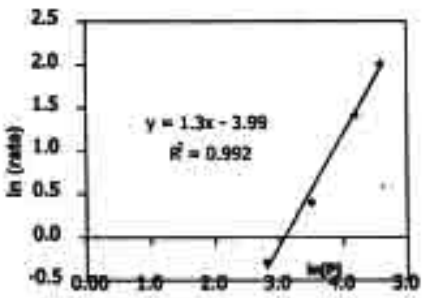

Fig. 7. Plot of ln(rate) vs ln[Phosphate].

3.4. Simultaneous determination of phosphate and silicate by stopped-FIA

The rate of the phosphate–molybdate–ascorbic acid reaction is faster than that of the silicate–molybdate–ascorbic acid reaction. It is then possible to determine phosphate and silicate simultaneously using the kinetic separation approach.

Fig. 3(b–d) show stopped-FIA recordings of phosphate, silicate, and a mixture of the two. By using the semi-automated stopped-FI Analyzer (Fig. 2), concentrations of phosphate and silicate in a mixture can be evaluated from I_P, I_{Si} and I_{mix} (signals at the first stopped points for phosphate, silicate and mixture, respectively, see Fig. 3), and D_P, D_{Si} and D_{mix} (signals at the last stopped points for phosphate, silicate and mixture, respectively, see Fig. 3).

Employing the conditions summarized in Table 3, calibration plots were obtained from the experimental results (Fig. 3). For phosphate standards (up to 15 μg P ml^{-1}):

$$I_P = 13.31[P] + 9.20,\ r^2 = 0.986 \quad (5)$$

Table 3
Conditions for simultaneous determination of phosphate and silicate with molybdate using the semi-automatic stopped-FI Analyzer

Condition	Value
Molybdate concentration, % w/v in 0.18 M HNO_3	0.6
Ascorbic acid concentration, % w/v	0.5
Injection volume, μl	35
Mixing coil length, cm	50
Flow rate (each line), ml min^{-1}	3.2±0.2
Travel time, s	2.5
Stop time, s	20
Wash time, s	15
Light source	Red LED (630 nm)

Table 4
Determination of phosphate and silicate by the proposed stopped-FI method and the standard method [23]

Water sample	Phosphate (μg P ml^{-1})		Silicate (μg Si ml^{-1})		Phosphate added[a] (μg P ml^{-1}) (*C*)	% Recovery of phosphate[b]	
	Stopped-FI method (*A*)	Standard method (*B*)	Stopped-FI method	Standard method		Stopped-FI method (*D*)	Standard method (*E*)
Reservoir	n.d.	n.d.	8.2	8.6	–	–	–
	1.6	1.8[a]	9.4	8.7	2.0	80	90
Pond	n.d.	n.d.	7.6	7.9	–	–	–
	5.7	3.5[a]	7.5	8.0	4.0	143	88
Irrigation canal	n.d.	n.d.	8.3	8.2	–	–	–
	6.5	5.7[a]	7.8	8.3	6.0	108	95
Moat	n.d.	n.d.	8.1	9.0	–	–	–
	6.3	7.9[a]	7.3	8.9	8.0	79	99
Drainage (from a dormitory)	1.1	1.2	11.9	13.9	–	–	–
	3.1	3.1[a]	11.2	13.9	2.0	100	95

[a] Total of present+added.
[b] % Recoveries evaluated by: $D = (A/C) \times 100$ and $E = (B/C) \times 100$.

For correlation of D_P (mV) and I_P (mV):

$$D_P = 4.65I_P - 3.38,\ r^2 = 0.998, \quad (6)$$

For silicate standards (up to 15 μg Si ml^{-1}):

$$D_{Si} = 35.82[Si] + 30.55,\ r^2 = 0.999. \quad (7)$$

The concentration of phosphate in a mixture or a sample is obtained from the calibration plot of phosphate standards (Eq. (5)) by using I_{mix} from the mixture or the sample for I_P (as at the first stopped point, there should be no contribution due to silicate). Then D_P is calculated from Eq. (6). In the signal profile of the mixture/sample, calculation for signal due to silicate at the last stopped point, $D_{Si,mix}$ is made from the correlation:

$$D_{Si,mix} = (D_{mix} - D_{P,mix}) + I_P. \quad (8)$$

$D_{P,mix}$ is the contributed signal due to phosphate in the mixture and can be evaluated from the Eq. (6). The last term (I_P) is for contribution from the initial (background) signal due to phosphate. Substituting the value of $D_{Si,mix}$ for D_{Si} in Eq. (7), one obtains the concentration of silicate in the mixture/sample.

The proposed procedure has been applied to several natural water samples. The results are compared to that obtained by the standard method [23], as summarized in Table 4.

4. Conclusion

A stopped FI system offers kinetic information on the phosphate–molybdate–ascorbic acid reaction. Simultaneous determination of phosphate and silicate in a mixture/sample is proposed by employing kinetic separation using molybdenum blue, and using a laboratory-made semi-automatic stopped-FI Analyzer with a microprocessor to control the system and for signal read-out. The proposed procedure has been demonstrated for its application to water samples by validation with a standard method.

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Sequential injection redox or acid–base titration for determination of ascorbic acid or acetic acid☆

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Abstract

Two sequential injection titration systems with spectrophotometric detection have been developed. The first system for determination of ascorbic acid was based on redox reaction between ascorbic acid and permanganate in an acidic medium and lead to a decrease in color intensity of permanganate, monitored at 525 nm. A linear dependence of peak area obtained with ascorbic acid concentration up to 1200 mg l^{-1} was achieved. The relative standard deviation for 11 replicate determinations of 400 mg l^{-1} ascorbic acid was 2.9%. The second system, for acetic acid determination, was based on acid–base titration of acetic acid with sodium hydroxide using phenolphthalein as an indicator. The decrease in color intensity of the indicator was proportional to the acid content. A linear calibration graph in the range of 2–8% w v^{-1} of acetic acid with a relative standard deviation of 4.8% (5.0% w v^{-1} acetic acid, $n = 11$) was obtained. Sample throughputs of 60 h^{-1} were achieved for both systems. The systems were successfully applied for the assays of ascorbic acid in vitamin C tablets and acetic acid content in vinegars, respectively.

Keywords: Sequential injection; Titration; Spectrophotometry; Ascorbic acid; Acetic acid

1. Introduction

Fast, economical and automated methods are especially required for routine analysis and process control. Flow injection analysis (FIA) has widely been used as it provides high sample throughput, low sample and reagent consumption, high reproducibility and simple automated operation [1]. More recently, sequential injection analysis (SIA)

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based on the similar principles as FIA has been introduced [2]. Normally FIA uses a multi-channel pump and unidirectional forward flow; in contrast SIA uses a single-channel pump to move the fluid zones in forward and reverse steps through a system consisting of a holding coil (HC), a multi-position valve and a detector. The multi-position valve acts as a central distributor through which required volumes of liquid segments are sequenced by aspiration into the HC and then flushed by a flow reversal into the detector. As only one pump is used to move the composite zone through the system, the sampling frequency of SIA is generally lower than the multi-channel pump FIA method. However, the SI system uses a smaller number of moving parts than a comparable FIA system and uses at least an order of magnitude less of reagents, on the order of microliters. Manipulation of solutions in an SIA system can be made via a computer keyboard using appropriate software.

Different methods were reported for the determination of ascorbic acid [3]. Most of them utilized the reducing property of ascorbic acid. A simple FI redox or acid–base titration of ascorbic acid with potassium permanganate or ammonia was proposed [4]. SI redox titration of ascorbic acid with cerium(IV) in sulfuric acid was applied for pharmaceutical products [5]

Acetic acid in vinegar was determined by a FI pervaporation system with acid–base titration [6]. FI titration of acetic acid could be performed under both non-steady and steady state conditions [7]. In the steady state condition, a high degree of mixing between the analyte and reagents is required which may be made on-line by use of a mixing chamber, and thus involves long analysis time. Monosegmented flow titration for determination of total acidity of vinegar with a sample throughput of 30 h^{-1} was proposed [8]. SI acid–base titration was developed to determine strong and weak acids [9].

In this work, SIA procedures for the determinations of ascorbic acid and acetic acid employing simple reagents with spectrophotometric detection have been developed. Both titrations were performed under a non-steady state condition, so they do not require a special mixing part apart from a mixing coil, and so provide short analysis times. The SIA system has robust hardware and flexible control software, which enables convenient optimization and operation of the system. On switching from the determination of ascorbic acid to acetic acid, no change in hardware configuration was required, only the program sequence for control of the instrument was modified. An analysis can be made rapidly with computerized control. Sample throughput of 60 h^{-1} was obtained for both systems. Sample, reagents and waste can be minimized.

2. Experimental

2.1. Chemicals and solutions

All chemicals were of analytical reagent grade, and deionized water was used throughout. Potassium permanganate, sulfuric acid, L-ascorbic acid, acetic acid, ethanol and phenolphthalein were obtained from Merck (Merck, Germany). Sodium hydroxide (AKZO Nobel, Sweden) was used. Vitamin C tablets and vinegar samples were from a local market.

A 0.1 M potassium permanganate solution was prepared by dissolving 1.58 g of potassium permanganate in 0.1 M H_2SO_4 solution and making up into a 100 ml volume. This stock solution was then further diluted for appropriated concentrations. Stock standard ascorbic acid solution (2000 mg l^{-1}) was prepared by dissolving 0.5000 g of ascorbic acid in deionized water in a 250 ml volumetric flask. Working standard ascorbic acid solutions were freshly prepared by diluting the stock solution.

The 0.2% w v^{-1} phenolphthalein in 50% v v^{-1} ethanol solution was prepared by dissolving 0.50 g of phenolphthalein in 50% v v^{-1} ethanol and made to a volume of 250 ml. This stock solution was then used to prepare various concentrations of phenolphthalein solutions. Standard acetic acid solution (20% w v^{-1}) was prepared by weighing 50.00 g of glacial acetic acid into a 250 ml volumetric flask and diluting it to the mark with deionized water. Working standard acetic acid solutions were freshly prepared by diluting the stock solution.

2.2. SIA manifold for determination of ascorbic acid

Fig. 1(a) shows a diagram of the SIA manifold used. A laboratory made SIA analyzer (Center for Biotechnology, University of Turku, Finland) consisting of a syringe pump, a HC (200 cm long), two six port selection valves, a reaction coil (100 cm long), a simple spectrophotometer (Spectronic 21, Bausch & Lomb, USA) with a 10 mm path length flow through cell (Hellma, Germany) and a personal computer was used. All tubings were 0.6 mm i.d. Teflon. An in-house made software (ANALYSIA, Center for Biotechnology, University of Turku, Finland) was used to control the system and collect data from the detector via a plug-in interface card (Lab PC+, National instruments, USA). The analysis was performed automatically following sequences that had been written as a computer program called ANALYSIA SCRIPT LANGUAGE (ASL).

After all lines were filled with water (also used as carrier), the standard/sample and reagents were sequentially aspirated into a HC. The sequence of solutions in the HC is shown in Fig. 1(b). Aspiration volumes of each solution and other conditions are summarized in Table 1. The zones

Table 1
The SIA conditions for ascorbic acid determination

Parameter	Studied range	Selected conditions
Concentration of potassium permanganate (mM)	1.0–5.0	5.0
Aspiration volume of acidic potassium permanganate (μl)	100–220	180
Aspiration volume of sample/standard ascorbic acid (μl)	80–120	80
Aspiration volume of 0.1 M sulfuric acid (μl)	–	50
Flow rate (μl s^{-1})	50–250	150
Analytical wavelength (nm)	–	525

were propelled through a mixing coil to the detector, while transmission of light at a selected wavelength was monitored (arbitrary units). The peak area was evaluated from the SIA profile obtained. A calibration graph is a plot of the peak area versus ascorbic acid concentration.

2.3. SIA manifold for determination of acetic acid

The same configuration manifold as for ascorbic acid determination was utilized, except with different standard/sample and reagents as shown in

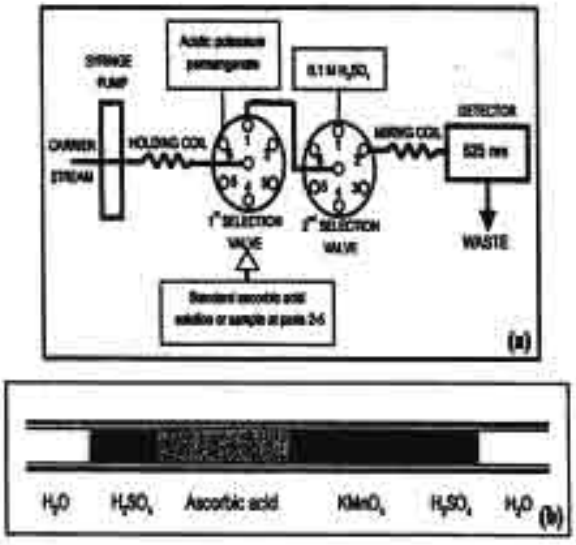

Fig. 1. (a) A schematic diagram of the SIA system for ascorbic acid determination; (b) a schematic diagram of order of the segments for ascorbic acid determination.

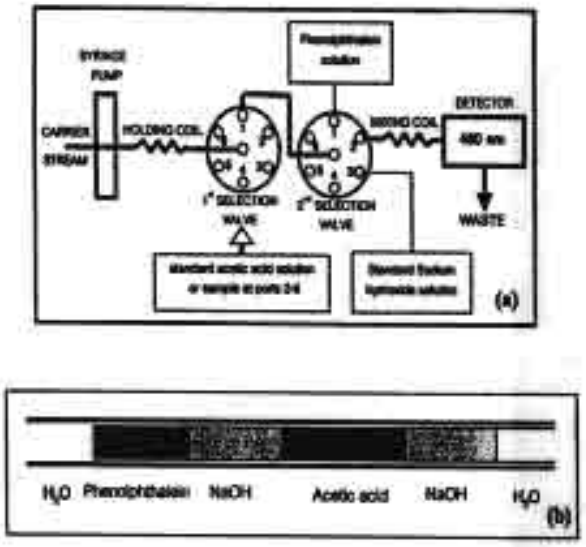

Fig. 2. (a) A schematic diagram of the SIA system for acetic acid determination; (b) a schematic diagram of the order of the segments for acetic acid determination.

Fig. 2(a). A similar operational sequence as above was also written as an ASL. The sequence of solutions in the HC is represented in Fig. 2(b). The aspiration volume of each solution and the other selected conditions are presented in Table 2.

3. Results and discussion

3.1. Optimization of SIA system for determination of ascorbic acid

A redox reaction of ascorbic acid with potassium permanganate was utilized in this system. The permanganate ion was also used as a color indicator, which was monitored for absorbance at 525 nm. A 0.1 M sulfuric acid solution was employed to prevent the conversion of permanganate to manganese dioxide precipitate. Various parameters such as concentration and aspiration volume of potassium permanganate, volume of sample, and flow rate of solution during propelling through the flow cell were investigated for the determination of ascorbic acid in range of 0–1200 mg l^{-1}. This concentration range was suitable for analysis of vitamin C tablets without difficulty in sample preparation. The studied ranges and the selected conditions are summarized in Table 1. The selected conditions were judged from a good slope and linearity of the calibration graph obtained, and a reasonable analysis time.

Table 2
The SIA conditions for acetic acid determination

Parameter	Studied range	Selected conditions
Concentration of sodium hydroxide (M)	0.2–1.0	0.8
Aspiration volume of sodium hydroxide solution (μl)	50–90	70
Concentration of phenolphthalein (% w v^{-1})	0.008–0.08	0.02
Aspiration volume of phenolphthalein solution (μl)	50–80	70
Aspiration volume of sample/standard acetic acid (μl)	40–70	50
Flow rate (μl s^{-1})	75–200	125
Analytical wavelength (nm)	–	480

Under the selected conditions, a linear calibration graph up to 1200 mg l^{-1} ascorbic acid ($y = -0.121x + 242.3$; $R^2 = 0.9933$) and relative standard deviations for 11 replicate determinations of 400 and 1200 mg l^{-1} ascorbic acid of 2.9 and 1.8%, respectively, were achieved. SIA profiles of a series of standard ascorbic acid solution are illustrated in Fig. 3. A sample throughput of 60 h^{-1} was obtained.

3.2. Analysis of vitamin C tablets

The system was applied to the determination of ascorbic acid in locally commercial vitamin C tablets. Twenty tablets of a vitamin C sample were weighed and ground in a mortar. After that a portion of the ground sample equal to about one tablet was weighed and then it was dissolved with deionized water to 1 l. The solution was then aspirated into the system using the same conditions as a standard. A titrimetric method [10] for reference purposes was also carried out. The results are summarized in Table 3. The comparison of the results obtained by the SIA method was evaluated by t-test [11]. The calculated t-test value was 0.67. The critical value of the t-test was 2.26 (9° of freedoms) at 95% confidence interval. Since the calculated t-test value is less than the critical value, the results from the two recommended methods were not significantly different.

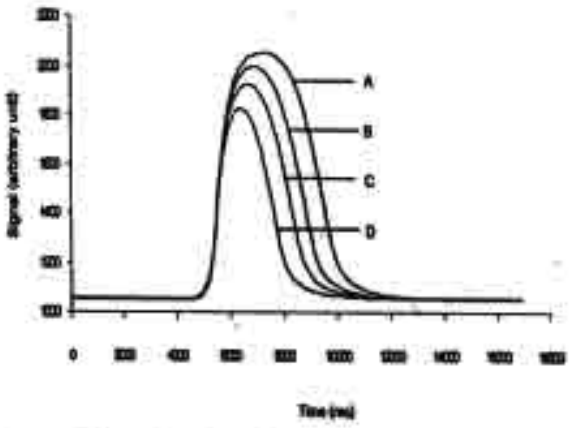

Fig. 3. SIA peaks for calibration graph of ascorbic acid determination; (A) blank, (B) 400 (C) 800 and (D) 1200 mg l^{-1} of standard ascorbic acid.

Table 3
Determination of ascorbic acid in vitamin C tablet samples

Sample code	Labeled amount (mg per tablet)	Ascorbic acid found (mg l^{-1})			
		Titrimetric method		SIA method	
		Milligram per tablet	Percentage label	Milligram per tablet	Percentage label
A	1000	1022	102	1014	101
B	1000	1013	101	892	89
C	500	477	95	520	104
D	100	101	101	101	101
E	60	69	115	72	120
F	1000	957	96	951	95
G	100	95	95	104	104
H	500	459	92	512	102
I	50	49	98	48	96
J	100	103	103	103	103

3.3. Optimization of SIA system for determination of acetic acid

Determination of acetic acid was based on acid–base titration of the acid with sodium hydroxide using phenolphthalein as an indicator. The physical configuration of the SIA system was the same as above for determination of ascorbic acid. The solutions around the selection valves and the computer program were changed (see Fig. 2). A similar optimization as in Section 3.1 was carried out for the determination of acetic acid in a range of 2–8% w v^{-1}. The studied ranges and the selected conditions are summarized in Table 2. The selected conditions were judged from a good slope and linearity of the calibration graph obtained with a reasonable analysis time.

Under the selected conditions, a linear calibration graph for 2–8% w v^{-1} acetic acid ($y = -37.709x + 390.9$; $R^2 = 0.9989$) was obtained. Relative standard deviations for 11 replicate determinations of 5 and 4% w v^{-1} acetic acid were 5.0 and 6.3%, respectively. Sample throughput of 60 h^{-1} was achieved.

3.4. Analysis of vinegar samples

The optimized procedure was applied to vinegar samples without any sample pretreatment. The acetic acid contents obtained from this procedure and titrimetry [12] are presented in Table 4. The results from the two methods were not significantly different (judged by t-test at 95% confident interval).

4. Conclusion

SI titration procedures for the determination of ascorbic acid and acetic acid have been proposed. The system is composed of robust hardware components and can be automatically controlled by a personal computer, with appropriate soft-

Table 4
Determination of acetic acid in local commercial vinegar samples

Sample code	Concentration of acetic acid (% w v^{-1}) by	
	Titrimetric method	SIA method
A	5.2	5.1
B	5.1	5.5
C	5.1	5.4
D	5.1	5.5
E	5.1	5.5
F	5.3	6.0
G	5.2	5.7
H	5.1	5.1
I	4.9	4.4
J	4.8	4.5
K	5.0	5.1

ware. Optimization of the systems can be conveniently carried out. No physical configuration changes of the system are required, but new programming sequences are needed in application of the system to determine a new parameter. Both procedures employed simple reactions with inexpensive and available chemicals. Chemical consumption was lower compared with batch and flow injection procedures, leading to lesser waste and more economical determinations.

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Exploiting flow injection and sequential injection anodic stripping voltammetric systems for simultaneous determination of some metals☆

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Abstract

Flow injection (FI) and sequential injection (SI) systems with anodic stripping voltammetric detection have been exploited for simultaneous determination of some metals. A pre-plated mercury film on a glassy carbon disc electrode was used as a working electrode in both systems. The same film can be repeatedly applied for at least 50 analysis cycles, thus reducing the mercury consumption and waste. A single line FI voltammetric system using an acetate buffer as a carrier and an electrolyte solution was employed. An injected standard/sample zone was mixed with the buffer in a mixing coil before entering a flow cell. Metal ions were deposited on the working electrode by applying a potential of −1.1 V vs Ag/AgCl reference electrode. The stripping was performed by anodically scanning potential of working electrode to +0.25 V, resulting a voltammogram. Effects of acetate buffer concentration, flow rate and sample volume were investigated. Under the selected condition, detection limits of 1 μg l^{-1} for Cd(II), 18 μg l^{-1} for Cu(II), 2 μg l^{-1} for Pb(II) and 17 μg l^{-1} for Zn(II) with precisions of 2–5% ($n = 11$) were obtained. The SI voltammetric system was similar to the FI system and using an acetate buffer as a carrier solution. The SI system was operated by a PC via in-house written software and employing an autotitrator as a syringe pump. Standard/sample was aspirated and the zone was then sent to a flow cell for measurement. Detection limits for Cd(II), Cu(II), Pb(II) and Zn(II) were 6, 3, 10 and 470 μg l^{-1}, respectively. Applications to water samples were demonstrated. A homemade UV-digester was used for removing organic matters in the wastewater samples prior to analysis by the proposed voltammetric systems.

Keywords: Flow injection; Sequential injection; Anodic stripping; Voltammetry; Cadmium; Copper; Lead; Zinc

1. Introduction

The determination of trace heavy metals in natural water and wastewater has been of concern because of their toxicities on man and the envir-

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onment. Anodic stripping voltammetry (ASV) has been widely used recently due to its speed, simplicity, low cost, high sensitivity and capability of multielement determination [1–3]. However, batch voltammetry consumes more time and chemicals. Therefore, flow techniques have been developed for voltammetric analysis to solve those drawbacks [4,5]. Differential pulse ASV in a flow system for determination of copper, lead, cadmium and zinc has been developed [6]. In our previous work [7], an on-line voltammetric system was set up for simultaneous determination of cadmium, copper, lead and zinc. A sample and electrolyte buffer were mixed together prior to aspiration into a flow cell where metals were in-situ pre-concentrated on the working electrode during the deposition step. The proposed system provides low detection limits and good precision but it concerns long analysis time and high reagent volumes.

Flow injection (FI) and sequential injection (SI) methods are more rapid, precise and consume lesser amounts of reagents [8]. FI with differential pulse voltammetric (DPV) detection using a static mercury drop electrode (SMDE) for determination of Cd(II) and Pb(II) was reported [9]. FI stripping voltammetry with wall-jet detector was applied for lead determination in blood [10]. SI-ASV was demonstrated for determination of copper in tap water. SI provided a convenient way to perform a medium exchange procedure for the stripping step, increasing selectivity of the method [11]. SI-ASV for determination of copper, lead, cadmium and zinc in river sediment extracts was reported [12]. SI with adsorptive stripping voltammetry was developed for determination of riboflavin and some heavy metals [13]. A mercury film electrode (MFE) is usually employed for ASV in flow systems due to that it provides better stability, well defined peaks of the voltammogram with high sensitivity and reduces risk of exposure to mercury vapour. The mercury film (MF) is usually plated in-situ with analytes during deposition step. However, the MFE preparation by the in-situ method produces mercury-contaminated waste.

In this work, FI and SI voltammetric systems using a pre-plated MFE as a working electrode were developed for simultaneous determination of cadmium, copper, lead and zinc. The same MF can be repeatedly used for several cycles, thus reducing the amount of mercury used. Peaks of metal ions obtained in a voltammogram were well resolved. Detection limits of a few $\mu g\ l^{-1}$ can be achieved. The proposed FI and SI systems were easily operated and required less sample and reagent than the batch and on-line systems. In addition, the systems are more rapid with high degrees of automation.

2. Experimental

2.1. Chemicals

Acetate buffer solution (pH 4.6), prepared from 100% glacial acetic acid (BDH, England) and sodium acetate 3-hydrate (BDH, England), was used for preparation of an electrolyte solution. Mercury(II) solution was prepared by dissolving Hg(II) nitrate (AJAX, Australia) in 0.1 M nitric acid (Merck, Germany). Metal standard solutions were prepared by diluting standard stock solution (1000 $mg\ l^{-1}$) (atomic absorption spectrometric grade, Merck, Germany). Water used throughout was from a Milli-Q water system (Millipore, Sweden). Nitrogen gas (99.999%) was used for purging oxygen in a solution.

2.2. Flow injection voltammetric (FIV) system

The FIV manifold is shown in Fig. 1(a). The system consists of a peristaltic pump (EYELA, Japan), a six-port injection valve (FIALab, USA), a mixing coil (PTFE, i.d. 0.8 mm) and a voltammograph with an electrochemical flow-cell (Bioanalytical System, USA). The flow-cell composes of a glassy carbon-working electrode (GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE). After the MF was coated on the GCE, the de-aerated electrolyte solution was pumped through the mixing coil and the flow-cell. A de-gassed standard solution or sample was injected into the carrier stream by the injection valve. When the sample zone reached the flow-cell, the potential of −1.1 V vs Ag/AgCl RE was applied to deposit the metals onto the MFE. After

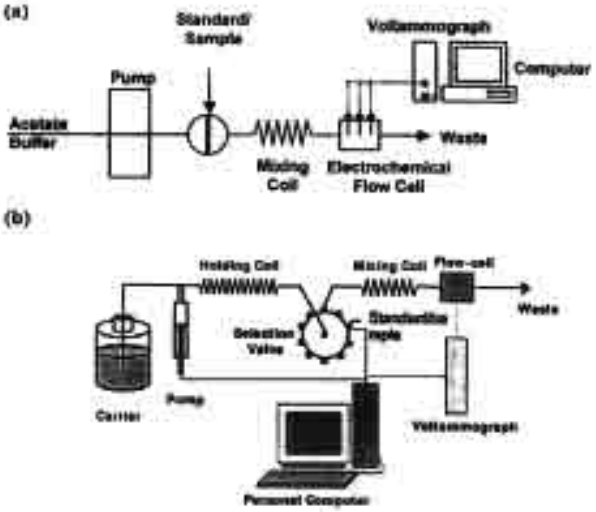

Fig. 1. Manifolds for simultaneous determination using voltammetric: (a) FI; and (b) SI.

the deposition step, the flow was briefly stopped to strip the metals out of the MFE by anodically scanning the potential to 0.25 V. The electrode was cleaned for 20 s by applying a potential of 0 V prior to performing the next analysis cycle.

2.3. Sequential injection voltammetric (SIV) system

The components of the SIV system (as shown in Fig. 1(b)) were similar to that of the above described FIV system except for the pump and valve. An autotitrator (Dosimat 765, Metrohm, Switzerland) was used as a syringe pump. A 10-port selection valve (Valco Instruments Co., Texas) was used. The pump and valve were controlled by a computer via an in-house written software based on LabVIEW® program (National Instruments Co., USA). The SIV flow conditions were set to be similar to the FIV system. Operation sequences were as followed: (1) 130 μl of standard/sample was aspirated into a holding coil; (2) the standard/sample zone was propelled through a mixing coil to a flow cell; (3) when the zone reached the flow cell, a potential of −1.1 V vs Ag/AgCl electrode was applied to the WE in order to deposit metals on the MFE; (4) the flow was stopped while a buffer was in the flow cell and stripping of the metals out of MFE was carried out; (5) finally, a potential of 0 V was applied for 20 s with the solution flowing to clean the MFE.

2.4. MF preparation

Mercury(II) 500 mg l^{-1} in 0.1 M HNO_3 solution was aspirated at a flow rate of 0.5 ml min^{-1} through the electrochemical flow-cell. A potential of −0.8 V vs Ag/AgCl RE was applied to the GCE for 10 min. The MF was then obtained on the GCE. Only 5 ml of the mercury solution was used for this purpose. The mercury solution from the waste out-let was collected for recycling.

2.5. A home-made UV-digestion unit

A home-made UV digester consists of two UV-C (180–280 nm) lamps, 15 W each, as depicted in Fig. 2. Aluminium foil, covered inside the digester house, was used for preventing the UV radiation. Sample trays were made by cutting a brown-coloured glass bottle into halves, which would increase the area of irradiation to sample in the tray. The trays were stored in nitric acid and rinsed with Milli-Q water before use. Hydrogen peroxide solution (30% v v^{-1}, 10 μl), as an oxidant, was

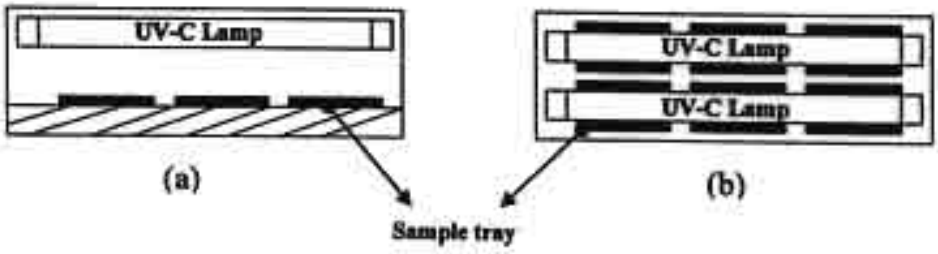

Fig. 2. A home-made UV-digestion unit: (a) side view; and (b) top view.

added to 10 ml of sample in the tray. The sample trays were then put in the UV digester.

3. Results and discussion

3.1. Study of MF preparation

Mercury(II) concentration, deposition potential and deposition time were considered for MF preparation. Mercury(II) concentrations of 500 and 1000 mg l^{-1} were tested. MF was obtained fully on the GCE by using 500 mg l^{-1} Hg(II), whereas using 1000 mg l^{-1} Hg(II) gave the film only on some area of the GCE. Deposition potentials of −0.8, −0.9 and −1.0 V were studied. It was found that mercury was adsorbed on the GCE at a potential of −0.8 V vs Ag/AgCl RE. On the other hand, at the potential of −0.9 and −1.0 V, mercury could not be coated on the GCE because H^+ in the solution could be reduced more at the GCE, at higher negative potentials. Higher peak currents (for the metals) were obtained when using the MF plated with a deposition time of 10 min compared with that obtained by using a 5 min deposition time. However, MF deterioration was observed. This should be due to that mercury did not adhere strongly to the glassy carbon and resulted in rapid decrease in sensitivity. Therefore, the MF was cleaned electrochemically at a potential of 0 V for 20 s before performing the next run. The proposed MF has been experimentally proven for reusing for more than 50 deposition-stripping cycles (as shown in Fig. 3), in comparison to the other reports that the MF was used only for one determination [10–12].

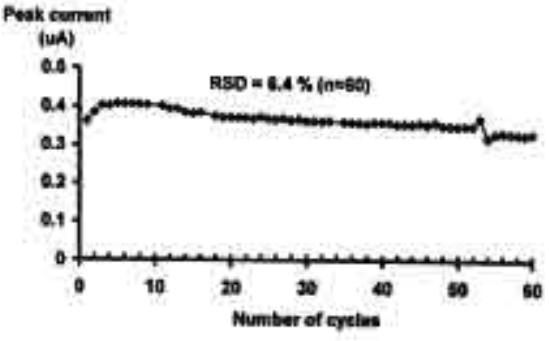

Fig. 3. Stability of the MF using electrochemical cleaning (60 analysis cycles of 10 μg l^{-1} Cd(II)).

3.2. Optimization of FIV system

3.2.1. FI parameters

3.2.1.1. Acetate buffer concentration. Electrolyte concentration affects the ionic strength of the solution and hence the peak currents of metals. Concentrations of acetate buffer (0.1, 0.2, 0.3, 0.5, 0.7 and 1.0 M) were varied while the flow rate, the sample volume and the length of mixing coil were fixed at 0.5 ml min^{-1}, 30 μl and 50 cm, respectively. It was found that the peak currents were fluctuated at an acetate buffer concentration less than 0.5 M. This could be due to that ionic strength of a lower concentration was not sufficient. A concentration of 0.6 M acetate buffer was chosen.

3.2.1.2. Flow rate. As expected, less dispersion was observed at higher flow rate. Peak currents of metals increased when the flow rate increased. However, at a higher flow rate than 0.5 ml min^{-1}, the deterioration of the MFE was obtained. Therefore, the flow rate of 0.5 ml min^{-1} was used in this FIV system.

3.2.1.3. Sample volume. A sample volume is related to the deposition time. At a deposition time of 20 s and a flow rate of 0.5 ml min^{-1}, approximately 167 μl of sample should be required. Injection volumes of 30, 80 and 130 μl were tested. It was found that the sensitivity obtained by using a volume of 30 μl were not different from that obtained by using a volume of 80 μl. However, using an injection volume of 130 μl yielded the worse precision. The precision of the currents obtained by using 30 μl injection volume was found to be the best. This could probably because less sample volume would provide better mixing pattern of sample and reagent and thus better reproducibility for the current observed. Using a sample volume of 30 μl also resulted in satisfactory current. Therefore, an injection volume of 30 μl was chosen.

Table 1
The voltammetric parameters used for the FIV and SIV system

Parameter	Condition
Deposition potential	−1.1 V
Deposition time	20 s
Sweep potential	−1.1–+0.25 V
Sweep mode	Square wave
Sweep rate	75 mV s^{-1}

3.2.1.4. Mixing coil length. For the low flow rate employed (0.5 ml min^{-1}), a length of 50 cm was used so that sample throughput and reproducibility of signal could be compromised.

3.2.2. Voltammetric parameters

The electrochemical parameters are represented in Table 1. Deposition times of 10, 20 and 30 s were tested. It was found that the longer deposition time, the higher peak current obtained, as expected. However, the deposition time of 20 s was chosen because it was faster and yielded enough sensitivity.

3.2.3. Characteristics of the developed FIV system

Voltammograms of metal standards obtained by the FIV system are depicted in Fig. 4. Calibration data, detection limits and precisions for the metal ions (Cd(II), Cu(II), Pb(II) and Zn(II)) are summarized in Table 2. The results illustrate that simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) can be performed by using the developed FIV system with a sample throughput of 20 h^{-1} and with better sensitivities than in other previous reports [6,10–13].

3.2.4. Applications to real samples

Six drinking water samples were analyzed by injecting them into the system. The metals in those samples were found to be below the detection limits. It indicated that the metal concentrations in the drinking water samples were below the values of maximum allowance of 0.010 mg l^{-1}, 1.0 mg l^{-1}, 0.1 μg l^{-1} and 500 mg l^{-1} for Cd(II), Cu(II), Pb(II) and Zn(II), respectively, which is announced by the Department of Environment Quality Standard, Ministry of Science, Technology and Environment of Thailand [14]. Wastewater

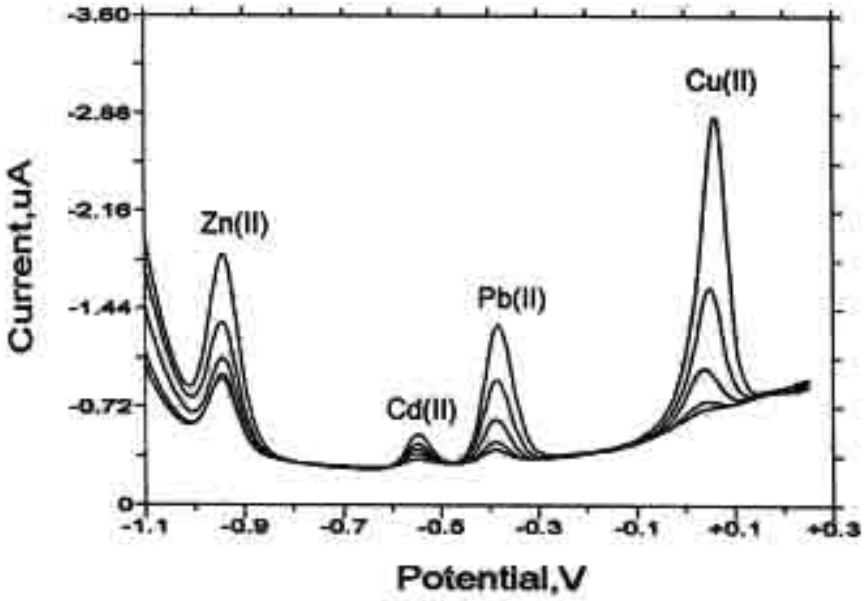

Fig. 4. Voltammograms of metal standards obtained from the proposed FIV system. (Concentrations of Zn(II), Pb(II), Cu(II): 10, 20, 50, 100 and 200 μg l^{-1}, and Cd(II): 5, 10, 15, 20 and 30 μg l^{-1}).

Table 2
Characteristics of the FIV system

Metal	Dynamic range (μg l^{-1})	Equation	R^2	Detection limit (μg l^{-1})	% RSD, $n = 11$
Cd	10–30	$y = 6.667x + 0.0235$	0.9990	1	3.6 (at 10 μg l^{-1})
Cu	20–200	$y = 11.569x - 0.1860$	0.9962	18	4.4 (at 75 μg l^{-1})
Pb	10–100	$y = 5.466x + 0.0171$	0.9997	2	1.8 (at 80 μg l^{-1})
Zn	50–200	$y = 4.338x - 0.0497$	0.9971	17	4.3 (at 100 μg l^{-1})

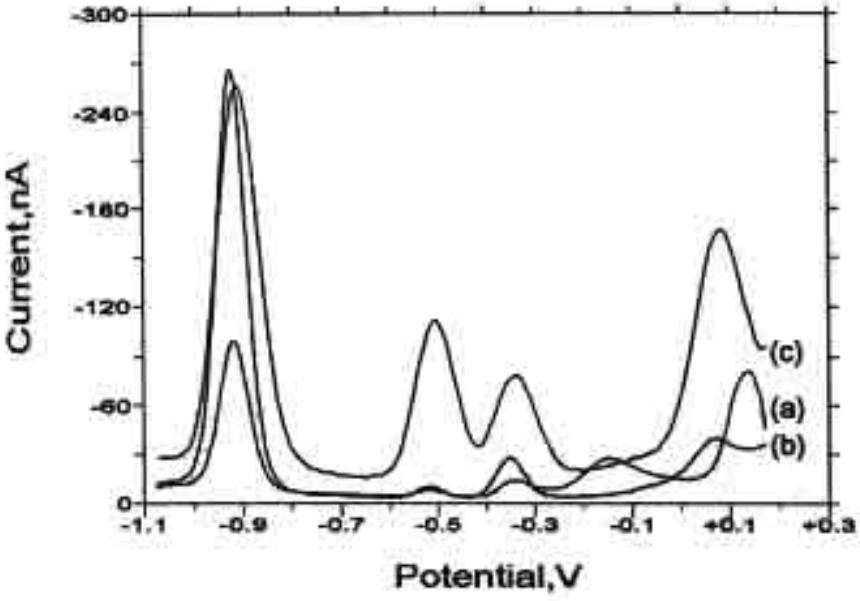

Fig. 5. Voltammograms of wastewater sample: (a) without; (b) with UV-digestion; and (c) a standard solution.

samples were also analyzed. The samples were digested for 2 h by using the home-made UV digester. UV-digestion can reduce some interferences in such a sample as illustrated in Fig. 5. Well-defined voltammetric peaks of metals were obtained for a sample with UV-digestion. Results are summarized in Table 3. The samples were also analyzed by ET-AAS [15]. The results obtained from the FIV method agreed with that by ETAAS. Zn and Cu may form an intermetallic Cu–Zn

Table 3
Examples of analysis of wastewater samples by the developed FIV system

Sample	Concentration found (μg l^{-1})							
	Cd		Cu		Pb		Zn	
	FIV	ETAAS	FIV	ETAAS	FIV	ETAAS	FIV	ETAAS
1	26	25	60	75	92	95	154	142
2	54	50	115	153	196	204	74	155
3	4	7	27	22	28	24	45	25

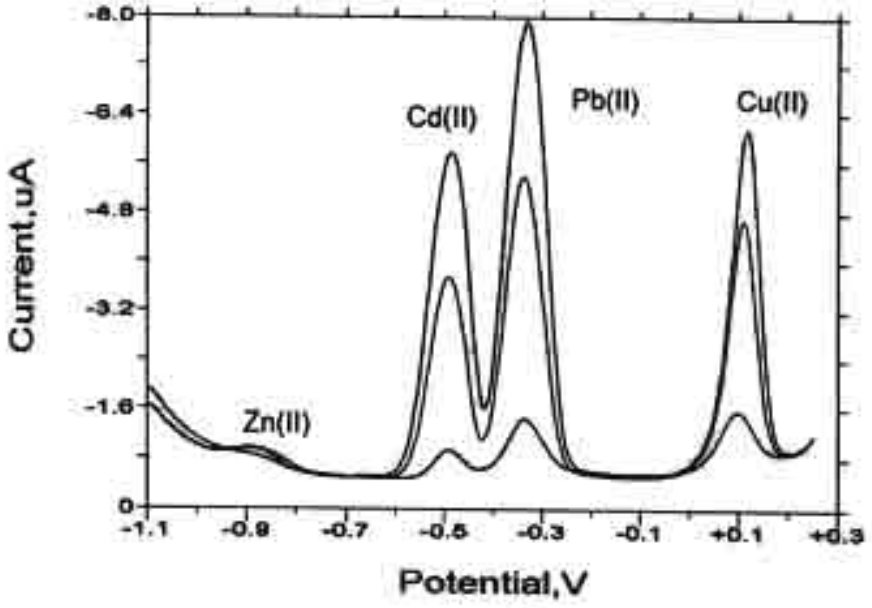

Fig. 6. Voltammograms of metal standards obtained from the propcsed SIV system. (Concentrations of Zn(II): 100, 700 and 1000 μg l^{-1}; Cd(II), Pb(II): 10, 70 and 100 μg l^{-1}; and Cu(II): 50, 150 and 200 μg l^{-1}).

phase in MF which may cause variation in FIV-results of these metals [12].

3.3. Preliminary study of SIV

The SI system with acetate buffer 0.6 M as carrier, was carried out for testing the performance of the system. By applying the experiences in the FIV system, similar conditions as employed in the FIV system were applied in the SIV system. Due to that the minimum pipette volume of the autotitrator used is 100 μl, a sample volume of 130 μl was aspirated into the SIV system. Dispensing volume was studied because it concerned the time to start the deposition step. Starting the deposition step at a volume of 0.30 ml or at a time of 36 s after starting dispensing the sample zone to the detector, the highest peak current was observed. Simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) can be performed by using the SIV system. The voltammograms of the metal ions are illustrated in Fig. 6. The results in Table 4 showed that the SIV system provided good precisions, linear calibrations and low detection limits for the metals, except the detection limit for Zn was high because of its low sensitivity and less reducibility. Due to the discontinuous nature of SI operation, reagent consumption and waste generation can be minimized and become advantageous. Further investigation on incorporation of on-line sample pre-treatment to the SIV system is in progress.

Table 4
Characteristics of the preliminary SIV system

Metal	Dynamic range (μg l^{-1})	Equation	R^2	Detection limit (μg l^{-1})	% RSD, $n=3$
Cd	10–70	$y=37.47x-0.051$	0.9976	6	9.8 (at 10 μg l^{-1})
Cu	50–200	$y=29.22x-0.537$	0.9999	3	1.0 (at 50 μg l^{-1})
Pb	10–100	$y=62.90x-0.191$	0.9946	10	2.6 (at 40 μg l^{-1})
Zn	470–700	$y=0.26x-0.0006$	0.9904	470	0.5 (at 700 μg l^{-1})

4. Conclusion

FI and SI systems with anodic stripping voltammetric detection for determination of some metal ions have been exploited. Employing the proposed conditions for making a pre-plated MFE as a working electrode, which can be utilized for several analysis cycles (at least 50 cycles), the mercury consumption can be minimized. The mercury waste solution was collected for recycling. The systems, therefore, produced less mercury waste. Detection limits of a few $\mu g\ l^{-1}$ of the metal ions can be achieved. The proposed single-line FI voltammetric system is cost-effective, very simple and easy to handle but achieves a high sensitivity simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II). The proposed SI voltammetric system, using an autotitrator as a syringe pump and a PC with an in-house software program, is an alternative for cost-effective higher degree of automation.

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Determination of trace iron in beer using flow injection systems with in-valve column and bead injection☆

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Abstract

Three flow injection (FI) systems were investigated for the determination of trace iron in beer: an FI-in-valve column-flame atomic absorption spectrophotometry (FI-FAAS) system, a spectrophotometric FI system with a column placed at the detection point, and an FI-spectrophotometric system with bead injection (FI-BI). Cationic exchange resin Dowex 50W X8 and iminodiacetate chelating resin, Chelex-100, were employed for the FI-spectrophotometric and FI-FAAS systems, respectively. The FI-in-valve column, packed with the resin, enhances the FAAS performance. The spectrophotometric FI system with a column (packed with Chelex-100) placed at the detection point (in a cell holder of a spectrophotometer) is based on the formation of iron (II)–1,10-phenanthroline complex sorbed onto the resin. No eluent has been found to be suitable. The FI-BI for renewable microcolumn has been proven to be an alternative. The FI-FAAS and FI-BI procedures provide online sample preseparation and preconcentration for the determination of iron in beer. Both are simple, rapid, and economical. The procedures also involve sample preparation (decarbonation and suppression of tannin interference by adding ascorbic acid) and standard addition. The results obtained by FI-FAAS and FI-BI agree with those of AOAC spectrophotometric method.

Keywords: Iron; Beer; Flow injection; Bead injection; Atomic absorption spectrometry; Absorptiometry; Spectrophotometry

1. Introduction

According to the AOAC standard method for the analysis of beer quality [1], various determinations are involved, namely for physical properties: color, total haze, refractive index, calories content, total acidity, foam collapse rate, beer bitterness,

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and for chemical assays: iron, copper, calcium, carbon dioxide, sulfur dioxide, alcohol, ethanol, glycerol, protein, starch, and proteolytic chill-proofing enzymes.

Iron influences beer quality such as taste [2], haze, the growth of yeast, the foaming quality, the flavor enhancement [3], and exhibiting of antioxidant activity [4]. Iron in beer is thus an important trace element to be assayed.

A standard method for the determination of iron in beer using conventional spectrophotometry based on complexation to 1,10-phenanthroline or 2,2′-bipyridine [1] is time consuming, with high chemical consumption and is tedious. Higher background from beer color (golden brown or black beer) and relatively high detection limits are found to be the drawbacks of the method.

Although using electrothermal atomic absorption spectrometry will improve sensitivity in terms of limit of detection [3], disadvantages of this method include relatively more expensive instrumentation, skill of operator required, matrix chemical modifiers needed, and complicated temperature programming.

Using a flow injection (FI) in-valve column-flame atomic absorption spectrometry (FI-FAAS) [2], flame interference by the matrix can be reduced by introducing low quantities of sample (200 μl of sample injection), and the problem of burner clogging could be overcome. Iron and some other elements such as Ag, Cd, Cu, In, Mn, Pb, Tl, and Zn were determined using an integrated-atom-trap system mounted on a standard atomic absorption air–acetylene flame burner [5]. Based on ion-exchange separation and size exclusion chromatography, copper, iron, and manganese in beer were investigated using electrothermal atomic absorption spectrometry [3].

Column techniques have been extensively utilized for metal preconcentrations. Even a conventional cation exchange resin used for online preconcentration of iron with spectrophotometric detection was described [6]. An iminodiacetate chelate resin such as Chelex-100 and Muromac A-1 played the role of high efficient solid-phase extractant for transition metals [7–9]. In addition, other packing materials in a sorption mechanism have been used for iron preconcentration; for instance, resin-immobilized 8-hydroxyquinoline [10], a cationic exchanger paper [11], C_{18}-bonded silica with 5,7-dichlorooxine [12], and Amberlite XAD-4 functionalized by *N*-hydroxyethylethylenediamine [13].

An in-valve column exhibited practical and convenient use for loading and eluting for metal preconcentrations [14–16]. Flow-based analysis with bead injection (BI) described as a renewable microcolumn has been applied for many detection systems such as amperometry [17], fluorescence [18–20], and UV–visible absorptiometry [21]. BI spectroscopy exhibited outstanding usefulness in terms of a renewable, small, and disposable sensing layer [22]. A simple FI-spectrophotometric system with bead injection (FI-BI) has recently been achieved for trace iron determination in water samples [23].

In this study, an FI-FAAS system with in-valve column, a spectrophotometric FI system with a column placed at the detection point, and an FI-BI system were investigated. The FI-FAAS and FI-BI systems were found to be useful for online separation and preconcentration of iron in beer so that the sensitivity and selectivity would be improved with possibility for automation. The in-valve column FI was coupled to flame atomic absorption spectrometry and a conventional cationic exchange resin was utilized. FI-BI was used for absorptiometry using 1,10-phenanthroline in conjunction with a renewable microcolumn packed with chelating resin.

2. Experimental

2.1. Materials and reagents

Two types of packing materials, conventional cationic exchange resin, Dowex 50W X8, 50–100 mesh (Fluka) and iminodiacetate chelating resin, Chelex-100, 50–100 dry mesh or 16–50 wet mesh (Bio-Rad) were used. By sieving the chelating resin, beads in the range 35–50 wet mesh were employed. For the FI system with in-valve column, the former was chosen in accordance with commonly used and inexpensive resins. The latter was used for both FI with column at detection

point and FI-BI due to its transparent characteristic.

Acetate buffer solution, 0.1 M, was prepared by dissolving 3.85 g ammonium acetate in water and adjusting to pH 4.5 by adding 2 ml of glacial acetic acid. Iron(III) stock solution, 1000 μg ml^{-1}, was prepared by dissolving 0.8634 g $NH_4Fe(SO_4)_2 \cdot 6H_2O$ (Merck) in water and adding 1 ml 4.5 M H_2SO_4 before making up to a volume of 100 ml with water.

Iron(II) stock solution, 1000 μg ml^{-1}, was obtained by dissolving 0.7020 g $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (BDH) in water and adding 1 ml 4.5 M H_2SO_4, then making up to volume of 100 ml with water.

Ascorbic acid 10% (w v^{-1}), using L-ascorbic acid (Merck) was freshly prepared. 1,10-Phenanthroline, 0.3% (w v^{-1}) in the acetate buffer solution was prepared by using solid chemical (Merck).

2.2. Sample preparations

Local commercial beer samples were taken for analysis. A sample was transferred into a larger container for decarbonation by stirring for 1 h. A portion of ascorbic acid solution (10%, w v^{-1}) was added to a known volume of the sample to give a final concentration of 1000 μg ml^{-1} ascorbic acid. A series of solutions for standard addition measurements for a sample was made to contain 0, 0.1, 0.25, 0.5 and 1.0 μg ml^{-1} Fe(II) standard added.

2.3. FI-FAAS with in-valve column

An acrylate column (0.3 mm i.d. and 40 mm long) was used in place of an injection loop in a 6-port valve (V-451, Upchurch). The column was packed with Dowex 50W X8 resins having Teflon wool as frits at both the ends.

As depicted in Fig. 1, the FI system with in-valve column consists of a peristaltic pump (FIAlab, USA), a 6-port valve, and a flame atomic absorption spectrometer (AA-670, Shimadzu, Japan). The FAAS conditions followed those recommended by the manufacturer with some adjusted parameters (burner height, ratio of fuel, and oxidant). After pumping water for conditioning the column, the three-way valve (V1) was switched to let standard/sample load onto the column at a certain loading flow rate and for a desired loading time. When reaching the duration of loading, V1 was switched back to the position that allowed water to flow in order to wash the column. By switching the 6-port valve (V2), sorbed iron will be released from the column by the eluent, countercurrent to the loading step. Opposite flow direction of loading and elution avoids problems due to tendency of clogging, which would affect column performance, as well as maximizes preconcentration [15]. The series of the standard addition set of solutions for a sample prepared as described earlier was injected in the FI manifold (Fig. 1) by employing the conditions in Table 1.

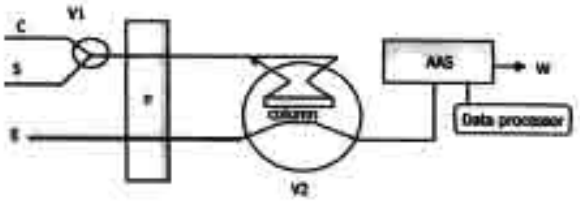

Fig. 1. Manifolds with in-valve column for FAAS; C = carrier (water or acetate buffer pH 4.5), S = standard or sample, E = eluent, P = peristaltic pump, AAS = atomic absorption spectrometer, V1 = three-way valve, V2 = 6-port valve, W = waste.

2.4. FI spectrophotometric system with bead injection

The instrumental setup for FI-BI was similar to that previously described [23]. Modification was made by inserting a three-way valve (V1) (Fig. 2) to get rid of pulsating and fluctuating signals caused by back-pressure. The peak-hold colori-

Table 1
Proposed method for FI system with in-valve column

Conditions	Studied range	Suggested value
Loaded solution (M HNO_3)	0.05–1.00	0.05–0.15
Eluent (M HNO_3)	1–6	4
Eluent flow rate (ml min^{-1})	3–8	4
Loading time (min)	15 s–10 min	15 s–10 min
Loading flow rate (ml min^{-1})	2–8	2–8

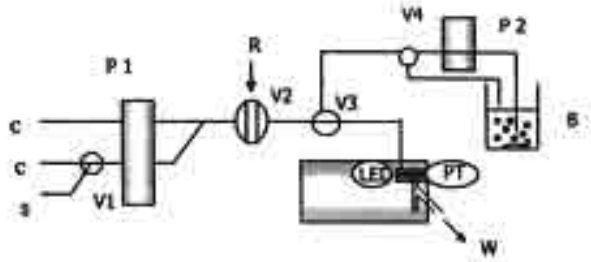

Fig. 2. FI-BI system; C = carrier (acetate buffer pH 4.5), S = standard or sample, P1 and P2 = peristaltic pumps 1 and 2, B = bead suspension, R = 1,10-phenanthroline, LED = light emitting diode, PT = phototransistor, V1, V3 and V4 = three-way valves 1, 3, and 4, V2 = 6-port valve, W = waste.

meter has also been modified so that a longer period (up to 5 min) for the maximum height of the output signal could be held for reading. After introducing the bead suspension at 16 ml min^{-1} for 15 s, acetate buffer as a carrier was propelled at 3 ml min^{-1} for 30 s to place beads into the flow cell. A standard/sample solution was pumped at 3 ml min^{-1} for a certain time. The beads in the cell were washed with the carrier, before 1,10-phenanthroline solution (0.3%, w v^{-1}) was injected via the injection valve V2 [23]. Signal due to increase in the color intensity of the Fe(II)–1,10-phenanthroline complex was monitored. Finally, the beads were discarded out of the cell. The set of standard addition solutions for a beer sample (described above) was analyzed by using a loading time of 30 s with a flow rate of 3 ml min^{-1}.

3. Results and discussion

3.1. Optimization of FI system with in-valve column (FI-FAAS)

Using the manifold in Fig. 1 and the range of conditions in Table 1, acidity of the loading iron(III) solution and of the eluent were studied, as summarized in Figs. 3 and 4. Lower acidity was suitable for loading, while higher acidity was proper for eluting conditions. This agrees with previous findings for the relationship of distribution ratio (D) and acidity [24]. The effect of eluent flow rate was found to be similar to the previous report [15] that a flow rate of eluent higher than an

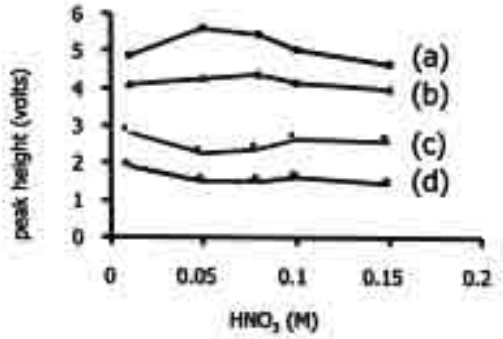

Fig. 3. Effect of acidity of loading solution for (a) 3 μg Fe, (b) 2 μg Fe, (c) 1 μg Fe, and (d) 0.5 μg Fe.

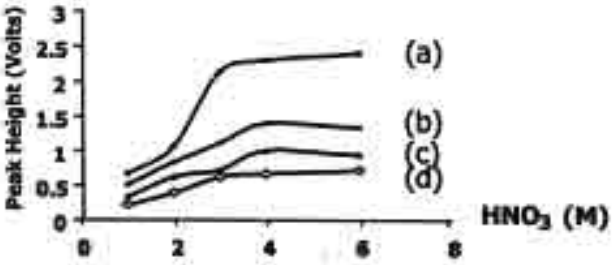

Fig. 4. Effect of acidity of eluent for (a) 1.2 μg Fe, (b) 0.4 μg Fe, (c) 0.08 μg Fe, and (d) blank.

uptake rate of FAAS (3.5 ml min^{-1}) would provide better signals. An eluent flow rate of 4 ml min^{-1} should be employed. Using the combined set of conditions in Table 1, single standard calibration could also be successfully applied (see Fig. 5). The maximum volume for sample loading was 80 ml. The detection limit was found to be 0.1 μg Fe (1.2 ng ml^{-1}; 80 ml loading).

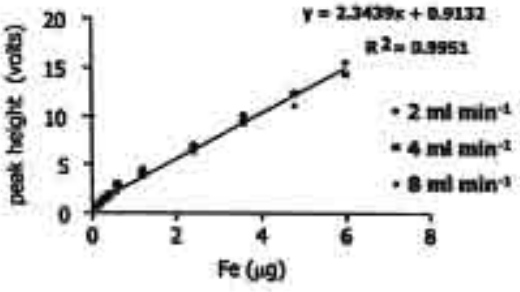

Fig. 5. Single standard calibration for FI-FAAS by volume-based loading (various time, and flow rate).

3.2. Spectrophotometric FI system with a column placed at detection point

A spectrophotometric FI system should also be an alternative for simple instrumental iron determination. Such a system was, therefore, investigated. In order to decrease the degree of dispersion of sorbed iron from elution, a resin was placed at the detection point in the spectrophotometer instead of at the injection valve as previously described. A glass column (3 mm i.d. × 40 mm long) was inserted at the position of the cell holder of a spectrophotometer (Spectronic 21, Spectronic Instruments, USA). The column was packed with the transparent chelating resin, Chelex-100. The chelating resin was expected to retain the iron complex. After passing a solution containing iron through the column (loading), 1,10-phenanthroline solution was injected via the injection valve into the flow, and the reagent formed a complex with the iron. Intensity of the colored complex (red) was continuously monitored at 505 nm.

Some eluents, namely solutions of NaCl (0.1 and 1 M), HNO_3 (2 M), EDTA (1×10^{-3}, 1×10^{-2}, and 1×10^{-1} M), and EDTA in 2 M HNO_3 (1×10^{-3}, 1×10^{-2}, and 1×10^{-1} M) were tried for eluting the complex from the column. None of the eluents was found to be suitable. The (sorbed) iron–1,10-phenanthroline complex retained very strongly on the column (see Fig. 6). Although HNO_3 (2 M) could elute free iron ions sorbed on Chelex-100 as reported previously [9], it is not an effective eluent to desorb the iron–1,10-phenanthroline complex. Even using another eluent, EDTA, which forms a more stable complex with iron(II) (the overall stability constant, $\log \beta = 21.3$ [25]) than iron(II)–1,10-phenanthroline (having $\log K_1 \approx \log \beta = 14.33$), the complex cannot be removed from the column.

3.3. Flow injection spectrophotometric system with bead injection

To overcome problem in not having a suitable eluent as described above, the FI-BI system (Fig. 2) with similar conditions for the determination of iron in water samples [23] was adopted for the determination of iron in beer. Beads were loaded into a cell for 15 s with 1.5% bead (Chelex-100) suspension at a flow rate of 16.0 ml min^{-1}.

Single standard calibrations were obtained by volume-based sample loading at two flow rates (3 or 6 ml min^{-1}) with the period of time 5–20 min or 150 s–10 min, respectively (Fig. 7). A detection limit (3σ) [26] was found to be 0.01 μg or 0.2 ng ml^{-1} with 60 ml sample loading.

3.4. Interference study

It was found in preliminary studies using iron spiked beer when applying without any sample preparation, very low recoveries, 10 and 2%, were obtained by using FI-FAAS and FI-BI, respectively. Non-retained iron from the column of FI-FAAS was found to be 90%. This indicated interference from sample matrices.

Some metal ions (Na^+, K^+, Ca^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Mg^{2+}) and some organic compounds (ethanol, cysteine and gallic acid) were studied for interference and found to have no effect in the range of concentrations presented in beer. Tannic acid was found to affect strongly the sorption efficiency of iron onto cationic exchange resin. There was a report [27] that gallic acid can be used as a standard for a spectrophotometric determination of tannic acid. Iron(II) and iron(III) can form complexes with both gallic acid and tannic acid at pH 4.5. Both complexes absorb at 595 nm. The former retains on the cation exchanger column whereas the latter does not. Iron–gallic acid complex could present in positively charged form [28] while iron–tannic acid complex should exhibit negative charge [3]. In this work, tannic acid cannot be replaced by gallic acid to represent an interference study by tannic acid.

Mixtures of ascorbic acid, 1000 μg ml^{-1}, and iron solutions spiked in beers resulted in better recoveries of iron: 30–45 and 60–66% for FI-FAAS and FI-BI, respectively. Using hydroxylamine could also yield 65% recovery of iron in the FI-BI procedure. Ascorbic acid could reduce iron(III) and/or tannic acid. Also ascorbic acid could possibly replace EDTA and form complex with iron [29].

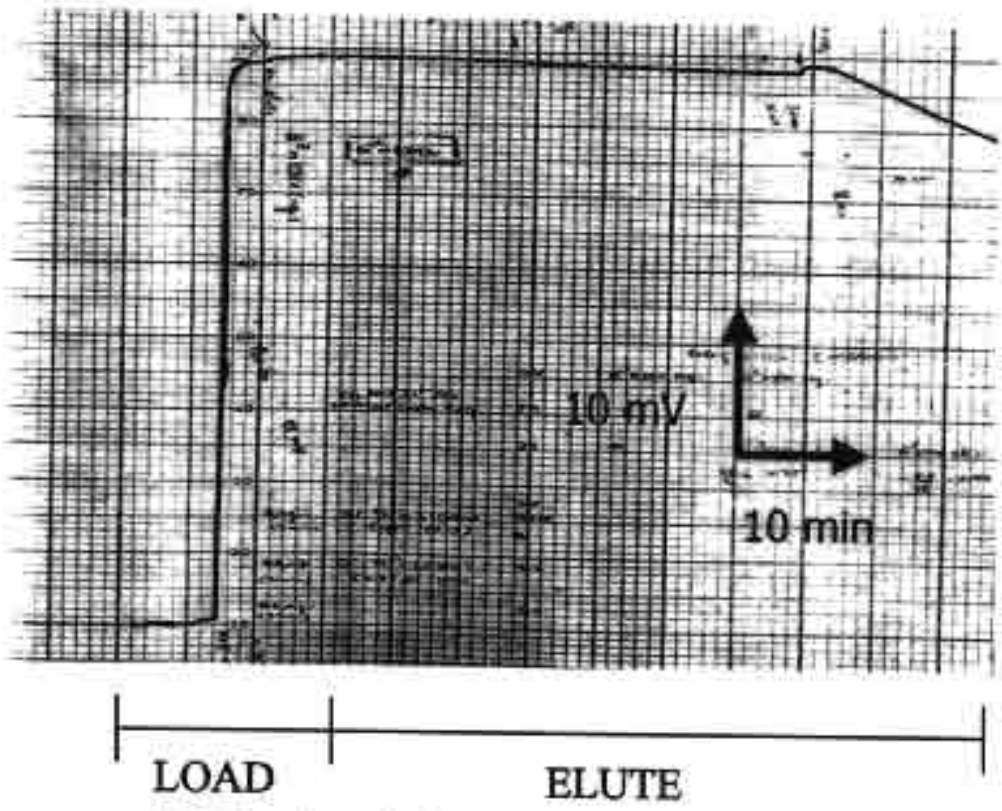

Fig. 6. Absorption profile of iron–1,10-phenanthroline (1 μg Fe ml^{-1}).

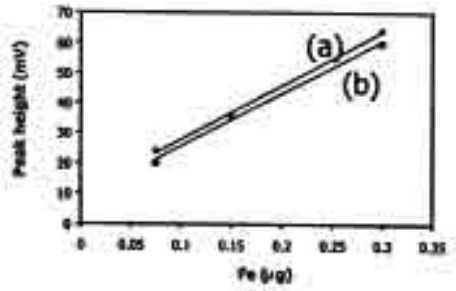

Fig. 7. Single standard calibration for FI-BI using iron(II) standard solution, 5 μg ml^{-1}: (a) at 6 ml min^{-1} for various loading times (2 min 30 s, 5 min, and 10 min); (b) at 3 ml min^{-1} for various loading times (5, 10, and 20 min).

In the FI-FAAS system, both Fe(III) in 0.1 M HNO_3 and Fe(II) in acetate buffer resulted in similar sorption efficiencies by the resin column. Tannic acid affected both iron(II) and iron(III) similarly. Therefore iron(II) can be used as standard iron for FI-FAAS method.

3.5. Determinations of iron in beer samples

Following the procedure for sample preparation, five beer samples were analyzed by the proposed FI-FAAS and FI-BI methods. The AOAC standard method [1] was also performed. All the methods were employed by using standard addition. Graphs of the standard addition method were plotted, of peak height (mV) or absorbance versus iron added (μg ml^{-1}). Iron contents in beer samples were calculated by extrapolating. The results are summarized in Table 2. The results obtained by the proposed FI-FAAS and FI-BI for traces of iron agree reasonably well with those of the AOAC method, being within tolerances required for the beer analysis. The color of a sample would affect the results obtained by the AOAC method because the absorbance measurement was performed using the whole solution while the resin column in the FI-BI suppresses the contribution

Table 2
Determination of iron contents in beer samples by FI and AOAC standard methods (all using standard addition)

Beer sample	Iron content (μg ml^{-1})		
	FI-BI	AOAC	FI-FAAS
Singha (Thailand)	0.089	0.067	0.103
Heineken (France)	0.111	0.085	0.086
Amstel (Holland)	0.021	0.035	0.039
Beck's (Germany)	0.130	0.101	0.103
Black Beer (Thailand)	0.071	0.174	0.117

due to the sample color. Both FI-FAAS and FI-BI can serve as alternatives for the assay of the iron content in beer. Although standard addition is still to be performed, in comparison to the AOAC spectrophotometric method [1], the procedures required much smaller volumes/amounts of sample and reagents. The operation time for an analysis is also reduced (24 and 30 injections h^{-1} for FI-FAAS and FI-BI, respectively). Less glassware is used. Both procedures offer a very cost-effective way to determine trace iron.

4. Conclusion

Three FI systems for trace iron determination were investigated: FI-in-valve column-FAAS, spectrophotometric FI with a column placed at the detection point, and FI-BI. FI-FAAS and FI-BI can be alternative procedures to assay iron contents in beer, which are cost-effective, rapid, and sensitive, especially in comparison with the AOAC standard method. Interference due to tannin is suppressed by adding ascorbic acid. The procedures incorporate standard addition technique.

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Sequential injection analysis with dynamic surface tension detection
High throughput analysis of the interfacial properties of surface-active samples☆

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Abstract

A sequential injection analysis (SIA) system is coupled with dynamic surface tension detection (DSTD) for the purpose of studying the interfacial properties of surface-active samples. DSTD is a novel analyzer based upon a growing drop method, utilizing a pressure sensor measurement of drop pressure. The pressure signal depends on the surface tension properties of sample solution drops that grow and detach at the end of a capillary tip. In this work, SIA was used for creating a reagent concentration gradient, and for blending the reagent gradient with a steady-state sample. The sample, consisting of either sodium dodecyl sulfate (SDS) or poly(ethylene glycol) at 1470 g mol^{-1} (PEG 1470), elutes with a steady-state concentration at the center of the sample plug. Reagents such as Brij®35, tetrabutylammonium (TBA) hydroxide and β-cyclodextrin were introduced as a concentration gradient that begins after the sample plug has reached the steady-state concentration. By blending the reagent concentration gradient with the sample plug using SIA/DSTD, the kinetic surface pressure signal of samples mixed with various reagent concentrations is observed and evaluated in a high throughput fashion. It was found that the SIA/DSTD method consumes lesser reagent and required significantly less analysis time than traditional FIA/DSTD. Four unique chemical systems were studied with regard to how surface activity is influenced, as observed through the surface tension signal: surface activity addition, surface activity reduction due to competition, surface activity enhancement due to ion-pair formation, and surface activity reduction due to bulk phase binding chemistry.

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1. Introduction

Multidimensional dynamic surface tension detection (DSTD) is based upon measuring drop pressure with a pressure sensor as a function of time during the formation of repeating drops with continuous flow [1–5]. Hence, DSTD measures the pressure across the air–liquid interface of drops that repeatedly grow and detach from the end of a capillary tip. The pressure signal is dependent upon surface tension properties of the solution. DSTD was first used with flow injection analysis (FIA) [1] for studying the characteristics of industrially relevant samples. A variety of useful information on dynamic surface tension was provided, such as the surface tension at the liquid–air interface and adhesion at the solid–liquid interface, for the rapid characterization of complex samples. DSTD has been coupled with hydrophobic interaction chromatography for determination of surface-active species in protein formulations [2]. A technique for calibration of dynamic surface tension by application of the Young–Laplace equation was recently created [3] facilitating the ease of application to FIA and HPLC. That work demonstrated that FIA/DSTD has promise as an investigative tool for the study of complex biological and industrial samples. The calibration procedure has been subsequently improved for rapid on-line calibration of DSTD [4]. The on-line calibration procedure is utilized in this paper. The new calibration procedure enables the measurement of a given surfactant solution in a flowing system without the need for optical measurements. These improvements to DSTD increase its potential as a detector for flow-based analytical techniques such as FIA and HPLC.

Because nearly all practical applications of surfactants involve the use of surfactant mixtures, the study of mixed surfactant solutions is of great interest [6–12]. In previous work, DSTD was used with FIA to study the interfacial properties of various surface-active analytes in mixtures [5]. A large volume of carrier (mobile phase) was needed for these experiments due to the continuous flow nature of the FIA technique. Also, individual concentrations of analytes were separately injected and processed, which was time consuming. From an analytical perspective it is desirable to further improve the implementation of DSTD to provide the desired analytical information while reducing the carrier (mobile phase) consumption and reducing analysis time. To this end, we report herein the integration of sequential injection analysis (SIA) with DSTD to study the interfacial properties of surface-active analytes in various concentrations of reagents. A concentration gradient of reagent was generated using SIA. In concert, a steady-state concentration of surface-active analyte was injected to blend with the reagent concentration gradient. Hence, a single concentration of analyte in various concentrations of reagent was achieved, analogous to running several reagent-to-analyte concentration blends in a relatively short period of time. Only a single concentration of reagent and sample was prepared instead of multiple concentrations as done with the typical FIA approach. In this manner, time, reagents, waste, and steps of data processing were reduced.

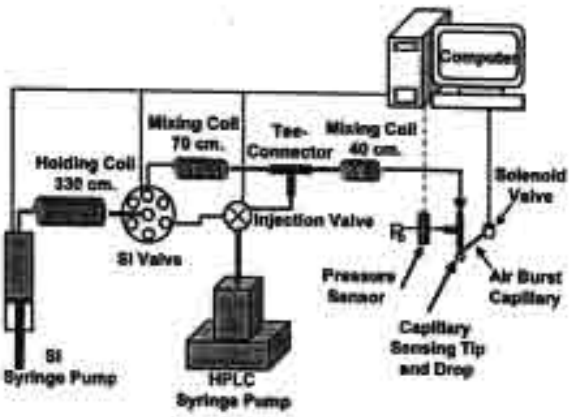

Fig. 1. The schematic diagram of SIA system with the dynamic surface tension detector.

To demonstrate the attributes of the SIA/DSTD system (Fig. 1), the concentration gradient of reagent was made using the SIA system, and the steady-state concentration of surface-active sample was injected to blend with the reagent gradient. A concentration gradient in the range of zero to the prepared concentration occurred by the SIA dilution phenomenon. After the blending of the reagent and sample (analyte) zones at a junction, the on-line mixture was blended and dispensed to DSTD to detect the surface pressure of the mixture. In this work, four unique chemical systems were selected and studied. A surface tension addition effect will be seen with a sodium dodecyl sulfate (SDS) sample mixed with a Brij®35 reagent gradient. A competition surface tension effect will be observed with a poly(ethylene glycol) at 1470 g mol^{-1} (PEG 1470) sample and a Brij®35 reagent gradient. A surface tension enhancement effect will be reported with an SDS sample combined with a tetrabutylammonium (TBA) hydroxide reagent gradient. Finally, a binding effect is observed with the SDS sample and a β-cyclodextrin (β-CD) reagent gradient. All experiments consumed lesser reagent and generated less waste, while requiring significantly less analysis time than traditional FIA/DSTD.

2. Theory

DSTD is a growing drop method utilizing a pressure sensor. The pressure signal measured is dependent on the surface tension properties of a sample solution drop that grows at the end of a capillary tip. The pressure sensor measures the internal pressure of a growing drop relative to atmospheric pressure as described in previous work [4]. Surface pressure measurements were related by the Young–Laplace equation. The time-dependent Young–Laplace equation [3] is shown in Eq. (1):

$$P(t) = \frac{2\gamma(t)}{r(t)} + P_C, \quad (1)$$

where P is the pressure signal, γ the surface tension, r the drop radius, and P_C accounts for viscous losses in the capillary tubing and the relative position of the sensor from the capillary tip. Note that P, γ, and r are all a function of time, t. Pneumatic detachment is used to remove the drops from the sensor capillary tip before gravity elongates the drop and reduces the applicability of the Young–Laplace equation [4,5]. Drops are detached in a size regime in which they are essentially spherical so one radius of curvature is sufficient to apply the Young–Laplace equation. The pneumatic detachment method will be explained in Section 3. The calculation of the dynamic surface pressure from the measured pressure signals has also been described in previous work [4,5]. First, the dynamic surface pressure of an analyte, $\pi(t)_A$, can be defined as the surface tension of an analyte, $\gamma(t)_A$, subtracted from the surface tension of the carrier (mobile phase), $\gamma(t)_M$. Thus, by application of Eq. (1), the dynamic surface pressure of an analyte in the mobile phase can be shown to be

$$\pi(t)_A = \gamma(t)_M - \gamma(t)_A = \frac{r(t)[P(t)_M - P(t)_A]}{2}. \quad (2)$$

To calibrate DSTD, a standard solution with a known surface tension is measured to collect the $P(t)_S$ signal. So, an equation similar to Eq. (2) can be written for a standard solution $\pi(t)_S$. Finally, the dynamic surface pressure of an analyte and standard solution can be related to the drop pressure data as shown in Eq. (3):

$$\pi(t)_A = \frac{\pi(t)_S[P(t)_M - P(t)_A]}{P(t)_M - P(t)_S}. \quad (3)$$

By using Eq. (3), the dynamic surface pressure of an analyte can be obtained from the drop pressure, $P(t)$ profile data from the mobile phase, M, the analyte, A, and the standard solution, S, of known surface pressure, $\pi(t)_S$. The high reproducibility of the experimentally determined surface pressure plots obtained using DSTD, either with or without kinetic effects observed, indicates that radius growth is very reproducible and thus Eq. (3) can be used to factor out the radius dependence term, $r(t)$. In previous work, we rigorously evaluated the time dependence of $r(t)$ for the mobile phase, typical analyte solution, and standard solution.

The results of the study indicated that $r(t)$ was essentially a constant function under a broad range of experimental conditions employed using pneumatic drop detachment [5]. In this regard, the role of convection is also factored out in the comparison of pressure signals of mobile phase, standard, and sample drops. On the other hand, convection is taking place with DSTD similar to the dropping mercury electrode (DME) experiment. While with DSTD convection of either analyte or standard occurs from within the growing drop and with the DME convection occurs from outside the growing drop, in both experiments useful diffusion and adsorption information is obtained in the presence of convection, since the convective process is reproducible.

Water was used as mobile phase, and 5% glacial acetic acid solution with surface pressure of 10.3 dyne cm^{-1} [13] was used as standard solution since it has a constant surface tension throughout drop growth [4]. Fig. 2A shows the drop pressure profile data of the mobile phase, $P(t)_M$, standard solution, $P(t)_S$, and analyte, $P(t)_A$. For this example, 3 mM SDS was used as the analyte. The three profiles shown in Fig. 2A were then used to calculate the dynamic surface pressure of 3 mM SDS as shown in Fig. 2B by application of Eq. (3). The resulting surface pressure plot in Fig. 2B for SDS is constant over the drop growth, indicating that the SDS surface activity is a fast process with no kinetic hindrance [5]. This rapid, on-line calibration technique enables the measurement of surfactant solutions in a flowing system without the need of cumbersome optical measurements and without the need for independently measuring the pressure offset, P_C.

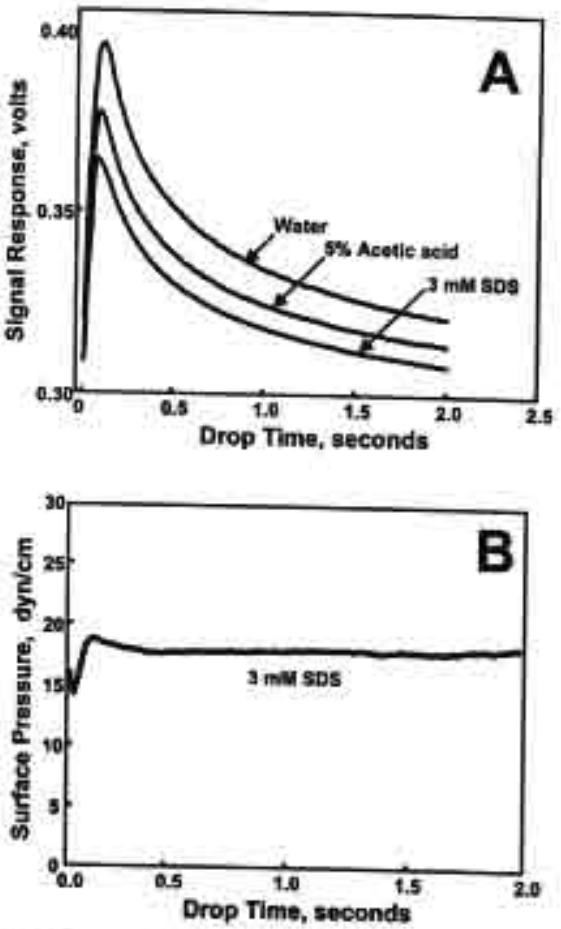

Fig. 2. Drop profile pressure data and application of the calibration procedure. (A) Overlay plots of individual drop profiles of water (mobile phase carrier), 3 mM SDS solution (analyte), and 5% (w/w) acetic acid (standard). (B) The dynamic surface pressure plot of 3 mM SDS solution after application of Eq. (3) to the drop profile data in (A).

3. Experimental

3.1. Materials

SDS (98% purity) and Brij®35 were obtained from Aldrich (Aldrich Chemical Co., Milwaukee, WI). Freshly prepared SDS solutions were used without further purification. SDS solutions handled by this procedure were previously found to produce DSTD surface pressure results that agreed with the literature [4]. PEG with an average molar mass of 1470 g mol^{-1} ($M_n = 1470$, $M_w/M_n = 1.02$) was obtained from Polymer Laboratories (Amherst, MA). Tetrabutylammonium hydroxide was obtained from Dionex (Dionex Corporation, Sunnyvale, CA). Acetic acid (glacial), of reagent grade quality, was obtained from Baker (J.T. Baker, Inc., Phillipsburg, NJ). All chemicals were used as received. Deionized (DI) water, demineralized to greater than 18 MΩ with a Millipore system (Millipore, Bedford, MA), was used in the preparation of all solutions. The water was degassed by using an ultrasonic bath (Cole-

Parmer Instrument Company, Vernon Hills, IL) for 15 min prior to use.

3.2. Instrumentation

A schematic diagram of the sequential injection dynamic surface tension detector (SIA/DSTD) analyzer is shown in Fig. 1. The SIA/DSTD system operates with three major operational stages. First, the SIA system produces the reagent concentration gradient and loads the sample solution into the sample loop. Second, the sample introduction system injects a plug of sample into the reagent concentration gradient. Last, DSTD collects dynamic surface tension information on each drop eluting from the system. A detailed diagram of DSTD can be seen in previous reports [4,5].

The SIA system employed consisted of a syringe pump (XP-3000 Syringe Pump with a valve; Cavro, San Jose, CA) and a six-port Lab on valve (FIA Lab Instrument, Bellevue, WA). For the flow lines, PTFE tubing of 0.76 mm i.d. and 1.59 mm o.d. was used. The holding coil was 330 cm and the two mixing coils were 70 and 40 cm. The first mixing coil was used for mixing the reagent concentration gradient and the second was used for mixing the concentration gradient and sample solution together before the mixed solution was detected by DSTD. A Tee connector (P-715; Upchurch, Oak Harbor, WA) was used to overlap the concentration gradient and sample solution before passing through the second mixing coil.

A syringe pump in the sample introduction system (Isco μLC-500; Lincoln, NE) was used to deliver the carrier, i.e. mobile phase (water) and sample solution to the system. Sample solutions were injected with a LabPRO two-position ten-port fluid processor (PR 700-102-01; Rheodyne, Cotati, CA). An injection volume of 500 μl was made from poly(etheretherketone) (PEEK) tubing (Upchurch, Oak Harbor, WA). Sufficient volume was injected so that the sample plug zone was not diluted by the water mobile phase and could be overlapped with the whole reagent concentration gradient zone. In all experiments, this pump was run at 1 μl s^{-1} unless otherwise stated.

In the DSTD portion of the system, the pressure sensor (Validyne P30 SD-20-2369; Northridge, CA) was configured with a sensing membrane (Validyne diaphragm 3-36) that was optimized for the measurements of interfacial kinetics at 120 μl min^{-1} total volumetric flow. The sensor capillary tip was made from a short piece of PEEK tubing, 457 μm i.d. and 635 μm o.d. Pneumatic drop detachment was performed at a rate of 0.5 Hz, corresponding to 2 s (i.e. 4 μl) drops at a volumetric flow rate of 120 μl min^{-1}. A skinner solenoid valve (MBD 002; Skinner valve, New Britain, CT) controlled by a computer did the pneumatic detachment. To record the concentration of any non-surface-active solution, a refractive index detector (HP 1047A; Hewlett-Packard Company, Germany) was used.

Data collection and instrument control were both done with a personal computer (600 MHz Pentium®; Intel Corporation, Santa Clara, CA) equipped with a data acquisition card (AT-MIO-16XE-50; National Instruments, Austin, TX). The data were averaged down to 100 points s^{-1} prior to saving. Data collection and instrument control were completed using LABVIEW (version 6; National Instrument, Austin, TX) with programs written in-house. Subsequent data analysis was performed using MATLAB 6.0 (Math Works, Natick, MA).

3.3. Procedure

The SIA/DSTD system was constructed as shown in Fig. 1. Deionized water was used as carrier solution (mobile phase). A reagent concentration gradient was introduced using the SIA system and passed through the first mixing coil to increase dilution, and then stopped in the front of the Tee connector. The sample was then introduced to the sample loop using the SIA system. After the reagent concentration gradient was achieved and the sample in the loop was ready, the injection valve was switched to inject the sample into the system and the syringe pump was started to push the reagent concentration gradient. The concentration gradient and sample were then overlapped at the Tee connector and transported to the second mixing coil to increase mixing. Finally, the mixed solution was dispensed

Table 1
SIA/DSTD method operations

Steps	SI valve*	Position of syringe pump	Operation	Injection valve position	Commentary
1	–	Valve in	Aspirate: 500 μl	Load	
2	2	Valve out	Dispense: 500 μl	Load	Flushes sample loop
3	5	Valve out	Aspirate: 700 μl	Load	Loads sample into sample loop
4	–	Valve in	Fill syringe	Load	Fills pump with water
5	2	Valve out	Dispense: 700 μl	Load	Fills the sample into loop
6	3	Valve out	Dispense: 300 μl	Load	Pushes solution residue to waste
7	4	Valve out	Aspirate	Load	Loads reagent
8	–	Valve in	Fill syringe	Load	Fills pump with water
9	1	Valve out	Dispense: 200 μl, 20 μl s^{-1}	Load	Makes a reagent dilution gradient
10	–	Valve in	Fill syringe	Load	Fills pump with water
11	1	Valve out	Dispense: 1000 μl, 1 μl s^{-1}	Inject	Detects the surface pressure
12	1	Valve in	Fill syringe	Inject	Fills pump with water
13	1	Valve out	Dispense: 1000 μl, 20 μl s^{-1}	Inject	Flushes system
14					Repeats n times from step 1

* SI valve positions: (1) to detector; (2) to sample loop; (3) to waste; (4) reagent; and (5) sample solution.

to DSTD for surface tension detection. A complete SIA cycle is described in Table 1.

The solution flowed out the end of the tip at a flow rate of 2 μl s^{-1} (each syringe pump was run with a flow rate of 1 μl s^{-1}). The key steps of the procedure are described in Table 1 and summarized here. In steps 1–6, the sample solution is loaded in the 500 μl sample loop by aspiration of 700 μl sample solution into the holding coil with SIA syringe pump. Then, the aspirated sample solution is dispensed to the sample loop. Thus, sample solution in the loop is ready and waiting for the injection step. In steps 7–10, the desired aspiration volume of reagent is aspirated to the holding coil and then the solution in the holding coil is dispensed through the first mixing coil with a flow rate of 20 μl s^{-1} and stopped in front of the Tee connector. Then, in step 11, multiple-drop pressure measurement of the solution is obtained. In this step, the SIA syringe pump with 1000 μl gradient solution at flow rate of 1 μl s^{-1} is started and the injection valve is switched to the injection position simultaneously, to allow the sample solution to overlap with the concentration gradient at the Tee connector. This mixture is then passed through the second mixing coil and then through DSTD. Finally, in steps 12 and 13, flushing of the flow lines is done with 1000 μl of deionized water used to flush the system.

4. Results and discussion

4.1. Kinetic surface pressure behavior of SDS with a Brij®35 concentration gradient

The development of SIA/DSTD for studying the interfacial properties of surface-active analytes and various mixture chemistries was investigated. The developed method was initially applied for the study of the interfacial properties of a surfactant mixture containing SDS and Brij®35. The signal profile results of Brij®35 concentration gradient mixed with an SDS sample are shown in Fig. 3. In Fig. 3A, surface pressure plots at drop detachment of water baseline, 2 mM SDS alone, 200 ppm Brij®35 alone, SIA on-line blending of SDS and Brij®35, and the signal addition of SDS and Brij®35 are shown. In this experiment, SDS elutes as a sample plug and contains a steady-state region in the center of the plug. Brig®35, on the other hand, elutes as a concentration gradient that begins after the SDS plug. Now, discussion of Fig. 3A is facilitated by dividing the plot into four time periods. In the first period, 0–200 s, the signal shows the dynamic surface pressure of water only, since neither the injection plug nor the concentration gradient has reached the detector. In the second period, 200–320 s, the initial SDS injection plug zone passes through the capillary tip and the