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Cross injection analysis: Concept and operation for simultaneous injection of sample and reagents in flow analysis

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ABSTRACT

This work presents a new flow injection strategy, called 'cross injection analysis' or CIA, an alternative cost-effective approach in flow analysis. The flow platform is made from a rectangular acrylic block, approximately $5 \times 3 \times 1.5$ cm ($x \times y \times z$), with crossing cylindrical channels drilled out along the *x*- and *y*-axis of the block. The outlet from the single *x*-axis channel is connected to a detector flow cell. This channel is filled with the carrier solution. The flow in the *x*-axis channel is driven by a computer controlled single-channel peristaltic pump. The multiple *y*-axis channels, running perpendicular to the *x*-channel, are connected to a multi-channel peristaltic pump. These channels contain the sample and reagent solutions that flow across the intersection zones of the channels. To mix the sample and reagent with subsequent detection of the reaction zone, flow is applied along the *x*-axis channel, while flow in the *y*-axis channels is stopped. We successfully demonstrated the validity of the CIA technique by the spectrometric determination of Fe(II) using 1,10-phenanthroline and the speciation of Fe(II) and Fe(III). To place the CIA technique within the context of flow analysis, a brief overview of the evolution of flow injection analysis and its later innovative development is included.

1. Introduction

Following the first report of flow injection analysis (FIA or FI) in 1975 by Ruzicka and Hansen [1], there have been a tremendous number of research papers published on new developments in this area [2–4]. FI can also be operated using the so-called 'reversed-FI' (rFI) [5,6], in which the reagent is injected into a flowing stream of the sample to improve the sensitivity over normal FI mode [7]. In 1990, Ruzicka and Marshall introduced sequential injection analysis (SIA or SI), the second generation of FI, that is fully controlled by the computer [8]. In SI technique, metered amounts of sample and reagent(s) are sequentially aspirated, through precise control of selection valve and the syringe pump (plunger in filling mode), as stacked layers into a holding coil. Finally, the reaction zone is propelled (plunger in delivering mode) to the detector. Ruzicka also proposed the lab-on-valve (LOV) technique that extends the feature of sequential injection to provide liquid handling in microliter and sub-microliter levels of sample and reagent(s) with a microfluidic platform made of acrylic or other polymeric material attached atop the selection valve [9–11]. The microfluidic platform accommodates connecting ports, working channels and flow through detection cell. The LOV technique is often called SI–LOV or μSI–LOV [12]. In 2004, Grudpan proposed an alternative cost-effective approach to SI called lab-at-valve (LAV) [13]. Liquid handling in LAV utilizes a syringe pump similar to the SI concept. However in LAV, there is no replacement of the stator plate of selection valve by the precisely machined microfluidic-platform. Instead devices such as in-house potentiometric flow cell [14] or pipette tip, as SI liquid–liquid separator [15], are attached or plugged directly onto the port(s) of the SI selection valve.

Cerdà et al. proposed 'multi-syringe' (also written as 'multi-syringe') flow analysis or MSFIA in 1999 [16]. The basic system of MSFIA comprises four syringes that are connected in a block to the same stepping motor. A three-way solenoid valve is fitted at the head of each syringe for selecting the flow path (reservoir or analytical flow path). These valves are commutation valves that can be separately controlled without having to stop the

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movement of the syringe plungers. MSFIA is a versatile technique and the basic system can be modified to add on other components, such as solenoid valve(s) for sample introduction to eliminate carryover of sample in the syringe barrel [17].

Reis et al. introduced 'multicommutation' flow analysis (MCFA) [18]. MCFA is a continuous flow strategy that utilizes computerized control of three-way solenoid valves and a peristaltic pump to insert sample and reagent plugs into the liquid flow path. In the arrangement of the MCFA system, the pump is placed at the end of the flow line after the detection cell and only a single pumping channel is used. Later in 2002, Santos and others from the same laboratory presented another strategy called 'multi-pumping' (also written as multipumping) flow system (MPFA) [19]. In MPFA, a set of solenoid micropumps are individually controlled by a computer to propel sample and reagent(s) by time-based or a pulse counting approach. With suitable arrangement of micropumps, use of solenoid valves and peristaltic pump is thereby eliminated.

Recently in 2010, a group in Japan [20] presented a hybrid system of FI, SI and multicommutation called 'simultaneous injection-effective mixing analysis' (SIEMA). A SIEMA system consists of a syringe pump as the liquid delivery module. One part of the three-way solenoid valve fitted to the syringe head is connected to the analytical flow path by a four-way cross-connector. The system also has separately controlled three-way solenoid valves for introducing sample and reagents into the central flow line when the syringe plunger is pulled down (reverse flow). The reaction solution is later pushed into and out of the mixing coil for detection (forward flow).

In this work, we present a new flow analysis technique called 'cross injection analysis' or CIA. The CIA platform has one channel as the analytical flow path (the *x*-axis channel) and four channels (*y*-axis channels) perpendicular to this channel. These channels are drilled out of an acrylic rectangular block. Both ends of the *y*-axis channels are connected with tubings to the same set of rollers of a peristaltic pump to form four flow lines. In CIA, the sample and reagents are introduced into the analytical flow path of the platform (*x*-axis channel) via individual pump tube connected to the *y*-channels of the platform. Unlike the SI and the multicommutation techniques, liquid introduction in CIA is simultaneous. We employ the cross-flow mode to improve the mixing to achieve desired sensitivity. Although CIA may be similar to the SI and the multicommutation in the way that liquid zones are inserted into analytical flow path of the system, the pattern of liquid flow in CIA is different to these previous techniques due to the platform design and the employed cross-flow. We exploit the characteristic of the peristaltic pump in which rollers are in 'press' mode when the pump is stopped. Thus pressure is maintained during the stopped-flow period holding the liquid inside the tubing or connected channels without the need to use syringe pump or solenoid valve as in the SI or the multicommutation techniques, respectively. In this design, using a peristaltic pump, with eight pump tubes placed on the rollers, is equivalent to operating a set of eight solenoid valves.

2. Experimental

2.1. Reagents and samples

All chemicals used in this work were AR grade. Deionized-distilled water was used throughout all the experiments. Standard stock solution of Fe(II) (1,000 mg L^{-1}) was prepared by dissolving an accurate weight of 4.98 g iron (II) sulphate heptahydrate (Fluka, Switzerland) in 1.00 L of 0.05 mol L^{-1} sulfuric acid. Standard stock solution of Fe(III) (1000 mg L^{-1}) was prepared by dissolving an accurate weight of 4.71 g iron(III) sulfate heptahydrate (Sigma-Aldrich, USA) in 1.00 L of 0.05 mol L^{-1} sulfuric acid. These stock solutions were calibrated against a certified atomic absorption standard (1002 ± 2 mg L^{-1} Fe) as $Fe(NO_3)_3$ in 0.5 mol L^{-1} HNO_3 (Merck, Germany) for accurate concentrations of iron. Working standard solutions of Fe(II) and Fe(III) were freshly prepared daily from the stock solutions by serial dilutions with 0.05 mol L^{-1} sulfuric acid. Acetate buffer pH 5.3, used as the carrier in the CIA system, was prepared by mixing 81.50 mL of 2.00 mol L^{-1} aqueous sodium acetate solution with 18.5 mL of 2.00 mol L^{-1} acetic acid in a one liter volumetric flask and making up to mark with water. The hydroxylamine solution was prepared by dissolving 19.8 g of hydroxylamine hydrochloride (Merck, Germany) in 250 mL of water. This solution was used to reduce iron (III) to iron (II) in the determination of total iron in the speciation analysis. The reagent for Fe(II) was 1,10-phenanthroline (Merck, Germany) in acetate buffer.

Five commercial products of multivitamin tablets were used in the validation study. Finely ground sample was weighed accurately (0.1 to 0.5 g) and dissolved in 100.0 mL of 0.05 mol L^{-1} sulfuric acid. After constant stirring for 30 min, the solution was centrifuged for 15 min at 3000 rpm. The supernatant was then filtered through a cellulose acetate membrane filter (0.45 μm). The filtrate was directly aspirated into the CIA system.

2.2. CIA platform

Fig. 1a is a photograph of a CIA platform, which is made from Perspex™. Fig. 1b shows the dimensions of a regular platform with cylindrical channels drilled out using computer controlled drilling bit. The CIA platform has a main channel, designated as the '*x*-channel', for the carrier stream. The crossing channels (labeled 1–4 in Fig. 1c) are drilled perpendicular to the *x*-channel. These channels are designated as '*y*-channels' and are used for the sample and reagents solution. We can reduce or add *y*-channels according to the number of reagents required. For determination of iron, we used the CIA platform as shown in Fig. 1 with four *y*-channels.

2.3. The manifold

Fig. 2 is a schematic diagram of the CIA flow-manifold employed for the determination of iron (II) and total iron. An Ismatec peristaltic pump (model ISM843, Switzerland), designated as P1 with capacity for accommodating eight pump tubes, was used for simultaneously filling or withdrawing the sample/standard (S) and the three reagents (R1, R2 and R3), in the *y*-channels (channels 1 to 4) of the CIA platform. P2 is a peristaltic pump used for driving the carrier solution (C) through the *x*-channel (designated as channel 5 in Fig. 1c and in Fig. 2). For this work, we employed an Ismatec pump (model IS7610, Switzerland). The system was also equipped with an Upchurch valve, V (model V-101L, USA). When the valve is set at the 'open' position (solid line), the flow line of the CIA platform in the *x*-direction is connected to the detection cell of the detector *D*, and then to waste (waste 2). When the valve *V* is set to 'close' position (dashed line), the carrier solution flows out of the platform via the *y*-channels to waste 1. A Jenway spectrophotometer (model 6450, UK) with 1-cm path length flow-through cell (Hellma, USA) was employed for absorbance measurement at 510 nm. Labview™ program, version 8.0, was used for data acquisition of the signal from the detector. An in-house CIA electronic board was built to control the peristaltic pumps P1, P2 and the valve V using software written in Visual Basic 6.0.

2.4. Investigated operating procedures

In this work, there are two operating procedures that were investigated as shown in Table 1. In procedure 1, flow in the

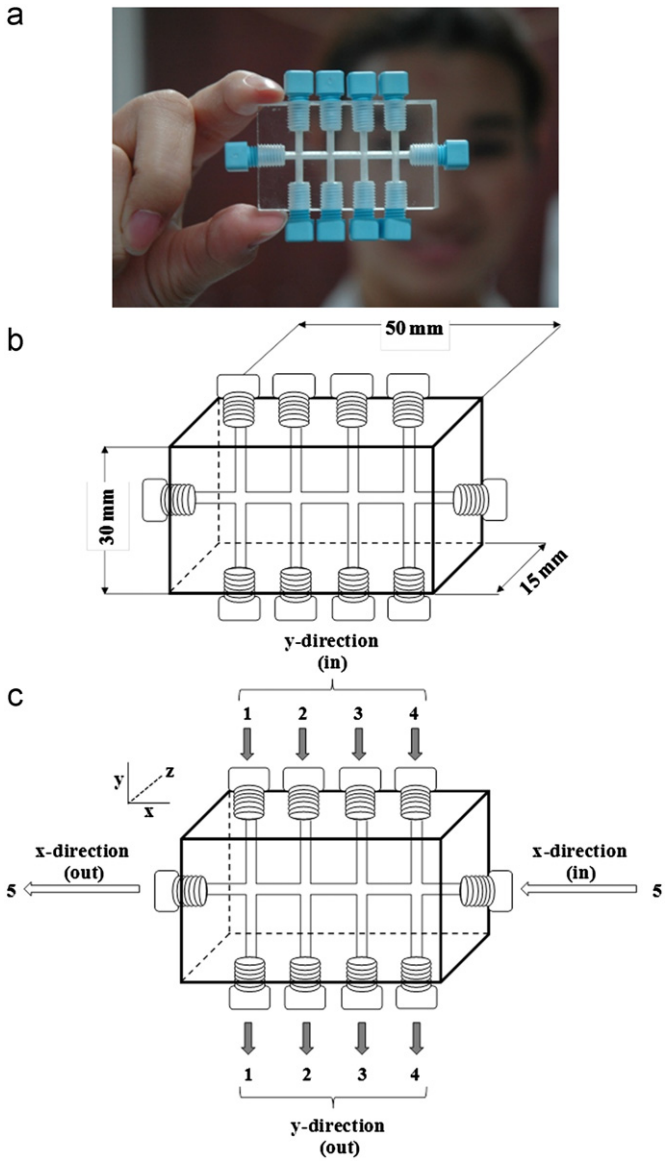


Fig. 1. (a) Photograph, (b) schematic diagrams of CIA platform with external dimension and (c) flow directions of liquids.

x- and *y*-channels was applied alternately. In step 1, pump P2 loaded the buffer carrier solution for 10 s into the *x*-channel of the CIA platform, with pump P1 stopped and valve *V* in 'open' position. In step 2, standard iron solution and reagents were simultaneously loaded into the CIA platform via channels 1 to 4 for 10 s, with switching valve, *V*, in 'close' position. Solutions in the *y*-channels flowed to waste 1 (Fig. 2). Using the flow rate of 1 mL min^{-1}, ca. 167 μL of liquid is dispensed in each channel during the 10 s loading time. In step 3, the switching valve V was set to 'open' position and flow of the carrier solution restarted. The plugs of sample and reagents at the four intersections of the channels were driven along the *x*-channel. The zones were mixed as they flowed to the detector. The flow was maintained for 50 s.

In procedure 2, we operated the system using simultaneous flow in step 2. That is, in step 2, both pumps P1 and P2 were operated. As described above valve V was closed, the carrier solution in the *x*-channel thus flowed out along the *y*-channels to waste 1. The sample and three reagents flowed along the *y*-channels in cross-flow manner. Precise control of the pumps ensured that this loading process was reproducible giving good precision for analysis (see Section 3.5).

3. Results and discussion

3.1. Selection of operating procedure

3.1.1. Sensitivity

We tested two procedures as given in Table 1 to operate the CIA system (Fig. 2) for monitoring the reaction of iron(II) with 1,10-phenanthroline. In Fig. 2, solutions of 1 mol L^{-1} hydroxylamine hydrochloride, acetate buffer (pH 5.3) and 0.015 mol L^{-1} 1,10-phenanthroline were employed as the reagents R1, R2 and R3, respectively. Standard Fe(II) solution (5 to 20 mg L^{-1} Fe(II)) was placed in the S reservoir for construction of the calibration curves for both procedures. The flows of reagents and standards were driven by the peristaltic pump P1. The flow of acetate buffer, carrier (C), was driven by peristaltic pump, P2.

The two procedures gave linear calibrations with comparable correlation coefficients ($r^2 \sim 0.99$). However, repetitive operations showed that the sensitivity of procedure 2 was greater than procedure 1 (see Table 1). The simultaneous flow in step 2 of procedure 2 had flow-characteristics that resulted in better sensitivity. Therefore, experiment was carried out to investigate this effect.

3.1.2. Flow characterization at the crossing-zones for the alternate and simultaneous flow mode

In order to investigate and compare the loading phenomena in step 2 of the two operational procedures in Table 1, the rapid formation of the pink color of 1,10-phenolphthalein with base was employed. The CIA system with the configuration shown in Fig. 2 was employed. The flow rate was set to 1.0 mL min^{-1}. In this particular experiment, pure water was loaded into all the reservoirs, C, S, R1, R2 and R3 (Fig. 2), and then pumped to fill all the channels. Pumps P1 and P2 were then turned off. The carrier reservoir (C) and sample reservoir (S) were then filled with 0.003 mol L^{-1} phenolphthalein and 0.4 mol L^{-1} potassium hydroxide, respectively.

When studying the alternate flow mode, the CIA system in Fig. 2 was operated using a different procedure to that given in Table 1. First, valve V was switched to the 'open' position (solid line), and pump P2 turned on for 20 s to fill channel 5 (*x*-channel) with the phenolphthalein solution. Valve *V* was then closed (dash line). Flow to channels 1 to 4 was then applied using pump P1. We began to see light pink color at the intersection of channel 4 and channel 5, indicating the arrival of the hydroxide solution Fig. 3a. The color became more intense as the flow continued. Fig. 3b is the photograph taken after 38 s.

The experiment with simultaneous flow was carried out by again first filling all the channels with water. The carrier (C) and sample (S) reservoir were filled with the phenolphthalein and potassium hydroxide solutions, respectively. Valve *V* was switched to 'open' position. Pump P2 was turned on for 20 s to fill channel 5 (*x*-channel) with phenolphthalein. Valve *V* was then set to 'close' position. Flows in the *x*- and *y*-channels were then applied simultaneously by operating pumps P1 and P2. Similar to the alternate flow mode, light pink color began to develop at the intersection of channel 4 and channel 5 (see Fig. 3c) and became more intense (Fig. 3d after 38 s). When compared with alternate flow mode (Fig. 3b), the length of the colored zone resulting from the simultaneous flow was significantly longer (Fig. 3d). Besides flowing directly across the intersection of channels 4 and 5, the KOH solution in this channel was also pushed by laminar flow along the *x*-channel towards valve *V*.

Using this simultaneous flow procedure, there is a larger volume of the crossing solutions injected into the carrier stream (*x*-channel). This was confirmed by the greater sensitivity observed

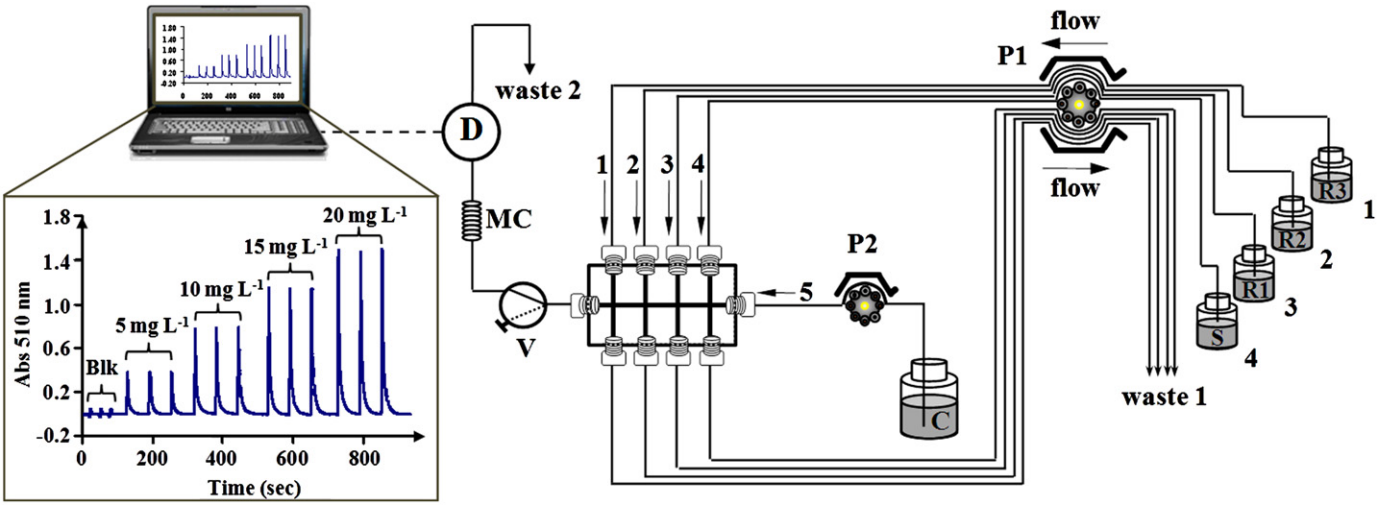


Fig. 2. Schematic diagram of the CIA flow manifold. P1 is the peristaltic pump with four flow lines for the sample S, and the reagents R1 to R3. P2 is the peristaltic pump with single flow line for the carrier solution C. V is the switching valve: solid line 'open'; dash line 'close'. MC is the mixing coil, 45 cm in length. D is the spectrophotometric detector. Insert shows a CIA signal profile for standard Fe(II) solutions, 0–20 mg L^{-1}.

Table 1
Operation procedures investigated for the CIA system in Fig. 1 and the resulting calibrations.

Step	Process (flow pattern in CIA platform)	Duration (s)	P1	P2	V
Procedure 1: Loading of sample and reagents using alternate flow					
1	Carrier loading (flow: *x*-direction only)	10	Off	On	Open
2	Sample+reagents loading (flow: *y*-direction only)	10	On	Off	Close
3	Zone flushing to detector (flow: *x*-direction only)	50	Off	On	Open
Repeat step 2–3 for next sample					
Calibration equation: $A_{510}=(0.050\pm0.005)$ mg L^{-1} Fe(II)+(0.090 ± 0.074): $r^2=0.991$					
Procedure 2: Loading of sample and reagent using simultaneous flow					
1	Carrier loading (flow: *x*-direction only)	10	Off	On	Open
2	Carrier+sample and reagents loading (flow: *x*-direction and *y*-direction)	10	On	On	Close
3	Zone flushing to detector (flow: *x*-direction only)	50	Off	On	Open
Repeat step 2–3 for next sample					
Calibration equation: $A_{510}=(0.073\pm0.003)$ mg L^{-1} Fe(II)+(0.041 ± 0.039)): $r^2=0.995$					

in the analysis of Fe(II) when using procedure 2 (simultaneous-flow mode).

Furthermore it could be observed (Fig. 3a and b) that the pink color for the alternate flow in step 2, was less intense than the color developed using the simultaneous-flow for loading (Fig. 3c and d). The laminar flow in *x*-direction dispersed and elongated the zones of liquid flow at the intersections of channels 1 to 4, enhancing the pre-mixing between iron(II) and reagents in *x*-channel. Thus, we selected procedure 2 in further experiments.

3.2. Zone sequence

In flow-based technique, the sequence of the sample/reagent zones may affect sensitivity. We examined two sequences as given in Table 2. In sequence a, the leading zone is 1,10-phenanthroline, followed by buffer, hydroxylamine (the reducing agent) and finally the Fe(II) standard solution. The zones are separated with zones of buffer carrier. In sequence b, the leading zone is Fe(II) standard solution, followed by hydroxylamine, buffer and finally the 1,10-phenanthroline. It was found that the slopes of the calibration lines obtained from the two sequences were not significantly different. However, we decided to use sequence 'a' since this sequence follows the sequence of mixing in a batch procedure.

3.3. Effect of channel diameter and use of mixing coil

Platforms A, B and C were made with various sizes of the channel, as shown in Table 3. The diameters of all channels for platform A and platform C are equal; that is, 2 and 3 mm, respectively. Platform B has the same size of the channels as platform A but with channel 4, the sample line, being 3 mm.

A series of iron(II) standard solutions were injected into the CIA system to compare the performance of the three platforms. The results in Table 3 clearly show increased sensitivity with increasing diameter of the sample channel (channel 4). Increasing the diameter of the channel increases the volume of solution introduced into the CIA platform. Thus by increasing the diameter of channel 4 from 2 to 3 mm in platform B, we increased the volume of iron(II). However increasing all the channels' diameter from 2 (platform A) to 3 mm (platform C) did not improve the sensitivity because the volume ratio of the solutions remained the same. In this work, we chose platform B in further experiments.

Using the CIA system in Fig. 2, the effect of employing a mixing coil placed before the detector was studied. The length of mixing coil was varied from 0 (no coil) to 90 cm. Injections of iron(II)

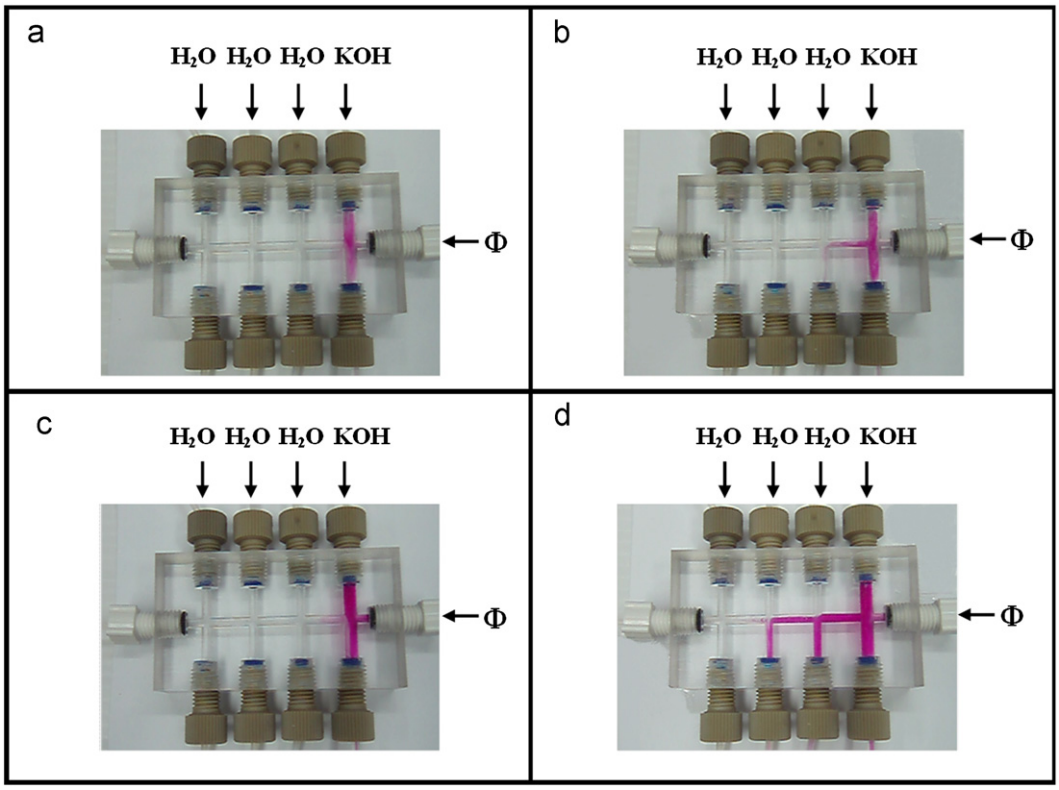
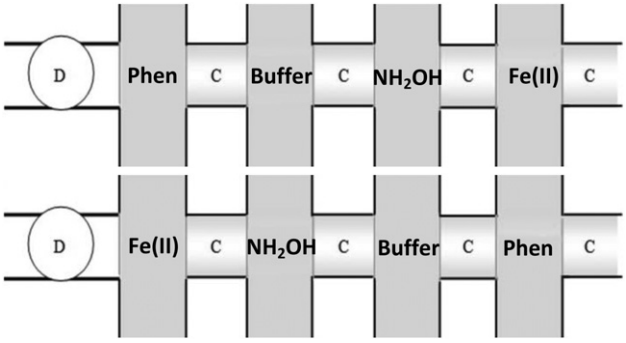


Fig. 3. Characterization of flow pattern of the crossing-zone using phenolphthalein (Φ) and KOH solutions. Fig. 3(a) and (b) employ alternate flow mode with flow only of H_2O and KOH for 22 and 38 s, respectively. Fig. 3(c) and (d) employ flow of the phenolphthalein and the H_2O/KOH channels (simultaneous flow) for 22 and 38 s, respectively.

Table 2
Zone sequence and linear calibration equations.

Zone sequence	Calibration; correlation coefficient
	$A_{510}=(0.033\pm0.004)$ mg L^{-1} Fe(II)$+(0.011\pm0.046)$; $r^2=0.994$
	$A_{510}=(0.034\pm0.001)$ mg L^{-1} Fe(II)$+(0.047\pm0.017)$; $r^2=0.994$

C, buffer: acetate buffer pH 5.3; NH_2OH: hydroxylamine hydrochloride; phen: 1,10-phenanthroline; *D*: Spectrophotometer (510 nm).
Note: Diameter of all CIA channels was 2 mm.

Table 3
Effect of channel diameter on the sensitivity of the CIA system in Fig. 2.

CIA platform	Channel diameter (mm)					Calibration; correlation coefficient
	CH 1 (R3)	CH2 (R2)	CH3 (R1)	CH4 (S)	CH5 (C)	
A	2	2	2	2	2	$A_{510}=(0.033\pm0.004)$ mg L^{-1} Fe(II)$+(0.011\pm0.046)$; $r^2=0.994$
B	2	2	2	3	2	$A_{510}=(0.074\pm0.001)$ mg L^{-1} Fe(II)$+(0.040\pm0.002)$; $r^2=0.999$
C	3	3	3	3	3	$A_{510}=(0.029\pm0.002)$ mg L^{-1} Fe(II)$+(0.068\pm0.017)$; $r^2=0.997$

CH: channel; C: carrier; S: iron standard/sample; R1, R2, R3: the reagents for iron(II).

solution (5, 10, 15 and 20 mg L^{-1} Fe(II)) were carried out in triplicate ($n=3$) for each coil length. It was found that sensitivity was enhanced with use of a mixing coil. The sensitivity increased with increasing coil length up to 45 cm. The highest sensitivity was achieved for the 45-cm coil, and was employed in the final flow manifold of the CIA system.

3.4. Concentrations of 1,10-phenanthroline and hydroxylamine hydrochloride

The concentration of 1,10-phenanthroline (R3) was varied from 5×10^{-3} to 1.5×10^{-2} mol L^{-1}. Iron(II) solution, at the maximum concentration (3.5×10^{-4} mol L^{-1}), was injected into the CIA system. The peak heights were constant for all concentration of the reagent, showing that 1,10-phenanthroline was in excess. The 1.5×10^{-2} mol L^{-1} 1,10-phenanthroline was selected to ensure sufficient excess of this reagent.

Hydroxylamine hydrochloride (R1) was used as the reducing agent for Fe(III). A Fe(III) standard solution, 20 mg L^{-1} Fe(III), was tested with various concentrations of the reducing agent (0.5–1.5 mol L^{-1}), and the peak height measured using the manifold in Fig. 2. The signals were constant and independent of the concentration of the reducing agent. The 1.1 mol L^{-1} solution was selected to ensure complete and rapid reaction.

3.5. Analytical performance in the determination of iron(II), iron(III) and total iron

When the CIA system in Fig. 2 was operated under the optimal condition discussed above for analysis of Fe(II), calibration was linear in the range 5–20 mg L^{-1} Fe(II) [$A_{510}=(0.0705\pm0.002)$ mg L^{-1} Fe(II)$+(0.031\pm0.011)$: $r^2=0.995$]. The limit of quantitation of this CIA technique for Fe(II) was 0.2 mg L^{-1} Fe(II) (10σ blank/slope), which is comparable to a multicommutation-based flow system [21] and a sequential injection method (SIA) using the same 1,10-phenanthroline reagent [22]. Since introduction of sample and reagents into our system is discrete, the volume employed in our method is therefore approximately three times less than in conventional FIA method for analysis of iron [23]. The consumptions of sample and reagents of the CIA system are 167 and 501 μL, respectively. This method provides throughput of up to 60 samples h^{-1}. With use of the electronic control, we could achieve precision with %RSD less than 2.6 (for 15 mg L^{-1} Fe(II), $n=10$).

The CIA system in Fig. 2 was also tested for its capability in speciation of Fe(II) and Fe(III). In order to determine only the Fe(II) species, the flow line for the hydroxylamine hydrochloride (R1) was replaced with water. The concentration of Fe(III) can be found by subtracting the concentration of Fe(II) from the total measured concentration of Fe. Table 4 shows that there is good agreement with the spiked amount and the measured value of Fe(II), Fe(III) and total Fe. The CIA system had good recoveries for all the iron species, as shown in Table 4.

Multivitamins also contain essential elements including I, Cl, Ca, Mg, Fe, Mn, Zn and Cu. Using the sample preparation procedure, concentrations of salts of the elements in the final solution are approximately 0.225 (KI), 36 (KCl), 162 ($CaHPO_4$), 100 ($MgSO_4$), 2 ($MnSO_4$), 20 ($ZnSO_4$) and 1 ($CuSO_4$) mg L^{-1}, respectively. We therefore investigated whether these salts may interfere in the analysis of iron. These salts were separately added to a standard solution of 5 mg L^{-1} Fe(II). There was no significant difference in the signal observed when compared to the pure Fe(II) standard even up to 1500 mg L^{-1}. Addition of mixture of these compounds at the levels expected in the final solution also gave no change. Therefore the CIA method for Fe(II) is not affected by common salts found in multivitamins.

3.6. Application to multivitamins

The developed method was applied to the determination of total iron in multivitamins (Fig. 4). Four different brands of vitamins (A–D) were analyzed in triplicate by CIA. The iron content found by our method is close to the label values except for brand D. However the results of the CIA method are comparable with those using flame atomic absorption spectrometry (FAAS). The paired t-test also confirm that the results of the CIA method are in significant agreement with the results of FAAS at 95% confidence level ($t_{stat}=0.16$, $t_{crit}=2.20$). The disagreement between the analytical results and the label content of iron for brand D may be due to the product itself.

The recovery of iron measurement in multivitamin tablets was investigated. We found that the recoveries of our method were in the range of 90.1–107%, indicating good accuracy for the CIA method.

3.7. Utilization of peristaltic pump

From the aforementioned literature, there is wider application of syringe pumps in FI-based/FI-related techniques due to its robustness, with precise and accurate fluid control even at microliter levels. Operation of a syringe pump can be completely automated with good reproducibility. Other types of pumps have been used in flow analysis to replace peristaltic pump: e.g., double plunger pump, employed in 'stopped-in-loop flow analysis' (SIL–FA) [24] and in 'all injection analysis' (AIA) [25]; solenoid pump [19]; piston pump [26]. In principle, these pumps provide consistent flow rates; they are suitable for heavy duty tasks, such as continuous monitoring in environmental or industrial process analysis. Although peristaltic pump has some drawbacks, it is still effective in the implementation of many flow-based techniques, including sequential injection analysis [27–29], all injection analysis [30], and stepwise injection analysis (SWIA) [31,32]. In this work, we employed the principle of peristaltic pump, by which the flow of liquid inside the flexible pump tube results from the alternate motion of 'press' and 'release' of rollers on the pump tube. If this motion is stopped, the flow ceases. We utilized this nature of the peristaltic pump in CIA both to flow and to retain liquid instead of using syringe pump coupled with selection valve or solenoid valves.

Table 4
Results obtained from speciation studies of Fe(II) and Fe(III) in four spiked solutions.

Spiked solution	Added (mg L^{-1} Fe)			Determined value (mg L^{-1} Fe)			%Recovery		
	Fe(II)	Fe(III)	Total Fe	Fe(II)	Fe(III)	Total Fe	Fe(II)	Fe(III)	Total Fe
A	3.00	12.0	15.0	2.81 ± 0.06	12.8 ± 0.21	15.6 ± 0.24	93.7	107	104
B	5.00	10.0	15.0	5.48 ± 0.11	9.05 ± 0.19	14.5 ± 0.22	110	90.5	96.9
C	7.00	8.00	15.0	7.32 ± 0.15	8.70 ± 0.19	16.0 ± 0.29	104	109	106
D	9.00	6.00	15.0	8.54 ± 0.17	6.65 ± 0.13	15.2 ± 0.21	94.9	111	101

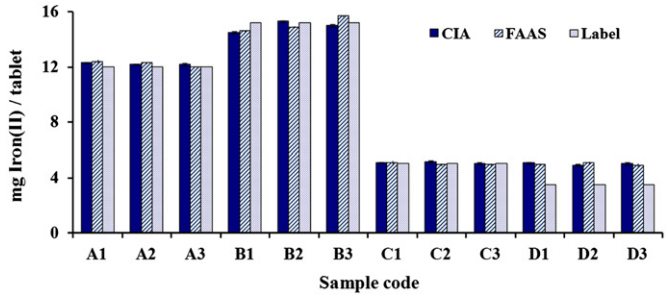


Fig. 4. Total iron content of four brands of multivitamin tablets (A–D) determined in triplicate by using the CIA system and by FAAS.

4. Conclusions

Most of the later flow analysis techniques, including CIA, exploit some of the concepts of FIA. However there is a major difference between CIA and SIA. In SIA, the sample and reagent zones are aspirated sequentially into the analytical flow path, with the need for selection valve and high precision pump, such as syringe pump. The mixing is usually enhanced by introducing flow reversal a few times in the holding coil before delivering the zones to detector. However, in CIA, we aspirate sample and reagent at the same time into the platform through the *x*- and *y*-channels using multi-channel peristaltic pump. We utilize the constant pressure maintained during the stopped-flow mode of peristaltic pump to retain the liquid inside the tubing, eliminating interference into the carrier stream. CIA requires only a multi-channel peristaltic pump for liquid delivery and for maintaining the system pressure. Thus, the number of hardware components in CIA is less than in the multicommutation technique.

The introduction of liquids into the analytical flow path of CIA is also different to that of the SIA and the multicommutation. When we simultaneously aspirate the sample and reagents through the *y*-channels (no. 1 to 4), we also aspirate at the same time the carrier flow through the *x*-channel (the analytical flow path). Some of the sample and reagents are partially dispensed at the *y*-outlets of the platform. This cross-flow results in prior mixing inside the platform, leading to a significant improvement in the sensitivity. Therefore flow-reversal as in SIA is not needed. Moreover when comparing with the multicommutation and multipumping techniques, we do not need to alternately aspirate sample and reagent slugs to form a tandem zone. The software command lines for CIA are therefore shorter and simpler. In CIA, we can modify the flow path simply by changing the tubing and the cross platform. In long-term use, CIA requires less maintenance costs than SIA, multicommutation and multipumping since the system consisted mainly of low-cost consumables such as pump tubes, tubings and the acrylic platform. There is only one switching valve that may needs the maintenance from time to time.

The CIA platform, with the four crossing channels, employed for determination of Fe(II), can also be applied to other analysis employing spectrophotometric measurements. The number of cross channels can be reduced or increased to suit the number of reagents. The CIA technique is a simple system that can be employed by other researchers working in the field of flow analysis.

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