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Exploiting sequential injection analysis with lab-at-valve (LAV) approach for on-line liquid–liquid micro-extraction spectrophotometry☆

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Abstract

Sequential injection analysis (SIA) with lab-at-valve (LAV) approach for on-line liquid–liquid micro-extraction has been exploited. Sample, reagent and organic solvent were sequentially aspirated into a coil attached to a central port of a conventional multiposition selection valve, where the extraction process was performed. The aqueous and organic phases were separated in a conical separating chamber LAV unit attached at one port of the valve. The organic phase containing extracted product was then monitored spectrophotometrically. The system offers a novel alternative on-line automated extraction in a micro-scale and has been successfully demonstrated for the assays of diphenhydramine hydrochloride (DPHH) in pharmaceutical preparations and anionic surfactant in water samples.

Keywords: Sequential injection analysis; Lab-at-valve; On-line liquid–liquid micro-extraction

1. Introduction

Liquid–liquid extraction is one of the most versatile techniques for sample matrix separation and/or analyte preconcentration. It has been applied to various analytical fields. For pharmaceutical applications, liquid–liquid extraction involves in the analytical processes for determination of active ingredient compounds such as cinnarizine [1], diphenhydramine hydrochloride (DPHH) [2,3] and paracetamol [4]. For environmental applications, it also exists in the determination of various compounds such as iron(II) and iron(III) [5], phenolic compounds [6] and surfactant [7]. However, manual extractions present a series of drawbacks such as high consumption of sample and toxic organic solvent, low sampling frequency, loss of analyte through manipulation and contamination of atmosphere by organic vapor. Many efforts have been made to overcome these inherent drawbacks. Among them, the successful techniques are probably the on-line liquid–liquid extraction using flow systems [8–10].

A variety of flow-based systems have been reported for the on-line liquid–liquid extraction. Flow injection liquid–liquid extraction was proposed in 1978 by Karlberg and Thelander [11] and Bergamin et al. [12]. Consumption of reagents and organic solvent are lower than those in manual procedures, consequently reducing the waste generated.

Based on this feature, different flow injection systems for liquid–liquid extraction were developed for a large number of analytical applications [7,13–18].

As another generation, the technique called sequential injection analysis (SIA) [19–22] has also been employed for on-line liquid–liquid extraction by sequential aspiration of a small volume of organic solvent and aqueous sample into contact before either resolve them using phase separator [23] or performing back extraction [24].

In the recent years, SIA has been modified into miniaturization with different concepts. Ruzicka proposed SIA with lab-on-valve (LOV) format in which a very precisely specific LOV module was mounted atop a selection valve to perform chemical reaction and monitor for a change in a conduit on such a modified multiposition valve [25]. SIA with a simple approach called lab-at-valve (LAV), firstly introduced by our group [26], is another approach of SIA which becomes an alternative cost effective micro total analysis system. SIA-LAV uses a designed LAV unit that can be fabricated using an ordinary less precise

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machine tool, to have a suitable function for chemistry of interest and can be easily attached at a port of the conventional selection valve in a usual way. This simpler approach was successfully demonstrated for potentiometric determination of chloride [27].

In the present work, SIA-LAV approach was investigated as a novel alternative for simple on-line liquid–liquid microextraction. A desired component, a separating chamber, was attached at one port of a conventional multiposition selection valve. Sample, reagents, and organic solvent were sequentially aspirated into an extraction coil (EC). By flow reversal, good extraction efficiency can be achieved. After that, the aqueous and organic phases were separated in a conical separating chamber attached at one port of a conventional multiposition selection valve ("Lab-at-Valve" concept). The organic phase containing extracted product was then monitored spectrophotometrically. Applications to the assays of diphenhydramine hydrochloride in pharmaceutical preparations and anionic surfactant in water samples were selected as models.

2. Experimental

2.1. Chemicals and reagents

All of reagents used were analytical reagent grade. Deionized water was used throughout the experiments. Diphenhydramine hydrochloride reference standard of 100% purity (Ministry of Public Health, Thailand) was used. Stock solution (1000 mg/l) of diphenhydramine standard was prepared by dissolving 0.1000 g of the reference standard in water and diluting to the mark of 100 ml volumetric flask. Working standards were freshly prepared by diluting the stock solution with water to obtain appropriate concentrations. A bromocresol green (BCG) solution (5×10^{-4} mol/l) was prepared by dissolving 0.0349 g BCG powder in water with addition of 0.2 ml of 0.1 mol/l sodium hydroxide and diluting to 100 ml with water. The solution was filtered before use. A phthalate buffer (pH 3) was prepared using 100 ml of 0.1 mol/l potassium hydrogenphthalate, 40.6 ml of 0.1 mol/l hydrochloric acid and diluting to 200 ml with water.

Stock solution (1000 mg/l) of sodium dodecylsulphate (SDS) was prepared by dissolving 0.1087 g of the standard in water and made up to a volume of 100.00 ml.

Stock solution (0.10%, w/v) of methylene blue was prepared by dissolving 0.10 g of the methylene blue in 100 ml of water.

2.2. Apparatus

The SI system used is schematically depicted in Fig. 1. It consisted of a modified autoburette Dosimat 765 (Metrohm, Switzerland) equipped with a 10 ml exchange unit, for a pumping system, and connected to a personal computer via RS232C interface, a 10-position selection valve VICI with a microelectric actuator (Valco Instruments, USA) and a Spectronic21 (Bausch & Lomb, USA) detector with a flow through cell (Hellma, Germany) of 1 cm light path. The autoburette was connected to the center of the selection valve via the EC (0.79 mm i.d. × 400 cm PTFE tubing) and a separating chamber was placed at port-1 of the selection valve. Both instrumental control and data acquisition were manipulated via a software using LabVIEW, developed in house and using a CYDAS ULV interfacing board (CyberResearch, USA). This software provided control of the volume to be dispensed or aspirated by the autoburette, flow rate, selection of the different valve positions and performed data acquisition. The data processing was computed by using Microcal Origin 6.0.

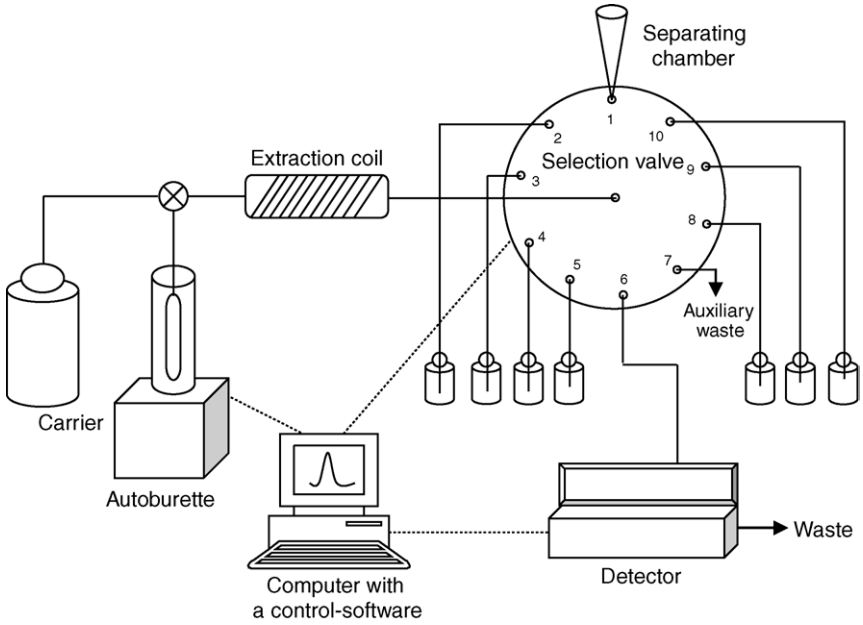

Fig. 1. Schematic diagram of SIA system.

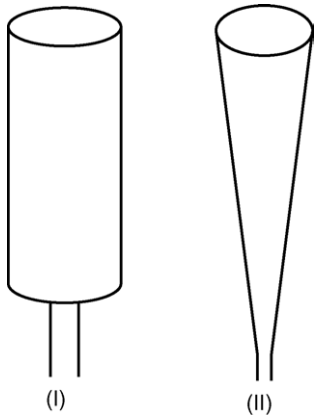

Fig. 2. Two types of separating chamber; I: cylindrical shape (8 mm i.d. × 5 cm long) and (II): conical shape (8 mm i.d. of the wider end × 7 cm long, modified from a 1 ml pipette tip (Eppendoft, Germany)).

3. Results and discussion

3.1. The assay of diphenhydramine hydrochloride

3.1.1. Optimization of the operational sequence

By using the separating chamber type I, as shown in Fig. 2, a sequence order of aspiration was firstly optimized. Three sequence orders (Fig. 3) were examined and a suitable one, as demonstrated in Fig. 3(c), which provided better sensitivity, was selected. BCG solution was firstly aspirated into the EC, then the standard/sample solution was introduced, an ion-association formed. A phthalate buffer (pH 3) and chloroform were then aspirated. At pH 3, a high extraction efficiency of ion-pair compound was obtained [2]. The extraction step was performed in the EC by programming the autoburette to aspiration and dispensed modes. This process determines the time and efficiency that the two phases are in contact and is a key parameter for better extraction efficiency. The solution was then dispensed into the separating chamber where the aqueous and organic phases were separated. The organic phase containing ion-association product was transported into the EC and transported into a flow through cell of the spectrophotometer. An absorbance at 415 nm was followed.

It should be noted that although the exchange unit of the autoburette was made from glass and furnished with PTFE plunger,

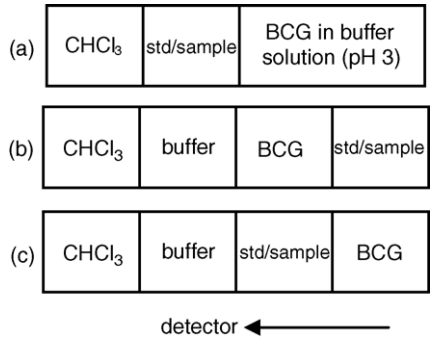

Fig. 3. Sequence orders of the SIA system for the determination of diphenhydramine hydrochloride.

manipulation of the organic extractant was accomplished by filling the syringe of the exchange unit with water. Before the detection step, the organic solvent was aspirated and stored in EC for use. It is necessary to avoid the introducing of aqueous into the flow cell of detector to prevent the change of baseline signal due to aqueous droplet.

3.1.2. Influence of the separating chamber shape

Two types of the separating chambers, as shown in Fig. 2, were examined to reach the best separation between the organic and aqueous phases. The results show that the better separation between two phases was obtained by using the conical shaped separating chamber. Therefore, the separating chamber type II was adopted.

3.1.3. Optimization of the chemical parameters

The concentration and volume of the reagents concerning in the reaction were optimized. According to the preliminary study, the concentration of BCG of 5×10^{-4} mol/l was excess to form the ion-association with diphenhydramine hydrochloride in the working range of 10–40 mg/l [2].

The selected conditions and operational sequence of the proposed system were summarized in Table 1. By fixing the aque-

Table 1
Selected conditions and operational sequence of the SIA system for the determination of diphenhydramine hydrochloride

Sequence	Valve position	Mode[a]	Volume (μl)	Description
1	5	PIP	170	Aspirate 5×10^{-4} mol/l BCG into EC
2	3–4, 8–9	PIP	170	Aspirate standard/sample into EC
3	2	PIP	150	Aspirate 5×10^{-4} mol/l buffer (pH 3) into EC
4	10	PIP	400	Aspirate chloroform into EC
5	1	PIP	1300	Extract
6	1	DIS C	2000	Extract
7	1	PIP	1100	Extract
8	1	DIS C	2000	Extract and propel to the separating chamber[b]
9	7	DIS C	3000	Clean EC
10	10	PIP	400	Aspirate chloroform into EC
11	1	PIP	250	Aspirate chloroform phase containing ion-association compound into EC
12	6	DIS C	40	Dispense chloroform phase containing ion-association compound through the selection valve
13	7	DIS C	3000	Clean EC
14	10	PIP	2900	Aspirate chloroform into EC
15	6	DIS C	2000	Dispense to detector with flow rate of 10 ml/min

[a] PIP = pipetting/aspiration, DIS C = cumulative dispensing.
[b] No standing time for phase separation.

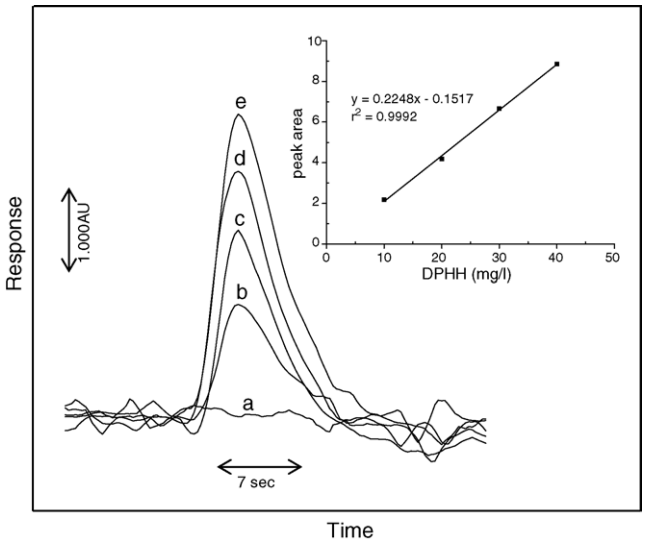

Fig. 4. Typical SI-grams and calibration graph of diphenhydramine hydrochloride; a = blank, b = 10, c = 20, d = 30, e = 40 mg/l.

ous volume (see Table 1), the volume ratio between aqueous to organic phases is of great importance in solvent extraction because it determines enrichment factor in the preconcentration of the analyte. The volume of chloroform used for the extraction was varied. The slopes of the calibration equation indicated that the sensitivity increased with decreasing the volume of chloroform: $y = 0.2248x - 0.1517$, $y = 0.1922x - 1.7877$ and $y = 0.0833x + 1.0726$ for 400, 450 and 470 μl, respectively. Using chloroform less than 400 μl, it was difficult to reach the complete separation between the two phases, i.e. aqueous droplets being entrapped within the organic phase. Better enrichment factor may be obtained by increasing the volume of the standard/sample solutions for a fixed volume of chloroform, but a larger separating chamber must be used.

3.1.4. Analytical characteristics

Fig. 4 shows the calibration graph and typical SI-grams of diphenhydramine hydrochloride. Calibration graph of diphenhydramine hydrochloride versus peak area was found to be linear in the range of 10–40 mg/l with the linear equation and correlation coefficient (r^2) of $y = 0.2248x - 0.1517$ and 0.9992, respectively. The limit of detection (LOD) calculated as three times the standard deviation was 1.90 mg/l. The relative standard deviation (%RSD) was less than 3.6 (n = 11, 20 mg/l diphenhydramine hydrochloride). The recovery was found to be 102%. Sample throughput of 5 h^{-1} can be achieved. A higher throughput may be possible if the modified autoburette would be replaced by another type of syringe pump, although it could be more expensive.

According to our previous study [2], using this reaction, the amount of the foreign compounds commonly presented together with diphenhydramine hydrochloride in preparations containing single tertiary alkylamine drug would not interfere in the determination.

3.1.5. Application to samples

The proposed SI method was applied to the determination of diphenhydramine hydrochloride in some pharmaceutical preparations (containing single tertiary alkylamine). Some locally commercial pharmaceutical preparations were taken as samples to be assayed. A weighed quantity of a sample was dissolved with deionized water, shaken for 15 min and adjusted to a volume with water to obtain a solution having a concentration in the range of a calibration graph. The solution was then filtered before analysis. The results were compared to those obtained by the standard method using high performance liquid chromatography [28], as summarized in Table 2. Evaluation by t-test at 95% confidence level indicates that there is no significant difference in the results obtained by the proposed SIA and the standard methods.

3.2. The assay of anionic surfactant

3.2.1. Optimization of the experimental parameters

The same SIA setup was used for the determination of anionic surfactant in water samples. The anionic surfactant reacts with methylene blue to form ion-association compound, which can be extracted into chloroform. The concentration of anionic surfactant can be determined by measuring the absorbance of the chloroform phase at 650 nm [29]. Some experimental parameters, i.e. operational sequence, volume and concentration of reagents were optimized. Zone sequence was chosen: standard/sample-methylene blue-chloroform. The aqueous sample containing anionic surfactant was firstly aspirated into the EC. Next, the methylene blue solution was introduced, the ion-association compound formed. Then, chloroform was aspirated and the extraction was done by programming the autoburette to aspiration and dispensed modes. After extraction in the EC, the solution was propelled to the separating chamber, where the separation between aqueous and chloroform phases occurred. The chloroform phase containing ion-association compound was re-aspirated into the EC for transportation to a detector.

The concentration of methylene blue solution was varied from 0.0038 to 0.02%. The sensitivity increased with increasing the methylene blue concentration and reached the maximum at

Table 2
Determination of diphenhydramine hydrochloride in pharmaceutical preparations by the proposed and standard methods

Sample	Label	Proposed method		Standard method [28]	
	(mg/5 ml)	Amount found (mg/5 ml)	% label	Amount found (mg/5 ml)	% label
1	12.50	12.57 ± 0.27	101	13.55 ± 0.49	108
2	12.50	12.81 ± 0.55	102	13.91 ± 0.12	111

Table 3
Selected conditions and operational sequence of the SIA system for the determination of anionic surfactant in water samples

Sequence	Valve position	Mode[a]	Volume (μl)	Description
1	2–4, 8–9	PIP	250	Aspirate standard/sample into EC
2	5	PIP	250	Aspirate methylene blue into EC
3	10	PIP	400	Aspirate chloroform into EC
4	1	PIP	1300	Extract
5	1	DIS C	2000	Extract
6	1	PIP	1100	Extract
7	1	DIS C	2000	Extract and propel to the separating chamber[b]
8	7	DIS C	3000	Clean EC
9	10	PIP	400	Aspirate chloroform into EC
10	1	PIP	250	Aspirate chloroform phase containing ion-association compound into EC
11	6	DIS C	40	Dispense chloroform phase containing ion-association compound through the selection valve
12	7	DIS C	3000	Clean EC
13	10	PIP	2900	Aspirate chloroform into EC
14	6	DIS C	2000	Dispense to detector with flow rate of 10 ml/min

[a] PIP = pipetting/aspiration, DIS C = cumulative dispensing.
[b] No standing time for phase separation.

0.01%. Using a methylene blue solution of higher concentration than 0.01% can cause higher background level due to methylene blue partition into chloroform phase. Therefore, methylene blue concentration of 0.01% was selected. The selected conditions and operational sequence of the SIA system for the determination of anionic surfactant are summarized in Table 3.

3.2.2. Analytical characteristics

Using the SIA setup shown in Fig. 1 and under the above-mentioned selected conditions, the linear calibration graph was obtained for SDS in the concentration range of 1–10 mg/l with the regression equation of $y = 1.0711x - 0.1356$ and the correlation coefficient of 0.9995, as shown in Fig. 5. The LOD of 0.48 mg/l was obtained. The RSD was less than 5% ($n = 11$, 5.0 mg/l SDS). A five samples per hour assay can be obtained. Increase of the throughput may be obtained by employing another type of pump.

3.2.3. Application to samples

The applicability of the proposed procedure was demonstrated for the determination of anionic surfactant in drainage water samples. The results were compared to those obtained by the methylene blue standard method [29], as summarized in Table 4. There is no significant difference in the results obtained from the proposed and the standard procedures.

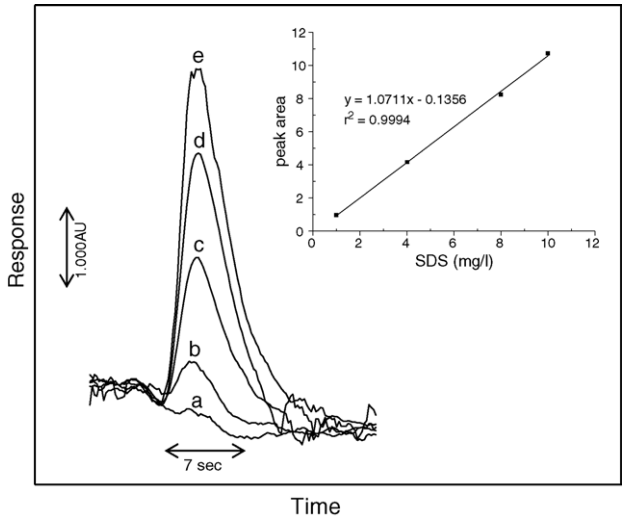

Fig. 5. Typical SI-grams and calibration graph of standard SDS; a = blank, b = 1, c = 4, d = 8, e = 10 mg/l.

Table 4
Determination of anionic surfactant in drainage water samples by the proposed and standard methods

Sample	Found (mg/l)	
	Proposed method	Standard method [29]
1	3.38 ± 0.01	3.42
2	2.76 ± 0.02	2.77

4. Conclusion

Sequential injection analysis with lab-at-valve approach for alternative simple on-line liquid–liquid micro-extraction was exploited. A simple fabricated LAV unit, a separating chamber, attached at a port of a conventional multiposition selection valve offers an on-line automated extraction in a micro-scale. Therefore, consumption of sample, reagent and organic solvent, also waste generation, is tremendously reduced. A cost effective LAV apparatus can be easily fabricated using a less precise machine tools. Applications for the assays of diphenhydramine hydrochloride in pharmaceutical preparations and anionic surfactant in water samples have been successfully demonstrated. Instead of using Spectronic 21 detector, investigation to plug a fiber optic spectrophotometric detection system to the conical separation chamber has been in progress to make such a system become a real SIA-LAV liquid–liquid extraction.

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