Sequential injection technique applied to pharmaceutical analysis

P. Solich, M. Polášek, J. Klimundová, J. Ruzicka

The versatility of sequential injection analysis (SIA), based on programmable flow, has been recently enhanced by the miniaturization implemented in lab-on-valve (LOV) format. This article discusses the implication of this development and the use of SIA for chromatography and for bead injection, identifying a trend towards development of a versatile, multi-purpose analytical system.

Keywords: Automation; Chromatography; Continuous monitoring; Microfluidics; Pharmaceutical analysis

P. Solich*, M. Polášek
Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, 500 05 Hradec Králové, and The Research Centre LN00B125, 500 05 Hradec Králové, Czech Republic

J. Klimundová
Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, 500 05 Hradec Králové, Czech Republic

J. Ruzicka
Department of Chemistry, Box 351700, University of Washington, Seattle, WA 98195, USA

*Corresponding author.
Tel.: +420 495067294; Fax: +420 495518718;
E-mail: solich@faf.cuni.cz

1. Introduction

Traditionally, pharmaceutical analysis relies heavily on chromatography, yet the assay of pharmaceuticals also frequently requires the use of reagent-based techniques with spectrophotometric, fluorescence or electrochemical detection. Automation and miniaturization of these solution-based assays to make them fast and efficient are essential for many routine and research tasks in pharmaceutical laboratories. Ideally, instrumental techniques should be versatile, capable of accommodating a wide variety of assays without the need for system reconfiguration, and compatible with both optical and electrochemical detectors.

As successor to flow injection analysis (FIA), SIA has been used for pharmaceutical assays for a number of years. Pharmaceutical applications of FIA were summarized in a monograph [1] and recent reviews [2,3]. An excellent comprehensive review has been published on the use of SIA technology in all areas [4]. The utilization of SIA in the pharmaceutical field is summarized in Tables 1–4.

The main purpose of this article is to highlight SIA along with its most recent version, micro-SIA carried out in LOV format (micro-SIA-LOV). In addition, it concludes with a brief discussion of recent variants of SIA [i.e. sequential injection chromatography (SIC) and bead injection (BI)], since these emerging techniques have considerable potential for pharmaceutical applications.

2. Principles of SIA

SIA and its microminiaturized variant micro-SIA operate on the same principle (Fig. 1). The core of the system is a computer-controlled, multi-position valve, the center port of which is connected via a holding coil to a high-precision syringe pump that is used to draw samples and reagents into the holding coil, to mix them and to transport the reacting mixture into the flow-through detector.

Micro-SIA systems in the LOV configuration have all the valve ports and the flow cell integrated within a transparent, monolithic structure mounted on top of the multi-position valve. This allows miniaturization of the flow channels with the result that the sample and reagent volumes are downscaled to the range 10–20 μL per assay, while waste production is typically 0.1–0.2 mL per assay. In contrast to the lab-on-chip design, the channels in the LOV system are much wider (0.5 mm I.D) so that the volume/surface ratio is large. As a result, LOV channels are not prone to clogging by real-life samples, which may contain solid particles. In the BI variant of micro-SIA, a suspension of microspheres (30–150 μm) is pumped through the LOV module.

As the name ''SIA'' implies, reagent and sample solutions are aspirated

 0165-9936/03/$ - see front matter © 2003 Published by Elsevier B.V. doi:10.1016/S0165-9936(04)00304-8

Table 1. Optical methods

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample through-put (h^{-1})	Ref.
Ascorbic acid (Vitamin C)	Drug formulations	Oxidation of Vitamin C by Ce(IV), spectrophotometric determination at 410 nm	30–200 mg/l	–	–	[28]
Ascorbic acid (Vitamin C)	Tablets and liquids	Absorbance of redox indicator ferroin incorporated into the membrane (redox optical sensor – optrode)	$2.5x10^{-3}$–$1x10^{-1}$ mol/l	–	17	[29,30]
Ascorbic acid (Vitamin C)	Tablets	Redox reaction between ascorbic acid and permanganate in acidic medium, spectrophotometric determination at 525 nm	0–1200 mg/l	–	60	[31]
Boron	Eye lotion	Complexation between D-sorbitol and boric acid, then acid-base reaction with methyl orange, spectrophotometric determination at 520 nm	0.06–12 mg/l	0.06 mg/l	30	[54]
Bromazepam	Tablets	Complexation reaction of bromazepam with iron(II) in hydrochloric acid, spectrophotometric determination at 585 nm	$5x10^{-4}$–$1.5x10^{-3}$ mol/l	–	–	[32]
Bromazepam	Tablets	Complexation with iron(II), spectrophotometric determination at 585 nm; chemometric optimization	300–1000 mg/l	–	–	[33]
Calcium	Effervescent tablets	Complexation reaction with cresolphthalein complexone; spectrophotometric determination at 578 nm	0–20 mg/l	0.05 mg/l	43	[34]
Ciprofloxacin	Tablets Infusion	Complexation reaction with iron(III), spectrophotometric determination at 447 nm	50–500 mg/l	–	–	[35]
Norfloxacin	Tablets	Complexation reaction with iron(III), spectrophotometric determination at 430 nm	50–400 mg/l	–	–	[35]
Iodide	Pharmaceutical preparations	Catalytic effect of iodide on the reaction between Ce(IV) and As(III), spectrophotometric determination at 410 nm	0.002–0.5 mg/l	1.5 µg/l	15	[36]

(*Continued on next page.*)

Table 1 (*continued*)

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample through-put (h^{-1})	Ref.
Iron (II)	Anti-anaemic pharmaceuticals	Complex of Fe(II) and 2,2-bipyridyl at pH 4.5, spectrophotometric detection at 523 nm	5.0–40.0 mg/l	0.97 mg/l	100	[37]
Iron(III)	Capsules Tablets	Complex with iron, spectrophotometric detection at 667 nm; dialysis	100–1000 mg/l	45 mg/l	8	[38]
Oxprenolol	Tablets	Oxidation of oxprenolol with Ce(IV), spectrophotometric detection at 480 nm	50–400 mg/l	–		[39]
Paracetamol	Tablets Powder Combined preparations	Oxidation of paracetamol by hexacyanoferrate(III) and reaction with phenol in the presence of ammonia, spectrophotometric determination at 630 nm	0–60 mg/l	0.2 mg/l	27	[40]
Promethazine	Tablets Syrup Elixir	Complexation reaction of promethazine hydrochloride with Pd(II) spectrophotometric determination at 540 nm	50–400 mg/l	–	–	[41]
Theophylline	Solution of theophylline and caffeine	Microcolumn packed with anion exchange beads; adsorbed theophylline eluted by HCl with spectrophotometric determination at 274 nm	0.022–0.28 mmol/l	–	–	[42]
Trimeprazine	Pharmaceutical preparations	Complexation with Pd(II), spectrophotometric determination at 515 nm	50–400 mg/l	–	60	[43]
Perphenazine	Pharmaceutical preparations	Complexation reaction with Pd(II), spectrophotometric determination at 560 nm	50–500 mg/l	–	–	[43]
Zinc	Pharmaceutical samples	Reaction with xylenol orange, spectrophotometric determination at 568 nm	10–60 mg/l	0.42 mg/l	30	[44]
Promethazin hydrochloride	Tablets	Chemiluminescence	$1.558x10^{-4}$–$1.870x10^{-3}$ mol/l	–	–	[53]
Sulphacetamide	Eye drops	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.012–1.20 mmol/l	0.012 mmol/l	120	[7]

(*Continued on next page.*)

Table 1 (*continued*)

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample through-put (h^{-1})	Ref.
Sulphafurazole	Tablets	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.009–0.88 mmol/l	0.009 mmol/l	120	[7]
Sulphanilamide	Standard solutions	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.015–1.50 mmol/l	0.013 mmol/l	120	[7]
Sulphathiazole	Standard solutions	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.009–0.92 mmol/l	0.009 mmol/l	120	[7]
Sulphadimidine	Standard solutions	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.035–0.87 mmol/l	0.030 mmol/l	120	[7]
Sulphamethoxypyridazine	Standard solutions	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.019–0.95 mmol/l	0.015 mmol/l	120	[7]
Trimethoprim	Tablets	Oxidation of trimethoprim by $KMnO_4$, chemiluminescence determination	20–100 mg/l	0.1 mg/l	30	[9]
Procaine hydrochloride	Injections	Oxidation of the analytes by permanganate, chemiluminiscence detection	0.5–50 mg/l	0.3 mg/l	120	[8]
Benzocaine	Babydent STADA solution	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.5–25 mg/l	0.3 mg/l	120	[7]
Tetracaine hydrochloride	Standard solutions	Oxidation of drug by permanganate in acidic medium, chemiluminiscence determination	0.2–25 mg/l	0.1 mg/l	120	[7]
Morphine	Morphine in non-aqueous solution	Chemiluminescence reaction of morphine with acidic potassium permanganate in the presence of sodium polyphosphate	0.01–0.1% m/v morphine	0.00003%	120	[6]
Morphine	Standard solutions	Chemiluminescence reaction of morphine with acidic potassium permanganate in the presence of sodium hexametaphosphate	2.5×10^{-6}–3.0×10^{-5} mol/l	10^{-8} mol/l	–	[4]

Table 2. Electrochemical methods

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample through-put (h^{-1})	Ref.
Azidothymidine	Capsules	Amperometric immunosensor, graphite paste with anti-AZT	80–2000 nmol/l	10 nmol/l		[52]
S-captopril	Tablets	Amperometric biosensor, based on L-amino acid oxidase; enantiopurity test	0.05–1.50 μmol/l	16 nmol/l	80	[10]
S-captopril	Synthesis process monitoring	Enantioselective membrane electrode based on maltodextrin, potentiometric determination	10^{-6}–10^{-3} mol/l	4.4×10^{-7} mol/l	38	[11]
R-captopril		Amperometric biosensor	10^{-7}–10^{-6} mol/l			
Captopril	Tablets	Potentiometric titration with Ag(I) solution	Linear to $1.4x10^{-3}$mol/l	–	–	[45]
S-perindopril	Standard solutions	Amperometric biosensors, based on L- and D-amino acid oxidase	60–800 nmol/l	40 nmol/l	30	[46]
R-perindopril			60–900 nmol/l	40 nmol/l		
Diclofenac	Tablets Injections Suppository	Potentiometric detection, Ion-selective electrode	$5x10^{-6}$–$1x10^{-2}$ mol/l	$2x10^{-6}$ mol/l	33	[47]
Potassium clavulanate	Tablets	Potentiometric detection of clavulanate anion and potassium cation simultaneously	$2x10^{-3}$–$1x10^{-1}$ mol/l	–	53	[12]
Riboflavin	Vitamin B tablets Multivitamin tablets	Adsorptive stripping voltammetry, determination and photodegradation study	0.0–0.8 μmol/l	0.07 μmol/l	–	[13]
Vitamin B6	Multivitamin formulations	Tubular, pyridoxin-sensitive electrode, potentiometric detection	$1.0x10^{-5}$–$5.0x10^{-4}$ mol/l	–	–	[48]

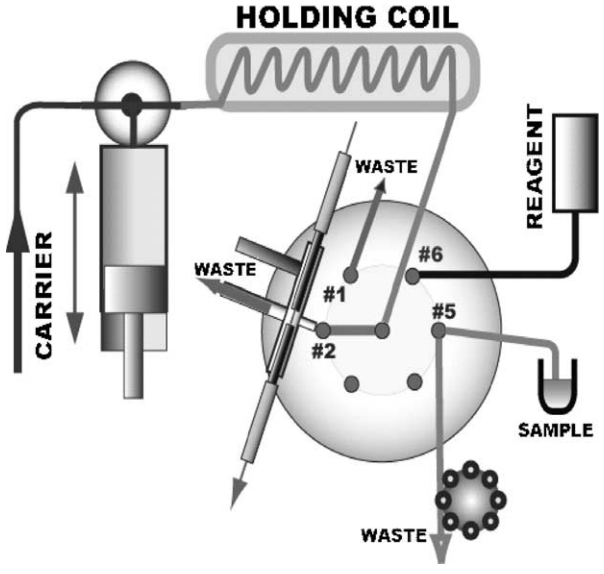

Figure 1. Micro-SIA system in LOV configuration. For continuous monitoring, the sample cup is replaced by the monitored vessel via sampling loop, which may be led back to the vessel rather than to waste.

sequentially into the holding coil and the resulting mixture is monitored within the flow cell. For fast chemical reactions, detection occurs ''on the fly'', as the reacted mixture flows through the detector. For slower reactions, the flow is stopped while the reacting mixture is held within the detector. This allows reaction rate measurements to be carried out – as often needed for enzymatic assays.

SIA is more versatile than FIA since it is based on programmable flow that allows reaction conditions (sample dilution, reagent addition, time for reaction to proceed) to be selected at will from a computer keyboard. However, FIA can do things that SIA cannot do very well, particularly monitoring some in-line processes requiring continuous flow for a long time.

Sample introduction into a LOV system is carried out via a flow-through port that can be connected to a sample changer or to process vessels for continuous monitoring (as required in dissolution studies or fermentation monitoring) or even filled manually by Eppendorf pipettes, when samples are few or of limited availability.

Table 3. Dissolution study

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample through-put (h^{-1})	Ref.
Ergotamine tartrate	Tablet	Fluorimetric determination ($\lambda_{ex} = 236$ nm; $\lambda_{em} \geq 390$ nm)	0.03–0.61 mg/l	0.01 mg/l	120	[19]
Acetylsalicylic acid	Tablets: normal, composed, effervescent, sustained release	In-line hydrolysis to salicylate and potentiometric determination by tubular salicylate-selective electrode	0.05–10 mmol/l	0.05 mmol/l	6	[18]
		Dissolution test of normal and sustained release tablets			20	
Aspirin	Compound aspirin tablets	Absorbance spectra (220–310 nm); simultaneous drug dissolution of three compounds	44–220 mg/l	–	45	[17]
Phenacetin			30–150 mg/l			
Caffeine			10–50 mg/l			
Ibuprofen	Normal tablets	Spectrophotometric determination at 222 nm	1–120 mg/l	1 mg/l	42	[16]
	Sustained-release capsules and controlled-release matrix tablets		2–350 mg/l	2 mg/l	42	

Table 4. Bioprocess monitoring

Analyte	Matrix	Detection method	Linear range	Detection limit	Sample through-put (h^{-1})	Ref.
Glucose	Cultures of penicillium chrysogenum	Enzymatic reaction using glucose oxidase – formation of H_2O_2, which is detected by chemiluminescence involving luminol	0.01–7.0 g/l	–		[21]
Penicillin		Formation of penicilloic acid by penicillinase; the acid is detected by chemiluminescence method or decolorization method	0.1–1.8 g/l	–		
Glucose	Broth fermentation process	Nylon tubular reactor with immobilized glucose oxidase – detection of H_2O_2 with luminol, chemiluminescence detection	30–600 μM	15 μM	54	[49]
Glucose	Penicillin cultures (also lactic acid)	Enzymatic reaction using glucose oxidase – formation of H_2O_2, which is detected by chemiluminescence involving luminol	5–100 mg/l	5 mg/l	20	[20]
Penicillin		Formation of penicilloic acid by penicillinase; the acid is prevents chemiluminescence generating reaction between iodine and luminol	10–1500 mg/l	10 mg/l		
Glucose	Standard solutions	Jet ring cell with a renewable support for amperometric detection	0.1–5 mM	0.1 mM		[50]
Glucose	Yeast fermentation (also total biomass)	Glucose determination by Trinder reaction, measurement of absorbance spectra	0–1200 mg/l		7	[51]

3. Pharmaceutical assays

The samples for pharmaceutical assays vary in form, being solids (tablets, capsules), pastes (ointments, creams) or liquids (emulsions, suspensions, solutions). In addition to the active ingredient, auxiliary substances are added to pharmaceutical preparations (e.g., fillers, binders, suspending agents and preservatives). In many cases, the non-active ingredients do not interfere with assay of the active component and can be removed easily. Another advantage is that the approximate composition of the assayed material is almost always known.

Systematic analysis of pharmaceuticals during their synthesis, extraction and purification is vital to assure their quality at all stages of production, since it affects

manufacturing capacity and production efficiency. Speed and high sensitivity are additional requirements for quality control in routine laboratory assays.

4. Detection techniques

4.1. Optical methods

Spectrophotometry, fluorescence and chemiluminescence have been used most frequently (Table 1). Since the analytical application of chemiluminescence gained momentum with the growth of flow methods [5], this section presents a short account of its uses.

Barnett determined morphine in aqueous and in non-aqueous solutions [6]. Detection was based on chemiluminescence produced by the reaction of morphine with acidic potassium permanganate in the presence of sodium hexametaphosphate.

Paseková and Polášek used chemiluminescence detection for the assay of sulphonamides [7] and local anesthetics [8]. They used sulphanilamide as a model substance. Luminescence was emitted during the oxidation of the analyte by potassium permanganate in sulfuric acid medium. A number of enhancers were tested (formic acid, sodium hexametaphosphate, glycolaldehyde, glutaraldehyde, Rhodamine B). Finally, 0.1 M glutaraldehyde was chosen to enhance the signal. The substances analyzed were sulphanilamide, sulphacetamide, sulphadimidine, sulphafurazole, sulphametoxypyridazine, sulphaguanidine. Assay of local anesthetics used the same principles. Procaine hydrochloride, benzocaine and tetracaine hydrochloride were oxidized by permanganate in aqueous sulfuric acid. Enhancers of cheminiluminescence intensity were 4-hydroxybiphenyl, Rhodamine B, glycolaldehyde, glutaraldehyde and formic acid, of which the latter (0.37 M) was found most effective. Hexametaphosphate was used as enhancer of chemiluminescence reaction between trimethoprim [9] and potassium permanganate. The method was applied to assay Triprim tablets for the active ingredient and for their uniformity tests.

4.2. Electrochemical detection

Potentiometric detection with ion-selective electrodes (ISEs) and titrimetry have been used most frequently (Table 2). For captopril [10,11], the determination procedure was based on colorimetric measurement of the captopril-palladium complex in acid and was compared with potentiometric titration of captopril with a 10^{-3} mol/l Ag(I) solution. Colorimetry has the advantage of selectivity even in the presence of high levels of matrix components, while potentiometric titration is more repeatable.

The potentiometric determination of captopril with an ISE based on cyclodextrin was compared with its fluorimetric determination. Also, the assay of potassium clavulanate [12] was based on the potentiometric detection of the clavulanate anion and the potassium cation by ISEs.

SIA with adsorptive stripping voltammetric detection has been proposed by Kubiak et al. [13]. The method was applied to determination of riboflavin [13] in vitamin pills and to study the photodegradation process of riboflavin in aqueous solutions. In general, however, electrochemical detection is not favored in pharmaceutical analysis, being viewed as the last resort.

5. Continuous monitoring

Since the first application of FIA for the on-line determination of enzymes and proteins [14], and the subsequent introduction of SIA, the advantages of flow injection as a tool for industrial process control have been recognized. With the advent of the micro-SIA concept, the automation of continuous monitoring has been even better, since the flow-through port of the LOV device (port 5, Fig. 1) can be supplied with constantly circulating solution from the monitored vessel.

Miniaturization of the flow system dramatically decreases sample consumption (to 10 μL per assay) and increases sampling frequency, yielding a response very close to the requirements of real-time monitoring. Also auxiliary reagents, when needed, are consumed in μL amounts, thus enabling continuous, prolonged monitoring with minimal reagent consumption and waste generation.

In addition to derivatization, micro-SIA technique allows automated sample dilution up to 5000 times, an important feature when the change of analyte concentration has to be monitored in a wide concentration range. The frequency of monitoring can be adjusted at will, since the system is under computer control.

Micro-SIA-LOV has been so far used for bioprocess monitoring of ammonia, glucose and glycerol [15].

5.1. Dissolution studies

The rate of release of active compounds from pharmaceutical formulations — to deliver the correct dosage of key ingredients to the target — is an essential parameter of pharmaceutical quality control. However, manual sampling of solutes and solvents is time- and labor-consuming, with poor resolution of the dissolution profile through restrictions on the number of samples, that can be withdrawn and analyzed. Dissolution tests ('in vitro' availability) are among the tests officially required by the majority of pharmacopoeias world-wide, so their automation is a major goal in pharmaceutical analysis.

The first automated system for drug-dissolution studies based on the sequential injection technique was described by Liu and Fang [16] for monitoring of

ibuprofen tablets, sustain release capsules and controlled release matrix tablets. The 800-μL sample of the assayed solvent from the dissolution vessel was aspirated into a holding coil through on-line filters. After delivering an aliquot (40 μL) of aspirated solution into the transfer line connected to the spectrophotometric detector, the residual solvent in the holding coil was returned to the dissolution vessel in order to clear the filter by flow reversal and to decrease sample consumption. Every hour, seven samples run in hexa-replicates were processed with total of 42 measurements. The system was very reliable, allowing continuous runs for 12 h and yielding an overall RSD of 2.18% (n=72) at a 50% final concentration level of the dissolution solution.

Later, the same authors published a paper [17] dealing with the simultaneous spectrophotometric monitoring of dissolution profiles of aspirin, phenacetin and caffeine in aspirin tablets.

Paseková and Sales [18] described an SIA-based dissolution method with a tubular salicylate selectrode suitable for potentiometric determination of acetylsalicylic acid. The flow-through electrode is formed by a PVC membrane containing 29.2% (w/w) PVC, 5.8% (w/w) tetraoctylammonium salicylate (ionic sensor), 58.5% o-nitrophenyloctylether (plasticizer) and 6.5% (w/w) p-tert-octylphenol (stabilizing additive which increases electrode selectivity). The electrode is used for sensing acetylsalicylic acid after its on-line chemical hydrolysis to salicylate.

Legnerová and Sklenářová [19] proposed an SIA method with fluorescence detection for monitoring dissolution profiles of ergotamine tartrate in pharmaceutical formulations (Bellaspon tablets). The results that they obtained correlated well with values required by the contemporary British Pharmacopoeia.

5.2. Bioprocess monitoring

This task is a complex since the assayed materials always contain a wide variety of species, including enzymes, proteins, carbohydrates, antibodies, yeast cells, and particulate matter (Table 2). Among the first SIA applications was the work of Min and Nielsen [20], who used an SIA set-up involving a 10-port multiposition valve and a chemiluminescence detector for monitoring glucose, lactic acid and penicillin. Glucose and lactic acid are the typical substrates and penicillin is major product of penicillium cultures. Glucose is converted to glucono-δ-lactone by glucose oxidase with the simultaneous formation of hydrogen peroxide that is detected by chemiluminescence generated in the presence of luminol. The penicillin analysis is based on formation of penicilloic acid.

Later, the same authors improved the spectrophotometric detection of penicilloic acid by decolorization of the iodine-starch complex [21].

5.3. Pharmacokinetic studies

Continuous monitoring of binding, release or consumption of drugs by tissues maintained *in vitro* is the central task of pharmacokinetic research. Sklenářová and Pávek [22] monitored the transporter-mediated passage of a model substrate, rhodamine 123 (Rho123), through human placenta was monitored by SIA technique. The method provided real-time monitoring of Rho123 concentrations in both maternal and fetal compartments using fluorescence detection. Transport of Rho123 was scanned under various conditions (ATP-synthesis inhibition) and several inhibitors of P-glycoprotein transporter were tested. Since the volumes of monitored perfusion liquids are often very small, use of micro-SIA for pharmacokinetic monitoring has an obvious advantage.

6. Recent developments and conclusions

Since its discovery [23], FIA has been a useful tool for pharmaceutical analysis and research. Along with a wide variety of reagent-based assays, including enzymatic assays, FIA has been found to have many uses in continuous monitoring and quality control. These FIA assay protocols can easily be adjusted to work in SIA format, albeit with substantially reduced reagent consumption. More importantly, however, the SIA concept, based on programmable flow offers as yet unexplored avenues for development of new methodologies applicable to pharmaceutical analysis.

6.1. Micro-SIA

In LOV format, micro-SIA is positioned between traditional flow techniques that operate on the mL scale and the more futuristic designs of the microTAS (micro total analysis system) concept that is supposed to function in the nL scale. By operating in the μL range, with channel diameters of 0.5 mm, micro-SIA-LOV systems are compatible with real-life samples that may often contain particles that would clog the μm-sized channels of nL-scale devices.

The choice of channel dimensions (i.e. a large diameter with a very short length) is the unique feature of the LOV concept, as it has two significant advantages:

- it features a large volume/surface ratio that minimizes the unwanted adsorption on channel walls that may result in carryover; and,
- even more importantly, this channel geometry allows LOV devices to be used as a platform for the BI technique.

6.2. BI

BI, another variant of the FIA technique, is the most advanced. In its simplest form, microspheres (Fig. 2A) are injected into a LOV conduit, where they are trapped in a flow cell. Next, the sample zone (Fig. 2B) is then injected and perfused through the beads, while the sample components react with functional groups on the bead surfaces (Fig. 2C). Retained analyte molecules are detected by spectroscopy in their native form, or reacted in situ with suitable color or fluorescent reagents (Fig. 2D). At the end of assay protocol, beads are discarded to waste (Fig. 2E).

The unique feature of BI is the high precision of the bead delivery, absence of carryover and the fast, automated renewal of the reactive solid phase that is delivered into the LOV in uniform composition throughout series of measurements [24].

Another application of the BI technique was described for determination of thiamine in pharmaceutical preparations [25].

6.3. SIC

SIC is based on using a monolithic separation column that is placed between the multi-position valve of the conventional SIA system and the flow cell (Fig. 3). Being made from a macroporous silica rod that allows coupling to a low-pressure SIA system, the monolithic column has a high resolving power but an extremely low flow resistance without any loss in performance. Surprisingly, the performance of the reverse phase monolith is equivalent to a typical C-18 5-μm particulate high-performance liquid chromatography column. The unique feature of SIC is the economy and speed of gradient elution that is achieved without any gradient device (e.g., four different pharmaceuticals could be separated by gradient elution with methanol/water eluent (Fig. 4 [26]).

More detailed information on monolithic columns is available on the Merck website [www.chromolith.com].

The material, presented in this article, leads to the interesting conclusion that the versatility of SIA technology, based on programmable microfluidic manipulations, allows, for the first time, the same instrument to be used for a diversity of tasks:

- serial assays;
- continuous monitoring;
- optical methods;
- electrochemical sensing;
- enzymatic assays; and,
- chromatographic separations.

In addition, micro-SIA has been also used as the ''front end'' to capillary electrophoresis and electrospray mass spectrometry [27].

For pharmaceutical analysis, the versatility of the SIA concept will lead to considerable savings in time and effort, since the use of the same methodology, the same instrumental set-up and software for different tasks is easier and more efficient than the present ''state

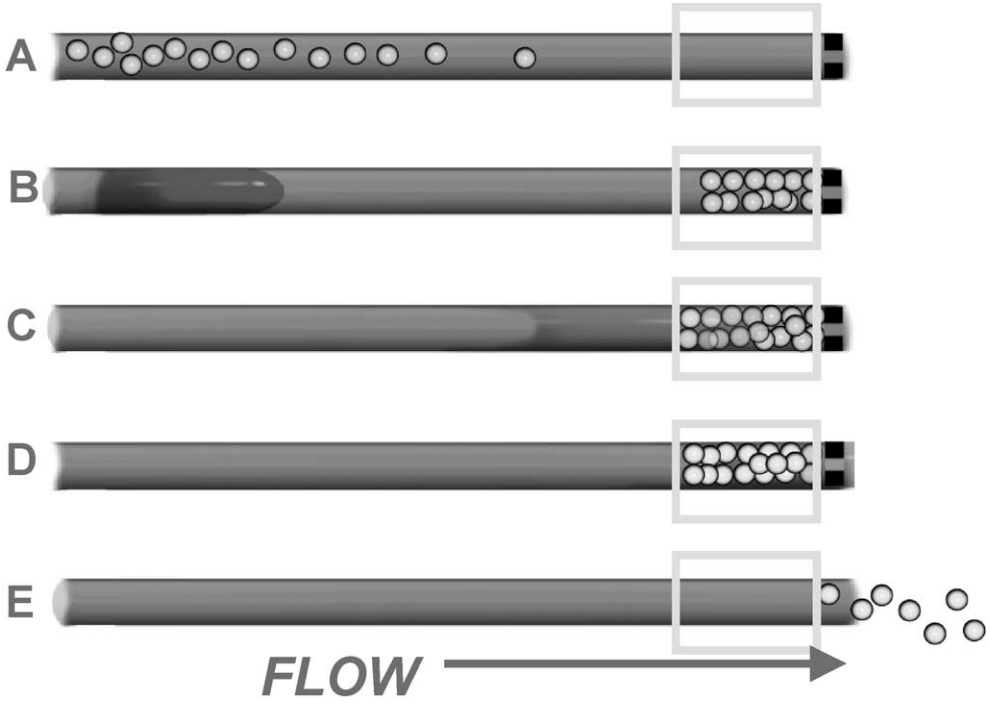

Figure 2. Principle of BI technique. (For details see text).

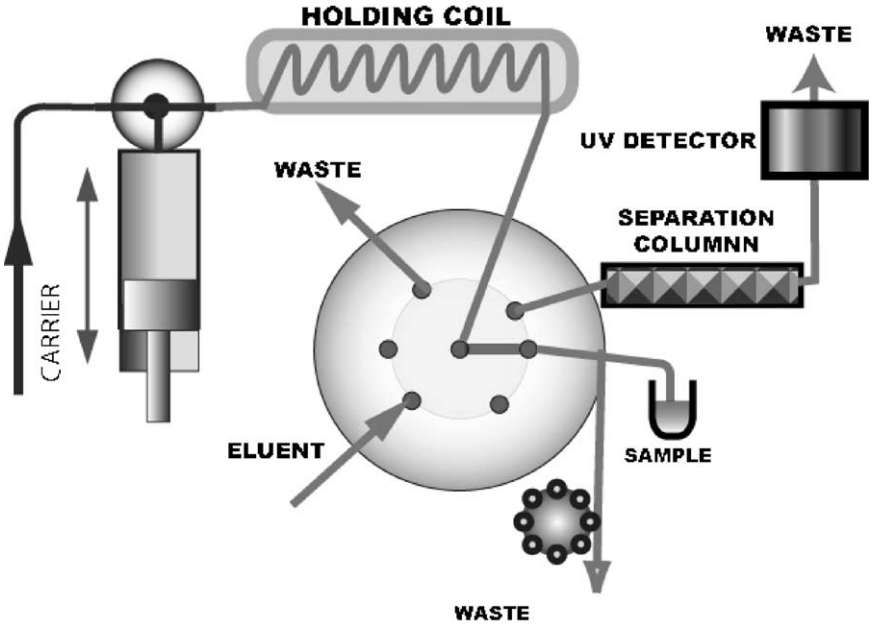

Figure 3. Manifold for SIC. Column length, 25 mm; Sample volume 50 μL; Eluent, methanol; Carrier, acetonitrile/water 40/70 + 0.05% triethylamine, pH 2.5. Chromatogram, see Fig. 4.

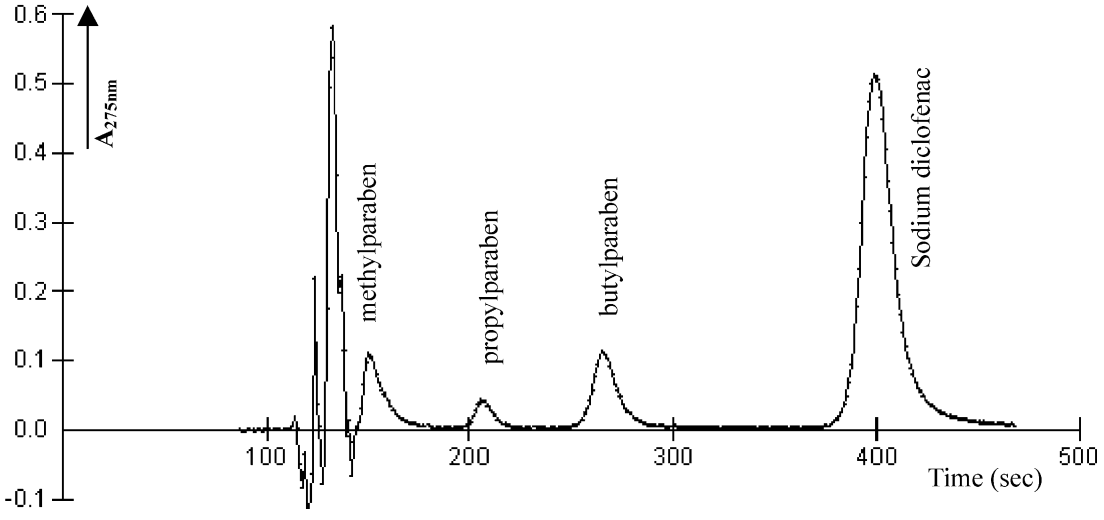

Figure 4. Separation of pharmaceuticals on Chromolith SpeedROD RP18e column (Merck) by SIC technique [26].

of the art'' that necessitates the use of different instruments, often controlled by different software, that are available in today's fractured market of analytical instrumentation.

Acknowledgements

The visit of J.R. to Czech Republic was sponsored by the Fulbright Scholar Program. The research of the Czech group was supported by grant GA UK 250/2002/B-CH/FaF and by The Research Centre LN00B125.

References

[1] J.M. Calatayud, Flow Injection Analysis of Pharmaceuticals, Taylor Francis, London, UK, 1996.
[2] P. Solich, H. Sklenářová, M. Polášek, R. Karlíček, J. Flow Inject. Anal. 18 (2001) 13.
[3] P. Solich, H. Sklenářová, M. Polášek, R. Karlíček, J. Flow Inject. Anal. 18 (2001) 118.
[4] C.E. Lenehan, N.W. Barnett, S.W. Lewis, Analyst (Cambridge, U.K.) 127 (2002) 997.
[5] D.J. Tucker, B. Toivola, C.H. Pollema, J. Ruzicka, G.D. Christian, Analyst (Cambridge, U.K.) 119 (1994) 975.
[6] N.W. Barnett, C.E. Lenehan, S.W. Lewis, D.J. Tucker, K.M. Essery, Analyst (Cambridge, U.K.) 123 (1998) 601.

[7] H. Paseková, M. Polášek, J.F. Cigarro, J. Dolejšová, Anal. Chim. Acta 438 (2001) 165.
[8] H. Paseková, M. Polášek, Talanta 52 (2000) 67.
[9] M. Polášek, M. Jambor, Talanta 58 (2002) 1253.
[10] R.I. Stefan, J.F. van Staden, H.Y. Aboul-Enein, Anal. Chim. Acta 411 (2000) 51.
[11] R.I. Stefan, J.F. van Staden, H.Y. Aboul-Enein, Talanta 51 (2000) 969.
[12] A.M. Pimenta, A.N. Araújo, M. Conceicao, B.S.M. Montenegro, J. Pharm. Biomed. Anal. 30 (2002) 931.
[13] W.W. Kubiak, R.M. Latonen, A. Ivaska, Talanta 53 (2001) 1211.
[14] H. Husted, K.H. Kroner, M. Kula, Biotech. Forum 2 (1985) 57.
[15] Ch. Wu, L. Scampavia, J. Ruzicka, B. Zamost, Analyst (Cambridge, UK) 126 (2001) 291.
[16] X.-Y. Liu, X.-L. Fang, Anal. Chim. Acta 358 (1998) 103.
[17] X.-Z. Liu, S.-S. Liu, J.-F. Wu, Z.-L. Fang, Anal. Chim. Acta 392 (1999) 273.
[18] H. Paseková, M.G. Sales, M.C. Montenegro, A.N. Araújo, M. Polášek, J. Pharm. Biomed. Anal. 24 (2001) 1027.
[19] Z. Legnerová, H. Sklenářová, P. Solich, Talanta 58 (2002) 1151.
[20] R.W. Min, J. Nielsen, J. Villadsen, Anal. Chim. Acta 312 (1995) 149.
[21] R.W. Min, J. Nielsen, J. Villadsen, Anal. Chim. Acta 320 (1996) 1999.
[22] H. Sklenářová, P. Pávek, D. Šatínský, P. Solich, R. Karlíček, F. Štaud, Z. Fendrich, Talanta 58 (2002) 1145.
[23] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 78 (1975) 145.
[24] J. Ruzicka, Analyst (Cambridge, U.K.) 125 (2000) 1053.
[25] H.L. Zhu, H.W. Chen, Y.G. Zhou, Anal. Sci. 19 (2) (2003) 289.
[26] D. Šatinský, P. Solich, P. Chocholouš, R. Karlíček, Anal. Chim. Acta (2003) (in press).
[27] J. Ruzicka, CD-ROM, FIA tutorial (www.flowinjection.com).
[28] S.M. Sultan, Y.A.M. Hassan, K.E.E. Ibrahim, Analyst (Cambridge, U.K.) 124 (1999) 917.
[29] D.T. Newcombe, T.J. Cardwell, R.W. Cattrall, S.D. Kolev, Anal. Chim. Acta 401 (1999) 137.
[30] D.T. Newcombe, T.J. Cardwell, R.W. Cattrall, S.D. Kolev, Lab. Rob. Autom. 12 (2000) 200.
[31] N. Lenghor, J. Jakmunee, M. Vilen, R. Sara, G.D. Christian, K. Grudpan, Talanta 58 (2002) 1139.
[32] S.M. Sultan, F.E.O. Suliman, Analyst (Cambridge, U.K.) 121 (1996) 617.
[33] S.M. Sultan, Y.A.M. Hassan, K.E.E. Ibrahim, Talanta 50 (1999) 841.
[34] J.F. van Staden, R.E. Taljaard, Anal. Chim. Acta 323 (1996) 75.
[35] F.E.O. Suliman, S.M. Sultan, Talanta 43 (1996) 559.
[36] J.A. Erustes, R. Forteza, V. Cerda, J. AOAC Int. 84 (2001) 337.
[37] P.C.C. Oliveira, J.C. Masini, Anal. Lett. 34 (2001) 389.
[38] J.F. van Staden, H. du Plessis, R.E. Taljaard, Anal. Chim. Acta 357 (1997) 141.
[39] F.E.O. Suliman, S.M. Sultan, Microchem. J. 57 (1997) 320.
[40] J.F. van Staden, M. Tsanwani, Talanta 58 (2002) 1095.
[41] S.M. Sultan, N.I. Desai, Analyst (Cambridge, U.K.) 122 (1997) 911.
[42] B. Dockendorff, D.A. Holman, G.D. Christian, J. Ruzicka, Anal. Commun. 35 (1998) 357.
[43] S.M. Sultan, F.E.O. Suliman, B.B. Saad, Analyst (Cambridge, U.K.) 120 (1995) 561.
[44] J.F. van Staden, M. Tsanwani, Fresenius' J. Anal. Chem. 371 (2001) 376.
[45] A.M. Pimenta, A.N. Araújo, M. Conceicao, B.S.M. Montenegro, Anal. Chim. Acta 438 (2001) 31.
[46] R.I. Stefan, J.F. van Staden, L.V. Mulaudzi, H.Y. Aboul-Enein, Anal. Chim. Acta 467 (2002) 189.
[47] A.M. Pimenta, A.N. Araújo, M. Conceicao, B.S.M. Montenegro, Anal. Chim. Acta 470 (2002) 185.
[48] R.N. Fernandes, M.G.F. Sales, B.F. Reis, E.A.G. Zagatto, A.N. Araújo, M. Conceicao, B.S.M. Montenegro, J. Pharm. Biomed. Anal. 25 (2001) 407.
[49] X. Liu, E.H. Hansen, Anal. Chim. Acta 326 (1996) 1.
[50] T. Lindfors, I. Lähdesmäki, A. Ivaska, Anal. Lett. 29 (1996) 2257.
[51] P.J. Baxter, G.D. Christian, J. Ruzicka, Chem. Anal. 40 (1995) 445.
[52] R.I. Stefan, R.G. Bokretsion, J.F. van Staden, H.Y. Aboul-Enein, Talanta 59 (2003) 883.
[53] S.M. Sultan, J.A.M. Hassan, A.M. Abulkibash, Talanta 59 (2003) 1073.
[54] J.F. van Staden, M. Tsanwani, Talanta 58 (2002) 1103.