Down scaling: From operation on lab bench space to manipulation at a valve

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Abstract

Down scaling for chemical analysis with flow-based techniques with the emphasis on sequential injection systems is reviewed. Sequential Injection (SI) systems with Lab-on-Valve (LOV) offer various advantages. SI with Lab-at-Valve (LAV) approach serves as an alternative cost effective means for automation and miniaturization in chemical analysis. Development of the techniques and their features are discussed.

Keywords miniaturization, flow based techniques, sequential injection analysis, lab-on-valve (LOV), lab-at-valve (LAV), cost effective analysis

1. Introduction

Research and development have favored the direction of "zero emission" and "greener" chemistry. This has also led to various approaches for cost effective chemical analysis. Flow-based techniques offer not only advantages gained in term of automation but also to down scaling in chemical analysis such as reduction in chemicals/ volumes used and waste, and in sample consumption; much less analysis time (leading to high sample through-puts), and less activities involving glassware cleaning. All concern cost effectiveness [1].

Flow injection analysis (FIA) may be known as an analytical technique based on microfluidic manipulation of samples and reagents. Usually, a sample is injected into a carrier/reagent solution which transports the sample zone into a detector to monitor changes due to the desired (bio)chemical reactions which have been taken place. Chemical information will be obtained from the detector response (absorbance, conductance, current, fluorescence, radioactivity, mass spectra, etc.), such as concentration from a calibration graph (signal value vs. concentration) [2, 3, 4].

Microfluidics have been employed for flow analytical techniques either in continuous flow modes, such as FIA and chromatography with constant unidirection (forward) flow of carrier to transport a sample from an injector to a detector, or programmable flow modes such as sequential injection analysis (SIA) with flow reversal to mix sample with reagent(s) or to stop flow to accommodate reaction time [2, 3, 5].

FIA was introduced by Ruzicka and Hansen as an approach for downscaling from batch analysis to flow analysis-from a beaker to microfluidics [3, 6, 7]. As the second generation, SIA was developed for more degrees in automation by having a bi-directional pump for programmable flow.

2. Lab-on-Valve (LOV) Approaches

Ruzicka [8] proposed a SI with "Lab-on-Valve" (LOV) system, as the third generation, to perform micro- or nanoanalysis by integrating all the necessary laboratory facilities for a variety of analytical schemes, including sampling, chemical reaction, and detection in a conduit at a multiposition valve. The conduit is a very precisely positioned engraved Plexiglas using computer-aided-design (CAD). This is mounted to replace the headpiece of a selection valve (see Figure 1 [3]). As SI-LOV is a system/device for analytical processes taking place, it could serve then as a type of micro-total analysis system (μ-TAS). SI-LOV has proven to have more tolerance than that of chip based approaches for real applications to dirty samples or samples with complicate matrices.

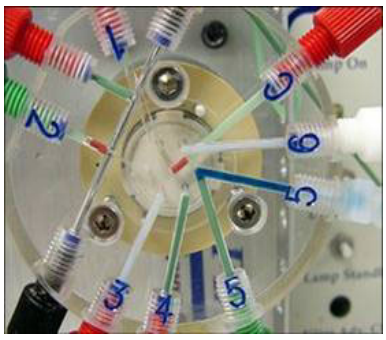

Fig. 1 SIA -Lab-On-Valve®
(more detail in www.flowinjection.com)

SI-LOV has become a platform for microSI manipulation using microliter volumes of sample and reagents per analysis. SI-LOV has been cooperated well with various techniques apart from UV-VIS such as bead injection, sequential injection affinity chromatography, mass spectrometry, capillary electrophoresis, atomic spectroscopy (AAS, ICP-AES and ICP-MS) and potentiometry.

Ruzicka has demonstrated the usefulness of SI-LOV systems to various applications [7, 9, 10, 11], including environmental analysis, pharmaceutical and bioanalytical assays.

Hansen, Wang and Miró have extensively reviewed the applications of the microSI-LOV systems with various sample

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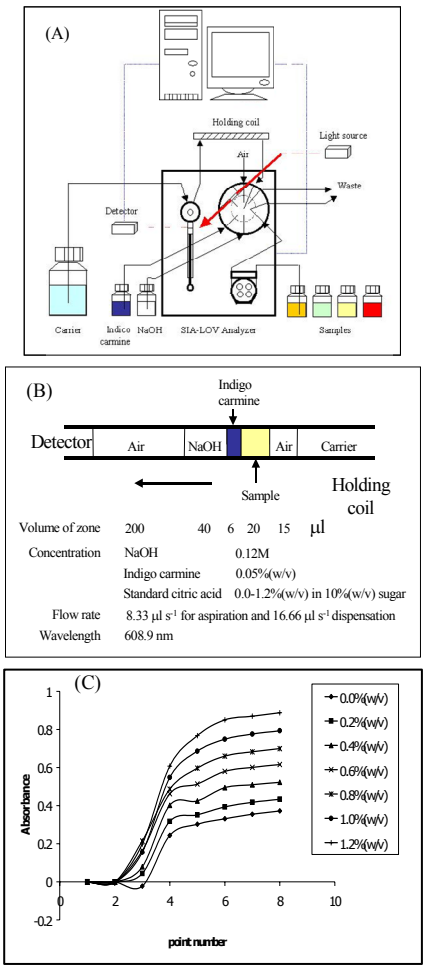

Fig. 2 Mono-segmented flow using SI-LOV for microtitration (adapted from ref 27): (A) the SI-LOV system, (B) the conditions and (C) the signal profiles.

pretreatment methods for determination of trace levels of elements using ETAAS and ICPMS. These sample pretreatments include sample dilution, dialysis, derivatization, hydride generation, liquid-liquid and solid phase extraction, chromatographic separation and preconcentration with beads and packed column [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23].

Coupling of the SI-LOV system with other detection systems such as AAS, FT-IR, electrochemistry has also been reviewed [24, 25].

Some previous reviews may be sources for information (Table 1). Table 2 summarizes some SIA-LOV developments.

Employing SI-LOV, micro-segmented microflow analysis [27] can be performed. Air segments are introduced to sandwich the sample-reagent plug to eliminate dispersion between the sample-reagent zone and the carrier stream. This enables promotion of mixing of analyte and reagent(s), with longer residence time and better sensitivity with low reagent consumption being possible. This was demonstrated by microtitration. By flow reversal, three solutions can be mixed together well in a mono-segmented zone. The air bubbles are to be discarded prior to absorbance measurement to obtain a smooth response. It should be noted that the signal profiles are not in the shape of peaks, which are different from normal SI-grams. (see Fig. 2). The concentration of a sample analyte can be obtained via a calibration graph. By the mono-segmented SI-LOV approach, the determination of copper using 2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene (Zincon) has been reported [32].

3. Lab-at-Valve (LAV) Approaches

As an alternative cost effective micro-total analysis system, the Lab-at-Valve (LAV) approach has been investigated. A LAV unit can be fabricated using an ordinary and less precise machine tool by designing for suitable function for the chemistry of interest, and for easy attachment at a port of a conventional selection valve in a simple usual way. There is no need to take out any part of a usually available selection valve [1, 47].

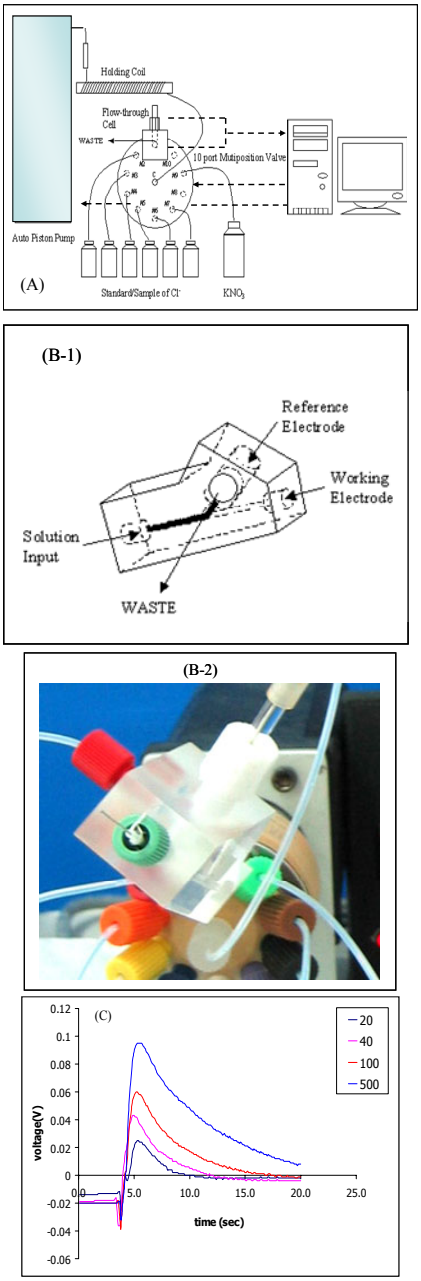

Fig. 3 SI-LAV for the potentiometric determination of chloride (adapted from ref 1, 47): (A) the SI-LAV system (B) the LAV flow cell and (C) the signal profiles

Table 1: LOV reviews and related articles

Analyte/sample/application field(s)	**Features (detection)**	**ref**
affinity separation, fermentation monitoring and functional cellular assay	µSI-LOV with various possible detectors for bionalytical assays	10
trace level metals	implementation of FI/SI-sample separation/preconcentration, mentioned LOV *(ETASS/ ICPMS)*	22
pharmaceutical samples	SI-LOV for chromatography and BI	9
trace level metals	progress in implementing miniaturized FI/SI systems for online separation and preconcentration, mention LOV *(ICPMS)*	19
ultra-trace level heavy metals	SI-BI-LOV for online solid phase extraction and preconc. *(ETASS/ ICPMS)*	20
trace level metals	SI-LOV with BI microcolumn for preconcentration *(ETASS/ ICPMS)*	21
	SI-LOV with other cost effective flow-based technique development	1
	impact of FI in modern chemistry including BI-LOV	17
	3 generations of FIA (FI, SI, BI-LOV)	18
	SIA for complicated online sampling manipulation (dilution, dialysis, gas diffusion, enzymatic and immunoassay) mentioned LOV *(ETAAS and ICPMS)*	25
trace level metals in complex matrices	FI/SI LOV for liquid-liquid extraction, precipitation, hydride generation, ion exchange/chelating packed column	14
	LOV mesofluidic system	15
	progress in implementing miniaturized FI/SI systems for online separation and preconcentration, mentioned LOV *(ETAAS)*	16
	programmable ufluidics to replace batch wet chem. *(various)*	7
nutrient (phosphorus) in fresh water and environmental solids	µSI-LOV microcolumn for dynamic fractionation *(spectrophotometry/ fiber optic)*	12
environmental field	SI with solid reactors, packed column for chem. derivatization, chrom. sep. and preconcentration including SI-BI with jet ring or LOV to accommodate dynamic fractionation of trace elements *(many)*	13
pharmaceutical samples	SIA (mentioned LOV) *(spectro, fluorescence, chemiluminescence, electrochem.)*	24
trace level metals	impact of FIA and SIA, LOV	23

Table 2: LOV with various detection techniques and applications

Detection	Analyte/sample	Features	ref
spectrophotometry	phosphate	molybdenum blue chemistry	8
	protease	enzymatic activity assay	
	immunoglobulin G	bioligand interaction assay protein G immobilized on Sepharose beads	
	ammonia, glucose, glycerol, free iron	μSI-LOV for offline and online monitoring of small scale fermentation	25
	nitrate, nitrite and orthophosphate	μSI-LOV with Cd foil filled microcolumn utilizing stopped flow	26
	acidity in fruit juice	mono-segmented flow micro-titration with SI-LOV	27
	DNA assay	crystal violet solution was de-colored inside the flow cell of the LOV at the presence of 5 l - DNA/HindIII within a certain pH range	28
	ketoprofen, naproxen, bezafibrate, diclofenac, ibuprofen and salicylic acid in surface water, urban wastewater, and urine	lab-on-valve (LOV) with bead injection (BI) for on-line solid-phase extraction (SPE) as a front end to highperformance liquid chromatography (HPLC)	29
	enzyme AChE and ACE	SI-LOV micro-reactor for enzyme kinetic and inhibition studies	30
	Cu (II) and Fe (II)	SI-LOV using reaction of 5-Br-PSAA) with Cu (II) and/or Fe (II)	31
	Cu (II)	SI-LOV with air segment using reaction of Cu(II) and Zincon	32
spectrofluorometry	nucleic acid sequences	SIA-LOV for sandwich hybridization of specific DNA probes to the target sequence	33
chemiluminescence	Tetracycline	LOV with bismuthate immobilized microcolumnfor in situ oxidation of KBr and generation of bromine as oxidant for the bromine-hydrogen peroxide-tetracycline (TC) chemiluminescent reaction	34
ICPMS	Ni (II) and Bi (II) in reference materials and spiked urine	SI-LOV with renewable ion exchange micro-column	35
UV-Vis and electrospray ionization MS	biotin containing conjugates and lysosomal-b-galactosidase in human cell homogenates	LOV-BI system for biotin conjuates using immobilized streptavidin	36
mass spectrometry	peptide mixture	affinity chromatographic-LOV system using multiple ligand affinities to proteins immobilized on beads.	37

Table 2 (con)

Detection	Analyte/sample	Features	ref
ETAAS	Pb (II)	SI-BI-LOV using Sephadex G-25 impregnated by dithizone	38
	Cd (II)	PTFE material for use as a means for separation and preconcentration of trace levels of metal ions with SI and SI-LOV	39
	Cd (II)	Octadecyl Immobilized Surface for Precipitate Collection with a Renewable Microcolumn in LOV	40
	Cr (VI) and Cr (III)	bead injection (BI) with renewable reversed-phase surfaces in a sequential injection-lab-on-valve (SI-LOV) mode - C18 sorption of Cr(VI) - DPC	41
	Cd (II), Pb (II) and Ni (II)	SI-LOV system using chelating Sepharose beads as sorbent material	42
	Ni (II) in saline matrices	Lab-On-Valve (μSI-LOV) for bead injection separation/pre-concentration of Ni-dimethylglyoxime (DMG) chelate	43
CVAAS	Hg (II)	SI-LOV for hydride generation	44
potentiometry	Ca (II)	SIA-LOV with solid contact ion selective electrode and pH electrode based on polyaniline	45
capillary electrophoresis	10 anions including chloride and sulfate	μSI-CE interfaced with LOV and demonstrated various injections such as electrokinetic, hydrodynamic and head column field amplification sample stacking injections	46

Table 3 Comparison of SI-LAV features to those of ion-selective electrode (ISE), ion chromatography (IC) and conventional titration with silver nitrate solution.

	LAV	ISE	IC	Titration with Ag^+solution	
Sample throughput	++++	+(+)	++	++	
Consumption:					
• sample	+	+++	+	+++	
• reagent	+	+	+++	+++	
Glassware	-	++	++	+++	
Cleaning activities	-	++	++	+++	
Automation	+++	++	+++	+	(conventional)
				+++	(auto-titrator)
Cost					
• system/ apparatus	++	++	+++	+	(conventional)
				+++	(auto-titrator)
• per sample	+	+	++	+++	

"+" refers to more scale

SI-LAV for chloride determination was proposed (Fig. 3) [47]. For potentiometry, a simple LAV flow through electrode system can be assembled. Both are Ag/AgCl electrodes, one a reference electrode (silver chloride activated surface-silver wire soaked in a constant chloride concentration in a small tube covered with a membrane), another as a working electrode situated in a flow channel. The potential difference due to the concentration cell effect is recorded as a peak. The SI-LAV provides a very simple, fast, precise, accurate, automatic and economical procedure for chloride determination and was applied to some water samples. Comparisons of features of SI-LAV to that of the conventional titration using silver nitrate solution, ISE, and ion-chromatography may be considered as illustrated in Table 3.

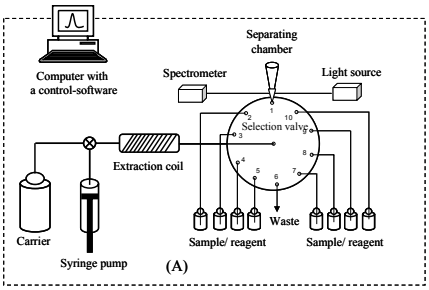

Fig. 4 SIA-LAV on-line micro solvent extraction (adapted from ref 48): (A) the SI-LAV system and (B) the LAV unit

The SI-LAV approach has been proposed for a novel alternative for simple on-line automated liquid-liquid microextraction [48, 49]. Sample, reagent and organic solvent are sequentially aspirated into an extraction coil connected to a central port of a conventional selection valve. Flow reversal enables good efficient extraction.

The aqueous and organic phases can be separated in a conical separating chamber, situated with a fiber-optic spectrophotometer to monitor the absorbance change in either the organic or aqueous layer. The unit (Fig. 4) is attached to one port of the valve. Applications have been made to the assays of anionic surfactant in water samples and diphenhydramine hydrochloride in pharmaceutical preparations by forming ion-association compounds with methylene blue and bromocresol green, respectively. Table 4 demonstrates some features of solvent extraction in comparison with conventional batch, FIA and SI-LAV formats.

Table 4 Features of solvent extraction performed in conventional batch, FIA and SI-LAV formats

	Conventional batch	FIA	SI-LAV
Consumption:			
reagents	+++	++	+
sample	+++	++	+
solvent	+++	++	+
Sample through-put	+	+++	++
Less hazardous (safety)	+	+++	+++
Cost			
• apparatus/ instrument	+	++	++
• per sample	+++	++	+

"+" refers to more scale

The SI-LAV setup for the previously described on-line solvent extraction may also be used for some applications employing stopped flow [51] (see Fig. 5(A)), such as the kinetic study of the persulfate-iodide reaction:

$$S_2O_8^{2-} + 2I^- \longrightarrow 2SO_4^{2-} + I_2$$

Employing a constant concentration of persulfate but varying the iodide concentration, signal profiles for a stopped flow mode can be obtained, as shown in Fig. 5(B). Rates for each reaction condition can be obtained from the slope of the peak profile. The order of reaction in a rate law can then be deducted from the graph (log [iodide] vs. log [rate], as illustrated in Fig. 5(C)).

Exploiting SI-LAV with air-segmented flow for automated on-line bead-based immunoassay has been made. The system provides precise delivery of micro-volumes of reagent and precise time of incubation and washing steps via manipulation of the syringe pump. Based on competitive enzyme linked immuno sorbent assay (ELISA), hyaluronan (HA), a biomarker, can be assayed by the competition of HA immobilized on beads and HA in the solution, to bind with a fixed amount of biotinylated HA-binding proteins (b-HABPs). After separation, anti-biotin conjugated with enzyme and a suitable substrate are introduced to follow the binding reaction of the immobilized HA and b-HABPs, whose degree of binding is indirectly proportional to the

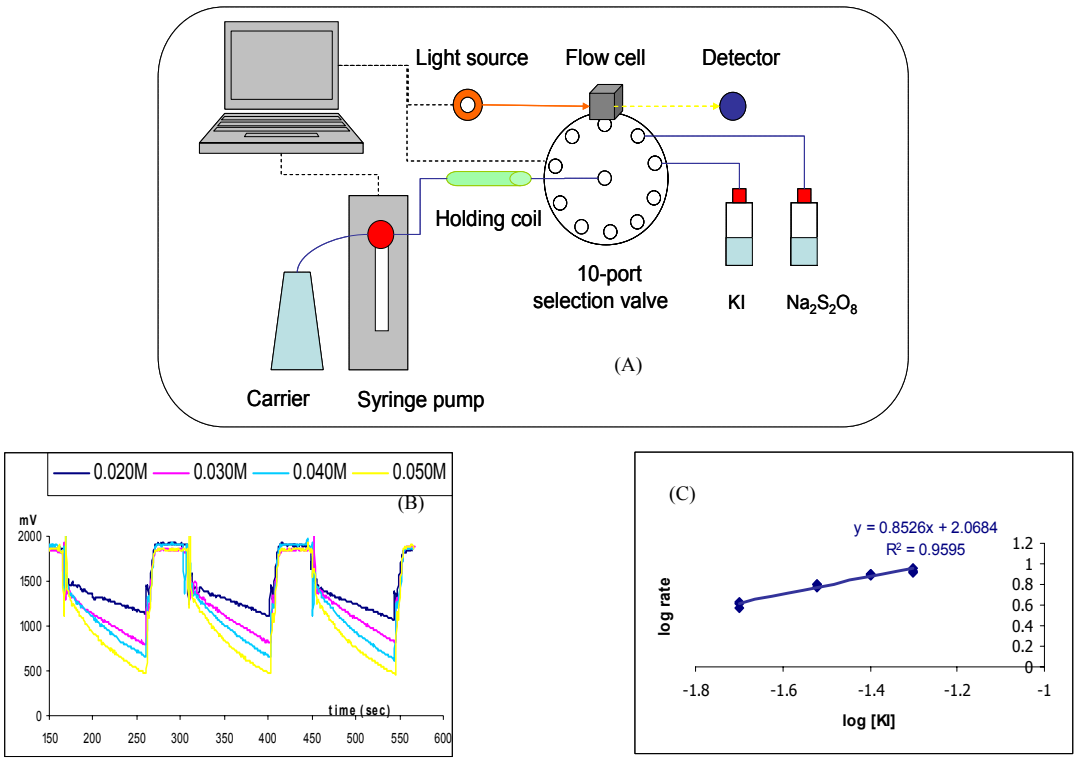

Fig. 5 SI-LAV for kinetic study of perfulfate-iodide reaction: (A) the SI-LAV system, (B) the signal profiles and (C) relation of log [Iodide] vs. log [Rate]

amount of HA in solution. Total analysis time for this automation is 30 min, comparing to 5-8 h for the conventional batch well ELISA. The system has been applied to human serum [52].

Investigation in progress is another SI-LAV system with bead injection for application to clinical analysis for the immunoassay for chondroitin 6-sulfate (C6S) as a biomarker for cartilage disease [53].

4. Conclusion

Flow based techniques provide advantages in automation and miniaturization, favoring "zero emission" and "greener" chemistry approaches. Sequential Injection (SI) with Lab-on-Valve (LOV) features give benefit in down scaling for chemical analysis. SI with Lab-at-Valve (LAV) approach may serve as a cost effective alternative. This enables down scaling chemical analyses, from operation on lab bench space to the manipulation at a valve.

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