Pure Appl. Chem., Vol. 84, No. 10, pp. 1983–1998, 2012.
http://dx.doi.org/10.1351/PAC-CON-11-11-14

Laboratory automation based on flow techniques*

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Abstract: Flow techniques have undoubtedly aroused special interest in relation to many other automatic methodologies of analysis. Ever since segmented flow analysis (SFA) was developed by Skeggs in 1957, flow techniques have been in continuous evolution toward new developments. There is no solid argument in favor of using any particular flow technique separately; rather, substantial advantages can be derived from their combination. Since flow-based methods are nonseparative tools, the advantages of combining flow techniques with separation techniques are noteworthy. High selectivity can be achieved by coupling them with liquid chromatography (LC), gas chromatography (GC), solid-phase extraction (SPE), or capillary electrophoresis (CE). Thus, a detailed description of flow techniques, their evolution, their hyphenation advantages, and a critical comparison between current developed methods exploiting flow techniques aimed at solving present analytical needs are reviewed in this article.

Keywords: analytical techniques; automation; chemistry; environmental chemistry; flow techniques; microfabrication.

INTRODUCTION

Advances in science and technology have raised an increasing demand for control analyses and posed various challenges to analytical chemists such as the need to develop new methods exhibiting as much selectivity, sensitivity, sample and reagent economy, throughput, cost-effectiveness, simplicity, and environmental friendliness as possible.

Moreover, the large number of samples with which analysts can be confronted imposes the use of expeditious analytical methods, currently automatic ones. Since the beginnings of the automation of analytical methods, various different flow techniques have been developed and used for analytical or monitoring applications. They have gained importance for clinical, industrial, and environmental purposes as they allow highly reproducible fast determinations. Automation and miniaturization of solution-based analysis are essential to make them fast and efficient for routine and research tasks [1]. Ideally, analytical equipment should be versatile, capable of accommodating a wide variety of assays without the need for system reconfiguration, and compatible with a wide range of detectors. Among the benefits of automation of analytical procedures, the increase of sampling frequency, minimization of sample contamination or alteration, miniaturization of the analytical system, and lower reagent and sample consumption, implying fewer personal and consumable costs, should be highlighted.

Pure Appl. Chem.* **84, 1973–2063 (2012). A collection of invited papers based on presentations on the Novelty in Green Analytical Chemistry theme at the 14th Asian Chemical Congress (14 ACC), Bangkok, Thailand, 5–8 September 2011.
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Automation of analyses involving liquid samples is facilitated by their usually adequate homogeneity and easy mechanical handling by use of peristaltic or piston pumps, or some other liquid management devices (e.g., a liquid driver). This is not the case with solid samples, analysis of which frequently involves their prior conversion into liquids by dissolution. The dissolution step is the bottleneck of analytical processes involving solid samples as it is frequently slow and must be performed manually.

The earliest automatic methods used dedicated devices suited to particular applications. This restricted their scope to very specific uses such as the control of manufacturing processes or to those cases where the number of samples to be analyzed was large enough to justify the initial effort and investment required. Flow techniques have undoubtedly aroused especial interest in relation to many other automatic methodologies of analysis. Ever since segmented flow analysis (SFA) was developed by Skeggs in 1957 [2], flow techniques have been in continuous evolution toward new developments such as those of flow-injection analysis (FIA) [3] by J. Ruzicka and E. H. Hansen in 1975; sequential injection analysis (SIA) [4] by J. Ruzicka and G. D. Marshall in 1990; multicommutated flow analysis (MCFIA) [5] by B. F. Reis et al. in 1994; and, more recently, multisyringe flow injection analysis (MSFIA) [6] by V. Cerdà et al. in 1999; multipumping flow systems [7] by R. Lapa et al. in 2002; lab-on-valve (LOV) [8] by J. Ruzicka in 2000; and chip-on-valve (ChOV), currently in development by V. Cerdà et al. There is no solid argument in favor of using any particular flow technique separately; rather, substantial advantages can be derived from their combination.

FIA is undoubtedly the most widely accepted flow technique. Its widespread success can be ascribed to its ease of implementation and, especially, to no need of a computer to control it. By contrast, computers are indispensable in all subsequent flow techniques. This initially hindered further development owing to unavailability of suitable commercial software and a general lack of experience in coupling personal computers to instruments. However, the advantages of current flow methods are also in part the result of the inception of computers; in fact, the flexibility of computers allows the same equipment (hardware) to be used with little or no alteration in order to implement the same analytical method on different types of samples simply by altering the software.

Flow techniques have gone through two generations. Initially, flow systems were operated exclusively by hand (e.g., in SFA and FIA). Subsequently, however, computers helped to develop variably automated techniques such as SIA, MCFIA, or MSFIA.

Since flow-based methods are nonseparative tools, the advantages of combining flow techniques with separation techniques are noteworthy. High selectivity can be achieved by coupling them with liquid chromatography (LC), gas chromatography (GC), solid-phase extraction (SPE), or capillary electrophoresis (CE).

This article reviews the state of the art of flow-based techniques, including the description of the principal flow techniques, their evolution as well as the most recent and relevant applications of them and their combination with most widely used separation techniques and detectors.

FLOW TECHNIQUES EVOLUTION

Skeggs' solution: Segmented flow analysis (SFA)

The need to seriously consider the development of automatic methods of analysis arose in the 1950s, when clinical tests started to be increasingly used for diagnostic purposes in medicine. This led to a rapid increase in the demand for laboratory tests which, for obvious economic reasons, could not be met simply by hiring more laboratory staff. The solution to this problem was provided by SFA [2], which afforded not only substantially increased throughput, but also substantial savings in samples and reagents. SFA laid the foundations for modern flow techniques. It should be noted that SFA was developed as a mechanical tool for automating a number of analytical methods. In many other areas, automation had to wait until the advent of microprocessors and computers.

SFA is an automatic continuous methodology. Its associated equipment (Fig. 1) usually includes a peristaltic pump for continuous aspiration of the sample and reagents, a series of plastic tubes (the manifold) intended to carry liquid streams and a detector. Once aspirated, samples are segmented by inserting air bubbles in the liquid streams that are subsequently removed before they can reach the detector. The air bubbles serve various purposes, namely:

(a) to avoid carry-over between samples, which is facilitated by inserting a segment of flushing water (W) between individual samples (S) in order to remove any residues of the previous sample potentially remaining on tubing walls,
(b) to prevent dispersion of the sample plug, and
(c) to facilitate the formation of a turbulent flow in order to homogenize the sample/reagent mixture in the plug sandwiched between each pair of bubbles.

The use of air bubbles has some disadvantages, such as their high compressibility results in pulsation; their injection and subsequent removal complicates the operational design; and their presence reduces the efficiency of separation techniques (dialysis, liquid–liquid extraction), hinders the implementation of stopped-flow methods, and precludes miniaturization in many cases. Because each individual segment is isolated from the neighboring segments of flushing water, the recording provided by the detector is roughly a rectangle, the height of which is proportional to the analyte concentration—as long as the reagents are permanently present in overstoichiometric amounts.

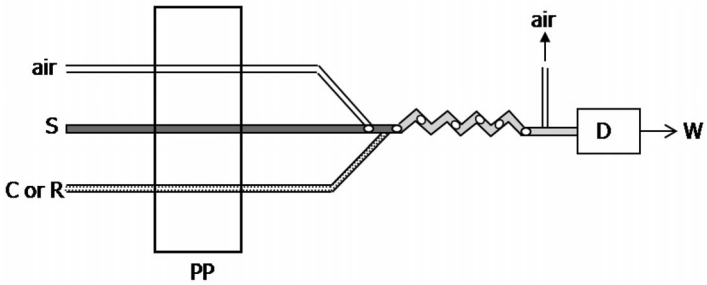

Fig. 1 Scheme of an SFA system. PP: peristaltic pump; S: sample; C: carrier; R: reagent; D: detector; W: waste.

Flow-injection analysis (FIA)

The name of this technique was coined by J. Ruzicka and E. H. Hansen in Denmark in 1975 [3]. While it initially resembled SFA, FIA is rather different from it in both conceptual and practical terms. Thus, basic components of FIA are virtually the same as those of SFA (see Fig. 2). Unlike SFA, the sample is not inserted by continuous aspiration; rather, a constant volume of sample is inserted into a stream of carrier liquid via an injection valve for merging with the reagents used by the analytical method applied. Tube lengths and the rotation speed of the peristaltic pump are dictated by the reaction time. Thus, if a long time is required for kinetic reasons, then a long piece of tubing is inserted—usually in coiled form—in order to increase the residence time of the sample and reagents in the reactor.

Unlike SFA, which operates under a turbulent flow regime, FIA uses laminar flow, which reduces the likelihood of carry-over between successive samples. Furthermore, separation of samples with intervening bubbles is not required in FIA, since it uses unsegmented flow.

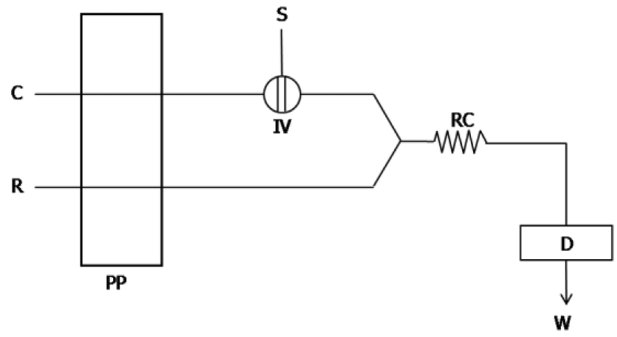

Fig. 2 Typical two-channel FIA manifold. C: carrier; R: reagent; PP: peristaltic pump; IV: injection valve; RC: reaction coil; D: detector; W: waste.

Table 1 summarizes the most salient features of FIA. As can be seen, the operating conditions are rather different from those of SFA. Thus, sample volumes are in the milliliter range in SFA and in the microliter range in FIA. Also, response times are substantially shorter and tubing diameters smaller.

Table 1 Figures of merit of some flow techniques.

	SFA	FIA	SIA	MCFIA	MSFIA
Sample volume (mL)	0.2–2	0.05–0.15	0.05–0.15	0.05–0.15	0.05–0.15
Response time (s)	120–1800	3–60	3–60	3–60	3–60
Tubing diameter (mm)	2	0.5	0.8	0.8	0.8
Detection conditions	Equilibrium	Equilibrium not needed	Equilibrium not needed	Equilibrium not needed	Equilibrium not needed
Throughput (inj h^{-1})	80	100	70	100	100
Precision (%)	1–2	1–2	1–2	1–2	1–2
Reagent consumption	High	Low	Very low	Very low	Very low
Flushing cycle	Essential	Unnecessary	Unnecessary	Unnecessary	Unnecessary
Kinetic methods	Unfeasible	Feasible	Feasible	Feasible	Feasible
Titrations	Unfeasible	Feasible	Feasible	Feasible	Feasible
Response type	Rectangle	Peak	Peak	Peak	Peak
Measured parameter	Peak height	Peak height or area	Peak height or area	Peak height or area	Peak height or area

While SFA usually requires that the analytical reaction reaches chemical equilibrium, FIA does not. In fact, FIA only requires a constant and reproducible extent of the reaction, which is facilitated by the high reproducibility of the hydrodynamic behavior of the system. Because FIA uses much thinner tubing and much lower flow rates, it consumes samples and reagents much more sparingly than does SFA. In addition, FIA is much more flexible than SFA and allows the implementation of a number of analytical methodologies unaffordable to the latter (e.g., kinetic methods, stopped-flow methods).

Another major advantage of FIA over SFA is its ease of implementation. In fact, a dedicated manifold can be readily assembled from fairly inexpensive parts (viz. a peristaltic pump, injection valves, flow-cells, Teflon tubing, and connectors) and available measuring instruments [e.g., spectrophotometers, potentiometers, ammeters, atomic absorption spectroscopy (AAS) equipment]. This propitiated a vast expansion of FIA among research laboratories and led to the development of a large number of applications relative to other, more recent techniques within a few years after its inception.

Sequential injection analysis (SIA)

SIA was developed by J. Ruzicka and G. Marshall [4] as an alternative to FIA. SIA has over time proved that its scope departs markedly from that of the earlier technique.

Figure 3 shows a schematic depiction of an SIA system. The central port of the valve is connected to a two-way piston pump, as are the side ports to the sample and reagent vessels, and also to the detector. The side ports can also be used for other purposes such as discharging waste or connection to other devices (e.g., a microwave oven, photooxidation system, or mixing chamber).

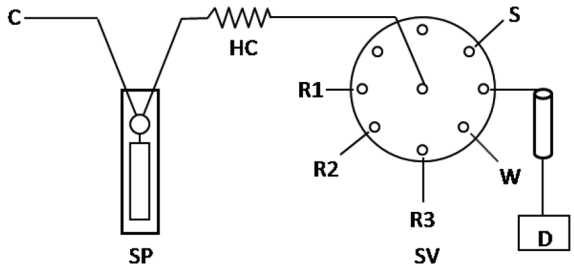

Fig. 3 Schematic depiction of an SIA system. SP: syringe pump; C: carrier; HC: holding coil; R: reagent; SV: selection valve; D: detector: S: sample; W: waste.

One of the essential features of SIA is its computerized control. The computer selects how the central port of the valve is connected to its side ports, starts and stops the pump in order to aspirate or dispense liquids, selects their volume, and adjusts the flow rate. Also, it acquires and processes data. All of this occurs under a laminar flow regime that facilitates dispersion of the sample and reagent plugs; as a result, the detector profile is no longer rectangular, but rather exhibits the typical asymmetric shape of FIA peaks.

Unlike FIA, SIA can be turned into a true multiparameter analysis system simply by using a switching valve with an appropriate number of channels to hold the different analytical reagents, delivery of which can be precisely programmed via the associated computer. In this respect, SIA is much closer than FIA to the original SFA that afforded the determination of up to 20 parameters per sample—except that SIA operates in a much simpler and, especially, more economical manner. Currently available switching valves can have more than 20 side ports. Also, the number can be increased by connecting a side port in a valve to the central port of several others. Such a high degree of expandability is exclusive of SIA, no other flow technique can match it in multiparameter determination capabilities.

Table 1 summarizes the characteristics of SIA, which are seemingly quite similar to those of FIA; careful scrutiny, however, reveals some differences between the two. One is the dramatically reduced consumption of sample and reagents in SIA; this, however, is not a result of using smaller injected volumes, but rather of the way a SIA system operates. In FIA operation, sample and reagent consumption are virtually independent of the analysis frequency as the peristaltic pump continuously propels the sample and reagents at a constant flow rate throughout. In SIA, however, the piston pump only works during the time strictly needed to aspirate or deliver the amount of sample and reagent needed for a given determination. As an example, an SIA monitor for determining ammonium ion in waste water uses 10 times less reagents than does a comparable FIA monitor [9]; this is of high economic and practical significance, especially with equipment that is intended to operate unattended over long periods (e.g., an automatic analytical monitor).

Because it uses piston pumps, SIA is more robust than other flow techniques that use peristaltic pumps. In fact, peristaltic pumps use tubing of materials that are relatively easily damaged by some fluids (viz. acids, bases, and, especially, solvents); by contrast, piston pumps only use glass or Teflon tub-

ing, which is highly inert and ensures a long service life. Also, in SIA, the sample, reagents, and solvents seldom reach the propulsion system, which just holds mostly the carrier solution.

One difficulty of SIA operation arises from the way plugs are stacked; this hinders mixing of the sample and reagents (especially with more than two, which require using a sandwich technique). One feasible solution in determinations involving many reagents is using a mixing chamber [10] in one of the side ports to homogenize the different sample/reagent mixtures with the aid of a magnetic stirrer for withdrawal of small aliquots as required.

One of the greatest initial hindrances to SIA development, one that, in contrast to FIA, resulted in the development of barely a few tens of methods during its first year of existence was the need to use a computer in order to govern the system. The scarcity of commercially available software and the lack of experience in interfacing computers to analytical instruments caused the slow development of SIA, despite its proven advantages. Only during the past decade, with the inception of commercial software, SIA has gained ground in the field of routine analyses.

On the other hand, the need to use a computer has been the origin of some advantages of SIA over FIA. Thus, residence times need no longer to be controlled via the length of the manifold tubes and the flow rates of a peristaltic pump; rather, they are controlled in a highly reproducible manner by the computer. Also, the ability to adjust the flow rate required in each step of the process and to change it at will at any time make SIA a highly flexible analytical tool. Thus, while using a different method in FIA very frequently entails altering the configuration of the manifold, switching to another method in SIA seldom requires more than using a different computer file containing the operational settings to be used with each procedure.

The incorporation of computers into SIA systems has facilitated the implementation of stopped-flow methods. It suffices to calculate the volume of carrier to be delivered and stop the system when a peak is obtained at the detector in order to readily implement various analytical methodologies including classical kinetic, spectrophotometric, polarographic, voltammetric, and anodic stripping methods. In addition, computers have increased the flexibility of analytical systems by allowing a number of operations mimicking those performed manually to be programmed for easy online implementation.

Multicommutated flow analysis (MCFIA)

The MCFIA technique, devised by B. F. Reis et al. [5] uses fast-switching three-way solenoid valves. The earliest MCFIA systems used a single-channel propulsion system to aspirate the liquids to be employed via individual valves. Because aspiration devices tend to insert air bubbles or degas liquids in the system, it is preferable to use liquid propulsion devices such as peristaltic or piston pumps instead.

The system depicted in Fig. 4 could be used for a number of purposes by rapidly switching the valves. By using a peristaltic pump and having solenoid valves V1, V2, V4, and V5 arranged in such a way that the propelled liquid is returned to the reagent reservoir while the valves are OFF, but inserted into the system while they are ON. By alternately switching V1 and V2 ON, one could dilute the sample to a preset extent with carrier. Because solenoid valves can be switched very rapidly, one can alternately insert variably thick slices of carrier and sample that will interpenetrate on their way through loop RC1. Valve V3 could allow the flow to be directed for example to a copperized cadmium column in order to reduce nitrates to nitrites, while switching to the lower channel would avoid this reduction reaction. Finally, valves V4 and V5 could be used to inject preset volumes by switching them ON over an appropriate interval. Loop RC2 is intended to facilitate homogenization of the diluted unknown sample with the reagents added in the last step.

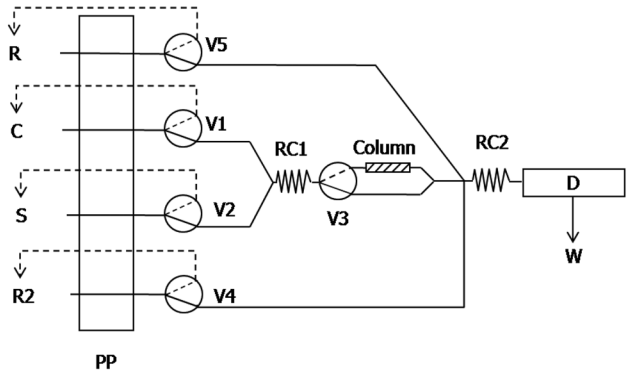

Fig. 4 MCFIA system. C: carrier; V: valve; PP: peristaltic pump; R: reagent; S: sample; RC: reaction coil; D: detector; W: waste.

One major shortcoming of solenoid valves is the unfavorable effect of the heat released by the solenoid coil when the valves are ON for a long time. The resulting increase in temperature can deform the Teflon inner membranes of the valves and render them unusable. Overheating here can be avoided by using an effective electronic protection system.

Table 1 summarizes the characteristics of MCFIA. As can be seen, it shares some advantages with FIA, such as an increased throughput (higher than that for SIA) and small consumption of reagents—a result of unused sample and reagents being returned to their respective reservoirs. However, MCFIA also shares one disadvantage of FIA, namely: the vulnerability of peristaltic pump tubing against aggressive reagents and, especially, solvents.

Multisyringe flow injection analysis (MSFIA)

This variant of FIA was developed in 1999 by our research group in cooperation with the firm Crison (Alella, Barcelona, Spain) [6]. The aim was to combine the advantages of the previous flow techniques while avoiding their disadvantages.

Figure 5 shows a typical multisyringe burette for use in MSFIA. The device consists of a conventional automatic titration burette adapted in such a way that the motor can simultaneously move the pistons of four syringes in order to avoid the need to have four separate burettes operating in parallel. This is accomplished by using a metal bar that is moved by the step motor of the burette, the bar accommodating the four syringes and each syringe head containing a fast-switching solenoid valve. Obviously, the motor moves the pistons of the four syringes simultaneously; this is equivalent to using a multichannel peristaltic pump in FIA but avoids the disadvantages of its fragile tubing. Solenoid valves located at the heads of the syringes (on: to the system; off: to the reservoir) allow four kinds of liquid displacement: On-dispense, Off-dispense, On-pick-up, and Off-pick-up (Figs. 5a,b). The ratio of flow rates between channels can be modified by using syringes of appropriate cross-sectional dimensions similarly to tubing diameters in FIA.

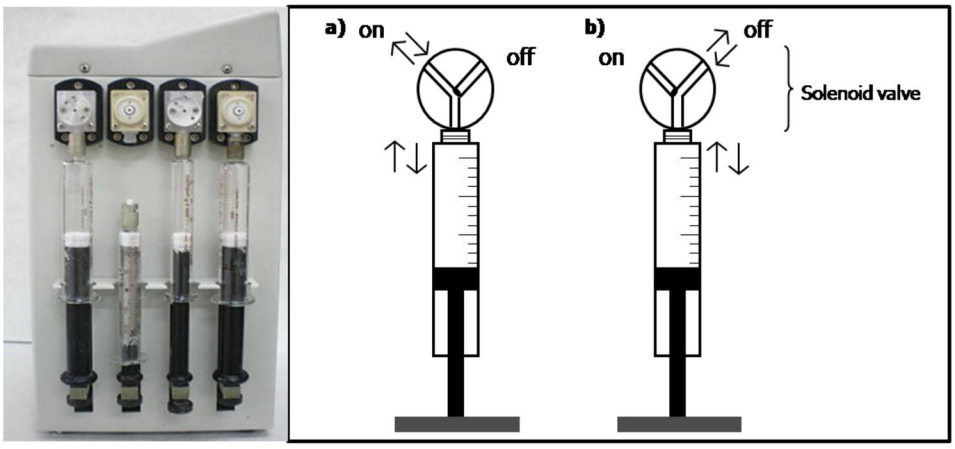

Fig. 5 Left: Frontal view of the multisyringe burette. Right: Schematic depiction of the solenoid valves placed at the head of each syringe. (a) Activated solenoid: "on" position and (b) Deactivated solenoid: "off" position.

MSFIA systems combine some of the advantages of the above-described flow techniques, namely:

(a) The high throughput of FIA, which is a result of sample and reagents being incorporated in parallel. This in turn leads to an improved mixing efficiency in relation to SIA.
(b) The robustness of SIA. In fact, liquids only come in contact with the walls of the glass syringes and Teflon tubing as no peristaltic pump tubes are used.
(c) The low sample and reagent consumption of SIA by virtue of the reaction ingredients being used in the amounts strictly required to perform a given determination. Also, reagents are only inserted when required.
(d) The high flexibility of SIA manifolds. Thus, residence times are not determined by tubing dimensions, but rather by commands of the computer used to govern the whole system, which sets the times and flow rates to be employed. Usually, switching to a different analytical method simply requires loading the file containing the appropriate settings for a new method and changing reagent vessels.
(e) The ability to use MCFIA solenoid valves, which can be actuated without the need to stop their pistons. Switching between valves is so rapid that no overpressure arises in the operation.

Unlike FIA, MSFIA requires the use of a computer to control the system. This, however, poses no special problem as a variety of affordable software for implementing any flow technique is by now available. A multisyringe is equipped with a four-outlet connecting strip supplying 12 V at up to 300 mA each in order to facilitate the control of single, double, and triple solenoid valves via the burette itself. The strip can also be used to govern other devices (e.g., relays, pumps) operating at the same voltage. Provided the maximum nominal current is not exceeded, each outlet can be used to connect several devices for synchronous operation (e.g., the pair of single solenoid valves needed for injection). The use of an independent burette results in substantially increased throughput. Alternate use of two burettes allows the throughput to be doubled (up to 200 injections/h, which can hardly be matched by any other flow technique). The only disadvantage of MSFIA in front of other flow techniques is the periodical syringe refilling, which causes a lower injection frequency than using an FIA approach. Table 1 summarizes the most relevant characteristics of MSFIA.

Multipumping flow systems (MPFs)

MPFSs, which were developed in 2002 [7] by two research groups at the Pharmacy Faculty of the University of Porto (Portugal) and the Piracicaba CENA (Brazil), are based on the use of solenoid piston pumps where each stroke propels a preset volume of liquid (3, 8, 20, 25, 50 μL), the flow rate of which is determined by the stroke frequency. Principal advantages of these systems are their high flexibility, easy configuration, robustness, and low cost—a result of the pump operating as both a liquid propeller and a valve. Like previous flow systems, MPFSs use samples and reagents very sparingly. Usually, they employ a combination of solenoid pumps and valves.

Analyte peaks provided by multipumping flow systems are higher than those obtained with other flow techniques. This can be ascribed to their pump piston strokes causing turbulences that facilitate mixing of sample and reagents. However, some disadvantages of the micropumps are the susceptibility to blockage by particles and to backpressure, requiring recalibration of the volume dispensed. A schematic depiction of the controller system designed and created by our group and commercialized by Sciware S.L. [11] (Palma de Mallorca, Spain) is shown in Fig. 6. This module can be controlled through the interface RS232.

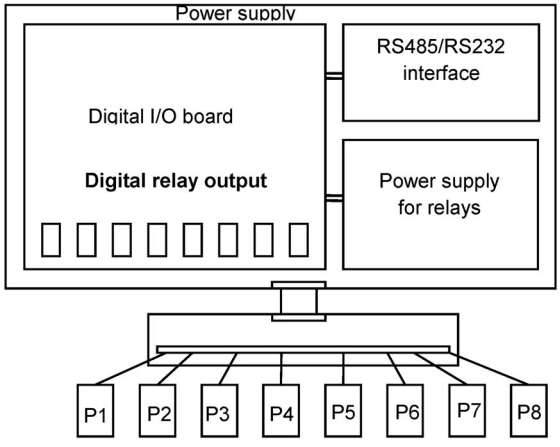

Fig. 6 Schematic depiction of the controller system of micropumps. P: micropumps.

A typical multipumping system is similar to an MCFIA system (Fig. 4). In fact, the former can also be used to implement MCFIA as it affords controlling any combination of valves and solenoid pumps. The primary difference between the two is that multipumping systems require controlling not only valve switching, but also the stroke frequency, in order to ensure reproducible flow rates. The simplicity and economy of MPFS should facilitate the development of portable equipment for field measurements.

Lab-on-valve (LOV)

LOV [8] (Fig. 7) is a novel methodology for downscaling reagent-based assays to micro- and submicroliter level, which significantly facilitates integration of various analytical units in the valve and provides great potential for miniaturization of the entire instrumentation.

It is shown that sample handling in the sequential injection mode, which employs forward, reversed, and stopped flow, can be programmed to accommodate a wide variety of assays within the same microfluidic device. Solution metering, mixing, dilution, incubation, and monitoring can be executed in any desired sequence in a system of channels, integrated with a multipurpose flow cell.

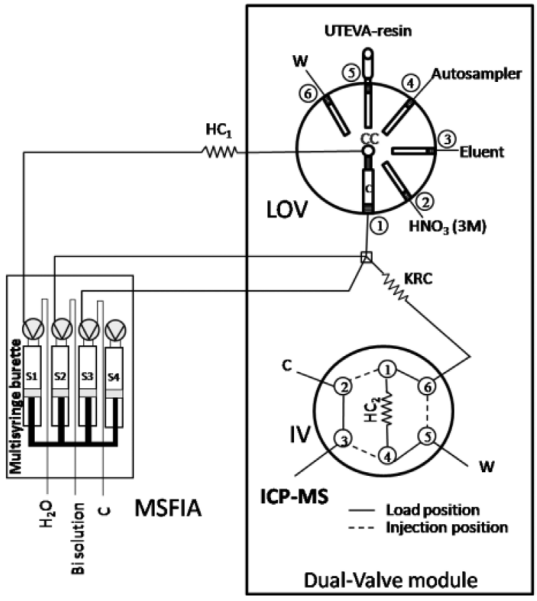

Fig. 7 Schematic depiction of a LOV-MSFIA-ICP-MS system used for isotopic analysis of Th and U. C: carrier; HC: holding coil; KRC: knotted reaction coil; W: waste.

It is noteworthy that LOV-based techniques [12,13] have not only been extensively employed in homogeneous solution-based assays, but have also shown promise in heterogeneous assays because flexible fluid manipulation is also suitable for delivering beads in flow-based manifolds [i.e., precise fluid manipulation by the LOV system and the channel configuration also make it a powerful platform for bead injection (BI)]. In combination with the renewable surface concept, BI has been widely exploited for separation and preconcentration of analyte in the presence of complex matrix components. Most importantly, the automated transport of solid materials in such a system allows their renewal at will and thus provides measurement, packing, and perfusion of beads with samples and reagents with a high degree of repeatability.

The channel system is fabricated as a monolithic structure mounted atop a conventional multiposition switching valve. In addition to compactness, the advantage of these "lab-on-valve" systems is the permanent rigid position of the sample processing channels, which ensures repeatability of microfluidic manipulations, controlled by conventional-sized peripherals. This provides proven robustness and reliability of operation, and makes the microfluidic system compatible with real-life samples and peripheral instruments.

Chip-on-valve (ChOV)

ChOV is a new microfluidic methodology that is at present being developed and studied by our group. A picture and a schematic depiction of one of the first prototypes are shown in Fig. 8. This technique is still in development but it can be anticipated that it is a step forward in automation and miniaturization of all the laboratory protocols. More ChOV prototypes have been designed to different analysis methods such as kinetic methods, some incorporating the sensor in the chip. Furthermore, in collaboration with a group from Mahidol University (Thailand), a chip applied to cross-injection analysis (CIA) has been developed. ChOV shares some characteristics with its predecessor LOV such as compactness, the permanent rigid position of the sample processing channels that ensures repeatability of microfluidic manipulations, controlled by conventional-sized peripherals, and it is also compatible with

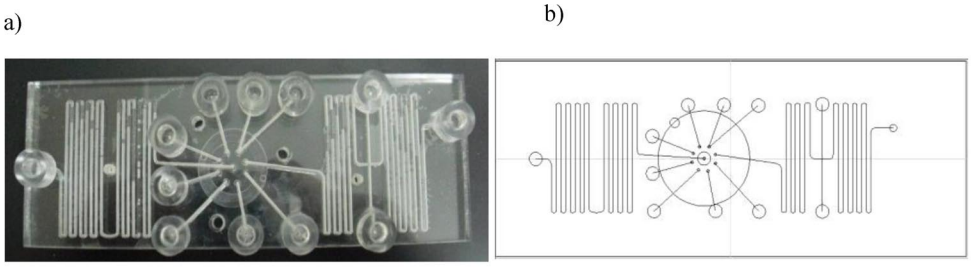

Fig. 8 (a) ChOV microdevice photograph. (b) Schematic depiction of the ChOVmicrodevice.

real-life samples and peripheral instruments. Some additional advantages of this novel methodology are its ease of use and the higher versatility compared with previous flow techniques. We are only at the beginning of the research that will prove all the advantages and possible limitations of this new and promising ChOV.

COMBINED USE OF MULTICOMMUTATED FLOW TECHNIQUES

Obviously, in developing a specific application more than one flow technique can be used in order to maximize the advantages of each individual choice. Multicommutated flow techniques have shown great potential in comparison with previous flow techniques in minimizing reagents consumption and waste production, providing more environmentally friendly methodologies. Thus, this section is focused on the advantages of the hyphenation of these multicommutated flow techniques. One potentially effective combination can be that of SIA and MSFIA [14]. In fact, SIA allows all preliminary operations on the sample (e.g., preconcentration on a solid support, photooxidation) to be conducted by mimicking the work of manual systems while MSFIA can contribute with increased precision injecting the reagents in parallel and hence more efficient mixing with the sample.

Another attractive combination is that of MSFIA with a module comprising two switching valves; this allows each valve to control two syringes and facilitates the simultaneous implementation of two improved SIA processes at no substantially increased cost. Our group is currently experimenting with this combination with a view to developing a monitor for energy cogeneration water from thermal power plants.

MSFIA/MPFS combinations are also interesting [15,16]. Our group has altered the dynamic link libraries (DLLs) for the multisyringe burette so that it can control the solenoid valves via the connecting strip at the back. Finally, MSFIA/LOV [17,18] is also a great combination, providing the full automation of all the steps including the column replacement. MSFIA is a very versatile flow technique that allows its easy hyphenation with LOV. Both flow techniques LOV-MSFIA complement each other, improving their individual advantages according to the required analytical needs. This allows drastic reduction of reagents consumption, waste generation, reduction of resin consumption, and time saving.

FLOW TECHNIQUES COUPLING TO SEPARATION TECHNIQUES

Flow-based methodologies can be readily coupled to a variety of detection techniques such as spectrophotometry, fluorimetry, AAS, inductively coupled plasma mass spectrometry (ICP-MS), etc. Flow-analysis equipment is fairly inexpensive but cannot by itself separate analytes in a mixture. The combination of different analytical techniques, such as flow-based methods and separation techniques, is a common way to improve the performance of the separation method. Sample preparation accounts for over 60–80 % of the total analysis time and normally is the main contributor to analytical uncertainty. Thus, automation of sample preparation is of great value in order to maximize throughput and minimize

costs, time, and analyst risks due to chemicals exposure. In this context, online coupling of separation techniques to different detectors represents the automation milestone, as the overall analytical protocol can be fully mechanized. The advantages of combining flow techniques with separation techniques are noteworthy. Some detection techniques are selective, but in any case the analytical performance can be enhanced by using online preconcentration and separation. High selectivity can be achieved by LC, GC, SPE, or CE.

Flow techniques: Liquid chromatography (LC)

Selectivity of flow techniques can be improved by chromatographic separation. The coupling of them to high-performance liquid chromatography (HPLC) raises its selectivity to required levels. Some examples of the implementation of different chromatographic approaches combined with flow techniques are described below. An FIA-HPLC hyphenated system was proposed for chloramphenicol preconcentration and determination in environmental samples [19]. In another application, a flow-injection online dialysis was developed for sample pretreatment prior to simultaneous determination of some food additives by HPLC (FID-HPLC) [20]. A hybrid FIA/HPLC system [21] equipped with a 5-mm-long C18 monolithic column was used for the determination of four parabens (methyl-, ethyl-, propyl-, and butylparaben). HPLC was coupled with SIA for the simultaneous determination of several heavy metals by means of nitro-PAPS (polyfluoroalkyl phosphate esters) complexes [22]. The same authors have recently published an LOV-HPLC system for online renewable micro-SPE of carbamate insecticides determination in food and environmental samples [23]. In both cases, the sequential injection system offers the means of performing automated handling of sample pretreatment, e.g., in the LOV system, sample preconcentration and matrix removal, achieving an enrichment factor between 20 and 125. An SIA-HPLC-AFS system has been recently proposed by Jesus et al. [24] for As speciation in seafood extracts, implementing standard addition method (SAM) for simultaneous quantification of four As species. An SI-HPLC with electrochemical detection for the determination of sulfonamides in shrimp has been lately proposed by Chantarateepra et al. [25] in which a homemade microcolumn SPE coupled to SIA was used to automate sample clean-up and extraction of sulfonamides. Some MSFIA-HPLC systems have been developed by our group, e.g., an MSFIA-HPLC system exploiting SPE was proposed for screening of phenolic pollutants in waters at ng mL^{-1} levels [26]. In any case, HPLC equipment is expensive. This fostered a search for more affordable alternatives, such as sequential injection chromatography (SIC) and multisyringe chromatography (MSC), which have proved to be an effective alternative to HPLC. These techniques are a result of the online coupling of SIA and MSFIA to monolith columns, respectively. Both are excellent tools that exploit the ability of monolithic columns of relatively large pore sizes to effect separations without the need for high-pressure pumps. The research in the field of LC columns has tremendously accelerated during the last few years. An important direction of this research is the development of monolithic columns with highly porous sorbent, permitting high flow rates of mobile phase at low backpressures without losing efficiency. SIC has been mainly applied to pharmaceutical analysis, e.g., Huclova et al. [27] proposed an SIC system for salicylic acid and its ester methylsalicylate determination in a topical pharmaceutical preparation, the separation was performed on a Chromolith® SpeedROD RP-18e, 50-4.6 mm column (Merck, Germany). Later, the same authors proposed an SIC system [28] using the same monolith column, but with a 10-mm precolumn to determine ambroxol hydrochloride, methylparaben, and benzoic acid in pharmaceutical preparations. In another application, SIC was used to study the binding between drugs and proteins, in particular between the antibiotic ciprofloxacin as model drug compound and bovine serum albumin, which was strongly retained on the monolith strong anion-exchanger [29]. SIC has also been applied to pesticide determination [30], using a miniaturized 10-mm monolithic column and spectrophotometric detection. MSC has been widely used in the last few years, an MSC system was proposed for the online SPE and determination of hydrochlorothiazide and losartan potassium in water samples [31]. Thiazide diuretics were determined, interfacing again SPE with MSC using chemiluminiscence

detection [32]. The same authors have recently published an MSC method for oxalate determination in beer and urine using also chemiluminiscence detection [33]. The combination of sample pretreatments in flow systems expands the applicability of low-pressure LC due to the isolation/preconcentration of the target compounds, enhancing selectivity and sensitivity. Another MSC system was satisfactorily applied for sulfonated azo dyes determination in environmental samples [34]. Another recent application of MSC is the simultaneous analysis of three herbicides (dicamba, 2,4-D, and atrazine) by online SPE coupled to MSC using UV detection [35]. For preconcentration purposes, a C18 (8 mm i.d.) membrane extraction disk was used. For the chromatographic separation, a C18 (25 × 4.6 mm) monolithic column was used. The separation of the three compounds was achieved in 10 min with a resolution >1.5. Some advantages of SIC and MSC are the possibility of two ways and stopped eluent-flow direction, the low eluent consumption (since the batch eluent-delivery mode), the short time of analysis, the low cost and the possibility of the analyzer portability due to their dimensions allowing on-field measurements. One additional advantage of MSC is the ability to perform multi-isocratic chromatographic development (i.e., by using different mobile phases without the need for gradients). However, in SIC and MSC the choice of flow rates for the mobile phase is limited by the highest pressures that the valves can withstand, and in addition, HPLC provides higher resolution of the peaks and higher robustness, being able to analyze samples of higher complexity. Furthermore, SIC and MSC would require a second piston pump, autoburette, or syringe to carry out chromatographic separations in the gradient mode.

Flow techniques: Gas chromatography (GC)

Sample treatment is also usually necessary before GC analysis. Its automation is of utmost interest in order to avoid tedious and time-consuming operations as well as sample contamination. This is in fact, the bottleneck in GC analysis. An example of this technique coupled with a flow technique is the one proposed by Quintana et al. [36], who developed an MSFIA-LOV-GC system for polychlorinated biphenyls (PCBs) determination in solid waste leachates, providing a renewable sorbent column and thus, avoiding the increase of backpressure.

Flow techniques: Capillary electrophoresis (CE)

CE technique has gained importance in the last years as it provides an efficient separation methodology for a wide variety of analytes in diverse matrices, low sample and electrolyte consumption, and experimental simplicity. Its hyphenation with flow-based techniques helps to improve its sensitivity [37]. As an example of FIA and CE hyphenation, an FI-CE system with contactless conductivity detection (C4D) for online analysis of metal cations (ammonium, potassium, calcium, magnesium, and sodium as complexes in aqueous 18-crown-6-ether-acetace electrolyte solution) was recently published [38]. An SIA-CE [39] using also C4D as a detector was successfully tested in the field for the determination of the concentration levels of major inorganic cations and anions in a creek over a period of 5 days. In another application, SIA-CE was coupled to laser-induced fluorescence via a valve interface for online derivatization and analysis of amino acids and peptides [39]. Another SIA-CE system was developed for As speciation coupled to an ICP sector field mass spectrometer (ICP-SFMS) [40] focused on the reduction of hazardous waste residues (ca. 87 %). An electronically controlled hydrodynamic injector was used to introduce microvolumes of solutions prepared by SIA into the CE capillary with precision better than 2 %. An MSFIA-CE system for preconcentration, separation, and determination of nitrophenols was proposed by Horstkotte et al. [41], the application of MSFIA allowed background operations and thus, higher sample frequencies. The advantage of the employment of a robust and multichannel syringe pump allowed the use of a very fine sorbent material, and parallel operations, which would not have been possible with a single syringe pump (SIA) or multichannel peristaltic pump (FIA) with the same obtained analytical performance and robustness.

Flow techniques: Solid-phase extraction (SPE)

Direct determination of trace elements, e.g., radionuclides, by instrumental techniques including selective detection techniques such as ICP-MS is still difficult because of insufficient sensitivity, lack of selectivity, presence of complex matrix, poor precision, and accuracy. To solve these problems, enrichment and separation techniques have been used in the analytical chemistry laboratories for trace element determinations. SPE is one of the most important preconcentration/separation procedures for trace heavy-metal ions, due to its simplicity and limited usage of the organic solvents. Among many others, ion exchange and extraction chromatography are very popular methods due to their applicability to both preconcentration and separation. Extraction chromatography combines the selectivity of liquid–liquid extraction with the rapidity of chromatographic methods. Moreover, preconcentration improves the detection limits, increases the sensitivity, and enhances the accuracy of the results. The implementation of highly selective chromatographic columns to flow-based methodologies allowed the automation of many analytical methods. Nowadays, there are specific resins for the determination of Ra, Ni, Pb, Th, U, Np, Pu, Am, Cm, Sr, Tc, ^{3}H, and Fe among others, which have been included in automated separation protocols [43–46]. Analytical expectations can be outranged if the total automation of the methodology is achieved. Comparing some separation/preconcentration flow systems with ICP-MS for analysis of U and Th in environmental matrixes [47,48], none of those systems is fully automated. Actually, there are few systems using SPE that are able to automate the resin replacement. Furthermore, these methods are based on the use of FIA technique, which although widely utilized, has several disadvantages in front of the use of multicommutated techniques [49]. Automation based on multicommutated techniques of the analytical method allows precise control of sample and reagent volumes and flow rates, which lead to improvement in reproducibility. Our group has recently developed a fully automated method for separation, preconcentration, and determination of Th and U in environmental samples exploiting extraction chromatographic materials in an LOV-MSFIA system coupled to ICP-MS [50] (Fig. 7).

CONCLUSIONS

Flow techniques have been in continuous evolution toward new developments ever since their inception and have proved to be excellent tools and to have potential in automating all the steps of analytical protocols.

The main advantages of flow-based systems compared to traditional manual methods could be summarized as: rapid separation, online sample pretreatment, online detection, minimization of reagent consumption and waste generation, reduction of cost per analysis, minimization of cross-contamination, minimization of sample and reagent handling, improvement of the analyst safety, analyzer portability, and low-cost equipment. Moreover, comparing with classical methodologies, the sample throughput is significantly increased. In all cases, isolation procedures are performed in minutes in contrast with long times required by conventional methodologies. Since all analytical steps of a method are carried out in a closed system, external contamination is avoided, which increases the precision of the method. All of these aspects contribute to the development of more environmentally friendly methodologies.

Substantial benefits can be derived from the hyphenation of different flow systems since they complement each other, improving their individual advantages.

The advantages of combining multicommutated flow techniques with separation techniques are noteworthy and indispensable to attend and automate current analytical needs.

ACKNOWLEDGMENT

This work was funded by Spain's Ministry of Science and Innovation (Project CTQ2010-15541).

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