Sequential Injection Analysis Hyphenated with Other Flow Techniques: A Review

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Online publication date: 18 February 2011
Flow and Sequential Injection—General Approaches

SEQUENTIAL INJECTION ANALYSIS HYPHENATED WITH OTHER FLOW TECHNIQUES: A REVIEW

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In the last two decades, sequential injection analysis (SIA) became an important automation tool in several analytical fields as it provided advantages in terms of versatility, robustness, and consumption of samples and reagents. The noteworthy versatility of an SIA selection valve allowed its association with several units, including devices typically associated with other flow techniques, resulting in generally better analytical performance. This review discusses the hyphenization of SIA with other flow approaches outlining its advantages and motivations, which are related mainly with sample manipulation and solutions management. The future trends and perspectives in this field are also addressed.

Keywords: Flow techniques; Hyphenization; Review; SIA

INTRODUCTION

Flow techniques are nowadays consolidated as powerful tools in analytical chemistry, mainly due to their simple configuration, easy operation, low cost, and compatibility with any detection system (Trojanowicz 2008; Cerdà and Cerdà 2009). The evolution of flow techniques in the last three decades illustrates their significance and importance in the field of automation and opened immense possibilities in analytical perspective. The origination of this family of analytical methods was flow injection analysis (FIA) in 1975 (Ruzicka and Hansen 1975), almost 20 years after the appearance of air-segmented flow systems (Skeggs 1957). By that time, FIA arose as a consequence of the ever increasing demands for analysis in several fields and industries. The appearance of sequential injection analysis (SIA), as an alternative to FIA, broadened the scope of flow techniques for chemical analysis by overcoming some of the drawbacks that hindered its routine utilization (Ruzicka

Received 9 February 2010; accepted 11 March 2010.
This paper was submitted as part of a Special Issue on Flow Injection Analysis.
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and Marshall 1990). Due to the simplicity of the manifolds and its inherent characteristics, SIA exhibited high potential for sample manipulation providing interesting possibilities in terms of automated analysis (Lenehan et al. 2002). In 1994, multicommutation appeared with the aim of increasing the versatility of flow systems, facilitating automation, and decreasing reagent consumption (Reis et al. 1994). This analytical strategy seemed to embrace some of the advantages of both FIA and SIA, overcoming, at the same time, their disadvantages. Multisyringe flow injection analysis (MSFIA) was developed in 1999, claiming to combine the advantages of the aforementioned techniques (Albertus et al. 1999). More recently multipumping flow systems (MPFS) were proposed as a novel strategy for solutions management based on pulsed flow.

Even though these modalities of flow analysis rely on the use of specific devices that are usually associated in the literature to specific flow approaches, they are all, in some way, based on the same initial principles inherent to FIA by its appearance: reproducible sample insertion, reproducible timing, and controlled dispersion (Ruzicka and Hansen 1978).

During the development of new methodologies, the search for solutions for concrete analytical problems has frequently justified the incorporation of concepts and devices of more than one solution management approach in the same manifold, in order to maximize the advantages of each one (V. Cerdà et al. 1999; A. Cerdà and Cerdà 2009). This hyphenization is usually associated with increased possibilities in terms of solutions and sample management and with better analytical performance of the resulting systems.

This review intends to summarize and discuss the motivations and advantages of the association of SIA with other flow techniques with the aim of further increasing research in the field of hyphenated techniques.

**Sequential Injection Analysis (SIA) in the Flow Context**

Even though the functioning of a SIA system relies on the same basic principles of FIA, based upon controlled dispersion of a sample introduced into a moving carrier stream, this technique soon proved to be a powerful and versatile solution handling approach. The basic functioning of a SIA system involves the sequential aspiration of solutions through a selection valve (Pérez-Olmos 2005). In several instances, this feature has been pointed out as a disadvantage in terms of mixing conditions. However, the operation mode, based on forward and reversed flow, facilitates the use of different chemistries and strategies without reconfiguration of the manifold so that the technique can constitute a useful analytical tool (Lenehan et al. 2002).

A typical SIA manifold is comprised of a selection valve, which operates in synchronization with a propelling device, and a detector (Fig. 1). The computer control mode operation of the systems allows the aspiration of precise volumes and an effective utilization of solutions, minimizing the consumption of sample and reagent solutions and the generation of effluents. Furthermore, the effective control of the most relevant analytical parameters at run-time assures a great operational flexibility and facilitates system optimization.

The most important feature of SIA is its versatility, which is centered on the selection valve whereby each port can be dedicated to a specific purpose or
operation. Thus, in this kind of system reagents, samples, standards, as well as detec-
tors and other devices are placed around the valve in order to accomplish a specific
task. Since the early utilization of SIA, researchers made use of this intrinsic charac-
teristic by coupling all kinds of devices to the selection valve. It is possible to find
SIA manifolds that incorporate gas diffusion units, solid phase and enzyme reactors,
mixing chambers and micro-wave ovens, among many others, with the aim of
increasing system profitability (Economou 2005).

Hyphenization of SIA with Other Flow Techniques

After several decades of remarkable evolution in the flow analysis area, the idea
that each flow technique is better than the last one is misplaced and must be reviewed. It
is obvious nowadays that flow techniques are all complementary, and their rentabiliza-
tion passes through their association in analytical systems that combine the most sig-
nificant features of the independent approaches, resulting in increased potential to
manipulate complex samples with high accuracy and analytical efficiency. This is
mainly related to the similarities among flow techniques, in terms of basic principles,
that will result, in the author’s perspective, in a relativization of the technique’s denomi-
nation and in a rational utilization of all the approaches in specific analytical situations.

As mentioned previously, the peculiar mode of operation of SIA and its inherent
versatility enable its association with all kinds of devices and flow approaches. This
association can be related to increased analytical efficiency but can also be the key
to solve some of the problems associated with the analysis in SIA systems, namely,
the difficulties arising from the sequential aspiration of solutions.

The next sections include a critical description of the flow approaches resulting
from the successful hyphenization of SIA with FIA, multicommutation, multi-
pumping, and multisyringe. This will embrace the methodologies in which the prin-
ciples and strategies of SIA were associated to that of other flow techniques in the same
flow assembly in order to solve concrete analytical problems, enhance the sensitivity
of the determinations, or enable the coupling to specific detection systems.

Hyphenization with Flow Injection Analysis (FIA)

The proposal of FIA in the 1970s as a tool for the automation of analytical
procedures represented a step forward in this challenging area. It relied on the
reproducible injection of a sample aliquot in a reagent flow stream enabling the analysis in the absence of chemical equilibrium (Ruzicka and Hansen 1978). This mode of operation, based on constant volume allowed the insertion of sample aliquots with high precision, resulting in procedures with less associated errors compared to those strategies in which solutions were inserted on a time basis. A typical FIA system incorporated an injection valve, whose loop defined the volume of the sample aliquot, a propulsion device, and a detector. The main drawbacks of FIA are related to the continuous flow of reagents that result in increased consumption and with the need of system reconfiguration for adaption to different analytical determinations that are not entirely acceptable in industrial analysis (Ruzicka and Marshall 1990).

The association of the FIA and SIA concepts in the same flow network has been performed several times in the last decades, resulting in manifolds with increased versatility and analytical efficiency. Several times, this hyphenization was performed, aiming the implementation of sample pretreatment procedures that resulted in methodologies with reduced operator intervention (Crespi, Forteza and Cerdá 1995, Oms et al. 1996a, Oms et al. 1996b, Costa Cardoso, and Araújo, 2003, Zárate, Araújo, and Pérez-Olmos 2004, Chen et al. 2008, Ma, Yuan, and Liang 2008, Costa et al. 2003, Chen et al. 2008, Ma et al. 2008, Oliveira et al. 1998, Oliveira, Sartini, and Zagatto 2000, Mateos et al. 2000). In these cases, the association allowed the simultaneous implementation of distinct operations in separate channels whereby the fluids could flow in opposite directions and increasing the separation rates. Moreover, it also permitted the connection of the flow manifold to some detection systems through the re-injection of treated samples in the detector with reduced dispersion (Wang and Hansen 2005).

Crespi, Forteza, and Cerdá (1995) developed a flow assembly in which the concepts of SIA and FIA were explored with the aim of preparing a reaction pre-form before injection in the amperometric detector. A mixer-reactor was connected to both selection and injection valves and worked in synchronization with a syringe pump. The configuration of the system based on the unidirectional circulation of the solutions in the reactor resulted in an efficient mixing of sample and reagent, as demanded by the reaction particularities, allowing the injection of the formed product in the detector with reduced dispersion.

One can find a couple of SIA systems in which an injection valve was connected to the selection valve as support of gas diffusion (Oms et al. 1996b) and preconcentration (Oms et al. 1996a) units being good examples of systems that allow the implementation of different tasks in distinct channels. In these systems the pretreatment devices were placed in the loop of the injection valve that was connected to the detector channel, enabling the re-injection of the concentrated sample in the detector pathway with reduced dispersion and high yield. Moreover, with this disposition, the acceptor channel of the analyte forms a closed loop avoiding problems related with pressure differences. Several authors adopted this strategy during the development of analytical methodologies based on preconcentration (Costa, Cardoso, and Araújo 2003; Zárate, Araújo, and Pérez-Olmos 2004; Chen et al. 2008; Ma, Yuan, and Liang 2008). In some of these methodologies, to reach an adequate sensitivity, volumes of sample in the mL range were introduced in the system by means of a peristaltic pump that was connected to the injection valve (Costa et al. 2003; Chen et al. 2008; Ma et al. 2008). As before, the loop accommodated the concentration
column and by continuous flow of the sample, the analyte was concentrated. Then, elution was performed by passage of the eluent by a reversed flow extraction scheme.

Two SIA methodologies involving wetting film extraction comprised the injection of air-segmented organic solvent in the extraction coil for the elution of the wetting film (Nakano et al. 1996, 1997). Other work from the same research group for the determination of Cr (VI and III) was based on a similar system and principle (Luo et al. 1997), but, in this situation, the system included a second injection valve, connected to a peristaltic pump, for the introduction of a sample/reagent mixture in the flow system.

Oliveira et al. developed two similar SIA procedures comprising on-line microwave sample digestion of food samples (Olivera et al. 1998; Oliveira, Sartini, and Zagatto 2000). The procedure involved the insertion of food samples as a slurry or suspension in a digestion bomb together with nitric acid. An injection valve, functioning as a two position valve was connected to the digestion bomb with the aim of closing it or connecting it to waste in order to eliminate gases resulting from the digestion process.

A flow assembly that associates the concepts of SIA and FIA was developed to perform analyte separation prior to radioactivity determination. The separation step was carried out by means of a MnO₂ impregnated cotton filter that was placed in the loop of an injection valve being the later connected to one of the inlets of the selection valve (Mateos et al. 2000). After adsorption, the analytes were removed from the cotton filter by the passage of hydroxylamine through the cartridge after changing the position of the injection valve. The obtained solution was then treated and analyzed outside the flow system.

Miro and co-workers proposed a SIA methodology based on flow reversal wetting-film extraction in which an injection valve, connected to the selection valve, was placed in the extraction coil outlet allowing the introduction of sample, in the mL range, by flow reversal (Miro et al. 2002). By changing the valve position, the sample was again dispensed to the extraction coil. This particular functioning allowed not only the reversal of the sample flow without disturbing the extraction process but also an additional washing out of the analyte by an organic solvent.

Several SIA methodologies involving extraction and preconcentration of metals prior to detection by ICP-MS (Wang and Hansen 2001; Jiménez, Velarte, and Castillo 2002; Wang and Hansen 2002, 2002, 2003; Sabarudin et al. 2006; Sabarudin et al. 2007) and ETAAS (Nielsen and Hansen 2000; Wang and Hansen 2000, 2002b, 2002d; Long et al. 2004; Chomchoei et al. 2005; Wang and Hansen 2005; Anthemidis 2008; Lemos et al. 2008; Anthemidis and Adam 2009; Anthemidis and Ioannou 2009) resorted to the injection of the treated sample into the detector by means of an injection valve. With this strategy, it was possible to avoid the insertion of the sample after flow reversal that could result in high dispersion of the analytes and, consequently, in a decrease of the sensitivity. This approach was also followed by several reports on miniaturized SIA systems, named lab-on-valve (LOV) by the original author (Ruzicka 2000), coupled to distinct detection systems, namely ICP-MS (Wang and Hansen 2001), ETAAS (Long et al. 2004), and spectrophotometry (Vogt et al. 2000). Similarly, Elsholz and co-workers developed a SIA system in which aliquots of sample and digestion reagent, consisting of a bromide/bromated mixture, filed the loop of the injection valve used (Elsholz et al. 2000). By changing the valve
position the treated sample was sent to detection by cold vapor atomic absorption spectrometry.

On an identical basis, several SIA systems coupled to high-performance liquid chromatography (HPLC) were responsible for the automated sample preparation; the final eluent filled the loop of an injection valve and was injected into the HPLC detector (Lenghor et al. 2003; Theodoridis et al. 2004; Zacharis, Theodoridis, and Voulgaropoulos 2004, 2006).

Anthemidis and co-workers proposed a methodology that hyphenates SIA and FIA in a very peculiar way (Fig. 2) (Anthemidis, Zachariadis, and Stratis 2004). The central channel of the selection valve was connected to a gas-liquid separator (GLS) and not to a propulsion device as usually occurs in these systems. Thus, it was necessary to couple a peristaltic pump to each solution to be inserted in the GLS, namely, a sample and reductant solution. An injection valve was used for sample insertion and GLS evacuation. A second injection valve managed the flow of the argon in the system avoiding its passage through the gas-liquid separator that would increase the analyte dispersion.

A SIA system interfaced with capillary electrophoresis made use of an injection valve to flush the sample through the interface between the system and the capillary (Kulka, Quintas, and Lendl 2006). This step was most important for the performance of the system as it ensured that the sample was close to the capillary inlet just before the pressurization of the system and injection.

Ayora-Cañada and Lendl (2000) developed a SIA methodology that was connected to a novel sheath-flow cell comprising three stream lines where solution flowed adjacent to each other in a laminar mode. The system incorporated an injection valve that was used as switching device, allowing all three flow lines to be

Figure 2. Scheme of the hyphenated SIA system developed by Anthemidis et al. (2004) for the determination of mercury. SV: selection valve; IV1, IV2: injection valve; PP1, PP2: peristaltic pump; GLS: gas-liquid separator; AAFC: atomic absorption flow cell; R: reagent; W: waste; DW: deionized water.
stopped simultaneously in the detection cell which avoided changes in the analyte concentration during measurement.

The sampling rate of two distinct SIA methodologies was increased resorting to the FIA concept in some of the essential steps of the analytical procedures (Guzman and Compton 1993; Segundo and Rangel 2002). Guzman and Compton (1993) proposed a system in which the main channel of the selection valve was not connected to the propulsion device but rather to an injection valve. Depending on the position of the valve, either a peristaltic or syringe pump was activated. The aspiration of sample and reagents was performed by activation of the syringe pump taking advantage of its high volume accuracy. The peristaltic pump was used to fill the system and clean it at the end of each analytical cycle. Furthermore, this disposition allowed the filling of the syringe at the same time that the system was being washed by pump functioning. Also, with the aim of increasing sample throughput, Segundo and Rangel (2002) developed a SIA system embracing an injection valve that supported two enzymatic reactors. This valve worked in synchronization with an auxiliary pump and while sample and reagent were sent to one reactor, the other was continuously washed with carrier propelled by the pump. This operation mode resulted in an increment of sample throughput as it allowed performing the detection of ethanol while the glycerol reactor was loaded.

The advantages of the hyphenization of SIA with FIA are very well illustrated in a methodology for the analysis of edible oils that intends to be a flow sampling strategy for the analysis of this kind of sample without pretreatment (Pinto, Saraiva and Lima 2006). The flow system was designed by incorporation of an injection valve that was responsible for the introduction of the sample in the system, avoiding the problems associated with its viscosity that resulted in inaccuracy of the inserted volumes.

The monosegmentation approach was explored in a novel strategy for calibration by the combined action of a selection and an injection valve in a system similar to the one developed by Guzman and Compton (1993) and described previously in this section (Kozak et al. 2008). A syringe pump was responsible for the aspiration of air, diluent, and sample from the selection valve to perform on-line dilution. At the same time the carrier was continuously propelled to the detector by the peristaltic pump through the injection valve. Then, the flow was reversed and the monosegment was partially sent to waste and homogenized. By changing the position of the injection valve, the monosegment was propelled to the detector by the peristaltic pump. By hyphenization of SIA with FIA, the authors managed to implement an automated calibration procedure with reduced associated errors by using one stock solution.

Hyphenization with Multicommutation

Since it was proposed in 1994 (Reis et al. 1994), multicommutation has been the basis for the development of numerous applications with increased complexity. The successful application of the multicommutation concept in distinct analytical situations has confirmed this approach as a versatile solution handling strategy. This diverse application profile is probably related with the different interpretations of multicommutation in the scientific community; it has been simply considered a methodology based on solenoid valves (Icardo, Mateo, and Calatayud 2002) and on the
other side has been explored, by some of the authors of the original proposal, in a much more broader perspective as a concept or attribute of a given flow system (Feres et al. 2008). The original authors mainly highlight the use of binary sampling, considering that tandem streams constitute a driving force toward improved mixing conditions. In this review, we tried to make a broad analysis of the multicommutation approach in order to contemplate its diverse interpretations.

The association of multicommutation with SIA in the same flow network has increased in the last years, probably, as the key to circumvent some of the limitations of SIA, mainly the ones associated with the mixing conditions of the solutions aspirated sequentially. As a result, the systems are generally more versatile, and it is possible to control the operations within the system more efficiently.

Since 1998, solenoid valves have been used in SIA systems and in its miniaturized versions as part of a mechanism which controls the peristaltic pump operation (Fig. 3) (Araújo et al. 1998; Araújo, Costa, and Lima 1999; Costa et al. 2000; Costa and Araújo 2001b; Costa et al. 2002; Costa et al. 2003; Pinto, Lima, and Saraiva 2003; Peña, Lima, and Saraiva 2005; Santos, Araújo et al. 2004; Passos et al. 2005; Pinto, Saraiva, Reis et al. 2005; Pinto et al. 2005; Garcia, Saraiva, and Lima 2006; Pinto et al. 2006; Amorim, Araújo, and Montenegro 2007; Amorim et al. 2008; Araújo, Saraiva, and Lima 2008; Passos, Saraiva and Lima 2008; Passos, Saraiva, Lima, and Korn 2008; Pérez-Olmos et al. 2008; Zárate et al. 2008; Passos et al. 2009; Zárate et al. 2009). With this mechanism it is possible to guarantee that in the beginning of each determination the position of the pump rollers is constant, thereby minimizing the effect of systems inertia at the beginning of the pump’s

Figure 3. Schematic representation of the mechanism for the control of peristaltic pump’s functioning. PP: peristaltic pump head; SV: selection valve; EC: electric contact.
rotation and guaranteeing reproducibility of the aspirated volumes, especially when dealing with reduced volumes. Thus, a magnet (Passos, Saraiva, Lima, and Korn 2008) or a copper wire (Araújo et al. 1998) was positioned on the head of the peristaltic pump; during the movement of the pump, these devices established a brief contact with two opposed electrical contacts that were connected to the digital-in port of the interface card and the ground. This contact originated an electric signal that was detected by the computer, which assures that in each step of the analytical cycle, the pump always started from the same position. Thus, in the beginning of each step the solutions circulated in a closed circuit until the activation of a 3-way solenoid valve placed between the pump and the holding coil, as schematized in Figure 3. At the end of each cycle, the solenoid valve changed its position, blocking the flow through the holding coil and the pump stops when the magnet and the electric device were in contact again.

Some of the aforementioned works explored multicommutation to solve specific issues related with the determinations. By implementation of the multicommutation concept in SIA systems, it was possible to introduce samples in the mL range, to perform preconcentration of specific analytes, overcoming the impossibility of performing such aspiration in a conventional SIA system (Araújo et al. 1999; Costa and Araújo 2001a, b Costa et al. 2002). The preconcentration device was placed into the detector channel, which allowed loading and elution steps to occur by propulsion of the solutions through the column. Two solenoid valves were connected by a confluence and one of them was linked to the detector. The analyte was eluted by propulsion of the eluent through the referred confluence point; by changing the position of the solenoid valve, the eluate was sent to detection.

The binary sampling strategy was implemented in a SIA methodology developed by the same group for the determination of chloride and iodide by titration (Araújo et al. 2004). The titration cycle was performed by intercalation of aliquots of sample and volumetric solutions by means of a solenoid valve positioned in one of the inlets of the selection valve. The mutually dispersed zones were sent to a detector enabling the titration to be carried out. This methodology overcame the limitations of the conventional method for the determination of chloride and iodide that could not discriminate between more than one halogen species.

Our research group also implemented the binary sampling strategy in SIA methodologies with the objective of overcoming the problems associated with the mixture of sequentially aspirated zones in SIA systems (Peña et al. 2004; Passos et al. 2005; Pinto et al. 2006). A tandem stream of sample and triton X-100 was generated by means of a solenoid valve placed in one of the inlets of the selection valve in a SIA system for the determination of cationic surfactants (Passos et al. 2005). With this it was possible to solubilize water insoluble complexes formed for high concentrations of cationic surfactants. This same approach was also followed for on-line reagent preparation to overcome a problem of instability of a derivatization reagent (Pinto, Saraiva, Santos et al. 2005). The very small volume of the inserted reagents segments, through the selection valve into the holding coil, facilitated mixing, and the formation of a homogenized reagents zone.

The same strategy was adopted in a series of works involving on-line preparation of analyte standard solutions from a concentrated solution (Fernandes et al. 2001; Pimenta, Araújo, and Montenegro 2002; Pimenta et al. 2004; Santos,
Montenegro et al. 2004; Pérez-Olmos et al. 2008). As before, the incorporation of the multicommutation strategy enabled the on-line mixing of concentrated solution and water or other dilution solution. This strategy further increased the automation potential of the developed systems.

In a slightly different approach, in a SIA system proposed by Peña et al. (2004), a tandem stream of chromogenic reagent and treated sample was generated by the insertion of reagent in a confluence point due to the impossibility of performing flow reversal of the treated sample through the enzymatic columns. This strategy guaranteed a good mixture of the different zones and a reduced dispersion of the reaction product.

In 1995 Shu, Hakanson, and Mattiasson (1995) developed a procedure based on a flow network in which the multicommutation concept was explored for the management of flow between the selection valve, enzyme and blank reactors, and spectrophotometric detector (Fig. 4).

The advantages related with the incorporation of the multicommutation strategy in SIA systems are very well illustrated in a system proposed by Luo and co-workers for the determination of gaseous ammonia in air samples using a glass diffusion denuder (Luo et al. 1995). Two solenoid valves allowed precise control of the sampling procedure through a gas permeation tube, redirection of samples to the detector, and system cleaning at the end of each analytical cycle. The peculiar mode of operation of the selection valve allowed the utilization of small volumes of reagent so that the color development was limited to a small zone, which resulted in higher sensitivity.

**Figure 4.** SIA system exploring the multicommutation concept for solution management (Shu et al. 1995). SV: selection valve; SP: syringe pump; SoV1, SoV2, SoV3: solenoid valve; ER: enzyme reactor; BR: blank reactor; D: detector; W: waste.
Hedenfalk and Mattiasson (1996) developed a SIA methodology integrating a solenoid valve, in the holding coil, that functioned as an interface between the selection valve and the enzymatic reactor. The methodology was applied to the determination of ethanol in fermentation broths and proved to be robust and easy to operate, leading to a reduction in the consumption of the expensive co-factor.

A SIA flow assembly exploiting the multicommutation concept was developed for the implementation of an on-line digestion procedure based on microwaves (Neira et al. 2000). In order to increase the number of solutions to be used, a network of solenoid valves was used for the selection of sample and reagents. The multicommutation approach allowed not only control of the conditions of the digestion procedure in the oven but also allowed the handling of the gases resulting from the entire process.

In the nineties, several works resorting to the hyphenization of SIA with multicommutation made use of a set of two-position valves to control the loading and washing of separation columns as well as the elution of the retained elements to the detector (Grate et al. 1996; Egorov et al. 1998; Grate and Egorov 1998; Egorov, Fiskum 1999; Grate, Egorov, and Fiskum 1999; Grate, Fadeff, and Egorov 1999). In a similar scheme, the same research group found the analytical potential of manifolds involving the hyphenization of SIA with the multicommutation concept through the development of a renewable separation column apparatus based on two two-position valves (Fig. 5) (Egorov, O’Hara 1999). These were connected by the column body and one of them was connected to two ports of the selection valve to collect sample, reagent, and sorbent slurries for the separation step. A frit disk was placed in one of the ports of the second valve to retain sorbent beads within the column. Sample and reagents placed in the selection valve were then introduced in the column and the analytes were separated and sent to the detector. By changing the valve position the sorbent beads were expelled from the system.

Bruckner-Lea et al. (2002) utilized the same concept in a SIA system developed with the aim of performing studies of DNA hybridization. In this case, the valve was connected to the selection valve through a coil and to a renewable column being responsible for the introduction of oligonucleotides solution in the system.

Figure 5. Schematic representation of the SIA system with a renewable separation column based on two two-position valves (Egorov et al. 1999). SV: selection valve; SP: syringe pump; TPV1, TPV2: two-position valve; FR: frit restriction; SCB: separation column body; D: detector; W: waste.
The monitorization of bioprocesses was tested by means of a SIA system in which the multicommutation strategy allowed the effective management of the solutions through the reactor (Shu and Ling 2000). A solenoid valve, positioned in one of the inlets of the selection valve, worked in synchronization with a peristaltic pump enabling the insertion of peroxidase substrate in the reactor pathway mixing it with the other solutions that were aspirated from the selection valve.

A binary sampling strategy was implemented in a SIA system by Paseková et al. (2001) with the aim of performing the alternated insertion of small slugs of buffer solution and sample. This approach allowed the on-line pH adjustment of the reaction zone, before its propulsion to the detector, which was mandatory for a successful application of the methodology to the determination of acetylsalicylic acid. The analytical efficiency of a SIA system for the determination of copper in seawater with sample pre-treatment was greatly enhanced by the incorporation of the multicommutation strategy (Yu, Du, and Wang 2007). A solenoid valve was placed in one of the inlets of the selection valve and was responsible for the addition of the complexing reagent to the sample just before the PTFE-beads-packed micro-column, where the complexed analyte was retained. With this configuration, it was possible to perform a prewashing procedure to eliminate sample matrix, which allowed the determination of ultra-trace copper in high-salinity seawater without dilution. A similar scheme was followed by Lee et al. (2005) that positioned a solenoid valve in one of the selection valve inlets and connected it to an on-line filtration unit coupled to a bioreactor. By means of an additional pump, a sample was aspirated, through the solenoid valve, from the bioreactor, in order to fill the tubing before sample insertion by the selection valve.

Lopes et al. (2007) conceived a fully automated procedure combining the special features of SIA with the automation potential of multicommutation. The methodology comprised an on-line sample clean-up/preconcentration step, and the system embraced two solenoid valves: one managed the propulsion of water and eluent from the peristaltic pump allowing a precise control of the conditions for the loading and elution of the column; the other valve was linked to the detector allowing the continuous aspiration of water to the nebulizer by means of a second peristaltic pump.

The combination of a selection valve, a solvent distributor, and six 3-way valves resulted in an automatic separation and preconcentration procedure of radio-nuclides in which the separation time was shortened (Kim et al. 2008). The solenoid valves were connected to the solvent distributor and were responsible for the management of the solutions coming into and out of the separation columns. The selection valve held the elution reagents and was linked to the solvent distributor that diverted the solution to the correct column. By increasing the number of columns and tubing lines, the system could process several samples simultaneously.

An interesting approach to increase the robustness of a SIA system applied in the determination of sorbitol in *Pichia pastoris* cultivation was designed by Horstkotte, Arnau et al. (2008). In this system, a solenoid valve was placed between the propelling device and the holding coil for the aspiration of acetonitrile to rinse the holding coil and to expulse stacked air bubbles.

The accuracy of a SIA methodology for the determination of both hypoxanthine and potassium in vitreous humor samples was greatly enhanced by the utilization of a solenoid valve that permitted interference elimination and enabled
the analysis of samples without drastic batch pretreatments (Passos et al. 2009). The determination of hypoxanthine was based on its oxidation to uric acid catalyzed by immobilized xanthine oxidase. The system also comprised a reactor with activated glass beads that was used to perform a sample blank. The referred solenoid valve, connected to both reactors, was used to direct the samples to the enzyme reactor or to the glass beads reactor in the case of the sample blank estimation.

Ogata et al. (2002, 2004) proposed two miniaturized SIA methodologies in which a solenoid valve allowed an effective management of the passage of the sample through the column and of the elution of the retained elements to the detector. The described systems showed excellent performances due to their versatility and reduced analysis time.

**Hyphenization with Multisyringe Flow Analysis (MSFIA)**

The MSFIA was introduced in 1999 as a robust multichannel manifold for performing process flow analysis. The authors initially proposed this strategy as an alternative to multicommutation whose typical functioning under negative pressure sometimes constitutes a major difficulty (Albertus et al. 1999). In this technique, a computer controlled multisyringe piston pump is employed as a multichannel device guaranteeing volume delivery within a very wide range of flow rates, readily stopped, or changed by software instruction. The authors considered that the most important features of MSFIA are its increased flexibility and the significant reduction in reagent consumption. However, the peculiar mode of operation of the technique demands that the forward movement of the solutions in the manifolds has to be stopped periodically to refill the syringes. This particularity introduces some delay in the analytical cycles resulting in lower analytical frequency.

The association of multisyringe modules to SIA has grown in the last years confirming the potential of this strategy. Generally, the hyphenization of these two techniques increases the versatility and flexibility of the resulting flow systems since it allows sample insertion on a time basis and so the analysis conditions can be changed without systems reconfiguration. At the same time, the carryover risk associated with the utilization of the syringes for sample introduction is reduced (Segundo and Magalhaes 2006).

A selection valve was attached to a multisyringe module in a chemiluminescence methodology for the detection of glucose. The valve was responsible for sample management and supported also a glucose oxidase reactor that was connected to the main flow line by confluence (Manera et al. 2004).

A selection valve and a supplementary syringe pump were associated with a multisyringe module in a flow assembly developed for on-line monitoring of orthophosphates in soils and sediments (Buanuam et al. 2007). The selection valve was used to select the appropriate extractant and accommodated several reagents and a soil microcolumn, being connected to the multisyringe by means of a solenoid valve.

A MSFIA system for the evaluation of hypochlorite in commercial products with on-line dilution incorporated a selection valve for the management of sample and standards (Soto et al. 2008). The selection valve accommodated a miniature dilution chamber and was connected to a cobalt catalytic column where the analyte was decomposed to chloride and oxygen. The analytical signal was established from
the difference in absorbance at 290 nm registered when the sample passed through the column or was sent directly to the detector.

In 2008, Horstkotte and co-workers coupled MSFIA to selection, injection and solenoid valves for the development of a methodology for the determination of nitrophenols by capillary electrophoresis (CE) that constitutes an excellent practical example of the benefits of flow techniques hyphenization (Fig. 6). The resulting system allowed the advantageous automation of the total analytic procedure including on-line sample modification, preconcentration and maintenance of the apparatus. In this system, an injection valve functioned as support of a solid phase extraction column and was connected to one of the inlets of the selection valve and to the CE system. The selection valve was responsible for the introduction of sample in the extraction column and accommodated also the reagents for column cleaning and elution. Auxiliary solenoid valves were used for pickup sample, redirect solutions to the SPE column, introduce air to reduce sample dispersion, and close the outlet of one of the implemented capillary flow interfaces for pressure build-up temporarily at the capillary inlet.

Maya, Estela, and Cerda (2008) developed a MSFIA methodology in which a selection valve was linked to one of the syringes and was responsible for the handling of sample, eluent, and rinsing solution. The other syringes operated in the forward flow mode avoiding the mixing problems typically associated with the sequential aspiration of solutions in reverse mode in the SIA technique. Thus, the reagents for oxidation and subsequent spectrophotometric determination of the analytes were

Figure 6. Scheme of the MSFIA-SIA hyphenated system for the determination of nitrophenols (Horstkotte, Arnau et al. 2008). SV: selection valve; IV: Injection valve; SoV1, SoV2, SoV3: solenoid valve; MS: multisyringe module; EL1, EL2: eluent; D: detector; W: waste; AC: acidifier; SB: separation buffer.
inserted, by means of confluences, in the detector channel (that was positioned in one of the inlets of the selection valve) before or after the photo-chemical reactor.

Very recently Mirabó, Forteza, and Cerdà (2009) developed a multisyringe sequential injection method for monitoring water quality. The SIA configuration minimized reagent consumption and the association with MSFIA maximized flexibility and robustness. The flow assembly comprised two 10-port selection valves that accommodated the reagents needed for the evaluation of eight important physico-chemical parameters in water (pH, specific and acid conductivity, hydrazine, ammonium, phosphate, silicate, and total iron) with high degree of automation in reduced time. The valves also supported an auto sampler, a spectrophotometric detector, a temperature probe, a cationic column, a pH meter, and a conductimeter. Two solenoid valves were responsible for the connection of the valves to the syringes of the multisyringe module.

In the last years, a few works involving the incorporation of multisyringe pumps in miniaturized SIA systems have been published (Long et al. 2006; Quintana et al. 2006; Quintana et al. 2009). The objectives of this association are similar to the ones described for conventional SIA systems and are related with the insertion of the sample in the flow networks and management of solutions within the system. The referred systems involved a pre-concentration or solid phase separation step by bead injection in which the beads are enclosed on a syringe in one of the inlets of the selection valve and other inlet served as microcolumn. One of the works was proposed by Long and co-workers in 2006 and was based on a multisyringe system with atomic fluorescence spectrometry detection for on-line bead injection pre–concentration. The methodology besides the MSFIA and SIA approaches embraced also the multicommutation concept that allowed the management of the passage of the solution through the microcolumn. The other works in the same line of research were developed by Quintana and co-workers and consisted of two methodologies also based on pre–concentration by bead injection prior to chromatographic separation (Quintana et al. 2006; Quintana et al. 2009). These systems also comprised an injection valve for quantitative injection of a metered eluate zone and one of them included also a solenoid valve to facilitate rinsing of the transfer line placed between the injection valve and chromatographic system (Quintana et al. 2009). The chromatographic systems in both cases are synchronized with the bead injection procedure allowing a sample to be analyzed while the ensuing one is being processed in the flow system. Due to their peculiar characteristics, the described systems showed to be extremely versatile and robust and opened new and interesting perspectives on the association of automated bead injection separation with gas and liquid chromatography.

**Hyphenization with Multi-Pumping**

The multipumping concept, proposed in 2002, revolutionized the concept of flow analysis since, in contrast to the typical flow systems, it is characterized by a pulsed flow that ensures a fast sample/reagent mixing which contributes to improving the reaction sensitivity even in situations of limited dispersion. (Lapa et al. 2002). This technique is based on the utilization of solenoid micropumps which are the only active devices acting simultaneously as liquid propelling units, sample insertion
ports, and commuting elements. Thus, the configuration and control of the flow system are greatly simplified.

The implementation of the pulsed flow approach in manifolds involving other flow strategies can result in systems with increased versatility in which the automation of chemical reactions can be performed with advantages in terms of mixture and, consequently, of sensitivity. Furthermore, the utilization of solenoid micropumps can result in many possibilities in terms of sample insertion including variable sample volume, binary sampling, and merging zones, which could be exploited to manipulate reagents addition, sample zone homogenization, and reaction development.

Regarding the association of the pulsed flow and SIA concepts there are two approaches in which two solenoid micropumps were used as propelling devices (Pinto et al. 2005; Takayanagi et al. 2009). This configuration was revealed to be an interesting tool to solve the mixing problems associated with the sequential aspiration of solutions in SIA, since the pulsed flow creates a chaotic movement of the solutions promoting a better mixture. Moreover, the use of solenoid micropumps as propelling devices helped to avoid some shortcomings related with the utilization of peristaltic and syringe pumps, such as periodic replacement of the tubing and interruption of the analytical cycle for filling or emptying the syringe, respectively. Pinto and co-workers developed a SIA system in which the utilization of two solenoid micropumps for propelling and aspirating solutions originated a pulsed flow that enhanced sample/reagent mixing (Pinto et al. 2005). This aspect was favorable for the implementation of an automated methodology for the fluorimetric determination of indomethacin in pharmaceutical preparations upon alkaline hydrolysis in micellar medium as it enhanced the intermixing of the used viscous solutions. The proposed flow system exhibited operational characteristics similar to those of a conventional SIA, and the obtained pulsed flow revealed both on the zone penetration studies and in the analysis of pharmaceutical preparations, an enhanced solutions intermixing aptitude that resulted in a significant increment in reaction zone homogenization, particularly in the case of low stroke volume micropumps.

The hyphenization of multipumping with SIA in a methodology for the evaluation of metals as catalysts in the reaction between luminol and hydrogen peroxide, confirmed the possibility of associating profitably of the pulsed flow technique with the chemiluminescence detection with a stable baseline (Takayanagi et al. 2009).

**CONCLUSIONS AND FUTURE TRENDS**

The large utilization of SIA for the implementation of analytical procedures over the last decades has confirmed this technique as a versatile and robust analytical approach. The potentialities of SIA are very well exemplified in the works discussed in this review. The association of SIA with devices or approaches typical from other flow techniques has led to the development of versatile flow methodologies for the determination of a large variety of analytes in distinct analytical fields.

Taking in consideration the strategies and devices of each flow technique, the possible instrumental configurations and operation modes are immense and can be adapted to the needs of specific determinations resulting in hyphenated techniques that permit the automation of complex procedures. Indeed, as can be confirmed
in the different sections of this review, the hyphenization of SIA with other flow techniques has allowed the automation of sample pre-treatment procedures such as gas-diffusion, pre-concentration and dialysis among others, reducing the analysis time and the errors associated with extensive operator intervention.

Furthermore, the hyphenization of SIA with flow injection and multicommutation approaches enables the coupling of flow networks to particular detection techniques such as ETAAS, ICP-MS, and HPLC. In these cases, injection and solenoid valves functioned as an interface between the detection and the SIA system that was responsible for sample treatment and preparation of the detector for the measurement step. With this, the advantageous features of the systems could be explored and the possible applications became uncountable.

A careful analysis of the literature reveals that the hyphenization of SIA with MSFIA is almost mandatory for the development of robust and versatile manifolds with improved analytical aptitude, constituting an import tool for the development of new MSFIA methodologies.

Even though some researchers have already explored the potential of miniaturized SIA hyphenated techniques, the investigation in this field is very promising. In detail, the association of these miniaturized systems with solenoid micropumps can constitute an interesting work basis.

It is our belief that in the near future, the fundamentalisms related with flow techniques and their denomination will naturally be absorbed by the need to solve concrete and complex analytical situations and by the high demands in the field of automation of procedures. This will result in a rational development of flow manifolds, involving the incorporation of specific approaches and devices according to the determination specifications, in a scheme that is independent from the researcher’s specialization or preferences.

REFERENCES


