Flow Analysis: A Critical View of Its Evolution and Perspectives

Marcela A. Segundo and António O. S. S. Rangel*

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

Abstract

In the present article, flow techniques developed for automatic chemical analysis are reviewed. Different aspects of several flow methodologies are discussed, namely their configuration and operation mode. Special emphasis is given to flow injection analysis and sequential injection analysis. Recent developments in this area are also discussed, including bead injection, multicommutation and multisyringe flow analysis.

Keywords Flow analysers, Segmented flow analysis, Flow injection analysis, Sequential injection analysis

1. Introduction

In the last five decades, several automatic techniques were developed for mechanisation of chemical analysis. Over the years, a high number of commercial analysers were available, mainly to perform routine analysis in clinical and environmental samples. Besides the first goal of enhancing throughput in routine analysis, automatic methods for chemical analysis had also an important role in industry, where real-time information is essential for process monitoring. The mechanisation of non-routinely work, especially in research areas, was also regarded as a convenient approach when large number of repetitive experiments were performed or when hazardous (*e. g.* radioactive) materials were handled.

The demand for robust and reliable analysers, adapted to the working conditions experienced in each field of application, generated a multitude of different techniques for mechanisation and automation of chemical analysis. Besides discrete and robotic analysers, which are out of the scope of this paper, flow analysers have been successfully used.

These analysers are characterised by the fact that the transport of samples and reagents along the system is effected by establishing a gas or liquid stream flowing through the tubes that constitute the manifold. Sample and reagents can be mixed in a number of ways, and a variety of intermediate operations from the mere halting of the flow to the incorporation of continuous separation units (dialysers, extractors, etc) can be implemented. In the following sections, several automatic flow methodologies used to develop this kind of analysers are described and compared.

2. Segmented flow analysis

Segmented flow analysis (SFA) was one of the first techniques widely used in laboratories requiring a large number of analysis. Described for the first time by Skeggs [1], SFA allowed the performance of the analytical process in a continuous fashion, inside the system conduits. The usual components of these systems (Fig. 1 a) are a sampling system, one or more propelling units, reaction/mixing coils and a flow-through detector.

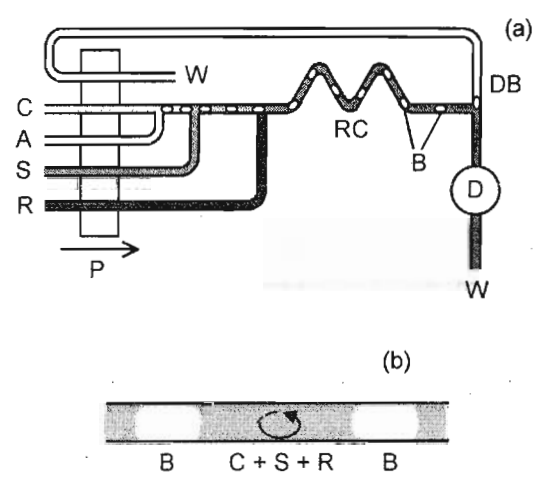

Figure 1. (a) Schematic representation of a segmented flow analysis (SFA) manifold. Samples are aspirated and introduced in a flow segmented by air. Reagent(s) is(are) introduced in confluence points and the mixture obtained is further propelled through a coil (RC) until the reaction is complete. Detection takes place in a flow through device (D), usually after elimination of air bubbles (DB). (b) Turbulent flow inside a liquid segment, intercalated between two air bubbles. C: carrier; A: air; B: air bubble; S: sample; R: reagent; P: pump; D: detector; DB: debubbler; RC: reaction coil; W: waste.

In these systems, the flow is segmented by air, nitrogen or even oil, establishing physical separation (segments) along the continuous flow stream. These bubbles are introduced in order to limit sample dispersion, to scrub the walls of the conduits and to promote homogeneous mixing by generating turbulent flow inside each segment (Fig. 1 b). Samples are introduced by aspiration through a moving articulated pipette, intercalated by a washing solution to avoid carry over between consecutive samples. Reagents are introduced in confluence points and a debubbler may be included to remove air bubbles before detection. If necessary, operations to separate the analyte from

*Corresponding author
E-mail: rangel@esb.ucp.pt

the sample matrix can be performed inside the system, such as dialysis, filtration, solvent extraction and gas-diffusion [2].

Similarly to discrete analysers, in air-segmented analysers measurements are carried out under dual equilibrium conditions: physical and chemical. Homogenous mixed solutions and steady state readout conditions were regarded as the only suitable way to attain reproducible measurements. These conditions restricted the efficiency of the systems, which required a long start-up time. Besides that, in systems based on slow kinetics reactions, the residence time of the samples is long, which can cause the loss of many samples and reagents if the system function is interrupted abruptly. Despite this, commercial analysers of this type have known considerable success.

3. Flow injection analysis

Although at a first glance flow injection analysis (FIA) systems look similar to SFA manifolds, the differences between these two methodologies are overwhelming. In FIA, the flow stream is not segmented by air. The conduits are narrower, through which the flow is of laminar type. The sample is not aspirated, but rather "inserted" in a carrier/reagent stream by means of a rotary valve. The signal detected is transient because neither physical equilibrium (homogenisation of sample and carrier/reagent) nor chemical equilibrium (reaction completeness) are attained.

FIA is based in three principles: (1) reproducible sample injection or insertion in a flowing carrier stream; (2) controlled dispersion of the sample zone; and (3) reproducible timing of its movement from the injector point to the detection system. A typical FIA manifold is depicted in Fig. 2 a.

Developed independently by Ruzicka and Hansen [3] in Denmark and by Stewart *et al.* [4] in USA, the concept of FIA has known some changes over the years. The first definition was suggested by the former authors, defining FIA as "a new concept of continuous flow analysis based on injecting the sample in a rapidly flowing carrier stream which has not been segmented by air". Most of the methodologies proposed in the first 10 years of FIA existence complied with this definition, aiming the automation of known chemical reactions, in order to compete directly with segmented flow analysers. During these years, solid reagents, immobilised enzymes and ion-exchangers were packed in miniaturised reactors and introduced in FIA systems to convert, catalyse or pre-concentrate the analyte. Moreover, solvent extraction, gas diffusion and dialysis were implemented in FIA manifolds in order to enhance selectivity and minimise matrix interference.

A more up-to-date definition, advanced by the same authors in 1988, classified FIA as a means of "information-gathering from a concentration gradient formed from an injected, well-defined zone of fluid, dispersed into a continuous unsegmented flow stream of a carrier" [5]. This definition is more comprehensive, also taking into account FIA techniques based on the exploitation of gradient formed by the dispersion process. Among these techniques, merging zones [6], zone sampling [7], penetrating (or chasing) zones [8], FIA titrations [9] and gradient dilution and calibration [10] were included. Stopped-flow FIA systems were also placed in this category [11]; they enabled reaction rate measurements and could be applied to enhance sensitivity of determinations, because dispersion ceased as the flow was stopped while the reaction continued.

Besides being a tool for serial analysis, FIA offered the opportunity to perform assays that were not feasible when carried out manually, by taking advantage of the reproducible timing attained in these systems. Two rather known examples illustrate this feature: the mechanisation of hydride generation for separating trace toxic metals from complex sample matrices prior to their detection by atomic absorption spectroscopy [12] and the use of methods based on transient light formation, generated by bio and chemiluminescence [13].

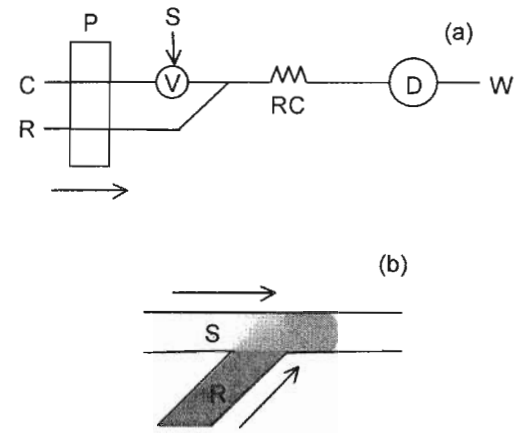

Figure 2. (a) Schematic representations of a flow injection analysis (FIA) manifold. Samples are introduced into the system through the injection valve (V); dispersion takes place inside the tube conduits and reagent is added through a confluence point (b). Reaction takes place in a coil (RC) positioned before the flow through detector (D). Detection can be performed before reaction completeness. C: carrier; V: injection valve; P: pump; D: detector; RC: reaction coil; S: sample; R: reagent; W: waste.

The compiled FIA bibliography, consisting of more than 10,000 papers, more than a dozen monographs and close to 150 PhD theses [14], confirms the wide acceptance of this technique among the scientific community. The feeling is not the same when industry and routine laboratories are concerned: the implementation of such systems are below expectations. The reasons leading to this situation can be: the lack of robustness of some manifold components, especially the tubes of peristaltic pumps and some types of injection devices; the high throughputs accomplished by FIA manifolds, surpassing the number of samples to be assayed or the capacity of external sample preparation (e.g. digestion, dilution).

Although FIA have been widely accepted, several flow methodologies were described during the 1980's as possible alternatives to this flow methodology. One of them was controlled dispersion flow analysis (CDFA), introduced by Riley *et al.* [15]. In this case, the sample is aspirated through a probe instead of being injected or inserted in the flowing stream. The advantages pointed out to this methodology are the elimination of injection/intercalation devices from the manifold and saving of sample, as just the required volume is aspirated. The applications resorting to this technique were mainly developed in the clinical chemistry field [16].

Monosegmented continuous flow analysis (MSFA) was proposed as an alternative for both SFA and FIA [17]. In this

case, the sample is introduced in the flowing stream between two air bubbles, initially by means of a special injection valve and recently by solenoid valves. Several applications of this technique were described in the literature [18]. In the past two years, the majority of the manifolds proposed associated this technique with the multicommutation concept, which will be further discussed in a following section.

4. Sequential injection analysis

Sequential injection analysis (SIA), conceived as a single pump, single valve, single channel technique, was introduced in 1990 by Ruzicka and Marshall [19] as a feasible and mechanically simpler alternative to FIA. The most basic system comprises a single bi-directional pump, a holding coil, a multi-position selection valve, a reaction coil and a detection system (Fig. 3 a).

In a typical analytical cycle, sample and reagent zones are sequentially aspirated through the selector valve into a holding conduit. In this way, a stack of well defined zones is obtained. By means of a flow reversal, a composite zone is formed in the holding coil, as sample and reagent zones penetrate mutually, owing to combined axial and radial dispersion. The combined zones are then propelled through the reaction coil and detection system, where the reaction product is monitored.

Although SIA is based on the same principles of FIA (precise sample introduction, controlled dispersion and reproducible timing), the differences between these techniques are remarkable, especially when considering the dispersion patterns inside the two systems. In FIA, reagents are normally added to the injected sample zone through confluence points (Fig. 2 b), resulting in a concentration gradient of analyte within a constant background of reagent. In SIA, an initial sharp boundary is formed between the adjacent sample/reagent zones stacked in the holding coil; even after the flow reversal, only a partial overlap of analyte and reagent zones is achieved in these systems (Fig. 3 b). This feature can be a source of inaccuracy, especially when sample is contaminated by interfering species that also consume reagent in the overlapping zone [20].

On the other hand, SIA can be considered more versatile than FIA as different methodologies can be implemented using the same manifold. Any changes (sample volume, reaction type, sample dilution and reagent to analyte ratio) are accomplished via flow programming rather than by physical reconfiguration of the flow path. In fact, computer control is essential in SIA in order to synchronise the valve and pump movements as well to enable precise timing events. Reagents saving is another advantage pointed out to SIA when compared to FIA, as just the required amounts are aspirated and carrier is not pumped continuously.

In the past eleven years, about 290 articles dealing with SIA were published, including two reviews about its application to process control [21, 22]. Almost half of the papers were published in the past three years, indicating that wide application of this technique is just beginning. UV/Vis spectrophotometry is the most used detection type, accounting for more than 50% of the systems described in the literature. Applications were developed in several different areas, mainly for analysis of environmental samples (29%), followed by food (19%) and clinical + pharmaceutical (15%) samples [23].

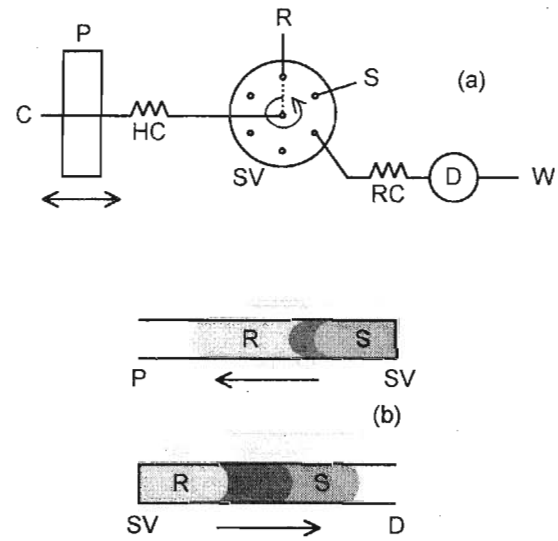

Figure 3. (a) Schematic representations of a sequential injection analysis (SIA) manifold. Sample and reagent are sequentially aspirated into the holding coil (HC) through the moving channel (dotted line) that connects the central port to the different side ports. After flow reversal, the zones are dispersed while they are propelled through the reaction coil (RC) towards the detector. (b) Representation of reagent and sample zones, stacked inside the holding coil and in the reaction coil, after flow reversal. The darker zones represent the overlap of reagent and sample zones, where reaction product is developed. C: carrier; P: pump; SV: selection valve; D: detector; HC: holding coil; RC: reaction coil; S: sample; R: reagent; W: waste.

5. Recent developments

In the following sections, a description of which can be considered recent developments in flow analysis is presented. The methodologies chosen have just been implemented by the research groups (or co-workers) where it was first described. Their status as "new flow methodologies" is not so clear as some of them can be considered an instrumental improvement or an operation mode of already existing techniques. However, it is undeniable that they bring some new features, opening up new possibilities in chemical analysis or even extending them to biochemical and biological fields.

5.1. Bead injection

Bead injection was developed to accommodate solid-phase chemistry in flow analysis [24]. This operation mode is based on the microfluidic manipulation of a precise volume of suspended beads that serve as a solid-phase carrier for reagents, reactive groups or even cells. The major benefit introduced by bead injection is automatic surface renewal, a critical feature when assay surfaces become contaminated or otherwise dysfunctional with repetitive use.

A typical analytical cycle is depicted in Fig. 4. Initially, an exact volume of a bead suspension is aspirated and loaded into a jet ring cell [25], where it is subsequently perfused by the analyte solution, buffers or auxiliary reagents. Chemical reactions or biological interactions occur at the bead surfaces and

can be analysed in real time, either directly on the solid phase or within the eluting liquid phase. A multi-parameter approach is also possible, by monitoring simultaneously the changes in the solid and liquid phases. At the end of a measurement cycle, the beads can be automatically discarded or collected for further analysis.

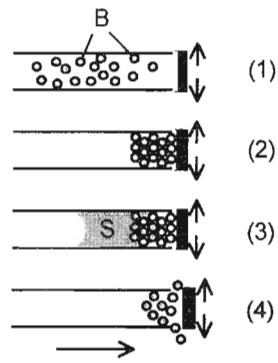

Figure 4. Schematic representation of an analytical cycle using bead injection. First, an exact volume of bead suspension is aspirated to the holding coil of a SIA manifold and then sent into a special flow cell (1), where they are trapped (2). Then, beads are perfused with analyte, reagent or carrier (3), depending on the determination aimed, as beads can adsorb analytes and reagents sequentially or carry previously immobilised reagents or cells. Afterwards, detection takes place by direct probing the bead layer or by probing the eluted phase. Finally, the configuration of the flow cell is changed and the beads are discarded (4). B: bead; S: solution (sample, reagent or carrier).

About 30 articles dealing with this technique have been published; almost all of them came from J. Ruzicka's group and co-workers. Applications were reported not only for chemical analysis but especially in the biological field. In fact, bead injection can be an invaluable tool in biochemical and cellular studies. Applications describing renewable surface immunoassay [26] and bioligand interaction studies [27] have been developed. Moreover, measurements of extracellular and intracellular pH [28], oxygen consumption [29] and variations in intracellular calcium concentration [30] were described, allowing assessment of cellular response in live cells, while they were exposed to different substances.

5.2. Multicommutation

Multicommutation is a novel approach in flow analysis, characterised by the use of individual commutation devices. The flow manifold is generally constituted of a set of solenoid valves, which can be arranged as depicted in Fig. 5, creating a flow network, where solutions can be accessed randomly.

Introduction of sample and reagents into the analytical path can be performed by aspiration through a single pump channel placed after the detection system, and by selecting the positions of the respective valves [31]. The introduction of solutions in the flow manifold can also be accomplished by placing the pumping device before the commutating valves; in this configuration, a multi-channel pump is required and solutions are propelled into the flow network or re-circulate to their own vessel, according to the position of the respective solenoid valve [32].

Although solenoid valves were already included in flow manifolds as a substitute for rotary valves for sample introduction [33] the multicommutation concept was first described by Reis *et al.* [31], associated to the binary sampling approach. The proposed system allowed the alternated insertion of small slugs of sample and reagent in the analytical path. When this string of sample in tandem with reagent was transported towards the detector, mutual dispersion occurred from the liquid interfaces, promoting conditions for development of chemical reactions.

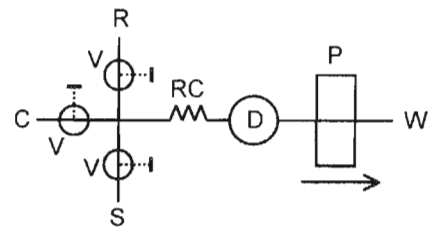

Figure 5. Schematic representation of a multicommutation flow system. The solutions can be accessed simultaneously and randomly. According to the position of solenoid valve (V), the solution vessel is connected to a blocked tubing (dotted line) or to the flow network (solid line). In this case, the solution is aspirated by the pump placed after the detector. C: carrier; P: pump; V: solenoid valve; D: detector; RC: reaction coil; S: sample; R: reagent; W: waste.

Until now, more than 40 articles dealing with this subject were published, describing different approaches on flow analysis, such as random reagent selection for sequential determinations [34], stream splitting for differential kinetic analysis [32], dynamical range expansion [35] and binary search for end-point determination in titrations [36].

Flexibility is, with no doubt, the main advantage of multicommutation over other flow techniques. In fact, Zagatto *et al.* [37] considered that multicommutation can unify all concepts already proposed in flow analysis, considering the possibility of accommodating different flow modalities (FIA, SIA) in a system with just solenoid valves.

5.3. Multisyringe flow analysis

Described for the first time by Cerdà *et al.* [38], multisyringe flow analysis relies on a device designated by multisyringe, manufactured by Crison. The multisyringe burette is a multiple channel piston pump, driven by a single motor of an usual automatic burette and controlled by computer software through a serial port. A two-way commutation valve is connected to the head of each syringe, allowing optional coupling to (or disconnecting from) the manifold lines, both in dispense or in pickup piston movements (Fig. 6). A wide range of flow rates, from values lower than 0.1 up to 72 ml min^{-1} can be obtained, according to the variable motor speed and the volume of the syringes.

This propulsion system opens up new possibilities, combining the multichannel operation of peristaltic pumps with the constant, pulseless and exactly known volume delivery achieved by piston pumps. Moreover, the use of a two-way commutation valve on each syringe introduces flexibility and reagents saving, since any

stream can be connected to the system or to the reagent vessel when required, without interfering with the other channels. However, it has the same disadvantage as for piston pumps that the forward movement must be stopped to reload the syringes, decreasing the sampling frequency. Until now, just a few applications were described, which are summarised in a review recently published [39].

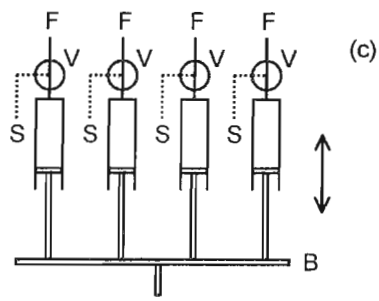

Figure 6. Schematic representation of a multisyringe. This device is a multiple channel piston pump, composed in this case by four syringes whose pistons are connected to the same bar. Each syringe is equipped with a solenoid valve which connects the syringe to the flow system (solid line) or to the solution vessel (dotted line). V: solenoid valve; F: flow system; B: bar that connects all pistons to the motor.

6. General overview

Considering all the choices available at the present moment to implement automatic analysis, it is not possible to state which one is better. It will depend on the specific analysis aimed and the features associated, such as the sample throughput, sample availability and reagents cost and toxicity. All these factors must be considered when choosing a particular flow methodology.

For instance, if sample consumption is not an issue and the reagents used are harmless and inexpensive, FIA would be an interesting alternative. On the other hand, SIA would be a more adequate choice if reagent and/or sample saving is required or if the waste generated should be minimised. These situations are found when reagent/sample are expensive or scarce or when toxic or organic compounds are used.

Other important aspect to be considered is the mixing conditions of solutions throughout the system. In FIA solutions can be added continuously in confluence points placed along the system while in SIA the mixture occurs essentially during flow reversal of the stacked zones in the holding coil. When considering reactions involving three or more reagents, SIA would be less suitable than FIA as efficient overlap of four zones (three reagents + sample) in the holding coil is not attainable. Nevertheless, SIA could still be applied by using a mixing chamber or by placing the different reagents in the same solution. In this context, the binary sampling approach associated to the multicommutation concept can also be an alternative to promote effective mixing between adjacent zones.

The recent developments described in the previous section will surely contribute to the production of new flow analysers. In the following years, we believe that the potential of this new methodologies will be further explored, aiming the development of computer controlled multi-parameter analysers. We also believe that more steps will be taken towards green analytical chemistry by using these methodologies to reduce reagent consumption and to minimise the amount of waste produced.

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M. A. Segundo

A. O. S. S. Rangel