Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 64 (2004) 1091–1098
Talanta
www.elsevier.com/locate/talanta

Multi-pumping flow systems: an automation tool☆

José L.F.C. Lima[a,*], João L.M. Santos[a], Ana C.B. Dias[b], Marta F.T. Ribeiro[a], Elias A.G. Zagatto[b]

[a] *REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, Porto 4050, Portugal*
[b] *Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, P.O. Box 96, Piracicaba 13400-970, Brazil*

Received 27 February 2004; received in revised form 16 April 2004; accepted 16 April 2004
Available online 28 July 2004

Abstract

Multi-pumping flow systems (MPFS) are one of the most recent developments in terms of the design, conception and implementation of continuous flow methodologies, for sample and reagent handling and for the automation of analytical procedures. Based on the utilisation of multiple solenoid micro-pumps they enable the configuring of fully automated and easily controlled and operated analytical systems since all the fundamental operations involved in carrying out a sample analysis, including sample insertion, reagent addition and signal measurement could be carried out by the same manifold component, reducing the number of system parts and minimising its control or the occurrence of mal-functions. On the other hand, micro-pumps actuation produce a pulsed flow characterised by a chaotic movement of the solutions, which contributes to a fast sample/reagent homogenisation with low axial dispersion yielding improved analytical signals. The combination of such advantageous features resulted in simple, compact, versatile, fast, low-cost analytical procedures, exhibiting low reagent and low sample consumption, reducing the production of undesirable wastes and minimising operator intervention.

1. Introduction

Since the conception of flow autoanalysers by Skeggs in 1957 [1], flow analysis systems have shown an accentuated evolution, especially in the advent of flow injection analysis [2], sequential injection analysis systems [3] and more recently, systems exploring discontinuous flows [4], "Lab-on-a-valve" type analysis modules [5], multicommutation [6,7] and multisyringe [8], among others. This evolution has arisen from the need to adapt to new analytical requirements (number, quality and diversity of assayed samples) and an increased availability in terms of instrumentation, particularly in relation to new systems of impulsion, insertion of samples and reagents, manifolds (miniaturisation), detectors and control interfaces.

Most of the already available flow methodologies although exploiting distinct flow strategies make use of similar components, namely in respect of the solutions' propelling systems and the insertion and commutation units. In general, these perform a specific function that markedly constrains the system's analytical performance.

Peristaltic pumps have been by far the most generalised means [9] of propelling solutions at constant flow rates, which are usually controlled as a function of the diameter of the pumping tube and rotation speed of the pump head [10]. They present some limitations, namely associated with the periodic substitution of the tubes, non-adherence to a nominal flow rate and production of rippled pattern streams. This latter limitation can be minimised using pulse dampeners [11] or temporal synchronisation [12,13] but this gives rise to a greater degree of system complexity.

The solutions can also be propelled by means of piston or syringes pumps that generate precise and pulseless flowing streams. However, these systems bring with them some limitations depending on how they are used. When the solutions are propelled it is necessary to have a syringe for each channel, whereas in the aspiration mode – requiring only a single syringe – the systems operate at a negative pressure. This makes it necessary to take greater care in the handling of the solutions in order to avoid the appearance of air bub-

☆ Presented as Plenary Lecture at the 12th International Conference on Flow Injection Analysis, Merida, Venezuela, 2003.

* Corresponding author. Tel.: +351 2 22078939; fax: +351 2 22004427.
E-mail address: limajlfc@ff.up.pt (J.L.F.C. Lima).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.04.034

bles in the interior of the manifold. Additionally, in any of the functioning modes syringes operation has to be periodically interrupted to fill up or empty these, which impaired sampling rate.

In addition to the abovementioned propelling units, other devices and strategies such as electro-osmotic pumps [14,15], gas displacement [16] as well as the exploitation of gravity [17] have been used. However, these alternatives have been scarcely used, due not only to the complexity of the flow manifold and respective control and operation, but also due to the elevated equipment costs or limited versatility.

The abovementioned propelling strategies were aimed at attaining laminar flow conditions. The pulses, which could eventually be derived from the propulsion processes under utilisation, were faced as a difficulty to be overcome.

Along with the solutions propelling units the mechanisms of insertion of samples and reagents also significantly condition the potentialities of the flow procedures. Distinct individual alternatives or the articulation of more than one device were proposed, although all have a specialised function. The more widely used insertion processes are based on the selection of fixed volumes (rotary valves) requiring an exchange of the sample loop in order to change the injected sample volume, or alternatively on the definition of time-based variable volumes (selector valve or solenoid valve). Irrespective of the insertion process under analysis, it is repeatedly stated in literature that the intercalation must occur without distortion of the hydrodynamic characteristics of the flow [18].

In 1980, a flow analysis system that utilised the insertion of small pulses at a constant pulse frequency was conceived [19]. The pulsed flow was obtained through a syringe coupled to a computer and the equipment designated as “pulsed-accelerated-flow” being used exclusively for the evaluation of the kinetics of several fast reactions [20–22].

Impulsion devices involving pumps with strokes varying between 0.01 and 5.0 ml capable of supplying a pulsed regime have also been proposed [23]. The authors conducted a detailed study of the physical characteristics of the mixture of two solutions originating from two independent channels. Additionally, using a FIA system with two channels, a comparative evaluation was made of the performance of the developed procedure when the two channels of the peristaltic pump of a FIA system were substituted by two of the referred pumps and maintaining the remaining components unaltered. Similarly, solenoid pumps were also used [24] in a flow injection system for the determination of some parameters of environmental interest.

The influence of pulsed flows in μ-flow systems was already evaluated [25] in comparison with continuous and stop flow techniques. Results obtained in this work with pulsed flow show no significant difference in comparison to the results obtained with continuous flow when the pulse frequency is above 1 Hz.

Exploitation of pulsed flows in flow analysis was recently reviewed [26] and strategies based on the use of small pumps to impel solutions in very small microconduits engraved in a plastic block or in a capillary tube where extraction processes take place [27–31] have been highlighted. Emphasis has also been given to pulsed flow in relation to chemiluminescent detection [32–34].

Multi-pumping flow systems (MPFS) have recently been proposed and their potentialities are discussed herein. In these systems, multiple micro-pumps are operated individually for the propulsion of liquids, introduction of sample and mono-commutation of reagents. The micro-pumps are characterised by a fixed stroke volume in such a way that a precise and effective control of the volume of sample and reagents, at a given flow rate, is accomplished by appropriate dimensioning of the frequency and number of strokes (pulses). Through individual control of each micro-pump, a selective and versatile introduction of reagents and samples can be explored.

2. Instrumentation

The MPFS manifold is made up of a series of solenoid micro-pumps, as many as the solutions required by the specific analytical method. The conception and establishment of the flow manifolds does not require the utilisation of other active devices for insertion and selection of solutions and samples although the use of solenoid valves for re-directing the solutions cannot be excluded in complex situations.

In the developed work were utilised micro-pumps (Bio-Chem Valve Inc. Bonton, USA) that dispense volumes of 3, 8, 25 and 50 μl of solutions by stroke with a dispensing precision of ± 2% of set volume. The frequency can be adjusted up to 250 strokes per minute, implying maximum flow rates of 0.75, 1.25, 2.0, 6.25 and 12.5 ml min^{-1} respectively. These micro-pumps are diaphragm pumps operated by solenoid in which the diaphragm is maintained closed by means of an inner spring mechanism. When voltage is applied, the solenoid coil is activated in order to open the diaphragm. This opening action permits fluid to be drawn into the pump chamber. The fluid is dispensed from the pump by dropping the applied voltage thus de-energising the solenoid coil; the spring then forces the diaphragm back to the closed status.

The micro-pump solenoids were controlled by TTL signals through the use of an interface card (Advantech) and required a power drive based on a UNL 2003 integrated circuit [35] or CoolDrive™ circuit (NResearch Inc). Data acquisition and control of the analytical system were accomplished by means of a microcomputer.

3. Manifolds

Experiments carried out with a dye solution demonstrated the characteristics of the flowing stream generated by the action of the micro-pumps. In these assays a flow manifold

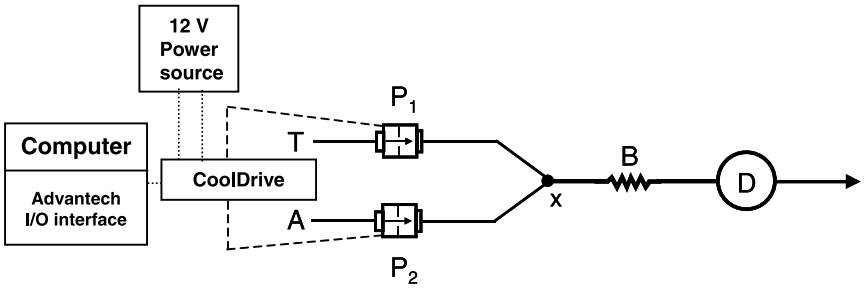

Fig. 1. Typical multi-pumping flow manifold: P1 and P2 – solenoid micro-pumps for carrier (T) and sample (A) insertion; X – confluence point placed at the lowest practical distance from the micro-pumps; B – reaction coil (0.8 mm i.d.); D – detector.

conceived with only two micro-pumps, responsible for the insertion of the dye solution and water that performed the role of sample and transporter solution, respectively, was used (Fig. 1). The obtained results showed that depending on the stroke volume of the micro-pumps and the sampling strategy followed, the mixture that occurs in confluence X can be considered as practically complete, minimising the length or the performance of reactor B, whose function is thus limited to the establishment of a physical connection between the confluence and the flow cell in the detector.

The actuation of the solenoid micro-pumps produces a pulsed flow evident when analysing the oscillating profile of the analytical signals obtained by intercalating, for example, a dye solution and water (Fig. 2). This profile is dependent on the volume of the stroke from the micro-pumps, pulse frequency, length of the reactor and internal volume of the flow cell, affecting the dispersion of the sample zone. The two latter parameters of the flow manifold, even when downsized to the lowest practicable values, will perform the role of dispersion elements, as in other continuous flow systems.

The effect of each pulse on the analytical signal profile is mainly dependent on the stroke volume and is more prominent in the zones of greater concentration gradient. Effectively, the analytical signal variation for each micro-pump pulse is less visible in the sample central zone and is greater in the sample zones of more pronounced concentration gradient (peak ascending and descending sections) increasing as well with pulse volume.

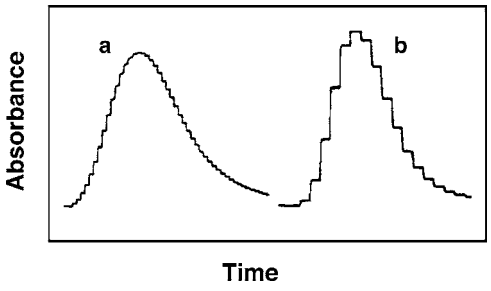

Fig. 2. Analytical signals obtained by inserting a Brilliant Green solution (95.0 mg l^{-1} in water, pH 6.1) by means of 8 μl (a) and a 25 μl (b) per stroke solenoid micro-pumps, using the flow manifold pictured in Fig. 1. Water was used as carrier solution.

The influence of stroke volume on the characteristics of the generated flow and on the capacity of mixture is also perceptible when considering the number of pulses necessary to transport the sample zone for detection. It was shown that with a 50 cm reactor (250 μl of internal volume for an internal diameter of 0.8 mm) the transport of a given sample volume required 83 pulses, when a micro-pump with a 3 μl stroke volume is used, and only 10 pulses when the stroke volume of the micro-pump was 25 μl. Bearing in mind that in both cases, the pulse frequency was adjusted in such a way to obtain the same flow rate, and therefore the same residence time, the efficiency of mixing obtained with the 3 μl pump was greater than that obtained with the 25 μl pump. This was confirmed by the attainment of an analytical signal with a less pronounced stair-like profile (Fig. 2). Moreover, with a 25 μl pump the sample segment completely crossed the detectors' flow cell after 19 pulses while, for example, an 8 μl micro-pump required 50 pulses. Additionally, the number of carrier pulses necessary for resolution of the analytical signal (baseline to baseline return) grew with the increase of sample volume intercalated and with internal volume of the flow cell.

The internal volume of the flow cell was another aspect that in the particular case of the spectrophotometric detection affected the profile of analytical signal obtained. Although the mixing between sample and reagents was extremely quick, the pulsed nature of the flow was visible at the time of detection, as a function of the stroke volume and optical volume of the flow cell. Indeed, whenever increasing the stroke volume or decreasing the optical volume of the flow cell, the stair-like profile of the analytical signal became more pronounced, which was particularly evident for cells with an optical volume lower than 18 μl (for larger flow cells the effect of chamber dilution predominates) and for stroke volumes greater than 25 μl.

4. Flow management in the MPFS

Considering that each micro-pump acts individually in the propulsion of the fluids, distinct sampling techniques such

as the single sample volume, binary sampling or merging zones, or even segmentation processes with gas bubbles, can be explored in a versatile and independent way. Utilising the system described in Fig. 1, the recourse to a strategy of sampling based on the insertion of a single sample volume, similar to that used in a conventional FIA system, would be easily implemented: pump P1 would be responsible for the propulsion of a dye solution, functioning as a sample, and pump P2 utilised to introduce water, which would perform the role of carrier solution and at the same time be responsible for the definition of the baseline. Consequently, the volume of sample to be inserted would be determined by the number of pulses while the flow rate would be controlled as a function of pulse frequency and internal volume of the selected micro-pump. Following sample insertion, the micro-pump propelling the carrier would be activated and after a given number of pulses (determined by the internal volume of the reaction coil), the sample zone would reach the detector. An immediate advantage of this system resides on the fact that the sample volume inserted into the manifold is not fixed and can be freely varied.

A second possible strategy would be the introduction of the sample through binary sampling [6]. Micro-pumps P1 and P2 would then be activated alternately based on an intercalation sequence established as a function of a given number of cycles and intercalation pulses, which would permit the definition not only of the volume of sample but also the volume and configuration of the reaction zone. Thereafter, interrupting the functioning of pump P1 and maintaining pump P2 functioning the process of transport of the sample zone to the detector, at a flow rate defined by both the volume and frequency of the pulse, would take place. An advantage of this procedure regarding the insertion of a single sample volume would be the establishment of a sample zone with a configuration that would present not two but multiple reaction interfaces, facilitating the sample/reagent mixture and the subsequent development of the reaction.

The mixture of the sample and carrier could also be carried out following a merging zones strategy. Under those circumstances, the P1 and P2 micro-pumps would be activated simultaneously. In this case, the volume of sample segment would be defined by the number of strokes of the two pumps. Deactivating P1 would terminate the introduction of sample and the segment to be transported to the detector by the solution originating from P2 would be defined.

In the MPFS, the utilisation of small stroke micro-pumps makes an almost immediate mixing between sample and reagent possible. However, due to the dependence of flow rate in relation to the stroke volume (and considering that from the technical point of view, the frequency of actuation of the micro-pumps has a limit value) the flow rate values that could be used in the process of transport of the reaction zone to the detector, and consequently the sampling rate, are therefore limited. This limitation and the form in which it can be overcome can be illustrated comparing the results

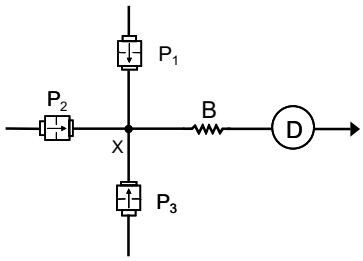

Fig. 3. Flow manifold used for bromhexine (reaction coil B = 50 cm; 0.8 mm i.d.) and phytic acid determination (reaction coil B = 20 cm; 0.8 mm i.d.) comprising three solenoid micro-pumps (P1, P2, P3), a confluence point (X) and a spectrophotometric detector (D).

obtained, in terms of sampling rates, with similar manifolds in the determination of bromhexine in pharmaceutical formulations [36] and phytic acid in plant extracts [37]. The flow manifold used in the two analytical applications was basically made up of three micro-pumps directly connected to a confluence point (X) and a small reactor that linked this point to the detector, as presented in Fig. 3. In the determination of bromhexine [36], two reagents were added through micro-pumps P1 and P3 and the sample through P2, all with a stroke volume of 8 μl. The solution added by pump P1 acted as carrier, responsible for the definition of the baseline and also as oxidant reagent of the reaction involved. Therefore, the directing of the sample segment to the detector was made at a flow rate limited by the volume and frequency of this micro-pump, obtaining a sampling rate of about 45 samples per hour.

In the case of phytic acid determination [37], the colour development reaction simply implied the mixture of the sample (P1, 8 μl) with a single reagent (P3, 3 μl) which as in the case of the determination of bromhexine [36], were mixed using small volume micro-pumps. However, the utilisation of a micro-pump P2 of greater stroke volume (25 μl) to propel, at an elevated frequency, a solution whose function was the rapid transport to the detector of the established sample zone, enabled the attainment of high sampling rates of about 150 determinations per hour.

5. Mixing in MPFS

The degree of mixing between fluids in the multi-pumping systems is superior to that verified in the manifolds with predominantly laminar regime, in which the interpenetration depends exclusively on the phenomena of dispersion and convection. Indeed, the differences that are known in the FIA systems for the single volume technique, binary sampling [6] and merging zones are less significant in the MPFS since the pulsed nature of the flow promotes an extensive mixture at the point where the confluence of the fluids occurs.

Experiments were carried out with a flow manifold similar to that presented in Fig. 1 and in which the connection

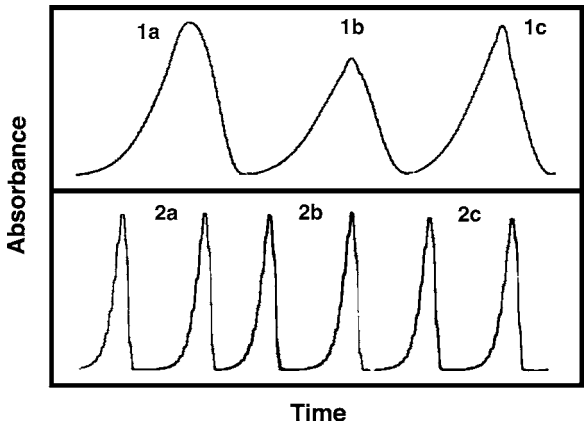

Fig. 4. Analytical signals obtained by using distinct sampling strategies: single volume (1a, 2a), binary sampling (1b, 2b) and merging zones (1c, 2c). Sample solution: Brilliant Green (15 mg l^{-1} in water, pH 9.0); carrier: sodium tetraborate (0.01 mol l^{-1} in water, pH 9.0); spectrophotometric detection at 617 nm; Solenoid micro-pumps of 8 μl (1a–1c) and of 50 μl (2a–2c) stroke volumes. The results of the 50 μl micro-pump are in duplicate.

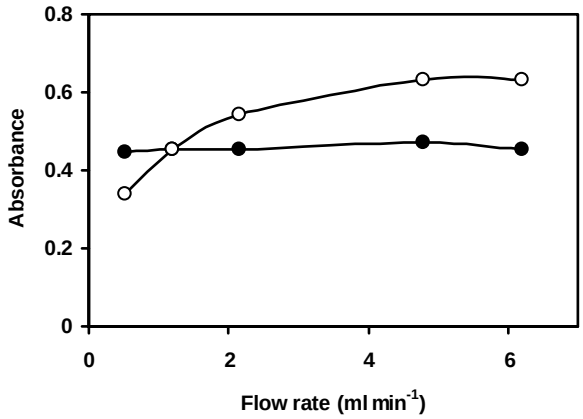

Fig. 5. Influence of carrier solution flow rate: analytical signals obtained with a FIA system (○) and with multi-pumping flow system (●). Sample and carrier solutions as in Fig. 4.

between the confluence point and the flow cell of the spectrophotometer was reduced to the minimum practical length (about 30 cm). By using micro-pumps of 8, 25 and 50 μl, a frequency of 4 pulses per second and a sample volume of approximately 100 μl, the single volume, binary sampling and merging zones were assayed in similar conditions (Fig. 4). For smaller pulses, for example 8 μl, the analytical signal supplied by the sample (dye) when intercalated by the binary sampling technique was lower, illustrating an extensive mixture at the point of confluence of the sample with the carrier, followed by dilution in the transport process. This effect was less evident when the stroke volume is greater.

In addition, comparisons were made in relation to the degree of mixing that was observed in the interior of the MPFS and in a flow manifold structurally similar but in which the single volume was introduced in the absence of pulses (FIA system). Here, the differences detected were significant. With this objective, temporal parameters (frequency of pulses or flow rate) and physical parameters (length of the reactor that establishes the connection between the confluence point and the detector) were varied, making the sample insertion either in the form of pulses (4 pulses with a 25 μl micro-pump) or as a single 100 μl segment defined by an injection loop.

Firstly, the influence of the flow rate of the carrier solution was evaluated by using 4 pulses of sample (100 μl) and a short reaction coil (30 cm length). Through variation in the pulsation frequency that corresponded to flow rates of 0.5 up to 6.2 ml min^{-1}, it was observed that the analytical signal remained practically constant in contrast to that of the FIA system (Fig. 5). This result can be explained by the strong influence in the degree of mixture of the pulsed flow particularly during the insertion of sample and carrier at the confluence point thereby conferring a secondary importance to the posterior mixing arising from the process of transport.

Since the mixing of the fluids occurred in a rapid and precise way, the influence of the sample pulse frequency (flow rate) on the mixture conditions at the confluence level was also evaluated. An experiment similar to that referred above was carried out using the 8 μl per stroke micro-pumps for propelling both the sample (dye) and carrier solutions, with a sample volume of 48 μl (6 pulses). The flow rate was fixed at 0.5 ml min^{-1} for the carrier and was varied from 0.25 to 1.6 ml min^{-1} for the sample solution with a reaction coil of 30 cm and applying the single volume strategy in the sampling. It was observed that by increasing the flow rate for sample insertion, a decrease in the analytical signal occurs, showing that a greater contact time of the sample with the carrier solution does not cause any increase in dilution. Nevertheless, these results could be explained by the utilisation of more elevated pulse frequencies, which would result in an increase in the mixing and therefore in the dispersion of the sample. In this way, the mixing conditions in this type of flow could be considered as more dependent on the pulse frequency during sample insertion at the confluence point and less on the flow rate of the carrier stream, as previously described, showing that the pulsed flow provides an almost instantaneous mixture. This dependence was observed for 8 μl as well as for 25 μl micro-pumps, with the mixture being more efficient at lower stroke volumes. The evaluation of the influence of the sampling techniques showed that no significant difference exists between the results obtained with the utilisation of binary sampling and the merging zones. However, these two sampling techniques revealed themselves as more efficient in terms of mixture capacity than the insertion of single volumes, mainly due to the greater contact between the sample and carrier streams.

The role of the reactors as promoters of the mixing between sample and reagents is less significant in the MPFS than in other FIA systems since control of the degree of mixing can be obtained at the expense of the control of the pulsed nature of the fluids in each confluence. Indeed, when comparing the performance of a multi-pumping sys-

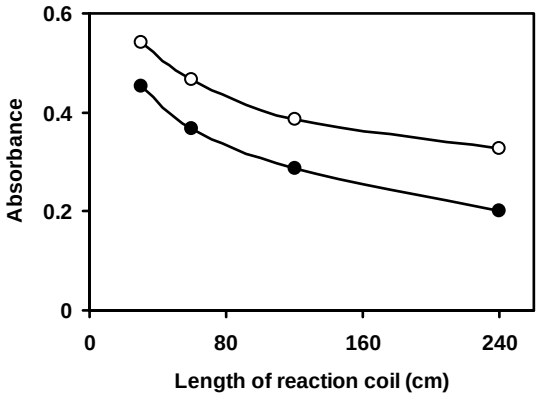

Fig. 6. Influence of reactor length: analytical signals obtained with a FIA system (○) and with a multi-pumping flow system (●). Sample and carrier solutions as in Fig. 4.

tem (Fig. 1) with a similar FIA system and fixing the flow rate of the carrier solution at 2.2 ml min^{-1} while varying the reactor length (30, 60, 120 and 240 cm), a greater dispersion was observed for the distinct analytical configurations of the multi-impulsion system regarding the FIA system. This was a consequence of the enhanced mixture that had already occurred at the confluence point (Fig. 6). Similar results were also obtained by fixing the distinct parameters of the flow system and varying the volume of the spectrophotometer flow cell.

The degree of mixing between solutions provided by the MPFS also permit to minimise the distortion of the analytical signals originated by the differences in the refraction indices (Schlieren effect) of the solutions intercalated in the system [38]. This occurs because the Schlieren effect is commonly associated with an insufficient mixture between sample and carrier. For example, in the determination of chromium(VI) in waters [39] or dipyrone [40] in pharmaceutical products, it was possible to very efficiently mix highly viscous acidic solutions with aqueous sample solutions. This capacity to mix solutions with different viscosities and different refraction indices, which is linked to the pulsed nature of the propulsion process, makes the carrying out of the determinations in very simple flow manifolds viable, without the need to resort to complementary strategies to compensate this phenomenon. These strategies include the determination of the absorbance at different wavelength or the increase in the reactor length which would result in a significant increase in sample dispersion and a reduction in sampling rate.

The degree of carry-over [41] in the multi-pumping systems is also lower when compared to systems in which the mixing of solutions is based on diffusion and convection phenomena. To evaluate the extent of this effect, experiments with the two previously referred systems were carried out, varying the flow rate of the carrier between 0.7 and 4.6 ml min^{-1} and intercalating 100 μl of sample. For all flow rate values studied, it was shown that the MPFS required a much shorter time for complete resolution of the analytical signal (Fig. 7).

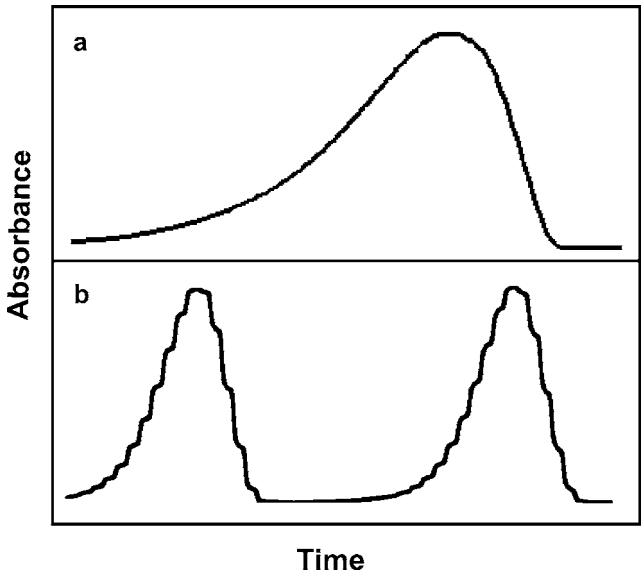

Fig. 7. Analytical signals obtained in a FIA system (a) and in multi-pumping flow system (b) by inserting the same sample volume (100 μl) and by using the same flow rate. Sample and carrier solutions as in Fig. 4.

6. Trends

The general functioning characteristics of the manifolds that incorporate micro-pumps as insertion process of samples/reagents, commutation and transport, permit to predict that in many circumstances, these systems could be used to advantageously implement determinations previously carried out by other continuous flow analysis systems. This is mainly due to the simple and controlled use of pulsed flows supplied by micro-pumps with independent operational regimes.

The control and versatility of adjustment of the pulse frequency provided by each micro-pump facilitates the amendment of distinct system analytical parameters, which is especially relevant in the case of the sample volume because it enables an effective manipulation of sample dispersion. This permits to anticipate that a MPFS system would be particularly adequate for the establishment of intelligent systems with feedback lines capable of adapting the concentration of the species under analysis to the working analytical range of the detection process.

The enhanced mixing capabilities exhibited by MPFS assured fast reaction zone homogenisation in the interior of the flow manifold, which makes it possible to predict that the quantity of reagents used per determination can be reduced to a minimum while the sampling rates can be increased, given the reduced degree of carry-over.

Flow manifolds comprising micro-pumps will be particularly useful in the implementation of methodologies in which the mixing regime is a critical parameter for the quality of the measurements, as is the case of pseudo-titrations [42] or even in other continuous flow titration models recently referred to in the literature [43,44].

The simplicity of the control of the sample zone transport for detection, either based on time or on a pulses counting, facilitates the effective monitoring of the position of the sample zone in the interior of the flow system. At the same time, it also permits controlled stops of the flowing stream, which can be of particular advantage in the implementation of kinetic methods and stopped-flow strategies.

It is predicable that the multi-pumping flow systems could have a great potential for the implementation of extraction processes in which the pulses, by producing a chaotic movement of the particles, will be promoters of a more profound contact between non-miscible fluids facilitating the transfer processes between phases of the species to be determined.

It is the belief of the authors that the use of the mechanical action of pulsed flows caused by the micro-pumps could be used for determinations that involve the use of exchange resins or immobilised enzymes. Using an ascending turbulent flow through the non-packaged reactors containing an exchange resin, or, for instance, spheres with immobilised enzymes, it would be possible to place the materials in suspension, making the bed fluid in nature and in this way magnifying the chemical processes that occur between the phases.

Given the well known influence of the mechanical action on the transport processes through membranes, it is possible to envisage the analytical potential of the association of pulsed flows and multi-pumping systems with dialysis membranes or gaseous diffusion.

Finally, it should be highlighted that the amount of work published with this type of flow systems is much reduced, being the detection process restricted to spectrophotometric measurements. However, the behaviour and implications of using multi-pumping flow systems with other detection techniques is already under evaluation and will the subject to further research. Nevertheless, given the ease in which the fluids are mixed and the great reagent saving they provide, it is possible to predict its great utility in chemiluminesence detection for which experimental evidence already exists [33,34]. Concerning the electrochemical measurements, it is possible to foresee the need to adapt the design of the voltammetric and potentiometric detectors, in the majority of cases wall jet type, to the predominantly pulsed nature of the transport process.

7. Conclusions

The MPFS is a flow strategy exploiting individual micro-pumps for liquid propulsion, sample and reagent introduction and component commutation, ensuring an effective and precise control of the sampled volume, either on a time-based or on a pulse counting-based strategy and its transport towards the detector.

The characteristics of MPFS make them very attractive for the implementation of automated analytical procedures, mainly due to the multi-tasks assigned to the solenoid pumps, resulting in flow manifolds that are simple and of easy conception, operation and control.

The individualised control of each pump responsible for the management of each solution, allows the operator to anticipate the functioning mode of the system, namely in relation to the volume and flow rates of the samples and reagents. The hydrodynamic characteristics of the pulsed flow are a valuable feature allowing a quick sample/reagent mixing even under limited dispersion conditions.

Additionally, it is easy through adequate system programming (and without altering the manifold) to condition the process of mixing the involved solutions either by using merging zones, binary sampling or single volumes, or even to control the experimental conditions for the implementation of stopped-flow strategies.

In mechanical terms the flow manifold is simple since in contrast to what occurs with other continuous flow systems, they only incorporate a single type of active elements, as the micro-pumps act simultaneously as processes of intercalation of samples and reagents, impulsion systems and commutation units.

The referred simplicity is also responsible for the high robustness of the analytical systems, namely in relation to reliability and compatibility, since the causes of deficiencies in the functioning of the systems are more reduced and controlled regarding other flow methodologies, in which at least impulsion and commutation are carried out by distinct units. These characteristics suggest that the MPFS systems could be advantageously used in the construction of portable units for fieldwork.

Other reasons that contribute to the simplicity of the manifolds are the ease in which differences between the refraction indices of the intercalated solutions can be minimised and, in equivalent circumstances, increase the sampling rates due to the decreased degree of carry-over.

The MPFS is a valuable flow methodology and could be an advantageous alternative to other available procedures, because it exhibits a high degree of automation, it is simple, fast, precise, accurate, requires low reagent consumption and minor operator intervention.

Acknowledgements

Partial support from a bi-national consortium GRICES (Portugal)/CAPES (Brazil) is greatly appreciated. A.C.B.D. and M.F.T.R. thank to FAPESP and FCT the Ph.D. grants.

References

[1] L.T. Skeggs, Am. J. Clin. Pathol. 28 (1957) 311.
[2] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 78 (1975) 145.
[3] J. Ruzicka, G.D. Marshall, Anal. Chim. Acta 237 (1990) 329.
[4] X.D. Wang, T.J. Cardwell, R.W. Cattrall, G.E. Jenkins, Anal. Commun. 35 (1998) 97.
[5] J. Ruzicka, Analyst 125 (2000) 1053.

[6] B.F. Reis, M.F. Giné, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, Anal. Chim. Acta 293 (1994) 129.
[7] F.R.P. Rocha, B.F. Reis, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, J.L.M. Santos, Anal. Chim. Acta 468 (2002) 119.
[8] V. Cerdá, J.M. Fortaleza, A. Cladera, E. Becerra, P. Altimira, P. Sitjar, Talanta 50 (1999) 695.
[9] R.C. Prados-Rosales, J.L. Luque-Garcia, M.D. Luque de Castro, Anal. Chim. Acta 461 (2002) 169.
[10] J. Ruzicka, E.H. Hansen, Flow Injection Analysis, second ed., Wiley, New York, 1988, pp. 172–185.
[11] R. Pereiro, in: A. Sanz-Medel (Ed.), Flow Analysis with Atomic Spectrometric Detectors, Elsevier, Amsterdam, 1999, pp. 34–63.
[12] B.F. Reis, M.F. Giné, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, Anal. Chim. Acta 293 (1994) 129.
[13] K.M. Pedersen, M. Kummel, H. Soeberg, Anal. Chim. Acta 238 (1990) 191.
[14] H. Wada, Y. Sawa, M. Morimoto, T. Ishizuki, G. Nakagawa, Anal. Chim. Acta 220 (1989) 293.
[15] S. Liu, P.K. Dasgupta, Anal. Chim. Acta 283 (1993) 739.
[16] P.K. Dasgupta, R.S. Vithanage, K. Petersen, Anal. Chim. Acta 215 (1988) 277.
[17] J.C. Andrade, M. Ferreira, N. Baccan, Quim. Nova 9 (1986) 123.
[18] R.C. Prados-Rosales, J.L. Luque-Garcia, M.D.L. Castro, Anal. Chim. Acta 480 (2003) 181.
[19] G.D. Owens, R.W. Taylor, T.Y. Ridley, D.W. Margerum, Anal. Chem. 52 (1980) 130.
[20] S.A. Jacobs, M.T. Nemeth, G.W. Kramer, T.Y. Ridley, D.W. Margerum, Anal. Chem. 56 (1984) 1058.
[21] M. Nemeth, K.D. Fogelman, T.Y. Ridley, D.W. Margerum, Anal. Chem. 59 (1987) 283.
[22] C.P. Bowers, K.D. Fogelman, J.C. Nagy, T.Y. Ridley, Y.L. Wang, S.W. Evetts, D.W. Margerum, Anal. Chem. 69 (1997) 431.
[23] T. Korenaga, X. Zhou, T. Moriwake, H. Muraki, T. Nalto, S. Sanuki, Anal. Chem. 66 (1994) 73.
[24] D.A. Weeks, K.S. Johnson, Anal. Chem. 68 (1996) 2717.
[25] E.B. van Akker, M. Bos, W.E. van der Linden, Anal. Chim. Acta 378 (1999) 111.
[26] P.S. Francis, S.W. Lewis, K.F. Lim, K. Carlsson, B. Karlberg, Talanta 58 (2002) 1029.
[27] K. Carlsson, H.S. Jacobsen, A. Lynggaard Jensen, T. Stenstrom, B. Karlberg, Anal. Chim. Acta 354 (1997) 35.
[28] K. Carlsson, L. Moberg, B. Karlberg, Water Res. 33 (1999) 375.
[29] K. Carlsson, B. Karlberg, Anal. Chim. Acta 423 (2000) 137.
[30] K. Carlsson, B. Karlberg, Anal. Chim. Acta 415 (2000) 1.
[31] K. Carlsson, B. Karlberg, Anal. Chim. Acta 434 (2001) 149.
[32] X.D. Wang, T.J. Cardwell, R.W. Cattrall, G.E. Jenkins, Anal. Commun. 35 (1998) 97.
[33] S.W. Lewis, P.S. Francis, K.F. Lim, G.E. Jenkins, X.D. Wang, Analyst 125 (2000) 1869.
[34] S.W. Lewis, P.S. Francis, K.F. Lim, G.E. Jenkins, Anal. Chim. Acta 461 (2002) 131.
[35] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, Anal. Chim. Acta 377 (1998) 103.
[36] A.C.B. Dias, J.L.M. Santos, J.L.F.C. Lima, E.A.G. Zagatto, Anal. Chim. Acta 499 (2003) 107.
[37] J.M.T. Carneiro, E.A.G. Zagatto, J.L.M. Santos, J.L.F.C. Lima, Anal. Chim. Acta 474 (2002) 161.
[38] E.A.G. Zagatto, M.A.Z. Arruda, A.O. Jacintho, I.L. Mattos, Anal. Chim. Acta 234 (1990) 153.
[39] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto, Anal. Chim. Acta 466 (2002) 125.
[40] J.L.F.C. Lima, S.M.O. Sá, J.L.M. Santos, E.A.G. Zagatto, J. Pharm. Biom. Anal. 32 (2003) 1011.
[41] S. Vicente, E.P. Borges, B.F. Reis, E.A.G. Zagatto, Anal. Chim. Acta 438 (2001) 3.
[42] J. Ruzicka, E.H. Hansen, H. Mosbaek, Anal. Chim. Acta 92 (1977) 235.
[43] P.B. Martelli, B.F. Reis, M. Korn, J.L.F.C. Lima, Anal. Chim. Acta 387 (1999) 165.
[44] C.M.N.V. Almeida, M.C.U. Araújo, R.A.S. Lapa, B.F. Reis, M. Korn, J.L.F.C. Lima, B.F. Reis, E.A.G. Zagatto, Analyst 125 (2000) 333.