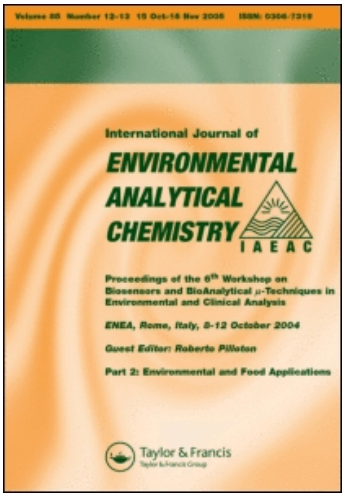

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Automatic pre-concentration and treatment for the analysis of environmental samples using non-chromatographic flow techniques

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Automatic pre-concentration and treatment for the analysis of environmental samples using non-chromatographic flow techniques

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In this work a brief description and a comparative review of different non-chromatographic flow analytical methods following their historical evolution are carried out with the aim to assess both the advantages and disadvantages of the methods.

In addition, a comparative study of different clean up and pre-concentration techniques used the above methods to achieve the selectivity and sensitivity required in environmental applications, is also carried out.

The possibilities offered by commuted flow techniques for the development of expert systems capable of selecting the most adequate procedures regarding the resolution of speciation problems – with or without involving pre-concentration of analytes, are considered.

Finally, several existing computer programs are referred to, discussing the advantages of those capable of satisfying the needs of all flow techniques, without requiring a specific program for each case.

The above-mentioned developments are illustrated by the description of various analytical methodologies.

Keywords: Flow analysis; FIA; SIA; MSFIA; MCFIA; MPS; Pre-concentration; Environmental samples

1. Evolution and comparative study of flow techniques

The need to consider seriously the implementation of automated methods in chemical analysis took place towards the fifties a time in which medicine underwent a great transformation since diagnosis of diseases became increasingly based on clinical analysis results. The former led to a fast increase in the demand of assays to be performed in the laboratory which could not be solved with an increase of laboratory personnel without difficult economical justification. The alternative solution to this problem arose with segmented flow analysis (SFA) [1], which can be considered the first flow technique universally accepted. Not only did it mean a relevant increase of the

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sample throughput, but also a considerable saving on samples and reagents. In this way, the first step for the development of the present flow techniques was taken.

It should be stated that segmented flow analysis was a mechanical solution to the automation process of the analytical methods. In many other fields true automation did not occur until the appearance of microprocessors and computers. The capacity of these two devices is such that an automated method can be easily re-programmed in order to be adapted to other needs, a versatility which simplifies the solutions and decreases considerably the costs. On the other hand, the advantages of the present flow methods are chiefly due to also the incorporation of computers, since the flexibility introduced by the possibility of re-programming enables a certain physical system to be easily adapted to different types of samples applying the same analytical method, or even to change the analytical methodology involving none or few modifications of the physical system (hardware or manifold) [2].

In the development of flow techniques two clearly differentiated steps may be considered. One first step in which the system can be easily controlled in an exclusively manual way, corresponding to segmented flow analysis (SFA) and flow injection analysis (FIA) techniques, and a second step in which the use of computers is essential, such as in the case of sequential injection analysis (SIA), multicommuted flow injection analysis (MCFIA), multisyringe flow injection analysis (MSFIA) and multi-pumping flow systems (MPFS).

1.1. *Segmented flow analysis (SFA)*

Segmented flow analysis (SFA) is a continuous automated method, proposed by Skeggs [1] in 1957, which usually consists of at the following components (see figure 1): a peristaltic pump which continuously aspires the sample and reagents, and a set of plastic tubes (manifold) which allow the transportation of liquids and a detector. The aspirated samples are segmented by injecting air bubbles, which should be eliminated before reaching the detector's inlet.

At that time segmented flow analysis happened to be a fair solution for the centres where numerous repetitive determinations were carried out throughout the day. However, its high cost hindered its use among the more modest laboratories. SFA is usually configured as a multichannel system, a manifold equivalent to that in figure 1

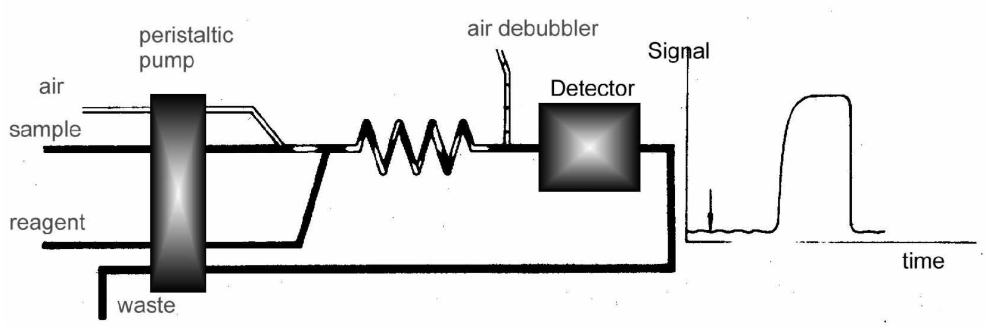

Figure 1. SFA system.

being required for each parameter to be determined. In this way, for example, the set up of a full analyzer of the different parameters of interest in blood is feasible.

1.2. *Flow injection analysis (FIA)*

The name of this technique appeared in 1975 proposed by Ruzicka and Hansen [3], however, there is evidence of similar references in articles by other authors such as those by Stewart [4].

Initially, it may resemble segmented flow analysis both conceptually and from a practical point of view, however, it leads to very different results.

Thus, the basic components are practically the same as those in SFA, since it consists of a peristaltic pump capable of propelling the sample and reagents and a set of plastic tubes (manifold) which transport the liquids towards the detector (figure 2). Unlike SFA, instead of the sample being aspirated continuously, a constant volume is situated in a carrier and is merged with the different reagents required in the analytical method. Both the length of the tubes and the rotation rate of the peristaltic pump determine the reaction rate. If long times are required due to kinetic problems, a long tube is placed, usually wound in the form of a coil, to increase the residence time.

In flow injection analysis the flow is of the laminar type and not turbulent as in SFA, thus, reducing the danger of mixing one sample with another. In FIA separation of samples by air bubbles is not required, and the flow is, therefore, not segmented.

Among the advantages of FIA against SFA the following can be stated: its lower instrumentation cost, the lower sample and reagents consumption, easy implementation and its high sample throughput. The disadvantages of FIA are similar to those of SFA, among which are the aging and short life of the peristaltic pumping tubes when aggressive reagents and organic solvents are used, low versatility, since basically each analytical method requires its own hardware configuration, as well as the difficulties involved to obtain multiparametric systems, which can be usually only achieved by the setting up of a FIA system for each parameter (however, exceptionally up to three parameters may be determined without varying the configuration [5]).

1.3. *Sequential injection analysis (SIA)*

This method was proposed by Ruzicka and Marshall [6] in 1990 as an alternative to FIA. It has been demonstrated that SIA is a technique which offers possibilities very different from those of FIA.

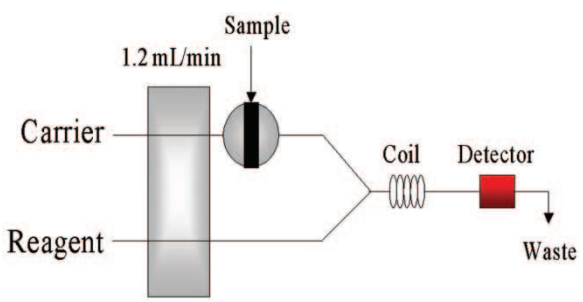

Figure 2. Double channel FIA configuration.

In figure 3 a sequential injection analysis system with spectrophotometric detection is depicted and schematized in figure 4.

This system is constituted by a selection valve, whose central port is connected to a bidirectional piston pump. The side ports of the valve are connected to the vessels containing the reagents required for the application of the analytical method, to the sample and to the detector which will carry out the corresponding measurements. The side ports can be also used for other purpose, such as waste, connection to other devices (microwave ovens, photoxidation systems, mixing chambers, etc.).

One of the basic characteristics of SIA systems is that most are controlled by a computer. Its function consists of selecting the connection of the central port of the valve with the side ports and giving instructions to the piston pump with regard to when aspiration or dispensing of the liquid is required, as well as the liquid volume and the working flow to be chosen. At the same time it carries out data acquisition and treatment.

SIA systems present several advantages against the previous techniques, among which the following should be mentioned:

– It's a very robust technique, since the sample and reagents are only in contact with glass or PTFE tubes, which solves the above-mentioned problems of peristaltic pumping tubes.

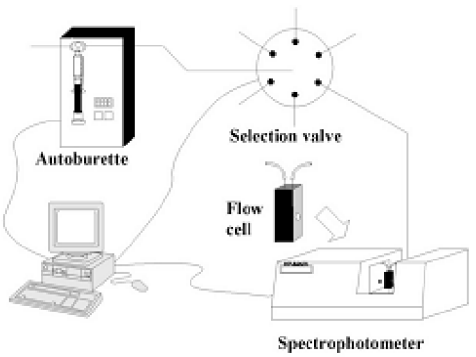

Figure 3. Analysis system by sequential injection.

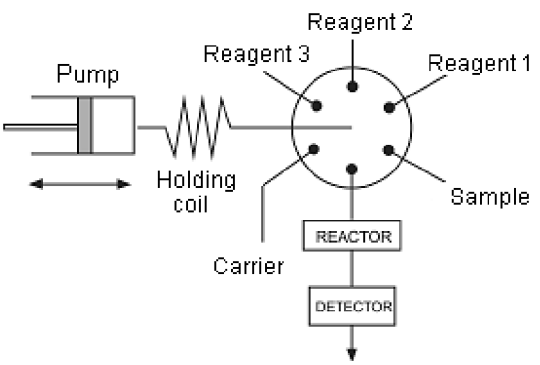

Figure 4. Schematic representation of a SIA.

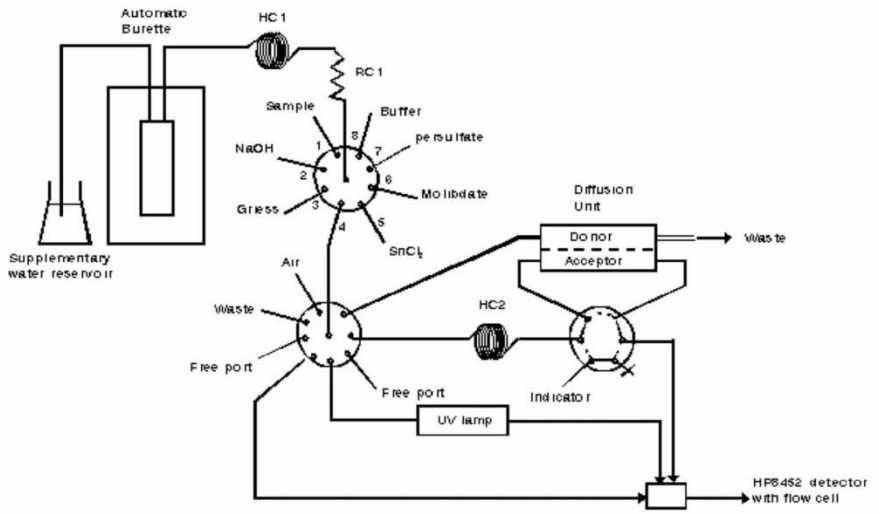

Figure 5. SIA system for monitoring of waste waters.

– Handling of the sample and reagents is very versatile and the operations performed in manual procedures can be simulated quite easily.
– It is the only flow technique truly multiparametric, since with a relatively simple system a large number of parameters can be determined, as demonstrated with the system depicted in figure 5, which represents a wastewater monitor capable of determining up to 12 of the most representative parameters within a sampling time of 15–20 min: NH_3, NO_2^-, N_{tot}, NO_3^-, PO_4^{3-}, P_{tot}, TSS, BOD, COD, detergents, etc. [7, 8].

The manifold of the SIA systems is far more versatile than those corresponding to the former techniques, since the delay times are not imposed by the tube lengths, but can be easily controlled by the computer's watch.

One of the inconveniences concerning SIA systems is the practically unavoidable use of computers or microprocessors in order to obtain a reproducible control of the different hydrodynamic variables. This inconvenience hindered seriously the initial development of this technique, due to the inexperience most scientists had in those days in the interconnection of computers with instrumentation, together with the lack of appropriate commercial software. As will be seen this inconvenience has been nowadays overcome, and the compulsory use of computers has become a clear advantage, since it allows not only the control of the systems but it has also enabled enormously the acquisition, digitalization and treatment of data. The easy control of the system is a fundamental feature in its application as a stopped flow technique.

Another fundamental advantage of SIA is that of the dramatic decrease on the sample and reagents consumption. Thus, unlike previous flow techniques, in which the peristaltic pumps are usually working non-stop independently of the required sample throughput, in SIA sample and reagents are aspirated only when determination is required.

A clear disadvantage of SIA against FIA should be attributed to the sequential conception of the first technique, whereas in FIA the management of the reagents is carried out in parallel. This explains the fact that the sample throughput of a SIA system is usually clearly lower than that of FIA (it can be estimated as 60% of the latter).

1.4. *Multicommuted flow analysis (MCFA)*

More recently, Reis *et al.* [9] have proposed a new flow technique in which quick commutation three way solenoid valves are used systematically. Figures 6 and 7 illustrate such systems. Different ways of management of the liquids have been proposed, either by aspirating through only one channel with a burette or peristaltic pump – which tends to produce a certain vacuum within the system and facilitates the appearance of bubbles or by dispensing each reagent and sample through a channel of a multichannel peristaltic pump. In this way, by placing the valves strategically, the management of each of the propelled liquids is feasible: the return of reagents to their corresponding containers when addition to the sample is not required, the selection of different paths with or without involving pre-concentration or sample treatment, etc.

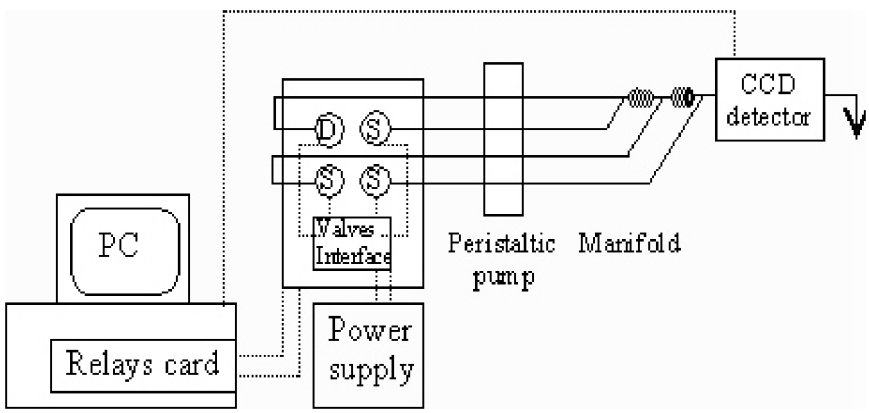

Figure 6. Scheme of a MCFA system.

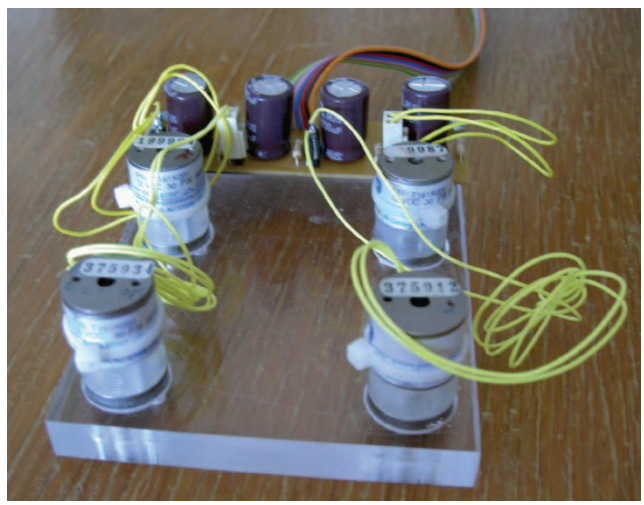

Figure 7. Details of a board with four quick commutation three way solenoid valves, with a protection electronic circuit to avoid overheating.

1.5. *Multipumping flow systems (MPS)*

The latest flow technique appeared, is the so-called multipumping flow systems (MPS) [10–15] by Costa Lima et al., in which solenoid pumps which act simultaneously as liquid drivers and commutation valves are used. These pumps are calibrated in such a way that each stroke propels a prefixed liquid volume (8, 20, etc. μL). By feeding these pumps with voltage pulses the dosage of both the amount of liquid propelled (according to the number of pulses sent) and the flow involved (pulses frequency) is feasible for each pump.

In figure 8 a multi-pumping system is represented in which, by controlling the pulses sent, the addition of a pre-determined volume of sample and reagent, whose mixture is subsequently propelled towards the detector by the carrier by another pump of a greater piston stroke, is feasible. A valve located between the reactor and the detector allows selecting whether the sample will pass through or not to the reducing cadmium column. The heart of a multipumping system is shown in figure 9.

The system is electronically and physically very simple, involving a considerable consumption reduction of sample and reagents, since these are only added when the measurements are required. On the other hand, all wet parts are made of Teflon and, therefore, the system is also very robust.

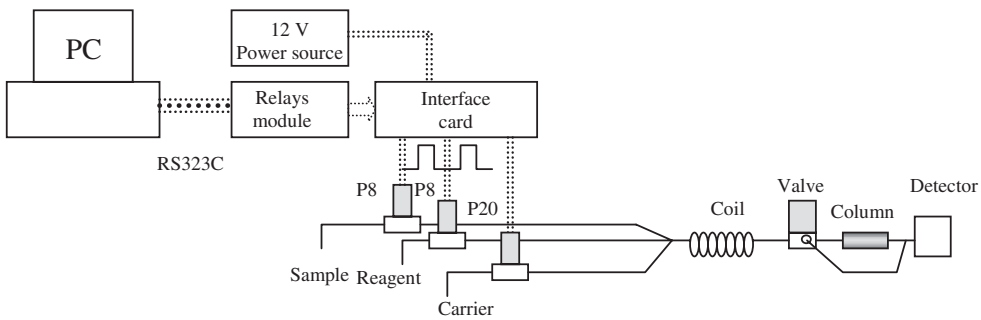

Figure 8. Multipumping system.

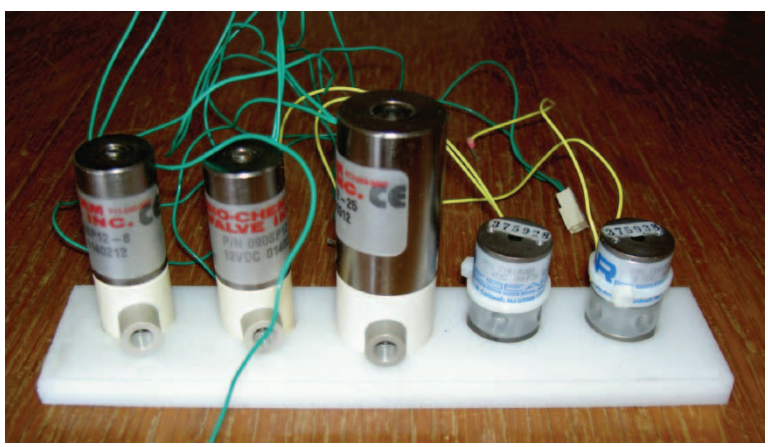

Figure 9. Detail of the multi-pumping system, in which two pumps of 8 μL, one pump of 20 μL and two quick commuted three-way solenoid valves are depicted.

1.6. *Multisyringe flow injection analysis system (MSFIA)*

This method was first introduced in 1999 and consists in a system integrating the advantages of several of the former flow systems [16].

- FIA high sample throughput using a parallel addition of sample and reagents
- SIA robustness (only glass and Teflon in contact with the liquids, no use of peristaltic pumps)
- SIA low sample and reagent needs
- SIA versatile manifold
- MCFIA very quick commutation valves

The result is represented in figure 10 and is constituted by a modified burette used in automatic titrations. The stepper motor moves an iron bar, where up to four syringes are attached. A quick three way solenoid valve is placed on the head of each syringe. Due to the high commutation quickness of these valves the flow of the dispensed liquid can be diverted for each syringe, without stopping its movement. In this way, it is possible to return the liquid to its container when not being required. On the other hand, the burette movement is only activated when a determination is required. These two properties lead to a substantial saving of reagents in relation to FIA: Moreover, the liquids are only in contact with glass and Teflon, providing the system a great robustness against aggressive reagents and organic solvents. In the third generation multisyringe burettes a four output control socket has been implemented in the rear panel, in such a way that each one can be independently programmed in order to provide an output of 12 V, allowing the control of additional solenoid valves, relays, or any other device which can be fed with this voltage.

1.7. *Combined flow systems*

The combination of several flow techniques may be of interest with the aim to benefit from several advantages which otherwise could not be obtained from each technique separately.

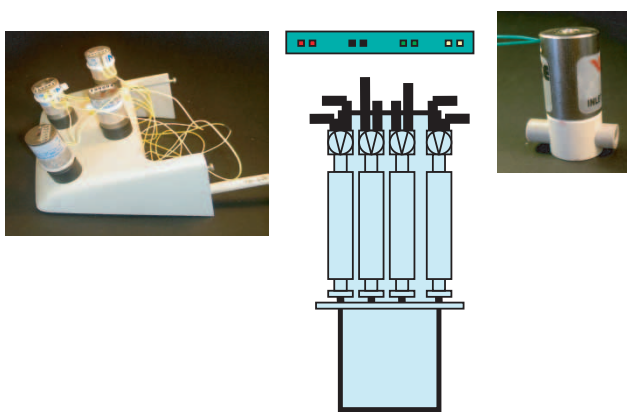

Figure 10. Multisyringe burette of third generation.

1.7.1. MSFIA–SIA combination. Since there are several modules of valves available on the market, some equipped with two multiposition selection valves, the combination of their use with a multisyringe burette to set up two SIA systems working in parallel is feasible and, thus, the obtaining of analytical data is significantly accelerated.

In figure 11 a system of this type constructed for monitoring the quality of waters used in closed circuits for the co-generation of energy in power stations-incinerator plants is shown (unpublished results).

One pair of syringes is used with one of the selection valves to determine several parameters, whereas, the other pair of the multisyringe burette is used with the other selection valve to determine the remaining parameters. Hence, 9 significant parameters can be obtained every 25 min regarding the prevention of the corrosion of the circuit (temperature, pH, conductivity, acid conductivity, hydrazine, ammonium, iron, phosphate and silicate).

1.7.2. SIA–MSFIA. It is possible to carry out a reverse combination, to benefit from the great versatility the SIA systems possess, for pre-treatment of the samples. Once appropriate conditions are found the pre-treated sample can be injected in the MSFIA system where the remaining required reagents can be injected both efficiently and quickly, for they are manipulated in parallel.

In figure 12 (unpublished results) a system used for multichannel detection by means of ion-selective electrodes is represented. Each syringe of the MSFIA system is used to inject one type of buffer of ionic strength (ISA) into one of the four channels. In each channel several electrodes compatible with the injected buffer are placed. A multiplexer performs a quick and periodical scanning measurement of each electrode against a common reference electrode located in a beaker acting as a drain of the liquids waste. The SIA system consisting of a monosyringe burette and a module of valves is used for taking a relatively large volume of sample and sequentially injecting it into each of the channels by means of quick commutation solenoid valves. The flow rate

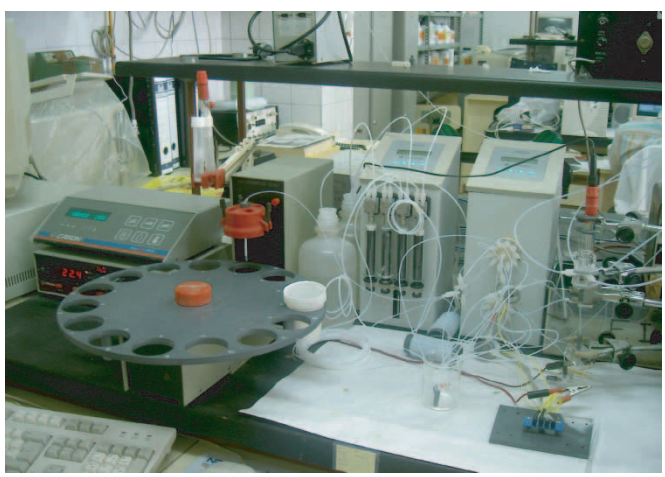

Figure 11. Combined MSFIA–SIA system.

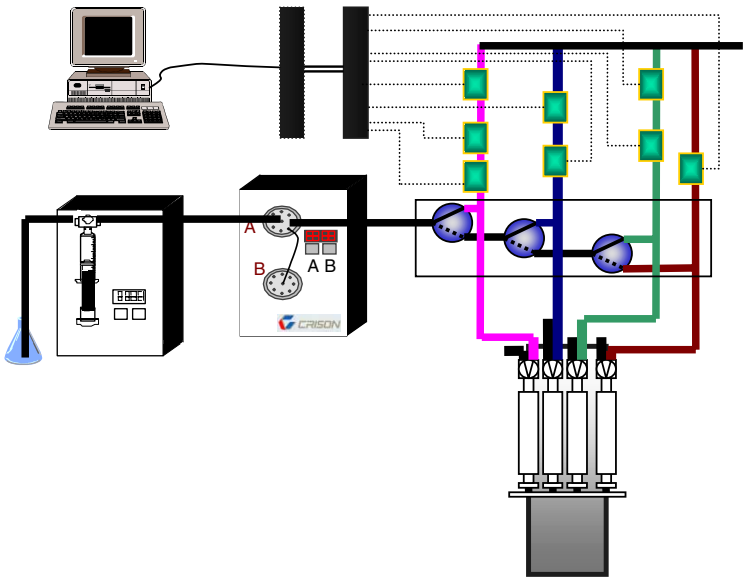

Figure 12. Multichannel potentiometric SIA–MSFIA system.

provided by the SIA system together with the selected times for each solenoid valve define the amount of sample to be injected into each channel.

2. Clean up and pre-concentration systems

Since non-chromatographic flow techniques are being considered, isolation of analytes of interest and/or elimination of interfering species are usually involved. On the other hand, the use of pre-concentration techniques is also frequently required in order to satisfy the determination limitations demanded by present regulations. By using appropriate techniques, it is sometimes possible to satisfy both selectivity and sensitivity demands. Next, a brief review of several of the former techniques will be carried out, underlying the corresponding advantages and disadvantages.

2.1. *Liquid–liquid extraction*

This technique can be used to increase the selectivity and/or sensitivity of flow techniques. It is here where one of the great advantages of the former techniques is stated, since time consumption and the levels of many millilitres of the solvent involved in manual methods are reduced, to attain a much higher sample throughput and a solvent consumption of the order of microlitres.

The process is still relatively complex when FIA is used for this type of treatment, since traditionally the alternative used is that of merging the extracting solvent with the sample properly treated, which gives place to a segmented flow in which the plugs of aqueous solution alternate with the segments of the solvent to produce a sufficiently large contact surface between both liquids [17]. This methodology implies further elimination of one of the phases before introducing the other phase in the detector flow cell.

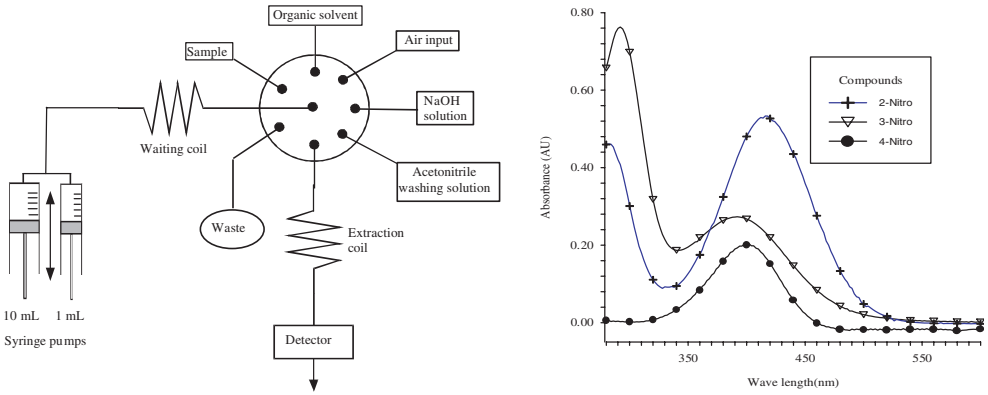

Figure 13. SIA set up for liquid–liquid extraction of phenols, which are resolved by multivariate analysis.

Much more simple is the alternative used by K.L. Peterson *et al.* [18]. If a solvent (pure or mixture) with an appropriate viscosity is selected (1 centipoise), it is then adhered to the Teflon tube due to the hydrophobicity of the latter, in such a way that a film which shifts more slowly than the flow of the aqueous solution is formed. Thus, the less polar compounds are extracted by the solvent film. Subsequently, a retro-extraction technique may be used to recover the analytes of interest, or to sweep the organic phase towards the detector by injecting an appropriate solvent (as for example acetonitrile).

In figure 13 a SIA system for the determination of phenols [19] is represented, in which the change of polarity of these compounds changes according to the acidity of the medium. Thus, a certain amount of acidified sample can be taken in order to obtain phenols without being dissociated as well as a certain amount of solvent (of the order of microlitres), to further propel both towards the extraction reactor. As the extracting solvent is being swept by the sample, the solvent is adhered in the form of a film throughout the tube. Finally, the carrier eliminates the excess of injected sample. By now taking a certain amount of sodium hydroxide, when the alkali is propelled towards the extraction coil, the extraction of the analytes in the form of phenolates takes place. Contrarily, non-polar compounds, without acidic character are retained by the organic solvent. When the phenolates arrive to the detector it is possible to obtain their corresponding spectrum at the peak maximum in the case of using CCD or diode array detectors, thus, allowing the application of a multivariate analysis technique to obtain the concentration of each of the phenols of a mixture without requiring physical separation. The organic solvent film can be eliminated by injecting a certain volume of acetonitrile. The same procedure has been used for the determination of mixtures of nitrophenols by MSFIA.

2.2. *Solid–liquid extraction*

Likewise chromatographic techniques, solid–liquid extraction is nowadays more popular than liquid–liquid extraction. The application of solid–liquid extraction is possible in different variants: packed column extraction carrying out the measurement on the subsequent eluate, packed column extraction and direct measurement on the

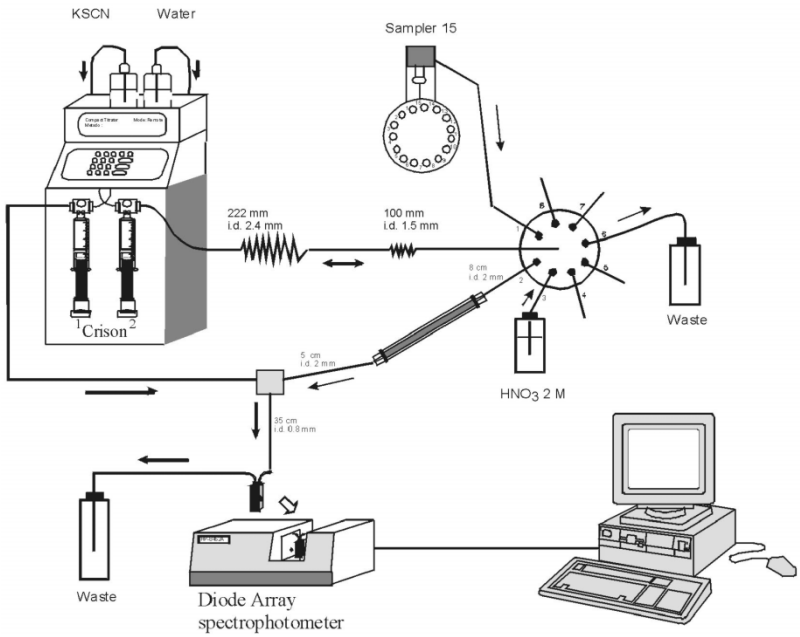

Figure 14. SIA system using a chelex 100 column for Fe(III) preconcentration and colorimetric determination with thiocyanate.

solid (optrode), solid phase extraction disk with elution and measurement of the eluate and solid phase extraction disk and direct measurement of the retained analyte by reflectance.

2.2.1. Extraction columns with elution of the retained analyte and measurement of the eluent. Extraction columns have been widely used in the pre-concentration and/or clean up of the analyte. In figure 14 a system for the spectrophotometric determination of iron with thiocyanate by a SIA system is represented [20].

In the first place, a considerable volume of sample (5–10 mL) is aspirated and propelled towards a column packed with Chelex 100 resin (iminodiacetic groups fixed onto the resin which act as ligands of metallic ions). Next, a small volume of nitric acid is aspirated, which when passing through the column, gives rise to a competitive equilibrium of the ligands with the metallic ions, thus, being the latter liberated from the resin. The eluted iron is merged with a thiocyanate solution and the red complex obtained is detected by a spectrophotometer. With this extraction system it is feasible to go from detection limit levels of the order of mg/L to μg/L. This same system has been used for the determination of Fe by atomic absorption spectroscopy [21].

In figure 16 a system for the determination of arsenic traces by means of a MSFIA system with atomic fluorescence is represented [22].

A certain volume of sample is merged with borohydride and the formed arsine is swept by argon from a phase separator to the atomic fluorescence detector in which the element is detected at μg/L levels.

Table 1. Comparative results between FIA, SIA and MSFIA for arsenic determination by atomic fluorescence [22].*

Parameters	FIA	SIA	MSFIA
Detection limit ($\mu g\,L^{-1}$)	0.05	0.67	0.07
Linear range ($\mu g\,L^{-1}$)	0.1–8	2.5–70	0.25–12
%RSD	–	1.9	4.9
Sample throughput per hour	–	6	36
Injection throughput per hour	45	33	113
NaHB4 concentration (%)	1.2	0.1	0.2
NaHB4 consumption (mL/inj)	4.7	0.5	0.3
HCl, KI/inj (mL/inj)	11.3	0.5	0.6
Sample volume/inj (mL/inj)	5.7	0.5	0.6
Sample volume needed (mL/inj)	11.5	0.5	0.6

*Results obtained in general for these flow techniques using different analytical methodologies applied to other analytes may be compared in references [23–25].

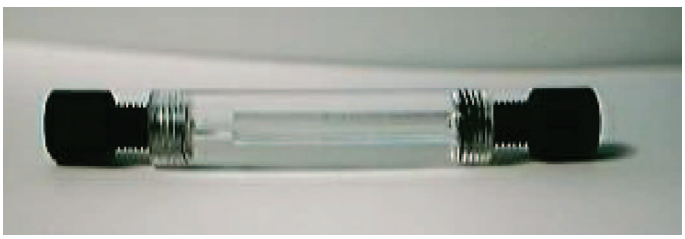

Figure 15. Preconcentration column for flow systems.

In table 1 the results for the different flow techniques in the determination of arsenic by atomic fluorescence are shown. It can be observed that MSFIA offers a higher sample throughput as well as a low sample and reagent consumption.

If before the reaction coil an anionic column is placed, as that represented in figure 15, the detection limits can be decreased even more [26]. In this case, the aim of the column is to sequentially retain borohydride and the anionic forms of arsenic. When subsequently passing a stream of HCl the reduction of the former species to arsine occurs. This compound is swept first by the liquid to the phase separator and next by argon to the atomic fluorescence detector. In this way, ppt levels are attained.

2.2.2. Extraction columns with direct measurement of the retained analyte: Optrodes. As a result of the elution, the analyte peak will undergo the typical phenomenon of dispersion, decreasing its height as a function of the distance existing from the outlet of the column to the detector. One way of avoiding this phenomenon of dilution is to perform directly a measurement of the analyte retained by the extracting resin. Thus, a flow cell (optrode) can be used for this purpose, in which the resin is located in a prismatic channel with a very narrow optical pathlength to minimise the strong attenuation of the radiation produced by the resin. The radiation can reach the cell by means of an optical fibre, and be further collected after passing through the resin by another optical fibre which transports the radiation up to the detector

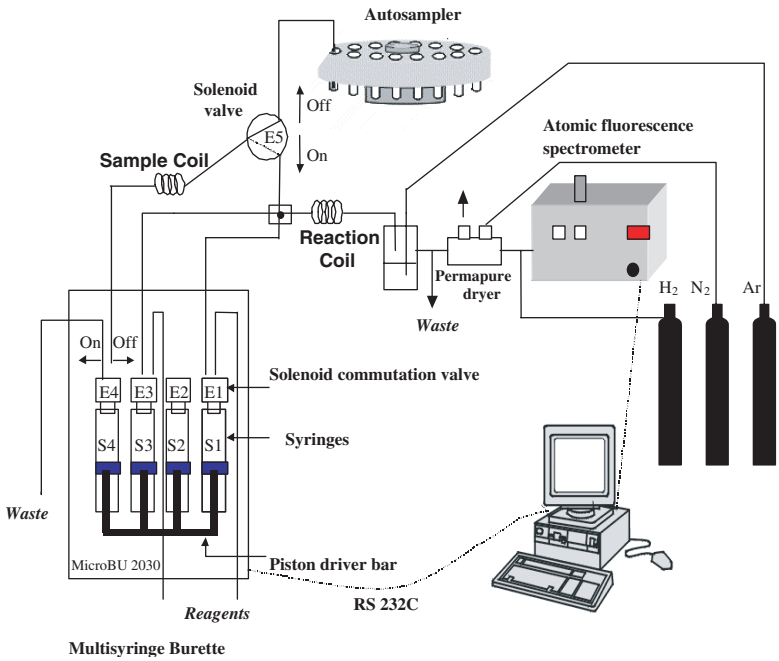

Figure 16. MSFIA system for arsenic determination with atomic fluorescence detection.

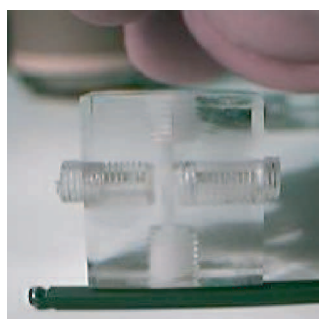

Figure 17. Optrode.

(Fig. 17). This device has been used with the aim to enhance the detection limits of nitrites in waters [27].

Another variant consists in using the flow cell in front of the photomultiplicator, which has been employed to carry out the chemiluminescence determination of phosphates with luminol, as depicted in figure 18.

In this case [28], the spiral flow cell located facing the photomultiplicator has been filled with a C18 resin. The sample is merged with molybdate and the heteropolyacid formed is retained in the cell. When subsequently sending a luminol stream, its oxidation produces a fluorescence emission which is detected by the photomultiplier. With this preconcentration system levels below μg/L for the determination of phosphates are attained.

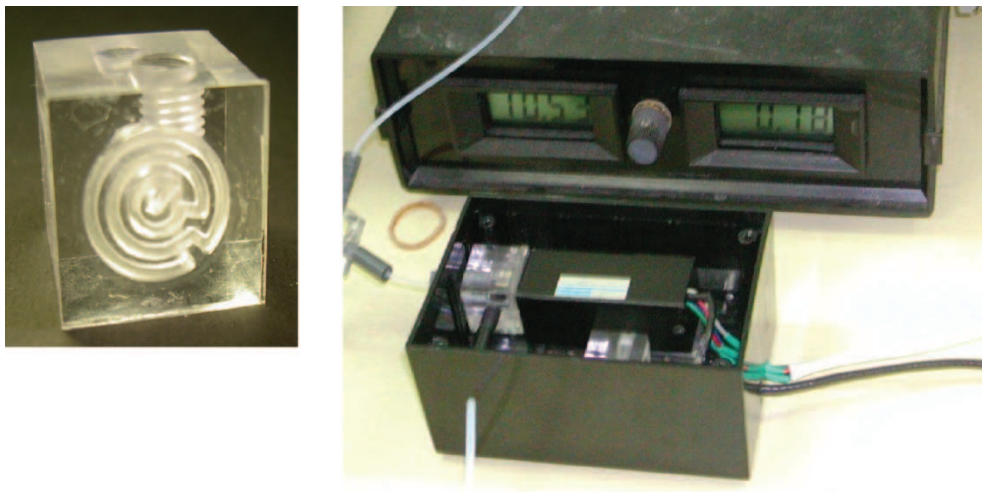

Figure 18. Chemiluminescence system using a spiral flow cell filled with C18 resin for phosphate preconcentration.

Figure 19. Holder for preconcentration disk filters.

2.2.3. Extraction disks with elution of the retained analyte. The use of packed solid phase columns requires a certain skill to obtain reproducible packing which does not cause excessive overpressures. Thus, a far better option is the use of modified filters which contain the extracting resin in a percentage which is sometimes close to 90%. Since they are industrially produced, disks with great reproducibility can be prepared, and their thickness being a few millimetres no overpressures occur. This system (Fig. 19) [29] has been used to retain Fe(III) with a filtrating disk of Chelex 100 and to determine the Fe(III)-thiocyanate complex subsequently formed. In this way, detection limits of the order of μg/L, with great reproducibility and without overpressures, can be achieved. Besides, the filters can be reused up to about 50 times by an appropriate cleaning after each injection. The filters can be easily changed in a reproducible way without requiring any special skill.

2.2.4. Extraction disks with direct measurement of the retained analyte by reflectance measurements. Again it is possible to avoid a decrease of the signal owing to the dispersion produced during the eluate travel by carrying out the measurements directly

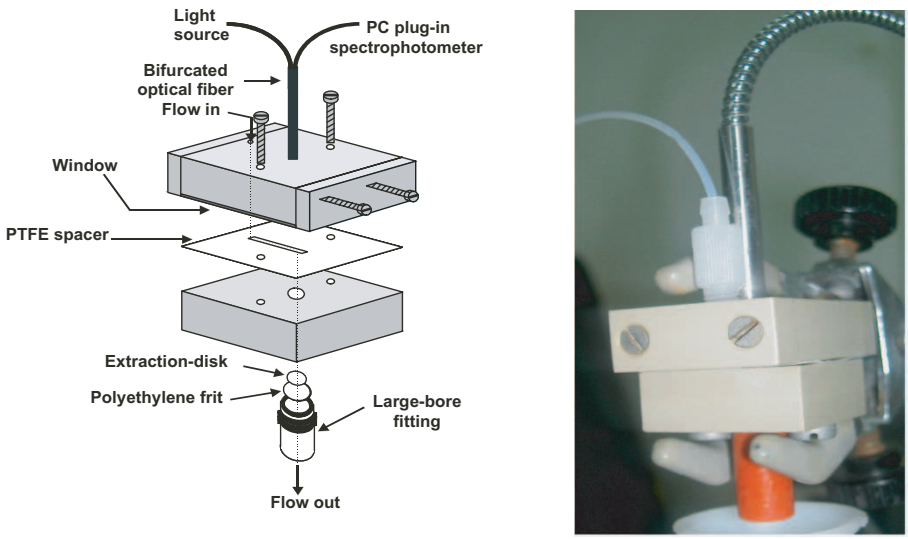

Figure 20. Reflectance flow through cell.

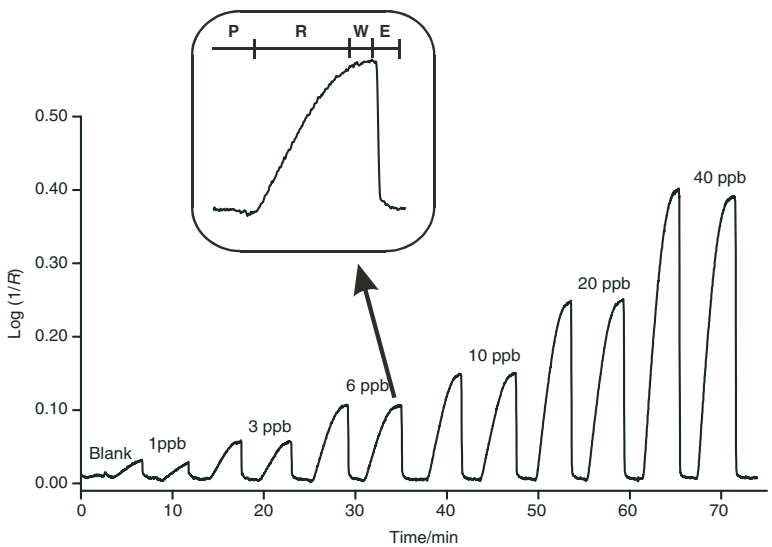

Figure 21. Flowgram obtained during the determination of nitrites in water, in which the height of the bands is proportional to the concentration of nitrites within the range of μg/L.

on the analyte retained in the solid phase. For this purpose, the system represented in figure 20 can be used.

In this system the analyte or a corresponding derivative is flowed through a filtering disk containing the retentive phase, and an increase of concentration can be achieved by means of reflectance measurements by using a bifurcated optical fibre (Fig. 21), in which the incident radiation reaches through one of the legs, and the radiation reflected is conducted through the other leg towards the CCD detector.

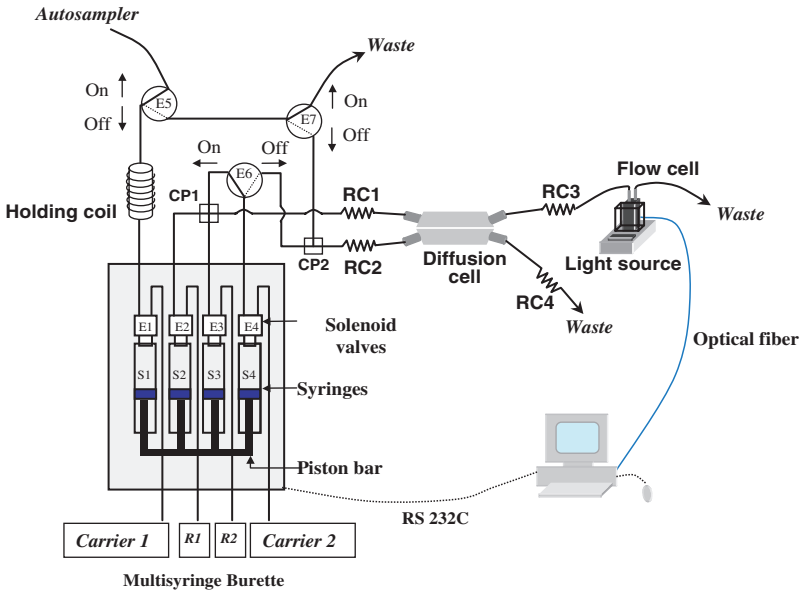

Figure 22. MSFIA system for sulphide determination using a gass difusión cell.

2.3. *Dialysis and gas diffusion cells*

A simple way of eliminating the effect of interferents consists of using gas diffusion or dialysis membranes. In the first case, Teflon membranes are usually employed, which being hydrophobic only allow gas compounds passing through (as such or dissolved gases), hindering the passing of water, as well as particles and solvated ions. Thus, the use of a low selective detector on the other side of the membrane (accepting solution) is feasible. In the case of dialysis, cellulose acetate membranes are usually employed.

In figure 22 a MSFIA system designed for the determination of sulphides in wastewater [30] based on the formation of methylene blue is represented. The selectivity of the method is based on the use of a gas diffusion cell with a Teflon membrane. The sample is acidified and further injected into the donor channel of the diffusion cell, and only (part of) the sulphydric acid formed is capable of passing through the membrane and being captured by an accepting solution of dimethyl *p*-phenyldiamine and Fe(III) in HCl to form methylene blue, which when detected by a CCD spectrophotometer of optical fibre gives rise to a peak whose height is proportional to the concentration of sulphide present in the wastewater.

3. Software

As above-mentioned, practically all flow techniques subsequent to FIA require the use of some kind of software for execution purposes, a feature which has been one of the limiting factors in their development due to basically the lack of experience in programming on behalf of the users together with the inexistence of commercialized programs.

This deficiency has been gradually overcome during the last decade with the appearance of more or less versatile programs.

One of the most widely used options has been the design of specific programs for the development of a particular application, frequently by using BASIC in its different variants. This implies the development of a new program for each application.

Although this alternative may be simple and easy to be applied by non-specialists in computers, however, it presents several inconveniences: continuous programming according to the applications to be developed, and the limitations of the operative system used. Initially, this was the alternative chosen, but, a better solution proved to be the creation of software libraries and to resort to the concept of Laboratory Unit Operations. Accordingly any analytical procedure can be considered as a combination of several standard laboratory operations appropriately coupled. This led us firstly to the development of a program (QuickBASIC) under MSDOS which we named DARRAY [31] (diode array), which subsequently evolved towards a much more versatile system, without the restraints of the low zone of memory when using the Windows operative system of 32 bits, a program which we have named AUTOANALYSIS.

In order to avoid reprogramming the main program according to the instrumentation used a software constituted by several layers of DLLs (Dynamic Link Libraries) has been designed, the main program being connected to the specific DLLs of each instrument and apparatus through a series of intermediate DLLs [32].

With this design the user is only required to learn the management of a sole program for any system needed to be automated by means of a flow technique, which facilitates considerably the user's work and avoids the learning of different programs according to the application to be developed.

The first step consists in defining the hardware configuration of the system to be used, taking into account which instrumentation is available and which types of interfaces the former possess to communicate with the computer. In this way, which communication channels to be used and the instrumentation to be hung from these channels are defined.

Thus, in figure 23 the hardware configuration menu of the AUTOANALYSIS program, in which a SIA system with spectrophotometric detection is being defined, is represented.

It can be observed that a HP diode spectrophotometer connected to a HP-IB parallel communication interface has been selected. On the other hand, two Crison modules which use a joint RS232C serial communication channel, an automatic burette defined as the first module of the chain, and a module of valves as a second element of the chain have been selected. At the top right of the menu the DLLs which can be chosen to define the different communication channels can be observed, whereas at the bottom the instruments and apparatus which can be used by these channels are depicted.

Once the hardware configuration has been defined, the user continues to define the analytical procedure through another ad hoc menu (see figure 24).

By using this menu, the subsequent steps which define the analytical procedure are being introduced, combining the use of the selected instrumentation with other typical programming instructions, such as the introduction of the waiting command, repetition loops of procedures, signal marks of the start of a procedure, conditional instructions, etc.

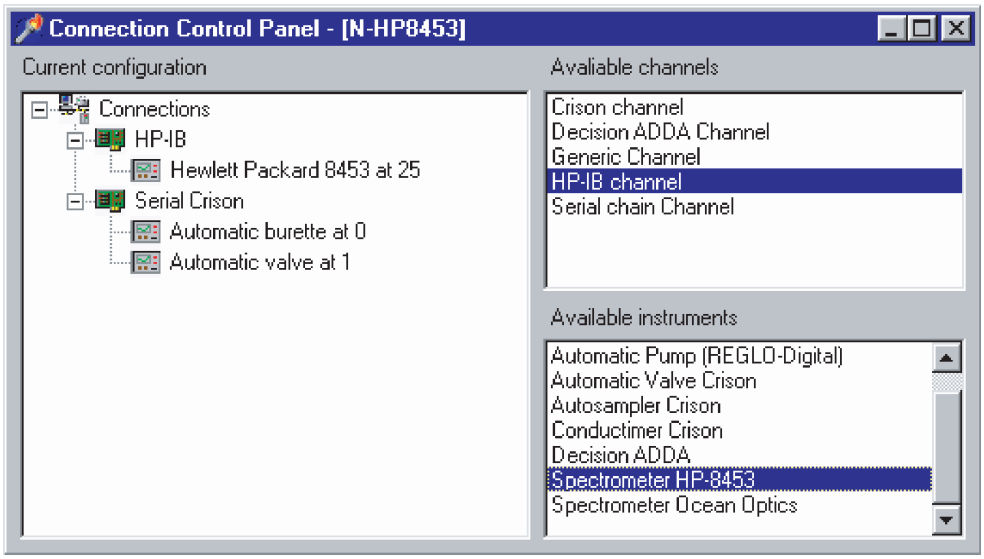

Figure 23. Hardware configuration menu of AUTOANALYSIS.

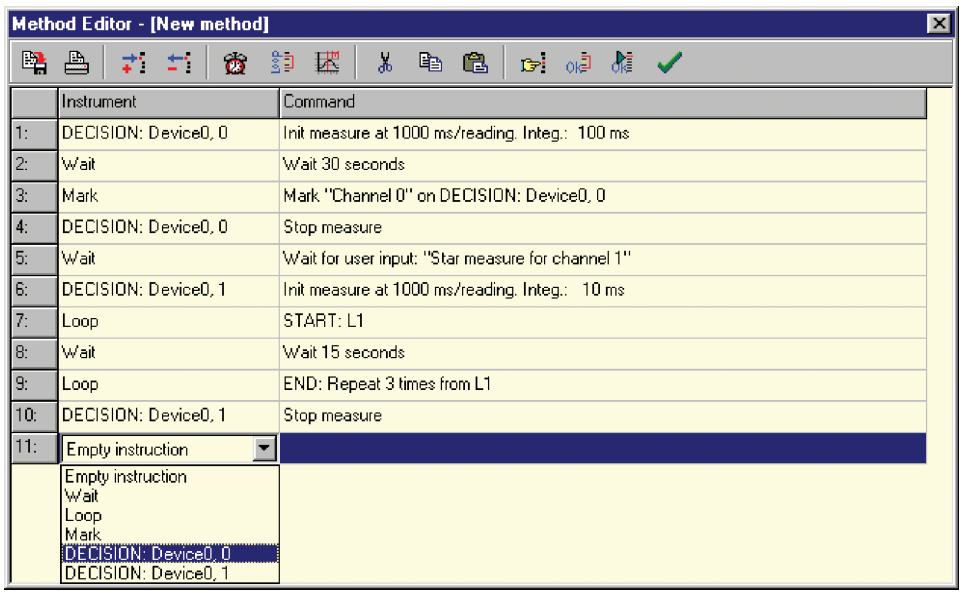

Figure 24. Procedure configuration menu of AUTOANALYSIS.

Since the iterative application of a certain procedure can give rise to unacceptable errors (for example, trying to inject different liquids surpassing the possibilities of the burette), a virtual simulation of the analytical procedure before putting it into practice, is feasible. Once the validation has been carried out, the method is executed and new screens are open in which the flow diagrams can be observed together with the representation in this case of the spectra obtained for each point of the former.

Table 2. Instrumental developed DLLs for AUTOANALYSIS.

Valves modules	Crison, Knauer
Monosyringe automatic burette	Crison
Multisyringe automatic burette provided with 4 output ports for solenoid valves and pumps	Crison
pH/potentiometer + temperature	Crison
Conductimeter	Crison
Autosampler	Crison
COMPACT Autosampler	Crison
Spectrophotometers	Ocean Optics
Fluorimeters	Perkin Elmerr
Atomic fluorescence	PS Analytical
Electrochemical system	AMEL
Microwave oven	Prolabo
Peristaltic pump	Ismatec
Chemiluminiscent detector	Sciware
A/D 14 bits, 16 channels card	Flytech, Decisión, Ibercom
I/O 48 lines, three counters 16 bits card	Flytech, Decisión

Once the program has been executed, it will automatically detect the peaks, calculate the areas and heights of the former and will be able to apply calibration curves if the use of appropriate calibration standards has been considered.

Nowadays, a great number of DLLs are available (see table 2) which can be combined to develop any kind of flow technique (including some of the chromatographic type), with the possibility of using very different types of detectors (fluorometric, spectrophotometric, atomic absorption spectrophotometry, atomic fluorescence spectrometry, conductimetric, amperometric, etc.). The program is versatile enough to be able to combine several flow techniques and even develop initially non-considered applications (such as, for example the use of different devices to maintain certain reaction variables constant, as in the case of requiring a pH within a certain range of a chemical reactor). In table 2 a list of DLLs is given, and has been developed for the control of a series of very different devices, from cards which are placed in the slots of the computer (A/D and D/A converters, digital inputs and outputs), to relatively complex instruments, such as the AMEL electrochemical system, with which amperometric measurements, a number of polarographic techniques, anodic stripping voltammetry, etc. can be carried out.

On the market are other programs for the control of several flow techniques, such as FIAlab for Windows (FIAlab Instruments Company) can be also found. As in AUTOANALYSIS, FIAlab for Windows is also ideal for online monitoring and process control applications.

The Global FIA Company commercialises the FlowTEK program, designed to be, a full feature FIA/SIA software package.

4. Applications

From the beginning, flow techniques have been extensively applied in very different fields, such as environment (29%), clinical assays (29%), agriculture (16%), pharmacy (14%), and industrial processes (9%). In figures 25, 26 and 27 the number

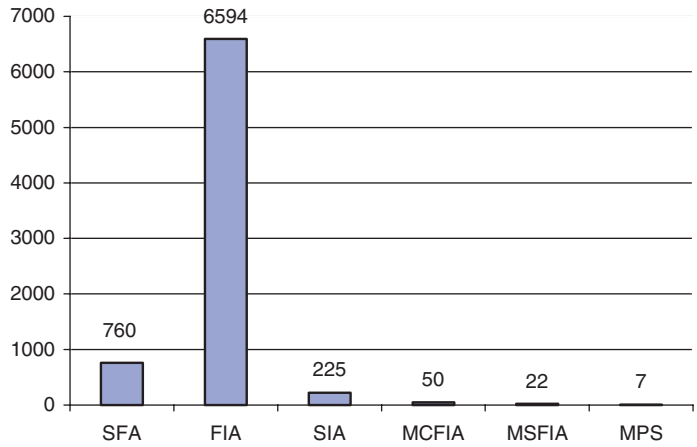

Figure 25. Number of works published for each flow technique since their appearance.

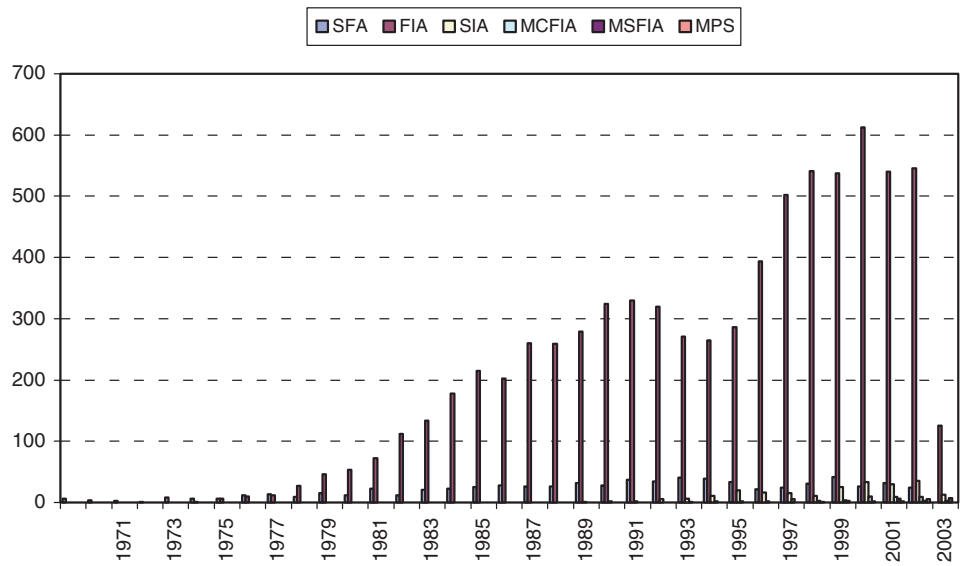

Figure 26. Evolution of number of publications of flow techniques from 1970 to 2003.

of publications carried out on these techniques and their evolution during the first years of existence are represented.

It can be observed that FIA has led by far with a greater number of publications than the remaining techniques, which can be easily explained. In the first place, SFA has been basically developed by commercial companies, perhaps far more interested in selling the instrumentation than in publications, the contribution of the non-commercially linked research groups being much more limited owing to especially the acquisition cost of the SFA apparatus. The great difference in publications of FIA with regard to the remaining flow techniques can be explained in terms of the easy manual implementation involved. The latter techniques, besides being more recent require experience in the connection of the instrumentation with computers.

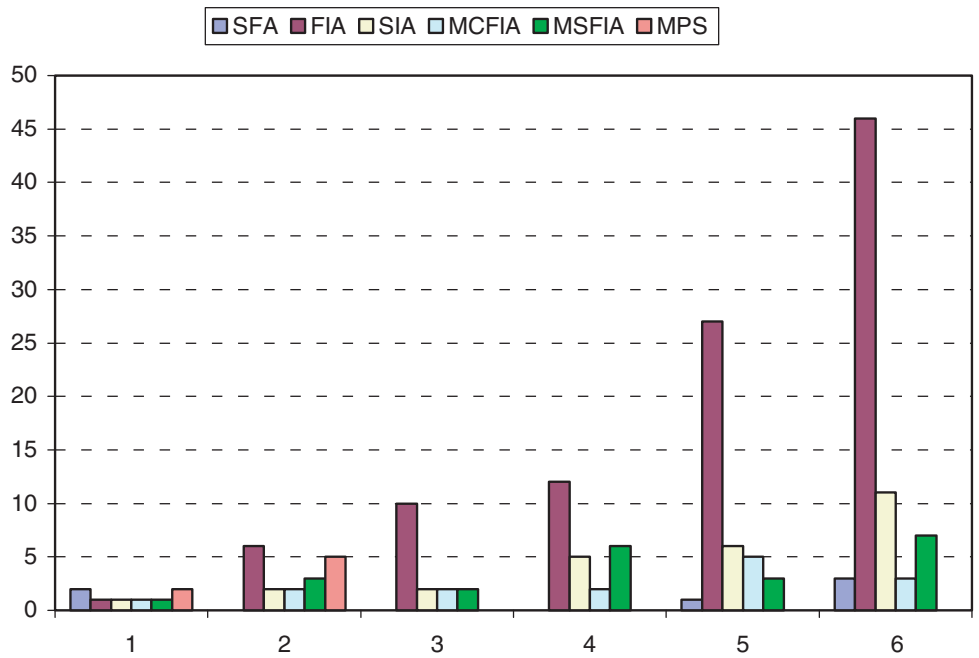

Figure 27. Evolution of each flow technique during the first six years.

Flow techniques have been fundamentally applied to the automation of the analytical methods used in the laboratory and, in less extent, to the development of online monitors in industrial processes and to measurements of environmental parameters, in which the SIA technique may be of special interest due to its multiparametric character. This same technique has aroused a great interest in the processes involving bioreactors, since rather than a high sample throughput what is really considered of interest is a multiparametric technique with low sample and reagent consumption.

Another field of interest is that of determination of radioactive isotopes, since these techniques allow the analyst to stay away from the working environment as much as possible. However, radioactivity detectors are usually very slow and, therefore, flow techniques are usually applied only in steps regarding previous treatment and sample conditioning, the measurement process being carried out subsequently in batch [33–35].

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