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FLOW INJECTION WINE ANALYSIS : A CRITICAL REVIEW AND FUTURE TRENDS

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Abstract : *For effective control of wine quality, a routine wine laboratory should be able to analyse rapidly and reliably a large number of samples. As flow injection analysis (FIA) is becoming a privileged way for automation of wet chemical procedures, a critical review of its present status for application to wine analysis is presented. We evidence the possibilities of this analytical technique in this area as well as its possible evolution trends, namely by using manifolds for multidetection.*

INTRODUCTION

A chemical analysis performed in a conventional way involves a set of operations basically composed by sampling, the sample manipulation in order to fit its characteristics to the detection system, the measurement of a physical property which can be related to the concentration, and the collection, treatment and registration of the results. Since the instrumentation (detection systems and computers) available nowadays allows the measurement, acquisition and data treatment in a quick and efficient way, the total time spent with the analysis is basically conditioned by the sample pre-treatment. These operations are still being performed with instruments which were designed even before this century, and suffered only small changes for making its use quicker and simpler.

The aforementioned changes have only minimized the difficulties in laboratories arising out from the increasing search for analytical information that has become accentuated during the last decade.

Considering that the expenses with personnel, conditioned by their number and qualification, is the most significant cost in a laboratory, one can easily conclude about the advantages in automating the analytical procedures. The automation of laboratories allows to make compatible two apparently opposed aspects: on one side the search for more and more determinations, and on the other side the need for decreasing costs of the analytical control.

Actually, the increasing tendency in using automatic methods of analysis is evident in all areas of chemical control, but has been more significant in areas that are related with public health, such as clinical, environmental and food chemistry. In this scenery, wine analysis is not an exception.

The large majority of the automatic methods are fundamentally based (and also inspired) in the replacement of corresponding manual operations by discontinuous or continuous operations prior to the measurement. In the discontinuous methods the samples keep their identity because they are placed in separate vessels where the different pre-treatment operations take place. In the continuous methods the sample preparation takes place in a liquid or gas flow, and the analyte concentration is measured in a flow mode.

In the scope of the continuous methods of analysis, systems like segmented flow (SFA) and, more recently, those of non-segmented flow (in particular flow injection analysis, FIA) have been the mostly used.

The segmented flow analysis systems were initially proposed by SKEGGS (1957) and are based on the segmentation of the flow with air, nitrogen or even oil bubbles. These systems aim to reproduce the typical concept and practices of classical analysis to keep the samples identity and perform the measurement under conditions of equilibrium and homogenization.

The segmented flow analysis systems are composed by sampling and propulsion units, devices for the introduction of air bubbles and intermediate devices for carrying out operations like dialysis, liquid-liquid extractions, heating, elimination of the air bubbles, detection and treatment of results systems. This kind of approach has been very successful and with recognized efficiency in wine analysis (CURVELO-GARCIA and BOUVIER, 1988). However, they present some disadvantages like being very complex and expensive, due to the technical difficulties associated with flow segmentation and to measurements performed under equilibrium and homogenization conditions.

As an alternative to these systems it was reported in the seventies (RUZICKA and HANSEN, 1975) a new methodology of continuous flow which revolutioned the concepts used so far in terms of automatic methods, and was designated as Flow Injection Analysis (FIA). This methodology, which was initiated simultaneously by RUZICKA and HANSEN in Denmark and by STEWART et *al.* (1976) in the United States, has broken the idea that the automatic methods should be simple adaptations of the classical methods to a flow system. In fact, in this new methodology there is no segmentation of the liquid flow, and the measurements are not performed under physical (homogenization) or chemical equilibrium conditions. In FIA the samples are inserted in a continuous liquid flow which promotes its transport towards the detector. During the transport process, the sample plug is dispersed in the carrier stream and reactions can occur with the carrier or with some other reagents strategically added to the system. Finally, when the species that produce the analytical signal reach the detector a transient signal is generated that can be related with the analyte concentration. The concept of homogenization and equilibrium associated with SFA is then replaced by the partial dispersion of the sample in the carrier, with a strict control of the time interval between

injection and measurement. Usually, a FIA determination is initiated with a calibration curve, then followed by the introduction of the samples and closing, if necessary, with another calibration to control an eventual change in the detector response. In this way it is guaranteed that all the parameters of the FIA system are kept constant during the analytical procedure. There is no need to perform the detection under physical or chemical equilibrium conditions since dispersion, and eventually the extension of the reaction that occurs, is reproducible for the successive standards and samples.

The simplicity, relative low cost, versatility and ease of construction has arisen an increasing interest in the application of this methodology to numerous areas, particularly in clinical analysis and also in environmental control. This paper presents a review on the application of flow injection analysis to wine determinations, and gives a perspective on its possible evolution.

FUNDAMENTAL CONCEPTS OF FIA

FIA is already a reasonably known methodology considering that the inevitable time gap between research in laboratories and its application in routine control is yet being transposed. The interest that FIA has gained can be perceived from the publication rate (about two articles a day), in the existence of a journal totally devoted to it (Journal of Flow Injection Analysis), as well as general books (KARLBERG and PACEY, 1989; RUZICKA and HANSEN,1988; VALCÁRCEL and LUQUE DE CASTRO, 1987) and one of them dedicated to FIA with a specific detection process (BRUGUERA,1989). As the general aspects of FIA were presented in detail in the aforementioned monographs, only a brief resume will be presented here.

FIA is essentially based on the combination of three principles: intercalation of the sample into the carrier, controlled dispersion of the injected sample zone and on the exact timing of its movement from the injection point to the detector. In order to accomplish these needs, a simple FIA manifold (figure 1) includes a propulsion unit, an injection device, a reactor (tube) where all the sample preparation operations take place and a detector connected to a data recorder system.

The propulsion system must force the carrier and the other solutions eventually added to the manifold to flow in a reproducible way. Peristaltic pumps, in spite of the slight pulses they introduce into the system, are the mostly used devices for propulsion. These pulses, however, can be minimized with adequate devices (e.g. ALEGRET et *al.*, 1987). Its use in FIA systems with potentiometric detection rise additional problems since the complexity of the FIA manifold is increased due to the need of eliminating the electronic noise introduced by the pumps (ALEGRET et *al.*, 1984; ALONSO et *al.*,1986). As an alternative, other propulsion systems can be used like piston pumps, gravity action devices or by using pressurized containers.

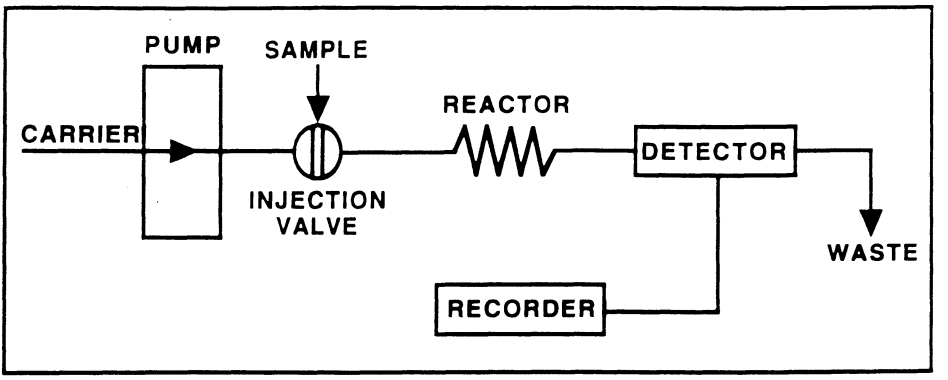


Fig. 1 — Simple flow injection manifold

The injection system must be able to introduce the sample in the carrier in a reproducible way without disturbing significantly the hydrodynamic characteristics of the flow. It is not needed to know the exact amount of sample injected into the system, regarded that the used volume is exactly the same for standard and sample solutions. Low pressure rotary valves and proportional injectors (commutators) are the injection devices more generally used, though the oldest FIA injection technique used a syringe furnished with a hypodermic needle. These traditional devices may present more complex aspects when special FIA modes like the sampling zone technique or multiparametric determinations are to be implemented.

The most common reactors in FIA are simple teflon tubes with inner diameters ranging from 0.5 to 1.0 mm, which can be straight, coiled, knotted or packed with small glass beads known as Single Bead String Reactors (SBSR) (REIJN et *al.*, 1981). Operations like dialysis, liquid-liquid extractions or ion exchange processes can be performed in FIA assemblies through the incorporation of appropriate units in the manifold.

Due to its versatile character, several types of detectors can be coupled to FIA systems (optical, electrochemical, enthalpimetric, photoaccustic, photoelectrochemical, piezoelectric, radiochemical) without the need for major technical modifications. A satisfactory flow injection detector should have a series of attributes: high selectivity, fast response, preferentially the signal should be independent of the flow rate and should not contribute to the enlargement of the transient signal by introducing additional dispersion zones.

The dispersion control of the concentration gradient corresponding to the sample plug flowing from the injection point to the detector is the basis of flow injection analysis. So, dispersion manipulation is determinant for the quality of the results.

At each instant, the sample plug dispersion profile is a consequence of two processes: convective and diffusional transport. In laminar flow conditions, convection generates a parabolic velocity profile where the solution film adjacent to the tube surface is practically stagnant and the solution that flows at the center has a velocity that doubles the average velocity. If this mechanism was the only one present, contamination between adjacent samples would occur and the signal would be too enlarged. However, diffusion also occurs as a consequence

of concentration gradients that are formed at different instants of convective transport. Diffusion can be axial (resulting from longitudinal concentration gradients) or radial (resulting from radial concentration gradients). Radial diffusion is important for the maintenance of samples identity since it forces particles to move from the walls to the center and vice-versa. This avoids sample contamination and allows a higher sampling rate by reducing the sample plug length.

Convective transport is the prevailing mode immediately after injection. As residence time increases, the diffusion process becomes more significant, being the dominant mechanism after some time. The FIA signal, resulting from the combination of these factors evidence a sudden rise when the sample reaches the detector, and returns to the baseline in a more relaxed way. When the sample residence time is relatively long, the analytical signal tends to a Gaussian-shaped curve.

The modular character of FIA systems confers them a great versatility allowing the use of manifolds with very different configurations and devices in order to perform different FIA modes. The stopped-flow mode is one example in which the flow is stopped when the sample plug is in the detector; the peristaltic pumps are then reactivated when the reaction extension is considered enough for the determination. This technique permits to perform the study of reactions kinetics, improve the measurements sensitivity by increasing the sample residence time inside the system (without increasing dispersion), and also the correction of the sample intrinsic signal.

Despite the low reagent consumption associated with the small tubes volume and the small flow rates used in a FIA system, the reagents economy can still be maximized with the use of techniques like the merging zones one (figure 2), in which sample and reagent plugs are injected in a synchronized way. This technique can also be used as a strategy to reduce the process sensitivity by forcing the overlap between the two sample plugs to occur in different degrees of coincidence.

When there is no restriction on the sample amount and one intends to save reagents, it is also possible to make use of the reversed FIA mode (figure 3) in which the sample works as a carrier and the reagents are inserted in it. This mode also allows the increase in the measurement sensitivity and also sequential determinations using injection valves positioned in series. However, the sampling-rate is necessarily reduced due to sample aspiration instead of sample injection.

In order to obtain high dispersion manifolds, the zone sampling mode can be the alternative (figure 4). The injected sample is dispersed along a portion of tube which heads it towards the loop of a second injection valve. When this second valve is activated it inserts a small section of the initial sample plug on another channel connected to the detector. The exact time control between the activation of the two valves permits the insertion of different amounts of the sample under analysis.

Another possibility associated with FIA systems is the execution of titrations under continuous flow, which is not possible in SFA systems. This capacity is due to the transient

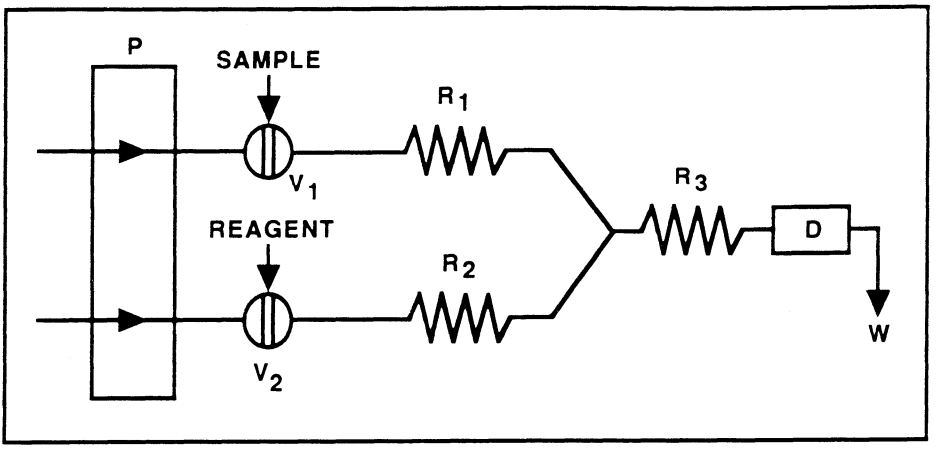


Fig. 2 — Merging zones FIA manifold

P: peristaltic pump; Vi: injection valves; Ri: reactors; D: detector; W: waste.

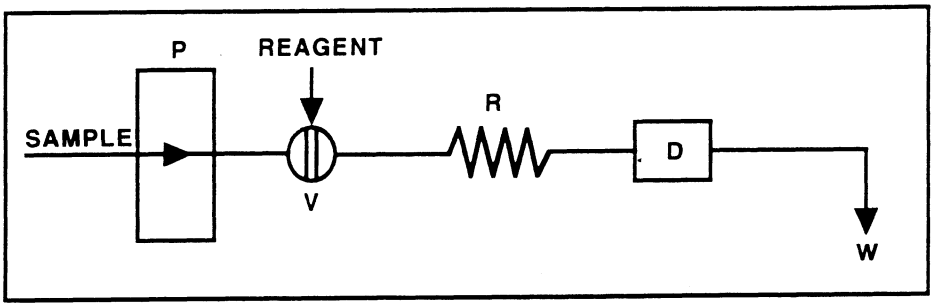


Fig. 3 — Reversed FIA system

P: peristaltic pump; V: injection valve; R: reactor; D: detector; W: waste

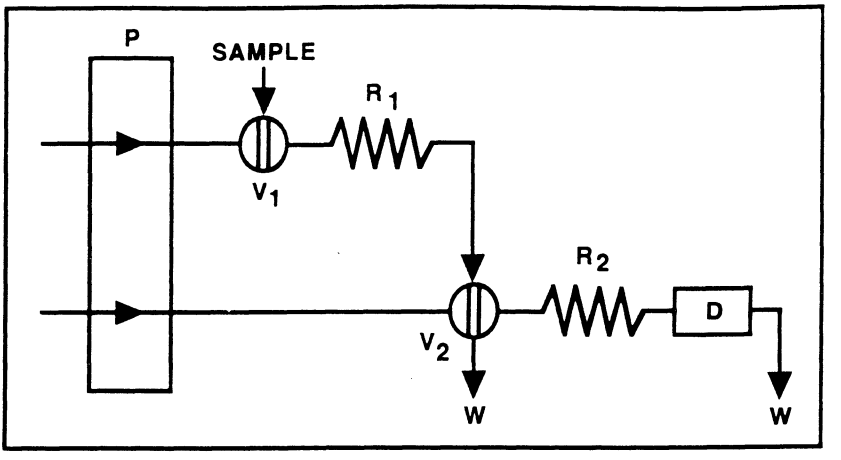


Fig. 4 — Zone - sampling flow injection system

P: peristaltic pump; Vi: injection valves; Ri: reactors; D: detector; W: waste

and controllable characteristics of the FIA signal. When a species that reacts quantitatively with another species present in the carrier stream is injected, it is possible to find two points in the obtained dispersion profile in which the concentration of both species is equal. The time interval between those two points is proportional to the concentration logarithm of analyte. The use of the transient peak width (always measured at a fixed value of the transient signal) as an analytical parameter is not exclusive of titrations; it can also be used to increase the linear range of the analytical methodologies due to the logarithmic dependency.

In addition to the previous modes, which are exclusive for FIA systems, separation modes can also be used like in SFA. The samples can be dialysed (Figure 5) from the channel sustaining the sample plug to another containing the acceptor stream. This technique is basically used to eliminate interferents, or to significantly decrease the amount of analyte that flows to the detector. The use of gas-diffusion units, in which the gaseous sample component diffuses from the donor channel to the acceptor through an hydrophobic membrane, is another possibility.

We can still refer the use of liquid-liquid extraction units with the aim of increasing determinations selectivity or sensitivity, and packed tubes with immobilized enzymes, ion exchange resin or with reducing agents.

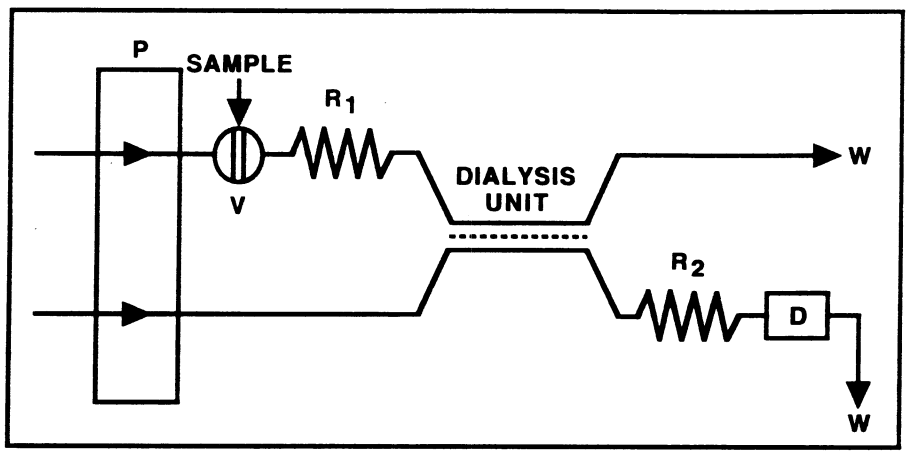


Fig. 5 — FIA manifold with a dialysis unit

P: peristaltic pump; V: injection valve; Ri: reactors; D: detector; W: waste.

COMPARING FIA AND SFA

SFA systems have been the most widely used automatic continuous methods. This tendency is also evident in wine analysis and this justifies a comparison presented here between these systems and those using flow injection analysis.

It was the firm Technicon that invested more in the development of the SFA technique and also the one that developed more efforts in constructing and divulging the first instruments to be commercialized under the name of "AutoAnalizers". This situation justifies the

current association of the word "autoanalizers" to the idea of segmented flow analysis.

Although the similarity between FIA and SFA apparatus, their characteristics, as well as their efficiency are quite different.

In FIA, the samples are injected and not aspirated as in SFA, permitting the use of very small sample volumes. This fact is not very important for the case of wine analysis but may assume a particular relevance in clinical analysis. The washing cycles, indispensable in SFA, are not needed in FIA because the solution corresponding to the blank assay flows between successive samples.

The consumption of reagents is remarkably smaller in FIA mainly due to the smaller volume of the tubes. The economy of reagents, which is critical when using very expensive reagents like enzymes, can still be maximized using techniques such as merging zones, or yet making use of immobilized enzymes or rotary valves that allow the reagents recirculation.

The possibility of performing titrations in continuous flow is another unique capacity of FIA systems, as well as the kinetic evaluation of a reaction.

FIA systems are simpler due to the absence of flow segmentation, more versatile and of lower cost, presenting results with equivalent quality to those supplied by the segmented flow systems but with higher sampling rates, except for analysis involving very long reaction times.

FIA APPLICATION TO WINE ANALYSIS

In what concerns the automation of wine determinations, the most routinely used systems have been the segmented flow ones. However, these systems have some limitations namely their high acquisition and maintenance costs, the need of being operated by specialized technicians and also the need to have a large number of samples to analyse in order to run the system with economical feasibility.

Due to its own characteristics, FIA is emerging as a methodology of great interest and potentiality for the automation of wine analysis. However, only recently attention has been devoted to it thus justifying the relatively short number of parameters that can be determined using this methodology (Table I). Furthermore, some of the systems already developed present some limitations, namely the fact that they were only tested with a certain type of wine, leaving unknown its applicability to other types. Additionally, some of the systems demand a previous treatment before the injection, thus reducing the advantage of its use.

In the following text it is described, in a resumed way, the FIA methodologies developed for wine analysis. Those methodologies are grouped by the detection processes that were used, as the FIA manifolds configurations are strongly dependent and conditioned by the characteristics and demands of the detection system.

I — UV/VIS SPECTROPHOTOMETRY

The detection system more widely used in flow injection systems for wine analysis has been UV/VIS spectrophotometry. The prevalence of this detection system can be explained by the easy coupling of a flow cell to the flow systems as well as by the possibility to determine a large number of species, whether through its intrinsic absorption properties or the so-called colour-development reactions.

SO_2 determination gave rise to the biggest number of publications, being the first one from 1980. In this paper RUZICKA and HANSEN (1980) developed a double-channel system based on the West-Gaeke reaction. The detection is performed under the stopped-flow mode, with the flow being stopped during 15 seconds. The increment of the analytical signal during this time interval is measured at a wavelength set at 580 nm.

Tecator firm presented a manifold including a gas-diffusion unit for total SO_2 determination (TECATOR, 1983a) based on the same chemical reaction, integrated on the FIA system commercialized by this firm. After the addition of a sodium hydroxide solution to release the combined SO_2, the sample is inserted in the carrier (water) and mixed with sulphuric acid at a confluence point. The released SO_2 flows towards the gas-diffusion unit where it diffuses through the hydrophobic teflon membrane to the acceptor stream with the colour reagent, and finally flows to the detector.

Using the same manifold described above, but with some slight changes, MOLLER and WINTER (1985) described the determination of SO_2, presenting a 1 p. cent relative standard deviation for the injection of a 10 mgL^{-1} standard, and a 90 samples h^{-1} sampling rate.

SULLIVAN et *al.* (1986) proposed a system for SO_2 determination also including a gas-diffusion unit but in which the measurement is based on the extension of malachite green decoloration. The sample, introduced in a sulphuric acid stream, is directed to the gas-diffusion unit where SO_2 diffuses to the acceptor channel, where the colour reagent flows.

SULLIVAN et *al.* (1990a) also described a system for total SO_2 determination including a gas-diffusion unit and based on the same colorimetric reaction. The wine sample is treated with a sodium tetrachloromercurate solution before its introduction into the system. Then, 50 µL of the resulting solution are inserted in the sodium hydroxide carrier solution to release bound SO_2. Sulphuric acid is added through a confluence in order to produce SO_2 gas which is directed to the gas-diffusion unit, following the sequence described before. Despite bad results obtained for red wines, good results were achieved for white wines with a relative standard deviation below 3 p. cent and a good correlation with the reference method.

SULLIVAN et *al.*(1990b) also developed a new methodology for the determination of SO_2 liberated at room temperature and at pH 2.2 which, according to the authors, corresponds to the free and weakly combined SO_2. The manifold is equal to that described on the previous paragraph, only replacing sodium hydroxide and sulphuric acid solutions by a solution of citric acid, and using an injection volume of 15 µL.

TABLE I

Flow injection analysis wine assays

Analysis	FIA mode	Method/ Detection	RSD[a] (%)	SR[b] (h^{-1})	Ref [c]
Acetaldehyde	Merging zones	Enz/Spect.	0.5	50	LÁZARO et *al.*, 1986b
Ca/K	Simple, two-line	Potentiometric	<2	30-60	ILCHEVA et *al.*, 1990
Ca	Mixing chambers	AAS	1.7	60	LIMA and RANGEL, 1991
	Flow splitting	AAS	1.3	240	LIMA and RANGEL, 1991
Ca, Mg, Na, K	Dialysis	AAS/FES	0.71-3.9	120-150	LIMA et *al.*, 1991a
Ca/K, Mg/Na, K/Na	Dialysis/Flow splitting	AAS/FES	<2.9	120	LIMA et *al.*, 1990b
Carbon dioxide	Gas diffusion	Spect.	1-2	120	TECATOR, 1983b
CO_2/SO_2	Gas diffusion	Poten/Spect.	<7	25	LINARES et *al.*, 1989
Chloride	Pseudo-titration	Potentiometric	<2	60-120	LIMA and RANGEL, 1989
Copper	Simple	AAS	0.82	360	LIMA and RANGEL, 1991
Ethanol	Simple, two-line	Enz/Spect.	___	120	WORSFOLD et *al.*, 1981
	Merging zones	Enz/Spect.	0.7	55	LÁZARO et *al.*, 1986b
	Gas diffusion	Potentiometric	0.8	25	OHURA et *al.*, 1990
	Simple, two-line	Enthalpimetric	<0.1	110	OLIVEIRA and PASQUINI, 1988

a) relative standard deviation; b) sampling rate ; c) references

TABLE I (next)

Flow injection analysis wine assays

Glucose	Merging zones	Enz/Spect.	2-3.1	40	RUZ et *al.*, 1988
	Simple, two-line	Enz/Spect.	2	___	TZOUWARA and CROUCH, 1990
	Zone sampling	Enz./Fluorim.	2	60	DREMEL et *al.*, 1989
Glycerol	Simple, three-line	Enz./Lumin.	<2.62	30	PUCHADES et *al.*, 1991b
Iron	Integrated retention	Spect.	___	<30	LÁZARO et *al.*, 1989
L(+) Lactic acid	Simple	Enz./Spect.	___	___	KOTORMÁN et *al.*, 1991
	Dialysis	Enz./Spect.	<2.5	20	LIMA and RANGEL, 1992
L(-) Malic acid	Stopped-flow/Merg.zones	Enz./Spect.	2.0	80	GARCÍA DE MARÍA et *al.*, 1991
	Dialysis	Enz./Spect.	<2.5	20	LIMA and RANGEL, 1992
	Simple/Zone sampling	Enz./Fluorim.	<2	12-20	CHEMNITIUS and SCHMID, 1989
Lactic /Malic acid	Simple/Sample splitting	Enz./Fluorim.	1.2/0.75	30	PUCHADES et *al.*, 1991a
Maltose	Simple, two line	Enz./Spect.	2	___	TZOUWARA and CROUCH, 1990
Metallic cations	Flow splitting	AAS/FES	<1.4	180-360	LIMA and RANGEL, 1990b
Na/Mg	Dialysis/splitting	FES/AAS	<3	180	LIMA et *al.*, 1990a
Phosphate	Flow splitting	Spect.	0.94	60	LIMA and RANGEL, 1991
Phosphorus	Flow splitting/Four-line	Spect.	<2	60-120	LIMA and RANGEL, 1990a
Polyphenols (total)	Simple, two-line	Spect.	3.4	200	BUITRAGO et *al.*, 1986
	Simple, two-line	Spect.	1.77	45	PERIS-TORTAJADA et *al.*, 1989

TABLE I (next)

Flow injection analysis wine assays

Reducing Sugars	Simple, two-line	Spect.	2.44-2.88	40	MAQUIEIRA et *al.*, 1987
	Simple, two-line	Spect.	1.50-1.61	50	MAQUIEIRA et *al.*, 1987
	Reversed FIA	Spect.	2.78-3.13	40	MAQUIEIRA et *al.*, 1987
	Dialysis	Spect.	1.72	40	PERIS-TORTAJADA et *al.*, 1991
Sucrose	Simple, two-line	Enz/Spect.	2	___	TZOUWARA and CROUCH, 1990
Sulphur dioxide	Stopped-flow	Spect.	___	105	RUZICKA and HANSEN, 1980
	Gas diffusion	Spect.	1	90	TECATOR, 1983a
	Gas diffusion	Spect.	1	90	MOLLER and WINTER, 1985
	Gas diffusion	Spect.	1-2	60	SULLIVAN et *al.*, 1986
	Gas diffusion	Spect.	___	___	SULLIVAN et *al.*, 1990a
	Gas diffusion	Spect.	5	___	SULLIVAN et *al.*, 1990b
	Simple, three-line	Spect.	1.5	250-300	HERNANDEZ-MENDEZ et *al.*, 1987
	Stopped-flow	Spect.	<3.1	35-55	LÁZARO et *al.*, 1987
	Gas diffusion	Spect.	0.4	20-50	BARTROLI et *al.*, 1991
	Gas diffusion	Spect.	<7	25	LINARES et *al.*, 1989
	Gas diffusion	Fluorim.	<2.0	___	HUANG et *al.*, 1992
	Gas diffusion	Amperometric	1.7-2.3	120	GRANADOS et *al.*, 1986
Tartaric acid	Simple, two-line	Spect.	0.79	50	LÁZARO et *al.*, 1986a
Volatile acidity	Gas diffusion	Spect.	___	60	BARROS and TUBINO, 1992
	Gas diffusion	Conduct.	___	80	BARROS and TUBINO, 1992

HERNANDEZ-MENDEZ et *al.* (1987) developed a FIA manifold in which they used an iodometric method for SO_2 determination. The previously diluted samples of white and rose wines are inserted in a carrier stream (water), suffer the addition of the reagent (I_3-) at a confluence point and flow towards the spectrophotometer. The reagent is formed inside the system in a previous confluence where a mixture of a iodate anion solution with a iodide solution in acidic medium occurs. This process is applicable in a concentration range between 0.2 and 15 mgL^{-1}, presenting a detection limit of 0.1 mgL^{-1}.

The simultaneous determination of free and total SO_2 without any previous treatment of the samples was described by LÁZARO et *al.*(1987) using an assembly named of doubly stopped-flow. The analytical reaction involves p-rosaniline and formaldehyde, as already described before, and a detection performed at 578 nm. The manifold includes a dual injection valve that inserts the sample into two different channels and equipment (microprocessor and respective interface, and a home made timer) which makes possible to control the start and stop of the peristaltic pumps, as well as the acquisition and treatment of the analytical signals. The two sample plugs injected simultaneously follow two different ways. The one devoted to the determination of free SO_2 (S_1) is inserted in water (the carrier), merges the channel where the reagent flows, then follows to the detector, being the first one to reach it. The plug devoted to the determination of total SO_2 (S_2) is injected into a sodium hydroxide solution to allow the hydrolysis of bound SO_2 and merges the reagent channel before the confluence point with the water carrier channel (the distance between the two confluence points is 4 cm). When plug S_1 reaches the detector, the flow is stopped, and the determination of free SO_2 is made by the quantification of the analytical signal increment during the halting of the flow. The peristaltic pumps are then reactivated until plug S_2 reaches the detector (in the meanwhile S_1 flows to waste), and the flow is stopped again. The increment of the analytical signal during this second stop allows the determination of total SO_2. With this system it is possible to perform determinations in a linear range between 1.0 and 16.0 mgL^{-1}.

BARTROLI et *al.* (1991) reported two manifolds for SO_2 determination. The color-development reagent is p-aminobenzene, and once again SO_2 is isolated by gas diffusion. Both manifolds are very similar, but the one devoted to the determination of total SO_2 presents a confluence point (prior to the injection valve) in which the sample is mixed with NaOH in a well-stirred mixing chamber.

The determination of total carbonate in beverages, like sparkling wines, was also studied by the TECATOR firm (1983b). In the developed manifold, which includes a gas-diffusion unit, 30 µL of sample are injected in a water carrier stream. It is then mixed with sulphuric acid to convert carbonates into CO_2, and this gas diffuses through the teflon membrane to the acceptor stream. The color change of the acceptor stream is measured and related with the sample concentration. The system is applicable to samples containing CO_2 in concentrations between 1 and 9 gL^{-1}.

The enzymatic determination of ethanol using FIA was described by WORSFOLD et *al.* (1981) with a system based on the catalytic oxidation of alcohol by alcohol dehydrogenase in the presence of coenzyme NAD^+, and the reduced form of the coenzyme (NADH) deter-

mined spectrophotometrically at 340 nm. The previously diluted sample is inserted in a buffer solution carrier stream and then mixed with the enzymatic reagent in a confluence point.

LÁZARO et *al.* (1986b) developed two systems for individual and simultaneous enzymatic determination of ethanol and acetaldehyde. The determinations are based on the oxidation of both compounds by NAD^+ in the presence of alcohol dehydrogenase for ethanol and aldehyde dehydrogenase for acetaldehyde. The NADH formed is monitored spectrophotometrically at 340 nm in both cases. The dual-channel system for uniparametric determinations makes use of a merging zones mode, in which the carrier is a buffer solution, and the sample and the enzymatic solution are injected in a synchronized way. In the ethanol determination, 30 μL of sample and 60 μL of enzyme are spent and 60 μL of sample and 90 μL of enzyme for acetaldehyde determination. The samples are previously diluted in an extension conditioned by the interval of applicability of the method (0.002-0.016 p. cent v/v of ethanol ; 1.0-8.0 μgL^{-1} of acetaldehyde). Concerning the ethanol determination, the authors still tested the dilution of the samples inside the manifold using asymmetric injections. This procedure permitted a linear calibration range and a precision comparable to the manual dilution procedure; however, they found a decrease in accuracy. They also tested the use of a "photometric dilution" thus achieving a decrease in the extent of manual dilution but not yet avoiding it. In the simultaneous determination of both components, they used a reversed FIA mode in which the carrier is the diluted wine sample, with the enzymes being injected simultaneously by two valves in series, originating two analytical zones reaching the detector in different times. They used a diode-array detector to make compatible the different dilutions needed for each determination, measuring both parameters at different wavelengths.

The use of a reactor with immobilized enzymes (hexokinase and glucose-6-phosphate dehydrogenase) was developed by RUZ et *al.* (1988) for glucose determination, by measuring the formed NADP. The direct connection between the two valves allow the insertion of three plugs of different solutions in the carrier stream. The procedure aimed, in this case, to avoid the use of the stopped-flow mode considering the significant and variable contributions of the matrix to the analytical sign*al.* Two manifolds are described, differing mainly in the position of the enzymatic reactors. In one of the configurations two sample plugs are introduced, and only one of them passes through the enzymatic reactor placed at the loop of one valve. Thus, two analytical signals are observed, one provided by the blank (sample plug that does not pass through the reactor), and the second provided by the blank plus the species to be measured (sample plug that flows along the reactor). In the other manifold the reactor is positioned after the injection system. Both sample plugs pass through it, being separated from each other by a certain volume of solution containing the coenzyme, placed at the loop of one of the valves. Under these conditions, there is a transient signal with three maximum points, two of them corresponding to the blank, and the central peak corresponding to blank plus species to be measured. The configuration with the reactor positioned on the valve allows the determination of glucose from 5 to 60 $\mu g\ mL^{-1}$. However, this system provides anomalous results when used with some red or sweet wines due to some colour compounds which are retained in the reactor. This problem does not occur in the configuration where the reactor is placed immediately before the detector, as both sample plugs flow through the reactor. This system is applicable to samples with a glucose concentration range between 10 and 75 $\mu g\ mL^{-1}$.

The individual determination of glucose, sucrose and maltose in food samples in a FIA system using a combination of immobilized and soluble enzymes was studied by TZOUWARA-KARAYANNI and CROUCH (1990). The samples are injected and pass through a SBSR containing the immobilized glucose oxidase enzyme. A reagent is added at a confluence point in order to convert the produced hydrogen peroxide into a red colour species. In order to determine glucose, the samples are previously diluted with a phosphate buffer, in the determination of sucrose with phosphate buffer and the invertase enzyme (to convert sucrose into glucose), and in the determination of maltose with a phosphate buffer and maltase (to convert maltose into glucose).

The determination of tartaric acid based on the Rebelein method was also studied by LÁZARO et *al.* (1986a) using a dual-channel manifold. Acetic acid flows as the carrier stream and is mixed with a reagent responsible for the production of the measured complex, sodium hydroxide (to form the buffer acetic acid/acetate anion in situ) and tartaric acid (in a low concentration), in a confluence point. The injected volume is 200 µL (wine samples previously diluted in acetic acid) providing measurements in a concentration range between 0.02 and 0.4 g L^{-1}.

The same research group described three systems for the determination of reducing sugars (MAQUIEIRA et *al.*, 1987) based on different classical reactions (picric acid method, Cu(II)-neocuproine method and Nelson-Somogyi method). The first one presents a dual-channel configuration in which the carrier stream is the reagent (picric acid), with the pH being adjusted with NaOH at a confluence point. 230 µL of previously diluted and filtered sample are injected. The reactor is thermostated, presenting the methodology two intervals of concentration where it is applicable, depending on the temperature (1.00-2.75 g L^{-1} at 80 °C and 0.3-0.5 g L^{-1} at 95 °C). The same configuration was used for the Cu(II)-neocuproine method, where 45.3 µL of sample were inserted in the reagent, after dilution and filtration through charco*al.* Again, in this case, two intervals of concentration are reported (1.0-7.0 g L^{-1} at 50 °C and 0.05-1.0 g L^{-1} at 75°C). Finally, a reversed FIA systems based on Nelson-Somogyi method is presented, in which the sample flows continuously and then is mixed with NaOH at a confluence. Then, the Fehling reagent is injected and the Nelson Somogyi reagent is added in another confluence. This system is applicable in samples with contents of reducing sugars between 3.0 and 6.0 g L^{-1} at 65 °C and between 1.0 and 2.8 gL^{-1} at 71 °C. This last method has the advantage of not needing sample decoloration.

PERIS-TORTAJADA et *al.* (1992) described a system based on the Cu(II)-neocuproine method, but in this case the decoloration with charcoal is unnecessary due to the incorporation of a dialysis unit.

The application of the flow injection technique to the determination of the total polyphenolic content of white wines was done by BUITRAGO et *al.* (1986) by resorting to the use of Jerumanis' method. They used a double-channel assembly, being the sample injected in the ferric reagent and later mixed with ammonia.

The same determination was described by PERIS-TORTAJADA et *al.* (1989), but via another colorimetric reaction, using the Folin-Ciocalteu reagent. The previously diluted

(1:5) sample (19.7 μL) is injected in a sodium carbonate solution and then mixed with the Folin-Ciocalteu reagent.

LÁZARO et *al.* (1989) proposed a system based on the Fe(III)-thiocyanate complex for the determination of iron. The innovation of this method lies in the fact that the complex is kept temporarily in an ion-exchange resin immobilized in the flow cell. A high sample volume (2 mL) is introduced into the eluent (that acts as the carrier) and then mixing with the thiocyanate occurs in a confluence. When the sample flows through the cell the complex is retained in the resin and the absorbance is registered, being maximum at the tail of the sample plug. After the sample has passed the detector, the eluent containing fluoride takes the complex from the resin and the signal returns to the baseline. However, it is not possible to apply this method to red wines since they contain species that inactivate the resins.

LIMA and RANGEL (1990a) proposed a system with two different methodologies for the determination of phosphorus, (total and in the inorganic form) based on the molybdenum blue complex reaction. In one of these approaches, it was possible to measure the quantity of total phosphorus, by introducing into the system previously incinerated samples. In the other approach, the determination of phosphate was performed without any prior treatment of the wine samples. The system shows a high dispersion coefficient (it has a flow splitting incorporated) in order to adjust the sample composition to the detection system characteristics and to minimize the intrinsic absorption of wines (especially in red wines). This system provides a linear response between 9 and 1000 mg L^{-1}, without the need of the sample previous treatment.

For the determination of lactic acid, KOTORMAN et *al.* (1991) developed a system employing an immobilized lactate dehydrogenase enzyme reactor, and using glycine buffer (pH=9.5) and hydrazine as the carrier solution. A linear calibration curve could be obtained up to 2.5 mM in lactic acid, which implies the need for dilution of the wine samples prior to their injection into the system.

GARCÍA DE MARÍA et *al.*, (1991) proposed a system based on the catalytic activity of malate dehydrogenase for the determination of free L(-)malic acid. The samples were initially subjected to alkaline hydrolysis while heating under a reflux condenser and later cooled down to room temperature and neutralized with sulphuric acid, prior to dilution with a buffer solution. All the determinations were done in a two-channel manifold using a stopped-flow mode and it was possible to detect concentrations of L(-) malic acid larger than 0.1 μg mL^{-1}.The calibration curve was linear between 0.5 and 41 μg mL^{-1}. With the aim of achieving an additional economy of reagent solution, a procedure using both merging-zones and stopped-flow techniques was tested. Identical volumes (125 μL) of samples and reagent solutions were injected simultaneously into carriers of buffer solution. Under these conditions, L(-)malic acid concentrations larger than 0.3 μg mL^{-1} could be detected and calibration was linear between 0.5 and 80 μg mL^{-1}.

LIMA and RANGEL (1992) described a flow injection system, which incorporates a dialysis unit, for the enzymatic determination of L(-)malic and L(+)lactic acids. The wine samples were introduced into the system without any prior treatment and a buffer solution was added

in a confluence before the dialysis unit. The quantity of each organic acid that diffuses to the acceptor stream reacts with the enzymatic solution that flows in the acceptor channel (L(-)malate dehydrogenase or L(+)lactate dehydrogenase) which links the dialysis system to the spectrophotometer. A blank measurement was performed (buffer solution in the acceptor stream) when the wines produced an intrinsic signal even after the larger dilution undergone in the dialysis process. This system provides a linear response for both acids in the range of concentrations between 0.02 and 4 gL^{-1}.

BARROS and TUBINO (1992) developed a method for the determination of volatile acidity. De-gassed wine (240 µL) was injected in the de-ionized water carrier stream. In the diffusion cell, acetic acid and other volatile components of the wine diffuse into a stream of Bromocresol Purple solution. Deviations from linearity, as a consequence of the limitation of Beer's law, were observed only above an acetic acid concentration of 0.12 p. cent w/v.

II — POTENTIOMETRY

The use of potentiometry as the detection method in FIA systems presents many potentialities, namely the possibility of making determinations over a wide range of concentrations with a constant sensitivity. Additionally the signal is not affected by colour or turbidity and complicated pre-treatment of the samples is not required. However, its use in FIA determinations has been scarce essentially because some mechanical difficulties arise from the incorporation of potentiometric detectors with a conventional configuration in FIA systems. Development of tubular configurations for these detectors could minimize these problems by keeping constant the characteristics of the flow and enabling sequential determinations.

LIMA and RANGEL (1989) developed a system to determine chloride in wines by potentiometric titration with silver (I) cation in a FIA system using a tubular electrode of silver/silver sulfide. The samples containing the chloride anion (the titrand in the sense of classic volumetry) were intercalated in the carrier stream containing the silver cation (the titrant). The concentration profile created in a well-stirred mixing chamber and generated by the decreasing silver cation concentration in the carrier stream, was monitored by the tubular electrode. The developed methodology is adequate for the determination of chloride in samples without any pre-treatment, with chloride contents between 5 and 600 mg L^{-1}.

OHURA et *al.* (1990) described a system for the determination of ethanol in alcoholic beverages with potentiometric detection. The determination is based on the oxidation by dichromate of the alcohol that is released by heating and which diffuses through an hydrophobic membrane, and subsequent reduction of the excess of dichromate with ferrous ion. The concentration of ferric ion formed is potentiometrically monitored with a platinum inert electrode and indirectly related to the alcohol content of the sample.

ILCHEVA et *al.* (1990) described a dual-channel flow manifold for the potentiometric determination of potassium and calcium in wines. The samples are injected in a water carrier stream, merged with a ionic strength/pH adjustment buffer in a confluence and subsequently flow to the detector cell. This cell comprises a calcium and a potassium selective electrodes in a serial arrangement and a reference electrode.

III — SPECTROPHOTOMETRY/POTENTIOMETRY

The use of two detectors in sequence (potentiometric and spectrophotometric) and of a gas-diffusion unit allowed LINARES et *al.* (1989) the construction of a system for the simultaneous determination of sulphur dioxide and carbon dioxide in wines. The previously diluted sample (150 µL) was injected in a sulphuric acid solution, and the volatile compounds (CO_2 + SO_2) diffuse to the acceptor stream that flows through the potentiometric cell (that includes a pH electrode) whose potential depends on these two constituents. Then the stream merges a p-rosaniline-formaldehyde solution in order to determine SO_2.

IV — ATOMIC ABSORPTION AND FLAME EMISSION SPECTROMETRY

LIMA and RANGEL (1990b) developed three flow-injection manifolds for the determination of several metallic cations using atomic absorption spectrometry (AAS) and flame emission spectrometry (FES). These assemblies, with different dispersion coefficients, allow the adequation of the characteristics of the samples to the requisites of the measuring system, and the eventual addition of reagents to eliminate chemical interferences. In order to attain the necessary analytical signal decrease for performing measurements in a linear response range, the authors used systems with a stream splitting and, additionally, a high flow rate was imposed at the entry of the nebulizer. This last aspect is important to minimize the influence of the physical characteristics of the matrices. With these systems, it was possible to make determinations in a linear response range without any sample pre-treatment in the concentration intervals of 1.4 to 200 mg L^{-1} for calcium, 0.9 to 200 mg L^{-1} for magnesium, 0.09 to 20.0 mg L^{-1} for iron, 0.03 to 2.0 mg L^{-1} for zinc, 0.1 to 6.0 mg L^{-1} for manganese, 0.04 to 4 mg L^{-1} for copper, 3 to 200 mg L^{-1} for sodium and 23 to 2000 mg L^{-1} for potassium.

The possibility of using an overpressure at the entrance of the atomic absorption nebulizer in order to minimize matrix physical interferences was demonstrated by LIMA and RANGEL (1991) by presenting the results obtained for the FIA-atomic absorption copper determination in wines. In the same paper the authors also described the usefulness of a stream splitting to obtain systems with high dispersion coefficients.

LIMA et *al.* (1990a) presented a system incorporating a dialysis unit that permits to partition the sample between two carriers, each one flowing towards a different detector, enabling the simultaneous determination of sodium (flame emission spectrometry) and magnesium (atomic absorption spectrometry).

LIMA et *al.* (1991a) also described the use of a dialysis unit for reaching large in-line dilutions in order to adjust the concentrations of calcium, magnesium, sodium and potassium present in wines, to the linear working range of the atomic absorption spectrometer and to the flame emission spectrometer.

The same authors (LIMA et *al.*, 1990b) explored the possibility of using a dialysis system to divide the sample among the donor and acceptor streams allowing the simultaneous determination of the following pairs of cations (Ca/K, Mg/Na and K/Na) using an atomic absorp-

tion spectrometer and a flame emission spectrometer in the two first cases and two flame emission spectrometers in the last one.

V — FLUORIMETRY

The use of fluorimetry as the detection mode in FIA pose no major problems. The possibility of coupling commercially available flow-cells to FIA assemblies may encourage further use of this detection process.

DREMEL et *al.* (1989) developed a system for the fluorimetric determination of glucose in wine and fruit juice based on a fiber-optic glucose biosensor with immobilized glucose oxidase (stable for more than 400 hours in continuous use). The sample (300 μL) was introduced into a first carrier stream and dispersed with the aid of a well stirred mixing chamber, being conducted to the loop of another injection valve. In order to further dilute the sample, they used a zone sampling technique that consists in the removal of 100 μL of this dispersed zone and subsequent introduction into a second carrier stream. The time span between the two injections (adjustable by a computer) conditions the dilution degree obtained. This system allows determinations in a linear range between 0.1 and 500 mM of glucose.

CHEMNITIUS and SCHMID (1989) developed a FIA system for the determination of L(-)malate in wines and fruit juices using an enzymatic method in which the malate dehydrogenase and asparte aminotransferase enzymes were co-immobilized in controlled pore glass. The sample and the NAD^+ coenzyme are simultaneously injected in two distinct channels (merging zones mode) where a phosphate buffer flows (pH = 7.0). After their mixing in a confluence, the sample flows through the enzymatic reactor to the fluorimetric detector where the measurement of the formed NADH takes place. As wines and fruit juices show self-fluorescence, the samples pass alternatively through the enzymatic reactor or a reference reactor, and the concentration is determined by the signals difference. This system has a linear response for concentrations between 5 and 100 μM, increased to 50 mM through the use of zone sampling. The authors refer a decrease in the activity of the bioenzyme reactor of less than 4 p. cent in three weeks, during which more than 1200 injections were done.

PUCHADES et *al.* (1991a) proposed a manifold for the simultaneous determination of L(-)malic acid and L(+)lactic acid in wines using enzyme-immobilized open tubular reactors coupled with fluorimetric detection. The samples were dialyzed on-line before its introduction in the FIA system. A T-connector was used after the sample injection point for dividing the sample plug into two portions. One of these passes through a blank reactor (without enzymes); the other one is again divided in two new portions, one of which flows through a reactor containing immobilized L(-)malate dehydrogenase and the other goes to another reactor with the L(+) lactate dehydrogenase enzyme. The three portions merge before the flow cell via T-connectors, reaching it at different times, and therefore three peaks are obtained: one corresponding to the blank, another for malic acid and the last one for lactic acid. The system presents a linear dependence (log-log calibration graphs) in concentrations ranging from 0.1 to 5 mM (relative standard deviation of 0.75 p. cent) and 0.05 to 3 mM (relative standard deviation of 1.2 p. cent) for malic and lactic acids, respectively.

VI — LUMINESCENCE

PUCHADES et *al.* (1991b) developed a method for the enzymatic determination of glycerol in wines using a reactor containing immobilized glycerokinase and glycerol-3'-phosphate oxidase. The sample was injected in a buffer and flows through the enzymatic reactor where production of hydrogen peroxide occurs. It was then mixed with an alkaline luminol solution containing potassium ferricyanide and the light emitted was measured in an home-made photomultiplier. With this method, linearity was obtained for concentrations ranging from 25 to 250 mg L^{-1}, for 29 µL injection volumes.

HUANG et *al.* (1992) developed the first method for sulphite determination based on the quantitative suppression of the luminol chemiluminescence by sulphite. In the free sulphite determination, the SO_2 produced from the sample reaction with sulphuric acid diffuses through a gas diffusion cell to an acceptor channel containing H_2O_2 and EDTA. Then a luminol solution is added in a confluence and the luminescence suppression is monitored in a spectrofluorimeter. For the total sulphite determination, the samples are injected in a NaOH solution and then receive the addition of sulphuric acid and are directed to the gas diffusion cell. Good linearity was observed between the suppression of light emission and sulphite concentration from 0.05 to 0.8 mM or from 10 to 200 µM.

VII — AMPEROMETRY

Amperometry is a detection method with many potentialities for wine analysis, especially because the signal is not affected by the colour of solutions.

However, only GRANADOS et *al.* (1986) developed a system for sulphur dioxide determination in wines using this type of detector. The system includes a gas-diffusion unit in order to isolate the sulphur dioxide. The donor and acceptor streams were both of sulphuric acid that flows to a wall-jet electrochemical cell. The samples were subjected to dilution prior to their injection in order to eliminate interferences in the diffusion membrane. Under 25°C the system showed two linear intervals: 0.06 - 6 mg L^{-1} and 12 - 110 mg L^{-1}, with a detection limit of 0.03 mg L^{-1}; at 50°C the linear range was from 0.04 to 5 mg L^{-1} and the detection limit was 0.02 p. cent.

VIII — ENTHALPIMETRY

Enthalpimetry has not been widely used as a detection method in FIA, possibly due to its non-selective nature.

OLIVEIRA and PASQUINI (1988) developed a flow injection enthalpimetric method for the determination of ethanol based on the heat of dilution of the samples. They used a dual-channel assembly using water as both carrier and diluent. Temperature changes were measured with a pair of twin thermistors. To avoid some interferences, the wine samples were previously destillated and then diluted with water in order that the final ethanol concentration would fall within the most precise range for measurement (around 12 p. cent).

IX — CONDUCTIMETRY

BARROS and TUBINO (1992) developed another method to determine the volatile acidity of wines, this time using a conductimetric detection. The method is based on the diffusion of acetic acid and other volatile compounds of wine through a diffusion cell into another de-ionized and de-gassed water stream. This stream flows through a conductimetric cell.

SOME NEW TRENDS IN WINE ANALYSIS

Due to the general characteristics of FIA systems already developed, mainly regarding large sampling rates and low reagent consumption, it is not surprising that most users are satisfied with already developed assemblies for determining one species. In fact, efforts to develop systems with a higher analytical capability, namely for multiparametric determinations, are still very scarce. However, it is our view that the development of systems for multiparametric analysis will become an important line of evolution of this methodology in the coming years, thus justifying special focus to be given in this paper.

The determination of more than one species present in a sample can be implemented by the use of multidetection or multidetermination. Each of these two modes can be obtained with simultaneous or sequential detection (RUZICKA and HANSEN, 1988).

In multidetection, two or more analytical signals are obtained from a single injection. These signals can result from the same detector at different times and experimental conditions (like wavelength), or from different detectors placed in series or in parallel.

The expression multidetermination corresponds to the determination of more than one species present in the sample. In a FIA system, measurements aiming multidetermination can be obtained sequentially, when the number of species to be determined is equal to the number of injections, or in a simultaneous way, when several species are determined from a single injection.

However, in automatic analysis it is hard to classify a system in a rigid way and so strictly separate these two terms. In fact, multidetermination can be achieved by multidetection although multidetection does not necessarily imply multidetermination. For example, obtaining two analytical signals corresponding to the sample and blank is a typical case of a multidetection, not a multidetermination.

Multidetection can be simply achieved by modifying different points of a FIA manifold developed for uniparametric determinations. This subject was discussed by VALCARCEL et *al.* (1989) for obtaining "multipeak recording in flow injection analysis". The peak recording could be derived from the propulsion system (interactive change of the flow direction or stopped flow), the injection system (multiple injection: series, parallel or with coupled valves), the transport and reaction system (several reactors in parallel or closed circuit reactors) or from the detector (double beam or fast response spectrophotometers). Actually, multide-

tection can be achieved by modifying one or more sectors of a FIA manifold such as the sample injection process, the transport and reaction system and the detection unit. It becomes clear that there are numerous possibilities to act over a conventional FIA manifold to convert it into a system capable of performing multiparametric determinations. In this paper, only those assemblies which seem to offer better perspectives for wine analysis were selected and focused.

I — USE OF DETECTORS IN SERIES

The intercalation of a set of detectors in series is perhaps the most obvious way for determining more than one analyte. Actually this type of assemblies were described when FIA was emerging as a new methodology of analysis. RUZICKA et *al.* (1977) proposed a simple manifold using sequential ion-selective electrodes in a cascade arrangement. However, since the solutions had to flow tangentially over the membranes surface and touch the reference electrode that was partially submerged in the waste container, a rather unstable assembly was produced. Due to these mechanical difficulties this type of arrangement has not often been used in the following years.

This problem was overcome together with the subsequent development of tubular shaped ion-selective electrodes (ALEGRET et *al.*, 1984; 1987) that enabled to rigidly place the electrodes in the manifold. Consequently, it became possible to use potentiometry in FIA manifolds devoted for routine analysis. The evolution of this type of electrode configuration made possible the accomplishment of multiparametric determinations, either using two tubular detectors placed sequentially (ALONSO et *al.*, 1985), or interposing a tubular electrode in a system with an atomic absorption spectrometer as the end-of-line detector (ALONSO et *al.*, 1986).

The use of serial detectors in wine analysis was reported by LINARES et *al.* (1989) and by ILCHEVA et *al.* (1990). The former reporting the simultaneous determination of CO_2 and SO_2 using potentiometry and spectrophotometry as detection systems and the latter for the determination of potassium and calcium using two serial indicator electrodes.

II — DETECTORS PLACED IN PARALLEL

In this case, multidetection is accomplished with several detectors (the large majority using two detectors), being the sample plug divided in a reproducible way in different plugs, each of them flowing through a channel with a detector.

One year after the first paper about FIA came out, STEWART and RUZICKA (1976) proposed a system with partition of flow, in order to obtain what they called simultaneous determinations. Although they used only one detector, they emphasized the possibility of using that FIA assembly with a double beam spectrophotometer where each beam would work as a detection system. BASSON and VAN STADEN (1981) described the first system where two detectors in parallel were really used and tested, allowing the chloride determination by photometric detection and sulphate by turbidimetry.

Another approach for sharing the sample plug between two different streams was proposed by (LIMA et *al.*, 1990a, 1990b), with the incorporation of a dyalisis unit in the flow system. In this case, the sample is not physically divided, but the species present in the plug diffuse through the membrane, impelled by concentration gradients between the two sides of the membrane. In these manifolds, the dialysis membrane was not only responsible for partition of the species between the two channels, but also for conditioning the quantities of each species that diffuses to the acceptor channel. As the efficiency of dialysis is low, it can the be used for adjusting the concentration of the analyte present on the channel that flows towards the detector (LIMA et *al.*, 1991a). The use of a dialysis unit for performing multidetection was, as far as we know, implemented for the first time in the determination of metallic cations in wine, by determining simultaneously sodium and potassium, calcium and potassium, magnesium and sodium, using a flame emission photometer and an atomic absorption spectrophotometer as detection systems.

III — SAMPLE PLUG SPLITTING, FOLLOWED BY A MERGING POINT

Most of the times, the process consists of dividing the sample plug in different channels which merge to a common stream before the detector. The different portions are then analysed in a sequential manner, since they reach the detector at different times. The number of peaks to be recorded is then equal to the number plugs obtained from the original sample bolus (STEWART and RUZICKA, 1976). The sample plug splitting process followed by a confluence was used in wine analysis by PUCHADES et *al.* (1991a) on the simultaneous determination of L(-) malic and L(+) lactic acids. The divided sample plug was forced to flow through two different reactors, each one with an immobilized enzyme, then converging via T-connectors and finally measured on the same detector at different times.

Alternatively, in order to obtain two sample plugs for independently flow through the manifold and reach the detector at different times, two injection valves that introduce two sample portions in different channels in a reproducible way can be used.

Every process described above for multiparametric determination present the advantage of using a single detection system, although it is limited to cases where the detection is performed at the same instrumental conditions. Otherwise, the detector would have to adjust itself after each measurement. Admitting that it is a spectrophotometric detection in the visible region, the coloured species should absorb at the same wavelength or, alternatively, at a wavelength that represents a compromise for every species. If these conditions are not met, it is necessary to use a detector capable of changing its wavelength rapidly, as is the case of a fast scanning or a diode array spectrophotometer.

IV — ZONE SAMPLING, USING TWO OR MORE DETECTORS

The zone sampling mode introduced by REIS et *al.* (1981) is based on taking a small zone of the sample plug to insert it into a new channel through each flows towards the detector. For implementing this FIA mode the authors used a commutator device (KRUG et *al.*, 1986). In the original paper the zone sampling approach was used to solve problems associated to samples that needed to be submitted to large dilutions inside the manifold, namely

when using Atomic Absorption Spectrometry as the detection system. The sample containing a high concentration of potassium was firstly introduced and dispersed. Then, by changing the commutator position, a small zone of the sample plug tail (where potassium concentration is lower) is transferred to another channel and then flows towards the atomic absorption spectrophotometer, reaching it with a concentration equivalent to a linear dependence range.

This reference to the zone sampling process seems to be sufficient to foresee the potentiality of this mode on the development of FIA methodologies in wine analysis, where the aforementioned problem is quite common.

The zone sampling mode may also allow the implementation of manifolds capable to perform simultaneous determinations, as it was demonstrated (ZAGATTO et *al.*, 1981) in the colorimetric determination of aluminum (534 nm) and in the atomic absorption spectrometric determination of iron species with an air/acetylene flame. It is easy then to predict that the principle the authors used for the determinations previously mentioned can be applied to a large number of pairs of species.

V — USING AN 8 PORT VALVE

As it was previously underlined, multidetection is not necessarily obtained by the use of different manifolds configurations, since it can be performed by changing the traditional way of sample insertion (6 port valves).

The huge possibilities that can be achieved by the adequate choice of the sample insertion process was referred by ERICKSON et *al.* (1987), by discussing the capacity of sample introduction with a home-made 12 port valve, with two 6 port blocks rotating in opposite directions.

Still in 1987 a new proposal with similar applicability, based on the use of the commercial 8 port, 4 ways valve has emerged. This valve allows the sample insertion between two solutions of different composition (sandwich technique), thus permitting to explore the analytical capacity of the two different interfaces (ALONSO et *al.*, 1987). It is important to stress that the use of this process of sample intercalation also presents other potentialities as is the case of the detection system systematic calibration in each injection (LIMA et *al.*, 1991b).

The first evaluation of the sandwich technique applicability for multiparametric determinations was described with the Cr (VI) and total iron sequential determination in natural and residual waters (ARAÚJO et *al.*, 1989). The possibility of using this technique for the determination of many other pairs of components, using a simple colorimetric detector, is now straightforward. However, this process is limited due to the need to select the pairs of species and corresponding colour-development processes, since the detection is performed at a certain fixed wavelength which obviously will have to be adequate for both species under analysis. This work made clear two important aspects when developing systems for sequential determinations using the sandwich technique: the selection of the sample volume to be inserted in the system is even more critical than in other FIA methodologies, and it can be complex to make compatible two reactions with very different kinetics. The rigorous selection

of the sample volume to insert in the system is essential to obtain convenient separation of the analytical signals and not to compromise the sampling rate. When colour-development reactions of very different kinetics are involved, the system optimization is complex because the time needed for a convenient colour-development in an interface may impose an excessive dilution on the other interface and consequently produce a decrease in the sensitivity of the corresponding determination. This justifies the mathematical modeling of the processes occurring inside the manifold in order to obtain optimization programs to follow and allow the development of manifolds with the best possible characteristics (MONTESINOS et. *al.*,1990;1991).

The possibilities of application of this methodology to wine analysis are wide, namely for the implementation of multiparametric systems based on different enzymatic reactions due to their specificity and also the fact that only one colored species is formed, allowing the detection at one fixed wavelength. Additionally, the 8 port valve can be used on the development of uniparametric manifolds capable of being operated with large economy of enzymatic reagents (ARAÚJO et. *al.* 1991a).

VI — USING A 14 PORT VALVE

In general, the major difficulty associated to the use of an 8 port valve is the reduced control over the amount of reagent in contact with both sample plug interfaces. The reagent responsible for the colour-development on the first interface act as carrier solution (thus being consumed in a larger extent) while the reagent volume in contact with the sample plug tail is controlled by the commutation time between the two valve positions.

The use of an 14 port valve allows the development of manifolds with similar characteristics to those obtained with an 8 port valve and, additionally, a better control over the reagents volumes involved in each interface is accomplished. This possibility is due to the use of three loops. On the valve filling position, the carrier flows directly towards the detector, and the three loops can be filled, for instance, with reagents in the extreme loops and sample in the middle one. On the injection position, the reagents and sample plugs are inserted and directed by the carrier towards the detection system. This way, the reagents and sample consumption is only conditioned by the volume needed to fill the loops. This process was used on the sequential determination of chromium (VI) and total iron (ARAÚJO et *al.*, 1991b), and on iron (II) and iron (III) determination (ARAÚJO et *al.*, 1991c). The results obtained on the sequential determination of the species referred are equivalent to those obtained with the reference procedures, with a maximum sampling rate of 70 samples h^{-1} and an analysis rate of 140 determinations h^{-1}. These examples, although presented in a summarized way, immediately evidence the advantages of the 14 port valve compared with the 8 port valve when used with the same aim. However, one should point out that for the time being, current use of the 14 port valves is more difficult as it is not yet commercially available unlike the 8 port ones.

VII — USE OF A MOBILE DETECTOR IN SEVERAL POINTS OF THE MANIFOLD

The use of FIA manifolds with a detector able to "move" on the manifold and thus able

to perform measurements in different points of the system was recently proposed by ZAGATTO et *al.* (1991; 1992). The application of the proposed methodology allows a sequential or parallel localization of the detector. This process contrasts with the procedures used so far, in which the detector operates in a static way, placed in a certain fixed point of the manifold. The role usually attributed to the manifold consists on the sample preparation and conduction towards the detector. In this new process, by coupling the detector to a commutator device, there is the possibility of performing measurements at different points of the manifolds using only one detector. The commutation system that was used to "move" the detector along the different points of the manifold was the one previously used to develop several processes for samples injection (KRUG et *al.*, 1986).

This "jumping" detector technique can be applied to many analytical situations already studied. Using the detector in a parallel position, different sample zones circulating in different channels could be monitored. Using a serial localization, there is only one channel and the sample plug is quantified in different points and times, and also in different conditions.

The various possibilities of the previously described systems were evaluated by performing the sequential determination of aluminum and iron and also in the iron speciation involving serial measurements.

It should be referred that the authors present other possible applications of the jumping detectors, namely the possibility to make the blank and the sample measurement from one single injection. In wine analysis, this capacity can allow to double the sampling-rate of some already developed FIA systems (applied to red table wines and Port wines) in which the blank assay is inevitable as it is the case of the determination of L(-) malic and L(+) lactic acids (LIMA et RANGEL 1992).

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