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Application of sequential injection analysis (SIA) to food analysis

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Abstract

For effective control of food quality, a laboratory should be able to analyse a large number of samples in an accurate, reproducible and quick way. As sequential injection analysis (SIA) is becoming an important tool for the automation of chemical procedures, this paper presents an overview of the applications of this emergent methodology to food analysis. SIA systems developed to date offer good characteristics for routine use and the analytical methods proposed show adequate precision and accuracy. In addition, the results obtained are in good agreement with those furnished by the application of the reference methods.

Keywords: Sequential injection analysis; On-line analysis; Food analysis

Contents

1. Introduction

In the seventies, flow injection analysis (FIA) appeared as a new concept that had a strong impact among automatic chemical analysis methods. This continuous flow analysis technique was initiated almost simultaneously by Ruzicka and Hansen (1975) in Denmark and by Stewart, Beecher, and Hare (1976) in USA. Taking into account its advantageous characteristics, subsequent adhesion to this technique was greater. In effect, in a review work Ruzicka and Hansen (2000) state that the application of FIA to the analysis of the

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most diverse matrices (waters, food, soils, drugs, biological fluids, industrial products, etc.), has generated about 10 000 articles, 10 books, monographs and reviews as well as over 100 doctorate theses (Ph.D). Currently, there is a research journal (Journal of Flow Injection Analysis), a congress (International Conference on Flow Injection Analysis) and a cycle of conferences (Flow Analysis) dedicated to this technique.

The application of the FIA technique to food analysis has received less attention than in other areas. The cause of this situation is probably the elevated complexity of the sample matrices, which basically made it necessary to carry out sample pre- treatments that are more difficult when solid samples are considered. López-Fernández, Ríos, and Valcárcel (1995) wrote an important review article about the quality of the analytical procedures, based on the FIA methodology, applied to food analysis. In spite of the aforementioned, it should be recognized that the majority of the work carried out is associated with research laboratories and that the technique has not been strongly implemented as a routine method in food control laboratories.

In the nineties, Ruzicka and Marshall (1990) proposed an important methodological innovation in the area of continuous flow analysis methods that maintained the advantages associated with flow injection analysis but reduced the inconveniences that hindered its utilisation as a routine tool. This new methodology was termed sequential injection analysis (SIA). Although the aspiration and propulsion systems in SIA are different to those used in FIA, the fundamentals and basic principles are to a large extent similar –that is, the sequential injection of well defined segments of samples and reagents, which disperse and penetrate, mutually allowing the attainment of a reproducible overlapping zone (Fig. 1). Consequently, the reaction products are formed in well-defined areas of concentration gradient and the transitory signals generated provide reproducible analytical results (Ruzicka, 1992).

Fig. 2 outlines a basic system founded on the SIA concept. It is formed by a propulsion device (peristaltic pump, automatic syringe or piston pump), a multi-position selection valve with various entry points (ports) to select fluids (substituting the injection valve in FIA), which operates in synchronisation with the pump, and a detector (any of the instrumental techniques utilized in FIA with that purpose). In the first manifold developed by Ruzicka and Marshall (1990) the detector was placed between the propulsion device and the selection valve, but nowadays in the most part, the SIA systems reported have the detector located in a port of the selection valve. With this configuration, the reaction product passes under positive pressure through the detector, avoiding bubble formation (Marshall & van Staden, 1992). Logically, the whole system is interconnected typically via Teflon tubing system, the holding and the reaction coils being highlighted. The complete system functions automatically and is controlled by computer.

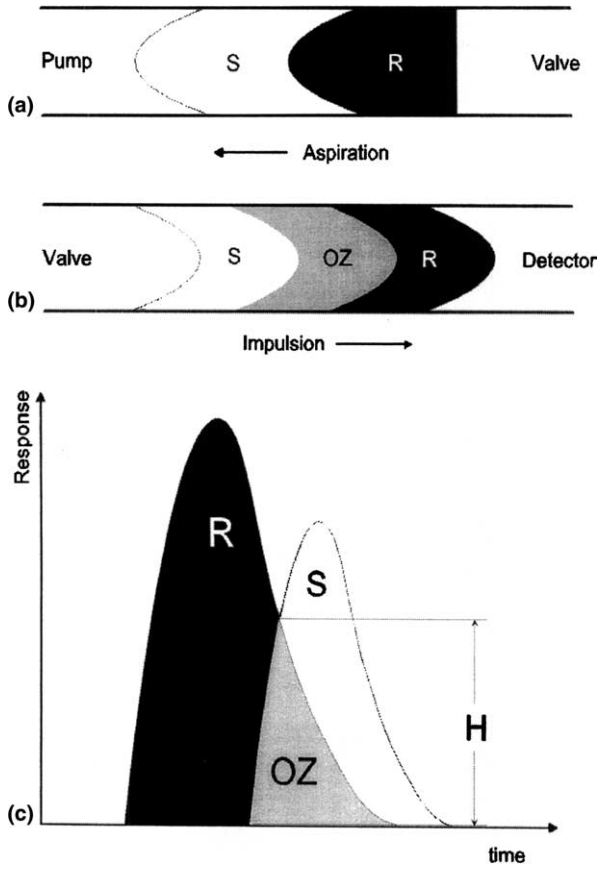

Fig. 1. Representation of reagent and sample zones: (a) stacked in the holding coil, (b) in the reaction coil after flow reversal, (c) concentration profiles as seen by the detector: R, reagent zone; S, sample zone; OZ, overlapping zone; H, peak height.

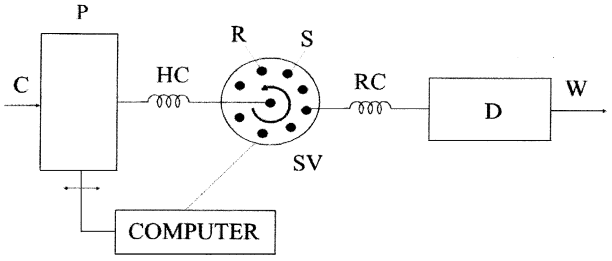

Fig. 2. Basic scheme of a SIA manifold: C, carrier; P, pump; HC, holding coil; SV, selection valve; R, reagent; S, sample; RC, reaction coil; D, detector; W, waste.

Initially, the SIA system promotes the aspiration of the carrier/washing solution to the holding coil, thus implying that the propulsion device undertakes this work in reverse form. Following this, a reduced volume of sample is aspirated in an exact and reproducible way through the appropriate positioning of the selection valve, that through one of its ports makes access to the holding coil viable. At this moment the propulsion

device is stopped to avoid an increase in pressure (Christian, 1994). Subsequently, through the rotation of the valve position, a small volume of the reagent solution is also aspirated and sent in the same direction. Finally, by rotating the valve position to the detector port, the direction of the flow changes and the segments of the different solutions that are in the holding coil are directed to the detector through the reaction coil.

Therefore, a complete analytical cycle, excluding the washing steps, can be simply made up of three steps. However, the cycle can be composed of a greater number of steps depending on the aspiration/propulsion device chosen and the type of operations to be carried out in order to obtain a detectable species. For this reason, the time required to carry out an analysis is the sum of the reaction period, the measurement period and the aspiration time of the different solutions. So, the sampling rate of SIA systems is lower than that of FIA systems for the same type of determination.

Due to dispersion, as much axial as radial, the sample and reagent zones that were aspirated sequentially and in close synchrony are intimately mixed. This generates a complex but reproducible concentration gradient, where a superimposed zone is created in which the sample was transformed into a detectable species and the generated transitory signal registered. Recently, Zagatto, Rocha, Martelli, and Reis (2001) have suggested that, even after the flow reversal, only a partial overlap of analyte and reagent zone is achieved. This feature can be a source of inaccuracy when the sample is polluted by the presence of interferents, that also consume reagent in the overlapping zone. An optimization between the sample and the reagent volumes permits large linear working intervals (Christian, 1992).

It has been pointed out that the penetration zone (related to dispersion) is the key parameter to design a SIA system and some authors have published several articles describing the most important parameters to be optimized (Gubeli, Christian, & Ruzicka, 1991; Ruzicka & Gubeli, 1991; Taljaard & van Staden, 1998; Zable, 1996). Almost without exception the following demonstrated to have a marked influence on the dispersion zone in a SIA manifold: The volumetric flow rate, tube diameter, length of the tubing, sample and reagent volumes, order of sample and reagent injection, flow reversal and geometry of the coils or reactors used.

The use of a selection valve with a greater number of ports allows the SIA systems to undertake more complex determinations without substantially increasing the overall complexity of the manifolds. In effect, in several situations it is necessary to aspirate the sample between two or more reagents that are indispensable for generating the chemical species capable of being detected. In this case, it is only necessary to connect auxiliary channels for the access of solutions to other ports of the valve. It is equally possible to carry out multiparametric determinations if several detectors are additionally coupled to the valve.

In SIA, in a similar way to FIA, the systems can be more complex, especially with a view to carry out operations such as the dilution of concentrated samples, the automatic calibration of the system, the utilisation of the standard addition method as analytical measurement technique, or carrying out on-line titrations. In these systems it is also possible to subject the samples to special treatments in the manifolds, which imply, for example, the use of gas-liquid separation cells, gas-diffusion and dialysis units, digestion minipumps made of Teflon in microwave ovens and minicolumns packed with ion exchange resins polymers or metal reductants (Saraiva, 2000; Segundo, 2002).

This new flow technique needs computer control to impose the performance of, the instruction sequence in well defined periods of time in order to achieve a reproducible process, and acquire and treat the data obtained. Guzmán and Compton (1993) have pointed out that the lack of availability of adequate programs to manage SIA systems has been one of the major difficulties that have retarded their initial development. Several authors developed their own programs with software written in different working environments (Turbo C++, Basic, Visual Basic and Lab VIEW). Ruzicka's group use a program called FIAlab (Baxter, Christian, & Ruzicka, 1994), the flowTEK package was design by Marshall and co-workers (Marshall, 1994), Cerdá's group initially developed the program DARRAY (Cladera, Tomás, Gómez, Estela, & Cerdá, 1995) and more recently the program AUTOANALYSIS (Becerra, Cladera, & Cerdá, 1999). Kullberg, Vilen, Sund, Talaslahti, and Sara (1999) wrote the program AnalySIA, Lenehan, Barnett, and Lewis (2002a) and Fletcher and van Staden (2003) have developed a software written within the National Instrument Lab VIEW graphical programming environment to fully automate SIA instrumentation and data acquisition. This fact explains why almost half of the approximately 300 articles published since the introduction of this recent technique have appeared in the past five years.

Recently, the need to design purpose-built software has been lessened as commercially available software has become more widely available in combination with instrumentation (Lenehan, Barnett, & Lewis, 2002b). Although the majority of the SIA systems have been developed by using modular components, at present it is possible to find some commercial analysers; for instance: Global FIA (www.globalFIA.com), FIAlab Instrument (www.flowinjection.com), formerly Alitea Instrument, and the analyser developed in the Turku

Centre for Biotechnology in Finland, commercialized by Arctic Instruments.

2. Applications to food analysis

Although only a decade has passed since the first application of this methodology to the analysis of foods, more than 30 papers have been published in this area. These are commented on below and the reader is referred to Tables 1 and 2, which provide a summary of the results obtained, the inorganic or organic nature of the compounds determined and the type of detection used.

The initial applications of the SIA methodology to food analysis were developed using simple matrices that allowed analytical determinations to be carried out in a direct way, without the need to accomplish any elaborate on-line treatment to the sample. In the cases, where direct determination was impossible, the samples were the target of a chemical pre-treatment before being introduced in the manifold. In this context, Van Staden and McCormack (1998) developed a SIA system that permitted the determination of the total edible amino acid content in liquidized soya bean samples. The chemical method underlying the determination was based on the derivatisation reaction involving the free ε-amino acid group and the 2,4,6-trinitrobenzenesulfonic acid (TNBS) which resulted in the formation of a yellow coloured compound detectable spectrophotometrically at 350 nm. Results obtained by applying this procedure to real samples were on average 10% lower than those furnished by the application of the manual method proposed by Carpenter (1960). The sampling rate of the SIA system proposed was clearly lower than that achieved by Sodek, Ruzicka, and Stewart (1978) for the same end purpose but with a manifold based on the FIA concept. However, it showed itself to be significantly more economical regarding the notably reduced consumption of the expensive TNBS reagent.

A sequential injection analysis method has been developed by Fletcher and van Staden (2003) for the determination of ethanol in spirit samples. The procedure was based on spectrophotometric monitoring (λ = 600 nm) of the Cr (III) formed when ethanol reacts with potassium dichromate in sulphuric acid media. Samples of vodka, gin and whisky were analysed using the SIA system after a 10% dilution and the results obtained showed a relative error less than 2% from the stated values by the manufacturers for all the samples analysed.

The determination of primary amino acids and ammonium, collectively referred to as yeast assimilable nitrogenous compounds (YANC) in grape juice and fermenting must has been carried out by Muik, Edelmann, Lendl, and Ayora-Cañada (2002) by sequential injection analysis with spectrophotometric detection. Isoindole-derivatives from the primary amino acid were formed by reaction with *o*-phthaldialdehyde and *N*-acetyl-L-cysteine and measured at 334 nm with respect to a baseline point at 700 nm to avoid the Schlieren effect. The method provides a fast and accurate determination of the YANC contained in grape juices of different white wines, which was demonstrated by comparison with HPLC measurements. A *t*-test showed no significant differences between both methods at a confidence level of 95%. Due to the long reaction time required for each analysis, the SIA system was configurated with four parallel reaction coils, so that four samples can be processed simultaneously.

March, Simonet, and Grases (2000) developed a SIA system to carry out the determination of phytic acid (inositol hexaphosphate) in granules or seeds of different plants such as walnuts, almonds, cocoa, coffee, etc. The analytical method was based on the reduction in the crystallisation velocity of calcium oxalate when in the presence of phytic acid. This velocity was evaluated by the increase in turbidity (observed at 550 nm) with time. Samples were analysed simultaneously by a manual spectrophotometric procedure, also proposed by March, Simonet, Grases, and Salvador (1998), based on the hydrolysis of phytic acid to phosphate and the liquid–liquid extraction of the phosphomolybdenum blue, clearly showing the attainment of concurring results. The SIA procedure proposed permitted measurements to be carried out with a sensitivity 1000 times greater than the only FIA system proposed for automating this determination, based on the interaction of phytic acid with molybdate (Kamaya, Furuki, & Nagashima, 1998).

Van Staden and van der Merwe (1998) have developed a SIA system for the determination of nitrite in cured meat based in the modified version of the Shinn method. Nitrite reacts with a primary amine to form a diazonium salt, which is then coupled to another aromatic compound to form a highly coloured azodye, the absorbance of which is measured at 540 nm. Samples were off-line pre-treated by extracting in a 1 mol/l; ammonium chloride solution (pH 9) and cooking in a microwave oven for 4 min. Finally, the samples were centrifuged for 5 min at 6000 rpm to separate the fats and the solids. When the developed method was applied on four meat samples, the results obtained were in good agreement with those furnished by a standard method not cited by the authors in their work. The SIA system proposed offers similar sampling rate and slightly lower precision when compared with a FIA system, based on the same method, previously developed by the same authors (Van Staden & Makhafola, 1996).

The multiparametric determination of glucose, fructose and sucrose in soft drinks was proposed by Schindler et al. (1998). In this work some additional potential uses of the SIA methodology were evident, in

Table 1
Determination of inorganic substances in food by using sequential injection analysis (SIA)

Analyte	Sample	Detector	Method	Linear range	Detection limit	Precision[a]	Accuracy[b]	Samples per hour	Reference
Iodide	Nutrition salts	UV–Vis $\lambda = 528$ nm	The determination was based on the catalytic effect of iodide on the oxidation of the chloropromazine by concentrated H_2O_2 under high acidity	10–100 μg/l	4.0 μg/l	<0.03	95–105[c]	24	Araújo et al. (1997b)
Iron	Table wines	UV–Vis $\lambda = 510$ nm	The sample was, in-line, digested in a microwave oven, reduced with ascorbic acid and a complex was formed by reaction with o-phenanthroline	2–14 mg/l	0.6 mg/l	2.3	98–101[c]	9	Oliveira et al. (2000)
Iron	Infant formulas	UV–Vis $\lambda = 480$ nm	Formation of the Fe(lll)/SCN^{-1} complex, previous digestion of the sample by dry ashing and treatment with HNO_3	0.5–20.0 mg/l	0.5 mg/l	<2	+0.5	100	Araújo et al. (1997a)
Iron	Soft drinks and milk	UV–Vis $\lambda = 510$ nm	The sample was, in-line, digested in a microwave oven, reduced with ascorbic acid and a complex was formed by reaction with *o*-phenanthroline	2.0–20.0 mg/l	–	<0.04	−0.98	9	Neira et al. (2000)
Calcium	Soft drinks and milk	UV–Vis $\lambda = 535$ nm	After, in-line, digestion of the sample, the Ca(ll)/*o*-cresolphtahleine complex was formed	15.0–150.0 mg/l	–	<0.04	+1.12	9	Neira et al. (2000)
Magnesium	Soft drinks and milk	UV–Vis $\lambda = 535$ nm	The same procedure was carried out but EGTA was added to protect the Mg(ll)/*o*-cresolphathleine complex by masking Ca(ll)	5.0–50.0 mg/l	–	<0.04	+0.89	9	Neira et al. (2000)
Nitrite	Cured meat	UV–Vis $\lambda = 540$ nm	Digested samples were aspirated and nitrite was coupled and diazotized with sulphanilamide and *N*-(l-naphtyl) ethylenediamine to form an azodye	0.1–50 mg/l	–	<1.7	−2.3[b]	49	Van Staden and van der Merwe (1998)
Phosphorous	Wines, fruit juices, milk	UV–Vis $\lambda = 619$ nm	The sample was, in-line, digested in a microwave oven and the molybdenum blue method was carried out by mixing the extract with ammonium heptamolybdate, ascorbic acid and NaOH	20–400 mg/l	0.6 mg/l	<3	97–107[c]	16	Oliveira et al. (1998)
Phosphorous	Milk	UV–Vis $\lambda = 710$ nm	The same spectrophotometric method that above but the in-line digestion was carried out by means of a UV-catalysed peroxodisulfate oxidation	–	2 mg/l	<2.0	−2.2	17	Lima et al. (2002)
Sulphate	Table wines	UV–Vis $\lambda = 420$ nm	Turbidimetric determination by precipitation with $BaCl_2$ in acid media. A mixing chamber was used	300–1500 mg/l	154 mg/l	2.2	+0.3	5	Silva et al. (2003)

(*continued on next page*)

Table 1 (*continued*)

Analyte	Sample	Detector	Method	Linear range	Detection limit	Precision[a]	Accuracy[b]	Samples per hour	Reference
Sulphur dioxide (free)	Table wines	UV–Vis λ = 580 nm	In a manifold with a gas diffusion unit, the sample, directly aspirated, reacted with formaldehyde and pararosaniline	2–40 mg/l	0.1 mg/l	1.2	−2.2	17	Segundo and Rangel (2001)
Sulphur dioxide (total)	Table wines	UV–Vis λ = 580 nm	The same procedure but the sample was processed after previous, in-line, hydrolysis of bound SO_2 with NaOH	25–250 mg/l	0.6mg/l	2.3	+1.1	16	Segundo and Rangel (2001)
Iron	Table and Port wines	FAAS λ = 248.3 nm	The sample was aspirated into the holding coil and directly impelled towards the nebulizator	up to 5.0 mg/l	0.03 mg/l	<3	−1.6	95	Costa et al. (2000)
Zinc	Table and Port wines	FAAS λ = 213.9nm	The sample was aspirated into the holding coil and directly impelled towards the nebulizator	up to 2.0 mg/l	0.002 mg/l	<3	−0.2	95	Costa et al. (2000)
Manganese	Table and Port wines	FAAS λ = 279.0 nm	The sample was aspirated into the holding coil and directly impelled towards the nebulizator	up to 3.0 mg/l	0.002 mg/l	<3	+2.3	95	Costa et al. (2000)
Copper (> 20 mg/l)	Table and Port wines	FAAS λ = 324.8 nm	The sample was aspirated into the holding coil and directly impelled towards the nebulizator	up to 2.0 mg/l	0.001 mg/l	<3	−1.5	75	Costa et al. (2000)
Copper (<20 mg/l)	Table and Port wines	FAAS λ = 324.8 nm	Formation of the Cu(ll)/DDTC complex and preconcentration, on a commercial cartridge packed with silica C_{18} before being impelled towards the nebulizator	up to 0.5 mg/l	0.0004 mg/l	<3	–	20	Costa et al. (2000)
Iron (III)	Table wines	FAAS λ = 248.3 nm	Formation of the Fe(lll)/ SCN^-complex and in-line liquid–liquid extraction with MIBK by using a home-made device	0.10–6.00 mg/l	0.03 mg/l	3.0	+0.1	18	Costa and Araújo (2001)
Iron (total)	Table wines	FAAS λ = 248.3 nm	The sample was aspirated into the holding coil and directly impelled towards the nebulizator	0.47–15.00 mg/l	0.14 mg/l	1.2	+0.6	18	Costa and Araújo (2001)
Mercury	Fish	CVAAS λ = 253.7 nm	The sample and $SnCl_2$ solution were impelled into a cylindrical gas–liquid separator and a N_2 flow swept the reduced Hg into the quartz cell	2–50 μg/l	0.34 μg/l	0.9	−1.6	30	Bauzá de Mirabó et al. (1997)
Mercury	Canned fish	CVAAS λ = 253.7 nm	The same procedure, above described, was carried out, but Ar instead of N_2 was used	1–20 μg/l	0 46 μg/l	0.9	–	45	Doering et al. (2000)
Arsenic	Table wines	HGAAS λ = 193.7 nm	The sample, previous microwave digestion, was aspirated and mixtured with $NaBH_4$. The AsH_3 formed was directed to the quartz cell	1.23–27.5 μ/l	0.37 μ/l	3.2	97–102[c]	23	Zárate et al. (2001)
Chloride	Milk	Conductimetry	The sample was directly aspirated and a dialysis unit was used to remove interferences and to facilitate automated dilution. A standard addition method was also used	–	0.025 g/l	<1.5	+0.6[d]	30	Silva et al. (1999)

Potassium (free)	Table and Port wines	ISE K^+	The sample was aspirated, mixtured with ISA and impelled through the tubular detector	39–3900 mg/l	0.8 mg/l	<2	98.4–106[c]	12	Zárate et al. (2003)
Potassium (total)	Table and Port wines	ISE K^+	The same procedure but the sample was previously, in-line, digested in a microwave oven	39–3900 mg/l	0.8 mg/l	<2	+0.1	12	Zárate et al. (2003)

[a] Relative SD (%).
[b] Mean relative error of the SIA method versus the reference method (%).
[c] Range of spike recovery (%).
[d] Relative error of the SIA method versus standard reference material (%).

relation to the possibility for automatic preparation of the multiple calibration solutions from individual standard solutions of each component. The utilisation of a multidimensional detector, as in the case of a FTIR spectrophotometer, made viable the processing of the spectra obtained as a function of time to achieve a multivariate calibration model based on partial least squares (PLS). The calibration model was obtained using two different procedures. When it was based on the total physical homogenization of the different standard solutions before being sent to the detector, it permitted the establishment of a linear relationship between the signal measured and the concentration up to about 50 g/l. Nevertheless, when a gradient mixture was created, the linearity reached attained 120 g/l. The precision values and the deviations relative to the results obtained by HPLC, using the differential refractometry for detection, were better with the first procedure and are reflected in Table 2. With the incorporation of a mixing chamber in the proposed SIA system, the same authors were able to determine in wines, in addition to the reducing sugars, other components such as ethanol, glycerol, and the following acids: Acetic, citric, tartaric, malic and lactic (Schindler, Vonach, Lendl, & Kellner, 1998). The mixing chamber with a capacity of about 400 μl was constructed in acrylic and could accommodate magnetic agitation. The wine samples were analysed simultaneously by HPLC with IR spectrophotometric detection and in the opinion of the authors, the results obtained were in relative agreement for the species present at concentrations greater than 3 g/l (ethanol, glycerol, glucose and fructose) or with characteristic IR spectra (lactic acid). However, there were discrepancies of results in the determinations of acetic, malic, citric and tartaric acids. The authors concluded, however, that in both studies the developed methodology is acceptable in the control of processes or for the purposes of "screening". They also state the belief in the need to improve the calibration model with the purpose of increasing the accuracy of the individual determinations, with the degree of precision being adequate.

LeThanh and Lendl (2000) have recently developed a SIA system that permits the determination of sugars and organic acids in soft drinks. To make the separation of both types of compounds viable, the manifold proposed differed from those previously described, in that it included a new solid phase extraction unit that employed convective interaction media (CIM) in the form of disks. These were initially designed for low volume stationary phases in HPLC, the active material (in the proposed application) being formed on one side by quaternary amines and by chloride ions as counter-ions. The samples were initially filtered to avoid the obstruction of the disks and diluted until they fell within the linear concentration range. After the pre-concentration of the acids in the CIM disks, they were eluted with an alkaline

Table 2
Determination of organic substances in food by using sequential injection analysis (SIA)

Analyte	Sample	Detector	Method	Linear range	Detection limit	Precision[a]	Accuracy[b]	Samples per hour	Reference
Aflatoxin B_1	Pistachio	UV–Vis $\lambda = 620$ nm	A suspension of acrylic beads with immobilized AFB_1–BSA conjugate was introduced into the jet ring cell and perfused with the mixtured sample and anti-rabbit alkaline-phosphatase conjugate and Bluephos as substrate. The development of colour was monitored	up to 20 μg/l	0.2 μg/l	–	–	6	Garden and Strachan (2001)
Aminoacids	Liquidized soya bean	UV–Vis $\lambda = 350$ nm	The determination was based on the reaction of the free ε-aminoacid group with 2,4,6-trinitrobenzenesulphonic acid	up to 10^{-2} mol/l	–	2.3	93–102[c]	15	Van Staden and McCormack (1998)
Ethanol	Distilled liquors	UV–Vis $\lambda = 600$ nm	After a 10% dilution, spirit samples were mixtured with acidified $K_2Cr_2O_7$ and the Cr(III) formed was monitored	0–6% (v/v)	0.09 (v/v)	<1	−1.1[d]	19	Fletcher and van Staden (2003)
Ethanol	Table and Port wine	UV–Vis $\lambda = 340$ nm	The determination was based on monitorization of NADH after oxidation of ethanol in the presence of NAD^+ by action of alcohol dehydrogenase immobilized in a microcolumn	0.1–0.5%	0.005%	3.4	+0.1	22	Segundo and Rangel (2002)
Glycerol	Table and Port wine	UV–Vis $\lambda = 340$ nm	The same procedure but the immobilized enzyme used was glycerol dehydrogenase	30–300 mg/l	8 mg/l	1.1	−0.2	22	Segundo and Rangel (2002)
Glucose	Milk	UV–Vis $\lambda = 510$ nm	The sample was, previously, deproteneized and after being aspired was mixtured with the glucose oxidase/peroxidase system, 4-chlorophenol and 4-aminoantipyrine. Again, the quinoneimine was monitored	up to 120 mg/l	7.0 mg/l	<2	−0.6	5	Saraiva (2000)
Glucose	Milk	UV–Vis $\lambda = 510$ nm	The same procedure but the enzymatic system was immobilized in a microcolumn	‘‘	18 mg/l	<2	+3.4	9	Saraiva (2000)
L(+)-Lactic acid	Table wines	UV–Vis $\lambda = 510$ nm	The sample was directly aspirated and propelled into a dialysis unit, afterwards was mixed with the lactateoxidase/peroxidase system, 4-chlorophenol and 4-aminoantipyrine. The reaction product, quinoneimine, was monitored	0.25–2.5 g/l	0.07 g/l	<2	+0.6	14	Araújo et al. (1997c)
D-Lactic acid	Pork meat	UV–Vis $\lambda = 340$ nm	The previously treated sample reacted in the presence of NAD^+ inside an enzymatic microreactor, packed with D-lactate dehydrogenase co-immobilized with L-alanine aminotransferase on porous glass. The NADH released was monitored	$(0.5–10.0) \times 10^{-3}$ mol/l	0.1×10^{-3} mol/l	< 3	95–105[c]	20	Shu et al. (1993)

L(−)-Malic acid	Table and Port wines	UV–Vis λ = 340 nm	The determination was based on the monitorization of the NADH after oxidation of L-malate in the presence of NAD^+ by action of a solution of L-malate dehydrogenase	0.01–0.15 g/l	0.009 g/l	–	3.4	22	Segundo and Rangel (2003)
Phytic acid	Corn, nut, peanut, almond, cocoa, coffee, chocolate, bread	UV–Vis λ = 550 nm	The determination was based on the diminution of the calcium oxalate crystallisation reaction rate in presence of phytic acid. The sample was previously purified by anion exchange chromatography	0.05–0.6 mg/l	0.03 mg/l	2.0	+0.2	20	March et al. (2000)
Phytic acid	Corn, rice, oat wheat, bean, pea lentil	UV–Vis λ = 525 nm	The extracted samples were transported to the ion-exchange resin beads packed in the minicolumn. The phytic acid retained was eluted and mixtured with a Fe (III)–salicylate coloured complex. The absorbance variation of the latter was monitored	50–200 mg/l	3.5 mg/l	<3.2	+0.3[b]	60	Sartini and Oliveira (2002)
Reducing sugars	Table wines	UV–Vis λ = 460 nm	The sample was directly aspirated and propelled into a dialysis unit to minimize colour interference and to dilute the sample. The method is based on the reaction of Cu(I) with neocuproine, after reduction of Cu(II) by reducing sugars	2–25 g/l	1.2 g/l	2.1	−0.2	14	Araújo et al. (2000)
Reducing sugars	Port wines	UV–Vis λ = 460 nm	The procedure remained the same, just a few operational parameters were changed	20–140 g/l	11.2 g/l	1.7	−0.2	18	Araújo et al. (2000)
Yeast assimilable nitrogenous compounds (YANC)	Grape juice and fermentation must	UV–Vis λ = 334 nm	Samples were initially diluted 1:1 with borate buffer and the α amino acid reacts with *N*-acetyl-L-cysteine (NAC) and *o*-phthaldialdehyde to form absorbing isoindole derivatives	28–140 mg/l (as N)	3.2 mg/l (as N)	1.5	−5.0	12	Muik et al. (2002)
Ethanol	Table wines	FTIR 970–1435 cm^{-1}	The sample procedure, above described, was carried out but the samples were, in-line, diluted using a mixing chamber	–	–	0.7	−0.4	20	Schindler et al. (1998)
Glycerol	Table wines	FTIR 970–1435 cm^{-1}	The sample procedure, above described, was carried out but the samples were, in-line, diluted using a mixing chamber	–	–	0.4	−3.2	20	Schindler et al. (1998)
Glucose	Table wines	FTIR 970–1435 cm^{-1}	The sample procedure, above described, was carried out but the samples were, in-line, diluted using a mixing chamber	–	–	1.3	−13.1	20	Schindler et al. (1998)

(*continued on next page*)

Table 2 (*continued*)

Analyte	Sample	Detector	Method	Linear range	Detection limit	Precision[a]	Accuracy[b]	Samples per hour	Reference
Fructose	Table wines	FTIR 970–1435 cm^{-1}	The sample procedure, above described, was carried out but the samples were, in-line, diluted using a mixing chamber	–	–	1.0	−6.8	20	Schindler et al. (1998)
Lactic acid	Table wines	FTIR 970–1435 cm^{-1}	The sample procedure, above described, was carried out but the samples were, in-line, diluted using a mixing chamber	–	–	0.8	+11.0	20	Schindler et al. (1998)
Glucose	Soft drinks	FTIR 971–1206 cm^{-1}	The determination was based on the direct chemometric evaluation by means of a PLS calibration model of the mid-IR spectra of the untreated sample	up to 50 g/l	–	2.5	+6.3	15	Schindler et al. (1998)
Fructose	Soft drinks	FTIR 971–1206 cm^{-1}	The determination was based on the direct chemometric evaluation by means of a PLS calibration model of the mid-IR spectra of the untreated sample	up to 50g/l	–	2.1	+10.0	15	Schindler et al. (1998)
Sucrose	Soft drinks	FTIR 971–1206 cm^{-1}	The determination was based on the direct chemometric evaluation by means of a PLS calibration model of the mid-IR spectra of the untreated sample	up to 50g/l	–	3.7	+4.1	15	Schindler et al. (1998)
Glycerol and 2, 3- butanediol	Table and Port wines	ISE IO_4^-	The determination was based on the diminution of potential signal corresponding to a IO_4^- solution, continuously monitored, when reacts with the glycerol contained in the sample previously, in-line, purified by anion exchange chromatography	–	220 mg/l	1.0	96–107[c]	33	Luca et al. (1998)
Total *N* Volatile *N* Acid detergent insoluble *N*	Silage	ISE NH_4^+	Taking into account the species to be determined, different, off-line, pre-treatments were carried out. After being mixtured with NaOH, the same NH_3/NH_4^+ conversion and potentiometric determination were accomplished	10–120 mg/l	3 mg/l	<2	−4.6	30	Silva et al. (2000a)
Sucrose	Silage	ISE IO_4^-	The determination was based on the diminution of potential signal corresponding to a IO_4^- solution continuously monitored when reacts with the reducting sugar contained in the sample previously enzymatic hydrolysed by a yeast crude extracts	–	0.13%	<2%	−0.3	24	Silva et al. (2001)
Urea	Milk	ISE NH_4^+	Urea contained in samples suffered, in-line, enzymatic hydrolysis and the gaseous NH_3 generated was transformed in NH_4^+ ions, which were potentiometrically monitored. A gas diffusion unit was used for the chemical conversion	–	6.0×10^{-4} mol/l	1.9	99–101[c]	20	Silva et al. (2000b)

Ethanol	Beer and wine	Amperometry	A suspension of acrylic beads with immobilized alcohol-oxidase was introduced into the jet ring cell, the diluted samples were aspirated and transported to the beads and the H_2O_2 liberated was detected measuring the oxidation current	7.8–78.7 mg/l	3.9 mg/l	2.9	−5.1[b]	–	Mayer and Ruzicka (1996)
Glucose	Beer and wine	Amperometry	The same principle but glucoseoxidase was the immobilized enzyme	1.8–270 mg/l	0.9 mg/l	2.9	−6.4[b]	–	Mayer and Ruzicka (1996)
Ethanol	Wines and fermentation musts	Amperometry	The quinoprotein alcohol dehydrogenase was cross-linked to a redox polymer and configurated as screen printed electrodes. The current originated was measured by this amperometric biosensor	0.05–11.5 g/l	0.05 g/l	<2.7	2.8[d]	–	Niculescu et al. (2002)

[a] Relative SD.
[b] Mean relative error of the SIA method versus the reference method (%).
[c] Range of spike recovery.
[d] Mean relative error of the SIA method versus concentration stated by the producers.

solution (pH 8.5) sodium chloride. The multivariate calibration models proposed, one for sugars and the other for acids, were established from standard solutions that contained all of the determined compounds. The results obtained were in agreement with those supplied by application of commercial enzymatic kits (Roche), with average percentage deviations of 4.7%, 4.4% and 3.7% for glucose, fructose and sucrose and 3.6% and 4.1% for citric and malic acids, respectively.

The simultaneous determination of citric, malic and tartaric acids in soft drinks has also been carried out by the same research group (Ayora-Cañda & Lendl, 2000). The method is based on pH modulation and evaluation of the induced spectral changes of the sample. The pH modulation took place in a novel sheath-flow cell with three stream lines flowing adjacent to each other in a strongly laminar fashion and the cell was connected to a SIA system with FTIR spectrophotometric detection. Stopped flow technique was applied and the region between 1180–1400 cm^{-1} was used to setup a partial least squares (PLS) calibration model. Eight samples containing 0.5 g/l of each acid were prepared and analysed by the developed method and the relative standard deviations of the predicted values using the PLS model were 1.8%, 2.4% and 2.5% for citric, malic and tartaric acids, respectively.

The determination of the iodide ion contained in iodised table salts was proposed by Araújo, Lima, Saraiva, Sartini, and Zagatto (1997b) using a SIA system that also utilized a small mixing chamber. This was coupled to one of the ports of the rotary valve with the objective of simplifying the mixture of solutions differing significantly in viscosity. In this way, an effective mixture was ensured without significantly increasing the sample dispersion, which could give rise to non-reproducibility of the baseline noise on the spectrophotometric detection. The basis of the analytical determination was the catalytic effect that the iodide ion exerts over the chloropromazine oxidation reaction by concentrated hydrogen peroxide in a strongly acid medium, which gives rise to the formation of a coloured intermediary species (λ = 528 nm). The possible interferent action of diverse anionic and cationic species were equally studied, two of which should be highlighted: The interferences due to Fe(III), when present at concentrations 20 times greater than iodide and the interference of iodate if present at concentrations 10% lower than iodide.

Silva, Segundo, and Rangel (2003) have developed a SIA system for the determination of sulphate in wines based on the precipitation of sulphate in the presence of barium chloride in acid media. The precipitate formed was turbidimetrically monitored at 420 nm. A mixing chamber, made of acrylic and furnished with a magnetic bar, was used to dilute and acidify the samples. If the chamber was located in one of the side ports of the selection valve, several aliquots could be drawn and ana-

lysed, allowing replicate measurements from the initial sample. This manifold also allowed time saving since washing of the mixing chamber was not required between several determination cycles. To avoid settling of the barium sulphate precipitate in the system, an alkaline buffer–EDTA solution was used to rinse the tubing between determinations, such as Van Staden and Taljaard (1996) had proposed when the procedure was applied to industrial effluents. The sample colour interference was eliminated by conjugating absorbance measurement at a fixed time and substraction of the absorbance of a blank assay. When the proposed SIA procedure and the reference gravimetric procedure (OIV, 1990a) were simultaneously applied on ten samples of different white and red wines, the results obtained were statistically comparable.

The determination of zinc, magnesium, iron and copper in wines was undertaken by Costa, Cardoso, and Araújo (2000) through two slightly different SIA systems. When the first three cations and copper were directly determined (the latter at concentrations greater than 0.20 μg/l), the manifold simply consisted of an atomic absorption spectrophotometer as a detector, a selection valve, a peristaltic pump as propulsion system and thereafter, a three way solenoid valve with the objective of ensuring reproducibility of the flow. Nevertheless, when copper was determined at concentrations lower than 0.20 μg/l it was necessary to couple a module that permits an additional on-line pre-concentration step. This was carried out in an heterogeneous phase using a commercial cartridge packed with silica gel spheres covered with C18 and using an aqueous DDTC solution as chelating agent. The authors demonstrated the versatility that the developed SIA system posessed by making viable the analysis of samples containing these metals in diverse linear concentration intervals. In this way, the multiparametric determinations could be accomplished without the need to physically reconfigurate the system. The results were in agreement with those obtained by the reference methods for zinc (OIV, 1990b) and iron (OIV, 1990c), those proposed by Ribéreau-Gayón, Peynaud, Sudraud, and Ribéreau-Gayón (1982a) for manganese, and the official Portuguese methods for copper (Curvelo-Garcia, 1987a).

Araújo, Gracia, Lima, Poch, and Saraiva (1997a) developed a SIA system that made possible the determination of iron contents in fortified infant food formulations. Here the analytical procedure was based on the attainment of the known coloured complex that is formed between Fe(III) and SCN^- and its spectrophotometric determination ($\lambda = 480$ nm). The solid samples were treated previously to their introduction in the interior of the manifold, bringing them to ashes and dissolving in 0.2 mol/l HNO_3. The samples were also equally analysed by flame atomic absorption spectrophotometry adopted as reference method and this provided similar results between the two methodologies. In the study on possible interferent substances, it was deduced that only phosphate showed adverse effects when present at concentrations greater than 100 mg/l.

The determination of mercury in waters and canned fish by the SIA methodology was undertaken by several authors using the cold vapour technique as the chemical basis of the analysis, and atomic absorption spectrophotometry with a quartz cell as the detection system. Initially, Bauzá de Mirabó, Thomas, Rubí, Forteza, and Cerdá (1997) developed a SIA manifold with two syringe pumps (automatic burette) as the propulsion system. Additionally, they used a cylindrical gas–liquid separation cell, made of acrylic, using Sn(II) chloride as reducing agent. More recently, Doering, James, and Echols (2000) optimized a simpler manifold that employed a single peristaltic pump, a simple piece of plastic in the form of a T as separation unit and the same reducing agent. In a similar way to the previous case, the solid samples were digested in an acid medium, prior to their introduction in the systems. Both methods were validated using reference materials. The results obtained, which are shown in Table 1, do not differ substantially between themselves and they show that both systems present higher detection limits and lower sampling rates than those attained in FIA (De Vargas & Romero, 1992).

Total arsenic contained in wines has been determined by using a SIA system which used the atomic absorption spectrophotometry with hydride generation as detector (Zárate, Araújo, Montenegro, & Pérez-Olmos, 2001). Initially, the samples were off-line treated in a microwave oven using concentrated nitric acid and hydrogen peroxide to destroy the organic matrix. The digested samples were aspirated and mixtured with sodium borohydride, in presence of hydrochloric acid, as reducting agent. The arsine generated was pushed out of the reaction chamber and by means of a gaseous current of argon was directed to the quartz cell of the spectrophotometer.

More recently, SIA systems have been developed which catered for on-line sample treatment; that is, in the interior of the manifold itself. In this manner, Oliveira, Zagatto, Araujo, and Lima (1998) developed a SIA manifold that permitted determinations of the total phosphorus contained in different types of food such as wines, milks, fruit juices and vegetables. Given that the analytical procedure was based on the well known method of the molybdenum blue, the detection system was spectrophotometric ($\lambda = 619$ nm). The digestion of the samples was carried out in the presence of a concentrated nitric acid flow in the interior of a self-constructed PTFE minidigestor of 3 ml capacity made of PTFE. This was introduced in a microwave oven and coupled to one of the ports of the rotary selection valve. The system also possessed a second control valve for the emis-

sion of gasses formed during the process, avoiding in this way the need to use a bubble elimination unit, previously used in the determination of phosphorus in residual waters by FIA (Benson, McKelvie, Hart, & Hamilton, 1994). The data from the analysed samples were in significant agreement with those obtained by the same method with external and manual digestion adopted as the reference technique (Zagatto, Krug, Bergamin, & Jorgensen, 1989).

The determination of phosphorous in milk has also been carried out by Lima, Fernandes, and Rangel (2002). Since in SIA methodology the flow can be stopped for a period of time, in-line digestion of complex matrices can be easily accomplished. In this occasion the method developed uses an in-line photo-oxidation digestion procedure (UV light) together with oxidizing and hydrolizing reagents (sulphuric acid and potassium peroxodisulphate) to convert all forms of phosphorous compounds to orthophosphate. After reaction with ammonium molybdate and stannous chloride as reductant the blue complex formed was determined spectrophotometrically ($\lambda = 710$ nm). Ten samples of cow milk were simultaneously analysed by the SIA method and a similar spectrophotometric manual adopted as reference (MSDA, 1973), and the results obtained were statistically concordant. If this SIA system is compared with the system previously described it is possible to affirm that the precisions, accuracies and sampling rates of both are similar but the latter showed higher detection limit.

Oliveira, Sartini, and Zagatto (2000) extended the application of the SIA system to the determinations of calcium, magnesium and iron in milk and fruit juices. The underlying chemical methods were based on the development of coloured complexes between the Fe(II) and *o*-phenantroline ($\lambda = 510$ nm) and between calcium and magnesium with *o*-cresolpthaleine ($\lambda = 535$ nm). To determine the magnesium in an isolated form, EGTA was added as the calcium chelating agent. To ensure that all the iron contained in the sample was in the ferrous form, the *o*-phenantroline solution also contained ascorbic acid. Although the proposed procedure operated with concentrated solutions, the Schlieren noise was absent, demonstrating that the digestion pump also acted as an efficient mixing chamber. On the other hand, the absence of abrupt variations in the register, after prolonged periods of functioning, evidenced the absence of air bubbles or particulate material in the interior of the system. Finally, the results from the system were in agreement with those obtained after the application of atomic absorption spectrophotometry as the reference technique and the manual digestion of samples.

Neira, Reyes, and Nóbrega (2000) conducted the determination of total iron in white wines. In this case, the developed manifold incorporated, in addition to the microwave-assisted digestion system, an ultrasonic bath that ensured the complete formation of the coloured complex resulting from the reaction between Fe(II) and *o*-phenantroline ($\lambda = 510$ nm). In the case of red wine samples, the authors proposed a more energetic treatment during the digestion or the utilisation of atomic absorption spectrophotometry as the detection system.

The elimination of interfering substances in the analysis of wines was carried out in other work through the utilisation of small ionic exchange columns. For example, the determination of glycerol and 2,3-butanediol in wines was proposed by Luca et al. (1998) employing a SIA system that used a periodate selective electrode as the detection system. This was made up of a tubular configuration, without internal reference solution, using bis(triphenylphosphoranylidene)ammonium chloride as the sensor immobilized in PVC. The technique used was similar to that previously described in FIA, but using a different sensor (Montenegro et al., 1993). With the objective of avoiding the interference of other reducing compounds present in wine, an anionic exchange minicolumn in hydroxylated form was incorporated into a side port of the valve. The analytical method was based on the existing relationship between the concentration of both polyols and the decrease in analytical signal generated by a periodate solution, when the reaction between this and the reducing compounds mentioned was carried out.

Silva, Souza, Ferraz, and Nogueira (1999) have designed a SIA system with conductimetric detection to carry out the determination of chloride in milk. The interferent action of the proteins present in the sample was eliminated in the interior of the manifold by using a small dialysis unit, such as it had been proposed for the first time, in SIA methodology, by Ivaska and Ruzicka (1993). This device consisted of two plates or blocks of perspex with a semicylindrical cavity that housed the dialysis membrane in its interior and small (entry and exit) channels for the liquids. With the objective of minimizing matrix interferences, the analytical procedure used the standard addition method put forward by Rizov and Ilcheva (1995). After an exhaustive study of potential interferents, the optimized SIA method were very reproducible and accurate, and the results obtained when applied to reference materials were in significant agreement with the certified values.

In addition to eliminating matrix interferences, the dialysis units were used simply to enable the on-line dilution of samples to be undertaken. Therefore, Araújo, Lima, Rangel, and Segundo (2000) carried out the determination of reducing sugars in wines. The basis of the analytical procedure consisted of reacting the reducing sugars with Cu(II) salts, the corresponding Cu(I) formed reacting with the neocuproine to form a coloured compound detectable spectrophotometrically at 460 nm.

The results obtained in the analysis of the samples were compared with those attained by applying a method consisting of an iodometric titration after distilling the wine, and showed concordance (Ribéreau-Gayón, Peynaud, Sudraud, & Ribéreau-Gayón, 1982b). This SIA system has resolved the problems that occurred when the same determination was carried out using FIA methodology: The necessity to filter the wine through active carbon (Maquieira, Luque de Castro, & Valcárcel, 1987); the lower efficacy using the dialysis unit in the treatment of wines with higher sugar concentrations (Peris-Tortajada, Puchades, & Maquieira, 1992) and the need to use different injection volumes; reconfiguring the manifold according to the sample contents (Lima, Neves, & Rangel, 1990).

In the SIA systems previously described in this work, the measurements have been based on evaluating the concentration of species obtained by a series of chemical reactions. Nevertheless, SIA systems were also developed that employed enzymatic reactions. The enzymes were used as much in solution as immobilized in the interior of minicolumns or reactors. The first work refered to the determination of D-lactic acid in pork meat (Shu, Hakanson, & Mattiasson, 1993). This pioneering work was based on the combination of a procedure to stop the flow with the use of immobilized enzymes. Two minicolumns were used, one with D-lactodehydrogenase (LDH) and the other with L-alanine aminotransferase (ALT). Due to the biochemical reactions that take place in the columns, NADH is released and monitored at 340 nm. The comparison of results obtained after sample analysis by SIA with those obtained by using commercial Boehringer enzymatic kits yielded the conclusion that they were approximately 10% greater. These authors also described that, from the practical point of view, the interferent action of the organic acids (citric, oxalic, succinic and piruvic) was negligible. The remarkable success of this system was based on the considerable reduction in the consumption of NAD^+, of which the enzymatic system was dependent, when compared with other FIA designs (Almuaibed & Townshend, 1988). For this reason the procedure proposed showed itself to be particularly suited to monitoring D-lactic acid in fermentation processes (Shu, Hakanson, & Mattiasson, 1995).

Araújo, Lima, Saraiva, and Zagatto (1997c) have determined L(+)-lactate in wines using a SIA system in which the interferent effect of some constituents present in the wines was reduced by using a dialysis unit connected to one of the ports of the fluid selection valve and whose membrane was substituted weekly. The difference in the work previously cited lay in the fact that the enzymes were not immobilized in an heterogenous phase, but were rather used in the solution, which was located between the pump and the central port of the selection valve. The upper channel of the dialysis unit was connected to the system, and the other was filled with the carrier solution and stoppered. Therefore, the aspirated sample remains in close contact with the dialysis membrane to permit sensitivity improvement. In this situation, the lactooxidase/peroxidase system was used. The former promoted the conversion of L(+)-lactate to pyruvate and hydrogen peroxide, the latter being together with 4-chlorophenol and 4-aminoantipirine, the substrate for the second. The reaction generated quinoneimine which was monitored spectrophotometrically at 510 nm. As in the previous case, the samples were analysed simultaneously with the Boehringer enzymatic kits adopted as the reference method.

The enzymatic determination of L(−)-malic acid in wines has been carried out by Segundo and Rangel (2003) using a SIA system with spectrophotometric detection. The method was based on the monitorization ($\lambda = 340$ nm) of NADH formed by oxidation of L-malate to oxaloacetate, catalysed by L-malate dehydrogenase in the presence of NAD^+. In order to avoid interference due to the intrinsic absorption of the wine matrix, a kinetic approach was implemented by stopping the flow when a solution plug containing sample, NAD and enzyme reached the reactor and the absorbance changes were monitored during a fixed period of time. Sixteen samples of table and Port wines were analysed so as to evaluate the applicability of the SIA system developed. The results obtained were comparable with those obtained by application of the Boehringer enzymatic kit adopted as reference method. In the present system enzyme consumption was 10 times lower than that obtained in the emerging zones system proposed by García de María, Manzano, Alonso, and Garcia de Maria (1991).

Saraiva (2000) subsequently developed a SIA system that permitted the enzymatic determination of glucose in milk. The basic fundamentals of the method consisted of coupling two enzymatic reactions catalyzed by two different enzymes. In the first place the glucose-oxidase catalyzed the reaction between the glucose and oxygen. The hydrogen peroxide formed subsequently reacted as in the case of determining lactic acid referred to above. Given the reduced glucose content in the samples and therefore the need to increase the sensitivity of the determination, the reagent solution was aspirated, placing itself in the storage reactor, between two large sample volumes. The samples were previously de-proteinised with barium hydroxide and zinc oxide. At the same time, these authors also optimized another SIA system for the same purpose, but in which the enzymes were immobilized in column. The results obtained, which are outlined in Table 2, yielded the conclusion that the degree of precision of both methods was similar, as well as the levels of agreement obtained against the Boehringer enzymatic kit. The main differences resided on the fact that the

method that used immobilized enzymes showed better detection limits and greater sampling rates.

Segundo and Rangel (2002) have designed a SIA system to carry out the enzymatic determination of glycerol and ethanol in wines. The method was based on the spectrophotometric determination of NADH at 340 nm after oxidation of glycerol and ethanol in the presence of NAD^+ by the action of glycerol dehydrogenase (GlyDH) and ethanol dehydrogenase (ADH) which were immobilized and packed in two minicolumns. The authors have included in the manifold an injection valve and an additional pump to enhance the throughput rate as it had been previously reported by Guzmán and Compton (1993). In this manifold, sample and reagent were aspirated through a selection valve as in SIA, but the stacked zones formed in the holding coil were sent to fill the loop of an injection valve, the loop content was then injected into a carrier stream and directed to the detector, as it occurs in FIA. The linear range obtained for the determination of glycerol was higher than the reported with the FTIR SIA system previously described (Schindler et al., 1998). With respect to the ethanol determination it is interesting to point out that the proposed method requires less NAD^+ than the SIA system developed by Hedenfalk and Mattison (1996). The results obtained, when the proposed methods were carried out on 15 wine samples, were in agreement with those obtained through the hydrometric method (OIV, 1990d) for the ethanol determination, and the Boehringer enzymatic kit for the glycerol determination, adopted as a reference method.

The determination of ethanol in wine samples and during wine fermentation has been carried out by Niculescu et al. (2002) using a SIA system with an amperometric biosensor as detector. The biosensor was constructed by cross-linking quinoprotein alcohol dehydrogenase to an Os-complex-modified poly(vinylimidazole) redox polymer using poly(ethylene glycol) diglycidil ether. The optimised configuration of the sensor was adapted to screen printed electrodes which were integrated in the manifold using specially designed miniaturized flow-through cell with a small dead volume. Three samples of wine were analysed using the developed system and the results obtained were in agreement with the stated values by the manufacturers.

Silva, Souza, and Nogueira (2001) have developed a SIA system for the enzymatic-potentiometric determination of non-structural carbohydrates content (expressed as percentage of sucrose) in sugar cane and maize to be used for silage production. The manifold was designed to automate the determination and to perform in-line the sucrose enzymatic-hydrolysis promoted by the use of a yeast crude extract (*Saccharomyces cerevisae*) as source of invertase. The same tubular potentiometric detector previously cited (Luca et al., 1998) was used for monitoring the periodate remained from the reaction between the reducing sugar monomer units and the periodate ions, in the same manner that it had been proposed using FIA methodology (Gomes-Neto et al., 1994). Samples were dried, milled and homogenized in water prior to being aspirated and introduced in the manifold. The SIA system developed presented some advantageous characteristics such as the use of only one low cost reagent and the agreement of the results obtained when compared with those furnished by application of the Authrone spectrophotometric method (Silva, 1981) adopted as reference technique.

Silva et al. (2000b) developed a SIA system to determine urea in milk, by enzymatic means, that used an ammonium tubular ion-selective electrode as detector. This tubular detector was constructed without internal reference solution and nonactine as the sensor in the PVC membrane (Alegret, Alonso, Bartrolí, & Fábregas, 1989). A gas-diffusion unit was placed between the sample/reagent flow channel and the detector channel to enhance the selectivity eliminating matrix interferences. The authors employed Jack bean meal (*Canavalia ensiformis* DC) crude extract as an inexpensive source of urease, directly aspirated by one port of the selection valve. The analytical procedure was based on the potentiometric monitoring of ammonia generated by enzymatic hydrolysis of urea in the donor channel which diffused through the gas-permeable membrane. The ammonia diffused is collected in the acceptor channel by a carrier stream of Tris–HCl buffer solution (pH 7.5) that promoted the conversion into ammonium ions. The method was applied simultaneously with a commercial enzymatic kit to various milk samples, yielding results that were in agreement.

With the aid of an appropriate flow cell, small amounts of bead suspensions can be manipulated by SIA systems to form renewable minicolumns that act as disposable reaction surfaces. The suspended beads can serve as solid-phase carriers for reagents, reactive groups or even cells. This new operation mode named as bead injection was used by Mayer and Ruzicka (1996), who developed a SIA system with amperometric detection for the determination of the glucose and ethanol contained in wine and beer. The principle of the method is based on the introduction of a defined volume of a suspension of non-conducting beads with immobilized alcohol or glucose oxidase into a SIA system, where the beads are trapped on the sensing surface of an amperometric detector accommodated into a jet ring cell previously designed by the authors (Ruzicka, Pollema, & Scudder, 1993). During the determination of alcohol, samples were diluted 1000–1700 times with phosphate buffer (pH 7.5) as carrier and in the case of glucose, samples were diluted 3–30 times with citric acid/sodium monohydrogenphosphate buffer (pH 5.9) as carrier. Concentrations were determined by

the standard addition method, and assays without enzyme beads trapped in the jet ring cell (blank signal) were carried out. Kits from Sigma, glucose determination, and from Boehringer, ethanol determination, were used as reference methods.

The bead injection principle has also been used by Garden and Strachan (2001) in a SIA system developed to determine aflatoxin B_1 (AFB_1) in pistachios. In this case the jet ring cell contained beads with AFBi–bovine serum albumin (BSA) conjugate immobilized on its sensing surface, which were also treated with BSA mixed with phosphate buffered saline (PBS). The beads were perfused with the sample mixture with antiAFB_1 and BSA–PBS followed by anti-rabbit alkaline-phosphatase conjugate antibodies and finally with Bluephos used as substrate. The development of colour was measured at 620 nm for 5 min. The samples were off-line treated by means of the extraction procedure proposed by Strachan, John, and Millar (1997) slightly modified. Spiked, pistachio nut samples were simultaneously analysed by the SIA system and by the microtitre plate enzyme-linked immunosorbant assay (ELISA) adopted as reference technique. The results showed that SIA was as sensitive as ELISA and took less time to perform a measurement.

Recently, a novel and interesting strategy for exploiting ion-exchange in SIA systems, using a modification of the bead injection principle, has been proposed by Sartini and Oliveira (2002). Its feasibility has been demonstrated by the in-line phytic acid separation and determination of phytic acid contained in different food samples. Initially, a defined volume of an ion-exchange resin (AG1-X8) suspension was introduced and packed in the analytical path, immediately before the flow cell, due to the presence of a circular nylon screen. Afterwards, a selected sample volume was directed to the resin minicolumn, where phytic acid was retained while the sample matrix was discarded. Finally, a defined volume of a solution containing chloride and Fe(III)–salicylate complex, used as eluant and spectrophotometric reagent, was directed towards the flow cell. The phytate ions were eluted, reacting with the coloured complex and the variation in the absorbance signal, proportional to the analyte concentration, was monitored at 525 nm. The results obtained when food sample extracts were processed by this method where precise and in agreement with those furnished by an official method (AOAC, 1997a). The SIA system developed showed higher sampling rate and lower detection limit that, the kinetic–turbidimetric method previously described in this review (March et al., 2000). On the other hand, in the present work the separation by the resin of the phytic acid contained in the extracted food samples was done in an in-line way, while in the other SIA system it was done in an off-line way.

The SIA methodology has also demonstrated itself to be an interesting tool in the field of speciation since the possibility of carrying out different on-line treatments allows it to determine different species. Therefore, the use of a gas-diffusion unit was again proposed by Silva, Nogueira, Souza, and Cruz (2000a) for the determination of total, volatile and acid detergent insoluble nitrogen, contained in silage destined for livestock. The manifold employed the same ammonium tubular ion-selective electrode as the detection system. The analytical procedure was based on the diffusion, in alkaline medium (NaOH 3.0 mol/l), of ammonia contained in sample extracts obtained prior to introduction in to the system. The diffusion took place through a Teflon hydrophobic membrane, subsequently producing the conversion of gaseous ammonia into ammonium ion by circulating a Tris–HCl buffer solution (pH 7.5) by the acceptor channel. The system was optimized taking into account the possible interfering effect that some components (also volatile) such as methanol, ethanol and acetic acid presented, by being capable of crossing the membrane. The results obtained were statistically in agreement with those achieved by the Kjeldhal method (AOAC, 1997b).

Regarding wines, Segundo and Rangel (2001) developed a SIA system that permits the determination of free and total sulphur dioxide. A gas-diffusion unit was incorporated with an hydrophobic membrane changed daily. To promote the formation of SO_2, a 4 mol/l solution of hydrochloric acid is added to the sample, before it passes through the acceptor channel in the diffusion unit. To determine the free sulphur dioxide, the sample was aspirated and sent to the storage channel while for the determination of total sulphur dioxide, an hydrolysis reaction of combined sulphur dioxide was produced in the interior of the system by addition of a 2.5 mol/l sodium hydroxide solution. The fundamentals of the method consisted of the formation of a coloured compound between the sulphur dioxide, the formaldehyde and the pararosaniline being detected spectrophotometrically at 580 nm. In other SIA systems previously developed, the two channels of the diffusion unit had been connected to two different selection valves (Lukkari, Ruzicka, & Christian, 1993) or to two independent pumps (Echols, James, & Aldstadt, 1997). In this way the transporter flowed simultaneously through the two channels as in a FIA manifold. On the other hand, in another SIA system the donor channel and the receptor channel were connected to two consecutive ports of an injection valve (Oms, Cerdá, Cladera, Cerdá, & Forteza, 1996). Therefore, the transporter flowed through the donor channel while the contents of the receptor channel were stopped. However, in this work, each channel was simply connected to different ports of a single selection valve. The results obtained for the

free and total SO_2 were in agreement with those obtained by the reference methods (OIV, 1990e). After studying the possible interferences, the authors concluded that this SIA system could not be used with sparkling wine samples.

The determination of Fe(III) and total iron in table wines by SIA with flame atomic absorption spectrophotometry, as detection system, has been carried out by Costa and Araújo (2001). The determination of Fe(III) is based on the extraction with MIKB of the complex formed between Fe(III) and thiocyanate. The manifold includes a device for on-line liquid–liquid extraction; it consists of a glass vial with a porous ceramic plate inside, which allows solvent less dense than water to float above it. The feasibility of this device had been previously demonstrated by the same authors in other SIA systems with the same type of detection (Araújo, Costa, Lima, & Reis, 1998), and they avoided the use of a phase-separation unit or large volumes of organic phase. The determination of total iron was accomplished by sending a small sample aliquot directly to the nebulizer of the spectrophotometer. The results obtained on ten wine samples were in accordance with those supplied by the colorimetric thiocyanate method (Ribéreau-Gayón, Peynaud, Sudraud, & Ribéreau-Gayón, 1982c) and with the atomic absorption spectrophotometric method (OIV, 1990b).

Zárate, Araújo, Montenegro, and Pérez-Olmos (2003) developed a SIA system that permits the determination of potassium that is permanently linked to the organic matrix of wines since the system can determine the total potassium and the existing free potassium to the same extent. The manifold uses a potassium selective electrode of tubular configuration as detection, without internal reference solution, which uses valinomycin as sensor in a PVC polymeric membrane (Alegret et al., 1987). At one of the ports of the selection valve, a PTFE minidigestor module was joined and introduced for 60 s at 700 W, into a microwave oven where the digestion of the wine occurs through the action of hydrogen peroxide, in order to determine the total potassium. The results obtained for total potassium were in agreement with those obtained through atomic emission spectrometry as reference method (Curvelo-Garcia, 1987b).

3. Conclusions

Sample injection, controlled dispersion and reproducible timing are the cornerstone of both FIA and SIA methodologies, but SIA systems are mechanically simpler and more universal than FIA manifolds since they use only a single pump, a single valve and a single channel. This methodology offers a great advantage since the systems can be easily adapted to different analytical situations, without the need for physical reconfiguration, just changing flow parameters or injection volumes via computer control.

SIA systems only perform when measurements are required and carrier is not pumped continuously like in FIA, thus sample and reagent saving and reduction of the waste produced are other possible advantages to point out when SIA is compared to FIA.

SIA systems present a great capacity in relation to solution handling operations and it is more flexible for applying stopped-flow and reversed-flow operations. Moreover, different types of devices, reactors and detectors can be clustered around the selection valve; for those reasons such systems can be designed to operate in a multiparametric way.

SIA is a robust technique which allows the choice of different types of liquid drivers, so they can manage aggressive reagents and solvents, and do not tend to present a faulty calibration, which can be easily performed using a dilution conduit and a single standard. solution. If standards are clustered around the selection valve the system might be automatically recalibrated as required.

Many of these advantageous attributes of the SIA methodology are of special interest to design and to construct environmental monitors and process analysers. However, SIA systems also present some disadvantages against FIA. Since SIA operates by aspirating sample and reagents one after the other, the sampling rate is considerably lower than in FIA methodology.

Taking into account than in SIA the mixture of sample and reagents takes place essentially during flow reversal of the stacked zones in the holding coil, when reactions involving three or more reagents are considered, efficient overlap of the zones is not attainable. Fortunately, this problem can be avoided by using a mixing chamber or by placing all the reagents in the same solution.

Although SIA was initially proposed as an alternative to FIA, experience demonstrates that both flow techniques are complementary, and it is not possible to state which one is better. It will depend on the specific analysis and the features associated, such as sampling rate, mixing conditions of solutions throughout the manifold, sample availability and reagents cost and toxicity. All these factors must be considered when choosing the most adequate methodology.

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