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Review

Application of sequential injection analysis to pharmaceutical analysis

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Abstract

Sequential injection analysis is a well established tool for automation of pharmaceutical analysis. A short historical background of this technique is given as well as a brief discussion on the basic principles and potentials. The current applications of SIA in the pharmaceutical analysis are also described and discussed. The manifolds developed offer good analytical characteristics and are suitable for analysis of drug formulations, process analysis, drug-dissolution, drug-release testing and functional assays for screening potential drugs. The results obtained are in good agreement with those furnished by the application of the reference methods presented in the pharmacopoeias.

Keywords: Automation; Sequential injection analysis; Pharmaceutical analysis; Drugs

Contents

1. Introduction

Analytical quality is a key factor in the success of programmes for development, production and quality control of pharmaceuticals. In recent years the regulations related to the quality control of medicines required by international pharmacopoeias have become strict, demanding modern pharmaceutical analysis rapid, reliable and economic results. These requirements are not indifferent either to the globalisation of the economy or to the adoption of automation strategies for information flows [1,2] and manipulation of the samples [3,4] on the part of the pharmaceutical industry.

Laboratory automation was initiated in the second half of the XX century, even before recourse to the computer means that we have today. Steward in the US as well as Ruzicka and

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Hansen in Denmark, created the flow injection analysis (FIA) technique for the automation of chemical procedures [5,6]. The introduction of this technique came to revolutionise the concept of automation in chemical analyses by allowing instrumental measurements to be carried out in the absence of physical equilibrium (without homogenisation of sample and carrier/reagent) and chemical equilibrium (without completing the reactions) [7–10]. FIA became popular since it made possible to automate routine procedures in a way that was simple and accessible to laboratories having sparse economic resources, with reduction in sample consumption and supplying high sampling rates. This was certainly not the only use found for the technique. The technique was also explored in other areas of chemical analysis, for example in the study of kinetic and mechanistic aspects of chemical reactions with a view to improving the selectivity of the analytical determinations [11,12] and the development of new chemical, enzymatic and immunological procedures as well as in live cell studies [13,14]. The number of scientific publications grew exponentially to register 15 000 scientific articles in 2004 [15] and the concomitant commercialisation of analysers based on this concept of automation [16]. Following the general application of computers in the routine laboratory, it was possible to cover up the deficiencies of the FIA technique in autonomy, given that automatic control of the different electric components in the mountings was possible. In that way, a second generation of flow analysis was proposed by Ruzicka and Marshall in 1990, designated sequential injection analysis (SIA) [17]. As with the FIA, this is a non-segmented continuous flow technique based on the same principles of controlled dispersion and reproducible manipulation of the FIA concept, but whose mode of functioning is based on the concept of programmable flow. A basic SIA system (Fig. 1) consists of a bi-directional propulsion device, a holding coil, a multiposition selection valve, a detector, tubing adequate for unifying all different components of the system and a microcomputer. This component makes the synchronised control of the propulsion device and the multiposition selection valve possible in such a way to define the volume and direction of stream of the different solutions. In a typical analytical cycle, precise volumes of sample and reagents are sequentially aspirated through the multiposition selection valve to the holding coil. By commutation of the selection valve and by inversing the direction of the stream, a zone composed by the overlapping of the sample and reagent zones is created, for which the radial and axial dispersion processes also contribute. When this overlapping zone is directed to the detector, a transient signal is obtained of a magnitude proportional to the concentration of the species under study. The introduction of SIA marked a new era in the development of flow analysis, by passing some important limitations associated with FIA [17]. Compared with FIA, the economy in reagents and the reduction in waste generation are some of the advantages of SIA, because a permanent flow of carrier solution ceases to be necessary and also because the reagents and samples are used in a more efficient form, given that only those volumes strictly necessary are processed. The economy in reagents alloyed to the high sampling rates (although the latter are less compared to FIA), make this technique an adequate tool when it is intended to undertake analytical measurements on a large scale. SIA can equally be applied successfully in the kinetic studies of chemical reactions, since the factors that affect the precision of the volumes and reaction times are rigorously controlled [18]. The ease with which the experimental parameters can be altered and controlled by computer, the knowledge of the flow rate profiles and the critical selection of both time/flow rate using just one propulsion unit, make these systems simple, robust, stable in the long term and with little maintenance requirements. The simplicity in configuration is one of its more relevant characteristics, not requiring significant physical reconfigurations when the analytical determination or the conditions under which this is carried out are altered. In that way, any alteration in the experimental conditions such as injection volume, reaction type, sample dilution or reagent/sample relation is achieved through an alteration of the instruction introduced by the computer keyboard. In this way, and in contrast to the complex FIA manifolds, there is no need to physically reconfigure the flow route. The robust nature of these configurations is of great use when it is intended to

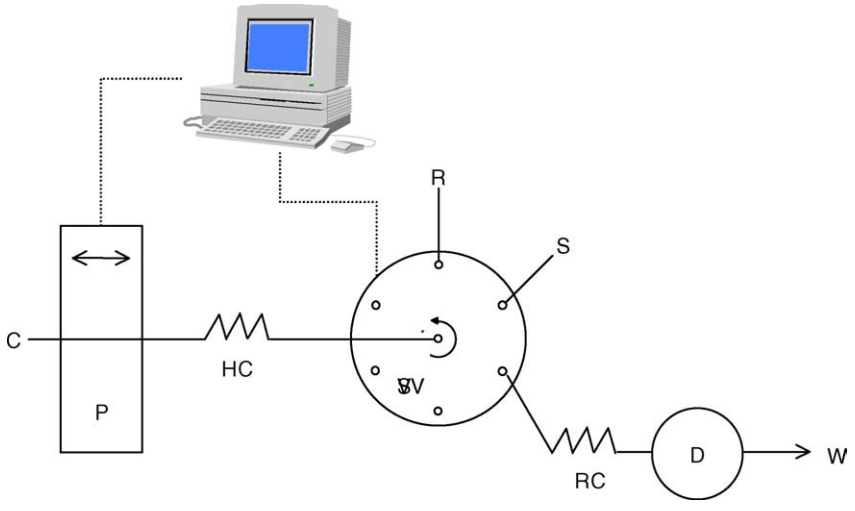

Fig. 1. Basic scheme of a SIA manifold: C, carrier; P, pump, HC, holding coil; SV, selection valve; R, reagent; S, sample; RC, reaction coil; D, detector; W, waste.

carry out determinations over long time periods, for example during the monitoring of industrial processes [19,20]. The versatility shown makes it possible to automate various steps of an analytical procedure, for example: sample dilution [21], automatic calibration [22], gas–liquid diffusion [23], liquid–liquid extraction [24], dialysis [25] and on-line digestion [26]. It is also an adequate technique for multiparametric determinations since different analytical determinations can be easily implemented in the same configuration [27].

The main limitation cited in relation to the FIA technique, is the reduction in the sampling rates by approximately 30–50%, since the time required for the analysis of a given sample results not only from the time required for the reaction and measurement, but also the time spent in the aspiration of the different solutions. Although the sampling rates are lower, this is not an important factor when applied to real situations and in a general way, the advantages outweigh the disadvantages.

In the space of 15 years, SIA has demonstrated itself to be a powerful and versatile instrument for the automation of diverse analytical procedures. More than 500 articles have been published and its advantages are frequently cited in review and trend articles in the field of analytical chemistry [28–46]. This technique has been associated with a large variety of detectors including UV–vis spectrophotometry, fluorescence, turbidimetry, electrochemical, atomic absorption and emission spectrophotometry, Fourier-transformed infrared spectrophotometry, chemiluminescence, mass spectrometry with radio-frequency plasma. This technique has been applied in chemistry areas as diverse as environmental, pharmaceutical, food and bevarage, biotechnology, radiochemical and metallurgic, kinetic studies of chemical reactions, chemometric studies as well as in industrial process monitoring [47]. To demonstrate its application in practice, five analysers are available and sold by three companies namely Global FIA (FloPro 9P and FloPro 4P) [48], FIAlab Instrument (FIAIab-3000 and FIAIab-3500) [15] and Arctic Instruments (SIAmate) [49].

Together with these flow techniques, others have been presented in the literature with proposals for new flow operation modes, or which have arisen as the result of instrumental improvements [41,50]. Within these new techniques we will only cite those that have been explored in conjunction with sequential injection analysis. Among these, an emerging technique is highlighted which has won over fans from day-to-day and whose mounting is based entirely on the manipulation of solutions by solenoid valves [51]. This technique is known by different designations, in the absence of consensus regarding its terminology since the authors have not as yet come to an agreement therein. The principle of its functioning, which is different to FIA, assumes the alternate introduction of small solution segments (lower than μl) by controlling (through a computer) the opening and closing times involved in the passage of the solutions. Recently the possibility of SIA miniaturisation has come to light, through the concept of "Lab on Valve" [52]. Miniaturisation is achieved using a monolythic structure mounted atop a conventional multiposition selection valve in a SIA mounting. This component is the heart of the LOV systems, designated the central sample processing unit (CSPU), which was strategically designed to incorporate a port for sample injection, access channels for reagents and a flow cell which makes detection possible in various configurations, for example absorbance, reflectance and fluorescence.

2. SIA systems for pharmaceutical analysis

The FIA technique has lent an important contribution to the development of automation in pharmaceutical analysis and its advantages are well documented in several review articles [53–58] as well as in a specialised monograph [59]. Despite the advantages presented and its relatively wide utilisation in research, its analytical applications in real situations did not have the expected acceptance. In the pharmaceutical industry its potential has not been conveniently explored, being occasionally used as an economic substitute for chromatography for large scale analysis [60]. This situation could be explained by the difficulty in profiting from a single mounting for multiple applications, as happens with the chromatographic equipment, random discrete batch analysis, and so on. The introduction of SIA has partially overcome this obstacle and has equally awakened the interest of the scientific community for automation in the pharmaceutical area [29]. Many articles dedicated to pharmaceutical analysis have been published, including two review articles [61,62], applying sequential analysis to a wide variety of matrices, such as solid matrices (tablets, capsules), pastes (ointments, creams), liquids (emulsions, suspensions, solutions) and covering various active ingredients with different therapeutic activities. By benefiting from the advantages in the economy of reagents and the elevated sampling rates, the majority of the applications are dedicated to the determination of active ingredients for quality control in pharmaceutical formulations. In addition to large scale analyses, other articles describe its use in kinetic assays, since the rigorous control of the volumes, times and conditions of the reaction allow reaction velocity measurements to be carried out with a high level of repeatability. Other applications make use of its robust nature and elevated efficiency characteristics over prolonged periods for the continuous monitoring of the processes of production of pharmaceutical compounds and for dissolution assays. By associating SIA with recourse to reagents in the heterogeneous phase (bead injection – BI) functional cellular assays have recently been described, which are important during the research phase of new pharmacological products. The advantages and potential of these applications will be described and discussed in detail in the following sections.

The ability to automate the SIA technique in pharmaceutical analysis is quite obvious. However, its potential mainly in the area of new drug research, pharmacological tests and in pharmaceutical technology studies has still not been fully explored. Possibly a more evident demonstration in these areas will have a greater impact on the pharmaceutical industry. Probably miniaturisation, which is already a reality with the recent LOV concept and the commercialisation of portable systems with defined assay protocols, will make it possible to definitely establish it in practice.

2.1. Automated analysis of drug formulations

One of the quality control tests in pharmaceutical formulations is the determination of active ingredient content. The analytical methods used for the determination of active substance must be selective, precise and accurate since auxiliary substances are also present (e.g., filters, binders, suspending agents and preservatives). Automation with the SIA technique satisfies all these requirements and has been explored using spectroscopic, luminescent and electrochemical detection (Table 1).

2.1.1. Spectrophotometry

The majority of the determinations of active ingredients in pharmaceutical formulations belong to this group of spectrophotometric determinations and within this group the majority of those described are based on chromogenic reactions or on the specific characteristics of light absorption by analytes. The extension of the application of SIA in its basic configuration to this type of reactions is as a norm, very simple. This involves selecting the nature and volume to adopt of one or more chromogenic reagents and the detection with spectrophotometers, equipment of which is economic and generally used in laboratories. The chromogenic reactions for drugs fit into these groups included: metal ion chelation reactions for the determination of organic compound or metal ion components, redox reactions, ion pairing, charge transfer complex formation reactions, etc. Obviously, it is possible to find procedures that use more than one type of reaction and on the other hand, it is not possible to find all example types because the SIA methodology is still relatively recent.

2.1.1.1. Methods without chemical derivatization of the analyte. In general terms, the native spectrum of a drug leads to analytical results of low sensitivity but which can be sufficient for the determination of drugs in pharmaceutical formulations available on the market. Another question that arises is selectivity, given that the active ingredient can be accompanied by other active substances, excipients, degradation products, etc., which have spectrums that are superimposed with that of the analyte. Consequently, the selectivity of the spectrum's direct measurement may not be sufficient and for this reason it is necessary to resort to alterations in pH, extraction with organic solvents and more recently (with integrated computer control in the instrumentation) to derivatization of the analyte spectrum (normally of low magnitude, not more than two or three) and other chemometric procedures. With the SIA methodology, some of these procedures have not been approached as yet.

In 2002, Chisvert et al. simultaneously determined the concentration of two UV-filters [63]. The system incorporated a SAX microcolumn and made it possible to extract benzophenone and phenylbenzimidazole sulphonic acid on-line, in the solid phase. These two spectrophotometric determinations were based on selective elution, obtained by varying the pH. This methodology did not have the interference of other compounds present in the samples and was successfully applied in the analysis of several sunscreen formulations.

Recently, Satinsky et al. used a new separation method based on a novel reversed-phase sequential injection chromatography technique for simultaneous determination of ambroxol hydrochloride and doxycycline in pharmaceutical preparations [64].

A SIA system was developed with a diode-array spectrophotometric detector to determine amoxicillin in pharmaceutical preparations [65]. By applying a series of chemometric techniques, this system evaluated the presence of interferents in unknown samples. When interferents were not observed or when these did not interfere in the selective spectrum area for amoxicillin determination, the analyte was quickly determined by monitoring the absorbance at 250 nm. In this work, it was not possible to determine the analyte in the presence of interferents without recurring to a multivariate calibration. Subsequently Pasamontes and Callao, with the same detection system previously presented, succeeded in determining amoxicillin in the presence of interferents using multivariate curve resolution with alternating least squares [66]. After optimising the chemical conditions of the system and the mathematical model applied for the treatment of the data, they determined amoxicillin in three tablets without any type of sample pre-treatment.

The use of chemometric techniques was revealed to be equally effective in the optimisation of the chemical processes prior to the determination. For example, Sultan et al. developed a chemometric optimisation for the spectrophotometric determination of bromazepam complexed with iron(II) in hydrochloric acid [67]. In this proposal, the optimum operational conditions were determined, both for the system and for the chemical variables. The conditions for the analysis were the most adequate and the sensitivity for the analyte was greater than that obtained previously in the kinetic determination of the analyte by the same methodology, but without the optimisation of the experimental variables [68]. The success of the results previously obtained led the same group to extend its application to the determination of oxprenolol [69]. This determination was based on the oxidation of oxprenolol by cerium(IV) in sulphuric medium, with monitoring of the absorbance of the oxidised drug at 480 nm. This method was applied to real samples and demonstrated a recovery of 99.45%, highlighting simplicity and quickness.

2.1.1.2. Metallic ion chelates.

2.1.1.2.1. Formation of metallic ion chelates for the determination of organic active principles. Due to relatively recent origins of the SIA methodology, it is still not possible to find an elevated number of methods and for this reason, the versatility in the formation of chelates with metallic ions is limited to some ions such as Pd(II), Ni(II) and Fe(II and III).

Sultan's research group, the first to describe a SIA method applied to the analysis of pharmaceutical products, proposed a series of methods for the determination of active ingredients in pharmaceutical formulations based on the complexation reactions of metallic ions with the active ingredients [67–72]. Trimeprazine and perphenazine were determined after complexation with palladium(II), in the presence of hydrochloric acid with spectrophotometric monitoring of the complexes formed at 515 nm in the case of trimeprazine and 560 nm for the deter-

Table 1
SIA systems for the active ingredient determination

Analyte	Matrix	Detection	Linear range	R.S.D. (%)	Sample through-put (h^{-1})	Reference
Alendronic acid	Tablets	S (240 nm)	1.0–60.0 mg/l	3	60	[111]
		F (λ_{ex} 340 nm; λ_{em} 455 nm)	0.13–10 mg/l	2	30	[111]
Ambroxol hydrochloride	Capsules; tablets	S (213 nm)	2–100 μg/ml	0.5–5.4	7	[64]
p-Aminobenzoic acid	Sunscreens; home-made samples	S (500 nm)	0–25 μg/l	2–6	13.8	[92]
	Lotain and sun milk; home-made samples	S (434 nm)	0–20 μg/l	3–7	13	[91]
Aminocaproic acid	Pharmaceutical formulations	F (λ_{ex} 350 nm; λ_{em} 450 nm)	4×10^{-7} to 6×10^{-5} mol/l	<2.0	40	[118]
Amoxicillin	Tablets	S (250 nm)	10–120 mg/l	9–16	20	[65]
	Tablets	S (210–340 nm)	10–60 mg/l	1.3	25	[66]
Azidothymidine	Raw materials; capsules	Amperiometric imunosensor	80–2000 nmol/l	1	38	[135]
Benzocaine	Injections	CL (≥390 nm)	0.5–25 μg/ml	<3.8	120	[112]
Benzophenone-4	Sunscreen sprays	S (286 nm)	20–120 μg/l	1–12	20	[63]
Bismuth	Tablets and capsules	S (548 nm)	0.0–75 mg/l	1.1	72	[86]
Bopindolol	Tablets	S (264 nm)	1–10 μg/ml	<1	40	[101]
Boric acid	Ophthalmic solutions	S (520 nm)	0–12 mg/l	<0.6	30	[100]
	Ophthalmic solutions; mineral waters	F (λ_{ex} 313 nm; λ_{em} 360 nm)	8–350 μg/l	2.7	55	[106]
Bromazepam	Tablets	S (585 nm)	5×10^{-4} to 1.5×10^{-3} mol/l	<1.2	36	[68]
			300–11000 pm	0.3	3	[67]
Calcium	Tablets; drinking water	S (573 nm)	0–20 mg/l	<1.4	43	[78]
R-captopril	Spiked samples	Amperiometric biosensor	120–950 nmol/l	<0.1	34	[129]
			100–1000 nmol/l	<0.1	38	[130]
S-captopril	Tablets	Amperiometric biosensor	0.05–1.50 μmol/l	<0.2	80	[128]
	Spiked samples	Amperiometric biosensor	0.4–1.6 μmol/l	<0.1	34	[129]
	Spiked samples	ISE	1–1000 μmol/l	<0.1	38	[130]
Captopril	Tablets	S (400 nm)	2×10^{-4} to 1.4×10^{-3} mol/l	>2	60	[75]
	Tablets	ISE	2×10^{-4} to 1.4×10^{-3} mol/l	>1	5	[75]
	Tablets	S (535 nm)	20–1000 mg/l	1.2	60	[99]
Chloride	Pharmaceutical formulations	Optode	1×10^{-3} to 1 mol/l	<1	14	[125]
		ISE	1×10^{-5} to 1×10^{-2} mol/l	<0.6	60	[125]
Ciprofloxacin	Tablets; infusion	S (447 nm)	50–500 ppm	<0.9	60	[71]
Clavulanate (potassium)	Tablets	ISE	2×10^{-3} to 1×10^{-1} mol/l	0.6	55	[124]
		ISE	2×10^{-3} to 1×10^{-1} mol/l	0.5	53	[124]
Diclofenac	Tablets; suppositories; injectables	F (λ_{ex} 305–95 nm; λ_{em} 430–70 nm)	1×10^{-5} to 1×10^{-4} mol/l	<0.6	32	[108]
		ISE		<0.4	33	[108]
Doxycycline	Capsules; tablets	S (213 nm)	2–100 μg/ml	0.5	6	[64]
S-enalapril	Raw material	Amperiometric biosensor	0.08–1.50 ppm	<0.1	75	[133]
Etilefrine hydrochbride	Pharmaceutical formulations	S (503 nm)	1–20 mg/l	<2.7	80	[103]
Fenoterol hydrobromide	Syrups	S (505 nm)	0.5–40 mg/l	1.8	60	[94]

Fluoride	Toothpaste; tap water	ISE	10^{-5} to 10^{-1} mol/l, 10^{-8} to 10^{-6} mol/l, 10^{-10} to 10^{-6} mol/l (depending on aspiration order of sample and buffer)	<0.003	30	[123]
Indomethacin	Tablets; capsules; extended release capsules	F (λ_{ex} 350 nm; λ_{em} 450 nm)	4×10^{-7} to 1×10^{-5} mol/l	<1.2	30	[117]
Iodide	Tablets; drops; drinking water	S (410 nm)	0.002–0.5 mg/l	<3.6	15	[96]
	Tablets	S (600 nm)	0.1–6.0 μg/l	>2	80	[97]
Iron(III)	Capsules; tablets	S (667 nm)	100–1000 mg/l	<1	8	[80]
	Pharmaceutical products; effluent streams	S (667 nm)	0.30–80.00 mg/l	<0.8	30	[94]
Iron(II)	Pharmaceutical formulations	S (523 nm)	5–40 mg/l	3–4	100	[82]
	Tablets; capsules; natural water	S (515 nm)	1–60 mg/l	<2.5	24	[81]
	Multi-vitamin tablets	S (512 nm)	0.25–5.0 mg/l	0.7–1.6	40	[83]
	Pharmaceutical products; effluent streams	S (667 nm)	0.15–100.00 mg/l	<1.3	30	[84]
Isoxsuprine hydrochloride	Tablets	S (507 nm)	1–60 mg/l	1.4–1.6	60	[104]
Lisinopril	Tablets and capsules	F (λ_{ex} 346 nm; λ_{em} 455 nm)	0.3–10.0 mg/l	2	60	[107]
L-methotrexate	Raw materials; tablets and injections	Amperiometric Biosensor; (E = mV)		<2	34	[132]
		Glox; 150	2–400 pmol/l			
		Glox; 650	10–2000 fmol/l			
		L-AAOD; 650	10–600 nmol/l			
		L-AAOD + HRP; 650	100–2000 pmol/l			
		L-AAOD + Glox; 360	100–8000 pmol/l			
		L-AAOD + Glox; 650	40–1000 fmol/l			
		L-AAOD + Glox + HRP; 240	60–1000 fmol/l			
		L-AAOD + Glox + HRP; 650	0.4–100 pmol/l			
		Glox + HRP; 650	6–800 pmol/l			
D-methotrexate		D-AAOD; 650	0.08–80 nmol/l			
		D-AAOD + HRP; 650	10–1000 nmol/l			
Norfloxacin	Tablets	S (430 nm)	50–400 ppm	<0.9	60	[71]
Magnesium	Tablets	S (570 nm)	0–2.0 mg/l	0.7–1.9	80	[85]
Metoclopramide	Tablets; injections	S (495 nm)	13–130 μg/ml (stopped-flow)	2.32	40	[98]
			3–42 μg/ml (continuous flow)	2.78	18	[98]
Naproxen	Tablets	F (λ_{ex} 280 nm; λ_{em} 356 nm)	4×10^{-7} to 1×10^{-5} mol/l	<2.1	70	[119]
Oxybenzone	Milk; oil; lotions	S (376 nm)	840 μg/l	3–8	24	[76]
	Lipsticks	S (376 nm)	840 μg/l	6–12	24	[77]
Oxprenolol	Tablets	S (480 nm)	50–400 ppm	0.88	120	[69]
Paracetamol	Tablets	S (630 nm)	0–60 mg/l	1.2	27	[93]
	Tablets, suspension, syrup	S (430 nm)	400–1000; 1000–2500 mg/l	3	60	[102]
			200–1000; 1000–2500 mg/l	4	15	[102]
Penicillin-G	Pharmaceutical formulations	ISE	2.0×10^{-4} to 1×10^{-2} mol/l	2	25	[126]
S-pentopril	Raw material	Amperiometric biosensor	0.2–6.0 μmol/l	<0.1	75	[133]
S-perindopril	Spiked samples	Amperiometric biosensor	60–800 nmol/l	<0.1	30	[133]

Table 1(*Continued*)

Analyte	Matrix	Detection	Linear range	R.S.D. (%)	Sample through-put (h^{-1})	Reference
R-perindopril			60–900 nmol/l	<0.1	30	[133]
Perphenazine	Synthetic samples	S (560 nm)	50–500 mg/l	<1	50	[70]
Phenylbenzimidazole sulphonic acid	Sunscreen sprays	S (300 nm)	50–80 μg/l	1–6	20	[63]
Piroxicam	Tablets and capsules	F (λ_{ex} 358 nm; λ_{em} 615 nm)	0.1–1.0 ppm	>3.8	60	[110]
Procaine	Oral solutions	CL (≥390 nm)	0.5–50 (g/ml	<3.8	120	[112]
Promethazine	Tablets, syrups; elixir	S (504 nm)	50–400 ppm	0.92	200	[72]
	Tablets	CL (200–750 nm)	1.6×10^{-5} to 1.9×10^{-3} mol/l	0.75	180	[115]
S-ramipril	Raw material	Amperiometric biosensor	0.12–0.60 μmol/l	<0.1	75	[133]
Sulphanilamide	Standard solutions	CL (≥390 nm)	0.015–1.50 mmol/l	<2.3	120	[113]
Sulphacetamide	Eye drops		0.012–1.20 mmol/l			
Sulphathiazole	Standard solutions		0.009–0.92 mmol/l			
Sulphadimidine	Standard solutions		0.035–0.87 mmol/l			
Sulphafurazole	Tablet		0.009–0.88 mmol/l			
Sulphamethoxypyridazine	Standard solutions		0.019–0.95 mmol/l			
Sulphaguanidine	Standard solutions		0.010–1.00 mmol/l			
Tetracaine	Standard solutions	CL (≥390 nm)	0.2–25 μg/ml	<3.8	120	[112]
	Powder	S (572 nm)	25–300 μg/ml	2.07	40	[98]
L-thyroxine (L-T4)	Tablets; injections	Amperiometric metric	10–780 ng/ml	>0.1	20	[134]
D-thyroxine (D-T4)	Tablets; injections	Immunosensor	50–500 nmol/l	>0.1	20	[134]
L-thriiodothyronine (L-T3)	Raw materials		15–380 ng/ml	>0.1	20	[134]
Trimeprazine	Synthetic samples	S (515 nm)	50–400 ppm	<1	50	[70]
Trimethoprim	Tablets	CL (370 nm)	0.5–100 μg/ml	<1	120	[114]
Warfarin	Spiked samples	F (λ_{ex} 310 nm; λ_{em} 385 nm)	0.1–1 μg/ml	1.5	180	[105]
Vitamin B_1	Multivitamin and Vitamin B tablets; multivitamin syrups	F (λ_{ex} 385 nm; λ_{em} 433 nm)	0.06–8.0 μg/ml	1.0	30	[109]
Vitamin B_2	Multivitamin and Vitamin B tablets	ASV	0.0–0.8 μmol/l	3	34	[120]
Vitamin B_6	Tablets, syrups; injections	ISE	1×10^{-4} to 1×10^{-2} mol/l	<0.47	50	[123]
Vitamin C	Tablets; effervescent tablets; capsules	S (510 nm)	20–300 ppm	<1.2	20	[87]
	Tablets; drops	S (410 nm)	30–200 ppm	<1.2	120	[88]
	Tablets	S (525 nm)	0–1200 mg/l	2.9	60	[89]
	Effervescent tablets; liquid preparation	Optode	2.5×10^{-3} to 1.0×10^{-1} mol/l	<2	17	[90]
		CL	5×10^{-8} to 1×10^{-5} mol/l	<4	180	[116]
Zinc	Supplement tablets; energy-boosting tablets	S (568 nm)	10–60 mg/ml	<1	30	[79]

R.S.D.: relative standard deviation; S: spectrophotometry; ISE: ion selective electrode; CL: chemiluminescence; F: fluorimetry, ASV: adsorptive stripping voltammetry.

mination of perphenazine [70]. These methods were successfully applied in the determination of these active ingredients in samples, which simulated the composition of pharmaceutical preparations. Rigorous control of the reaction volumes and times also permitted the determination of the reaction stoichiometry. SIA revealed itself to be equally useful for a complete investigation of the reactions involved: determination of the reaction order with respect to each reactant, determination of the kinetic energy and the postulation of a reaction mechanism. The reaction studied involved the complexation of bromazepam with iron(II), in hydrochloric medium and determination of the absorbance of the complex formed at 585 nm [68]. After the kinetic parameters were known, a kinetic method was developed for the determination of bromazepam. Fluoroquinolone antibiotics such as ciprofloxacin and norfloxacin were also determined as iron(II) complexes in sulphuric acid medium. This sequential injection technique was also employed for stoichiometric studies [71]. A kinetic approach, with the same potential as that previously described [71], was presented for the determination of promethazine [72]. The chemical reaction was based on the complexation of promethazine with palladium(II) in hydrochloridic medium and measurement of absorbance at 504 nm. The performance parameters of the proposed method were greater than those founded with other FIA methods previously described [73,74].

A SIA system was developed for the determination of captopril using two different modes of detection [75]. One of the captopril determination procedures was based on the colorimetric measurement of captopril–palladium complex in acidic medium while the other procedures involved the potentiometric titration of the analyte with an Ag solution, used as titrant. This system check the accuracy of the results in real time, by simultaneously comparing the results obtained with two independent analytical methodologies.

Chisvert et al. explored the bathocromic shift in the absorption maximum of oxybenzone in the presence of nickel(II) and ammonia, for its quantitative analysis in five sunscreen formulations [76]. Two methodologies, a FIA and a SIA, based on this reaction were compared. The sample throughput for the FIA configuration was three times higher but the SIA proposal was more economic in relation to the consumption of reagents and had a high level of automation due to the control of the system by the software. Also, in the same year the same authors proposed a similar procedure for the analysis of oxybenzone in sunscreen lipsticks. This analysis protocol required a sample pre-treatment out of the system for the extraction of the analyte [77].

2.1.1.2.2. Determination of metallic ions in pharmaceuticals. The determination of metals in pharmaceutical samples is normally carried out through the formation of coloured chelates, the only exception being iron determination which is determined as Fe(II) or as Fe(III) and which requires prior oxidation or reduction.

The determination of calcium in water, urine and pharmaceutical samples, based on its complexation with cresolphthalein and with spectrophotometric detection of the complex formed at 573 nm, was proposed by Van Staden and Taljaard [78]. These authors showed that the baseline shift due to a background, originating from coloured free reagent species, could be minimised by an optimised injection sequence of samples and reagents.

Van Staden and Tsanwani determined zinc in vitamin supplements, essential elements supplements and energy-boosting tablets based on the reaction of the analyte with xylenol orange and on the spectrophotometric monitoring of the complex formed at 568 nm [79]. The interference of copper(II) and lead(II) was masked by aspirating a sodium thiosulfate solution before detection of the coloured complex. It was not possible to eliminate the interference of nickel(II) but it was possible to successfully apply this methodology to six diverse products, since the presence of nickel in these samples was negligible.

For the determination of iron(III), the same group used a flow-through dialyser in a SIA system to separate the colorimetric and turbidimetric interferents present in pharmaceutical samples [80]. After on-line dialysis, the iron(III) was complexed with tiron and the resulting complex monitored spectrophotometrically at 667 nm. This methodology determined the total iron as iron(III), since iron(II) was oxidised to iron(III) before the samples being introduced into the system. The determination of total iron in pharmaceutical samples but this time as iron(II) was also shown [81]. The system incorporated a solid phase reactor which was constructed with cadmium granules and was used for the on-line reduction of iron(III) to iron(II). The iron(II) reacted with the 1,10-phenanthroline and the complex formed was monitored at 515 nm. This system presented a sample throughput of 24 samples/h. However, an excessively slow sample pre-treatment was necessary (more than 12 h). Oliveira and Masini proposed a methodology for the determination of iron(II) in pharmaceutical formulations without turbidity [82]. The determination was based on the reaction of iron(II) with 2,2-bipyridyl and monitoring of the complex formed. The only pre-treatment required was an appropriate sample dilution and the sampling rate was $100\,h^{-1}$. Another proposal for the determination of iron(II) used 1,10-phenanthroline as the complexing reagent [83]. Compared with the 2,2-bipyridyl procedure, this system showed a lower sampling rate, but was more sensitive and the detection limit was lower, making it more adequate for the determination of iron(II) in pharmaceutical formulations containing a low content of this element. The feasibility of Fe speciation in a simple way and with a high sampling frequency was also shown [84]. Initially the iron(III) was determined by comlexation with the tiron and monitoring of the complex formed. Subsequently, total iron was determined as iron(III), after the oxidation of iron(II) to iron(III) by additional injection of hydrogen peroxide. The iron(II) could be determined by subtracting the quantity of iron(III) initially calculated from the quantity of total iron.

Considering the favourable characteristics of the SIA technique, Tesfaldet et al. determined magnesium masking the interfering species on-line [85]. The method was based on the reaction between *o*-cresolphthalein complexone and Mg(II) in alkaline media, yielding a pink coloured complex with absorption maximum at 570 nm. The interference of calcium and zinc was greatly reduced by the use of masking-buffer solution and the correct order of sequential aspiration of the solutions into the system.

The spectrophotometric method reported by Tzanavaras et al. for the bismuth determination was based on the absorbance of the Bi(III)–methylthymol blue complex which was monitored at 548 nm [86]. The method was applied to the analysis of various pharmaceutical formulations containing Bi(III) and the validity of the method was tested by comparing the obtained results with those found using atomic absorption spectrometry as the reference. According to the authors the reagent consumption was considerably reduced compared to conventional flow injection systems as only 150 μl of methylthymol were consumed per sample.

2.1.1.3. Redox reactions. Numerous methods have been described in the analytical literature for the determination of drugs using oxidants that are not necessarily strong, due to the reducing capacity of the greater majority.

Sultan and Desai investigated the chemical reaction kinetics of Vitamin C oxidation and based on the data from the reaction orders with respect to each of the reagents involved, established a reaction mechanism [87]. In this reaction, iron(III) was used as oxidant, 1,10-phenathroline as indicator and the resulting complex was monitored spectrophotometrically at 510 nm. The system incorporated a mixing chamber and the sampling rate was 20 samples/h. However, the rate could have been increased without loosing reliability, in the absence of the mixing chamber. In 1999, Sultan et al. were the first to describe a spectrophotometric titration employing the SIA technique [88]. In this innovative work, Vitamin C was determined after its oxidation with cerium(IV) in sulphuric acid medium, measuring the decrease in absorbance of cerium(IV) at 410 nm. The mode of operation of this system was optimised using a chemometric design and after determining the optimum conditions, was applied to real samples. Compared with the conventional titration method and with the reference method from the British Pharmacopoeia, this proposal was superior in terms of precision and operator autonomy. The sample throughput was considerably greater compared with the previous determinations. As an alternative, a titration procedure was proposed based on the oxidation–reduction reaction between the ascorbic acid and the potassium permanganate in acidic medium and on measurement of the decrease in color intensity of potassium permanganate, monitored at 525 nm [89]. Newcombe et al. used an optode in a flow cell of a SIA system for the determination of Vitamin C [90]. The optical sensor was constructed incorporating a redox indicator, tris(1,10-phenantroline)-iron(II) in a Nafion membrane, which was inserted into a flow through cell. The analytical results from this application were in good agreement with the results obtained with a conventional iodometric titration. This permitted the automation of a titration in an economic way but according to the authors, a subsequent optimisation of the sensor and the operational conditions of the system were necessary to improve the sensitivity of the procedure and the sample throughput.

Salvador et al. developed a procedure for indirect spectrophotometric determination of *p*-aminobenzoic acid [91]. This determination was based on the reaction of the analyte with hypochlorite in acidic medium and the subsequent measurement of the residual chlorine through the reaction with *o*-tolidine. The performance of this system was identical, in terms of sampling rate and precision, to a system proposed in the previous year and which was based on the diazotization of the analyte, coupling with 8-hydroxyquinoline and the subsequent formation of a coloured product [92].

For the analysis of paracetamol in pharmaceutical formulations, a procedure was proposed by Van Staden and Tsanwani, involving its oxidation with potassium hexacyanoferrate(III) and the subsequent reaction with phenol in the presence of ammonia [93]. The results obtained were in agreement with a FIA procedure based on the same reaction although they were obtained in a more robust and economic way.

Beyene et al. used the ferricyanide-aminoantipyrine reaction for the sequential-injection spectrophotometric determination of fenoterol hydrobromide in pharmaceuticals [94]. Reagent consumption was significantly reduced when compared to the reported FIA method based on the same reaction [95] and waste generation was extremely minimised.

Erustes et al. determined the iodide in Pharmaceuticals and in drinking water samples. The method was based on the catalytic effect of iodide on the redox reaction between Ce(IV) and As(III) [96]. The calibration curve was constructed by measuring the decrease of Ce(IV) absorbance versus iodide concentration. This reaction showed interference with the halides (fluoride, chloride, bromide) and metals (Ag^{+} and Hg^{2+}) and for this reason, it was necessary to use a standard addition approach to eliminate the matrix effects. The pharmaceutical formulations that contained ascorbic acid and/or glucose had to be subjected to an additional pre-treatment since these components were interferents in the reaction used. Tesfaldet, Van Staden and Stefan reported the implementation of a SIA based procedure for the determination of trace amounts of iodide [97]. This was based on the catalytic effect of iodine on the oxidation of 4,4′-methyllenebis(*N*,*N*-dimethylaniline) (tetra base) by chloramines-T in acidic solution. The resulting compound was monitored spectrophotometrically at 600 nm. The procedure was selective, presented a high sampling frequency and was successfully applied in iodide determinations at μg level.

Recently, Fan and co-workers explored the reproducibility characteristics offered by SIA technology in the monitoring of the transient signals for the quantitative determination of analytes in non-equilibrium conditions [98]. In that work, metoclopramide and tetracaine were determined spectrophotometrically, based on the detection of an unstable red intermediate compound resulting from the reaction of analytes with potassium dichromate in the presence of sodium oxalate in sulphuric acid solution. The related reaction mechanisms were also investigated and two different sequential injection models were proposed. The results presented indicated that the continuous flow model had the wider linear range and the higher sampling frequency, whereas the stopped-flow model had the higher sensitivity.

Tzanavaras et al. proposed two flow configurations for the determination of captopril; one FIA and the other SIA, both based on the classic procedure of oxidation of the drug with the ferric ion [99]. The resulting ferrous ion was complexed with 2,2′-dipyridyl-2-pyridylhydrazone in acidic medium, giv-

ing rise to a coloured compound that was monitored at 535 nm. After optimisation of both methods, captopril was determined in commercial anti-hypertensive formulations, whose normal excipients do not interfere in the methodology.

2.1.1.4. Other methods. Van Staden and Tsanwani described a SIA system for the determination of boron as boric acid in eye lotions [100]. The method was based on the complexation reaction between D-sorbitol and boric acid followed by the reaction with methyl orange.

The bopindolol was determined using a color reaction of its indole group with Ehrlich's reagent in acidic medium [101]. This method was revealed to be quite selective and despite the reaction speed being slow, when compared to the other methods published for the determination of this active ingredient, was considered quite rapid.

For the analysis of sunscreen formulations, a SIA system was developed for the spectrophotometric determination of *p*-aminobenzoic acid [92]. The method was based on the diazotization of the analyte, the subsequent complexation with 8-hydroxyquinoline and monitoring of the complex formed. This method did not require any sample pre-treatment and was validated using a HPLC procedure.

Burakham et al. proposed a methodology for the determination of paracetamol based on the nitrosation reaction [102]. The paracetamol was nitrosated on line with the sodium nitrite in an acidic medium and then the formed nitroso derivative species reacted further with sodium hydroxide to convert it to a more stable compound that was monitored continuously at 430 nm. Two SIA and one FIA configuration modes were tested. Comparatively, the FIA configuration required a manual operation and an extra dilution was required for sample handling. The second SIA manifold proposal was less complicated since it used an autoburette instead of a syringe pump and only one selection valve instead of the two required by the first proposal. The analytical quality obtained with the three methodologies was very similar and the results coincide with the method proposed by the American Pharmacopoeia.

Beyene et al. converted a FIA system previously published for the determination of etilefrine hydrochloride, based on its condensation with 4-aminoantipyrine, to a SIA system [103]. Comparatively, this system showed a lower consumption of reagents and minimum waste generation. Latter, Beyen et al. described the determination of isoxsuprine hydrochloride based on its condensation with 4-aminoantipyrine in the presence of potassium hexacyanoferrate [104].

2.1.2. Fluorescence

With the reduced cost of instrumentation, fluorimetry has had a more widespread use. The procedures involved elevated selectivity determinations to be carried out since the displacement of the Stokes is characteristic for the analyte measured. The sensitivity is equally more elevated than in the spectrophotometric procedures both by the lower interference of the matrix and by the proportionality between the intensity of the radiation source and the analytical signal measured. The analytical range of the two or three tenths of concentration is equally another characteristic of the fluorimetric procedures that permits the pre-processing of the samples to be simplified.

Tang et al. proposed a SIA system for the determination of warfarin exploring the phenomenon of fluorescence enhancement which is observed in organised mediums; in this case the molecule forms an inclusion complex with the β-cyclodextrin [105]. The sampling rates were in the order of 180 determinations/h and were applied to the analysis of water samples spiked with warfarin.

Recently, Economou et al. presented a system with fluorimetric detection for the determination of boric acid [106]. This method relies on enhancing the fluorescence of chromotropic acid as a result of its complexation with boric acid. The method was applied to the determination of boron in natural waters and pharmaceutical products, with recoveries in the range of 96–106%.

The same group extended the application of SIA to the determination of lisinopril in two pharmaceutical formulations [107]. This method was based on reaction of the analyte with *o*-phthalaldehyde in the presence of 2-mercaptoethanol at a pH of 10.6 with borate buffer medium. The method was adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples.

Diclofenac was determined by fluorescence after on-line irradiation with UV light [108]. This system also included a potentiometric determination with an ion selective electrode based on a cyclodextrin. Both determinations were aimed at exploring the concept of accuracy assessment previously described by the same authors.

Zhu et al. adapted the sequential injection renewable surface technique to solid-phase spectrofluorometry for thiamine determination [109]. The fluorimetric determination required the conversion of the analyte to fluorescent thiochrome by hexacyanoferrate(III) oxidation in alkaline solution and the isolation of the produced thiochrome from the reaction medium by solid-phase extraction. The SIA system on-line coupled to a chip-based flow-through cell was employed to handle the chemical reaction, bead injection and discharging as well as adsorption of thiochrome. Compared with the conventional thiochrome method which requires the extraction of thiochrome with organic solvents, this method avoided the use of flammable and toxic organic solvents and had the merit of higher analytical speed, better reproducibility and reduced manual operation.

Kindy et al. developed a sensitive and robust microanalysis procedure for the determination of piroxicam in pharmaceuticals using sensitized europium fluorescence [110]. The method was based on the luminescence sensitization of europium by complexation with piroxicam and was successfully applied for the analysis of piroxicam in capsules and tablets.

Recently a normal spectrophotometric and stopped-flow spectrofluorimetric method have been developed for the determination of alendronic acid in tablets [111]. The spectrophotometric assay was based on the reaction of the analyte with Cu(II) ions in acidic medium and the spectrofluorimetric method was

based on the reaction of alendronic acid with *o*-phthalaldehyde in the presence of 2-mercaptoethanol in basic medium.

2.1.3. Chemiluminescence

Chemiluminescent processes can be classified into two groups. One of the groups covers the methods in which the analyte itself is the emission specie through the direct action of common strong oxidants (mainly permanganate, although other reagents such as cerium(IV) are also used). The other group covers the indirect methods in which the analyte interferes in some way (as a catalizer, inhibitor, etc.) in the oxidation reaction of the chemiluminescent precursors such as luminol, lucigenine, etc. These latter methods are the "classics" but the current interests in liquid phase analytical chemiluminescence are dedicated to finding new chemiluminescent reactions directly over the analyte. Those that utilize the SIA methodology belong to this group.

Paseková and Polásek used a SIA system for the determination of procaine, benzocaine and tetracaine with chemiluminescence detection [112]. Chemiluminescence was emitted during the oxidation of the analytes by potassium permanganate in aqueous sulphuric acid in the presence of various chemiluminescence enhancers. The monitoring of the respective reaction kinetics revealed different intensity–time profiles for the three analytes with the sensitivity of the measurements being greater for procaine and benzocaine.

Subsequently, Paseková et al. related the chemiluminescence of the permanganate to the determination of a series of sulphonamides [113]. In this work, the effect of various enhancers used in the chemiluminescence assay with potassium permanganate was studied with glutaraldehyde having been selected for subsequent assays. These authors presented determination methodologies for various sulphonamides but only the determination of sulphacetamide and sulphafurazole were applied to real samples.

Polásek and Jambor determined trimethoprim based on its oxidation reaction with potassium permanganate and using hexametaphosphate as enhancer [114]. This reaction was accompanied by the emission of chemiluminescence at 370 nm. The sulphonamides were strong interferentes and their presence in the samples made the determination of trimethoprim impossible. In the majority of formulations presented in the market, this active ingredient is found in association with sulphonamides and for this reason, this methodology was only successfully applied in the analysis of formulatioins containing just trimethoprim as active ingredient.

For the determination of promethazine, Sultan et al. developed a flow system with chemiluminescent detection based on the measurement of emission intensity produced as result of its oxidation reaction with permanganate in sulphuric acid medium [115]. This combined FIA/SIA manifold set-up utilized a peristaltic pump for the carrier flow and a syringe pump for injecting the analyte directly into the carrier to monitor the short life chemiluminescence reaction. The chemical and physical conditions of the system were optimised using a chemometric method.

Anastos et al. compared the determination of ascorbic acid with chemiluminescence detection using soluble manganese(IV) and acidic potassium permanganate as reagents in a FIA and SIA system [116]. The detection limits for the four methodologies were greater using the reaction with permanganate and using the FIA systems. The ascorbic acid was determined in Vitamin C tablets using the standard additions calibration, since this provided the means of overcoming the matrix interferents observed with external standardisation in the four methodologies.

Pinto et al. reported the development of a SIA system based on the utilisation of pulse generating solenoid micro-pumps, which replace the conventional solutions propelling units commonly use. This controlled pulsed flow was favourably applied in the implementation of a SIA methodology for the fluorimetric determination of indomethacin in pharmaceutical preparations upon alkaline hydrolysis in micellar medium [117]. Latter, the same authors proposed a methodology for the fluorimetric determination of aminocaproic acid [118]. The procedure was based on the derivatisation reaction of the aminocaproic primary amine with *o*-pthalaldehyde and *N*-acetylcysteine and the fluorimetric detection of the formed product. This system included an on-line dilution strategy that enabled the expansion of the analytical working range of the methodology, and thus its application in dissolution studies, without manifold reconfiguration.

Zisiou et al. developed a sensitive methodology for determination of naproxen based on its complexation with β-cyclodextrin yielding an enhanced fluorimetric signal [119].

2.1.4. Electrochemistry

Electrochemical methods are adequate for flow analysis due to their favourable characteristics of sensitivity, controllable selectivity, good precision and accuracy, simplicity and easy of signal handling and automation. However, the sensors are difficult to conceive and develop and the electrochemical methods often suffer from interaction between the sensor and the test liquid, leading to poor stability and reproducibility of the active electrode surface. These problems can be overcome by rigorously selecting the experimental conditions and in a general way, the sensors associated with the SIA give rise to robust, portable, low-cost and high-throughput systems to perform multicomponent determinations. SIA systems with voltametric, potentiometric and amperiometric detection are found in the literature.

Kubiak et al. developed a SIA system with adsorptive stripping voltammetric detection for the determination of Vitamin B_2 in tablets, based on the adsorption of this molecule on the mercury electrode [120]. The same system was used to study the process of photodegradation of this vitamin in aqueous solutions.

Recently Gutés et al. developed a SIA system to be used with higher dimensional data generated using electrochemical sensors [121]. The manifold included a magnetically stirred mixing cell with the aim of obtaining an automatic way of preparing standards needed in the study of higher dimensional data systems. Different analytical techniques were used: automated potentiometric calibrations with and without interference, determinations of selectivity coefficients, simultaneous voltammetric determinations of lead and cadmium and a voltammetric electronic tongue for the determination of oxidizable species. The authors used an artificial neuronal network (ANN) to design the

electronic tongue and solve the overlapped signal of ascorbic acid, 4-aminophenol and paracetamol. The final analysis system quantified these three compounds in test solutions but was not applied to real samples.

Van Staden et al. utilized a fluoride-selective membrane electrode for the determination of this ion in toothpaste and tap water [122]. This work presented the evaluation of the peak profiles of four different buffer-sample SIA configurations (buffer-sample, sample-buffer, buffer-sample-buffer, sample-buffer-sample). For the determination of fluoride in toothpaste, the best performance characteristics of the methodology were obtained with the buffer-sample configuration. The sandwich configuration was more convenient for the determinations of low concentration levels of fluoride, as occurs in drinking water samples.

Fernandes et al. determined Vitamin B_6 in pharmaceutical formulations by direct potentiometry using a Vitamin B_6 selective electrode with a PVC membrane, without internal reference solution [123]. In this work the authors proposed the coupling of a solenoid valve to a conventional SIA mounting. The potential of this system included: calibration, electrode characterisation procedures, standard addition technique and a potentiometric titration. The fully automated procedures were carried out from a single standard solution and did not require any physical reconfiguration of the system.

A system with two potentiometric detectors was used for the determination of potassium clavulanate in pharmaceutical formulations [124]. The simultaneous attainment of the two measurements, one of clavulanate and the other potassium, permitted the standardisation of the results in real time, the detection of faults in the procedure and monitoring the chemical stability of clavulanate. By further exploring this concept, a system was constructed for the determination of chloride using an electrode and an optode based on the same ionophore [125]. The better performing determinations were achieved using the ion selective electrode.

Santos et al. constructed ion selective electrodes for penicillin-G based on a metalloporphyrine and evaluated their performances using different polymeric matrices and different additives [126]. The electrode which supplied the best results in relation to reproducibility as well lower response time was coupled to a SIA system. This system was applied to the analysis of penicillin-G injections although when the lower limit of linear response for the electrodes was greater than the solubility of the penicillin salts, their determination was not possible.

In a review article, Stefan et al. analysed the state of the art in the process of construction and utilisation of enantioselective sensors in flow analysis systems for the analysis of pharmaceutical products [127]. In this context, they described an amperiometric biosensor based on L-amino acid oxidase (L-AAOD) immobilized on a graphite paste electrode for the enantioselective determination of *S*-captopril [128]. The recoveries for this analyte in the presence of four molar equivalents of *R*-captopril, proline or polyvinylpyrrolidone were in the order of 100%. In the same year, these authors utilised the same previously described detector and another biosensor based on D-amino acid oxidase (D-AAOD) for the simultaneous determination of *S*- and *R*-captopril [129]. This system determined the active ingredient (*S*-captoril) as well as one of its synthesis impurities (*R*-captopril, without pharmacological activity). According to the authors, this system could be used for the on-line monitoring of the process of synthesis of this quiral drug. Also in the same year, these authors presented another system with simultaneous detection of *S*- and *R*-captoril [130]. In this work, they utilised an enantioselective membrane electrode based on maltodextrin for the potentiometric detection of *S*-captopril and the amperiometric sensor previously described for the determination of *R*-captopril. The two amperiometric detectors previously described (based on D- and L-amino acid oxidase) were also applied in the simultaneous determination of *S*- and *R*-perindopril [131]. These two detectors were incorporated in two flow analysis systems (FIA and SIA). The SIA configuration was considered more efficient due to the greater precision and accuracy as well as the lower consumption of sample and carrier. In 2003, some of these authors simultaneously determined L- and D-methotrexate (Mtx) using a sequential injection analysis/amperiometric biosensors [132]. Various biosensors were developed and studied based on L-AAOD or/and L-glutamate oxidase (L-Glox) and horseradish peroxidase (HRP) for the detection of L-Mtx and D-AAOD and HRP for the D-Mtx assay. The linear concentration ranges were from pmol to nmol magnitude order with very low limits of detection. These sensors were used to evaluate the enantiopurity of raw materials and pharmaceutical formulations. In the following year, some of these authors returned to use amperiometric biosensors based on L-AAOD for the determination of *S*-enantiomers of enalapril, ramipril and pentopril [133]. Stefan, van Staden and Aboul-Enein developed a SIA system designed for the simultaneous assay of L-thyroxine (L-T_4), D-thyroxine (D-T_4) and L-thriiodothryonine (L-T_3) [134]. For the L-T_3 assay they utilised an amperiometric biosensor based on (L-AAOx) and for the determination of L-T_4 and D-T_4 they utilised immunosensors constructed on a chemically modified carbon paste that contained anti-L-T_4 and anti-L-T_3, respectively. The system incorporated the three sensors and detected the three analytes in raw materials, tablets and injections. Later, they developed another amperiometric immunosensor based on the physical immobilisation of anti-azidothymidine in a carbon paste for the analysis of AZT in raw materials and pharmaceutical formulations [135]. This sensor could be utilised in batch analysis or with the SIA system.

2.2. Automated process analysis in pharmaceutical prodution

The analytical determinations carried out in monitoring the chemical and biochemical reactions involved in the production processes should systematically supply quantitative measurements of the various parameters that control those reactions [136,137]. These determinations must eliminate, or at least minimise, the sample preparation steps to in that way rapidly obtain a report on the state of the production process. The attainment of this chemical information in real time is extremely useful for improving the consistency, quality and quantity of the productive processes. Process control can be utilised to carry out corrections

Table 2
SIA systems for process monitoring

Analyte	Matrix	Detection	Linear range	R.S.D. (%)	Sample throughput (h^{-1})/assay (h)	Reference
Morphine	Water streams of *P. somniferum*	CL (250–650 nm)	2.5×10^{-6} to 3.0×10^{-6} mol/l	1.4	100/144	[141]
Morphine	Water immiscible streams of *P. somniferum*	CL (250–650 nm)	0.001–0.1% m/v	6	120/144	[142]
Penicillin, glucose, lactic acid	*P. chrysogenum* cultivations	CL	0.010–1.200 g/l, 0.010–7.000 g/l, 0.0050–5.000 g/l	2.06, 2.56, 2	30/160, 20/160, 20/160	[139]
Penicillin, glucose	*P. chrysogenum* cultivations	CL, S, QL	0.10–1.8 g/l (QL), 0.10–1.8 g/l (S), 0.010–7.000 g/l	–	–/425, –/350, –/45	[140]

R.S.D: relative standard deviation; S: spectrophotometry; CL: chemiluminescence.

to the productive processes if necessary, being of vital importance for the efficient use of energy, time and raw materials. For its robustness, capacity to collect the sample directly from the reaction medium and possibility of determining the different species, the SIA technique has been revealed to be adequate for the control of industrial production processes [39,138]. The SIA systems were also applied in the monitoring of the production processes of some drugs (Table 2).

Rong et al. demonstrated the potential of SIA in the monitoring of the fermentation process used for the production of penicillin [139]. The SIA system was developed for the monitoring of glucose, penicillin and lactic acid during cultivations of the filamentous fungus *Penicillium chrysogenum.* These were the three crucial parameters in the production process, since the glucose acted as a source of carbon and energy and had to be maintained at controlled levels during the long phase of production, the lactic acid was an important criteria for the control of the bioculture and the penicillin was the product to be obtained. For the determination of glucose and lactic acid, glucose oxidase (GOD) and lactate oxidase (LOD) were immobilised in enzymatic reactors to catalyse the oxidation of the β-D-glucose and lactic acid to β-D-glucono-δ-lactone and pyruvate, respectively. These two reactions concomitantly gave rise to the formation of hydrogen peroxide which was detected by chemiluminescence after addition of luminol and the $K_3Fe(CN)_6$. The determination of penicillin was based on the formation of penicilloic acid through its catalytic hydrolysis in a reactor immobilised with penicillinase. The penicilloic acid reacted with the iodine and partially extinguished the signal obtained through the chemiluminescence reaction generated between the iodine and the luminol. The system continually monitored the fungi cultures in the course of 140 h but was not explored to its full potential, since the sample collection was carried out outside the system. The measurements obtained were comparable to those obtained with the application of the HPLC method. The analysis times were considerably higher than those obtained with the same procedures in a FIA configuration. Despite this disadvantage, they were considered advantageous and acceptable for analysis on an industrial scale. In an attempt to improve the previously proposed system, these authors described a mounting that permitted the on-line analysis of glucose and penicillin [140]. The glucose and penicillin were determined by the same previously mentioned methods while the penicillin was also analysed through the reaction of penicilloic acid with the iodine and measurement of the consumption of the latter, by the decrease in the absorbance of an iodide–starch complex. In this system, the samples were introduced directly and any sample dilution step was not necessary since the volumes could be automatically adjusted. The reliability of the system in the long term permitted penicillin to be determined through more than 400 h of fermentation. The results of the three on-line determinations were in agreement with those obtained by a HPLC procedure, off-line, but the iodimetric method for the determination of penicillin was simpler and yielded better results.

Barnet et al. described two SIA systems to monitor and control the process of morphine production [141,142]. The production of this alcaloide consisted of its extraction form the *Papaver somniferum* plant and involved a series of extractions in various aqueous and non-aqueous solvents. Control of the concentration of morphine in these extracts directly influenced the efficiency of the process and was determined based on the chemiluminescence reaction with acidic potassium permanganate in the presence of sodium hexametaphosphate. The emission of chemi luminescence was monitored at 610 nm transferring the light from the flow cell, with a fibre optic, to a photomultiplicator detector. In the first system presented [141], the morphine was determined in aqueous extracts and the results obtained were compared with a HPLC-independent procedure. The results of some extracts were in agreement while others revealed some matrix effects. Two years later, the same authors determined the concentration of morphine in non-aqueous extracts [142]. This SIA methodology presented a weak repeatability compared with the HPLC methodology due to the heterogeneous nature of the reaction mixture. Nevertheless, the results were comparable and the sample throughput was higher than those managed with the HPLC methodology used in industrial control. However, these authors failed to explore the potential of SIA, since the measurements were carried out off-line, requiring a manual pre-treatment of the sample before being introduced into the system.

2.3. Automated drug-dissolution and drug-release testing

The dissolution assays of pharmaceutical formulations are important for quality control of pharmaceuticals. When these tests supply detailed information on the dynamic characteristics

Table 3
SIA systems for drug-dissolution and drug-release testing

Analyte	Matrix	Detection	Linear range	R.S.D. (%)	Sample throughput (h^{-1})/assay time (min)	Reference
Acetylsalicylic acid	Conventional tablets Sustained release tablets	ISE	0.05–10 mmol/l	<2	20/30 6/9	[146]
Aspirin Phenacetin Caffeine	Multi-component tablets	S (220–310 nm)	44–220 mg/l 30–1500 mg/l 10–50 mg/l	0.5 0.4 0.7	15/60	[145]
Ergotamine	Conventional tablets	F (λ_{ex} 236 nm; λ_{em} 390 nm)	0.03–0.61 mg/l	<0.86	120/20	[147]
Ibuprofen	Conventional tablets; sustained-release capsules; controlled-release matrix tablets	S (222 nm)	0–0.12 g/l 0–0.35 g/l	0.5 0.3	42/80 42/720	[144]
Indomethacin	Gels and ointments	F (λ_{ex} 330 nm; λ_{em} 385 nm)	0.05–10 mg/l	2.3	120/360	[150]
Prazosin hydrochloride	Conventional tablets	F (λ_{ex}244 nm; λ_{em}, 389 nm)	0.02–2.43 mg/l	1.89	70/60	[148]
Salicylic acid	Ointments	F (λ_{ex} 297 nm; λ_{em} 405 nm)	0.05–10 μg/l	0.52	120/360	[151]
Rutin trihydrate	Multi-component tablets	S (262 nm)	2–20 μg/ml	0.4	26/72	[149]
Vitamin C	Multi-component tablet	S (262 nm)	0–100 μg/ml	0.7	26/72	[149]

R.S.D: relative standard deviation; ISE: ion selective electrode; S: spectrophotometry; F: fluorimetry.

of the dissolution process, they are also useful for accompanying the studies on the development of new dosage forms. The majority of the official assays described in the pharmacopoeias are based on the manual collection of the sample from the dissolution medium for some, or just one, pre-determined period of time. The analytical information obtained in that way is insufficient to describe the kinetic dissolution process, making a contribution in the studies of the conception and evaluation of new dosage forms impossible. Furthermore the manual batch analytical procedures present low sampling rate, are not very practical and particularly difficult, or even impossible to execute, when various tests are running in parallel in the multidissolution vessels (usually six).

The continuous monitoring of the active ingredients in the dissolution mediums for this reason requires its automation. The following features for an ideal automated system have been called for to fulfil such tasks [44]:

(1) fast determination and on-line sample pre-treatment permitting almost real-time monitoring;
(2) high sampling frequency allowing high resolution of the dissolution processes;
(3) low sample consumption, introducing minimum disturbance to the volume of the dissolution medium, particularly when high sampling frequencies are involved;
(4) parallel testing in multivessels using a single detector;
(5) simultaneous determination of multicomponents in a dosage form;
(6) highly stable sample pre-treatment and detection capable of continuous operation over extended periods;
(7) continuous baseline monitoring and on-line recalibration of the detection system;
(8) low reagent consumption.

The SIA systems can bring together these characteristics. Recently, the advantages of direct sample collection in the dissolution medium (through on-line filtration), the carrying out of parallel tests in the multi-dissolution vessels, the application of chemometric methods for the determination of multicomponents were explored with sequential injection technique (Table 3).

Although official methods have been developed for dissolution test studies of solid dosage forms, no official rule for the performance of release testing in semi-solid dosage forms is given. The only existing model tool for in vitro monitoring of the liberation process, recommended by FDA [143], was recently automated connecting the proposed device with a SIA system.

The first SIA system used for dissolution assays was described by Liu and Fang and was used to evaluate the dissolution profiles of ibuprofen tablets, sustained-release capsules and controlled-release matrix tablets [144]. In this system, the samples were collected after on-line filtration and the ibuprofen determined by monitoring the absorbance at 222 nm. The system carried out six dissolution tests in parallel, with a sampling rate of 12 samples/h while the consumption of the sample over a period of 12 h represented just 1.6% of the total volume of the dissolution vessel. The excellent long-term precision (relative standard deviation of 2.18%) demonstrated the stability and robustness of the automated system.

Subsequently, these authors took advantage of the versatility and flexibility of this system for simultaneously monitoring aspirin, phenacetin and caffeine in compound aspirin tablets [145]. For the simultaneous determination of these three compounds, a partial least-squares calibration technique was used. When the sample aliquot reached the detector, the flow stopped and the scan of the spectrum was initiated in the region between

220 and 310 nm. The sampling rate was 15 samples/h and the sample consumption was 0.2 ml per determination. This system did not require the separation of the analytes, was quick, easily adapted to monitoring various dissolution vessels and permitted the attainment of results in real time, with an elevated resolution of the dissolution kinetics in the three analytes.

Paseková et al. utilised a salicylate-selective electrode for the determination of acetylsalicylic acid after its on-line chemical hydrolysis, in pharmaceutical formulations and in dissolution assays [146]. The results obtained in the acetylsalicylic acid dosage assays in various pharmaceutical formulations, including composed and effervescent tablets, were in agreement with the reference HPLC methodology. The same methodology was applied to evaluate the dissolution profiles of conventional and composed aspirin tablets (30 min of analysis, and 10 determinations) and a sustained release tablet was also tested (90 min of analysis and 9 determinations). The profiles obtained were comparable to those obtained with the dissolution assays described in the American Pharmacopoeia. These two methodologies selectively determined the acetylsalicylic acid even in the presence of other active substances.

Legnerová et al. applied the SIA concept in the monitoring of the dissolution assays of ergotamine tartrate in pharmaceutical formulations [147]. The samples were filtered and aspirated by the system and sent for fluorescent detection. These dissolution assays had a duration of 20 min, a sampling rate of 120 samples/h and a sample consumption per measurement of 50 μL. Various dissolution tests of ergotamine tablets were carried out and the results obtained were in agreement with the British Pharmacopeia reference methodology. Later, they proposed the fully automated approach for monitoring the profiles of prazosin hydrochloride by fluorescence [148]. These dissolution tests had a duration of 60 min, a sampling rate of 70 samples/h and a sample consumption per measurement of 20 μL.

A new methodology was proposed for the simultaneous separation and determination of the two active ingredients in a combined pharmaceutical formulation, using a solid phase extraction microcolumn coupled to a SIA system [149]. This methodology was based on the spectrophotometric separation and determination of ascorbic acid and rutin trihydrate and was applied to the dissolution test and assay. This combination of chromatographic sorbent with SIA yielded a hybrid technique which exhibits the advantages: analyte separation, full automation and on-line performance of dissolution studies, high sample throughput and non-continuous flow, and drastic decrease in organic waste generation.

To test the extent of liberation of the active ingredients in semi-solid formulations, Klimundová et al. coupled the SIA mountings to a liberation apparatus known as the *Franz cell* [150]. In this work, membranes from different materials (poly-carbonate, mixed esters of cellulose, teflon, silicon, poly-vinylidene fluoride) and different pore diameters were studied, to compare the dissolution profiles of indomethacin 1% gel. After these studies, a poly-carbonate membrane was selected (pore diameter 0.4 μm), to compare the liberation rates of indomethacin in gels and ointment prepared by different technological procedures. The liberation profiles of the three semi-solid pharmaceutical preparations were monitored during the whole 6 h cycle without any necessity of human control.

Recently, the same authors applied the same system to evaluate the drug release from three different topical semisolid formulations containing 3% salicylic acid [151]. This system did not require any human control during the experiments and the possibility of coupling 6 Franz diffusion cells makes this system a useful tool for the release tests used during manufacturing process control, monitoring pre- and post-changes in product properties, monitoring batch uniformity as well as preformulation screening and product development.

2.4. Functional cellular assays for screening potential drugs

Ligand/reagent binding to a cell receptor site is not indicative of a subsequent physiological response. The identification and characterization of drug candidates require that the efficacy of a drug be determined. Such assays are referred to as functional assays and are designed to classify a drug as agonist or antagonist, depending on whether it evokes or inhibits a biological response. If the biding of the drug to the receptor induces a cellular response, this can be evaluated by monitoring suitable parameters such as release of cytosolic Ca^{2+}, intracellular pH, acid release, lactate extrusion, glucose consumption, oxygen consumption or even the potential of the membrane. Functional assays carried out using manual techniques are usually conducted by repeatedly exposing the same biological material to different concentrations of drug, rather than using fresh material for each dose. This situation can corrupt the cells' physiological responses, reducing the sensitivity of the sensors or even destroying the biological materials. Automation of the biological assays by the SIA technique through using reagents in the heterogeneous SI–BI phase [42,152–154] was recently proposed using various cell lines and measuring various evaluation parameters of the cellular response (Table 4). The cells used in these assays are adsorbed to the surface of the microbeads, with it being possible to obtain a representative sampling using a small precise volume of a medium with these particles in suspension. Also, because each injection of stimulant reacts with a fresh portion of cells through controlled exposure periods, the response is very reproducible and the biological variability that occurs when cells are cultivated by the traditional way is eliminated. In addition, BI provides kinetic information that cannot be gleaned when stimulants are manually added to a cell culture. Based on the kinetic behaviour, with respect to the initial onset receptor interaction, the initial rate, the duration and the maximum response, it is possible to differentiate, classify and determine the efficacy among various agonists.

Hodder and Ruzicka used Chinese hamster ovary cells transfected with the rat muscarinic receptor (CHO M1) [155] in a functional assay of agonists. These cells were cultivated in microbeads, loaded with calcium intracellular sensitive fluorescent probe (fura-2-am) and finally suspended in a buffer. The BI–SI system automatically aspirated and captured a small volume of this suspension of particles to a jet-ring chamber.

Table 4
SIA systems for drug discovery functional assays

Analyte	Matrix	Detection	Linear range	Assay time (s)	Reference
Intracellular calcium	CHO M1 cells exposed to acetylcholine, pilocarpine, atropine	Fluorescence measure with fura-2	NA	500 s	[155]
Glucose	TABX2S cells in basal metabolism and with azide	S (340 nm)	0.1–5.6 mmol/l	2 min	[158]
Glucose lactate	TABX2S cells in basal metabolism and with azide	S (340 nm)	0.1–5.6 mmol/l, 0.05–1.00 mmol/l	10 s, 30 s	[159]
Oxygen	CHO M1 cells exposed to amobarbital	Fluorescence measure with Pt–porphyrin complex	NA	3 min	[156]
Intracelular pH acidic extrusion	CHO M1 cells exposed to carbacol	Fluorescence measure with BCECF-AM	NA	700 s	[157]

N/A: not applicable; S: spectrophotometry.

The cells captured in the microcolumn were subsequently perfused with the drugs. The responses caused by the drugs were evaluated by monitoring the transitory liberation of cytosolic calcium, resulting from the stimulation of the receptor. In the final stage of each analytical cycle, the cells were automatically discarded and the analysis could again be repeated using new cells. The cellular responses to various concentrations of two agonists (acetylcholine and pilocarpine) and an antagonist (atropine) were studied. Dose-response curves constructed discriminated the partial agonists (pilocarpine) from the full agonists (acetylcholine), classifying these drugs according to their pharmacological properties. This system also evaluated the influence of muscarinic antagonists on the intracellular calcium response. This was achieved by analysing a series of samples that consisted of a mixture of acetylcholine at a constant concentration, and antagonist at a variable concentration. The decrease in the intracellular calcium response was dependent on the dose of the antagonist and its efficacy could also be determined. The results of the pharmacological parameters evaluated were compared with those described in the literature. These data were considered useful for the discovery of neurological and gastrointestinal Pharmaceuticals, since the muscarinic receptors are important pharmacological targets of this group of drugs.

In the same year, Lahdesmaki et al. proposed a SI–BI system to measure the cellular stimulation through metabolic oxygen consumption [156]. Oxygen has a central role in the aerobic metabolism of cells, functioning as a final electron acceptor in the aerobic oxidation route and the consumption measurements can be used to study the biochemical events when the aerobic pathway is affected. Detection of oxygen consumption is achieved trough measurement of the extracellular oxygen consumption, i.e., by probing another quantity of the bulk solution surrounding the cells. The proposed system was used to study the effect of amobarbital and acetylcholine in the CHO M1 cells through monitoring the consumption of oxygen. The sensing is based on oxygen-dependent quenching of phosphorescence in a Pt–porphyrin complex immobilized on microcarrier beads, which were also used as the cell culture substrate. This work demonstrated that the stimulation of the cells' muscarinic receptors increased the consumption of oxygen, reflecting a disturbance in the energetic metabolism caused by some drugs.

Lahdesmaki et al. presented a similar SI–BI system to measure the cellular responses caused by drug exposure through evaluating the intra- and extracellular pH [157]. The generation of acidic products form part of the metabolic energetic process of all living cells. Consequently, the intra- and extracellular pH values can be monitored to study the events that disturb cellular metabolism, for example during the stimulation of the cells by a chemical agent. In the presented work, it was possible to measure these two parameters using the same system. For intracellular pH measurement, the cells were cultivated on microbeads and were subsequently stained by incubating them in a solution of fluorescent pH indicator. These microbeads were packed in a microcolumn through the BI system and exposed to the stimulant. Alterations in the intensity of the fluorescence were proportional to the intracellular pH variations. For the evaluation of extracellular pH, an insoluble fluorescent indicator was immobilised at the surface of the microbeads and only afterwards were the cells on these marked particles cultivated. These microbeads were inserted into the BI system in a similar way and the extracellular pH response was monitored by fluorescente detection. No physical alteration of the system was necessary to carry out these two measurements—the only difference was the placement of the indicator: either on the inside of the cells to measure the intracellular pH or on the outside, adjacent to the external membrane of the cells, to measure the extracellular pH. The study was undertaken with CHO M1 cells and utilising carbachol as stimulant. Exposure to carbachol resulted in a subtle increase in acid release and in a transitory acidification in the cytosol. The two alterations were dose-dependent, making it possible to obtain dose–response curves. Subsequently, the efficacy of carbachol as a stimulant of the CHO M1 cells was determined. The effective agonist concentration corresponding to half-maximal response (EC_{50}) calculated was comparable with the value obtained using another procedure described in the literature. Recently, Schulz and Ruzicka demonstrated that the lab on valve (LOV) concept can also be linked to BI to evaluate the consumption of glucose in situ of living cells [158]. Glucose is the major source of carbon and energy utilised by living cells and its consumption can

be used to assess the metabolic state of the cells after chemical stimulation or to study the metabolic effects before, during and after the occurrence of a hypoxic event in the cells. In this first application of LOV to biological assays, the murine hepatocyte cells (TABX2S) were cultivated on microbeads and thereafter packed and perfused in a microcolumn with a microbioreactor, under controlled temperature conditions. The glucose consumed was determined by evaluating its concentration in the perfusate through an enzymatic process which took place in the flow cell equipped with fibre optics. Glucose sensing was based on an enzymatic reaction which occurred in two steps and gave rise to NADH as the reaction product, which was monitored at 340 nm. These tests were also conducted in the presence of azide, which is a cytochrome *C* oxidase inhibitor involved in the processes of glucose degradation via the aerobic pathway. In the presence of azide, the glucose could only be degraded anaerobically and consequently, the cells increased the consumption of glucose to compensate the lower energetic return obtained by this pathway. These results validated those previously obtained.

Also in the same year, Schulz et al. proposed a LOV system linked to BI for the determination of glucose consumption and lactate extrusion of cultured cells [159]. The rate of cellular lactate extrusion combined with the rate of cellular glucose consumption, provides a means to access the metabolic regime of a cell culture. By comparing these values, an estimate of the portion of energy that is being generated by anaerobic and aerobic metabolism can be made. The two pieces of information arising from this can be used to classify the metabolic regimes of the different cell lines. In a similar way, the metabolic regimes can be used to distinguish primary cultures from secondary cultures, since the primary tend to be much more aerobic, or even to elucidate the effect of chemical compounds in live cells. The effect of chemical agents on cellular metabolism can be evaluated by comparing the consumption of glucose and lactate extrusion in the basal state and in the presence of these compounds. These studies can also be used to study the environmental effects that induce metabolic disturbances. For example, an ischemic event (as occurs in strokes or cardiac arrests) can be simulated in a BI system degasifying the carrier. The system developed by Schulz et al. was used to compare the metabolic regime of two different hepatocyte cell lines, the TABX2S cells which over-express the Bcl-xL protein, an outer mitochondrial membrane protein which regulates the permeability of important metabolites going into and out of the mitochondria, and the TABX1A cells which do not express this protein. The cells were cultured, packed and perfused to a microculumn with a microbioreactor, in a form identical to that previously described. The metabolic regime was evaluated monitoring the glucose consumed and the lactate extruded in the perfusate through quantitative assays that utilised the same type of enzymatic chemistry (involved with the production of NADH). Results from the assays demonstrated that the TABX2S cells consumed glucose and extruded lactate at greater velocities when compared with the TABX1A cells. These tests were also carried out in the presence of azide. The results for the consumption of glucose and the extrusion of lactate in the two cell line types were greater in the presence of this compound, since the metabolism of the glucose was displaced to the anaerobic pathway.

3. Conclusions

The great majority of the SIA procedures presented in the literature aim at the routine analysis of pharmaceutical formulations, in that way demonstrating its potential as automatic equipment for aleatory multiparametric analysis. The potential for these systems is quite apparent in this area of application since these systems process an elevated number of samples, with reduced consumption of sample and reagents and consequently, reduced analysis costs. In addition, the analysis protocols developed have shown themselves to be favourable relative to the procedures proposed by the official codes, relating to the analysis of pharmaceutical products, which refer to tedious stages of sample pre-treatment and the need for sophisticated equipment and qualified operators for its execution.

The instrumentation used for this type of mounting is economical, robust and simple, which gives rise to mechanically stable analytical systems, with little maintenance requirements and tailored for functioning over prolonged periods of time. For this reason, the versatility and the advantages of these automatic systems are still being explored in other areas of quality control of medicines of which dissolution assays, process control and functional assays are examples. By contributing to the success of programmes for development, production and control of Pharmaceuticals, these aspects could be determinants for the definite establishment of this technique in pharmaceutical analysis.

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