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Application of Flow-Injection Spectrophotometry to Pharmaceutical and Biomedical Analyses

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Abstract

The discovery of new drugs, especially when many samples have to be analyzed in the minimum of time, demand the improvement or development of new analytical methods. Various techniques may be employed for this purpose. In this context, this chapter gathers the collection of paper and represents the review of past work on spectrophotometric technique coupled to a continuous flow system to determine low concentrations of several chemical species in different kinds of pharmaceutical and biological samples. A short historical background of the flow-injection analysis technique and a brief discussion of the basic principles and potential are presented. Part of this chapter is devoted to describing the sample preparation techniques, principles, and figures of merit of analytical methods. Representative applications of flow-injection spectrophotometry to pharmaceutical and biomedical analysis are also described.

Keywords: pharmaceutical, biomedical samples, flow-injection, spectrophotometry

1. Introduction

The monitoring of chemical species in pharmaceutical and biomedical samples is a field in which analytical chemistry plays an important role, contributing new procedures of analysis and instrumentation. Many methods have been developed for pharmaceutical and biomedical analysis including chromatographic, electrophoretic, and spectrophotometric methods. However, there are inherent difficulties associated with the types of samples involved. The

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© 2017 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. most practical difficulty encountered is the preservation and integrity of the species during sampling, storage, and sample pretreatment. In the medical area, the main matrices are blood, serum, and urine, while in the pharmaceutical industry, there are many types of samples and variations in their compositions. This hinders the application of analytical techniques for the fast and accurate monitoring of pharmaceutical and biomedical species in real samples.

Spectrophotometry is the technique most commonly employed in chemical analysis, and it provides advantages in terms of the availability of instruments, simplicity of procedures, speed, precision, accuracy, and applicability to a wide range of bio-medically important substances. Due to recent advances, increasing attention is being given to the coupling of a spectrometer to a continuous flow system to determine low concentrations of several chemical species in different kinds of pharmaceutical and biological samples. The flow-injection analysis technique has found wide application, which can be mainly attributed to its versatility, ease of automation, high sampling frequency, and the requirement for minimum sample treatment prior to injection into the system.

This chapter draws attention to some of the important and unique aspects of the applications of flow-injection spectrophotometry, addressed within the context of pharmaceutical and biomedical analysis. A short historical background of the flow-injection analysis technique and a brief discussion of the basic principles and potential are presented.

A notable feature of this chapter is the large number of papers on chemiluminescence discussed herein. In addition, considerable attention is given to sample preparation techniques and the characteristics of analytical methods such as precision, accuracy, and sampling frequency. Representative applications of flow-injection spectrophotometry to pharmaceutical and biomedical analysis are also described.

2. FIA origin and development

The process of flow-injection analysis (FIA) was initially proposed in the 1970s by Prof. Dr. Jaromir Ruzicka of the Technical University of Denmark and was subsequently consolidated as a state-of-the-art technology for the automation and mechanization of chemical systems. At that time, the cited researcher spent a year in Brazil advising on the installation of the Laboratory of Analytical Chemistry at the Center of Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP), where pioneering work was carried out that led to the FIA process becoming very well established. Since its introduction, more than 20,000 papers have been published reporting the development of advanced instrumentation methods in the context of chemical analysis, which are available for environmental, food, and clinical services involving pharmaceutical and biomedical samples [1, 2].

The FIA process involves the insertion of the sample into a carrier fluid that transports it to a suitable detection system. During this process, the sample can be brought into contact with reagents that are also inserted by propulsion, resulting in a controlled dispersion of the sample. The processes that characterize FIA systems have gained great prominence in contemporary analytical chemistry since several limitations have been overcome in the development of improved analytical procedures.

The origin and development of flow analysis systems was strongly influenced by the work of Skeggs, who proposed an approach called continuous flow analysis (CFA) [3]. For approximately 20 years, it was accepted that segmentation by dividing the flow into small regular compartments separated by air bubbles was the best strategy to avoid contamination and the widening of the discrete zone of the sample along the course, which is known today as dispersion [3–5]. It was only in the mid-1970s that it was widely accepted that segmentation could be omitted following an innovative proposal for a method employing the continuous flow of the samples and reagents with adequate dimensions and flow rates. The system was subsequently simplified, increasing the frequency of samples analyzed per unit of time, referred to as the analytical frequency. Due to the advantage of good reproducibility offered by FIA systems, it has also become possible to quantify the analyte even before the reaction between the sample and the reagent reaches equilibrium since the interval between the injection and detection is the same for the standard solutions and the sample.

In general, the FIA process consists of fluid propulsion, usually performed by a peristaltic pump that operates at constant flow, sample injection, reaction promoted in a homogenizing mixing coil with suitable geometry and a compatible detection technique, such as molecular spectrometry and atomic, chromatographic, and electroanalytical techniques.

Initially, Ruzicka used a hypodermic syringe to promote the injection of the sample, which gave rise to the name of the process [6]. Since then, other devices have been proposed for the insertion of the sample into the loader fluid, such as the proportional commutator injector and the rotary valve. The FIA systems have thus evolved and independent injections by multicomutation can be performed, enabling binary sampling [7]. In recent years, FIA systems have evolved in ways that have led to the development of sequential injection analysis (SIA) systems. In this case, the injection of the sample and the contact with the reagent flow occur through the selection of the port of a central selector valve in which the mixture is provided with bi-directional movement, alternating the propulsion direction occurring in a single line, in the absence of confluences [2, 8]. Due to these characteristics, SIA systems can be considered as differentiated from conventional FIA systems.

The classification of FIA systems has become necessary considering the variety of analytical procedures available. This can be based on the way in which the sample is introduced (continuous or intermittent) and on the flow characteristic (segmented, monosegmented, nonsegmented). **Figure 1** shows a classification scheme for flow analysis methods.

FIA systems have become commonly used and a number of variations in the configurations have been proposed in order to minimize the consumption of sample and reagents and to enhance the sensitivity of detection and the selectivity and precision of the analytical measurements.

The simplest configuration is the single-line flow diagram, where the loading fluid is the reagent itself, and the mixing occurs exclusively by dispersion. When the ratio between the volumes of the injected sample aliquot and its pathway is inappropriate, the addition of reagents by confluence may provide a more effective reaction where inert solutions, such as carriers, are employed. In order to overcome the excessive waste of reagents, which are continuously consumed, the system of flow injection through coalescing zones was proposed,



Figure 1. Schematic diagram of classification of flow analysis procedures.

thus minimizing the amount of waste generated. Since then, FIA systems have proven to be highly versatile and robust, making it possible to obtain strategically various arrangements and configurations that have been satisfactorily employed for extractive, separation, and preconcentration purposes. In addition, there are a number of approaches through which clinical formulation products can be efficiently monitored for the certification of their quality.

3. Spectrophotometric flow-injection procedures for pharmaceutical samples

Spectrophotometric methods are the most commonly used techniques in chemical analysis due to the availability of instruments, simplicity of procedures, precision, and wide applicability. Based on the laws governing absorption and emission phenomena, it is possible to determine the concentrations of compounds in solutions, notably those of biological, chemical, or pharmaceutical interest [9].

Drug analysis, involving the pharmaceutical preparations or the raw materials used for their production, and the determination of drugs together with metabolites in biological samples (serum, plasma, saliva, urine, and some secretions) constitutes a large part of the activities carried out by pharmaceutical and clinical laboratories.

Spectrophotometry is the most commonly used technique for the determination of drugs, and it is based on chromogenic reactions or light absorption by the analyte. Chromogenic reactions for drugs include metal-ion complexes, redox reactions, and the formation of chargetransfer complexes. Some of the flow-injection spectrophotometry procedures for the quantification of pharmaceutical samples [10–14] are detailed in **Table 1**. An important observation is the choice of carrier, aiming to avoid matrix effects and even clogging of the flow channels due to precipitation [14, 15].

Flow techniques, characterized by great flexibility, versatility, and ease of automation, allow the development and implementation of many analytical systems, which are compatible with a wide range of sample manipulation techniques, under highly reproducible conditions. Some flow-injection procedures are based on oxidation-reduction reactions. For the determination of N-acetyl-L-cysteine [16], this procedure involves the oxidation of the analyte of interest by Fe(III). The Fe(II) produced can be determined using 1,10-phenanthroline, and the chromophore formed is analyzed at 510 nm. On-line oxidation by Ce(IV) in acid medium—a procedure based on oxy-reduction—has been used to determine pyrazine. The colored free radical produced by the reaction was monitored at 510 nm [17].

Other procedures are based on the formation of a colored complex between the analyte of interest and metal-ions. For example, the determination of cimetidine with Cu(II) in acetate buffer (pH 5.9) can be carried out at a wavelength of 330 nm [18] and epinephrine with Fe(II) in amino acetic carbonate buffer (pH 8.3) at a wavelength of 530 nm [19].

A procedure for the determination of paracetamol (4-acetaminophen) has been described by Fatibello-Filho and Vieira [20]. The method is based on paracetamol oxidation by sodium hypochlorite, and the determination of the oxidant using o-toluidine dichloride as the chromogenic reagent at 430 nm. The analytical curve for paracetamol was linear in the concentration range of 8.50×10^{-6} to 2.51×10^{-4} mol L⁻¹ with a detection limit of 5.0×10^{-6} mol L⁻¹. The relative standard deviation was less than 1.2% for a paracetamol solution of 1.20×10^{-4} mol L⁻¹ (n = 10).

Analyte	Methodology	Detection limit	Ref.
Ketoprofen	Distilled water as a carrier for gels and citrate buffer, pH 6.5, for ampoules at 261 nm.	0.436 and 0.303 g ml ⁻¹ for gels and ampoules, respectively.	[10]
Lansoprazole	0.01 mol L ⁻¹ NaOH as carrier at flow rate of 1 ml min ⁻¹ and wavelength of 292 nm.	5.8 × 10 ⁻⁷ mol L ⁻¹	[11]
Diazepan	0.1 mol L ⁻¹ HCl as carrier at flow rate of 6.8 ml min ⁻¹ and wavelength of 360 nm.	0.6 mg L ⁻¹	[12]
Gemfibrozil	0.1 mol L ⁻¹ NaOH as carrier at flow rate of 1 ml min ⁻¹ and wavelength of 276 nm.	1.4 mg L ⁻¹	[13]
Cefuroxime	Methanol:water (10:90 v/v) as carrier at flow rate of 1.0 ml min ⁻¹ and wavelength of 281 nm.	1.31 × 10 ⁻⁷ mol L ⁻¹	[14]

 Table 1. Flow-injection spectrophotometry procedures for the quantification of pharmaceutical samples.

Many methods for the determination of pharmaceuticals also involve the flow-injection procedure based on homogeneous reactions. For example, the indirect determination of olanzapine from the reaction with hexacyanoferrate (III) in which the measurement of the unreacted

Analyte	Methodology		Ref.
Levofloxacin	Oxidation with N-bromosuccinimide.	3.0	[23]
Benzylpenicillin	Derivatization with 4,6-dinitrobenzenofuroxane.	0.14	[24]
Isoproterenol	Oxidation by polyphenol oxidase immobilized on controlled-pore silica.	13.2	[25]
Paracetamol	Reaction with sodium hypochlorite followed by reaction with sodium salicylate.	0.4	[26]
Dipyrone	Reaction with ammonium molybdate to produce molybdenum blue.	32	[27]
Vitamin B complex (B1, B6, B12, and benfotiamine)Multicomponent spectrophotometric analysis using Multivariate Curve Resolution Alternating Least Squa (MCR-ALS) algorithm.		0.0009–0.016	[28]
Promethazine and trifluoperazine	Bead injection spectroscopy-flow injection analysis (BIS- FIA) system and spectrophotometric detection.	0.00009-0.00014	[29]
Flutamide	Detection by electrospray ionization mass spectrometry.	0.001	[30]
Lansoprazole	Detection by electro spray ionization mass spectrometry.	0.0055	[31]

Table 2. FIA procedures using based on color-forming reactions.

oxidant is collected at 425 nm [21]. Diclofenac and mefenamic acid can also be oxidized in a flow system by hexacyanoferrate (III) and spectrophotometrically determined [22]. Other analytes that can be determined in homogeneous reactions are shown in **Table 2**.

4. Spectrophotometric flow-injection procedures for biomedical samples

The FIA technique can be coupled to various detection systems, such as a spectrophotometer, which allows a wide range of analytical devices to be combined [32].

A flow-injection analysis procedure using spectrophotometry was used to determine urea in blood plasma, employing the legume *Cajanus cajan* as a source of urease enzyme, in a minicolumn coupled to the FIA collector. A confidence level of 90% and a relative standard deviation of 1.4% (n = 12) were obtained [33]. In another study, the development of a flow analysis procedure for the determination of total protein in a bovine blood plasma was carried out using the Biuret method. Samples of bovine plasma with 12.5 and 100.0 g L⁻¹ of total protein were analyzed, and the analytical range was 2.5–20.0 g L⁻¹. The relative standard deviation of the procedure was 2.8%, and the analytical frequency was 76 determinations per hour. The results were compared with the traditional method of analysis (Biuret), and no statistically significant differences were observed at the 95% confidence level [34].

Sensors based on optical techniques are widely applied in different types of analysis, including biomedical sensing, and when connected to flow-injection analysis, a much faster analysis procedure is obtained [35]. In this context, a multicomutation flow system was used, which incorporates a sol-gel optical sensor (sensor: base catalyzed 4-(2-pyridylazo) resorcinol (PAR)) for the spectrophotometric determination of Cu(II) in urine samples using a photodiode detector with a maximum absorbance at 500 nm. The results were in agreement with those obtained by inductively coupled plasma mass spectrometry (ICP-MS), with a confidence level of 95% [35].

A FIA system was used to determine copper and zinc in water, pharmaceuticals, soils, and human hair samples. The product of the reaction with 2-carboxyl-2-hydroxy-5-sulfoformazylbenzene (Zincon) was introduced into a stream of carrier solution in the flow system. A sequential reaction of Cu(II) and Zn(II) was performed using Zincon, with the formation of two complexes and monitoring at 627 nm [36].

A flow procedure with spectrophotometric detection to determine bromopride in different matrices has been studied [37]. To increase the sensitivity of the reaction, a micellar medium (sodium dodecyl sulfate—SDS) was employed. Factorial planning was carried out to optimize the experimental parameters. The limit of detection was 1.07×10^{-7} mol L⁻¹. The method developed was satisfactorily applied in the determination of bromopride in pharmaceuticals and human urine, and recoveries were in the ranges 99.6–101.2 and 98.6–102.1%, respectively.

The application of a very sensitive and selective on-line flow-injection method for the determination of thorium(IV) after preconcentration in a minicolumn with N-benzoylphenylhydroxylamine-impregnated XAD-4 resin to biological samples has been described [38]. Sample rates of 40 and 11 h⁻¹ were obtained at the 60 and 300 seconds preconcentration times, respectively; the preconcentration factors were 32 and 162, with detection limits of 0.76 and 0.150 μ g L⁻¹, respectively [38].

Sarcosine has been investigated as a new marker for prostate cancer. A method for detecting sarcosine in biological samples (urine or blood plasma) has been proposed [39]. Ion exchange liquid chromatography with photometric detection at 570 nm was used as a separation method, which proved insufficient for the detection of sarcosine (70 μ M). An off-line approach to the ninhydrin derivatization of the fractions collected was optimized, after which a known amount of ninhydrin was added followed by incubation of the mixture under the optimized temperature and time conditions. FIA system with electrochemical detection was used. In this case, 5 μ L of sample was injected through a manual valve with a cell phase flow rate of 1 mL min⁻¹ and spectrophotometric detection in the wavelength range of 450–800 nm. A detection limit of 1.7 μ M was obtained for sarcosine [39].

5. On-line sample processing methods for in flow analysis

Flow analysis systems are widely used in analytical chemistry, contributing to increased reproducibility and accuracy of the methods. They also enable a reduction in the reagent consumption and the development of cleaner methods, meeting the requirements of "green" chemistry.

However, there are still limitations inherent to the procedures involved in preparing the samples, and these need to be suitable for each matrix.

Several procedures for analyzing pharmaceutical and biological samples have been developed where the sample preparation method performed on a laboratory bench is replaced by a flow procedure coupled directly to the instrument (spectrometer, chromatograph, electrophoresis unit, etc.). This increases the reliability of the method since it minimizes the potential for contamination inherent to the analysis, increases the reproducibility of the results, and increases the analytical frequency. These characteristics are due to the automation and processing of samples in closed systems under highly reproducible mixing and timing conditions. One of the factors that contributes to the success of this sample processing procedure carried out in closed systems is the combination of techniques and methodologies, known as hyphenation, which promotes faster analysis that is more efficient with less interference. The pretreatment of pharmaceutical and biological samples in flow is an important step in closed systems. Due to the complexity of these samples, the determination of chemical species presents significant challenges [40]. Thus, different types of procedures can be developed for the preparation of on-line samples for each matrix, according to its characteristics, such as solid phase extraction, solid phase microextraction, liquid-liquid microextraction, and chemical derivatization.

Some articles using on-line processes for the determination of compounds in pharmaceutical and biological samples are shown in **Table 3**. Two on-line procedures have been reported for the determination of ranitidine: chemiluminescence and UV-Vis detection [41, 42]. Several methodologies for the on-line preparation of saliva samples with detection by UV-Vis [43], ICP-OES [44], and AFS [45] are described in the literature.

Sample	Detection technique	Strategies for analysis	Ref.
Ranitidine and salbutamol	FIA- chemiluminescence	For Ru(bipy) ₃ ²⁺ chemiluminescence, a sulfuric acid carrier stream was employed into which Ru(bipy) ₃ ³⁺ and sulfuric acid was injected (20 μ L), while a second stream delivered the analyte standard and sample solutions.	[41]
Ranitidine	UV-Vis	Injected samples were analyzed by spectrophotometry at 313 and at 615 nm after reaction with 3-methyl-2- benzothiazolinone and ferric chloride.	[42]
Sulfamethoxazole and trimethoprim	UV	Analytical procedure was solid phase extraction.	[46]
Saliva	UV-Vis	Analytical procedure involving extraction and preconcentration using 5-BrDMPAP.	[43]
Saliva	ICP-OES	The analytical procedure involved extraction by sorption and elution of the analytes.	[44]
Saliva	AFS	The flow system was equipped with a microwave and an ultraviolet photo-oxidation system.	[45]

Table 3. On-line treatment procedures for pharmaceutical and biological samples using spectrometric techniques.

The complexity of the matrices of the pharmaceutical and biomedical samples requires an efficient decomposition process, without losing the necessary characteristics for a precise quantification, maintaining the integrity of the analyte. The use of microwave radiation energy was found to be an efficient alternative to conventional sample preparation methods since the processing time is reduced, minimizing problems associated with the loss of the more volatile components. In spite of these advantages, the process requires the manual transfer of volumes, addition of reagents, and excessive dilutions, which are all potential sources of errors, for instance, contamination. The mechanization of the microwave sample preparation processes in a continuous stream decomposition system has contributed to improving the sample processing and, therefore, the analytical performance of the method.

The use of flow systems coupled to a microwave oven for the preparation of samples was first proposed by Burguera *et al.* [47], where urine samples were decomposed for further determination of lead. A volume of up to 100 μ L of the sample was decomposed using a home microwave oven with a maximum power of 700 W and a 100 μ L mixture of 0.4 M HNO₃ and 0.3 M HCl. The application of this system allowed an analytical frequency of 80 samples per hour. Since the first work of exploring the coupling of a microwave oven and a flow system, several systems have been developed and applied to a wide variety of samples, for instance, water, effluents, plants, food and minerals, along with biological fluids and tissues. The analysis of biological fluids is of great importance since it allows the diagnosis of various diseases, nutritional, and metabolic research, therapeutic monitoring involving the biological action of some metals, such as calcium, magnesium, iron, cobalt, zinc, and manganese, and the detection of some drugs (including cocaine and marijuana) [48, 49].

Coelho and collaborators developed an on-line decomposition system for urine samples using a microwave oven prior to the determination of calcium and magnesium by flame atomic absorption spectrometry (FAAS). The decomposition efficiency allowed a rapid treatment of the urine sample with an analytical frequency of 45 samples per hour. The system consisted of three decomposition coils inserted into the cavity of the microwave oven and a valve that allowed the interruption of the passage of the flow and confined the sample to the inside of the oven [50].

The preparation of flow samples in biomedical and pharmaceutical matrices employing spectroscopic techniques remains a challenge, and few studies have been reported in the literature when compared with the chromatographic methods. When spectrometric techniques are subjected to hyphenation, they are promising for the preparation of one or more samples, since the on-line detection systems cited in the literature favor a decrease in the use of batch procedures, thus minimizing the potential for contamination and automating the sample processing procedure.

6. Combination of FIA and other analytical systems

The advance of laboratory research has enabled the identification and quantification of analytes, individually or simultaneously.

One of the techniques that has contributed to the simultaneous determination of analytes is FIA system combined with other analytical systems, such as high performance liquid chromatography (HPLC), enzymatic reactions, gas chromatography, biosensors, electrophoresis, electrochemical, and immunoassays. According to Saurina [51], in most cases, these combinations enable analysts to detect and quantify up to three compounds simultaneously. The methods required to increase this number may not be compatible with the physical resources used in systems involving flow injection.

The combination of the FIA system with other analytical techniques enables reductions in the analysis time and the reagent/sample consumption and improved accuracy, sensitivity, selectivity, and sampling frequency. In addition, the analyst's contact with the sample is minimized, decreasing the potential for contamination. Thus, by combining the pretreatment (digestion, preconcentration, sample clean-up, and solvent-solvent extraction, etc.) with on-line sample introduction, the FIA system becomes a very efficient and advantageous technique [52–57].

In this context, the possibility of detecting multianalytes using various techniques involving the combination of the FIA system with other traditional analysis systems should be highlighted. Different separation and sample pretreatment procedures can be performed using detection techniques such as fluorescence, spectrophotometry, and electrochemistry, enabling the detection of innumerable analytes, including those present in samples involved in biological applications [58–63].

Several approaches to detection have been used, and electrochemical detectors are prominent in the scientific literature, notably in studies involving conventional amperometric detection coupled to an FIA system. The main characteristics of this combination are increased sensitivity, minimized contamination of the surface of the working electrode, the presence of negligible capacitive current and *in situ* measurement, etc. A limitation associated with this system is the instability of the electrochemical signal during the determination of some compounds, compromising the repeatability of the response and the reproducibility of the results [64].

Another example of combining FIA and an electrochemical system is found in the studies of Chaves *et al.* [65] in which three compounds were determined simultaneously: caffeine, ibuprofen, and paracetamol. The authors report results obtained by combining FIA with multiple pulse amperometry (MPA) using a wall-jet flow cell with a boron-doped diamond electrode. In this analysis, cyclic voltammetry (50 mVs⁻¹) was used.

According to Llorent-Martinez *et al.* [66] and Oliveira *et al.* [67], most of the methods involving detection by UV-Vis using flow procedures offer many advantageous of this combination, being simple, fast and direct methods offering good selectivity and sensitivity in the separation and/or preconcentration steps.

Vidal *et al.* [68] address the simultaneous determination of a mixture of three analytes that are often combined in pharmaceutical formulations: two analgesics (paracetamol and propyphenazone) and a stimulant drug (caffeine). The quantification was performed by separating the three compounds using an FIA system combined with a precolumn containing C18 silica gel to avoid spectral overlap of the compounds under analysis. The detection was conducted with a spectrophotometric detector through UV absorbance measurements. The

results were satisfactory, since the compounds were quantified at low concentration ranges, that is, 25–350 μ g mL⁻¹ for paracetamol, 5–75 μ g mL⁻¹ for caffeine, and 15–150 μ g mL⁻¹ for propylphenazone. Also, the proposed method provided low detection limits ranging from 0.65 to 7.5 μ g mL⁻¹.

Pistonesia et al. [69] carried out the simultaneous analysis of levodopa and benserazide in tablets of pharmaceutical formulations. The samples were not subjected to pretreatment, and the reaction mixture containing the sample and potassium periodate was directed to a flow cell (8 µL inner volume) inserted in a spectrophotometer with a UV-Vis diode array detector. The concentrations used for the construction of the calibration curves analyzed were 4.1×10^{-4} to 2.03×10^{-3} M for levodopa and 8.5×10^{-5} to 4.25×10^{-4} M for benserazide. During the analysis, the FIA system variables (flow velocity, reactor length, and injected volumes) were optimized and the validation of the method (considering the robustness, repeatability, reproducibility, and accuracy) was studied. The kinetic-spectrophotometric data provided by the diode array detection were treated applying partial least squares (PLS) multidimensional regression. Samples were injected into the HPLC system using preoptimized conditions. The results obtained using the HPLC analysis (197 and 49 for levodopa and benserazide, respectively) and the FIA/PLS system (200 and 48 for levodopa and benserazide, respectively) showed no discrepancy. Thus, a simple, fast, and direct method was obtained through the implementation of a flow-injection system coupled to UV-visible diode spectrophotometry.

Regarding the analysis of biological samples, Reis and Luca [70] described a procedure for the determination of total protein in bovine blood plasma using a FIA system. The use of the FIA system enabled the in-line dilution of bovine plasma samples containing between 12.5 and 100.0 g L⁻¹ total protein. The conditions for the flow analysis were optimized and the results, when compared to those obtained with the traditional method (Biuret), did not indicate significant statistical differences (*t*-paired test) at the 95% confidence level. The proposed method provided fast results, low reagent consumption, and minimization of the sample handling, as well as an analytical frequency of 76 determinations per hour.

In addition to the combination of the FIA system with innumerous detectors, the coupling of flow analysis with pretreatment and separation systems has been employed in some studies, especially FIA combined with capillary electrophoresis (CE). The first studies involving this coupling were described in 1997 by Kuban *et al.* [71] and Fang *et al.* [72]. They described this as an advantageous combination, capable of overcoming certain limitations presented by CE, such as low sensitivity, precision, and analytical frequency. An important feature is that the sample pretreatment step in the FIA-CE system is performed online, minimizing the potential for sample contamination.

Numerous studies involving FIA-CE have been reported in the literature notably: Kuban and Karlberg [73] carried out the determination of small anions through dialysis; Chen and Fang [74] performed the preconcentration of samples; Arce *et al.* [75] determined cations and anions; Chen and Fang [74] monitored multi-components in drugs; Kuban *et al.* [76] determined trimethoprim and sulfamethoxazole in drug samples; and Kuban and Karlberg [77] determined pseudoephedrine in human plasma.

An example of an FIA-CE system is also described in a paper by Liu *et al.* [78], which proposes a method developed through the combination of flow injection and CE for the separation and determination of paracetamol (Par), pseudoephedrine hydrochloride (Pse), dextromethorphan, potassium bromide (Dex), and chlorphenamine hydrogen maleate (Chl) using uncoated fused silica capillaries. Detection was performed on a UV detector at 214 nm. During the analysis, a flow-injection analyzer was used to transport the background electrolytes and the samples. The system consisted of a double piston, a 16-way automatic switching valve with three sample loops, and a peristaltic pump. The limits of detection (LOD) values were 0.22, 0.29, 0.42, and 0.70 μ g ml⁻¹ for the compounds Dex, Chl, Pse, Par, respectively. The low LOD values, the separation of the baseline of the peak of each analyte and the low cost of this FIA-CE system are characteristics that indicate that the proposed system is suitable for the identification and quantification of the compounds investigated.

Kuban *et al.* [76] described the determination of small inorganic cations (K⁺, Na⁺, Mg²⁺, and Ca²⁺) in blood, milk, or plasma samples by electrokinetic injection using an FIA-CE system. Since the undesirable adsorption of proteins onto the capillary wall during electrophoresis was inhibited, pretreatment of the samples was not necessary, and they could be injected directly into the system. In the initial stage, two injection modes were tested for all electrolyte and standard solutions: electrokinetic (EK) and prehydrodynamic (HD). The results indicated that EK injection was the better option because it showed high sensitivity and low matrix effects, with good repeatability of the cation migration times, mainly in the case of human plasma samples. In addition, a better performance was observed for the FIA-CE system when compared to the commercial CE system.

Other researchers have reported the determination of multianalytes using the FIA system combined with other systems of separation, identification, or quantification (detectors). These include the following: an immunoassay system using detection by chemiluminescence [79]; an electrochemiluminescence immunosensor for the detection of tumor markers [80]; biosensors with the use of enzymes [81]; the quantification of carbohydrates with amperometric biosensors [82]; and the analysis of pharmaceutical formulations combining FIA with HPLC or CE [83]. Thus, it is clear that FIA coupling with other analytical techniques allows the detection/quantification of multianalytes in pharmaceutical and biological samples, etc., either for the development of methods in laboratory research or in routine analysis.

Tzanavaras and Themelis published a review on the application of flow injection to pharmaceutical analysis that covers the topics of spectrophotometric determination of active pharmaceutical ingredients [84]. According to Tzanavaras and Themelis [84], the discovery of new drugs, especially when many samples have to be analyzed in the minimum of time, demand the improvement or development of new analytical methods.

7. Conclusions and future prospects

Many methods aimed at the monitoring of chemical species in pharmaceutical and biomedical samples have been developed and investigated in recent decades. This is a field in which analytical

chemistry plays an important role, contributing new analysis procedures and instrumentation. However, methods for the determination and monitoring of pharmaceuticals are still scarce.

Although some progress has been made in the development of methodologies for the monitoring of chemical species in pharmaceutical and biomedical samples, some important points still need to be addressed, such as the sample pretreatment.

In this context, a further challenge has emerged for scientists, which is the development of new clean environmentally acceptable technologies with commercial feasibility. Thus, laboratory researchers need to improve the techniques for the identification and quantification of analytes, individually or simultaneously, with a focus on this challenge.

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Abbreviations

atomic fluorescence spectrometry
bead injection spectroscopy-flow injection analysis
capillary electrophoresis
Center of Nuclear Energy in Agriculture
continuous flow analysis
flame atomic absorption spectrometry
flow injection analysis
high performance liquid chromatography
inductively coupled plasma mass spectrometry
inductively coupled plasma optic emission spectrometry
multivariate curve resolution-alternating least squares
multiple pulse amperometry
4-(2-pyridylazo) resorcinol
partial least squares
sodium dodecyl sulfate
sequential injection analysis

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