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Review

Flow injection analysis: a new approach to pharmaceutical determinations

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Abstract: A review is presented in which the fundamental principles, instrumentation and practical aspects of flow injection analysis (FIA) are discussed. A particular focus is put on applications in pharmaceutical chemistry, which offers a wide scope to the new technique.

Keywords: *Flow injection analysis; pharmaceutical dosage form analysis; trace organic analysis.*

Introduction

The versatility and simplicity of the FIA technique allows its adaptation at relatively low cost to the different requirements of a diverse range of analytical problems. In this context its application to pharmaceutical analysis, although important, has not yet been exploited to the extent indicated by the great potential that this technique offers in general to analytical chemistry. FIA is a recently invented methodology [1], revolutionary from the point of view of its simple background, inexpensive instrumentation and easy sample handling, all of which can provide excellent results. Since the initial work in 1975 [1, 2], its development has been spectacular, leading to the publication of about 450 articles, several reviews [3–11] and some recent monographs [12, 13].

FIA is a mode of continuous flow analysis (CFA) [14] in which the flow is not segmented by air bubbles. A quantity of dissolved sample, accurately measured, is injected or introduced into a carrier stream flowing through the system tubing, with or without additional processes, such as chemical reaction, extraction, etc., occurring between the sample and the carrier. The transport process is strictly controlled in such a way that the dispersion can be suitably manipulated. As the analyte (or its reaction product) passes through the continuous detector, a transient signal is generated and recorded. Measurement is carried out under non-equilibrium conditions since neither physical homogeneity nor chemical equilibrium has been attained by the time of

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detection. FIA techniques can therefore be considered as kinetic methods of analysis in the fixed-time mode.

Fundamental aspects of FIA

Basic components

The general scheme of an FIA system is shown in Fig. 1. These systems are adaptable to automation, as indicated by the dashed lines. The basic scheme for a non-automated system consists of the following elements.

Propulsion system. This establishes the solution stream which may contain the dissolved reagent or may act as a simple carrier. The pump can be based on an inert gas, whose pressure over a solution in a closed vessel induces it to circulate through the system tubing. Usually, a peristaltic pump is used for economic reasons and also to ensure reproducible higher flow-rates (generally between 0.5 and 4.0 ml. min^{-1}). Single- and double-piston pumps have also been used, as well as gravity flow.

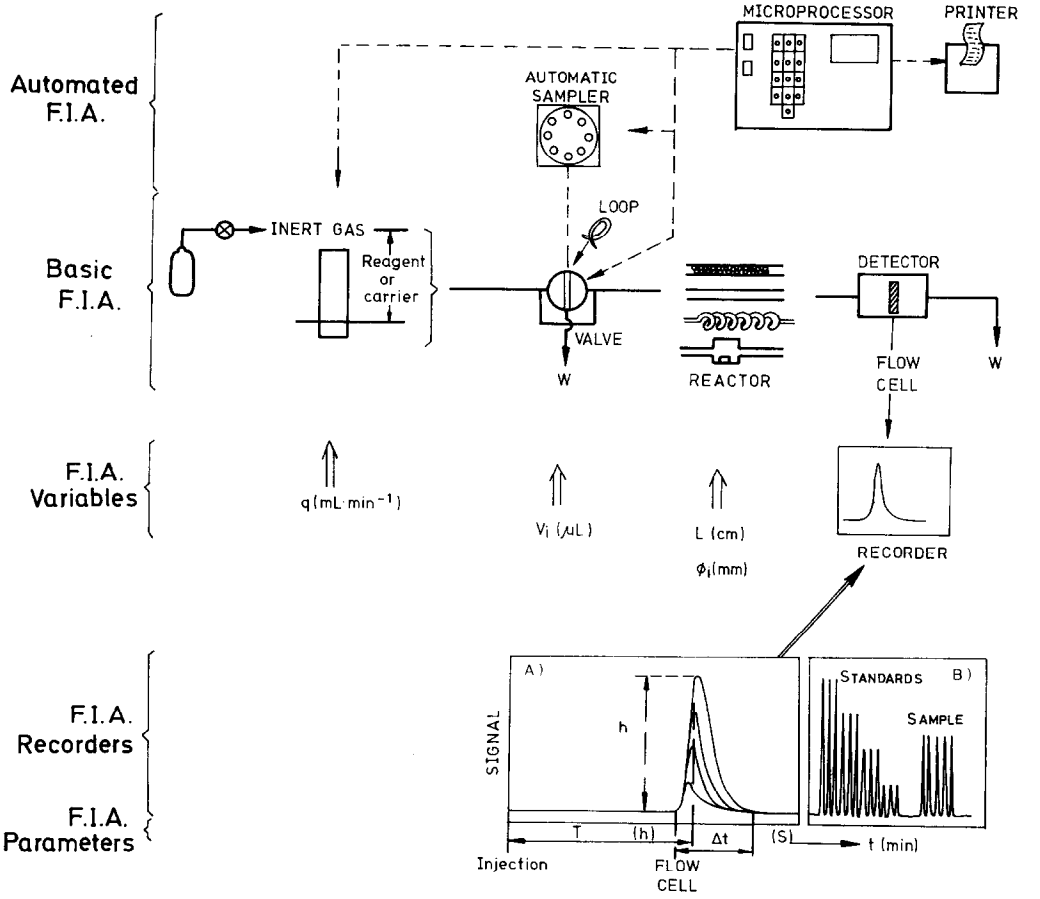

Figure 1
General scheme of an FIA system and readout of the transient signal at different scan-rates: (A) fast; (B) normal rate, with triplicate injection for calibration. The dashed lines indicate the possibility of automating the system.

Tubes. The tubes used in FIA are of two types: (a) flexible peristaltic pump tubes with variable inner diameter selected to allow different flow-rates and made of various materials (PVC, silicon rubber, tygon, etc.) depending on the properties of the fluids concerned; (b) the reactor tubing is generally made of teflon or polyethylene, with an inner diameter (ϕ_i) between 0.3 and 1.0 mm, its length being a function of the chemical processes involved. Various connections between tubes are used, it being important that they have a small dead volume.

Sample injection system. The purpose of this device is to introduce a discrete sample volume into the carrier stream, in such a manner that its properties are not altered. The volumes injected (20–100 μl) should be completely reproducible. In the earliest FIA systems a hypodermic needle was employed; however, the injection system most widely used at present is the six-way rotary valve of variable, but known volume.

Reactor. This can comprise straight tubing, mixing chamber or a coil and is a function of the additional processes involved; it can be packed with either inert or chemically active material.

Detector. The detection system may be any measuring device, optical or electrical, which incorporates a small-volume flow-cell, permitting the measurement of a transient signal, which is then transduced to an x–t recorder. The curve shape is similar to that obtained by other analytical techniques providing transient readouts. The characteristic parameters of these curves, as illustrated in Fig. 1, are: (a) peak height or area, related to the analyte concentration; (b) residence or response time, T, during which the analyte is in the reactor (from injection to the top of the response curve); (c) peak–base width, Δt, or the time elapsed between the beginning and the end of the curve.

Automated FIA systems incorporate a microprocessor and a passive interface, which collects and processes the analytical data (peak height, area, width, peak-to-peak distance) and calculates and prints the concentration of each sample. An active interface allows the system to be controlled by means of the microprocessor. These systems are especially interesting for the analysis of a large number of samples, since they can control the sampler, injection valve, and the pump(s).

With these basic components several FIA configurations can be designed, adapted to the requirements of each type of analysis. Three schemes of pharmaceutical interest have been selected, each differing in complexity as shown in Fig. 2. One of these (2C) has been applied to the determination of corticosteroids [15] and is completely automated.

Comparison of FIA with CFA and HPLC

Since FIA is a mode of continuous flow analysis (CFA) and taking into account the similarity between this technique and high-performance liquid chromatography (HPLC), it is of interest to establish the most significant differences between them. Table 1 summarizes in a comparative way the most important features of these methodologies. From one viewpoint, FIA can be regarded, at least in principle, as a hybrid between CFA and HPLC. In fact, of the two pioneering groups in this technique, whereas the Danish-FIA team designed their first FIA systems from CFA components [1], the American-FIA team employed modifications of HPLC equipment [2].

The similarity between FIA and HPLC from an operational point of view has, in some cases, permitted their complementary coupling. The most significant difference between

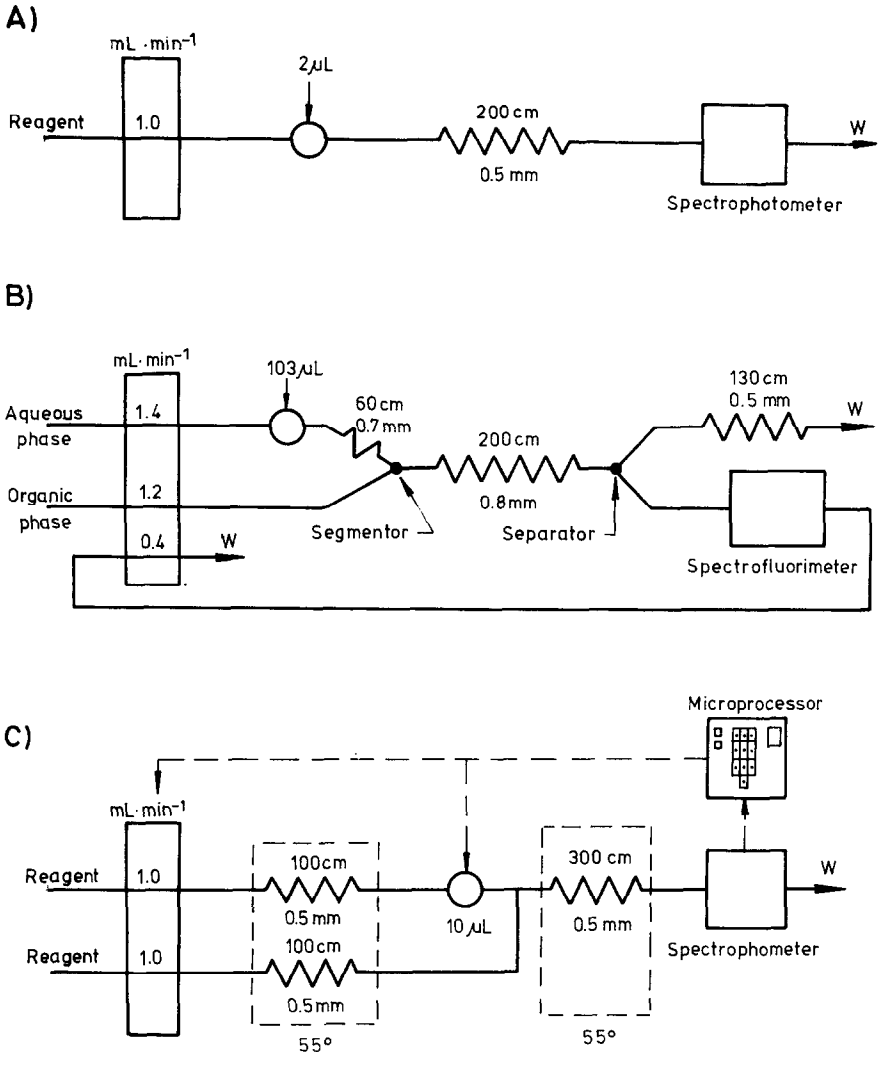

Figure 2
Some examples of the flow manifolds employed:
(A) Simple FIA determination of water in organic solvents by the Karl Fischer reagent [108]. (B) Multichannel FIA fluorimetric determination of vitamin B, by thiochrome method and extraction into chloroform [79].
(C) Automated FIA rapid determination of corticosteroids in pharmaceutical products based on the reduction of tetrazolium blue by the steroids to form a highly coloured formazan. In this manifold the possibility of total automation has been included [15].

FIA and HPLC is, fundamentally, their specific objective. Whereas in HPLC the separation or resolution of a mixture is paramount, in FIA the determination of one or more known components in a sample is the key objective. The operating pressure in both techniques is in fact very different.

With reference to CFA, it is noteworthy that FIA has a higher sampling capacity, shorter response time, needs smaller washing volumes, provides more analytical data and shows considerable potential for titrations or stop-flow methods. In CFA the air bubbles, in addition to segmenting the stream, avoids the dilution or dispersion of

Table 1
Comparison of the principal features of continuous flow analysis, HPLC and FIA

Feature	CFA	HPLC*	FIA
Sample introduction	Aspiration	Injection	Injection
Sample volume	0.2–2.0 ml	10–200 μl	10–200 μl
Flow type	Segmented	Non-segmented	Non-segmented
Response time	2–30 min	3–60 s	3–60 s
Internal diameter of tubing	>2 mm	1–7 mm	0.35–0.70 mm
Detection	Equilibrium (homogenization)	Not reaction	Partial dispersion
Capacity	Up to 80 h^{-1}	Up to 30 h^{-1}	Up to 500 h^{-1}
Precision (RSD)	1–2%	1–2%	1–2%
Reagent consumption	High	Low	Low
Washing cycle	Essential	Not essential	Not essential
Differential kinetic analysis	Not possible	Not possible	Possible
Titrations	Not possible	Not possible	Possible
Data acquired	Peak height	Peak height	Peak height
		Peak area	Peak area
			Peak width
			Peak–peak distance

* NB The primary analytical objective is to separate the sample components.

samples in the stream; it also generates a turbulent flow, thanks to which physical (homogenization) as well as chemical equilibrium is reached. Similar precision is achieved by both techniques (Table 1).

Theoretical Considerations

The physical principles of FIA are related to dispersion, which is defined as the dilution undergone by a sample volume injected into a stream. It is characterized by the concentration-profile of the plug. Thus, the shape of an FIA recording shows the dispersion attained at the detector cell. In FIA systems this dispersion is 'controlled and partial', as opposed to segmented continuous methods, where the dispersion or dilution of the sample inserted between two air bubbles is 'controlled and complete'. Moreover, in FIA it is necessary to take into account the chemical factors if a chemical reaction between sample and carrier takes place. Therefore, it is essential to know the contribution of chemical kinetics to the dispersion [16–18], which contributes to the shape of the FIA recording. Consequently, the total dispersion, D, observed in a FIA system is the sum of the dispersion due to physical phenomena (D_p) and that due to the chemical reaction (D_c):

$$D = D_p + D_c.$$

The term 'practical dispersion' coined by Růžička and Hansen [12] is valid only if no processes other than transport occur, and is defined as 'the ratio between the signal from the non-dispersed analyte, S_o, and that of the analyte after transport through the FIA system, S', i.e. the S_o/S ratio.

Several theoretical models have been applied (Taylor's model [12, 17–19], the tanks-in-series model [12, 20], the mixing chamber model [21–23] and a general model [24, 25]). Different equations for disperson have also been suggested (Růžička *et al.* [26], Vanderslice *et al.* [25], Valcárcel *et al.* [27]), in order to study dispersion and to establish

the influence of various factors, such as geometry, hydrodynamic effects, sample volume etc. In these models and equations, a laminar flow, in which sample dispersion into the stream is controlled by convection–diffusion phenomena, must be assumed. For these reasons the classical expressions which regard transport as due only to pure diffusion or convection are not valid [19]. In this respect, Stewart *et al.* have suggested several equations to account for transport phenomena in FIA [2].

Principal features

Several features permit this methodology to be considered as revolutionary, both analytically and instrumentally (Table 2). From an analytical point of view, although its *sensitivity* is less than that of conventional techniques, due to dispersion, in general FIA shows higher *selectivity*. This last fact is due to the kinetic nature of FIA, which makes it possible to manipulate the design and working conditions and to dramatically reduce interference by foreign species, especially if the interference is related to the equilibrium of the chemical reaction. It is also worth mentioning the high *reproducibility* achieved by FIA methods (comparable to that achieved by some other analytical techniques), in spite of the fact that measurements are carried out under conditions of chemical and physical imbalance.

Other important features are as follows: *fast operation* is probably one of the most significant features in routine control analyses. In addition to its simple background, the use of simple manifolds and their easy handling turn FIA into a technique characterized by its *simplicity* as opposed to some other recent analytical methods. FIA is as inexpensive as it is simple, from the point of view of reagent and sample consumption, as well as from the instrumentation viewpoint. *Versatility* is another major feature of FIA, which allows the substitution of some components to adapt to specific requirements and to incorporate common laboratory instruments.

Thus it can be understood that FIA permits a wide range of experiments to be performed from titrations to stopped-flow and differential kinetic determinations. Elements that can be incorporated range from liquid–liquid extraction units to inert or active reactors (redox, ion-exchangers, immobilized enzymes, etc.). It can be said that the only limitation of this technique is the researcher's ingenuity.

Finally, it is necessary to add the fact that easy adaptation of FIA to automated methods is possible. This implies an important FIA contribution to the future development of analytical methods. Its capability for miniaturization can also be mentioned as another important aspect of relevance to special situations.

FIA modes

The FIA modes so far reported in the bibliography are numerous and very different. FIA systems can be divided into two large groups, depending on whether or not processes in addition to transport (e.g. chemical reactions) take place. The FIA systems involving electroanalytical and atomic techniques may be considered as non-reaction processes. When an additional chemical reaction is exploited, this may involve complex formation or redox reaction, although catalytic, enzymatic and dye formation reactions have been also reported.

For clarity, the principal FIA modes have been classified as 'conventional' and 'new approaches', to illustrate current development trends in FIA.

Conventional FIA modes. The earliest systems based on FIA were characterized by their simplicity. They consisted of single or multiple reagent channels and one or several

Table 2
Principal characteristics of FIA

Principles	Components	Additional process to transport	Detection system	FIA modalities	Features	Applications
Kinetic method	Propulsion unit	Complex formation	Optical:	Simple	Speed	Environmental
Non-segmented flow	Injection system	Redox reaction	Photometry	Closed loop	Reproducibility	Agricultural
Sample injection	Tubes	Catalytic/enzymatic reaction	Fluorimetry (laser)	Intermittent pump	Selectivity	Clinical
Controlled-partial dispersion	Connectors	Dye formation	Chemiluminescence	Merging zone	Simplicity	Pharmaceutical
	Detector			Zone trapping	Versatility	Industrial
Reproducible operation time		Precipitation	Nephelometry			
	x–t recorder			'Reversed' FIA (rFIA)	Low cost	Food
		Acid–base reaction	Turbidimetry			
	(Sampler)			Miniaturized FIA	Miniaturization	
	(Microprocessor)		Atomic absorption	Sampling zone	Ease of automation	
	(Separation units)		Inductively coupled plasma	Gradient		
				Titration		
			Electrochemical:	Stopped-flow		
			Potentiometry			
			Voltammetry			
			Coulometry			
			Stripping modes			
			Others			

reactors, in addition to the usual basic components. An interesting variant of these systems are the closed-loop modes, as suggested by Bergmeyer [28] and later developed by Mottola and co-workers [29–35]. These systems are characterized by a closed, recirculating stream, possibly with a regeneration unit located after the detector.

Another attractive possibility is to control the pump(s), triggered by a timer synchronized to the injection system [36]. Thus, a single pump can be stopped when the sample plug is located at the detector, to give the stopped-flow mode, a method of great interest to kinetic (differential) analysis. Two synchronized pumps permit the sampling rate to be increased (by increasing the washout speed). Two pumps allow hydrodynamic injection to be performed [37]. The 'merging zone' mode [38, 39], involving the confluence of two symmetrical sample and reagent 'plugs' simultaneously injected into two channels, is very useful when either the reagents or the sample are expensive or scarce. For concentrated samples an asymmetric confluence of the two 'plugs' may be chosen [40]; alternatively measurement may be performed at points other than at the peak maximum (so-called 'electronic dilution').

FIA also allows the incorporation of special units developed for separation, such as extraction [41–44], ion-exchange [45], dialysis [46, 47] and distillation [48, 49]. These are of great interest to pharmaceutical analysis, where prior separation is often required. Unlike CFA methods, FIA permits titrations to be performed [50, 51], using mixing minichambers or, more recently, coils.

New FIA approaches. Gradient modes, which have been recently reviewed by Růžička [52], entail a very interesting feature — reversed FIA (rFIA), in which the sample circulates through the system, while the reagents themselves are injected [53] to give a sharp increase in sensitivity [54, 55].

To exploit the possibility of miniaturizing the FIA system two new modes have been developed: capillary FIA [56, 57], and integrated microconduits as recently designed and patented by Růžička *et al.* [58, 59]. The advantages of these approaches lie in the small sample and reagent consumption, as well as in an increase in the sampling rate.

Sampling zone or re-injection techniques are based on the introduction of a portion of an accurately selected zone of the analyte already in the flowing stream, into a second line of the system [60], thus allowing the simultaneous determination of species in the two parts of the sample bolus, as well as the possibility of 'electronic dilution'.

The zone trapping mode is used to select a portion of a processed sample zone and to remove it. After a given period of time, the zone is reintroduced into the same carrier stream and directed towards the detector with minimal dispersion [61].

Recently, the adaptation of dynamic detection systems [62] has allowed the readout scan to be performed in a considerably shorter time. Thus absorbance can be monitored at several wavelengths simultaneously as demonstrated for HPLC [63] (optical methods); or current intensity can be recorded at several potentials simultaneously (electroanalytical methods). This points to promising possibilities for multicomponent analysis, as well as for the presentation of three-dimensional absorbance–wavelength–time data [63] or of current intensity–potential–time recordings [62].

FIA Applications to Pharmaceutical Analysis

An important function of quality control in pharmaceutical industry is the assay of the active ingredient in each batch of tablets, capsules, etc. This is usually carried out by