

2 Flow Injection Analysis

2.1 Introduction

Flow injection analysis (FIA) is a simple, rapid, and versatile technique that is now firmly established, with widespread application in quantitative chemical analysis. This is apparent from the number of related papers that have appeared in the technical press since 1975, and since then the scope of the method has grown at an unprecedented rate. Over 10,000 papers devoted to FIA have been published in scientific journals up to now [8], with two papers being published each day. The literature on FIA doubles approximately every two years, whereas analytical literature as a whole doubles only every 14 years and general literature every 15.

The designation of FIA was proposed in 1975 by Ruzicka and Hansen [9]. The inclusion of the term injection in the name of this technique occurred because the technique originally entailed using a syringe to inject a sample through a septum into a reagent flow. Currently, rotation valves are mainly used for this purpose.

FIA may be defined as [10] the sequential insertion of discrete sample solution into an unsegmented continuously flowing stream with subsequent detection of the analyte. Even this definition, however, is frequently made obsolete by new developments. The first definition, given by Ruzicka and Hansen [11] was *“A method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode potential, or any other physical parameter, as it continuously changes as a result of the*

passage of sample material through the flow cell". This definition, however, was soon considered obsolete and was revised [9] to describe a technique for *"information gathering from a concentration gradient formed from an injected, well-defined zone of a fluid, dispersed into a continuous unsegmented stream of a carrier"* in order to accommodate new developments in stopped-flow FIA, merging zones, zone sampling and other gradient techniques. This new definition was soon challenged by FI systems which were segmented in one way or another, or which dealt with samples eluted from columns without well-defined boundaries. Furthermore, Fang [12] defines FIA as *"A flow analysis technique performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions"*

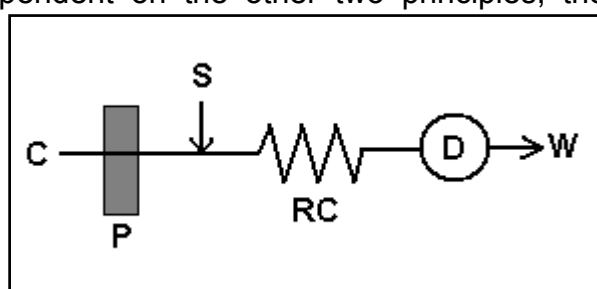
The simplest flow analyzer consists of a pump, which is used to propel the carrier stream through a narrow tube, an injection valve, a microreactor in which the sample zone disperses and reacts with the components of the carrier stream, forming a species that is sensed by a flow-through detector and recorded. In the case of the FIA technique the physical equilibrium (flow homogenization) is never reached at the moment of detection. Moreover, it is not necessary for the chemical equilibrium to be obtained at the moment of detection.

The concept of FIA depends on a combination of three factors: reproducible sample injection volumes, controllable sample dispersion, and reproducible timing of the injected sample through the flow system. Except for detector warm-up, the system is ready for instant operation as soon as the sample is introduced. FIA offers several advantages in term of [10]: considerable decrease in sample (normally using 10 to 50 μL) and reagent consumption, high sample throughput (50 to 300 samples per hour) reduced residence times (reading time is about 3 to 40 s), shorter

reaction times (3 to 60 s), easy switching from one analysis to another (manifolds are easily assembled and/or exchanged), reproducibility (usually less than 2% RSD), reliability, low carry over, high degree of flexibility, and ease of automation. Perhaps the most compelling advantage of the FIA technique is the great reproducibility in the results obtained by this technique that can be set up without excessive difficulties and at very low cost of investment and maintenance. These advantages have led to an extraordinary development of FIA, unprecedented in comparison to any other technique.

2.2 Principle of the FIA

The three principles or cornerstones of FIA were identified by Ruzicka and Hansen [11] as sample injection, controlled dispersion of the injected sample zone, and reproducible timing of the movement of the injected zone from the injection point to the detector. More recent developments of FIA showed that sample injection should be understood in a much broader sense. It follows that neither the timing of zone movement nor the control of dispersion should be restricted to those of injected samples. Although dependent on the other two principles, the central issue of FIA is the



control of dispersion, which is the very basis for extraction of analytical data under non equilibrium conditions.

Figure 2.1 Schematic diagram of the basic FI system
(C=carrier; P=pump; S=point of sample injection;
RC=reaction coil; D=detector; W=waste)

In the simplest form of FIA the sample is injected into a continuous flow of reagent solution (carrier), dispersed, and transported to detector. Sample

dispersion is controlled through the suitable choice of the injected sample volume, flow rate of carrier, length of the reaction coil, and diameter of the tubing used. A schematic diagram of the basic FI system is shown in Figure 2.1.

2. 3 Dispersion in the FIA

As stated previously, the control of dispersion is the most important aspect of FI systems. The dispersion of a fluid zone reproducibly introduced into a non-segmented flow stream (carrier) during transport of the zone to the detector is the most important physical phenomenon in all FI systems. The specific feature of dispersion processes in FIA is that they are reproducible and controllable through the manipulation of flow parameters and geometrical dimensions of flow conduits. The driving forces active in dispersion of the injected zone into the carrier stream are molecular diffusion and convection, but the effects of convection dominate, and the effects of molecular diffusion may be neglected in most cases. Convection occurs both as result of linear flow-rate differences of fluid elements located at different points along the radial axis of the conduit and as a result of secondary flows created by centrifugal forces perpendicular to the flow direction in non-straight conduits. A convex parabolic front of the injected zone and a concave parabolic tailing edge are developed with penetration into the carrier stream, the extent increasing with the distance traveled. Thus, under the specific conditions applied in FIA and with a fixed conduit, the acting forces are well under control, so that no random turbulence occurs. The result is that perfectly reproducible concentration-time relationships may be obtained which, when recorded and superimposed, precisely overlap each other to form a single curve. This provides the basis for extracting reproducible readout under both

physically and chemically non-equilibrium conditions. The dispersion process typical of FIA system is shown in Figure 2.2.

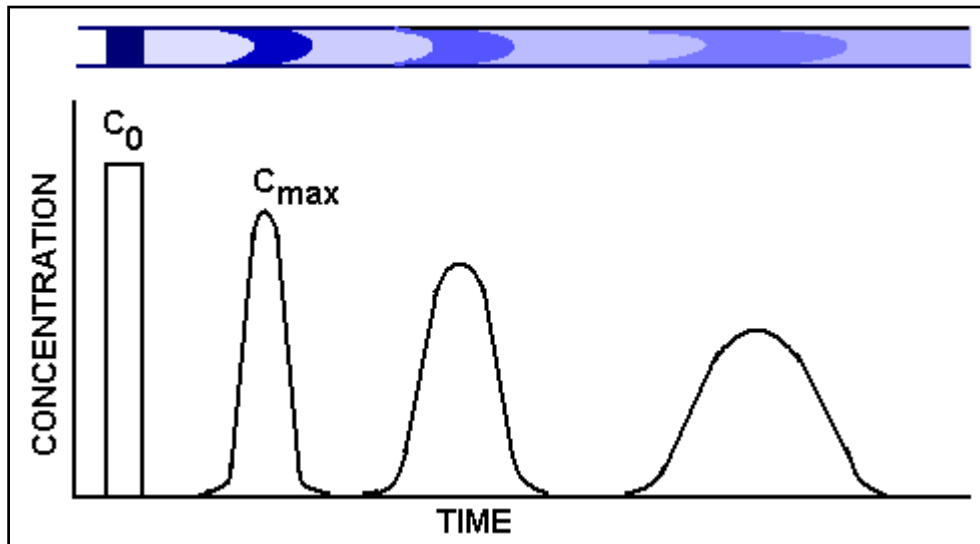


Figure 2.2 The dispersion process typical of FIA system

The injected fluid zones in a non-segmented flow stream can be manipulated reproducibly to produce various degrees of dispersion. In order to provide a quantitative criterion evaluating the extent of dispersion, the term dispersion coefficient (D) was introduced that being defined as the ratio of the concentration of the constituent of interest in a fluid element of the injected zone before and after dispersion, expressed by:

$$D = C_0/C \dots\dots\dots 2.01$$

where C_0 is the original concentration of the constituent in the solution before dispersion, and C is the concentration of that fluid element of the dispersed fluid zone from which analytical readout is extracted. When the fluid element with the highest concentration is used for readout, equation 2.01 is expressed as:

$$D = C_0/C_{\max} \dots\dots\dots 2.02$$

where C_{\max} is the concentration of the constituent at peak maximum.

D is a dimensionless value, which is equivalent to the dilution factor of the fluid element under consideration. For example, if the sample is diluted 1:1 by carrier, thus the dispersion coefficient is 2.

FI systems are categorized into high, medium, and low dispersion systems depending on the degree of dispersion of the injected zone at the read out point. Systems with D above 10 are classified as high, those between 2 and 10 as medium, and those below 2 as low dispersion system.

The main experimental parameters influencing the dispersion of an injected fluid zone include sample volume, flow rate of carrier and merging fluid streams, geometrical dimensions and configuration of transport conduits and on line reactor, and pattern of flow segmentation in system with two immiscible phases. The volume of the injected fluid zone, which most cases is the sample, is important factor influencing its dispersion. The dispersion decreases with an increase in sample volume.

Ruzicka and Hansen [9] stated that dispersion diminishes with a decrease in flow rate. This happens because decreasing flow rates increase the retention time of the sample awaiting transport to the detector. In this phase the reaction between sample and reagent almost reaches the equilibrium. Hence, the peak signal will be higher in a slower flow rate. Fang [12] said that those conditions are only valid at extremely low flow rate where the rate of molecular diffusion approaches that of convection. This has been experimentally demonstrated by Karlberg and Pacey [2]:

with a fixed manifold, dispersion is minimally influenced by flow rate variations within a wide range of 1.6-4.0 mL/min.

The geometrical dimensions and configurations of components constituting a FI manifold are important factors influencing the dispersion. The dispersion of the sample zone increases with the square root of the distance traveled through an open narrow tube. This rule is valid only for straight conduits. When the conduits are coiled for the sake of tidiness or knotted to improve radial mixing, the intensity of dispersion is decreased to different degrees, depending on the radius of the coil or knots. This is due to the generation of secondary flows, which limit the axial dispersion while promoting radial dispersion.

The dispersion of injected zones is enhanced with increases in the inner diameter of the conduit. No generally applicable quantified relationships are available, however, owing to the complexity of the influences from other parameters.

2.4 Detection in the FIA

The usual location of a flow injection manifold is on-line and placed before a detector system in order that proper monitoring of the species is performed after it has been subjected to the step developed in a continuous fashion. While the interface between an flow injection (FI) system and a conventional detector is a commercial or laboratory-made flow cell (or simple aspiration to the flame in the case of atomic techniques assisted by this source), the interface to the high resolution detector has a decisive influence on the performance of the hyphenated system, as analytical quality parameters such as reproducibility, accuracy, sensitivity and selectivity are highly dependent on how the coupling is accomplished.

The complexity of the interface is very different depending on whether the measurement is performed in solution, plasma, or vacuum.

The pre- or post-column coupling of FI to LC (usually HPLC) depends on the pursued objective: a pre-column position is mainly used for implementing a continuous separation step prior to chromatographic individual separation and, in a smaller extension, for developing pre-column derivatizing reactions, meanwhile a post-column position is most often used for derivatizing purposes.

Precolumn (FI-HPLC) assemblies in which the FI manifold includes a separation unit have been mainly devoted to the use of microcolumn with the following objectives:

- (1) trace preconcentration, using different materials such as ion-exchangers, ion-pairing reagents, ligand-exchangers or size-exclusion gels which provide preconcentration factors up to 10,000;
- (2) sample clean-up, by taking advantage of the differences in the interaction between the components of a given sample and the sorbent (e.g., weakly retained phenol compounds or acid substances can be readily separated from phthalate esters strongly adsorbed on a non polar sorbent);
- (3) sample storage, by taking advantage of the relatively inert character of many sorbent materials. This is of special interest when samples have to be collected in remote places;
- (4) protection of the analytical system, as the solid-phase microcolumn acts as a protective filter, lengthening the usable lifetime of the separation unit;
- (5) pre-column derivatization, by using sorbent impregnated with the reagent, a solid redox agent, or a support for retention of a (bio)catalyst

Also other separation steps such as liquid-liquid extraction [13], membrane extraction [14], and dialysis [15] have been coupled to HPLC through a FI manifold. The solution containing the analytes once separated from the matrix is injected into the chromatograph.

Inexpensive flow-cells (either conventional or demountable micro-flow cells) with KBr windows and different thickness spacers (0.015-0.22 mm, 0.159 μL volume) have been used in FI-FTIR coupling when organic solvent carriers are involved [16]. The use of thick spacers yield a higher contribution to the blank measurement from the carrier solvent, so poorer detection limits are obtained. More sophisticated cells are used with uncommon solvents, as in the case of a 25 μL micro circle cell equipped with a zinc selenide crystal, used for aqueous samples in FI-FTIR [17], and a high pressure flow cell (2 μL , 1 mm optical path length and 2 mm^2 cross sectional area) used when supercritical CO_2 was the carrier [18].

Special attention has been paid to interfacing FI systems and Inductively Coupled Plasma (ICP). A general interface to introduce a liquid into a plasma consists of a nebulizer, a spray chamber and a separator. The main shortcomings of using conventional interfaces in FI-ICP couplings related to continuous sample aspiration are the large dead volume and the sample loss involved, as well as the band broadening. Miniaturized interfaces such as the microconcentric nebulizer [19] and micro glass frit nebulizers [20] have been designed in order to overcome this drawback. A jet separator [21], a condenser [19], or a membrane dryer separator have been used to remove the majority of the solvent and convert the sample into a dried and desolvated aerosol in a flow of argon.

Flow injection and mass spectrometry have used either membrane introduction or ion spray systems as interfaces. The former, suitable for

volatile analyte, consists of a membrane probe directly inserted into the mass spectrometer ion source: the carrier solution flows across the inside surface of the membrane while the outside surface is exposed to the vacuum of the mass spectrometer. In ion spray systems, the interface is most often a fused-silica tubing [22].

In dealing with conventional spectrometry detectors, one of the most clear integrating effects is achieved when chemiluminescence reactions are involved, as the sample-reagent mixture reaches the luminescence detector in a time as short as necessary for proper detection of the transient emission from the deactivation of the product. Thus, the method for the simultaneous determination of nitrate and nitrite in water in gas phase [23], the chemiluminescence sensor for the determination of vitamin B₁₂ [24], and the photometric biosensor for determining the activity of urease [25] are basic examples of the integrating effect achieved.

Spectrometer array detectors enable more information to be collected faster from a sample injected into the FI manifold, which can be used for improving precision, for enlarging the linear range of the calibration curve, and for multi determinations, among the most important applications. The faster response of charge-coupled detectors also allows the acquisition of chemiluminescence spectra profiles [26].

Lasers provide an excellent excitation source for the typical small volumes in FI. Thus, the determination of dissolved silica [27] and that of total phosphorus [28] both in water, together with that of sulphide [29], are examples of the enhanced sensitivity of fluorescence when assisted by laser excitation. A laser based technique known as thermal lens spectrometry has been used in flow injection determination of ascorbic acid dehydroascorbic acid [30].

Atomic spectrometry, either those providing single or multi information, have been widely coupled to FI. Moreover, the multiple aspects of this integration have been exhaustively explained elsewhere [31]. Some examples of: (a) the use of FI coupled to laser-enhanced ionization detection, either for reducing electrical interference or providing optimum dilution levels in matrix interfered determinations and for on-line separation concentration prior to laser assistance; and (b) flow injection for arsenic and antimony hydride generation prior to glow-discharge atomic emission spectrometry detection and for the elimination of the matrix effect in a preconcentration step before flame atomic absorption spectrometry, are described in [32] to show both the versatility and integrated effect of these couplings.

Electroanalytical techniques are even more prolific in innovations due to the wider possibilities for the design of probe-sensors that can easily be converted into flow-through sensors through insertion in the appropriate flow cell. Potentiometric electrode arrays have been coupled to FI manifolds with the aim either of improving the determination of a single analyte in case of determination of ammonium [33], or simultaneous measurements based the enzyme field-effect transistor sensor array for monitoring cultivation processes [34].

Both voltammetric and amperometric measurements have been implemented in FI from the very beginning of the technique. Both conventional and new excitation modes have been applied to both commercial unmodified and chemically modified electrodes. The conventional FI methods have been proposed for the determination of a number of analytes, such as trace metals [35], glycerol [36], and acid phosphatase activity [37]. Fast-scan and dual-pulse voltammetries have been used in FI for selective determination of methylmercury and ethanol

in beer [38]. Arrays of gold microelectrodes modified by electrodeposition of palladium for the determination of ascorbic and uric acids in urine [39] or by electrocatalysis for carbohydrates and amino acids [40] have also been reported.

Stripping modes of electroanalysis have been coupled with FI as a means of preconcentrating the analyte, removing the sample matrix, and performing simultaneous determinations. FI-stripping also gives other advantages, such as (a) in situ regeneration of film-mercury electrodes by passage through the flow cell of the appropriate dissolution of Hg(II) using a switching valve for introduction; (b) ability to exchange the stripping solution in order to avoid interferences during this step; and (c) ability to stop the flow during stripping for increasing reproducibility.

In stripping voltammetry, mercury film electrodes have been the most commonly used due to their ability to regenerate the film in a continuous way [41]. Potentiometric stripping has most often used in situ formation of the amalgam by introducing the Hg(II) to the sample [42]. Adsorptive stripping voltammetry with different types of electrodes depending on the analyte nature and different voltammetry modes has also been coupled with FI to produce significant integrating effects. An example of this phenomenon is a calibrationless determination of mercury based on FI-stripping coulometry. But conductometry and coulometry are less frequently coupled with FI.

The FI-ICP-AES coupling has been reviewed [43]. More recent and less common is the arrangements of FI with instruments such as MS, FTIR, and NMR. Recent contributions which exploit known and new integrating aspects of FI-FTIR are the determination of oil in water [44], determination of dithiocarbamate pesticides [45], the FTIR enzymatic determination of

glucose, and the simultaneous determination of organic acids in sugars and soft drinks with prior automated solid phase extraction [46].

Examples of using nuclear magnetic resonance in combination with FI are the studies of the interaction of catechol and ascorbic acid with the crown ether [47].

2.5 Application of the FIA

The diverse nature of the samples encountered, as well as the specific methodologies dictated in many cases by regulation bodies, requires that FIA methods be quite versatile. Industrial, clinical, research-oriented, environmental, agricultural, metallurgical, geological, food mixture relevant, pharmaceutical, and biotechnological applications of FIA have been described in over 10,000 papers and numerous books [9-12, 48-51].

FIA methods of analysis could be very useful for water analysis. The collection of water samples from a stream, river, lake, or effluent provides the largest potential source of error in the chemical analysis of water. Continuous sampling would be required to obtain really representative measurements. In most cases, the cost of equipment and the amount of labor involved make this impossible. Ideally, the chemical composition of water should be measured in situ, thereby being subject to fewer external influences. Although there are electrochemical methods that can be used for certain parameters, for example, pH, conductivity, oxygen, ion selective electrodes, they do not cover at all the range of analysis required. One possibility is the use of field application of FIA that are now available. The application of FIA to water analysis has been reviewed by several authors [9, 10, 51-53].

Valcarcel and colleagues present [54] the results of an interesting method for assessing analytical quality in water analysis by flow injection analysis. The evaluation of 225 papers regarding accuracy, applicability, precision, selectivity, sensitivity, determination range, and sample throughput indicated that:

- In the literature a large number of papers regarding FIA for water analysis were published, confirming the potential of this technique for performing routine analysis in an automated and simple way;
- The overall quality of the application was quite acceptable;
- Many of the surveyed applications were concerned with inorganic analysis and photometric detection;
- It is expected that there would be a much greater implementation of these procedures in control laboratories in the near future;
- Trends in the field should be focused on organic analysis especially for monitoring one or two compounds in water analysis.

During twenty-nine years of its existence, the FIA technique became a versatile instrumental tool that contributed substantially to the development of automation in pharmaceutical analysis. This is well documented by a number of reviews on the use of FIA in the analysis of drugs [1, 55-58].

The specific features of the flow injection analysis of pharmaceuticals are associated with the necessity of determining analytes in complex matrices and at a trace level. Thus, one of the problems that restricts the application of FIA to pharmaceutical analysis is the necessity of selective determination of substances with high sensitivity. The application of highly selective detectors enables the determination of medicinal substances without derivatization. Such variants include determination of nucleic acids based on the oxidation of their guanidine constituent on a carbon paste

electrode [59], and determination of paracetamol in medicine using FTIR spectrometry [60].

The FIA of pharmaceuticals can be carried out with the application of several detection systems. Spectrophotometry is the most widespread method for the detection of pharmaceuticals in FIA because of its versatility and low cost. In number of cases, masking agents are used to eliminate the interference from the matrix. For examples, ascorbic acid was determined by its absorption in the UV region using a subtraction method and a dual-channel FIA system after the alkaline decomposition of the pharmaceutical [61]. The use of diazotization reaction [62] in the flow injection determination of sulfanilamides in a flow containing sodium dodecylsulfate results in a tenfold decrease in the limits of detection compared to similar measurements in non-micellar solutions [63]. An increase the reaction coil to 2.5-4.5 m increases the yield of colored products in the reactions of catecholamine oxidation [64]. The use of the pH gradient in the flow system improves the selectivity of methionine and cysteine determinations in pharmaceutical preparations [65].