

FLOW INJECTION ANALYSIS: AN OVERVIEW

ADITYA. A. KULKARNI^a, ITISHREE. S. VAIDYA^b

¹Department of Quality Assurance, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, ²Department of Pharmaceutical Chemistry, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India
Email: adityakulkarniak@gmail.com

Received: 29 May 2015 Revised and Accepted: 10 Sep 2015

ABSTRACT

The manual handling of the solutions remains the base of modern analytical instrumentation. Flow Injection Analysis (FIA), an automated technique, and the automated techniques being the need of the hour, has had a profound impact on how the modern analytical procedures are implemented. The FIA is a promising technique with well-defined principles of operation. The article provides an overview of the FIA.

Keywords: Analysis, Analytical methods, Flow injection analysis.

INTRODUCTION

Continuous Flow analysis (CFA) has given a new dimension to the chemical industries, which have to follow stringent international regulations. One of the landmarks in analytical development, with respect to analytical instruments, is the development and utilization of Continuous Flow Analysis for automation of the wet chemical method of analysis. Wet chemical analysis is the term used to refer to chemistry generally done in the liquid phase, also known as bench chemistry as many of the tests are performed on a lab bench. It is a technique whereby the sample is placed in a flowing analytical carrier to react with the sample. Reagents can be added to carrier to react with the sample.

The application of CFA for automation of wet chemical methods of analysis did not become practical until the work of Skeggs. He introduced an air bubble into the analytical stream to divide the sample into segments to preserve its identity. This technique was called as segmented continuous flow analysis (SFA). Pioneering work of Ruzicka and Hansen in Denmark and Steward in USA led to application of CFA to analytical streams unsegmented by air bubbles i.e. devoid of air bubbles. This technique was called non-segmented continuous flow analysis by Steward and Flow Injection Analysis (FIA) by Ruzicka and Hansen [1].

The FIA is a CFA technique whereby a highly reproducible volume of sample is injected into a flowing analytical stream which is unsegmented by air bubbles. On preliminary examination, it may appear that the major difference between FIA and SFA is the presence or absence of air bubbles in flowing stream; however the FIA differs from SFA in three important ways:

Sample introduction

Sample is introduced in FIA by injecting a reproducible volume at precisely timed intervals into a flowing stream in such a way that the flow of the stream is not disturbed. In SFA, the sample is aspirated into the stream. The net result is sharp peaks in FIA and flat topped peaks in SFA. Sampling rates in the FIA analyzer can approach 200–400 samples per hour with baseline resolution, while in the SFA analyzer are generally 100 samples per hour. Higher sampling rate is obtained in SFA but baseline resolution is affected.

System timing

Reproducible timing is mandatory in FIA as any variation in time a sample spends in the analyzer will directly affect the peak height due to dilution with the carrier stream. Timing is not critical in SFA.

Controlled dispersion

FIA is based on the precise control of flow induced dispersion of sample zone as it passes through the analyzer. Mixing occurs by

dispersion and flow profile is laminar. Conversely, SFA is based on elimination of the sample induced dispersion by the addition of air bubbles and flow profile obtained is turbulent. Sample integrity in FIA is maintained by precise control on sample dispersion.

The FIA technique was defined by Ruzicka and Hansen as, "A method based on injection of a liquid sample into a moving unsegmented continuous stream of suitable liquid. The injected sample forms a zone, which is then transported towards 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 was rephrased to read, "Information-gathering from a concentration gradient formed from an injected, well-defined zone of fluid, dispersed into a continuous unsegmented stream of carrier." [2].

Principle and theory [3]

The FIA is a kinetic method of analysis. Even for the simplest possible FIA system, such as injection of the sample into an inert carrier stream to transport it to the detector, is not in equilibrium. The detector response function is time dependent and depends on the physical process of the dispersion of sample bolus into surrounding carrier. If the analyte now reacts with the carrier and the reaction product is detected, the chemical kinetics of the reaction also affects the detector response function. In this system, the reagent carrier and the analyte form gradients in the reaction zone that depend on the sample dispersion. The reagent: analyte ratio varies throughout the length of the sample zone.

Even for the simpler systems, it has not been possible to describe properly the dependence of the detector output function on system operating parameters such as manifold design, flow rate, etc., due to complexity in describing the two simultaneous physical and chemical processes. An understanding of the theoretical basis for FIA is important in order to optimize system design and performance.

Dispersion

A sample injected into a carrier stream flowing through a narrow bore straight section of tube initially exists as well a well-defined plug. As the plug travels downstream it disperses and mixes with the carrier stream. A well-defined concentration gradient is formed. If the sample dispersion is due to convection, the flow profile is parabolic head and tail. This type of flow is called as laminar flow and is characteristic of most FIA systems. In laminar flow, the liquid can be thought to be divided into an infinite number of velocity profiles and maximum velocity is observed at the center of a flowing stream. The velocity at the wall of the conduit (tube) is zero. Two additional mass transport processes are operational, which are: molecular diffusion in the longitudinal direction (parallel to the direction of flow) and molecular diffusion in the radial direction (perpendicular to the direction of flow).

Dispersion is dependent on tube radius in laminar flow conditions and increases as the square of the radius. These parameters can be manipulated to obtain the required degree of dispersion for analytical application. As dispersion increases, the analyte bolus is diluted and spread out. This leads to decreased sensitivity and sample throughput. Therefore, dispersion has to be minimized with respect to an analytical application when designing an FIA system. Dispersion in the FIA system can be reduced by coiling the tube.

Dispersion has been defined as, "ratio of the sample concentration before and after the dispersion process has taken place in the element of fluid that yields analytical results."

In a single-line flow injection manifold, the extent of dispersion and mixing and hence the dilution, of the injected sample zone that occurs as it is transported through the conduit is expressed in terms of the dispersion coefficient, D , which is defined by,

$$D = C_0 / C_{max}$$

Where, C_0 is an original concentration of an injected sample, C_{max} is the maximum concentration of the sample zone after it has undergone all dispersive process and is passing through the detector.

When a FIA cell is to be fabricated one must know to what extent the original solution is diluted while flowing through to the detector and time between the sample injection point and read out point. Hence dispersion coefficient (D) is used to decide these dimensions. D affected by: Sample volume, tube length, flow rate, tube internal diameter.

Three general categories of dispersion are:

1. Limited dispersion: FIA system with $D = 1$ to 3.
2. Medium dispersion: FIA system with $D = 3$ to 10.
3. Large dispersion: FIA system with $D > 10$.

Sample volume

The volume of sample injected is a powerful variable that can be used to alter the sample dispersion. As the volume of the sample increases, so does the peak height and thereby increasing sensitivity. Paralleling the peak height, there is increase in peak width, i.e. dispersion, which results in decreased sample throughput. As the volume injected approaches the volume of the system, steady state is obtained and peak becomes flat topped.

An important value is half-volume, $S_{1/2}$, given by the equation,

$$S_{1/2} = 0.693/K$$

This is the volume of solution required to reach 50% of the steady state detector response value. At one $S_{1/2}$ unit, $C_{max} = 50\% C_0$. If the tube radius is halved, the sample will occupy a portion of the tube four times longer, thereby reducing the area of the sample-carrier interface. The net result is decreased dispersion.

Flow rate

As the flow rate is increased in a narrow bore tube (internal diameter < 1 mm) under laminar flow conditions, the parabolic profile becomes more pronounced. The concentration gradient stretches along the tube length. The parabolic head and concentration gradient continue to increase in magnitude as flow rate increases until the laminar flow pattern is broken by turbulence.

At a low flow rate, radial mixing begins to nullify the formation of the parabolic velocity profile caused by forward motion of liquid. If the injection of the sample is stopped, dispersion of sample zone essentially ceases, except for a small contribution due to radial molecular diffusion.

At higher flow rate, dispersion increases, leading to decreased residence time and increased reagent consumption. If a chemical reaction is involved, the extent of reaction decreases at higher flow rate as time for the reaction is less. Sensitivity decreases as the sample is diluted by increased dispersion. At low flow rates, it is exactly opposite.

Instrumentation

Ideally, the FIA system should be constructed in such a manner that:

1. The carrier stream flows through a narrow tube of uniform internal diameter, including injection and detector section.
2. Sample solution is injected as an instant pulse of exact volume and in a short duration of time, in such a way that the movement of the carrier stream remains undisturbed.
3. Side streams are added to the main stream in an easily reproducible manner.
4. The flow of all streams is pulse free and their movements can be started and stopped instantaneously.
5. Detector instantly and selectively responds to analyte concentration with maximum signal yield [4].

The basic FIA system consists of a pump, an injection valve, an analytical manifold, a detector and a recorder. In addition, an auto-sampler can be used for automating sample injection and data processing devices.

Two parameters must be controlled to ensure a high degree precision. First, reproducible timing is essential. As the system is not at equilibrium and the degree of dispersion depends on the sample residence time, the system flow rate must be precisely controlled. Secondly, the sample volume and injection timing must be controlled precisely so that a highly reproducible sample bolus is placed in the carrier stream.

Pumps

An ideal pump would provide a pulse free constant flow of carrier and reagents. Syringes, pressure bottle, reciprocating and peristaltic pumps have all been used in the construction of FIA systems. The type of pump selected depends on the application and objective of the system.

The most widely used is the peristaltic pump. It is a multichannel pump and variable flow rates can be obtained for each channel by varying the internal diameter of pump tubes used in each channel. Modern pumps have 8-10 rollers, arranged in a circular manner, such that half are squeezing the tubing at any given time [5].

Injectors

The injection valve should be designed to place a highly reproducible wave bolus of sample into the carrier stream in such a fashion that the flow of the stream is not changed by an injection event. Sample size for flow injection analysis ranges from less than 1 μ l to 200 μ l, with 10 to 30 μ l being used for most applications.

Two types of injection valves are found to have widespread utilization: rotating and sliding valves. Rotary valve e. g. six port HPLC rotary valves can be used for sample injection. The sample is injected by rotation of the valve so that the sample loop connects to the carrier stream. Slider valves, e. g. four part slider valve can be utilized. The two valves are required. Once the sample loop is loaded, the valve state is changed so the carrier flow is directed through the sample loop.

Manifold

The manifold is the heart of FIA system. Its design is dependent on the application. For simple chemical reactions, it can be constructed from tubing. For applications like solvent extraction, dialysis, etc. specialized modules are required.

Manifold coils are usually constructed from polypropylene, polyethylene or Teflon tubing. Teflon is considered as ideal for tubing. Tubing connections to each other and to other system components are made using standard chromatographic plastic ferrules and washer. When constructing a manifold, dead volume must be minimized since they increase dispersion and produce badly tailing peak.

Wide variety of tube sizes are available commercially (i.d. = 0.25-4 mm), which allow the flow rates as small as 0.0005 ml/min to as large as 40 ml/min.

Detectors

Practically any detector described for use of HPLC can be used for FIA system.

The main criteria for an FIA detector are that the response time of the detector is fast and volume of the detector is small. Since the peak width of most FIA is for a few seconds, the detector should have response time less than one second. A slower response time can affect peak shape.

Some detectors used in FIA systems are: amperometric, atomic absorption, chemiluminescence, coulometric, fluorometry, potentiometric, pH, ion selective electrode, spectrophotometric, nephelometry, flame [3, 5].

Techniques used in FIA [3, 5, 6]

FIA systems have been described for automating sample dilution, sample transport and for a variety of analytical systems utilizing chemical reactions to enhance detection or to eliminate interference in the measurement step. FIA systems have also been described for reducing reagent consumption to as little as micro liters

Solvent extraction

It is a well-established and effective separation/preconcentration technique. A fixed volume of sample is injected into a reagent stream, where it is converted to an extractable form.

Dialysis and diffusion

They are effective techniques for separation of low molecular weight species or volatile compounds from macromolecules. The separator design is similar to membrane solvent extractor except a dialysis membrane or a gas permeable membrane is used. The sample passes through the separator where the analyte migrates by diffusion to donor stream which is pumped to the detector.

Merging zone

In FIA systems described so far, the sample is injected into continuous flowing streams of reagents which fill the entire system even when a sample is not in the system. One approach towards minimization of reagent consumption is to simultaneously inject the sample and reagent into inert stream and mix these segments in the manifold where the carrier is wash solution or water. This technique is called as merging zone technique, significantly reduces reagent consumption to as little as the few microliters per sample. This becomes important if an expensive reagent is used.

FIA titration

Titration methods based on complexometric, redox and neutralization are frequently employed for determination of acid, bases and metals in a variety of samples. Titrations are typically performed in batch mode, i.e. the titrating vessel is filled, the measurement performed and the vessel is emptied, cleaned and reused.

Solid phase extraction

Solid phase extraction (SPE) is a sample preparation tool with extensive application in analytical chemistry, mainly due to its

simplicity and the avoidance of the toxic organic solvents used in traditional solvent extraction. The concept is based on the immobilization of the reagents-that flow in a separate stream or channel in normal FIA-at a predefined point of the manifold. Despite of its apparent advantages, SPE operation in batch mode is a tedious and time consuming process.

Online digestion/hydrolysis/photolysis

The principle of these methods is simple and is based on the cleavage of C-P or C-O-P bonds and subsequent determination of the yielded orthophosphate ions by molybdenum blue approach. C-P bonds can be cleaved online by thermal digestion induced at 90 °C in the presence of persulphate ions. C-O-P bonds can be cleaved either by same procedure or enzymatically by alkaline phosphatase. Photolysis process can be carried out by using a light source like UV lamp and advantage of this process is the lack of reagents, manifold simplicity and enhanced selectivity.

Stopped-flow method

Dispersion in smaller i.d. tubing decreases with the decrease in flow rate and ceases almost entirely when the flow is stopped. These phenomena are used to increase the sensitivity of measurements by allowing time for reactions go towards completion without dilution of the sample zone by dispersion. In this technique, a timing device is required to switch off the pump at regular predetermined time interval.

Another application of this technique is for kinetic measurements. In this, the flow is stopped with the reaction mixture in the flow cell wherein the changes in the concentration of reactants and/or products can be monitored as a function of time.

FIA AND HPLC

Conceptually, the FIA can be seen as High Performance Liquid Chromatography (HPLC) without column. In HPLC, the column provides the specificity. While in FIA, a combination of the chemistry and detectors provide specificity. Chromatography theory is based on the mass transport and dispersion in a straight and narrow tube, can also be applied to the FIA.

FIA resembles HPLC instrumentation. Recorder output has in both cases, in the form of a peak, height of which is the basis of analytical readout (although in chromatography, measurement of peak area is often preferred).

Some of the dimensional and operational parameters of both systems are also similar-volume of sample injected, flow velocities and flow through detector-as are the requirements with regards to the response speed of the electronic components and recording equipment attached. Therefore, a simple FIA apparatus can be constructed by using components of HPLC systems, and wide experience with HPLC led Stewart and his colleagues to design a nonsegmented continuous flow analyzer. Basically, however, the FIA and HPLC are two quite different techniques, because their principles and purposes are dissimilar.

Table 1: Differentiation between FIA and HPLC

HPLC	FIA
Differences	
<ul style="list-style-type: none"> Columns The pressure of about 7000 psi is required as sample passes through tightly packed column Separation of samples due to different velocities of migration as a consequence of difference in equilibrium distribution Band broadening by any of the components outside the column must be minimized, and the velocity of the liquid stream must be optimized with respect to column performance rather than to chemical reactions in carrier stream. 	<ul style="list-style-type: none"> Reaction coils The pressure of about 1000 psi is required as sample passes through a short length of narrow tubing having internal diameter 0.5 mm The sample zone travels at the same speed as the carrier stream through an open narrow tube usually at conditions of essentially laminar flow so that the parabolic flow reaches the detector before the material situated close to the walls. Band broadening-or sample zone dispersion-is controlled so that its degree-high or low-exactly suits the detection method and chemistry associated with it.
PURPOSE	
<ul style="list-style-type: none"> The main purpose is to obtain adequate resolution of several components, originating from a single injected sample, in minimum time. 	<ul style="list-style-type: none"> The purpose is analyzing the maximum number of discrete samples using the minimum amount of time, reagent and the sample solution.

Advantages of FIA [7, 8]

It is easy to see that compared to manual analyses; the tubing lines serve as solution containers and transfer vessels, the injection valve serves as a micropipette, and the pump replaces the lab technician using all this lab ware.

The FIA has been very successful in simplifying chemical assays. The main reasons for the success are the following advantages of FIA over conventional manual techniques:

1. Automation in sample preparation and detection.
2. Simplicity of FIA configuration.
3. High sampling rate (typically 100-300 samples per hour).
4. Fast response time (often less than 1 min) with good reproducibility.
5. Rapid start-up and shutdown times.
6. Smaller sample and reagent consumption and hence less waste generation.
7. Simple, flexible and low cost instrumentation.
8. Reduced analyses and labour cost when a lot of samples have to be analyzed.
9. Increased precision compared to batch methodologies.
10. Ease of implementation of continuous separation techniques on flow injection manifolds.

Applications of FIA

Typical applications of flow injection analysis include the following fields:

1. Pharmaceutical application.
2. Environmental analysis.
3. Food analysis.
4. Biological material.
5. Mineral material.
6. Clinical assay.
7. Bio-analytical chemistry.
8. On-line monitoring in Biotechnology.
9. Monitoring waste and its treatments.

Examples of applications**Pharmaceutical application****Dissolution testing**

FIA can act as an excellent interface between dissolution apparatus and instruments (e. g. HPLC). The FIA can be used in derivatization, preconcentration, on-line separation process, dilution, selectivity and/or sensitivity enhancement, near real time response and for analysis unstable active components [9].

Determination of thiamine HCl (THC) in pharmaceuticals

Simple and sensitive, normal and reverse flow injection methods for spectrophotometric determination of THC was developed and optimized. Both methods are based on the reaction between THC and diazotized metoclopramide in alkaline medium. Limits of detection of normal and reverse phase were 2.118 and 0.839 µg/ml and the sampling rates were 80 and 95 injections per hour respectively [10].

Environmental**Determination of cationic surfactants**

A method for the determination of four cationic surfactants, found in environmental (i.e. Ground, surface, municipal waste water) and

commodity (i.e. Detergent, shampoo, soap), was developed on the FIA. Determination was based on enhancement of the colour intensity of Fe (III)-SCN-complex [11].

Analysis of stable and radioactive strontium sample

A multisyringe FIA method using solid phase resin was developed for determination of stable and radioactive strontium. This method was applied to different samples of environmental interests (water, milk and soil) [12].

Food analysis**Determination of dibutyl phthalate (DBP) in wine**

A sensitive method for determination of DBP in wine using FIA-chemiluminescence was developed for the first time. Chemiluminescence was based on quenching effect of DBP on luminal-myoglobin system. Limit of detection was 0.03 picogram/ml. The method was also applicable to human serum and urine [13].

Simultaneous determination of anti-oxidants, preservatives and sweeteners

Mixture of aspartame, acesulfame/saccharin, methylparaben, ethylparaben, propylparaben, butyl paraben (BP), propylgallate and butylhydroxyanisole was separated on a monolithic C18 silica column attached to the FIA. The method was applicable to food and cosmetic samples [14].

Biological material**Determination total nitrogen content in plant material**

A FIA system with an AgCl(s) reactor was used for spectroscopic determination of total nitrogen in Kjeldahl digest of plants. Limit of detection was 0.2% w/w nitrogen [15].

Determination of plant sulphur and sulphate-sulphur

FIA along with turbidimetric detection was proposed in this method. The method can favourably be compared to other methods of sulphur and sulphate-sulphur detection. Limit of detection was 0.6 mg/kg SO₄-S [16].

Mineral material**Determination of gold in the mineral**

The method was based on the fluorescence enhancing reaction of 2-Pyridine carboxaldehyde furfuralhydrazone with potassium bromate, catalyzed by Au³⁺, in aqueous medium at pH 4.20 and 35° C. Gold was separated on a micro polyamide resin column and collected [17].

Determination of Cu, Ni and Zn in alloy

Copper and nickel are determined by simple dissolution of alloy in a nitric acid-phosphoric acid mixture, and are determined as Cu (II) and Ni (II) aquo-complexes. For zinc, the alloy is treated with thiosulphate-acetate buffer, and absorbance of Zn-xyleneol orange is measured. The method has been applied to standard copper-base alloys [18].

Clinical analysis**Determination of metformin**

A simple, fast and sensitive quantification method for the drug Metformin in dog serum were developed using flow injection analysis (FIA)-tandem mass spectrometry (MS/MS). The total data acquisition for this method was merely 2 min [19].

Determination of total cholesterol

Normal FIA and stopped-flow FIA in combination, either with photometric and fluorimetric detection were developed for the determination of total cholesterol [20].

Bioanalytical chemistry**Determination of amino acids**

Determination of different amino acids using FI-spectrophotometric method was carried out by reacting with Cu (II). The amino acid-Cu

(II) complex was then treated with zinc in basic medium to produce blue colour, which was monitored [21].

Determination of glucose

Determination of glucose in biologic samples in a non-enzymatic way was developed using FIA. This method was based on electrocatalytic oxidation of glucose at nickel electrode [22].

On-line monitoring in biotechnology

On-line determination of beta-galactosidase activity

A FIA method for on-line monitoring of intracellular beta-galactosidase production during cultivation of recombinant *E. coli* was developed. FIA assay in conjunction with cell disintegration step could also be applied for on-line monitoring of intracellular protein formation [23].

Monitoring of bioprocess using sensors

A micro fluidic chip, integrating amperometric enzyme sensors for the detection of glucose, glutamate and glutamine in cell-culture fermentation processes was developed. The biosensor chip was coupled to a flow-injection analysis system for electrochemical characterization of the sensors [24].

Monitoring wastes and its treatment

Monitoring of Ag (I) and iodine residues in recycled water

A laboratory built FIA was reported for monitoring of the drinking water disinfectants Ag (I) ions and iodine in water produced from NASA's water recovery system [25].

Monitoring of phosphorus and nitrogen in waste water

A FIA system was used for monitoring and control of a biological waste water treatment plant with biological removal of phosphate and nitrate. The chemical methods used were based on the classical calorimetric method [26].

Pharmaceutical application [2]

Pilot screening tests in the discovery of drugs of natural origin

The main goal of preliminary stage large-scale screening tests for discovery of drugs of natural origin is to find sources of compounds with desired biological activities (prospective drugs or lead

structures). An automated FIA system equipped with a mass spectrophotometer was devised for rapid monitoring and evaluation of antioxidants and radical scavenging activity of plant materials. This method was based on the known reaction of the stable 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) with antioxidants in organic or aqueous-organic media resulting in bleaching of DPPH.

Screening test for drug-bioligand interaction

In pharmacological screening the drug candidates can be identified and characterized at cellular or molecular biology level by biological tests called "functional assay". Functional assays permit to recognize if a potential drug inhibits or elicits certain biological response through interactions with living cell receptor sites. An automated FIA system with online solid phase extraction and simultaneous fluorescence and mass spectrophotometric detection was used to study the interaction of G-protein coupled receptors (e. g. histamine H2 receptor) with inhibitors (e. g. Fluorescent-labeled receptor ligands) as potential drugs. The system allowed sensitivity down to 5 f mol of the ligand studied.

Study of drug-protein binding

After administration, most drugs enter the blood stream where they are transported partly in unbound form and partly reversibly bound form. A microdialysis probe integrated in a FIA manifold to determine amperometrically the concentration of streptomycin not bound to bovine serum albumin (BSA) at pH 13. The data obtained were used for the determination of the streptomycin-BSA association constant and the number of binding sites.

Process monitoring during drug production

Process Analytical Technology (PAT) can be conducted: (a) off-line — the analysis utilizing discrete samples is carried out in a laboratory away from the production process; (b) at-line — the analysis of discrete samples is carried out next to the production process; (c) on-line/in-line — continuous analysis takes place in a side stream and (d) in situ — continuous analysis takes place directly in the reaction vessel. The FIA has become a successful example of an in-line PAT implementation owing to its versatility, possibility for miniaturization and ease of handling of a large number of discrete samples to be analyzed in preset time intervals. The potential of the FIA in utilizing bio-enzymatic analytical micro bioreactors for glucose, lactate, ethanol, galactose and L-amino acid monitoring of cell culture media has been demonstrated recently.

Table 2: Examples of pharmaceutical applications of FIA [4]

S. No.	Sample	Method	Sensitivity	Samples per hour
1	Arsenic (V)	The sample is reduced to Arsenic (III) by reacting with hydrazine. Excess reagent is removed with a cation exchange column. The product is detected amperometrically using a platinum wire flow through the detector.	1 ppb	30
2	Caffeine	The sample is extracted using chloroform and is detected spectrophotometrically.	200 ppm	75
3	Codeine	Codeine in aqueous sample is extracted as its picrate ion pair into chloroform and after phase separation, is measured spectrophotometrically at 335 nm.	5x 10 ⁻⁵ M	60
4	Corticosteroids	Blue tetrazolium is reduced by steroids in alkaline medium, forming a highly coloured formazon which is measured spectrophotometrically at 525 nm. (The method has been especially studied for a typical corticosteroid, methyl Prednisolone acetate, but has been extended to additional twelve steroidal drugs.)	0.1 mg/ml	100
5	L-Dopa	Aqueous samples are injected into an aqueous supporting electrolyte solution and measured amperometrically by reticulated vitreous carbon flow through the detector. (An identical system has been used for epinephrine, ascorbic acid and ferricyanide with similar levels of sensitivity and sampling frequency.)	0.3 ng	264
6	Glycine	Glycine forms a strong fluorescent species with o-phthalaldehyde which can be measured at excitation and emission wavelengths of 337 nm and 455 nm respectively.	2 pg/ml	180
7	Glucose	Glucose is degraded enzymatically by glucose dehydrogenase in the presence of a co-enzyme, nicotinamide adenine dinucleotide, which serves as a chromogen, colour of which is measured spectrophotometrically at 340 nm.	1 mM	120
8	Hydrazine	Hydrazine reacts with 4-dimethyl aminobenzaldehyde in acidic medium, yielding a yellow p-quinone like compound which is measured spectrophotometrically at 460 nm.	0.02 ppm	350
9	Glycerol	Solution of glycerol in water is injected into the aqueous stream of a buffer containing a colour indicator, and the dispersion of the sample zone is measured spectrophotometrically. The decrease in absorbance is then a linear function of the log of viscosity of injected sample.		120
10	Meptazinol	A meptazinol containing the sample is injected into a carrier stream of electrolyte and determined voltammetrically by means of a glassy carbon electrochemical detector based on wall-jet principle.	0.01 mg/ml	80

Trends and future of FIA

Future advances in methodology must extend the residence time of the sample in the system. FIA is based on controlled dispersion. For those applications involving modification of the sample before detection, there is a tradeoff between sensitivity and sample throughput. The detector output depends on two kinetic processes: reaction of the sample with reagent and physical dispersion of the sample zone. One approach to reduce dispersion is by breaking up the laminar flow pattern that develops as the sample plug travels down the conduit. The application of packed bed reactors and pearl string reactors shows promise for extending the residence time of the sample while maintaining the sample frequency of several hundred samples per hour.

FIA systems designed to greatly reduce reagent consumption will extend applications to the systems that require expensive reagents. Systems which utilize immobilized enzymes and that regenerate the reagent in situ are also being explored.

It is possible to further miniaturize the FIA instrument. This would reduce the space required for an instrument and also reduce reagent consumption. Application of miniaturized systems may be hindered by the back pressure as conduit radius decreases and the increased tendency for its blockage by particles.

Increased theoretical understanding of the dispersion process should lead to additional applications. Exploitation of the sample/reagent interface for analytical methods is a new and promising concept in the analysis.

One potential application of the FIA in the pharmaceutical laboratory is in the automation of dissolution testing. A simple FIA manifold can be used for rapidly analyzing the large number of samples generated in a typical dissolution laboratory. Such a system would require microliters of sample.

In summary, a large variety of analytical problems can be solved by the FIA. Despite its great potential, it has not been fully exploited, probably because of its less commercial popularity in some countries and because analysts are not yet fully aware of its great possibility as an efficient tool for routine analysis. The FIA can make automation cost effective in small laboratory or in those laboratories where a small number of similar samples are available. Continuing advances will produce additional applications and make the technique more attractive.

Despite being one of the most popular flow analysis techniques, the discussion of FIA in instrumental books is rare. Almost all the books practically skip this topic. FIA can be introduced in under-graduate laboratory using low cost instrumentation. It can be used to bring the automation used in the real world into the analytical chemistry laboratory, which can help the students learn about the multidisciplinary character of modern analytical science and techniques.

CONFLICT OF INTERESTS

Declared None

REFERENCES

- Luque De, Castro MD, Valcarcel M. Flow injection analysis for pharmaceuticals. *J Pharm Biomed Anal* 1989;7:1291-300.
- Kolev SD, Mckelvie ID. editors. *Advances in flow injection analysis and relate techniques*. 1st ed. United Kingdom: Elsevier Science; 2008.
- Landis JB. Flow injection analysis. In: Munson JW. editor. *Pharmaceutical Analysis Modern Methods Part B*. Marcel Dekker Series, Mumbai: International Medical Book Distributors; 2001. p. 217-77.
- Ruzicka J, Hansen EH. *Flow Injection Analysis*. 2nd ed. NY: John Wiley and Sons; 1981.
- Skoog DA, Holler FJ, Crouch SR. *Automated methods in analysis*. In: *Instrumental Analysis*. Indian ed. New Delhi: Cengage Learning; 2010. p. 1017-27.
- Tzavaras PD, Themelis DG. Review of recent applications of flow injection spectrophotometry to pharmaceutical analysis. *Anal Chim Acta* 2007;588:1-9.
- Pharik A, Patel K, Patel C, Patel BN. Flow injection: A new approach in analysis. *J Chem Pharm Res* 2010;2:118-25.
- Lazaro F, Luque de Castro MD, Valcarcel M. Novel strategies in flow injection analysis. *J Pharm Biomed Anal* 1988;6:585-98.
- Luque de Castro MD, Valcarcel M. Automation of pharmaceutical dissolution testing by flow injection analysis. *J Pharm Biomed Anal* 1990;8:329-36.
- Al Abachi MQ, Hadi H. Normal and reverse flow injection-spectrophotometric determination of thiamine hydrochloride in pharmaceutical preparations using diazotized metoclopramide. *J Pharm Anal* 2012;2:250-355.
- Patel R, Patel KS. Simple and specific method for flow injection analysis determination of cationic surfactants in environmental and commodity samples. *Talanta* 1999;48:923-31.
- Fajardo Y, Gomez E, Mas F, Garcias F, Cerda V, Casas M. Multisyringe flow injection analysis of stable and radioactive strontium in samples of environmental interest. *Appl Radiat Isot* 2004;61:273-7.
- Guo J, Luo K, Chen D, Tan X, Song Z. A rapid and sensitive method for determination of dibutyl phthalate in wine by flow-injection chemiluminescence analysis. *J Food Compos Anal* 2013;31:226-31.
- Garcia-Jimenez JF, Valencia MC, Capitan-Vallvey LF. Simultaneous determination of antioxidants, preservatives and sweetener additives in food and cosmetics by flow injection analysis coupled to a monolithic column. *Anal Chim Acta* 2007;594:226-33.
- Carneiro JMT, Sartini RP, Zagatto EAG. Spectrophotometric determination of total nitrogen content in plant materials using a flow-injection system with an AgCl(s) reactor. *Anal Chim Acta* 2000;416:185-90.
- Morante C. Determination of plant sulphur and sulphate-sulphur by flow-injection analysis using a two-line manifold. *Anal Chim Acta* 1991;249:479-88.
- Tang B, Zhang H, Wang Y. On-line separation, preconcentration and determination of trace amounts of gold in mineral sample by flow injection catalytic kinetic spectrofluorimetry. *Anal Chim Acta* 2004;525:305-11.
- Kuroda R, Mochizuki T. Continuous spectrophotometric determination of copper, nickel and zinc in copper-base alloys by flow-injection analysis. *Talanta* 1981;28:389-93.
- Michel D, Casey Gaunt M, Arnason T, El-Anead A. Development and validation of fast and simple flow injection analysis-tandem mass spectroscopy (FIA-MS/MS) for the determination of metformin in dog serum. *J Pharm Biomed Anal* 2015;107:229-35.
- Fernandez-Romero JM, Luque De Castro MD, Valcarcel M. Determination of total cholesterol in serum by flow injection analysis with immobilized enzymes. *Clin Chim Acta* 1987;167:97-104.
- Zamora LL, Calatayud JM. Flow-injection spectrophotometric determination of amino acids based on an immobilised copper (II)-zincon system. *Anal Chim Acta* 1993;28:601-5.
- Zhao C, Shao C, Li M, Jiao K. Flow-injection analysis of glucose without enzyme based on electrocatalytic oxidation of glucose at a nickel electrode. *Talanta* 2007;71:1769-73.
- Kracke-Helm HA, Brandes L, Hitzmann B, Rinas U, Schugerl K. On-line determination of intercellular β -galactosidase activity in recombinant *Escherichia coli* using flow injection analysis (FIA). *J Biotechnol* 1991;20:95-104.
- Backer M, Rakowski D, Poghosian A, Biselli M, Wagner P, Schoning MJ. Chip-based amperometric enzyme sensor system for monitoring of bioprocess by flow-injection analysis. *J Biotechnol* 2013;163:371-6.
- Williamson JP, Emmert GL. A flow injection analysis system for monitoring silver (I) ion and iodine residuals in recycled water from recovery systems used for spaceflight. *Anal Chim Acta* 2013;792:72-8.
- Pedersen KM, Kummel M, Soeberg H. Monitoring and control of biological removal of phosphorus and nitrogen by flow-injection analysers in a municipal pilot-scale waste-water treatment plant. *Anal Chim Acta* 1990;238:191-9.