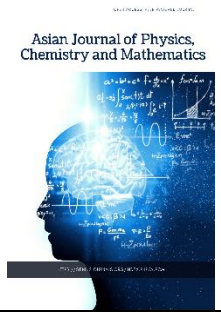

Review of Flow Injection Analysis

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ABSTRACT

The first generation of FIA techniques, Flow Injection (FI), is still the most extensively utilized today since the sample zone is injected into a flowing carrier stream of reagent. As the injected zone moves downstream, the sample solution disperses into the reagent, and a product forms at the point where the sample and reagent zones meet.

Keywords:

1.1 Review of Flow Injection Analysis

The first generation of FIA techniques, Flow Injection (FI), is still the most extensively utilized today since the sample zone is injected into a flowing carrier stream of reagent. As the injected zone moves downstream, the sample solution disperses into the reagent, and a product forms at the point where the sample and reagent zones meet. A detector placed downstream detects a change in color or another feature as the derivatives sample material travels past the flow cell. Hansen and Ruziicka have been together for a long time [1], see (Fig.1.1).

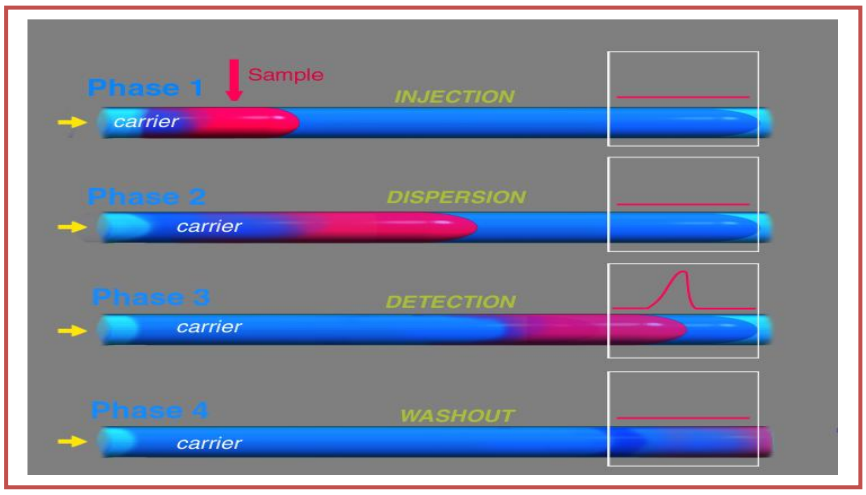

Fig. 1.1 The four phases of FIA

Analysis of Flow Injection (FIA) is a well-known flow-based approach that has revolutionized the analytical laboratory speed, automated solution handling, compactness, and cheap cost throughout the preceding 30 years. There are around 15000 references in the FIA literature [2] , Pharmaceutical assays [3], environmental research [4], Some of the disciplines in which we operate include oceanography, process control, agriculture, drug development, and clinical testing [5,6] are among the tests and applications covered. Because it provides sample pre-treatment, matrix removal, and automatic detector recalibration, For a wide range of spectroscopic and electrochemical equipment, The front end or solution handling system has been FIA [2].

A small, carefully determined amount of sample is injected into a reagent-flowing stream in FIA. Because the gas—usually air in ordinary continuous-flow systems—is not introduced into the streams, the FIA apparatus is less complicated and sophisticated than gas-segmented analytical systems. The reaction products are monitored before "steady-state" conditions are attained, and in many studies, the readout is available within seconds after inputting the sample, allowing for quick sample throughput. When adopting the "merging zones" strategy, the amount of sample and reagent required is little, and accuracy and precision are great. A simple yet versatile FIA system is easy to develop and adapt, and this novel way allows for a lot of flexibility[7].

However, only a few clinical chemists have experimented with this unique concept thus far, and we hope that our findings will stimulate their curiosity. Since the development of chromatographic technologies, gas chromatography, and then "high-performance" liquid chromatography, samples of analytical interest have been injected into nonsegmented flowing streams and the concentration profile monitored using suitable flow-through detectors. In analytical chemistry systems, the use of non-segmented liquid reagent flowing streams was also studied[1, 8-15].

The need to thoroughly mix the samples with reagents by utilizing pulsers [8,9,15] or integrating mixing chambers [10] resulted in peak widening and limited sample throughput in some of the early systems. As a result, it's customary to believe that in continuous-flow systems, there are only two strategies to avoid carryover: sectioning of gas [16] or turbulent flow [1,17] . Because of the parabolic velocity profile, this should result in less sample zone dispersion than laminar flow (Fig.1.3). A more radical technique depends on the dispersion patterns created under laminar flow conditions for analysis instead of properly mixing the sample and reagent[7].

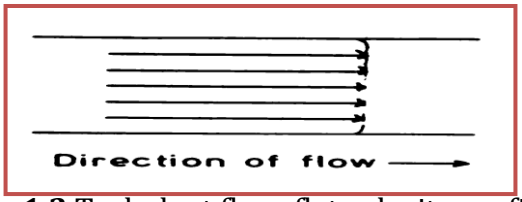

Fig.1.2 Turbulent flow, flat velocity profile

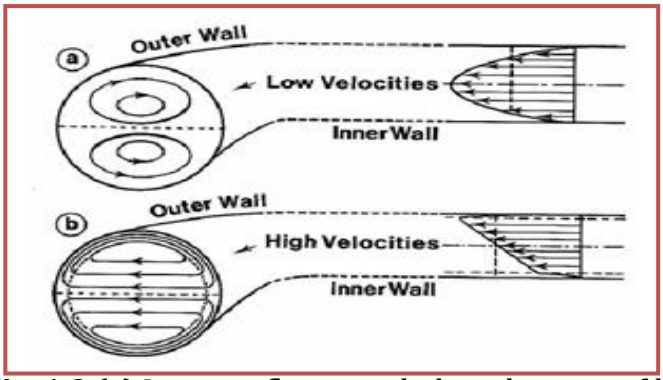

Fig. 1.3 (a) Laminar flow, parabolic velocity profile
(b) Velocity profile in coiled tube at high flow velocity

White and Fitzgerald and Bergmeyer and Hagen [11] were early proponents of this concept [12]. The new interest in this approach may be credited in large part to Stewart's and his colleagues' efforts in the United States, as well as Ruzicka and Hansen [7] in Copenhagen, who are responsible for coining the phrase "Analysis of flow-injection." These two factions, working independently, have made numerous advances in our theoretical knowledge of the In nonsegmented flow systems, a dispersive process occurs. As a result, the conceptual framework that is progressively emerging should make it easier to design systems that are optimal Sensitivity, sample rates, precision, and accuracy are all factors to consider.

1.2 Fundamentals of FIA

The FIA system is depicted in its most basic form in (Fig.1.4). The FIA system consists of a reagent carrier stream (R) driven by a pump (P) injection system, flow-through a reaction coil (RC), detector (D), and recorder (R) or other data-handling device. The method is based on a blend of the three notions described below[7].

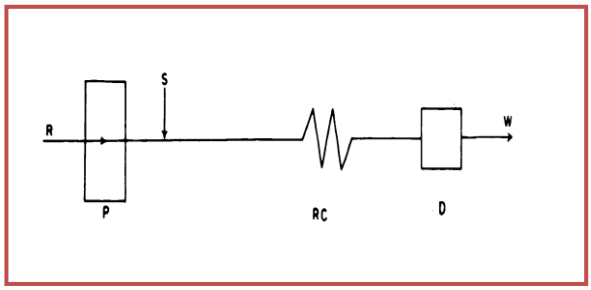

Fig. 1.4 Simple FIA system

1

.2.1 Sample Injection

Sample injection is the procedure for introducing a discrete "slug" of sample into a continuously running carrier stream while maintaining the stream's velocity. The sample amount does not need to be precise, However, it must be precisely injected into the carrier stream in order to reproduce the slug's volume and length from sample to sample. In the beginning, samples were injected through a flap valve with a syringe [18]. Later advancements included rotary valves with sample containers in the shape of bores, as well as other types of loop valves used in liquid chromatographic systems [2,19,20].

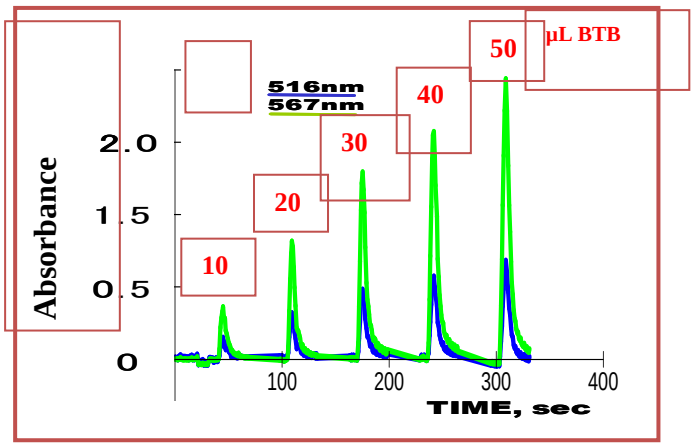

Fig. 1.5 Automated injection of increasing sample volumes µL

The ability to select injected sample quantities is a valuable tool for optimizing all FIA procedures. It enables for the setting of sensitivity and detection limit on spectrophotometry of a Bromothymol blue dye (BTB) 0.002 percent, carrier 0.005 M sodium tetraborate, monitored at two wavelengths[2], as illustrated in (Fig.1.5).

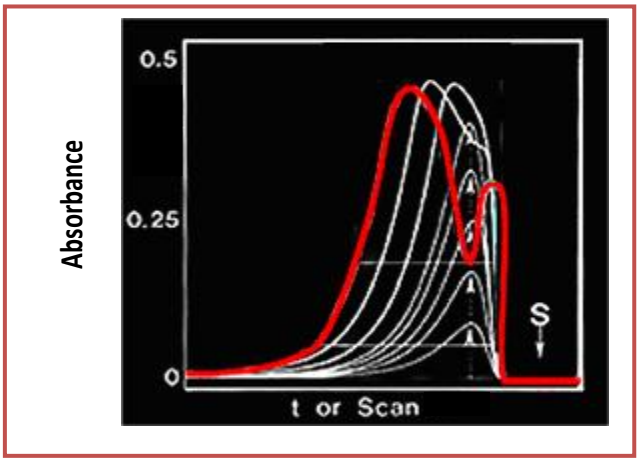

Fig. 1.6 Double peaks effect

The creation of a double peak effect is caused by the injection of increasing sample quantities (Fig.1.6). If the injected sample volume causes a shortage of reagent in the zone's center, a double peak will be recorded in a single stream manifold. To prevent this issue, either inject a lower sample volume or use a two-stream system with a confluence point. [2].

When water flowed slowly and steadily through a conduit, frictional forces between the layers of flowing liquid generated a longitudinal velocity profile, according to Sir Isaac Newton. Under such laminar flow circumstances, the liquid layer in contact with the tube surface is nearly immobile, and the velocity of centrally positioned molecules is double the liquid's mean velocity [21].

1.2.2 Controlled Dispersion

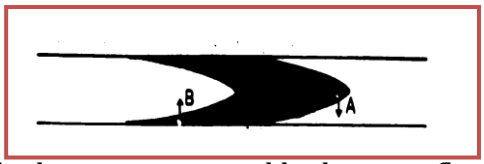

Fig. 1.7 Sample dispersion caused by laminar flow without diffusion

A parabolic velocity profile results as a result of this (Fig.1.3a). A sample put in a flowing stream would quickly take on the form indicated in the absence of molecular diffusion (Fig.1.7). Under these theoretical conditions, the sample would have an unacceptably long tail by the time it reached the detector, resulting in unacceptable carryover between samples. Fortunately, due to molecular diffusion, this scenario does not arise. The convective dispersion is limited by molecular diffusion between the carrier and sample bolus, which effectively mixes the sample and reagent. Consider the fate of molecules A and B in (Fig.1.7). A sample molecule (A) placed in the center of the bolus will diffuse into a lower-concentration zone, increasing the possibility that it will migrate into a layer of liquid moving at a slower longitudinal velocity, which is mostly radial diffusion.[21,22] . It modifies the form of the sample bolus head when applied perpendicular to the flow direction. A molecule at position (B) also diffuses into the carrier solution, but this time it collides with a layer of

faster-moving liquid, forcing it away from the tube walls and into the sample zone's centerAs a result, the reagent, sample, and carrier molecules are all mixed together. The longitudinal dispersion created by carrier flow is moderated by diffusion-induced dispersion (Fig.1.8), which explains why FIA provides for little carryover and high sample throughput. The sample dispersion seen by the detector would be affected by changes in mean flow velocity, tube diameter, monitoring distance, analyte diffusion coefficient, or any combination of these. Previously, FIA's poor carryover was blamed on turbulent flow.[1,14], ,however it is now understood that FIA is only effective in laminar flow [1, 23].

Fig. 1.8 Sample shape resulting from laminar flow with molecular diffusion

In FIA systems, dispersion and concentration characteristics must be described. Ruzicka and Hansen [23] adapted the chemical engineering concept of "tanks-in-series" and identified the primary factors that may be modified to obtain the required degree of mixing. Reijn et al. took a similar technique. [24] The injection and detection procedures utilized in FIA have been theoretically outlined. Despite the fact that these authors have produced useful guidelines, the implications of which will be discussed further in the design of FIA manifolds, more sophisticated models that account for both chemical and physical mechanisms will be required to aid in the design of more efficient analytical systems. The longitudinal dispersion of the sample zone should be kept as low as feasible to generate large signals and allow high sample throughput; coiled tubes are preferable than straight tubes for this. As the length of a tubular channel rises, the peak height decreases, but the peak form changes from asymmetrical to symmetrical. At the same time, the resident length of the peak maximum rises as the distance traveled increases, and the peak base spreads. as seen in (Fig.1.9).

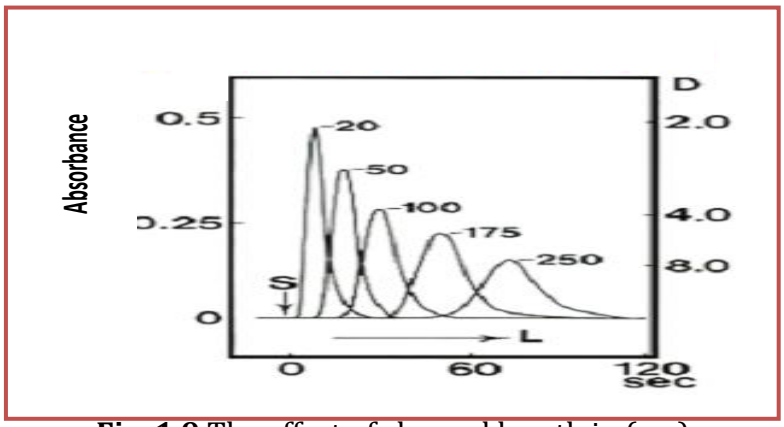

Fig. 1.9 The effect of channel length in (cm)

As shown in, blue dye injected into a colorless carrier stream running through clear 0.5 mm (i.d.) tubing has an impact on zone dispersion (Fig.1.10). Laminar flow dominates in a straight tube, as illustrated in (Fig.1.10 b), and the observed peak profile (A) is asymmetrical.

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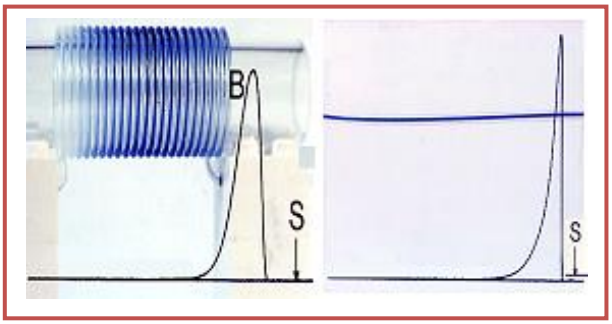


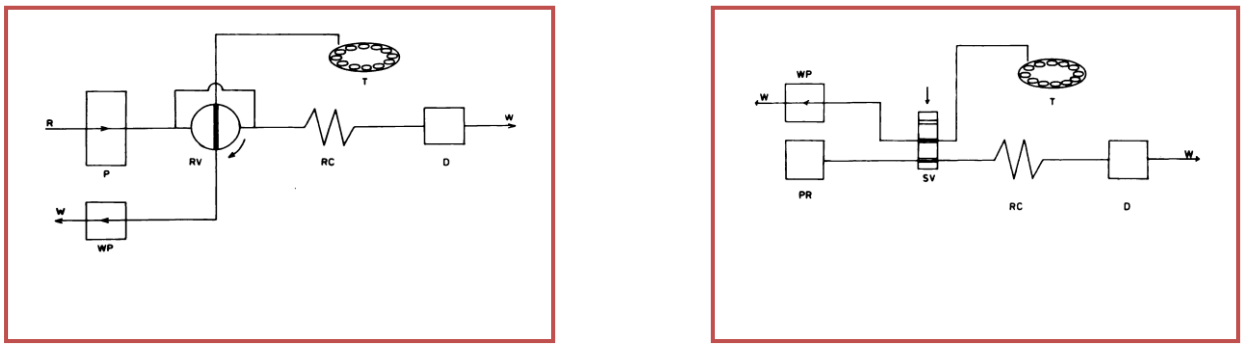
(a) (b)

Fig. 1.10 (a) Dispersion in coil (b) Dispersion in straight tubing

Radial dispersion is increased by coiling the tubing as seen in (Fig.1.10 a), resulting in a more symmetrical peak. A colorimetric flow cell installed at the downstream end of a 500 cm tube recorded the peak profile (B)[2].
In tightly coiled narrow-bore tubes, centrifugal forces create a secondary flow perpendicular to the primary axial flow at high flow velocities (Fig.l.3b). Because axial flow velocity is highest at the tube's center, centrifugal forces will drive the fluid at the center towards the outer tube wall, resulting in greater flow rates at the outer tube wall. As a result, the velocity profile becomes more linear, reducing sample dispersion while allowing for exceptionally high sample throughput. With 20-m-bore (i.d.) coiled tubes, Tijssen [25] proved that sample frequency > 600 samples/h is attainable, and subsequently Hungerford [26] established that the optimal flow rate for a microminiaturized system is roughly 1 nL /sec for a tube diameter of 125 m.

1.2.3 Reproducible Timing

In FIA, the value of repeatable time cannot be overstated. The period between when the sample is injected into the carrier stream and when it is identified is totally dependent on pumping speed because there are no compressible gas bubbles in the system. With typical peristaltic pumps, great temporal repeatability is easy to accomplish since there are no compressible gas bubbles in the system. By adjusting the experimental variables, the residence time may be adjusted to provide the required sample dispersion as well as enough time for the reaction to occur. It is feasible to maintain regular residency times for both long and short periods of time. Because the response curves do not reach the steady-state plateau and the samples pass through the detector quickly, the notion of "steady state" is abolished and the reaction monitored before equilibrium is reached, the curves appear to have abrupt peaks. Typical residence periods range from 3 to 30 seconds. Inaccurate peak heights will arise from any flow rate variations that impact the sample's residence duration in the system . Both companies created realistic unsegmented flow systems; however they differ in significant ways. Ruzicka and Hansen invented a low-pressure technique [1,27] Peristaltic pumps are used to inject samples, which can be done manually or automatically.



(a) (b)

Fig. 1.11 (a) Automated FIA system (b) Original automated system of Stewart

Reagent (R), sample (S), pump (P), reaction coil (RC), waste (W), detector (D), motorized rotary valve (RV), withdrawal pump (WP), slide valve (SV), pressurized reagent reservoir (PR) [7]

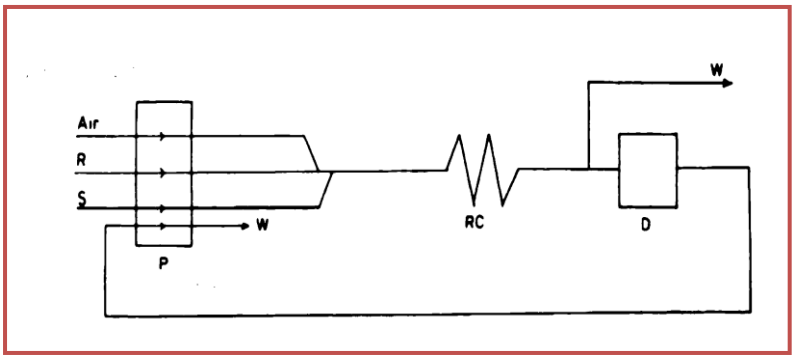
Stewart et al. developed technology based on their knowledge with (high-pressure liquid chromatography), as shown in (Fig.1.4, Fig.1.11a) (Fig.1.11b). In the early versions, the sample was transported from a vial on a turntable into the chamber of a sliding valve using a peristaltic pump[14].

A predetermined volume of sample was delivered into the reagent stream, which was fed from a reservoir pressurized to 400-500 psi. One or more high-pressure pumps replaced the pressurized reagent reservoir in subsequent systems (28). To distinguish their method from simpler systems, Stewart et al. (29) term it "Automated Multiple Flow Injection Analysis" (AMFIA). Both systems have successfully sampled at speeds exceeding 120 samples per hour using a variety of detectors. Simple flow-injection devices have also been used to estimate colorimetric measurements of albumin in serum (28) total protein, glucose, urea, uric acid, potassium, calcium, and serum pH. There have been reports of analysis rates as high as 700 samples per hour. [30].

1.3 Comparison of FIA with Gas-Segmented Flow Analysis

Skeggs [16] He was the first to introduce gas-segmented continuous flow systems to medical laboratory analysis in 1957, and now, equipment based on this principle can be found in practically every clinical chemistry lab. Prior to the introduction of FIA, many professionals believed that successful continuous-flow analysis required air segmentation and a steady-state signal. Ruzicka and Hansen's [1] initial flow-injection system utilized a lot of reagent, and Stewart et alapparatus.'s used a lot of reagent as well [14]. High pressures were necessary. Following that, merging zones were introduced [33,34] Shorter, thinner reaction tubes were used to counteract FIA's following criticism [31] as well as the debate over the advantages of gas segmented vs. non-segmented systems [31,32]. FIA and gas-segmented flow analysis (SFA) are similar in that they are both continuous-flow approaches that have been used to automate chemical experiments. A lot of comparisons have been made between the two approaches [35-38] Table-1.1 compares and contrasts the two methods. Simple systems, such to those shown in (Fig.1.4, Fig.1.12), were assumed for the purposes of comparison. The numbers given are typical, however the exact values of the experimental variables may differ depending on the research type.

Fig. 1.12 Simple gas-segmented system



Both approaches have their own set of benefits and drawbacks. Because of its increased sampling rate, practically immediate availability of the analytical output, decreased sample and reagent volume requirements, and short start-up and close-down periods, FIA is better suited to most of the extremely rapid analytical procedures widely used in clinical chemistry[7].

Table 1. 1 Comparison of operation characteristics for (SFA) and (FIA) [7]

Parameter	SFA	FIA
Start up time	20 min	20 s (useful for stats)
Reagent stream	Gas-segmented (Air- bubble)	Nonsegmented
Reagent consumption	Higher	Low (extremely low when merging zones are used)
Manifold	Relatively complicated to allow for introduction and removal of air	Simple
Conduits, (i.d.)	2 mm	0.5 mm
Sample introduction	Aspiration	Injection
Sample volume	Large (200 µL)	Small (50 µL)
Sample rate (Sample/h)	Typically 60	150 or greater
Sample mixing	Through turbulent flow generated by gas bubble and tube wall friction	Through controlled dispersion process
Steady state	Usually required	Not required
Readout time	Minutes	Seconds
Wash cycle	Essential	Not required

Dialysis / solvent extraction	Possible	Possible
Titrimetry	Not possible	Possible
Continuous kinetic analysis	Not possible	Possible with stopped flow
Shut down	Slow, several minutes	Fast (10 s)
Data acquisition	Recorded peak height	Peak height , area, width

SFA systems, which employ gas bubbles to prevent sample dispersion, would, nevertheless, give a greater throughput in investigations that need extended incubations, as Snyder [37] emphasizes The existence of gas in Skeggs is surprising. [39] Because the compressibility of the gas bubble causes pulsing flow and the "lag phase" associated with gas-segmented systems, this type of equipment hinders both high sample throughput and downsizing. Bubble-segmented systems are designed to obtain at least 95 percent of the steady-state (plateau) signal to avoid inconsistencies in sampling time caused by uneven pumping (generated by pulsation), changing volume heights of sample liquid in the sample cups, and uneven timing of the sampler. In comparison to FIA, this necessitates longer sampling periods and bigger sample volumes. The FIA system is basically ready to use as soon as the pump is switched on, with the exception of the time it takes for the detector to warm up. As a result, even a tiny sample set may be evaluated in a short period of time[7].

1.4 FIA Instrumentation

Most large clinical chemistry laboratories have standard equipment that can be quickly and easily converted into an experimental flow-injection analyzer. A pump, an injection valve, a manifold with transport and reaction tubes, a detector with a low volume flow-through cell, and a recorder are the basic minimum [7].

1.4.1 Pumps

There are several techniques to create the consistent, steady flow of fluids required for repeatable FIA. Various pumps were utilized, including peristaltic pumps (see Fig.1.13 a). Pulsation is never a big issue because the system is devoid of gas bubbles, although some employees consider it a small issue, and depulsing devices have been suggested [40,41].According to Vanderslice et al. [22] a small amount of pulsation in the flow may improve the peak forms produced during an analysis. The majority of clinical chemistry laboratories have a spare peristaltic pump on hand that would be great for a test FIA system. Pumps designed for liquid chromatography can also be used, however each stream will require its own pump. The majority of FI systems use peristaltic pumps, which allow carrier and reagent flow rates to be controlled by adjusting pump rotation rate and pump tube diameter. Individual streams flow at 0.5-1.5 ml/min on average. [7].

Fig. 1.13 (a) Peristaltic pump (b) Stepper motor (c) Syringe pump (d) Solenoid pump

As samples and standards are serially injected, the pump propels solutions constantly in a forward direction, giving a predictable time frame. As a result, all samples and standards are processed in the same way, and the standards provide a readout that can be used to create a calibration curve[2].

Stepper motor-driven syringe pumps, such as those depicted in (Fig. 1.13 b and c), produce a highly repeatable and computer-controllable flow. Because the piston speed and syringe size may be adjusted, they can handle a broad range of flow rates. They are long-lasting and chemically robust, with the primary negatives being the high cost and inability to create a continuous flow beyond the syringe's capacity. Micro pumps with solenoid activation create flow by delivering well-defined pulses, the frequency and volume of which govern the flow rate, as indicated in the diagram (Fig. 1.13 d). [42]

1.4.2 Injection valves

The most often used instrument for the injection of well specified sample quantities is a two-position, six-port injection valve with a fixed loop, as shown in Fig.1.14. By adjusting the length and i.d. of the loop tubing, the capacity of the external loop may be adjusted from 20 to 100 liters. The valve may be manually or automatically switched from load to inject mode, and the loop can be filled manually with a syringe or automatically with a pump from an autosampler. In order to preserve sample material, it is critical to maintain the length of the conduit between the sample container and port # 4 as short as feasible. and to avoid cross-contamination between samples. The use of an air bubble and a wash between samples is beneficial, but it necessitates precise timing to ensure that the injected volume is air-free and includes undiluted material. [2]

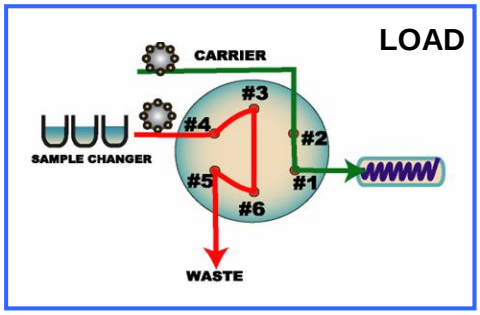


Fig. 1.14 Injection valve

There have been reported single [14] and multi-injection valves [19] built particularly for FIA. Plastics may be used to make these injectors, & At the very least one of the designs is for sale (Bifok, Sweden). In many laboratories, injection ports and valves used in liquid chromatography may be used in FIA systems. A standard "high-performance liquid chromatography" valve, for example, can reliably feed small volumes of serum (normally 10 L) into a carrier stream [34]. To minimize stream surging when the valve is opened and to allow the carrier solution to flow through the valve while the sample is being filled into the loop, the valve should ideally be equipped with a bypass of larger hydrodynamic resistance than the volumetric bore [2].

Using a flow arrangement similar to, a standard continuous-flow sampler may automatically charge a rotary valve with sample (Fig.1.11a). Two distinct flow streams are employed in this system. When the valve is in the "fill" position, the sample is inhaled into it, and the carrier flow in the analytical stream is maintained via the bypass tube. The sample is put into the carrier stream when the valve is spun through 900; the valve can then be rotated back to its original position, cleaned, and filled with a new sample while the analysis proceeds [41].

For this function, a variety of valves have been described. A multi-channel analyzer can be fed by connecting sampling valves in series (14, 20,27,43), and a special "injector commutator" has been devised. Stream splitting has also been used to feed separate channels [45, 46] By precisely synchronizing the sample tray, sample probe, and sample insertion valve, a microprocessor control system has been proposed to promote high sample throughput[44].

1.4.3 Design of FIA Manifolds

Many clinical chemistry analytical methods may be automated simply by placing the sample in a moving reagent stream that can be prediluted if necessary (Fig.1.11a). By carefully building the manifold and adjusting flow parameters, the dispersion of the sample in the analytical conduits may be regulated to match the demands of each analytical operation. Controlling injection volume, flow rate, and tube dimensions yields a wide range of highly repeatable sample-dispersion patterns, allowing for optimal response with least time and reagent. The band broadening is kept low for maximum sampling frequency by selecting the smallest sample size consistent with sufficient sensitivity; the tube length and radius should also be kept short[7].

Examine the effect of various tubing lengths on the detector response to determine the shortest tubing length required for the reaction. For clinical work, they recommend 0.5 or 0.8 mm (i.d.) polytetrafluoroethylene tubing, according to their expertise. Reaction tubes with a diameter of less than 0.3 mm are easily blocked, and at lengths of more than 1 or 2 m, they generate enough back pressure to cause problems with peristaltic pumps. Tubes with a diameter greater than 1.0 mm (i.d.) are not recommended due to high carryover. The tube wall must be strong, especially when employing the stop-flow method, to prevent the tube diameter from expanding and contracting when the pump is turned on and off. The manifold may include pretreatment equipment like as dialyzers and solvent-extraction components. These modules, as well as the connecting fittings, should have a low dead volume and cause as little turbulence in the stream as feasible [7].

Because the core of the stream flows at double the rate of mean flow velocity, a zone of dye (A) injected into a carrier stream initially takes the shape of a hollow cone (B), while friction at the tube walls results in the creation of a trailing edge, as illustrated in Figure 1. (Fig.1.15). Radial dispersion reshapes the hollow cone as the zone advances downstream. As a result, the asymmetric (B) initial square input (A) becomes a Gaussian output (C) (C). Radial mixing is required for all types of flow-based assays, and it is facilitated by channel geometry that is well-designed and flow programming [2].

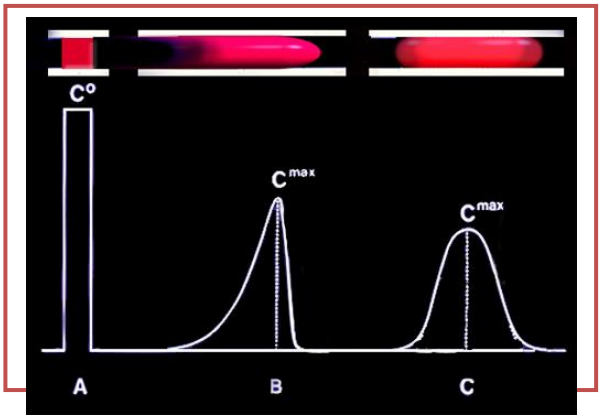


Fig. 1.15 Injection and dispersion

Dispersion coefficient (D) is defined by Ruzicka and Hansen[23] as the ratio of the concentrations of the sample solution before (C^0) and after (Cmax) the dispersive process, as

demonstrated in (Fig.1.15). This provides a simple and practical approach for evaluating dispersion and comparing different flow conditions. In colorimetry, for example, In the colorimeter cuvette, a dye solution could be placed, and the peak height corresponding to C^0 could be measured. When a sample of the dye solution is put into the system under working conditions, the peak height that corresponds to Cmax is determined. D = 5 for a sample solution diluted five times by the carrier stream. The three types of dispersion are limited dispersion (D = 1-3), medium dispersion (D = 3-10), and high dispersion (D = 10-10000). Here are a few instances of how different degrees of dispersion are used.

Because the sample undergoes little dispersion, high sampling rates are possible in estimations where little or no dilution of the sample is required, such as pH determination, the use of ion-selective electrodes, and the introduction of samples into flame photometers or atomic absorption spectrophotometers. Most analytical methods have relied on medium dispersion manifolds, In this step, the sample zone is mixed with the carrier stream (and perhaps a number of reagents) to create a product detectable by a flow through detector. There must be sufficient mixing between the sample zone and the reagent, as well as sufficient time between the sample zone and the detector for optimum product synthesis. Fortunately, the majority of colorimetric techniques are based on quick reflexes, making them appropriate for this strategy. The residence time is enhanced for slower reactions by lowering the flow velocity rather than extending the length of the coil, which would enhance dispersion and band widening [3] Large dispersion can be used to generate a sufficient sample dilution or to lengthen the duration of a concentration gradient. A blood sample that is too concentrated for direct examination can be diluted by feeding it into a manifold that provides a high dispersion. When doing flow-injection titrations, more highly dispersed patterns, best achieved by placing a mixing chamber between the injection port and the detector, are advantageous[47, 48].

The mixing chamber holds the majority of the system's volume and provides a concentration gradient between the carrier and sample streams (for example, a strong base carrying an indicator) (acid). In this technique, the peak breadth (in time) is more analytically significant than the peak height. When the peak width is plotted against the A straight line is formed by taking the logarithm of the sample concentration. 1 minute is the typical titration time [10].

The sample is injected into a stream of water that merges with a stream containing a "linear buffer" solution, and peak height is measured potentiometrically in a flow-through cell with a glass electrode, according to Astrom.[49]. The buffer is set up to produce a linear change in pH as a function of sample concentration, and the procedure can handle up to 720 samples per hour. pH gradients can be utilized for a variety of applications. A pH gradient, for example, has been used to do multi-element analysis in a single bolus [50-52] . The pH absorbance curve for a given metal ion/reagent combination is highly characteristic; additionally, if the sample contains a mixture of ions, the absorbances across a pH gradient are additive, allowing quantitative determination of the mixture's components based on the peak profile information. This method has been used to test Lead and Vanadium [47] compounds as well as Copper and Manganese [49].

The manifolds classified into two types as follows:

1.4.3.1 Single Stream Manifold

Peristaltic pump, manually operated two-position injection valve, manifold of connections, tubing and reactor, and flow-through detector make up the simplest manually operated system (Fig.1.16). [2]

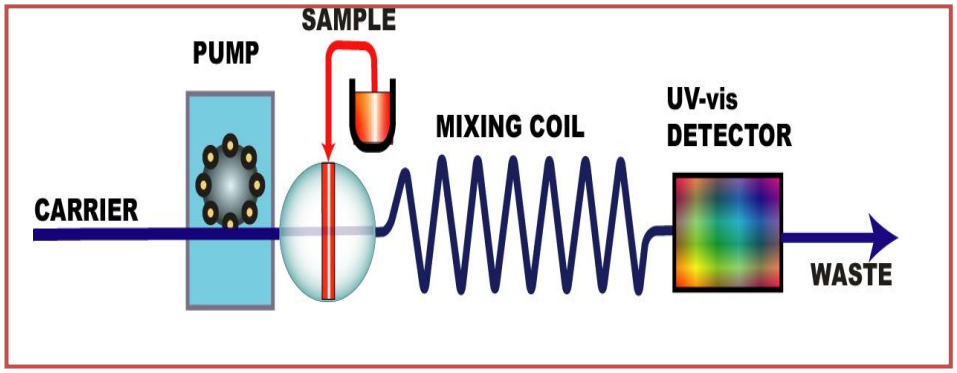


Fig. 1.16 Single stream manifold

1.4.3.2 Multistream Manifold

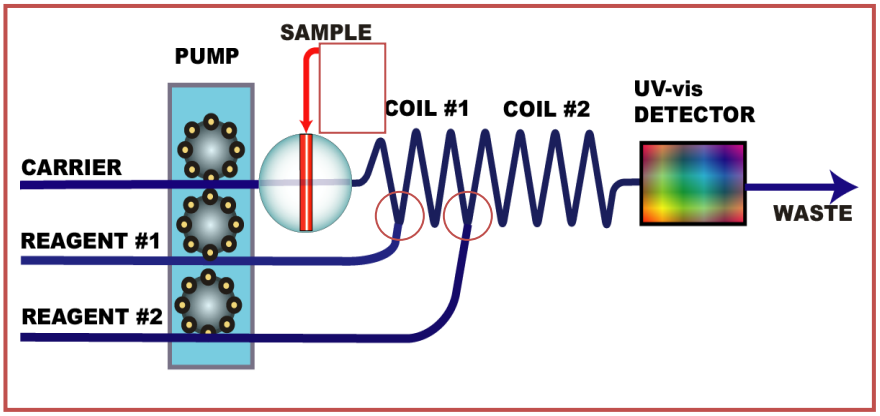


Fig. 1.17 Multistream manifold (two reagent, three stream FI system)

Because many experiments require many chemicals to be introduced in a certain order, FI systems employ multichannel pumps to drive the carrier stream alongside the reagent streams. This allows reagents to be continuously provided to the injected sample at a predefined concentration, allowing reactions to proceed in sequence as the sample zone passes through the first and second reactors, with the injected volume generally ranging from 25 to 100 liters. As illustrated in (Fig.1.17), FI systems are frequently constructed as multichannel systems, with each channel dedicated to a separate chemical test. Phosphate, nitrate, and nitrite may all be measured concurrently in water and soil samples using a three-channel system. Solvent extraction, dialysis, and gas diffusion-based tests may all be automated with multistream FI systems. The majority of FI tests use continuous flow, with the carrier and reagents injected at the same time at a consistent rate. The sample is injected into a water or suitable buffer carrier stream, and the reagent streams are added at the confluence point, as indicated in (Fig.1.18).

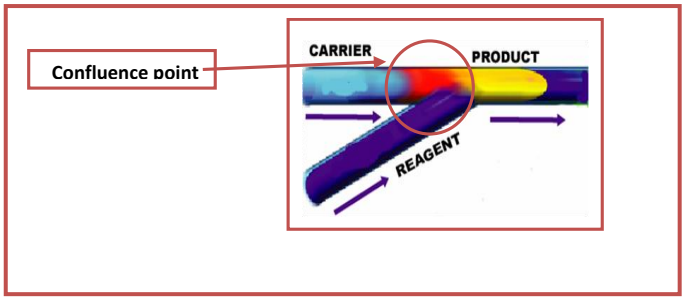


Fig. 1.18 Y- Junction

The following are some of the benefits of this method: even reagent addition over the whole sample zone length, a consistent baseline, little carryover, and the operation's ease and transparency to the user. To move carrier and reagent streams that join at confluence sites, where the reagent merges with the sample zone, almost all FI instruments use multichannel peristaltic pumps. [2]

1.4.4 Detectors

Flow injection analysis may be performed with almost any flow-through detector. The technique determines the kind. In an ideal world, the detector would provide an electrical signal (proportional to analyte concentration) that could be used to power an output recording or data-processing device, such as a potentiometric recorder or a computer. High stability and a quick response time are required. The device's flow channels should not cause turbulence in the flowing stream, and the flow cell should have a low dead volume. Poppe has researched these requirements as well as the optimal flow-through detection system design [53].

Colorimeters and spectrophotometers with small-volume flow-through cuvettes (about 8 L) are the most often used detectors in clinical chemistry. Fluorometry [33, 54, 55], Atomic absorption spectrophotometry [43, 56-59], Flame emission spectrophotometry [46] Chemiluminescence [60-62], Turbidimetry [63, 64], and Ion-specific electrodes are some of the additional detection techniques utilized in FIA. The assay of volatile compounds of Ag, Co, Cu, Ni, and Zn has been described using hydride generation-based atomic spectroscopy for the analysis of traces of As, Bi, Ge, Hg, Pb, Se, Sn, and Te The gas-liquid separator is the most critical component of the design. [65 - 67]

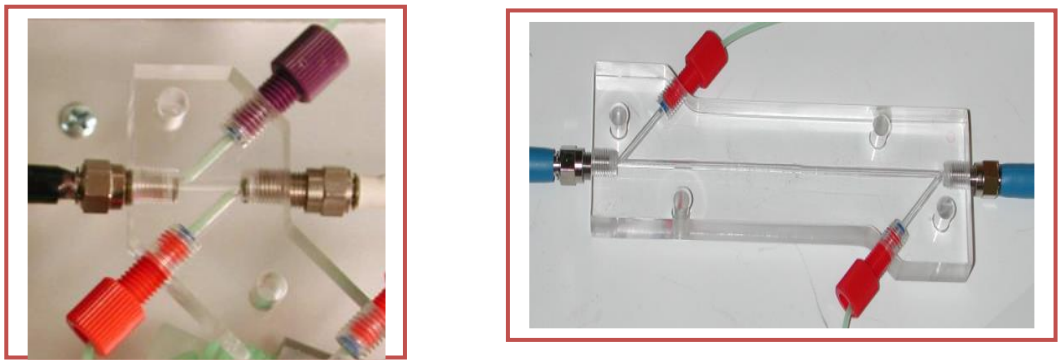


Fig. 1.19 (a) Z- flow cell with 10 mm light path (b) Z- flow cell with 10 cm light path

Fiber optics and solid-state spectrophotometers have transformed the way FIA methods are performed since they allow us to deliver light and capture data at any point along the sample flow route. While this development has had a significant influence on Sequential Injection, more classic FI systems can also benefit from fiber optic technology's adaptability and resilience. A common setup includes a "Z-type" flow cell (Fig. 1.19) coupled to a spectrophotometer and a tungsten or deuterium light through quartz fibers. A light emitting diode is put directly on the flow cell for a single-purpose system [2].

1.4.5 Readout and Continuous flow

The peak height (H) is most commonly utilized to generate a calibration curve (Fig.1.20). This readout may be provided in less than 30 seconds after the sample injection, depending on the flow rate and response rate. Thousands of samples are examined each week in regular laboratories, where FI systems are generally paired with an autosampler, with sampling frequencies of up to 120 samples per hour. The readout for a FI titration is peak width (W), although peak area (A) is rarely utilized. [2]

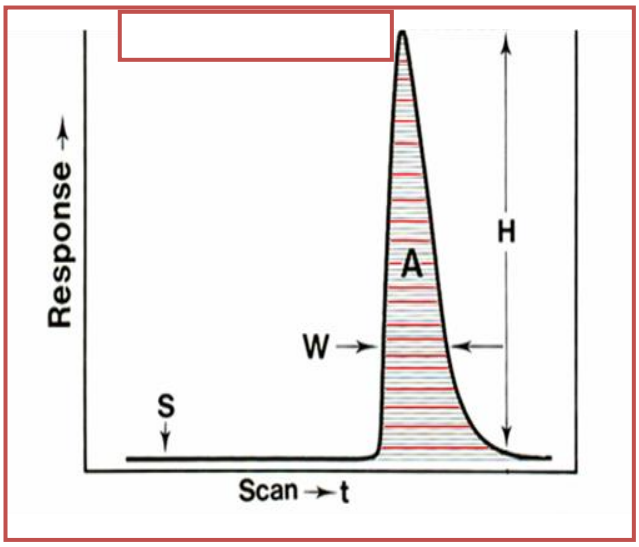


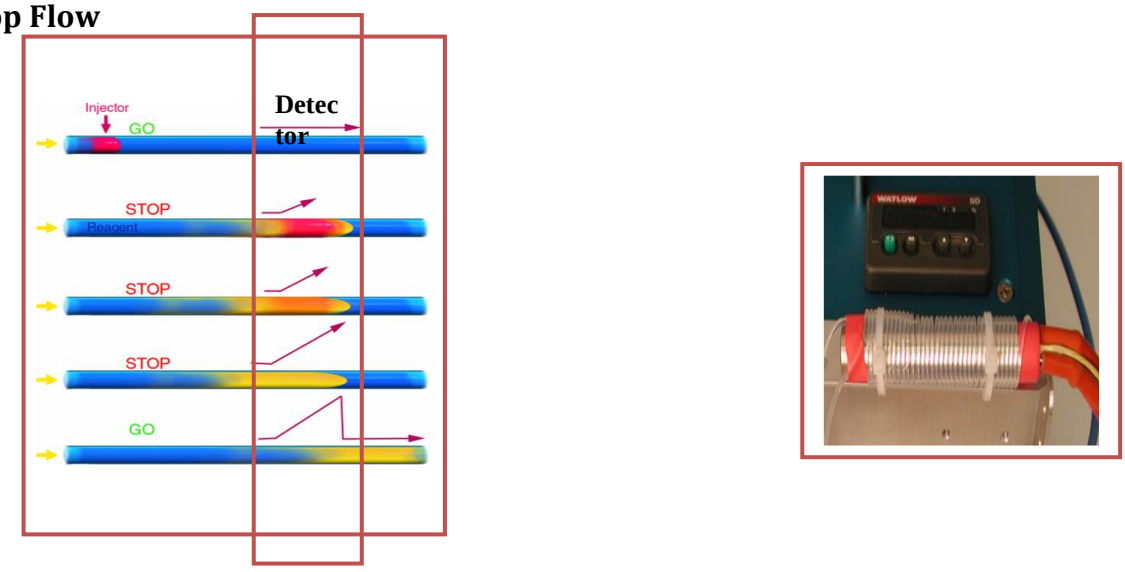
Fig. 1.20 Peak readout

Peak-height measurements in FIA are simple using a recorder with a fast reaction time, ideally less than 0.5 seconds for full-scale deflection. If necessary, a chromatographic integrator can be utilized to calculate peak regions. Peak heights have been measured using microprocessors that have been programmed [69] and peak widths [70], A circuit has been created in amperometric measurements to record the largest peak current produced [68]. [44] .

Intermittent pumping of the sample bolus through the reaction tubes may be precisely controlled until the reagent is thoroughly mixed with the sample, at which point the flow can be stopped to enable the reaction to take place in the colorimeter cuvette, as shown in (Fig.1.21a) There is no movement of liquid in the conduits since no air or other gases have been fed to the manifold after the pump has been turned off, allowing reaction kinetics to be followed[33].

1.5 Flow Injection Analysis Techniques

1.5.1 Stop Flow



(a) (b)

Fig. 1.21 (a) Stop flow technique (b) Heated reactor coil with a temperature controller

This method may also be used to improve the sensitivity of a slow reaction by stopping the flow in the reaction coil (which can be heated) and allowing enough reaction products to build before pumping the sample zone through the detector, as illustrated in (Fig.1.21b)[33] .

1.5.2 Merging Zones

Traditional continuous-flow instruments have the issue of constantly using reagent even when there is no sample in the analysis system. Although this is less of an issue in FIA, since the total volume of the system is rarely more than a few hundred microliters, "merging zones," which limit the use of costly reagents, can be employed to save even more money. Bergamin et al. were the first to describe this approach. [70] who used a twin injection valve to inject the sample and reagent into separate, inert carrier streams The reagent and sample slugs combine and partially mix when the carrier streams converge. The sample/reagent zone is routed through a reactor coil before entering the detector for additional mixing and reaction development. As the carrier in both streams, distilled water, dilute buffer, or detergent solution can be used; consequently, reagent usage per determination can be extremely low. This approach was used to minimize the quantity of lanthanum reagent required for calcium and magnesium determination using atomic absorption spectrometry. Plant-digest samples were examined at a rate of 300 samples per hour using just 50 L of reagent. [56].

A novel approach to dealing with concentrated samples based on the merging zone being slightly out of sync, such that when the streams combine, the reagent zone just happens to be in the center. This approach, which overlaps the sample zone's somewhat dilute tail, was used to quantify serum albumin at a pace of 300 samples per hour without predilution.

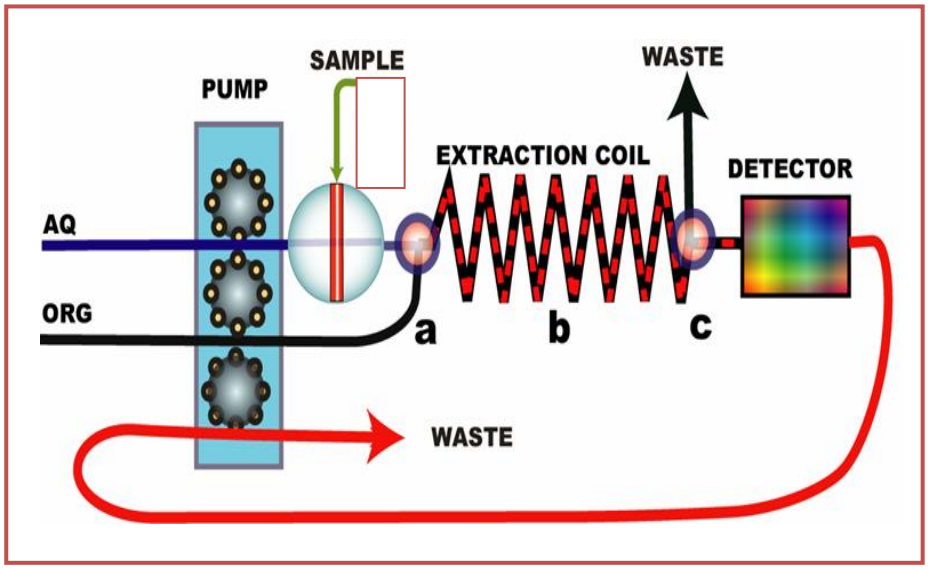
By combining the principles of merging zones and halted flow, kinetic measurements using extremely tiny amounts of sample and reagent may be achieved. For enzymic determination of glucose, Ruzicka and Hansen [34] Combining these methods; They used a 10-liter sample volume and just 26.5-liters of glucose dehydrogenase reagent per test; the carrier flow was switched off, and the sample/reagent zone was held in the spectrometer cuvette for 20 seconds while the reaction rate was monitored. With a sample analysis rate of 100 samples per hour, the analytical readout was available within 30 seconds after sample intake.

1.6 Separation Methods in FIA

The method described thus far includes injecting a sample into a single flowing carrier stream, resulting in a detectable signal. When more chemicals are required, convergent reagent carrier streams can be employed to introduce them sequentially. For example, urea has been measured by converting it to ammonia with urease; the ammonia produced is then calculated using the indophenol blue reaction, with all of the required chemicals provided downstream in the correct sequence[27] . Other separation and pretreatment procedures usually associated with gas-segmented systems have been modified for use with FIA, in addition to the usage of merging zones and stopped flow, which are unique to FIA[7] .

1.6.1 Automated Solvent Extraction

(A)



(B)

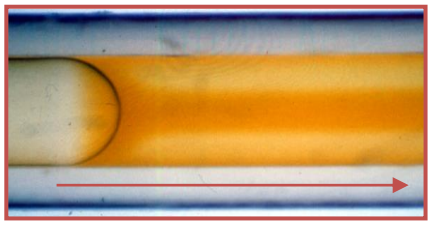


Fig. 1.22 (A) Solvent extraction (B) Circulation of extracted dye

Two-stream manifold (Fig.1.22 A) for automated solvent extraction. The sample is injected into an AQ carrier stream, which is mixed with an organic phase (ORG) and pushed through a Teflon extraction coil (a) (b). AQ is sent to garbage in the separator (c), whereas ORG is directed to a detector. As illustrated in (Fig.1.22 B), the circulation of extracted dye in a section of the organic phase as it goes through a Teflon tube provides a hint to the solvent extraction hydrodynamics mechanism. [71]. Karlberg and Thelander [72] (1978) revolutionized the solvent extraction technique, which was previously primarily done by hand. Hormones, medications, and a range of hydrophobic compounds may all be tested using this method. Miniaturization and automation of solvent extraction reduces exposure to harmful solvents, reagent consumption, and the production of hazardous waste. Because the aqueous phase (aq) sticks to glass and the organic phase attaches to Teflon, the materials used for manifold components and their orientation are crucial (Fig.1.23). The organic phase enters a segmenter through a glass fitting and clings to Teflon tubing (1).

A thin Teflon strip (3) passes the organic phase through a glass T piece in a separator. A Teflon membrane in a membrane separator permits only the organic phase to pass through hydrophobic holes, while the aqueous phase is rejected, as illustrated in the diagram (Fig.1.23) [73,74]

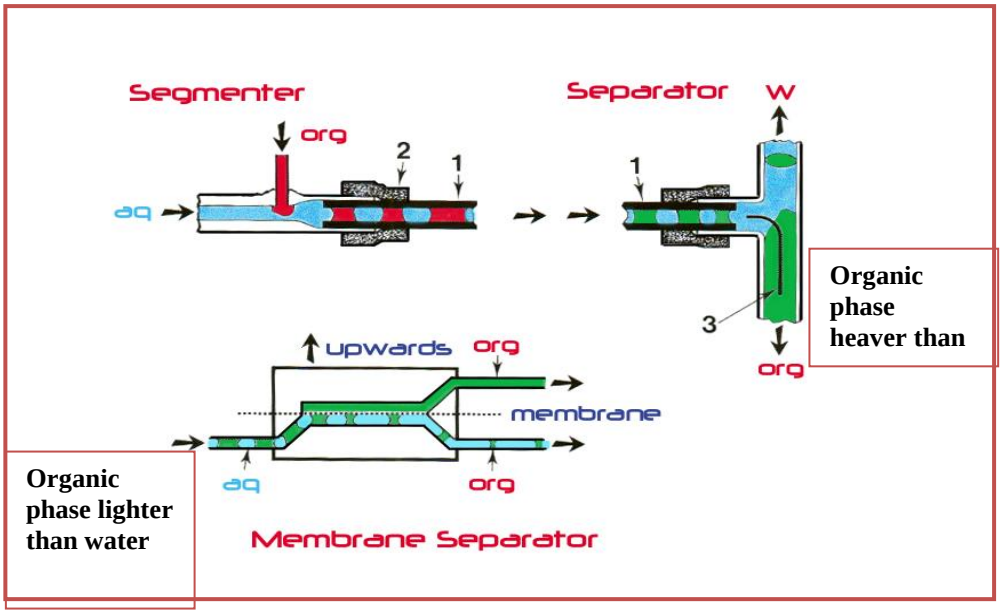
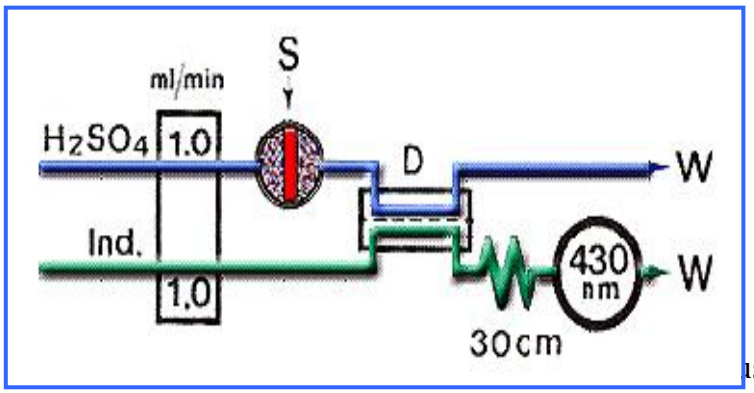


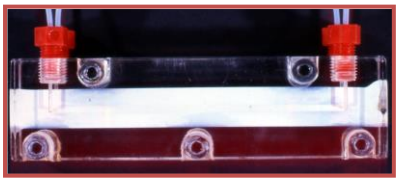
Fig. 1.23 Segmenter, Separator, Membrane separator.

1.6.
2 Gas diffusion

(Fig.1.24a) A two-stream FI system sample containing carbonate (or dissolved carbon dioxide) is acidified, releasing carbon dioxide that diffuses from a donor (blue) to an acceptor (green) stream, changing the color of an acidobasic indicator (Ind.) measured at 430 nm. Teflon membranes are hydrophobic, with up to 50% porosity, forming an air gap between the carrier and donor streams, allowing gases such as ammonia, sulphur dioxide, chlorine, ozone, and volatile compounds to quickly permeate into an acceptor stream, where they can be detected with the right reagent [75].



D



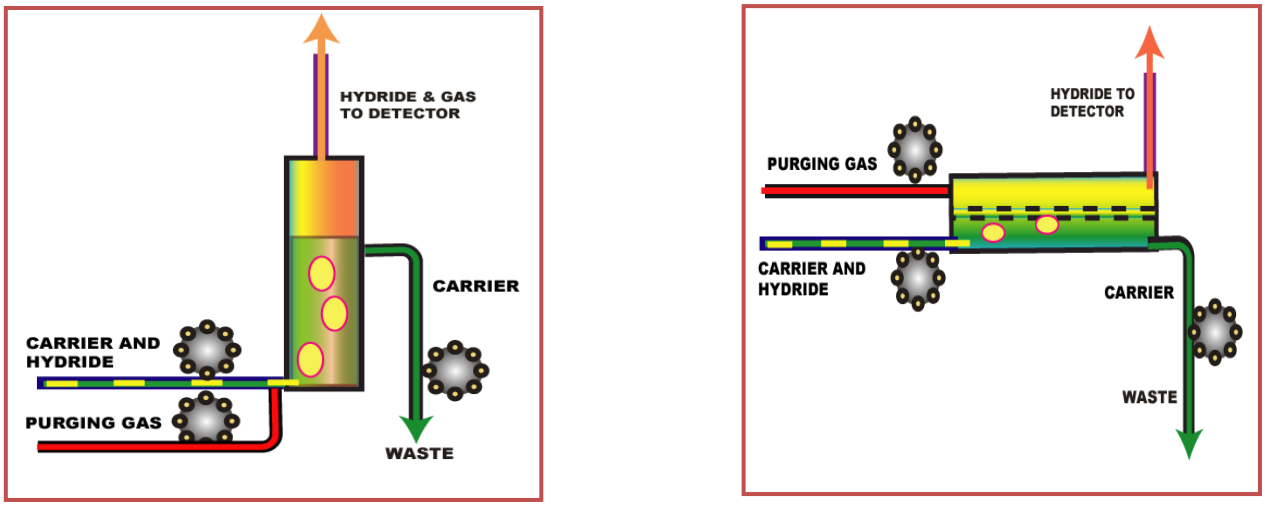
(b)

The flat plate diffuser (Fig.1.24 b) is simple to put together. The disadvantage of hydrophobic membranes is that surfactants can dirty them, destroying the air gap barrier. [76]

1.6.3 Gas-liquid separation

Gas expansion and membrane separators are shown in Figure 1.25. Gas expansion separators are the most commonly utilized because they are durable and simple to build and maintain. Because a hydrophilic glass surface aids in gas-liquid separation, the whole separator (Fig.1.25 a), Glass, or at least its vertical tubular body, is generally partially filled with large glass beads. To sweep the liquid in the separator and transport the released volatiles to the detector, a purging gas (air, nitrogen, or argon) is mixed with a

carrier/hydrogen/hydride stream. An external pump keeps the liquid level in the separator constant. The total flow rate of sample, reagent, and carrier in gas expansion separators can reach 15 ml/min, and a purging gas flow rate of 30 ml/min is relatively uncommon. Membrane separators operate by allowing gas to pass across a hydrophobic membrane, and because their internal gas volume is significantly smaller, they offer higher sensitivity at lower flow rates (Fig.1.25 b).



(a) (b)

Fig. 1.25 (a) Gas expansion separator (b) Membrane separator

Different gas/liquid separation devices were compared for hydride-generation and cold-vapour atomic absorption spectrometry (A.A.S.). Combinations of column preconcentration with hydride-generation and cold-vapour (A.A.S.) provided good sensitivity and detection limits for ultra-trace selenium and mercury determination[77] .

1.6.4 Packed-Bed Reactor Tubes

In theory, packed-bed reactors—tubes filled with inert impermeable particles (glass beads)—are superior than open tubular reactors in minimizing band widening in flowing systems [25,78]. The increased flow resistance of packed-bed reactors, on the other hand, places additional strain on the pump's pressure capabilities, the injection valve, and the connections between the different flow-injection system parts. Peak spreading is efficiently reduced while flow resistance is maintained low by filling the reactor tube with relatively big glass beads [78] . Three or four samples can be kept without much overlap in a 2m tube filled with large glass beads (a "single bead string reactor"). Nonsegmented flow systems were first developed by analytical chemists who took advantage of the advantages of immobilized enzyme preparations [1, 13, 79-84] . Some of these procedures may be covered by the FIA. The sample is injected into a stream of buffer solution, then passed through a tube laden with particles attached to an enzyme, and finally through a detector to quantify the reaction products catalyzed by the enzyme.

Fig. 1.26 Coil tube packed with glass beads

Bergmeyer and Hagen [11] a glucose oxidase column was used to measure An oxygen electrode was used to measure the following drop in oxygen tension, which was proportional to the glucose content of the injected sample, and the subsequent drop in oxygen tension, which was proportional to the glucose content of the injected sample. The use of immobilized enzymes has the potential to be significantly less expensive than continuous-flow analytical devices. Immobilized enzymes, on the other hand, take a long time to manufacture since few appropriate preparations are commercially available, Their activity diminishes with time and use. Because of the merging zone modification to FIA, it is now feasible to use extremely small quantities of soluble enzyme reagent, making immobilized enzyme columns an uneconomical alternative for many experiments. The combination of merging zones with stop-flow kinetic analysis is a far more profitable technique. Nonenzymic column packing was used to colorimetrically determine nitrate after reduction to nitrite by passing through a mini-column containing copper-coated cadmium. [4,85,86]

1.7 The Recent development of Flow Injection Instruments

From 1974 through the mid-1980s, the majority of Flow Injection devices were made from components found in the laboratory or bought piecemeal. This was owing to the fact that many researchers appreciated the challenge of creating their own systems, as well as the comparatively high cost of commercially available systems. However, with the introduction of computers, a dramatic shift occurred, and software became an essential component of effective design. Designing and writing software that controls instrument operations, enables adjustable scheduling of events, and controls peripherals such as spectrophotometers, external pumps, and valves while gathering and assessing data in real time is not an easy process. To handle peripherals added to the main instrument, commercial software is now available. A high-quality multichannel peristaltic pump, an injection valve, a coiled reactor, a detector such as a photometric flow cell, and an autosampler are typical components of a contemporary Flow Injection Analysis system. A flow through heater, columns to reduce sample size, debubblers, and filters to remove particles are all feasible additions. [2]

Because of its open architecture, a FI instrument may be configured for almost any research job or specific test. "Patches" are available for sophisticated detectors that allow us to bridge the gap between FIAlab software and detectors that use proprietary software drivers. As a result, spending time writing software applications is no longer essential. Commercial instrument kits from FIAlab are available for regular, serial tests such as ground water or environmental assessments. Recently, the costs, specific features, and available peripherals of commercially accessible FI instruments were examined. [87]

Sequential Injection (SI), Bead Injection (BI), and Sequential Injection Chromatography have all joined Flow Injection (FI) in recent years (SIC). SI and BI allow for downscaling from milliliter to microliter scales, leading in

increased reagent efficiency and reduced waste formation. SI, BI, and SIC have the disadvantage that their software methods are sophisticated and must be built separately for each test, utilizing existing analytical techniques as templets. [2]

1.7.1 The FIAlab-2500 System

The FIAlab-2500, as depicted in Fig.1.27, offers comprehensive flow injection analysis automation, including FIA lab for Windows control software, UV/VIS spectrometer, flow cell (1.5 mm to 10 cm optical path length), light source (Tungsten, Deuterium), fiber optics, tubing, connections, and a four-channel peristaltic pump.The FIAlab-2500's low price makes it desirable to academic teaching and research laboratories. It's the finest solution for commercial laboratories doing routine analysis because of its strong architecture and versatility. Up to four systems can be linked together to execute four separate tests at the same time. There's no need to prepare several standards when the SCSP-2500 autodilutor, which is available as an optional component, can handle it for you.

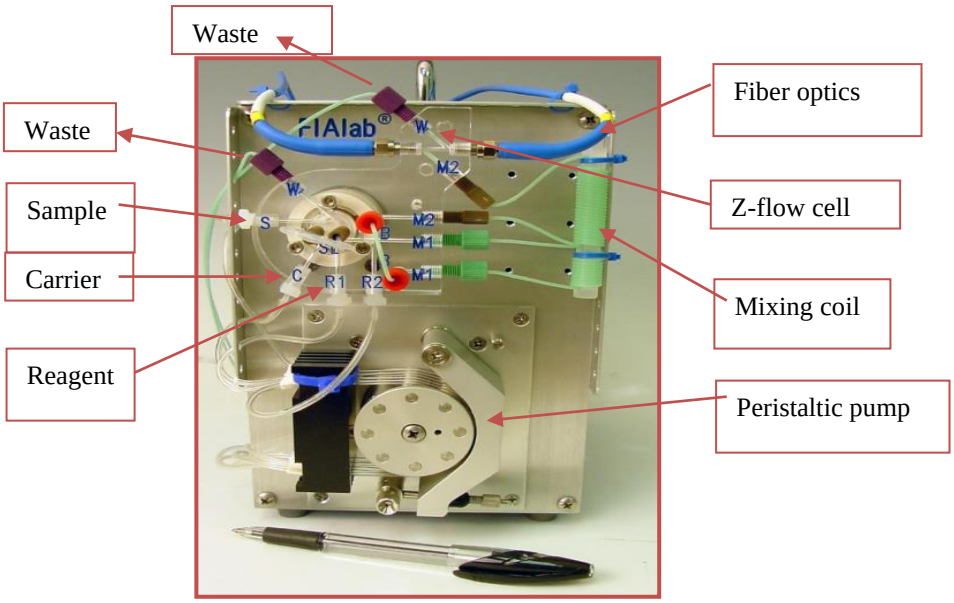


Fig.1.27 FIAlab-2500

The SCSP-2500 aspirates standards and samples from the optional component autosampler, dilutes them to the required concentration, and distributes the diluted sample through the FIAlab-2500 manifold, allowing for exact dilutions down to a factor of twenty. You may easily create a calibration curve from a single standard, or you can automatically dilute samples to concentration levels within the detector's dynamic range. When a high sample is out of range, the FIAlab software will automatically dilute and re-measure it using the SCSP-2500.[88]

1.7.2 The FIAlab-2700 System

The FIAlab-2700 Multi-Syringe Flow Injection Analysis System, as shown in the picture, is the first commercially available FIA system with a four-channel syringe pump for aspiration/dispensing carrier, sample, and reagents (Fig.1.28). Because it employs the same four-channel peristaltic pump as the FIAlab-2500, it may be used in regular peristaltic pump-based FIA mode or in combination with the syringe for more sophisticated studies. Japanese researchers identified the benefits of employing syringe pumps for FI applications a long time ago[89], and the usage of Multisyringe Flow Injection Systems (MSFIA) has been recommended in various articles. [90]

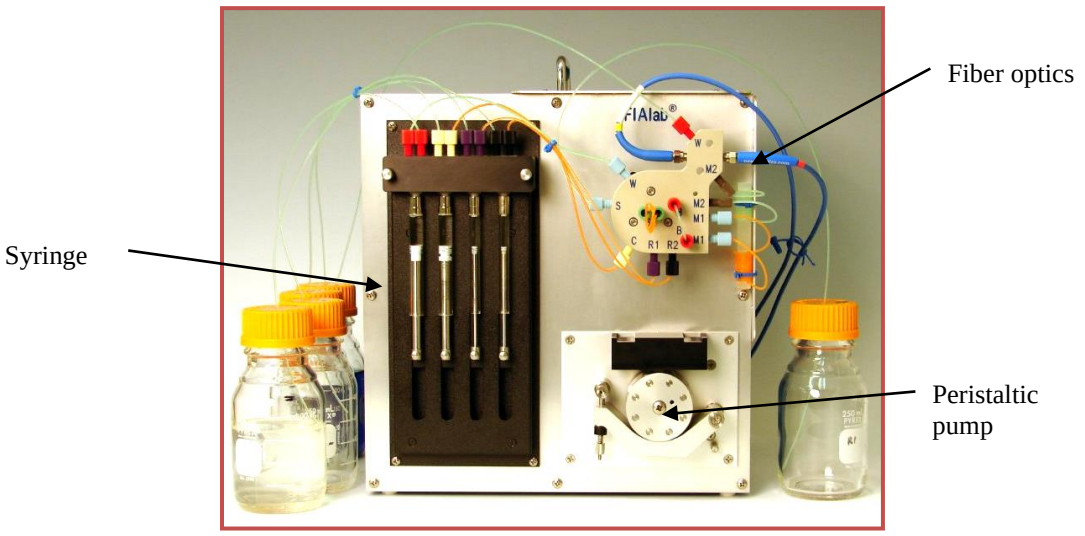


Fig. 1.28 FIAlab-2700

The Multi-Syringe Flow Injection Analysis System (MSFIA) has the following benefits: No expensive peristaltic pump tubing to replace, simple computer control of each pump individually or all pumps simultaneously, resistance to corrosive chemicals, precise speed and volume control, programmable flow capability, including stop flow FI for reaction rate measurement, no expensive peristaltic pump tubing to replace. (91)

1.7.3 Sequential Injection Analysis (SIA)

Ruzicka and Marshall (92) The University of Washington developed SIA in response to industry need for a more robust automated wet chemistry approach than FIA. Industry leaders urged teachers and researchers at the Center for Process Analytical Science (CPAC) to take FIA to the next level. Some say that it is only an extension of FIA because it shares many of its properties. Despite this, since the initial study in 1990, SIA has been the subject of over 100 academic articles. While it is essentially similar to FIA in that it is based on the dispersion of zones in a moving stream, the practice of SIA is conceptually distinct. Let's look at a simple FIA experiment and evaluate how it stacks up against its SIA cousin. Consider a single-line FIA experiment that involves injecting a sample into a reagent-carrier stream. The carrier is continually flowing through the detector in the FIA manifold while a sample is injected into the sample loop of a two-position injection valve (Fig.1.29). The length of the sample loop determines the volume of sample injected. After loading the sample loop, the valve is opened and the sample is injected into a flowing carrier stream. The carrier transports the sample from the reactor (often a reaction coil) to the detector. The sample interacts with the reagent during transportation, producing a detectable species. As the detectable species travels through the detector's flow cell, it generates a peak(92).

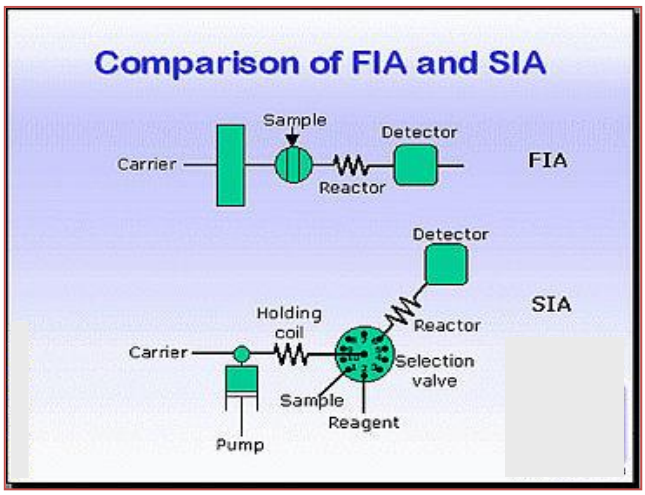


Fig. 1.29 Comparisons of FIA and SIA

REFERENCES

1. Ruzicka J. and Hansen E. H., **Anal. Chim. Acta 78**, 145 (1975).
2. Ruzicka J., ***Flow Injection Analysis***, CD-ROM tutorial, 3rd edn (2005).
3. Al-Abdullah Z. T., MSc. Thesis, Basra University (1998).
4. Al-Maliki A. D., MSc. Thesis, Basra University (1999).
5. Al-Shaheen Q. H., MSc. Thesis, Basra University (2000).
6. Al-Shemmary M. A. K., MSc. Thesis, Basra University (2000).
7. Rocks, B. and Riley C., **Clin. Chem. 28/3**, 409-421 (1982).
8. Blaedel, W. J., and Hicks, G. P., **Anal. Chem. 34**, 388-394 (1962).
9. Hicks, G. P., and Blaedel, **Anal. Chem. 37**, 354-358 (1965).
10. Nagy, G., Fehr, Z., and Pungor, E., **Anal. Chim. Acta 52**,47-54 (1970).
11. White, W. R., and Fitzgerald, J. M., **Anal. Chem. 44**, 1267-1269 (1972).
12. Bergmeyer, H. U., and Hagen, **Anal. Chem. 261**, 333-336 (1972).
13. Miller, J. N., Rocks, B. F., and Thorburn Burns, D., **Anal. Chim. Acta 86**, 93-101 (1976).
14. Stewart, K. K., Beecher, G. R., and Hare, P. E., **Anal. Biochem. 70**, 167-173 (1976).
15. Blaedel, W. J., and Hicks, C. P., **Anal. Biochem. 4**, 476-488 (1962).
16. Skeggs, L. T., **Am. J. Clin. Pathol. 28**, 311-322 (1957).
17. Pretorius, V., and Smuts, T. W., **Anal. Chem. 38**, 274-281 (1966).
18. Betteridge, D., **Anal. Chem. 19**, 832A-841A (1978).
19. Mindegaard, J., **Anal. Chim. Acta 104**, 185-189 (1979).
20. Baadenhuijsen, H., and Seuren-Jacobs, H., **Clin. Chem. 25**, 443-445 (1979).
21. Taylor, C., **Proc. R. Soc. London Ser. *A* 219**, 186-203 (1953).
22. Vanderslice, T. J., Stewart, K. K., Rosenfeld, A. G., and Higgs, D. J., **Talanta 28**,11-18 (1981).
23. Ruzicka, J., and Hansen, E., **Anal. Chim. Acta 99**, 37-76 (1978).
24. Reijn, J. M., Van der Linden, W. E., and Poppe, H., **Anal. Chim. Acta 114**, 105-118 (1980).
25. Tijssen, R., **Anal. Chim. Acta 114**, 71-89 (1980).
26. Hungerford J., *Ph. D. Thesis, Dept. of Chemistry, Univ. of Washington* (1986).
27. Ruzicka, J., and Hansen, E., **Anal. Chim. Acta 114**, 19-44 (1980).
28. Renoe, B. W., Stewart, K. K., Beecher, G. R., Wills, M. R., and Savory, J., **Clin. Chem. 26**, 331-334 (1980).
29. Stewart, K. K., and Beecher, G. R, 176th National Meeting of the ACS, Miami, FL, paper 87, (1978).

30. Wolff, C., and Mottola, H. A., **Anal. Chem. 50**, 94-98 (1978).
31. Margoshes, M., **Anal. Chem. 49**, 17-19 (1977).
32. Ruzicka, J., Hansen, E. H., Mosback, H., and Krug, J. F., **Anal. Chem. 49**, 1858-1862 (1977).
33. Lim, C. S., Miller, J. N., and Bridges, J. W., **Anal. Chim. Acta 114**, 183-189 (1980).
34. Ruzicka, J., and Hansen, E. H., **Anal. Chim. Acta 106**,207-224 (1979).
35. Ruzicka, J., and Hansen, E., **Chem. Technol. 9**, 756-764 (1979).
36. Ranger, C., **Anal. Chem. 53**, 20A-32A (1981).
37. Snyder, L. R., **Anal. Chim. Acta 114**, 3-18 (1980).
38. Ranger, C. B., *Multiparameter analysis*. Industrial Research Development 134-137 (Sept 1979).
39. Thiers, R. B., Reed, A. H., and Delander, K., **Clin. Chem. 17**, 42-48 (1971).
40. Stewart, K. K., **Anal. Chem. 49**, 2125-2126 (1977).
41. Bergamin, H., Reis, B. F., and Zagatto, E. A., **Anal. Chim. Acta 97**, 427-431 (1978).
42. Rangel A.O.S., Santos J. L. M. Clausse, A. Lima J. L. F. C., and saraiava M. L. M. F. S., **Aalyt. Sci. 21**, 46 (2005).
43. Basson, W. D., **Lab. Pract. 26**, 541-545 (1977).
44. Stewart, K. K., Brown, J. F., and Golden, B. M., **Anal. Chim. Acta 114**, 119-127 (1980).
45. Ruzicka,J., Stewart, J. W. B., and Zagatto, E. A., **Anal. Chim. Acta 81**, 387-396 (1976).
46. Basson, W. D., and Van Staden, F. J., **Anal.Chem. 302**, 370-374 (1980).
47. Ruzicka, J., Hansen, E., H., and Mosbaek, H., **Anal. Chim. Acta 92**, 235-249 (1977).
48. Watson, B., Stifel, D. N., and Semersky, F. E., **Anal. Chim. Acta 106**, 233-242 (1979).
49. Astrom, 0., **Anal. Chim. Acta 105**, 67-75 (1979).
50. Betteridge, D., and Fields, B**., Anal. Chem. 50**, 654-656 (1978).
51. Baban, S., **Anal. Proc. 17**, 535-537 (1980).
52. Betteridge, D., Dagless, E. L., Fields, B., Sweet, P., and Deans, D. R., **Anal. Proc. 18**, 26-31 (1981).
53. Poppe, H., **Anal. Chim. Acta 114**, 59-70 (1980).
54. Braithwaite, J. I., and Miller, J. N., **Anal. Chim. Acta 106**,395-399 (1979).
55. Ishibashi, N., Kina, K., and Goto, Y., **Anal. Chim. Acta 114**,325-328 (1980).
56. Zagatto, E., Krug, F., Bergamin, H., Jdrgensen, S., and Reis, B. F.,**Anal. Chim. Acta 104**, 279-284 (1979).
57. Wolf, R. W., and Stewart, K. K., **Anal. Chem. 51**, 1201-1205 (1980).
58. Nord, L. A., and Karlberg, B., **Anal. Chim. Acta 125**, 199-202 (1981).
59. Mindel, B. D., and Karlberg, B., **Lab. Pract. 30**, 719-723 (1981).
60. Burguera, J. L., and Townshend, A., **Proc. Anal. Div. Chem. Soc. (London) 16**, 263-264 (1979).
61. Rule, G., and Seitz, R. W., 25, 1635-1638 (1979).
62. Burguera, J. L., Townshend, A., and Greenfield, S., **Anal. Chim. Acta 114**, 209-214 (1980).
63. Van Staden, J. F., and Basson, W. D., **Lab. Pract. 29**,1279-1280 (1980).
64. Baban, S., Beetlestone, D., Betteridge, D., and Sweet, P., **Anal. Chim. Acta 114**,319-332 (1980).
65. Fang, Z-L., ***Flow Injection Atomic Spectrometry***, Wiley, Chichester, (1995).
66. Sanz-Medel, A., (Ed.), ***Flow Analysis with Atomic Spectrometric Detectors***, Elsevier, Amsterdam (1999).
67. Burguera, J.L., (Ed.) ***Flow Injection Atomic Spectroscopy***, Marcel Dekker, New York, (1989).
68. Strohl, A. N., and Curran, D. J., **Anal. Chem. 51**, 1045-1049 (1979).
69. Ramsing, A., Ruzicka, J., and Hansen, E. H., **Anal. Chim. Acta. 114**, 165-181(1980).
70. Bergamin, H., Zagatto, E., Krug, F., and Reis, B. F., **Anal. Chim. Acta 101**, 17-23 (1978).
71. Nord L., and Karlberg B., **Anal. Chim. Acta 164**, 233 (1984).

72. Karlberg B.and Thelander S., **Anal. Chim. Acta 98**, 1 (1978).
73. Karlberg B., Pacey C.E., ***Flow Injection Analysis, A Practical Guide***, Elsevier, Amsterdam (1989).
74. Fang Z-L., ***Flow-Injection Separation and Preconcentration***,VCH Verlagsgesellschaft Weinheim, (1993).
75. Baadenhuijsen H., and Seuren-Jacobs H.E.H., **Clin. Chem. 25**, 443 (1979).
76. Luque de Castro M. D. and Papaefstathiou I., **TRAC, 17**, 41 (1998).
77. Fang Z-L., Zhu Zhaohai, Zhang Suchun, Xu Shukun, Guo Lei and Sun Lijing, **Anal. Chim. Acta. 214**, 41-55 (1988).
78. Reijn, J. M., Van der Linden, W. E., and Poppe, H.,. **Anal. Chim. Acta 123**, 229-237 (1981).
79. lob, A., and Mottola, H. A., **Anal. Chem. 52**, 2332-2336 (1980).
80. Bowers, L. D., Canning, L. M., Sayers, C. N., and Carr, P. W. **Gun. Chem. 22**, 1314-1318 (1976).
81. Bowers, L. D., and Carr, P. W., **Clin. Chem. *22***, 1427-1433 (1976).
82. Rocks, B. F., **Analytical Proc. Soc. Anal. Chem. 10**, 164-165 (1973).
83. Miller, J. N., Rocks, B. F., and Thorburn Burns, D., **Anal. Chim. Acta 93**, 353-356 (1977).
84. Gorton, L., and Bhatti, K. M., **Anal. Chim. Acta 105**, 43-52 (1979).
85. Anderson, L., **Anal. Chim. Acta 110**, 123-128 (1979).
86. Gine, M.,F., Bergamin, F., Zagatto, E. A., and Reis, B. F., **Anal. Chim. Acta 114**, 191-197 (1980).
87. Smith J.P. and Hinson-Smith V., **Anal. Chem. 74**, 385A (2002).
88. Scampavia Louis D. and Ruzicka Jaromir, *Micro-sequential Injection: A multipurpose Lab-on-Valve for the advancement of Bioanalytical Assays,* **Analytical Sciences,** 17 Supplement (2001).
89. Yoza, N., Ishibashi, K., and Ohashi, S. J. **Chromatography 134**, 497 (1977).
90. Cerda V., Estela J.M., Forteza R., Cladera A., Becarra E., Altimira P., and Sitjar P., **Talanta 50**, 695 (1999).
91. Miro M., Estela J.M., Cerda V., **TRAC 21**, 199 (2002).
92. www.globalfia.com