Flow Injection Analysis (FIA)

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Summary: Flow injection analysis (FIA) is a simple analytical sample processing technique, based on the injection of a definite volume of liquid sample solution in a continuously flowing unsegmented carrier stream, followed by the quantitation of species of interest. It offers many advantages over manual methods of analysis, like: flexibility, simplicity, selectivity, reproducibility, higher sampling rate, rapid start-up and shutdown time and economy.

Injection volume, operation timing and dispersion must be carefully controlled to achieve precise results.

Consumption of reagents is a serious problem in certain cases which can be controlled by the introduction of different FIA techniques. Instrumentation of FIA is simple. A simple manifold can be quickly assembled.

This technique has numerous areas of applications such as environmental pollution, biotechnology, agricultural, pharmaceutical and clinical analysis.

Introduction

Flow injection analysis (FIA) was conceived in 1975 [1]. Since that time a lot has been written about this technique especially by the leading practitioners [2,3]. It is defined as an automated or semiautomated analytical sample processing technique which is based on injection of a definite volume of liquid sample solution into a continuously flowing unsegmented carrier stream, followed by the quantitation of species of interest at a downstream detection area [4]. A simple flow injection manifold is shown in Fig. 1.

Advantages and limitations of flow injection systems

Flow injection analysis has the drawback of being less sensitive than manual analytical methods for the following reasons.

1. Short reaction time; reaction may not reach equilibrium.
2. Dilution of the sample in the carrier results in a decrease in signal intensity.

However FIA has great flexibility and the sensitivity of the system can be increased by making modifications, which are described later.

FIA has many advantages:

1. Selectivity

FIA may show higher selectivity than conventional methods. As the manifold can be changed according to requirements, interferences

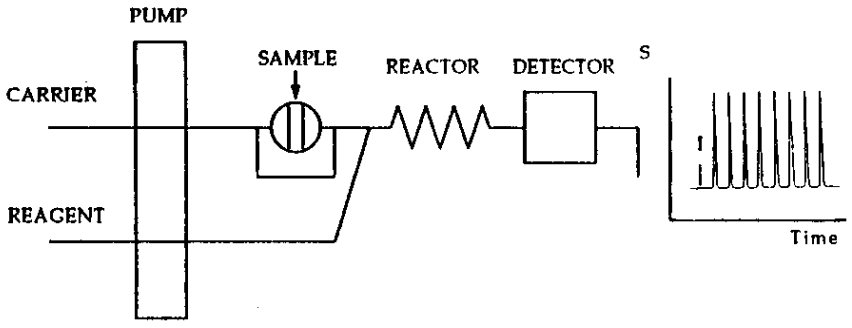

Fig. 1: Simple flow injection manifold used in FIA. Adapted from (3).

due to other species can be avoided by controlling the coil length so that their reaction does not develop. Because it is an enclosed system, FIA also plays an important role in the development of methods which need an inert atmosphere or must avoid exposure to the atmosphere and the environment.

2. Reproducibility

Many factors affect the reproducibility of the FIA signal such as temperature, mode of injection, and especially flow rate. Flow rate is a critical factor which affects the FIA signal, which can be controlled:

(I) by using a constant flow rate pump;

(ii) by replacing pump tubing frequently.

3. Higher sampling rate

FIA is remarkably rapid compared to conventional techniques. The sampling rate is increased by increasing the flow rate, but in a system where a chemical reaction takes place, sensitivity decreases with increase in flow rate. In the absence of a chemical reaction both sensitivity and sampling rate increase with increase in flow rate.

4. Simplicity and flexibility

The system manifold is very simple to assemble and can easily be handled. It offers a vast range of possibilities for changing the manifold according to requirements.

5. Rapid start-up and shutdown

A flow injection system is usually very simple to start up. One only needs to turn the pump on and leave the carrier stream to flow for a few minutes. And it is even simpler to close down the system by just stopping the pump.

6. Economical

Another important property of FIA is its low cost, due to the construction of home-made manifolds from inexpensive components.

The ability of FIA to handle microliter volumes has made the system more economical especially while working with enzymes and compounds which are not easily available.

Essential features of FIA

According to Ruzicka and Hansen [2] FIA has three essential features.

1. Sample injection

The purpose of sample injection is to pass a definite volume of sample into a continuously flowing carrier stream so that the flow of the carrier stream is not disturbed. Injection is possible manually and by the help of a syringe, injection valve or automatically by a pump.

2. Reproducible operational timing

Reproducible timing is essential in FIA. In order to get a constant residence time, a peristaltic pump with minimum pulsation is normally applied to provide a constant flow rate.Since precision depends upon a well-defined volume of sample and a reproducible flow rate, both factors need particular attention [5].

3. Controlled dispersion

This is the most important factor in FIA. Dispersion (dilution) occurs when the injected sample is carried by the carrier stream toward the detector. Dispersion affect both peak height and peak shape.

Ruzicka and Hansen [2] have defined dispersion D as the ratio of the concentration of sample before and after the dispersion process:

$$D = C_o/C \text{ (max)}$$

where C_o is the original concentration of the injected sample and C(max) is the concentration which corresponds to the peak maximum.

Factors affecting dispersion

1. Sample volume

Dispersion decreases with increase in sample volume. As the sample volume increases the signal increases until a limit is reached where the dispersion is 1.0. This "steady state" signal

corresponds to the concentration of undiluted sample.

2. Flow rate

Dispersion is directly proportional to the flow rate F:

$$D = KF$$

where K is a constant. Commonly used flow rates in FIA are between 0.5 and 5.0 ml min^{-1}.

3. Mixing coil diameter and length

Dispersion is proportional to the square root of the length of the tubing between the point of injection and the detector. Dispersion also increases with increase in diameter of the mixing coil tubing. These parameters can be manipulated to achieve the required dispersion that will be suited to a particular FIA system.

Types of Dispersion

Generally, dispersion can be classified as limited, medium or large and can be employed in the following ways:

1. Limited dispersion

Limited dispersion is used in a flow system where no chemical reaction needs to take place and the sample is simply transported to the detector without chemical reaction. It finds application in electrochemical, atomic absorption and pH monitoring [6].

2. Medium dispersion

Medium dispersion is more common in FIA because it allows a chemical reaction to take place by mixing the sample components with the reagent present in the carrier stream in order to get a measurable amount of product before reaching the detector. Medium dispersion has found its application in spectrophotometry and chemiluminesence detection [7].

3. Large dispersion

Large dispersion is important in slow chemical reactions, for diluting concentrated samples and for some other special measurements [8].

FIA Techniques

1. Merging zones techniques

A disadvantage of continuous flow injection analysis is the continuous consumption of the reagents. If this problem is serious it can be minimized by the introduction of the merging zones technique, which can be achieved by occasional pumping [2] (Fig. 2a or by multiple injection valves originally described by Mindegaard [9] and Bergamin [10]. In a multiple injection system there are two choice, in one, two injection valves are placed in parallel with each other Fig. 2b; sample and reagent are injected into the separate streams and then merged at the mixing point. In the other, the injection valves are placed in series and sample and reagent are injected into the same carrier stream; the two zones move downstream and merge before passing through the detector. The purpose of this technique is to reduce the consumption of expensive reagents and to create a composite zone that is information rich. In this research a multiple injection technique was adopted in which two injection valves were placed in series in order to minimise the high cost of reagents, enzymes and coenzymes.

2. Stopped flow technique

A most useful manoeuvre in FIA is stopped flow. This has been exploited in two situations. Firstly, when a period of incubation is required to increase sensitivity without increasing reaction coil length, without increasing dispersion and also without simply decreasing the flow rate. Secondly, where kinetic studies are to be made. A variety of stopped flow manifolds has been reported for determination of organic and inorganic species [11-13].

The stopped flow technique is not only important for the above purpose but it can also help to eliminate background signals and to reduce reagent consumption by making use of it only when it is required rather than continuously [14]. In spite of all these advantages this technique is not widely utilized, possibly because of lack of suitable instrumentation and control system to produce a

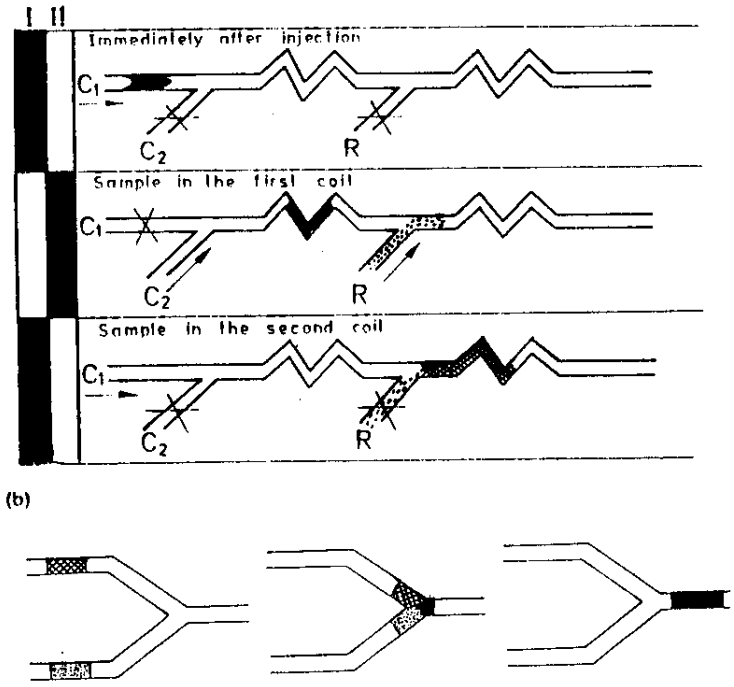

Fig. 2: (a) Merging zones with intermitent pumping, (b) merging zones with two injection valves. Adapted from (3). R = reagent, C_1 and C_2 are carriers 1 and 2 respectively (1) and (11) shows two pumps.

reproducible stop time and because the analysis time is lengthened. Christian and Ruzicka [15] have discussed a variety of problems, especially in process control, that have been solved by stopped flow.

Sequential analysis

The concept of sequential analysis was reported by Ruzicka *et al.*, [14] and has been explored in many assays [15-18]. Sequential analysis is simpler than the other techniques; precisely measured volumes of carrier solution, sample solution and reagent solution are aspirated into a holding coil by means of a pump, which could be a syringe pump or peristaltic pump. Due to its simplicity this technique will find wide application in process control and in the laboratory. Although the technique is simpler than conventional FIA, the complexity of gradients formed by zone penetration with reverse flow is still an unsolved problem [14].

Flow injection instrumentation

The instrumentation of a flow injection system is simple. A basic system requires no more than a means of propelling the stream of reagents, reaction coil tubing, some means of sample injection and a detector with a flow-through cell connected to a recorder for recording the signal. Despite the commercial availability of FIA equipment, most workers have preferred the use of home-made manifolds. A simple manifold can quickly be assembled from the following basic components.

1. Pump

A pump is an important device which provides the means for propelling the carrier stream at a constant flow rate with minimum pulsation. Various types of pump have been used in FIA. These include peristaltic pumps [19,20], syringe driven pumps [21, 22,15] and pressurised gas pumps [23,24]. The most common means of carrier stream propulsion is the peristaltic pump. This provides a low pulsation flow but the flow is never pulse-free. Pulsation is usually not a problem, but some workers do consider it to be a minor difficulty and depulsing devices have been used [25,26]. Vanderslice et al., [27] suggested that a slight degree of pulsation in the

flow may have beneficial affects on the peak shape produced during the analysis.

2. Injection port

The design of the injection device is critical for the effectiveness of any flow injection system. It is very important to inject a defined volume of sample to achieve reproducibility in the results. In the earliest methods the sample was injected from a syringe through the wall of the tube, through a septum [28] or through a flap valve [29-31]. Nowadays different types of injection valves are available, such as a rotary valve [11,3], hydrodynamic valve [32] and chromatographic injection valve [33]. Most workers have preferred to use rotary valves of the type described by Ruzicka and Hansen [2] and Mindegaard and Anderson [9]. Riley et al., [34] have described a motorised version of the valve. Fig. 3 shows the operation of such a rotary valve. There are two positions, in the load (filling) position a definite volume of the sample is loaded and at the inject (emptying) position the carrier stream is passed through the sample loop. Thereby sample is inserted into the carrier stream from where it travels to the detector.

3. Detector

Any detector which can be equipped with a flow-through cell can be used in FIA. A good detector should have only a small dead volume, high stability and fast response. A wide range of detection systems has been developed in combination with FIA, among which spectrophotometry [35], fluorimetry [36] amperometry [37] and chemiluminèsence [38] are well known.

A simple small scale amperometric detector has been constructed by Masoom and Townshend which was used for the determination of glucose [39], sulphite [40] and cholesterol [41].

4. Other components

Usually in a FIA manifold, PTFE tubing with i.d. 0.3-0.7 mm is used for transportation and for mixing coils. The flow line must be held constant in order to avoid any disturbance in the flow. Ready-made tubing with different lengths fitted with connectors is available, and new connections can be made according to need with the help of a flanger (supplied by firms like Tecator). A flanger consists of a cylindrical heating wire slightly smaller in diameter than the bore of the tubing. The end piece of the tubing is introduced onto in heating wire, which slowly warms it up and helps in its easy flanging. Nowadays a wide range of connectors is also available, details are given in [2]. Different types of reactors like open tubular, packed bed or single bead string reactors are also employed according to the particular need of the system [42].

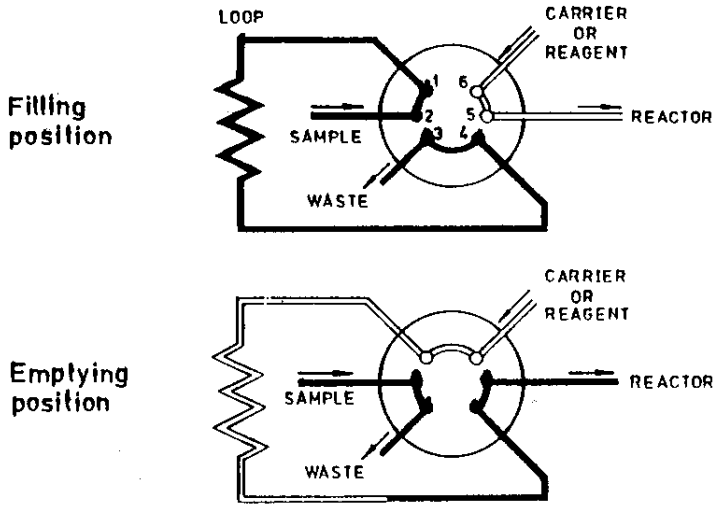

Fig. 3: Four-way rotary injection valve at its load and inject position, comonly used in FIA and also used in this research. Adapted from (3).

Data processing

In FIA the analyte concentration is usually estimated by peak height and less frequently by peak width or peak area. This kind of data output is usually produced on a chart recorder which has quite a fast response. Microcomputers are also used to collect and a process the data, and the analytical results are printed out in an organised format [43,44].

Multicomponent determinations in FIA

A typical solution some times needs to be analysed for more than one species, which is important, for example, in food processing, in clinical and industrial analysis and in process control. In order to eliminate the limitation of FIA for the determination of only one species at a time many attempts to achieve multicomponent analysis have been made with a variety of procedures and manifolds, for example by using a single injection with several detectors in series or parallel [45,46]. Enzymatic assays have particular importance in food and clinical analysis. A number of enzyme reactors have been reported to be used for simultaneous determination of more than one component in a mixture [47-49]. By the implementation of the pH gradient technique, which is based on the formation of complexes of various cations in different parts of the sample zones according to the pH, more than one component can be determined in an assay mixture [50]. Marcos *et al.*, reported the simultaneous determination of metal ions in this way [51]. More recently an automated flow injection method was developed for the determination of six different analytes simultaneously, by using six enzyme reactors in parallel, with a single detector and a single injection valve [52].

Applications of FIA

As FIA is compatible with a variety of chemical processes and detection systems and also offers ease of automation, it has numerous areas of applications such as environmental pollution, biotechnology, agricultural, pharmaceutical and clinical analysis. Up to the end of 1992 FIA had been described in 4000 papers and six monographs and discussed in many meetings [14]. Different features of FIA have been exploited from time to time in clinical analysis. A list of clinically relevant species determined by FIA has been compiled by Ruzicka and Hansen [2], and Rocks and Riley [53]. The use of immobilised enzymes in FIA has also gained attention for the determination of enzyme inhibitors like paraoxons and durgs [54,55]. A review of applications of FIA in clinical analysis is also given by Valcarcel and Luque de Castro [3], divided into sections on enzymatic and non-enzymatic methods in clinical analysis.

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