FLOW INJECTION ANALYSIS

PRESENTED BY:

ARPIT BANA

& SAGAR MASANI

GUIDED BY:

Dr. R.C.MASHRU

Faculty of Pharmacy,

M. S. University

INTRODUCTION

FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector

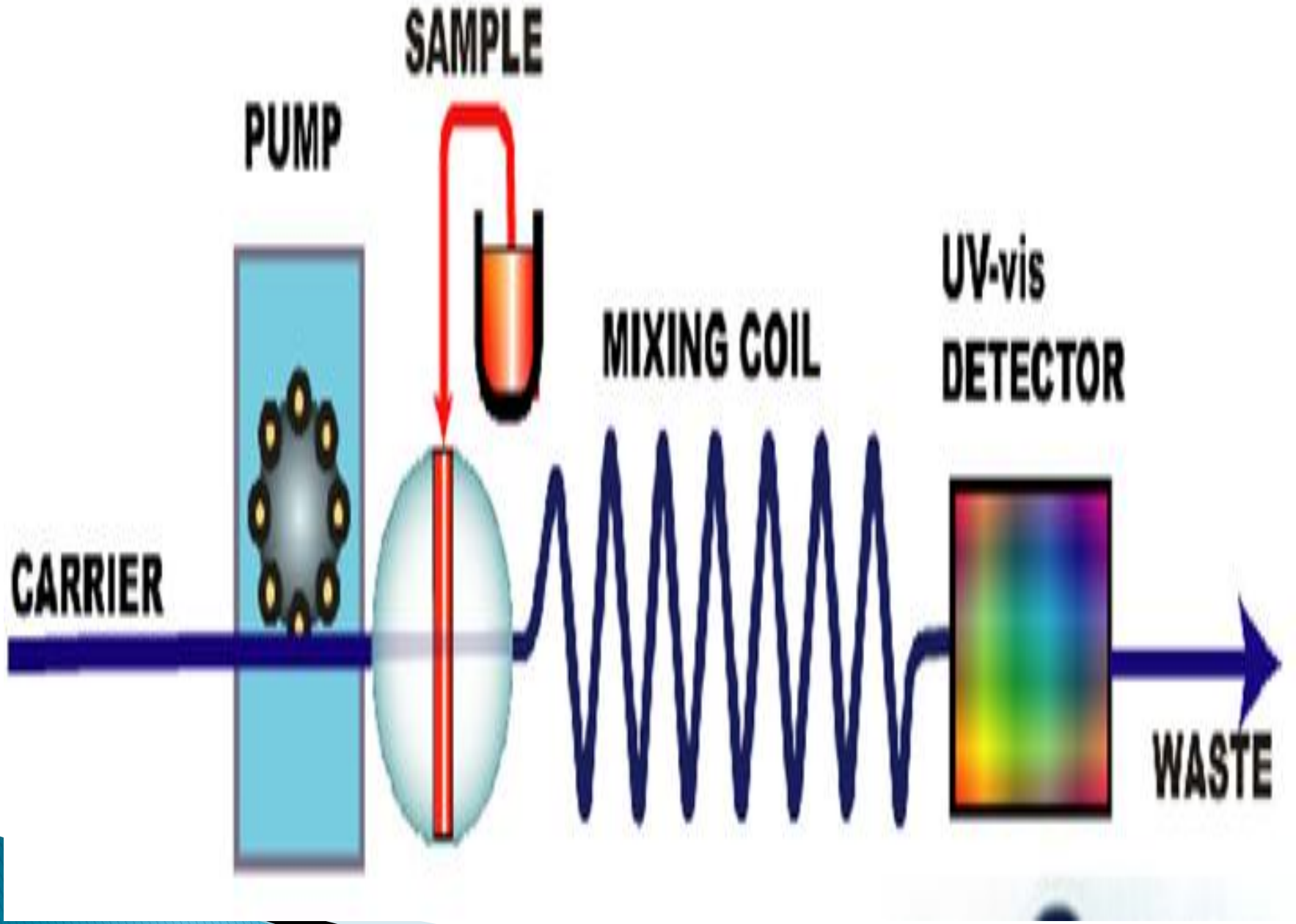
Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

- Flow Injection evolved into Sequential Injection and Bead Injection which are novel techniques based on computer controlled programmable flow.
- Flow injection methods were first developed by Ruzicka & Hansen in Denmark in the mid 1970.
- Flow injection methods are an outgrowth of segmented flow procedures which widely used in clinical laboratories for determination of variety of species in biological fluids for medical & diagnostic purposes.

DEFINITION

A typical definition describes FIA as:

- *"*A simple and versatile analytical technology for automating wet chemical analysis, based on the physical and chemical manipulation of a dispersed sample zone formed from the injection of the sample into a flowing carrier stream and detection downstream".


SAMPLE
PUMP
UV-vis
DETECTOR
MIXING COIL
CARRIER
WASTE

Flow Injection (FI), *the first generation of FIA techniques, is the one most widely used. In its simplest form , the sample zone (red) is injected into a flowing carrier stream of reagent (blue). As the injected zone moves downstream, the sample solution disperses into reagent, while a product begins to form at interfaces between the sample zone and the reagent. A detector placed downstream records a change of color or of another parameter as it changes due to the passage of the derivatized sample material through the flow cell* (Ruzicka & Hansen 1975).

- ***FIA techniques fall into four categories:***

- **Flow Injection (FI),**
- **Sequential Injection (SI),**
- **Bead Injection (BI),**
- **Sequential Injection Chromatography (SIC).**

- FI is based in sample injection into a unidirectional flowing carrier stream to which reagents are added. So, the concentration gradient is formed by dispersion of the sample zone alone, signal reflects the gradient of the sample zone, as it passes through the detector.
- SI and BI are based on sequential injection of sample and reagents, into carrier stream, followed by flow reversal that promotes mutual dispersion of injected zones. The flow of the carrier stream is programmed, to optimize the assay protocol.
- While SI is designed to process liquids, BI uses suspensions of suitable materials in bead form to assemble columns with solid reagents or suitable ligands. SIC(sequential injection chromatography) is emerging chromatographic technique that uses programmable flow to separate components, based on difference in migration velocities, on short columns.

Types of continuous Flow method

(1) Conti. mixing methods:- sample is injected in to the system, mixing it with carrier or reagent, measure the plug flow as it passes through a suitable detector and either sending to the waste or recirculating it.

(2) Stopped flow conti. mixing methods:- the flow is stopped at various stages during the process in order to prevent air from entering the system between the system aspiration & reagent washing. Flow is stopped in between these two operations.

(3) Continuous flow titrations:- large dispersion technique, here mixing chambers are used instead of mixing coils to promote large dispersion. The sample is mixed with the reagent containing indicator and then the color is detected.

Segmented Flow Analysis

- **Segmented flow analysis** is characterized by the use of one or several liquid streams where the samples are introduced by sequential aspiration and are separated by means of air bubbles so, the final liquid stream is segmented into small discrete liquid by bubbles of air .

- Air-segmented flow analysis (SFA) is a method that automates a large number of chemical analyses.

- SFA was first applied to analysis of sodium and potassium in human serum, with a flame photometer as the detection device, by removing protein interferences with a selectively porous membrane (dialyzer).

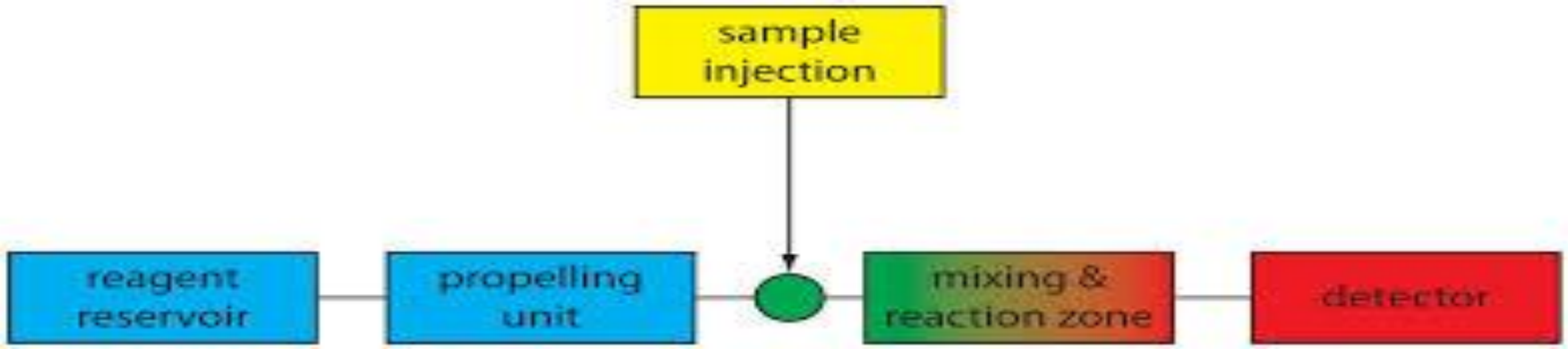
Principles of Operation.

- A sample (analyte) is injected into a carrier solution which mixes through radial and convection diffusion with a reagent for a period of time.
- Before the sample passes through a detector to waste, a peristaltic pump is the most commonly used pump in FIA instruments.
- New variants of FIA technique use computer controlled syringe pumps that generate discontinuous flow .
- Decrease of sample and reagent consumption and waste generation.

Inject the liquid sample in to moving conti. carrier stream of a suitable liquid.

Injected sample forms a zone.

- Then zone is transported toward detector conti. & record the changes in absorbance, electrode potential or other physical parameter resulting from the passage of the sample material through the flow cell.



Description of FIA

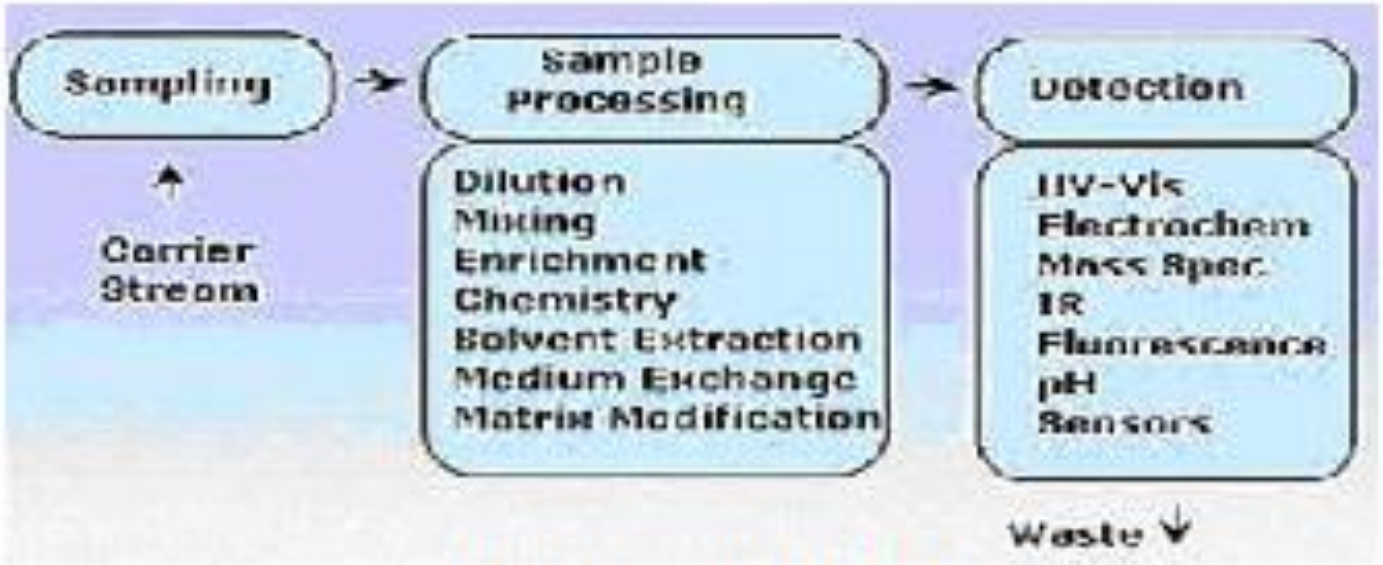


Fig 1: Description of FIA

Sampling:

➢First is sampling, where the sample is measured out and injected into the flowing carrier stream (thus, the name Flow Injection Analysis). This step is generally performed with a sample injection valve.

Sample processing

➢Second stage is sample processing. The purpose of this step is to transform the analyte into a species that can be measured by the detector and manipulate its concentration into a range that is compatible with the detector, using one or more of the indicated processes.

Detection

➢The third stage is detection where the analyte, or a derivative of it, generates a signal peak that is used to quantify the compound being determined. As indicated, a large variety of detectors can be used in FIA. The power of FIA as an analytical tool lies in its ability to combine these analytical functions in a wide variety of different ways to create a broad range of different methodologies, and perform these methodologies rapidly and automatically with minute (μL)amounts of sample. The first and last stages are, largely, conventional technology.

Principle of FIA

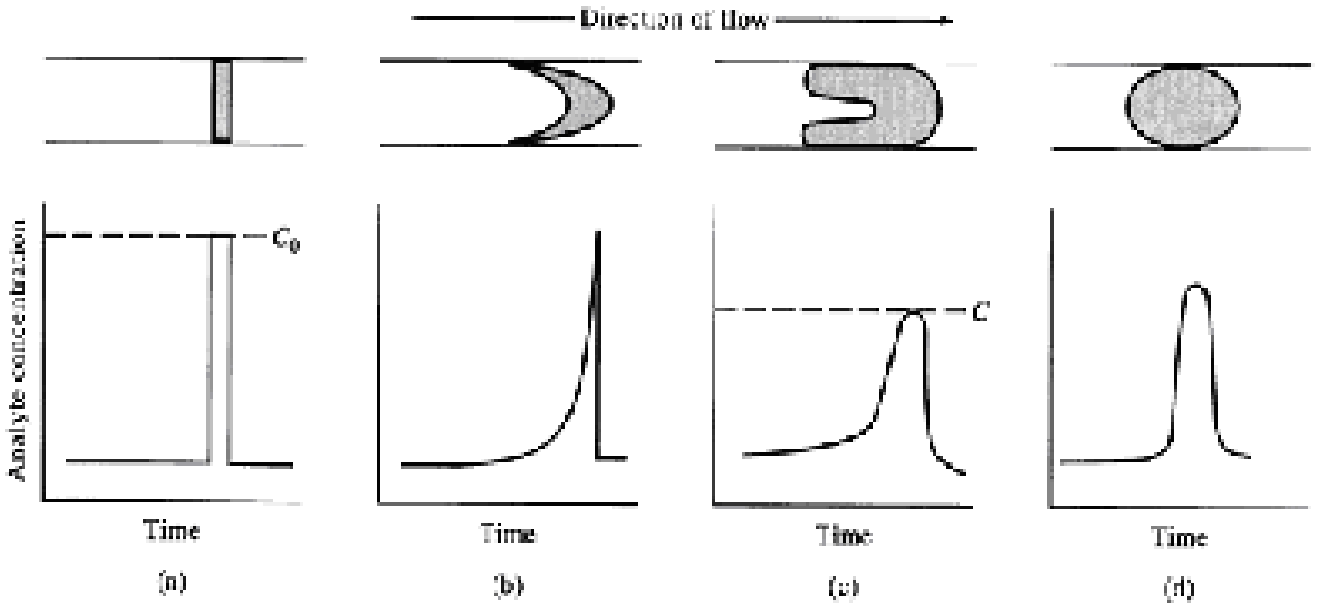


Fig 2: Effect of convection on concentration profiles of analytes at the detector, (a) no dispersion, (b) dispersion by convection, (c) dispersion by convection and radial diffusion, (d) dispersion by diffusion.

A FIA peak occurs due to two processes :

•one involving the simultaneous physical process of zone dispersion and

•the second involving the chemical process resulting from reaction between sample and reagent species. A difference in the concentration gradient is thus generated.

Immediately after injection with a sampling valve, sample zone (plug) concentration profile is rectangular shown in figure

As it moves through the tubing, band broadening or dispersion takes place. The shape of the resulting zone is determined by two phenomena.

The first is **convection** arising from laminar flow in which the centre of the fluid moves more rapidly than the liquid adjacent to the walls,

thus creating the parabolic shaped front and the skewes zone profile had shown in figure

Broadening also occurs as a consequence of **diffusion**.

Two types of diffusion can, in principle, occur:

1. Radial, or Perpendicular to the flow direction
2. Longitudinal or parallel to the flow.

Dispersion

The extent of dispersion of dilution is measured in terms of dispersion coefficient (D). D is the ratio of the concentration of the sample before and after the dispersion process takes place.

Dispersion coefficient (D) is defined by the equation

$D = C_0/C$

Where C_0 = concentration in injection volume,

C =peak concentration at 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 id

Three types of sample dispersion

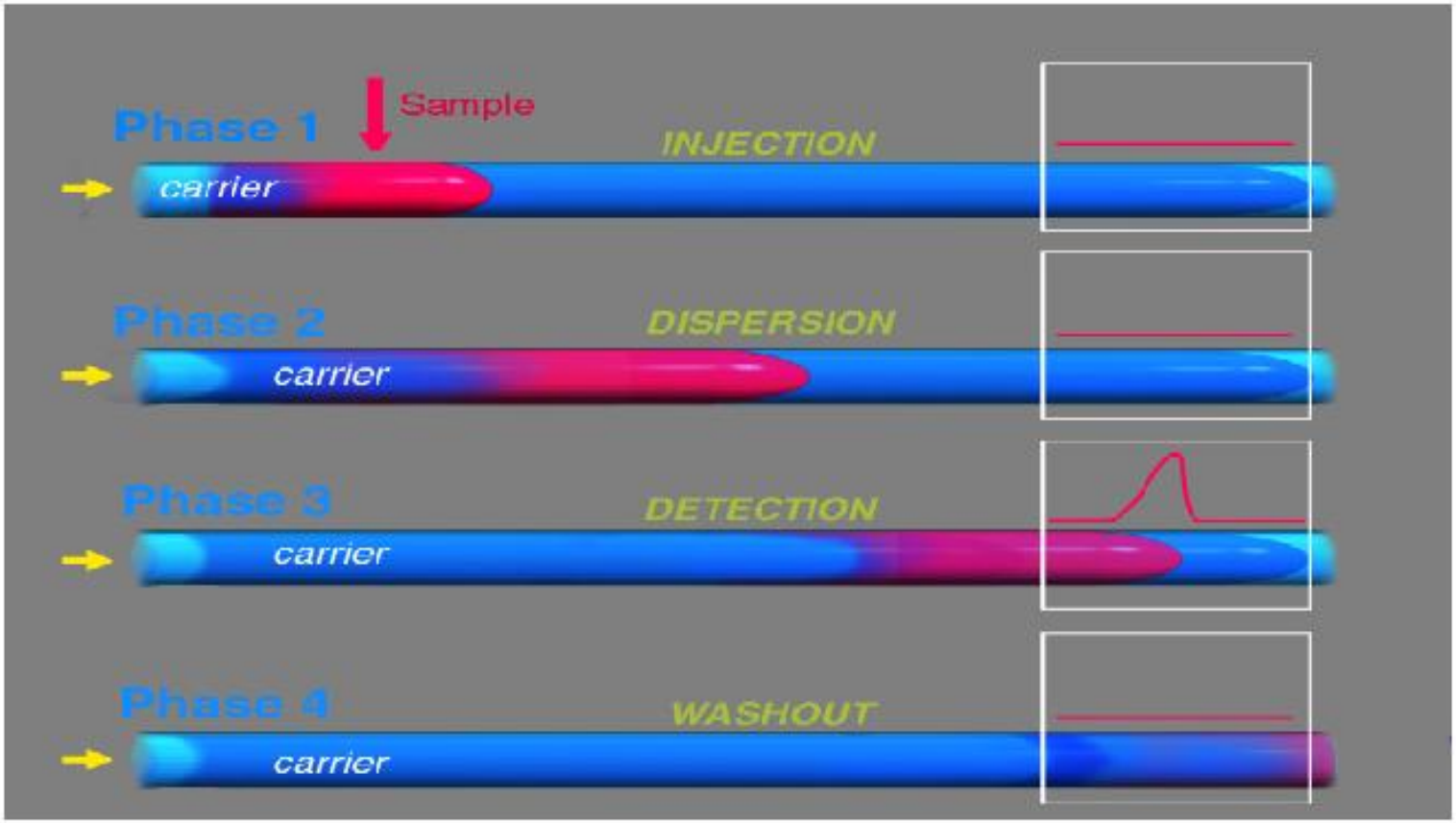
1. Limited dispersion if D=1 to 3,
2. Medium dispersion if D=3 to 10,
3. Large dispersion if D>10

- **Out of three limited dispersion is preferred**
- **Four stages of dispersion process:**

  injection, dispersion, detection, washout

1.2.4.

DISPERSION of SAMPLE ZONE



Four stages of dispersion process shown above depict the underlying physical principle of FI at continuous forward flow. For success of a reagent based assay, it is essential to design the flow system in such a way that the degree of the axial dispersion is controlled to suit the purpose of a planned assay, while the radial dispersion is designed to provide an efficient mixing of sample zone with reagent supplied by carrier stream.

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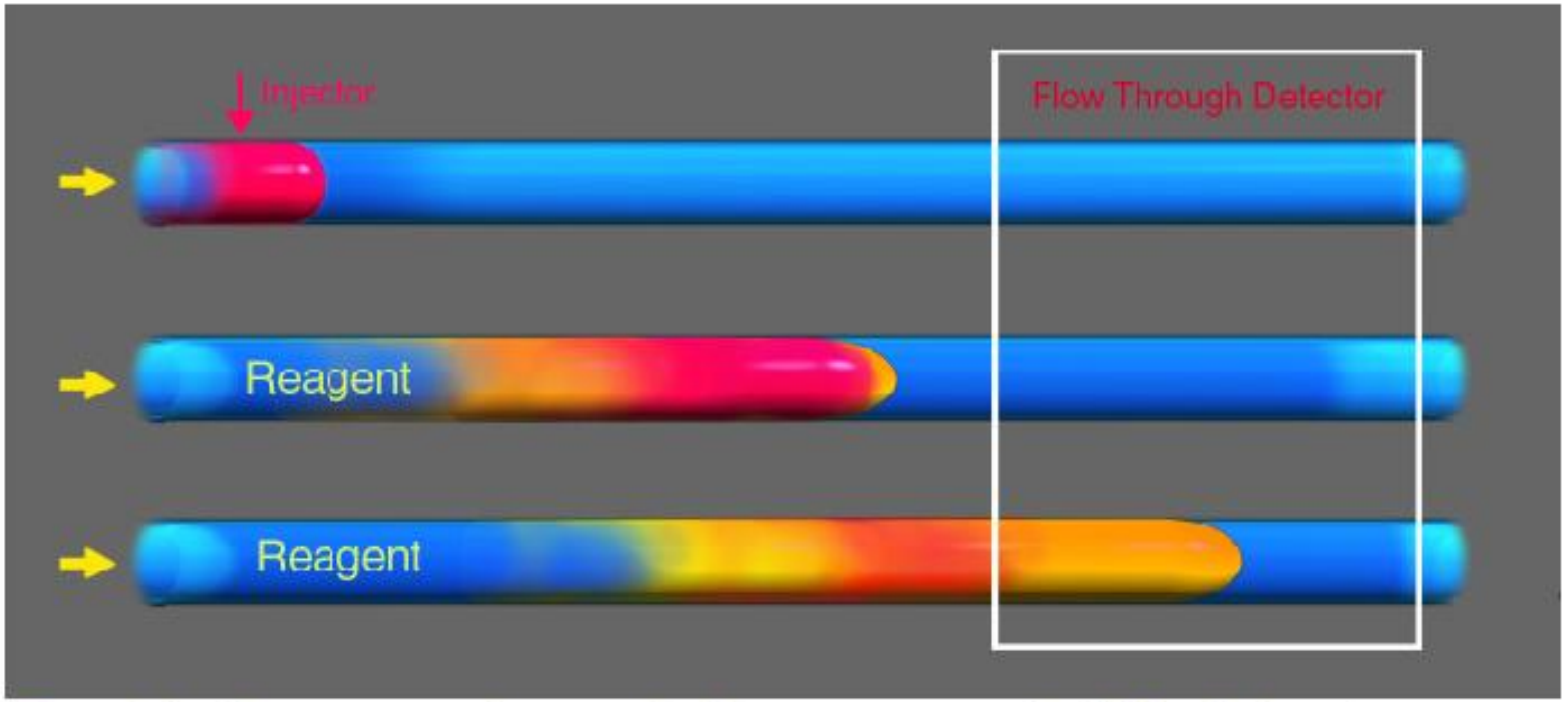
1.2.5.

DISPERSION and REACTION



Flow Injection response is a result of two processes, both kinetic in nature: the physical process of dispersion of the sample zone and the chemical process of formation of a detectable species. These two processes occur simultaneously and they yield , together with the dynamic characteristics of the detector the FI response curve. Note that the reaction product (yellow), is gradually formed at the interface between the sample zone (red) and carrier stream of reagent (blue).

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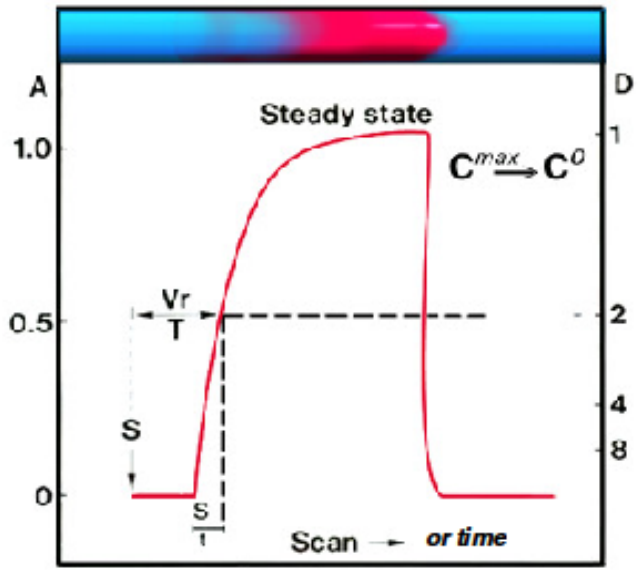
1.2.8.

LIMITED DISPERSION

When the distance between injector and detector is minimized in a single stream FI system, injection of a sufficiently large sample volume will produce a "square" peak that will have a horizontal "steady state" section with C max concentration situated at its top.
Systems with limited dispersion are designed for automation of all reagent based assays.



- *conductivity measurement*
- *for direct sample injection in high sensitivity ICP and AA based assays*
- *for bioligand interaction studies by surface plasmon resonance (BIA)*
- *for functional receptor binding assays on live cell for drug discovery.*
- *pH measurement*

Note that in a multistream system, D value is always > 2 as the flow rates of at confluence points have to be taken into account.

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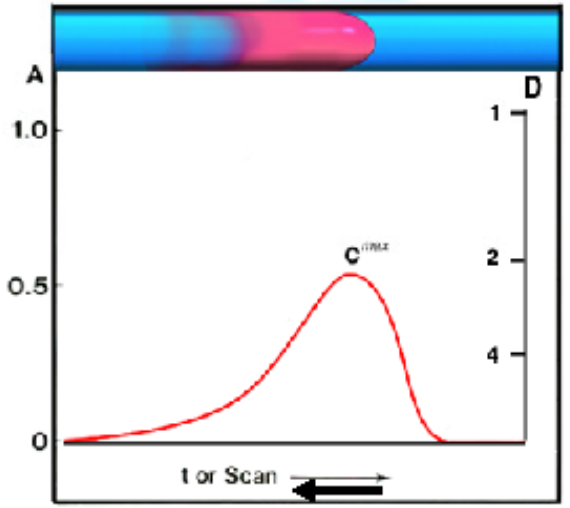
1.2.9.

MEDIUM DISPERSION



By adjusting the volume of injected sample, of the volume of reactors between injector and flow cell and of flow rates in single or multistream manifolds, dispersion can be adjusted to a medium value. Resulting concentration gradient will have a form of a smooth peak that will be only slightly skewed.

Systems with medium dispersion are designed for automation of all reagent based assays. Note that in order to reach high sensitivity of a colorimetric assays:

- *sample volume should be maximized*
- *reagents should be added by reagent streams via confluence points*
- *long path flow cell should be used*
- *dispersion coefficient should be adjusted to between 2 and 5*

NOTE: Sensitivity and detection limit of reagent based assays can be further enhanced by Bead Injection Technique .

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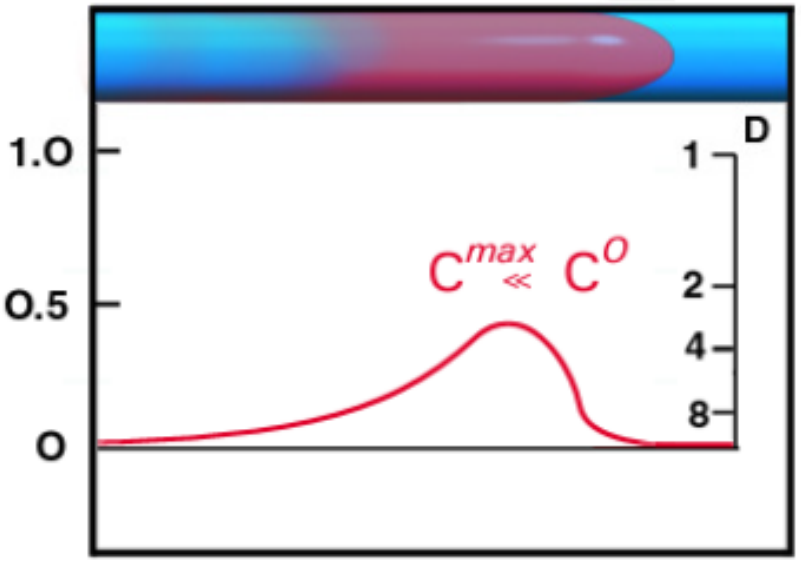
LARGE DISPERSION

By decreasing volume of injected sample, and by increasing the volume of conduits between injector and flow cell, dispersion can be increased to a large value. Resulting concentration gradient will have a form of a smooth long peak that will have an exponentially decreasing tailing edge. In order to obtain very large D values a **mixing chamber** should be integrated into flow manifold.



If the volume of a mixing chamber dominates the volume of the flow channel the resulting concentration gradient will have a exponentially decreasing trailing edge if the system is operated t continuous constant flow rate. Systems with large dispersion are used for process control monitoring when extensive sample dilution is required and for automated titrations.

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INSTURMENTATION

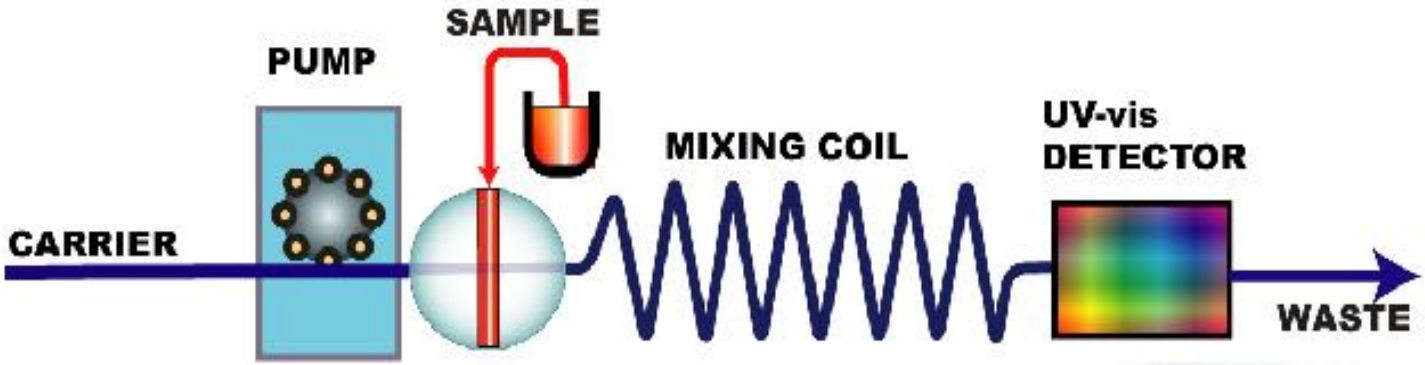
Instrumentation of FIA

- **Injection valve,(sample injector)**
- **peristaltic pump,**
- **Coiled reactor,**
- **Tubing manifold**
- **Detector**
- **Auto sampler**

Additional components may include a flow through heater to increase the speed of chemical reactions, columns for sample reduction, de bubblers, and filters for particulate removal.

1.1.1

BASIC SETUP



SINGLE STREAM MANIFOLD

SIMPLEST , MANUALLY OPERATED SYSTEM
IS COMPRIZED OF:

- peristaltic pump
- manually operated two position injection valve
- manifold of connectors tubing and reactors
- flow through detector

Basic FI instrument furnished with a tungsten light source and spectrophotometer, is well suited as a training tool in an undergraduate laboratory or for assay of small sample series.

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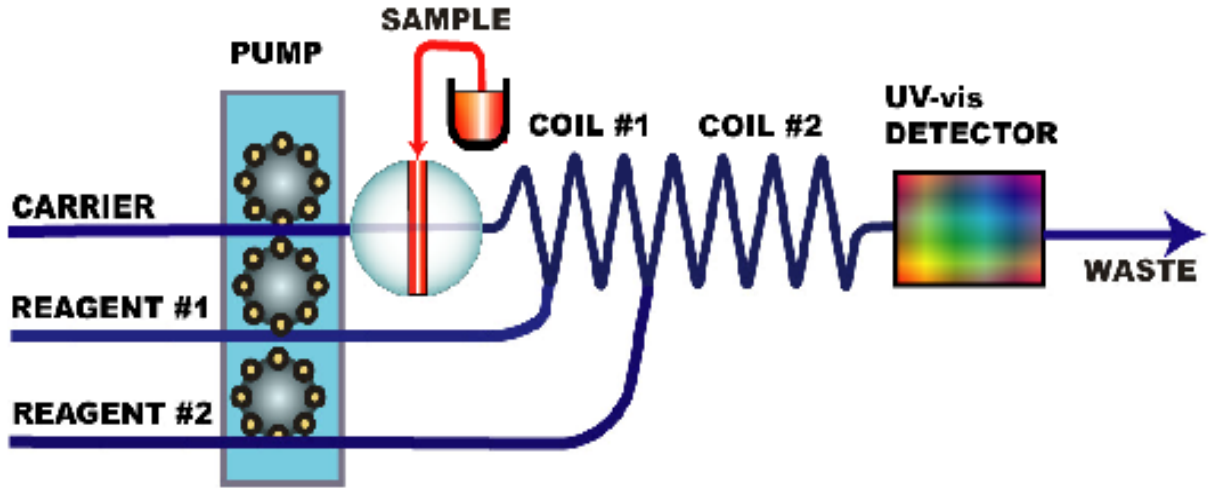
1.3.2.

MULTISTREAM FI SYSTEMS



A majority of FI assays are carried at continuous flow, when carried and reagents are pumped simultaneously at a constant flow rate. Sample is injected into carrier stream of water (or appropriate buffer) while reagent streams are added at confluence points. Advantages of this approach are:

- *even addition of reagents to entire sample zone length*
- *steady baseline*
- *minimized carryover*
- *simplicity of operation and transparency to user.*

The main drawback of FI is continuous reagent consumption and waste generation

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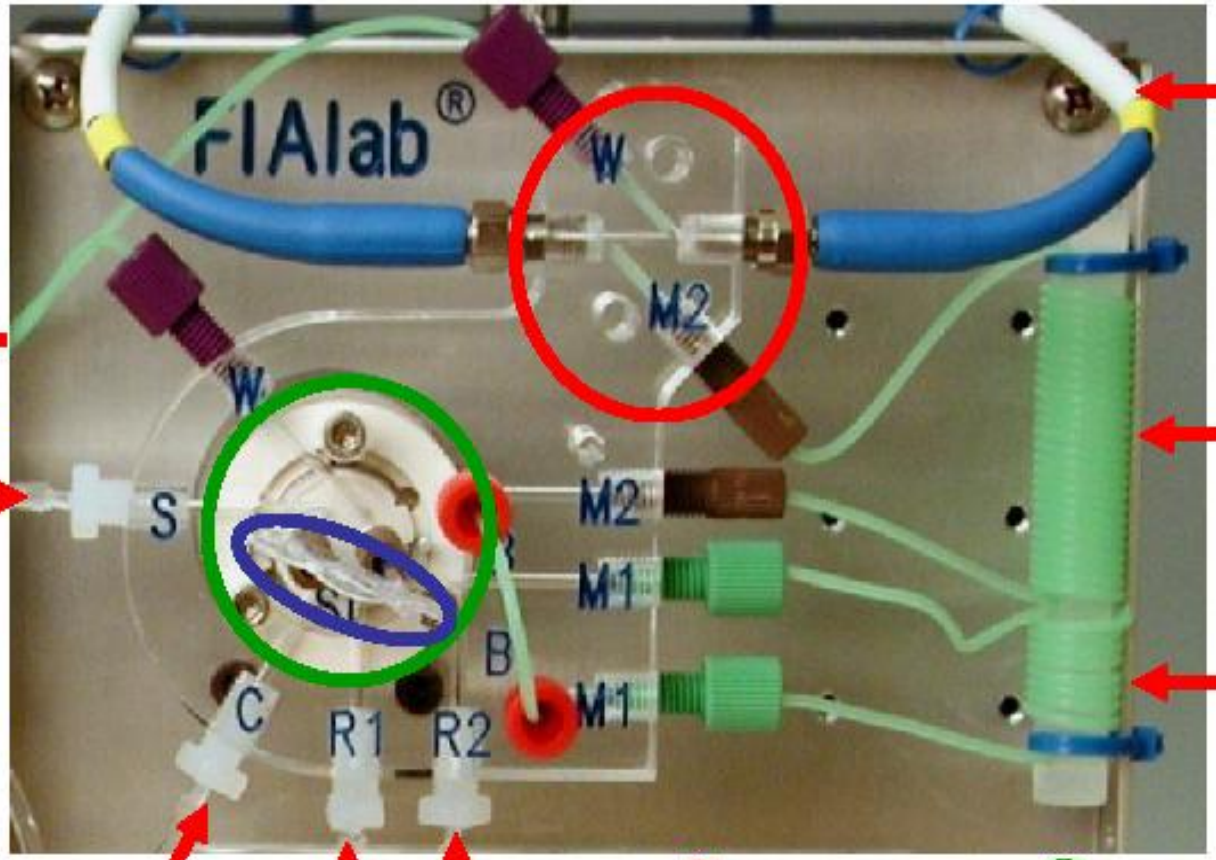
1.1.6

FI in LAB-ON-VALVE CONFIGURATION



WASTE

SAMPLE

SAMPLE LOOP

OPTICAL FIBER

MIXING COIL #2

MIXING COIL #1

CARRIER

REAGENTS

FLOW CELL

INJECTION VALVE

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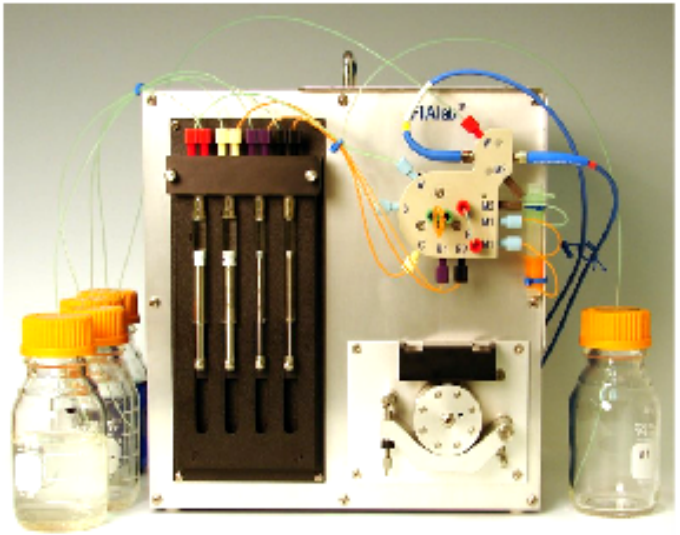
1.4.4.

microFI-LOV INSTRUMENT

Replacing peristaltic pump with four channel syringe pump is a logical extension of FI-LOV instrument development. Advantages of using syringe pumps for FI applications have been recognized by Japanese researchers long time ago (Yoza 1977) and the use of Multisyringe Flow Injection Systems (MSFIA) has been proposed in numerous publications (Cerda 1999). However, use of peristaltic pumps for FI applications is deeply entrenched, and it is likely to prevail in routine laboratories, because of cost, convenience of operation, and ease of replacement of peristaltic tubing. Yet, an instrument build around individually driven syringe pumps combined with solvent resistant LOV module has following advantages:

- *resistance to corrosive chemicals*
- *precise control of liquid delivery and manipulation*
- *capability of programmable flow, including stop flow FI for reaction rate measurement.*



The main drawback of using multiple syringes is mechanical complexity, as compared to the conventional FI system. Also microSI instrument, is far less complex as it operates with a only a single pump and a single valve. Indeed, unless all four pumps will be run in a fully synchronized and automatically cycled mode, the flow programming of this novel instrument configuration will be a challenging task.

Yoza N., Ishibashi K., Ohashi S. J. Chromatography134, 497 (1977)

Cerda V. et. al. Talanta 50, 695 (1999) **Miro M., Estela J.M., Cerda V., TRAC 21, 199 (2002)**

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OPTIMIZATION PARAMETERS

Sample Volume

Increase injection of sample volume increase the peak height until to the steady state is reached. In single stream system peak height is increase linearly with the injected sample volume.

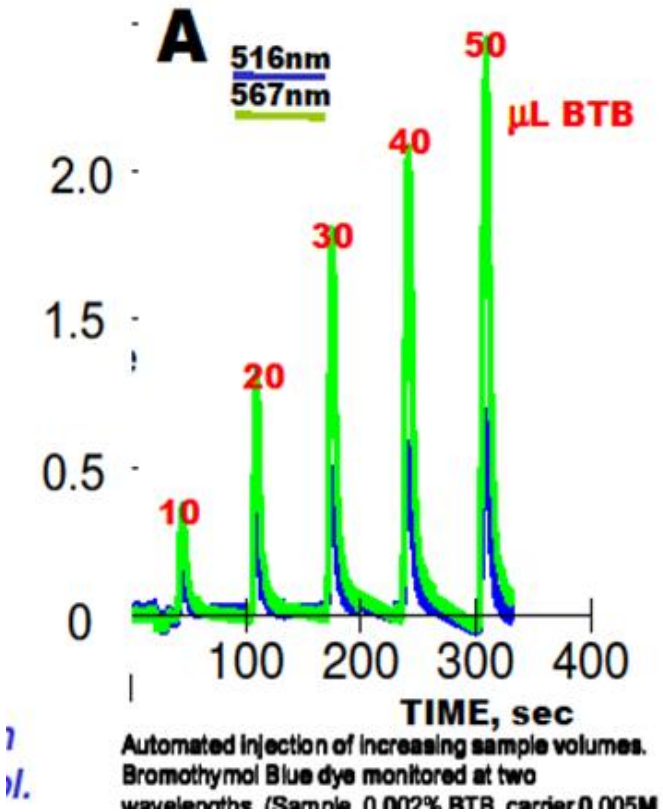
The steady state depends on volume geometry & flow rate between injection and flow cell.
For conventional FIA system this value is around 50µL & for the micro system its as low as 5µL.

Sample Volume & peak Height

•Volume of sample solution injected in to conventional FI system is accomplished by manually changing the volume of sample loop.

•Selection of sensitivity & detection limit of detector.

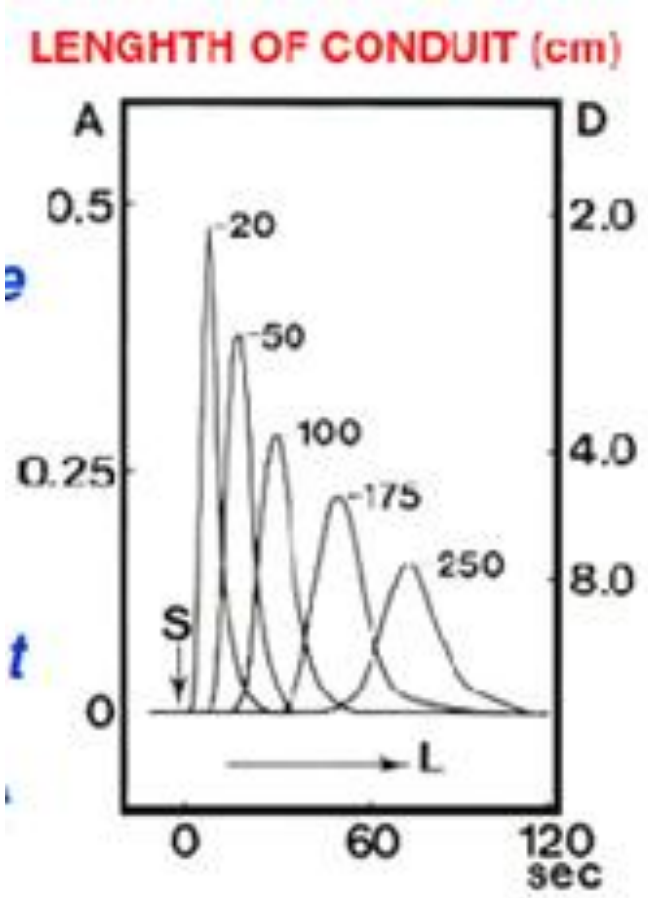
•Identification of linear range of detector.
•Automated dilution of sample material.



Automated injection of increasing sample volumes. Bromothymol Blue dye monitored at two wavelengths (Sample 0.002% BTB, carrier 0.005M

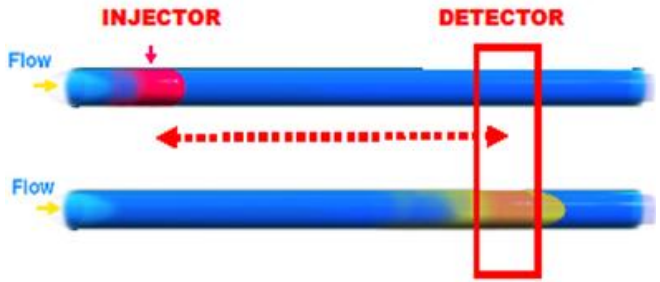
Channel Length

Increasing length of channel decreases peak height while peak shape undergoes a change from symmetrical shape.
At the same time the resident time of the peak maximum increases with the distance travelled and the peak broadens.
This is the principal limitation of FI based on constant flow rate limits the incubation time for chemical reaction to about 20 seconds, with total length of conduit of 250cm and combined flow rate around 3mL/min.

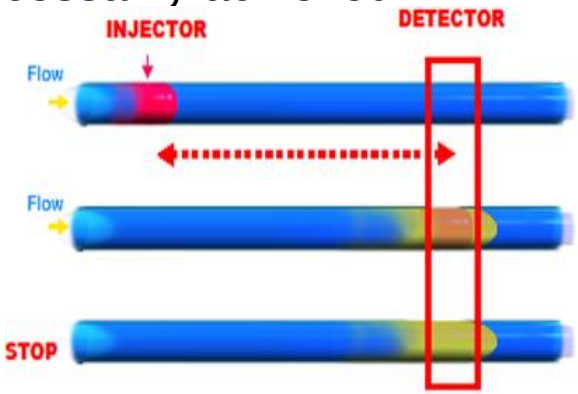


Residence Time

All chemical reactions are time dependent.
In this format reaction equilibrium is not necessarily achieved.



At continuous flow the time interval available for chemical reaction to take place is defined by linear flow velocity and is limited by length of conduit between point of injection and detector.



Stop flow allows longer reaction time with penalty of dilution, thus yielding higher sensitivity.
It saves reagent and generates less waste than continuous pumping.

INSTRUMENTATION AND WORKING

- **The modern Flow Injection Analysis system usually consists of:**

- · An injection valve,
- · A high quality multi channel peristaltic pump,
- · A coiled reactor,
- · A tubing manifold
- · A detector
- · An auto sampler

Additional components may include a flow through heater to increase the speed of chemical reactions, columns for sample reduction, de bubblers, and filters for particulate removal.

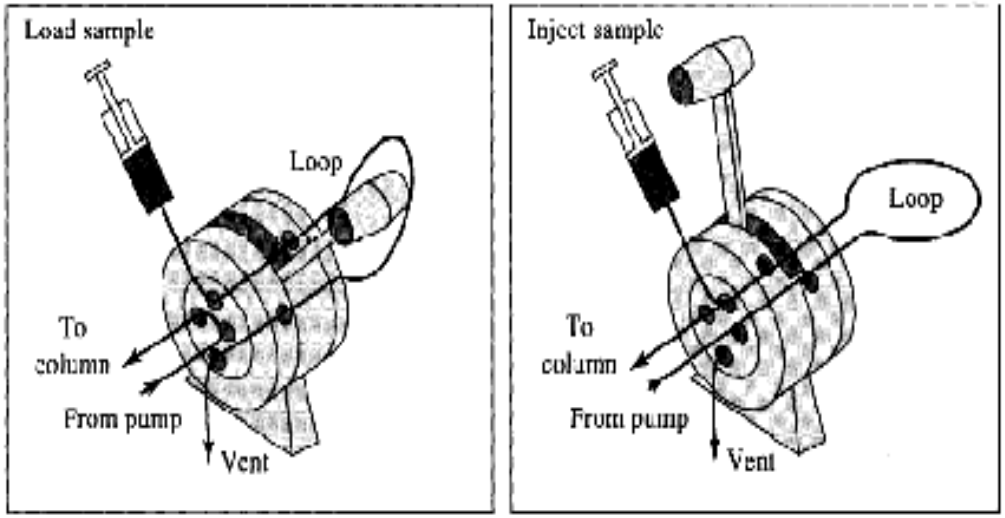


Fig 4: An injection valve

Sample Introduction

Injection valve

Important features of valves suitable for FIA:

- High precision,
- Fast switching,
- Pressure limits of about 100 psi,
- Ability to inject sample volumes from a few micro litres to several hundred micro litres,

and in some cases fractions of a micro litre.

PUMPS

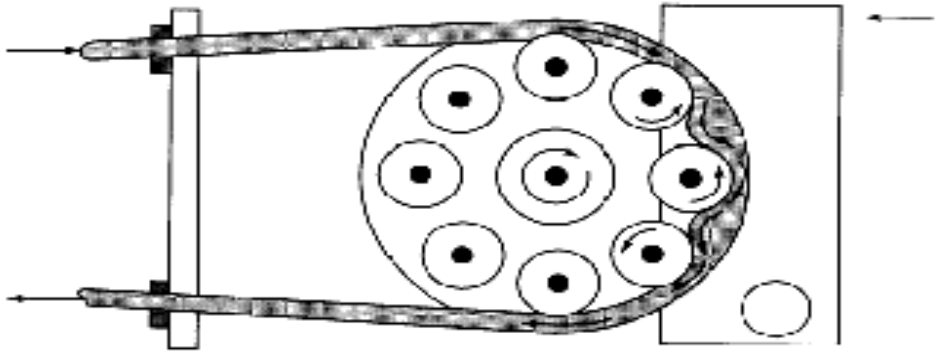


Fig 5: Peristaltic pump

(I) Variable speed peristaltic pump

(ii) Flow rate (0.0005 to 40 ml/min) controlled by pump speed and tube id

(iii) The pump is used to propel one or more streams through the Detector via narrow bore (0.5 –0.8 mm ID) tubing. These streams may be reagents, solvents, or some other medium such as a buffer. This design leads to a flow that is relatively pulse free. The flow rate is controlled by speed of motor, speed is >30 rpm and the inside diameter of the tubing.

1.4.6. PUMPS

Peristaltic pumps are still the most frequently used drives for FI systems, since they generate continuous flow in any desired number of parallel channels. while the flow rates can be easily adjusted by rotation rate and I.D of peristaltic tubing. It is important to use a pump furnished with at least eight rollers, in order to generate a flow with small **regular** pulses – as otherwise resulting irregular flow rate will affect dispersion and repeatability of assay. Contributing factor to popularity of peristaltic pumping is its apparently low cost, although cost of peristaltic tubing exceeds many times the price of a pump over its lifetime. The largest drawback of peristaltic pumping is due to elasticity of peristaltic tubing as the flow rates gradually change as the tubing is stretched out, requiring frequent recalibration of the analyzer.

Stepper motor driven syringe pumps generate highly reproducible flow that can be computer controlled in a programmable way. They cover a very wide range of flow rates as the piston speed and syringe size can be varied. They are durable and chemically resistant, their only drawbacks being cost and inability to generate continuous flow beyond the capacity of the syringe – that has to be refilled.

Solenoid activated micro pumps generate flow by delivering well defined pulses the frequency and volume of which controls the flow rate. A typical FI pulsed flow system **(Rangel 2005)** used 8μL pulses in three stream, three pump system generating flow between 0.48 to 1.92mL/min., depending on pulsing frequency (60 to 240 pulses/min). The weakness of this truly innovative approach is durability of these pumps that must generate about 300.000 pulses/day while exposed to aggressive chemicals.

Solenoid Pump.

Santos J.L.M, Clausse, A.,Lima J.L.F.C., Saraiava M.L.M.F.S.,Rangel A.O.S., Analyt. Sci. 21, 461 (2005)

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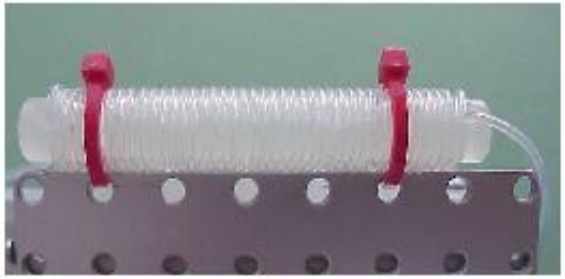
1.4.7. CONNECTORS and REACTORS

While I.D. of 0.5mm to 0.8mm is typical for majority of FI and SI systems, there is a wide variety of tubing materials available for constructing reactor coils and connection lines. Teflon and Peek are the most frequently used polymers. Stainless steel is yet another material that has advantage of heat conductivity gas impermeability and surface properties that minimize protein adsorption. A majority of polymer made tubing is transparent and often available
color coded, so that tubing I.D. can be identified at glance.
Connectors made of colored coded polymers are fitted with ferrules that are designed to grip tubing while the connector nut is being tightened. Since all FIA systems operate at a low pressure, there is not necessary to use connectors designed for HPLC. It is, however very important to use nuts, ferrules and fitting from a single manufacturer as products from different sources are often incompatible, resulting in a leak.

Tubing connectors, ferrule and T-connector



Teflon made reactor coil .

Heated reactor coil with temperature controller

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DETECTORS

- Detection is most frequently photometric (UV/VIS and more recently IR).
- In the field of life sciences, different luminescence techniques are gaining popularity.
- Electrochemical techniques such as amperometry, and potentiometer, have gained new life by coupling them to flow-based sample handling techniques such as FIA.
- Even AAS, ICP-MS and ICP-AES, and even GC have been coupled to FIA manifolds.

Factors Affecting Calibration Standards in FIA

concentration in a sample is usually estimated from the peak heights, or less frequently peak areas, by using calibration curves prepared from standards.

If the refractive index of the sample slug is different from that of the carrier solution, then, because of the parabolic shape adopted during flow, the slug will act as a lens. This liquid lens can cause convergence or divergence of the light from the light source. This effect can be maximized, and has been used in the determination of re fractive index (67). For most clinical chemistry analyses this effect is of little significance, being only a small fraction of the total absorbance, but for analysis of samples producing a low absorbance (e.g., <0.05), it may present a problem.

In such cases the refractive index of the carrier solution should be

similar to that of the samples and standards.

The temperature of the carrier, standards, and sample should also be the same,

the rate of reaction and the refractive index both being temperature dependent.

The effect of refractive index can also

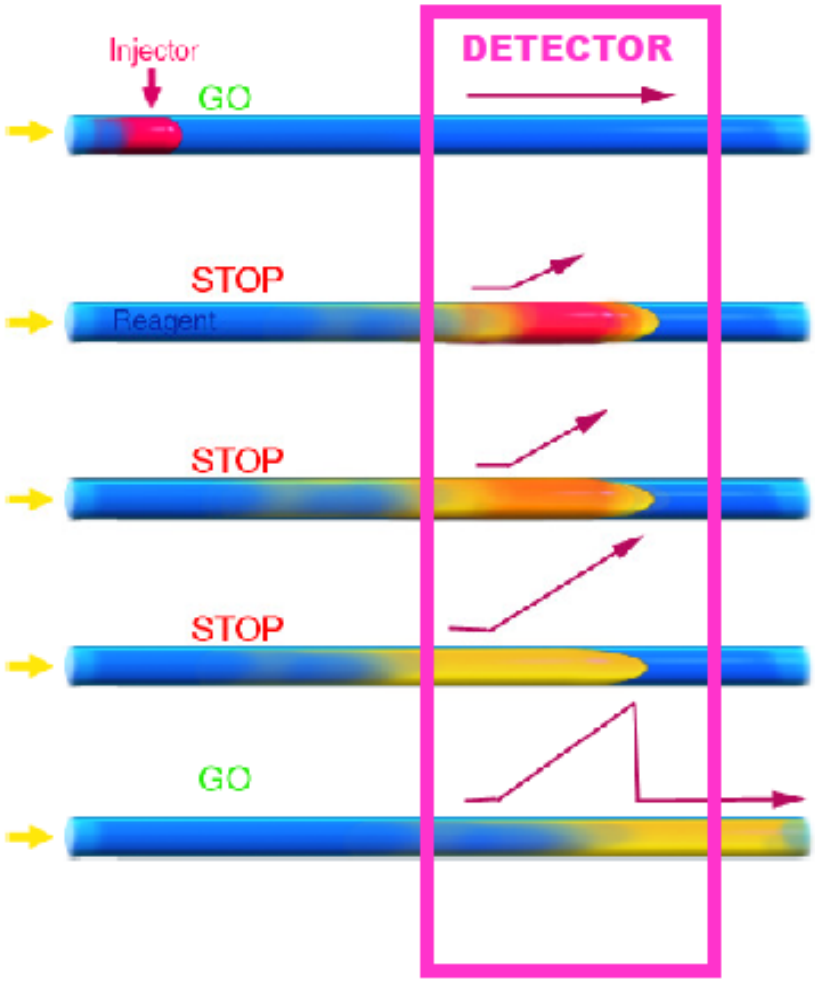
be minimized by diluting samples and using appropriate flow-cell design. The higher the viscosity of a particular sample, the lower its dispersion therefore, a more viscous sample zone will be less effectively mixed with the carrier stream. Often the viscosity of serum samples is decreased before analysis by dilution, so that the differences in viscosities

between sera and aqueous standards do not influence the analytical results;

REFINEMENTS TO BASIC FLOW INJECTION ANALYSIS

1.2.15.

STOP FLOW



The stop flow mode is based on arresting a selected portion of the sample zone in the detector. Provided that the reaction did not reach equilibrium while the zone was on the way to detector, reaction rate curve will be recorded while the reaction product (yellow) is being formed in the detector.

Next, flow is resumed and reacted sample zone is flushed out of the detector, while the baseline is restored.

While stop flow technique has been used in FI format, it is difficult to carry out reproducibly when peristaltic pumps are used propel carrier and reagent streams. Syringe driven systems either FI or SI are reliable and their use in stopped flow mode is highly recommended.

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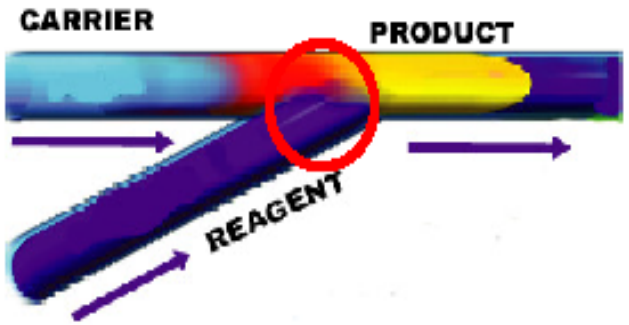
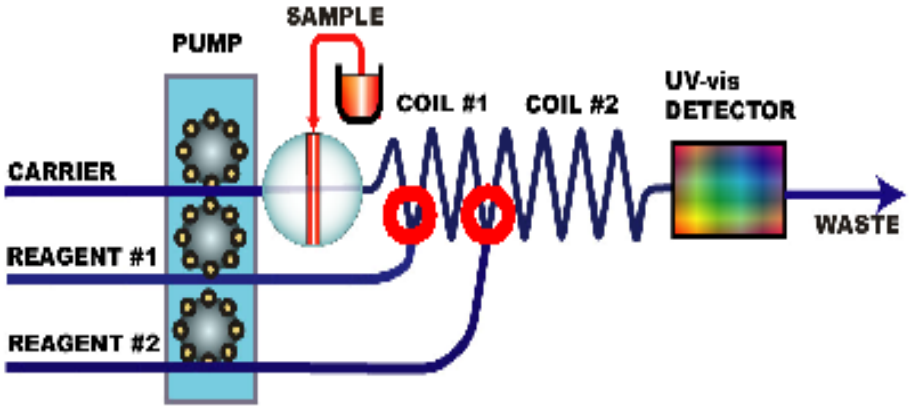
1.3.3.

MIXING @ CONFLUENCE POINT



Merging of reagent and carrier stream



Two reagents, three stream FI system

Almost all FI instruments employ multichannel peristaltic pumps to move carrier and reagent (R1,R2) streams that merge at confluence points (O) where reagent merges with sample zone. Sample is injected by means of a two position injection valve with a fixed injection loop. The valve is furnished with a bypass (not shown) that allows carrier solution to pass through the valve, while the sample is being filled into the loop. The pump moves solutions continuously in forward direction, thus providing a repeatable time frame for samples and standards as they are serially injected. In this way all samples and standards are processed in exactly the same way and the standards yield a readout used for construction of a calibration curve.

Separation In FIA

Separation in FIA is done by,

1. Dialysis
2. Liquid/liquid extraction,
3. By gaseous diffusion

Dialysis

✓**Dialysis is a simple process in which small solutes diffuse from a high concentration solution to a low concentration solution across a semi permeable membrane until equilibrium is reached**

✓**Since the porous membrane selectively allows smaller solutes to pass while retaining larger species, dialysis can effectively be used as a separation process based on size**

Dialysis cont…

Dialysis is often used continuous-flow methods to separate inorganic ions, such as chloride or sodium or small organic molecules, such as glucose, from high-molecular-weight species such as proteins.

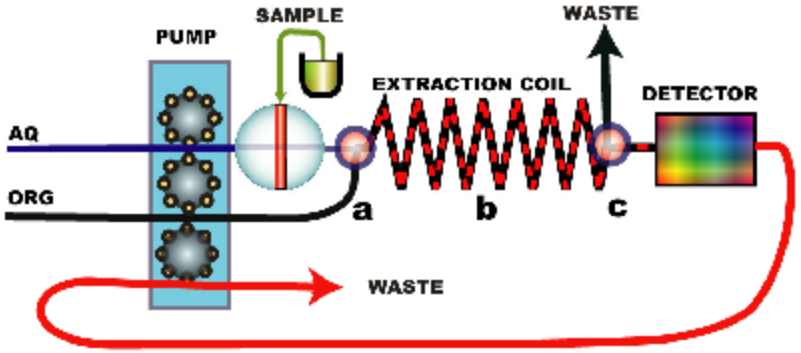
Extraction

extraction, is technique used to ***separate analytes from interferences in the sample matrix by partitioning the analytes between two immiscible liquid.***

Extraction cont…

1.3.11.

SOLVENT EXTRACTION



Two stream manifold for automated solvent extraction. Sample (S) is injected into a moving carrier stream of water (AQ), which is merged (a) with an organic phase (ORG) and pumped through a Teflon made extraction coil (b). In separator (c) the aqueous phase is discarded into waste, while organic phase is led into a flow cell. Detail showing circulation of extracted dye within segment of organic phase **(Nord & Karlberg 1984),** *as it moves through a Teflon tubing, provides clue to mechanism of hydrodynamics of solvent extraction.*

This method, applicable to assay of hormones, pharmaceuticals and numerous hydrophobic compounds, **(Karlberg & Thelander 1978),** *revolutionized solvent extraction technique, that up to that time was mostly carried manually. Miniaturization and automation of solvent extraction minimizes exposure to harmful solvents and reduces consumption of reagents and generation of hazardous waste.*

B. Karlberg & S.Thelander, Anal. Chim. Acta 98, 1 (1978) L. Nord & B. Karlberg, Anal. Chim. Acta, 164, 233 (1984)

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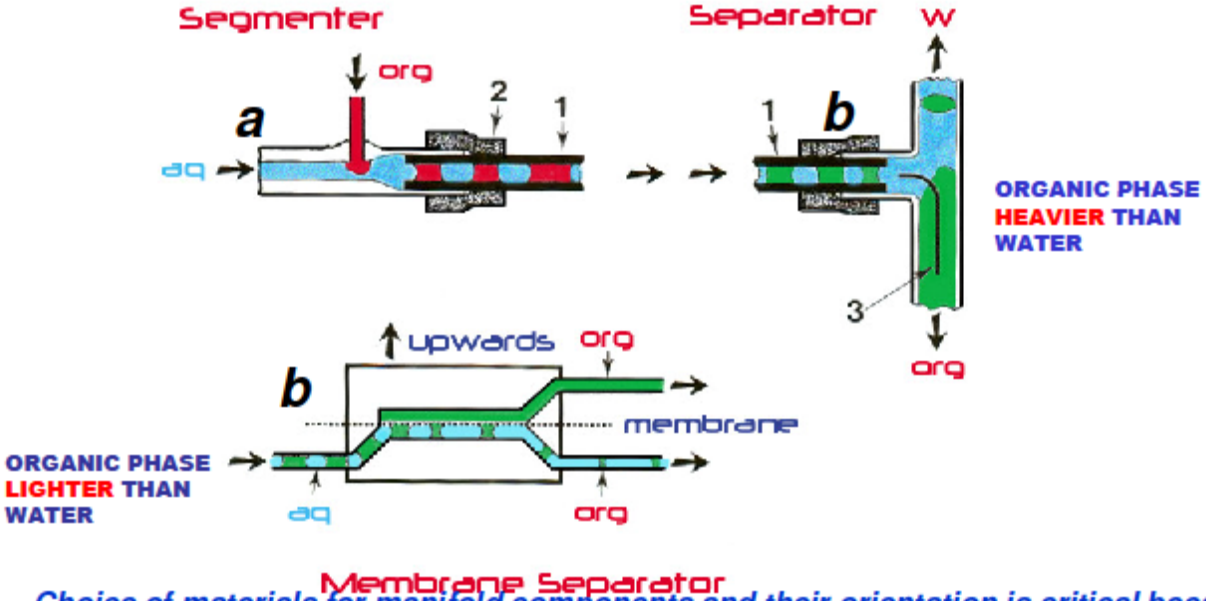
Extraction cont…

1.3.12.

SOLVENT EXTRACTION



Membrane Separator

Choice of materials for manifold components and their orientation is critical because aqueous phase (aq) adheres to glass, while organic phase adheres to Teflon.

In segmentor organic phase enters through a glass fitting and adheres to Teflon tubing (1). In separator a thin Teflon strip (3) serves to guide organic phase through a glass made T piece. In the membrane separator Teflon made membrane allows only the organic phase to penetrate through hydrophobic pores, while aqueous phase is discarded.

Karlberg B. Pacey C.E.: Flow Injection Analysis, A Practical Guide, Elsevier, Amsterdam, 1989.

Fang Z-L.:, Flow-Injection Separation and Preconcentration, VCH Verlagsgesellschaft Weinheim, 1993.

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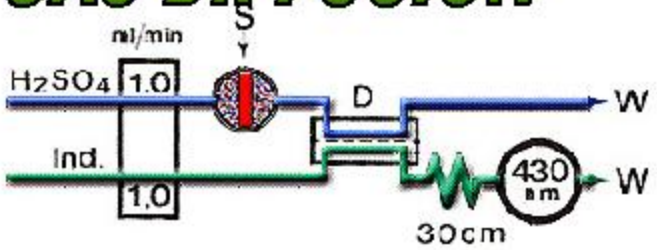
1.3.9.

GAS DIFFUSION



In a two stream FI system, sample containing carbonate (or dissolved carbon dioxide) is acidified, releasing carbon dioxide, that diffuses across a silicone rubber made membrane from a donor (blue) to an acceptor (green) stream changing color of an acidobasic indicator, monitored at 430nm **(Baadenhuijsen 1979).** *Membranes made of Teflon are hydrophobic, with up to 50%porosity, forming an air gap between carrier and donor stream through which gases like ammonia, sulphur dioxide, chlorine, ozone or volatile compounds rapidly permeate into an acceptor stream where they are detected by means of a suitable reagent. Flat plate diffusers, (as the one shown above) are easy to assemble. The drawback hydrophobic membranes is that they can be fouled by surfactants that destroy the air gap barrier. When miniaturized and integrated with a fiber optic detector, placed into acceptor channel, a "sandwich cell" construction allows increase of sensitivity of an assay.*

Another, innovative approach to gas separation is gas pervaporation, that offers a robust alternative to gas diffusion in parallel plate diffuser **(Castro 1998)**

H. Baadenhijsen & H.E.H. Seuren-Jacobs, Clin. Chem. 25, 443, (1979)

•M. D. L. de Castro & I. Papaefstathiou, TRAC, 17, 41, (1998)

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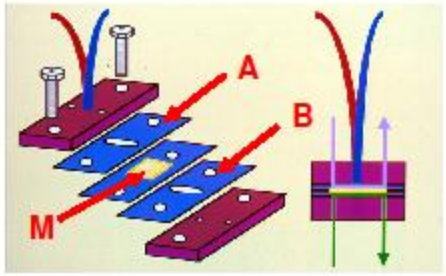
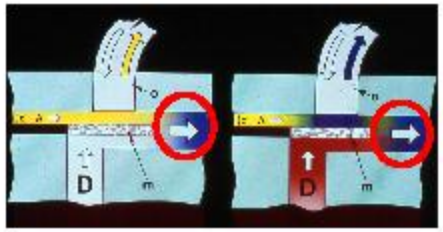
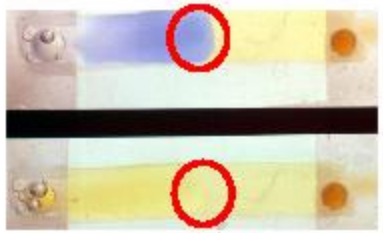
1.3.10.

GAS DIFFUSION in SANDWICH CELL

This flow cell design uses a bifurcated optical cable to illuminate a white surface and to collect reflected light as it passed twice through the monitored aqueous layer. This flow cell can be used to monitor either a single , liquid stream, or if furnished with a gas permeable membrane, (M) mounted between two spacers (A,B) it is useful to monitor volatile species emanating from a donor stream. Note that Teflon membrane may be furnished with an opening (O) situated downstream from the fiber, to alleviate pressure differences between acceptor and carrier streams. Note that stopping the flow of acceptor (indicator) stream allows accumulation of analyte and increase of sensitivity of measurement. **(Pavon et. al. 1992)**







J.L.P.Pavon et.al. Anal. Chem. 64, 923 (1992)
C. G. Pinto, M. E. F. Laespada, J. L. P. Pavon and B. M. Cordero Analytical applications of separation techniques through membranes Lab. Autom. Inf. Managem., 34(2) 115-130 (1999)

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It is important that none of the separation procedures in FIA methods is ever complete. The lack of completeness is of no consequence, however, because unknowns and standards are treated an identical way.

Segmented Flow Analysis

Segmented Flow Analysis

Air-segmented flow analysis (SFA) is a method that automates a large number of wet chemical analyses. An SFA analyzer can be thought of as a “conveyor belt” system for wet chemical analysis, in which reagents are added in a “production-line” manner.

SFA was first applied to analysis of sodium and potassium in human serum, with a flame photometer as the detection device, by removing protein interferences with a selectively porous membrane (dialyzer).

❑ ***Principle:*** *A rudimentary system (Figure 4120:1) contains* four basic components:

✓a sampling device,

✓a liquid transport device such as a peristaltic pump,

✓the analytical cartridge where the chemistry takes place

✓the detector to quantify the analyte.

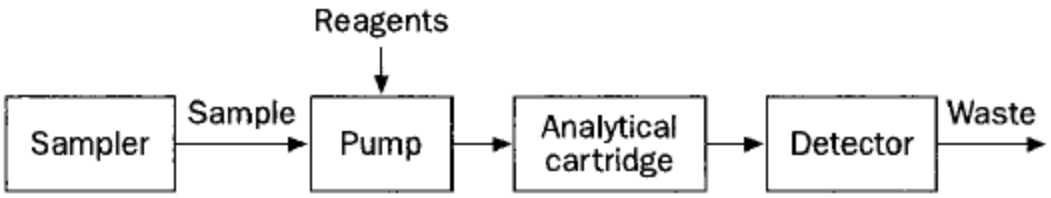


Figure 4120:1. Schematic of a segmented flow analyzer.

Configurations of different FIA systems and of a gas-segmented system

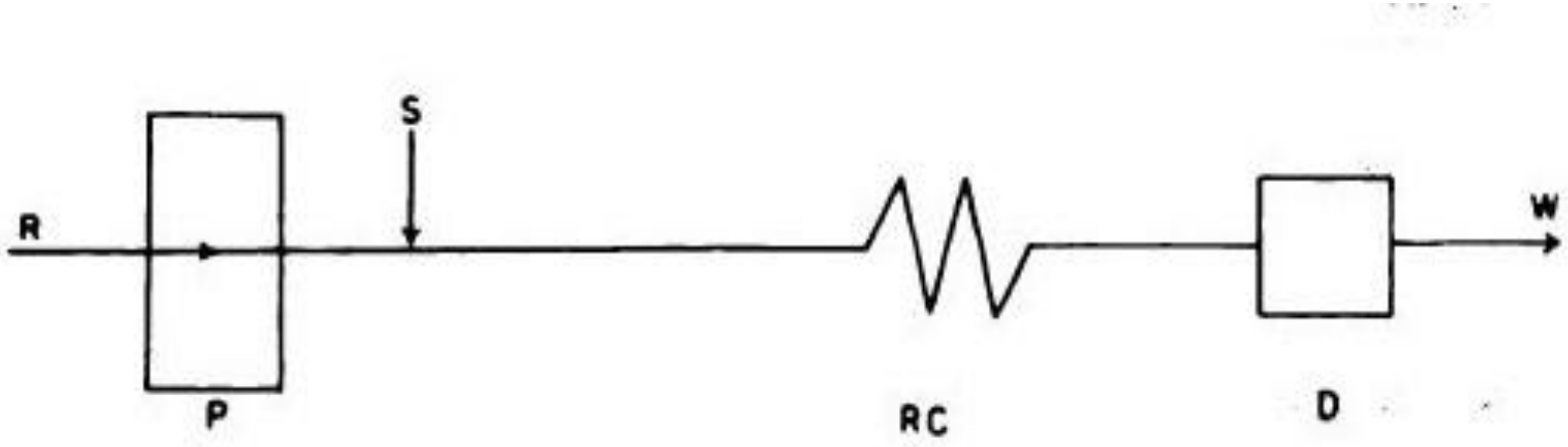


Fig. a: Simple FIA system

sample (S)
reagent (R)
a pump (P)
reaction coil (RC)
detector(D)
waste (W)

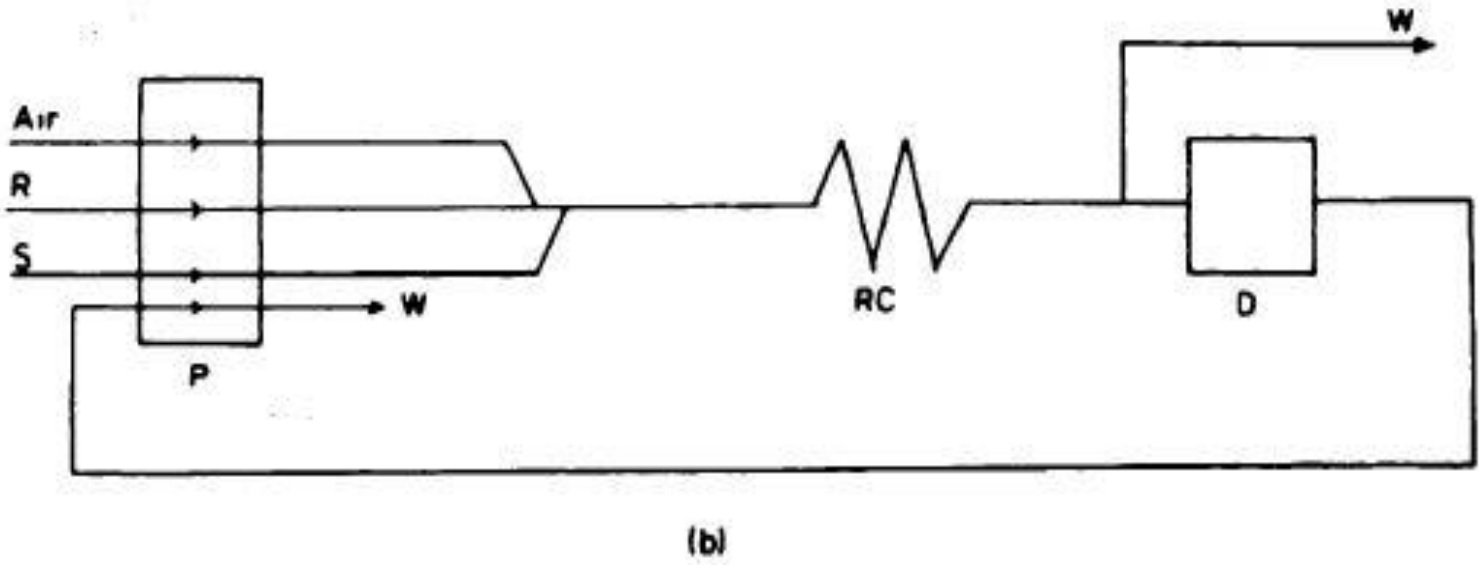


Fig. b: Simple gas-segmented system.

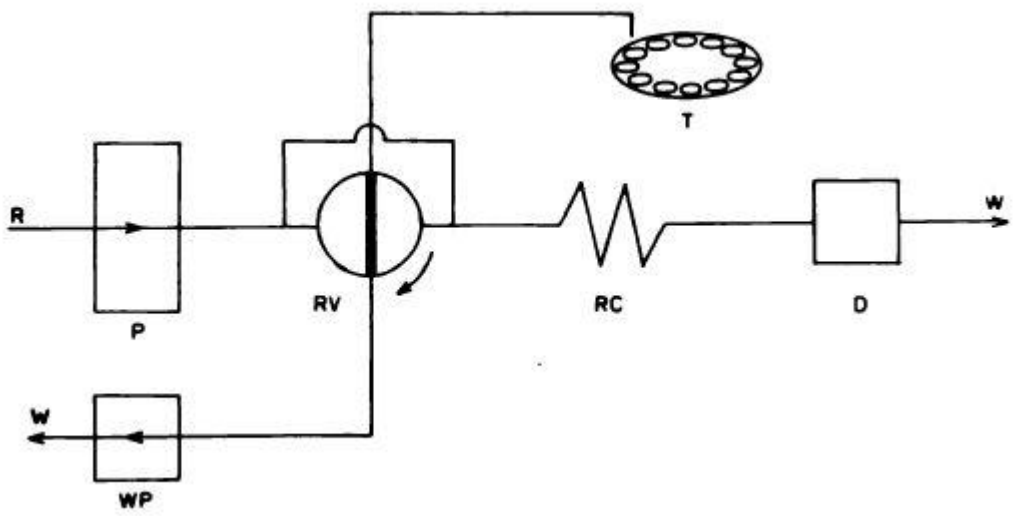
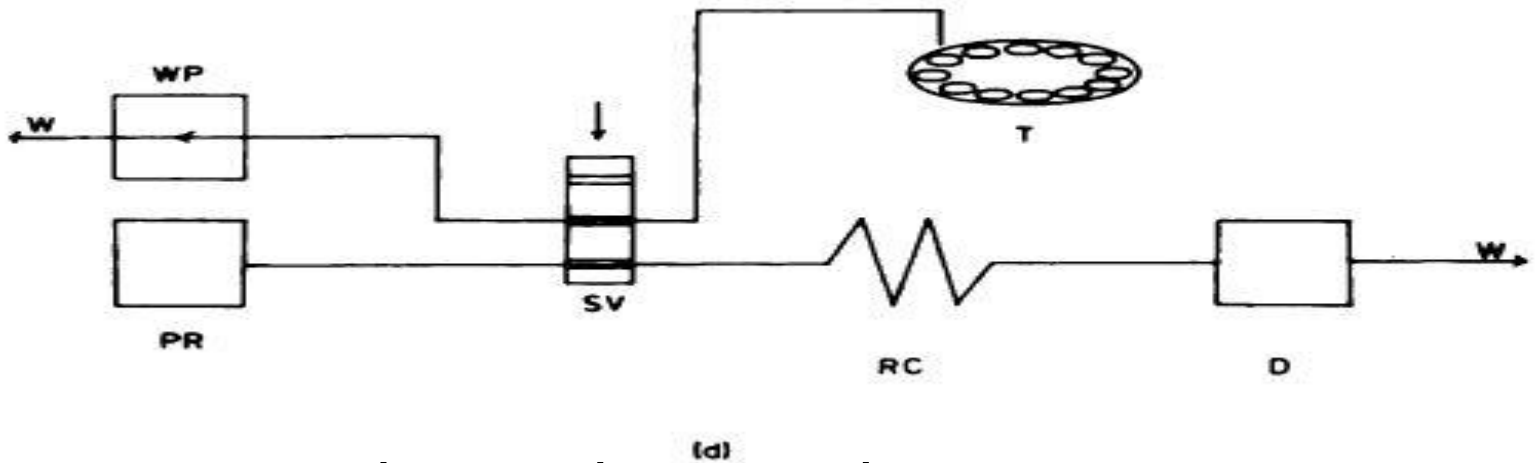


Fig.c: Automated F1Asystem

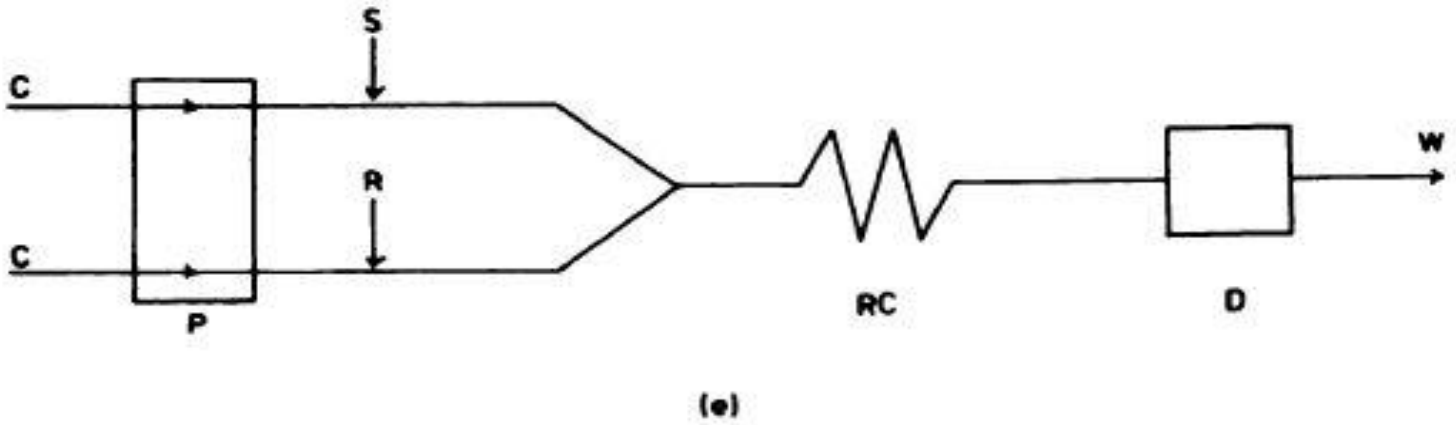
A sample-withdrawal pump (WP) aspirates the sample from the sample changer (T) Into the volumetric cavity of a motorized rotary valve (RV).After a preset time, when the valve cavity has been charged with sample, the valve is automatically rotated Into the inject position *(horizontal) and the sample is swept through the reaction coil by the carrier solution (sample and reagent circuits may also be propelled* by a single peristaltic pump).



(d)

Fig. d: Original automated system

A withdrawal pump (WP) was used to charge a slider valve (SV), shown in the fig c, with sample. When the valve moves downwards, the sample is introduced into the carrier stream. PR is a pressurized reagent reservoir.



Fid. E: Synchronous merging zone FIA

C is an inert carrier solution into which sample and reagent are injected.

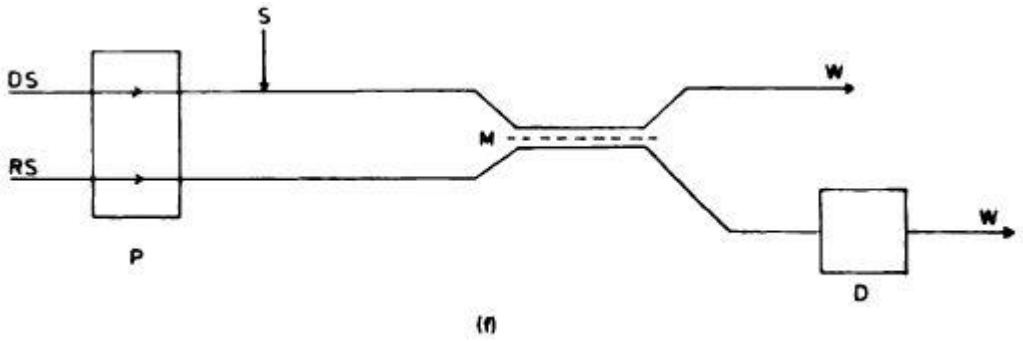


Fig. f: Dialysis manifold

donor stream (DS),
recipient stream (RS),
dialysis membrane (M).

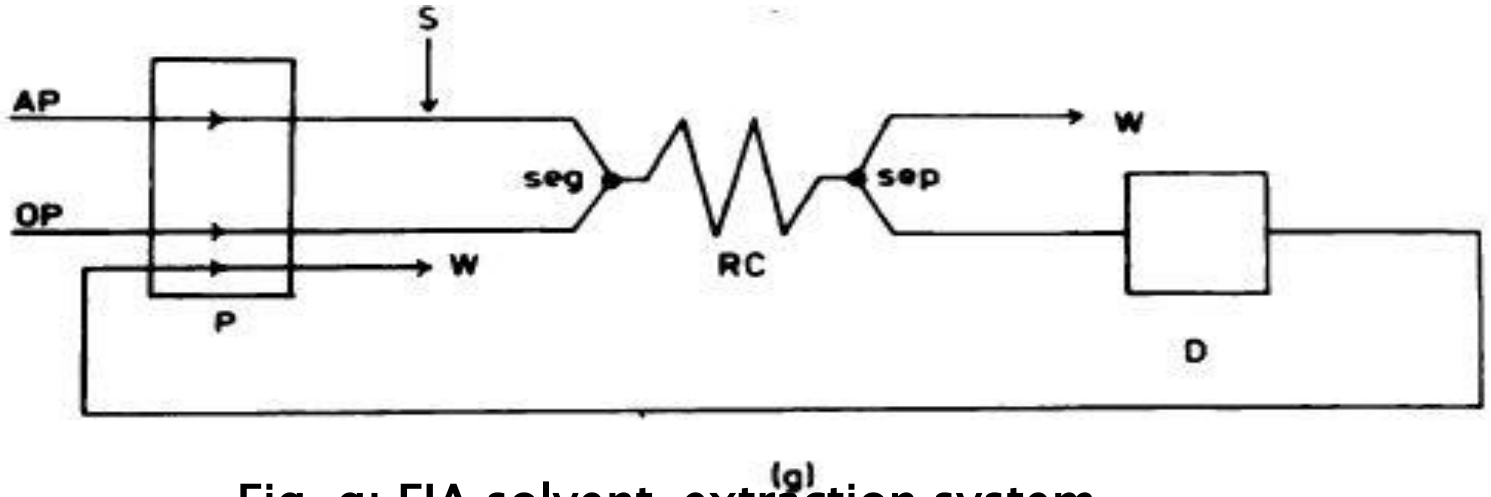


Fig. g: FIA solvent-extraction system

aqueous phase (AP),
organic phase (OP), segmentor
device (seg), phase separator (sap).

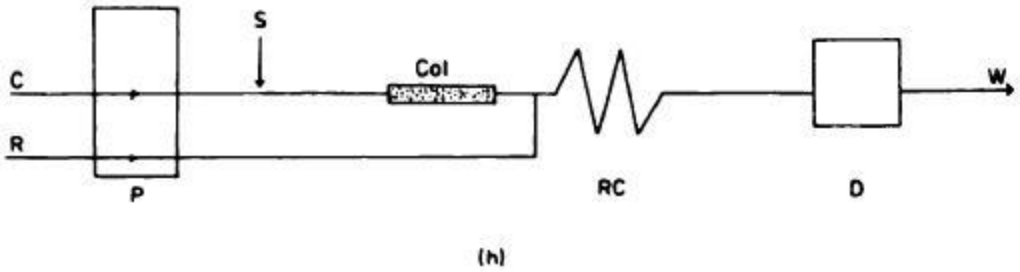


Fig. h: FIA system with a column (Col).

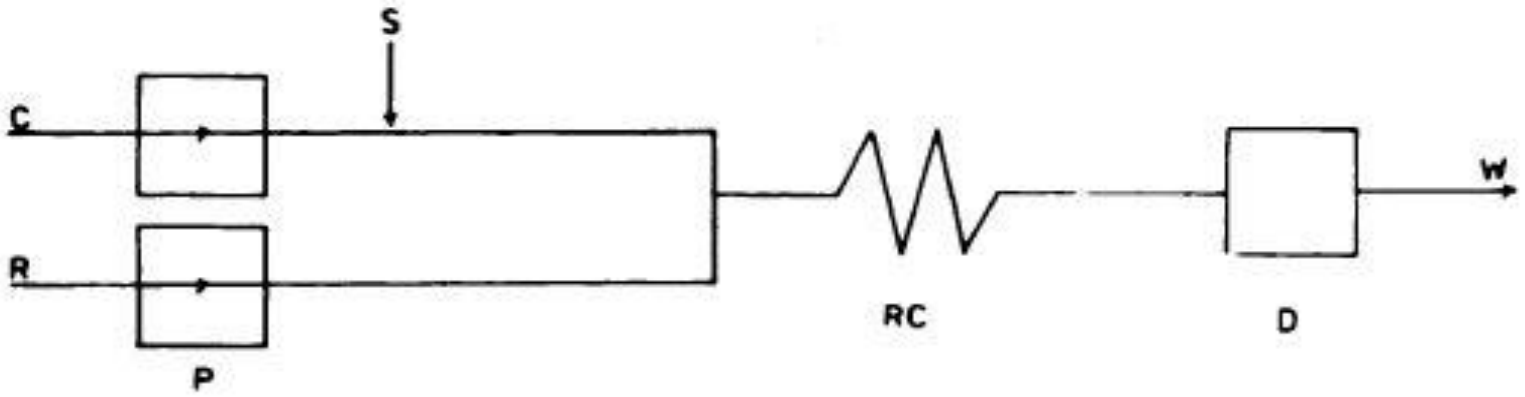


Fig. I: Configuration for automatic dilution of serum samples in inert carrier (C) before they mix with reagent stream (R).

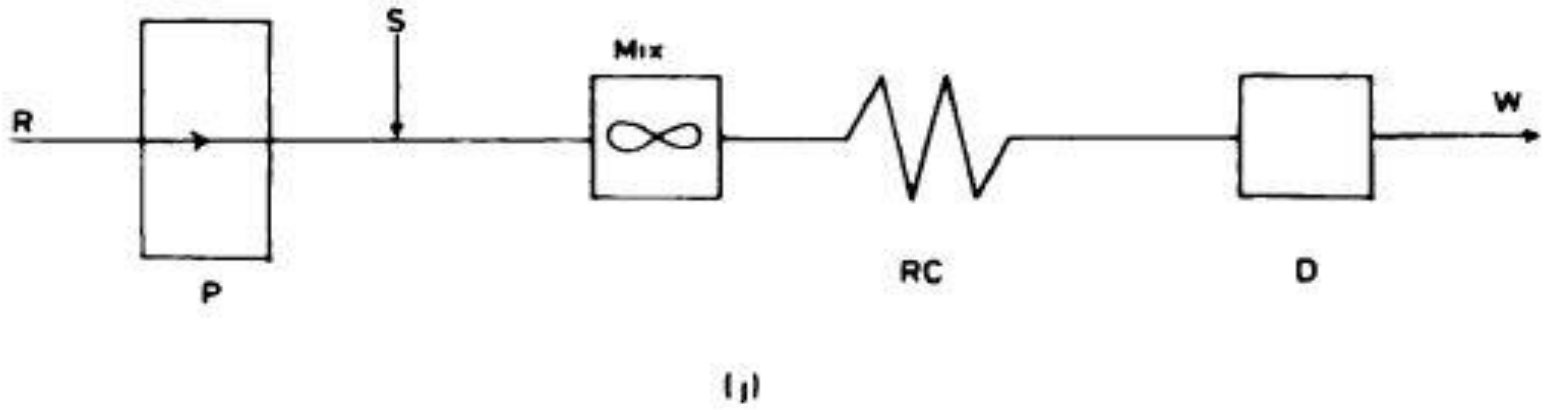


Fig. J: FIA system with mixing chamber (Mix)

Comparison b/w SFA & FIA

SFA	FIA
Start up time: 20 min	20 sec
Reagent stream: Gas-segmented	Non segmented
Reagent consumption: higher	Slow
Manifold: Relatively complicated to allow for introduction and removal of air	Simple
Internal dm: 2 mm	0.5 mm

SFA	*FIA*
Sample Introduction: aspiration	Injection
Sample volume: 200 µL	50 µL
Sampling rate: Typically 60 (150)	150 or greater
Sample mixing: Through turbulent **flow generated by** gas bubble and tube wall friction	through control dispersion system

SFA	*FIA*
Lag phase: significant	Negligible
Steady state: Usually required	Not attained
Readout time: min	Sec
Wash cycle : essential	Not required
Reproducibility: Better than 1%	Better than 1%
Possibility of long Incubation times: suitable	Not as suitable

SFA	*FIA*
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 or peak width

The advantages of segmented flow

- compared to the manual method,
- ✓reduced sample and reagent consumption,
- ✓ improved repeatability,
- ✓minimal operator contact with hazardous materials.
- ✓A typical SFA system can analyze 30 to 120 samples/h.
- ✓Reproducibility is enhanced by the precise timing and repeatability of the system. Because of this, the chemical reactions do not need to go to 100% completion.

The advantages of segmented flow cont…

- ✓Decreasing the number of manual sample/solution manipulations reduces labour costs,
- ✓improves workplace safety, and improves analytical precision.
- ✓Complex chemistries using dangerous chemicals can be carried out in sealed systems.
- ✓An SFA analyzer uses smaller volumes of reagents and samples than manual methods,
- ✓producing less chemical waste needing disposal

Applications

Determination of Phosphate by FIA

Description of Method:

The FIA determination of phosphate is an adaptation of a standard spectrophotometric analysis for phosphate. In the presence of acid, phosphate reacts with molybdate to form a yellow-coloured complex in which molybdenum is present as Mo(VI).

$$H_3PO_{4(aq)} + 12H_2MoO_{4(aq)} = H_3P(Mo_{12}O_{40})_{(aq)} + 12H_2O_{(l)}$$

In the presence of a reducing agent, such as ascorbic acid, the yellow-coloured complex is reduced to a blue-coloured complex of Mo(V).

Determination of Phosphate by FIA cont...

❖ ***Procedure:***

✓*Prepare reagent solutions of 0.005 M ammonium molybdate in* 0.40 M HNO_3, and 0.7% w/v ascorbic acid in 1% v/v glycerine.

✓Using a stock solution of 100.0-ppm phosphate, prepare a set of external standards with phosphate concentrations of 10, 20, 30, 40, 50, and 60 ppm. Use a manifold similar to that shown in Figure with a 50-cm mixing coil and a 50-cm reaction coil.

✓Set the flow rate to 0.5 ml/min. Prepare calibration curve by injecting 50 mL of each standard, measuring the absorbance at 650 nm. Samples are analyzed.

Determination of captopril by FIA

- ✓A schematic diagram of the flow manifold is shown in Figure 1.
- ✓The injector- commutator (I) is in the injection position.
- ✓In this position, the reagent (L1, 250 μL) and the sample or reference solution (L2, 150 μL) were simultaneously injected and propelled by carrier streams (5.0 × 10-3 mol L-1 acetate buffer solution (pH 4.6)), merging at point X. Captopril oxidation by Fe(III) occurs in reactor coil B1 (50 cm) producing Fe(II) ions that meet the chromogenic reagent stream (1,10-phenanthroline) at confluence point Y, with the subsequent chelation of Fe(II) by 1,10-phenanthroline in reactor coil B2 (70 cm).
- ✓This product is monitored spectrophotometrically as a stable tris(1,10-phenanthroline)iron (II) complex at 540 nm.

Determination of captopril by FIA cont...

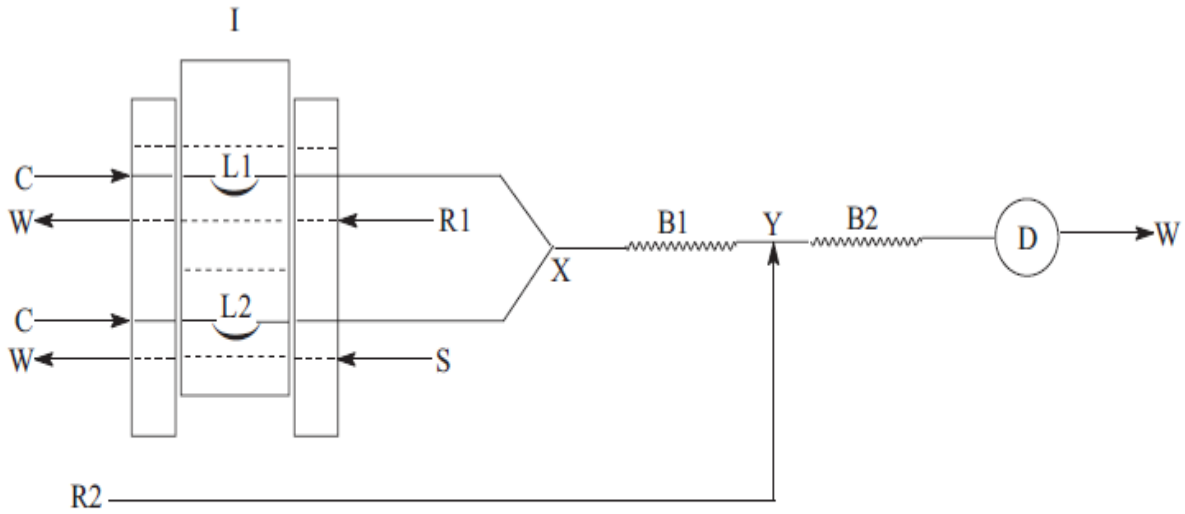


Figure 1. Schematic diagram of the flow injection system used for the spectrophotometric determination of captopril. The central bar of the manual injector-commutator (I) shows the injection position. S, sample or reference solution; R1, $Fe(NO_3)_3.9H_2O$ (5.0×10^{-3} mol L^{-1} in 5.0×10^{-4} mol L^{-1} nitric acid); R2, 1,10-phenanthroline solution (5×10^{-3} mol L^{-1} at a flow rate of 1.3 mL min^{-1}); L1, reagent loop (150 µL); L2, sample loop (250 µL); C, carrier solution (acetate buffer solution (pH 4.6) at a flow rate of 1.2 mL min^{-1}); B1, reactor coil length (50 cm); B2, reactor coil length (70 cm); X and Y, confluence points; D, spectrophotometer (540 nm) and W, waste. X is the confluence point placed 5 cm from the injector-commutator.

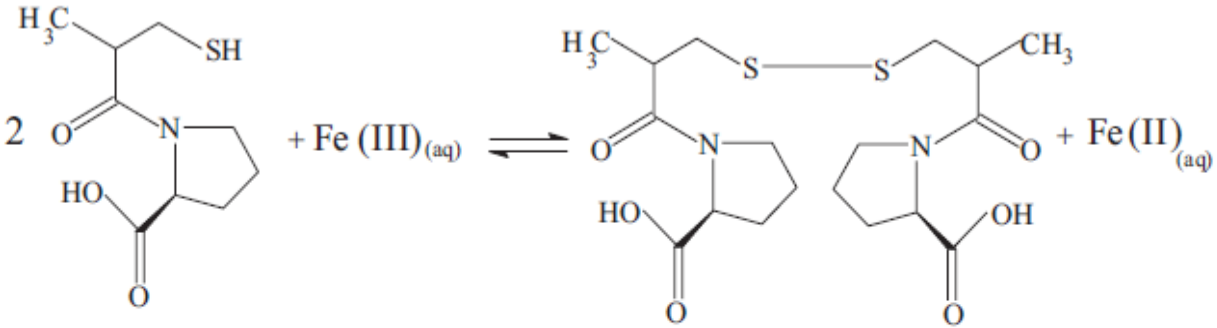
Determination of captopril by FIA cont...

In this work, the development of the reaction occurs in two steps:

✓the first step is the oxidation of captopril by Fe(III) producing Fe(II) ions.

✓In the second step Fe(II) is chelated by 1,10-phenantroline, and the product is monitored spectrophotometrically as a stable Tris(1,10-phenanthroline)iron (II) complex at 540 nm (Scheme 1).

Determination of captopril by FIA cont...



Scheme 1. Reduction of iron (III) by captopril (equation 1) and chelation reaction of iron(II) by 1,10-phenanthroline (equation 2).

Determination of NITRAZEPAM by FIA

FI method using spectrophotometric detection at 613 nm is described for the determination of NZP. The batch method was adopted as a basis to developed FIA method.

The method is based on oxidative coupling reaction of reduced NZP with Promethazine hydrochloride in the presence of sodium periodate to form a green solution.

The FI method has been successfully applied to the determination of NZP in pharmaceutical tablets.

Determination of NITRAZEPAM by FIA cont...

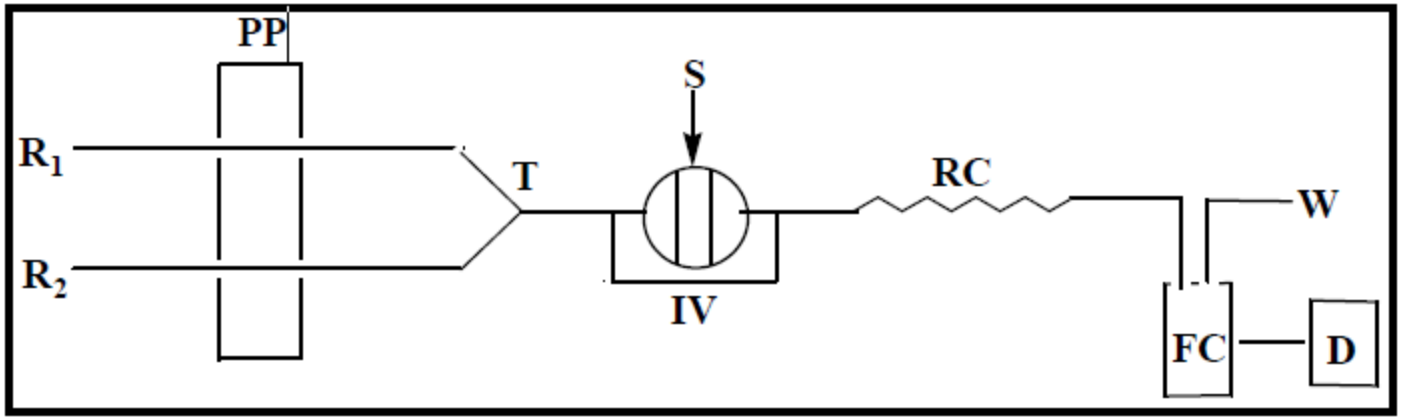
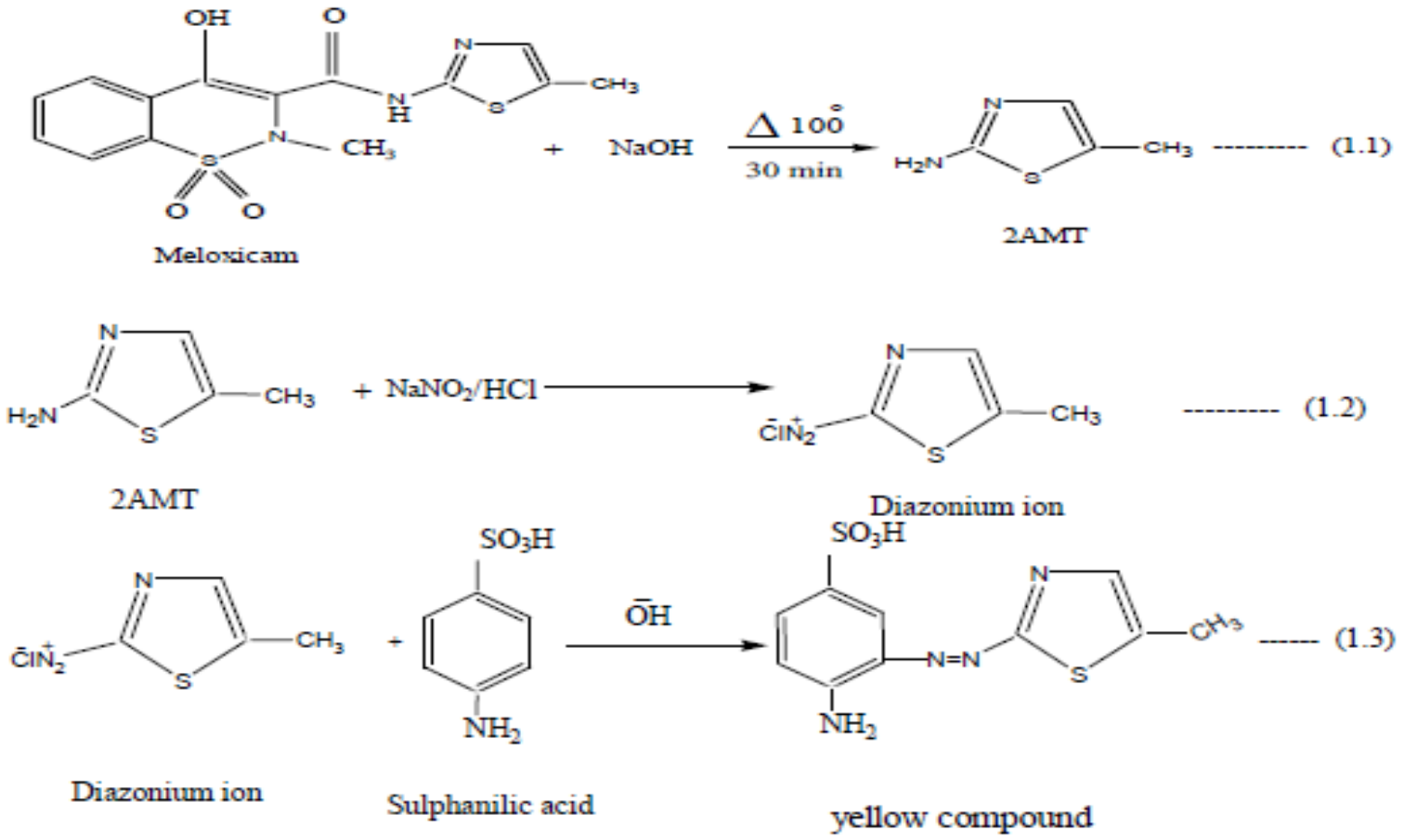


Figure (1): FI manifold for determination of NZP
(R_1 = PMH, R_2 = SPI, S = Sample injection, PP = Peristaltic pump, IV = Injection valve, T = T-link, RC = Reaction coil, FC = Flow cell, D = Detector and W = Waste)

Determination of MELOXICAM by FIA

The methods were based on azo-coupling reaction of meloxicam with sulphanilic acid in the presence of sodium nitrite in alkaline medium after converted to primary aromatic amine.

Determination of MELOXICAM by FIA cont...



FIA assays with UV/VIS spectrophotometric detection

drug	matrix	λ (nm)	reagents/ technique	linear range (mg/l)	detect. limit (mg/l)	ST (h^{-1})
acetylsalicylic acid	tablets	525	Fe^{3+}	25 - 250	4	90
adrenaline	pharmaceuticals	287	solid phase detection	1 - 12	0.17	12
ambroxol	tablets	209			25 µM	50
amiloride	solution	545	methylene blue, Ce^{4+}	up to 120		30
ammonium	pharmaceuticals	636	phenantroline	1 – 20	0.35	
amoxycillin	pharmaceuticals		solid phase detection	0.5 – 50		
ascorbic acid (L-)		480	Cu^{2+}	5 - 40 µM	0.3 µM	80
ascorbic acid (L-)		265	ion pairs	10 - 100 µM	2 µM	30
ascorbic acid	pharmaceuticals	528	Rhodamine 6G	0.1 – 4	80 ng/ml	100
ascorbic acid	vitamin tablets	525	$KMnO_4$, indirect	up to 200 ppm		90
ascorbic acid	pharmaceuticals	267	solid phase detection	0.2 – 20	0.02	
benzocaine novocain	biological fluids	310	dinitrobenzofuroxan derivatives	0.08 – 5.0	0.04 0.05	
benzodiazepine	human urine	550, 600	$NaNO_2$, naphtol	2.5 – 13 µM	0.3–1µM	
bismuth	pharmaceuticals	363, 505	tetraphenylarsonium	2.3 µM – 0.15 mM	1.5 µM	40
busulphan	tablets	570	dissolution	4 – 24		
caffeine acetylsalicylic acid paracetamol	pharmaceuticals	220 – 300	diode-array			
caffeine dimenhydrinate acetaminophen	pharmaceuticals	245 – 310	multivariate calibration			
cefadroxil	pharmaceuticals	600	phenylenediamine, Fe^{3+}	80 – 320	40	
cephalosporines	human urine	330	Pd^{2+}	5 – 60	2	
chlorhexidine	pharmaceuticals		extraction	10 – 100 µM		40
chlorpromazine	solution	340	enzyme inhibition	20 – 100 µM	20 µM	
cysteine	pharmaceuticals	360	Co^{2+}	1 – 90		90

diclofenac	tablets	412	acridine yellow	8 μM – 0.2 mM		40
diclofenac	human urine	380	Ce^{4+}	0.2 – 8	0.023	
diclofenac	pharmaceuticals	281	solid phase detection	0.5 – 140		
L-dopa carbidopa	tablets	370, 500		0.4 – 10 mM	0.2 μM 0.15 μM	26
dopamine methyldopa	pharmaceuticals	360		0 – 0.2 mM 0 – 0.3 mM	3.5 μM 0.43 μM	
epinefrin isoprenaline	pharmaceuticals	330	Fe^{3+}	5 – 200 10 – 300	1 1	
etambutol	tablets	420	dissolution	50 – 300		20
fluticasone	solution		o-phtalaldehyde			
furosemide sulphathiazole	tablets	410	Pd^{2+}	20 – 400 μM 50 – 300 μM	55 μM 14 μM	50
glycine	pharmaceuticals	235	immobilised $CuCO_3$	50 μM – 1 mM		
heparin	solution		thiamine dyes	0 – 12	0.1	
hydrochlorothiazid	pharmaceuticals	220 – 350				
iron	vitamin tablets		thiocyanate			
iron	drugs		photometric diode	1.6 – 4.0		133
iron	tablets	440	dihydroxybenzaldehyde	up to 8 ppm		20
iron	pharmaceuticals	396	dihydroxyphenylacetate	up to 8.3 ppm		
iron	pharmaceuticals	520	phenantroline			54

drug	matrix	λ (nm)	reagents/ technique	linear range (mg/l)	detect. limit (mg/l)	ST (h^{-1})
iron	drugs	360		1 – 10 μM	0.2 μM	
lactate	pharmaceuticals	562	ferrozine	0.3 – 90	50 ng/ml	30
minoxidil	pharmaceuticals	282	solid phase	0.05 – 7	6 ng/ml	
morphine	solution	480	$NaNO_2$	2 – 40	0.6	
neostigmine	pharmaceuticals	610	extraction	0.1 – 0.5 μM	18 nM	48
nitroglycerine isosorbide dinitrate	tablets injections	615	stopped flow	2 – 80 mg/l		15
paracetamol	pharmaceuticals	264		0.5 – 8.0	0.022	40
paracetamol caffeine acetylsalicylic acid	pharmaceuticals	240 – 350	simultaneous determination			
paracetamol salicylamide	pharmaceuticals	300	solid phase		0.104 0.35	36
peroxide hydroperoxide	pharmaceuticals	350	acetic acid, propanol	up to 300 mM		
phenothiazines	tablets	500	MnO_2	5 – 250		
phenothiazines	pharmaceuticals	526	Fe^{3+}	250 – 500	5	
pindolol	solution	655	Fe^{3+}	5 – 120		30
promazine	injections	512	Fe^{3+}, phenantroline	2 – 12 ppm	0.1 ppm	163
pyridoxine	pharmaceuticals	450	cetylpyridinium	up to 1 mM	50 μM	60
tenoxicam	pharmaceuticals	540, 355	methanolic medium HCl medium	7 – 320 0.5 – 8.5		
theophylline	solution	270	solid phase extraction	1 – 100	0.5	
thiamine	tablets, ampoules	420	turbidimetry	50 – 300 μM	10 μM	90
thiamine	vitamin tablets	369	hexacyanoferrate	2.5 – 50	1	
thioridazine	solution	470	PbO_2	0.25 – 5		39
thioridazine	pharmaceuticals	662		10 – 60	0.5 μg/l	50
zinc	pharmaceuticals		thiazolazonaphtol	0.04 – 4	0.01	45

FIA assays with chemiluminescence detection

drug	matrix	reagents/ technique	linear range (mg/l)	detection limit (mg/l)	ST (h^{-1})
pyridoxine	tablets	H_2O_2, oxalate	10 – 250		
quinine	pharmaceuticals	Co^{3+}	0.1 – 100	0.033	
ranitidine	pharmaceuticals	$Ru(bipy)_3$	0.001 – 1 mM	0.6 µM	
salbutamol			50 nM– 0.1 mM	25 nM	
reserpine	injections	H_2O_2, $KMnO_4$	1 – 80	0.3	
reserpine rescinnamine yohimbine	pharmaceuticals	$KMnO_4$ phosphoric acid	0.05 – 3.0		
riboflavine	tablets, injections	$KMnO_4$	0.7 – 10	62 ng/ml	
riboflavin	tablets, injections	$KMnO_4$ phosphoric acid	0.1 – 10	30 ng/ml	
rutin	extracts	luminol hexacyanoferrate	0.02 – 8	6.7 ng/ml	
rutin	pharmaceuticals	hypochlorite	40 pg/l – 10 ng/l	13 pg/l	
rutin	extracts	luminol, H_2O_2, Cr^{3+}	0.02 – 10	7 ng/ml	
salicylamide	urine	$KMnO_4$	up to 8	20 ng/ml	142
sulphite	injections	Rhodamine 6G	0.01 – 5		
tetracyclines	solution	$KMnO_4$	1 – 1000	0.4	
tetracycline + metabolites	solution	hexacyanoferrate	0.04 – 2 µg		
thiamine	solution, tablets	Fe^{3+}		20 µM	
thioridazine	pharmaceuticals	$KMnO_4$		1.2 µM	110
tiopronin	pharmaceuticals	Ce^{4+}	1 – 400 µM	0.34 µM	
trimipramine	pharmaceuticals	$KMnO_4$	0.02 – 0.22		
vitamin B_6	tablets	$KMnO_4$	0.1 – 80	58 ng/ml	
vitamin B_{12}	pharmaceuticals	H_2O_2, luminol	0.001 – 10	0.35 µg/l	60
vitamin K_3	pharmaceuticals	immobilised Rhodamine 6G	0.5 – 10	2,6 µg/l	
vitamin K_3	pharmaceuticals	Rhodamine 6G	0.05 – 50	0.01	
dipyrone	biological fluids	Tween 80	0.05 – 10	0.003	
vitamin K_3	injections	bisulfite, Ce^{4+}	0.01 – 10	2 ng/ml	
vitamin K_3	pharmaceuticals	photoreactor	0.1 – 500 µM	2.03 nM	30

drug	matrix	reagents/ technique	linear range (mg/l)	detection limit (mg/l)	ST (h^{-1})
atropine scopolamine	pharmaceuticals	luminol micelar medium	0.01 – 100	1 ng/ml	
aztreonam penicillin G	solutions	luminol hexacyanoferrate		100 60	
beta-lactam antibiotics	pharmaceuticals	luminol hexacyanoferrate	0.2 – 200 ng	60 pg – 4.5 ng	
chloramphenicol	pharmaceuticals	luminol, Co^{2+}	up to 0.3 µM	3 nM	60
chlorpromazine	solution	H_2O_2, luminol	0.01 – 10 mM		
chlorotetracycline	solution, urine	H_2O_2	40 nM – 4 µM		
ciprofloxacine	tablets, capsules	Ce^{4+}	1 – 20	0.27	
clavulanic acid sulbactam	pharmaceuticals blood serum	H_2O_2 luminol	0.1 – 150 0.01 – 12	0.05 0.01	
codeine	pharmaceuticals	$Ru(bipy)_3$		50 µM	
codeine	solution	$Ru(bipy)_3$			
cysteine glutathione	pharmaceuticals	Ce^{4+}	1 – 100 µM 2 – 100 µM	1.4 µM 0.2 µM	
cytarabine	solution	hypochlorite	10 – 100 ng/l	8 ng/l	
dipyridamole	pharmaceuticals	$KMnO_4$	0.2 – 80	58 ng/ml	11
dopamine	pharmaceuticals	lucigenin	10 – 200 nM	2 nM	40
L-dopa	tablets	$KMnO_4$	0.4 – 80	62 µg/l	120
emetine	solution	$Ru(bipy)_3$	1 – 10 µM	0.1 nM	
ergonovine	pharmaceuticals	hexadecylpyridinium	0.07 – 1000 ppb	0.07 ppb	118
flufenamic acid mefenamic acid	pharmaceuticals biological fluids	$Ru(bipy)_3$	0.07 – 6.0 0.05 – 6.0	3.6 nM 0.21 µM	
fluoroquinolones	pharmaceuticals	Ce^{4+}, sulphite	0.04 – 30	0.016	
folic acid	tablets	$KMnO_4$ formaldehyde	0.1 10 µM	24 nM	
furosemide	pharmaceuticals	Ce^{4+}, Rhodamine 6G	1 – 50 µM	0.22 µM	

gentamycin	pharmaceuticals	Co^{2+}	0.01 – 80	5 ng/ml	
hydrochlorothiazide	solution, tablets	Ce^{4+}, rhodamine	0.33 – 130 μM	7.5 pM	
imipramine	tablets	$KMnO_4$	40 ng/ml – 1	12 ng/ml	
isoniazid	solution	Mn^{2+}, luminol	0.1 – 10	30 ng/ml	
isoniazid	pharmaceuticals	Mn^{3+}	0.1 – 10	0.003	
isoniazid	pharmaceuticals	hypobromite	0.02 – 1	7 ng/ml	60
isoniazid	pharmaceuticals	hypochlorite	10 nM – 1 μM	6 nM	
mercaptoethane-sulfonate	pharmaceuticals	Ce^{4+}, quinine	0.29 – 2.21 ng	0.21 ng	
methotrexate	pharmaceuticals	hypochlorite	20 – 400 μg/l	10 μg/l	
morphinan alcaloids	injections, tablets	H_2O_2, luminol		60 ng/ml	
nitroprusside	injections	H_2O_2	0.2 μM – 0.1 mM	9 nM	40
ofloxacin	tablets, injections	Ce^{4+}, sulphite	0.04 – 4.0	0.016	
perphenazine	pharmaceuticals	$KMnO_4$	50 – 350 ppm		110
persantin	tablets	hypobromite	0.01 – 2	4 μg/l	
phenacetin	pharmaceuticals	Ce^{4+}	0.004 – 1 μM	1 nM	
phenothiazines	tablets plasma, urine	Ce^{4+} rhodamine-B	0.5 – 90	0.01	129
phenothiazines	pharmaceuticals		2 – 20 μM		110
prednisone	solution, tablets	Ce^{4+}	0.2 – 20	31 μg/l	
promethazine	solutions, tablets	$KMnO_4$	0.1 – 6 μM	35 nM	

drug	matrix	reagents/ technique	linear range (mg/l)	detection limit (mg/l)	ST (h^{-1})
pyridoxine	tablets	H_2O_2, oxalate	10 – 250		
quinine	pharmaceuticals	Co^{3+}	0.1 – 100	0.033	
ranitidine salbutamol	pharmaceuticals	$Ru(bipy)_3$	0.001 – 1 mM 50 nM– 0.1 mM	0.6 µM 25 nM	
reserpine	injections	H_2O_2, $KMnO_4$	1 – 80	0.3	
reserpine rescinnamine yohimbine	pharmaceuticals	$KMnO_4$ phosphoric acid	0.05 – 3.0		
riboflavine	tablets, injections	$KMnO_4$	0.7 – 10	62 ng/ml	
riboflavin	tablets, injections	$KMnO_4$ phosphoric acid	0.1 – 10	30 ng/ml	
rutin	extracts	luminol hexacyanoferrate	0.02 – 8	6.7 ng/ml	
rutin	pharmaceuticals	hypochlorite	40 pg/l – 10 ng/l	13 pg/l	
rutin	extracts	luminol, H_2O_2, Cr^{3+}	0.02 – 10	7 ng/ml	
salicylamide	urine	$KMnO_4$	up to 8	20 ng/ml	142
sulphite	injections	Rhodamine 6G	0.01 – 5		
tetracyclines	solution	$KMnO_4$	1 – 1000	0.4	
tetracycline + metabolites	solution	hexacyanoferrate	0.04 – 2 µg		
thiamine	solution, tablets	Fe^{3+}		20 µM	
thioridazine	pharmaceuticals	$KMnO_4$		1.2 µM	110
tiopronin	pharmaceuticals	Ce^{4+}	1 – 400 µM	0.34 µM	
trimipramine	pharmaceuticals	$KMnO_4$	0.02 – 0.22		
vitamin B_6	tablets	$KMnO_4$	0.1 – 80	38 ng/ml	
vitamin B_{12}	pharmaceuticals	H_2O_2, luminol	0.001 – 10	0.35 µg/l	60
vitamin K_3	pharmaceuticals	immobilised Rhodamine 6G	0.5 – 10	2,6 µg/l	
vitamin K_3 dipyrone	pharmaceuticals biological fluids	Rhodamine 6G Tween 80	0.05 – 50 0.05 – 10	0.01 0.003	
vitamin K_3	injections	bisulfite, Ce^{4+}	0.01 – 10	2 ng/ml	
vitamin K_3	pharmaceuticals	photoreactor	0.1 – 500 µM	2.03 nM	30


Thank You... ☺