CHAPTER 1

Sequential Injection Analysis (SIA)

From homogeneous to heterogeneous system

1.1 Introduction

The need for process analysers that are able to process large amounts of samples continuously and automatically and deliver accurate and precise results with regard to sample contents are ever growing. Sequential injection analysis (SIA) as a process analyser satisfy these requirements and its popularity has never been better, since its introduction in 1990 [1,2].

Effective cost control is also an important aspect that demanded serious attention. It does not only affect efficient management of industrial, agricultural, clinical and pharmaceutical processes, but it forms the cornerstone in the provision of high quality value-added products in a highly competitive world where clean and sustainable environment must be maintained [3]. These needs triggered the decision to develop automated systems capable of fulfilling the set requirements of real time monitoring and control of process on an analytical base.

The advantages of SIA were exploited by several laboratories and research groups [4-11]. Several reasons can be given for the employment of flow and sequential injection (SI) systems as process analysers. These reasons include: reduction in cost (personnel, equipment and

following aspects must always be highlighted:(i) the components it was constructed of, (ii) the specific manifold dimensions, as well as (iii) the device sequence or method construction (See addendum A).

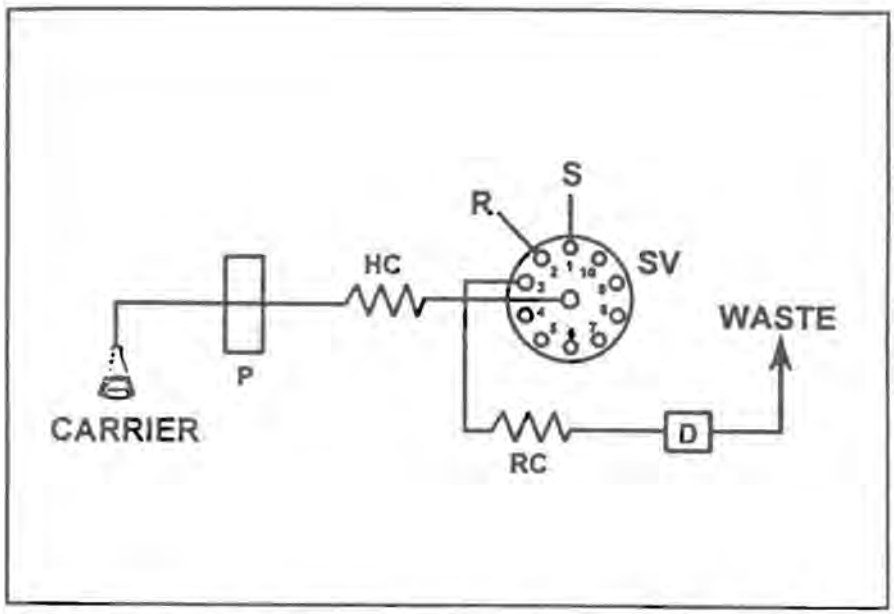

Fig. 1.1 A schematic flow diagram of a typical SIA system. P - pump, HC - holding coil, RC -reaction coil, S - sample, R - reagent, SV - selection valve, D - detector.

There are presently two draw backs of SI to be mentioned. Firstly, since aspirations of the wash solution and sequencing of the zones in the holding coil take some time (typically 30 seconds) the sampling frequency of the SI system is presently half that of a conventional flow injection (FI) systems where filling of the injection valve is a matter of a few seconds. Secondly, SIA requires specialized software, since the sequencing, injection and data collection are entirely computer driven. The mentioned draw backs are however, not an obstacle in using the SIA technique [4].

Automatic methods of analysis have been especially influential in clinical chemistry. Since

for hospitals to obtain a large number of analytical data as quickly and inexpensively as possible. The situation is much the same in most industries where quality control laboratories have become much more important than they were a few decades ago.

Most of the process control systems currently used are based on physical measurements such as flow rate, pressure, electrical resistance, etc. While this has resulted in processes which are operated under statistical control, verification of the process performance can only really be achieved by chemical analysis, usually in a remote plant laboratory. This approach is seen as unacceptable in the design of quality management systems for the production process. In such systems the emphasis is on quality assurance during the process rather than after-the-fact.

Process analysis brings the process controller a step closer to ensuring excellent control of the plant and real time quality assurance. At this stage, lengthy development times, the cost of these analysers and their maintenance requirements mean that only a few critical streams are monitored. The demands in this field are dictated by the large number of samples to be analysed, especially in on-line control of automized manufacturing processes and the quality now required in manufactured products. It is therefore necessary to control not only the raw products, but also the intermediate and end products.

Sequential injection systems have been proven to be suitable as on-line process analyser for most single component [9,10,23] and multi-component analysis [27]. Studies revealed that reduced numbers of samples can be used when applying SIA systems as process analysers, provided a correct regression model is used [23]. The flow systems already adapted to SIA systems involved very simple methods and operations in a homogeneous medium.

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However, the need for analysis when the reagent is expensive, only slightly soluble or only available in the solid form, has necessitated the introduction of solid-phase reactors (enzymatic, immuno-assay, ion exchange or redox) into the SIA manifold.

1.2 Solid-phase reactors in flow systems

Solid-phase reactors can be classified into two distinct groups, namely, reactors in which a chemical reaction takes place to derivatise the analyte, and reactors in which no derivatisation reaction takes place. The first group includes enzyme reactors while the second group consists mostly of reactors used for pre-concentration.

The introduction of solid-phase reactors in flow systems represents a high achievement for on-line determination of different substances [28,29]. These type of heterogeneous reactions have been converted for use in FI systems with some success [30,31]. It has enhanced such basic analytical parameter such as sensitivity and selectivity. Fig.1.2 illustrates the most common solid-phase reactor incorporated in a FIA manifold.

The use of reagents, particularly enzymes, in the solid-phase has been known from the early part of the 20th century. These enzymes were immobilized on a variety of support materials for a number of reasons [32] and offered a greater degree of control over the relevant reactions.

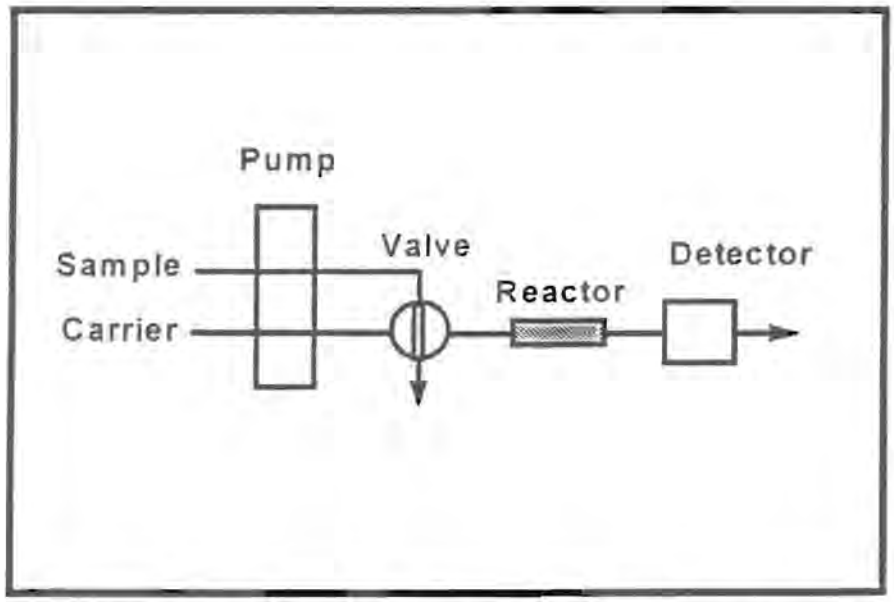

Fig. 1.2 Diagram of the most common location for a solid-phase reactor.

In addition to the excellent analytical features already available with normal FIA systems, systems which incorporate a solid-phase reactor offer further advantages with respect to miniaturisation, simplification and cost reduction. The different types of immobilisation techniques for solid reagents, the location of the reactors in the flow system, along with the various shapes and types of reactors used and its various applications is fully discussed in Chapter 4.

The introduction of solid-phase reactors in a SIA manifold heralded another dimension in process analysers. Since most of the SIA systems originate from conventional FI methods, likewise the incorporation of solid-phase reactors in a SIA manifold is an improvement on existing FIA methods and a further boost for process analysers. Fig.1.3 illustrates a typical SIA system incorporating a solid-phase reactor.

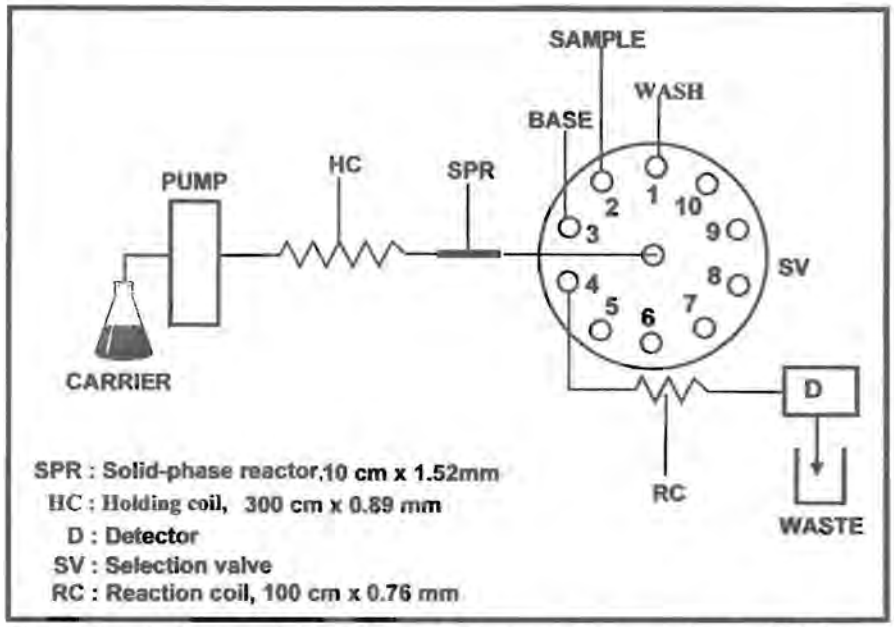

Fig.1.3 A typical sequential injection analysis system incorporating a solid-phase reactor in its manifold.

SIA systems used, incorporating solid-phase reactors in its manifold, include a spectrophotometric method [33] for the determination of lactic acid from industrial inorganics, simultaneous monitoring of glucose, lactic acid and penicillin [34], glucose and penicillin [35], theophylline and caffeine [36] and the separation of radionuclides [37].

1.3 Aim of this study

The study was first devoted at evaluating super Serpentine reactor types for their sensitivity and precision in the SIA system [38]. They are four super Serpentine reactors (I, II, III & IV). A comparative study of these reactors was done.

Due to the need for safe analysis of trace elements, which are toxic at certain concentrations when consumed, cost reduction and robustness, the application of SIA to real samples was considered. The incorporation of a solid-phase reactor (redox reactor) in a SIA manifold was used to enhance the analyses of manganese, iron, nitrate and nitrite as well as chromium in natural, effluent and electroplating waters and pharmaceutical products. The use of solid-phase (redox) reactors in SIA to determine these components were considered since it has not been attempted yet.

Manganese (II) [39] was spectrophotometrically determined in tap water and effluent streams using a redox solid-phase reactor. Manganese (II) from a sample was oxidised by a PbO_2 (solid-phase reactor) embedded in silica gel beads to produce the permanganate ion which is then detected with a UV/VIS spectrophotometer. The reaction is pH dependent.

Total iron as Fe (II) [40] was determined in pharmaceutical products and effluent streams using a solid-phase cadmium reactor incorporated into a SIA system. The reactor reduced the Fe (III) to Fe (II) and was complexed with 1.10 Phenanthroline as Fe $(phen)_3^{2+}$ complex. This was determined with a UV/VIS spectrophotometer.

Oxidised nitrogen (nitrate + nitrite) [41] was determined in natural water as nitrite. The nitrate in water was reduced to nitrite using a cadmium reactor. The nitrite produced was diazotised with sulphanilamide and coupled with ethylene diammonium dichloride to form a highly coloured azo dye which was detected with a UV/VIS spectrophotometer.

Chromium [42] as chromate was determined spectrophotometrically in electroplating and

natural waters. The sample was oxidised with ammonium cerium (IV) sulphate to dichromate/chromate. In a basic medium the redox solid-phase reactor, PbO_2 converted the dichromate to chromate. The chromate was released in excess ammonium hydroxide and was detected using a UV/VIS spectrophotometer.

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CHAPTER 2

Sequential Injection Analysis (SIA)

2.1 Introduction

It is a decade since the introduction of sequential injection analysis (SIA) as a process analyser. Since its introduction in 1990 [1,2], its growth has exceeded expectations. This signifies recognition of the tremendous versatility of this method originally designed as a mere tool for automation of serial assays [3].

Increasing pressure on the chemical manufacturing industry to provide higher quality products in an economically viable and environmentally acceptable manner, has increased the requirements to maintain strict control of plant conditions throughout the production process. Hence, the use of process control strategies represent a significant shift in the thinking of many process control engineers.

SIA is a technique of flow analysis whose roots can be traced back as far as 1974 [3]. It has introduced a new dimension to flow analysis due to the simplicity of its flow channel, the reduction in both sample and reagent consumption and the efficiency with which the hydrodynamic variables can be controlled. Although SIA is very characteristic of a flow system, especially with regard to the dispersion taking place in the flow conduit, it also has certain inherent characteristics. It is these characteristics that distinguishes SIA from conventional flow

systems, namely the introduction of sample and reagent into the flow conduit as zones as they are propelled towards the detector.

The demand for mechanically simple and robust flow injection methodology has been the driving force behind the development of the sequential injection (SI) technique. The simplicity of the SI manifold and its low need for maintenance makes it an ideal tool in process analysis. As miniaturization and reduction of reagents consumption are also ultimate goals in chemical sensing, it is useful to review the use of combined injection and programmed flow as a central issue in designing chemical sensors and structurally simplified chemical analysers.

Various parameters were assigned to the characteristics of the SIA system and in depth studies were conducted to investigate the result of the stacking of the zones, the influence of the various operational parameters, deformation and dispersions of the zones and subsequent zone penetration [5-10].

Extraction, separation, pre-concentration, dialysis, titrations, dilutions and redox methods were adapted for use in SIA manifolds. Hence, the design of a SI manifold can be considered as the search for optimum dispersion characteristics. Colorimetric, electrochemical and other detectors equipped with a suitable flow-through cells, were also incorporated into these manifolds. A new scope of SIA manifolds were developed for use in both industrial applications as well as in the laboratory. The use of biosensors and the incorporation of solid-phase reactors in SIA manifolds has further enhanced the potential of SIA as a process analyser.

2.2 Historical background

The introduction of SIA in 1990 [1,2] ushered in a new dimension in flow analysis. Although the technique is just a decade old, the number of journals and reviews published bear testimony of its tremendous growth. SIA was born from flow injection analysis (FIA), which can be traced back to 1974 [4]. Hence the discussion that follows will briefly start at FIA after which the development of SIA will flow from it.

FIA is an analytical technique that relies on the injection of a well defined sample zone into moving carrier stream and the subsequent detection of a signal which has been modulated by a combination of physical and chemical interactions. The technique relied on a constant flow rate.

The successful operation of any injection analyser requires that the sample and reagents are brought together, mixed and allowed to react in a perfectly reproducible manner. To obviate the need for frequent re-calibration, it is necessary to maintain reproducible flow conditions for extended periods of operation. This has led to the practice of using unidirectional monotonous flow, because as long as constant flow rate is maintained, the sample may be injected into the system at any time.

The use of an unidirectional monotonous flow rate has been the prevailed practice in FIA, which unlike chromatography does not aim at separation of the analyte components, but rather at their effective chemical conversion into detectable species. Therefore the key issue in FIA is the reproducible dispersion of the injected zone into the carrier stream and timing of the arrival of the reacted zone at the detector. If all critical parameters (reproducible injection, controlled

reaction time and controlled dispersion) are held within certain tolerance levels, the result will be reproducible [11].

The instrumentation needed for an FIA system are a multichannel pump, an injection valve, a flow through detector and a recorder. Save for the last component which was replaced when computers were introduced in laboratories some years later, the basic flow scheme remained essentially unchanged [12]. When FIA research became orientated towards the exploitation of concentration gradients formed by the dispersion process [13], new techniques using stopped flow, reversed flow, sinusoidal flow, reagent injection, sequential injection and single solution calibration were developed.

Use of a flow programme, rather than constant monotonous flow, requires synchronization of sample zone injection with the start of each flow cycle. A system configuration was required which will allow sample zone injection, reagent addition, mixing, measurement, and ejection of the reacted mixture by a combination of forward and reversed flow steps.

While linear flow programming traditionally refers only to the flow pattern employed, i.e. the rate and direction of flow, the inclusion of a mechanism of selecting different streams to be subject to the flow programme may also be added. The group at the University of Washington [14] used these ideas for the basis of an extension of FIA, which was called SIA [15].

The transformation of FIA into SIA stems from the random walk model. By using the random walk model as a basis, it has been postulated that there is no net flow needed for the successful operation of a flow injection system [2]. Růžička *et al.* [1] discussed the consequences of the

latter observation and the role of the random walk model in FIA. It was this discussion that led to defining the principle of SIA. The random walk model suggested that mixing, the fundamental requirement for both FIA and SIA, could take place with no net displacement of sample. The transformation of FIA into SIA signifies recognition of the tremendous versatility of this method originally designed as a mere tool for automation of serial assay [3]. With the introduction of SIA, the basic parameters of flow injection were assumed to be applicable, because the same basic components were used.

Sequential injection is mechanically simpler than flow injection for it uses only a single channel. SI uses a selection valve (rather than an injection), through which precisely measured volumes of sample and reagent solutions are aspirated into a holding coil by means of a pump that is capable of a precisely controlled stop-go-forward-reverse movement [5, 16-18].

A SI manifold must be designed to achieve mixing between reagent and analyte such that the reagent is in sufficient excess at the maximum of the profile to ensure the greatest degree of reaction. Dilution of the formed product zone should, however be minimized so as to avoid an unnecessary loss in sensitivity. The design of a SI manifold can be considered as the search for optimum dispersion characteristics.

A basic sequential injection analysis manifold is shown in Fig. 2.1. Mainly four different liquid drivers were used in SIA. The sinusoidal flow piston pump [6, 17, 19-21] was specially designed for SIA. The flow rate is dependent on the rotation angle, the radius of the pump, the cam and the frequency of the pump. Its repeatability and reproducibility are good, but it is difficult to maintain a constant flow rate during analysis.

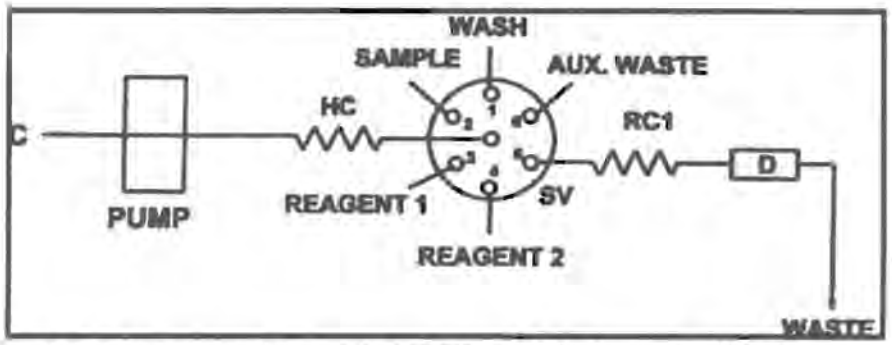

Fig 2.1 A basic sequential injection analysis manifold. C - carrier, HC - holding coil, RC - reaction coil. SV - selection valve and D - detector.

Ivaska and Růžička [22] investigated the performance of peristaltic pumps. These pumps are suitable for SIA applications when used with Neoprene tubing. Automatic burettes that are computer programmable and have variable speed are mainly used by the Spanish group. However a drawback of these liquid drivers is that it is impossible to use flow rates lower than 2 mℓ/min. Finally field-decoupled electro-osmotic pump is described by Liu and Dasgupta [23, 24] as on ideal pumping system for SIA, because the flow direction is readily and reproducibly reversed and the flow rate can be maintained with a high degree of reproducibility.

Optimum exploitation of these flow techniques in automated modes of operation necessitates computer control. Specialized software packages were designed to control both the movement of the apparatus (pumps and valves) and to handle data acquisition and storage. The FlowTEK package (obtainable from MINTEK, Randburg, South Africa) was designed by Marshall and Co-workers [16, 25] and is widely used. The Spanish group uses the program DARRAY, obtainable

from SCIWARE, Palma de Mollorca, Spain [26]. Růžička's group [27] uses a program called FIALab for control and data aquisition.

The advantages of SIA have been discussed in detail by Růžička and Gübeli [19] and in comprehensive reviews and congresses [2, 4-5,14,16,18, 29-30]. A valuable contribution by the group at the University of Washington was the exploitation of new sensor system which broadened the scope of SIA tremendously and opened new horizons in the field of flow analysis.

2.3 Basic principles

The concept of SIA is based on the mixing of a samples zone with a reagent zone in order to produce a measurable response [22]. This necessitates solution handling operations such as sample injection, reagent injection, sample and reagent mixing and detector wash and reconditioning. The SIA system used a selection valve, compared with the injection valve used in FIA. The selection valve is used to aspirate precisely measured volumes of carrier solution, sample solution and reagent solution into a holding coil. The aspiration of the zones is achieved by means of a pump which is capable of a precisely controlled stop-go-forward-reverse-movement [18].

Following the first step of zone sequencing, during which the sample and reagent zones are stacked in the holding coil conduit to each other, the valve is switched to the detector position (Fig. 2.2A). In the next step, the flow is reversed so that the stacked zones are propelled through the valve and the reactor to the detector (Fig. 2.2B). As the central streamline moves at a rate twice the velocity of the mean flow velocity, whereas the elements of fluid more adjacent to the

walls move at lesser rates, the cores of the sequenced zones penetrate each other [31]. During this movement the flow reversal creates a complex region within the analyte which is transformed into a detectable species (Fig. 2.2C). The fundamental requirement for SI to succeed is to achieve maximum zone penetration through a deliberate increase in axial dispersion, obtained by means of the flow reversal and channel design [1,19,32].

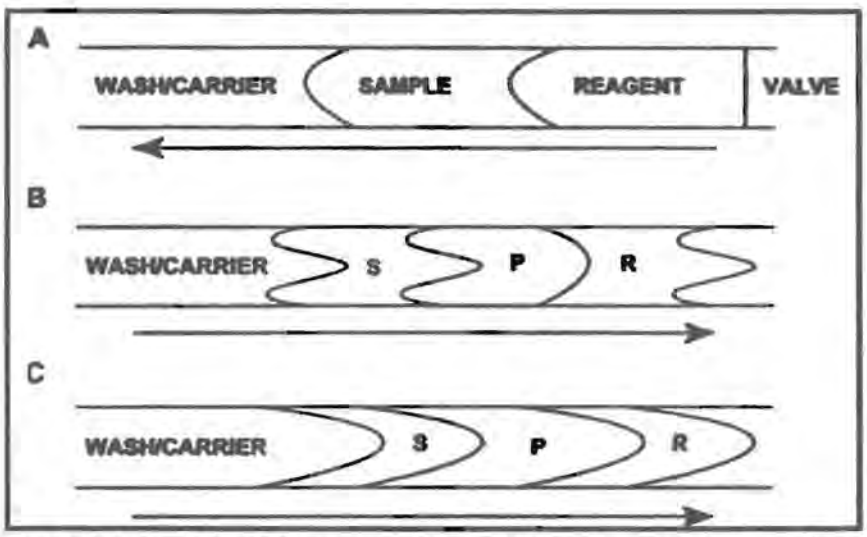

Fig 2.2 Flow profiles of the sequenced (A) and injected zones (B - immediately after flow reversal and C - in reaction coil). S - sample, R - reagent and P - formed product zone.

Reproducible dispersion is the basis for analysis by flow injection methods. Dispersion is the result of all the physical forces acting on the injected zones. It is the process by which the zones transform from homogeneous, geometrically well defined zones at the moment of injection to the final zone that is detected downstream. The dispersion coefficient is the ratio of the detector response of the injected solution in the absence of these forces to that of the solution due to these

forces. Růžička and Hansen [31] defined the conceptually simple and practically useful dispersion coefficient, $D = C^0/C$, where C^0 is the detector response of the undispersed solution zone and C is the detector response of the dispersed element of fluid that yields the analytical readout. Because there is generally a direct relationship between the property used for detection, the magnitude of the transduced signal recorded and the concentration of the sample or its reaction product, the dispersion coefficient can be taken as the height ratio of the signal [16,31]. The random walk model was also used by Růžička and Marshall [1] to describe dispersion in the SIA analyser channel.

Thus, in addition to reproducible timing and sample injection, controlled dispersion is important in flow systems. The purpose of controlling dispersion in a flow system is to optimize the chemical reactions taking place between the sample and reagents. In essence, what has been called "controlled dispersion" is in fact the result of the sample is reproducibly diluted as it travels down the tubing. Dispersion is characterized by the concentration profile adopted by a zone or plug inserted at a given point in the system without stopping the flow.

Two processes are responsible for dispersion in the flow conduit, namely the physical process of material dispersion due to hydrodynamic processes taking place in the flow through system and the chemical process of formation of chemical species.

Although Růžička and Gübeli [19] stated that "for a rational design of the sequential injection analyser, the degree of sample dispersion must be considered as main design guideline", zone penetration (related to dispersion) is found to be the key parameter, the control of which is essential to the successful execution of sequential injection [6]. The importance of zone

penetration can be ascribed to the fact that this influence has a dramatic impact on the surface area over which a concentration gradient exists and therefore over which axial mixing takes place. It follows from the foregoing that, for reagent-based chemistries, a region of mutually inter-dispersed sample reagent zones must be identified, within which D is larger than 2 and where at the same time sufficient excess of reagent is present. Analogous to the definition of resolution, zone penetration is defined as:

$$P = 2W_0/(W_s + W_r) \qquad (2.1)$$

Complete overlap is obtained for $P = 1$, zero overlap for $P = 0$ and for values in between, partial overlap will be obtained. This approach yields useful results although it is difficult to determine the value automatically [17]. An isodispersion point, I_d, is observed in cases where $1 > P > 0$ (Fig. 2.3). At this point the dispersion of the sample and reagents zones are identical and the ratio of sample and reagent concentrations is the same as their ratio prior to injection ($C_s/C_r = C_s^0/C_r^0$).

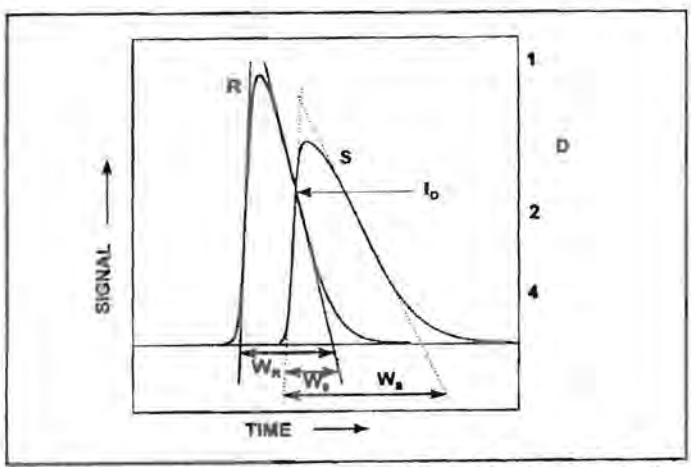

Fig. 2.3 Schematic representation of zone penetration showing the isodispersion point (I_D). R - reagent zone, S - sample zone, D - dispersion coefficient, W_R - baseline width of reagent zone, W_S - baseline width of sample zone and W_0 - baseline of the overlap.

The isodispersion point is independent of concentration, but studies done by van Staden *et al.* [38] illustrate the shift of the isodispersion point due to the difference in concentration gradients when different volume ratios of sample and reagent were employed. These studies also showed that the position of penetration and the sequence of introduction of samples and reagents for different sample and reagent volume ratios in a total constant volume has a major influence on the response of the final peak profile. This is illustrated in Figs. 2.4 and 2.5, where Fig. 2.4 represents the injection order of first the metal followed by the ligand and Fig. 2.5 represents the reversed injection order.

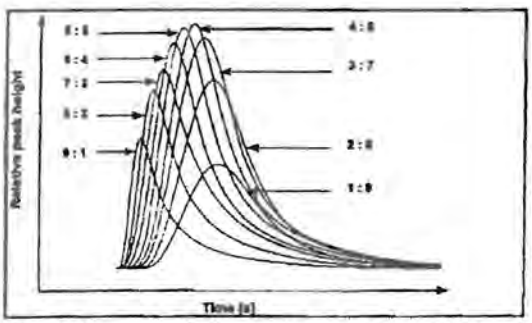

Fig. 2.4 Schematic representation of the influence of different sample : reagent ratios in a total constant volume. The figure represents the injection order of first the metal followed by the ligand.

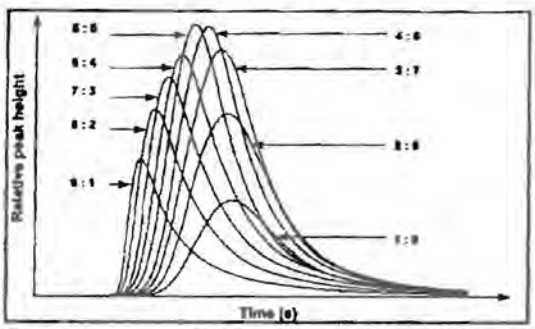

Fig. 2.5 Schematic representation of the influence of different sample : reagent ratios in a total constant volume. The figure represents the injection order of first the ligand followed by the metal (reversed order).

The response of the final peak profile depends largely on the kinetics involved in both the ascending and tailing parts of the sample and reagent zones at the isodispersion point. Zable [11] found mathematical equations to calculate the maximum zone overlap. The larger this number,

the greater the degree of zone penetration. This approach too suffers from certain limitations as it does not indicate the sensitivity of the measurement, because it does not take the concentration of the sample and reagent into account.

2.4 Operational parameters

When applying the SI technique, it is imperative to understand the principles on which it is based in order to do subsequent analysis. The greatest challenge is the theory of flow dynamics, which will lead to optimisation of flow systems based on flow stopping and reversal. Many researchers generally set up a system without regard to the dispersion of the individual components or some of the general rules for optimising the system. There are a number of publications describing different techniques of optimisation [34-37] and standardization [38] as well as systems able to diagnose multivariate responses, with the aim of detecting faulty responses [39,40].

The extent of dispersion that the product peak will undergo is essentially influenced by the operational parameters that govern the SIA flow conduit. Although some would argue that SIA is simply a variation of FIA, there are certain fundamental differences in the use and control of the operational parameters used in SIA.

A number of papers were published that described the most important parameters optimised [6, 9, 11, 16, 17, 41]. Almost without exception the following parameters had been shown to have a marked effect on zone dispersion in an SIA system: the volumetric flow rate, tube diameter length of flow path from injection to detection, sample and reagent volumes, order of sample and reagent injection, flow reversal and to a lesser extent reactor geometry. The use of mixing

chambers in the flow conduit and their influence on dispersion was studied by van Staden and Botha [9, 60]. To evaluate the influence of every parameter, a non-reactive dye was used as sample and reagent zone respectively in a series of experiments

The *volumetric flow rate* includes both the loading and forward flow rates and is also referred to as the combined effect of pump speed and the internal diameter of the pump tubing when using a peristaltic pump [9, 11,16, 60]. In correlation with the Vanderslice expression, $D = k'q$, where q is the flow rate in mℓ/min, the dispersion of the different zones decreases as the flow rate is increased [42]. The dispersion coefficient decreases with increasing flow rate because the residence time decreases, in a non-linear fashion, with increasing flow rate [16]. A linear relationship consists between the pump speed and flow rate; therefore the flow rate can be altered by changing the pump speed [9, 16,17, 70]. At high flow rates a deterioration in sensitivity and reproducibility is experienced due to the higher back-pressure [16,43]. It is preferred that the loading flow rate should be faster than the forward flow rate to ensure higher sample throughput, provided that the pump will allow this [17].

The *length of the tubing* is dictated by the experimental requirements [16]. Longer tubing leads to longer residence times and therefore larger dispersion. Zable [11] stated that the dispersion must be proportional to the square root of the tube length, but experimental data had shown that there was a linear relationship between dispersion and path length. The mixing height (number of plates or tanks) is defined as the average length of tubing for each mixing stage [31].

In SIA the manifold tubing is divided into two parts: the holding coil which is the tubing between the liquid device (pump)and the selection valve and the reaction coil which is the part connecting

the selection valve with the detector. The holding coil primarily acts as a reservoir and should be large enough to prevent the sample and reagents from entering the pump conduit. The reaction coils should not exceed one-third of the volume of the washing solution, thereby ensuring that they are adequately flushed during every experiment [17]. Reaction coils are usually kept as short as possible to avoid excessive dilution of the formed product zone. The length is governed by the physical distance between the valve and the detector [6,19].

A knowledge of the reaction rate is of particular value when adapting a method to sequential injection analysis, because the time spent in the manifold can be too short to ensure complete colour development. Due to the discontinuous nature of SIA, stopped-flow periods can easily be incorporated to enlarge reaction times [44-46]. Van Staden and Taljaard [44] used a stopped-flow period of 80 seconds to ensure adequate formation of indophenol during the determination of ammonia.

Related to the Dean number the *tubing diameter* had a dramatic influence on the dispersion of the different zones [11]. The dispersion is found to be proportional to the fourth root of the coil diameter. Several factors should come to mind when considering the optimum tube diameter. These include the resultant back-pressure in a length of tubing, the vulnerability to blockage and the degree of radial dispersion attainable [16,21]. Wider tubing is usually used for the holding coil, because of its promotion of axial dispersion and, therefore, zone penetration. Narrower tubing is used for the reaction coils to prevent excessive dilution of the former product zone. Wider tubing is used for the uptake tubes to prevent any back-pressure.

Gübeli *et al.* [6] have conducted an in depth study on the effect of *sample and reagent volume*

on zone penetration and sensitivity. Their conclusions can be summarized in three rules:

1. Changing of the sample zone volume is an effective way of changing the sensitivity of measurement. Dilution of concentrated samples is best achieved by reducing the injected sample volume.

2. Injecting at least twice as large reagent zone volume as sample zone volume, while keeping the volume of the sample zone less or equal to 0.5 $S_{1/2}$, allows the optimum conditions for single based chemistries to be met. ($S_{1/2}$ is defined as the sample volume required to yield a dispersion factor of two in the manifold).

3. Two reagent chemistries can be accommodated provided that the sample volume is kept below the $S_{1/2}$ value, so that the sample zone is surrounded by reagent zones and that the concentration of the injected reagents are sufficiently high.

Van Staden *et al.* [33] found that the best sensitivity was obtained when a 1:1 sample to reagent ratio was used. At this ratio, the two zones experienced almost the same axial dispersion and penetration occurred almost at the maximum of the descending sample zone as well as the maximum of the ascending reagent zones. Gübeli *et al.* [6] found that increasing zone volumes at equal volume ratios caused zone overlap to decrease from nearly complete overlap (with small equal volumes) to a partial one (with relatively large equal volumes). While keeping the reagent volume constant, the authors [6] also varied the zone volume ratios by increasing the sample volume from less than the reagent volume to one where the sample volume was in excess of the reagent zone. This also resulted in a decrease in zone overlap.

Optimum sample and reagent zone volumes can be determined by plotting log [1 - (A_{max}/A_0)] versus sample volume ($\mu\ell$), where A_0 is the absorbency corresponding to the case where the element of fluid undergoes no dispersion. The $S_{1/2}$ value can be determined from the slope of the linear relationship. Experimental results gave good correlation with calculated values [9, 60]. $S_{1/2}$ values are, however, influenced by a number of experimental parameters. Cladera *et al.* [7] and Araujo *et al.* [47] showed that the ionic strength or electrolyte concentration of the medium influenced the $S_{1/2}$ value. The flow rate (slower flow rate result in higher $S_{1/2}$ values), the number of flow reversals and the dimensions of the reactor loop also influence the value [7].

A simple and convenient method for the determination of injection volumes in sequential injection analysis is presented by van Staden and Malan [48]. It is based on comparing the dilution of the injected dye with a standard calibration curve. The proposed colourimetric method gave the volume of the whole injection device more accurately than methods where the inner dimensions of the injection device are not precisely known. The colour methods is within the 95% confidence level with an RSD of 0.8%. Sampling strategies in sequential injection analysis were also investigated by Vieira *et al.* [49]. These techniques were exploited using a mono segmented-flow approach.

In a publication of Mas-Torres *et al.* [50], the authors discussed a new approach to sequential injection analysis. This approach involved the use of the sample as carrier stream. Although this technique may render good results, it removes one of the main advantages of sequential injection analysis - the fact that it uses minimized amounts of sample [16].

The importance of the correct order of sample and reagent injection is highlighted in a number

of publications [17, 33, 45, 51-53]. The order which the different of reagents are drawn up are very dependent on the reactions involved. The residence time of a specific zone also depends on its position in the reagent sequence. The zone that is drawn up first reaches the detector last due to the flow reversal. This zones has the longest residence time of all the zone and is therefore more dispersed [11, 16, 17]. The following must be considered: when sensitivity is important, the reagent, at a sufficiently high concentration, should be introduced first and allowed to penetrate the sample zone, which will experience minimal dispersion. If buffering of the sample by the wash solution is required, the order must be reversed. If solubility considerations prevent the reagent concentration from being increased, sandwiching of the sample between two reagent zones is an option to be considered [17].

Since the duration of flow reversal is the most effective in providing mutual zone penetration [6,17], more than one flow reversal was needed in the determination of ammonia, (due to insufficient mixing of the adjacent zones because of their different viscosities). The stack of zones was subjected to three flow reversals before the product zone was propelled to the detector. Multiple flow reversals were also used to better the transfer of ammonia over the membrane in a coupled gas-diffusion-SIA system [54] as well as to improve iron dialysis through the membrane during the determination of iron (III) [4].

Various reactors have been described in the literature on FIA manifolds [31]. Where the reactor consists of a length of tubing, various geometries have been proposed. Three were evaluated to establish the effect of reactor geometry on zone penetration and geometry. Studies done by Taljaard [16] and Marshall and van Staden [17] showed that reactor geometry does not have a marked effect on sensitivity or precision. Straight tubes are, however, preferred in SIA manifolds

due to the better axial dispersion obtained.

It should be noted that only the physical dispersion of all the above mentioned parameters were highlighted. The influence of a chemical reaction on dispersion is not even mentioned. The optimum values for each parameter will to a large extent depend on the specific reaction conditions and do not only depend on maximum sensitivity, but also on the reproducibility of measurements (%RSD). A set of parameters resulting in high sensitivity can be rejected if the relative standard deviation is too high, as shown by van Staden and du Plessis [55] and by Nakano *et al.* [56]. It is however surprising that such good precision is attained in SIA systems, because the reaction takes place at an interface with steep concentration gradient.

2.5 Operational techniques

2.5.1 Simple SIA systems

SIA provides a robust methodology for performing automated wet-chemical analysis. Reagents, samples and wash solutions are selected sequentially using a selection valve and are drawn into a holding coil. The well stacked zones are then propelled through a reaction coil. The reaction products are then expelled through the flow cell of a suitable detector giving rise in the measurable signal. The selection valves used are multi port (up to 20 ports), meaning that single to multi zone SIA techniques may be employed.

Single zone: The analysis of chemical species that can be measured directly, such as those that

have a high molar absorptivity of light at a specific wavelength (e.g. concentrated hexavalent chromium) can be analysed using single zone sequential injection analysis [11]. The technique can also be used for pH determinations or methods where detection is done using non-selective electrodes or chemical sensors. In these types of analysis the sample is the only zone injected.

Double zone: Double zone sequential injection analysis depends on the addition of a single reagent. In this type of analysis, the sample and the reagent solution are the only two zones injected. Reaction stoichiometries of different complexes can easily be determined when using a two zone system [36, 51]. The main advantage of these methods above their FIA counterparts is their tedious and time-consuming process of changing the sample loops for every different ligand: metal ratio, is eliminated. Double zone SIA was also used by others [57-60].

Three zones: In a three zone sequential injection analysis systems, a sample and two reagents are involved. The order in which the different sequences of reagents are drawn up depends very much on the reactions involved. In the determination of ammonia using the indophenol blue method [45] a three zone sequence of sample, phenol and hypochlorite reagents was used. This sequence is in contrast with the manual and flow injection methods [61], where the hypochlorite reagent is first added to the sample. The importance of the correct zone sequence is also highlighted in the determination of phosphate [52], where the sample had to be drawn up first to ensure minimum reaction time between the molybdenum and ascorbic acid reagents. Three zone SIA was also applied by other researchers [62-64].

Multi zones: Although it is stated that three zones were the maximum to ensure effective mixing [4,16,19], Guzman and Compton [65] published an article where six zones were used in the

part of segmented auto analysers, flow injection and sequential injection systems [73]. In this work the influence of various parameters and dialysis efficiency was studied [43].

Dilution: On-line dilution with SIA has been evaluated using a dilution step as part of the timing sequence [16,72,74,75]. The manifold of the SIA system with the dilution coil is more complicated than the system including the dilution step. Control over there magnitude and range of dilution is effected by three volume parameters: sample volume, transfer volume and analysis volume [72].

Mixing chambers: Mixing chambers are often used in SIA when it is necessary to add several reagents serially [32] or when extensive dilution of the analyte is required [76]. The influence of a mixing chamber on dispersion and zone penetration in a SIA manifold has been studied [9,10, 60].

Although mixing chambers have contained undesired properties such as large dead volume and causing hold up effects, it offers some distinct advantages, for example, in the use of mixing liquids with different viscosities [77]. Mixing chambers were essentially used in SIA to dilute highly concentrated samples [72,75,78] or to ensure adequate mixing when three or more zones were involved [32,65,67,79]. Recently, mixing chambers were used to improve the degree of mixing even for cases where only two zones were used [51]. Increased mixing leads to an increase in dispersions [9,60].

Higher volume of mixing chambers is also an important parameter, since larger volumes resulted in larger dispersion, which is not always desirable [9,67,60].

Extraction: Peterson *et al.* [80] described a flow-based extraction method where an aqueous sample and organic solvent were sequentially injected into an extraction coil, mixed and separated due to the differential flow velocities of the aqueous and organic phases. The sequential injection extraction manifolds are much simpler than the ones used for FIA extractions, since no segmenters or phase separations were needed.

Sequential injection extraction (SIE) provides more economical use of reagent and sample solutions as well as simplified manifolds compared to those of flow injection analysis. SIE manifolds do not need phase segmenters and separators which do not only simplify the manifolds, but also exclude extensive dilution and other problems associated with these devices [81]. Despite the use of toxic organic solvents, the role of liquid-liquid extraction SIA in some areas will become indisputable.

2.6 SIA - Application

Since its birth SIA has been used extensively in evaluation of operational parameters (see 2.4), operational techniques (see 2.5) and applications to industrial, clinical agricultural, environment and pharmaceutical products. These applications range from simple to complex method designs, which may involve the incorporation of additional components, such as dialysers, hydride generators, mixing chambers, jet ring cell and currently solid - phase reactors in the SIA manifold.

The group at the University of Washington made a valuable contribution with the exploitation

of new sensor systems which broadened the scope of SIA tremendously and opened new horizons in the field of flow analysis.

First Scudder *et al.* [81] developed a fountain cell in fluorescence microscopy. A chemiluminescence system that combines the simplicity and reproducibility of SIA with the unique radial flow properties of the fountain cell was then successfully employed for the chemiluminescence determination of hydrogen peroxide and glucose [82]. The fountain cell design was further used as basis for a perfusion chamber to perform the characterization of planar concentration gradients in a sequential injection system for cell perfusion studies [83]. The group [15, 84] also innovated and designed a novel jet ring cell which was incorporated into a sequential injection system for automated immuno-assays and for pre-concentration of analytes on sorbents with *in situ* spectroscopic detection. The jet ring cell with a renewable solid support was connected to a sequential injection system to determine glucose amperometrically [85]. A renewable gas sampling interface (liquid droplet) coupled with a SIA analyser was used to determine ammonia [86].

SIA was also applied for the determination of total ammonium nitrogen and free ammonia in a fermentation medium [27], nitrites and nitrates [87-89], D-lactic acid in pork [90], glucose using sensor injection and amperometric detection [91] and cyanide using ion-selective electrodes [92]. Wine [93] and sugar [94] analysis were done using sequential injection (SI)-FTIR spectrometry. Sequential injection manifolds were also used to handle reagents for flourescence microscopic measurements [4].

Coupling of sequential injection analysis with inductively-coupled plasma mass spectrometry as

an analytical tool for trace element detection was used by Al-Swaidan [95]. The technique was applied for the determination of lead, nickel and vanadium at the part per billion level in sample solutions of Saudi Arabian crude oils. Hydride-forming elements were determined by direct current plasma atomic emission spectrometry based on a modified version of the sequential injection technique [96].

SIA was also employed as a sample preparation device, especially for high-performance liquid chromatography [97, 98]. Lukkari *et al.* [97] used solid-phase extraction on Aluminium oxide in a sequential injection system to purify pyrocatechol, protocatechuic acid, pyrogallol and gallic acid in black liquor. Sequential injection systems for the determination of mercury by cold-vapour atomic absorption spectroscopy [99,100] used special gas-liquid separation units for effective analysis.

Application of the SIA technique to anodic stripping voltammetry (ASV) alowed the on-line plating of the mercury film and therefore substantially reduced the generation of mercury waste [101]. Other potentiometric applications include the determination of glycerol and 2.3-butanediol in wine [102]. Primary explosive azides in environmental samples were determined amperometrically using a SIA system [103]. A sequential injection system used in speciation studies employed two detectors in series, namely a potassium ion-selective electrode and a flame emission spectrometer [104].

SIA was also extensively used for the monitoring of bioprocesses [6, 105-107], enzyme activity [108,109] and fermentation processes [110,111]. Immobilized enzyme reactors played a great part in sequential injection analysis [90, 112-116] as well as systems for medical and

pharmaceutical uses [117-123]. Other SIA methods were also used for medical and pharmaceutical uses [124, 125]. Vitamin C was monitored photometrically in a kinetic application involving an iron (II)-iron (III) reaction [126], while morphine was determined with a SIA system employing chemiluminescence detection [127]. Van Staden and McCormack [128] used a SIA system to determine amino acids spectrophotometrically. Chemiluminescence detection was employed in the preliminary analytical evaluation of novel reagents using a SIA system [129]. A method for determining the bromine (Br) number by coulometric flow-injection titrations, using sequential injection with sinusoidal flow is described by Tayler [130]. SIA was even applied to determine 90 Sr in nuclear waste [131].

Iron (II) was separated from a sample matrix by dialysis in an SI system [43]. The dialysed iron was complexed with Tiron and the resulting complex was monitored spectrophotometrically at 667nm. A sample frequency of 8 samples per hour and a detection limit of 45mg/ℓ iron (III) were obtained.

Dialysis was also used in the spectrophotometric determination of L(+)-lactate in wines [132]. In the determination of total ammonium-nitrogen and free ammonia in a fermentation medium, a two channel sequential injection system was used [21]. The streams were propelled by an *Alitea S2-V* two channel piston sinusoidal flow pump equipped with two cam driven parallel syringes. Two electrically actuated multi-position valves, a six port valve on the donor line and an eight port valve on the acceptor line, were used to direct the flow streams. A *Celgard 2400* hydrophobic membrane was used in the combined gas diffusion unit flow cell. Luo *et al.* [133] described the determination of gaseous ammonia using a glass diffusion denuder in an SI system.

Coupling of gas-diffusion separation and sequential injection analysis is applied to determine ammonia in aqueous environmental samples [54]. [The sample and an alkaline solution are sequentially aspirated using an automatic burette and mixed by flow reversal while being propelled to a gas-diffusion unit.]

Van Staden and du Plessis [55] described a sequential injection titration system for the titration of a strong acid with a strong base. The concept is based on the sequential injection of a base titrant, and analyte and a second base titrant zone into a distilled water carrier stream. A titration method without mixing or dilution is described by Holman *et al.*[134]. This method also involves the use of chemical sensing membranes.

In the determination of phosphate, in bio-processes, dilution (when required) was performed in a mixing chamber connected to the selector valve [79]. In the spectrophotometric catalytic determination of iodide in nutrition salts, Lima *et al.* [116] used a SIA system with mixing chamber for handling high concentration solutions.

Barbiturates (phenobarbital, amobarbital, pentobarbital and secobarbital) and serontonin re-uptake inhibitors (SRIs) - venlafaxine, paroxetine, sentraline and norsertraline - were extracted as model acidic and basic compounds from urine into a 1:4 (V:V) mixture.

Nakano *et al.* [56] combined wetting film extraction with colorimetry to determine nanogram amounts of molybdenum (VI). Using a very simple manifold a highly sensitive sequential injection system was developed. Molybdenum (which reacted with thiocyanate) was extracted in the first step into a toluene film as an ion paired complex. The thiocyanate ligands were

displaced by 1.5 diphenylcarbazone (DPC) to form intensely coloured product which was measured at 540 nm. Wetting film extraction was also used in the photometric determination of vanadium (IV) and vanadium (V) [136] and chromium (VI) and chromium (III) in water [136].

Grate and Taylor [137] described an on-line soil extraction procedure employing SIA. On-line extraction was performed with the soil placed in an open-ended column attached to the sample line.

Rubi *et al.* [138,139] described a sequential injection assembly for the determination of iron in natural waters. Iron was pre-concentrated on a micro-column packed with a chelating resin (chelex 100) that was inserted into the manifold. The SIA system offers automatic pre-concentration, elution, detection and data acquisition. Using a simple sequential injection method, ammonia was determined with conductometric detection [140]. The ammonia permeated through a gas permeable membrane and was collected (pre-concentrated) in a static acceptor stream.

In the determination of 90Sr, the 90Sr was separated from other radionuclides using a sorbent micro-column containing a resin that selectively binds 90Sr as a crown ether under acidic conditions [131]. The isolated 90Sr was then detected on-line with a flow through liquid scintillation counter.

In a SIA process control application, a mixing chamber connected to a fibre optic detector has proven to be successful for the determination of total biomass [27,14]. This mixing chamber or cell was used both as a dilution chamber and detection cell. In the simultaneous determination

of cobalt and nickel, a mixing chamber with a volume of 500 mℓ was used to ensure adequate mixing of the reagents before splitting the product zone in two [67]. A sequential injection analysis was developed for the spectrophotometric determination of thiocynate [143].

Taljaard [142] developed a sequential injection analyser to monitor iron and sulphate concentration in aqueous solutions and an sequential injection extraction system for the simultaneous determination of mercury (II) and cobalt (II) ; cadmium (II) and mercury (II) as well as the thiozone metals (lead (II) , copper (II) , zinc (II) , cobalt (II) , cadmium (II) , iron (II) and mercury (II)) in aqueous and soil samples.

A method for the resolution of binary mixtures of cobalt and nickel at low levels was performed by employing coupled on-line complex formation and ligand substitution reactions in a sequential injection analyser [67, 142].

The use of amperometric biosensors based on modified graphite paste has been used as detectors in the SIA. The enantioselective analysis of chiral drugs needs reliable methods, hence an automated system for the enantiopurity test of S-Captopril based on the concept of SIA with an amperometric biosensor as detector was developed [143]. A SIA method for the simultaneous determination of S- and R- captopril is proposed, an amperometric, enantioselective membrane electrode based on maltodextrin for the assay of S-captopril and an amperometric biosensor for the assay of captopril was developed [144]. Through using electrochemical sensors and biosensors, the reliability of the enantiomers assay is improved. The use of SIA has led to a reduction in reagents which are very expensive.

The use of solid-phase reactor incorporated into the SIA manifold is one of the areas in which very little has been done. Lukkari *et al.*[97] incorporated a solid - phase (Aluminium oxide) in a sequential injection system to purify pyrocatechol, protacatechuic acid, pyrogallol and gallic acid in black liquor. Shu *et al.* [145] developed a spectrophotometric method for the determination of lactic acid from industrial inorganics. A method for the simultaneous monitoring of glucose, lactic acid and penicillin [146] by SIA with glucose oxidase or lactate oxidase immobilised onto nylon tubing was developed. The online monitoring of glucose and penicillin [147] with immobilised glucose oxidase and penicillinase on a piece of nylon tubing from industrial organics was accomplished spectrophotometrically. Theophylline and caffeine [148] using a microcolumn packed with Micro High Q anion exchange beads were determined spectrophotometrically. The separation of radionuclides [149] using Sr-resin, TRU-resin and TEVA resin beads as slurry packed into a microcolumn was developed.

2.7 Conclusion

Sequential injection analysis introduced a new approach in conducting analysis by the sequencing of the sample and reagent zones in the flow conduit. Since, its introduction into the analytical field , it is evident from the amount of work done, that by careful manipulation of the system parameters, successful analyses can be conducted with SIA by controlling the degree of zone penetration and dispersion.

Sequential injection analysis has reached the point where a manifold that does not need changing can be designed and adapted for multi-reagent techniques and multi-detection systems without the need of reconfiguring the manifold. Different sample handling techniques were successfully

adapted to SIA with the incorporation of different components within the manifold to perform certain function.

The advantages of SIA over conventional FIA , is its more cost effectiveness in the use of reagents and samples, its versatility, robustness, flexibility for applying stopped-flow and reversed flow operations and the use of multiport selection valve. However, the sample throughput frequency of an SIA system is normally less than that of the conventional FIA system.

Further developments in SIA, involves the incorporation of solid - phase reactors in the SIA manifold, the use of biosensors as well as using versatile controlling software to manipulate sample and reagents in novel ways to achieve desired sample handling procedures.

SIA, surely has a very useful and bright future lying ahead if properly explored, especially with the use of biosensors and the incorporation of solid - phase reactors in the SIA manifold which is gradually gaining momentum. This, however, does not mean that SIA will replace FIA.

2.8 References

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CHAPTER 3

Super Serpentine reactors in Sequential Injection Analysis Systems

3.1. Introduction

Sequential injection analysis (SIA) is well known for its use as an analytical technique, having the advantage of reduced sample and reagent consumption compared to flow injection analysis (FIA). An important aspect that needs critical attention in the optimization of this system is the dispersion which could have a direct effect on zone penetration that takes place within the SIA conduit.

Reactors provide an extensive range of control of dispersion for the development and application of FIA/SIA methodologies. Control of dispersion is key in both the development and optimization of its analytical performance.

There are various reactors that have been used in SIA , straight tubes, helically coiled tubes, knotted tubes, mixing chambers, solid phases, single bead string reactors and Serpentine 8. Solid phase reactors will be discussed in full in chapter 4 and the other reactors will be briefly discussed in this chapter. However, this chapter will mainly focus on super Serpentine reactors, where the influence or effect of reactor type and length will be investigated on sensitivity and precision. Dispersion and zone overlapping will be paramount in this investigation.

3.2 Dispersion

An important cornerstone of the family of flow systems, in addition to reproducible timing and sample injection, is the concept of controlled dispersion. The purpose of controlling dispersion in a flow system is to optimize the chemical reactions taking place between the sample and reagents. In essence, what is called "controlled dispersion" is in fact the recognition that the sample is reproducibly diluted as it travels down the tubing. Dispersion is characterized by the concentration profile adopted by a zone or plug inserted at a given point in the system without stopping the flow.

Dispersion is the result of all physical forces acting on the injected sample zone. It is the process by which the sample zone transforms from a homogeneous geometrically well defined zone at the moment of injection, to the final zone that is detected down stream. Two processes are responsible for dispersion in the flow conduit, namely the physical process of material dispersion due to hydrodynamic processes taking place in the flow-through system and the chemical process of formation of chemical species. Dispersion of the sample is the product of three processes: laminar flow, secondary flow and molecular diffusion which results from the transportation of the sample.

3.2.1 Transport

The transport of matter along the tubes of a flow system is said to take place essentially by laminar flow [1] and not as a result of turbulent flow as thought earlier [2]. There are two

mechanism that contributes to dispersion of the injected sample, namely convective transport and diffusional transport [3].

(i) *Convective* transport occurs under laminar flow conditions. It yields a parabolic velocity profile with sample molecules at the tube walls having zero linear velocity and those at the centre of the tube having twice the average velocity. Fig 3.1 illustrates the two types of convective transport, namely turbulent and laminar flow. Laminar flow is also referred to as the axial dispersion and it is the result of viscous shear forces near the tube wall.

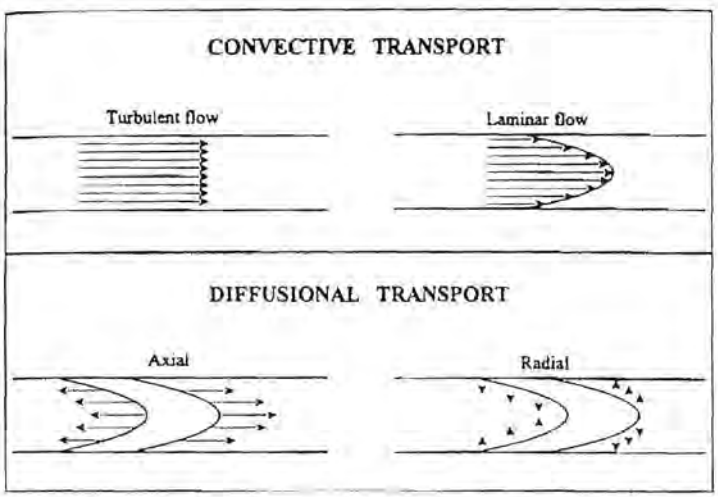

Fig. 3.1 General types of transport in closed tubes

(ii) *Diffusional* transport gives rise to axial and radial diffusion (Fig. 3.1) due to the presence of concentration gradient at the leading and trailing edges of the injected sample zone. It contributes insignificantly to the overall dispersion. Radial diffusion results from concentration differences perpendicular to the overall dispersion. It tends to balance concentrations in such a manner that the molecules located at the tube walls tend to move to the centre, whereas those

at the centre travel outwards. Indeed this motion slows down convective transport, thus hindering progressive dilution of the zone in the carrier stream.

3.2.2 Theoretical models

First of all, it is salutary to explain the difficulties involved in the theoretical predictions of the behaviour of the injected sample or reagent plug in practical systems. It is not an easy task to define the contributions of the elements such as the injection operation, connectors or geometry of the flow cell to the dispersion. All relations are therefore empirical in nature, nevertheless, several models have been developed for laminar conditions, to define the theoretical principles and derive mathematical expressions accounting for the physical behaviour of the injected plug. A number of these models are described in the following paragraphs. Table 3.1 gives the list of symbols that will be used in the discussion.

TABLE 3.1 List of symbols

Name	Symbol	Name	Symbol
Concentration (M)	C	Partial tube radius (mm)	r
Injected volume (mℓ)	V_i	Partial tube length (cm)	l
Initial concentration	C_p	Flow rate (mℓ/min)	q
Concentration at signal peak maximum	C_{max}	Linear velocity (cm/sec)	u
Molecular diffusion coefficient (cm^2/sec)	D	Maximum linear velocity (cm/sec)	u_o
Overall tube length (cm)	L	Mean residence time (sec)	$\bar{t}$
Tube diameter (mm)	d	Dispersion coefficient	**D**

Volume of system (mℓ)	V_r	Baseline-to-baseline time (sec)	Δt
Travel time (sec)	t_a	Vanderslice's accommodation factor	f
Tube radius (mm)	R	Residence time (sec)	T

3.2.2.1 Taylor' models

This model only holds for low flow rates and very long reactors, which help to compensate for radial concentration changes and favour the prevalence of diffusion phenomena. Taylor's model [3] is applicable to a Gaussian distribution defined by $C = f(t)$ in a form which depends on the chosen parameters, namely:

$$C = \frac{m}{4r^2\pi Dt} \exp\left[\frac{-(x - L)^2}{4Dt}\right]$$

and

$$C = \frac{C_0 V_i}{q\sigma(2\pi)^{1/2}} \exp \frac{(t - \bar{t}_r)^2}{2\sigma^2}$$

where m is the injected solute mass ($m = C_o V_i$), σ is the parameter corresponding to the standard deviation characteristic of a Gaussian distribution and x is the axial distance from the injection point. This model is only applicable if the injected volume is practically negligible compared with the reactor volume, thus $V_i \ll V_r$.

3.2.2.2 Tanks-in-series model

This model is analogous to the description of liquid chromatography in terms of theoretical plates. It relies on the assumption that the fluid flow passes sequentially through a large number (N) of mini chambers in which stirring is perfect (instantaneous mixing). The mathematical expression derived from this model [2] is:

$$C = \frac{1}{(\bar{t}_r)_N} \left[\frac{t}{(\bar{t}_r)_N}\right]^{N-1} \frac{1}{(N-1)!} \exp\left[\frac{-t}{(t_r)_N}\right]$$

$(\bar{t}_r)_N$ being the mean residence time of an element of fluid in a given tank. The larger N, the more Gaussian the profiles of the $C = f(t)$ curves become. Under this conditions, the variance is given by:

$$\sigma^2 = N(\bar{t}_r)_N^2 = \frac{(\bar{t}_r)^2}{N}$$

since the overall mean residence time is $\bar{t}_r = N(\bar{t}_r)_N$. The suitability of this model is rather questionable for small N values, i.e for reactors that are not very long.

3.2.2.3 Mixing chamber model

Pungor *et al.* [4] developed a mathematical model to describe the dispersion when there is a mixing mini chamber positioned close to or in the reactor itself. The concentration of a substance in the mixing chamber can be described as a function of time by:

$$\frac{d\Delta C_t}{dt} = \frac{V}{W}\left[\Delta(C_s)_t - \Delta C_t\right]$$

where t is the time (sec) from the moment of injection, $\Delta C_t = C_t - C_o$; C_t is the actual analyte concentration in the carrier stream on entry to the mixing chamber, C_o is the analyte concentration before injection, V is the flow rate (mℓ/min) and W is the volume of the mixing chamber.

Mixing chambers are often used in SIA when it is necessary to add several reagents serially [5] or when extensive dilution of the analyte is required [6]. The influence of a mixing chamber on dispersion and zone penetration in an SIA manifold has been studied [7, 8].

3.2.2.4 General model

The expression which takes place strictly into account both convective and diffusional transport and therefore best describes the overall physical dispersion phenomena is:

$$\frac{\delta C}{\delta t} = D\left[\frac{\delta^2 C}{\delta l^2} + \frac{\delta^2 C}{\delta r^2} + \frac{1\delta C}{r\delta r}\right] - u_0\left(1 - \frac{r^2}{R^2}\right)\left(\frac{\delta C}{\delta l}\right)$$

This expression takes into account axial and radial concentration gradients, as well as flow profiles under laminar flow regime. The left-hand side corresponds to diffusional transport, the

first term within the brackets accounting for axial diffusion (dependence of C on l) and the other two for radial diffusion (dependence of C on r). The first term on the right -hand side corresponds to build-up of matter, which only occurs in a non-safety regime, and the second term accounts for the contribution from convective transport for which the velocity profile is parabolic in shape and given by:

$$u = u_0(1 - \frac{r^2}{R^2})$$

The molecules at the tube walls ($r = R$) have zero velocity ($u = 0$), whereas those at the centre ($r = 0$) have the maximum velocity ($u = u_0$).

3.2.3 Practical definition of dispersion

The dispersion or dilution at the detector, of a sample injected into the flow, is given by the position and shape of the analyte signal band. Therefore, in practice it is the parameters characterizing the transient signal which are chosen to define the dispersion. An FIA peak is characterized, at least qualitatively, by:

a) its position, as defined by the travel time, t_{av}

b) its bandwidth, characterized by the baseline-to-baseline time, Δt, and

c) the co-ordinate of the band maximum (T, C_{max}).

Bearing in mind the general theoretical considerations and the description of the different models used to define dispersion, it is easy to understand the difficulty involved in relating them

to the experimental observations in a straightforward way.

3.2.3.1 Růžička's dispersion coefficient

This is the earliest parameter used to characterize passage of the sample through the system. The dispersion coefficient at the peak maximum (**D**) is defined as the ratio between C_0, the concentration of the dye injected into the system as it passes through the detector in an FIA system and when at maximum, C_{max} [3, 9], i.e.

$$D_{max} = \frac{C_o}{C_{max}}$$

The dispersion coefficient is useful in that it allows comparisons of different manifolds [9]. Furthermore, it provides a means to verify and monitor the degree of sample dilution resulting from any changes made to the manifold during method development. It is important to note that the definition of the dispersion coefficient considers only physical process of dispersion and not the ensuing chemical reactions.

3.2.4 Influence of various factors on the dispersion

The parameters that influences the amount of dispersion to be achieved in a flow conduit has been studied for both FIA [3, 11] and SIA [7, 12, 13]. These parameters essentially affects the sensitivity of the measured signal and the success of the reaction between the sample and reagent components. The effect of these parameters on dispersion in both FIA and SIA are very

similar as will follow clearly from the following discussion. The effect of the physical parameters on dispersion is usually investigated by injecting (FIA) or aspirating (SIA) a well-defined volume of a dye solution into a colourless carrier stream and evaluating the resulting signal of the dispersed dye zone, which is an indication of the amount of dispersion the sample has undergone.

It is clear that the analyst has complete control over the amount of sample dispersion or dilution that occurs as the sample passes through the manifold. This control originates from the way in which the manifold is designed. In the discussion below the factors that influences sample dispersion in the flow conduit are discussed. The experimental parameters have been classified according to whether they are representative of the sample, geometry of the system or hydrodynamic working conditions.

3.2.4.1 Sample volume

The introduction of increasing volumes of dye into an FIA system revealed the following information [3]:

a) the travel time is independent on the injected sample volume,
b) the residence time, and hence t', increases with the injected volume,
c) the baseline-to-baseline time also increases with the injected volume and
d) the dispersion coefficient decreases with increasing sample volume.

According to Růžička, **D** and V_i are inversely proportional to each other, i.e.

$$D = \frac{k}{V_i}$$

Růžička *et al.* [10] formulated the following rule to define the behaviour of different sample volumes in the flow conduit:

"**Rule 1.** Changing the injected sample volume is a powerful way to change dispersion. An increase in peak height and in sensitivity of measurement is achieved by increasing the volume of the injected sample solution. Conversely, dilution of overly concentrated sample material is best achieved by reducing the injected sample."

A similar conclusion was drawn from the dispersion experiments in an SIA system [7, 13], especially with regard to increased sensitivity, and decreased dispersion, with increasing sample volumes. It was also stated that the increase in sensitivity of the measurement will be possible only when sufficient reagent in excess is available in the element of fluid situated at the peak maximum [13].

3.2.4.2 Hydrodynamic factors

The flow rate *(q)* was related to the travel and baseline-to-baseline times through the following equations [3]:

$$t_a = \frac{k}{q^{1.025}}$$

and

$$\Delta t = \frac{k'}{q^{0.64}}$$

where the constants k and k' include, among other parameters, the accommodation factor, f, which is independent of the flow rate. As can be seen, t_a and Δt, and hence the dispersion, should decrease with increasing flow rate. Valcárcel *et al.* [3] indicated that the dispersion coefficient does indeed decrease with increasing flow rate contrary to Růžička *et al.* [11] who stated that:

"Rule 3. The dispersion of the sample zone increases with the square root of the distance travelled through the tubular conduit and decreases with decreasing flow rate. Thus, if the dispersion is to be reduced and the residence time is to be increased, the tube dimensions should be minimized and the pumping rate should be decreased..."

The increase in sensitivity with increasing flow rates has been demonstrated for SIA [7], thus confirming that which had been observed by Valcárcel *et al.* [3]. They stated further that the decrease in the dispersion coefficient with increasing flow rate, occurs in a non-linear fashion, except for large L and q values, in which case a slight increase in **D** with increase in q is observed.

3.2.4.3 Geometric factors

This section deals with the influence of the reactor shape (open, coiled, packed) its dimensions and the presence of a mixing chamber on the dispersion in a flow system. The function of these reactors is to increase the intensity of radial mixing, by which the parabolic velocity profile in the axial direction is reduced when the sample zone is injected into a laminar flow carrier stream [11]. Thus, the reagent becomes more readily mixed with the sample and the axial dispersion of the sample zone is reduced.

Relaxation of the laminar profile in the radial direction is best achieved by creating a local turbulence whereby the direction of flow is suddenly changed. This causes the elements of fluid that are falling behind because they are close to the walls of the channel to be moved into the rapidly advancing central streamline. The elements of fluid that have been advanced in an axial direction because they are close to the central streamline are repositioned closer to the tube wall. The more frequently this process is repeated, the more symmetrical the concentration gradient within the dispersed sample zone will be, and the peak shape will change from an asymmetric to a symmetric (Gaussian) one.

3.2.4.3.1 Straight tubes

Straight tubes represent the simplest situation. The influence of the reactor length, L, on t_a and Δt is predicted by the Vanderslice's expression[3]:

$$t_a = k L^{1.025}$$

and

$$\Delta t = k' L^{0.64}$$

It is evident from these expressions that an increase in reactor length is accompanied by an increase in t_a and Δt . The dispersion coefficient also increases with increasing reactor length, which is consistent with Růžička's expression,

$$\boldsymbol{D} = K L^{1/2}$$

The influence of the reactor diameter (R = d/2), is in agreement with Vanderslice's prediction [1], and it is evident that t_a and Δt are directly related to the diameter.

$$t_a = k d^2$$

and

$$\Delta t = k' d^2$$

The residence time and the dispersion coefficient both increased, with increasing tube diameter [3]. In SIA however, the tube internal diameter has been shown to have a more pronounced effect on the dispersion obtained than the length thereof [7, 14, 15].

3.2.4.3.2 Coils

When the reactor tube is coiled helically, the centrifugal force originating from the circulation of a fluid through the tube results in a radial-type flow as illustrated in Fig. 3.2. At low flow rates, the centrifugal force is not very great and the velocity profile is practically parabolic. At high flow rates the profile is completely different since molecules at the tube walls travel at a higher velocity than those at the centre of the tube.

Both situations result in a split circulation, symmetrical with respect to the ideal central plane of the tube. This circulation, especially fast at high flow rates, has been termed "secondary flow" [3]. It has the same effect as radial diffusion, thus tending to decrease the dilution or dispersion of the injected sample, a feature of great importance. It is observed that the smaller the coil diameter, the smaller the dispersion [3].

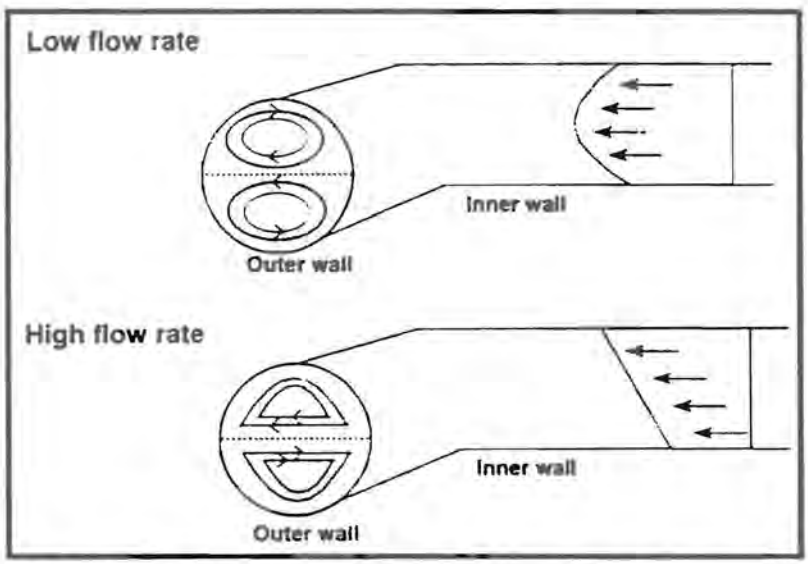

Fig. 3.2 Fluid dynamics corresponding to various in flow rate.

A coiled tube is the most frequently used reactor geometry, since it can conveniently accommodate any length of tubing in an experimental setup and also because secondary flow within the coiled tubing promotes mixing in the radial direction. The tighter the coiling of the tube is, the more pronounced this effect will be.

3.2.4.3.3 Knotted reactors

This type of reactor is also known as a three dimensionally disoriented (3-D) reactor. It is formed when a length of flexible tubing is knotted from end to end, resulting in a drastic decrease of dispersion. This may be due to the intensification of the dispersion-reducing effect introduced by coiling, since in effect the knots are very tight coils. The chaotic movement of the carrier stream through a spatially disoriented path promotes radial dispersion. The Serpentine 8 and super Serpentine reactors falls in this category. The Serpentine 8 reactor provides less axial dispersion and more radial dispersion compared to coiled reactors which are used when larger dilutions are required providing largely axial dispersion with minimal radial dispersion. It is intended for methodologies where less dilution, greater sensitivity, and higher sample throughput is desired. A single reactor may be wound on the frame as two separate reactors on the same frame. The reactor is easy to wind and knit to any length. The super Serpentine reactors will be discussed in detail later in this chapter.

3.2.4.3.4 Normal packed tubes

The behaviour of packed reactors is a well known in chromatography . The ratio, τ = tube diameter : particle diameter is a useful parameter to describe dispersion. For τ between 5 and

50 the axial dispersion is directly related to the particle size, the smaller the particle diameter, the smaller the dispersion. A major drawback though of this type of reactor results from the hydrodynamic resistance of the system, requiring high pressures and hence a technical sophistication.

3.2.4.3.5 Single bead string reactor

The single bead string reactor (SBSR) consists of ordinary Teflon tubes packed with tiny glass beads having diameters that are 60 - 80% of that of the tube. The chief effect of the beads is to increase radial dispersion, which reduces dilution of sample in the flow line, and therefore decreases dispersion [3]. This reactor provides several advantages, e.g. a significantly high sampling rate and dispersion that is approximately one -tenth of that corresponding to open tubes with the same dimensions. It does though also have certain disadvantages namely that small air bubbles and solid particles tend to be trapped in the SBSR, which may increase carry-over and flow resistance.

3.2.4.3.6 Mixing chamber

A mixing chamber with a magnetic stirrer is sought to achieve homogeneous mixing of sample and reagent. It has been shown that the mixing chamber contributes considerably to increasing dispersion zones both in FIA [11] and SIA [7, 8, 15] when introduced in the flow conduit. Růžička *et al.* [11] drew following conclusion regarding the use of a mixing chamber:

"Rule 4. Any continuous flow system that includes a mixing chamber generates larger

dispersion and yields a lower meuasurement sensitivity than a corresponding channel without a mixing chamber. A system with mixing chamber will also have a lower sampling frequency, unless the pumping rates are increased, which, in turn, requires large sample and reagent volumes."

The use of a mixing chamber is justified for the following reasons: first, substantial variations of injected volume, hold-up volume of the chamber and flow rate do not adversely affect the precision over a large dynamic range of dispersion coefficients (D up to 2000) [11]. The sample conditioning (density, viscosity, ionic strength, buffer capacity, etc.) is very efficient, because active stirring provides better compensation of matrix effects than the confluence technique commonly used with tubular reactors.

3.3 Super Serpentine reactors

3.3.1 Introduction

The super Serpentine reactor has four stitch patterns, hence super Serpentine I, super Serpentine II, super Serpentine III and super Serpentine IV (Figs. 3.3 -3.6). The stitch patterns have been designed (Global FIA) for optimum radial dispersion with minimal axial dispersion. It is intended for applications, such as trace analysis where high sensitivity and high sample throughput is desired. It provides the most narrow and highest peaks compared to Serpentine 8 reactors. It can be custom made to any length [17]. Marshall and van Staden [12] investigated the influence of pump speed, tubing diameter, reactor tube geometry and order of reagent and

sample zone injection on dispersion in the SIA manifold. Although Marshall and van Staden [12] studied the influence of the holding coil and reactor length have on dispersion, it was van Staden and Botha [7, 15] who made an in depth study thereof by also including their internal diameters. They [7, 15] also included flow rate, sample and reagent zone volumes. Gübeli *et al.* [13] also carried out studies on the effect of certain operational parameters on the dispersion in a SIA conduit. Van Staden and McCormack also investigated SBSR [16] as well as mixing chambers [8] for sensitivity and precision.

The objective of this study was to investigate the effect or influence reactor type and lengths have on sensitivity and precision [17]. Hence six reactors, straight tubes, helically coiled tubes, super Serpentines I, II, II and IV were investigated and their peaks overlayed for comparative studies. A Tiron-iron(III) complex solution was used. The dispersion profiles obtained were key to the results that follow in this Chapter.

3.3.2 Experimental

In this section the reagents and reactor preparations, instrumentation as well as procedure will be discussed.

3.3.2.1 Reagents and solutions

All reagents were prepared from analytical reagent grade unless specified otherwise. All aqueous solutions were prepared using de-ionised water from a Modulab system (Continental Water System, San Antonio, Texas).

3.3.2.1.1 Stock Iron(III) solution

A 7.2340 g of iron (III) nitrate [$Fe(NO_3)_3.9H_2O$] was dissolved in 0.01 mol/ℓ of perchloric acid. and made up to a litre. Working standards were prepared by suitable dilution of the stock solution with 0.01 mol/ℓ perchloric acid solution.

3.3.2.1.2 Perchloric acid solution

A 0.01 mol/ℓ perchloric acid solution was prepared by diluting 4.4 m ℓ of $HClO_4$ (Merck, GPR 70%) to 5 ℓ with double deionised water. The 0.01 mol/ℓ perchloric acid solution was also used as carrier.

3.3.2.1.3 Tiron solution

A 0.1 mol/ℓ Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid) stock solution was prepared by dissolving 3.3120 g of Tiron in 100 mℓof a 0.01 mol/ℓ perchloric acid solution. A 0.025 mol/ℓ Tiron solution was prepared by suitable dilution of the stock solution with 0.01 mol/ℓ perchloric acid solution.

3.3.2.2 Reactor preparation

The super Serpentine reactors were made in house from FEP tubing obtained from Global FIA. The platens on which the tubings were knitted were purchased from a local electronic shop (Pretoria, South Africa). The super Serpentine I stitch pattern is similar to a knotted membrane

reactor with "all overhand knots". Each repetitive stitch is horizontally joined.

Fig. 3.3 A diagram of a super Serpentine I stitch pattern

Super Serpentine II is similar to a knotted membrane reactor with alternating "overhand and underhand knots". Each repetitive stitch pattern is diagonally joined.

Fig. 3.4 A diagram of a super Serpentine II Stitch pattern

Super Serpentine III and IV do not really have a knotted reactor, however their unit stitch patterns are twice the size of the unit stitch pattern of super Serpentine II. They have knotted reactors with alternating outer and underhand knots.

To form a single unit stitch pattern of super Serpentine III, two units of super Serpentine II were horizontally joined. To form a single unit stitch pattern of super Serpentine IV, two units stitch pattern of super Serpentine III were diagonally joined.

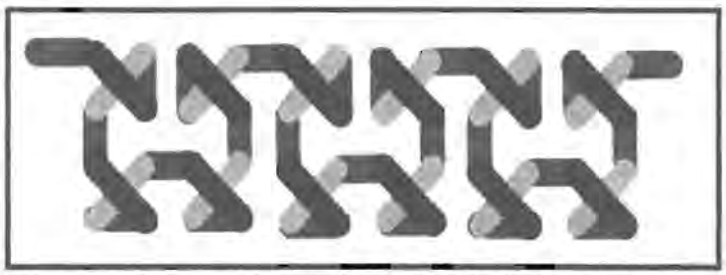

Fig. 3.5 A diagram of a super Serpentine III stitch pattern

To build a specific length of super Serpentine III, each stitch pattern unit of super Serpentine III was repetitively joined from inside to the outside alternately. To build a specific length of super Serpentine IV, each stitch pattern unit of super Serpentine IV was repetitively joined from outside.

Fig. 3.6 A diagram of a super Serpentine IV stitch pattern

3.3.2.3 Instrumentation

The sequential injection system depicted in Fig. 3.7A was constructed from the following components: A Gilson minipuls peristaltic pump (Model M 312, Gilson, Villiers-le-bel, France); a 10-port electrically actuated selection valve (Mode ECSDIOP; Valco Instrument, Houston,

Texas); and a Unicam 8625 UV/VIS spectrometer equipped with a 10 mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell (volume 80 $\mu\ell$) for absorbance measurements. The absorbance of the Tiron-iron (III) complex was measured at 667 nm.

The holding coil was of 3.0 m x 0.76 mm internal diameter coiled Tygon tubing. The reactors were: a straight tube, a coiled tube and super Serpentine I, II, III, and IV of various length (50 cm; 60 cm; 80 cm and 90 cm) constructed from a 0.76 mm inner diameter FEP tubing.

Computer aided flow analysis was carried out using a general purpose analogue and a digital input/output PC30-B interface board (Eagle Electric, Cape Town, South Africa) and a FlowTEK [17] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition.

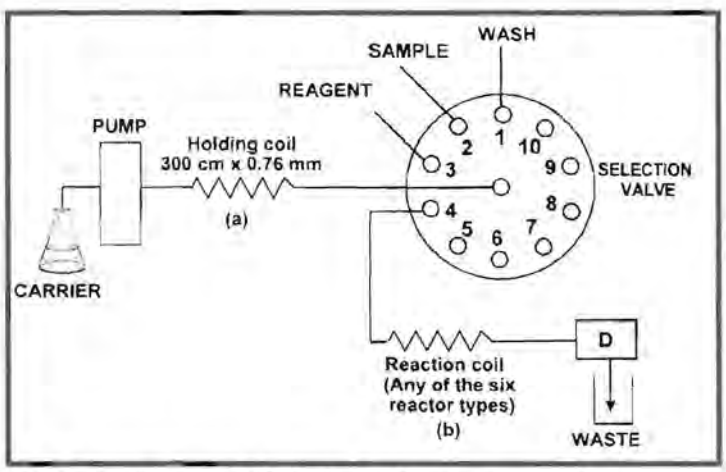

Fig. 3.7A A sequential injection analyses system used.

3.3.2.4 Procedure

Six reactor types were used in the SIA system. The carrier stream was a 0.01 mol/ℓ perchloric acid. The sample solution was iron (III) and the reagent Tiron. The concentration of both sample and reagent were kept constant during the whole process. The sample/reagent volume ratio was 1:1.25.

The experimental work was carried out by first drawing the wash solution (deionised water) followed by the sample solution [iron(III)] and then, the reagent solution (Tiron). These were flushed to the detector by the carrier (0.01 mol/mol/ℓ perchloric acid). The iron (III)-Tiron complex was detected at 667 nm using a UV/VIS spectrometer. This wave length was the maximum obtained when the solution was scanned between 100 and 1100 nm. The device sequence is shown in Fig. 3.7B and Table 3.2.

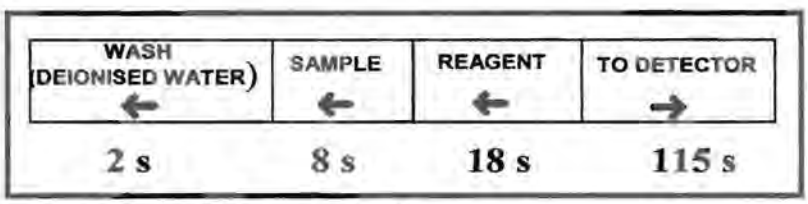

Fig. 3.7B A device sequence for one cycle.

The experiments were performed with the reactors incorporated in one of the two positions in the SI manifold shown in Fig. 3.7A. The two positions were: (a) between the selection valve and the pump, replacing the normal holding coil and (b) before the detector, replacing the normal

reaction coil between the valve and the detector. In position (a) the sample and reagent zones were stacked into the reactor as is the case with the normal holding coil. In position (b) the selection valve is switched to the detector and due to flow reversal the stack of zones from the holding coil mutually disperse and penetrate on their way to the detector. Four different flow rates were investigated (0.6; 1.2; 2.2 and 4.2 mℓ/min)

TABLE 3.2 Device sequence for one cycle

Time (s)	Pump	Valve	Description
0	Off	Position 1	Pump off. Select wash solution.
1	Reverse		Draw up wash solution.
3	Off		Pump stop.
4	Off	Position 2	Select sample stream.
5	Reverse		Draw up sample.
13	Off		Pump stop.
14	Off	Position 3	Select reagent stream. Valve
15	Reverse		Draw up reagent.
33	Off		Pump stop
34	Off	Position 4	Select detector line.
35	Forward		Pump all zones to detector.
150	Off	Position 1	Valve return home

3.3.3 Results and discussion

When studying sensitivity in SIA, the degree of sensitivity can easily be approximated by using the response which manifest itself in peak heights. It is known from FIA that the degree of

dispersion that occurs in an FIA flow conduit is equal to the inverse of the peak height. This means that the longer the peak height the smaller the resultant dispersion. Since SIA is seen as an extension of FIA, the same conclusion regarding the relationship between peak height and dispersion can be applied. Thus, by studying the resultant peak heights obtained, it becomes possible to make certain conclusions regarding sensitivity. The precision of the results was measured by determining the relative standard deviation of ten measurements at a given concentration of the iron (III)-Tiron complex.

The response and precision were studied as a function of length and reactor type using an iron (III)-Tiron complex. The results were the mean of 10 measurements. An investigation showed that no significant difference in the response was obtained for the different type of reactors when incorporated into position (a) between the selection valve and the pump. The results, however, showed that the reactor type had an influence on sensitivity when placed before the detector.

3.3.3.1 Influence of reactor type on ***sensitivity and precision***

Figs. 3.8A - 3.11D show the overlay of peaks that was investigated for sensitivity and precision. When each reactor type was considered separately, there was a general decrease insensitivity with increase in flow rate. The high response obtained at the highest flow rates corresponded to sharp peaks. This confirmed work already done [6, 11, 14]. However, when the six reactor types were compared to one another(overlay of peaks) for a given reactor length there was no definite pattern with the change in sensitivity with increase in flow rate. The reactor that gave the best response at the lowest flow rate (compared to other types of the same length), did not necessarily maintain that as flow rate increased. The behaviour of the different types of reactors

was first grouped according to reactor length and the observation accordingly discussed below for each reactor length.

3.3.3.1.1 50 cm reactors (Figs. 3.8A- 3.8D)

Figs. 3.8A to 3.8D shows the overlay of peaks for 50 cm reactors at flow rates of 0.6, 1.2, 2.2 and 4.2 mℓ/min respectively. The results show clearly that for the lower flow rates (0.6 mℓ/min, Fig. 3.8A; 1.2 mℓ/min, Fig. 3.8B) super Serpentine IV reactor gave the best response.

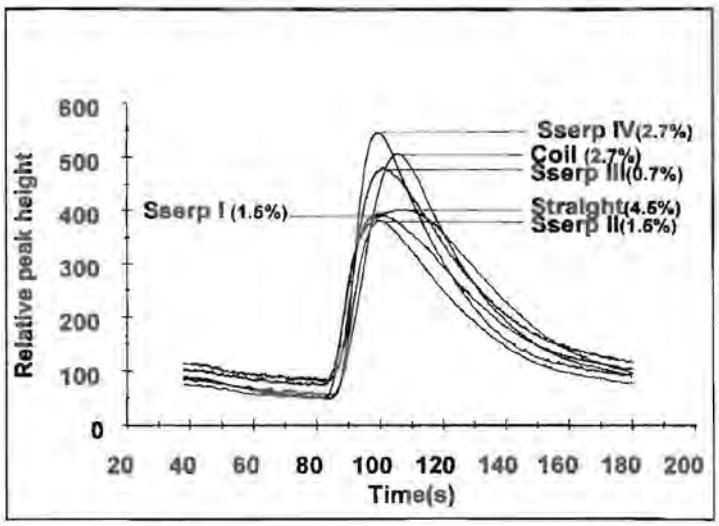

Fig. 3.8A Influence of 50 cm reactor types on response and precision at 0.6 mℓ/min

However, at 0.6 mℓ/min (Fig. 3.8A) super Serpentine III gave the best precision. When the flow rate was increased to 1.2 mℓ/min the precision of all reactors improved (Fig. 3.8B), with super Serpentine I giving the best precision.

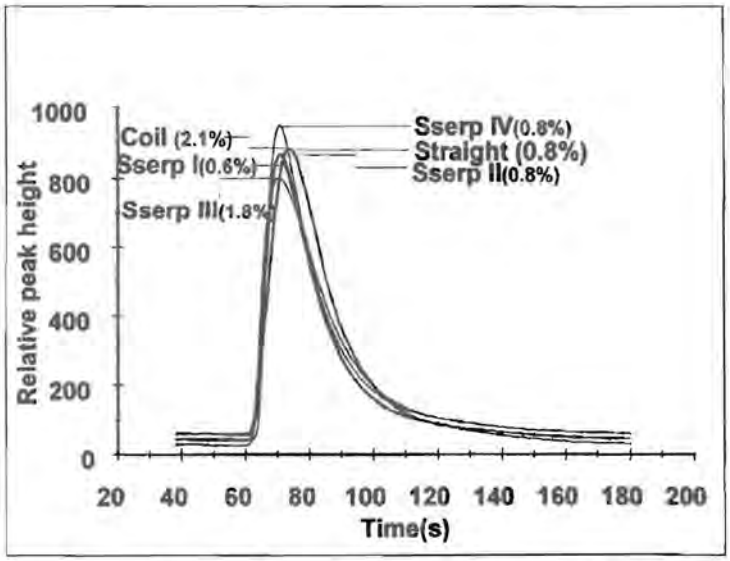

Fig. 3.8B Influence of 50 cm reactor types on response and precision at 1.2 mℓ/min

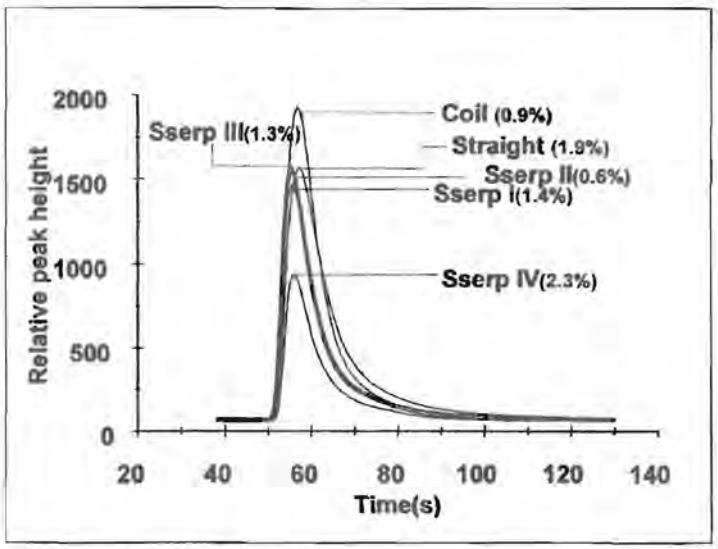

Fig. 3.8C Influence of 50 cm reactor types on response and precision at 2.2 mℓ/min.

At a flow rate of 2.2 mℓ/min (Fig. 3.8C) and 4.2 mℓ/min (Fig. 3.8D), it was the coiled tube reactor that gave the best response. It was, however, super Serpentine II (Fig. 3.8C) and super Serpentine III (Fig. 3.8D) that gave the best precision respectively. From these observations it is clear that super Serpentine III was on average the best reactor for this length. However, super Serpentine IV emerged the best at a flow rate of 1.2 mℓ/min in both precision and response.

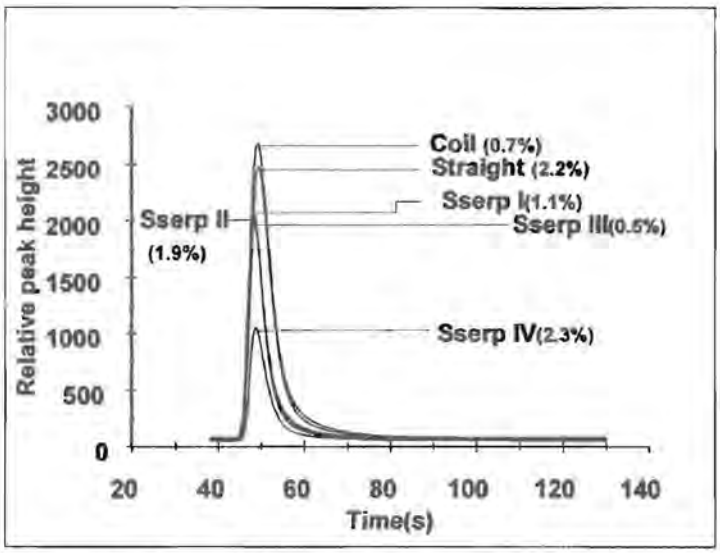

Fig.3.8D Influence of 50 cm reactor type on response and precision at 4.2 mℓ/min.

3.3.3.1.2 60 cm reactors (Figs. 3.9A-3.9D)

Figs. 3.9A to 3.9D shows the overlay of peaks for 60 cm reactors at flow rates 0.6, 1.2, 2.2 and 4.2 mℓ/min respectively. It is clear from the results obtained (Figs. 3.9A- 3.9D) that super Serpentine IV is by far the most reliable reactor with the best precision for the 60 cm reactor at flow rates between 0.6 and 4.2 mℓ/min. At flow rates of 0.6 and 1.2 mℓ/min, super Serpentine II

gave the best response, but a bad precision. On the other hand, super Serpentine IV gave a better precision (Figs. 3.9A and 3.9B).

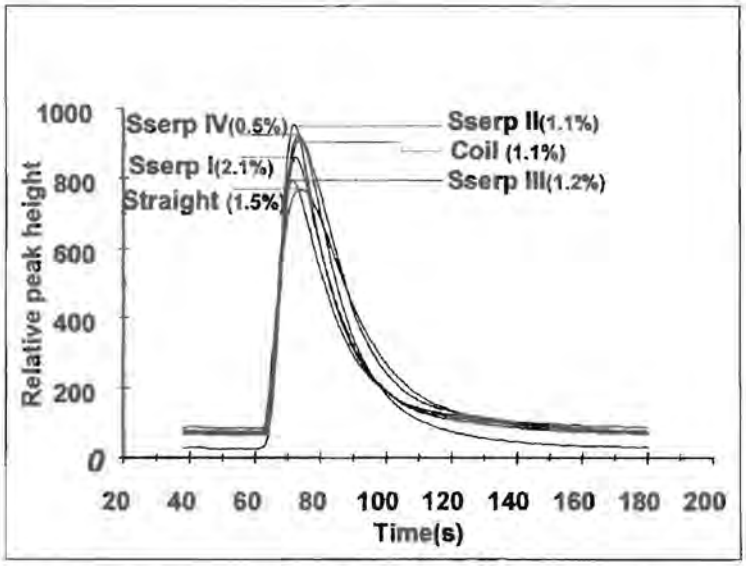

Fig. 3.9A Influence of 60 cm reactor types on response and precision at 0.6 mℓ/min.

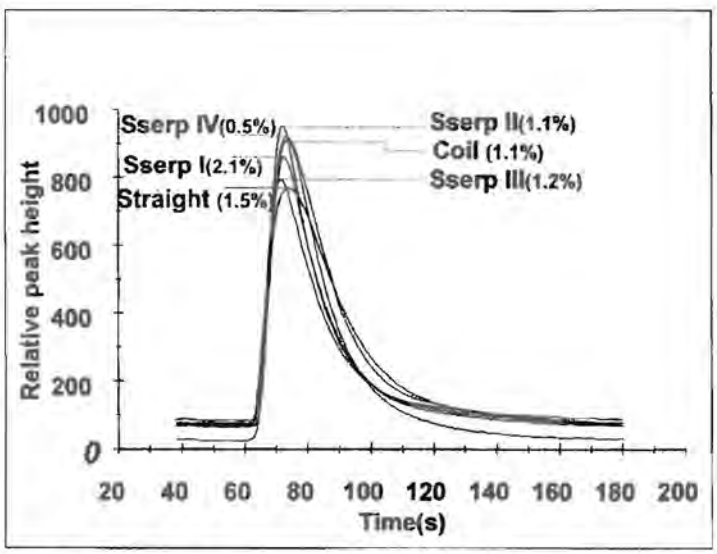

Fig. 3.9B Influence of 60 cm reactor types on response and precision at 1.2 mℓ/min.

At a flow rate of 2.2 mℓ/min, the coiled tube reactor gave the best response. However, it was super Serpentine IV with the second best response that gave the best precision (Fig. 3.9C).

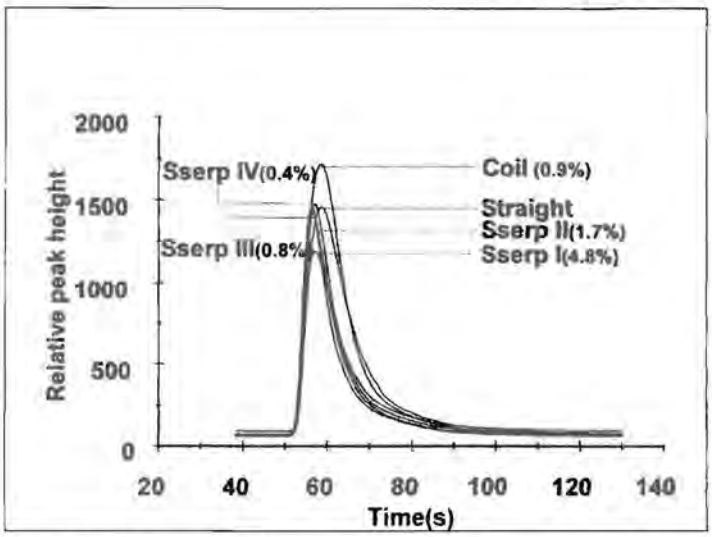

Fig. 3.9C Influence of 60 cm reactor types on response and precision at 2.2 mℓ/min.

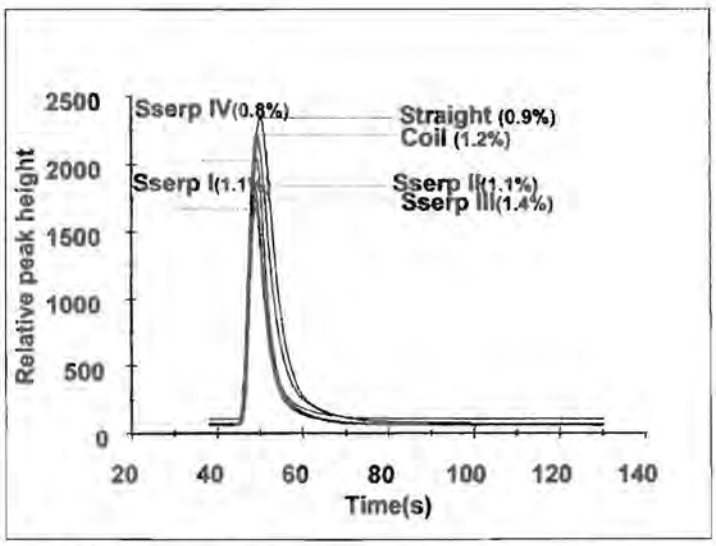

Fig. 3.9D Influence of 60 cm reactor types on response and precision at 4.2 mℓ/min.

When the flow rate was increased to 4.2 mℓ/min, the best response came from the straight tube reactor and the best precision from super Serpentine IV (Fig. 3.9D).

3.3.3.1.3 80 cm reactor (Figs. 3.10A-3.10D)

Figs.3.10A to 3.10D shows the overlay of peaks for 80 cm reactors at flow rates 0.6, 1.2, 2.2 and 4.2 mℓ/min respectively. Super Serpentine IV gave the best response at a flow rate of 0.6 mℓ/min. However, the percent relative standard deviation (%RSD) for all the reactors at this flow rate was above 0.9% (Fig. 3.10A).

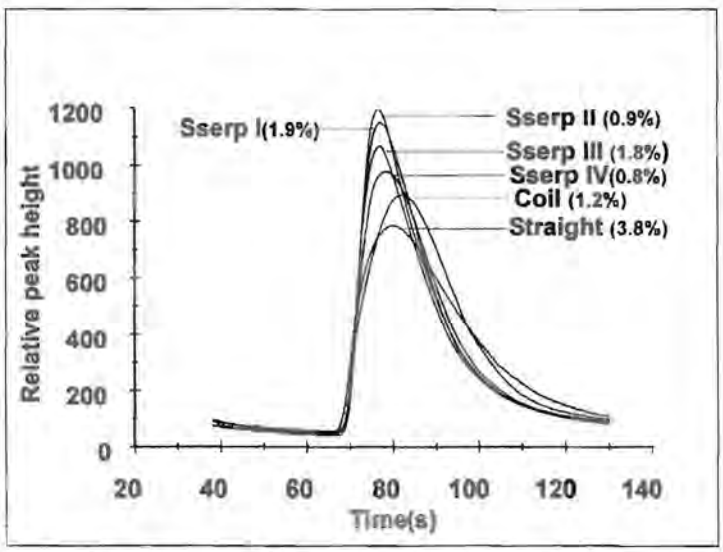

Fig. 3.10A Influence of 80 cm reactor types on response and precision at 1.2 mℓ/min.

When the flow rate was increased to 1.2 mℓ/min, super Serpentine II gave the best response (Fig. 3.10B). When the flow rate was increased to 2.2 mℓ/min, the coiled tube reactor gave the best response (Fig. 3.10C). At a flow rate of 4.2 mℓ/min, the straight tube reactor gave the best

response and super Serpentine III gave the best precision (Fig. 3.10D).

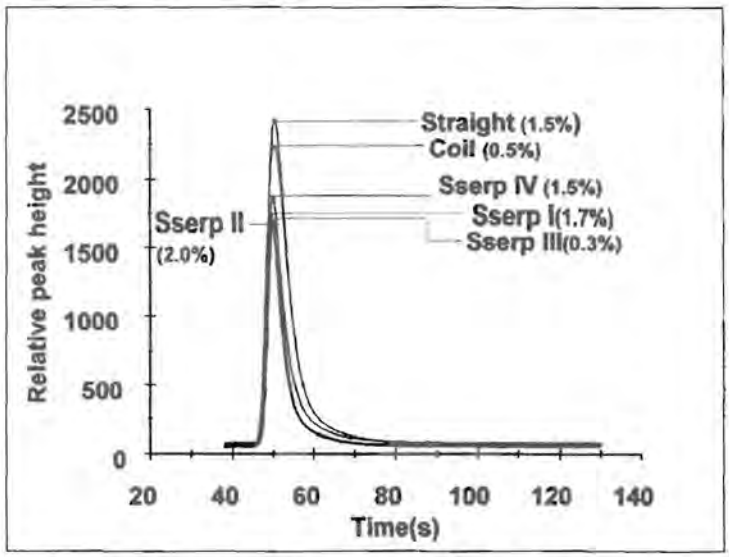

Fig. 3.10B Influence of 80 cm reactor types on response and precision at 1.2 mℓ/min.

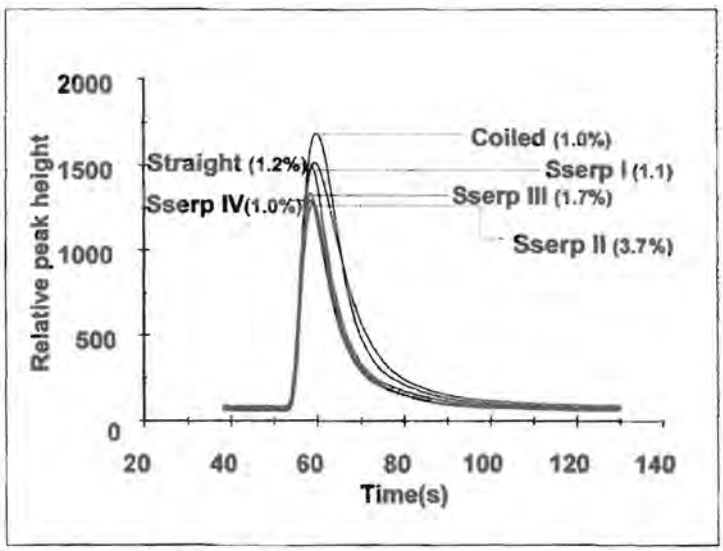

Fig. 3.10C Influence of 80 cm reactor types on response and precision at 2.2 mℓ/min.

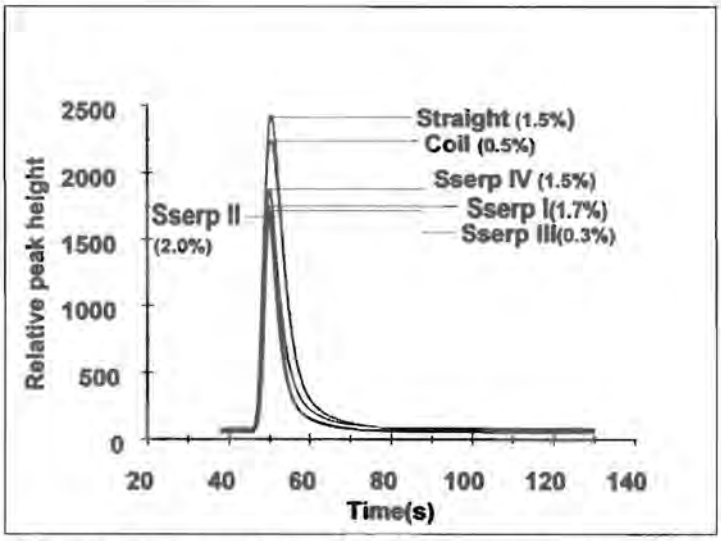

Fig. 3.10D Influence of 80 cm reactor on response and precision at 4.2 mℓ/min.

3.3.3.1.4 90 cm reactor (Figs. 3.11A -3.11D)

Figs. 3.11A to 3.11D shows the overlay of peaks for 90 cm reactors at flow rates 0.6, 1.2, 2.2 and 4.2 mℓ/min respectively. At a flow rate of 0.6 mℓ/min, super Serpentine III gave the best response and super Serpentine I gave the best precision (Fig3.11A). When the flow rate was increased to 1.2 mℓ/min super Serpentine II gave the best response and the coiled tube reactor the best precision (Fig. 3.11B).

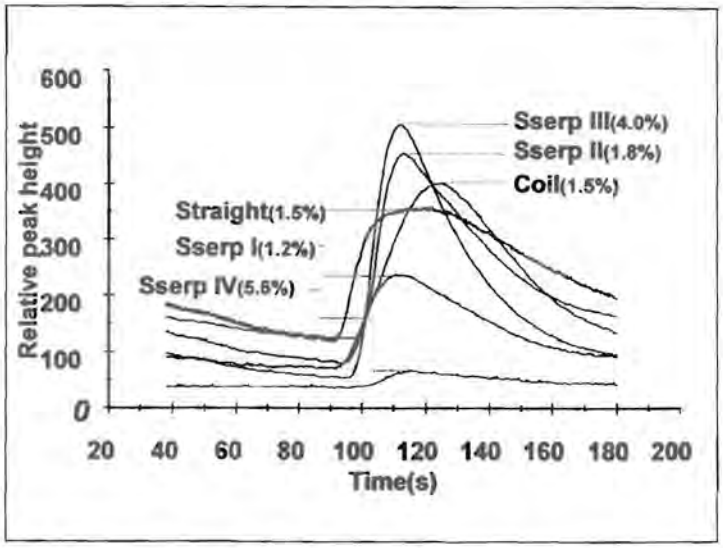

Fig. 3.11A Influence of 90 cm reactor types on response and precision at5 0.6 mℓ/min.

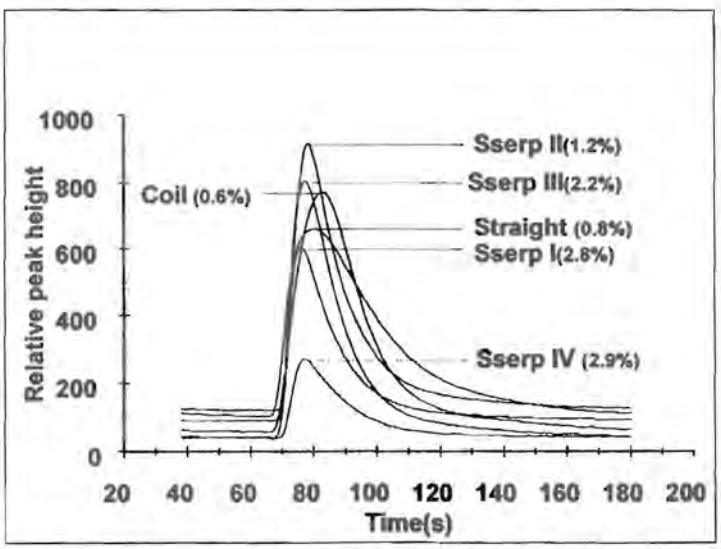

Fig. 3.11B Influence of 90 cm reactor types on response and precision at 1.2 mℓ/min.

At a flow rate of 2.2 mℓ/min the coiled tube reactor gave the best response, while super Serpentine

I gave the best precision (Fig 3.11C). When the flow rate was increased to 4.2 mℓ/min the straight tube reactor gave the best response (Fig. 3.11D).

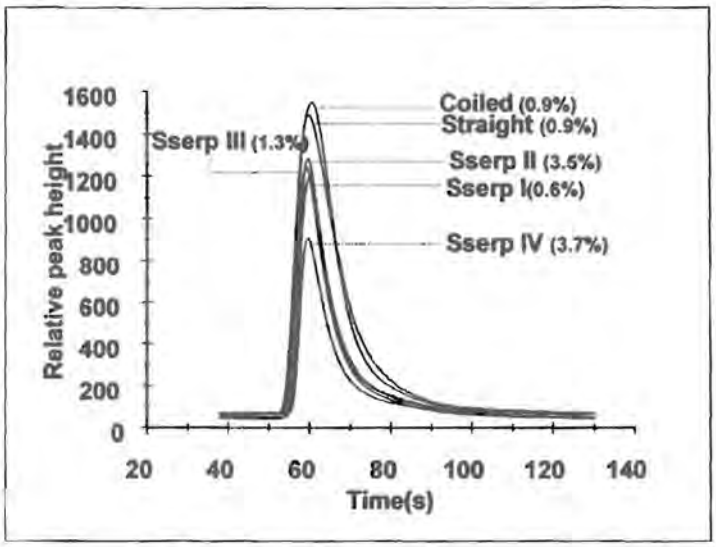

Fig. 3.11C Influence of 90 cm reactors on response and precision at 2.2 mℓ/min.

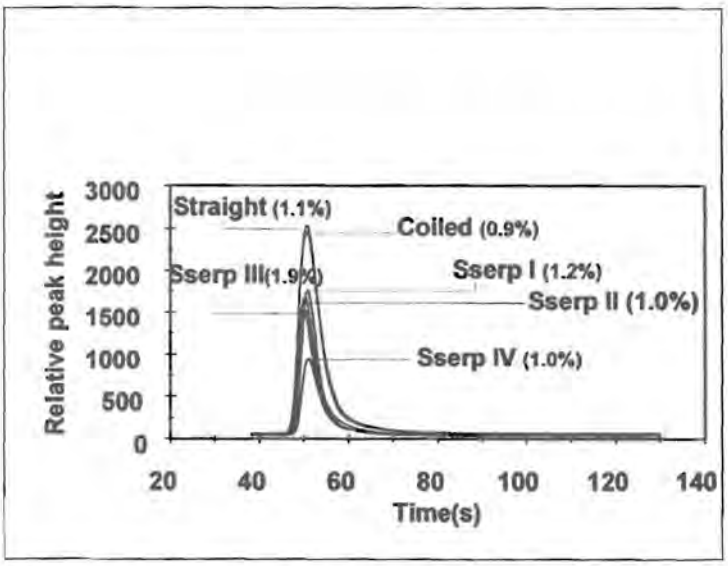

Fig. 3.11D Influence of 90 cm reactor types on response and precision at 4.2 mℓ/min.

Despite all these, the precision shown by super Serpentines was very good except for super Serpentine III where %RSD was close to 2%.

3.4 Conclusion

When the six reactor types of the same lengths were compared by overlaying their peaks, no consistency was observed with increase in the flow rate. The response from each reactor type also differed at different flow rates. However, some reactors did show some consistency just for two consecutive flow rates. The reactor with the highest response did not necessarily give the best precision.

Furthermore, when overlaying peaks of each super Serpentine of different lengths at a given flow rate, there was no clear correlation between the response obtained and the reactor length. Similar observations were made with the precision.

The overlayed peaks revealed that the longest super Serpentine reactors gave the highest response at the lowest flow rate. The shortest super Serpentine reactors gave the highest response at the highest flow rates with a relatively good precision.

From all these, it may be said that the choice of a suitable super Serpentine reactor for the development of method for trace analysis is important. A specific reactor has to be defined with regard to length and flow rate at the same time. Super Serpentine reactors were intended for applications such as trace analysis where high sensitivity and /or high throughput is desired

(Global FIA). Thus one has to know the type of trace analyte intended, throughput, sensitivity and precision so desired to effectively accomplish the analysis. Hence the simultaneous choice of reactor type and length plays an important role with regard to sensitivity and precision and hence dispersion. The sensitivity and precision obtained for the various super Serpentines may with proper choice of the reactor accomplish this objective.

3.5 References

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