

**Sequential Injection Analysis: Evaluation of operational parameters and
application to process analytical systems**

by

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SYNOPSIS

The increasing amount of research that is done on the development of process analysers, verifies the importance thereof in the various fields that require analytical measurements to be performed. Analyses need to be done faster, more precise, accurate and economically. The strict requirements that must be submitted to for a process analyser to be acceptable in the process environment, places an enormous responsibility on researchers to surpass themselves when developing new systems.

The development of a process analyser is based on a certain need that is expressed by either the clinical, pharmaceutical, industrial or agricultural fields. The control of their processes and products are essential since it undoubtedly influences the quality and safety of human lives, either directly or indirectly.

The aim of this research was to investigate the application of the sequential injection analysis technique as a process analyser for the determination of copper(II) and thiocyanate as industrial pollutants and subsequently for environmental monitoring. In the first instance it was necessary to evaluate the parameters that influence the system since these directly affect the success with which analysis can be performed.

Although the sequential injection technique delivers a lower sampling frequency compared with its parent technique flow injection analysis, it does present several excellent features and advantages which are highlighted during the course of this study.

**Sekwensiële-inspuitanalise: Evaluering van sisteem parameters en
toepassing as proses-analitiese-sisteme**

deur

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SAMEVATTING

Die toenemende hoeveelheid navorsing wat op die ontwikkeling van prosesanaliseerders gedoen word, bevestig die belangrikheid daarvan in die onderskeie velde wat analitiese metings vereis. Dit is nodig om analyses vinniger, meer presies, akkuraat en ekonomies uit te voer. Die streng vereistes waaraan prosesanaliseerders moet voldoen om aanvaarbaar te wees in die prosesomgewing, plaas 'n groot verantwoordelikheid op navorsers om hulself te oortref by die ontwikkeling van nuwe sisteme.

Die ontwikkeling van 'n prosesanaliseerder is gebaseer op 'n spesifieke behoefte wat deur die kliniese, farmaseutiese, industriële of landbougebiede weerspieël word. Die beheer van prosesse en produkte is belangrik, aangesien dit die kwaliteit en veiligheid van menselewens, direk of indirek, beïnvloed.

Die doel van die navorsing was om die toepassing van die sekwensiële-inspuitanalisetegniek as 'n prosesanaliseerder vir die bepaling van koper(II) en tiosianaat in industriële besoedeling en gevolglik as omgewingsmonitor te ondersoek. In die eerste instansie was dit nodig om die parameters wat die sisteem beïnvloed te evalueer, aangesien dit die sukses waarmee analyses uitgevoer word, direk beïnvloed.

Al lewer die sekwensiële-inspuitanalise tegniek 'n laer monsterfrekwensie as vloeï-inspuitanalise, het dit wel verskeie uitstaande kenmerke en voordele wat uitgelig word met verloop van die studie.

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“The LORD himself will lead you and be with you. He will not fail you or abandon you, so do not lose courage or be afraid” : Deuteronomy 31:8

BOTHA, A.

SEQUENTIAL INJECTION ANALYSIS: EVALUATION OF
OPERATIONAL PARAMETERS AND APPLICATION TO
PROCESS ANALYTICAL SYSTEMS

MSc

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Sequential Injection Analysis: Evaluation of operational parameters and application to process analytical systems

by

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CHAPTER 1

Sequential Injection Analysis: The New Approach Towards Performing Flow Analysis

1.1 Introduction

The ever increasing demand for analyses in clinical, agricultural, pharmaceutical, industrial and process analytical control played an important role in the development of flow analysis systems. The need for process analysers that were able to process large amounts of samples continuously and automatically and deliver accurate and precise results with regard to the sample contents were ever growing.

Effective cost control was also an important aspect that demanded serious attention. Not only does it affect the 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 a clean sustainable environment must be maintained [1]. These needs triggered the decision to develop automated systems capable of fulfilling the set requirements of real time monitoring and control of processes on an analytical base.

Any measurement in a chemical laboratory involving liquid comprises the following operations: solution handling, analyte detection, data collection and computation of results. There was no shortage of computers and sophisticated detectors to aid chemists in performing the latter two tasks, but solution handling required an arsenal of skills that seemed virtually impossible to automate. The problems of the incompatibility of modern analytical instrumentation with the unwieldiness of manual solution handling (“beaker” chemistry) had to be faced.

Flow operations were relatively easy to automate, since they replaced the mechanical handling of oddly shaped (and often fragile) containers by sequential movements of liquids in tubes. Flow operations were also much easier to miniaturize by using small bore tubing and the micro-volumes could be conveniently manipulated and metered by pumping devices, which (unlike pipettes) were not affected by surface tension (or by shaking hands) [2]. Flow operations were much easier to control in space and time, since using closed tubing avoided evaporation of liquids, provided exactly repeatable paths through which measured solutions moved, and provided an environment for the highly reproducible mixing of components and formation of reaction products.

Flow analysis is nowadays a well used technique employed in many branches of chemistry. Since the original paper published by Skeggs [3] on multi-segmented continuous flow analysis (CFA), many improvements and even simplifications have been made. Flow injection analysis (FIA), introduced by Růžicka and Hansen [4] was a very important one and due to its simplicity, versatility and low cost achieved great acceptance. CFA differed conceptually from FIA because in CFA the measurements could be made under conditions of chemical equilibrium, steady state and with minimized sample dispersion. In fact, the segmentation of the sample by air was used

to provide these facilities.

With FIA though, which does not employ air segmentation, measurements could be performed without attaining chemical equilibrium and, therefore, simpler manifolds could be designed, enabling higher sampling frequencies with lower reagent and sample consumption. In 1985, Pasquini *et al* [5] proposed monosegmented-flow analysis (MSFA), which embodied the advantageous features of both FIA and CFA. In MSFA, sample and reagents are injected into a reaction coil between two air bubbles, providing facilities to maintain various sample zones in the analytical path, with insignificant carry over. Under these conditions, long residence times (up to 10-15 minutes) could be achieved, with minimal sample dispersion and a high sampling frequency. High sampling frequencies and low reagent consumption were among the main objectives to be attained by flow analysis, from the mid-70s to the end of the 80s [6].

Despite the numerous advantages that characterized flow injection analysis, it did though present a few obvious drawbacks like the sometimes excessive consumption of sample and reagent and the complexity of the flow manifolds that necessitated the use of multi-channel pumps and plumbing schemes. Although these devices functioned well in the laboratory, they were not entirely acceptable in the industrial environment [7].

Consequently Růžička and Marshall [7] developed the principle of sequential injection analysis (SIA) to circumvent some of the obstacles presented by its predecessors. Robustness, versatility and simplicity are the main features of SIA and makes it ideally suited as a process analyser. The sampling frequency is however lower than that of the usual flow systems.

It is useful to briefly review the batch and continuous flow analysers that were initially designed to perform the automated analysis and in certain instances used as process analysers. This will allow us to identify some of their advantages and shortcomings and to support the development of SIA.

1.2 Discrete analysers

Batch methods are one of the first mechanized versions of manual procedures. In a batch analyser, each discrete sample is assigned a container (sample cup or sample bag), within which it is held through all the steps necessary to perform the analysis. The different analytical stages (dilution, reagent addition, mixing, heating) take place through mechanical processes, which transfer each cup in turn to a unit that dispenses the reagent(s), dilutes and mixes the solution, and then to the detector (colorimeter, potentiometer, etc.) where a signal corresponding to the analyte concentration is obtained and recorded [8]. This approach is illustrated in Fig. 1.1.

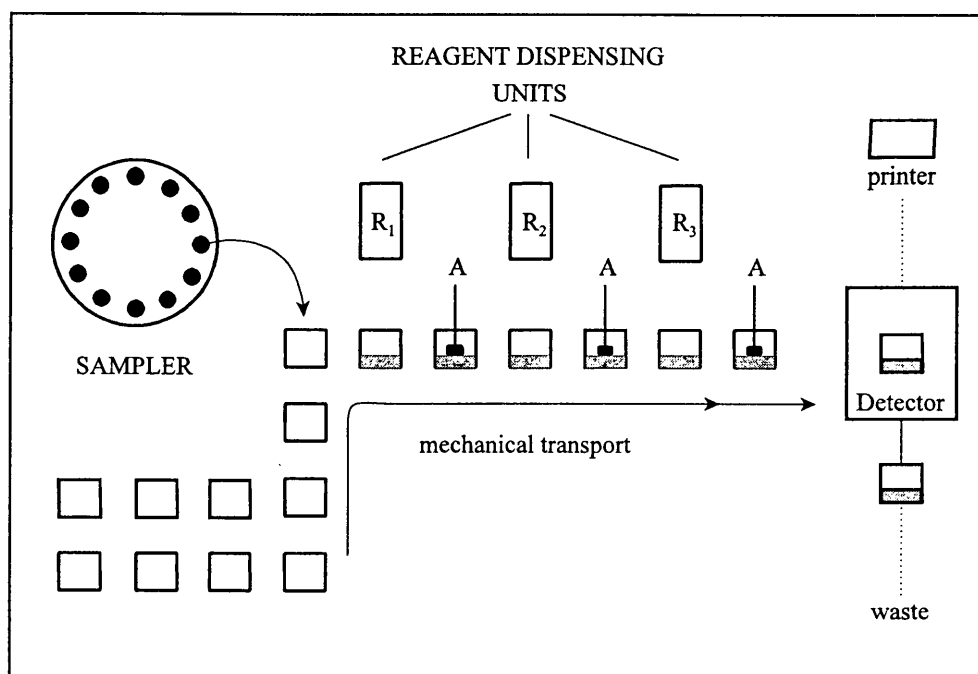


Fig.1.1 Schematic diagram of the batch automatic method of analysis.

An advantage of this approach is that cross-contamination between samples is not possible. The samples preserve their identity and cannot be mismatched, since each container carries an identification label.

Despite the advantages of the discrete approach, such as the ability of some of the instruments to perform assays via random access and the capability of stat operation, it does though have certain drawbacks. The disadvantages of the batch analysers include mechanical complexity, several moving parts that may become worn, components that must be precisely machined and the high cost of operation. This results in the limited lifetimes thereof [2] and that the cost of the various components of these systems were sometimes above the acceptable limit for the strained budgets of most clinical laboratories. In addition, these machines are seldom used outside the laboratory.

1.3 Continuous flow analysis

Continuous flow analysis constitutes a group of techniques that has automated sample handling. In its broadest context, the term continuous flow analysis refers to any process in which the concentration of analyte is measured uninterruptedly in a stream of liquid (or gas) [2]. For serial assays, successive samples are introduced into the same path (length of tubing) in the analyser, with the reagents being added at strategic points and the mixing and incubation taking place while the sample solution is on its way towards a flow-through cuvette, where the signal is monitored continuously and recorded.

The greatest difficulty to overcome in continuous flow analysis was to prevent intermixing of adjacent samples during their passage through the analyser conduits. Continuous flow methods presented an added advantage to automated analysis in that it can perform dialysis, distillation, solvent extraction and other types of separations that could not be achieved by the standard batch methods. Also, the continuous flow analyser is made with fewer moving parts, because it is the liquid that is in motion and therefore the instrument is mechanically much less complex and easier to construct than a batch analyser.

The greatest drawback of the continuous flow analyser is the inability to randomly access the assay. A single channel analyser requires a large number of samples to be collected for an assay of a single analyte. Another analyte in the same batch of samples, can be analysed only after the analyser has been reprogrammed (by changing manifold components, reagents etc.). It was subsequently necessary to design multi-channel analysers with a large number of parallel channels, each for a different analyte. The aspirated sample is split into parallel channels which

run simultaneously all the time. The unfortunate feature of such a design, besides its complexity, is its inability to perform a single selected test [2].

There are two general types of continuous flow methods: segmented and unsegmented. A short discussion on both types follows.

1.3.1 Segmented continuous flow method

Segmented continuous flow includes the introduction of an air segment [9] that divides the flowing stream into a number of compartments that are separated by air bubbles, as illustrated in Fig. 1.2. In such systems segmenting allowed the identity of each individual sample plug to be preserved (limited sample dispersion). Additionally, the segmented CFA is normally operated under steady-state chemical conditions.

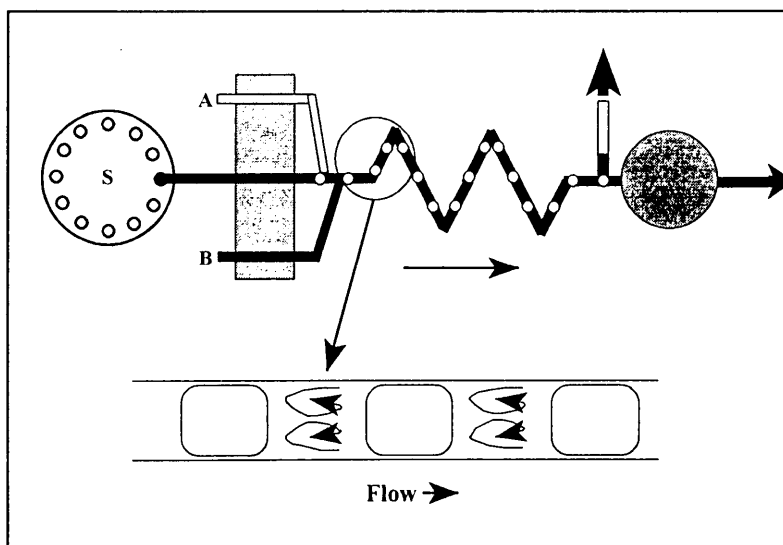


Fig.1.2 A typical continuous flow air-segmented manifold where sample, air and reagent are pumped to provide segmentation between the air and sample-reagent segments. The bottom picture depicts the mixing process.

The primary drawback to air-segmented systems is the bubble. These air bubbles are compressible, thereby creating pulsations in the flowing stream. In addition, for most detectors, the air bubble must be removed before the sample passes through the detection cell. It has also been difficult to control the precision of bubble size. This variation in bubble size adds to the irreproducibility of the system [10]. The lack of exact timing, which is mainly due to air segmentation and to sample transport through the pump, leads to a loss of sampling frequency. These drawbacks made this type of system inappropriate for application as reliable and precise process analysers.

1.3.2 Unsegmented continuous flow method

The term unsegmented flow methods refer to the flow injection analysis methods and differ from segmented flow methods in that the flow is not segmented by air bubbles. The sample is injected instead of aspirated into the flow conduit and neither physical (flow homogenization) nor chemical equilibrium has been attained by the time the signal is recorded. The injected sample forms a zone that disperses and reacts with the components of the carrier stream as it is transported through the flow system, to form species that is sensed by a flow-through detector that continuously records the absorbance, electrode potential, or other physical parameter as it continuously changes due to the passage of the sample material through the flow cell.

The simplest flow injection analyser, shown in Fig. 1.3, consists of the following components:

- a pump that is used to propel the carrier stream through a narrow tube,
- an injection port, by means of which a well-defined volume of a sample solution is injected into the carrier stream in a reproducible manner and
- a micro-reactor in which the sample zone disperses.

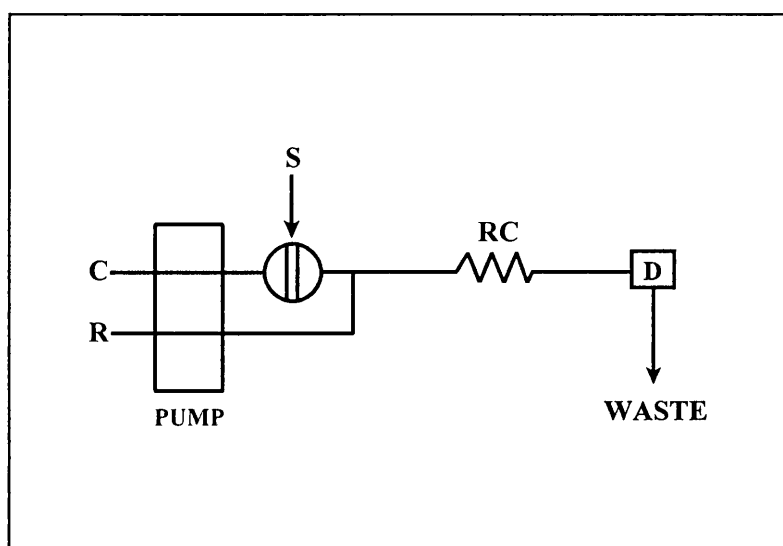


Fig.1.3 General schematic flow diagram of a flow injection system. C - carrier stream, R - reagent, S - sample, RC - reaction coil, D - detector.

This new approach to performing flow analysis proved that air segmentation, homogeneous mixing of sample with reagent solution, and attainment of a steady state, which were thought to be essential prerequisites for performing continuous flow analysis, could be replaced by the concept of FIA. What remained unchanged however is that all samples were sequentially processed in exactly the same way during passage through the analytical channel or in other words: “*what happens to one sample, happens in exactly the same way to any other sample*” [2].

Thus in contrast to all other methods of instrumental analysis, the chemical reactions are taking place while the sample material is dispersing within the reagent, that is, while the concentration gradient of the sample zone is being formed by the dispersion process.

The three basics of FIA, as defined by Ružička *et al* [2], are reproducible timing, sample injection and controlled dispersion. Without question reproducible timing is the critical principle, since it leads to reproducible physical conditions. The primary benefit of reproducible timing and controllable sample dispersion in FIA is that steady-state conditions are unnecessary. As a result, the relative standard deviations of signals from the same sample or standard are around 1.0% [10,11].

It was observed that the injected sample zone always seemed to spread out inside the FIA manifold in a reproducible manner. By observing the peak shapes and heights it was clear that the sample zone expanded reproducibly or diluted in the flowing stream. This process was called dispersion and is discussed in detail in chapter 2 and 3. In essence, what has been called “controlled dispersion” is in fact the recognition that the sample is reproducibly diluted as it travels down the tubing. The reproducible timing allows for this reproducible physical mixing and dilution, and the dispersion is thus controlled.

Several advantages characterize FIA, e.g. high sampling frequency, good reproducibility (compared with that of the segmented continuous flow analyser) and computer-compatible hardware which ensures that process analysers are conveniently incorporated into existing or planned distributed process control systems [12].

Although FIA uses pump speeds typically of the order of 1.0 ml/min, which translates into about 10 l of reagent per stream per week [12], it still consumes excessive amounts of reagent. To overcome this disadvantage, reversed FIA was suggested [13]. In this technique the roles of “sample” and “reagent” are reversed, the method being based on the injection of reagent(s) into a carrier stream of sample. This approach was still though characterized by the large consumption of the reacting species.

1.4 Sequential injection analysis

While the “classical” or continuous flow injection systems can operate well without the aid of a computer, they are uneconomical in terms of reagent consumption and waste generation because all solutions are pumped continuously. In contrast, the recently designed variant of flow injection, sequential injection [7] (Fig. 1.4), is based on discontinuous flow and consumes reagents only when the sample is being treated by exploiting a combination of stopped, reversed as well as forward flow in the micro-litre scale.

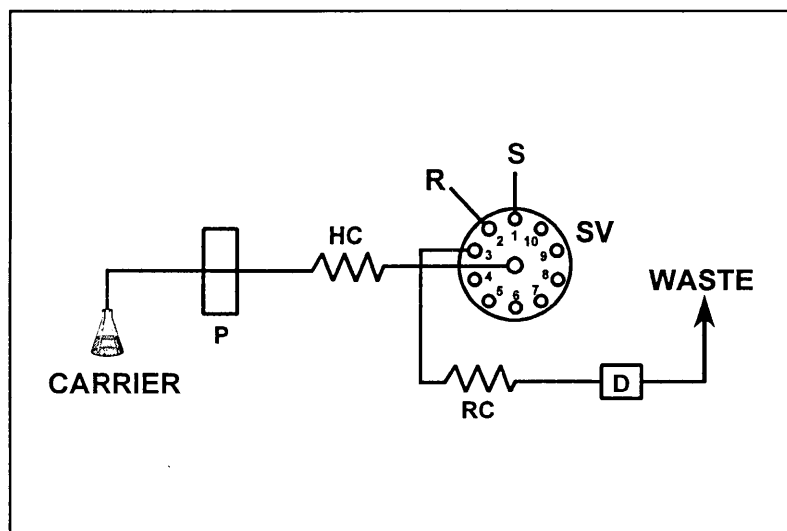


Fig.1.4 A schematic flow diagram of a typical SIA system. P - pump, HC - holding coil, RC - reaction coil, S - sample, R - reagent, SV - selection valve, D - detector.

SIA is based on the sequential aspiration of a sample and reagent zone into a holding conduit (HC) through a selector valve. In this way a stack of well-defined zones is obtained, which in the second step is injected into a reactor and detector by means of a flow reversal (Fig. 1.5). The flow reversal creates a composite zone in which sample and reagent zones mutually penetrate each other, owing to combined axial and radial dispersion. The concentration profiles that result due to the dispersion of the zones are shown in Fig. 1.6. In contrast to FIA where the carrier flows from the pump through the valve, reactor and detector continuously and unidirectionally, in SIA the selector valve is the hub through which the zones are moved back and forth in a programmed fashion by means of a series of precisely controlled flow reversals [7,11].

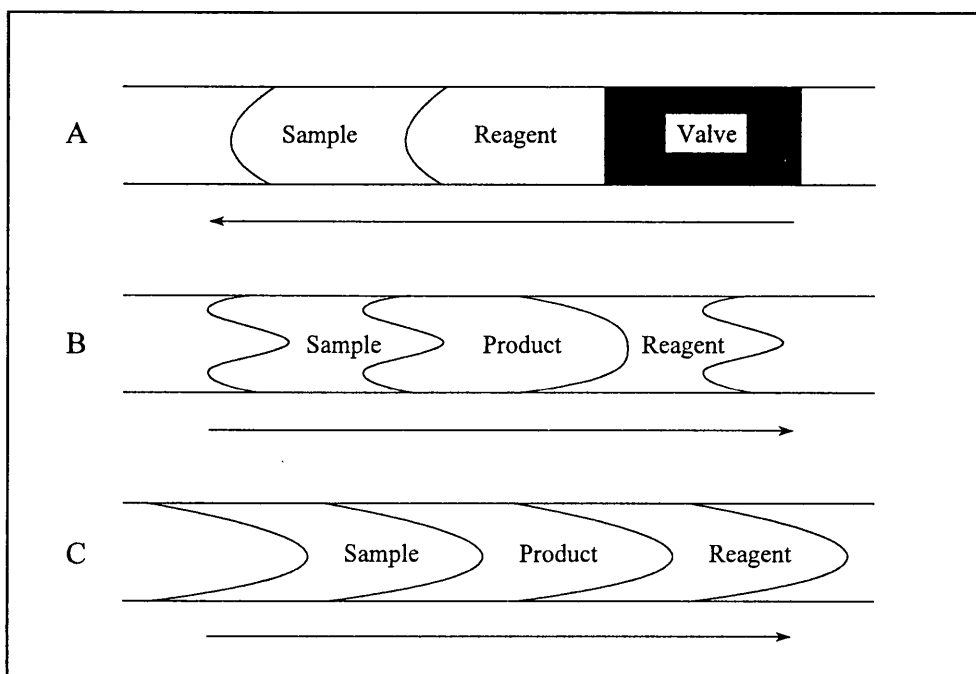


Fig.1.5 Principle of sequential injection. Profile of zones after injection (A), immediately after flow reversal (B) and in reaction coil (C).

The complex reagent/product/analyte zone can be either propelled through the detector continuously, or stopped within the detector, resulting in measurement of the rate of formation of the reaction product. In this way the stopped-flow approach yields kinetic information and allows for longer reaction times. The usefulness of this approach has been successfully illustrated before [14] and in the present work (chapter 4). The ability of SIA to perform reaction rate measurements is an added advantage compared with FIA in which this is achieved more difficultly.

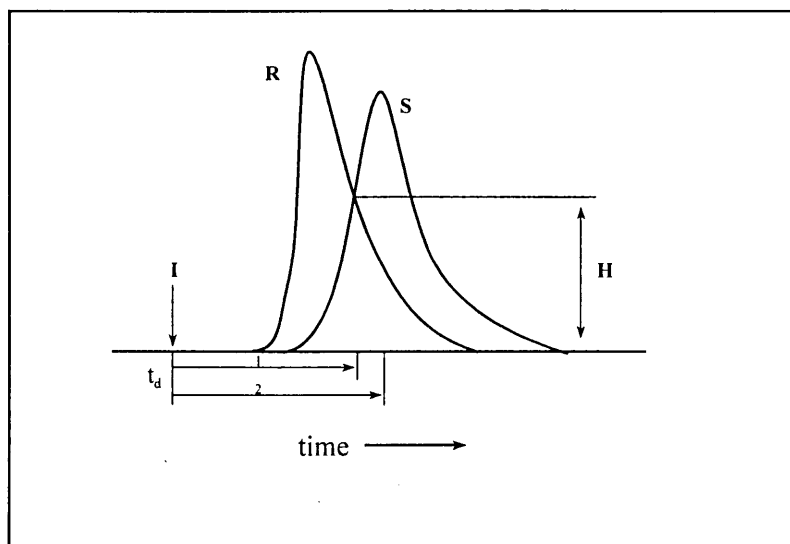


Fig.1.6 Concentration profiles as seen by the detector. R - reagent, S - sample, H - peak height, I - point of injection.

The simplicity of the sequential injection flow channel can be said to bring flow injection from the era of “spaghetti”-like plumbing to a streamlined flow-through system where all experimental parameters can be changed and controlled from the computer keyboard and there is thus no need for the physical reconfiguration of the flow path. Any changes (injected sample volume, reaction time, sample dilution and reagent:analyte ratio) are thus accomplished via flow programming. This is one of the biggest advantages of SIA compared with FIA.

In the sequential injection mode the sampling cycle equals the residence time that the injected zone spends while travelling from the injector throughout the system and detector, but as only one pump is used to propel the composite zone through a tortuous path as the complexity of the system increases, the sampling frequency of SIA is always lower than that of the corresponding FIA method [15]. This is one of the major disadvantages of SIA. Also, although the sequential injection system always has fewer moving components than a comparable flow injection system

and uses at least an order of magnitude less of reagents (as reagents are injected and not pumped continuously), it will require more complex software.

Whereas the concept of dispersion, controlled within space and time, is the central issue of FIA [2], zone penetration or overlap of the adjacent zones was identified as an essential parameter characterizing SIA [12]. 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. The fundamental requirement thus for SIA to succeed is to achieve maximum zone penetration. The degree of zone penetration, and dispersion of the zones, is dependant on the relative volumes, in addition to the usual parameters of tubing size and length, reaction coil geometry, flow rate and so forth. The influence of these parameters has been studied in depth [16-20] for a better understanding of the SIA system.

Automated control of FIA manifolds is not mandatory, since many manual systems have been used successfully in a variety of applications. This is not the case for SIA. Because the sample and reagent volumes are determined by the time that the pump is drawing up solution from a selected stream, reproducible timing is imperative. This kind of accuracy can only be achieved by micro-processor-based control. This has been identified as a disadvantage of SIA [19]. Nowadays, the proliferation of PCs and the availability of dedicated and object oriented software make SIA widely accessible.

1.5 Rationale for automation and the development of process analysers

Several arguments contributed to the decision in automating existing analytical methods [10].

These include:

- Reduction in personnel cost
- High sample throughput
- Situations that are too hazardous for human exposure.

The single major problem that can be addressed by automation is the sample matrix. Real world samples are seldom in a clean, easy to handle matrix. Instead the sample is usually in a highly complex matrix that may not only contain potential chemical interferents, but may also exhibit properties such as turbidity, viscosity or salt content that make the standard method of analysis useless.

Automatic methods of analysis have been especially influential in clinical chemistry. Since health is one of the major concerns of our society, it is easy to understand the imperative need for hospitals to obtain a large number of analytical data as quickly and cheaply as possible. Automation is thus essential in this context.

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. The demands in this field are dictated by the large number of samples to be analysed, especially in on-line control of automatized manufacturing processes and the quality now required in manufactured goods. It

is therefore necessary to control not only the raw materials, but also the intermediate and end products.

Present-day society's increasing awareness of ecological problems has also potentiated the development of automated analytical methods for monitoring pollution levels in air and in all types of waters, in urban environments and in marshes, rivers, seas, mountains and forests, where the ecosystems may be altered by the action of man.

There are certain prerequisites necessary for a process analyser to be implemented successfully for process control and monitoring and in the planning and design of instrumentation of dynamic systems. The following points should be considered [1]:

- selection of the process variable (characterising the quality of the process)
- quantitative relation between the measured and controllable properties
- place of sampling (or analysing) points
- frequency of the measurements required
- duration time of the measurements (time elapsed from sampling until result)
- tolerance limits (upper and lower) of the measured variable
- selection of sensing or analysing instrument(s)
- cost and maintenance of instruments
- calibration frequency of the instruments used
- total cost of the measurements and regulations
- reliability, ease of operation and simplicity

It is evident that there is an enormous demand for the development of process analysers and that the requirements thereof are extremely high to comply with.

1.6 On-line, at-line and off-line determinations

In process monitoring the point of sample acquisition is traditionally defined with the terms, on-line, at-line and off-line. The extreme case of process monitoring and analysis is off-line analysis where the sample is acquired and then taken to a measurement laboratory, although the actual measurement system can be the same as the on-line or at-line system. Usually calibration will be performed using traditional analytical procedures. This approach though leads to a time-delay monitoring of the operating system involved and the time lapse from sampling to corrective action may take several hours and even days.

Occasionally, analysis may require that the sample be removed from the process completely but measured at-line. This approach is necessary when, for example, there is incompatibility between the process and the analysis system. This could be for safety or chemical reasons (e.g. solvent or pH incompatibility). While the measurement in such cases can be made at the process site, the actual monitoring system can be essentially the same as that used for the on-line measurements. Only the sample introduction needs to be modified.

Only an on-line analyser requires some form of direct automated determinations and represents an “ideal” situation for measurements. From a practical perspective near-real-time measurements are made with on-line process monitors [10]. The on-line process monitors utilize sensors in the

sample stream with calibration usually performed by moving the sensors into a separate calibration stream. This requires some redundancy in the system if continuous monitoring is needed. Direct measurements can be made in sample streams or in a slip stream, where all the sensing is performed. The automated feedback to the operating system allows for the immediate adjustment and corrective action.

The ultimate is however in-line analysis where the analysing probe is situated inside the operating system (or plant) as part of the operating system (or process stream). Analysis is done inside the operating system with a feedback to the processor outside the operating system with an automatic adjustment and corrective action [1].

It is evident that the preferred approach is on-line and in-line analysis since it saves a lot of time regarding completion of the actual analysis and delivering of results. SIA is a technique that has great potential for on-line measurements due to the simplicity and convenience with which sample manipulations can be automated.

1.7 Aim of this study

For the application of flow injection analysers in process control it was necessary to simplify the system design thereof. Robustness, reliability, long-term stability and low frequency of maintenance of process analysers is an essential requirement. While manual reconfiguration of the flow channel, high reagent consumption, frequent servicing of peristaltic pumps and frequent recalibration of the system is acceptable in laboratory applications, these are prohibitive in terms of cost and manpower in a process environment. By conceptualizing a novel system comprising

only a single piston pump, single valve and single channel, the sequential injection technique has been conceived for application as a process analyser.

This study was first devoted at evaluating the physical parameters of the SIA system. Despite the fact that zone penetration is the most important aspect that needs to be considered, dispersion of the zones also have an important influence on the aspirated zones in the flow conduit. All the operational parameters of the SIA system has not yet been studied that intensely and the decision was made to conduct an in depth study into these parameters and their influence on dispersion in the SIA system.

The application of SIA to real systems was then considered. Due to the increasing demand of conducting environmentally safe analysis two components were identified as potentially hazardous if large amounts thereof should be released into the environment, namely copper(II) and thiocyanate. The use of SIA to determine these components were considered since it has not been attempted yet.

Copper(II) was spectrophotometrically determined by its reaction with the diethyldithiocarbamate reagent, based on the approach by Wang *et al* [21]. This approach excluded the use of an extraction step that characterises most of the methods that are currently available for the determination of copper(II). This determination was done both in the presence and absence of interferences and is discussed in detail in chapter 4. The spectrophotometric determination of thiocyanate was based on the reaction of thiocyanate with iron(III) to form the iron(III)-thiocyanate complex. This was based on the approach of Jones *et al* [22] who developed a similar method with FIA. This is discussed in chapter 5.

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CHAPTER 2

Theoretical Background

2.1 Introduction

Sequential injection analysis 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 sequential injection analysis 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 distinguished SIA from the conventional flow systems, namely the introduction of sample and reagent into the flow conduit as zones and the subsequent deformation and mutual penetration of the zones as they are propelled towards the detector.

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 various operational parameters, deformation and dispersion of the zones and the subsequent zone penetration [1-5]. Since the foundation of certain aspects of the flow systems were already laid down, especially the dispersion that characterises flow through these systems, it was only necessary to build forth on this to characterize the SIA system more thoroughly.

This chapter is devoted at outlining the most important characteristics of the SIA system that not only characterizes flow in the flow conduit, but also essentially distinguishes it from its predecessors. It is obvious that the discussion will start at the flow injection analysis characteristics after which the development of sequential injection analysis will follow from it.

2.2 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 has been 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.

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. The influence of these processes on dispersion is discussed below. Table 2.1 lists the symbols, and their definitions and units, where applicable, used in this chapter.

TABLE 2.1 **List of symbols**

Sample	Reactor geometry	Hydrodynamic aspects	FIA signal
C = concentration (M)	L = overall tube length (cm)	q = flow rate (mℓ/min)	D = dispersion coefficient
V_i = injected volume (mℓ)	d = tube diameter (mm)	u = linear velocity (cm/sec)	Δt = baseline-to-baseline time (sec)
C_0 = initial concentration	V_r = volume of system (mℓ)		T = residence time (sec)
$C_{\max}$ = concentration at signal peak maximum	R = tube radius (mm)	u_0 = maximum linear velocity (cm/sec)	t_a = travel time (sec)
D = molecular diffusion coefficient (cm ² /sec)	r = partial tube radius (mm)	$\bar{t}_r$ = mean residence time (sec)	f = Vanderslice's accommodation factor
	l = partial tube length (cm)		

2.2.1 Transport

The transport of matter along the tubes of a flow system is said to take place essentially by laminar flow and not as a result of turbulent flow as thought earlier [6]. There are two mechanisms that contributes to dispersion of the injected sample:

2.2.1.1 Convective transport

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. 2.1 illustrates the two types of convective transports

namely turbulent and laminar flow.

2.2.1.2 Diffusional transport

This type of transport gives rise to axial and radial diffusion due to the presence of concentration gradients in the convective transport regime.

- i) Axial diffusion, illustrated in Fig. 2.1, exists due to the horizontal concentration gradients at the leading and trailing edges of the injected sample zone. It contributes insignificantly to the overall dispersion.
- ii) Radial diffusion, that results from concentration differences perpendicular to the direction of the flow, makes an important contribution 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.

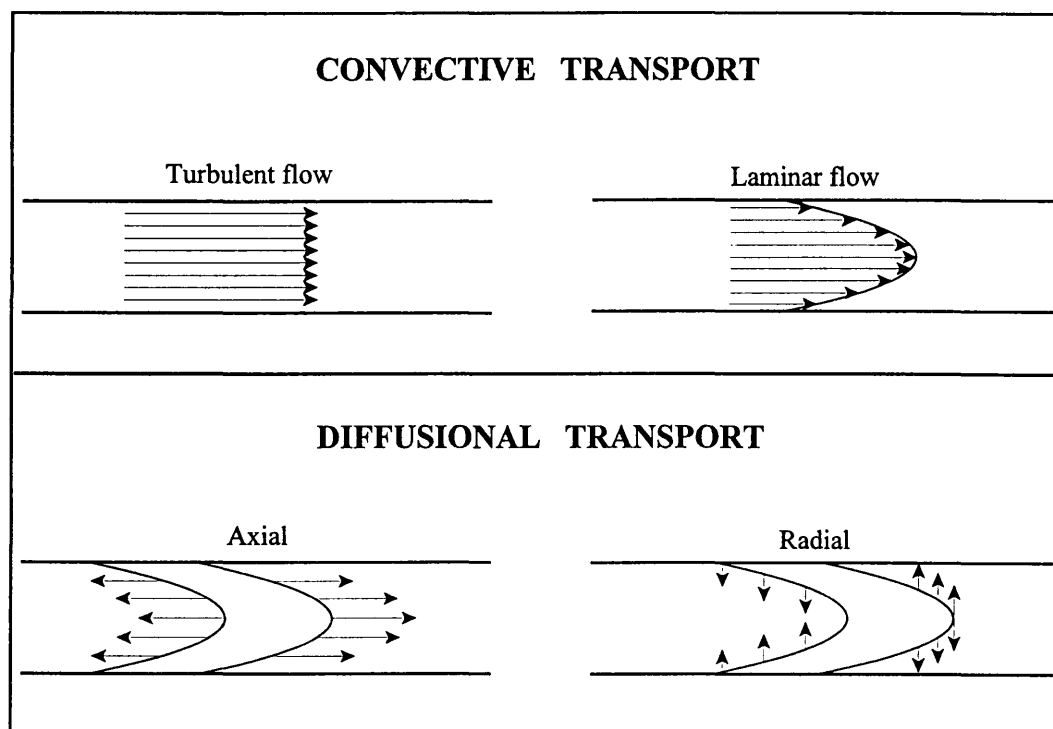


Fig.2.1 General types of transport in closed tubes.

2.2.2 Theoretical models

Various attempts have been made to derive a general expression $C = f(t)$ accounting for the signal profile and relating its characteristics (travel, residence and baseline-to-baseline times, and peak height or area) to the experimental parameters of the FIA system (flow rate, reactor length, etc.). It is important to note the difficulties involved in deriving theoretical predictions of the behaviour of an injected plug in practical systems, since it is not an easy task to define the contribution of elements such as the injection operation, connectors or geometry of the flow cell to the dispersion. A discussion follows on the models that have been developed for laminar flow conditions, to define the theoretical principles of FIA and derive mathematical expressions, of the form $C = f(t)$, accounting for the physical behaviour of an injected plug.

2.2.2.1 Taylor's model

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 is applicable to a Gaussian distribution defined by $C=f(t)$ in a form which depends on the chosen parameters, namely

$$C = \frac{m}{r^2} \frac{1}{4\pi Dt} \exp \left[-\frac{(x - L)^2}{4Dt} \right] \quad (1)$$

and

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

where m is the injected solute mass ($m = C_0 V_i$), 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 $V_i \ll V_r$.

2.2.2.2 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 minichambers in which stirring is perfect (instantaneous mixing). The mathematical expression derived from this model [7] is

$$C = \frac{1}{(\bar{t}_r)_N} \left(\frac{t}{(\bar{t}_r)_N} \right)^{N-1} \frac{1}{(N-1)!} \exp \left[\frac{-t}{(\bar{t}_r)_N} \right] \quad (3)$$

$(\bar{t}_r)_N$ 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 these conditions, the variance is given by

$$\sigma^2 = N(\bar{t}_r)_N^2 = \frac{\bar{t}_r^2}{N} \quad (4)$$

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.

2.2.2.3 Mixing chamber model

Pungor *et al* [8] developed a mathematical model to describe the dispersion when there is a mixing minichamber 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

$$\frac{d\Delta C_t}{dt} = \frac{V}{W} \left[\Delta(C_s)_t - \Delta C_t \right] \quad (5)$$

where t is the time (sec) from the moment of injection, $\Delta C_t = C_t - C_0$; C_t is the actual analyte concentration in the carrier stream on entry to the mixing chamber, C_0 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 [9] or when extensive dilution of the analyte is required [10]. The influence of a mixing chamber on dispersion and zone penetration in an SIA manifold has been studied [5,11].

2.2.2.4 General model

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

$$\frac{\delta C}{\delta t} = D \left(\frac{\delta^2 C}{\delta l^2} + \frac{\delta^2 C}{\delta r^2} + \frac{1}{r} \frac{\delta C}{\delta r} \right) - u_0 \left(1 - \frac{r^2}{R^2} \right) \frac{\delta C}{\delta l} \quad (6)$$

This expression takes into account axial and radial concentration gradients, as well as flow profiles under a 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-steady regime, and the second term accounts for the contribution from convective transport for which the velocity profile is parabolic in shape and given by:

$$u = u_0 \left(1 - \frac{r^2}{R^2} \right) \quad (7)$$

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

2.2.3 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_a ,
- b) its bandwidth, characterized by the baseline-to-baseline time, Δt , and
- c) the co-ordinates 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.

2.2.3.1 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 a pure dye, and $C_{\max}$, the concentration of the dye injected into the system as it passes through the detector in an FIA system [6,12], i.e.

$$D = \frac{C_0}{C_{\max}} \quad (8)$$

The dispersion coefficient is useful in that it allows comparisons of different manifolds [13]. 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 the physical process of dispersion and not the ensuing chemical reactions. This relationship is discussed in further detail in chapter 3 where dispersion in an SIA system is investigated in depth.

2.2.4 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 [6,14] and SIA [1,2,5]. 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.

2.2.4.1 Sample volume

The introduction of increasing volumes of dye into an FIA system revealed the following information [6]:

- 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 Růžicka, D and V_i are inversely proportional to each other, i.e.

$$D = \frac{k}{V_i} \quad (9)$$

Růžicka *et al* [14] 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 volumes.”

A similar conclusion was drawn from the dispersion experiments in an SIA system [2,5], especially with regard to increased sensitivity, and decreased dispersion, with increasing sample volumes. It was also stated that an increase in sensitivity of the measurement will be possible only when sufficient reagent excess is available in the element of fluid situated at the peak maximum [2].

2.2.4.2 Hydrodynamic factors

Flow rate (q) was related to the travel and baseline-to-baseline times through the following equations [6]:

$$t_a = \frac{k}{q^{1.025}} \quad (10)$$

and

$$\Delta t = \frac{k'}{q^{0.64}} \quad (11)$$

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* [6] indicated that the dispersion coefficient does indeed decrease with increasing flow rate contrary to Růžicka *et al* [14] 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 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 [5], thus confirming that which had been observed by Valcárcel *et al* [6]. 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.

2.2.4.3 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, formed when the sample zone is injected into a laminar flow of carrier stream, is reduced [14]. Thus,

- a) the reagent becomes more readily mixed with the sample, and
- b) 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 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.

2.2.4.3.1 *Straight tubes*

The simplest configuration of the tubes in a flow system is that of the straight tubes. The influence of the reactor length, L , on t_a and Δt is predicted by Vanderslice's expressions:

$$t_a = k L^{1.025} \quad (12)$$

and

$$\Delta t = k' L^{0.64} \quad (13)$$

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,

$$D = k L^{1/2} \quad (14)$$

The influence of the reactor diameter was also studied and it was found that t_a and Δt are directly related to the diameter ($R = d/2$), in agreement with Vanderslice's predictions:

$$t_a = k d^2 \quad (15)$$

and

$$t = k' d^2 \quad (16)$$

The residence time and the dispersion coefficient both increased, with increasing tube diameter [6]. In SIA however, the tube internal diameter has been shown to have a more pronounced effect on the dispersion obtained than the length thereof [5,15].

2.2.4.3.2 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. 2.2. 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.

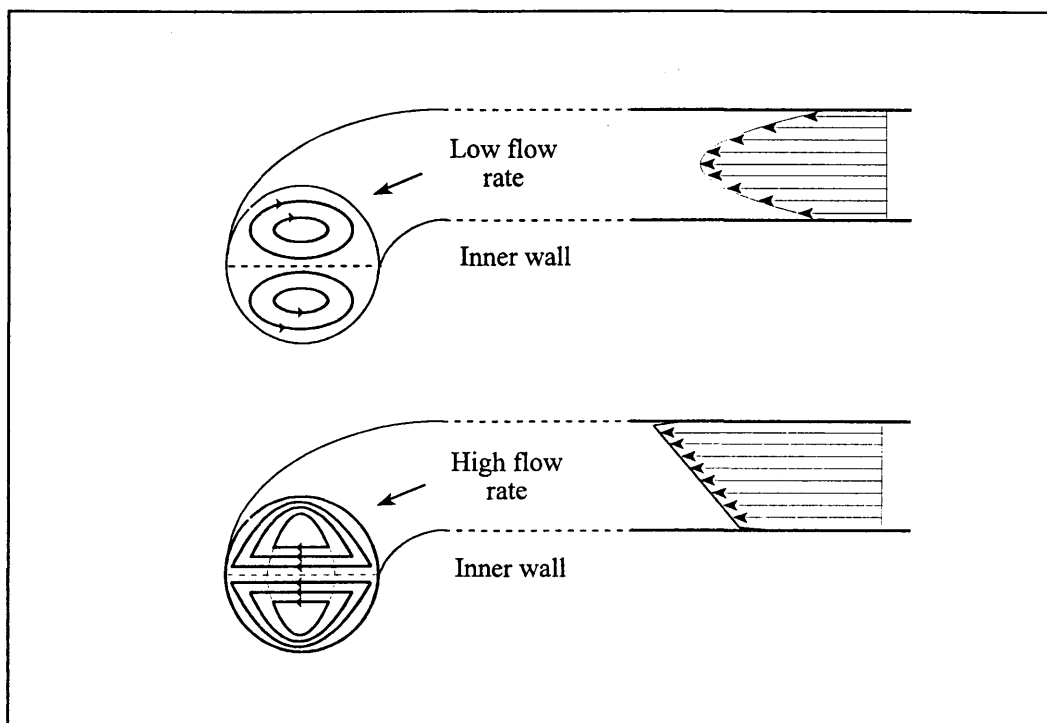


Fig.2.2 Fluid dynamics corresponding to variations in the flow rate.

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” [6]. 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 was observed that the smaller the coil diameter, the smaller the dispersion [6].

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.

2.2.4.3.3 *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 the 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.

2.2.4.3.4 *Normal packed tubes*

The behaviour of packed reactors is a well known concept in chromatography and is defined by the following ratio:

$$\tau = \frac{\text{tube diameter}}{\text{particle diameter}} \quad (17)$$

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.

2.2.4.3.5 *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 the sample in the flow line, and therefore decreases dispersion [6]. 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.

2.2.4.3.6 *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 of the zones both in FIA [14] and SIA [5,11] when introduced in the flow conduit. Růžicka *et al* [14] drew the 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 measurement 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.”

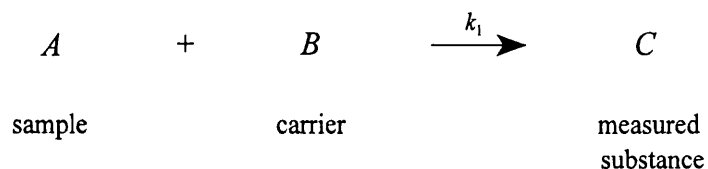
The use of a mixing chamber is justified for the following reasons: first, substantial variations of injected volume, hold-up volume of the chamber and flow rate do not adversely affect the precision over a large dynamic range of dispersion coefficients (D up to 2000) [14]. The sample conditioning (density, 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. The influence of mixing chambers on dispersion in SIA are illustrated in chapter 3.

2.2.5 Contribution of chemical kinetics to dispersion

Dispersion can not be considered as a purely physical phenomenon when a chemical reaction takes place in a flow system. The peak which is obtained as a result of a chemical reaction taking place in the flow system is thus the result of two processes, namely physical dispersion and subsequent chemical reactions [14]. These two kinetic processes occur simultaneously in any flow system. The dispersed zones are not homogeneously mixed, in both FIA and SIA, but are composed from concentration gradients that are formed by gradual penetration of the reacting species in both the axial and radial direction. Reaction rates should thus be considered in FIA (and SIA) since the measurement is made under non-equilibrium conditions.

A chemical reaction may modify the value of the dispersion coefficient in two ways [6]:

- a) When a certain property of the reaction product in the flow is measured, the chemical contribution results in a decrease in the practical dispersion.



The higher the reaction rate constant, k_1 , the smaller **D** becomes.

- b) When a property of one of the reactants (A, above) which diminishes with time is measured, the chemical contribution increases the dispersion coefficient. The higher k_1 , the larger **D** will become.

It is thus evident that when a chemical reaction is considered in a flow system, and a change in dispersion and sensitivity is observed, that this change can not be ascribed solely to the influence of physical dispersion. It is important to have in depth knowledge in the reaction you are dealing with to better understand and explain the results delivered by the flow system.

2.3 From FIA to SIA via the random walk model

The random walk model is based on a probabilistic approach and offers a more realistic insight into the micro-structure of a stratified flow such as that experienced in FIA [14]. By using the random walk model as a basis it has been postulated that no net flow is needed for the successful operation of a flow injection system [16]. Růžicka *et al* [17] discussed the consequences of the latter observation and the role of the random walk model in FIA. Their discussion lead to defining the principle of SIA. A detailed explanation follows below. It is important to

understand the underlying concept of SIA to apply it as successfully as FIA has been applied up to date.

The successful operation of any injection analyser requires that the sample and reactants are brought together, mixed and allowed to react in a perfectly reproducible manner. It is thus necessary to maintain reproducible flow conditions for extended periods of operation so as to obviate the need for frequent recalibration. This has led to the practice of using unidirectional monotonous flow, because as long as a constant flow rate is maintained the sample may be injected into the system at any time. In this way samples injected into the carrier stream at different times can be treated identically. To fulfil these criteria, strict control of the flow rate must be maintained throughout the entire analysis period.

Since the theoretical description of the zone dispersion process in the analyser channel is a random process, it can be described by the random walk model. Giddings [18] used this model to describe the dynamics of chromatographic processes, whereas Betteridge and co-workers [19,20] applied it to the description of zone dispersion in FIA by considering monotonous flow.

The random walk model allows one to view the element of fluid as being displaced during the dispersion process in any direction by a step of fixed length. The direction of the step is determined entirely by chance. If the chance of moving in any direction is equal, then the random walk is symmetrical. As all molecules within a said element of fluid cannot be expected to move through the same step of fixed length (their movement is erratic), the length of a fixed step must be taken as the average length of actual displacement (viz., its square root). Thus, if a large number of molecules start together on a random walk of many steps, the standard deviation, σ ,

of the resulting (Gaussian) concentration profile is

$$\sigma = ln^{1/2} \quad (18)$$

where l is the fixed step length and n is the number of steps taken. It is noteworthy that the spread of the zone and the resultant degree of mixing with the surrounding carrier stream are proportional to the individual step length, l , which is easy to understand as the final displacement of the molecule from the origin depends on step length in any one direction. The final zone displacement however, increases only with the square root of the number of steps, n , taken. This is to be expected as a cancellation effect must be considered in which the molecule is equally likely to move in the opposite direction to the original displacement.

If several random walks occur simultaneously or consecutively, as they do in chromatography and FIA, the final displacement of any given molecule is determined by the sum of the displacements in all directions. Therefore,

$$\sigma^2 = l^2n_1 + l^2n_2 + \dots = \sigma_1^2 + \sigma_2^2 + \dots \quad (19)$$

so that components of the process (variances σ^2) are additive.

Two driving forces are responsible for the movement of the molecules and the spreading of the zone: an internal one which is symmetrical and caused by molecular diffusion, and an external one caused by the flow imposed on the liquid which transports the zone through the system. If the flow is unidirectional and if the molecular diffusion and/or radial mass transfer (caused by coiling or packing the tubing) does not compensate for the asymmetry resulting from Poiseuille flow, the resulting concentration profile is asymmetric. Consequently, the result of such a random process is asymmetric.

The accumulated distance of migration, L , imposed by the flow, can be traversed in many ways, each of which constitutes a different number of random steps depending on the flow geometry. This relationship has been expressed in chromatography by means of the height equivalent to a theoretical plate,

$$H = \frac{\sigma^2}{L} \quad (20)$$

which is a measure of the generation of variance per unit length of a column travelled by the dispersing zone. It is evident that the zone spreading will increase with the square root of the distance travelled (L), while separation of the components increases with the number of theoretical plates unidirectionally traversed, and therefore linearly with distance travelled.

The use of a unidirectional monotonous flow rate has been the prevailing 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. The key issue in FIA is therefore reproducible dispersion of the injected zone into the carrier stream and timing of the arrival of

the reacted zone at the detector. The random walk model is useful for visualization of the degree of mixing, since the transition of the original square input, through skewed peaks, and finally to Gaussian-shaped peaks is indicative of the degree of mixing of the reacting components [14,16].

As it is well known that the skewness of the peak is due to initial acceleration and is further amplified by Poiseuille flow, the value of the symmetrical random walk is that it leads to the conclusion that efficient mixing of the reacting components can be achieved without actually travelling any net distance, L . By moving the injected zone forward and back in several steps (n) of sufficient length (l) an efficient mixing of sample zone and carrier stream components may be achieved through a combination of external and internal forces (equation 18) (although this movement is not strictly random, the same principles hold). By selecting the step length and number of flow reversals, any degree of mixing and any desired length of reaction time can be conveniently obtained simply through flow reversal.

The need to travel the length of a channel in one direction in classical flow injection systems is only necessary because the sample zone has to be transported from the injector, past a mixing point (where confluence of reagent and sample zone bearing carrier stream occurs), through the reaction coil and detector to waste. Hence it is the physical size of the system and the configuration of its components (pump → injector → confluence point → reactor → detector → waste) which require a net flow of the carrier stream larger than the minimum volume required by the reacting species. As forward flow is used to promote mutual dispersion of reacting species, the consumption of liquids is mainly determined by the flow channel dimensions and flow rates. The required volume is therefore much larger than if a net zero forward flow took place. On the other hand, as flow reversal yields a zero net flow, a very high linear flow velocity

can be imposed on the selected elements of fluid and thus a turbulent flow may be achieved if necessary.

Use of a flow pattern, rather than constant monotonous flow, requires synchronization of sample zone injection with the start of each flow cycle. Therefore, a system configuration must be conceived which will allow sample zone injection, reagent addition, mixing, measurement and ejection of reacted mixture by a combination of forward and reversed steps. It follows from the foregoing that these steps may have different length, duration and speed, provided that they always follow the same pattern. It is important that the pattern is strictly repeatable in each measuring cycle then the sample zone dispersion, being composed of individual variances, will always be the same (equation 19) and so will the reaction time.

To achieve an absolute zero net flow is, of course, not practical, but sample and reagent consumption can be drastically reduced and the flow system can be dramatically streamlined if mutual dispersion of the sample and reagent zones, their reaction to yield detectable species and detection of these species occur within the same section of the flow conduit.

2.4 Manifold design in SIA

The concept of SIA is based on the mixing of a sample zone with a reagent zone in order to produce a measurable response [17]. This necessitates solution handling operations such as sample injection, reagent injection, sample and reagent mixing and detector wash and reconditioning. The SIA system uses 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 [21].

After the first step of zone sequencing, during which the sample and reagent zones are stacked in the holding conduit adjacent to one another (Fig. 2.3), the valve is switched into the detector position. In the following step, the flow is reversed so that the stacked zones are propelled through the valve and the reactor into the detector, while they mutually interdisperse. It is during this movement that the flow reversal creates a complex concentration gradient in which the sample and reagent zones mutually penetrate, forming a composite region within which the analyte is being transformed into a detectable species.

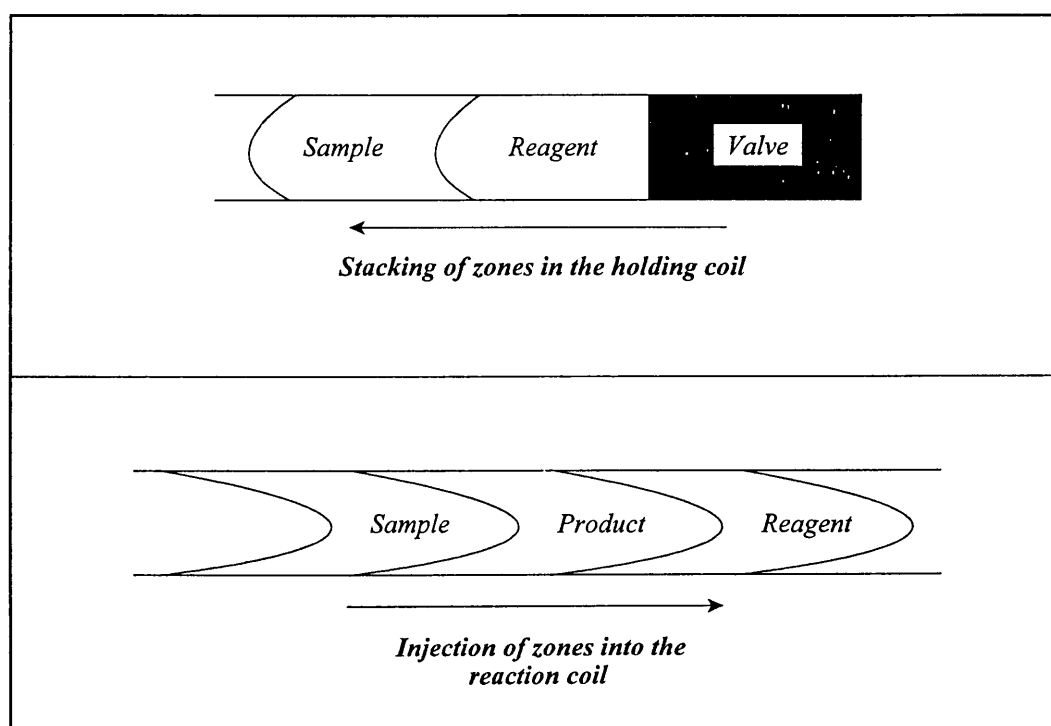


Fig.2.3 Structure of stacked and injected zones.

It is important to understand the working of the liquid drive and selection valve that characterizes the SIA system setup and that especially distinguishes SIA from FIA. A discussion on these parameters follows.

2.4.1 Liquid drives

Whereas FIA uses a multichannel pump and unidirectional (forward) flow, SIA uses a single-channel pump to move the column of liquid in reverse and forward steps through a channel [22]. It has been well established that all components of a flow injection system must be non-elastic, as elements such as air bubbles or unsecured sections of plastic tubing adsorb and release mechanical energy, resulting in uncontrolled flow pulsations. This is also a critical requirement for sequential injection.

During the original evaluation of the SIA system [1,2], the flow rate was controlled with a unique cam-driven piston pump illustrated in Fig. 2.4. Růžička [21] did though indicate that a peristaltic pump (Fig. 2.4) can in principle be used for controlling the flow rate. An important requirement of the liquid drive is that it must allow sufficiently precise forward-reverse-stop-go movement of liquids.

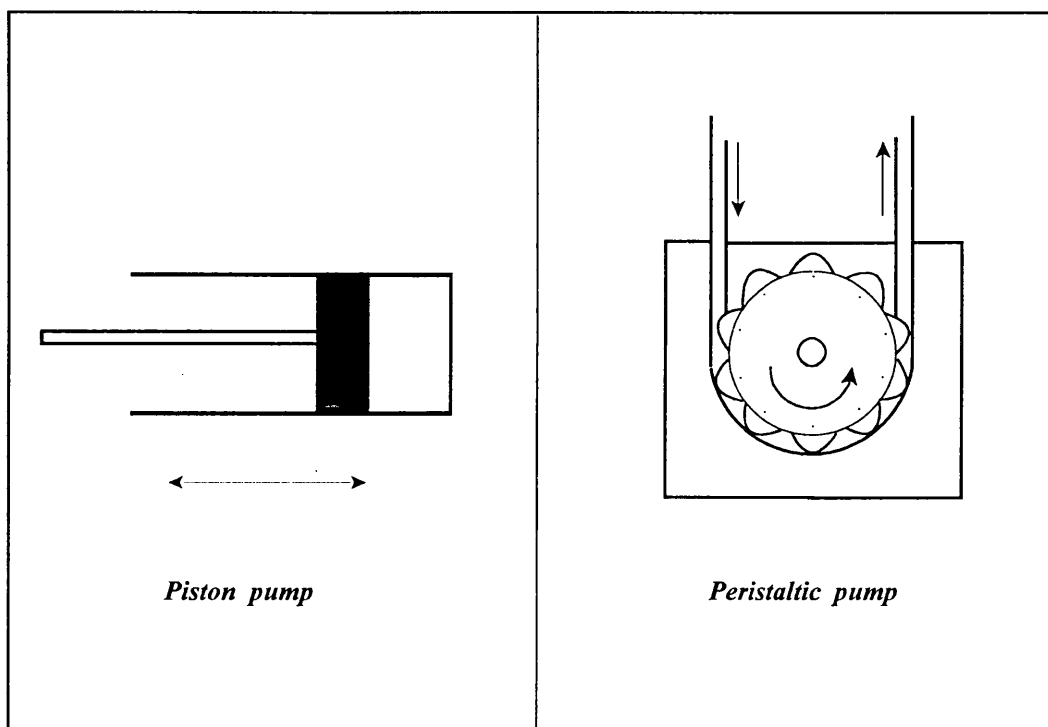


Fig.2.4 Liquid drives commonly used in SIA.

As timing and precise liquid manipulation are far more critical for sequential injection than flow injection, the sinusoidal flow piston pump, specially designed for experimental verification of the sequential injection concept, has been used. Although the syringe pump is inherently more precise than the peristaltic pump, and has therefore been used, its disadvantage is that it has to be recharged with the carrier solution at the beginning of each measuring cycle, an additional operation which takes time and therefore decreases sample throughput [23].

The widespread availability of peristaltic pumps and their convenience in use prompted Ivaska *et al* [22] to investigate the applicability thereof for sequential injection. They subsequently conducted a comparison study of the two types of liquid drives and set guidelines for the evaluation of the suitability of different propulsion systems.

The obvious difference between the piston and peristaltic pump propulsion is in the way in which the liquid is being propelled. A low-pressure syringe provides a sinusoidal flow as a result of the non-uniform motion of the piston in the cam-driven piston pump. The peristaltic pump consists of a set of rollers that simultaneously squeezes a number of parallel flexible tubes arranged at right angles to the rollers, so that the successive flexions of the tubes force various fluids through the tubes. This motion provides a pulsating flow.

The piston and barrel of the syringe pump are rigid, and therefore do not deform during prolonged use. Ivaska *et al* [22] was therefore surprised to observe that also the elastic, deformable peristaltic pump tubing (the best results were achieved with thick-walled tough elastomer tubing such as Norprene, whereas the regular Tygon peristaltic tubing was found to be less stable and durable) fulfilled the stringent requirements of sequential injection. This was a valuable observation since there are several advantages to the use of a peristaltic pump for sequential injection:

- 1) the sampling cycle is considerably shorter as there is no need for wash aspiration;
- 2) the system is easier to configure and simpler to design, initiate and operate;
- 3) peristaltic pumps are easy to handle and widely available.

In addition, as sequential injection requires only one channel and as the pump propels only pure solvent (usually water), the pump components are not exposed to corrosion being insulated from the reagent- and sample-handling part of the system by a column of water or other inert liquid. Various peristaltic pump tubing is though available, the properties of which makes them suitable for use with a specified range of fluids, organic or inorganic, as set out in Table 2.2. Hence, for

laboratory or portable instruments, a peristaltic pump is useful even for sequential injection methodology.

TABLE 2.2 Peristaltic pump tubing

Classes of liquids and solutions	Tubing material (Trade name)
Aqueous solutions	PVC, Tygon
Dilute ethanol solutions	PVC, Tygon
Dilute acids and bases	PVC, Tygon, silicone rubber
Concentrated acids and bases	Fluoroplasts ("Acidflex" from Technicon)
Alcohols	Modified PVC ("Solvaflex" from Technicon)
Lower alcohols	Silicone rubber
Formaldehyde, acetaldehyde	PVC, Tygon
Acetone	Silicone rubber
Acetic acid and anhydride	Silicone rubber
Aliphatic hydrocarbons	Modified PVC ("Solvaflex" from Technicon)
Aromatic hydrocarbons	Fluoroplasts ("Acidflex" from Technicon)
Chloroform	Fluoroplasts ("Acidflex" from Technicon)
Carbon tetrachloride	Modified PVC ("Solvaflex" from Technicon)

Cladera *et al* [24] evaluated the use of an autoburette actuated by a stepper motor as liquid driver in sequential injection analysis, to circumvent the short lifetime which is characteristic of peristaltic pump tubing. In addition to the longer life of the autoburette, it is also easy to operate and more widely available. An autoburette also frees one of the channels of the switching valve as it can be loaded with carrier by using its own two position valve.

The reproducibility delivered by the approach of Cladera *et al* was ~ 1.0% which is identical to that achieved by using a peristaltic pump on stabilization and somewhat lower than that provided

by the sinusoidal pump (0.3%) [24]. They stated that the precision of the proposed system could be increased by using low-volume burettes, which would allow smaller volumes to be measured more reliably. Their low capacity would however have prevented the reactants from reaching the detector or hindered channel flushing unless a very short length of tubing had been used.

2.4.2 Selection valve

The selection valve or multiposition valve serves as a central distributor through which appropriate volumes of liquid segments are sequenced by aspiration into the holding coil, and then propelled by a flow reversal into the detector. This valve allows multi-reagent chemistries and multi-detector assays to be carried out in a single sequential injection system, by simply adding additional reagents, reactors and detectors around the valve to suit a particular analysis [21].

Indeed a sequential injection cluster may serve as a single- and a multichannel analyser. Alternatively, a series of standards can be permanently nested around the valve, being ready for automated recalibration whenever needed. The only apparent limit is the number of ports available.

2.5 Fundamentals of SIA

Zone sequencing and mutual dispersion of the zones are the key operations in SIA [2]. These have to be carried out in such a manner that a desired degree of penetration of the sample and reagent zones will be achieved, within an adequate resident time, while yielding satisfactory sampling frequency. Maximum zone penetration is achieved through a deliberate increase in axial dispersion, obtained by means of the flow reversal and channel design [21]. A discussion follows on the parameters which govern and characterize penetration of the sequentially injected zones.

2.5.1 Zone overlap

The control of zone overlap is essential for the successful execution of sequential injection. For reagent-based chemistries, a region of mutually interdispersed sample and reagent zones must be identified, within which D_s (dispersion coefficient of the sample defined in chapter 3) is larger than 2, and where at the same time sufficient excess of the reagent is present. A parameter describing the degree of mutual zone penetration had to be established in order to identify a time interval within which a meaningful readout, such as peak height or peak area, could be obtained. In formal analogy with the definition of resolution as used in chromatography, the concept of zone penetration was introduced by defining:

$$P = \frac{2 W_0}{(W_s + W_r)} \quad (21)$$

where:

P	=	zone penetration
W_0	=	baseline width of the zone overlap
W_s	=	baseline width of the sample zone
W_r	=	baseline width of the reagent zone

These concepts are illustrated in Fig. 2.5. A complete zone overlap is obtained for $P = 1$ (when $W_s + W_r = 2W_0$), zero zone overlap would be at $P = 0$, and partial zone overlaps will attain values between these two extremes.

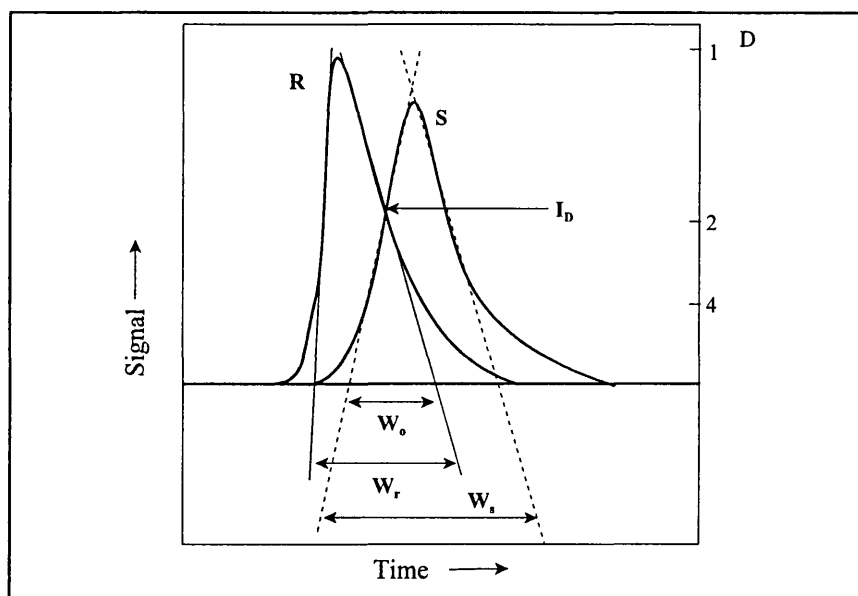


Fig.2.5 Description of zone penetration. S = sample zone, R = reagent zone, I_d = isodispersion point, W_r , W_0 and W_s = respective baseline widths of the reagent, overlap and sample.

Gübeli *et al* [2] have studied zone penetration as a function of relative volumes of sample and reagent zones by injecting a non-reactive dye into a colourless carrier stream and recording the dye as a sample and reagent peak in two independent experimental runs respectively. In this way,

two concentration profiles were recorded, one for the reagent (R) zone and the other for the sample (S) zone, allowing their mutual overlap to be visualized. In all their experiments the R-zone was less dispersed than the S-zone, since it was stacked as the second one and therefore being closer to the valve was subjected to less flow reversal and travelled a shorter distance than the S-zone.

They found that increasing zone volumes at equal volume ratios resulted in decreased zone penetration. This decrease, from $P = 0.796$ to $P = 0.498$ [2], is illustrated in Fig. 2.6 and Fig. 2.7.

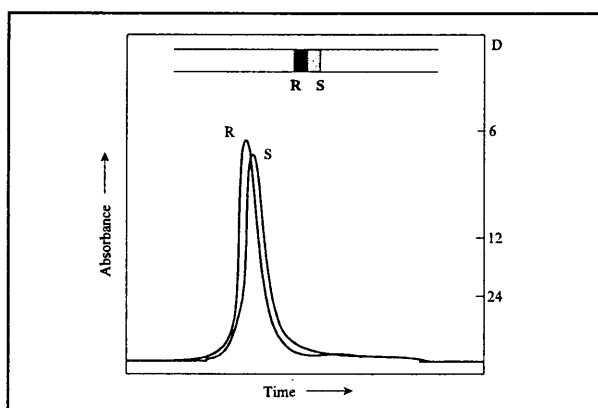


Fig.2.6 Penetration of sample (S) and reagent (R) zones for $R_v = S_v = 0.25S_{1/2}$ and $P = 0.796$.

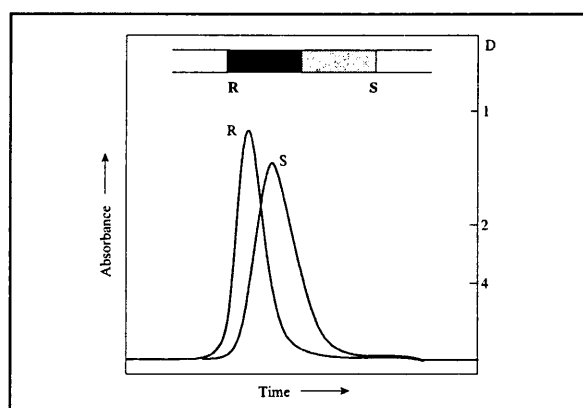


Fig.2.7 Penetration of sample (S) and reagent (R) zones for $R_v = S_v = 2.5S_{1/2}$ and $P = 0.498$.

They concluded that:

“simultaneous increase in sample and reagent volumes above one $S_{1/2}$ value is not an effective way to increase peak height and sensitivity of a measurement.”

The increase in peak height, thus decreased dispersion, that was obtained with increasing zone volumes at equal volume ratios was ascribed to the substantial increase in sample, reagent and time consumption. The increase of the sample and reagent volumes above one $S_{1/2}$ value (Fig. 2.7) does though create a situation for sample dilution by the gradient approach and for stopped flow reaction rate measurement.

Gübeli *et al* also evaluated the effect of varying the zone volume ratios on zone penetration [2]. They observed a decrease in zone penetration for the larger zone volume ratios illustrated in Fig. 2.8 and Fig. 2.9. They concluded that when complete overlap is achieved (Fig. 2.8) the conditions are closest to a conventional flow injection technique where all the elements of the dispersed sample zone are mixed with an adequate amount of reagent solution. At these conditions the influence of kinetics of chemical reactions will be minimized.

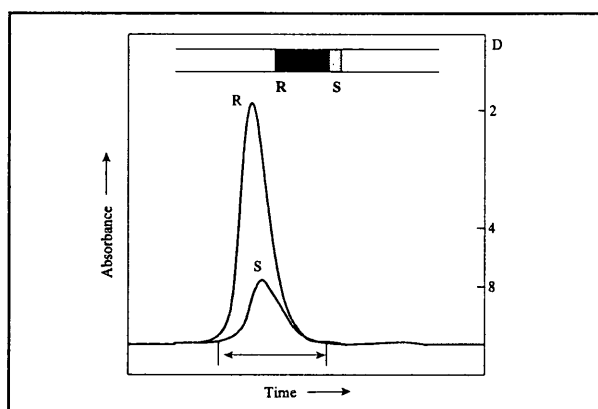


Fig.2.8 Penetration of sample (S) and reagent (R) zones for $R_v = S_{1/2}$, $S_v = 0.25S_{1/2}$ and $P = 0.806$.

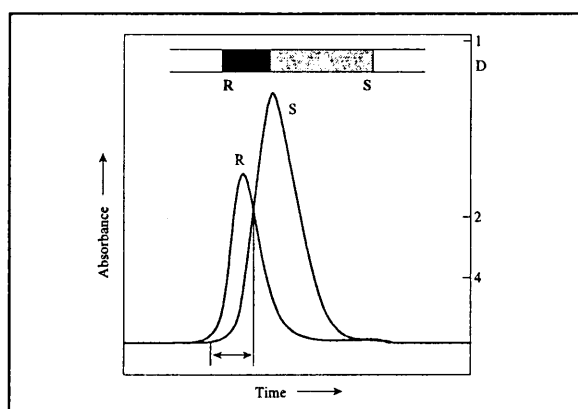


Fig.2.9 Penetration of sample (S) and reagent (R) zones for $R_v = S_{1/2}$, $S_v = 2.5S_{1/2}$ and $P = 0.518$.

The partial overlap (Fig. 2.9), having a low P value, yields only a limited range of time slices (situated in front of I_D toward the centre of the R-zone) at which the vertical readout will yield a meaningful result. The part of the response curve past I_D is starved for reagent and the very end of the zone is devoid of it. Fig. 2.9 also provides a wide range of information since the analyte readouts can be obtained within the time span indicated by the arrow ($\leftrightarrow$) and the sample blank is also accessible at the very tail of the sample zone.

The influence of tube diameter and reactor geometry on zone penetration has been studied by Marshall *et al* [1]. They indicated that a decrease in tube diameter resulted in a noteworthy increase in zone penetration. The reactor geometry also affected the zone penetration but to a lesser degree compared with that of the tube diameter. The zone penetration increased slightly from a straight to a coiled to a knitted reactor [1].

2.5.2 Isodispersion point

In the absence of complete zone overlap, an isodispersion point, I_D , is observed as is clear from Fig. 2.5. The isodispersion point is observed where the dispersions of the sample and reagent are identical. This point is independent of concentration. In the element of fluid corresponding to I_D , the ratio of sample and reagent concentrations is the same as their ratio prior to injection since they are equally dispersed. Thus,

$$\frac{C_s}{C_r} = \frac{C_s^0}{C_r^0} \quad (22)$$

Since dispersion describes the physical process only, the chemical reaction will of course affect this ratio further. In the case of a completed reaction and equal (equivalent) input sample and reagent concentrations, a maximum of product concentration will be observed at the isodispersion point, and if the product is detected, the peak maximum will appear at time t of I_D .

Since C_r^0/C_s^0 is usually much larger than 1 due to an excess of reagent normally used, the position of the peak maximum will move from the I_D point toward the centre of the sample zone. This shift continues until the reagent gradient causes the value of C_r to drop to the point where the equivalent sample concentration exceeds it (Fig. 2.9), and the concentration of the monitored product no longer increases, but decreases.

This simplification does not take reaction kinetics into consideration, and therefore, the position of I_D along the time axis can serve only as a guide for system design and to estimate how input changes of reagent concentration will affect the effective zone overlap and sensitivity of the determination.

Van Staden *et al* [4] used different volume ratios, while keeping the total volume of sample and reagent constant, to illustrate the shift of the isodispersion point. It was found that the I_D point shifted to the right (larger times) for the smaller reagent:sample ratios. The I_D point was virtually absent at a reagent:sample ratio of 2:8. They concluded that the position of penetration for different sample and reagent volumes has a major influence on the final peak profile. This largely depends on the kinetics involved in both the ascending and tailing parts of the sample and reagent zones at the I_D point.

2.5.3 Sample volume

This parameter requires consideration since the selection thereof is the main tool for optimizing readout in conventional flow injection. The injected sample zone can be viewed originally as a rectangular block input function, transformed during the passage through the flow channel, which is visualized as consisting of a number of mixing stages (N). For a single mixing stage [14]

$$\frac{C^{\max}}{C^0} = 1 - \exp\left(\frac{-0.693 S_v}{S_{1/2}}\right) \quad (23)$$

where S_v is the injected sample volume and $S_{1/2}$ is the volume necessary to reach $D^{\max} = 2$, where $D^{\max}$ is the dispersion in the element of fluid corresponding to the peak maximum. For an increasing number of mixing stages, the skewness of the flow injection response curve decreases and at $N = 10$ approaches a Gaussian shape. Therefore, for large values of N equation 23 is replaced by an error function [14], which for $S_v = S_{1/2}$ leads to

$$S_{1/2} = \left(\frac{V_r}{2}\right)\left(\frac{N}{2\pi}\right)^{1/2} \quad (24)$$

where V_r is the reactor volume. For $S_v < S_{1/2}$, in other words $D > 2$, the peak height (C/C^0) increases linearly with injected sample volume. It follows from equations 23 and 24 that $S_{1/2}$ is the parameter which describes dispersion of the zone in any flow injection system, regardless of its geometry and type of flow, since it is tied to the variance which describes zone broadening via the N value. Note that

$$\sigma^2 = Nl^2 \quad (25)$$

where σ^2 is the variance of the peak width, l is the length of a mixing step, and N the number of steps [18]. Indeed the peak height increases with the injected sample volume, in the same way as described for conventional flow injection. As predicted by theory, a plot of S_v versus $-\log(1-A_{\max}/A_0)$ yields a straight line. $S_{1/2}$ can be obtained from the following equation

$$-\log\left(1 - \frac{A_{\max}}{A_0}\right) = \frac{0.693 S_v}{2.303 S_{1/2}} \quad (26)$$

where $A_{\max}$ is the maximum absorbance for each peak, A_0 is the initial absorbance (without dispersion), S_v is the sample volume. The $S_{1/2}$ value was calculated from the slope of equation 26. The increase in peak height with increasing sample volume and the determination of $S_{1/2}$ is also illustrated in chapter 3.

The influence of propulsion rate and the instrumental design parameters on dispersion in an SIA system was studied via $S_{1/2}$ by Cladera *et al* [3]. They found that the correct determination of $S_{1/2}$ entailed using controlled conditions as regards the type of dye, its concentration and ionic strength of the solution in order to avoid a potential effect of the dye concentration on $S_{1/2}$ values.

Decreasing flow rates resulted in increasing $S_{1/2}$ values, the effect being more marked at the lower flow rates. They did find however that the $S_{1/2}$ values obtained at high flow rates hindered correct identification of the peak maximum since the peaks were too narrow at the maximum acquisition rate available (2.5 data/s). The reaction tube inner diameter and length also had a

noticeable effect on $S_{1/2}$. Increasing diameter and length resulted in higher $S_{1/2}$ values due to increased residence time and longer path travelled by the dye inside the system.

Cladera *et al* [3] concluded that two systems that have identical $S_{1/2}$ values obtained from different combinations of instrumental parameters may lead to different dispersion profiles and therefore to a different extent of sample-reagent interdispersion. $S_{1/2}$ values seemingly do not allow for direct extrapolation of the results to different systems and while $S_{1/2}$ is a measure of dispersion in a given system, it provides no information on geometric factors (particularly symmetry). Furthermore, though $S_{1/2}$ can be used as a guidance to choose a starting point, it seemed that each particular system must inevitably be optimized specifically.

2.5.4 Three-zone penetration

The stacking of three zones adjacent to one another in an SIA flow conduit offers a different approach in performing SIA. A third zone can be introduced into the SIA system by two possible mechanisms:

- a) draw up an additional zone next to the standard configuration of two zones or
- b) stack two zones, mix them and then add the third zone to the mixture

Obviously, the second mechanism (b) will closely resemble the two-zone configuration discussed so far. Gübeli *et al* [2] investigated the coalescence of three simultaneously stacked zones. They conducted the experiments by injecting two zones of equal volumes ($S_v = R_v = S_{1/2}$) of non-reactive dye into a colourless carrier stream in two different experimental runs and by recording

separately the absorbance of the injected dye zone as a sample and then as a reagent peak.

They introduced the third zone, known as a “spacer”, in each of the two runs (Fig. 2.10 and Fig. 2.11) as a zone of a colourless carrier stream, between the sample and reagent zones. This allowed them to visualize the overlap of the sample and reagent zones in the presence of two different volumes of spacer. The spacer remained invisible, but its presence was apparent by an increasing gap as the spacer volume was increased.

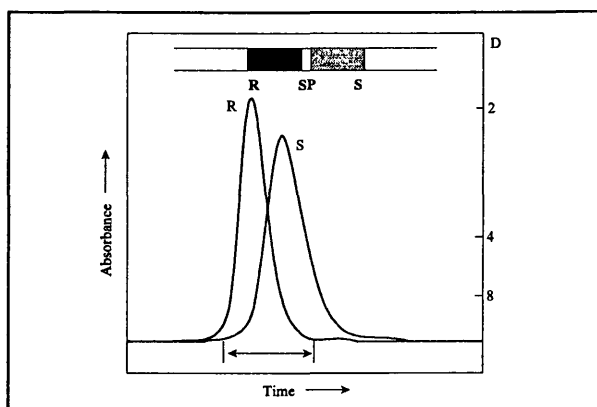


Fig.2.10 Penetration of sample (S) and reagent (R) zones for $Sp_v = 0.25S_{1/2}$ and $P = 0.571$.

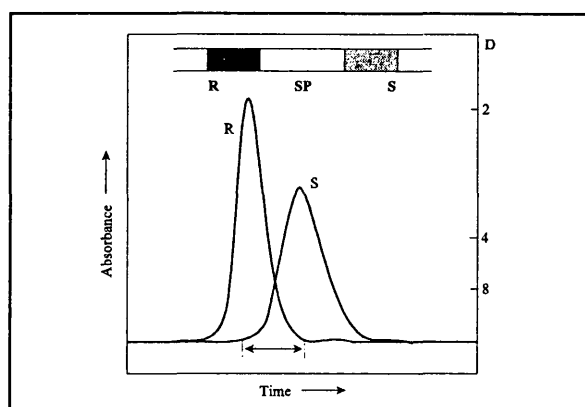


Fig.2.11 Penetration of sample (S) and reagent (R) zones for $Sp_v = 2.5S_{1/2}$ and $P = 0$.

It was evident that an increase in the spacer volume resulted in a decrease of the outside zone overlap between the sample and reagent zones. This approach illustrates the feasibility of performing two reagent chemistry. When the sample zone is sequenced in the spacer position and the two complementing reagents are sequenced as sample and reagent zones, while a sample volume of $0.5S_{1/2}$ is not exceeded, the thus sequentially stacked and injected zones will sufficiently overlap to form a product peak which will yield a meaningful readout. The peak maximum will be close to the I_D point, provided that the original concentrations of injected

reagents (C_{r1}^0 and C_{r2}^0) are much higher than the concentration of the sample material (C_s^0). Only under these conditions will the sample zone be supplied with sufficient excess of both reagents.

Gübeli *et al* concluded that:

“two-reagent chemistry can be accommodated in the sequential injection system, provided that the sample volume is kept below the $S_{1/2}$ value, that the sample zone is surrounded by the reagent zones, and that the concentrations of injected reagents are sufficiently high”

This approach can also be applied to overly concentrated sample zones. A dilution effect can be established by introducing a water zone as the third zone (spacer) between the sample and reagent and by appropriate dilution of the sample, it could then react with the reagent to deliver a measurable detectable specie.

A three zone configuration is though the limited amount of zones that can be mixed by flow reversal in a tubular reactor [25]. Consequently, if several reagents need to be added serially, as is often the case in enzymic assays, the only alternative in SIA is the use of a mixing chamber [9].

2.5.5 Flow reversal

The term flow reversal refers to exactly that, namely reversing the flow by a forward and backwards propelling motion of the liquid drive used in SIA. This action effectively contributes to zone penetration and allows more effective mixing of the reacting components. Clark *et al* [26] indicated the usefulness of flow reversal in FIA-titrations and also demonstrated that thereby

the amount of mixing of the zones could effectively be controlled.

It follows from the random walk model that effective mixing is obtained by a suitable combination of a number of steps (reversals), n , of length l . An increasing number of flow reversals of sufficient amplitude, l , will lead to thorough mixing of sample and reagent at their adjacent interfaces [17].

The influence of flow reversal on zone penetration has been studied by Gübeli *et al* [2]. They indicated that it is the length of a step (l , equation 25) which causes zone broadening more effectively than the number of steps (N). The influence of an increased number of reversals at a constant step length is illustrated in Fig. 2.12. For this purpose a three-zone sequence was stacked and then injected into the reactor (R2), between the valve and detector. The flow was then reversed to return the mixed zones into the reactor (R1) between the holding coil and valve and was reversed again to inject the zones through R2 into the detector. The increasing number of flow reversals indeed contributed to increased mutual zone penetration ($P = 0.383$ for a single reversal (A), while $P = 0.738$ for seven reversals (D) [2]).

Gübeli *et al* concluded that:

“ ... it is the first flow reversal and its length which is most effective in providing mutual zone penetration, and since multiple flow reversals increase overall dispersion - and time to complete a measuring cycle - their use will remain restricted for difficult solution handling tasks, such as mixing of zones of very different viscosities.”

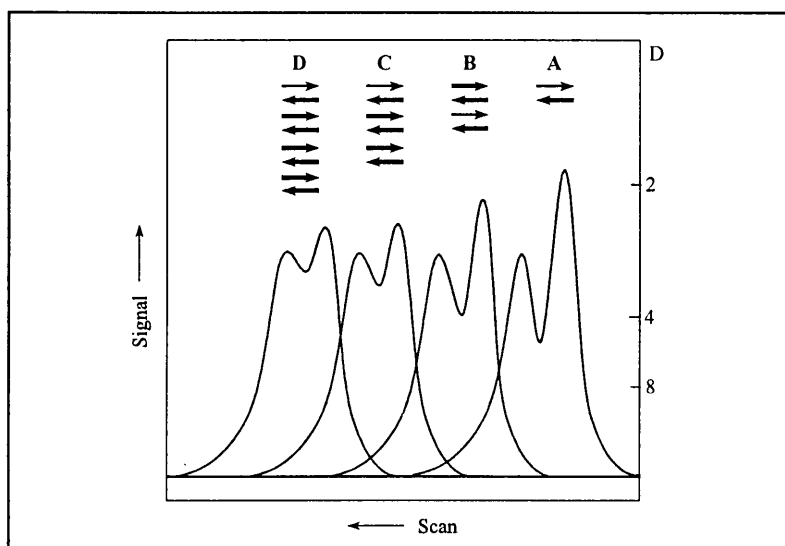


Fig.2.12 Influence of repeated flow reversals on the mutual penetration of sample and reagent zones ($S_v = R_v = S_{1/2}$ and $Sp_v = 1.5S_{1/2}$). The top arrow indicates the stacking direction, the additional arrows indicate the number of flow reversals and their direction.

Flow reversals have also been successfully applied in increasing the mixing between the reacting components. This is illustrated in chapter 4 where the EDTA/citrate masking agent action was improved by introducing multiple flow reversals in the SIA procedure.

2.6 Conclusions

Sequential injection analysis introduced a new approach in conducting analysis by the sequencing of the sample and reagent zones in the flow conduit, compared with the injection of the sample into a reagent carrier stream with flow injection analysis. Not only did this approach result in an enormous reduction of sample and reagent consumption, but it also brought a new dimension to computer controlled analysis. Despite the fact that SIA delivers similar dispersion patterns than those that have been found for its predecessors, it does though have certain inherent

characteristics. Zone penetration was identified as an important aspect of the SIA system besides dispersion which has been studied as an essential parameter for the previous flow systems.

Since its introduction into the analytical field a lot of work has been done on the parameters that governs zone penetration in the SIA flow conduit. It was evident that the success of this technique was dependent on the successful attainment of zone penetration. A major advantage of SIA is that any changes that are to be made are accomplished via flow programming rather than physical reconfiguration of the flow path, as was custom with FIA. The selection valve allows for multi-reagent chemistries and multi-detector assays to be carried out, which is an added advantage.

It is evident that by careful manipulation of the system parameters successful analysis can be conducted with SIA by controlling the degree of zone penetration and dispersion.

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CHAPTER 3

Evaluation of the Operational Parameters Affecting Dispersion in Sequential Injection Analysis

3.1 Introduction

The physical foundations of flow injection analysis are related to dispersion that is defined as the dilution undergone by a sample volume injected into the flowing stream [1]. 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. The development of flow injection analysis has been characterized by an in depth study into the variables that affects dispersion in a manifold and hence its effect on the analytical signal. As knowledge of the dispersion process increased, the concept of flow injection analysis was refined further. In the years that followed several models have been used to gain greater understanding of the dispersion process and have been discussed in several monographs' [1-3].

The development of sequential injection analysis from its mother technique flow injection analysis has also resulted in the extensive investigation of the physical parameters that depicts the flow system [4,5]. Both flow injection analysis and sequential injection analysis operate on identical underlying principles: sample injection, controlled dispersion and reproducible

timing [6]. It is evident that though dispersion dominates the theory of flow injection analysis [2], it has been found for sequential injection analysis that zone penetration, in addition to the dispersion of the zones, is also of fundamental importance [2].

Despite the growing importance of zone penetration, sequential injection analysis does though rely on the mutual dispersion of the reactant segments since it also contributes to the signal to be recorded. Controlling dispersion in a given system is important with view to its characterization and optimization [7]. Despite the number of papers that have appeared on SIA, not that many have appeared on the basic principles that depict dispersion in the SIA system [4,5]. It was subsequently decided to conduct a study on those parameters that essentially influences dispersion in an SIA manifold and to contribute to the existing knowledge regarding the effect of the operational parameters on an SIA system.

3.2 Theoretical aspects of dispersion

As is already known, sequential injection analysis consists of the sequential aspiration of a sample (S) and reagent (R) zone into a holding coil. In this way a stack of well-defined zones is obtained which, after reversal of the flow, is propelled through a reactor into a detector. The flow reversal creates a composite zone in which the sample and reagent zones penetrate one another due to combined axial and radial dispersion. This gives rise to the formation of a so-called product zone that will be observed by the detector and subsequently deliver a Gaussian peak.

The extent of the dispersion that the sample and reagent zones will undergo to result in the formation of the product peak, is essentially influenced by the operational parameters that govern the SIA flow conduit. Since zone sequencing and mutual dispersion of the zones are the key operations [5], it is imperative that the parameters which determine the extent of dispersion are optimized to the point where the required amount of dispersion is achieved.

The earliest parameter used to characterize passage of the sample through the system was proposed by Ružička *et al* [8] and was called a dilution factor or dispersion. It is a direct measure of the extent of dilution undergone by a particular part of the sample zone between injection and detection and is defined as the ratio of the concentrations before (C_0) and after (C) transport through a given FIA system:

$$D = \frac{C_0}{C}$$

Thus, each point on the signal record has a corresponding dispersion coefficient. At the signal maximum the relationship between C_0 and C is given by:

$$D_{\max} = \frac{C_0}{C_{\max}}$$

Since there is generally a direct relationship between the property used for detection (absorbance, pH, potential, fluorescence intensity), the magnitude of the transduced signal recorded and the

concentration of the sample or its reaction product, the dispersion coefficient can be determined from the ratio of the height of the signal found in the absence of dispersion (i.e. for continuous flow of undiluted sample) to that of the FIA signal. Thus

$$D_{\max} = \frac{k_o h_o}{k_l h_{\max}} = \frac{h_o}{h_{\max}}$$

where k_o and k_l are proportionality constants, identical if the signal height is linearly related to concentration over the range considered [1]. Hence, the dispersion coefficient can often be taken as the signal height ratio. From its definition it follows that the dispersion coefficient always exceeds unity. Qualitatively, the greater the dispersion (dilution), the flatter the resulting curve. The dispersion coefficient has similarly been defined for sample dispersion ($D_s = C_s^0/C_s$) and reagent dispersion ($D_r = C_r^0/C_r$). These simple relations allow estimation of the concentrations of reacting components in the relevant element of the dispersed sample zone in SIA [5].

The value of the dispersion coefficient is dictated by the physical parameters of a system and has to be adjusted accordingly to suit the requirement of the intended measurement. Three categories can be identified [5]:

- *Limited dispersion* ($D \rightarrow 1$) : pH or conductivity measurements
- *Medium dispersion* ($D = 2 - 10$) : reagent-based chemistries
- *Large dispersion* ($D > 10$) : extensive sample dilution

It is thus evident that the amount of dispersion can be controlled to deliver adequate sensitivity, depending on the requirements of the system.

3.3 Approach towards evaluation of the operational parameters

Several authors have successfully attempted to evaluate different aspects of the sequential injection analysis system [4-7,9-11] since its introduction into the analytical field in 1990 [12,13]. A short discussion follows on certain studies that have been done up to date and that resulted in the decision to conduct the dispersion study described below.

Gübeli *et al* [5] conducted a study on the variables governing zone penetration in an SIA flow conduit. These variables included the sample volume and $S_{1/2}$ ($S_{1/2}$ is the volume necessary to reach $D^{\max} = 2$, where $D^{\max}$ is the dispersion in the element of fluid corresponding to the peak maximum), zone penetration as a function of relative volumes of sample and reagent zones, increasing zone volumes at equal volume ratios, varying of zone volume ratios, three-zone penetration and flow reversal.

The effect of certain operational parameters on an SIA system was evaluated by Marshall and van Staden [4]. The following parameters were under consideration: tubing diameter, reaction tube geometry, pump speed and the order of sample and reagent zone injection. It was not specified whether the tubing diameter was studied specifically for the RC or HC. The decision was made to conduct an in depth study regarding the effect of both the internal diameter (i.d.) and length of the RC and HC on the dispersion to be obtained in an SIA flow conduit. Although the

effect of the RC and HC i.d. had been studied previously [4], the length thereof did not come under consideration and it was decided that the evaluation of the RC and HC length could contribute to understanding the effect thereof on dispersion in an SIA system.

The range of flow rates, encountered at a fixed pump speed, studied by Marshall and van Staden [4] was conducted with a dual-piston sinusoidal-flow syringe pump. These results were obtained for different cam positions. A previous study by Ivaska *et al* [6] proved that peristaltic propulsion is just as feasible and practical as the piston pump propulsion. They indicated that the peristaltic pump was successful in complying with the strict requirements of SIA. In the present study a peristaltic pump was used to regulate the flow rate. A range of flow rates can be achieved with a peristaltic pump by changing the pump tubing internal diameter (i.d.) and pump speed (these two parameters combined determine the flow rate given by a peristaltic pump) respectively. It is evident that the proposed action delivers a different approach to evaluating the effect of flow rate on dispersion in an SIA system.

Taljaard [14] discussed the effect of pump speed, pump tubing i.d., holding coil (HC) length and i.d., reaction coil (RC) length and i.d., sample and reagent zone volumes, order of sample and reagent zone injection, reactor geometry and flow reversal on zone dispersion and zone penetration in an SIA system.

Although mixing chambers have been used before in flow systems [15-18], the influence thereof on dispersion in an SIA flow conduit has not been evaluated previously and it was subsequently included in the present study.

The studies done by Marshall and van Staden [4] and Gübeli *et al* [5] are the only ones documented regarding the effect of certain operational parameters on the dispersion and zone penetration to be achieved in an SIA flow conduit. There were clearly a number of variables that had not been studied yet, e.g. HC and RC lengths and a mixing chamber in the SIA manifold, and thus needed further attention.

The operational parameters that were studied are: pump speed, pump tubing i.d., holding coil length and i.d., reaction coil length and i.d., sample and reagent zone volumes and mixing chambers. The approach by which the parameters were studied might differ from that already done, thus the results and conclusions drawn from these results could also differ from those already made [4,5,14]. The study was carried out by aspirating a dyestuff solution (Bromothymol Blue (BTB) in Borax) into a water carrier stream at a pH ensuring maximum absorbance.

It is believed that the results obtained could contribute significantly in understanding SIA systems and therefore to the optimization of subsequent SIA systems, especially those that are dependent on the degree of dispersion that takes place.

3.4 Experimental

3.4.1 Reagents and solutions

All reagents were prepared from analytical-reagent grade chemicals unless specified otherwise. All aqueous solutions were prepared using de-ionised water from a Modulab system (Continental Water Systems, San Antonio, Texas, USA). The de-ionised water used as carrier stream and to prepare the aqueous solutions with, was degassed, by heating to boiling point and cooling before the solutions were made up.

Borax solution: A 1.0×10^{-2} mol/l Borax solution was prepared by dissolving 19.068 g disodium tetraborate in an adequate volume of water and diluting the resulting solution to 5.0 l with water.

Bromothymol Blue solution: A 2.1×10^{-5} mol/l BTB solution was prepared by dissolving 0.0263 g Bromothymol Blue in 15.0 ml of ethanol and diluting the resulting solution to 2.0 l with the 1.0×10^{-2} mol/l Borax solution.

3.4.2. Instrumental

The sequential injection system schematically outlined in Fig. 3.1 was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-le-Bel, France); a 10-port electrically actuated selection valve (Model ECSD10P; Valco Instruments,

Houston, Texas, USA); and a UNICAM 8625 UV-visible spectrometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell (volume 80 μ l) for absorbance measurements.

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (Mintek, Randburg, South Africa). The FlowTEK [19] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All the data given (relative peak heights) are the mean for $n = 10$.

The absorbance of the Bromothymol Blue solution used to study the dispersion in an SIA manifold, was measured at 620 nm. The decision to conduct measurements at this wavelength was based on a scan of the specified solution over the 200 to 1100 nm range with a Spectronic Genesys 5 spectrophotometer (Milton Roy Company, USA). The optimum wavelength was found to be 620 nm [20].

The mixing chambers used were made in-house of Perspex and the volumes studied were: 0.5; 1.0; 3.0 and 3.1 ml. Tygon tubing was used for the holding coils and reaction coils.

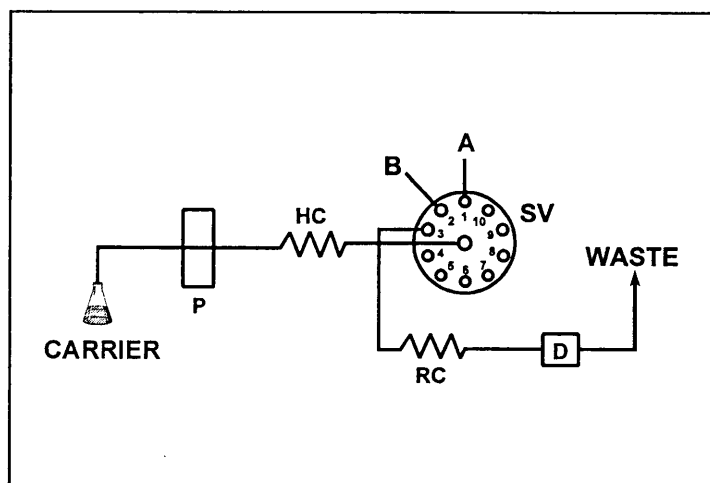


Fig. 3.1 A schematic diagram of the SIA system used for the evaluation of the operational parameters with BTB. P - pump, HC - holding coil, RC - reaction coil, SV - selection valve and D - detector, A & B = BTB or Borax solutions.

3.4.3 Procedure

The measuring cycle of the SIA system for evaluation of the operational parameters comprised of the following operations: (1) pump off and selection of BTB (i) or Borax (ii) stream at valve position 1, (2) aspiration of BTB (i) or Borax (ii) as the sample solution by reversed action of the pump for a specified time, (3) pump off and selection of Borax (i) or BTB (ii) stream at valve position 2, (4) aspiration of Borax (i) or BTB (ii) as the reagent solution by reversed action of the pump for a specified time, (5) pump off and selection of detector line at valve position 3, (6) pump forward for a specified time and expulsion of the sample and reagent through the detector (where a signal is measured) to waste, (7) pump off after completion of the experimental run.

3.5 Evaluation of the operational parameters

When studying dispersion in SIA, the degree thereof can easily be approximated by using the analytical signal that is obtained experimentally [1]. It is known from FIA that there is an inverse relationship between dispersion and the resulting signal, meaning that the larger the dispersion the less intense the recorded signal (peak height in this instance) will be. Since SIA is seen as an extension of FIA, the same conclusion regarding the relationship between the recorded signal and dispersion can be applied. This relationship will also be evident from the results that were obtained from this study. Thus by studying the peak heights that were obtained by varying the operational parameters of the SIA system, it will be possible to make certain conclusions regarding the effect of these parameters on dispersion.

In the following study relative peak height (the quantification of the recorded signal) was studied when varying the operational parameters in the SIA system. The precision of the experimental results was determined by calculating the relative standard deviation of ten measurements at a given concentration of BTB. The effect of the operational parameters on dispersion was subsequently evaluated by using both peak height and the reproducibility of the obtained experimental results.

A Bromothymol Blue solution was used to study the dispersion taking place, bearing in mind that it simply reflects the dispersion process taking place in the manifold and not any chemical reaction that might occur in a real system. Two measurements, illustrated in Fig. 3.2, were carried out for each variation of a parameter. In the first type of measurement, the dye was selected first and the buffer solution second (a). When selecting the dye first it referred to the dye

being drawn first into the holding coil and the buffer solution secondly. In this instance the dye solution represented the sample zone. This sequence was reversed for the next measurement with the buffer solution being drawn up first and the dye solution second (b). Now the dye solution represented the reagent zone.

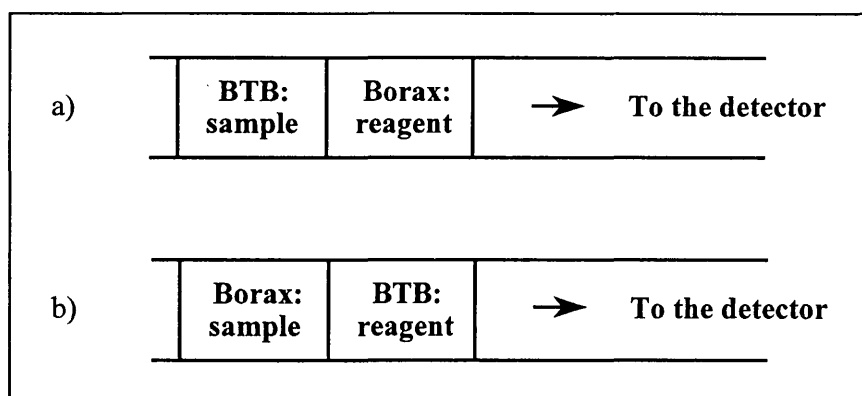


Fig. 3.2 Arrangement of BTB and Borax zones in the holding coil as the reagent and sample respectively.

The actual dispersion that was experienced in the SIA system was calculated from the recorded peak heights ($D_{\max} = h_0/h_{\max}$). By pumping the $2.1 \times 10^{-5} \text{ mol/l}$ BTB solution continuously through the SIA system and recording the resulting peak height (h_{BTB}), h_0 could be quantified after subtraction of the background ($h_0 = h_{\text{BTB}} - h_{\text{background}}$). This value corresponded to the peak height where BTB does not experience any dispersion at all. The peak height obtained as a result of the BTB dispersion due to the system parameters, is $h_{\max}$.

The original system from which further studies were carried out, was composed as follows: HC length 2.50 m; HC i.d. 1.14 mm; RC length 1.00 m; RC i.d. 0.76 mm (these parameters were chosen arbitrarily). These choices were based on the fact that the HC length should be adequate

enough so that the drawn up zones will, under no circumstance, penetrate the pump tubing since the dispersion experienced in the pump tubing is different from that experienced in the HC itself. The HC i.d. was chosen to be wide enough to prevent blockage of the tubing and narrow enough to prevent the reaction products from penetrating the pump tubing. The RC dimensions were chosen according to the results in [14].

3.5.1 Results and Discussion

3.5.1.1 Pump speed and Pump tubing internal diameter (flow rate)

A study regarding the effect of pump speed has previously been conducted by Marshall and van Staden [4]. They stated that flow rate could be varied by changing the pump speed. In conclusion to their study it was found that a range of flow rates could be encountered at a fixed pump speed, for a dual-piston sinusoidal-flow syringe pump, at different cam positions. The flow rate of a peristaltic pump though, is altered by changing one of two parameters namely: pump speed and/or pump tubing i.d. This combination of parameters has not been studied yet and the influence of the two variables on dispersion in an SIA system was subsequently evaluated.

The pump tubing i.d.'s studied was: 1.65; 1.85; 2.06; 2.29 and 2.79 mm. For each one respectively, the pump speed (rpm) was varied as follow: 10.0; 12.0; 15.0; 18.0; 20.0 and 22.0 rpm. Each separate combination of pump speed and pump tubing i.d. gave rise to a specific flow rate, since the variation of any one of these two parameters influence the flow rate directly. A plot of pump speed vs flow rate for each pump tubing i.d. gave rise to a straight line

relationship.

The influence of pump speed on peak height, for each of the pump tubing i.d.'s, is shown in Fig. 3.3 and Fig. 3.4 for the sample and reagent zones respectively. It is evident from these figures that an increase in pump tubing i.d. and pump speed respectively resulted in increased peak heights for both the sample and reagent zones. The increase in peak height was attributed to a decrease in dispersion as the flow rates were increased [1].

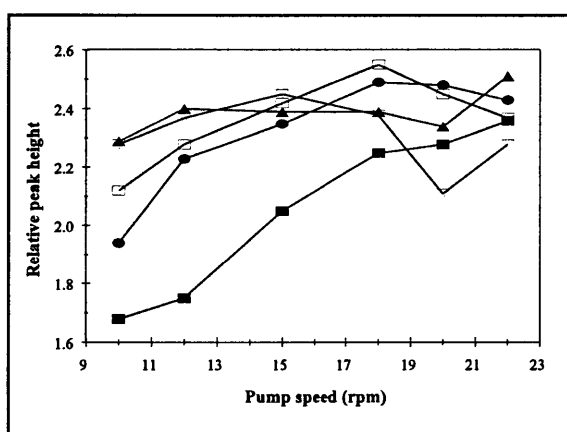


Fig. 3.3 Peak height for different pump tubing i.d.'s (mm) [■, 1.65 mm; ●, 1.85 mm; □, 2.06 mm; ▲, 2.29 mm and ▽, 2.79 mm] at various pump speeds, for BTB drawn up as the sample zone.

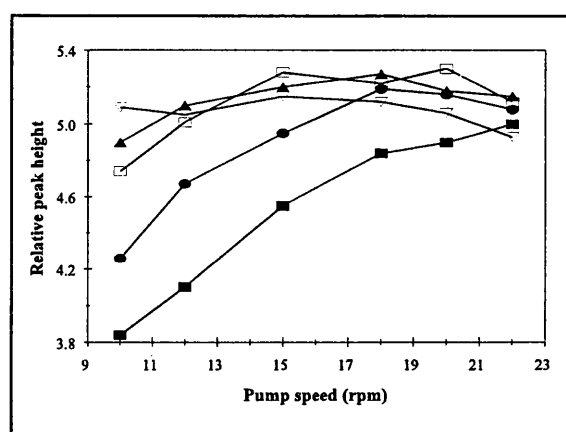


Fig. 3.4 Peak height for different pump tubing i.d.'s (mm) [■, 1.65 mm; ●, 1.85 mm; □, 2.06 mm; ▲, 2.29 mm and ▽, 2.79 mm] at various pump speeds, for BTB drawn up as the reagent zone.

The peak height vs pump speed curve flattened out for the largest i.d. and pump speed, which was attributed to the fact that the maximum obtainable peak height was achieved at these conditions, and that any further increase in the i.d. and pump speed would not have a noteworthy effect on the peak height. In Fig. 3.4 a decrease in peak height was obtained for BTB as the reagent zone, over the entire pump speed range, for the largest pump tubing i.d. (2.79 mm). This follows clearly from the above reasoning.

From the results obtained the largest peak height (maximum response) was achieved with a pump speed of 18.0 rpm and a pump tubing i.d. of 2.06 and 1.85 mm (S-zone) and at 20.0 rpm for an i.d. of 2.06 and 2.29 mm (R-zone). These flow rates thus delivered the best sensitivity and subsequently the lowest amount of dispersion. The decision regarding an optimum pump tubing i.d. and pump speed was made by evaluating the reproducibility of the optimum conditions just mentioned.

Considering the reproducibility of the results in Fig. 3.5 for the sample zone, it was evident that a pump tubing i.d. of 1.85 mm, at a pump speed of 18.0 and 20.0 rpm, not only gave the best but also better reproducibility than an i.d. of 2.06 and 2.29 mm. In Fig. 3.6 (R-zone) on the other hand an i.d. of 1.85 mm gave fairly good reproducibility at 18.0 rpm, while an i.d. of 1.65 mm gave better reproducibility at 20.0 rpm than the 1.85 mm i.d..

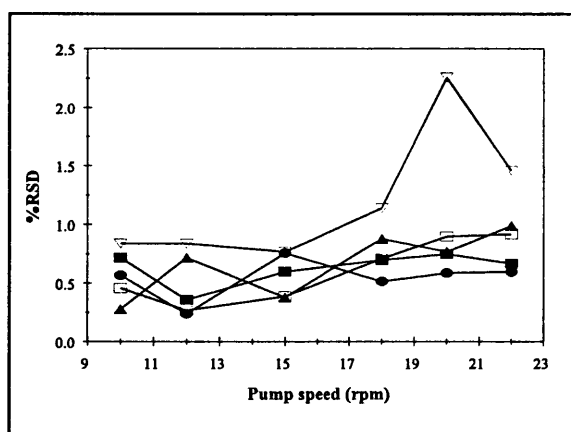


Fig. 3.5 %RSD for different pump tubing i.d.'s (mm) [■, 1.65 mm; ●, 1.85 mm; □, 2.06 mm; ▲, 2.29 mm and ▼, 2.79 mm] at various pump speeds, for BTB drawn up as the sample zone.

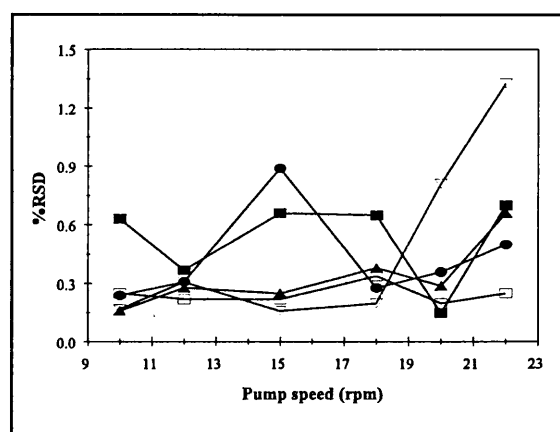


Fig. 3.6 %RSD for different pump tubing i.d.'s (mm) [■, 1.65 mm; ●, 1.85 mm; □, 2.06 mm; ▲, 2.29 mm and ▼, 2.79 mm] at various pump speeds, for BTB drawn up as the reagent zone.

The reproducibility was relatively constant for all the pump tubing i.d.'s over the entire range of pump speeds for both the sample and reagent zones, except for a pump tubing i.d. of 2.79 mm at pump speeds above 18.0 rpm. It seems as if it became increasingly difficult to deliver reproducible dispersion of the zones in the flow conduit at very high flow rates, compared with the lower flow rates, that are determined by smaller pump tubing i.d.'s and lower pump speeds. A possible explanation for this is that the back-pressure in the system became too high at excessively high flow rates [2] and subsequently had a negative influence on reproducibility.

When deciding on an optimum it seems as if the best sensitivity and reproducibility were obtained at a pump speed of 18.0 rpm and a pump tubing i.d. of 1.85 mm. This optimum corresponds to a flow rate of approximately 4.8 ml/min as well as with the maximum flow rate of 5.2 ml/min used during a previous study [5].

An interesting observation that is made when considering the sample and reagent zone overlap, illustrated in Fig. 3.7, is the positional change of the isodispersion point when changing the flow rate. It is evident in Fig. 3.7 that for a constant pump tube i.d. (2.79 mm) and decreasing pump speed, the isodispersion point moves to larger times. This is ascribed to the increase in dispersion of the respective zones with decreasing flow rates.

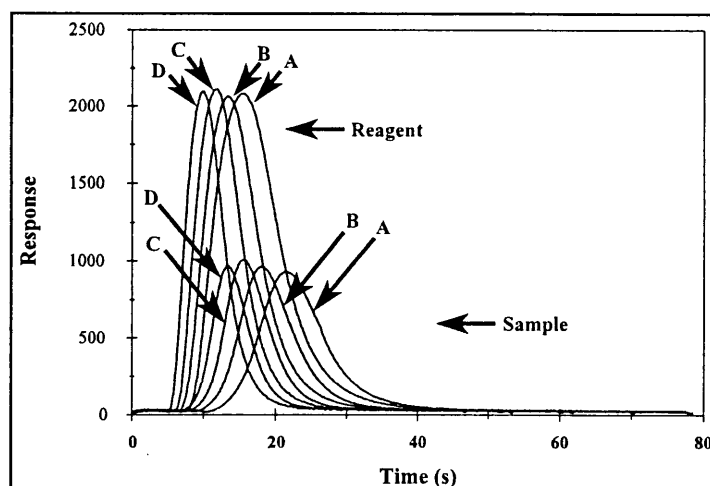


Fig. 3.7 Shifting of isodispersion point with a change in flow rate.
A - 10.0 rpm, B - 12.0 rpm, C - 15.0 rpm and D - 18.0 rpm.

The optimum pump tubing i.d. and pump speed was used to evaluate the following operational parameters.

3.5.1.2. Sample and reagent zone volumes

Gübeli *et al* [5] have already conducted a study on the effect of sample and reagent zone volumes on zone penetration in an SIA flow conduit. The decision to conduct the study described below was not aimed at repeating work that had already been done, but to determine optimum sample- and reagent-zone volumes for the SIA system optimized so far. It was decided to take the reagent volume (V_r) as twice that of the sample volume (V_s), thus: $V_r = 2 V_s$, according to [5] which states that at these conditions the optimum conditions for sequential injection single reagent based chemistry is met. Although the present study does not focus on actual reactions taking place, the S:R zone volume ratio mentioned is a good choice to build forth on.

In the study conducted on flow rate (3.5.1.1) the sample volume was taken as 350 μl and the reagent volume as 700 μl [11]. In the following study, these volumes were varied in a 1:2 (S:R) ratio. The effect of increasing the sample volume, and subsequently also the reagent volume, on the dispersion of the resulting product peak was studied and conclusions were drawn regarding the effect thereof. From previous studies [5] it was evident that an increase in the S- and R-zone volumes gave rise to a decrease in the dispersion of the product peak. The reason therefore was because of the inverse relationship between the volume of sample/reagent (V_i) drawn up and the dispersion taking place along the flow conduit [1]:

$$D \propto \frac{1}{V_i}$$

In Table 3.1 the dispersion, obtained by converting peak height measurements to dispersion, is depicted together with the corresponding %RSD for the BTB drawn up as the sample and reagent zone respectively. The results are documented for different sample volumes not neglecting the fact that the reagent volume was also varied simultaneously in a 1:2 (S:R) volume ratio. When the BTB solution was regarded as the S-zone the sample volume would refer to the volume of BTB solution drawn up, but when the BTB solution was regarded as the R-zone the sample volume would refer to the volume of Borax solution drawn up.

TABLE 3.1. Influence of the sample and reagent zone volumes on the dispersion and %RSD obtained for BTB drawn up as the sample and reagent zone respectively.

Sample volume ($\mu\ell$)	BTB the sample		BTB the reagent	
	zone		zone	
	Dispersion	%RSD	Dispersion	%RSD
100	8.27	1.65	2.79	1.16
150	4.15	0.79	2.19	0.61
250	3.37	0.67	1.57	0.38
350	3.04	0.54	1.37	0.41
450	2.61	0.78	1.24	0.14
600	2.46	0.30	1.16	0.15
750	2.19	0.37	1.11	0.18

It is evident from Table 3.1 that by increasing the volume of sample and reagent drawn up, dispersion decreased for both the S- and R-zones. The best reproducibility was obtained for the larger volumes of S- and R-zone drawn up. This was ascribed to the fact that the larger volumes were drawn up more accurately compared with the smaller volumes. It was noticeable that the dispersion obtained for the S-zone was larger than that of the R-zone. The reason therefore was that the reagent zone was drawn up secondly and the distance that it travelled to the detector was smaller compared with that of the sample zone drawn up first, and thus undergoes less dispersion [9].

The optimum sample- and reagent-zone volumes were calculated by plotting: $-\log [1 - A_{\max}/A_0]$ (A_0 is the absorbance that corresponds to the instance where the Bromothymol Blue solution does not undergo any dispersion in the SIA flow conduit) vs sample volume ($\mu\ell$). A linear regression

was subsequently carried out on the obtained data and from the slope of the calculated regression [21] it was possible to determine $S_{1/2}$ (corresponding to a maximum dispersion of 2). The $S_{1/2}$ value was calculated to be 1270 $\mu\ell$.

According to Gübeli *et al* [5] it was necessary to keep the sample zone volume less than or equal to $0.5S_{1/2}$ and the reagent volume twice as large as the sample volume, thus less than or equal to $S_{1/2}$. This allows for the optimum conditions of sequential injection single reagent based chemistry to be met. This resulted in a sample volume of 635 $\mu\ell$ and a reagent volume of 1270 $\mu\ell$.

When considering Table 3.1, it is evident that minimum dispersion (thus optimum peak height) and good reproducibility was obtained at a sample volume of 600 $\mu\ell$ (thus 1200 $\mu\ell$ reagent volume) for both the sample and reagent zones studied. These results compare well to the calculated values deducted above. Too large S- and R-volumes are not always practical since it is not always the most effective way of increasing the sensitivity of the measurements. Although smaller volumes would have been expected because of the better zone penetration [5], too small volumes are not always effective since the dispersion of the resulting zones become too big and reproducibility of the measurements decrease.

A S-zone volume of 600 $\mu\ell$ and R-zone volume of 1200 $\mu\ell$ was taken as the optimum.

3.5.1.3 HC length and internal diameter

The holding coil plays an important role in the dispersion of the sample and reagent zones in SIA. Both the length and i.d. of the holding coil affects the resulting dispersion, as both the S- and R-zones are drawn up into it before the flow is reversed, after which the zones are then propelled towards the detector.

According to a previous study that has been done on these variables [14], the i.d. has a greater effect on the dispersion that takes place in an SIA flow conduit, compared with the effect of the tubing length on the resulting dispersion. The peak height results for BTB drawn up into the HC as a S- and R-zone respectively are shown in Table 3.2. The results were obtained by studying the effect of six HC lengths: 2.50; 3.00; 3.50; 4.00; 4.50 and 5.00 m at three HC i.d.'s: 1.02; 1.14 and 1.60 mm respectively.

Although there was an apparent decrease in peak height for the longer tubing lengths, the numerical differences between these peak heights were quite small so that the actual influence of changing the HC length was not very noticeable. This was confirmed by earlier studies [14]. The effect of the HC i.d. on the resulting dispersion was more apparent.

TABLE 3.2. Comparison of peak height obtained for holding coils of different i.d. and at various holding coil lengths for BTB drawn up as the sample and reagent zone respectively.

HC length (m)	Internal diameter (mm)					
	BTB the sample zone			BTB the reagent zone		
	1.02	1.14	1.60	1.02	1.14	1.60
2.50	2.97	2.82	2.65	5.83	5.71	5.62
3.00	2.89	2.79	2.56	5.83	5.72	5.72
3.50	2.91	2.72	2.59	5.83	5.79	5.69
4.00	2.87	2.71	2.56	5.87	5.78	5.65
4.50	2.78	2.71	2.48	5.70	5.76	5.67
5.00	2.71	2.72	2.46	5.66	5.74	5.54

It is also known that an increase in the HC i.d. contributes to a decrease in peak height, thus an increase in dispersion, because of the frictional effect of the tubing walls which decreases with an increase in tube diameter [2]. This effect results in reduced axial dispersion that accordingly gives rise to smaller peaks. This phenomenon is evident from Table 3.2 for both the sample and reagent zones.

Larger HC i.d.'s are preferred because they are not as prone to blockages as the narrower tubes. They could also give rise to more effective mixing of the zones because of the extent of the dispersion that the zones will undergo in the larger tubing i.d.'s. The HC i.d. must also be narrow enough to prevent the reaction products from penetrating the pump conduit where the dispersion can differ from that, which is achieved in the HC itself. This can effectively be achieved by controlling the volume of the HC that is determined by the length and i.d. thereof.

The optimum HC i.d. with regard to dispersion was 1.60 mm. It gave rise to smaller peaks, for both the S- and R-zones (Table 3.2), thus the resulting dispersion will be the larger that is an important requirement in this part of the flow conduit. The reproducibility of the experimental results is depicted in Fig. 3.8 and Fig. 3.9, for the S- and R-zones respectively. It is evident from Fig. 3.8 that the best overall reproducibility was achieved for an i.d. of 1.02 mm with an i.d. of 1.60 mm not far behind. There is not a definitive trend regarding the reproducibility of the measurements that were obtained for BTB drawn up as the S-zone.

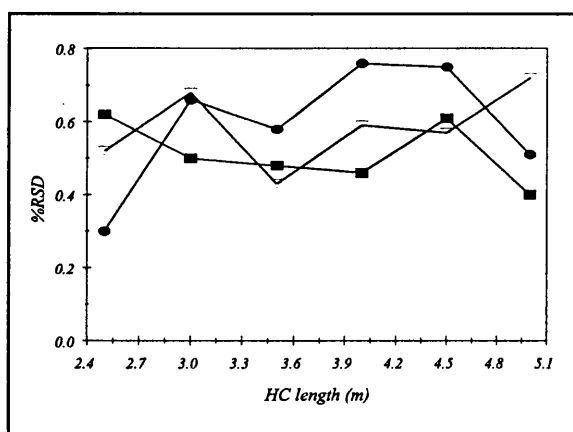


Fig. 3.8 Comparison of the %RSD obtained for HC's of different i.d. (mm) [■, 1.02 mm; ●, 1.14 mm and ▽, 1.60 mm] at various HC lengths for BTB drawn up as the sample zone.

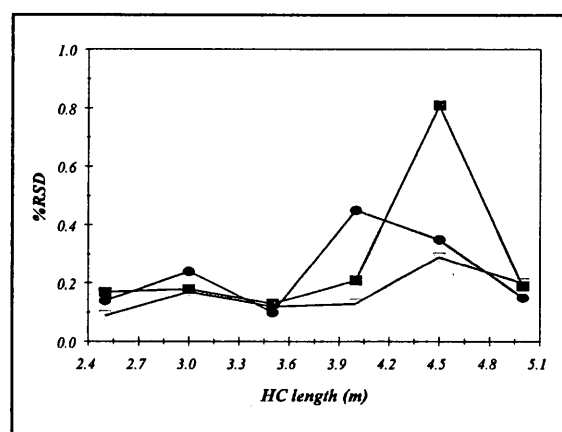


Fig. 3.9 Comparison of the %RSD obtained for HC's of different i.d. (mm) [■, 1.02 mm; ●, 1.14 mm and ▽, 1.60 mm] at various HC lengths for BTB drawn up as the reagent zone.

Fig. 3.9 indicates a decrease in reproducibility for the measurements above a HC tubing length of 4.00 m. For an i.d. of 1.60 mm the best reproducibility was achieved for tubing lengths of 2.50 and 3.50 m. The 3.50 m length gave good results for the corresponding S-zone (Fig. 3.8). Although the reproducibility for the 1.14 mm i.d. and 2.50 m length tubing seems better than that of an i.d. of 1.60 mm at the same HC length, the corresponding dispersion was not high enough (Table 3.2) to result in the effective mixing of the zones. This should be taken into account when

deciding on an optimum.

The peak height must be small enough (a large dispersion thus) to achieve a large enough degree of mixing between the “reacting” zones. Although it is necessary to achieve a large degree of dispersion in the HC it must not be so big that the dilution effect could result in the unnecessary decrease in sensitivity of the measurements. A compromise should be made between dispersion (dilution) and sensitivity.

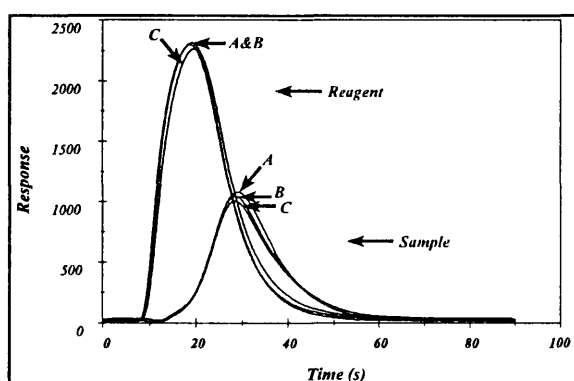


Fig. 3.10 Shifting of isodispersion point when changing the HC length. A - 2.50 m, B - 4.00 m, C - 5.00 m

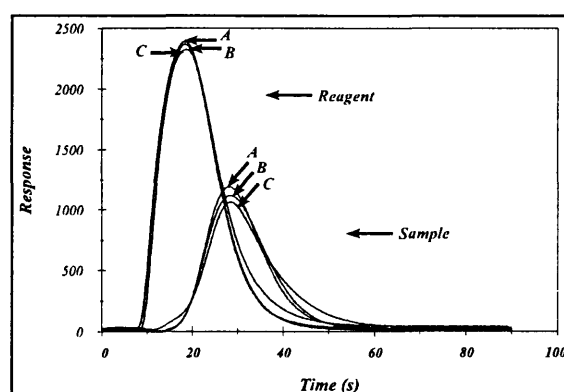


Fig. 3.11 Shifting of isodispersion point when changing the HC i.d. A - 1.02 mm, B - 1.14 mm, C - 1.60 mm

It is evident for the cases illustrated in Fig. 3.10 and Fig. 3.11 that there is no obvious shift of the isodispersion point for a constant HC i.d. (1.60 mm) and varying HC lengths (Fig. 3.10) and for a constant HC length (3.50 m) and varying HC i.d.'s (Fig. 3.11). This is due to the limited affect of the HC dimensions on the dispersion of the respective zones.

The optimum HC dimensions used for further work was an i.d. of 1.60 mm and a tube length of 3.50 m.

3.5.1.4 RC length and internal diameter

The sample and reagent zones have undergone an appreciable amount of dispersion by the time it reaches the reaction coil (RC) due to the system variables. It is necessary to minimise further dispersion of these zones, especially the formed product zone, so that adequate sensitivity can be achieved. An important requirement of the RC dimensions is thus minimization of the product zone dispersion. A short RC tube length and/or a small i.d. would thus be desirable to fulfill the requirements of minimum dispersion.

Marshall and van Staden [4] have previously conducted a study on the effect of the RC i.d. on the resulting dispersion in an SIA system. In the study described below, both the i.d. and length of the RC came under consideration.

Two possibilities exist when studying the effect of the RC dimensions on dispersion in an SIA system. The RC can be placed at one of two possible positions in the SIA flow conduit: (i) connecting the holding coil with the selection valve or (ii) connecting the selection valve with the detector. The decision was made to only study the second option since the conclusion drawn from this, as described below, corresponds to that which had been obtained for the first option during an earlier investigation [14].

The RC dimensions were studied by varying the RC length for three respective i.d.'s. The i.d.'s that were studied are: 0.64; 1.02 and 1.60 mm, at seven arbitrarily chosen lengths: 0.55; 0.70; 0.85; 1.00; 1.10; 1.15 and 1.30 m respectively. Table 3.3 depicts the peak heights obtained for the respective RC lengths and i.d.'s for BTB studied as the S- and R-zone respectively. It is

evident from Table 3.3 that a decrease in the RC tube lengths resulted in increased peak heights, due to the shorter distance that the drawn up zones had to travel before reaching the detector and as a result a decrease in dispersion was observed. This was observed for both the S- and R-zones.

TABLE 3.3. Comparison of peak heights obtained for reaction coils of different i.d and at various reaction coil lengths for BTB drawn up as the sample and reagent zone respectively.

RC length (m)	Internal diameter (mm)					
	BTB the sample zone			BTB the reagent zone		
	0.64	1.02	1.60	0.64	1.02	1.60
0.55	2.36	2.49	2.25	5.64	5.51	5.03
0.70	2.32	2.52	2.20	5.71	5.61	5.00
0.85	2.24	2.45	2.14	5.76	5.43	4.68
1.00	2.13	2.38	1.98	5.77	5.38	4.47
1.10	2.07	2.45	-	5.77	5.44	-
1.15	1.99	2.41	1.99	5.76	5.45	4.35
1.30	1.91	2.35	1.93	5.74	5.30	4.18

It is noticeable that the change in numerical values of the peak heights are very small for an i.d. of 1.02 and 1.60 mm (S-zone) and 0.64 and 1.02 mm (R-zone) at different lengths of tubing respectively. It is thus evident that the influence of the RC length on dispersion is not that important.

A conclusion that can be drawn from the results in Table 3.3 is that a decrease in the RC i.d. resulted in increased peak heights. This was especially noticeable when decreasing the i.d. from 1.60 mm to 1.02 mm, for both the S- and R-zones. This was ascribed to the same reason as explained for the HC i.d., namely the frictional effect with a change in tubing i.d. [4].

Another interesting observation is that the peak heights that were obtained for the S-zone, at the various RC lengths, were smaller for a RC i.d. of 0.64 mm than that for 1.02 mm. This was contrary to that expected namely an increase in peak height with a decrease in tubing i.d. [4]. A possible explanation for this is that the S-zone, undergoing the dispersion, can not achieve a dispersion that is lower than the minimum, thus maximum peak height, and then the reversed effect is observed, namely a decrease in sensitivity. It is important to notice that the S-zone is already subject to a large degree of dispersion, compared to the R-zone that is drawn up last and undergoes less dispersion. This is possibly why a similar observation was not made for the R-zone.

The RC i.d. that delivered minimum dispersion for the S-zone was an i.d. of 1.02 mm and for the R-zone an i.d. of 0.64 mm. The reproducibility results obtained for the respective RC i.d.'s at various lengths are shown in Fig. 3.12 and Fig. 3.13 for the S- and R-zones respectively. It is evident that if an i.d. of 0.64 mm should be chosen as optimum it could not deliver the same reproducible results for the S-zone compared to an i.d. of 1.02 mm (Fig. 3.12). The numerical difference between the R-zone peak heights obtained for 0.64 mm and 1.02 mm i.d. are however also relatively small. The decision was thus made to rather choose the 1.02 mm i.d. as optimum for both the S- and R-zones.

The following conclusions were drawn from the reproducibility results for the R- and S-zones respectively. It was evident for the R-zone (Fig. 3.13), that RC tube lengths greater than 1.00 m delivered more precise results than the lengths below 1.00 m. The S-zone seemed to have delivered optimum precision at a RC tube length of 1.00 m.

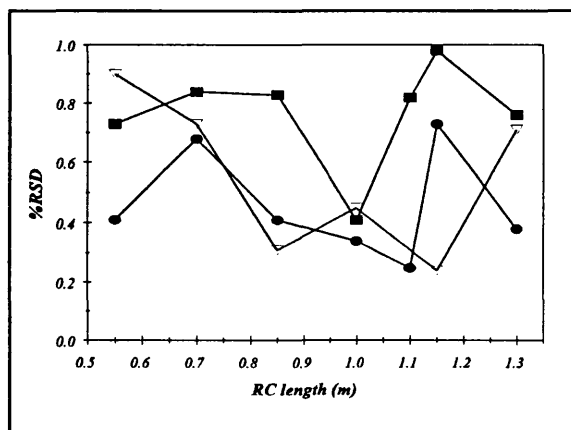


Fig. 3.12 Comparison of the %RSD obtained for RC's of different i.d. (mm) [■, 0.64 mm; ●, 1.02 mm and ▽, 1.60 mm] at various RC lengths for BTB drawn up as the sample zone.

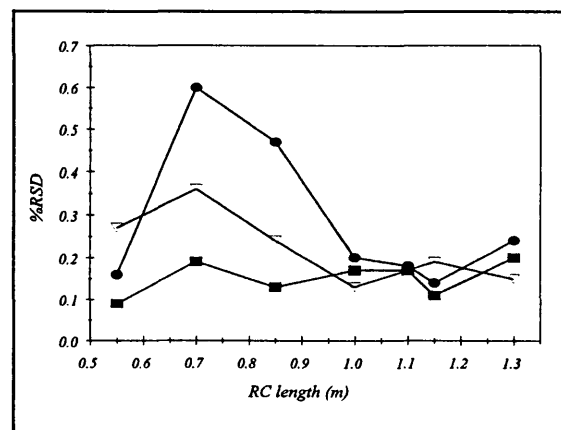


Fig. 3.13 Comparison of the %RSD obtained for RC's of different i.d. (mm) [■, 0.64 mm; ●, 1.02 mm and ▽, 1.60 mm] at various RC lengths for BTB drawn up as the reagent zone.

When deciding upon an optimum RC tube length, the reproducibility results of the optimum RC i.d., namely 1.02 mm, were considered. This i.d. gave fairly good reproducibility for the R-zone at a RC length of 1.10 m and 1.15 m (Fig. 3.13). The reproducibility though for the S-zone at an i.d. of 1.02 mm and 1.15 m length (Fig. 3.12) is quite high ($> 0.70\%$ RSD) whilst the reproducibility is quite good at a 1.10 m length.

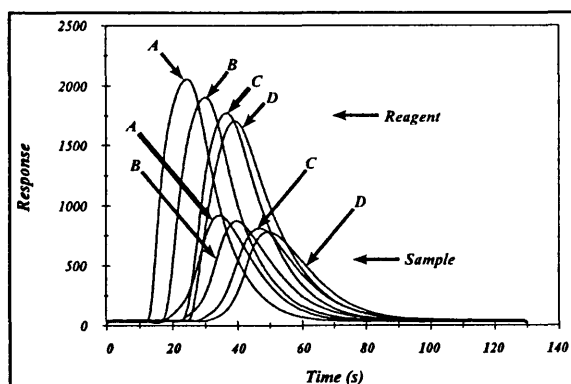


Fig. 3.14 Shifting of isodispersion point when changing the RC length. A - 0.55 m, B - 0.85 m, C - 1.15 m and D - 1.30 m.

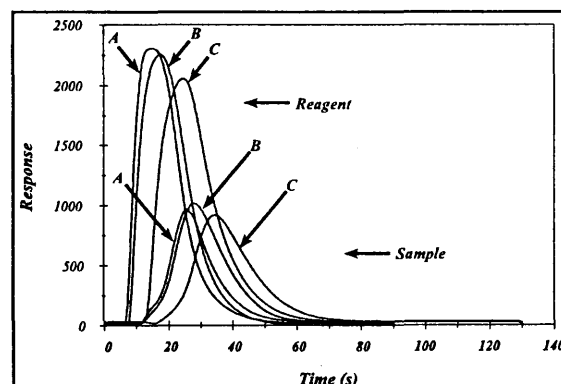


Fig. 3.15 Shifting of isodispersion point when changing the RC i.d.. A - 0.64 mm, B - 1.02 mm and C - 1.60 mm

Fig. 3.14 and Fig. 3.15 illustrates the shift of the isodispersion point when changing the length of the RC while keeping the i.d. fixed (1.60 mm) and when changing the i.d while keeping the length fixed (0.55 m) respectively. The shift is evident and can be ascribed to the change in dispersion of the respective zones with changing RC dimensions.

The optimum RC dimensions is thus an i.d. of 1.02 mm and a length of 1.10 m.

3.5.1.5 Mixing chambers

The use of mixing chambers in a flow conduit is not a new concept. Their use can be found as far back as the seventies [22]. Despite the fact that they have certain unfavourable properties such as a hold up effect, they offer some distinct advantages e.g. in the use of mixing two liquids with differing viscosities [22]. In this case the stirrer levels the small differences in dispersion. Similarly, the effects of sample in specific gravity, temperature, detergent content, etc., on the concentration profile can be remarkably reduced by introducing a mixing chamber into the flow system. A mixing chamber has also been applied in a system where several reagents had to be added serially, e.g. the determination of factor XIII, that was based on the mixing of six reagents [16].

In the present study, the influence of a mixing chamber in an SIA system was evaluated for four different mixing chamber volumes namely: 0.5; 1.0; 3.0 and 3.1 ml. It was decided to evaluate the influence a mixing chamber could have on the dispersion when placed at two different positions in an optimized SIA system, for the Bromothymol Blue solution drawn up as a S- and R-zone respectively. The positions at which the mixing chambers were placed at respectively,

are (i) in the position between the holding coil and valve (HC position) and (ii) between the valve and the reaction coil (RC position).

The optimum SIA conditions at which the mixing chambers were evaluated are given in Table 3.4.

TABLE 3.4. Optimum SIA conditions

Operational parameter	Dimension
Pump tubing i.d.	1.85 mm
Pump speed	18.0 rpm
Sample volume	600 $\mu\ell$
Reagent volume	1200 $\mu\ell$
HC i.d.	1.60 mm
HC length	3.50 m
RC i.d.	1.02 mm
RC length	1.10 m

When placing the mixing chamber between the holding coil and valve the zones will be drawn up through the mixing chamber into the holding coil and then be propelled through the mixing chamber again to the detector. For the mixing chamber positioned between the valve and reaction coil the zones will be drawn into the holding coil and then be propelled first through the mixing chamber and then through the reaction coil to the detector. A lot more dispersion is expected for the mixing chamber in the HC position since the zones enter the mixing chamber twice and because of increased mixing, an increase in dispersion is expected.

The influence of the mixing chamber volumes on dispersion is illustrated in Fig. 3.16 and Fig. 3.17 for the R- and S-zone respectively. It is evident that an increase in dispersion was achieved, for both the zones, with increasing mixing chamber volumes. This could be ascribed to the fact that increased mixing of the zones resulted in increased dispersion and subsequently decreased peak height intensity. The increased S-zone dispersion compared with that of the R-zone is also very evident.

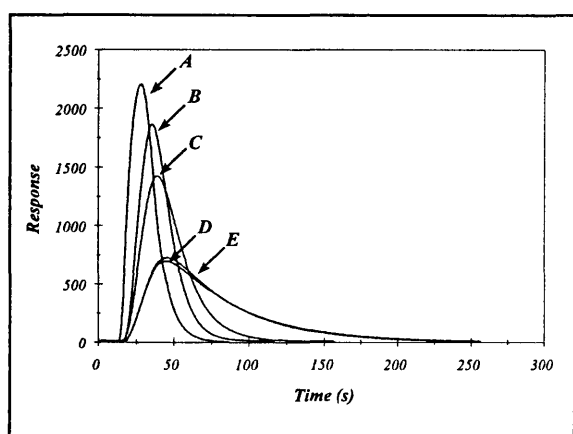


Fig. 3.16 Influence of mixing chamber volume on the R-zone dispersion. Mixing chamber volumes: A = 0 ml; B = 0.5 ml; C = 1.0 ml; D = 3.0 ml; E = 3.1 ml.

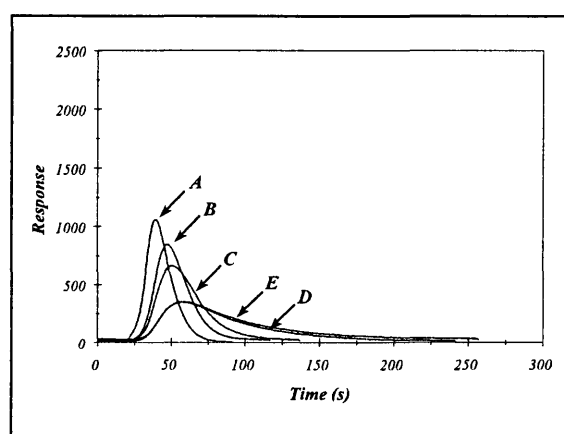


Fig. 3.17 Influence of mixing chamber volume on the S-zone dispersion. Mixing chamber volumes: A = 0 ml; B = 0.5 ml; C = 1.0 ml; D = 3.0 ml; E = 3.1 ml.

Table 3.5 depicts the dispersion and reproducibility results obtained for the BTB solution drawn up as the R-zone and the mixing chamber in the RC- and HC positions respectively. The dispersion of the zones in the RC position is larger than that obtained for the HC position. This could be due to the tubing connecting the selection valve with the mixing chamber in the HC position. The entire R-zone (drawn up secondly) does not enter the mixing chamber completely when the mixing chamber is in the HC position. This is due to the connecting tubing which prevents the entire zone from entering the mixing chamber directly.

Since the R-zone is not drawn into the mixing chamber completely, it does not undergo the same degree of dispersion achieved when the mixing chamber is in the RC position. When the mixing chamber is in the RC position, the entire R-zone will be propelled through the mixing chamber when the zones are propelled forward to the detector. Thus the entire R-zone will be mixed and the degree of dispersion will be greater than that achieved with the mixing chamber in the HC position.

TABLE 3.5. Dispersion and %RSD obtained when placing the mixing chamber in either the RC- or HC position of the SIA manifold for BTB drawn up as the reagent zone.

Mixing chamber volume (mℓ)	Dispersion		%RSD	
	RC	HC	RC	HC
0	1.29	1.29	0.22	0.22
0.5	1.53	1.47	0.12	0.41
1.0	2.01	1.86	0.23	0.08
3.0	4.04	3.44	0.90	0.22
3.1	4.17	3.48	1.51	0.33

The mixing chambers of volume 3.0 and 3.1 mℓ have approximately the same volume and only differ in their dimensions. Their heights differ with 1.60 cm for the 3.0 mℓ volume and 0.90 cm for the 3.1 mℓ volume respectively. The mixing chamber radii were changed accordingly. The influence of these dimensions was studied to see whether changing it would influence the resulting dispersion. It does not seem to have had a noticeable effect (Table 3.5).

The precision of the results obtained for the R-zone are also depicted in Table 3.5. For the mixing chamber in the RC position, a decrease in precision (larger %RSD values) was achieved with increasing mixing chamber volumes. The reproducibility of the results obtained for the RC position is also lower than that of the HC position. This could be ascribed to the fact that with an increase in dispersion, as with the RC position results (Table 3.5), it becomes more difficult to obtain reproducible results.

The dispersion and reproducibility results obtained for the S-zone are shown in Table 3.6. The opposite effect, compared with the results depicted in Table 3.5, was obtained regarding the dispersion obtained for the mixing chamber in the RC- and HC positions respectively. It is evident from Table 3.6 that the dispersion obtained for the RC position is smaller than that of the HC position. This could be due to the fact that in this case, where the BTB solution is drawn up first as the S-zone, the entire BTB zone will be drawn into the mixing chamber (in the HC position) and the Borax zone (drawn up secondly) will be partially in the connecting tubing.

TABLE 3.6. Dispersion and %RSD obtained when placing the mixing chamber in either the RC- or HC position of the SIA manifold for BTB drawn up as the sample zone.

Mixing chamber volume (ml)	Dispersion		%RSD	
	RC	HC	RC	HC
0	2.72	2.72	0.42	0.42
0.5	3.45	3.75	0.36	0.47
1.0	4.43	4.85	0.56	0.40
3.0	8.68	8.92	1.42	0.56
3.1	8.66	9.26	1.02	1.02

Since the BTB zone will be propelled twice through the mixing chamber in the HC position, compared with when the mixing chamber is in the RC position, the degree of dispersion will be greater than that achieved for the mixing chamber in the RC position. The actual difference in the obtained dispersion for the RC- and HC positions is very small compared with the differences in Table 3.5.

The same tendency was observed for the precision of the results for the S-zone (Table 3.6) when compared with those of the R-zone (Table 3.5).

When considering the SIA profiles for the system containing no mixing chamber (Fig. 3.18) and the system containing a 3.0 ml mixing chamber (Fig. 3.19), it is evident that the amount of sample and reagent zone overlap increased with increasing mixing chamber volume. Another interesting observation of the larger mixing chamber volumes, was the disappearance of the iso-dispersion point.

Although the amount of mixing between the zones were increased by introducing larger mixing chamber volumes, it also gave rise to decreased sensitivity due to increased dispersion. The dilution effect of the mixing chambers is also very pronounced and it is evident that it can be applied to the dilution of overly concentrated samples. This has been done for the dilution of analyte for process control applications [15] and for handling highly concentrated solutions [18].

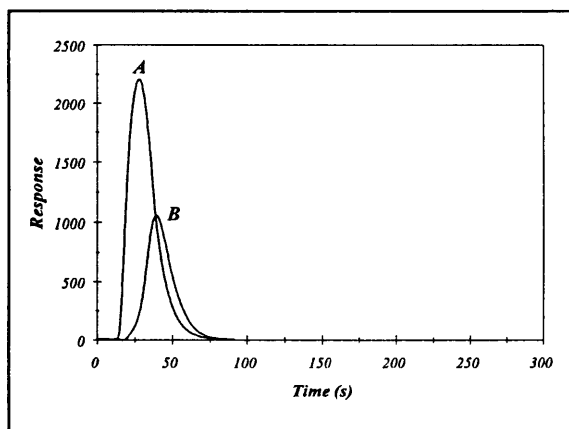


Fig. 3.18 Reagent- (A) and sample-zone (B) overlap in the absence of a mixing chamber.

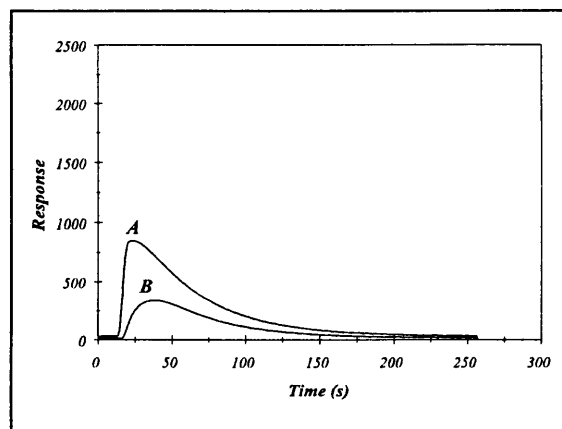


Fig. 3.19 Reagent- (A) and sample-zone (B) overlap in the presence of a 3.0 ml mixing chamber.

The length of the sampling cycle was increased by a factor three when introducing the 3.0 ml mixing chamber. It is evident that the use thereof must be avoided if serial assays are to be attempted or whenever the sampling frequency is the highest priority [3].

Fig. 3.20 illustrates the shifting of the isodispersion point for different mixing chamber volumes. The disappearance of the isodispersion point with increasing mixing chamber volumes is very obvious, since the reagent zone increasingly overlays the sample zone. This is different than that observed for the reaction coil where the isodispersion point simply changed position in time and did not disappear completely.

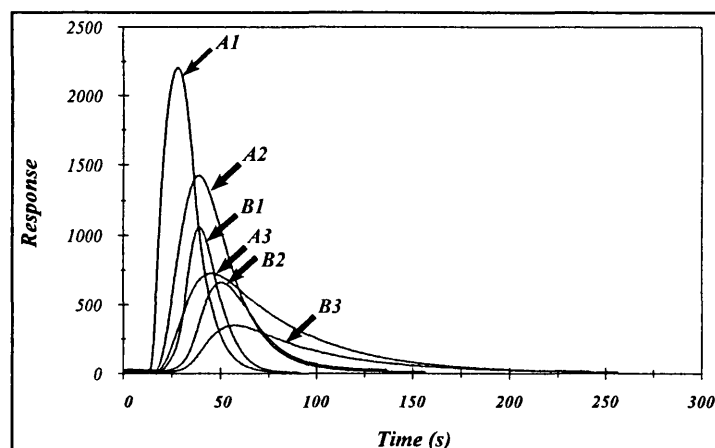


Fig. 3.20 Shifting of isodispersion point for different mixing chamber volumes. A - reagent, B - sample, 1 - 0 ml, 2 - 1.0 ml and 3 - 3.0 ml.

3.6 Conclusions

It is evident that the amount of dispersion in an SIA system can be controlled by adjusting the operational parameters accordingly. If the analysis requires a great deal of mixing between the reacting components, this can easily be achieved by altering certain operational parameters in the SIA system to increase mixing.

The following conclusions can be drawn from the study described above. Although some of the conclusions have already been drawn from earlier work [4,5] some are just a more intense study into that, which has already been done.

The flow rate can be altered by changing both the pump speed and the pump tubing i.d. of a peristaltic pump. An increase in pump speed gives rise to an increase in flow rate and thus a decrease in the dispersion of the product zone. By increasing the pump tubing i.d. the resulting

dispersion also decreases.

The i.d.'s of the holding and reaction coils have a more pronounced effect on the dispersion than its length. For both, an increase in i.d. resulted in increased dispersion. The sample and reagent zone volumes also have a marked influence on the dispersion. The larger the volumes that are drawn up, the smaller the resulting dispersion ($D \propto 1/V_i$) [1].

After these optimum conditions were determined, it was possible to evaluate the influence of a mixing chamber in the SIA flow conduit. An increase in dispersion was achieved with the larger mixing chamber volumes because of the increased mixing of the zones. The position of the mixing chamber in the SIA manifold determines the extent of the dispersion that the sample and reagent zones will undergo. The reproducibility of the obtained results decreased for larger mixing chamber volumes because replicate results are not that easily obtained for large dispersion of the zones.

It is thus essential to carefully optimize those parameters that have a pronounced influence on the dispersion in an SIA manifold, to achieve the required amount of dispersion under certain specified conditions.

3.7 References

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CHAPTER 4

Spectrophotometric Determination of Cu(II) with Sequential Injection Analysis

4.1 Introduction

One of the first metals known to mankind is copper. The earth's crust contains about 68.0 mg/ℓ of copper that is found mainly as the sulphide, oxide or carbonate [1]. Although copper does not occur abundantly in nature, many copper-containing ores are known. Copper is principally extracted from copper pyrites (chalcopyrite), CuFeS_2 , which is estimated to account for about 50% of all copper deposits [1]. Other major copper ores include copper glance (chalcocite), Cu_2S ; cuprite, Cu_2O and malachite, $\text{Cu}_2\text{CO}_3(\text{OH})_2$. Some copper is also found in native (metallic) form, which resulted in its classification as a siderophilic element (an element in its uncombined, native form) [2].

Copper is an essential trace element in life and a component of several metalloenzymes and other proteins. It is present in all organisms, land and marine. Table 4.1 shows the distribution of copper in the environment [2]:

TABLE 4.1. Copper concentrations in the environment

Location	Concentration
Crust	50 mg/ℓ
Sea water:	
surface	0.008 µg/ℓ
deep	0.02 µg/ℓ
Fresh waters	1 - 10 µg/ℓ
Soils:	
average	30 mg/ℓ
contaminated	100 - 10 000 mg/ℓ
Human body	1 mg/ℓ

The threat of metal pollution to public health is an age-old, but unappreciated, problem. The environmental levels of metals rarely produce acute injury, and any chronic damage may not be evident for months or years and may barely be manifested by any pathological symptoms. Over the past decades, the natural quality of water sources has been altered by the impact of various human activities and water uses. The five sources that are primarily responsible for heavy metals in inland waters are [3]:

- Geological weathering,
- Industrial processing of ores and metals,
- The use of metals and metal compounds,
- Leaching of metals from domestic wastes and solid waste dumps, and
- Heavy metals in animal and human excretions.

The water quality guidelines regarding the permissible levels for selected inorganic micro pollutants are given in Table 4.2 [3]:

TABLE 4.2. Water quality guidelines for various uses (mg/ℓ)

Inorganic micro pollutant	Drinking water	Irrigation water ^a		Livestock watering
		No use restriction	Severe restriction	
Cadmium	0.005	0.01	0.05	0.05
Copper	1.0	0.2	5.0	1.0
Lead	0.5	5.0	10.0	0.1
Iron	0.3	5.0	20.0	-

^aOnly indicative - highly dependant on plant species

The number of trace metal analyses increased over the years due to improvements in analytical techniques that have significantly lowered the cost and increased the efficiency. There is though still an increasing demand for the development of reliable, cost-effective methods that could assist health authorities in determining whether the toxic metal levels are not exceeded in drinking water sources.

4.2 Properties of copper

Copper is an attractive reddish coloured metal, which is soft and extremely malleable and ductile. Its electrical and thermal conductivities are second only to those of silver. Copper has a melting point of 1083°C and a density of 8.94 g/cm³ [4]. An outstanding feature of copper is its resistance to chemical attack that is reflected in its positive electrode potential.

The metal is slowly attacked by moist air and its surface gradually becomes covered with an attractive green layer of basic copper carbonate. At approximately 300°C it is attacked by air or oxygen and a black coating of copper(II) oxide forms on its surface; at a temperature of about 1000°C copper(I) oxide is formed instead [4]. Copper is also attacked by sulphur vapour, with the formation of copper(I) sulphide, and by the halogens that form the copper(II) halide. The metal is not attacked by water or steam and dilute non-oxidising acids.

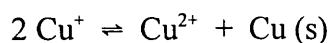
Copper has two stable isotopes, and a slight variability in its abundance prevents its atomic mass being quoted with greater precision.

4.3 Compounds of copper

Copper exhibits the following oxidation states:

- +1 : only stable as the complex ion,
- +2 : most common,
- +3 : a few compounds are known for this state, although it is not very common (e.g. K_3CuF_6).

In aqueous solution the hydrated copper(I) ion is very unstable with respect to disproportionation:



largely because of the high heat of hydration of the divalent ion. Nevertheless, copper(I) can be stabilized either in compounds of very low solubility or by complexing with ligands having π -acceptor character. Copper(I) is colourless, unless coordinated to easily polarized ligands. Although the chemistry of copper(I) is largely that of its water insoluble compounds and of its stable complexes, other copper(I) compounds are perfectly stable in the absence of moisture, e.g. copper(I) sulphate (Cu_2SO_4).

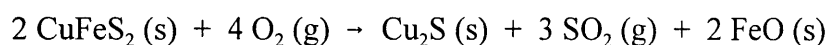
Copper(II) forms simple salts with most anions, except CN^- and I^- , which instead form covalent copper(I) compounds that are insoluble in water. The copper(II) salts are predominantly water-soluble, the blue colour of their solutions arising from the $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ ion, and they frequently crystallize as hydrates. The aqueous solutions are prone to slight hydrolysis and, unless stabilized by a small amount of acid, are liable to deposit basic salts. Basic carbonates occur in nature, basic sulphates and chlorides are produced by atmospheric corrosion of copper, and basic acetates (verdigris) find use as pigments.

Copper(III) is generally regarded as uncommon, being very easily reduced, but it has recently received attention because of its apparent involvement in some biological processes [1].

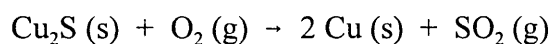
4.4 Uses of copper

A few of the oxide ores of copper can be reduced directly to the metal by heating with coke. The bulk of production is though from sulphide ores containing iron. The extraction of copper from copper pyrites will be considered.

The pulverised ore is concentrated by the ore flotation process and roasted in a limited supply of air to convert the iron into iron(II) oxide:



After the addition of silica, SiO_2 , the mixture is heated in the absence of air to convert the iron(II) oxide into a slag of iron(II) silicate, FeSiO_3 , which is poured away. The copper(I) sulphide is now reduced to copper by heating in a controlled amount of air:



Copper is refined electrolytically to give a product of about 99.95% purity [4].

Copper plays an important part in power stations where most of a country's electricity is generated. Nuclear power stations use heat exchangers made of copper because of its excellent heat conducting properties. It is also used for the windings of dynamos. Coppers' resistance to chemical attack and its high thermal conductivity make it a useful metal for the construction of condensers for chemical plants and car radiators [4].

Copper is unusual among the base metals in that it is largely used in its pure, unalloyed state. Virtually all the copper used in electronics is pure, but for engineering and building purposes, where strength and durability are necessary, alloys of copper are often used [5]. Copper will mix readily with other metals. This generally makes it harder, although it does reduce its conductivity. The first copper alloy to be used was bronze.

The term “bronze” covers an enormous variety of alloys. Bronze, an alloy of copper and tin, is used for fabricating bearings and ships’ fittings. Phosphor bronze, which contains about 0.04% phosphorus, is used for watch springs and galvanometer suspensions. Bronzes with up to 20% lead in them, the leaded bronzes, are becoming increasingly important in the manufacture of bearings for modern car and aircraft engines. Aluminium bronze, which is not really a bronze but a mixture of copper and aluminium, is used for wrought and cast work and is in general, three times as strong as copper. It is widely used in shipbuilding, particularly propellers.

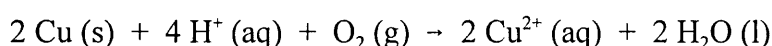
Brass was the second of the copper alloys to be discovered. Zinc in combination with copper forms brass and the amount of each determines the quality of the brass. The alloy with the greatest amount of copper (95%) is called cap copper and is used for the sensitive caps that detonate ammunition. Brass with a slightly increased zinc content is ideal for gilding jewellery. Cartridge brass that consists of 70% copper and 30% zinc, is used to make cartridge cases for modern bullets and shells [5].

Copper is not just found and used as a metal. It has many different chemical compounds, some of which are found naturally, and some of which can be produced for special purposes. Tiny traces of copper are vital to the health of humans, animals and plants. It is one of the “trace

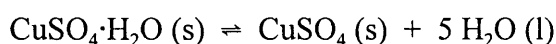
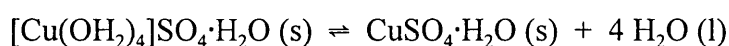
elements” (others include iron, magnesium and calcium) without which we could die. It has been demonstrated that copper, in addition to iron, is necessary for blood cell formation [6,7]. Copper also plays an important role in nerve myelinization and pigmentation [7].

Copper also plays an important role in modern agriculture. Centuries of ploughing and grazing have robbed farming areas of essential elements such as copper. This stunted the growth of crops. The copper has though been returned to the soil by spraying it with copper salts.

The best-known copper salt is the sulphate pentahydrate (“blue vitriol”), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Copper(II) sulphate is prepared on an industrial scale by forcing air through a hot mixture of copper and dilute sulphuric acid:



Copper(II) sulphate pentahydrate loses four of its molecules of coordination water on heating to about 100°C ; the fifth molecule of water crystallization is lost at a temperature of about 250°C :



Copper(II) sulphate is widely used in electroplating processes and as an algicide in water purification. Gardeners and farmers who grow potatoes use a preparation of copper salts (called the “Bordeaux mixture”), to spray their crops. This mixture is also used to spray grape vines to

prevent mildew. This fungicide contains 1.0 - 2.5% copper(II) sulphate solution neutralized with hydrated lime [6]. Copper sulphate is also used as an antidote for phosphorus poisoning. Phosphorus is removed through the formation of insoluble copper phosphide. In addition to these uses, copper(II) sulphate is also used in textile dyeing and as a timber preservative.

A variety of copper compounds are also employed to eradicate fungi and protect materials from rot and decay. These include, e.g. copper chloride, oxide, sulphate, phosphate and silicate [7]. Certain fertilizers also contain small amounts of copper, in addition to the nitrogen, phosphorus and potassium compounds.

Certain hair-colouring agents, which are used to tint and dye hair, have also been known to contain toxic metallic preparations such as copper [7]. An incident concerning copper poisoning as a result of spraying metallic streaks on hair has been reported [6].

Another interesting use of copper is in paints. Bronze powder, used for gilding Christmas cards, is in the form of thin flakes consisting of 70% copper, 30% zinc and a trace of stearate lubricants [7]. The fumes are toxic if the paint is not sprayed in a properly ventilated room.

It is evident that copper is a very versatile element and that is nearly impossible to cover all its modern uses.

4.5 Biochemistry of copper

Copper occurs in all bodily tissues. The distribution varies with age, species and diet. The liver, heart, brain, kidney and hair contain high concentrations compared with other tissues [6]. A human adult contains around 100 mg of copper, mostly attached to protein, an amount exceeded only by iron and zinc among transition metals. The required daily intake of copper is about 3-5 mg [1].

Enzymes that contain copper include: e.g. tyrosinase, ascorbic acid oxidase and monamine oxidase. Several manifestations of copper deficiency in animals appear to be related to decreased tissue concentrations of certain of these enzymes [6].

Abnormally high copper levels in liver are characteristic of certain disease states in humans. Conditions that manifest increased copper content in liver include tuberculosis, hemachromatosis, cirrhosis and yellow atrophy of liver. Wilson's disease is also characterized by abnormally high copper levels and is ascribed to the congenital inability to excrete copper, resulting in its accumulation.

Although copper occurs naturally in human organs, the ingestion of copper through other means can also result in the increase of its levels in humans or animals. An example is the ingestion of water. Drinking water that originates from natural sources could contain elevated concentrations of copper due to, e.g. the use of copper salts to control plant growth in reservoirs or from the corrosion of copper, brass or bronze pipe fittings. There are cases where mining operations have led to localized cases of extreme copper pollution [8].

An excessive intake of copper results in its accumulation in the liver. Drinking water that contains an abnormal high copper content due to, e.g. the reasons given above, could cause ptyalism, nausea, vomiting, epigastric burning and diarrhea if ingested [9].

4.6 Choice of analytical method

The development of systems to conduct routine analysis is subject to stringent requirements. Low cost instrumentation and minimum sample and reagent consumption are important requirements.

Several automated methods have been successfully developed for the determination of copper, in various samples, by AAS detection [10-15] and ICP [16], with a flow injection analysis system. These systems are very fast and deliver a high sample frequency that makes it ideally suited for process analysis. These detection systems are however expensive when compared with UV/Vis spectrophotometers and as a result do not comply with the cost-effective requirements.

The flow injection analysis systems that have been developed for the determination of copper(II), with spectrophotometric detection [17-22], also deliver high sample frequencies. A disadvantage though of these flow injection analysis systems is the enormous reagent consumption (and sample consumption in the case of reversed flow injection analysis) that characterizes it.

To comply with the set requirements, it was decided to develop a procedure for the determination of copper(II), with sequential injection analysis, by spectrophotometric detection. A short discussion follows on a selection of methods that have been developed up to date for the

determination of copper(II) with spectrophotometric detection.

Ríos *et al* [22] developed a reversed flow injection analysis system for the determination of copper(II) by its reaction with cuprizone [oxalic acid bis(cyclohexylidene hydrazine)]. The blue coloured complex, which formed as a result of the mentioned reaction, was spectrophotometrically monitored at 595 nm. The procedure was linear over the 0.3 - 4.0 mg/l copper(II) concentration range and the relative standard deviation for a 1.5 mg/l copper(II) solution was 0.51%. The system processed 44 samples per hour. A disadvantage of this system is that it consumes enormous amounts of sample and buffer.

A method based on the catalytic effect of copper(II) on the oxidation of quinol by hydrogen peroxide, was reported by Yuan and Qu [23]. The formed quinone was spectrophotometrically determined at 420 nm. The calibration graph was linear up to 50.0 µg/l of copper(II) with a detection limit of 0.7 µg/l. The coefficient of variance was 2.90% for 40.0 µg/l of copper(II).

Szpunar-Łobińska and Trojanowicz [20] developed a flow injection extraction procedure for the determination of copper(II). This procedure was based on the extraction of copper(II) with a chloroform solution of lead diethyldithiocarbamate (DDTC), and measurement of the absorbance of the resulting complex formed between Cu(II) and DDTC at 436 nm. The calibration graph was linear up to 2.0 mg/l of copper(II) with a detection limit of 0.04 mg/l. The sample throughput was 64 samples per hour with a precision of 2.20%. A drawback of this procedure is that it uses a toxic organic solvent to perform the extraction. This does not comply with conducting environmentally safe analysis.

The sequential determination of copper and nickel by reversed flow injection spectrophotometry was reported by Wang *et al* [21]. The formation of a complex between copper(II) and sodium diethyldithiocarbamate in a slightly basic medium was used as the indicator reaction for the determination of copper(II). The absorbance of the aqueous solution was measured at 460 nm. This procedure improved on the procedure discussed above [20] by excluding extraction of the complex and subsequently also the use of toxic organic substances. The procedure was linear over the 0.04 - 4.0 mg/l copper(II) concentration range with a relative standard deviation of 0.75% for a 2.0 mg/l copper(II) concentration. The sample frequency was 60 per hour. Although Wang *et al* [21] succeeded in reducing the consumption of reagent, the procedure is still largely dependant on the availability of large volumes of sample.

The method proposed by Wang *et al* [21] was used as basis for the development of a sequential injection analysis system for the determination of copper(II) by complexation with DDTC. This procedure indicated that Fe(II) interfered tremendously, even from the smallest interfering ion : Cu(II) ratio. It was therefore decided to optimize the complexation reaction between Cu(II) and DDTC in the presence of Fe(II) as interferent (4.9). By optimizing the sequential injection analysis system in the presence of an interferent, a procedure could be developed for application to real samples that may contain high levels of interfering species. Since samples with minimal to negligible interference also exist it was decided to develop a second SIA system for the determination of Cu(II) with DDTC in the absence of interferences (4.8).

4.7 Principle of the reaction between copper(II) and DDTC

Metal dithiocarbamates are widely used for analytical purposes due to its characteristic colours [24]. Diethyldithiocarbamate (DDTC) $[(C_2H_5)_2NCSS]^-$ is often used as reagent for the selective determination of copper(II) since the stability of the complex formed is the highest, next to silver, compared with the stability of the complexes formed with other metals [20]. DDTC coordinates with copper(II) through the two sulfur atoms to form a four-membered ring chelate (Fig. 4.1) [25,26].

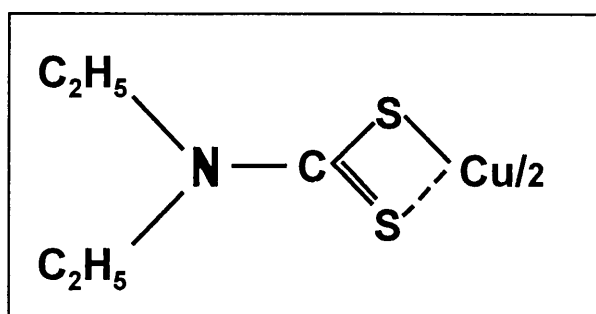
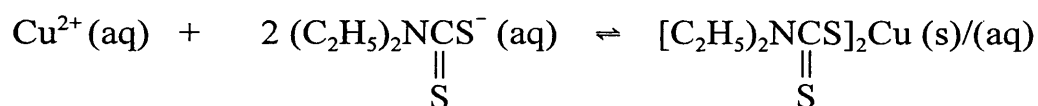


Fig. 4.1 Formation of a four-membered ring chelate between DDTC and Cu(II).

An extensive study has already been conducted on the analysis of copper(II) using its reaction with DDTC and the subsequent extraction of the formed complex into an organic medium [20,27-33]. Extraction of the complex formed between Cu(II) and DDTC is preferred since it is sparingly soluble in water and dissolves more readily in organic solvents [25,26]. The preferred organic solvents that are commonly used for the extraction are carbon tetrachloride and chloroform [25]. A disadvantage though of these organic solvents is that it is classified as toxic and environmental pollutants. In addition to the toxicity of it, carbon tetrachloride and chloroform have also been listed as carcinogens by the Environmental Protection Agency (EPA)

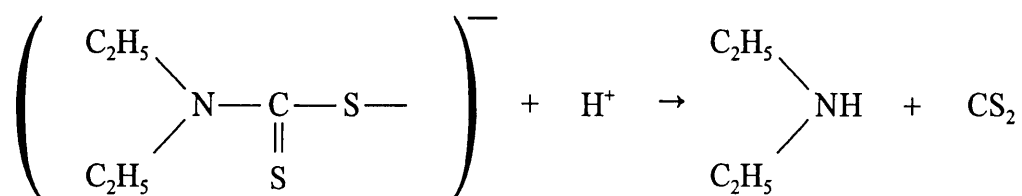
[34]. A lot of emphasis is placed on conducting environmentally friendly analysis and eliminating the use of harmful substances like those mentioned.

Spectrophotometric analysis of the complex, without solvent-extraction, has also been successfully conducted by measurement of the coloured complex in aqueous medium [21,34-37]. The system developed in this study was based on the spectrophotometric measurement of the complex formed between Cu(II) and DDTC in an aqueous medium according to the following reaction equation:



It is possible to monitor the complex spectrophotometrically at 460 nm since it is slightly soluble in aqueous medium.

The DDTC-reagent solution should be stored in dilute alkali (pH ~ 9) [26], since it decomposes rapidly below a pH of 6 [25]. The rate of decomposition of DDTC solutions, into diethylamine and carbon disulphide, is directly proportional to the hydrogen ion concentration and is given by the following reaction equation:



DDTC is a group reagent. The selectivity of the method for individual determination of copper(II) can be enhanced considerably by using EDTA as a masking agent and by appropriate choice of pH. In a tartrate or citrate medium at pH 8 - 9 it masks interfering species very effectively [26].

4.8 The determination of copper(II) with SIA in the absence of interferences

4.8.1 Experimental

4.8.1.1 Reagents and solutions

All reagents were prepared from analytical-reagent grade chemicals unless specified otherwise. All aqueous solutions were prepared using de-ionised water from a Modulab system (Continental Water Systems, San Antonio, Texas, USA). The de-ionised water used as carrier stream and to prepare the aqueous solutions with was degassed, by heating to boiling point and cooling before it was used.

Standard Cu(II) stock solution, 1000.0 mg/l: Pure copper metal coarse chips were used in the preparation of the Cu(II) stock solution. The copper metal was cleaned to remove any oxides and dissolved by heating 1.0 g of the copper metal in 10 ml 55% HNO₃ (m/m) and *ca.* 10 ml of water. The subsequent solution was cooled and then diluted to 1000 ml with water. The 3.0 mg/l Cu(II) working solution was prepared by appropriate dilution of the stock solution.

DDTC reagent solution: 0.100% (m/v) sodium diethyldithiocarbamate was dissolved in 50 ml water by heating the solution to 60°C. After the DDTC solution was cooled, 9.5 ml of a 2 mol/l NH₄Cl solution was added to it and the pH of it adjusted to 9.2 by adding an appropriate volume of 2 mol/l NH₃. The final solution was made up to 100 ml with water.

4.8.1.2 Sample preparation

The tap water that was analysed was allowed to run from the tap for 10 minutes before samples were taken. A known concentration of Cu(II) was added to each of the respective tap water samples and the final solution made up to 100 ml with the tap water.

4.8.1.3 Apparatus

The sequential injection system depicted in Fig. 4.2 was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-le-Bel, France); a 10-port electrically actuated selection valve (Model ECSD10P; Valco Instruments, Houston, Texas, USA); and a UNICAM 8625 UV-visible spectrometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell (volume 80 μl) for absorbance measurements.

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (Mintek, Randburg, South Africa). The FlowTEK [38] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All the data given (relative peak heights) are the mean for $n = 5$.

pH measurements of the DDTC solution were conducted with an Orion pH meter (Model 420 A; Orion Research Inc., USA) and an Orion pH TriodeTM electrode.

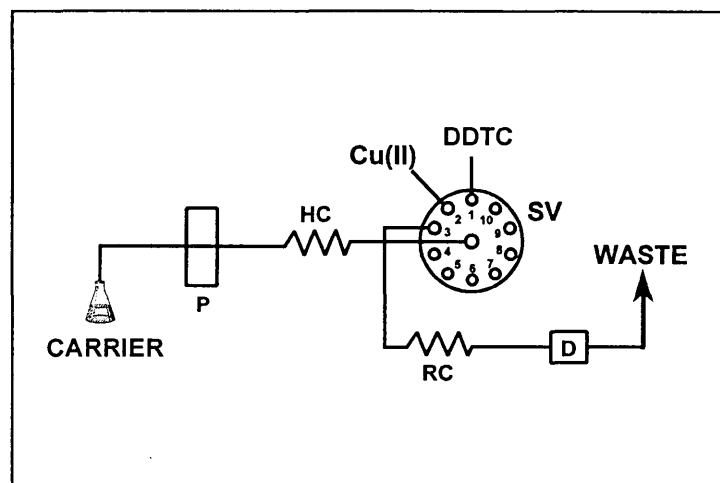


Fig. 4.2 A schematic diagram of the SIA system used for the determination of Cu(II). P - pump, HC - holding coil, RC - reaction coil, SV - selection valve and D - detector

4.8.1.4 Manifold

The components of the SIA system were arranged as illustrated in Fig. 4.2. The holding coil consisted of 3.5 m x 1.60 mm i.d. Tygon tubing and the reaction coil of 1.1 m x 1.02 mm i.d. Tygon tubing. A flow rate of 5.8 ml/min was used.

4.8.1.5 Procedure

The device sequence for the determination of Cu(II) by sequential injection analysis in the absence of interferences is given in Table 4.3.

TABLE 4.3. Device sequence for one cycle of the sequential injection system

Time (s)	Pump	Valve	Description
0	Off	DDTC	Pump off, select DDTC stream (valve position 1)
5.0	Reverse		Draw up DDTC solution
7.1	Off		Pump stop
8.1		Cu(II)	Select Cu(II) stream (valve position 2)
9.1	Reverse		Draw up Cu(II) solution
17.4	Off		Pump stop
18.4		Detector	Select detector line (valve position 3)
19.4	Forward		Pump stack of zones to detector
240.0	Off	Home	Pump off, return valve to starting position (valve position 1)

4.8.2 Method optimization

4.8.2.1 Physical parameters

The physical parameters of the sequential injection analysis system were optimized by regarding the effect of flow rate, reagent volume and sample volume on sensitivity and reproducibility. A decision is made regarding an optimum when a specific physical setup delivers optimum sensitivity and reproducibility. The effect of analysis time is also considered since this could have an important effect on the feasibility of the procedure.

4.8.2.1.1 Flow rate

The influence of flow rate was studied to determine whether it affects the amount of complex that will form between Cu(II) and DDTC. Optimum sensitivity will be achieved when the flow rate results in optimum complex formation. Flow rates ranging from 2.7 - 6.8 ml/min were studied and its effect on sensitivity and reproducibility evaluated. The results (Fig. 4.3) clearly indicate an increase in sensitivity as the flow rates increased from 2.7 - 5.0 ml/min after which the sensitivity decreased slightly and then more evidently for flow rates above 5.0 ml/min.

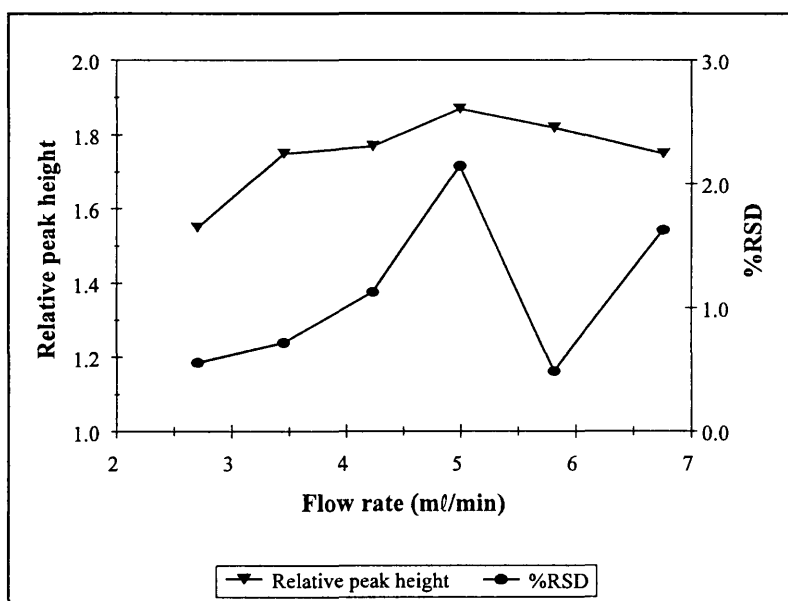


Fig. 4.3 Influence of flow rate on sensitivity and precision.

The increase in sensitivity for flow rates from 2.7 - 5.0 ml/min was attributed to a decrease in dispersion. The decrease in dispersion with increasing flow rates is characteristic of an SIA system [39] and it can be concluded that the same amount of complex is formed for flow rates up to 5.0 ml/min since the dispersion effect is predominantly responsible for the increase in

sensitivity. For flow rates above 5.0 ml/min the sensitivity decreased due to decreased complex formation. At very high flow rates, the sample and reagent zones are propelled very fast to the detector with the result that an inadequate amount of time is available for the sample and reagent to react and form sufficient detectable complex.

Although the flow rates below 4.2 ml/min delivered precision comparable to a flow rate of 5.8 ml/min it has the distinct disadvantage of delivering very low sample frequencies and result in low sensitivities compared with a flow rate of 5.8 ml/min. The optimum flow rate was chosen as 5.8 ml/min due to good sensitivity and reproducibility and a high sample frequency.

4.8.2.1.2 Reagent volume

The effect of reagent volume on sensitivity and precision was evaluated while keeping the sample volume constant (400 μl). Reagent volumes ranging from 50 - 1000 μl were studied. Fig. 4.4 illustrates the effect of reagent volume on the amount of complex formed and the reproducibility of the measurements. The sensitivity increased slightly from 50 - 200 μl , after which it remained relatively constant for increasing reagent volumes.

The increase in sensitivity with increasing reagent volumes was attributed to a larger amount of reagent available to react with the sample, which is not completely used up by the small reagent volumes that contain less reagent. With further increasing reagent volumes (> 200 μl), an excess of reagent is available to react with the sample. Since there is only a limited amount of sample available to react with, no increase in peak height is achieved for the larger reagent volumes.

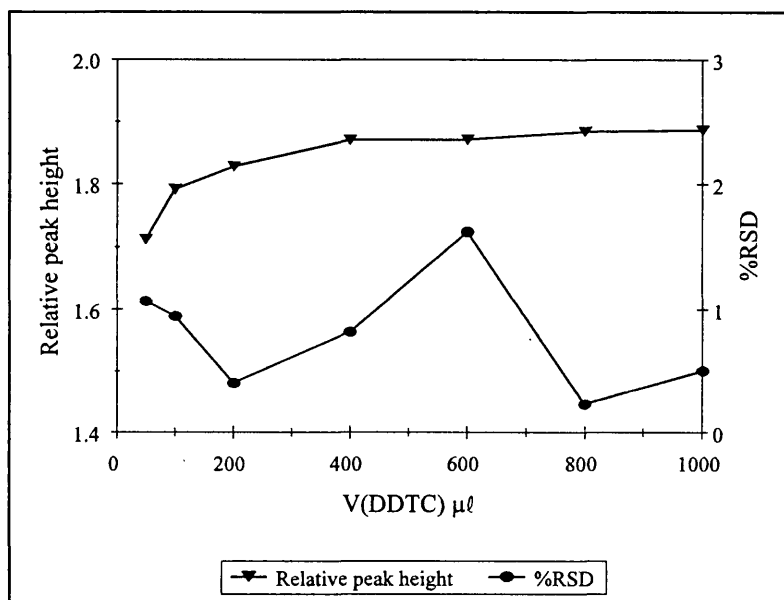


Fig. 4.4 Influence of reagent volume on sensitivity and precision.

The reproducibility is very low at reagent volumes below 200 μl since it is difficult to draw up small volumes accurately. The low precision at 600 μl is probably because it is not the optimum condition to work at. A reagent volume of 200 μl is chosen as optimum since it delivers good sensitivity and precision. This optimum results in a high sample frequency since little time is necessary to draw up reagent and flush the system afterwards, compared with a reagent volume of 800 μl that delivers similar sensitivity and slightly better reproducibility.

4.8.2.1.3 Sample volume

The sample volume determines the amount of sample that is available for the reagent to react with. The effect of sample volume on sensitivity and precision is illustrated in Fig. 4.5. Sample volumes ranging from 50 - 1200 μl were studied, while keeping the reagent volume constant (200 μl). It is evident that an excess of reagent is available in the reagent volume since the peak

height increases continuously up to 800 $\mu\ell$ for increasing sample volumes. It seems that the optimum amount of reagent has reacted with sample volumes at and above 800 $\mu\ell$ and thus the peak height stays relatively constant.

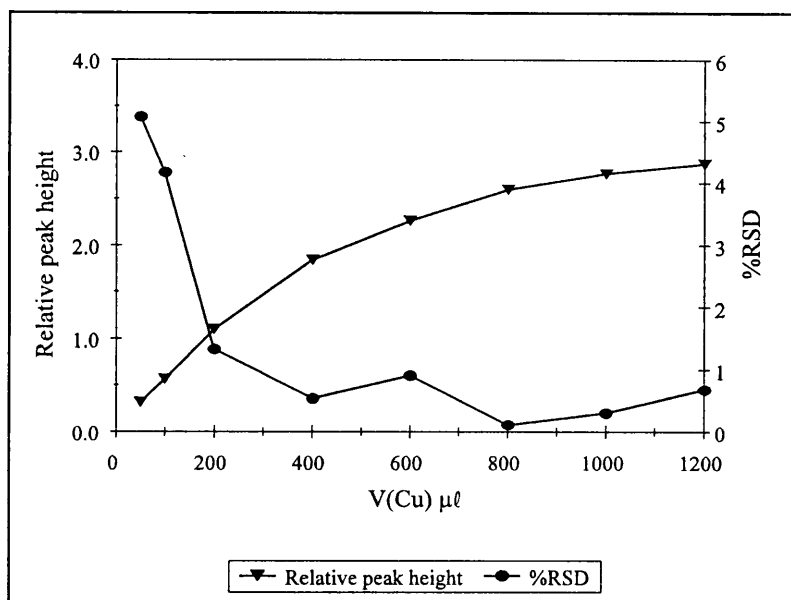


Fig. 4.5 Influence of sample volume on sensitivity and precision.

It will not be advantageous to choose a sample volume smaller than 400 $\mu\ell$ since the reproducibility is very low, due to the difficulty in drawing up very small volumes. The precision only becomes acceptable at sample volumes above 200 $\mu\ell$. Although a small sample volume would contribute considerably to a higher sample frequency, it does not deliver adequate sensitivity and reproducibility and this could become a problem when analysing very low Cu(II) concentrations. A sample volume of 800 $\mu\ell$ delivers close to optimum sensitivity and very good reproducibility and was thus chosen as optimum.

4.8.2.2 Chemical parameters

It is important to optimize the chemical parameters that affect complexation of Cu(II) with DDTC to deliver optimum detectable complex because the colours of metal complexes with DDTC are not very intense [26].

4.8.2.2.1 Sodium diethyldithiocarbamate concentration

In addition to the reagent volume, the reagent concentration ([sodium diethyldithiocarbamate]) also affects the amount of reagent that will be available to react with the sample. Reagent concentrations ranging between 0.010 - 0.300% (m/v) were studied and their effect on sensitivity and precision evaluated. The results are given in Table 4.4. It was expected that the peak height would increase with increasing reagent concentrations due to the increased amounts of reagent that would be available for complexation. This was confirmed by the obtained results.

TABLE 4.4. Influence of sodium diethyldithiocarbamate concentration on sensitivity and precision

% [sodium diethyl- dithiocarbamate] (m/v)	Relative peak height	%RSD
0.010	1.58	0.71
0.025	2.29	0.25
0.050	2.48	0.36
0.075	2.60	0.63
0.100	2.69	0.13
0.200	2.70	0.46
0.300	2.66	0.38

Sensitivity increased up to a concentration of 0.100% reagent after which it stabilized for the higher concentrations. The peak height remained constant for reagent concentrations above 0.100% since there was only a limited amount of sample available with which to react. When the available amount of sample was depleted, increasing reagent concentrations could not result in increased complex formation.

The reproducibility indicated no extreme changes when changing the reagent concentration. A reagent concentration of 0.100% was chosen as optimum with regard to sensitivity and reproducibility. A disadvantage of too high reagent concentrations is that it results in the formation of large amounts of precipitate that increases the time to flush the system and as a result decreases the sample frequency. This though was not a problem at the optimum reagent concentration.

4.8.2.2.2 pH of DDTC solution

It is important to control the pH to obtain optimum complex formation for spectrophotometric detection. A pH range of 6.4 - 10.2 was considered. The results are shown in Table 4.5. A gradual increase in sensitivity was observed from pH 6.4 - 9.2. Although the difference in sensitivity is very small, it is evident that less complex is formed at lower pH's. This can be ascribed to the possible decomposition of the reagent in more acidic solutions [26].

TABLE 4.5. Influence of pH of DDTC solution on sensitivity and precision.

pH	Relative peak height	%RSD
6.4	2.51	0.46
7.3	2.58	0.42
8.2	2.59	0.59
9.2	2.69	0.32
10.2	2.61	0.41

The difference in reproducibility is almost negligible and it can be concluded that pH does not affect precision. A pH of 9.2 was chosen as optimum. At this pH decomposition of the reagent could be minimized.

4.8.3 Method evaluation

The proposed SIA system was critically evaluated with regard to linearity, accuracy, precision, detection limit, sample interaction (carry-over) and sampling frequency.

4.8.3.1 Linearity

The response of the proposed SIA system for the spectrophotometric determination of copper(II) was evaluated under optimum conditions given in Table 4.6.

TABLE 4.6. Optimum SIA conditions

Operational parameter	Dimension
Flow rate	5.8 ml/min
Reagent volume	200 µl
Sample volume	800 µl
[sodium diethyldithiocarbamate]	0.100%
pH of DDTC solution	9.2

The following relationship was obtained for relative peak height versus copper(II) ion concentration:

$$y = 0.8130x + 0.1415; r = 0.9950$$

where y = relative peak height, x = copper(II) ion concentration in mg/l and r = correlation coefficient. A linear relationship, indicated by r , between relative peak height and copper(II) ion concentration was obtained from 0.2 to 7.0 mg/l.

4.8.3.2 Accuracy

Five tap water samples were analysed with the proposed SIA system and the results compared with the results obtained for the samples by direct measurement of copper(II) with flame-AAS. By comparing the SIA results with the results obtained by flame-AAS, the accuracy of the proposed system could be evaluated. The flame-AAS was calibrated with a range of copper(II) standards ranging from 0.5 - 9.0 mg/l before sample measurements were taken. The SIA results, shown in Table 4.7, are in good agreement with the results obtained by flame-AAS.

TABLE 4.7. Comparison of results obtained by the proposed SIA system and flame-AAS.

Samples	AAS (mg/l)	SIA (mg/l)
A	1.18	1.10
B	2.95	2.78
C	4.98	4.81
D	5.45	5.26
E	6.41	6.23

The proposed SIA system was successful in analysing real samples without the need to introduce masking agents to mask possible interfering species. The system can thus be applied with confidence to samples where interference is negligible.

4.8.3.3 Precision

The precision of a procedure gives an indication of the reproducibility of the measurements. A high reproducibility reflects the ability of a system to deliver reproducible zone penetration and mixing between sample and reagent zones and the subsequent formation of a coloured complex. The precision was determined by 5 repetitions of the standard and sample solutions in the linear range of the proposed SIA system. The results are shown in Table 4.8. In all of the cases the RSD was < 3.65%.

TABLE 4.8. Precision of the proposed SIA system

Standard/Sample (C in mg/l)	%RSD
0.2	1.24
0.5	1.80
1.0	0.29
3.0	0.69
5.0	1.02
7.0	0.99
Sample A	3.65
Sample B	0.55
Sample C	1.03
Sample D	0.84
Sample E	0.79

4.8.3.4 Detection limit

The detection limit was calculated with the following formula:

$$\text{Detection limit} = \frac{(3 s_b + I_b) - k}{m}$$

where: s_b = standard deviation of the background signal

I_b = relative peak height of the background signal

k = intercept of the calibration curve for the proposed SIA method

m = slope of the calibration curve for the proposed SIA method

The detection limit was calculated to be 0.1 mg/l Cu(II).

4.8.3.5 Sample interaction

The interaction between samples was calculated by using the following formula:

$$Interaction = \frac{(A_3 - A_1)}{A_2} \times 100$$

where: A_1 = relative peak height of a sample containing 0.5 mg/l Cu(II),

A_2 = relative peak height of a sample containing ten times more Cu(II) (5.0 mg/l),

A_3 = relative peak height of a sample containing the same amount of Cu(II) as A_1 .

Due to partial formation of a precipitate as a result of the complex formed between Cu(II) and DDTC, carry-over might be expected. This was minimized by thorough rinsing of the sequential injection system. Sample interaction was calculated to be less than 1.0%, which is negligible.

4.8.3.6 Sample frequency

It took 240 seconds to complete one cycle of the sequential injection analysis procedure. This resulted in the processing of 15 samples per hour. The analysis time may be lengthy but it is necessary for drawing up sample and reagent (10.4 seconds in total) and to rinse the sequential injection system, thus minimizing carry-over between samples.

4.9 The determination of copper(II) with SIA in the presence of interferences

4.9.1 Experimental

4.9.1.1 Reagents and solutions

All reagents were prepared from analytical-reagent grade chemicals unless specified otherwise. All aqueous solutions were prepared using de-ionised water from a Modulab system (Continental Water Systems, San Antonio, Texas, USA). The de-ionised water used as carrier stream and to prepare the aqueous solutions with, was degassed, by heating to boiling point and cooling before it was used.

Standard Cu(II) stock solution, 1000.0 mg/l: Pure copper metal coarse chips were used in the preparation of the Cu(II) stock solution. The copper metal was cleaned to remove any oxides and dissolved by heating 1.0 g of the copper metal in 10 ml 55% HNO₃ (m/m) and *ca.* 10 ml of water. The subsequent solution was cooled and then diluted to 1000 ml with water.

Standard Fe(II) stock solution, 100.0 mg/l: 0.0702 g Fe(NH₄)₂(SO₄)₂·6 H₂O was dissolved in *ca.* 20 ml of water with 1.1 ml of 18.4 mol/l H₂SO₄ (98%, m/m). The final solution was diluted to 100 ml with water.

Cu(II)/Fe(II) working solution: A solution containing 3.0 mg/l Cu(II) and 15.0 mg/l Fe(II) was prepared in order to study the effect of Fe(II) as the largest interferent on the reaction between

Cu(II) and DDTC [21] in the presence of the analyte. This solution was made by appropriate dilution of the 1000.0 mg/l Cu(II) and 100.0 mg/l Fe(II) standard solutions respectively.

DDTC/EDTA/Citrate reagent solution: 0.100% (m/v) sodium diethyldithiocarbamate was dissolved in 50 ml water by heating the solution to 60°C. After the DDTC solution was cooled, 1.2 g ethylene-diamine-tetra-acetic acid disodium salt (EDTA) together with 1.5 g tri-ammonium citrate was dissolved in 25 ml water and added to the DDTC solution. 9.5 ml of a 2 mol/l NH₄Cl solution was then added to the DDTC/EDTA/Citrate solution and the pH adjusted to 8.3 by adding an appropriate volume of 2 mol/l NH₃. The final solution was made up to 100 ml with water.

4.9.1.2 Sample preparation

Cu(II) was determined in plant food (water soluble samples) and water samples. A predetermined mass of plant food was weighed, dissolved and finally diluted to 100 ml. The water samples were spiked with a known concentration of Cu(II).

4.9.1.3 Apparatus

The sequential injection system depicted in Fig. 4.6 was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-le-Bel, France); a 10-port electrically actuated selection valve (Model ECSD10P; Valco Instruments, Houston, Texas, USA); and a UNICAM 8625 UV-visible spectrometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell (volume 80 µl) for

absorbance measurements.

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (Mintek, Randburg, South Africa). The FlowTEK [38] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All the data given (relative peak heights) are the mean for $n = 5$.

The wavelength of maximum absorbance was identified by scanning the complex solution over the 300 to 700 nm range with a Spectronic Genesys 5 spectrophotometer (Milton Roy Company, USA). The optimum wavelength was chosen as 460 nm that corresponds to the wavelength used by previous authors conducting the same determination [21,35].

pH measurements of the DDTC/EDTA/Citrate solution were conducted with an Orion pH meter (Model 420 A; Orion Research Inc., USA) and an Orion pH TriodeTM electrode.

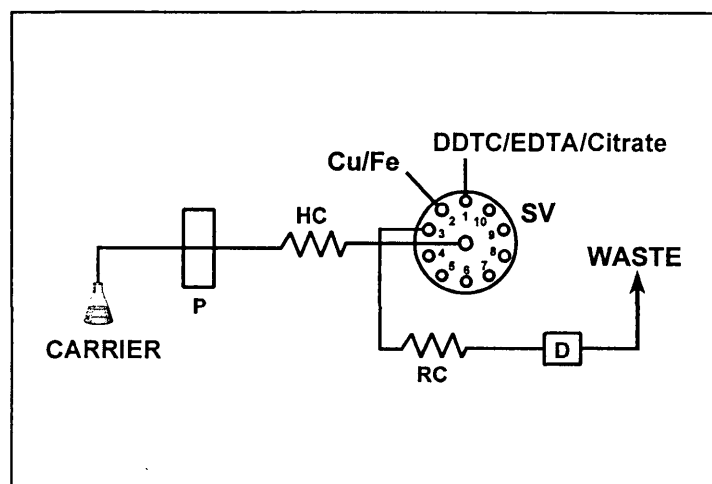


Fig. 4.6 A schematic diagram of the SIA system used for the determination of Cu(II). P - pump, HC - holding coil, RC - reaction coil, SV - selection valve and D - detector

4.9.1.4 Manifold

The components of the SIA system were arranged as illustrated in Fig. 4.6. The holding coil consisted of 3.5 m x 1.60 mm i.d. Tygon tubing and the reaction coil of 1.1 m x 1.02 mm i.d. Tygon tubing. A flow rate of 1.4 ml/min was used.

4.9.1.5 Procedure

The device sequence for the determination of Cu(II) by sequential injection analysis in the presence of an interferent is given in Table 4.9.

TABLE 4.9. Device sequence for one cycle of the sequential injection system

Time (s)	Pump	Valve	Description
0	Off	DDTC/EDTA/ Citrate	Pump off, select DDTC/EDTA/Citrate stream (valve position 1)
5.0	Reverse		Draw up DDTC/EDTA/Citrate solution
15.7	Off		Pump stop
16.7		Cu/Fe	Select Cu/Fe stream (valve position 2)
17.7	Reverse		Draw up Cu/Fe solution
49.8	Off		Pump stop
50.8		Detector	Select detector line (valve position 3)
51.8	Forward Reverse		29 Flow reversals of 4s each (2s forward and 2s reverse)
167.8	Forward		Pump stack of zones to detector
519.0	Off	Home	Pump off, return valve to starting position (valve position 1)

4.9.2 Method optimization

The initial approach regarding optimization of the physical parameters of the sequential injection analysis system was that the masking agents would be introduced through a third zone, in addition to the sample and reagent zones. This approach is illustrated in Fig. 4.7. Two possible arrangements of the three zone configuration were considered:

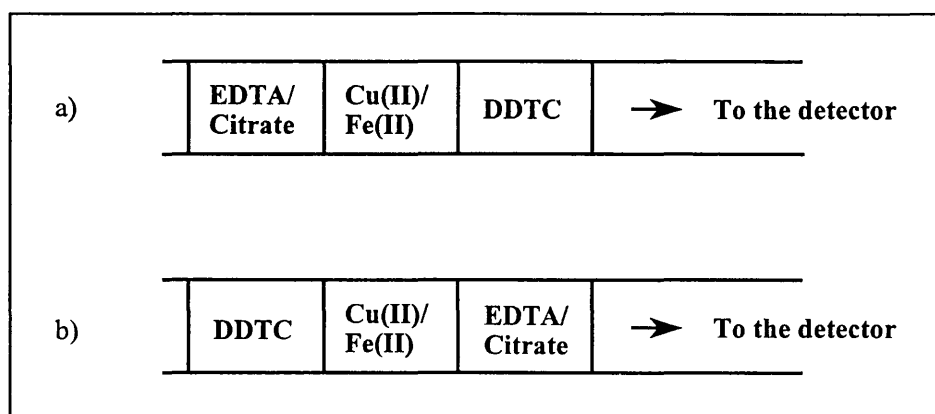


Fig. 4.7 Arrangement of the zones in a three zone configuration.

Preliminary experiments regarding the proposed arrangements lead us to the conclusion that this approach would not be suitable. Although this approach succeeded in reducing the interference to a slight extent, it was found that it would require enormous volumes of masking agent to reduce the interference to a larger extent. This would result in high analysis times due to the time necessary to draw up the zones and rinse the system. It appeared that the sample and masking agent zones did not effectively penetrate one another with the proposed arrangement that resulted in the inadequate reaction between the zones. The masking agents could thus not act effectively in reducing interference through the amount of mixing that was obtained by this approach. It was decided to combine the masking agents and reagent as one solution so that it would only be necessary to draw up one zone in addition to the sample zone (two zone configuration).

Although interference was more effectively reduced by combining the masking agents and reagent as one (with less masking agent/reagent volume needed than before), the reduction of interference was still not sufficient enough. Since it is known that the attainment of equilibria between dithiocarbamates and metal ions are slow [24], it was concluded that the masking agents needed more time to replace the DDTC that might have complexed with the interfering ion.

Another possible explanation for the inability of the masking agents to effectively reduce interference is that effective zone penetration and mixing of the zones are not achieved with the current physical setup of the sequential injection analysis system.

Two techniques are known to contribute to increased reaction time (stopped-flow [40]) or increased mixing (flow reversals [41]) if it is not effectively achieved by the standard physical setup. Stopped-flow consists of stopping the flow for a fixed time after flow had been reversed. By stopping the flow, increased time would be available for the masking agents to react more effectively with the interfering components.

Flow reversals offer two possibilities: (i) changing the number of flow reversals while keeping the length of the flow reversals fixed or (ii) changing the length of the flow reversals while keeping the number of flow reversals fixed. Flow is reversed by changing the direction of the flow, thus either forward or backwards. If the number of backwards and forward motions are increased, the number of flow reversals is also increased. The length of the flow reversal is changed by increasing or decreasing the time it takes to complete one reversal. Flow reversals affect the amount of mixing obtained between sample and reagent [41]. By increasing the amount of mixing between the zones, the reagent/masking and sample zones would be allowed to react more effectively.

Preliminary experiments were conducted by incorporating these techniques into the sequential injection analysis procedure. Since the proposed techniques could contribute to dispersion of the product zone, it was taken into account that a decrease in relative peak height as a result of the technique did not necessarily result in the decrease in interference. The experiments were

conducted in such a way that physical dispersion was accounted for. Preliminary experiments showed that interference could be reduced effectively by introducing either of the techniques. These techniques were subsequently included in the optimization of the physical parameters.

4.9.2.1 Physical parameters

It has been shown that the physical parameters of an SIA system influence the sensitivity and precision of measurements [39,41,42]. These parameters also have a direct influence on the sensitivity and precision obtained when studying a chemical reaction. Since the colours of metal complexes with DDTC are not very intense [26], it is necessary to optimize the physical parameters to obtain optimum sensitivity.

4.9.2.1.1 Comparison of different techniques to reduce Fe(II) interference

It has already been mentioned that optimization of the basic physical components of a sequential injection analysis system does not necessarily provide all the adequate zone penetration and mixing of the zones. Two physical techniques were studied that could contribute to increased reaction time, mixing and zone penetration of the zones and as a result contributes to reducing the interference. The techniques that were studied include: stopped-flow period and flow reversals (length and number). The results are given in Table 4.10.

The stopped-flow period was implemented for a fixed time directly after the flow was reversed. The length of the stopped-flow period was evaluated. A decrease in Fe(II) interference was observed for the longer periods, due to increased time available for the masking agents to react

with the interferent. A disadvantage of the stopped-flow period was however, a decrease in sample frequency due to the extensive analysis times that characterized the long periods.

TABLE 4.10. Influence of different techniques to reduce interference from Fe(II)

Stopped-flow period (s)	% Interference
0	31.8
120	27.9
240	10.4
Flow reversals (#)	% Interference
1	52.0
10	32.2
20	28.6
30	13.6
Length of flow reversal (s)	% Interference
none	65.2
4	59.2
10	43.9
20	12.6

Flow reversals were obtained by propelling the flow forward and backwards in the flow conduit. By repeating this action more than once, zone penetration was enhanced and a mixing effect was obtained which allowed more effective mixing of the sample and reagent zones. This action contributed to a larger reaction between the masking and interfering components. The length of the flow reversals was kept constant (2s forward and 2s backwards) while changing the number of flow reversals. The results show that the Fe(II) interference decreased for a larger number of

flow reversals. Although sensitivity decreased due to increased dispersion of the zones, as a result of enhanced mixing of the zones for the larger number of flow reversals, suitable analysis times and adequate sensitivity were still achieved.

The length of a fixed number of flow reversals was changed by changing the time it takes for each reversal (counting one forward and backward motion as one reversal) to be completed. The number of flow reversals was kept constant at five while evaluating the length of it. Increasing the length of the flow reversals effectively reduced interference although dispersion of the zones increased dramatically and sensitivity decreased as a result. This approach is not suitable since the sensitivity of the complexation reaction between Cu(II) and DDTC is already very low [26].

Thirty flow reversals were chosen as optimum to reduce interference effectively and still give adequate sensitivity and analysis times.

4.9.2.1.2 Flow rate

The effect of flow rate on peak height and precision was studied for the 0.9 - 4.8 ml/min range. The results are shown in Fig. 4.8. A decrease in peak height was observed for flow rates above 1.4 ml/min. This was attributed to the low reaction rate between Cu(II) and DDTC [24].

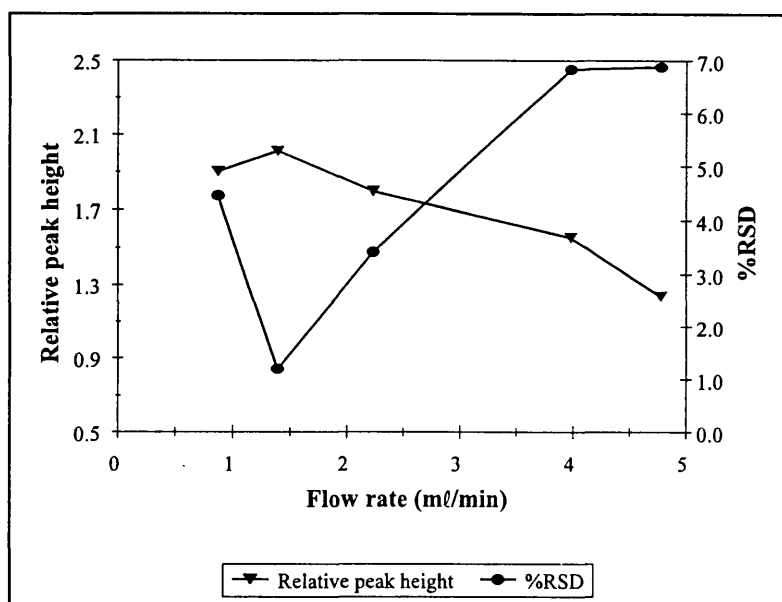


Fig. 4.8 Influence of flow rate on sensitivity and precision

The change in sensitivity for different flow rates can be explained as follow. At low flow rates adequate time is available for equilibrium to be attained. An adequate amount of complex is thus formed which results in the high peak heights obtained. The decrease in peak height below 1.4 ml/min is attributed to a large degree of dispersion that characterizes the lower flow rates.

An increase in flow rate causes the formed product zone to reach the detector much faster. When the flow rate is increased, dispersion of the penetrated zones decreases [39], which results in increased peak heights. This was not observed in this case due to the reaction rate of the complex formation that dominated the decreased dispersion effect. The time available for complex formation was thus shortened by increased flow rates and as a result, the sensitivity decreased. A similar observation was made by Szpunar-Łobińska *et al* [20].

Although higher flow rates result in higher sample frequencies, it was characterised by a loss of sensitivity and decreased precision. The high flow rates are evidently not the best choice regarding an optimum. A flow rate of 1.4 ml/min, which gave the best sensitivity and precision compared with other flow rates, was chosen as optimum.

4.9.2.1.3 Reagent volume

The reagent volume was studied by aspirating increasing reagent volumes (50 - 750 μl) into the sequential injection system and evaluating its effect on sensitivity and precision while keeping the sample volume constant (500 μl). An initial increase in sensitivity was observed as the reagent volume was increased up to 200 μl and reached a plateau (thus no further increase) for the larger reagent volumes (Fig. 4.9).

The optimum reagent volume was a choice between 250 μl and 500 μl . These two volumes delivered adequate sensitivity and the best overall reproducibility. The low reproducibility of reagent volumes below 200 μl was attributed to the difficulty in drawing up small volumes accurately. An optimum reagent volume of 250 μl rather than 500 μl was chosen although the sensitivity for 500 μl was higher. This decision can be substantiated by the following explanation.

By using the large reagent volume (eg. 500 μl) an excess of DDTC reagent would be added to the system, and subsequently a larger sample volume would be necessary to react with all the reagent to deliver a plateau (no further increase in sensitivity with increasing sample volume) in an increasing sample volume graph. Systems with such large sample and reagent volumes would

require longer analysis times for drawing up sample and reagent and for rinsing the system. This will result in lower sample frequencies that are not acceptable for on-line process analysers.

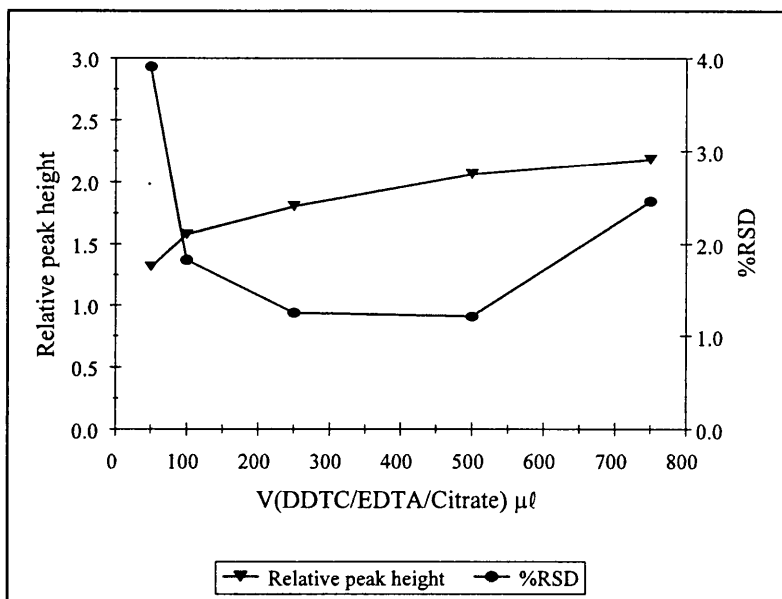


Fig. 4.9 Influence of reagent volume on sensitivity and precision

With smaller reagent volumes (eg. 250 μl), smaller sample volumes would be necessary to deliver a plateau in an increasing sample volume graph. A distinct advantage of SIA is the reduction in reagent and sample consumption. By choosing a smaller reagent volume as optimum (250 μl) a slight decrease in sensitivity would be inevitable, but the analysis time could be kept as short as possible and reagent and sample consumption would be reduced.

4.9.2.1.4 Sample volume

Fig. 4.10 illustrates the effect of sample volume on sensitivity and precision while keeping the reagent volume constant (250 μl). The range of sample volumes that were evaluated is: 100-1000 μl . The increase in peak height with increasing sample volume was attributed to the larger quantity of analyte available to react with the excess of reagent resulting in increased formation of the detectable specie. A plateau was reached at volumes above 500 μl . This plateau corresponds to optimum sensitivity and is reached when the sample reacts with most of the reagent in the reagent volume. Optimum sensitivity and precision were obtained for 750 μl of sample and was thus chosen as optimum.

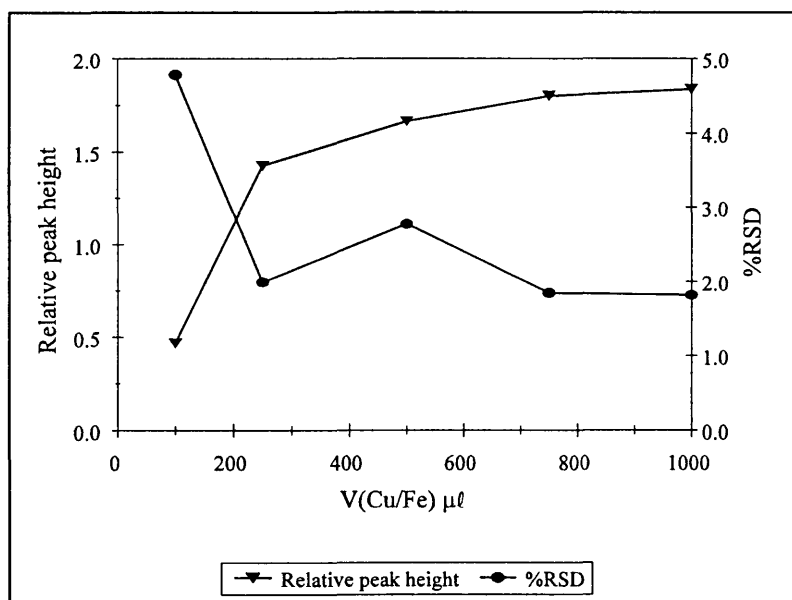


Fig. 4.10 Influence of sample volume on sensitivity and precision

4.9.2.2 Chemical parameters

It is necessary to optimize the chemical parameters that affect the reaction between Cu(II) and DDTC to obtain optimum sensitivity and precision. Preliminary experiments showed that these parameters also affect the amount of interference experienced and it was subsequently necessary to optimize the chemical parameters to the point where interference from Fe(II) was minimized. The chemical parameters that affect the complexation reaction between Cu(II) and DDTC are [21]: DDTC concentration, pH, EDTA and citrate concentration.

4.9.2.2.1 Sodium diethyldithiocarbamate concentration

The sodium diethyldithiocarbamate concentration affects the amount of reagent that is available for complex formation. The influence of the sodium diethyldithiocarbamate concentration on sensitivity, precision and interference was studied for the 0.050 - 0.400% (m/v) concentration range. The detector response is usually expected to stay constant for the very high reagent concentrations due to all the sample being used up by the excess reagent. The results obtained in this instance proved the contrary.

Increasing the % sodium diethyldithiocarbamate concentration resulted in increased peak heights (Table 4.11) for the Cu(II)/Fe(II) working solution. The relative constant peak height obtained for the reaction between Cu(II) and DDTC (in the absence of Fe(II)) suggested that all the Cu(II) had reacted and that by increasing the sodium diethyldithiocarbamate concentration no significant increase in complex formation was achieved.

An increase in peak height was, however, observed for the DDTC and the Cu(II)/Fe(II) sample. This increase was attributed to Fe(II) which was added as interferent to the Cu(II) sample. In this instance Fe(II) was also available to react with the DDTC after an optimum of Cu(II) had reacted. In addition to the detected complex formed between Cu(II) and DDTC, the complex formed between Fe(II) and DDTC might also contribute to the peak height.

TABLE 4.11. Influence of sodium diethyldithiocarbamate concentration on sensitivity, precision and interference

%[sodium diethyl- dithiocarbamate] (m/v)	Relative peak height (Cu)	Relative peak height (Cu/Fe)	%RSD (Cu/Fe)	%Interference
0.050	1.75	1.41	1.47	-19.22
0.100	1.74	1.57	2.86	-9.86
0.200	1.83	1.83	3.52	0.02
0.300	1.88	2.06	4.49	9.47
0.400	1.85	2.16	3.08	17.07

It is also evident from Table 4.11 that the % interference changes from a large negative to a large positive value with increasing sodium diethyldithiocarbamate concentrations. This is due to the possibility that Fe(II) might have formed different complexes at various sodium diethyldithiocarbamate concentrations. Increased sodium diethyldithiocarbamate concentrations could affect the coordination of DDTC towards Fe(II) to form different complexes with differing absorption characteristics.

Choosing an optimum was based on a compromise regarding sensitivity and % interference and gaining with respect to precision. The 0.100% sodium diethyldithiocarbamate concentration was chosen as optimum. The 0.200% concentration gave better sensitivity with less interference, but the precision was not that satisfactory. The interference experienced for the 0.100% concentration can be reduced further by optimization of the masking agent concentrations.

4.9.2.2.2 pH of the DDTC/EDTA/Citrate solution

The effect of solution pH on the ability of the reagent to form metal complexes in aqueous solution varies from metal to metal [43]. It is thus important to control the pH of the aqueous solution since it could affect with which metal the reagent will complex predominantly. An important feature of the dithiocarbamate ion is its protonation in acidic solutions and the subsequent decomposition into carbon disulphide and the protonated amine [24-26].

Preliminary experiments indicated that complex formation between Cu(II) and DDTC did not occur at a pH below 6, possibly due to the rapid decomposition of the reagent below this pH [25]. Complex formation did though occur at a pH above 6. It is thus necessary to maintain a high enough pH to prevent decomposition of the reagent since it could reduce the amount of reagent that will be available for complex formation and as a result influence the sensitivity.

Due to possible interference of Fe(II) on the spectrophotometric determination of Cu(II), the pH range between 6 - 10 was studied to see whether pH might influence the sensitivity, precision and interference of Fe(II).

TABLE 4.12. Influence of pH of DDTC/EDTA/Citrate solution on sensitivity, precision and interference

pH	Relative peak height (Cu)	Relative peak height (Cu/Fe)	%RSD	%Interference
6.3	1.64	1.47	1.89	-10.52
7.3	1.65	1.67	3.42	0.95
8.3	1.59	1.54	1.34	-2.84
9.2	1.60	1.50	1.84	-6.11

It is evident from Table 4.12 that a change in pH had a minor affect on the sensitivity of the measurements. The pH did however influence the amount of interference experienced. Minimum interference was observed between a pH of 7.3 - 8.3. It can be concluded that the masking agents performed best in the mentioned range and that pH definitely affects the complexing ability of the masking agents, especially EDTA, with other metals [44]. The very high pH ranges should be avoided in any case due to the possible formation of metal hydroxo complexes that may cause the metal to precipitate, thus making it inaccessible to complexation [43].

Precision obtained at pH 8.3 was better than at pH 7.3 and was thus chosen as the optimum working condition. This compares well to the optimum pH used in previous studies [21,35].

4.9.2.2.3 Masking agents concentration

The selectivity of the complexation reaction between Cu(II) and DDTC is enhanced considerably by using EDTA in combination with citrate as masking agent [21,35,44]. Increasing the EDTA concentration did not affect the sensitivity and had a minor influence on precision. The results are shown in Table 4.13. As expected, the % interference decreased with higher EDTA concentrations. The decrease in interference is ascribed to a larger amount of the interfering ion being complexed by the EDTA. A 1.2% (m/v) EDTA concentration was chosen as optimum concentration for masking interferences.

TABLE 4.13. Influence of EDTA concentration on sensitivity, precision and interference

%[EDTA] (m/v)	Relative peak height (Cu)	Relative peak height (Cu/Fe)	%RSD	%Interference
0.3	1.64	1.49	1.61	-9.14
0.6	1.62	1.52	1.36	-6.24
1.2	1.57	1.50	1.18	-4.35

The citrate concentrations that were evaluated covered an extensive range (1.5 - 10.0% (m/v) citrate). This wide range was chosen because preliminary experiments indicated that there might be an increase in interference with increasing citrate concentrations, compared with the trend observed for EDTA. It is usually expected that an increasing amount of masking agent will reduce interference and not really contribute to it. The results are given in Table 4.14.

An increase in sensitivity was observed with increasing citrate concentrations, both in the presence and absence of Fe(II). This observation was attributed to the fact that the citrate might react with either Fe(II) or Cu(II) to form complexes that absorb at the wavelength of interest to contribute to the absorption signal of the complex formed between Cu(II) and DDTC. This also explained the increase in % interference with increasing citrate concentrations. Least interference was experienced at 1.5% (m/v) citrate and this concentration was therefore chosen as optimum.

TABLE 4.14. Influence of tri-Ammonium citrate concentration on sensitivity, precision and interference

%[Citrate] (m/v)	Relative peak height (Cu)	Relative peak height (Cu/Fe)	%RSD (Cu/Fe)	%Interference
1.5	1.54	1.52	2.25	-1.05
3.0	1.60	1.71	2.01	7.11
6.0	1.72	1.86	1.10	7.98
10.0	1.82	1.99	1.45	9.05

4.9.3 Method evaluation

The proposed SIA system was critically evaluated with regard to linearity, accuracy, precision, detection limit, sample interaction (carry-over), sampling frequency and interferences.

4.9.3.1 Linearity

The response of the proposed SIA system for the spectrophotometric determination of copper(II) was evaluated under optimum conditions given in Table 4.15.

TABLE 4.15. Optimum SIA conditions

Operational parameter	Dimension
Flow reversals	30 with a length of 4s each
Flow rate	1.4 ml/min
Sample volume	750 µl
Reagent volume	250 µl
[sodium diethyldithiocarbamate]	0.100%
pH of DDTC/EDTA/Citrate solution	8.3
[EDTA]	1.2%
[Citrate]	1.5%

The following relationship was obtained for relative peak height versus copper(II) ion concentration:

$$y = 0.4984x + 0.1367; r = 0.9964$$

where y = relative peak height, x = copper(II) ion concentration in mg/l and r = correlation coefficient. A linear relationship, indicated by r , between relative peak height and copper(II) ion concentration was obtained from 0.5 to 5.0 mg/l.

4.9.3.2 Accuracy

The accuracy of the proposed SIA system was evaluated by analysing two plant food samples and five tap water samples. The results obtained by the SIA system were compared with the results obtained for the samples by direct measurement of copper(II) with flame-AAS. The flame-AAS

was calibrated with a range of copper(II) standards ranging from 1.0 - 5.0 mg/ℓ before sample measurements were taken. The SIA results, shown in Table 4.16, are in good agreement with the results obtained by flame-AAS. It is evident that the proposed system allows the accurate determination of copper(II) in real samples.

TABLE 4.16. Comparison of results obtained by the proposed SIA system and flame-AAS

Samples	AAS (mg/ℓ)	SIA(mg/ℓ)
A	1.30	1.27
B	1.68	1.82
C	2.87	2.66
D	3.29	3.16
E	4.18	4.28
Plant food : A	4.88	4.47
Plant food : B	2.69	2.47

4.9.3.3 Precision

The precision was determined by 5 repetitions of the standard and sample solutions in the linear range of the proposed SIA system. The results are shown in Table 4.17. In all cases the RSD was < 4.55%. The %RSD reflects the capability of the proposed SIA system to deliver reproducible and precise measurements. This system was relatively successful to this regard.

TABLE 4.17. Precision of the proposed SIA system

Standard/Sample ([] in mg/l)	%RSD
0.5	0.75
1.0	2.52
2.0	4.13
4.0	1.51
5.0	1.55
Sample A	3.98
Sample B	1.78
Sample C	1.72
Sample D	4.54
Sample E	2.29
Plant food: A	2.76
Plant food: B	2.02

4.9.3.4 Detection limit

The detection limit was calculated to determine the lowest concentration of copper(II) that can be detected by the proposed SIA system. The detection limit was calculated with the following formula:

$$\text{Detection limit} = \frac{(3 s_b + I_b) - k}{m}$$

where: s_b = standard deviation of the background signal
 I_b = relative peak height of the background signal
 k = intercept of the calibration curve for the proposed SIA method
 m = slope of the calibration curve for the proposed SIA method

The detection limit was calculated to be 0.2 mg/l Cu(II). Though the proposed system is not as sensitive as previously developed procedures [20,21], it does compare favourably.

4.9.3.5 Sample interaction

The sample interaction was determined for three consecutive samples. This was done by first analysing a sample containing a very low concentration of copper(II), followed by a sample containing ten times the amount of copper(II) than the first sample and lastly a sample containing the same amount of copper(II) as the first. Interaction is experienced between consecutive samples when for example the high concentration is not effectively rinsed out of the system and as a result contributes to the relative peak height obtained for the smaller concentration sent through last. The sample interaction was calculated by using the following formula:

$$Interaction = \frac{(A_3 - A_1)}{A_2} \times 100$$

where: A_1 = relative peak height of a sample containing 0.5 mg/l Cu(II),

A_2 = relative peak height of a sample containing ten times more Cu(II) (5.0 mg/l),

A_3 = relative peak height of a sample containing the same amount of Cu(II) as A_1 .

The sample interaction was calculated to be less than 1.0%, which is negligible.

4.9.3.6 Sample frequency

It took 519 seconds to complete one cycle of the sequential injection analysis procedure. This resulted in a sample frequency of 7 samples per hour. The lengthy analysis time was ascribed to the time necessary to draw up the sample and reagent, time needed to perform the flow reversals and to effectively rinse the system to minimise possible carry-over between consecutive experiments.

4.9.3.7 Interferences

Interference is experienced in the determination of copper(II) with DDTC when an appreciable amount of certain metals are present in the samples to be analysed. The main interfering metals in the determination of copper(II) with DDTC are Fe, Bi, Mn, Ni, Co, Cr, Mo and U, which forms coloured complexes. The selectivity of the method is though considerably enhanced by using EDTA as masking agent and by appropriate choice of pH [26]. In a citrate medium at pH 8 - 9, Fe, Mn, Ni and Co are presumably masked by EDTA, as are Cd, Pb, Zn and In which forms colourless complexes with DDTC [26]. It is important to note that though the list of interfering ions is quite extensive, not all are commonly found in plant food and water samples.

Wang *et al* [21] evaluated the interference of certain ions that could probably affect the spectrophotometric determination of Cu(II) with DDTC. The interferences of these ions were studied with the proposed SIA system and the results are highlighted in Table 4.18.

The interference study was conducted by adding a predetermined amount of the interfering ion to a solution containing 3.0 mg/l Cu(II). The effect of the interfering ion was evaluated by comparing the resulting peak height with the peak height obtained for a 3.0 mg/l Cu(II) solution containing no interfering species. The ratio of (x mg/l interfering ion) : (1.0 mg/l Cu(II)) was used to indicate at which levels severe interference started. The interfering ions interfered by either reducing or increasing the signal of the complex formed between Cu(II) and DDTC.

It is evident that the lowest level of tolerance is a 5:1 (foreign ion to Cu(II) ion) ratio for Fe(II) and Fe(III). The Fe(II) ion interfered by reducing the relative peak height by 7.1%. This reduction in peak height is probably due to the formation of a complex formed between Fe(II) and DDTC with absorption characteristics that differ from those of the complex formed between Cu(II) and DDTC. Fe(III) on the other hand interfered by increasing the peak height, probably due to the formation of a complex with DDTC with absorption characteristics similar to those of complex formed between Cu(II) and DDTC.

TABLE 4.18. Interference from foreign ion species studied for a 3.0 mg/ℓ Cu²⁺ solution. The ratio of foreign ion to Cu(II) is indicated as x:1.

Tolerance ratio of foreign species (x)	Foreign species
600	Mg ²⁺
300	SO ₄ ²⁻ , Mn ²⁺
200	NO ₃ ⁻
150	Ca ²⁺
40	Cl ⁻
20	Zn ²⁺ , K ⁺
10	CO ₃ ²⁻
5	Fe ²⁺ , Fe ³⁺

The proposed system is though much more tolerant towards the interference of Mn(II), Zn(II), sulphate, calcium, magnesium and nitrate than the procedure described by Wang *et al* [21].

4.10 Conclusion

The sequential injection analysis system that was developed for the determination of Cu(II) in the presence of an interferent, proved to be successful in analysing Cu(II) in plant food and water samples. The lengthy analysis time that contributes to the low sample frequency was due to the flow reversals necessary to reduce interference and to flush the system. The flow reversals successfully reduced interference. The calibration curve is linear from 0.5-5.0 mg/ℓ with a detection limit of 0.2 mg/ℓ. The system processes 7 samples per hour with a relative standard deviation of < 4.55%.

The sequential injection analysis system that was developed for the analysis of Cu(II) in the absence of interferences was also successful in conducting analysis on real samples. A disadvantage of this system is that it will not be able to deliver accurate results for samples containing high levels of interfering species. Despite this, the proposed system delivers a higher sample frequency (15 samples per hour) than the above-mentioned system with a precision of < 3.65%. The linear range extends over the 0.2 - 7.0 mg/l Cu(II) concentration range with a detection limit of 0.1 mg/l.

In both cases analysis was done without the necessity of introducing an extraction step thus complying with the need of conducting environmentally safe analysis. The systems are also fully computerized and can be incorporated on-line if required.

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CHAPTER 5

Spectrophotometric Determination of Thiocyanate with Sequential Injection Analysis

5.1 Introduction

Carbon is the third or fourth most abundant element in the universe and the major element of life. On earth it is relatively much less abundant, with the earth crust consisting of about 480.0 mg/l of carbon [1]. Although it is not one of the most abundant elements on earth, it remains a crucial element in the environment. Carbon occurs in compounds principally in the +IV oxidation state. It occurs both as the free element (graphite and diamond) and in combined form (mainly as the carbonates of Ca, Mg, and other electropositive elements). It also occurs as CO₂, a minor but crucially important constituent of the atmosphere and is abundant as the bicarbonate ion (HCO₃⁻) in all natural waters. In addition to its natural occurrence as the free element, carbon is widely distributed in the form of coal and petroleum. It appears that around 80% of the carbon on earth is oxidised while the other 20%, mostly hydrocarbons in sedimentary rocks, is in a reduced chemical state [1].

The unusual diversity of carbon chemistry stems partly from the ability of a carbon atom to form strong covalent bonds, particularly between two carbon atoms, which are resistant to attack. Carbon is found directly linked to H, N, O, S and occasionally other elements in certain compounds that are present in living systems [1]. Carbon has a dual role in life: as an element required in the organic molecules of life, and as a component of the redox reactions which supply free energy.

Of the carbon compounds generally considered inorganic, the cyanides (CN^-) and the thiocyanates (SCN^-) are of great importance [2]. The toxicity of the cyanides is well known and though not as toxic as cyanide, thiocyanate is harmful to aquatic life.

The increasing demand for stricter control over the outlet of harmful substances into the environment, increases the responsibility of the health authorities to enforce restrictions. When the public demand what they describe as "pure" water, what they are really referring to is "safe" water. This "safe" water may only contain a minimum amount of salts, and should be free from those substances that might be harmful or cause disease.

Natural waters may contain many substances, even poisons, attributed to the deliberate discharge of domestic and industrial wastes into the water sources. Many effluents, particularly from industry, contain toxic substances. Some of these substances, including phenols and thiocyanates, are usually broken down by bacteria, particularly if they are mixed and diluted with ordinary sewage that promotes rich bacterial growth [3]. If thiocyanate is not completely broken down by this process and it should enter the aquatic environment, much harm can be done to both humans and animals.

It is thus important to monitor and control the level of toxic substances in natural water streams and industrial effluents. Although the toxicity of thiocyanate is relatively low, the environmental effect of it should not be underestimated. The development of a process analyser to monitor the thiocyanate level in water is thus necessary.

5.2 Properties and occurrence of thiocyanate

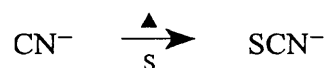
The behaviour of thiocyanate can be explained by its unique properties. A discussion of some of its more distinct properties follows.

Thiocyanate is classified as a pseudo-halide. This group resembles the halide groups to some extent in their properties. The term pseudo-halide was first applied to a series of nitrogen-containing groups, particularly CN, OCN, SCN, SeCN and N₃, but other groups such as (CF₃)₂NO, NSF₂ and FSO₃ are sometimes also classed with the pseudo-halides. The pseudo-halides resemble the halides for three main reasons [4]:

- a) Although the groups may not be thermodynamically stable with respect to their elements, they contain strong bonds and have a considerable degree of kinetic stability that enables them to retain their identity as monovalent groups in a variety of chemical environments.

- b) The ease of oxidation of the pseudo-halides to free pseudo-halogens (or their decomposition or polymerization products) is similar to the ease of oxidation of halides to halogens [approximate sequence $F < Cl < NSF_2 < (CF_3)_2NO < Br < CN < SCN < SeCN$].
- c) The size, charge and polarizability of the pseudo-halide ions and groups are sufficiently similar to that of bromide or iodide to make physical properties such as the solubility of metal salts or the volatility of covalent compounds quite comparable.

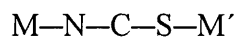
Thiocyanate can be prepared by fusing the corresponding cyanide with S:



The thiocyanate ligand has been studied extensively and is of particular interest because it may coordinate to metal atoms in three distinct ways, giving rise to the possibility of "ligand isomers" [5]:

- a) By formation of a metal-nitrogen bond — such complexes are called *isothiocyanates* and occur most frequently, though not exclusively, among elements of the first row of the transition series;

- b) by formation of a metal-sulphur bond (called thiocyanato complexes), usually found for second- and third-row transition elements;
- c) by formation of bridge complexes, in which both sulfur and nitrogen atoms donate:



The resonance possibilities that exist for the free and coordinated SCN^- ion are given in Fig. 5.1:

<i>Free ion</i>	<i>N-bonded</i>	<i>S-bonded</i>
(a) $\text{N}\equiv\text{C}-\bar{\text{S}}$	(d) $\text{M}-\text{N}^+\equiv\text{C}-\bar{\text{S}}$	(g) $\text{M}-\text{S}-\text{C}\equiv\text{N}$
(b) $\bar{\text{N}}=\text{C}=\text{S}$	(e) $\bar{\text{M}}=\text{N}^+=\text{C}=\text{S}$	(h) $\text{M}-\text{S}^+=\text{C}=\bar{\text{N}}$
(c) $\text{N}^{2-}-\text{C}\equiv\text{S}^+$	(f) $\text{M}-\text{N}=\text{C}=\text{S}$	(i) $\bar{\text{M}}=\text{S}^{2+}=\text{C}=\bar{\text{N}}$

Fig. 5.1 Resonance possibilities for the thiocyanate ligand

Linear bonds are to be expected for *isothiocyanates* unless there is a substantial contribution from form (f), and in the case of the S-bonded complexes, form (g) should predominate [5].

It has already been mentioned that the first-row transition elements, except Cu(II), form *isothiocyanates* and the second- and third-row transition elements to form thiocyanates. In general, metals that form bonds having a considerable degree of ionic character might be expected to favour N- over S-bonding. This is because sulphur is more polarisable than nitrogen, and the lone-pair dipole on nitrogen is greater than it is on sulphur. As expected, there exists a rough correlation between CHATT's "class (a)" metals (which form their most stable complexes with donor atoms from the first row of the Periodic Table - N, O, F) and the formation of *isothiocyanates*, and the "class (b)" elements (those forming their most stable complexes with donor atoms of the second and third rows of the Periodic Table) and formation of S-bonded thiocyanates. There are a few exceptions to this rule [5]. It should be stressed that kinetic factors and relative thermodynamic stability, steric influences, electronic factors and solvent effects affect the bonding mode of thiocyanate. The interrelation of these various factors are summarized in Table 5.1:

TABLE 5.1. Mode of bonding in thiocyanate complexes

Metal type ^(a)	σ -Donor ligand	High- ϵ solvent	Low- ϵ solvent	π -Acceptor ligand
Class a	-NCS	-NCS	-SCN	-SCN
Class b	-SCN	-SCN	-NCS	-NCS

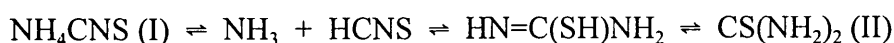
^(a) Sometimes discussed in terms of "hard" and "soft" acids and bases

A preliminary indication of the mode of coordination can sometimes be obtained from vibrational spectroscopy since N coordination raises both $\nu(\text{CN})$ and $\nu(\text{CS})$ relative to the values of the uncoordinated ion, where S coordination leaves $\nu(\text{CN})$ unchanged and increases $\nu(\text{CS})$ only somewhat. The bridging mode tends to increase both $\nu(\text{CN})$ and $\nu(\text{CS})$. These "group vibrations" are however in reality appreciably mixed with other modes both in the ligand itself and in the complex as a whole, and vibrational spectroscopy is therefore not always a reliable criterion. Increasing use is being made of ^{14}N and ^{13}C nmr data but the most reliable data, at least for crystalline complexes, comes from X-ray diffraction studies [6].

5.2.1 Occurrence of thiocyanate

The thiocyanates find application in several fields either as their salts or as the anion. Ammonium and alkali thiocyanates are used in photography, catalysis, agricultural chemicals (notably in weedkillers), dyeing and printing of textiles, and rust inhibition (hence in paints) [7].

Ammonium thiocyanate is an important starting material in the manufacture of thiourea (thiocarbamide). Thiourea (II) may be obtained from ammonium thiocyanate (I) by an isomeric change [8]:



The thiocyanate ion also appears in foods and feeds as a result of the decomposition of isothiocyanates, e.g., from the indolyl glucosinolates and *p*-hydroxybenzyl-GS. Analysis of cabbage and related edible plants disclosed the presence of SCN^- . Ingestion of these *Brassica* vegetables caused a rise in thiocyanate in the blood followed by its appearance in the urine of animals and humans [9].

Sodium and potassium thiocyanate are obsolete hypertensive drugs that have been largely replaced by safer and more effective compounds. These salts have a low therapeutic index, the fatal serum level being 20 mg/100 ml. Thiocyanate is reabsorbed by the renal tubules and renal excretion is the only method for eliminating these ions from the body [10]. In giving human patients thiocyanate to treat hypertension it was discovered that the thiocyanate ion causes thyroid enlargement because it inhibits the uptake of iodine by the thyroid [9]. The principal manifestations of thiocyanate poisoning are caused by stimulation of the central nervous system. The symptoms of thiocyanate poisoning include nausea, vomiting, diarrhea, acute gastric pain, acute depression and exhaustion and signs of hypothyroidism.

Aliphatic thiocyanates (rhodanates) are used in certain household agents for fly and mosquito control [10]. The organic thiocyanates were important insecticides before DDT and the chlorinated cyclodienes came into widespread use. In general, the thiocyanates produce rapid knockdown and paralysis of insects and have been especially used for fly sprays. No insects have developed resistance to these compounds. Thiocyanates are mild general poisons and are self-warning. Methyl- and ethylthiocyanates have been used as fumigants. They are however also toxic to plants,

which limits their use somewhat [11]. Many of the thiocyanates are commercially known as Lethanes:

TABLE 5.2. Properties of the Lethane insecticides

Insecticide	Chemical name	Toxicity	Acute oral LD ₅₀ (mg/kg)
Lethane 60®	2-thiocyanatoethyl laurate	moderately toxic	500
Lethane 384®	2-(2-butoxyethoxy)ethylthiocyanate	toxic	90

A thiocyanate with a rapid knockdown of houseflies is Thanite®. The structure of this compound is given in Fig. 5.2:

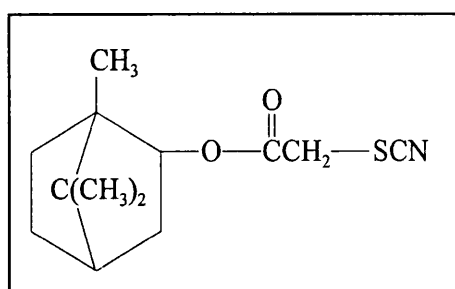
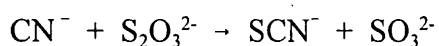


Fig. 5.2 Thanite: Isobornyl thiocyanoacetate

The thiocyanate ion is also partially excreted by the human body, through saliva or urine, due to the detoxification of cyanide. Thiosulphate is responsible for the conversion of inorganic cyanides to thiocyanates. The reaction is catalysed by the enzyme rhodanese, which is widely distributed in several organs. As it involves an approximately 200x reduction of toxicity, it is among the most efficient detoxification reactions known. This reaction:



detoxifies the limited amounts of cyanide that are normally formed in the organism (e.g., from degradation of vitamin B₁₂ or from the glycoside occurring in almonds, amygdalin) and the cyanides formed through incomplete combustion of tobacco products [12]. The thiocyanate concentration in the saliva of cigarette smokers is high enough to give a distinctly visible colour reaction with the ferric ion.

5.3 Choice of analytical method

Several approaches have already been described for the determination of thiocyanate in various materials. These include the determination of thiocyanate in waste waters [13-16], biological samples [16-24], metallurgical processes [25] and food [26,27]. A variety of detection system was applied in these applications for monitoring the level of thiocyanate in samples. The detection systems that were used in these applications include spectrophotometers [13,14,16,19,21,23,25], ion-

selective electrodes [15,28], chromatography [17,18,22,27], voltammetry [26], fluorimetry [17], amperometry [20] and flame-AAS [24]. Evidently, spectrophotometry has frequently been used as the detection system.

Although extensive research has already been done on the determination of thiocyanate, there are very few procedures available for its determination in waters and hydro metallurgical solutions. The demand for automated, preferably on-line, analysers that are cheap and affordable to install and maintain are ever increasing. The aim of this study was to develop an automated on-line procedure for the determination of thiocyanate in process solutions. The literature survey was aimed at finding procedures and methods that had already been developed for the determination of thiocyanate in waste waters, effluents and process solutions. By using existing methods a new procedure was developed. A discussion of the few limited procedures that are available for the determination of thiocyanate in waters follows:

The indirect determination of thiocyanate by atomic absorption spectrometry (AAS) and solution spectrophotometry was reported by Stratis and Vasilikiotis [29]. This determination involved first the formation and then the extraction of the 2,2'-dipyridyl-2-pyridylhydrazone (DPPH)—Cu—SCN complex from aqueous solution (pH 6.25) into a methyl isobutyl ketone layer. The organic layer was then aspirated into the flame of an AAS and the atomic absorbance of copper was measured at 324.7 nm or the absorbance of the organic layer was measured at 510 nm. The procedure has a detection limit of 0.05 mg/l, but has a narrow linear range (0.1 - 2.5 mg/l) which requires the dilution of samples with higher thiocyanate concentrations. The RSD of the measurements was 1.16% by

AAS and 1.18% by spectrophotometric detection. A drawback of this method is its narrow linear range and that it has to be operated manually.

An automated procedure was developed by Korenaga [30] using a thiocyanate ion-selective electrode with liquid membrane. Liquid membranes using long-chained quaternary ammonium salts as the exchange sites were prepared by using ion-association extraction systems and their performances were investigated for the analysis of thiocyanate. A tetradecyldimethylbenzylammonium cation was used as the exchange site in the liquid membrane using 1,2-dichloroethane. A disadvantage of this procedure was that the membrane potentials were not stable for extended periods of time. The calibration graph extended from 1.0 - 100.0 mg/ℓ which made it suitable for the determination of high levels of thiocyanate.

Mohammad and Chahar [13] reported the use of a manual TLC-colorimetric method for the determination of thiocyanate in photogenic waste, river and seawater. Thiocyanate was separated from the interfering ions by TLC and determined by spectrophotometry at 460 nm using acidic FeCl_3 solution as chromogenic reagent. Beer's law was followed up to 11.5 mg/ℓ thiocyanate. The relative standard deviation ranged from 5.42 - 10.70% for the samples studied. It is evident that though the proposed method is sensitive it is not very precise.

Ensafi *et al* [16] reported a manual procedure for the kinetic determination of thiocyanate based on the catalytic effect of thiocyanate on the oxidation of sodium azide by iodine at pH 5. The reaction was spectrophotometrically monitored at 348 nm (absorption maximum of iodine). The calibration

curve was linear in the range 1.0 - 2000.0 $\mu\text{g}/\ell$ with a detection limit of 0.3 $\mu\text{g}/\ell$. Measurements were very sensitive and precise with RSD's less than 1.90%. A few drawbacks of this procedure are the numerous interfering species and that it is manually operated.

Ensafi [14] adapted the kinetic procedure described above [16] for use in flow injection analysis. This made the procedure more useful as a process analyser. The procedure is very sensitive with the linear range extending over 5.0 - 1800.0 $\mu\text{g}/\ell$ and with a limit of detection of 0.6 $\mu\text{g}/\ell$. The sampling rate was ~ 40 samples per hour. The precision on the sample measurements was good with RSD's $< 2.50\%$.

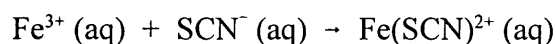
The iron(III) nitrate colorimetric procedure is one of the most rapid spectrophotometric methods available for the determination of thiocyanate. This method is straightforward since the reaction between sample and reagent leads to the immediate formation and detection of the coloured complex, compared with the more complex extraction procedure described by Stratis and Vasilikiotis [29]. A disadvantage of the iron(III) method is the colour instability and the wide range of interfering species. Jones and Hemmings [25] developed a flow injection analysis method for the iron(III) nitrate procedure, which effectively excluded any problems that might arise from the colour instability. The procedure was applied to the determination of thiocyanate over the range 10.0 - 200.0 mg/ℓ . Although the linear range is wider than the procedures described above, the proposed procedure is not very sensitive for small thiocyanate concentrations. The proposed method is precise with a relative standard deviation of 0.007 at a thiocyanate concentration of 12.5 mg/ℓ . A sampling rate of 60 samples per hour was achieved which is an improvement on the method discussed

above [14].

A disadvantage of the flow injection procedures described above is that it involves an enormous consumption of reagent since the reagent is pumped continuously.

5.4 Principle of the spectrophotometric determination

The spectrophotometric determination of thiocyanate is based on the reaction of thiocyanate ions in a moderately acidic medium with ferric ions to yield the red coloured $\text{Fe}(\text{SCN})^{2+}$ complex:



In general, thiocyanate will react with iron(III) to form a series of consecutive complexes represented by $[\text{Fe}(\text{SCN})_n]^{+3-n}$ where $n = 1-6$ [31]. The thiocyanate concentration will determine the number of thiocyanate molecules that will coordinate around each iron(III) atom and the specific complex that will predominate. Thiocyanate can be determined as the $\text{Fe}(\text{SCN})^{2+}$ complex when an excess of iron(III) is present [2]. The colour of the formed $\text{Fe}(\text{SCN})^{2+}$ complex is measured at 480 nm [25].

From the practical standpoint it is of significance that thiocyanic acid is highly ionized. This means that the thiocyanate method can be applied in mineral acid medium. Thiocyanic acid is one of very few organic reagents of the type HR forming complexes at high acidities. It is important that the

aqueous solution is sufficiently acidic to prevent appreciable hydrolysis of iron(III), which begins even below pH ~ 3.0 [2]. The solution should not however be too acidic otherwise the concentration of thiocyanate may be reduced by polymerization.

A factor requiring attention in the proposed thiocyanate method is the colour instability of the aqueous iron(III)-thiocyanate solutions. A change in colour intensity may result from the reducing properties of thiocyanate or its decomposition products (causing fading of the ferric thiocyanate colour) or, more often, from the slow decomposition of thiocyanate in acidic medium. The colour can fade by a few per cent in 30 minutes and by 50% in six hours [2]. It is thus important to control those parameters that can influence the colour instability namely, the time lapse before measurements are made and the acid concentration.

5.5 Determination of thiocyanate with sequential injection analysis

5.5.1 Experimental

5.5.1.1 Reagents and solutions

All reagents were prepared from analytical-reagent grade chemicals unless specified otherwise. All aqueous solutions were prepared using de-ionised water from a Modulab system (Continental Water Systems, San Antonio, Texas, USA). The de-ionised water used to prepare the aqueous solutions was degassed, by heating to boiling point and cooling before the solutions were made up with it.

Iron(III)-reagent solution: 3.5 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was dissolved in *ca.* 25 ml water. A 10 ml volume of 65% (m/m) HNO_3 was added to the iron solution after which it was diluted to 100 ml with water.

Thiocyanate solutions: A 1000.0 mg/l thiocyanate stock solution was prepared by dissolving 0.1311 g NH_4SCN in *ca.* 25 ml water and diluting the resulting solution to 100 ml. This solution was standardized by the Volhard method. A 50.0 mg/l thiocyanate working solution was prepared by appropriate dilution of the stock solution.

Carrier solution: A 0.36 mol/l HNO_3 carrier solution was prepared by diluting 50 ml of 65% (m/m) HNO_3 to 2.0 l.

5.5.1.2 Apparatus

The sequential injection system depicted in Fig. 5.3 was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-le-Bel, France); a 10-port electrically actuated selection valve (Model ECSD10P; Valco Instruments, Houston, Texas, USA); and a UNICAM 8625 UV-visible spectrometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell (volume 80 μl) for absorbance measurements.

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (Mintek, Randburg, South Africa). The FlowTEK [32] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition.

The wavelength of maximum absorbance was identified by scanning the $\text{Fe}(\text{SCN})^{2+}$ complex solution over the 300 to 800 nm range with a Spectronic Genesys 5 spectrophotometer (Milton Roy Company, USA). The optimum wavelength was chosen as 480 nm that corresponds to the wavelength used by previous authors conducting the same determination [25].

The results obtained by the proposed SIA system were verified with a Hewlett Packard 8453 diode array spectrometer (Germany). The diode array spectrometer was calibrated with thiocyanate standard solutions and the concentration of thiocyanate in the tap water samples, which contained

dilutions from the thiocyanate stock solution, were subsequently determined.

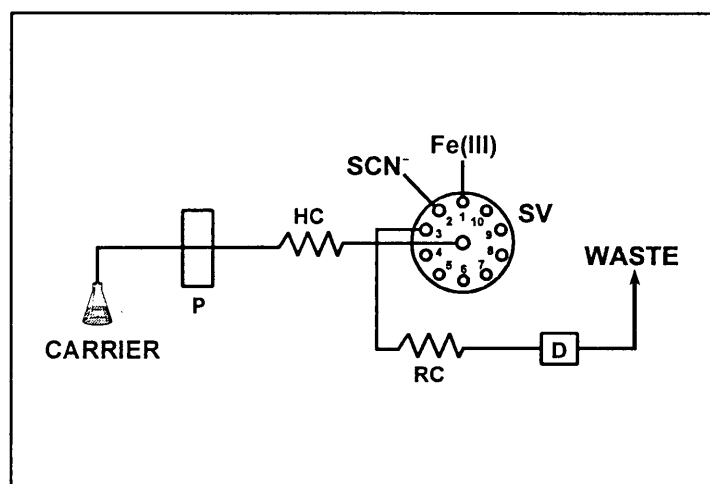


Fig. 5.3 A schematic diagram of the SIA system used for the determination of thiocyanate. P - pump, HC - holding coil, RC - reaction coil, SV - selection valve and D - detector.

5.5.1.3 Manifold

The components of the SIA system were arranged as illustrated in Fig. 5.3. The holding coil consisted of 1.00 m x 0.89 mm i.d. Tygon tubing and the reaction coil of 0.60 m x 0.89 mm i.d. Tygon tubing. A flow rate of 4.7 ml/min was used.

5.5.1.4 Procedure

The device sequence for the determination of thiocyanate is given in Table 5.3.

TABLE 5.3. Device sequence for one cycle of the sequential injection system

Time (s)	Pump	Valve	Description
0	Off	$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$	Pump off, select $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ stream (valve position 1)
5.0	Reverse		Draw up $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ solution
6.8	Off		Pump stop
7.8		NH_4SCN	Select NH_4SCN stream (valve position 2)
8.8	Reverse		Draw up NH_4SCN solution
9.7	Off		Pump stop
10.7		Detector	Select detector line (valve position 3)
11.7	Forward		Pump stack of zones to detector
150.0	Off	Home	Pump off, return valve to starting position (valve position 1)

5.5.2 Method optimization

5.5.2.1 Physical parameters

The physical parameters of an SIA system affect the degree of dispersion and the zone penetration to be obtained when the reagent and sample zones are propelled to the detector along the flow conduit. It is important to optimize these parameters (flow rate, sample and reagent volumes, holding coil length and i.d. and reaction coil length and i.d.) to obtain optimum mixing of the zones and deliver an appreciable amount of the detectable specie.

5.5.2.1.1 Flow rate

The reaction between iron(III) and thiocyanate is very fast, resulting in the immediate formation of the $\text{Fe}(\text{SCN})^{2+}$ complex. At high flow rates the time available for complex formation is much less than at the lower flow rates. The result is that an inadequate amount of $\text{Fe}(\text{SCN})^{2+}$ complex is formed for spectrophotometric detection at the high flow rates. That is why a decrease in sensitivity was observed for the higher flow rates (Fig. 5.4).

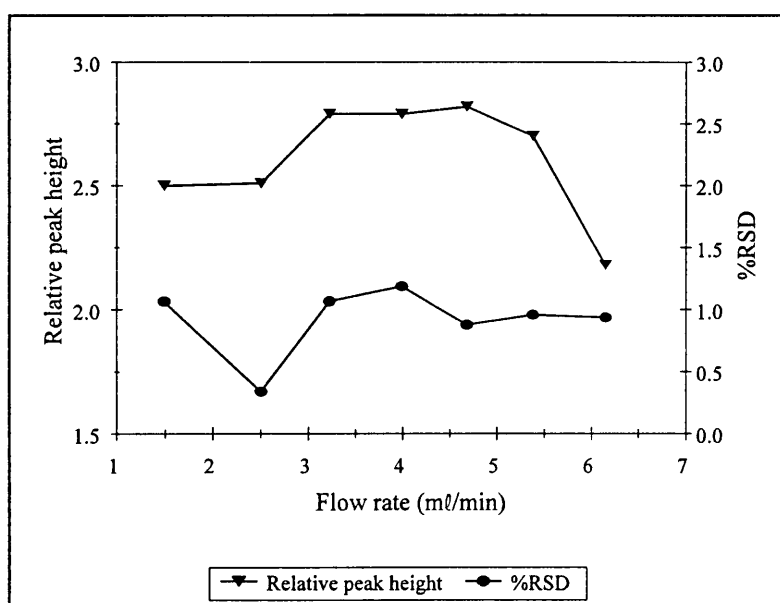


Fig. 5.4 Influence of flow rate on sensitivity and precision

The decrease in sensitivity observed below 3.0 ml/min is ascribed to the high degree of dispersion that characterizes low flow rates [33]. The dispersion decreases for higher flow rates which accounts for the increase in sensitivity at flow rates above 3.0 ml/min. Optimum sensitivity was obtained for

flow rates between 3.0 and 5.0 ml/min.

The optimum flow rate was chosen as 4.7 ml/min. A flow rate of 2.5 ml/min may have given better reproducibility than 4.7 ml/min, but it also caused a decrease in sensitivity and sample frequency compared to 4.7 ml/min. Then again, an increase in sample frequency is obtained for the higher flow rates but it is also characterized by decreased sensitivity. A flow rate of 4.7 ml/min is the best choice regarding the best sensitivity and sample frequency.

5.5.2.1.2 Sample volume

The sample volume was optimized by varying it for three fixed reagent volumes respectively. The effect of sample volume on the linear range of thiocyanate was studied. The aim was to obtain optimum sensitivity for the small thiocyanate concentrations including the widest linear range possible. The results are summarized in Table 5.4. For each fixed reagent volume, the sensitivity increased and the linear range decreased for the larger sample volumes. The decrease in linear range was due to the absorbance that became too high for the spectrophotometer to detect.

TABLE 5.4. Optimization of the sample volume for three fixed reagent volumes. The effect of the sample volume on the thiocyanate linear range is shown.

V(reagent) ($\mu\ell$)	V(sample) ($\mu\ell$)	Relative peak height			
		[SCN ⁻] (mg/l)			
		10	50	100	200
100	50	0.41	1.72	3.25	5.51
	100	0.51	2.43	4.41	7.40
	200	0.82	3.52	6.34	-
140	70	0.61	2.62	4.86	8.17
	140	0.82	3.95	6.83	-
	280	1.25	5.42	-	-
200	50	0.54	2.56	4.62	8.05
	100	0.84	3.74	6.72	-
	200	1.16	4.95	8.57	-
	400	1.70	6.74	-	-

The results that were obtained for the different fixed reagent volumes can be compared by choosing identical reagent : sample volume ratios. For a 2 : 1 reagent : sample volume ratio (e.g.), an increase in sensitivity was observed when increasing the reagent volume from 100 to 200 $\mu\ell$, for each of the respective thiocyanate standards. This increase was more pronounced for the higher thiocyanate concentrations. For the larger volume ratios much more reagent and sample was available to react, thus an increase in complex formation and sensitivity was obtained. Although larger volume ratios increase sensitivity, a disadvantage thereof, as well as the larger reagent volumes, is that it increases

the analysis time due to the time that is necessary to draw up the solutions and rinse the system. This effectively decreases the sampling frequency. The larger volume ratios also decreased the linear range.

An optimum sample volume was chosen by regarding the results which were obtained for a fixed reagent volume of 140 $\mu\ell$. A fixed reagent volume of 140 $\mu\ell$ gave the best results regarding a high sample frequency and a wide linear range with good sensitivity for the smaller thiocyanate concentrations, compared to the other fixed reagent volumes which were studied. The optimum sample volume was chosen as 70 $\mu\ell$.

5.5.2.1.3 Reagent volume

The reagent volume was optimized for an optimum sample volume of 70 $\mu\ell$. The results are shown in Table 5.5. For reagent volumes smaller than 140 $\mu\ell$ no response was obtained due to the inadequate overlapping of, and reaction between, the sample and reagent zones. An increase in sensitivity was observed for all the thiocyanate standard solutions when increasing the reagent volume from 140 to 210 $\mu\ell$ and a decrease in sensitivity from 210 to 280 $\mu\ell$. It is evident that an increase in reagent volume, which contains increasing amounts of reagent that could react, does not necessarily promote increased complex formation. The linear range also decreased with increasing reagent volume. A reagent volume of 140 $\mu\ell$ was chosen as optimum as it gave good sensitivity for the smaller thiocyanate concentrations and a wide linear range.

TABLE 5.5. Optimization of the reagent volume for a sample volume of 70 $\mu\ell$. The effect of the reagent volume on the thiocyanate linear range is shown.

V(reagent) ($\mu\ell$)	Relative peak height			
	[SCN ⁻] (mg/ ℓ)			
	10	50	100	200
140	0.66	2.75	5.27	8.35
210	0.82	3.40	5.97	-
280	0.63	3.28	5.95	-

5.5.2.1.4 Holding coil

The main function of the holding coil is to prevent the stacked zones, which are drawn up into the holding coil before it is propelled to the detector, from being drawn up into the pump tubing. This is achieved by maintaining a large enough holding coil volume. The holding coil dimensions, internal diameter and length (which determine the holding coil volume), affects the dispersion of the sample and reagent zones to a certain extent [33]. If the holding coil volume is not large enough to house the stacked zones, and the zones are drawn up into the pump tubing by accident, deformation of the zones could take place.

The effect of internal diameter on sensitivity and reproducibility was studied by keeping the volume of the holding coil constant and changing the internal diameter. Tube diameters between 0.76 and 1.60 mm were studied. The results are depicted in Table 5.6. The effect of internal diameter on sensitivity is negligible since there is not much change in peak height for different internal diameters.

The reproducibility decreased for the larger internal diameters with 0.76 mm giving the best results. The optimum internal diameter was chosen as 0.89 mm since a lot of back pressure was experienced in the flow conduit for the 0.76 mm internal diameter.

TABLE 5.6. Effect of holding coil internal diameter on sensitivity and precision.

HC internal diameter (mm)	Relative peak height	%RSD
0.76	2.99	0.30
0.89	3.07	1.37
1.14	2.99	1.55
1.60	2.97	1.53

The holding coil length was studied at the optimum holding coil internal diameter. Holding coil lengths between 0.75 and 1.50 m were studied (Fig. 5.5). A decrease in sensitivity was observed for longer holding coils with the best reproducibility being obtained for holding coil lengths between 1.00 and 1.25 m. A holding coil length of 1.00 m was chosen as optimum since the best sensitivity and reproducibility was obtained for it.

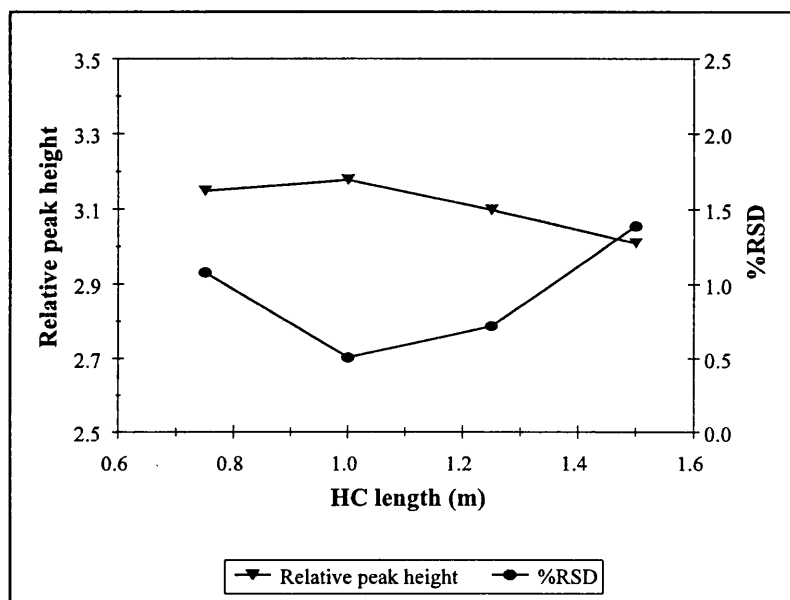


Fig. 5.5 Influence of holding coil length on sensitivity and precision

5.5.2.1.5 Reaction coil

The reaction coil has a more pronounced effect on the reagent and sample zone dispersion than the holding coil. The zones are propelled via the reaction coil to the detector and the reaction coil dimensions, internal diameter and length, must be chosen to minimise dispersion of the formed product zone and to deliver optimum sensitivity and reproducibility. Dispersion should be minimised in the reaction coil since the reaction between sample and reagent has reached completion by the time it reaches the reaction coil. By minimising the dispersion, sensitivity can be optimized. The dispersion can be minimised through a short reaction coil length and a small internal diameter [33]

The reaction coil internal diameter was studied by keeping the volume of reaction coil constant and changing the internal diameter. Tube diameters between 0.64 and 1.14 mm were evaluated. The results are given in Fig. 5.6. As expected, the sensitivity decreased for larger internal diameters. This decrease was due to the frictional effect of the tubing walls. The frictional effect decreases with larger internal diameters with the result that axial dispersion is reduced [34]. This contributes to the decrease in sensitivity. An internal diameter of 0.89 mm was chosen as optimum due to good sensitivity and the best reproducibility obtained.

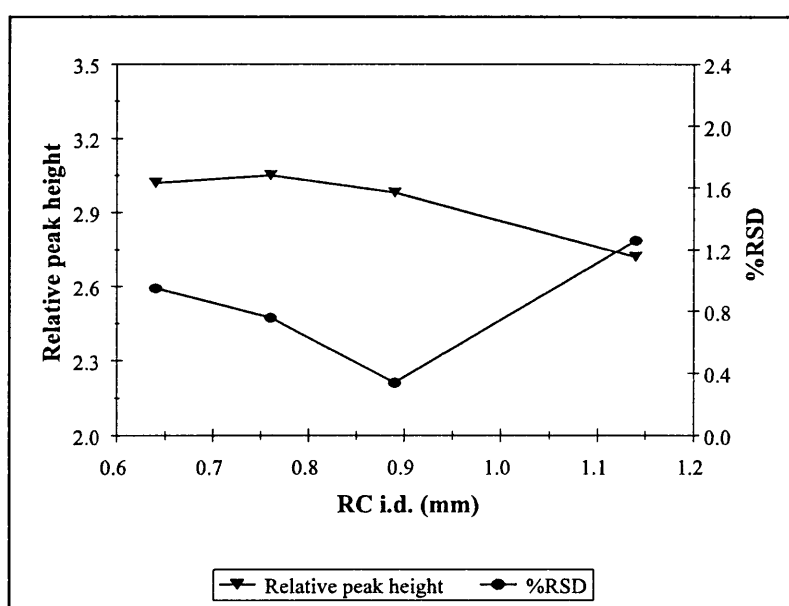


Fig. 5.6 Influence of reaction coil internal diameter on sensitivity and precision.

The length of the reaction coil determines the amount of dispersion that the sample and reagent zones will undergo on the way to the detector. Table 5.7 depicts the change in sensitivity for different reaction coil lengths. Line lengths between 0.40 and 1.00 m were evaluated. The longer

the length of the reaction coil, the higher the dispersion of the zones will be [33]. This is confirmed by the results as a decrease in sensitivity was observed for the longer reaction coil lengths due to increased dispersion. A reaction coil length of 0.60 m gave good sensitivity and the best reproducibility and was chosen as the optimum.

TABLE 5.7. Effect of reaction coil length on sensitivity and precision.

RC length (m)	Relative peak height	%RSD
0.40	2.94	0.91
0.50	3.11	1.02
0.60	2.93	0.84
0.80	2.64	1.12
1.00	2.67	1.26

Although a length of 0.40 m also delivers good sensitivity and reproducibility, the practical implication thereof should be considered. The selection valve and detector are connected by the reaction coil. If the reaction coil is too short, it would be difficult to connect these two devices. A reaction coil length of 0.40 m is on the verge of being too short for its intended purpose. Thus, the 0.60 m length was chosen.

5.5.2.2 Chemical parameters

5.5.2.2.1 Reagent concentration ($Fe(NO_3)_3 \cdot 9H_2O$)

The reagent concentration affects the amount of detectable specie that will be formed. It is necessary to optimize the reagent concentration to achieve optimum sensitivity on especially the small thiocyanate concentrations that are difficult to detect.

The reagent concentration was optimized by studying its effect on the thiocyanate linear range. Reagent concentrations ranging from 10 - 75 g/l was evaluated. An increase in sensitivity was observed for increased reagent concentrations (Table 5.8). The increase in sensitivity with increasing reagent concentration was more pronounced for the higher thiocyanate concentrations due to the excess of sample that was available to react with the reagent, compared with the limited amount of sample available at the lower thiocyanate concentrations.

TABLE 5.8. The effect of the reagent ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) concentration on the thiocyanate linear range.

[Reagent] (g/l)	Relative peak height				
	[SCN ⁻] (mg/l)				
	10	50	100	150	200
10	0.32	1.71	3.20	4.55	5.91
20	0.50	2.34	4.41	6.31	7.50
35	0.59	2.90	5.16	7.30	9.28
50	0.63	2.89	5.49	7.94	-
75	0.61	3.16	5.86	8.66	-

A decrease in the thiocyanate linear range was also observed for the higher reagent concentrations, due to the absorbance of the coloured complex that became too high for the spectrophotometer to detect. A 35 g/l reagent concentration, which gave the widest thiocyanate linear range and the best sensitivity for this linear range, was chosen as optimum. Maintaining an excess of iron(III) is important to assure the formation of the $\text{Fe}(\text{SCN})^{2+}$ complex specifically.

5.5.2.2.2 Nitric acid concentration

The effect of the nitric acid concentration was studied by changing the concentration thereof in the reagent solution and the carrier stream. Four acid concentrations were studied: 0.36, 1.44, 2.88 and 5.76 mol/l. For each of the four carrier stream acid concentrations, four reagent solutions with the four different acid concentrations mentioned, were studied. In total sixteen different combinations were studied. The results are summarized in Table 5.9.

For each respective reagent solution a decrease in sensitivity was observed for higher carrier stream acid concentrations. A possible explanation for this decrease is the slow decomposition of thiocyanate in acidic medium. This decomposition, usually described as a polymerization of thiocyanic acid, results in the formation of yellow products, especially in fairly strong acidic solutions [31]. The conclusion that can be drawn from this explanation is that the stronger the acidic solution is, the faster the decomposition of thiocyanate will be, thus reducing the amount of coloured complex that will be formed and subsequently detected.

TABLE 5.9. Effect of the nitric acid concentration, in the reagent solution and carrier stream respectively, on sensitivity.

		Relative peak height			
		[HNO ₃] in carrier stream (mol/l)			
		0.36	1.44	2.88	5.76
[HNO ₃] in reagent solution (mol/l)	0.36	2.77	2.34	1.95	1.36
	1.44	2.76	2.45	2.19	1.75
	2.88	2.75	2.46	2.29	1.95
	5.76	2.75	2.66	2.43	2.17

When keeping the carrier stream concentration constant, an increase in sensitivity was observed for the higher reagent solution acid concentrations. This increase was more pronounced for the higher carrier stream concentrations and negligible for the small carrier stream concentrations.

A 0.36 mol/ℓ carrier stream concentration gave the best sensitivity for the iron(III) and thiocyanate complexation reaction. This concentration is also the better choice when considering the physical aspects of the flow conduit. High acid concentrations can attack the Tygon tubing thus reducing its life span.

Since the difference in sensitivity between the reagent solutions were negligible for a 0.36 mol/ℓ carrier stream concentration, it did not really matter upon which reagent acid concentration was decided. A 1.44 mol/ℓ reagent acid concentration was chosen as optimum. If the acid concentration is too low, the reduction of iron(III) cannot be effectively prevented, and a too high acid concentration can reduce the rate of complex formation.

5.5.3. Method evaluation

The proposed SIA system was critically evaluated with regard to linearity, accuracy, precision, detection limit, sample interaction (carry-over), sampling frequency and interferences.

5.5.3.1 Linearity

The response of the proposed SIA system for the spectrophotometric determination of thiocyanate was evaluated under optimum conditions, shown in Table 5.10.

TABLE 5.10. Optimum SIA conditions

Operational parameter	Dimension
Flow rate	4.7 ml/min
Sample volume	70 µl
Reagent volume	140 µl
HC i.d.	0.89 mm
HC length	1.00 m
RC i.d.	0.89 mm
RC length	0.60 m
[Fe(NO ₃) ₃ ·9H ₂ O]	35 g/l
[HNO ₃] in carrier stream	0.36 mol/l
[HNO ₃] in reagent solution	1.44 mol/l

The following relationship was obtained for relative peak height versus thiocyanate ion concentration:

$$y = 0.04934x + 0.02138; r = 0.9993$$

where y = relative peak height, x = thiocyanate ion concentration in mg/l and r = correlation coefficient. A linear relationship, indicated by r, between relative peak height and thiocyanate ion concentration was obtained from 2.0 to 150.0 mg/l.

5.5.3.2 Accuracy

The proposed SIA system was evaluated with regard to accuracy by analysing seven tap water samples. These samples were analysed with the SIA system and a diode array spectrometer respectively. The results obtained for the two systems are shown in Table 5.11. There is a good correlation between the results obtained by the SIA system and the diode array spectrometer. It can be concluded that the proposed SIA system delivers accurate results with regard to the analysis of samples.

TABLE 5.11. Comparison of results obtained by the proposed SIA system and diode array.

Sample	Diode array (mg/l)	SIA (mg/l)
A	6.4	6.9
B	28.7	30.4
C	67.7	69.9
D	89.0	95.1
E	106.8	107.9
F	116.5	121.8
G	132.6	130.4

5.5.3.3 Precision

The precision was evaluated by considering the calculated percentage relative standard deviation (%RSD) of the measurements that were obtained for the samples and standards respectively. The precision was determined by 10 repetitions of the standard and sample solutions in the linear range of the proposed SIA method. The results are shown in Table 5.12. In all cases the %RSD was < 1.20%, except thiocyanate concentrations below 10.0 mg/ℓ (an explanation follows). Since the relative peak height is very small at analyte levels below 10.0 mg/ℓ (< 0.5 compared with a relative peak height of ~ 2.6 for a 50.0 mg/ℓ thiocyanate standard solution), a small change in relative peak height delivers a high %RSD compared with a small change in relative peak height for higher analyte levels.

The relatively low %RSD obtained for the proposed method, indicates good reproducibility and compares well to [16,25] and even better than [13,14] some of the methods that have already been developed for the determination of thiocyanate.

TABLE 5.12. Precision of the proposed SIA system

Standard/Sample ([] in mg/l)	%RSD
2	6.55
5	5.78
10	0.42
50	1.13
100	0.61
150	0.36
Sample A	3.30
Sample B	0.80
Sample C	0.71
Sample D	0.35
Sample E	0.45
Sample F	0.49
Sample G	0.38

5.5.3.4 Detection limit

The detection limit gives an indication of the lowest thiocyanate concentration that can be distinguished from the background signal with absolute certainty. The detection limit is calculated by using the following formula:

$$\text{Detection limit} = \frac{(3 s_b + I_b) - k}{m}$$

where: s_b = standard deviation of the background signal
 I_b = relative peak height of the background signal
 k = intercept of the calibration curve for the proposed SIA method
 m = slope of the calibration curve for the proposed SIA method

The detection limit for the proposed SIA system was calculated to be 1.1 mg/l SCN⁻.

5.5.3.5 Sample interaction

Sample interaction is investigated to determine whether carry-over takes place between successive samples. This feature is characteristic of systems which are not effectively rinsed after each experimental run. Rinsing is an important part of the experimental procedure since it prevents any sample and reagent from remaining in the flow system that can affect the outcome of the following experiment. Reagents and samples that can adhere to the tubing of the flow conduit and could be washed out in later experiments or the precipitate of a turbidimetric procedure are a few of the possible causes of carry-over.

The sample interaction was determined by using the following equation:

$$Interaction = \frac{(A_3 - A_1)}{A_2} \times 100$$

where: A_1 = relative peak height of a sample containing a low thiocyanate concentration (10.0 mg/l),
 A_2 = relative peak height of a sample containing ten times more thiocyanate (100.0 mg/l), and
 A_3 = relative peak height of a sample containing the same amount of thiocyanate as A_1 .

The sample interaction between samples was calculated to be 0.8%, which is negligible. The small interaction between samples is attributed to the effective rinsing of the flow conduit after each experimental run.

5.5.3.6 Sample frequency

It took 150 seconds to complete a single experimental run which means that 24 samples can be processed per hour.

5.5.3.7 Interferences

The practicality of a procedure is evaluated by its ability to function in the presence of interferences. A few species are known to interfere in the determination of thiocyanate by the iron(III) procedure [7]. An important feature of this method is the non-interference of cyanide. This makes the proposed SIA procedure ideally suited for application in hydro metallurgical solutions. Certain

metals (e.g. Mn, Zn, Cu, Bi, Ti, Hg(II)) interfere due to its complexation with thiocyanate. Judging from the high stability constant of the iron(III)-thiocyanate complex compared with the thiocyanate complexes of other metals, the reaction of thiocyanate with Fe(III) is very selective [35]. Although Ag(I), Au(I) and Hg(II) form more stable complexes with thiocyanate than Fe(III) [35], they are not common constituents of water streams. Problems arise when appreciable amounts of the interfering metals are present. It is then when thiocyanate does not react selectively with Fe(III) and form other thiocyanate complexes that affect the colour of and ease of complex formation of the detectable specie.

Iron(III) on the other hand is also known to form stable complexes with certain anions (e.g. fluoride, oxalate, sulphate and pyrophosphate) in acidic solution [31]. When appreciable amounts of these anions are present interference may also be experienced. Addition of a miscible organic solvent with a low dielectric constant (water-acetone solution) has shown to reduce interference from such ions as fluoride and oxalate [31].

Metal-cyanide complexes are common constituents of hydro metallurgical solutions and have also been shown to interfere in the determination of thiocyanate [25]. The metal-cyanide complexes that seem to have the largest influence on the iron(III)-thiocyanate determination are: $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Fe}(\text{CN})_6^{3-}$ [25]. Fluoride, sulphate and oxalate were also tested though they are not commonly found in process solutions. The results of the interference study are highlighted in Table 5.13.

TABLE 5.13. Interference from foreign ion species studied for a 8.61×10^{-4} mol/ℓ (50.0 mg/ℓ) thiocyanate solution. The ratio of foreign ion to thiocyanate is indicated as x:1. The mol ratio is indicated.

Tolerance ratio of foreign species (x)	Foreign specie
1000	Ni^{2+} , SO_4^{2-}
700	Zn^{2+}
300	Mn^{2+} , $\text{C}_2\text{O}_4^{2-}$
50	Cu^{2+}
30	F^-
0.66	$\text{Fe}(\text{CN})_6^{3-}$
0.44	$\text{Fe}(\text{CN})_6^{4-}$

Interference from the $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Fe}(\text{CN})_6^{3-}$ complexes at ratios of 0.44 and 0.66 (these ratios correspond to 80.0 and 120.0 mg/ℓ respectively) are acceptable since the concentrations of these metal complexes are generally below a ratio of 0.55 (100.0 mg/ℓ) depending on the composition of the hydro metallurgical solutions [25]. The large interference of fluoride is ascribed to strong complex formation with Fe(III) and of Cu(II) due to its interaction with thiocyanate. Interferences from Ni(II), sulphate, Zn(II), Mn(II) and oxalate is negligible at the levels that were studied.

5.6 Conclusion

Sequential injection analysis was successfully applied to the determination of thiocyanate in water samples. The simplicity of the manifold makes it ideally suited for use as an on-line analyser for the monitoring of thiocyanate in effluent streams and hence the control of environmental pollution. The system is robust, simple to operate and capable of rapid automatic analysis when computer controlled. The calibration curve is linear from 2.0 - 150.0 mg/ℓ with a detection limit of 1.1 mg/ℓ. The system processes 24 samples per hour with a relative standard deviation of < 1.20% for samples and standards with thiocyanate concentrations above 10.0 mg/ℓ. An increase in RSD was observed for thiocyanate concentrations below 10.0 mg/ℓ. This was ascribed to the difficulty in obtaining reproducible results at very low analyte levels.

5.7 References

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CHAPTER 6

Conclusions

The importance of developing process analysers capable of on-line monitoring with a high sample throughput, minimum sample and reagent consumption that are robust, reliable and requires a low frequency of maintenance has become very evident through the course of this work. FIA was only moderately successful in complying with these strict requirements and there were still certain aspects that needed improvement. FIA manifolds require physical manipulation of the manifold when changing from one configuration to another. Very often multiple lines are required that makes the system inconvenient to operate and more prone to failures. Simply changing a chemistry can be time consuming.

The introduction of SIA was an attempt to make injection techniques more rugged for process control application. This single-line system that is completely computer controlled, can be configured to perform most operations of conventional flow injection analyses with no or minimal physical reconfiguration of the manifold. This system contributes considerably to reducing reagent and sample consumption which is one of its greatest attributes. The simplicity of the SIA system is attributed to the multi-port selection valve by which the samples, reagents, reaction lines and detector line are accessed.

It has been shown that there are various parameters that influences the results that flow systems deliver. The importance of controlling these parameters is important in the development of sensitive and precise flow systems. In FIA, where attaining a certain degree of dispersion is of prime importance, the system parameters must be carefully controlled. High sensitivity is obtained by minimising the dispersion in the flow manifold, but it is also important to achieve an adequate amount of dispersion so that the sample zone can react effectively with the surrounding reagent. It has though been indicated that for SIA zone penetration plays an important role in the sample and reagent interaction. Those aspects that affect zone penetration has been thoroughly studied to lay the foundation for the development of other SIA systems.

The development of SIA systems is aimed at providing the industrial, agricultural, clinical and pharmaceutical fields with reliable, precise and cost-effective instrumentation for performing the analyses they require. The increasing awareness regarding environmental pollution and the regulation of the effluents that are released into the environment, places an enormous responsibility on analysts to develop methods that can be effectively applied to determining the actual amount of the polluting elements that are discharged.

In this study the various system parameters that characterises the SIA system were evaluated with regard to their influence on the amount of dispersion to be attained in the system. Although this has been studied previously to a certain extent, there were various aspects that needed more attention. The parameters that were neglected in the previous studies were identified and together with the known ones, the parameters were studied to give in depth information regarding their effect on dispersion in an SIA system. Although dispersion only partially contributes to the success of the SIA analysis, in addition to zone penetration, it does though have an important

effect on the amount of zone penetration to be attained.

The theoretical study was followed by the application of SIA to real systems. By applying SIA to real systems, its usefulness as a process analyser becomes more evident. Two elements, copper(II) and thiocyanate, were identified regarding their potentially hazardous influence on the environment. The determination of these elements had not been attempted previously by SIA and the studies were subsequently undertaken.

Several methods have been developed for the determination of copper(II), but most of them include the extraction of a complex of copper(II). The system that was developed in this instance was based on the spectrophotometric determination of the complex formed between copper(II) and diethyldithiocarbamate. This determination was done both in the presence and absence of interferences depending on the type of sample that was to be analysed. The application of flow reversals to increase the performance of the masking agents were also effectively illustrated. Although not as sensitive as previously developed methods, the proposed system is accurate and precise and offers considerable advantages with regard to its application as a process analyser.

The determination of thiocyanate is based on the well known reaction with the iron(III) reagent. The proposed method is especially advantageous with regard to the reduction in the consumption of reagent compared with the method developed for FIA where the reagent is pumped continuously. The influence of various physical and chemical parameters on both the sensitivity and linear range of the thiocyanate concentration is clearly illustrated.

The proposed methods clearly indicate that SIA can be successfully applied to the monitoring of certain selected components. Despite the disadvantages that have been mentioned to discard SIA as practical in the process environment, it has excelled and proven to be just as reliable and useful as many of its predecessors.

APPENDIX A

APPENDIX A

Publications and Presentations

Publications

1. J. F. van Staden and A. Botha, *Evaluation of the Operational Parameters Affecting Dispersion in Sequential Injection Analysis*, **S. Afr. J. Chem.**, **51(2)** 1998 100.
2. J. F. van Staden and A. Botha, *Spectrophotometric Determination of Cu(II) with Sequential Injection Analysis*, **Talanta**, In Press, (1999).
3. J. F. van Staden and A. Botha, *Spectrophotometric Determination of Thiocyanate with Sequential Injection Analysis*, Submitted to **Anal. Chim. Acta**, (1999).

Presentations

1. Spectrophotometric determination of Cu(II) with sequential injection analysis
A. Botha
Young Chemists Symposium: The Gauteng South Section of the South African Chemical Institute. Johannesburg. South Africa. 26 June 1998.
2. Spectrophotometric determination of Cu(II) with sequential injection analysis.
A. Botha and J. F. van Staden.

7th International Chemistry Conference in Africa and 34th Convention of the South African Chemical Institute. Durban. South Africa. 6 - 10 July 1998.

3. Spectrophotometric determination of Cu(II) with sequential injection analysis.

A. Botha and **J. F. van Staden.**

ICFIA 98. Ninth International Conference on Flow Injection Analysis. Seattle. Washington. USA. 23-27 August 1998.

4. Spectrophotometric determination of Cu(II) with sequential injection analysis.

A. Botha and J. F. van Staden.

Euroanalysis X. Working Party on Analytical Chemistry of the Federation of European Chemical Societies and the New Swiss Chemical Society. Basel. Switzerland. 6 - 11 September 1998.

5. Spectrophotometric determination of Cu(II) with sequential injection analysis.

J. F. van Staden and A. Botha.

SCAR'98 XIVth National Conference on Analytical Chemistry. Division of Analytical Chemistry of the Federation of European Chemical Societies and the Romanian Society of Analytical Chemistry. Piatra Neamt. Romania. 24-26 September 1998.

6. Spectrophotometric determination of thiocyanate with sequential injection analysis.

J. F. van Staden and A. Botha.

SCAR'98 XIVth National Conference on Analytical Chemistry. Division of

Analytical Chemistry of the Federation of European Chemical Societies and the Romanian Society of Analytical Chemistry. Piatra Neamt. Romania. 24-26 September 1998.

7. Spectrophotometric determination of Cu(II) with sequential injection analysis.

A. Botha and J. F. van Staden.

ANALYTICA '98. Third national symposium on analytical science. S A Chemical Institute. Midrand. 12 -14 October 1998.