# Application of sequential injection dialysis systems in the assay of food and fertilizer products

by

**Supi Isaiah Tlowana**

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**Supi Isaiah Tlowana**

**Supervisor: Professor Jacobus F. van Staden**

**Department of Chemistry**

**University of Pretoria**

**Pretoria**

**Degree: Magister Scientiae**

## Synopsis

Sequential injection analysis (SIA) is a stopped flow technique developed in 1990 on the basis of flow injection analysis (FIA). SIA is based on the reproducible sample injection, controlled dispersion, and accurate timing. The control of variables makes this technique very versatile and can be automated. Samples can be easily modified by introducing various sample modifying techniques in the SIA system, such as analyte preconcentration, sample dilution and analyte separation. Passive

dialysis is one of the techniques based on the barrier separation process. In this technique unwanted solutes and impermeable colloidal matter are rejected. The separation barrier is a neutral semipermeable membrane. The theory of dialysis, membranes and membrane processes are discussed. The study of movement of ions, in solution and across the passive membrane is given. The following factors influencing the efficiency of passive dialysis in the SIA system were discussed. The flow rate of the donor and the acceptor channels, volume of an undialysed sample, dialysis time, time for a single flow reversal during agitation. The systems that were developed are the following: The determinations of chloride in drinking, mineral and ground waters, chloride in milk, zinc in fertilizers and total acidity in fruit juices. The study of dialysis is also given.

# Toepassing van sekwensiële inspuiting dialisesisteme in die essaiëring van voedsel en kunsmis produkte

deur

Supi Isaiah Tlowana

Studieleier: Professor Jacobus F. van Staden

Department Chemie

Universiteit van Pretoria

Pretoria

Graad: Magister Scientiae

## Samevatting

Sekwensiële inspuitanalise (SIA) is 'n stop vloeitegniek wat in 1990 ontwikkel is en gebaseer is op vloei inspuitanalise (VIA). SIA is gebaseer op die herhaalbare inspuit van 'n monster, beheerde dispersie en akkurate tydsbereking. Die beheer van veranderlikes maak die tegniek veelsydig en kan

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# Contents

# CHAPTER 1

# Introduction to sequential injection analysis

## 1.1 Introduction

Current advances in analytical chemistry rely on complex techniques as well as on sophisticated instruments. However the purchase, maintenance cost and difficult operation of which makes them inaccessible to numerous scientists and quality control laboratories. In fact this type of innovation can often only be tackled by teams of electronic and mechanical engineers, who are only affordable by large commercial firms. Flow injection analysis (FIA) is characterised by its simple basis, relatively less expensive equipment, handy operation, rapidity, and precision. The technique can be readily adopted to meet any type of requirement without introduction of complex technical changes. The term flow injection analysis was coined by Ruzicka and Hansen [1] in 1975. The technique was introduced due to the ever increasing demand for analysis in clinical, agricultural, pharmaceutical, industrial, and process analytical control.

A dramatic decrease in reagent consumption and design of more effective flow injection systems has resulted from the introduction of flow programming, first as stopped flow and then followed by the flow reversal and recently as sinusoidal flow. The reduction of reagent consumption and programmed flow lead to the development of sequential injection analysis (SIA). An SIA system fulfilling these requirements has been developed [2]. In principle each measurement starts with the aspiration of a wash solution, followed by aspiration of the sample, reagent aspiration and the

measurement of the analytical profile. In this fashion, well-defined zones are aspirated sequentially into the conduit that serves as a holding reservoir, and by reversing the flow the zones passed through a conduit that serves as a reactor where they mutually disperse and react to form a detectable species that is eventually detected in a flow-through detector. The measurement cycle is terminated after the reversal and dispersion of the zones and propelled back through the system and finally to the waste reservoir [2].

Although originally FIA was devoted for the analysis of agricultural, samples, its application in clinical chemistry was also widely accepted. Unlike other assays (i.e. plant and soil), the representative samples for clinical materials are homogenous (i.e. blood serum and urine). The nature of the samples imposes serious restrictions such as: -

a) The amount of sample available is always limited,

b) The samples usually differs from the standards used for the calibration, thus introducing an error inherent to the analytical system,

c) A dialysis or dilution process is employed to avoid interferences from proteins and other large molecules or to accommodate the signal into the detector range,

d) Fats and proteins tend to deposit into the walls of the conduits.

The earliest application of liquid dialysis in flow systems was reported by Hansen and Ruzicka [3] for the determination of inorganic phosphates using an FIA system with a dialyser incorporated into the tubes of the system. It is also possible to incorporate a dialysis chamber into the conduits of an SIA system for the analysis of clinical, pharmaceutical and agricultural samples which are turbid and contain many undissolved substances in their matrices.

## 1.2 Basic components of sequential injection analysis with a single dialyser

Dialysis is a valuable tool that fulfills a very important role for on-line separation as part of the sequential injection system. It is very attractive, because of its simplicity, repeatability reliability, compatibility, robustness, requires little or no pretreatment of the sample and shows no interferences from sample colour and turbidity [4].

This type of sequential injection system (Fig 1.1), consists of the following components, two pumps, two selection valves, a flow through detector, and a minichamber fitted with a dialysis membrane. The minichamber usually consists of the inlet for the donor stream and the outlet for the acceptor stream. The first pump and the valve are usually used to aspirate and propel the naturally occurring sample into the donor stream at a certain flow rate. The second pump and the valve are used to aspirate the dialysed sample from the acceptor stream into the holding coil. The selection valve switches from one port to another with the pump alternately aspirating the different reagent at different ports into the holding coil to setup the programmed flow and sequence of the reagents and analyte. The valve then selects the detector channel and the pump propels the stack of zones to the detector and eventually to waste.

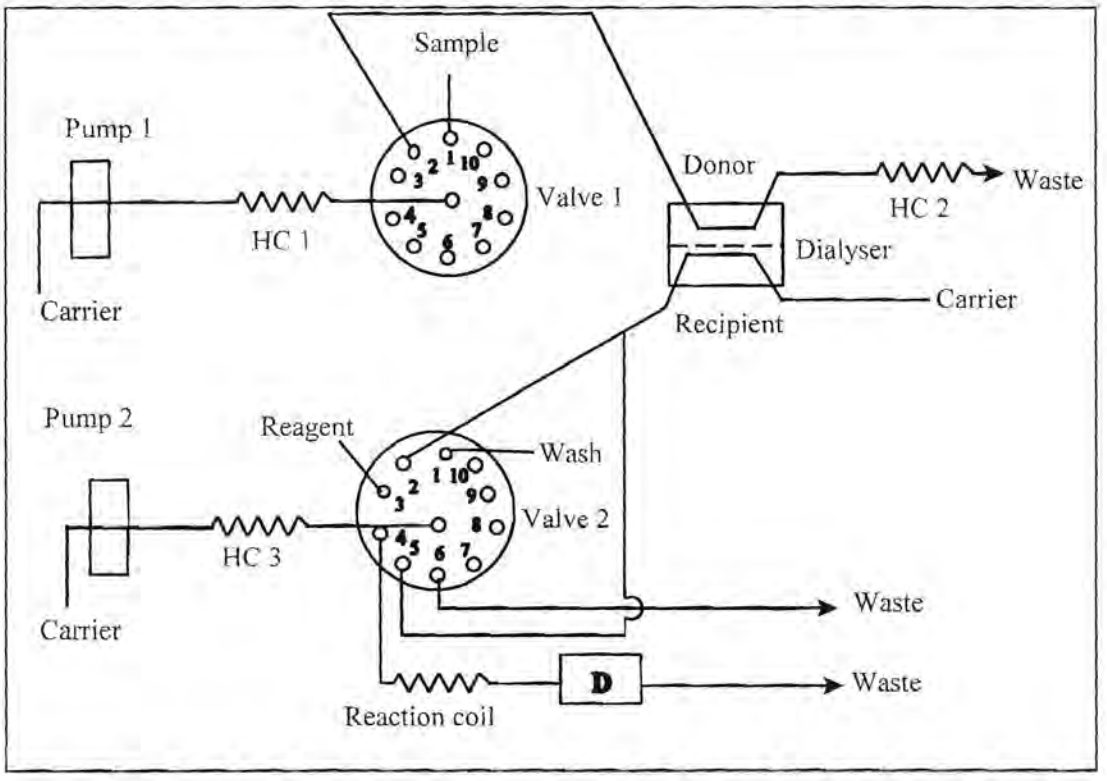


**Fig 1.1** Schematic diagram of a basic SIA system with an incorporated on-line dialyser, D=detector and HC = holding coil.

Unlike FIA, as stated before [2], sequential injection (SI) uses a selection (rather than an injection) valve through which precisely measured volumes of samples and reagent solutions are aspirated in the conduit that act as a holding reservoir alternating with the pump, which is capable of a controlled stop-go-forward-reverse movement.

The major difference of FI and SI is their dispersion pattern, which are observed in the channels of the two techniques. During the flow injection (FI) dispersion pattern, the injected sample zone flow past the confluent point where the regent and the carrier stream carrying the sample merge synchronously and an equal volume of reagent solution is added to each element of the passing carrier stream. The result is a concentration gradient of an analyte within the constant background

of the reagent solution. This type of dispersion pattern is totally different to the one that occurs in SI, where the multiport selection valve is used to place the zones sequentially into the holding conduit. The valve cannot serve as a confluent point because it connects two channels at a time. Initially a sharp boundary between adjacent zones is formed and therefore only partial overlap of analyte and reagent peaks are possible. There is a limitation of the number of zones that can be mixed in the flow reversal. When many reagents need to be added, and when very intensive dilution is needed [2] the use of a mixing chamber will be a good alternative.

In SIA once the manifold is set and optimised there is: -

i) Less servicing of the devices,

ii) less calibration of the system,

iii) no limit of the solutions (samples, reagents and standards) or devices (pumps, mixing chambers, dialyzers and detectors) that can be incorporated in to the manifold,

iv) no need for change of the physical configuration of the flow path,

v) fully computer compatible, and

vi) relatively less expensive devices are used.

The major drawback of SI is low sampling frequency compared to the conventional FI systems. This is because in the former technique the aspiration of the solutions and the sequencing or the programming of the flow takes some time whereas in the latter the filling of the injection valve is a matter of a few seconds [5].

## 1.3 Aims of the study

Automation is one of the key concepts for the development of tools to streamline the analytical procedure. Sequential injection analysis lends itself to a great extent to be automated. One of the means that SIA was made more streamlined is the introduction of tools for on-line sample preparation. The tools of interest include the incorporation of a dialyser unit equipped with a passive neutral membrane into the conduits of the SI system for sample dilution and clean up.

By introducing a passive membrane into a dialyser unit one should be able to increase the mass transfer of the analyte across the membrane, and by introducing the concept of multiple flow reversals a massive increase in the percentage dialysis can be expected and the sample clean up can be enhanced. It will be possible to remove the interfering species to a great extent if there is a mass difference between the analyte and the interfering species present in the sample matrix.

Since the proposed dialysis system will operate on-line in the SIA system, automation will be easily introduced. Systems satisfying the above requirements were assembled and tested on the analysis of chloride in milk, zinc in fertilizers and total acidity in fruit juices.

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# Chapter 2

# Historical background, basic principles and the essential parameters in SIA

## 2.1 Introduction

Since sophisticated instrumentation of laboratories facilities are unlikely to be suitable for manufacturing environments, dedicated systems offering long term dependability must be developed. The demand for the simple, rapid, robust and less expensive flow-injection methodology has been the driving force behind the development and exploitation of the SI technique. This made SIA to be seen as a new generation of FIA [1]. SIA is based on the same principle as FIA, i.e. controlled dispersion, reproducible aspiration of the sample, and exact timing of different operations. The ease of sample handling make SIA more versatile than FIA. The presence of digitally controlled pump and a multiport selection valve into the SIA manifold enhance the versatility of the system. Timing has to be accurate, precise and easily adjustable and therefore the system requires an interfaced personal computer. The other additional devices like tubing, the detectors, dialyser, etc. are the same as in FIA [2].

The simplicity of the SI manifold and its low need for maintenance makes it ideal for process analysis. Most SIA methods are designed to deliver analytical results for just one analyte at a time, but multiple analyses are feasible. SIA is suited for kinetic determinations due its discontinuous nature [3].

## 2.2 Historical Background

### 2.2.1 What is SIA?

Sequential injection analysis SIA since it was conceived in 1990 from its mother technique, FIA, is a simple and convenient concept of flow analysis. To understand where SIA fits in, it is best to start at the very beginning.

FIA is an analytical technique that is based on injecting a known volume of a sample, with a geometrically well defined shape, into a moving unidirectional carrier or reagent stream. In this moving stream the sample undergoes physical and chemical transformation until a detectable species causes a detector response down the stream of the injection point. If all critical parameters (reproducible sample injection, reaction time and dispersion) are controlled and held within certain tolerance levels the results of the analysis will be reproducible.

The instrumentation needed for the FIA system are, a multichannel pump, an injection valve, a flow through detector and a recorder. The recorder was replaced when the computers became mostly used in the laboratories. The basic flow technique remained basically unchanged.

The basic parameters for FIA were assumed to be applicable in SIA because the same basic components (with minor changes) were used.

SIA differs from FIA in the sense that, SIA is mechanically simpler than flow injection for its use of a single pump and a selection valve. Mainly five liquid drivers/pumps are used in SIA, namely: -

1. A piston pump,
2. a peristaltic pump,
3. a Milligat pump,
4. an automatic burette, and
5. an electro osmotic pressure pump.

These pumps are suitable for SIA especially when they are using Neoprene tubing [4].

An ideal liquid driver should satisfy the following requirements: -

i) Able to withstand continual use for extended periods of time with minimal scheduled maintenance.

ii) Device actions rapid and without significant inertia.

iii) Flow rate in the range 0.5-15 mL $min^{-1}$.

iv) Constant and reliable flow rates over an extended period.

v) Smooth, reproducible and pulseless flow.

vi) The pump should be self priming .

vii) Option of inherent safety even in the hands of an inexperienced user.

viii) Small and compact in size.

ix) Low power consumption.

x) Be able to withstand pressures as high as 700 kPa.

Not every pump will satisfy the above requirements. Some of the requirements will be needed by the request of the purchaser. Only the piston pump and the peristaltic pump will be discussed briefly because they are the ones mainly used.

1) Piston pump

Papers dealing with its application used a sinusoidal flow piston pumps especially designed for flow systems [5]. With this pump the flow rate is dependant on the rotation angle, the radius of the pump, the cam and the frequency of the pump. Repeatability and reproducibility are good with this pump. This pump offers a flow rate in the range 0.2-7.0 mL $min^{-1}$, 0.1-3.0 mL $min^{-1}$, and 0.5-5.0 mL $min^{-1}$.

2) Peristaltic pump

This pump is built up of: -

i) A solid plate,

ii) rollers,

iii) a drum, and

iv) pump tubing.

Advantages of a peristaltic pump are: -

i) They are suitable for flow rates between 0.05-25.0 mL $min^{-1}$.

ii) They are considerably shorter in length.

iii) Very flexible with multichannels.

iv) Configuration easier, design and operation.

v) Already widely available.

The selection valve can be switched forward and backward to the desired position. The main function of the selection valve is to select flow from a number of samples and reagents solutions and they are as well applied in process analysis.

An ideal selection valve should satisfy the following requirements [6]: -

i) Able to withstand continual use for extended periods of time with minimum scheduled maintenance.

ii) Wetted parts must be resistant to corrosive solutions, organic solvents and acids.

iii) Minimal effect of flow channels on flow pattern.

iv) Ports must be the same diameter as the manifold tubing.

v) Availability on various flow patterns.

vi) Easily controlled via transistor-transistor logic or switch control.

vii) Low power consumption for the valve to be able to be incorporated into portable systems for field works.

viii) Able to withstand high pressures.

ix) Switching from one port to another should be rapid, and reproducible to eliminate cross contamination of the solutions.

x) It must have an option of inherent security even in the hands of and inexperienced user.

xi) It must be small and compact in size.

It is unlikely that every valve would satisfy the above requirements.

Any detector is suitable to be incorporated into the flow system if it has got a flow through, detection path, quick response time and it must be rugged. Detection in flow systems has been

carried out by atomic absorption, emission instruments, fluorometers, electrochemical systems, and spectrophotometers. The last two are the most commonly used [7-14]. Taking into consideration, the advantages of the technique, it has been applied by many scientists. Silva *et al.* [8] used SIA for determination of chloride in milk. The authors used conductrimetry as their chloride detector. In this method the interferences were removed by the incorporation of an on-line dialyser. Galhardo and Masini [15] used the technique for *in situ* monitoring of iron (II), iron (III), nitrite, and nitrate in natural and waste waters. They used a syringe pump as a liquid driver. The paper demonstrates the ability of the technique for multicomponent determination. Van Staden *et al.* [2] studied the influence of different volume ratios on the isodipersion point in SIA. The work confirmed that in order to single reagent chemical assays in an SIA system, attention should be given to the following: -

i) The sequence of introduction of samples and reagents,

ii) The impact of zone penetration for the specific system,

iii) The position of zone overlap, and

iv) The rinsing time between consecutive samples.

Van Staden and McCormack [16] studied the use of the SIA technique to evaluate the effect of mixing chambers on zone penetration. The study revealed that, placing the mixing chamber between the holding coil and the selection valve causes too much dispersion and loss of sensitivity, whilst having a small volume mixing chamber positioned just before the detector aids zone penetration and increases sensitivity. Ivaska and Kubiak [1] applied the technique to anodic stripping voltammetry.

Thomas *et al.* [11] developed a new concept for monitoring basic water pollution based on the SIA provided with two simple spectrophotometric detectors. The main concern of the study was to estimate total organic carbon (TOC), chemical oxygen demand (COD) and particulate pollution (TSS, total suspended solids). Nitrogenous and phosphorus compounds were also determined. Alpizer *et al.* [17] used SIA for the simultaneous determination of chloride and pH in waste waters. Taljaard and van Staden [3] applied SIA for the simultaneous determination of cobalt(II) and nickel(II) in water and soil samples. The system used, involved a mixing chamber incorporated into the conduits of the system. The method permitted the resolution of binary mixtures of cobalt and nickel at low levels without prior separation. Van Staden *et al.* [9] used a dialysis chamber in a sequential injection system for the determination of iron(III) in pharmaceutical samples using Tiron as a reagent. Dialysis efficiency was improved by the use of multiple flow reversals.

## 2.3 Basic principles of SIA

After the first step of zone sequencing, during which the sample and reagent zones are stacked in the holding coil, the valve selects the detector line. In the next step the flow is reversed so that the stacked zones are propelled through the valve and the reaction coil to the detector. During this movement the flow reversal creates a complex region, within which the analyte is transformed into a detectable species. The fundamental requirement for SI to succeed is to achieve maximum zone penetration through a deliberate increase in axial dispersion obtained by means of the flow reversal and channel design [4].

Reproducible dispersion is the basis for analysis by flow-based methods. Dispersion is the result of all the physical forces acting on the aspirated zones. It is the process by which the zones

transform from homogenous, geometrically well defined zones at the moment of injection to the final zone that is detected downstream. The dispersion coefficient $D$ is the ratio of the detector response of the injected solution in the absence of any physical forces to that of the solution in the presence of the forces. Ruzicka and Hansen [18] defined the conceptually simple and practically useful dispersion coefficient $D$; which is given by the relation:

$$D = \frac{C^{\circ}}{C}$$

Where C° is the analyte concentration of the injected sample or the detector response of the undispersed solution zone and C is the peak concentration at the detector or the detector response of the dispersed element of fluid that yields the analytical readout.

Dispersion is controlled by three interrelated and controllable variables: Sample volume, tube length, and the manifold flow rate. With large sample volumes the dispersion becomes unity. In this instance, no sample dilution has occurred.

Although Ruzicka and Gubeli [19] stated that " for a rational design of the sequential injection analyser the degree of sample dispersion must be considered as main design guideline" zone penetration (related to dispersion) is the key parameter. The control of which is the key to the successful execution of sequential injection. Zone penetration can be ascribed to the fact that it has a dramatic impact on the surface area of which a concentration gradient exists of which axial mixing takes place.

Analogous to the definition of resolution, zone penetration is defined as:

$$P = \frac{2W_{ov}}{(W_s + W_r)}$$

Complete overlap is obtained when $P = 1$, zero overlap when $P = 0$, and for values in between partial overlap will be obtained. Isodispersion point, $I_D$ is observed in cases where $1 > P > 0$ (Fig.2.1). At this point the dispersion of the sample and reagent zones is identical and the ratio of reagent and sample concentration is the same as their ratio prior to injection $C_s / C_r = C_{so} / C_{ro}$. Studies done by van Staden *et al.* [2] illustrated the shift of the isodispersion point due to the difference in concentration gradients when different volume ratios of sample and reagent were employed. The studies also showed that the position of penetration and the sequence of introduction of samples and reagents for different sample and reagent volume ratios has a major influence on the response of the final peak profile.

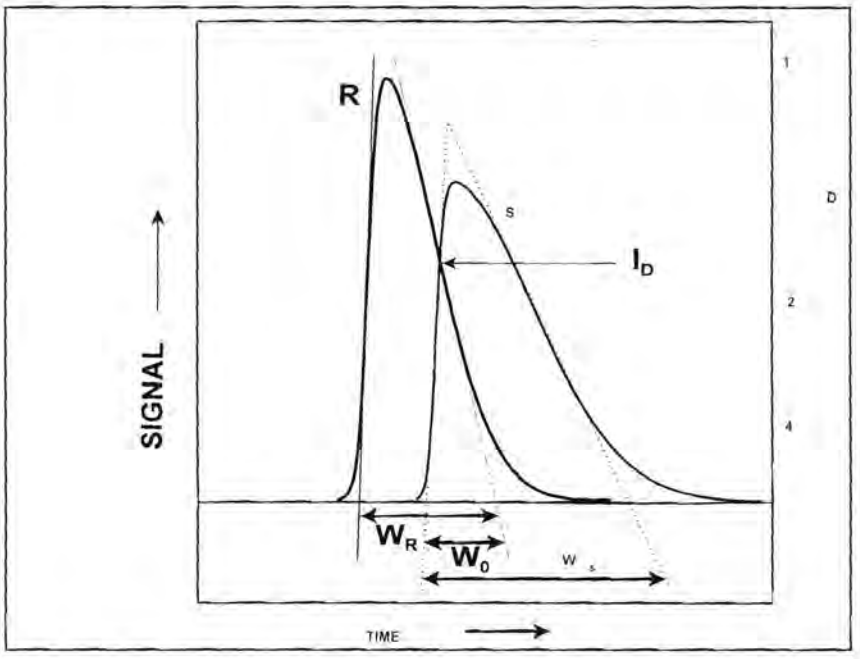


**Fig. 2.1** Schematic representation of zone penetration showing the isodispersion point ($I_D$), R, reagent zone; S, sample zone; D, dispersion coefficient; $W_R$, baseline width of reagent zone; $W_S$, baseline width of the sample zone; and $W_O$, baseline width of the overlap.

## 2.4 Operational parameters

A number of articles where published that describes the most important parameters to be optimised [20-23]. The following parameters have shown to have an influence on zone dispersion in an SIA system: -

i) The volumetric flow rate,

ii) order of sample and reagent introduction,

iii) tube diameter,

iv) Length of flow path from injection to detection,

v) sample and reagent volumes,

vi) flow reversal,

vii) and to lesser extent reaction coil geometry.

The volumetric flow rate is referred to as the combined effect of pump speed and the internal diameter of the pump tubing when using a peristaltic pump. The relationship of flow rate and dispersion is commonly expressed as $D = k'q$, where $q$ is the flow rate in mL $min^{-1}$ and the relation suggest that the dispersion of the different zones decreases as the flow rate is increased [21]. The dispersion coefficient decreases with increasing flow rates [20]. A linear relationship exists between the flow rate and pump speed, therefore the flow rate can be altered by changing the pump speed. At relatively high flow rates there is substantial deterioration of sensitivity and reproducibility, due to the back pressure [9, 20].

The length of the tubing is dictated to the experimental requirements. Longer tubing leads to longer residents times and therefore larger dispersions. The SIA manifold tubing is divided into two parts, namely: - the holding coil, which is the channel connecting the liquid driver (pump) and the selection valve and the reaction coil which is the channel connecting the selection valve and the detector. The holding coil acts as a reservoir and should be long enough to accommodate the sample and reagent zones. And prevent them from entering the pump tubing. The reaction coils are usually kept as short as possible to avoid excessive dilution of the formed product by the carrier stream.

Gubeli *et al.* [23] have conducted the in-depth study on the effect of reagent and sample volume

on zone penetration and sensitivity. Their conclusions were as follows:

1. Changing the sample volume is an effective way of changing the sensitivity of the measurement. Reduction of the aspirated sample volume is another way of achieving dilution of the concentrated samples.
2. For the reagent volume the first prerequisite was that a certain amount of reagent solution was necessary in the centroid of the stack of zones for optimum production of the measured reaction product. They reported that for an optimised region of naturally interdispersed sample and reagent zones with high precision, the volume of a reagent should be at lest twice that of the sample.
3. Two reagent chemistries can be accommodated provided that the sample volume is kept below $S_{1/2}$ value so that the sample zone is surrounded by the reagent zone and that the concentrations of the aspirated zones are sufficiently high.

The order at which different sequences of reagents are drawn depend on the reactions involved. The residence time of the zone in the system also depends on the sequence of aspiration. The zone that is aspirated first has got a long residence time and it undergoes more dispersion that the other zones. The zone that is aspirated first reaches the detector last due to the flow reversal. If reagents of significantly different viscosities are aspirated the zone may be subjected to a few multiple flow reversals before the start of the detection.

Various reactors have been described in the literature on FIA manifolds [18]. The reactor usually consists of length of tubing of a certain inner diameter. Various reactor coil length geometries have been proposed. They were evaluated to establish the effect of reactor coil geometry on zone

penetration and geometry. Studies done by Taljaard [20] and Marshall and van Staden [22] showed that the reactor geometry does not have much influence on sensitivity and precision.

## 2.5 Single zone sequential injection analysis

The analysis of chemical species that can be measured directly such as those that have a high molar absorptivity of light at a specific wavelength can be analysed (e.g. concentrated hexavalent chromium) using single zone SIA. In this type of analysis the sample is the only zone aspirated.

## 2.6 Two-zone SIA

Two-zone SIA involves the addition of a single reagent to the carrier stream conveying the sample. In this type of analysis or study, the sample and the reagent solutions are the only zones aspirated into the holding conduit. McCormack and van Staden [7] used two-zone SIA to evaluate the effect of mixing chambers on zone penetration in SIA. The two zones which were used were iron(III) and tiron. Iron(III)-tiron zones were again used by van Staden *et al.* [2] in the study of the different volume ratios on the isodispersion point in SIA. The study of reaction stoichiometries, kinetic aspects of some reactions and the influence of some of the important parameters of the SIA system can be carried out by using two-zone SIA.

## 2.7 Three-zone SIA

In this type of an SIA system two reagents and a sample zone are aspirated into the holding conduit. The three-zone SIA was employed by Nyman and Ivaska [24] in the determination of calcium in paper machine white paper by sequential injection analysis. In this study the calcium reacted with o-cresolpthalein complexone. Magnesium is the major interfering species. The third zone, 8-hydroxyquinoline was added as the third reagent to mask the magnesium. Munoz *et al.* [25] used two reagents, a solution of malachite green and a mixture of malachite green and tin chloride in the evaluation of spectrophotometric methods for the determination of orthophosphates by SIA.

## 2.8 More than three-zone SIA

Although it was emphasized that three-zone SIA is the maximum number of zones one can work with for achieving effective mixing of the three-zones [20], Guzman and Compton [26] used a SI technique and robotics for the automation of rhXIII (recombinant human factor thirteen) flourimetric activity assay. The mixing chamber was introduced into the manifold to ensure thorough mixing of the different multiple zones. This was done after the SI method was simulated to a large extend to the chronology of the manual procedure.

## 2.9 Multicomponent techniques in SIA

Most determinations which were carried out by SIA were based on the determinations of a single

component. Only a few number of multicomponent techniques were published. Alpizer *et al.* [17] used the technique for the simultaneous determination of chloride and pH in waste waters using potentiometric detection. The method uses a separate flow cell to measure the pH by stopping the flow during the determination of chloride. Alpizer and co-workers [27] also described the simultaneous determination of chloride and fluoride in water samples using ion selective electrodes. Luo *et al.* [28] applied a SI wetting film extraction to the spectrophotometric determination of chromium(VI) and chromium(III) in water. In the work reported the product of chromium(VI) and diphenyl carbazide was ion-paired with perchlorate and extracted into an organic wetting film consisted of octanol and 4-methyl-2-pentanone on the inner wall of a Teflon tube. Chromium(III) was previously oxidized to chromium(VI) and calculated as the difference between total chromium and chromium(VI).

Galhardo and Masini [15] studied the *in situ* monitoring of iron(II), iron(III), nitrate and nitrite in natural and waste waters with SIA. Determination of nitrite was based on the Griess-Ilosuay reaction and the iron(II) determination on the reaction with 1.10-phenanthroline. Iron(III) and nitrite were determined after their reduction, in Jones and copperized cadmium columns. Van Staden and Taljaard [3], used the SI concept for the simultaneous determination of cobalt(II) and nickel(II) in water and soil samples. In their study the authors used the different rates of the pseudo first order dissociation of the citrate complex of the two ions at pH 8.00. This lead to the kinetic-based determination of the elements at trace levels in water and soil extracts. The reactions were followed by measuring the absorbance of the complexes of the metal ions with 4-(2-pyridilazo) resorcinol (PAR) which were formed in the subsequent rapid reactions.

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# CHAPTER 3

# Membrane separations in flow systems: Dialysis

## 3.1 Introduction

The use of membranes for on-line separation in flow through dialysers as part of the flow system is extremely useful. Dialysis membranes have a very important role to play in automated sample preparation and processing before a final processed sample is delivered under well defined and interferences free conditions for detection. The technique is simple, repeatable, reliable, compatible, requires little or no pretreatment of the sample and shows no interference from sample colour and turbidity. In most cases there is no reaction in the membrane interface involved. Dialysis can be interfaced in the continuous flow, flow injection, stopped flow, sequential injection and even process analysis modes.

Although dialysis was the first membrane separation process to be discovered and studied [1], it has never really become one of the applications in the industrial field. Dialysis was however seen as an important tool in the medical field where haemolysis is used for the treatment chronic and acute anaemia and for the removal of hazardous substances from the blood stream. The main reasons why dialysis was never given much attention in industry were: -

1. It was seen as a slow process, because it was driven by concentration differences which are usually set by the system.

2. It is not highly selective. Species that are similar in chemistry or molecular size cannot be cleanly separated by dialysis.

## 3.2 Fundamental aspects

In analytical chemistry separation can be regarded as splitting a representative mixture (sample) into at least two parts of different composition in order to enrich one of the parts or fractions with one composition in relation to the rest. The main concern of this technique being a result of selectivity and sensitivity afforded by these techniques as many analytical methods lack adequate levels of this prime feature. During these process the different components should be finally pulled apart and through a space, physical displacement should take place.

Dialysis is the selective separation of the species through a semi permeable membrane which separates two fluids. The process is primarily based on the separation of the analyte of interest from the sample matrix [1]. The separation of the different solutes relies on the difference between the rates at which they are transferred across the semi-permeable membrane. In an equilibrium and conventional classical batch dialysis process with two compartments separated by a semi-permeable membrane, the process is normally allowed to develop until mass transfer equilibrium is reached.

In a continuous flow, flow injection or sequential injection/dialysis combination system, the system is not developed until mass transfer equilibrium is reached due to the dynamics of the system and scrupulous timing should be employed. Agitation of a fixed volume of one or both phases can be employed to increase the mass transfer [2] and to facilitate dilution. In a continuous flow, flow

injection or sequential injection/dialysis system, two channels are involved. In one channel the sample donor stream flows over the second channel containing a recipient (an acceptor stream). The two channels are physically separated by a semi-permeable membrane barrier.

The system is designed so that the analyte is transferred to the recipient stream, leaving interfering ions or molecules and impermeable colloidal matter in the donor stream. The transport of the sample plug along a donor channel depends on flow rate with the concentration profile governed by convective or diffusional transport in the conduits of the donor channel. The rate of the solute transfer across the semi-permeable membrane is dependant on the differential transport governed by Fick's law of molecular diffusion [3]:

$$\frac{dm}{dt} = -DA\frac{dc}{dx}$$

where, $D$ = the diffusion coefficient of the solutes through the membrane,

$A$ = the membrane surface area,

$\frac{dm}{dt}$ = mass flow diffusion rate, and

$\frac{dc}{dx}$ = concentration gradient.

In a dialysis process the nature (membrane thickness, membrane surface, and porosity) of the membrane barrier plays an important role and it determine the features of the transfer process and therefore the nature and size of the ions and molecules diffusing through it.

The classification of dialysis process as passive or active (Donnan) depends on the nature of the membrane. Passive dialysis involves the transfer of particles within a given range of molecular weight across a neutral semi-permeable membrane. In active (Donnan) dialysis, ions with a given type of charge are transferred across an ion exchange membrane. The nature of some samples and also the amount used may result in fouling of the membrane which may have an adverse effect on the diffusion process. It is possible to increase the diffusion rate by increasing the ratio of the membrane surface area to sample volume, by designing a suitable dialyser system.

## 3.3 Theoretical principles

Valcarcel and Luque de Castro [3] briefly described the operating efficiencies of a dialyser expressing the ratio between the rate and concentration gradient in terms of a non-dimensional parameter. $D_A/\gamma_S$

Where $D_A$ is the composite mass transfer dilution coefficient, and

$\gamma_S$ is the donor channel flow rate.

$D_A$ in corporate all the following physical parameters of the system such as, membrane surface, membrane area, membrane porosity, membrane thickness, temperature, electrical effects, pressure, flow rates of solution, geometry, viscosity and tortuosity of the membrane.

Fig. 3. which gives the general schematic representation of a dialyser incorporated into the conduits of a sequential injection analyser, the analyte enters the top donor channel at a flow rate $q_d$ and a concentration of $Cd_1$ but leaves at the same flow rate but less concentration $Cd_2$. At the

bottom of the acceptor (recipient) stream, the flow rate is $n_1$ and the analyte concentration $Ca_1$ and at the exit the flow rate is the same but the concentration has increased to $Ca_2$.

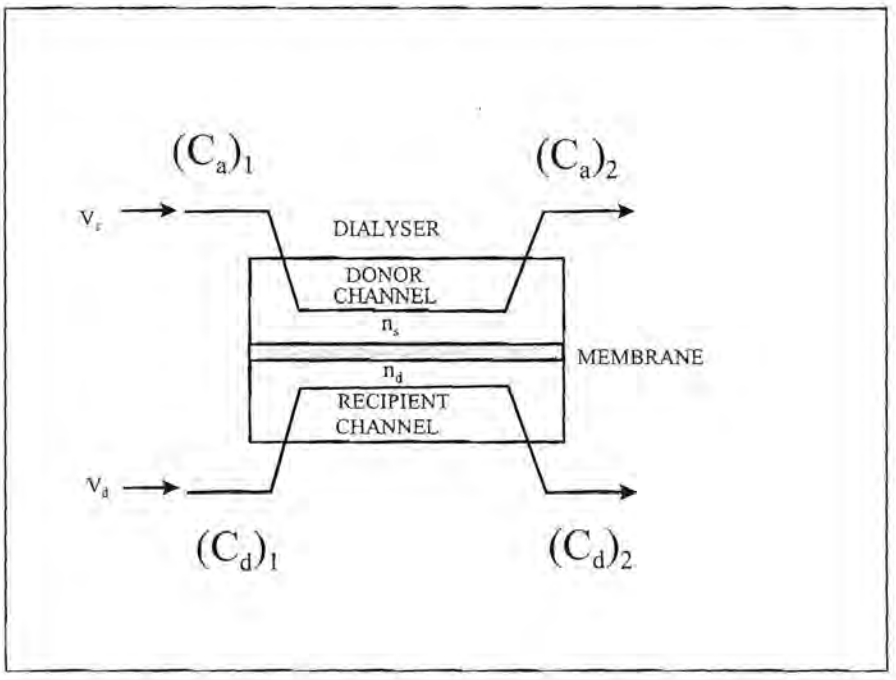


**Fig. 3.1** Schematic illustration of a dialyser unit incorporated into the conduits of a sequential injection analyser (symbols are defined in text).

If $Ca_1 = 0$, then the efficiency of the dialysis process in terms of non-dimensional parameter is:

$$\frac{D_A}{\gamma_s} = \frac{[(Ca)_1 - (Ca)_2}{(Ca)_1}$$

which relates the efficiency (in absolute value) to the flow rate of the donor phase. The mass transfer efficiency may also expressed using the percentage of dialysis and multiplying by 100.

$$\%Dialysis = \frac{Cd}{Ca} \times 100\%$$

## 3.4 Basic components

The dialyser unit with the membrane forms the heart of the separation process in dialysis. The dialyser unit is incorporated somewhere in the conduits of a sequential injection analyser.

### 3.4.1 Dialyser units

One of the oldest dialyser units is the circular form or round dialyser. This type of a dialyser was very popular and used in clinical laboratories coupled to segmented flow systems where a long path length was required. Most conventional micro and miniature dialysers are of the sandwich type and are designed in a rectangular form. The membrane is fitted between the mating surfaces of the upper and lower plates. The carrier stream of sample containing the analyte zone is channelled along the groove of the donor block. At the same time, water or reagent stream moves along the grooves of the recipient block. As soon as the analyte zone enters the donor channel, it starts diffusing through the membrane to the bottom recipient stream.

### 3.4.2 Membrane materials

The dialysis membrane is the key contributor in the successful operation of a flow injection/dialysis, continuous flow/dialysis, sequential injection/dialysis or process/dialysis analyzer. To obtain a high efficiency of the dialysis process, the membrane should be semi-permeable. The membrane surface should be mechanically and chemically resistant and suitable for along period of time. The rate of mass transfer across the membrane should be fast enough to cope with the required sample throughput of a specific analyzer.

Porosity is the key factor in membrane separations and most manufacturers classify the membranes according to their pore size. The porosity of the membrane should be kept constant throughout the dialysis process. This can be achieved by strictly avoiding any mechanical strain on the membrane. It is possible to adjust the pore size effectively by using a stretching procedure [4]. Controlled acetylation reduces the pore size or by controlled swelling through treatment with a concentrated zinc(II) chloride solution.

Dialysis membranes are usually hydrophilic in nature, and are typically made of cellophane, cellulose acetate, polycarbonate, polyvinyl chloride, polyamides, polysulphone, polyvinylidene fluoride, copolymers of acrylonitrile and vinyl chloride, polyelectrolyte complexes, cross linked polyvinyl alcohols as well as acrylic coplymers such as Nafion [1]. Regenerated cellulose or cellophane is the most popular material used in flow-based methodologies. Cellophane is a compact material in the dry state, it swells considerably to form a sponge like hydrogel containing up to 50 % water after covering with water. A very important property of the gel is that it behaves as a substance with pores of more or less fixed size ranging from 3-5 nm. Practically cellophane carries no fixed charges, which makes it ideally suitable for passive dialysis and mass transfer is based entirely on molecular diffusion.

Dialysis membranes are classified as microporous, homogenous or ion-exchange [3, 5]. Microporous membranes are rigid and highly voided with randomly interconnected pores of smaller size (10- 100 A°). Microporous membranes and conventional filters operate basically on the same principle. A microporous membrane only allows particles of different sizes to be separated. Mass transfer across the membrane occurs via molecular diffusion and diffusivity of the species across the membrane. In passive dialysis this type of membrane can be viewed as a

partition between the two aqueous phases where the solute molecules will flow from the solution with high concentration to the one with the low concentration. Particles larger than the largest pore size of the membrane will be retained. These types of membranes are not suitable in passive dialysis and are not used very often.

Homogenous or non-porous membranes are made of homogenous films [3] with interfaces distributed uniformly through the membrane. Species are transferred across the membrane by molecular diffusion where the dialysis efficiency depends on the solubility and diffusivity of the species across the membrane interface. In this process the membrane is seen as a partition between two aqueous phases and the solutes diffuse from a region of high concentration to the one with the low concentration across the membrane.

Ion-exchange membranes are of submicroporous nature. This type of membrane has no conventional microscopic pores, but consist of film forming polymers with positively or negatively charged ions fixed to the pore walls. The mass transfer efficiency not only upon the solubility and diffusivity of the species across the membrane, but also upon the ion exchange properties of the membrane. The positive and negatively charged ions act as cations and anions respectively. In active (Donnan) dialysis this type of membrane can be viewed as the partition between two aqueous phases where the solute ions flow from the solution in the donor phase containing a high ionic strength solution to the recipient phase containing a low ionic strength solution.

## 3.5 Factors influencing dialysis efficiency

The mass transfer efficiency across the membrane is normally expressed as the percentage

dialysis.There are two major influences on the dialysis which must be taken into account [3]. Variables associated with the membrane and the parameters of the particular experimental procedure used in the flow injection analysis.

### 3.5.1 Membrane variables

#### 3.5.1.1 Type of membrane

The structure and the chemical composition of the membrane material are of extreme importance for the purpose for which the membrane is used and the mass transfer efficiency obtained for the period applied. The exact compositions of the membranes are not known due to patents and it is therefore difficult to judge the membrane according to their structural composition. Van Staden and van Rensburg [3] studied different types of membranes to demonstrate their dialysis efficiency between different dialyser dimensions of different types and various variables were evaluated. The results which were reported revealed that there is a negligible difference between the different membranes. Membranes act according to pore-mechanism, which means that in dialysis, the membrane functions as a simple sieve which transmits the different materials according to their molecular size.

#### 3.5.1.2 Membrane path length

The mass transfer efficiency increases with an increase in path length from 70-300 nm with a dialyser unit having a semi tubular groove with an inner diameter of 0.500 mm [3]. With the path lengths of longer than 300 mm, the flow injection systems tend to change irregularly, for example

due to differential pressure changes, and, as a result, the mass transfer efficiency changes accordingly. The dialysate concentration also depends on the donor analyte concentration.

#### 3.5.1.3 Membrane porosity

The membrane used in dialysis such as homogeneous membranes, the mass transfer across the membrane occurs via molecular diffusion and the pore size of the membrane plays an important role in the diffusivity of the species. Mass transfer across the membrane increase with increase in path length [3].

#### 3.5.1.4 Membrane thickness

Van Staden and van Rensburg [3] reported that the mass transfer efficiency increases with a decrease in membrane thickness proving the assumption that diffusion inside the membrane is the rate determining step of the dialysis process.

#### 3.5.1.5 Membrane geometry

An increase in the membrane contact area influences the mass transfer efficiency and it is assumed that an increase in membrane contact area will lead to better dialysis efficiency. The convenient to use of the dialysers are the parallel plate one [3].

## 3.6 Experimental parameters

### 3.6.1 Flow direction

Skeggs [6] observed that when the donor and recipient streams flowed through the dialyser in the same direction (concurrent flow), less material was generally recovered than when the opposing (countercurrent flow) was used. Bernhardsson *et al.* [7] preferred concurrent flow over countercurrent flow owing to the smaller differential pressures across the membrane and much smaller dispersion in a long dialyser. Van Staden and van Rensburg [3] worked with relatively high flow rates (1-4 mL $min^{-1}$) in order to obtain a maximum sample throughput and could not detect any differences with the change in flow direction. The authors also reported that with countercurrent flow the precision of the result decreased markedly.

### 3.6.2 Flow rates

Van Staden and van Rensburg [3] reported that it is desirable to have equal flow rates of the donor and the recipient stream, As the flow rate decreases the mass transfer efficiency increases.

### 3.6.3 Analyte solute concentration

Van Staden and van Rensburg [3] found that the ratios of the membrane surface area to sample in flow injection and continuous flow for their systems were such that differences in dialysis efficiency were negligible for the concentration ranges used.

### 3.6.4 Sample volumes

Sample volumes have no effect on dialysis efficiency and it may be concluded that, for a dialyser system with fixed parameters, the ratio of the membrane surface area to sample volume has no influence to the dialysis efficiency [3].

### 3.6.5 Composition of the donor and recipient stream

Van Staden and van Rensburg [3] found that the use of cresolpthalein complexone as the recipient stream increases the dialysis process very significantly in some cases. This is due to the different osmotic pressures. It is advisable to use the reagent as the recipient stream and water as the donor.

### 3.6.6 Temperature

Dialysis is a kinetically controlled process and the slight change in temperature lead to significant changes in the mass transfer efficiency. Working at ambient temperatures will avoid variation in results [3].

## 3.7 Dilution

A dialyser as part of the manifold system does not only remove the interferences such as suspended solids and unwanted macromolecules but also facilitates automated dilution. This feature makes it possible to use dialysis for automated on-line dilution to give rapid and reliable results. The class of membrane (passive or active) used, plays a very important role in the dialysis

efficiency and therefore the amount of dilution obtained. In the passive process where the allowed species within a given range of the molecular mass diffuse across a neutral membrane, the concentration gradient and the contact time determine the amount of analyte that dialysed across the membrane surface. Solutes diffuse from a region of high concentration to a region of low concentration. Applying Fick's law of molecular diffusion it is assumed that diffusion in the membrane is the rate determining step. It is possible to control the amount of analyte that dialyses through the membrane by careful control of the flow dynamics of the flow system and by using various approaches in the configuration of the system.

Lima *et al.* [8] incorporated a dialysis unit into an FIA manifold to obtain large in-line dilutions before transporting the analyte to atomic absorption and emission spectrometers. The authors used the manifold with a single dialyser to determine magnesium, calcium, sodium, and potassium in various types of Portuguese wines. Van Staden [9] used automated dilution in FIA with two on-line dialysers in tandem mode for the determination of chloride at levels up to 60 g $L^{-1}$ in industrial affluents and plating solutions. The two dialysers were incorporated in succession in the FIA manifold.

## 3.8 References

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# Chapter 4

# Spectrophotometric determination of chloride in minerals and drinking waters using sequential injection analysis

## 4.1 Introduction

Chloride is widely distributed in nature at levels from almost zero to as much as 1 000 mg $L^{-1}$ depending on the type of natural water; it is, therefore a very important aspect of the quality of mineral and drinking water. Quality control of mineral and drinking water is of prime importance for human health. Reliable, rapid and accurate analytical methods are, therefore necessary in the analysis of this type of water.

The most standard basic method used for the determination of chloride is the Volhard titration [1] method. Although this standard method is accurate, it is time consuming and expensive.

Numerous flow injection systems [2-12] using UV spectrophotometry for detection have been developed for the indirect monitoring of chloride. Spectrophotometric measurement at 480 nm is based on the detection of the red iron(III) thiocyanate complex formed as follows:

$$Hg(SCN)_2 + 2\ Cl^- \rightarrow HgCl_2 + 2\ SCN^-$$

$$SCN^- + Fe^{3+} \rightarrow [Fe(SCN)]^{2+}$$

Since it was conceived in 1990 by Ruzicka and Marshall [13] sequential injection analysis (SIA) has been widely accepted because of its simplicity, high analytical throughput, low reagent consumption sensitivity, and low running costs [14-19]. The technique involves the sequential injection of wash, sample and reagent solutions into a holding coil where well defined zones are formed. When the flow is reversed the reagent and the sample merges and as dispersion occurs the analyte is transformed into a detectable species.

This article reports the indirect determination of chloride in drinking mineral, natural and ground waters by measurement of the red iron-thiocyanate complex at 480 nm.

## 4.2 Properties of chloride

Chloride (Cl) has a covalent radius of $0.99 \times 10^{-10}$ and an atomic mass of 35.54 g $mol^{-1}$. It is situated in the 7th main group on the periodic elements. It has got the first electronegativity after flouride. The electronegativity is because of the electronic configuration:

$$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^5$$

Chloride can easily accept an electron when it is in the chloride ion form $Cl^-$ and it can also form covalent bonds with different elements such as hydrogen (H) and oxygen (O). Chloride can also exist as a homogenous molecule in the form of $Cl_2$, which is a pale green gas. It can also form part of organic molecules such as in chlorobenzene, trichloroethylene, tetrachloromethane, etc.

The chloride ion form favours the following oxidation states: $Cl^{1-}$, $Cl^{3-}$, $Cl^{5-}$ and $Cl^{7-}$. The best examples of these oxidation states are oxyanions: $ClO^-$ (hypochlorite), $ClO_2^-$ (chlorite), $ClO_3^-$ (chlorate), and $ClO_4^-$ (perchlorate). The most significant forms of chloride are HCl, NaCl, HClO

and $Cl_2$.

## 4.3 Biochemistry of chloride

Chloride is an anion mostly combined with sodium in the extracellular fluid and, to some extent, it is also found with potassium in the cells. Chloride ions can pass freely through the cell membrane to balance the osmotic pressures of the cells. The movement of the chloride ions are compensated by those of sodium. The movement between the blood plasma and erythrocytes, where chloride ions travel rapidly in and out of the cells is an exchange of bicarbonate, thereby enhancing the capacity of the red blood cells to carry carbon dioxide from the tissues to the lungs and aiding in the maintenance of the acid-base balance in the body fluids.

During digestion, some of the chloride ions are used for the formation of hydrochloric acid by the gastric glands, and secreted to the stomach where it functions temporarily with the gastric enzymes and is then reabsorbed into the blood stream along with other nutrients.

## 4.4 Choice of analytical method

The most common basic standard methods for the determination of chloride are the Volhard and the Mohr methods [1]. Although this standard methods are accurate they are time consuming and expensive. Ion-selective electrodes have been used for numerous manual determinations of chloride [20-22]. Direct analysis of chloride in milk by ion-selective electrodes is not possible. This is caused by the interference of proteins such as casein, globulins and α-lactoglobulins. Van

Staden [23] eliminated the interferences by developing a rapid and reliable automated procedure for the direct measurement of chloride content in milk using flow injection analysis with a dialyser and a coated tubular inorganic chloride-selective electrode as a sensor.

## 4.5 Experimental

### 4.5.1 Reagents and solutions

All reagents where prepared from analytical reagent grade chemicals unless specified otherwise. Deionized water from the Modulab system ( Continental Water System, San Antonio, Texas, USA) was used throughout. The water was tested before hand for traces of chloride. Before measurement all solutions were degassed with a vacuum pump system. The main solutions were prepared as described below.

#### 4.5.1.1 Chromogenic reagent

The chromogenic reagent was prepared by dissolving mercury(II) thiocyanate (126 g) in methanol (300 mL). Deionized water (1L) was added, then nitric acid (specific gravity 1.45: 8 mL) and $Fe(NO_3)_3\,9H_2O$ (31,00 g). The solution was finally diluted to 2 L with distilled water and any undissolved matter was removed by filtration. If stored in a dark bottle at room temperature this reagent is stable for several months.

#### 4.5.1.2 Standard chloride solutions

A stock solution of 10 000 mg $L^{-1}$ chloride was prepared by dissolving sodium chloride (dried in an oven at 110°C for 3 h; (16.484 g) and quantitatively diluted to a total volume of 1 L. Working standard solutions ( 0-50 mg $L^{-1}$) were prepared by appropriate dilution of the stock solution.

### 4.5.2 Equipment

The SIA system (Fig. 4.1) was constructed from a Gilson Minipuls peristaltic pump, A Valco Instruments model E-10 10-port electrically actuated selection valve, and a Unicam (Cambridge, UK) 8625 spectrometer equipped with a 10-mm (80 $\mu$L) Hellma flow-through cell for absorbance measurement. Tygon tubing was used for the holding and the reaction coils. The coils were constructed by winding the tubing on the Perspex rods. Data acquisition and device control were accomplished by use of a PC30-B interface board ( Eagle Electric, Cape Town, South Africa) and an assembled distribution board (Mintek, Randburg, South Africa). The Flowtek [16] software package (obtainable from Mintek) was used throughout for computer-aided flow analysis, device control, and data acquisition.

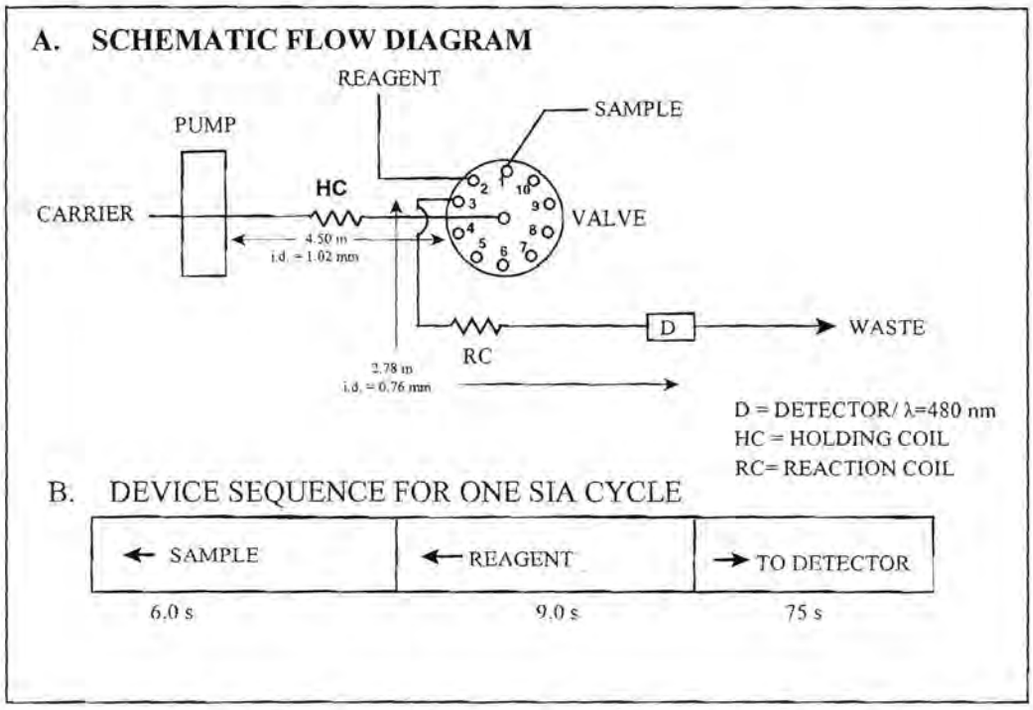


**Fig. 4.1.** Schematic representation of the SIA system for the determination of chloride (A) and device sequence for one cycle of the SIA system (B).

### 4.5.3 Device sequence

Device sequence for the determination of chloride by SIA is fully outlined in Table 1.

**Table 4.1.** Device sequence for one full cycle of the sequential injection system used fro the determination of chloride.

| Time (s) | Pump | Valve | Description. |
|---|---|---|---|
| 0 | Off | Home | Pump off, Valve select a sample stream. Valve position 1 |
| 1.0 | Reverse | | Draw in sample solution. |
| 10.0 | Off | | Pump stops. |
| 16.0 | | Advance | Valve select the reagent line. Valve position 2. |
| 17.0 | Reverse | | Pump draw up the reagent solution |
| 18.0 | Off | | Pump stops. |
| 19.0 | | Advance | Valve select the detector line. Valve position 3. |
| 20.0 | Forward | | Pump stacks of zones to the detector. |
| 95.0 | Off | Home | Pump stops, valve return to position 1 |

## 4.6 Results

### 4.6.1 Method optimization

To determine the optimum conditions for the determination of chloride the flow rate of the SIA system was studied between 1.95 and 3.95 mL $min^{-1}$ using a 4.50 m × 1.02 mm holding coil and a 2.78 m × 0.76 mm reaction coil. A flow rate of 3.21 mL $min^{-1}$ (Fig. 4.2) gave the best results and was chosen as the optimum flow rate. Different sample volumes were investigated (Fig. 4.3) to ensure that the predominantly red iron(III) thiocyanate complex [17] was formed. Although a sample volume of 640 $\mu$L (Fig. 4.3) gave the best zone penetration and sensitivity, 320 $\mu$L resulted in the most reproducible zone penetration, and the latter was selected as the optimum.

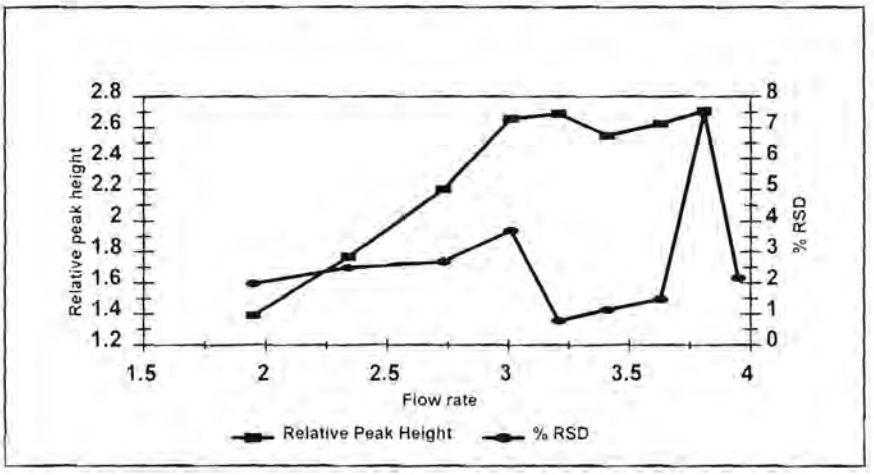


**Fig. 4.2.** The effect of carrier stream flow rate (mL $min^{-1}$) on relative peak height and % RSD. $[Cl^-]$ = 50 mg $L^{-1}$.

Gubeli et al. [18] and Tucker et al. [19] have conducted the in-depth study of the effect of reagent volume on zone penetration and sensitivity. The first prerequisite was that a certain amount of reagent solution was necessary in the centroid of the stack of zones for optimum production of the measured reaction product. They reported that for an optimised region of mutually interdispersed sample and reagent zones with high precision, the volume of the reagent should be at least twice that of the sample.

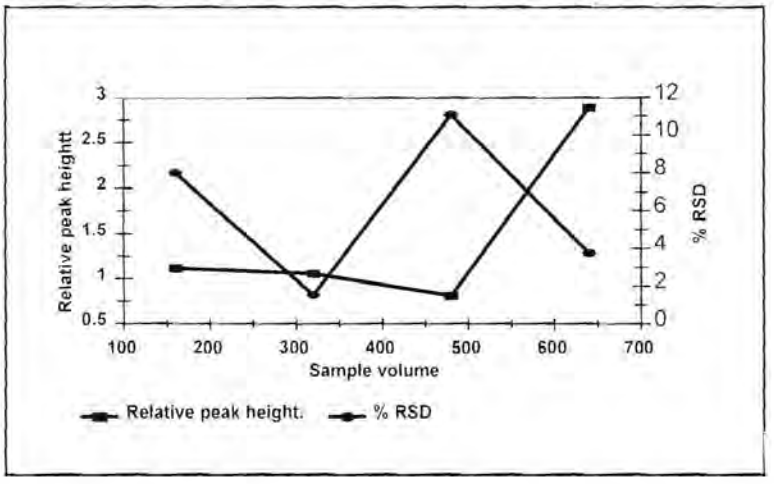


**Fig. 4.3.** The effect of sample volume ($\mu$L) on relative peak height. [$Cl^-$] = 50 mg $L^{-1}$.

Because two chloride ions are necessary for the release of one thiocyanate ion from the mercury(II) thiocyanate [17], the same volume should be used for the reagent and sample zones. This was evaluated and compared. For the best reproducibility the optimum reagent volume was found to be 480 $\mu$L, as depicted in Fig. 4.4. The volume of the reagent must be slightly in excess to ensure total conversion of the chloride to the predominantly red iron(III) thiocyanate complex [17]. Reaction coils of different lengths were studied, because length has a major influence on the dispersion of the final analytical profile. The standard curve for the determination of chloride tends to be non-linear [17] when the final product is measured as the red-coloured complex ion $[Fe(SCN)]^{2+}$. It was found that a reaction coil of 2.78 m gave the best results (including linearity), as shown in Fig. 4.5, and was chosen as optimum.

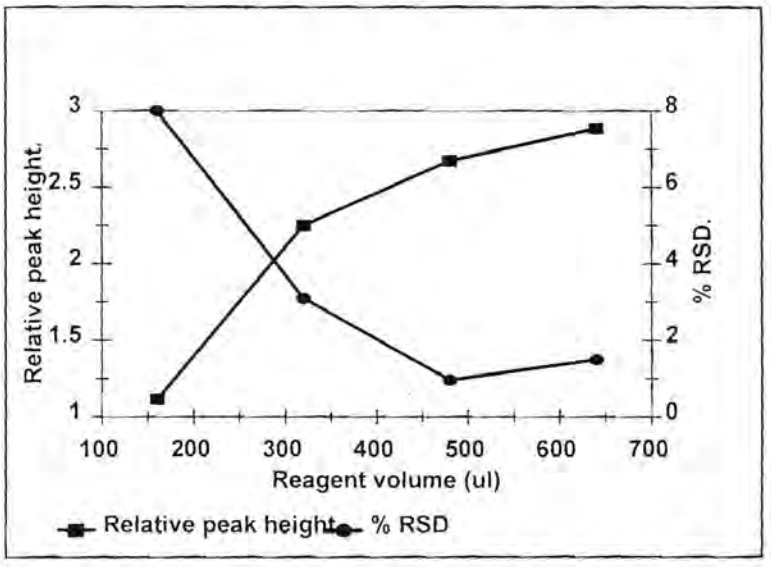


**Fig. 4.4.** The effect of reagent volume ($\mu$L) on relative peak height.[$Cl^-$] = 50 mg $L^{-1}$.

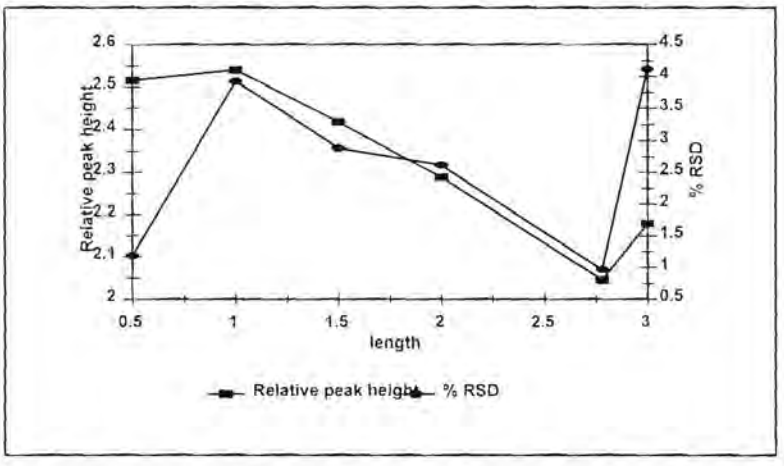


**Fig. 4.5.** The effect of reaction coil length(m) ( i.d.=0.76 mm) on relative peak height.

[$Cl^-$] = 50 mg $L^{-1}$.

### 4.6.2 Method evaluation

The proposed SIA system was critically evaluated with regard to linearity, accuracy, precision, sampling frequency, and interferences. The response obtained from the proposed SIA system was linear between 0-50 mg $L^{-1}$, and the relationship between relative peak height and chloride ion

concentration were y=0.1313x + 0.275 (r=0.9971; n=10) where y=relative peak height and x=chloride ion concentration in mg $L^{-1}$. The accuracy of the proposed SIA analyser was evaluated by comparing results obtained from mineral and drinking water samples and the values obtained by the standard Volhard titration method [1]. The results shown in Table 4.2, revealed good correlation between SIA and the values obtained by the standard method. The accuracy was also determined by spiking real water samples with standard chloride solutions. The recovery efficiency of the proposed system varied between 99 and 101%. Precision<2.50% was obtained for the SIA system (Table 2) at a sampling frequency of 37 samples $h^{-1}$. The calculated detection limit was 3.01 mg $L^{-1}$ chloride. The t-test with multiple samples (paired by differences) was used to examine whether the two methods differed significantly at 95 % confidence level [24, 25]. Because of the calculated value of 0.5629 is less than the critical value t-value 2.447, it cannot be rejected; It follows that there is no significant difference between results obtained by use of the two techniques. Iron(III) might be a possible interferant, but it was eliminated because of the amount of iron(III) added as reagent.

**Table 4.2.** Performance of the proposed SIA system for the determination of chloride

| Sample | Proposed SIA system | | Standard manual method |
|---|---|---|---|
| | [$Cl^-$] Method mg/L | % RSD*. | [$Cl^-$] in mg/L |
| Mineral water. | | | |
| Sample 1 | 23.46 | 1.90 | 23.54 |
| Sample 2 | 5.11 | 1.41 | 5.38 |
| Ground water. | | | |
| Sample 3 | 12.66 | 1.84 | 13.00 |
| Sample 4 | 10.74 | 1.99 | 10 |
| Sample 5 | 42.74 | 2.12 | 42 |
| Sample 6 | 22.72 | 2.09 | 23.00 |
| Sample 7 | 29.22 | 0.73 | 29 |

*n=10

## 4.7 Conclusion

System is suitable for determination of chloride levels as low as 5 mg $L^{-1}$ and below. The main advantage of the method compared with FIA is the very low reagent consumption and sampling frequency of 37 samples $h^{-1}$. The method is economical and can be used for routine laboratory analysis. It can be easily adopted for on-line analysis of chloride. Samples with chloride concentrations>50 mg $L^{-1}$ should be diluted by incorporation of a dialyser unit or a mixing chamber into the conduits of the system before analysis in routine laboratory analysis.

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# Chapter 5

# The use of dialysis membranes for on-line separation and determination of chloride in milk by sequential injection analysis with the aid of multiple flow reversals.

## 5.1 Introduction

The use of membranes for on-line separations in flow through systems [1, 2] has been seen and regarded as a valuable tool. The technique is simple, reproducible, reliable, agreeable and requires little or no pretreatment of the sample and shows no interferences from sample color and turbidity. The coupling of dialysers with flow through systems proved to be useful in this regard.

Dialysis is a separation technique employed mainly to provide interference free analysis. In a continuous flow, flow injection or sequential injection / dialysis combination the donor stream flows over the acceptor stream the two streams are separated by a semipermeable membrane. The separation process depends on various aspects such as molecular size and ionic charges. The nature of the membrane ( i.e., type of membrane, membrane thickness, membrane surface and the porosity of the membrane) determines the features of the transfer process across the membrane. The dialyzer also facilitates on-line sample dilution [3]

Since it was conceived SIA [3, 4] proved to be a valuable and useful technique for on-line determinations in routine laboratory works. The concept is based on the sequential injection of a

wash solution, sample, and regent(s) into a single channel [4] with the use of a single carrier stream. The basic components of an SIA manifold are a peristaltic pump with a single carrier channel, a multiport selection valve and a single detector. After a stack of well-defined zones are formed adjacent to each other, the valve selects the detector channel. By reversing the flow, the zones mutually disperse and penetrate each other to form a new detectable product and after detection they are pushed through to waste.

This chapter describes the incorporation of a dialyzer into the conduits of SIA system with the use of multiple flow reversals to enhance the percentage dialysis and to minimize too much reagent consumption. The system is employed for the determination of chloride in milk samples.

Chloride determination in milk is very useful to establish the degree of mastitis that can occur in cattle [5]. The determination can be qualitative, comparative, manual or with the use of extremely well developed techniques using numerous detectors. Milk being a very complex matrix usually requires pretreatment.

## 5.2 Properties and biochemical importance of chloride

The properties some biochemical importance of chloride are discussed in the previous chapter.

## 5.3 Choice of the analytical method

Milk consist of proteins (such as casein, globulins and α-lactoglobulins), fats and undissolved

suspended solids. Most analyses of chloride were performed by the Volhard and the Mohr standard manual methods. Direct manual determination of chloride in milk using ion-selective electrodes has been used . Results obtained by de Clercg *et al.* [6] showed substantial error. This was due to the interference of the factors listed above.

UV-VIS spectrophotometry as detection system in FIA [7-9] was developed for the indirect monitoring of chloride which was based on the indirect determination of red iron(III) thiocyanate complex. Applying this procedure in flow analysis still does not solve the problems due to the same interfering species listed above.

To remove the interfering species, a dialyser fitted with a semi-permeable membrane was incorporated into the conduits of a flow system for sample pretreatment and conditioning.

## 5.4 Experimental

### 5.4.1 Reagents and solutions

All the reagents were of the analytical reagent grade unless specified otherwise. Deionised water from the Modulab (Continental water system, San Antonio, TX, USA) was used throughout. The water was tested before usage for any chloride traces.

#### 5.4.1.1 Chromogenic reagent

Dissolve 1.26 g of mercury(II)-thiocyanate in 300 mL of methanol, add 1 L of water and shake

well to mix. Then add 8 mL of nitric acid (specific gravity: 1.42) and 31 g of $Fe(NO_3)_3 .9H_2O$. Shake well until dissolved and dilute to 2 L with water. Filter off any undissolved matter in the solution. When stored in a dark bottle at room temperature this reagent is stable for several months.

#### 5.4.1.2 Standard chloride solutions

A stock solution of 10 000 mg $L^{-1}$ chloride was prepared by dissolving 16.484 g of sodium chloride which was first dried in an oven at 110 °C for three hours and dilute to a total volume of 1 L with water. Working standard solutions were prepared by appropriate dilution of the stock solution to cover the range from 40-90 mg $L^{-1}$ .

### 5.4.2 Sample pretreatment

The milk samples were first manually diluted with water before they were injected. The dialyzer unit, which was connected in series with the sequential injection system was equipped with a neutral passive, semipermeable, type 'C' Technicon pre-mounted dialysis membrane to separate any interfering species in the sample matrix.

### 5.4.3 Equipment

The SIA manifold (Fig. 5.1) was assembled using the following components. Two peristaltic pumps (Gilson Villiers Le Bel, France), two ten ports electrically actuated selection valves (Valco Instrument Co. Inc.), an 80 mm dialyzer chamber equipped with a type 'C' cellulose membrane (Technicon) and a Unicam 8625 spectrophotometer (Cambridge, UK) equipped with a 10 mm light

path Hellma flow through cell with a volume of 80 $\mu$L. Tygon tubes were used for the liquid channels. The holding and reaction coils were constructed by winding the tubes on perspex rods. Data acquisition and device control were accomplished using a PC-30B interface board (Eagle electric, Cape Town, South Africa) and assembled distribution board (Mintek, Randburg, South Africa). The Flowtek software package [10] (obtainable from Mintek) for computer-aided flow analysis, device control and data acquisition was used throughout.

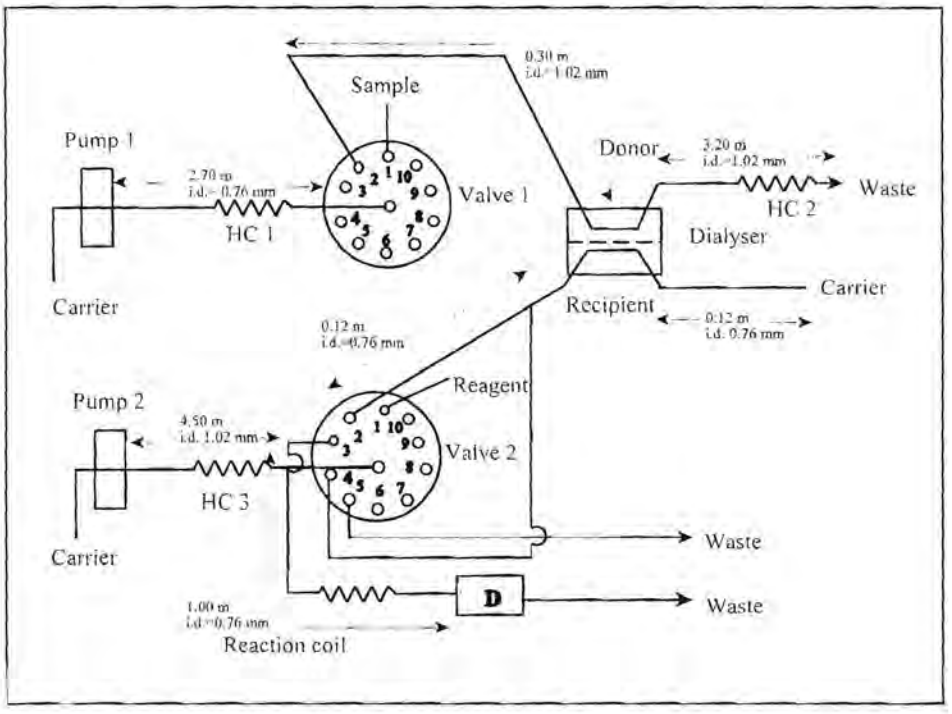


**Fig. 5.1.** Schematic representation of the SIA system for the determination of chloride, D= detector at λ=480 nm and HC= Holding Coil.

### 5.4.4 Device sequence

The device sequence for the determination of chloride by the sequential injection system is fully outlined on Table 5.1

**Table 5.1.** Device sequence for one full cycle of the SIA system for the determination of chloride.

| Time (s) | Pump 1 | Valve 1 | Pump 2 | Valve 2 | Description |
|---|---|---|---|---|---|
| 0 | Off | Home | Off | Home | Pumps off, valve 1, position 1. |
| 1.0 | Reverse | | | | Draw up the sample. |
| 19.0 | Off | | | | Pump 1 off. |
| 19.5 | | Advance | | | Select the dialyser through port 2. |
| 20.0 | Forward Off | | | | Propel sample over the membrane. |
| 40.0 | Off | | | | Pump 1 off. |
| 40.5 | Reverse | | | | Propel sample over the membrane |
| 50.5 | Forward | | | | Agitation to enhance mass transfer. |
| 60.5 | Reverse | | | | Agitation to enhance mass transfer. |
| 70.5 | Forward | | | | Agitation to enhance mass transfer. |
| 80.5 | Reverse | | | | Agitation to enhance mass transfer. |
| 90.5 | Forward | | | | Agitation to enhance mass transfer. |
| 100.5 | Reverse | | | | Agitation to enhance mass transfer. |
| 110.5 | Forward | | | | Agitation to enhance mass transfer. |
| 120.5 | Reverse | | | | Agitation to enhance mass transfer. |
| 130.5 | Forward | | | | Agitation to enhance mass transfer. |
| 140.5 | Off | | | | Agitation to enhance mass transfer. |
| 141.0 | Reverse | | | | Agitation to enhance mass transfer. |
| 161.0 | Off | | | | Draw the sample into the holding coil1. |
| 162.0 | | | Reverse | | Pump 2 draw up reagent, valve 2 at port 1. |
| 167.0 | | | Off | | Pump 2 off. |
| 168.0 | | | | Advance | Valve 2 select the dialyzed sample. |
| 169.0 | | | Reverse | | Pump 2 draw up sample, valve 2 at por 2. 2. |
| 175.0 | | | Off | | Pump 2 off. |
| 176.0 | | | | Advance | Valve 2 select the detector channel at port 3. |
| 177.0 | | | Forward | | Pump 2 propel the zones to the detector. |
| 244.0 | | | Off | | Pump 2 off. |
| 245.0 | | | | Advance | Valve 2 select the recipient stream at port 4. |
| 246.0 | | | Reverse | | Pump 2 draw up the carrier. |
| 266.0 | | | Off | | Pump 2 off. |
| 267.0 | Forward | | | | Pump 1 rinse the donor stream of the dialyser. |
| 268.0 | | | | Advance | Valve 2 selects the waste at port 5 |
| 269.0 | | | Forward | | Rinse the SI system |
| 350.0 | Off | Home | Off | Home | |

## 5.5 Results and discussions

### 5.5.1 Method optimization

The parameters outlined below were studied in order to attain the optimum conditions of the method for chloride determination. A slower pump speed of 10 rpm was used to propel the donor stream and it was found that a little faster pump speed of 15 rpm was used to propel the recipient stream as shown on Figs. 5.2 and 5.3. When looking at Fig. 5.2, at flow rates above 1.6 mL $min^{-1}$ there is a decrease in peak height, this is due to the insufficient time for the sample to diffuse through the membrane. Looking at the recipient stream flow rate, where the reaction of the sample and the reagent take place, good sensitivity is attained at higher flow rates as depicted on Fig. 5.3. The flow rates of 2.0 and 3.0 mL $min^{-1}$ were chosen as the optimum flow rates for the donor and the recipient streams respectively.

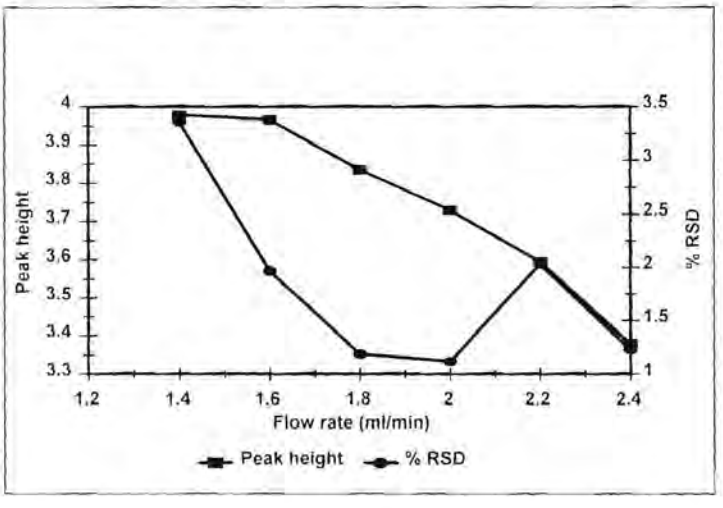


**Fig. 5.2** The effect of donor stream flow rate on the relative peak height and % RSD. $[Cl^-] = 100$ mg $L^{-1}$

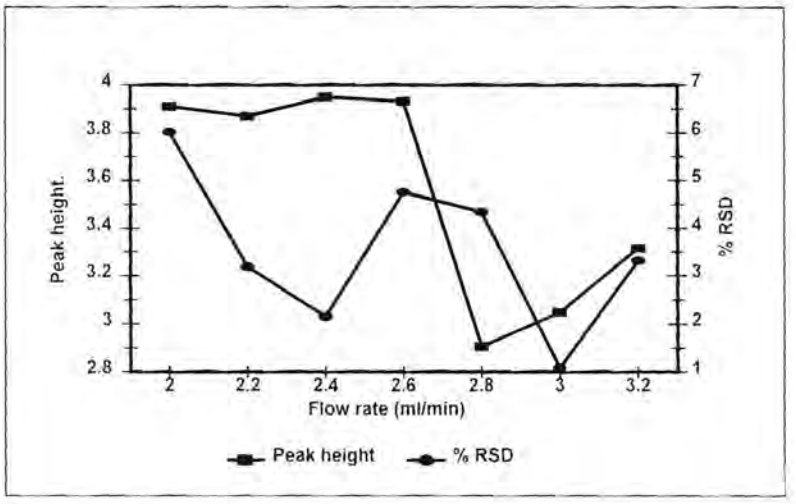


**Fig. 5.3** The effect of the recipient stream flow rate on the relative peak height. $[Cl^-] = 100$ mg $L^{-1}$.

In this type of sequential injection system, two types of sample volumes are involved. The sample volume from the sample reservoir drawn up via valve1 and the dialyzed sample volume from the recipient stream drawn up via valve 2. Sample volumes from the reservoir ranging from 150 to 1000 $\mu$L were evaluated as highlighted on Fig. 5.4. Although sample volumes higher than 450 $\mu$L gives very constant peak heights they possess inconsistent reproducibility. There is an abrupt increase in peak height for the volumes higher than 450 $\mu$L. This shows that larger sample volumes resulted in a higher degree of dialysis and shows a good reproducibility of the peak profile. Although sample volumes below 450 $\mu$L's gave very constant peak profiles at different volumes, a volume of 700 $\mu$L was chosen as the optimum owing to the good reproducibility.

The dialyzed sample volumes were evaluated and the results are shown on Fig. 5.5. This clearly shows that at volumes higher than 100 $\mu$L the sensitivity increases until a certain point where the plateau is reached. Owing to the good sensitivity and the constant reproducibility it gave the sample volume of 300 $\mu$L's was chosen as the optimum. Various reagent volumes were evaluated and the results are outlined

on Figure 5.6. The trend shows that an increase in reagent volume leads to increase in sensitivity. It shows that at volumes greater than 300 $\mu$L a plateau will be reached. The volume of 250 $\mu$L was chosen as the optimum volume due to the best reproducibility of the peak profile.

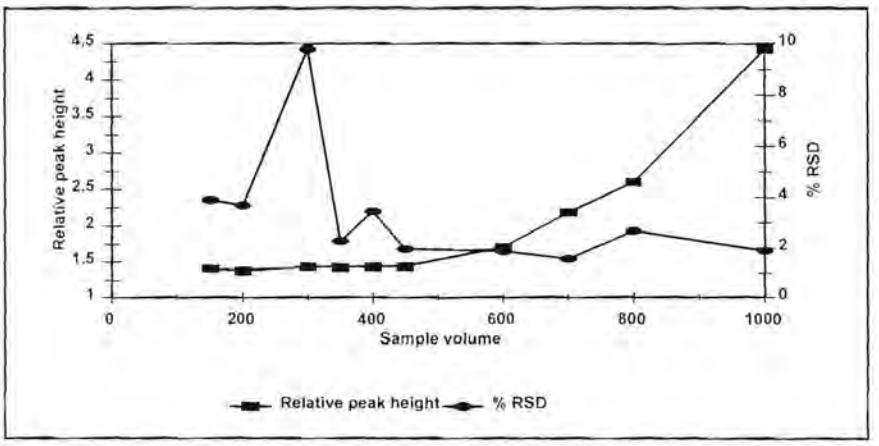


**Fig. 5.4** The effect of sample volume ($\mu$L) drawn from the sample reservoir via valve 1 on the relative peak height.[$Cl^-$] = 100 mg $L^{-1}$.

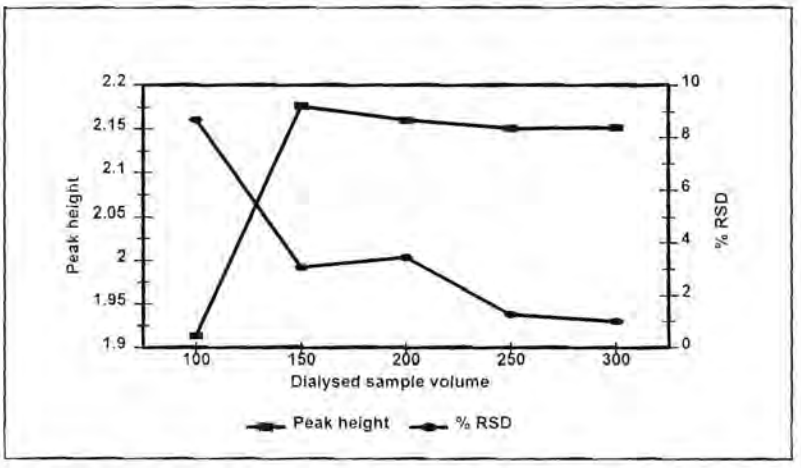


**Fig. 5.5** Influence of dialyzed sample volume ($\mu$L) on the relative peak height and % RSD. [$Cl^-$] = 100 mg $L^{-1}$.

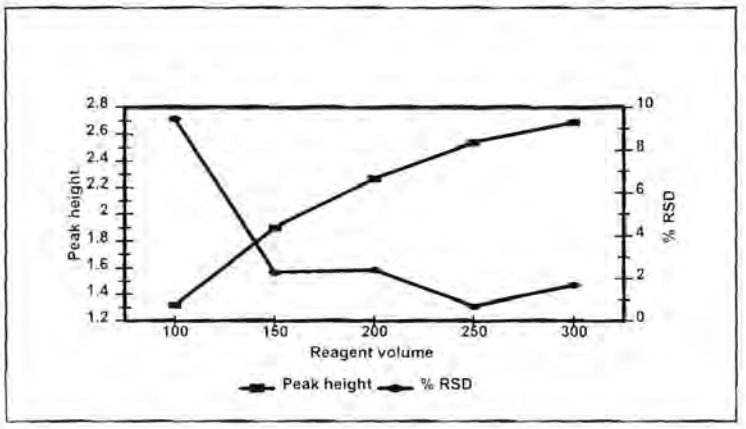


**Fig. 5.6** The influence of reagent volume ($\mu$L) on peak height and percentage RSD. [$Cl^-$] = 100 mg $L^{-1}$.

Looking at Fig. 5.7, as the reaction coil length is increased there is a steady decrease in peak height this might be due to the fact that at early stages the sample is converted to a product and thereafter eventually disperse in the carrier before reaching the detector. A reaction coil of 1.00 m was chosen as the optimum considering the better sensitivity and the reproducibility.

When a dialysis membrane is incorporated into the channels of a sequential injection system, the contact time of the sample with the membrane is having much influence on the quantity of the sample dialyzed through the membrane [4]. By propelling the sample zone forward for various periods of time over the membrane it showed that the whole sample zone was propelled over the membrane in about 20 s. This forward pumping time was done to ensure that the most concentrated part of the sample is over the membrane before the start of the multiple flow reversals.

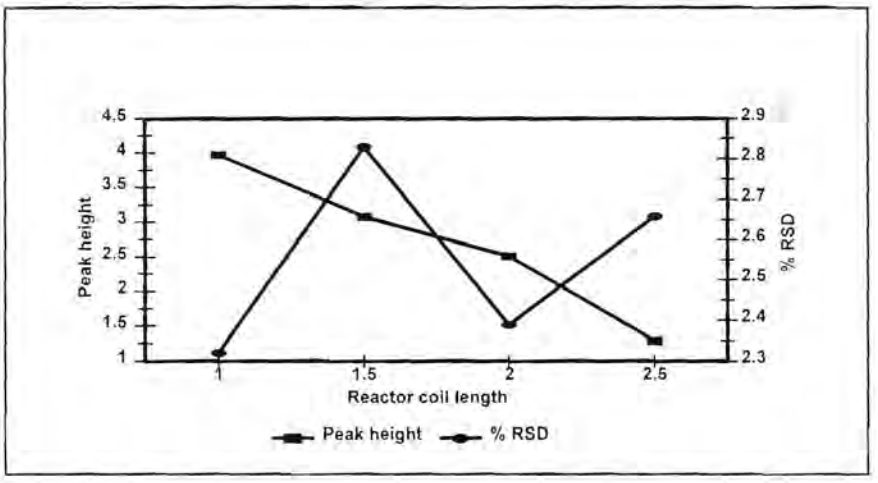


**Fig. 5.7** The effect of reaction coil length (m) on relative peak height and % RSD (i.d. =0.76 mm). $[Cl^-] = 100$ mg $L^{-1}$.

Multiple flow reversals [11] were then used to enhance the dialysis time. The time between the sequential flow reversals was 10 seconds for the forward and 10 seconds for the reverse step. The dialysis time was increased by adding the forward and the reverse step simultaneously as illustrated on Fig. 5.8. The relative response was increasing with the dialysis time. The percentage dialysis was increasing with the increasing dialysis time. The evaluated period varied from 20 -100 seconds. Longer dialysis times will lead to the reduction in the sampling frequency. The dialysis time of 100 seconds was chosen as the optimum due to the high percentage dialysis it possessed. The percentage dialysis was 4.62. After the multiple flow reversals the sample was pumped backwards for a period of 20 seconds to ensure that the sample is held in Holding Coil 1 before the dialyzed one is drawn into Holding Coil 2. The time of the multiple flow reversals was evaluated. The time was reduced from 10 - 2 seconds as outlined on Table 4. The stopped flow of 100 seconds between the first 20 seconds forward step, and the last 20 seconds reverse step was also evaluated.

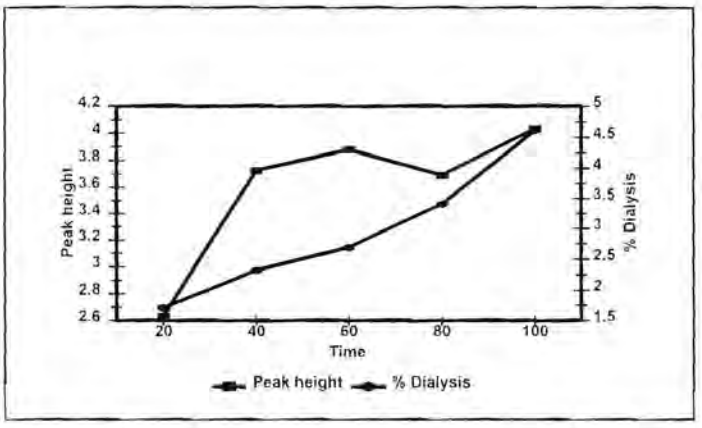


**Fig. 5.8** The influence of dialysis time (s) on the relative peak height and % dialysis. $[Cl^-] = 100$ mg $L^{-1}$.

The fully optimized system consisted of the first forward step of 20 seconds, 10 multiple flow reversals alternating 10 seconds reverse and 10 seconds forward and the last reverse step of 20 seconds to draw the sample back to Holding Coil 1.

### 5.5.2 Method evaluation

The resultant SIA method was critically evaluated with regard to reproducibility, linearity, detection limit, accuracy, sample interaction, sampling frequency and interferences. To test for the reproducibility of the system, 10 repetitive analysis cycles of the samples were performed and the percentage RSD of all the samples was better than 1.80 percent as shown on Table 5.2. Under the optimum conditions the proposed SIA method was found to be linear from 40 - 90 mg $L^{-1}$ of chloride ($y = 0.035x + 1.82$), $r = 0.9994$, $n=5$, here $y$= peak height and $x$= chloride concentration in mg $L^{-1}$. The detection limit was calculated using the formula $[(3\sigma + k) - c]/m$, where $\sigma$ (0.44) is the relative standard deviation of the baseline, k (0.5708) is the average signal value of the baseline and c (1.8221) and m (0.034) is the intercept and the slope of the calibration graph respectively. The calculated detection limit was found to be 2.01 mg $L^{-1}$. The comparison was done between the SIA and the Mohr titration method (Table 5.2). The comparison was

done to establish whether the SIA system can be accepted as giving reliable results in the determination of chloride. The null hypothesis was used. The t-test with multiple samples (paired by difference) was applied to examine whether the two methods differed significantly at 95% confidence level. The null hypothesis $H_o$: $\zeta = 0$ against the alternative $\zeta \neq 0$, where $\zeta$ the population paired difference [7, 8]. Thus $t_{calc} = |\bar{x}_d| \times \sqrt{n} / s_d$ and from Table 2 n=3, $s_d$ = 4.21 and $\bar{x}_d$ =1.45 substituting for $t_{calc.}$ we find $t_{calc} = 1.45 \times \sqrt{3} / 4.21 = 0.5965$. At 95 % confidence level the critical values are therefore $\pm$ 4.303. Since the calculated value is less than the critical value, $H_o$ cannot be rejected and it follows that there is no statistically significant difference between the two techniques. The accuracy of the proposed SIA analyzer was evaluated by comparing the results from the SIA system with the values obtained by the Mohr titration method [12, 13]. The results shown on Table 5.2 revealed good correlation between the values obtained with the SIA system and that of the standard manual method.

**Table 5.2** The accuracy of the proposed SIA analyser compared with the standard manual method.

| **Sample name** | **[Cl⁻] (mg L⁻¹) SIA system** | **[Cl⁻] (mg L⁻¹) Mohr method** | **% RSD * SIA system** | **% RSD * Mohr method** |
|---|---|---|---|---|
| Sample 1 | 425.29 | 429.91 | 0.71 | 2.02 |
| Sample 2 | 893.54 | 896.61 | 1.26 | 1.01 |
| Sample 3 | 623.40 | 620.07 | 1.61 | 0.75 |

***n=5**

The accuracy was further evaluated by spiking real milk samples with standard chloride solutions (Table 5.3). The recovery efficiency of the proposed method varied from 96.38 - 99.03 %. The samples were spiked by the addition of 5 mL of 20 mg $L^{-1}$ chloride standards. Sample interaction or the carry over effect was investigated by analysing a sample with a low analyte concentration followed by that of the

high analyte concentration and then that of the low analyte concentration. A sample containing 40 mg $L^{-1}$ of chloride was used to represent the low analyte concentration and 90 mg $L^{-1}$ was used for the high analyte concentration.

**Table 5.3** Analysis of spiked real milk samples.

| Sample name | Expected [$Cl^-$] (mg $L^{-1}$) | Dialyzed [$Cl^-$] (mg $L^{-1}$) | % Recovery |
|---|---|---|---|
| Sample 1 | 405.99 | 321.28 | 96.38 |
| sample 2 | 851.94 | 840.60 | 98.67 |
| Sample 3 | 594.67 | 588.88 | 99.03 |

**Table 5.4.** The effect of the multiple flow reversals on peak height, % RSD and % Dialysis.

| Time (s) | Peak height | % RSD * | % Dialysis |
|---|---|---|---|
| 2 | 2.8078 | 4.17 | 2.30 |
| 5 | 2.9927 | 4.76 | 1.44 |
| 7 | 3.3740 | 2.74 | 2.30 |
| 10 | 3.5088 | 2.03 | 1.04 |
| stopped flow | 2.8491 | 2.23 | 1.48 |

*n=5

Sample interaction was then calculated using the following formula:

$$\text{Sample interaction} = \frac{A_3 - A_1}{A_2} \times 100\%$$

where $A_1$ =relative peak height of the analyte containing 40 mg $L^{-1}$ chloride, $A_2$ = relative peak height of the analyte containing 90 mg $L^{-1}$ of chloride and $A_3$ = relative peak height of the analyte containing the same amount of chloride as $A_1$ and their relative peak heights were

$A_1 = 3.1948$

$A_2 = 4.9643$

$A_3 = 3.2080$

The samples carry over effect was 0.28 % (Table 5.3).

The experimental time for one complete analytical cycle was 350 s. This gave the overall sampling rate of 10 samples per hour. Milk contains fats, proteins and many other undissolved substances. This may act as possible interferences to the method. This potential interferences were removed by the use of a passive, neutral, semipermeable membrane incorporated into the conduits of the SIA analyzer.

## 5.6 Conclusions

The fully automated sequential injection system proved to be suitable to determine chloride in full cream milk samples at low reagent consumption levels. This was accomplished by the use of the feature of multiple flow reversals which again improved the dialysis and dilution. This method is suitable for analysis of samples with high chloride concentrations due to the pre-valve dilution which is made possible by the use of the dialyzer and the multiple flow reversals.

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# Chapter 6

# On-line separation and spectrophotometric determination of zinc in fertilizer samples using xylenol orange as a complexing agent

## 6.1 Introduction

Numerous methods for the determination of zinc in diverse samples by using metalloenzymes and complexing agents have been described [1-6]. When dealing with environmental, agricultural, pharmaceutical and natural samples, separation and dilution of the analyte from a complex sample matrix are needed. This is usually accomplished by the incorporation of a dialyzer unit [7-10] or ion-exchangers. Few spectrophotometric techniques have been described with ion-exchangers because they usually require alternating additions of concentrated solutions, which results in very pronounced concentration gradients. These gradients are associated with pH which is the limiting factor in the system design [7].

Zinc is of prime importance in diverse fields, such as environmental, domestic and waste water control, metallurgy, galvanizing and alloy manufacturing, agricultural (soil analysis), pharmaceutical and clinical. The listed fields require a sensitive, selective (interferences free), rapid, accurate, rugged and less expensive technique [5]. Dithiozone and zincon methods are well established and officially adopted for the analysis of zinc in water, soil and pharmaceutical samples. Nevertheless, no reagent is selective toward zinc. In many cases/analysis separation, extraction and masking steps are required with the

control of pH as an intrinsic parameter. A few external parameters like temperature have been reported.

Many sophisticated techniques have been employed, e.g., XFS, FAAS and ICP-AES, for the determination of the total amount of metals in a diversity of complex materials. The techniques are expensive and could not be applied directly to real/natural samples.

Sequential injection analysis (SIA) coupled with a spectrophotometric detector is a very valuable technique. It is simple, rapid, less expensive and a useful tool for the quantification of samples in a variety of real natural samples. If there is a need for separation or dilution, a well-designed dialyzer equipped with a semipermeable neutral membrane can be incorporated into the conduits of the SIA manifold for on-line dilution and pretreatment of the sample(e.g., on-line separation).

In the case of passive dialysis little work have been done to circumvent the disadvantage of dilution of the analyte during the passage over the membrane. Methods have been developed where the sample can be pre-concentrated after dialysis and before detection. Some workers compared various cation resins which are commercially available [11].

## 6.2 Properties of zinc

Zinc is a metallic chemical element with a commonly used symbol Zn, with an atomic number of 30 and an atomic weight of 65.38 g $mol^{-1}$. Zinc has a melting point 419.58 °C and a boiling point of 907 °C, with a specific gravity of 7.133 at 25 °C. Zinc has a got a valence of 2. It is a transition metal with the following electronic configuration:

$$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}$$

Zinc can loose two electrons and become $Zn^{2+}$. The pure metal form dissolves very slowly in strong acids and alkalis. The dissolution occurs in dilute $H_2SO_4$, HCl and $HNO_3$. A typical reaction of zinc is the following:

$$Zn\ (s) + HCl \rightarrow ZnCl_2 + H_2$$

Zinc is a lustrous bluish-white metal. It is found in group IIB of the periodic table of elements. It is brittle and crystalline at ordinary temperatures, but when heated to temperatures between 110°C and 150°C it becomes ductile and malleable, it can then be rolled into sheets. Although it is not abundant in nature, it is of great commercial importance.

## 6.3 Importance of zinc in agriculture

Zinc deficiency is widespread in intensive crop production. This is due to the hastened removal of available zinc from the root zone, sometimes to erosion of the topsoil and with it soil organic matter which is important in facilitating the uptake of zinc by plants. Zinc deficiencies were reported in sandy soils and it is dependant on the pH of the soil.

Another factor inducing zinc deficiency is a high level of available phosphates in the soil. Zinc fertilization is a common practice when soil or plant analysis indicates a deficiency. The most commonly used fertilizer is the zinc sulphate, either monohydrate or heptahydrate containing about 35 % and 23 % Zn respectively.

Another source of applying zinc to soils is with zinc chelates. The chelating helps to shield the zinc from

undesirable reactions in soils. Zinc may also be applied to soils as a foliar spray.

Zinc deficiency led to corn failing to develop all the kernels in the ear which is a detrimental effect to the corn production [12].

## 6.4 Biochemistry of zinc

Zinc is involved in many biochemical processes and contributes significantly to human health. Zinc is essential for functioning of over 70 enzymes that control protein synthesis and the growth and the repair of cells. It plays an important role in the environment and in both the animals and plants, and it is associated with the activities and the absorption of vitamin A and B complexes.

Another role that zinc play in biochemical processes such as, sexual development, physical growth, wound healing and tissue repair, and acts as a cofactor for many enzymes involved with metabolism.

Zinc is a constituent of insulin, which is used in the treatment of diabetes. The most reliable sources of zinc are animal food, especially muscle meat, wheat germ and bran.

## 6.5 Choice of analytical method

The determination of zinc is of prime importance in an array of fields such as, environmental chemistry, industrial and domestic water control, agriculture, metallurgy and in the health industry. In all the above listed fields the selectivity, sensitivity and rapidity are essential for the determination of zinc. The most

commonly used methods are atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, titration with EDTA and the dithiozone method.

A flow injection method using xylenol orange as a reagent and thiosulphate as the masking reagent for copper(II) and lead(II) has been described [13] although nickel(II) still interferes. Zinc analysis in many samples such as soil, fertilizer, blood, etc. is a problem owing to the fact that these samples have proteins and many undissolved matters suspended in their sample matrices that act as potential source of interferences. They may be effectively removed by incorporation of a dialyser unit fitted with a neutral semipermeable membrane into the conduits of the FI system.

This chapter deals with the analysis of zinc in fertilizers using sequential injection analysis with a dialyser fitted with a neutral semipermeable membrane incorporated into the channels of the system. The dialyser is employed to remove the undissolved matter present in the fertilizer samples. The zinc that has diffused through the membrane reacts with xylenol orange to form a maroon-red zinc-xylenol orange complex. The thiosulphate was used as a masking reagent for cooper(II) and lead(II).

## 6.6 Experimental

All the reagents were of an analytical reagent grade unless specified otherwise. Deionised water from the Modulab (Continental water system, San Antonio, TX, USA) was used throughout.

### 6.6.1 Reagents

#### 6.6.1.1 Chromogenic reagent

A 0.012 % (m/v) aqueous solution of xylenol orange was prepared.

#### 6.6.1.2 Masking reagent-buffer solution

The masking reagent-buffer solution was prepared by mixing 3 mL of 99.99 % (v/v) of acetic acid, 2 g of sodium hydroxide and 5 g of sodium thiosulphate in a 500 mL volumetric flask and diluted to the mark with deionised water. This gives an acetic acid-sodium acetate buffer of pH about 5.9 with sodium thiosulphate and the masking reagent for the interfering nickel(II), copper(II) and magnesium(II).

#### 6.6.1.3 Carrier solution

The carrier solution was a 0.14 mol $L^{-1}$ aqueous nitric acid.

#### 6.6.1.4 Zinc stock and working solutions

A 1991.7 mg $L^{-1}$ stock solution of zinc(II) was prepared by dissolving 1.9917 g of zinc pellets in 20 mL of 7 mol $L^{-1}$ nitric acid and diluted to 1 L with water. Working standard solutions were prepared by the appropriate dilution of the stock solution.

## 6.7 Equipment

The SIA system (Fig.6.1) was assembled using the following apparatus. Two peristaltic pumps (Gilson, Villiers Le Bel, France), two ten ports electrically actuated selection valves (Valco Instrument Co. Inc.), an 80 mm dialyzer equipped with a type 'C' cellulose membrane (Technicon) and a Unicam 8625 spectrophotometer (Cambridge, UK) equipped with a 10 mm light path Hellma flow through cell with a volume of 80 $\mu$L. Tygon tubes were used for the liquid channels. The holding and reaction coils were constructed by winding the tubes on perspex rods. Data acquisition and device control were accomplished using a PC-30 interface board (Eagle Electric, Cape Town, South Africa), an assembled distribution board for the conversion of the analogs to digital or vice versa (Mintek, Randburg, South Africa). The Flowtek software package [14, 15] obtainable from Mintek for computer-aided flow analysis, device control and data acquisition was used throughout.

## 6.8 Device sequence

The device sequence for the determination of zinc by sequential injection system is fully outlined in Table 1.

**Table 6.1** The device sequence for the determination of zinc by SIA.

| Time (s) | Pump 1 | Valve 1 | Pump 2 | Valve 2 | Description |
|---|---|---|---|---|---|
| 0.0 | Off | Home | Off | Home | Pumps off, valve 1 select sample through port 1. |
| 2.0 | Reverse | | | | Draw up the sample. |
| 8.0 | Off | | | | Pump 1 off. |
| 9.0 | | Advance | | | Select the dialyser donor stream through port 2. |
| 10.0 | Forward | | | | Propel the sample over the membrane. |
| 25.0 | Off | | | | Pump 1 off. |
| 26.0 | Reverse | | | | Pumps sample over membrane to enhance dialysis. |
| 36.0 | Forward | | | | Agitation of the sample to enhance mass transfer. |
| 46.0 | Reverse | | | | Agitation of the sample to enhance mass transfer. |
| 56.0 | Forward | | | | Agitation of the sample to enhance mass transfer. |
| 66.0 | Reverse | | | | Agitation of the sample to enhance mass transfer. |
| 76.0 | Forward | | | | Agitation of the sample to enhance mass transfer. |
| 86.0 | Reverse | | | | Agitation of the sample to enhance mass transfer. |
| 96.0 | Forward | | | | Agitation of the sample to enhance mass transfer |
| 106.0 | Reverse | | | | Agitation of the sample to enhance mass transfer. |
| 116.0 | Forward | | | | Agitation of the sample to enhance mass transfer. |
| 126.0 | Off | | | | Agitation of the sample to enhance mass transfer. |
| 127.0 | Reverse | | | | Draw the sample into holding coil . |
| 142.0 | Off | | | | Pump 1 stops. |
| 143.0 | | | Reverse | | Pump 2 draw up the sample, valve 2 at port 1. |
| 152.0 | | | Off | | Pump 2 off. |
| 153.0 | | | | Advance | Valve 2 select the reagent solution, and valve 2 at port |
| 154.0 | | | Reverse | | Pump 2 draw up the reagent solution. |
| 163.0 | | | Off | | Pump 2 off. |
| 164.0 | | | | Advance | Valve 2 select the masking reagent-buffer solution. |
| 165.0 | | | Reverse | | Pump 2 draw up the masking reagent-buffer solution |
| 172.0 | | | Off | | Pump 2 off. |
| 173.0 | | | | Advance | Valve 2 select the detector channel at port 4. |
| 174.0 | | | Forward | | Pump 2 pushes the formed zones to the detector. |
| 175.0 | Forward | | | | Pump 1 rinses the donor stream of the dialyser. |
| 274.0 | | | Off | | Pump 2 off. |
| 275.0 | | | | Advance | Valve 2 select the acceptor stream of the dialyser. |
| 276.0 | | | Reverse | | Pump 2 draw up the carrier solution. |
| 297.0 | | | Off | | Pump 2 off. |
| 298.0 | | | | Advance | Valve 2 select waste stream at port 6. |
| 300.0 | | | Forward | | Pump 2 rinse the acceptor stream. |
| 360.0 | Off | Home | Off | Home | Pumps stopped, valves at home. |

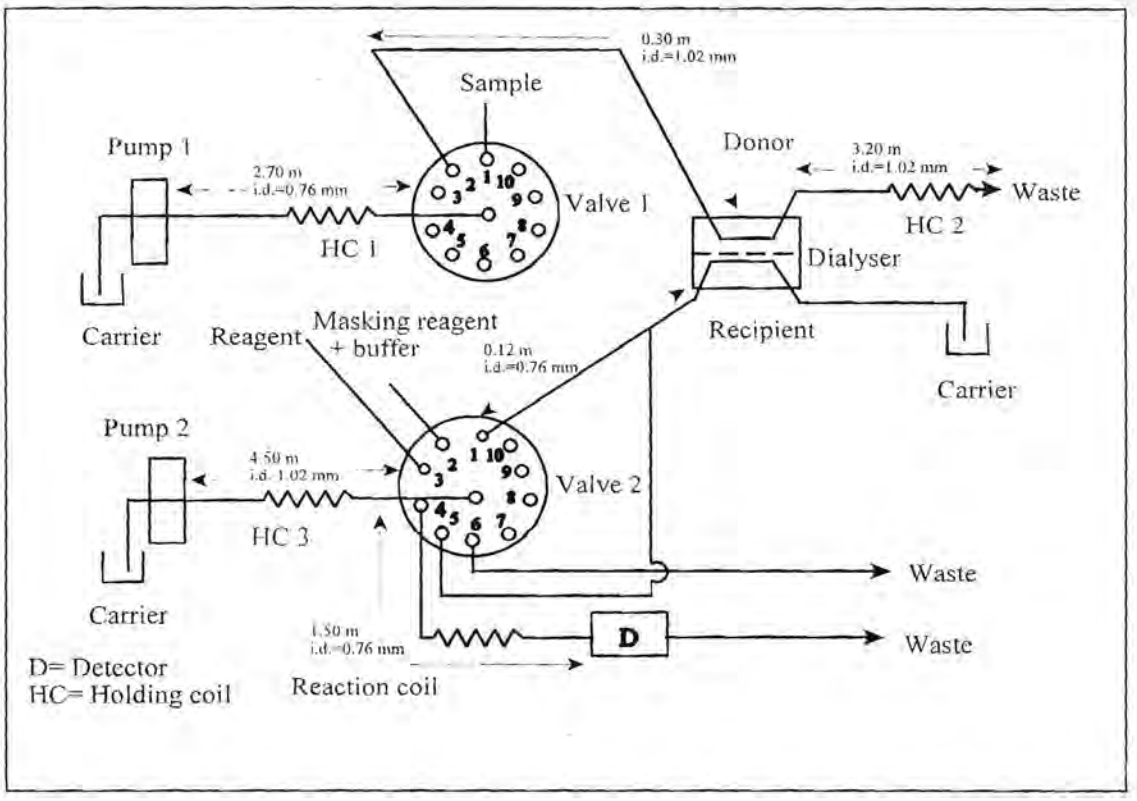


**Fig. 6.1** Schematic representation of the SIA system for the determination of zinc in fertilizers, D= detector at λ=568 nm and HC= Holding coil.

## 6.9 Results and discussions

### 6.9.1 Method optimization

The flow rate of the donor stream was an important parameter which had an influence on the dialysis efficiency. High flow rates prevented the transfer of bulk zinc ions through the membrane into the acceptor stream. The flow rate of the donor channel was varied from 1.0 to 2.24 mL $min^{-1}$. The influence of flow rate was evaluated on the basis of percentage RSD as illustrated in Fig. 6.2.The best % RSD was obtained at a flow rate 1.0 mL $min^{-1}$. Looking at Fig 6.2, it is also apparent that the lower the flow

rate the higher the sensitivity. The best compromise of the percentage RSD was chosen at a flow rate of 1.0 mL $min^{-1}$. Looking at Fig.6.3, good sensitivity and reproducibility are attained at a flow rate of 2.8 mL $min^{-1}$. Sample volumes ranging from 100 to 600 $\mu$L were evaluated based on the reproducibility as depicted on Fig 6.4. Although volume higher than 300 $\mu$L gives high sensitivity values, the problem is that there is loss of reproducibility.

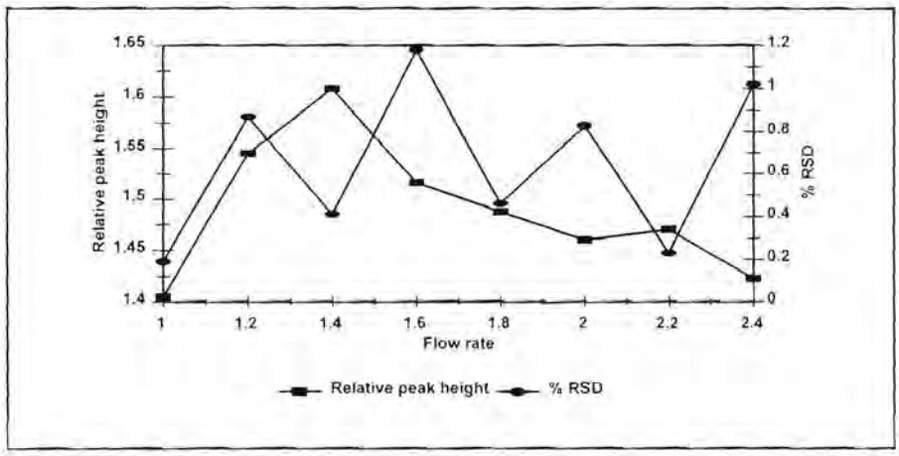


**Fig. 6.2.** The influence of donor stream flow rate (mL $min^{-1}$) on relative peak height and % RSD. [Zn] = 50 mg $L^{-1}$

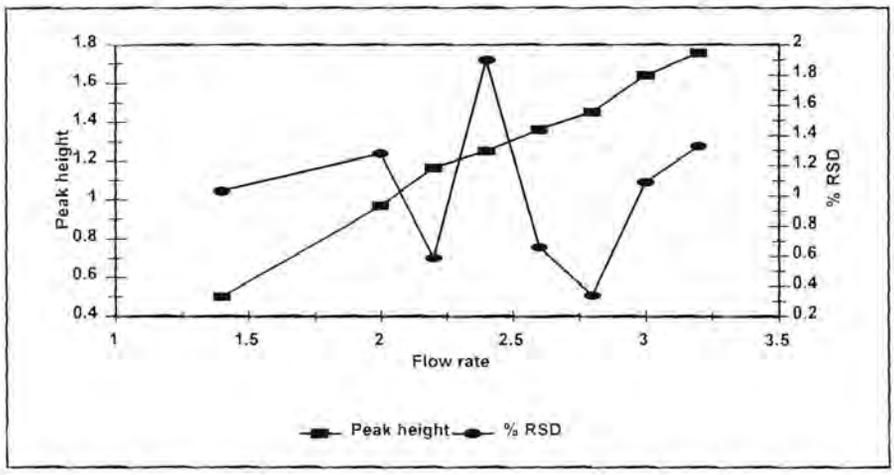


**Fig. 6.3.** The effect of acceptor stream flow rate (mL $min^{-1}$) on relative peak height and %RSD. [Zn] = 50 mg $L^{-1}$.

The volume of 200 $\mu$L was chosen as the optimum owing to the best reproducibility. In order to enhance the transfer of the zinc ions through the passive neutral membrane, optimum conditions are of utmost important in the design and operation of the system. Firstly, the zinc sample must be drawn and placed on the passive neutral membrane. In this case the time needed to propel the sample over the membrane was 15 seconds. This was done to ensure that the most concentrated part of the sample is located over the membrane. Multiple flow reversals were then used to enhance the mass transfer of the zinc ions across the membrane. The optimum time for the flow reversals was 10 seconds for the reverse step and 10 s for the forward step. The time was increased by adding the forward and the reverse steps simultaneously as illustrated on Fig 6.5. The sensitivity was increasing with the addition of steps. The total time for the dialysis was evaluated ranging from 20 to 100 seconds. The dialysis period of 100 seconds was chosen as the optimum due to the high sensitivity and good reproducibility. After the mass transfer period the sample was then pumped back in Holding Coil 1 to ensure that the sample is held back before the dialyzed one can be drawn. The time for the single step of the flow reversals was then evaluated. Time evaluated varied from 10-2 seconds including the stopped flow of 100 seconds between the first and the last 15 seconds.

**Table 6.2** The influence of multiple flow reversals as well as stopped flow period on response, % RSD and percentage dialysis for the dialysis time of 100 seconds

| Time (s) | Peak height | % RSD * | % Dialysis |
|---|---|---|---|
| Stopped flow | 1.3379 | 0.56 | 0.06 |
| 2 | 1.4214 | 1.04 | 3.91 |
| 5 | 1.3374 | 1.85 | 4.04 |
| 7 | 1.4736 | 1.1 | 6.32 |
| 10 | 1.5288 | 1.27 | 8.86 |

*n= 5

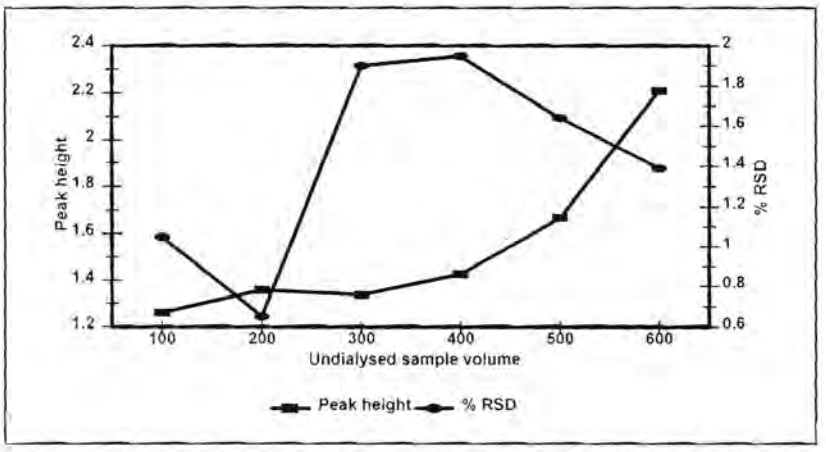


**Fig. 6.4.** The influence of undialysed sample volume ($\mu$L) on the relative peak height and % RSD. [Zn] = 50 mg $L^{-1}$.

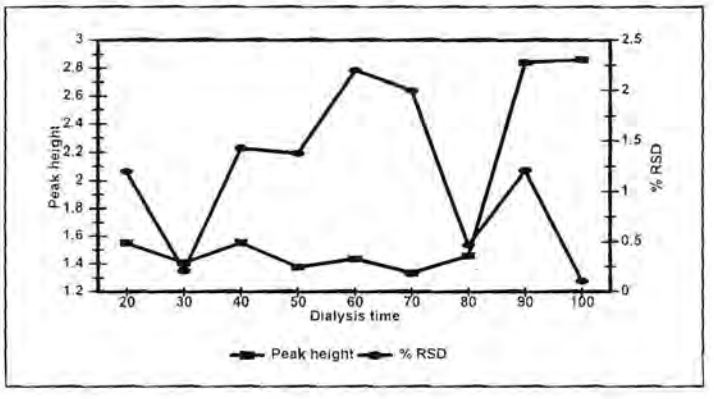


**Fig. 6.5** The effect of dialysis time (s) on peak relative peak height and % RSD. [Zn] = 50 mg $L^{-1}$.

The dialyzed sample volumes from 195 to 780 $\mu$L were also evaluated (Fig 6.6). A volume of 585 $\mu$L was chosen as the optimum volume owing to the best % RSD. Volumes higher than 585 $\mu$L shows lost in the reproducibility. Reagent volume ranging from 195 to 715 $\mu$L were evaluated ( Fig.6.7). This shows an increase in sensitivity as the reagent volume is increased. This is due to the fact that more sample is converted into a product. A volume of 455 $\mu$L was chosen as the optimum considering the best reproducibility of the peak profile.

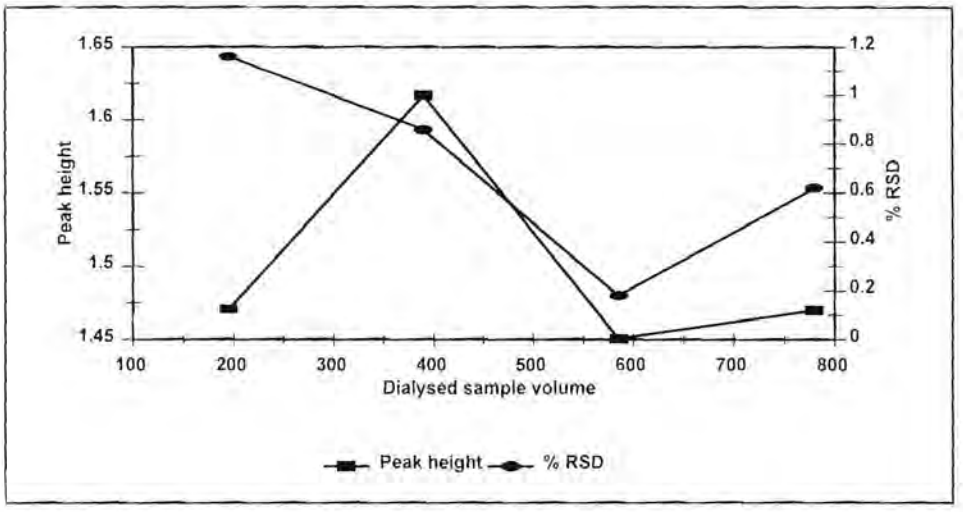


**Fig. 6.6** The influence of dialyzed sample volume ($\mu$L) on relative peak height and % RSD. [Zn] = 50 mg $L^{-1}$.

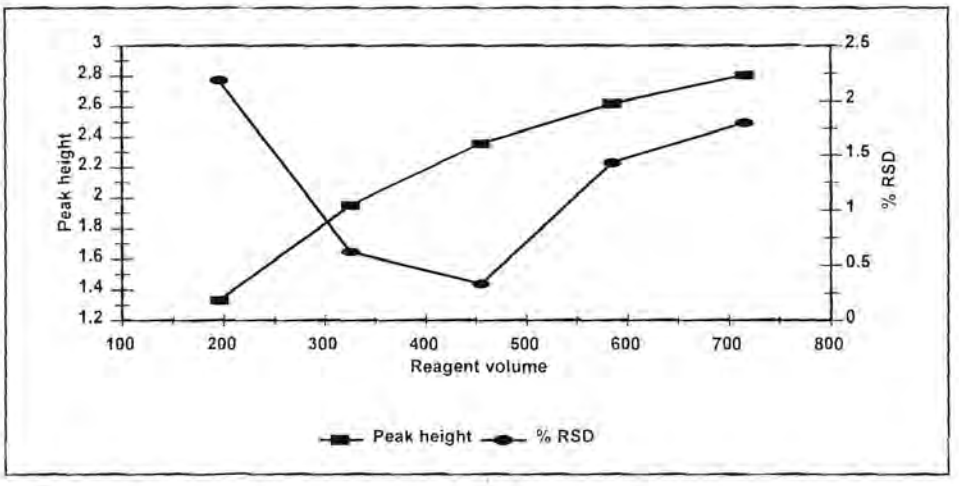


**Fig 6.7 .** The effect of reagent volume ($\mu$L) on relative peak height and % RSD. [Zn] = 50 mg $L^{-1}$.

Different lengths of the reaction coil ranging from 1.00 to 2.50 m were experimented and evaluated (Fig. 6.8). At lengths above 1.50 m there is a lost in sensitivity. This is due to the fact that at early stages the sample and the reagent mutually merge and disperse as the reaction is taking place to form a product, and therefore the product eventually disperses into the carrier stream before reaching the detector. A

dispersion coil of 1.50 m was compromised for, owing to the best sensitivity and reproducibility of the peak profile.

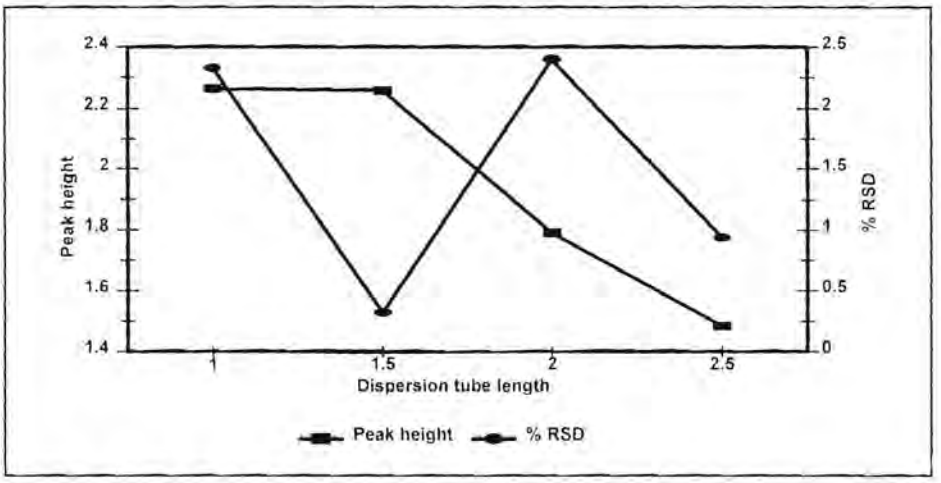


**Fig. 6.8** The influence of reactor coil length (m) on relative peak height and % RSD. [Zn] = 50 mg $L^{-1}$.

Nickel (II), copper (II) and lead(II) are possible interfering species to this method. The interfering species was eliminated by the addition of sodium thiosulphate as the masking agent, and the control of pH of the buffer solution. The undissolved materials which are suspended in the sample matrix were eliminated by the passive neutral membrane incorporated into the conduits of the system.

The fully optimized system is summarized as follows:

| | |
|---|---|
| Donor stream flow rate: | 1.00 mL $min^{-1}$. |
| Acceptor stream flow rate: | 2.80 mL $min^{-1}$. |
| Undialysed sample volume: | 200 $\mu$L. |
| Dialysis time: | 100 s. |
| Time for one flow reversal step: | 10 s. |
| Dialyzer sample volume: | 585 $\mu$L. |

| | |
|---|---|
| Reagent volume | 455 $\mu$L. |
| Dispersion tube length: | 1.50 m. |

### 6.9.2 Method evaluation.

The resultant SIA analyzer was critically evaluated with regard to reproducibility, linearity, detection limit, accuracy, sample interaction and sampling frequency. To test the reproducibility of the SI analyzer, five repetitive analysis cycles of the sample were determined and the %RSD of the sample was better than 0.55 as shown in Table 6.2. Under the optimum conditions, the calibration curve was linear between 10 and 50 mg $L^{-1}$ with the relationship between peak height and zinc(II) ion concentration given by the relation $y = 0.0434x + 1.3365$, where y = peak height and x = zinc ion concentration and a linear regression coefficient $r = 0.998$, $(n = 5)$.

The detection limit was calculated using the relation $[3(\sigma + k) - c]/m$, where $\sigma$ (0.0001) is the relative standard deviation of the baseline sensitivity, k (0.5005) is the average signal value of the baseline and c (1.3365) and m (0.0434) is the intercept and the slope of the calibration curve respectively. The calculated detection limit was found to be 4.75 mg $L^{-1}$.

The accuracy of the proposed SIA analyzer was evaluated by comparing the results with those obtained by the standard flame atomic absorption spectrometric method. The results shown in Table 6.2 revealed good correlation of the two methods. Sample interaction or the carry over effect was investigated by analyzing a sample with a low analyte concentration followed by that of high concentration and again that of low concentration. A sample containing 8.0 mg $L^{-1}$ was used to represent the low analyte concentration and that of 40 mg $L^{-1}$ for the high analyte concentration. Sample interaction was then

calculated using the following relation:

$$\text{Sample interaction} = \frac{A_3 - A_1}{A_2} \times 100\%$$

Where $A_1$ is the sensitivity of the analyte containing 8.0 mg $L^{-1}$ zinc, $A_2$ is the sensitivity of the analyte containing 40 mg $L^{-1}$ zinc and $A_3$ is the response of the analyte containing 8.0 mg zinc. The calculated carry over effect was found to be 0.0487 %.

The experimental period for one complete analytical cycle was 360s. This gave the overall sampling rate of 10 samples per hour.

The comparison was done between the SIA method and FAAS (Table 6.3). This comparison was done to establish whether the SIA system can be accepted as giving reliable results in the determination of zinc. The null hypothesis was used. The t-test with multiple samples (paired by difference) was applied to examine whether the two methods differed significantly at 95% confidence level. The null hypothesis Ho: $\zeta = 0$ against the alternative $\zeta \neq 0$. Where $\zeta$ is the population paired by difference [16, 17]. At 95 % confidence level, it was found that the critical values are ± 3.182 and the calculated value is 2.67. Since the calculated value is less than the critical value $H_o$ cannot be rejected and it follows that there is no statistically significant difference between the two methods.

Table 2. Comparison of results obtained for the samples by SIA and FAAS standard method

| Sample | FAAS method | | SIA method | |
|---|---|---|---|---|
| | [Zn] mg $L^{-1}$ | % RSD* | [Zn] mg $L^{-1}$ | % RSD* |
| 1 | 39.1 | 2.5 | 36.28 | 0.16 |
| 2 | 42 | 0.9 | 42.25 | 0.35 |
| 3 | 41.8 | 2.1 | 38.47 | 0.53 |
| 4 | 37.7 | 1.4 | 34.08 | 0.51 |

* n=5

## 6.10 Conclusion

The proposed system is suitable for the analysis of turbid samples with undissolved particles suspended in the sample matrix. The particles are of a nature that continuously block the inlets of the tubes and also interfere in the reaction. There are numerous papers reported on the analysis of zinc with the FIA method using xylenol orange as the color reagent with high sampling frequencies. Although the analytical throughput of this method is low, it still outclasses the FIA counterparts owing to the fact that it is interference free, it use cheap and readily available equipment, it uses microlitres of the reagents and solutions per analytical cycle, full automated and very simple to assemble and operate. The method has got a linear range of 10 to 50 mg $L^{-1}$ with a detection limit of 4.75 mg $L^{-1}$ and consumes 200 $\mu$L of the sample per analytical cycle.

## 6.11 References

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# Chapter 7

# On-line dialysis and sequential injection acid-base titration: Determination of total acidity in fruit juices

## 7.1 Introduction

Titration is one of the most used techniques in the field of industrial process monitoring. Time consumption, difficulties in sample collection and collecting and plotting of the calibration graphs large amounts of reagent consumption [1] are the major drawbacks of these procedures as analytical tools. Since it was first reported by Ruzicka *et al.* [2] in 1977, several systems based on flow injection (FI) titration has been developed. With the help of FI titration many of the problems listed above were circumvented. Although they are rapid and they involve less human interaction. One of the draw backs of FI titration is high reagent consumption.

Since it was conceived in 1990 by Ruzicka and Marshall [3] sequential injection analysis (SIA) is a simple and convenient concept of flow analysis developed primarily to circumvent the high reagent consumption experienced in flow injection analysis [4].

Determination of acidity and alkalinity in food and other turbid and highly coloured with macromolecules and suspended solids in the sample matrix require extensive pretreatment of the representative samples. The coupling of dialysers with flow through systems proved to be useful in this regard. Dialysis is a

separation technique employed mainly to provide interference free analysis. In a continuous flow, flow injection or sequential injection/ dialysis combination where the donor stream flows over the acceptor stream and the two streams are separated by a semi-permeable membrane. The separation process and the mass transfer process depend on various aspects such as molecular size and ionic charge, the nature of the membrane (i.e., type of membrane, membrane thickness membrane surface and porosity). Dialysis also facilitates sample dilution.

Typically dialysis is utilized when analyte constituents in complex matrices such as serum, proteins or blood are of interest, permitting diffusable low molecular weight species to be separated from macromolecular components [5]. Van Staden and Basson [6] in developing their FIA procedure for turbidimetric determination of urinary inorganic sulfates, exploited the dilution capability of the dialysis unit.

This chapter describes the incorporation of a dialyser into the conduits of the SI system with the use of multiple flow reversals [4] to enhance the mass transfer and dilution and to eliminate the interfering species. The system is employed for the determination of the total acidity in fruit juices. The acid upon reacting with sodium hydroxide in the presence of the indicator the orange colour end point is formed and it was detected at 420 nm.

## 7.2 Importance of pH in fruit juices

Acid is of fundamental importance to living animals and plants. Citric acid and ascorbic acid are the main sources of acidity in fruit juices. Citric acid is responsible for the production of energy by means of the Krebs cycle and several processes such as the metabolism of fatty acids, carbohydrates and amino acids.

Since it is a harmless substance, it is used in food industry as an antibacterial agent and additive for pH control. Ascorbic acid and citric acid are naturally found in many citrus fruits. Sodium citrate, which is the salt of citric acid is used as an additive in sodas.

Citric acid has been described as nature's acidulent occurring in a wide variety of fruit. In short the main functions of citric acid in fruit juices are: -

a) As a flavour adjunct, to improve taste,
b) as a pH control agent, for gellation control, buffering and preservative enhancement, and
c) as a chelating agent, where it improves the action of antioxidants, and prevents spoilage of foods such as seafood

## 7.3 Choice of analytical method

The determination of acidity in food and beverages is of utmost importance. The selectivity, sensitivity and rapidity are essential for the determination of the pH control of fruit juices. The most commonly used methods are: Titration and the use of pH meters. This methods are prone to interferences arising from sample turbidity, colour and the present of many undissolved matter suspended into the sample matrix which lead to unclear endpoints in the case of titrations.

This chapter deals with the analysis of total acidity (pH) in fruit juices using sequential injection analysis with an on-line dialyser unit fitted with a neutral semi-permeable membrane incorporated in to the channels of the system for sample conditioning. The conditioning of the sample involve the dilution of the sample to obviate colour intensity and turbidity and removal of colloidal matter in the sample.

## 7.4 Experimental

### 7.4.1 Reagents

All chemicals were of an analytical reagent grade unless stated otherwise and all the solutions were made up with double de-ionized water (Continental water system, San Antonio, TX, USA).

The colour developing reagent sodium hydroxide solution (0.05% m/v) containing 1 mL of the prepared indicator per 250 mL of the base, which was standardized against the potassium hydrogen phthalate. The bromothymol blue indicator stock solution was prepared by dissolving 0.4011g of an indicator in 25ml of 96 % ethanol and diluting to 100 mL with water. The citric acid standards from the range 0.8-2.0% (m/v) were prepared by the appropriate dilution of a 30 % (m/v) stock solution of the acid. To evaluate the system, two fruit juices were analysed as they were without any manual dilution or filtration.

### 7.4.2 Equipment.

The SIA manifold (Fig. 7.1) was assembled using the following apparatus: Two peristaltic pumps (Gilson Villiers Le Bel, France) two ten ports electrically actuated selection valves (Valco instrument Co. Inc.), an 80 mm dialyser equipped with a Type "C" cellulose membrane (Technicon) and a Unicam 8625 spectrophotometer (Cambridge UK)equipped with a 10 mm Hellma flow through cell with an internal volume of 80 $\mu$l. Tygon tubes were used for the liquid channels. The holding and the reaction coils were constructed by winding the tubes on perspex rods.

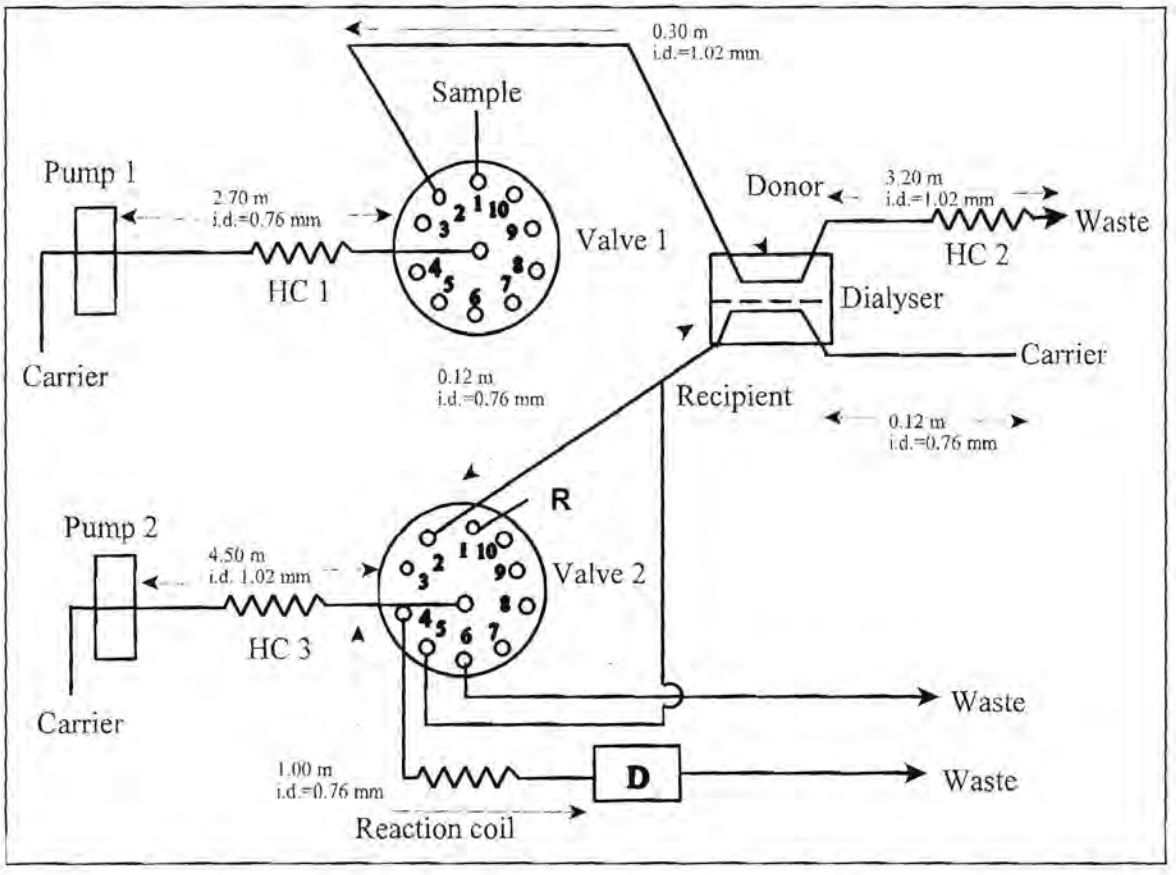



**Fig.7.1** Schematic representation of the SIA system for the determination of total acidity in fruit juices, R=reagent, HC = holding coil and D= detector at λ=430 nm.

Data acquisition and device control were accomplished by using a PC-30 interface board ( Eagle electric, Cape Town, South Africa), an assembled distribution board (Mintek, Randburg, South Africa) The Flowtek soft ware package[7] obtainable from Mintek for computer-aided flow analysis, device control and data acquisition was used throughout.

### 7.4.3 Device sequence

Device sequence for the determination of acidity in fruit juices by sequential injection analysis is fully

outlined in Table 1.

**Table 7.1**. Device sequence for one cycle of SIA fro the determination of total acidity.

| Time | Pump 1 | Valve 1 | Pump 2 | Valve 2 | Description |
|---|---|---|---|---|---|
| 0 | Off | Home | Off | Home | Pumps off, valve 1 select sample through port 1. |
| 1 | Reverse | | | | Draw up the undialyzed sample. |
| 4.33 | Off | | | | Pump 1 off. |
| 6 | | Advance | | | Select the dialyser donor stream through port 2. |
| 7 | Forward | | | | Propel the undialyzed sample over the membrane. |
| 22 | Off | | | | Pump 1 off. |
| 23 | Reverse | | | | Pumps sample over membrane to enhance dialysis |
| 28 | Forward | | | | Agitation of the sample zone to enhance mass transfer. |
| 33 | Reverse | | | | Agitation of the sample zone to enhance mass transfer. |
| 38 | Forward | | | | Agitation of the sample zone to enhance mass transfer. |
| 43 | Reverse | | | | Agitation of the sample zone to enhance mass transfer. |
| 48 | Forward | | | | Agitation of the sample zone to enhance mass transfer. |
| 53 | Reverse | | | | Agitation of the sample zone to enhance mass transfer. |
| 58 | Forward | | | | Agitation of the sample zone to enhance mass transfer. |
| 63 | Reverse | | | | Agitation of the sample zone to enhance mass transfer. |
| 68 | Forward | | | | Agitation of the sample zone to enhance mass transfer. |
| 63 | Off | | | | Pump 1 stops |
| 68 | Reverse | | | | Pump 1 draw the sample into holding coil 1 |
| 74 | Off | | | | Pump 1 stops |
| 75 | | | Reverse | | Pump 2 draw up the dialysed sample, valve 2 at port 1. |
| 77.7 | | | Off | | Pump 2 off. |
| 79 | | | | Advance | Valve 2 select the reagent solution, and valve 2 at port 2 |
| 80 | | | Reverse | | Pump 2 draw up the reagent solution. |
| 82 | | | Off | | Pump 2 off. |
| 83 | | | | Advance | Valve 2 select the detector channel at port 4. |
| 84 | | | Forward | | Pump 2 pushes the formed zones to the detector. carrier. |
| 85 | Forward | | | | Pump 1 rinses the donor stream of the dialyser. |
| 185 | | | Off | | Pump 2 off. |
| 186 | | | | Advance | Valve 2 select the acceptor stream of the dialyser. |
| 187 | | | Reverse | | Pump 2 draw up the carrier solution. |
| 207 | | | Off | | Pump 2 off. |
| 208 | | | | Advance | Valve 2 select a waste stream at port 6. |
| 209 | | | Forward | | Pump 2 rinse the acceptor stream. |
| 260 | Off | Home | Off | Home | Pumps off, valves at home. |

## 7.5 Results and discussions

### 7.5.1 Method optimisation

Various factors influence the peak width of the acid sample in the sequential infection titration. i.e. the flow rates of the donor and the acceptor streams, zone volumes of the dialyzed acid and the base (titrant), the volume of the acid sample injected to the donor stream and the reaction coil length and therefore were optimized for the system.

Different flow rates have been studied and compared to. To compare the different flow rates it was it was important to keep the volumes of the reagent and sample constant. This was made possible by increasing the draw up times for the different zones as the flow rates are increased. A slower flow rate of 1.60 mL min was found to be the optimum flow rate for the donor stream owing to the best reproducibility of the peak profile. (Fig. 7.2). It was discovered that a little faster flow rate of 1.8 mL min for the acceptor stream gave the best precision and it was chosen as the optimum flow rate (Fig.7.3 ).

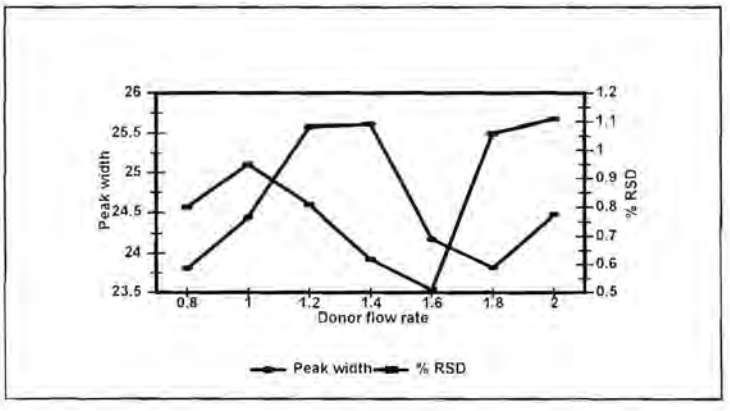



**Fig. 7.2** The effect of donor flow rate (mL $min^{-1}$) on relative peak width and % RSD. [Citric acid]=1.2 % (w/v).

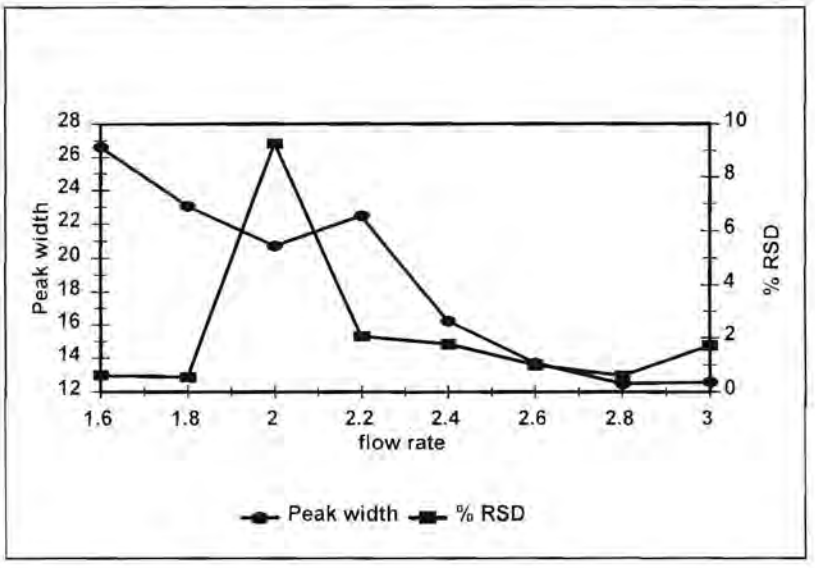



**Fig. 7.3** The influence of acceptor stream flow rate (mL $min^{-1}$) on relative peak width and % RSD. [Citric acid]=1.2 % (w/v).

In this type of sequential injection system, two types of sample volumes are encountered. The undialysed sample volume and the dialyzed sample volume. Different undialysed sample volumes were studied and it was found that a sample volume of 100 $\mu$L gave the best precision and it was chosen as the optimum. (Fig. 7.4 ). Dialyzed sample volumes were also evaluated and the sample volume of 80 $\mu$L was chosen as the foremost due to the best reproducibility (Fig. 7.5). Various reagent volumes were evaluated (Fig. 7.6) The volume of 60 $\mu$L was found to be the optimum volume considering the high reproducibility of the analytical profile.

As the reaction coil length is increased, the peak width increased. This is due to the fact that the sample volumes injected react with the reagent solution and eventually disperse into the carrier stream before reaching the detector. A reactor coil length of 1.00 m was chosen as the optimum owing to the best

sensitivity and precision (Fig. 7.7).

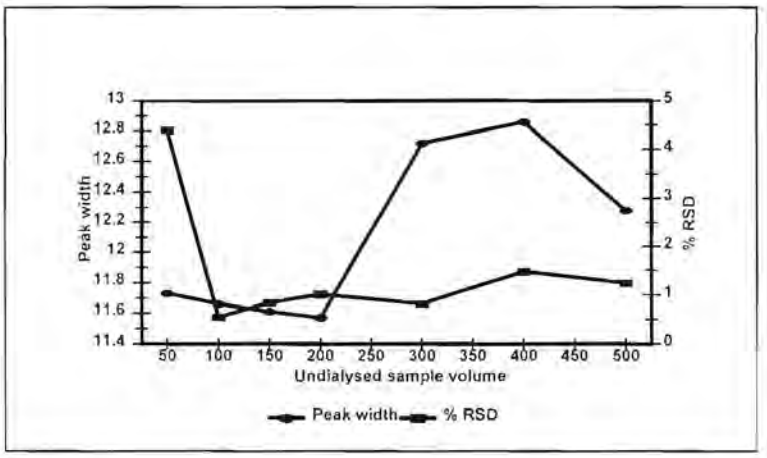



**Fig. 7.4** The influence of undialysed sample volume ($\mu$L) on relative peak width and % RSD. [Citric acid]=1.2 % (w/v).

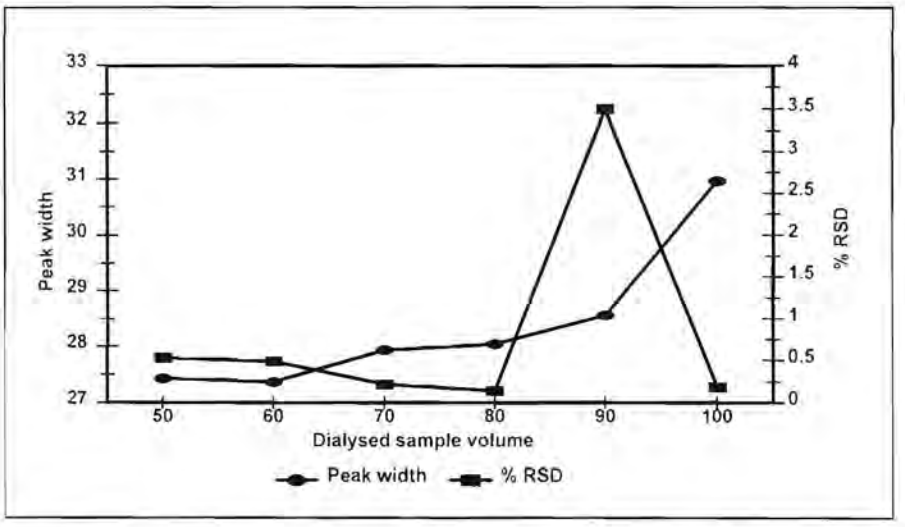



**Fig** 7.5. The effect of dialysed sample volume ($\mu$L) on relative peak width and % RSD. [Citric acid]=1.2 % (w/v).

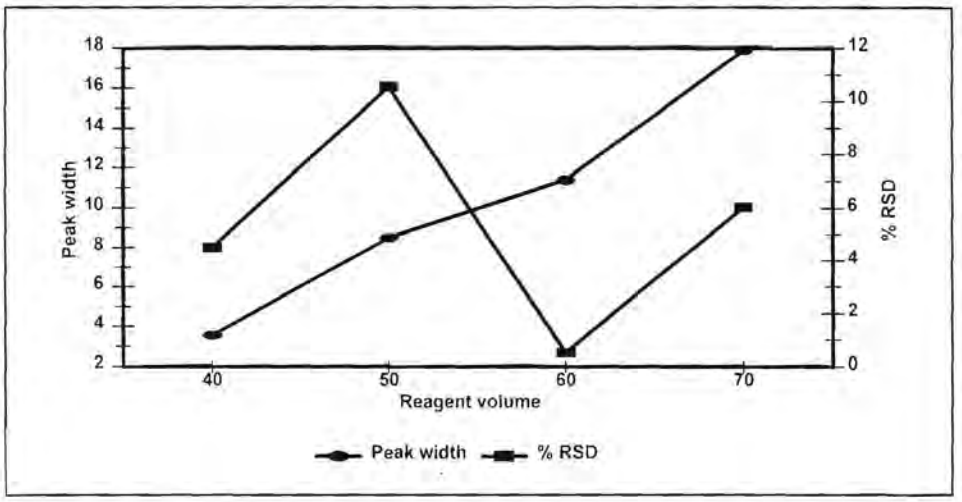



**Fig 7.6.** The influence of reagent volume ($\mu$L) on relative peak width and % RSD.[Citric acid]=1.2 % (w/v).

When a dialyser fitted with a dialysis membrane is incorporated into the conduits of a sequential injection analyser, the contact time of the sample with the dialysis membrane is having much influence on the quantity of the sample dialyzed [3]. By propelling the sample zone forward into the donor stream for 15 s over the dialysis membrane the most concentrated part of the sample was placed on top of the membrane before the start of the multiple flow reversals.

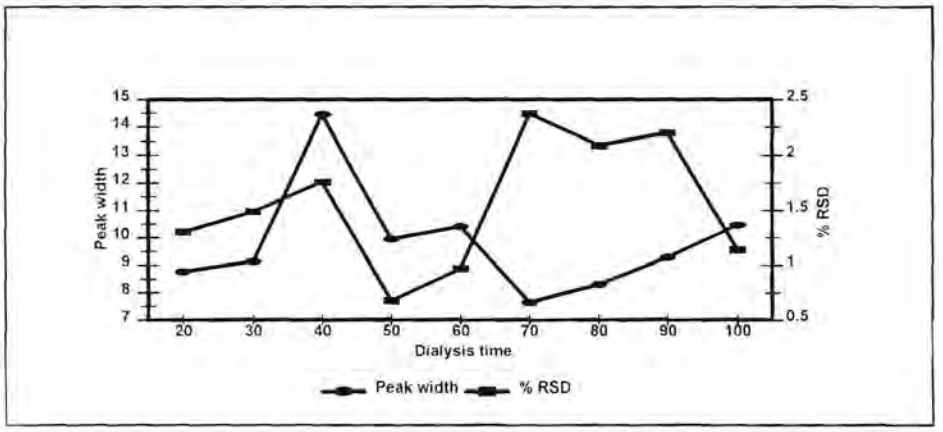



**Fig** 7.7. The influence of dialysis time (s) on relative peak width and % RSD. [Citric acid]=1.2 % (w/v).

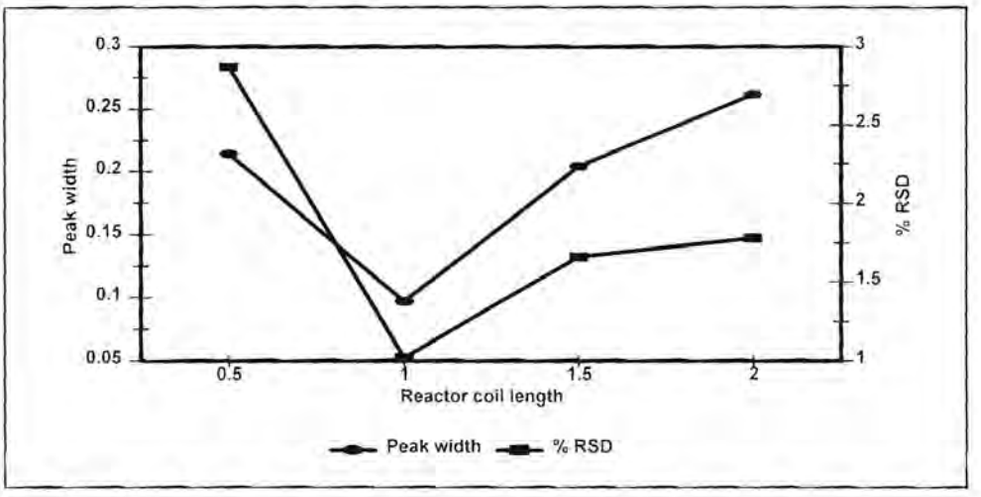



**Fig. 7.8.** The influence of reaction coil length (m) on peak width and % RSD. [Citric acid]=1.2 % (w/v).

Multiple flow reversals [3] were then used to enhance the mass transfer across the membrane and the dilution of the sample with the carrier stream. The optimum time between the flow reversals was found to be 5s for the reverse step and 5s for the forward step (Table 7.3). The total dialysis time was increased by either adding the forward or the reverse step. A total dialysis time of 50 s was found to be the optimum due to the consistent peak width. After the multiple flow reversals the sample was drawn backwards for a period of 15s ensure that the sample is held into holding coil 1 (Fig. 7.1) before the dialysed sample is drawn and held in holding coil 2.

**Table 7.2** The effect of multiple flow reversals on peak width including the stopped flow.

| **Time (s)** | Stopped flow | 2 | 5 | 7 | 10 |
|---|---|---|---|---|---|
| **Peak width** | 4.17 | 9.95 | 11.19 | 8.5 | 6.98 |
| **% RSD*** | 3.74 | 1.42 | 1.23 | 1.4 | 1.28 |

*n=10

**Table 7.3**. Comparison of the SIA results with those obtained by the manual titration method.

| **Sample name** | **SIA method** | | **Titration method** | |
|---|---|---|---|---|
| | **% Acidity** | **%RSD*** | **% Acidity** | **% RSD*** |
| Sample 1 | 0.89 | 1.22 | 0.85 | 0.81 |
| Sample 2 | 1.41 | 1.44 | 1.33 | 1.12 |
| Sample 3 | 1.42 | 1.28 | 1.51 | 0.69 |

* n=5

The experimental time for one complete analytical cycle was 260 s and this gave an overall sampling rate of 13 samples per hour. Although the sampling frequency is much lower compared to the high speed FI titration [2], the reagent consumption is lower than those of the FI titrations.

The comparison was done between the SIA and the standard titration method. The comparison was performed to establish whether the SIA system can be accepted as giving reliable results in the determination of the total acidity in substances. The null hypothesis was used. The t-test with multiple samples (paired by difference) was applied to examine whether the two methods differed significantly at 95 % confidence level. The null hypothesis $H_o$: $\zeta=0$ against the alternative $\zeta\neq0$ where $\zeta$ is the population paired difference [8 , 9 ]. The calculated t value ($t_{calc}$) was found to be 0.204. The tabulated t value at 95 % confidence level for two degrees of freedom is 4.303, therefore the $t_{calc} < t_{table}$ and this conclude that there is no significant difference between the two methods and the SI analyser is accepted as giving reliable results in the determination of the total acidity of substances.

### 7.5.2 Method evaluation

The proposed SI titration system was critically evaluated with regard to linearity of the calibration curve, accuracy, precision, sampling frequency and interferences. After a parameter was optimised, it was then immediately incorporated as part of the system.

Under optimised conditions the calibration graph was found to be linear from 0.8-2.0 % (w/v) citric acid, r= 0.997, n=5, y=36.47x +10.11. The calibration graph was plotted as peak width as the function of the logarithm of concentration.

The detection limit of the system was evaluated using the formula $[(3\sigma + k) - c]/m$, where $\sigma$ ( 0.00) is the standard deviation of the of the base line, k (0.00) is the average signal value of the baseline and c ( 10.11) and m (36.47) are the intercept and the slope of the of the calibration curve respectively. The calculated detection limit of the SI analyser was found to be 0.277 % (m/v).

The accuracy of the optimised SI analyser by comparing the results of the system and those obtained by the standard titration method. The results and excellent agreement between the two methods.(Table 7. 3). In both methods the % RSD > 1.50 was obtained. To test for the precision of the system, five repetitive analysis cycles of the true samples were determined and the % RSD of the samples was better than 1.3 0%.

Fruit juices usually contain many undissolved substances and they are very colourful. Their sample matrix is a source of potential interference to the method. The suspended solids, colour intensity and the turbidity of the samples were removed by a dialyser coupled to the SI titration system. Multiple flow reversals were introduced to enhance the dilution factor of the samples.

## 7.6 Conclusions

The results obtained with novel dialysis SI titration agree with its feasibility for the use in industrial beverage analysis, which could replace more complex, time consuming and expensive methods for the acidity control of many fruit drinks. The main features of the proposed SIA method are: Low detection limit, good precision and low reagent consumption. The fully automated method can be used in many routine laboratory analyses for the monitoring of the acidity of beverages and the samples are injected as they are occurring no pretreatment is necessary unless they are extremely turbid and colourful.

## 7.7 References

1. J.F. van Staden and H. du Plessis, **Analytical Communications,** 34 (May 1997) 147
2. J. Ruzicka and G. D. Marshall, **Anal Chim. Acta**, 237 (1990) 329
3. J.F. van Staden, H. du Plessis and R.E. Taljaard, **Anal. Chim. Acta,** 357 (1998) 141
4. J. Ruzicka, E.H. Hansen and H. Mosbaek, **Anal Chim. Acta** (1997)
5. J. Ruzicka and E.H. Hansen, **Flow Injection Analysis.** 2nd Edition, John Wiley & Sons, (1988).
6. J.F. van Staden and W.D. Basson, Lab. Prac., 29 (1980) 1279.
7. G.D. Marshall and J.F. van Staden, **Process Control and Quality,** 3 (1992) 251.
8. G.D. Christian, **Analytical Chemistry**, 5th Edition, Wiley (1994) 34.
9. D. McCormack and A. Roach, **Measurement, Statistics and computation. Analytical Chemistry by Open Learning,** Wiley (1997) 239.

# Chapter 8

# Conclusions

The aim of the research was to incorporate a dialysis minichamber fitted with a passive neutral membrane into the channels of the SI system and the use of multiple flow reversals to enhance separation and dilution in the analysis of food and fertilizer products. All extra parameters brought into the system by the incorporation of the dialysis minichamber were taken into consideration and were critically evaluated. To test for the success of the system analysis of food samples and fertilizer samples was performed and the results obtained were compared with those obtained with the standard methods.

The analysis of the product was successfully executed and it was found that there was no significant difference on the methods developed and their corresponding standard methods of analysis. It was proved that incorporating a dialysis system incorporated with a neutral semipermeable membrane into the conduits of an SIA system shown much improvement in the efficiency of dialysis process, good reproducibility, enhanced dilution and a vast reduction in sample volume and interfering species with the reduction of the reagent volume as well.

It was again taken into consideration that longer dialysis time lead to the reduction in analytical through put. The system was well rinsed after every measurement cycle and thus eliminating the sample carryover effect.

The system was fully computer compatible and can be readily adopted for on-line and routine laboratory analysis. Dialysis can be employed in flow systems to separate the analyte of interest from the sample matrix and can serve as a dilution chamber in analysis of very concentrated samples.

# Addendum 1

## Method construction

Chemistry is a subject that has many areas of interest. This is because chemistry has roots in fields such as quantum mechanics to molecular organization of life, and it deals with a variety of problems in diverse fields, such as analytical, synthetic, physical, and industrial chemistry.

One of the revolutions to affect the field of chemistry is the advancement of spectroscopic techniques. These spectroscopic revolutions it is already undergoing its own revolution as a consequence of new advance affecting the lives of all chemists. The new advance is of course the effect of the computer sciences on chemistry.

For this reason all the experimental works done in this project wa accomplished by the aid of the computer. The software package used was a FlowTEK program developed for FIA and SIA by G.D. Marshall [1]. To explain the use of the FlowTEK program, as it was used in the experimental work of this SIA project. The devices operated from the FlowTEK program were two peristaltic pumps, two selection valves and a UV-VIS spectrophotometer.

## Detector

The UV-VIS detector was changed from the default setting (the default setting converts the peak directly as the signal is received from the detector, thus maximum output from the detector will be at maximum). The change was executed as follows:

Select Setup (S) from the main menu and from the setup menu select Detectors (D). Then the prompts that follow must be answered in the following way.

| | |
|---|---|
| Enter No. of detectors | 1 |
| Enter signal transformation for detector | N (Normal) |
| Enter analog input for detector | 1 (indicates the position where the detector is interfaced to the distribution box). |

## Pumps and the injection valves

The operation of the pumps and the selection valves were accomplished by making use of the method. The dialysis and the detection steps were incorporated into the constructed method. The method for device control and data acquisition were constructed in the following way:

From the Main menu select Method (M) for the method to recognize and identify the devices used.

From the Method menu select Type device (T) and answer the following prompts:

| | |
|---|---|
| Enter No. Of devices | 4 (two pumps and two selection valves) |
| Enter type device 1 | GP (Gilson pump) |
| Enter digital output point for GP | 1 (indicates the position on the distribution box) |

| | |
|---|---|
| Enter type device 2 | SV ( selection valve) |
| Enter digital output point for SV | 3 |
| Enter type device 3 | GP |
| Enter digital output point for GP | 5 |
| Enter type device 4 | SV |
| Enter digital output point for SV | 7 |

Upon completion of the above questions, the screen will be divided into four horizontal columns, each representing one of the devices in the method construction. The horizontal solid yellow line in each column shows that the Gilson pumps are in off position and the selection valves are at home positions.

To specify the total experimental time, go to the Method menu select Exp time (E) and answer the following questions.

| | |
|---|---|
| Enter time to start data collection | 170 |
| Enter experimental time | 360 |

Now the actions of the devices can be entered. A better way of introducing and deleting events is to activate the Num. Lock key on the key board of the Personal Computer. At this point the red square

will flash at the bottom right corner of the screen. To introduce an event, first move the cursor to the column representing the appropriate device using the navigation keys on the keyboard and secondly select Insert (I) from the Method menu. The following example shows the insertion of an event for the selection valve and the pump.

For the selection valve:

| | |
|---|---|
| Enter device No. | 3 |
| Enter time of event | 21 |
| Enter event (SH) | S (step indicates step to the next port and H indicates home port) |

For the pump:

| | |
|---|---|
| Enter device No. | 1 |
| Enter time of event | 1 |
| Enter event (RFO) | R (R indicates reverse, O indicates Off and F indicates Forward) |

To delete an event move the cursor to the column representing the device you want to delete an event from and enter Delete (D) form the Method menu and answer the following questions.

| | |
|---|---|
| Enter device No. | 3 |
| Enter time of event | 21 |

Press the return key then the event will be deleted.

Various events were inserted into the method to fulfill the requirements of the experiment in question and after the construction of the method the method has to be saved. This was accomplished by selecting File (F) from the Method menu and answer the following questions:

| | |
|---|---|
| Save, Retrieve or Erase (SRE) | S |
| Enter method file name | C:\ Flowtek\Supi\zinc\dialysis.met (.met is the suffix for the method file) |

The screen will look like in Fig. A.

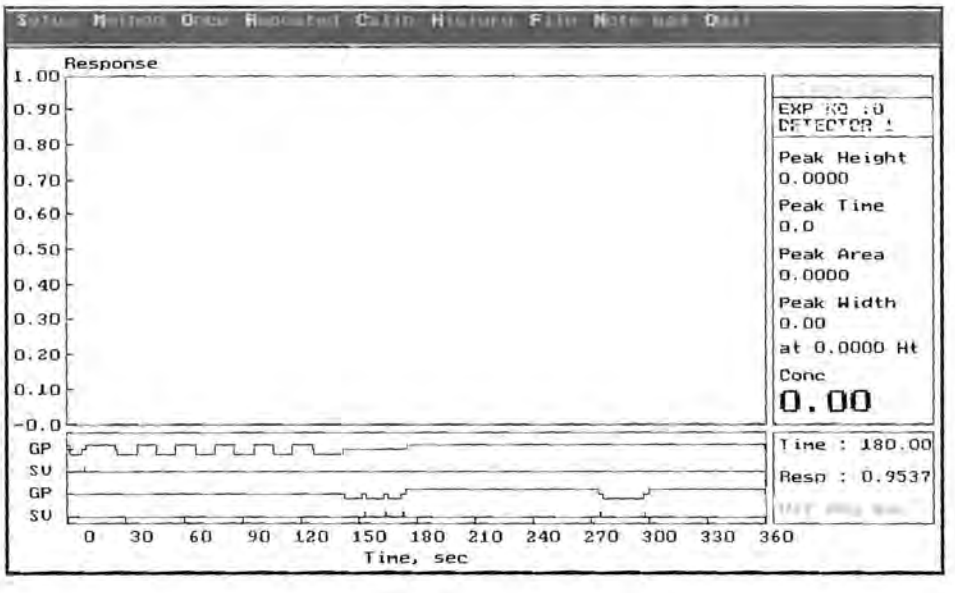



**Fig. A.** A schematic outline of the screen after the method has been saved. Picture taken from [1].

The method constructed can be applied directly as it is or can be build into a procedure. To urn the method directly Select Once (O) from the main menu and the program shall run the method once.

The procedure can be build as follows:

From the main menu select Repeated (R). From the Repeated menu select Build Proc (B) an answer te following questions and commands.

| | |
|---|---|
| Enter procedure file name | C:\flowtek\Supi\zinc\dialysis.pdr<br>(.pdr indicates the procedure file) |
| Enter procedure or method file name | C:\flowtek\supi\zinc\dialysis.met |
| Enter number of repetitions | 1 |
| Enter procedure or method file name | Wait |
| Enter time to wait (in seconds) | 20 |
| Enter procedure or method file name | C:\flowtek\supi\zinc\dialysis.met |

To terminate the definition of a procedure use the ESC key on the keyboard. Thereafter the main procedure must be selected. In the repeated menu select Main Proc. (M) and answer the following command:

| | |
|---|---|
| Enter main procedure file name: | C:\flowtek\supi\zinc\dialysis.pdr |

For saving all the experimental work and results select the option Red. Data file on the Repeated menu. Therefore, answer the following prompts:

| | |
|---|---|
| Enter reduced data file name: | C:\flowtek\supi\zinc\dialysis.red |

On the Repeated menu there is an option for the Profile file which selects the file root name for

storing all the profile data. The extension name gives the number of the experiment number. To execute the main procedure select the option Go (G) from the Repeated menu and to abort press ESC.

To build up the calibration file, Select Calib. (C) from the main menu and select Setup (S) and answer the following prompts.

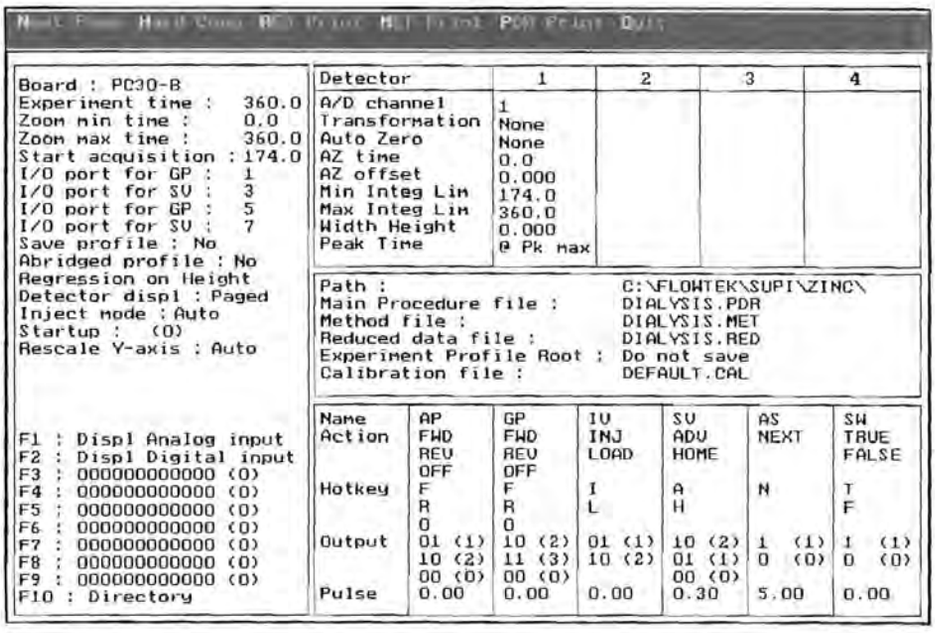
| | |
|---|---|
| Enter No. Of calibration standards | 5 |
| Enter No. Of replicates for each standard | 5 |
| Enter concentration units | mg/L |
| Now enter the concentrations you prepared | |
| Enter reduced data file (ESC for manual input) | ESC |

Select Peak param. (P) from the Calib menu and answer the following questions:

| | |
|---|---|
| Enter peak parameter (H A W T) | A |
| Std. to edit (1-5; 0 for all; ESC) | 0 |
| Enter the calibration model (L Q T E H A) | L |

To save the built calibration file, select File (F) on the Calib menu.

| | |
|---|---|
| Save, Retrieve, Erase (S R E) | S |
| Enter calibration file name | C:\flowtek\Supi\zinc\dialysis.cal |



| | |
|---|---|
| Board | PC30-B |
| Experiment time | 360.0 |
| Zoom min time | 0.0 |
| Zoom max time | 360.0 |
| Start acquisition | 174.0 |
| I/O port for GP | 1 |
| I/O port for SV | 3 |
| I/O port for GP | 5 |
| I/O port for SV | 7 |
| Save profile | No |
| Abridged profile | No |
| Regression on Height | |
| Detector displ | Paged |
| Inject mode | Auto |
| Startup | (0) |
| Rescale Y-axis | Auto |

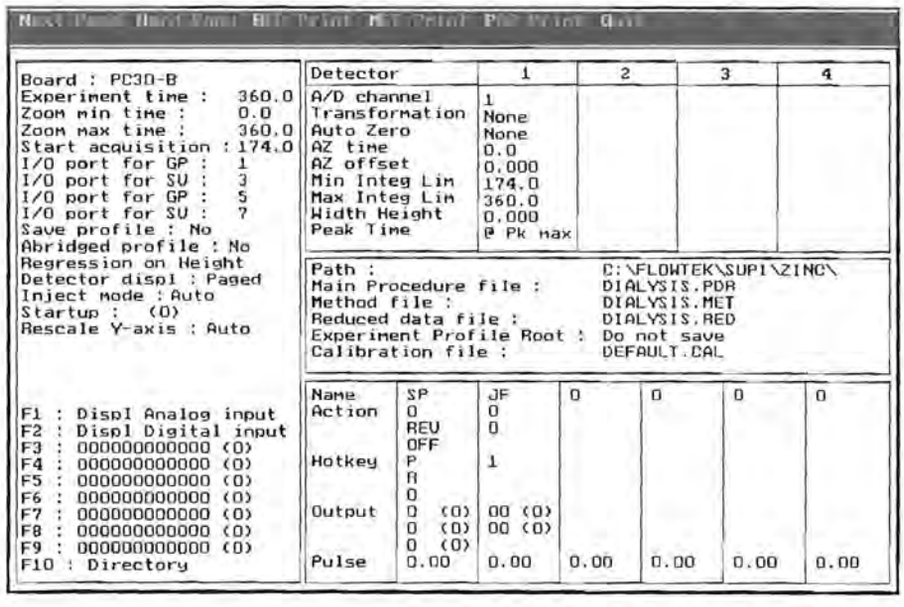
| Detector | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A/D channel | 1 | | | |
| Transformation | None | | | |
| Auto Zero | None | | | |
| AZ time | 0.0 | | | |
| AZ offset | 0.000 | | | |
| Min Integ Lim | 174.0 | | | |
| Max Integ Lim | 360.0 | | | |
| Width Height | 0.000 | | | |
| Peak Time | @ Pk max | | | |

| | |
|---|---|
| Path | C:\FLOWTEK\SUPI\ZINC\ |
| Main Procedure file | DIALYSIS.PDR |
| Method file | DIALYSIS.MET |
| Reduced data file | DIALYSIS.RED |
| Experiment Profile Root | Do not save |
| Calibration file | DEFAULT.CAL |

| | | | | | | |
|---|---|---|---|---|---|---|
| Name | AP | GP | IV | SV | AS | SW |
| Action | FWD | FWD | INJ | ADV | NEXT | TRUE |
| | REV | REV | LOAD | HOME | | FALSE |
| | OFF | OFF | | | | |
| Hotkey | F | F | I | A | N | T |
| | R | R | L | H | | F |
| | 0 | 0 | | | | |
| Output | 01 (1) | 10 (2) | 01 (1) | 10 (2) | 1 (1) | 1 (1) |
| | 10 (2) | 11 (3) | 10 (2) | 01 (1) | 0 (0) | 0 (0) |
| | 00 (0) | 00 (0) | | 00 (0) | | |
| Pulse | 0.00 | 0.00 | 0.00 | 0.30 | 5.00 | 0.00 |

F1 : Displ Analog input
F2 : Displ Digital input
F3 : 000000000000 (0)
F4 : 000000000000 (0)
F5 : 000000000000 (0)
F6 : 000000000000 (0)
F7 : 000000000000 (0)
F8 : 000000000000 (0)
F9 : 000000000000 (0)
F10 : Directory

(i)



| | |
|---|---|
| Board | PC30-B |
| Experiment time | 360.0 |
| Zoom min time | 0.0 |
| Zoom max time | 360.0 |
| Start acquisition | 174.0 |
| I/O port for GP | 1 |
| I/O port for SV | 3 |
| I/O port for GP | 5 |
| I/O port for SV | 7 |
| Save profile | No |
| Abridged profile | No |
| Regression on Height | |
| Detector displ | Paged |
| Inject mode | Auto |
| Startup | (0) |
| Rescale Y-axis | Auto |

| Detector | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A/D channel | 1 | | | |
| Transformation | None | | | |
| Auto Zero | None | | | |
| AZ time | 0.0 | | | |
| AZ offset | 0.000 | | | |
| Min Integ Lim | 174.0 | | | |
| Max Integ Lim | 360.0 | | | |
| Width Height | 0.000 | | | |
| Peak Time | @ Pk max | | | |

| | |
|---|---|
| Path | C:\FLOWTEK\SUPI\ZINC\ |
| Main Procedure file | DIALYSIS.PDR |
| Method file | DIALYSIS.MET |
| Reduced data file | DIALYSIS.RED |
| Experiment Profile Root | Do not save |
| Calibration file | DEFAULT.CAL |

| | | | | | | |
|---|---|---|---|---|---|---|
| Name | SP | JF | 0 | 0 | 0 | 0 |
| Action | 0 | 0 | | | | |
| | REV | 0 | | | | |
| | OFF | | | | | |
| Hotkey | P | 1 | | | | |
| | R | | | | | |
| | 0 | | | | | |
| Output | 0 (0) | 00 (0) | | | | |
| | 0 (0) | 00 (0) | | | | |
| | 0 (0) | | | | | |
| Pulse | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

F1 : Displ Analog input
F2 : Displ Digital input
F3 : 000000000000 (0)
F4 : 000000000000 (0)
F5 : 000000000000 (0)
F6 : 000000000000 (0)
F7 : 000000000000 (0)
F8 : 000000000000 (0)
F9 : 000000000000 (0)
F10 : Directory

(ii)

**Fig. B.** Schematic illustration of the two note pad page, (i) is the first page and (ii) is the second page.

To verify the device descriptions and the file name and the saving modes can be viewed under the Notepad menu by selecting Notepad (N) from the FlowTEK main menu. The notepad menu consists of two pages (Fig. B.).

# References

[1] G.D. Marshall and J.F. van Staden **Anal. Instrum.** 20 (1992) 79

[2] FlowTEK reference manual, **Device control and data acquisition software Version 1.1** Mintek, (1993

# Addendum 2

## Publications

Spectrophotometric determination of chloride in drinking mineral and ground waters using sequential injection analysis.

J.F.van Staden and S.I. Tlowana

Fresenius J. Anal. Chem., 371 (2001) 396.

**Submitted**

I. The use of dialysis membranes for on-line separation and determination of chloride in milk by sequential injection system with the aid of multiple flow reversals.

II. On-line separation and spectrophotometric determination of zinc in fertilizer samples using xylenol orange as a complexing agent.

III. On-line dialysis and sequential injection acid-base titration: Determination of total acidity in fruit juices.

# Conference proceedings

## Poster presentation

Instrumental methods of analysis 2001, University of Ioannina, Greece (September 2001).

**Title:** Spectrophotometric determination of chloride in mineral and drinking waters using sequential injection analysis.

## Oral presentation

South African Chemical Institute (SACI) Young Chemist Symposium, Vaal Triangle Technikon, South Africa, (October 2001) oral presentation.

**Title:** Sequential injection dialysis systems.