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Application of Sequential Injection Systems in the Assay of Pharmaceutical Products

by

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SYNOPSIS

The concept of sequential injection analysis (SIA), introduced as a simple and convenient principle, has established itself as a well defined analytical technique suitable for routine laboratory analysis. This technique is fully computerized and reliable with a reasonable sample frequency, low sample and reagent consumption and low frequency of maintenance.

In pharmaceutical based analysis SIA can be used at each step through the entire production process, from raw material to the final consumer product. With its substantial advantages, an SIA system can replace sophisticated instrumentation facilities, which are unlikely to be used for manufacturing environments.

The aim of this study was to investigate the application of the sequential injection analysis technique in the determination of selected substances of biological importance from the pharmaceutical industry (zinc, paracetamol and boron). It is important to control the level of zinc and boron in human, animals and plants. Overdose of paracetamol is a problem in our body. That is why the uniformity tests of paracetamol must be very accurate and precise. The aim was successfully achieved.

The results obtained for all substances proved the high reliability of the SIA technique.

Toepassing van sekwensiële inspuitanalise in die essaiering van farmaseutiese produkte.

deur

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SAMEVATTING

Sekwensiele inspuitanalise (SIA) is 'n eenvoudige en gerieflike beginsel wat alreeds gevestig is as 'n goed gedefinseerde analitiese tegniek wat geskik is vir roetine laboratoruims. Hierdie tegniek is ten volle gerekenariseerd en betroubaar met 'n redelike monsteruitset, lae monster en reagensverbruik en 'n lae onderhoudfrekwensie.

SIA kan in die farmaseutiese bedryf aangewend word dwarsdeur die hele produksieproses vanaf rou materiaal tot by die finale produk. SIA met sy besondere voordele kan gesofistikeerde instrumentele fasiliteite vervang. Laasgenoemde is nie altyd geskik in die vervaardigengsomgewing nie.

Die doel van hierdie studie was om in met behulp van SIA die bepaling van geselekteerde stowwe, wat biologies belangrik is die farmaseutiese industrie (sink, parasetamol en boron), te ondersoek. Dit is belangrik om die sink-en boronvlakke in mense, diere en plante te kontroleer. 'n Oordosis parasetamol is 'n probleem in die menslike liggaam. Dit is hoekom eenvormige toetse van parasetamol akkuraat en presies moet wees. Die doel is suksesvol bereik.

Die resultate van alle stowwe wat verkry is bevestig betroubaarheid van SIA.

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CHAPTER 1.

Sequential injection analysis - Promoting flow analysis to a new dimension.

1.1. Introduction.

Sequential injection analysis (SIA) has received considerable attention in many fields of chemistry since its introduction in 1990 by Ruzicka and Marshall [1]. SIA has been developed as an alternative to the well known flow injection analysis (FIA). It is a technique that has great potential for on-line measurements due to the simplicity and convenience with which sample manipulations can be automated. SIA offers several advantages to be used in small laboratories or big laboratories. Following below are some of the reasons why sequential injection analysis can be used as a process analyzer [2] without any hesitations.

1. *Cost reduction.*

This is attributed to low sample and reagents consumption. As the reduction of reagent consumption is becoming world-wide a major issue due to the environmental impact of chemical waste, the more cost effective use of reagents is becoming a major advantage of SIA. The instrumentation is inexpensive and easy to assemble making the system an ideal technique for emerging companies with small laboratories.

2. *Reduction or partially elimination of human intervention.*

The reason for the reduction of human intervention in the laboratories has been to increase the objectivity of the analysis. Human intervention is seriously reduced in sequential injection analysis

because the system is fully computerized.

3. *Versatility.*

The system can be easily adapted to a variety of fields, extending from simple single analysis to more complex simultaneous multi-determination analysis.

4. *High sample throughput.*

The system can analyze many samples in a short period of time.

The need to develop a manifold whereby several analysis could be performed simultaneously on a single test specimen arose by the early 1960s. Since then, the use of a single-channel AutoAnalysers became well known by many chemical laboratories [3]. Early studies showed that SIA has the ability to perform determinations of different analytes simultaneously employing a single channel manifold [1]. The versatility of this system is centered around the selection valve and the pump. The selection valve consists of 4-10ports and each port can be dedicated to a specific purpose depending on the chemistry desired. The pump of the SIA system operates in a different way from the pump of FIA from which it evolved. An SIA pump can reverse and forward where as an FIA pump operates only in one way. Because the SIA system is fully computerized the pump and the selection valve are both controlled by a computer. All essential conditions can be changed merely by using a computer keyboard [4]. The system enables the use of different analytical conditions without the need to reconfigure it.

Sequential injection analysis (SIA) and flow injection analysis (FIA) systems operate in complete different ways but they are based upon more or less the same principles. In both systems the controlled partial dispersion is defined in almost the same way. One of the main differences between

the two systems lies in the way the samples and the reagents are introduced into the system [5,6].

In flow injection analysis (Figure 1.1), the sample zone is injected into a flowing carrier stream and the auxiliary reagents are merged with it on the way to the detector. The injected sample forms a slug or zone that is mixed with the carrier and the reagent solutions. Dispersion occurs at the same time. The sample and the reagent solutions form a product that is transported into a flow-through detector for measurement and then to waste.

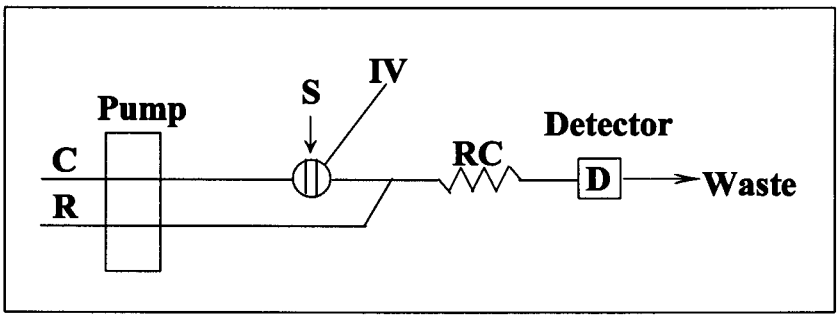

Figure 1.1. General schematic diagram of a flow injection system.
C -carrier, R - reagent, S - sample, RC - reaction coil, IV - injection valve, D - detector.

In sequential injection analysis (Figure 1.2), the sample and the reagent solutions form a stack of well-defined unsegmented zones in a holding coil by using a selection valve and a liquid driver or a pump operating in a reverse mode [4-8]. Dispersion occurs when the sample and the reagent solutions are propelled by the pump towards the detector. The product is measured as the zones pass through a suitable flow through cell in a detector and then to the waste. The order in which the

sample, the reagent solutions and the detector line are placed around the selection valve depends on the desired chemistry.

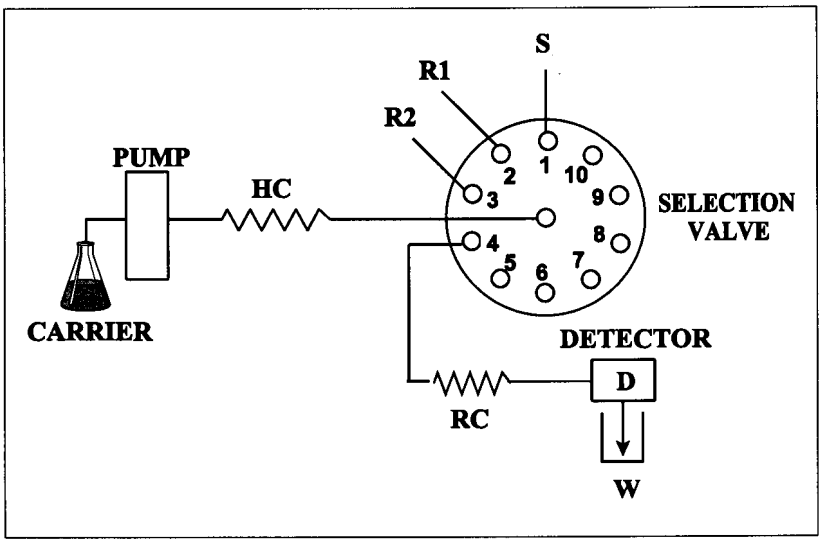

Figure 1.2. General schematic flow diagram of a sequential injection analysis system.

S - sample, R1 - reagent solution 1, R2 - reagent solution 2, HC - holding coil, RC - reaction coil, W- waste.

In both SIA and FIA, dispersion is affected by the change in the following,

1. Injected sample volume.
2. The flow rate of the carrier and the reagent streams.
3. The length and the inner diameter of the reaction coil.

Sequential injection analysis has become a well established and a powerful system for both laboratory analysis and process analytical chemistry. This new generation of flow systems can be

applied in industrial and environmental situations [9]. The limited number of samples that can be analyzed in a particular time by traditional process analytical chemistry can result in an incomplete chemical profile and therefore only partial understanding of the process being monitored. With the use of a sequential injection analysis, rapid process monitoring can be achieved.

1.2. **Flow methods.**

Nowadays, flow analysis is a widespread technique employed in many branches of chemistry. Since the original paper published by Skeggs [10] on the multi segmented continuous flow analysis (CFA), many improvements and even simplifications have been made. Flow assembles is made of manifold through which the sample and the reagent solutions are propelled by the pump towards the detector. Flow methods differ from the batch methods because in batch methods a known amount of the sample and the reagent solutions are held in the individual beakers and then brought together to be mixed in a common beaker. The measurements are taken after chemical equilibrium has been reached. The reproducibility of the signal for the reagent and the sample complex at a given time is dependent on the rate of mixing and consistency of pressure, temperature and the kinetic reaction rates. In flow methods the sample and the reagent solutions are inserted into a chemical stream. A chemical reaction occurs from the point of insertion on the way to the detector. Flow methods can be classified as follows [11]:

1. Segmented continuous-flow methods.
2. Unsegmented continuous-flow methods.
3. Sequential injection analysis.

1.2.1. **Segmented continuous flow methods.**

The term continuous flow method apply to analytical procedures in which the analyte concentration is measured without stopping the flow [12]. In segmented-flow methods, the flowing stream is segmented by air bubbles. The main function of air bubbles is to avoid the carry-over between successively processed samples. The instrumentation required includes the following essential elements:

1. Propulsion unit.
2. Sample and air bubble insertion system.
3. Reactor.
4. Detector.

The signal produced by these assemblies corresponds to a time after which equilibrium is reached. Both chemical and physical equilibrium are reached.

1.2.2. **Unsegmented continuous flow methods**.

Unsegmented continuous flow methods differ from segmented flow methods in that the flow is not segmented by air bubbles, the sample is injected instead of aspirated and neither physical (flow homogenization) nor chemical equilibrium has been attained by the time the signal is recorded. In continuous flow methods (segmented and unsegmented), the sample is introduced at a regular interval into a channel through which flows a liquid containing the reagents. The flow then passes through the flow-cell of a detection system. Flow injection analysis can be classified as unsegmented

continuous flow method with injection of the sample. Besides flow injection analysis (FIA), other flow methods based on unsegmented streams without injection have been described in literature [13].

1.2.3. **Sequential injection analysis.**

This new generation of flow methods differs from continuous flow methods because of the difference in the treatment of the sample and the reagents. In sequential injection analysis the introduction of the reagent solutions and the sample into the system is discontinuous resulting in a minimal consumption of the reagent solutions and the sample. Continuous flow methods are uneconomical in terms of the sample and reagents consumption because the reagents are pumped continuously.

1.3. **Aim of this study.**

Many of the manufacturing industries may be faced with an increasing pressure to produce high quality products in an economically viable and environmentally acceptable manner. This kind of pressure increases the need to maintain and strictly control the plant conditions throughout the production process. This study was aimed at developing simple, rapid, sensitive, accurate and inexpensive methodologies which are economically viable and environmentally acceptable in chemical laboratories.

The simplicity of the SIA manifold and its low need for maintenance makes it an ideal tool for use

in pharmaceutical companies, hospitals and any other chemical companies. In pharmaceutical based analysis SIA can be used at each step through the entire production process, from raw material to the final consumer products. With its substantial advantages, SIA systems can replace the sophisticated instrumentation facilities, which are unlikely to be suitable for manufacturing environments.

The application of an SIA system was exploited in the analysis of the real pharmaceutical samples in comparison with the standard methods. This study presents the analysis of zinc, boron and paracetamol in pharmaceutical products. It is important to control the level of zinc and boron in human, animals and plants. Overdose of paracetamol is a problem in our body. That is why the uniformity tests of paracetamol must be very accurate and precise.

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CHAPTER 2.

Theoretical Background.

2.1. Introduction.

The design of a sequential injection analysis system must be in such a way that mixing of the sample and the reagent solutions is achieved in order to attain maximum degree of dispersion and penetration. Sequential injection analysis has replaced the old ways of handling solutions. The use of beakers and fragile containers has been replaced by the sequential movements of the liquids in tubes. This concept has changed the attitude of many chemists who thought the batch approach, where homogeneous mixing is thought to be the only way of bringing reagents together is the only suitable way in which a reproducible measurement can be taken. The concept of sequential injection analysis has been accepted world wide due to the simplicity of its flow channel.

The way in which the sample and the reagent solutions are introduced into the flow conduit as zones and the mutual penetration of zones as they are propelled towards the detector distinguishes sequential injection analysis (SIA) from the conventional flow system. Much significant research has been done in developing and characterizing sequential injection analysis. It was, therefore, decided to focus this work on the application of the sequential injection analysis in pharmaceutical productions. The following discussion covers some of the important concepts of the theoretical background of sequential injection analysis.

2.2. **Dispersion.**

In addition to the reproducible timing and sample injection, controlled dispersion is regarded as the cornerstone of the family of flow systems. Early studies of FIA showed that controlled dispersion is recognized as being fundamental to the optimum design and understanding of this technique[1]. In sequential injection analysis (SIA), it was noticed that zone penetration in addition to dispersion is of paramount importance.

In contrast to other methods of instrumental analysis, the chemical reaction in both SIA and FIA takes place while the concentration gradient of the sample zone is being formed by the dispersion process. What happens to one injected sample happens in exactly the same way to all other subsequently injected samples. The preceding processes are strictly reproducible in all sequentially occurring sampling cycles.

2.2.1. **Transport.**

Earlier workers thought that the transport of matter was due to turbulent flow but it was not the case since the transport of matter along the tubes is due to laminar flow [1]. The dispersion of the injected sample occurs as a result of the following transport mechanisms:

(1) Convective transport.

(2) Diffusional transport.

2.2.1.1. **Convective transport.**

Convective transport yields a parabolic velocity profile with sample molecules at the tube walls having zero linear velocity where as those at the center of the tube have twice the average velocity. This type of transport occurs under laminar flow conditions (Figure 2.1). Transport of the injected zones in SIA occurs via laminar flow. During zone reversal a short period of turbulent flow occurs, but after a few seconds laminar flow is restored.

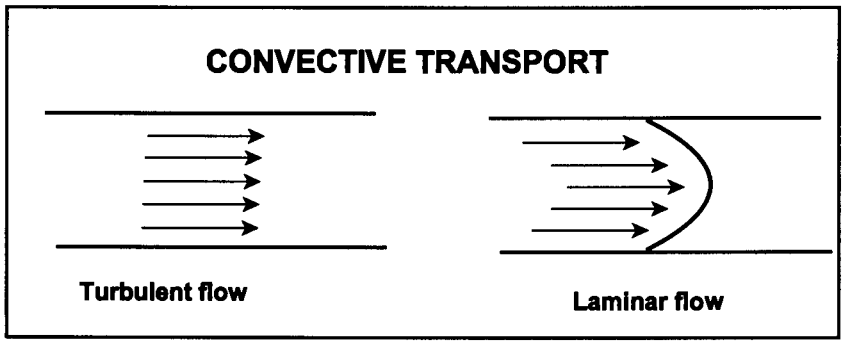

Figure 2.1. Convective transport.

2.2.1.2. **Diffusional transport.**

Diffusional transport causes radial and axial diffusion due to the presence of concentration gradients in its regime. Figure 2.2 illustrates the differences between the radial and the axial diffusion.

1. *Axial diffusion.*

This type of diffusion which has an insignificant contribution to the overall dispersion exists due to the horizontal concentration gradients at the leading and trailing edges of the injected sample zone.

2. *Radial diffusion.*

Radial diffusion as shown in Figure 2.2 results from concentration differences perpendicular to the direction of the flow. It has a significant contribution to the overall dispersion.

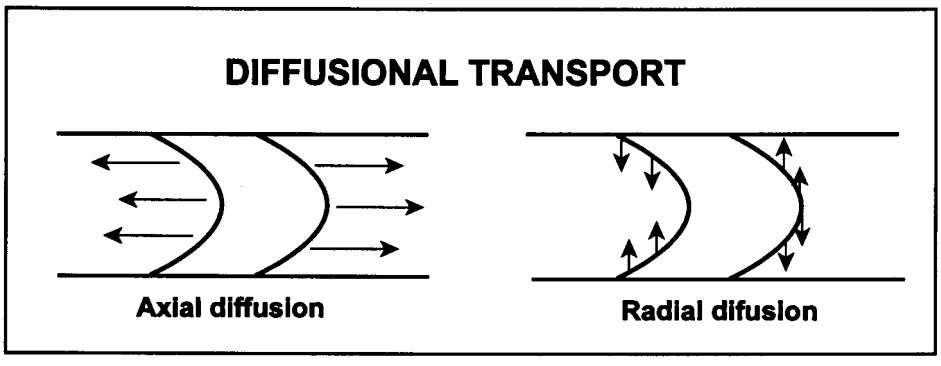

Figure 2.2. Diffusional transport.

2.3. **Quantitative evaluation of dispersion.**

Various attempts have been made to derive a general expression C = *f(t)* accounting for the signal profile and relating its characteristics (travel, residence, and baseline-to-baseline times, and peak height or area) to the experimental parameters of the FIA system (flow rate, reactor length, etc.). A discussion follows on the models that have been developed for laminar flow conditions, to define the theoretical principles of FIA and derive mathematical expressions, of the form C = *f(t)*, accounting for the physical behavior of an injected plug. The list of symbols used in this discussion is given in Addendum C.

2.3.1.. **Taylor's model.**

Taylor's model [1] is applicable if the injected volume is practically negligible compared to the reactor volume ($V_i << V_f$). It only holds for low rates and very long reactors, which help to compensate for radial concentration changes and favor the prevalence of diffusion phenomena. It is applicable to Gaussian distribution defined by C = *f(t)* in a form which depends on the chosen parameters, namely

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

and

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

where m is the injected solute mass ($m = C^o V_i$), σ is the parameter corresponding to the standard deviation of a Gaussian distribution and x is the axial distance from the injection point. This model is not applicable under normal SIA conditions.

2.3.2. **Tanks-in series model.**

This model is based on the view that the liquid flows through a series of ideally stirred tanks of equal size or through a network of parallel ideally stirred tanks. The mathematical expression derived from this model is [3],

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

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

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

Since the overall mean residence time is $\bar{t}_r = N(\bar{t}_r)_N$ equation (4) becomes,

$$\sigma^2 = \frac{(\bar{t}_r)^2}{N} \qquad (5)$$

2.3.3. **Mixing chamber model.**

This is a derivative of the Tank's-in series model and was devised by Pardue and Fields [4]. It relies on the following steps:

(1) Chemical reactions should be fast.

(2) Sample reaches the mixing chamber undiluted.

(3) Immediate mixing of analyte and reagent inside the chamber.

(4) There is further dilution between the mixing chamber and the detector.

Pungor *et al* [5] developed a mathematical model to describe the dispersion when there is a mixing minichamber positioned close to or in the reactor itself. The concentration of a substance in the mixing chamber can be described as a function of time by the following equation,

$$\frac{d\Delta C_t}{dt} = \frac{V}{W}[\Delta(C_s)_t - \Delta C_t] \qquad (6)$$

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

Mixing chambers are used in sequential injection analysis mainly to dilute highly concentrated samples [6] or to influence the dispersion and zone penetration.

2.3.4. **General model.**

The expression below (7) takes into account both convective and diffusion transport and describes the overall physical dispersion phenomena. It also takes into account axial and radial concentration gradients, as well as flow profiles under a laminar flow regime.

$$\frac{\delta C}{\delta t} = D\left(\frac{\delta^2 C}{\delta l^2} + \frac{\delta^2 C}{\delta r^2} + \frac{1}{r}\frac{\delta C}{\delta r}\right) - u_o\left(1 - \frac{r^2}{R^2}\right)\frac{\delta C}{\delta l} \qquad (7)$$

The left hand side corresponds to diffusional transport, the first term within the brackets accounting for axial diffusion (dependence of C on l) and the other two for radial diffusion (dependence of C on r). The first term on the right hand side of equation (7) corresponds to a build-up of matter, which only occurs in a non-steady regime. The second term accounts for contribution from the convective transport for which the velocity profile is parabolic in shape and given by,

$$u = u_o\left(1 - \frac{r^2}{R^2}\right) \qquad (8)$$

The molecules at the walls ($r = R$) have zero velocity ($u = 0$), where those at the center ($r = 0$) have the maximum velocity ($u = u_o$).

2.3.5. **Practical definition of dispersion.**

The dispersion can be explained as the dilution of the sample or any reagent injected into a flowing carrier stream. This concept is given by the position and shape of the analyte signal band. It is defined from the parameters characterizing the transient signal, since the shape and position of this signal are directly related to dispersion of the sample at the detector. An FIA and SIA peak is characterized, at least quantitatively, by

(1) its position, as defined by the travel time, t_a,

(2) its bandwidth, characterized by the baseline-to-baseline time, Δt,

(3) the co-ordinates of the band maximum (T, C^{max}).

2.3. 6. **Ruzicka's dispersion coefficient.**

This concept represents the ratio between the concentrations before (C^o) and after (C) the dispersion process has taken place.

$$D = \frac{C^o}{C} \qquad (9)$$

It is important to note that the dispersion coefficient (D) only considers the physical process of dispersion and not the ensuing chemical reactions.

2.4. **Influence of geometric and hydrodynamic aspects on dispersion.**

These parameters essentially affect the sensitivity of the measurements and the success of the reaction between the sample and the reagent components. The effects of these parameters are similar in both SIA and FIA as shown clearly in the following discussion.

2.4.1. **Hydrodynamic factors.**

2.4.1.1. **Flow rate.**

The flow rate, q, can be related to the travel and baseline-to-baseline times through

$$t_a = \frac{k}{q^{0.125}} \qquad (10)$$

and

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

It can be seen that t_a, Δt , and hence the dispersion, decrease with increasing flow rate.

2.4.2. **Geometric factors.**

This factor explains the influence of the reactor shape (open, coiled, packed) and its dimensions on

the dispersion. The function of these reactors is to increase the intensity of radial mixing, by which the parabolic velocity profile in the axial direction, formed when the sample zone is injected into a laminar flow of a carrier stream, is reduced [7]. A detailed discussion is given by Taljaard [2] who explained the influence of the straight tubes, coils, knitted reactors, normal packed tubes, and single bead string reactors on the dispersion.

2.5. **Conclusion**.

Although zone penetration was discovered as being of fundamental importance in SIA, reproducibility of zone dispersion is still a central issue. In contrast to the other methods of instrumental analysis, the chemical reaction in both SIA and FIA takes place while the concentration gradient of the sample is being formed by the dispersion process. The dispersion coefficient can be compared with the sensitivity and sampling rate in analytical methodology. The larger the value of D, the less intense the signal (sensitivity) and the broader the curves recorded.

2.6. **References.**

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CHAPTER 3.

Sequential injection analysis.

3.1. **Introduction.**

Like in any other field of science and technology, scientists in the field of analytical chemistry have embarked on a process of transforming old concepts and technologies into new concepts, which are easy and economically viable. Since the introduction of flow injection analysis (FIA), there has been a considerable reduction or partial elimination of problems such as time consumption and tedious measurements.

Sequential injection analysis (SIA), introduced in 1990 [1, 2] is a simple and convenient concept of flow analysis transformed from FIA. The inception of a sequential injection analysis (SIA) system in turn is the result of a long search for a better analytical technique which could offer great improvement in the performance as compared to old techniques. The transformation of FIA into SIA signifies recognition of the tremendous versatility of this method originally designed as a mere tool for automation of serial assay. A basic sequential injection analysis manifold is shown in Figure 1.2. The difference between the FIA and SIA systems revolves around the valve and a pump. Instead of an FIA injection valve which uses the inject and load steps, SIA uses a selection valve consisting of 4-10 ports. The selection valve and the pump are both computer controlled.

3.2. **Basic principles.**

Sequential injection analysis uses a selection valve and the pump to aspirate the sample and the reagent solutions into the channel. Following the first step of zone sequencing, during which the sample and the reagent zones are stacked in the holding coil conduit adjacent to each other, the valve is switched to the detector line (Figure 3.1. A, B). In the next step, the flow is reversed so that the stacked zones are propelled through the valve and the reactor to the detector (Figure 3.1. C) As the central streamline moves at a rate twice the speed of the mean flow velocity, whereas the elements of fluid more adjacent to the walls move at lesser rates, the cores of the sequenced zones penetrate each other [3]. During this movement, the flow reversal creates a complex region within which the analyte is transformed into a detectable specie (Figure 3.1. C).

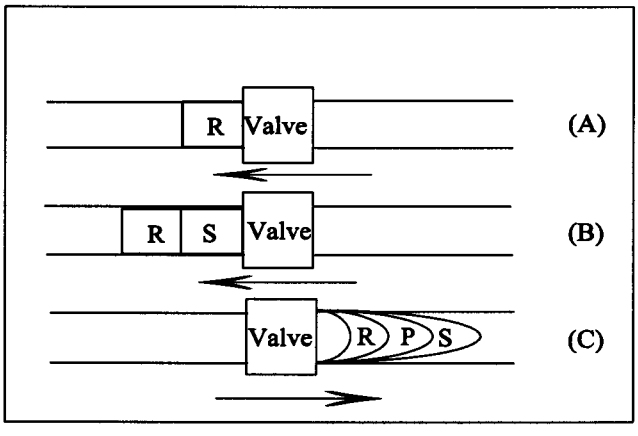

Figure 3.1. (A and B) Sequential stacking of sample and reagent in holding coil.
(C) Merging of zones on flow reversal to give zone product.
Arrows show direction of low.

Reproducible dispersion is the basis for analysis by flow-injection methods. Dispersion is the result of all the physical forces acting on the injected zones. It is the process by which the zones transform from homogeneous, geometrically well-defined zones at the moment of injection to the final zone that is detected downstream.

The formation of the zone product is due to the combined axial and radial dispersion of the zones. A Gaussian peak (Figure 3.2) is observed on the computer screen as the zone of the product passes through the flow through cell at the detector.

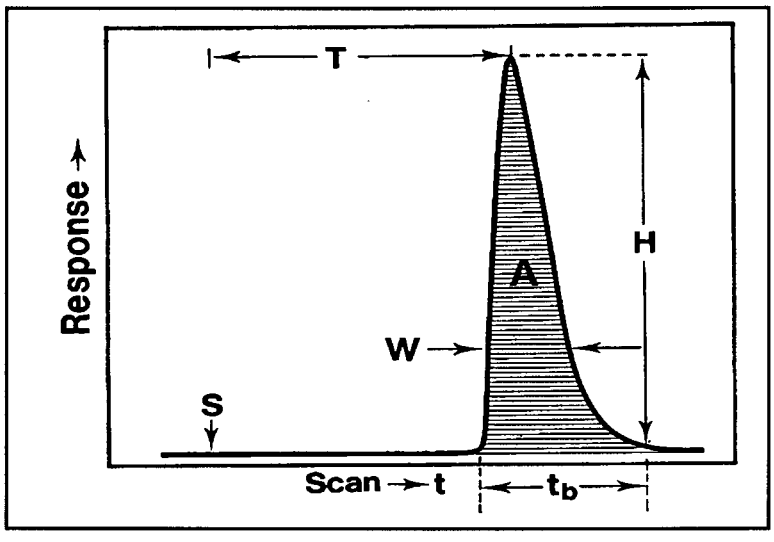

Figure 3.2. The analog output in a form of a peak, the recording starts at S (time of injection t_o). *H* is the peak height, *W* is the peak width at a selected level, and *A* is the peak area, *T* is the residence time corresponding to the peak height measurement, and t_b is the peak width at the baseline.

3.3. **Operational parameters.**

Several researchers have successfully attempted to evaluate different operational parameters which govern the performance of an SIA system [4, 5]. They reported that both FIA and SIA operate on identical underlying principles which includes controlled dispersion, reproducible dispersion and reproducible timing [6].

When applying sequential-injection technique, it is imperative to understand the principles on which it is based in order to do subsequent analysis. The extent of dispersion that the product peak will undergo is essentially influenced by the operational parameters that govern the SIA flow conduit. The following parameters have been shown to have a tremendous effect on the zone dispersion and zone penetration that takes place in sequential injection analysis flow conduit: flow rate, tube-diameter, tube-length, sample and reagent volumes, and sequence of sample and reagents.

3.3.1. **Flow rate.**

In correlation with the Vanderslice expression, $D = K'q$, where q is the flow rate in mL/min, the dispersion of the different zones decreases as the flow rate is increased [7]. The dispersion coefficient decreases with increasing flow rate because the residence time decreases, in a nonlinear fashion, with increasing flow rate [8].

Marshall and van Staden investigated the effect of the flow rate on the dispersion with a dual piston sinusoidal flow syringe pumps [4]. The result of this investigation was that the flow rate can be

changed by varying the speed of the pump. It was also found that the flow rate changes at different cam positions at a constant pump speed.

Botha and van Staden conducted the investigation of the effect of the flow rate on the dispersion using a peristaltic pump [9]. It was mentioned in their report that a range of flow rates can be achieved with a peristaltic pump by changing the pump speed and the pump tubing internal diameter, respectively. The relative peak height increases with an increasing flow rate at a fixed time of drawing up the solutions. When optimizing the flow rate using a peristaltic pump, it is vitally important to look at the effect of the internal diameter of the pump tubing on the dispersion.

3.3.2. **Tube internal diameter**.

The dispersion is highly affected by the internal diameter of the tubing used in SIA. The internal diameter of the pump tubing, holding coil, reaction coil and "up take tubes" must be carefully looked at when building up a SIA system. The dispersion is found to be proportional to the fourth root of the coil diameter. Several factors should come to mind when considering the optimum tube diameter. These include the resultant back-pressure in a length of tubing, the vulnerability to blockage, and the degree of radial dispersion attainable [8]. The internal diameter of the holding coil must be large enough in order to promote axial dispersion and zone penetration. The internal diameter of the reaction coil should be narrow to prevent unnecessary dilution of the product zone. Wider tubing can be used for the "up take tubes" to prevent any back-pressure. The flow rate is also affected by the internal diameter of the pump tubing.

3.3.3. **Tube length.**

The length of the tubing is dictated by the experimental requirements. Longer tubing leads to longer residence times and therefore larger dispersion. The length of the holding coil does not have an important role on the dispersion of both the sample and the reagent solutions but, it must be long enough to be able to accommodate all solutions drawn up. A short reaction coil length is mostly preferred because the sample and the reagent solutions undergo quite a degree of dispersion to form a product zone by the time they reach a reaction coil. It is also evident that the product zone can undergo unnecessary dilution before it reaches the detector if the reaction coil is too long.

3.3.4. **Sample and reagent volumes.**

The sensitivity of the SIA measurements is highly affected by the amount of sample and reagent solutions injected into the system. If necessary, dilution of the concentrated samples can be achieved by reducing the injected sample volume. Injecting at least twice as large reagent zone volume as sample zone volume, while keeping the volume of the sample zone less or equal to 0.5 $S_{1/2}$, allows the optimum conditions for single-based chemistries to be met [5]. ($S_{1/2}$ is defined as the sample volume required to yield a dispersion factor of 2 in the manifold).

Van Staden *et al.* [10] found that the best sensitivity was obtained when a 1 :1 sample : reagent ratio was used. At this ratio, the two zones experienced almost the same axial dispersion, and penetration occurred almost at the maximum of the descending sample zone as well as ascending reagent zones.

Optimum sample and reagent zone volumes can be determined by plotting $\log[1 - (A_{max}/A_0)]$ versus sample volume (μL), where A_0 is the absorbance corresponding to the case where the element of fluid undergoes no dispersion.

3.3.5. **Sequence of sample and reagents**.

The order in which the different sequences of the reagents are drawn up depends very much on the reaction involved. Sensitivity of the sequential injection analysis measurements may be affected by the change in different sequences of the sample and the reagent solutions. The zone that is drawn up first reaches the detector last due to the flow reversal. To obtain an appreciable sensitivity, the reagent at a sufficient high concentration should be introduced first and allowed to penetrate the sample zone, which will experience minimal dispersion.

3.4. **Instrumental set-up**.

Early work has revealed that a single sequential injection analysis manifold is sufficient irrespective of the chemistry to be employed [1]. The reason for proposing this technique was to satisfy the demands for mechanical simplicity in flow injection techniques. The instrumentation is easy to assemble and to work with. This system does not occupy a lot of space and can be placed easily on top of the table. Unlike other sophisticated instruments, sequential injection analysis system is easy to operate and can be understood in a short time.

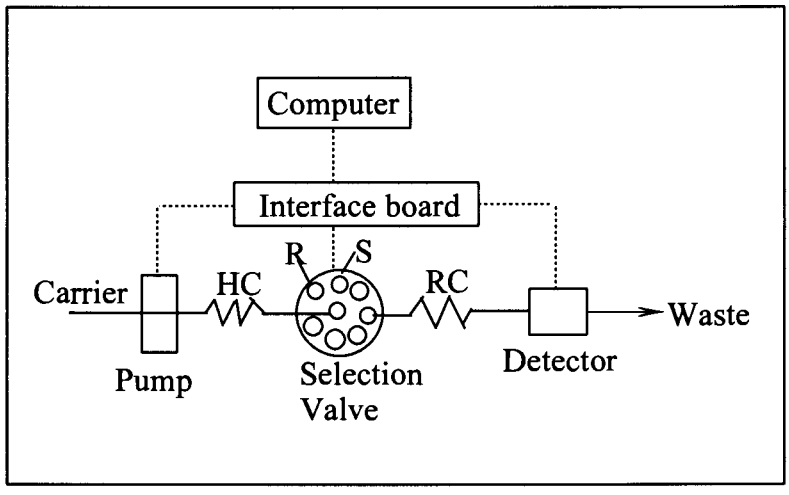

Figure 3.3. Representation of a typical SIA instrumental set-up.

HC is the holding coil, R is the reagent, S is the sample and RC is the reaction coil.

Figure 3.3 shows a typical sequential injection analysis instrumental set-up. This set-up consists of three main components, which are the pump, the selection valve and the detector. All these three main components are linked together by the interface board. The interface board enables a good communication amongst the pump, the selection valve, the detector and the computer. This technique uses a special designed software package to control the movement of both the pump and the selection valve. The software is also used for data acquisition. There is no need for physical reconfiguration of the system since it is fully computer controllable.

3.4.1. **Pumps.**

Whereas FIA uses a multichannel pump and unidirectional (forward) flow, SIA uses a single-channel pump to propel the column of a liquid in reverse and forward steps [6]. Throughout this study a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-le-bel, France) was used to propel the zones forward and backwards in the sequential injection manifold.

Advantages of using a peristaltic pump are listed below:

1. The sampling cycle is considerable shorter.
2. The system is easier to configure, simpler to design, initiate and to operate.
3. This pumps are easy to handle and widely available.
4. They require low maintenance.

Included in the specifications for the ideal pump are the following:

I. robust-able to withstand continual use for extended periods of time with little or no scheduled maintenance,
II. all wetted parts must be able to withstand corrosive solutions and organic solvents,
III. not prone to blockage,
IV. be easily controlled using transistor-transistor-logic (TTL) or switch controls (forward, reverse and stop),
V. device actions should be rapid and without significant inertia,
VI. flow rates in the range 0.5 to 15 ml/min,
VII. constant flow rate over extended periods,

VIII. smooth, reproducible and pulse less flow,

IX. pressures up to 700 kPa,

X. small and compact in size,

XI. the pump should not be adversely affected if it runs dry. It should be self priming,

XII. low power consumption, and

XIII. have the option of inherent safety.

3.4.2. **Selection valve**.

The valve used in sequential injection analysis is a multi-port valve which is able to link different manifold sites and is fully computer controlled. The ports of the multi-position selection valve are connected to sample and reagent reservoirs. Aliquots of the sample and the reagent solutions are sequentially aspirated into a holding coil connected to the middle port of the selection valve by operating the pump in the reverse mode. The pump changes the direction in order to push the sample and the reagent solutions towards the detector, via another port on the valve. Specifications for the ideal valve have been discussed in details by Taljaard [8].

3.4.3. **Detector**.

The most widely used detector in sequential injection analysis are those which measures the absorbance of the light by a coloured solutions. Other type of detectors which have been used includes: fluorescence [11], chemiluminescence [12] and diode array spectrophotometer [13,14]. One of the most important components of any sequential injection analysis detector is the flow-cell.

The flow pattern in SIA is different from the one in FIA. In FIA the flow is continuously pumped through the detector. In SIA, however, the flow is stopped while the sample and the reagents are aspirated into the holding coil. Therefore, the detector in SIA has to reach a stable baseline very fast after directing the flow through the detector again. This important when using detectors such as potentiometric and voltametric, where the response is based on the interaction of the components in the flow and the sensor surface.

3.5. **Fundamental of SIA.**

A typical SIA measuring cycle involves zone sequencing in which the sample and the reagents are stacked adjacent to each other in a holding coil. Zone dispersion and zone penetration occurs on the way to the detector. It is desired to achieve a maximum zone penetration of the sample and the reagent zones. Maximum zone penetration which is a key operation in SIA is achieved through a deliberate increase in axial dispersion obtained by means of the flow reversal and channel design.

3.5.1. **Zone overlap.**

In SIA, zone penetration is of paramount importance because of the impact that it has on the surface area over which a concentration gradient and the axial mixing exist [13]. The concept of zone penetration can be defined similar to the definition of resolution as applied in chromatography. Zone penetration can be expressed as:

$$P = \frac{2W_o}{W_s + W_r}$$

where: P = zone penetration.

W_o = baseline width of the zone overlap.

W_s = baseline width of the sample zone.

W_r = baseline width of the reagent zone.

The parameter, P, which describes the degree of mutual zone penetration has been established in order to identify the time interval within which a meaningful readout, such as peak height and peak area can be obtained. A complete zone overlap is obtained for P = 1, zero zone overlap would be at P = 0, and partial zone overlap will attain values between those two extremes.

3.5.2. **Isodispersion**.

Isodispersion point, I_D, is a point observed where the dispersion of the sample and the reagent zones are identical (Figure 3.4). The maximum product concentration is observed at this point. The isodispersion point is independent of concentration, but studies done by van Staden *et al.* [10] illustrate the shift of the isodispersion point due to the difference in concentration gradients when different volume ratios of sample and reagent were employed.

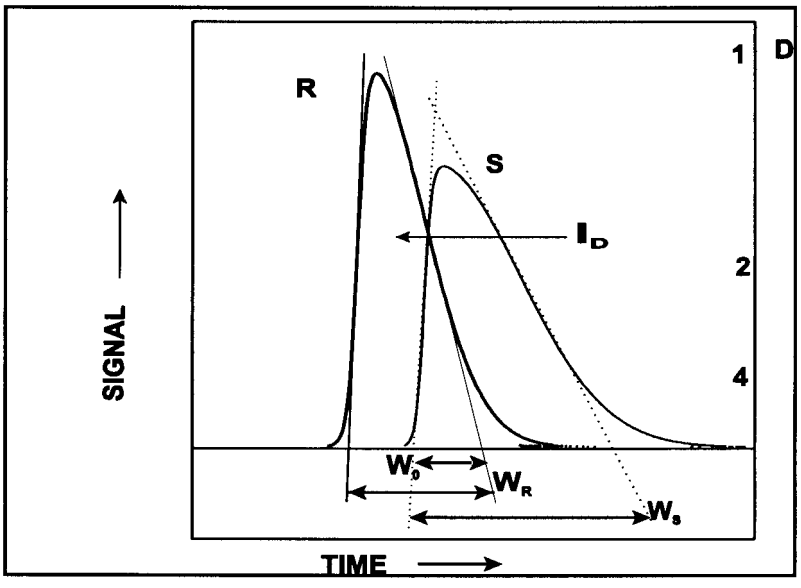

Figure 3.4. Description of zone penetration, S = sample zone, R = reagent zone, I_D = isodispersion point, W_r, W_0, W_s = respective baseline widths of the reagent, overlap and sample.

3.5.3. **Single zone SIA.**

In this type of analysis, the sample is the only zone injected into the system. Only chemical species with a high absorptivity of light at a specific wavelength can be analyzed. The sample is diluted by the carrier on its way to the detector.

3.5.4. **Two zone SIA.**

In this type of analysis, only the sample and the reagent zones are injected into the system. Merging of the sample and reagent zone is sufficient. Reaction stoichiometries of different complexes can

easily be determined when using a two-zone system.

3.5.5. **Three zone SIA.**

The sample and two reagents are injected into the system in this type of analysis. Introduction of the third zone can be done in two ways:

1. By drawing up the three zones (sample zone and the reagents zones) sequentially into the system or.
2. By stacking the two zones first, mix them and then add the third zone to the mixture.

Two reagents or more can be accommodated in sequential injection analysis provided that the sample volume is kept as small as possible and the concentration of the injected reagents should be sufficiently high. Whether the sample should be introduced into the system first or between the reagents depends pretty much on the desired chemistry. The importance of the correct zone sequence is highlighted in the determination of paracetamol where the sample had to be drawn up first to for it to be oxidized with potassium hexacyanoferrate(III) before it reacts with phenol (Chapter 5).

3.5.6. **Flow reversal.**

Flow reversal effectively promotes zone penetration. It creates a composite zone in which sample and the reagent zones penetrate each other due to combined axial and radial dispersion. Gubeli *et al.* [3] investigated the effect of the number of flow reversal at a constant step length and zone sequence composition. The authors concluded that an increase in a number of flow reversals

increases mutual zone penetration (Figure 3.5). The top arrows in Figure 3.4 indicate the number of flow reversals and their direction. Multiple flow reversals can be used when working with solutions which are difficult to handle, such as mixing zones of different viscosities.

In analogy with chromatography, other ways of measuring zone penetration, P, can be based on considering overlapping peak areas rather than their widths and also considering peak asymmetry using further approximations.

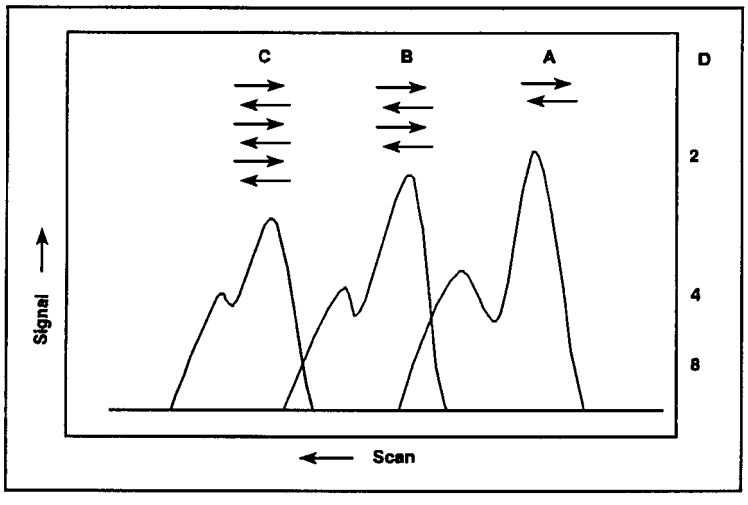

Figure 3.5. Influence of repeated flow reversals on the mutual penetration of the sample and the reagent zones.

3.6. **Device control and data acquisition.**

Data acquisition and device control were achieved using a PC-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (MINTEK, Randburg, South Africa). The FlowTEK [15] software package (obtained from MINTEK) for computer-aided flow-analysis was used throughout for device control and data acquisition. The basic components of SIA are computer controlled. The response which is displayed on the computer monitor can be recorded as peak height, width, peak time and concentration. The results can be easily saved within the computer for future reference. The computer fitted interface card is one other component that is essential to the operation of SIA.

3.7. **Conclusion.**

Controlled dispersion and reproducible sample handling is an integral and indispensable prerequisite for the success of both FIA and SIA. Since SIA operates on a stack of well defined sample and reagents zones, the concept of zone penetration was identified as being of paramount importance. Zone penetration has a substantial impact on the surface area over which the concentration gradient exists and therefore over which the axial mixing takes place. Since zone sequencing, mutual dispersion and zone penetration are the key operations in SIA, it is imperative that the parameters which determine the extent of dispersion and penetration be optimized to the point where the required amount is achieved.

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CHAPTER 4.

Determination of zinc in pharmaceutical products by use of a sequential injection analysis system.

4.1. **Introduction.**

Zinc plays an important role in many biological activities. It is an essential element for normal sexual development, wound healing and taste acuity [1]. Research scientists have now found that active zinc ions acts as the natural protectors of the cell membranes and are an essential component of the body's defence system [1]. Determination of zinc is very important in different fields of analytical chemistry such as environmental analysis (e.g. waste water control), process control (e.g. metallurgy and alloys manufacturing) and clinical analysis [2].

Many methods, based on different techniques, have been described for the determination of zinc: molecular spectrophotometry [2- 4], atomic absorption spectrometry [5], plasma atomic emission spectrometry [6] and stripping potentiometry [7]. Of all these methods, atomic absorption is the most sensitive analytical technique, but the instrumentation is rather expensive [8, 9], and our laboratory has been approached on several occasions to develop a cheap robust analytical system, with special request to replace atomic absorption spectrophotometry by a cheaper detector.

Several flow injection analysis (FIA) methods have been reported in literature [10-12], but some involve the use of toxic reagents and the high consume of sample and reagents makes the technique expensive.

Since its introduction in 1990 [13], sequential injection analysis (SIA) has received considerable attention owing to its simplicity, low sample and reagents consumption, and, most important, the use of inexpensive technique. The SIA system enables different conditions of analysis to be attained without the need to reconfigure the system, all the essential conditions can be changed merely by using a computer keyboard [14]. This is successfully achieved with the help of a selection valve which sequentially aspirates the sample zone and the reagent zones into in a stack of well defined zones situated in a holding coil. When the valve is switched to the detector position the flow is reversed and the zones mutually disperse and penetrate each other as they pass through the detector. The difference between FIA and SIA, and the performance and real advantages of SIA have been discussed in detail [15-20].

This chapter proposes the use of an SIA method for the spectrophotometric determination of zinc using xylenol orange as a reagent. Although many spectrophotometric methods have been employed for the determining zinc in alloys and pharmaceutical samples, the method using xylenol orange has been selected for its simplicity and its sensitivity [16]. This procedure does not involve toxic reagents, which makes it readily suitable for automated routine operation. The method has been applied to the analysis of zinc in pharmaceutical samples.

4.2. **Properties of zinc.**

Zinc is a silvery white metal with a relatively low melting point (419.5°C) and boiling point (907°C). Expect when very pure, zinc is brittle at room temperature, but malleable above 100°C.

There are no natural radioactive isotopes of zinc. The normal valence states are Zn(0) and Zn(II). Compounds of Zn(I) do not exists naturally, although ZnH and ZnX (X = Br, Cl) are known as spetrographic species. Zinc is generally divalent, and can give up two outer most electrons to form an electrovalent compound, for an example zinc carbonate ($ZnCO_3$). It may also share those electrons as in $ZnCl_2$ in which the bonds are partly ionic and partly covalent.

Dry air has little attack on zinc at room temperature, but above 200°C oxidation occurs rapidly. The metal is readily dissolved by most mineral acids. Dissolution occur in dilute sulphuric acid, hydrochloric acid and in nitric acid.

A typical reaction for zinc in hydrochloric acid is the following:

$$Zn(s) + HCl(aq) \longrightarrow ZnCl_2(aq) + H_2(g).$$

4.3. **Natural occurrence.**

In total, vast quantities of zinc occur in the earth's crust. It is present in small amounts in igneous rocks and sedimentary rocks. Zinc is mostly found in association with some other elements and

substances. The deposits almost always contain lead and cadmium, and minerals containing copper, silver and manganese are frequently present. Zinc ores are widely distributed and they are common in mines.

Zinc occurs as 0.003 % (w/w) in humans.

4.4. **Biochemistry of zinc.**

The presence of small quantities of zinc is essential to both animal and plant life. It has been estimated that most normal diets contain 10-15 mg of zinc per day, most of which originates in the protein intake.

The absorption of zinc in the body is influenced by low molar mass ligands, e.g., organic acids and peptides promoting or facilitating absorption as well as complexing agents like phytic acid depressing absorption. The absorption of zinc from the cereal based meals varied from 8 % (w/w) in an oatmeal meal with phytic avid content of 615 μmol to 27 % (w/w) in a meal with a phytic acid of 100 μmol. From the high protein meals the absorption varied from 19-325 depending on the zinc and protein content.

The most reliable source of zinc is animal food especially muscle meat, wheat germ and bran.

4.5. **Experimental.**

4.5.1. **Reagents.**

All reagents were prepared form analytical-reagent grade chemicals unless specified otherwise. Deionized water from a Modulab system (Continental Water System, San Anytorio, TX, USA) was used throughout. The water was tested beforehand for traces of chloride. All solutions were degassed before measurements with a vacuum pump system. The main solutions were prepared as follows.

4.5.1.1. **Standard zinc solution.**

A stock solution of zinc (2000 mg. L^{-1}) was prepared by dissolving zinc metal (Merck; 0.2 g) in nitric acid (7 mol L^{-1}, 20 mL) and diluting to 100 mL with deionized water.

4.5.1.2. **Combined masking agent-buffer solution.**

Sodium thiosulphate (10 g) was dissolved in an acetic acid - sodium acetate buffer solution (pH 5.6, 1 mol L^{-1}) and diluted to 1L with the acetic acid - sodium acetate buffer solution.

4.5.1.3. **Xylenol orange solution.**

0.012 g of xylenol orange was dissolved in deionized water (100 ml).

4.5.1.4. **Carrier.**

A solution of 0.014 mol L^{-1} nitric acid was used as a carrier.

4.5.1.5. **Preparation of pharmaceutical samples.**

The samples chosen were different vitamin supplement, essential elements supplement and energy boosting tablets. Two tablets of each sample were dissolved in deionized water and filtered through an 11.0 cm Whatman filter paper. The filtrate was diluted quantitatively to 1 L in a volumetric flask.

4.5.2. **Apparatus.**

The sequential injection system was constructed from a Gilson Minipuls peristaltic pump, a 10-port selection valve (VICI Valco model E10-230, USA), and a Unicam 8625 UV-visible spectrophotometer equipped with a 10 mm Hellma type flow-through cell for absorbance measurements. For device control and data acquisition a PC 30-B interface board (Eagle Electric, Cape Town, South Africa) and FlowTEK [17] software package (obtained from MINTEK) for computer-aided flow analysis were used throughout.

4.5.3. **Manifold.**

The components of the SIA system were arranged as illustrated in Figure. 4.1. The holding and reaction coils (Tygon tubing) were 350 cm x 1.14 mm and 300 cm x 0.64 mm internal diameter,

respectively.

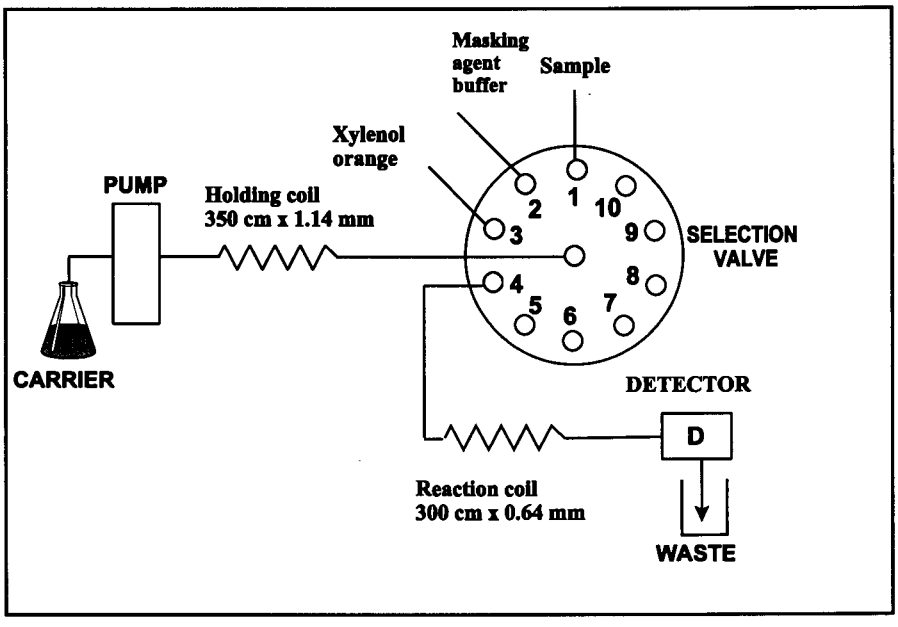

Figure 4.1. Schematic representation of the sequential injection analysis system for the determination of zinc.

4.5.4. **Procedure.**

The device sequence for the determination of zinc is illustrated in Table 4.1. The sample, combined masking agent-buffer, and the calorimetric reagent were aspirated through a selection valve into a holding coil resulting in a stack of well defined zones. The pumping direction was then reversed and the zones penetrate each other as they pass through the reaction coil to the detector, as illustrated in Figure 4.1. Because xylenol orange is too sensitive to be applied directly to the analysis of zinc, the sample was diluted within the system with a combined masking agent-buffer solution. The

optimum pH for the formation of the complex was between pH 5.6 and pH 5.8. The absorbance of the complex is measured at 568 nm.

Table 4.1. Sequence for one cycle of the SIA system for the determination of zinc.

Time (s)	Pump	Valve	Description
0	Off	Sample	Pump off, valve select sample stream. Valve position1.
1	Reverse		Draw up the sample solution
6	Off		Pump stops.
7		Buffer	Selects the buffer stream. Valve position 2
8	Reverse		Draw up the buffer solution
18	Off		Pump stops
19		Reagent	Selects a colour reagent stream. Valve position 3
20	Reverse		Draw up colour reagent
27	Off		Pump stops
28		Detector	Selects the detector stream. Valve position 4
29	Forward		Pump stack of zones to the detector
130	Off	Home	Pump stops. Valve return to position 1.

4.6. **Results and Discussion.**

The determination of zinc is based on the reaction of zinc in the sample with xylenol orange to form a zinc-xylenol orange complex which is measured spectrophotometrically at 568 nm.

4.6.1. **Method optimization.**

Various factors that influence the performance of the proposed SIA method were optimized.

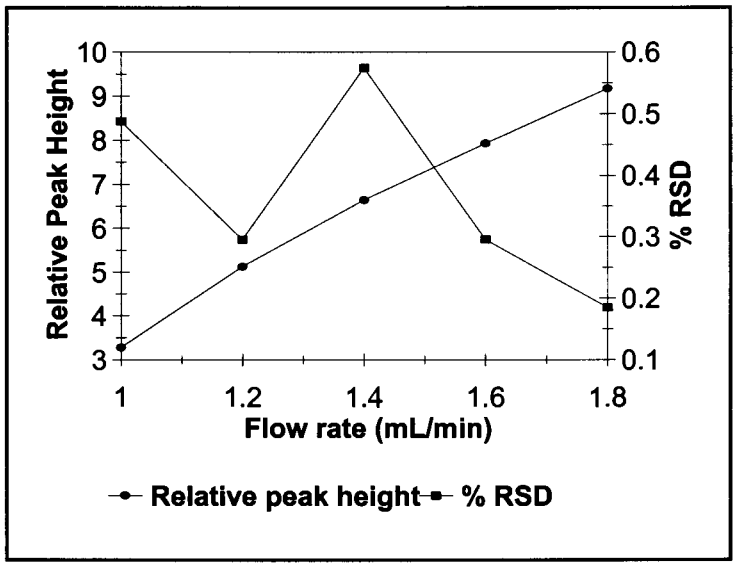

Figure 4.2. Influence of the carrier stream flow rate on relative peak height.

4.6.1.1. **Flow rate.**

The effect of the flow rate between 0.5 and 2.4 $mLmin^{-1}$ on the relative peak height (Figure 4.2) was evaluated to obtain the optimum working conditions. As expected, the relative peak height increases with increasing flow rate up to 1.8 mL min^{-1}. As shown in Figure 4.2, the best precision was obtained at a flow rate of 1.8 % $mLmin^{-1}$. At higher flow rates the precision deteriorated.

4.6.1.2. **Zones volume.**

Selection of the sample injection and reagents volumes is one of the main means of optimizing a sequential injection analysis system. This conditions were investigated here because zones volumes play a vital role in the extent of zones dispersion and zones penetration. A standard solution of 50 mg L^{-1} was used to optimize the sample volume. As illustrated in Figure 3, the sample volume has a significant effect on the relative peak height. The relative peak height increased substantially as the volume was increased from 120 μL (relative peak height1.1408) to150 μL (8.6528). Good precision and sensitivity were obtained with a volume of 150 μL and this was regarded as the optimum volume. To optimize the volume of the colour reagent, the optimized sample volume was used. The optimum volume of the colour reagent was 210 μL. It was found to be necessary to optimize the volume of the combined masking agent-buffer solution. This was achieved by using the optimized volumes of the sample and colour reagent. The optimum volume of the buffer solution, in terms of the sensitivity relative standard deviation, was found to be 300 μL.

4.6.1.3. **Holding and reaction coil.**

A holding coil of 350 cm with an internal diameter of 1.14 mm was used. The length of the reaction coil has a significant effect on the sensitivity of the method. Increasing the length of the reaction coil resulted in reduced peak height but improved reproducibility. It was then decided to use a reaction coil of 300 cm with an internal diameter of 0.64 mm.

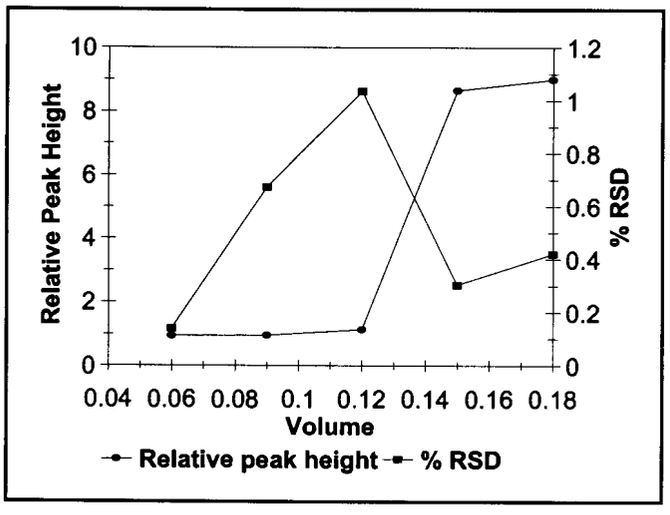

Figure 4.3. Influence of the sample volume on relative peak height.

4.6.2. **Method Evaluation.**

The proposed SIA system was critically evaluated with regard to linearity, accuracy, precision, detection limit, sample frequency and interferences.

4.6.2.1. **Linearity.**

The calibration graph is linear for zinc concentration between 10 and 60 $mL.min^{-1}$. The relationship between the peak height and the zinc concentration was given by the equation:

$$H = 0.060x + 0.728, \qquad r = 0.997$$

where H is the relative peak height and x is the concentration of zinc in $mg.L^{-1}$.

4.6.2.2. **Accuracy.**

The accuracy of the proposed SIA system was evaluated by comparing the results obtained with the proposed SIA method in the analysis of pharmaceutical samples with the values obtained by a standard AAS method [23]. The results (Table 4.2) revealed a good correlation between the SIA system and the values obtained by the standard method.

4.6.2.3. **Precision.**

The reproducibility of the proposed method was evaluated by 10 replicate determinations of several zinc standard solutions and pharmaceutical samples within the linear concentration range of the method. The results obtained by utilisation of the proposed SIA system on real samples are given in Table 4.2. The relative standard deviation was always less than 1 %.

Table 4.2. Comparison of results by the proposed SIA system and by a standard AAS method for the determination of zinc in pharmaceutical samples (*n = 10).

Sample	Proposed SIA system		Standard method
	([Zn^{2+}] in mg/L)	% RSD *	([Zn^{2+}] in mg/L)
SA	19.2	0.78	19.8
SB	27.9	0.82	28.3
SC	19.6	0.55	20
SD	19.9	0.81	20.8
SE	29.4	0.3	30
SF	25	0.93	25.6

4.6.2.4. **Detection limit.**

The detection limit was calculated by use of the formula:

$$\text{Detection limit} = \frac{3 \times s_k \times k}{100}$$

where s_k = 1.4113 is the relative standard deviation of the lowest zinc standard concentration.

k = 10 mg.L^{-1} is the lowest concentration investigated. The value was found to be 0.42 mg.L^{-1}, which indicated that the proposed SIA system functions well above the detection limit.

4.6.2.5. **Statistical comparison.**

The comparison was done between the SIA and the standard atomic absorption spectrophotometry method. The 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 differ significantly at 95 % confidence level. The null hypothesis was H_0: $\zeta = 0$ against the alternative hypothesis H_t : $\zeta \neq 0$, where ζ is the population paired difference [21, 22]. The test is two tailed, as we are interested in both $\bar{x}_d < 0$ and $\bar{x}_d > 0$, where $\bar{x}_d$ is the mean difference between the methods. The calculated value of t was found using the formula, $t_{calc} = \left|\bar{x}_d\right| \times \sqrt{n}/s_d$

, where s_d is the standard deviation. Substituting for the calculated value of t, we found 1.0119, where $\bar{x}_d$ was -0.42, s_d was 1.0167 and n was 6. At 95 % confidence level $t_{0.05,5}$ was found to be 2.571. The critical t-values are therefore ± 2.571. Since the calculated value of t is less than the

critical t-value, H_o it cannot be rejected and it follows that there is no statistically significant difference between the two techniques.

4.6.2.6. **Sample frequency.**

The time needed to complete one cycle was 130s, resulting in a sample frequency of 30 samples per hour.

4.6.2.7. **Interferences.**

Copper (II), lead (II), and nickel (II) are possible interferences in the determination of zinc with xylenol orange [23]. These interferences were throughly investigated and it was found that copper (II) and lead (II) can be effectively masked with sodium thiosulphate but nickel (II) still interferes seriously. The amounts of these compounds in pharmaceutical samples were negligibly small and they did not, therefore, pose any threat to the proposed SIA system. Use of the combined masking agent-buffer solution for pharmaceutical samples that might contain traces of the interferents is, however, recommended.

4.7. **Conclusion.**

The results obtained by sequential injection analysis technique confirm its feasibility for use in process analysis. The reagent consumption with this method is much lower than the previously proposed FIA method. This method can be used to determine zinc at a frequency of 30 samples per hour. Because an SIA system is fully computerized it is simple and easy to handle.

4.8. **References.**

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CHAPTER 5.

Determination of paracetamol in pharmaceutical samples using a Sequential Injection Analysis system.

5.1. **Introduction**.

Paracetamol is an extensively employed antipyretic analgesic frequently prescribed solely or with other related drugs. It is used extensively in the treatment of mild to moderate pain and fever. In case of an overdose, it may cause hepatic necrosis but it is a very safe analgesic at therapeutic doses [1]. Paracetamol has been determined using various analytical methods such as fluorimetry [2], voltammetry [3], Fourier transform infrared spectrometry [4,5], spectrophotometry [6-9] and also by chromatography [10-15]. Flow injection analysis [FIA] procedures have also been proposed for the determination of paracetamol [16-18]. Many of the FIA methods are associated with the problem of high consumption of the sample and reagents which makes the technique expensive.

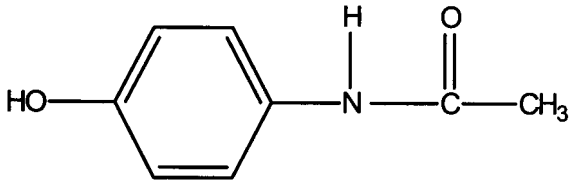
Since its introduction by Ruzicka and Marshall at the University of Washington in 1990 [19], sequential injection analysis SIA has become a widespread technique employed in many branches of chemistry. This technique has received a considerable attention and great acceptance owing to its simplicity, versatility, low sample and reagent consumption and its low cost. In contrast to flow injection analysis, sequential injection analysis employs a computer controlled multi-position valve and a pump (generally peristaltic) operated synchronously. This enables a

system to perform determinations of different analytes employing a single channel manifold with minor modifications [20, 21].

In this chapter we propose a simple, speedy and inexpensive sequential injection analysis technique for the determination of paracetamol. This method is based on the oxidation reaction of paracetamol with potassium hexacyanoferrate (III) followed by the reaction with phenol at elevated temperature in aqueous ammoniacal solution.

5.2. **Properties of paracetamol.**

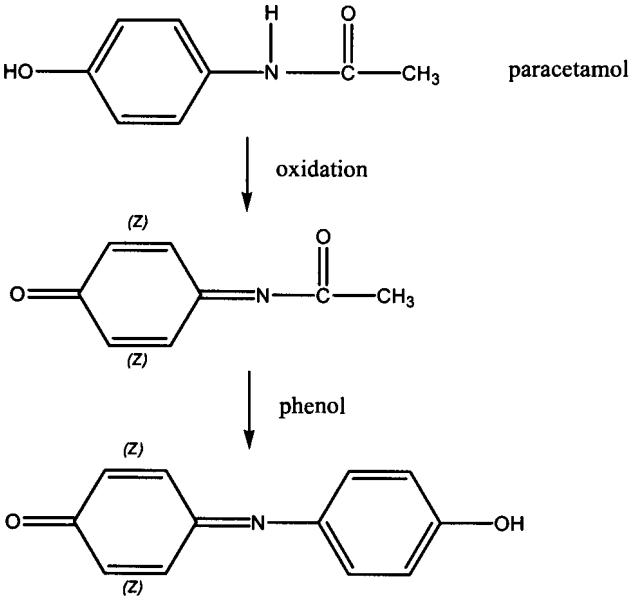
Paracetamol is 4-acetaminophenol ($C_8H_9NO_2$) and may be represented by the following formula:



Paracetamol ia a white, odourless crystalline powder with a bitter taste. This compound has a melting point of 169-172 °C. Paracetamol is soluble in 70 parts of water (1 in 20 boiling water), 7 parts of alcohol (95 %), 13 parts of acetone, 40 parts of glycerol, 9 parts of propylene glycol, 50 parts of propylene glycol, 50 parts of chloroform, or 10 parts of methyl alcohol. It is also soluble in solutions of alkali hydroxides. It is insoluble in benzene and ether. A saturated aqueous solution has a pH of about 6 and is stable (half-life over 20 years) but stability decreases in acid or alkaline conditions, the paracetamol being slowly broken down into acetic acid and p-aminophenol.

5.3. **Principle for the assay of paracetamol.**

The reaction is based on the oxidation of paracetamol with potassium hexacyanoferrate (III). The product formed reacts with phenol in the presence of ammonia to form a blue product (Figure 5.1) measured spectrophotometrically at 630 nm [22].



N-[p-hydroxyphenyl]-p-benzoquinoneimine (blue colour)

Figure 5.1. Colour development for the assay of paracetamol.

5.4. **Uses of paracetamol.**

Paracetamol relieves pain and fever in adults and children, and it is the most widely accepted medicine fir this purpose. It is used mainly for its pain relief properties either as a medicine prescribed by a doctor or it can be purchased as an over-the-counter medicine both in retail pharmacies or grocers shops.

There are virtually no groups of people who should not take paracetamol, and interactions with other treatments are not a problem. When taken at the recommended dosage, there are virtually no side-effects.

Its pain relief (analgesic) and fever relief (antipyretic) effects are similar to those of aspirin and it works in a similar, though not identical, way. Unlike aspirin, however, increases the dose does not result in clinically useful anti-inflammatory activity.

Paracetamol can be combined with decongestant ingredients to help relieve the symptoms of the common cold, influenza and sinusitis by relieving headache, general aches, nasal congestion and fever.

Paracetamol and its combinations are mainly available as tablets for immediate consumption or for dissolving in water before consumption. It is suitable for all age groups including the very young for whom it may be used following immunization procedures, and it is available in liquid formulations for young children.

5.5. **Experimental.**

5.5.1. **Reagents.**

All reagents were prepared from analytical reagents grade unless specified otherwise. Deionized water from a Modulab system (Continental Water System, San Antorio, TX, USA) was used throughout to prepare all solutions. All solutions were degassed before measurements with a

vacuum pump system. The main solutions were prepared as follows.

5.5.1.1. **Standard solution.**

0.05 g of pure paracetamol (Merk, Germany) was dissolved in 250 mL of de-ionized water. The equivalent volume of ammonia solution was added to obtain a concentration of 0.4 $mol.L^{-1}$ of ammonia in solution.

5.5.1.2. **Potassium hexacyanoferrate (III).**

1 g of potassium hexacyanoferrate (III) was dissolved in 250 mL of de-ionized water and then diluted to a final volume of 500 mL.

5.5.1.3. **Phenol solution.**

This solution was prepared by dissolving 10 g of phenol (Merk) in 500 mL of de-ionized water.

5.5.1.4. **Carrier.**

A solution of 0.4 $mol.L^{-1}$ ammonia was used.

5.5.2. **Sample preparation.**

For the determination of paracetamol in pharmaceutical formulations (tablets), the required

amount of powdered tablets were dissolved in de-ionized water and filtered. The filtrate was diluted to a final volume of 500 mL with de-ionized water into which the equivalent volume of ammonia was added to obtain a concentration of 0.4 mol.L^{-1} in solution.

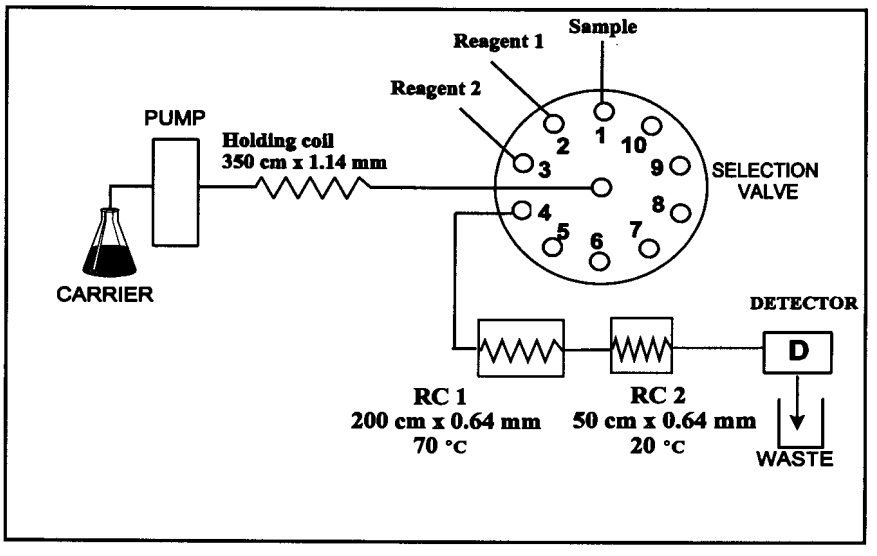


Figure 5.1. A schematic diagram of an SIA system used for the determination of paracetamol. Reagent 1 = potassium hexacyanofferate (III) solution, reagent 2 = phenol solution, RC 1= reaction coil 1 at 70 °C and RC 2 = reaction coil 2 at 20 °C.

5.5.3. **Apparatus.**

The sequential injection analysis system (Figure 5.1) was constructed from the following components: a Gilson Minipuls peristaltic pump; a 10- port selection valve (Model E10-230, VICI Valco Instruments, USA); and a Unicam 8625 UV-Visible spectrophotometer equipped with a 10 mm Hellma type flow through cell for absorbance measurements; Two thermostat water circulating baths one fixed at 70 °C and the other one at 20 °C. For the device control and

data acquisition a PC 30-B interface board (Eagle Electric, Cape Town, South Africa) and FlowTEK [23] software package (obtained from MINTEK) for computer aided flow analysis were used throughout.

5.5.4. **Manifold**.

The components of the SIA system were arranged as illustrated in Figure 5.1. A 350 cm holding coil (Tygon tubing) with 1.14 mm internal diameter was used. The reaction coil RC1 was 200 cm and RC2 was 50 cm long both with the same internal diameter of 0.64 mm.

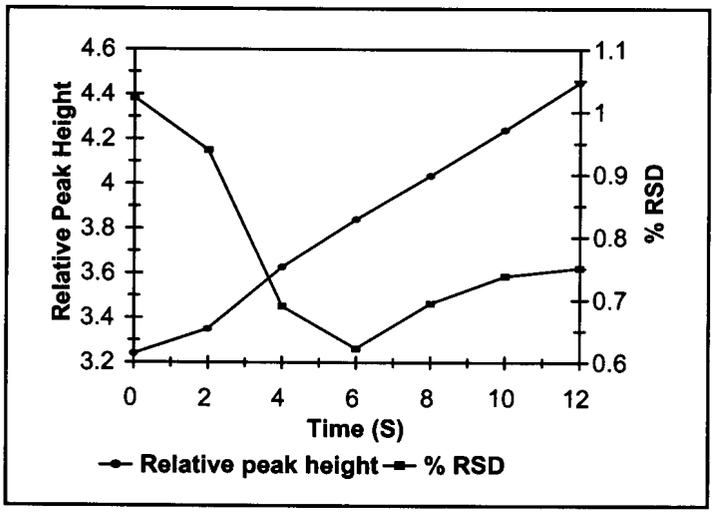


Figure 5.2. Influence of stopping the stack of zones in the hot water bath on the relative peak height.

5.5.5. **Procedure**.

Illustration of the device sequence for the determination of paracetamol is given in Table 5.1. The sample and the reagent solutions were aspirated into the holding coil and then propelled by

the peristaltic pump into the reaction coil RC1 where the stack of zones stayed for 6 seconds improving the sensitivity of the method in the hot water circulating bath fixed at 70 °C as shown in Figure 5.2. The zones were then passed through the reaction coil RC2 placed in cold water bath fixed at 20 °C towards the detector where the absorbance of the blue complex was measured at 630 nm.

TABLE 5.1. Device sequence for one cycle of the SIA system for the determination of paracetamol.

Time (s)	Pump	Valve	Description
0	Off	Sample	Pump off, valve selects sample stream (valve position 1).
1	Reverse		Draw up the sample solution
7	Off		Pump stops
8		Reagent 1	Valve select reagent 1 stream (valve position 2).
9	Reverse		draw up reagent 1 solution.
14	Off		Pump stops.
15		Reagent 2	Valve select reagent 2 stream (valve position 3).
16	Reverse		Draw up reagent 2 solution.
19	Off		Pump stops.
20		detector	Select the detector line (valve position 4).
21	Forward		Pump stack of zones into the water bath fixed at 70°C.
35	Off		Stack of zones stop in the water bath fixed at 70°C.
41	Forward		Pump stack of zones to the detector.
130	Off	Home	Pump stops. Valve returns to position 1.

5.6. **Results and discussion.**

The reaction is based on the oxidation of paracetamol with potassium hexacyanoferrate (III) followed by a reaction with phenol in the presence of ammonia. The reaction is monitored at 630 nm.

5.6.1. **Method optimization.**

Various factors that influence the performance of the method were optimized.

5.6.1.1. **Flow rate.**

Optimization of the flow rate is important because of its great effect on the dispersion and penetration of the reaction zones.

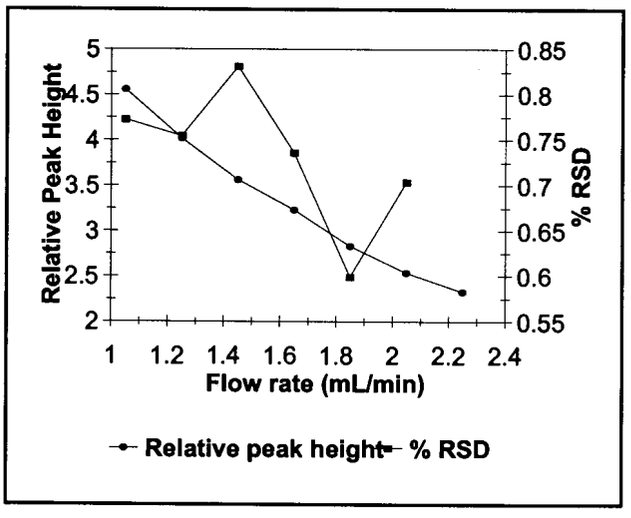


Figure 5.3. Influence of the carrier stream flow rate on the relative peak height.

Figure 5.3 shows a decrease in the sensitivity of the method with an increase in the flow rate which can be due to the decrease in the oxidation time of paracetamol at a fixed temperature of 70 °C. A flow rate of 1.85 $mLmin^{-1}$ was used as the optimum flow rate throughout because of its better precision.

5.6.1.2. **Sample and reagents volumes.**

A study of the effect of the sample and reagent volumes showed that this parameter plays a vital role in the degree of zones dispersion and penetration. A concentration of 50 mg. L^{-1} was used to obtain the optimum volume of the sample. The peak height increases only between 60 μL and 185 μL with an increase in the sample volume as shown in Figure 5.4. The sample volume of more than 190 μL was unnecessary. A 185 μL was used as an optimum volume and this volume was used to optimize the volume of the potassium hexacyanoferrate (III).

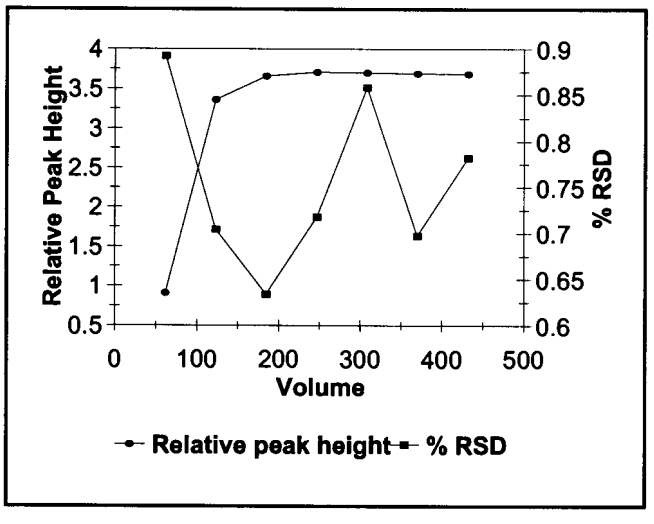


Figure 5.4. Influence of the sample volume (μL) on the relative peak height.

Figure 5.5 shows that the sensitivity of the method increases with an increase in the volume of this reagent. 154 μL was found to be the optimum value. The optimum volume of the phenol reagent was found using the optimized volumes of the sample and potassium hexacyanoferrate (III) reagent. It was found that the peak height increases with an increase in the volume of the phenol reagent. An optimum volume of the phenol reagent was found to be 93 μL as shown in Figure 5.6.

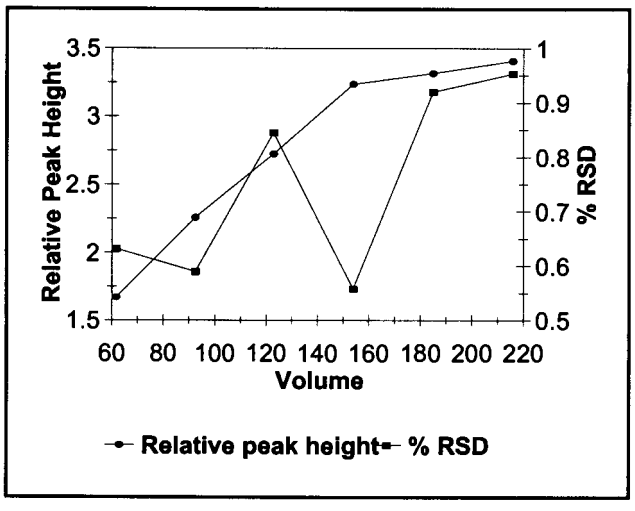


Figure 5.5. Influence of the potassium hexacyanoferrate (III) volume (μL) on the relative peak height.

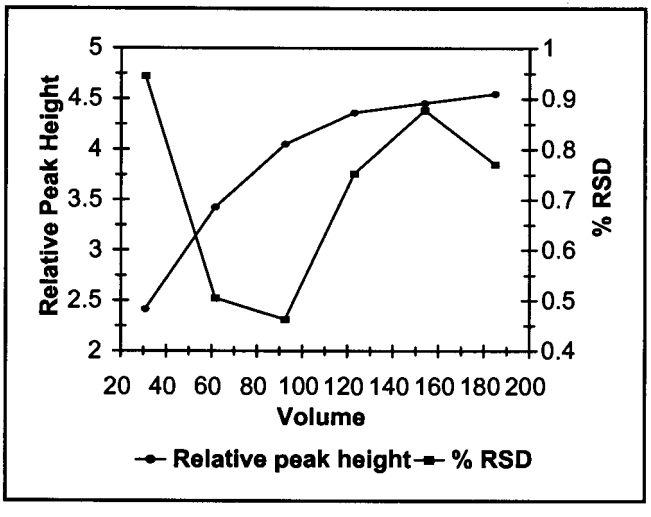


Figure 5.6. Influence of phenol reagent volume (μL) on the relative peak height.

5.6.1.3. **Sequence of sample and reagents.**

The order in which the different sequences of the sample and reagents are drawn up plays a vital role in this reaction. The best sequence was found to be: sample, potassium hexacyanoferrate (III) and then lastly the phenol reagent. Other sequences had a poor sensitivity because the sample has to be oxidized by potassium hexacyanoferrate (III) before it reacts with phenol.

5.6.1.4. **Holding and reaction coil.**

A holding coil of 350 cm with an internal diameter of 1.14 mm was used. The sensitivity of the method increases with an increase in the reaction coil RC1. The longer the reaction coil RC1 the better the sensitivity of the method. A better % RSD was found with a reaction coil RC1 of 200 cm inserted at 70 °C. The reproducibility was poor with a reaction coil RC1 of longer than 300 cm. The other part of the reaction coil RC2 was 50 cm long. This part was inserted in a water bath fixed at 20 °C in order to cool the reaction before it is detected to avoid air bubbles.

5.6.1.5. **Effect of the temperature.**

A study of the effect of the temperature between 30 °C and 100 °C showed that the peak height increases with an increase in the temperature of the reaction coil RC1 as illustrated in Figure 5.7. A better % RSD was found with a temperature of 70 °C. The air bubbles were seriously produced at high temperatures but were tolerable at 70 °C.

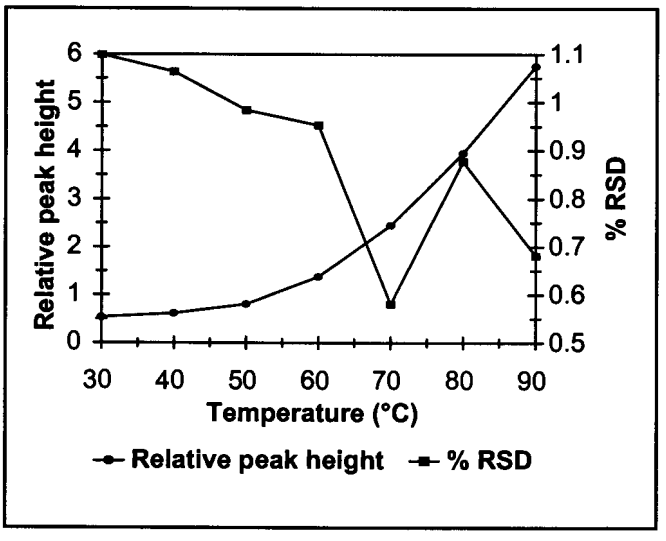


Figure 5.7. Influence of temperature (° C) on the relative peak height.

5.6.2. **Method evaluation.**

The proposed SIA method was critically evaluated with regard to the linearity, accuracy, precision, detection limit and sample frequency.

5.6.2.1. **Linearity.**

Standard solutions of pure paracetamol were used to evaluate the linearity of the method. The calibration curve is linear up to 60 $mg.L^{-1}$. The relationship between the peak height and the concentration is given by the equation below,

$$Y = 0.0297x + 0.5441 \qquad r = 0.9995$$

where Y is the relative peak height and x is the concentration of paracetamol in $mg.L^{-1}$.

Table 5.2. Comparison of the results obtained by the proposed SIA method with those specified by the manufactures.

Sample	**Proposed SIA system.** $mg.\ L^{-1}$	**Manufactures specification** $mg.\ L^{-1}$
Panado	499	500
Grand-pa	502	500
Base max	498	500
Paramed	496	500
Compral	97	100
Pain powder	275	276

*n = 10

5.6.2.2. **Accuracy.**

The accuracy of the proposed method was evaluated by comparing results obtained with those specified by the manufactures. The results as given in Table 5.2 revealed a good correlation between the results obtained with SIA and the manufactures specifications.

5.6.2.3. **Precision.**

The precision of the proposed SIA method was evaluated by 10 repetitive analysis of a number of samples and standard solutions. A relative standard deviation of less than 1.2 % was obtained in all cases.

5.6.2.4. **Detection limit.**

The detection limit of the proposed method was determined using the following equation.

$$\text{Detection limit} = \frac{(3\delta + K)(K - c)}{m}$$

where $\delta = 0.0124$ is the relative standard deviation of the baseline, $K = 0.5564$ is the average signal value of the baseline, $m = 0.0297$ is the slope of the calibration curve and c = 0.5441 is the y -intercept of the calibration curve. The value of the detection limit was found to be 0.2458 $mg.L^{-1}$.

5.6.2.5. **Sample frequency.**

It took 130 s to complete one analytical cycle resulting in a sample frequency of 27 samples per hour.

5.7. **Conclusion.**

The proposed sequential injection analysis (SIA) method for the determination of paracetamol in pharmaceutical formulations gives accurate and precise results. The method is simple, rapid and inexpensive. 27 samples can be analyzed per hour with a precision of less than 1.2 %. The results given by the proposed method were in good correlation with those specified by the manufactures on the samples.

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CHAPTER 6.

Determination of boron in eye lotions using a sequential injection analysis system.

6.1. Introduction.

Although no essential biochemical function of boron has yet been positively identified to establish its essentiality to animals and human beings, its excess has been proved to be toxic. The tendency of boron to accumulate in animals and vegetable tissues constitute a potential hazard to the health of those consuming food and water with a high boron content [1].

The determination of boron is very important in a variety of fields such as nuclear energy, industrial metallurgy, pharmacy and agriculture [2-5]. Recently there has been considerable research on the development of new and improved methods for the determination of traces of boron. Most spectrophotometric methods and other fluorimetric methods based on the reaction of boron with reagents such as azomethine-H [6-9], crystal violet [10], alizarin red [11] have also been developed and used to some extent. Some of these methods suffer from numerous interferences and have low sensitivity and precision.

Several flow injection analysis (FIA) methods for the determination of boron have been reported in literature [7-13] but some of them are fairly time consuming. The reagent consumption in flow injection analysis is very high making the technique expensive. Compared with flow injection

analysis (FIA), sequential injection analysis (SIA) system has an advantage of reduced samples and reagent consumption [14]. The simplicity of sequential injection analysis (SIA) system makes it readily suitable for automated routine operation. With the help of the selection valve, the SIA system enables different conditions of analysis to be attained without reconfiguring the system [15]. All essential parameters can be changed using a computer keyboard and this allows a simple and straight forward way of optimizing the system.

The use of boron in cleansing formulations and boric as a preservative often results in the necessity of analyzing traces of boron in various samples such as water and food. Since boric acid is a weak acid it cannot be titrated directly with a standard alkali solution. However some polyhydroxy compounds react with boric acid to form complexes which are sufficiently acidic to be titrated directly [16].

A sequential injection analysis (SIA) method for the determination of boron as boric acid is presented in this chapter. The method was successfully applied to the determination of boron in eye lotions using methyl orange as an indicator. Common cations and anions were found not to affect the determination of boron since the method is based on the acid-base reaction of methyl orange (MO) [17].

6.2. **Properties of boron**.

Boron is a non-metal with several allotropes. It is an extremely hard refractory solid of high melting point of 2573 K and a boiling point of 3931 K. Boron has a low density and a very low electrical conductivity.

The chemical reactivity of boron is influenced primarily by its small size and high ionization energy, and these factors coupled with the similarity in electronegativity of B, C and H, lead to an extensive and unusual type of covalent (molecular) chemistry. Amorphous boron is dark powder unreactive to oxygen, water, acids, and alkalis.

The small size of boron enables many interstitial alloy-type metal borides to be prepared, and the range of these is considerably extended by the propensity of boron to form branches and unbranched chains, planar networks, and three-dimensional arrays of great intrinsic stability which act as host frameworks to house metal atoms in various stoichiometric proportions.

6.3. **Natural occurrence.**

Boron is comparatively unabundant in the universe. It constitutes only about 0.001% of the earth's crust. It occurs to the extent of about 9 ppm in crustal rocks and is therefore rather less abundant than lithium (18 ppm) or lead (13 ppm) but is similar to praseodymium (9.1 ppm) and thorium (8.1 ppm). It occurs almost invariably as borate minerals or as borosilicates.

6.4. **Uses of boron.**

Amorphous boron is used in pyrotechnic flares (distinctive green colour), and rockets (as an igniter). Boric, or boracic, acid, is used as a mild antiseptic. Borax is a cleansing flux in welding and is a water softener in washing powders. Boron compounds are used in production of enamels for covering steel of refrigerators, washing machines, etc. Boron compounds are also used in the manufacture of enamels and borosilicate glasses.

Boron deficiency in plants may result in reduced growth, yield loss, and even death, depending on the severity of the deficiency. Excess boron is toxic to plants and animals. The tendency of boron to accumulate in animal and vegetable tissues constitute a potential hazard to the health of those consuming food and water with a high boron content.

6.5. **Experimental.**

6.5.1. **Reagents.**

All reagents were prepared from analytical-reagent grade unless specified otherwise. De-ionized water from a Modulab system (Continental Water System, San Antorio, TX, USA) was used throughout to prepare all solutions. All solutions were degassed before measurements with a vacuum pump system. The main solutions were prepared as follows.

6.5.1.1. **Standard boron solution.**

The standard boron solution (1000 $mg.L^{-1}$) was prepared by dissolving 5.715 g of boric acid in 1 liter of deionized water. This solution was stored in a polythene container. Working standard solutions were prepared from the stock solution by appropriate dilution with water.

6.5.1.2. **Methyl orange solution (MO).**

A stock solution of pH indicator was prepared by dissolving methyl orange (MO) in water to give a concentration of 2.0×10^{-3} $mol.dm^{-3}$.

6.5.1.3. **The D-sorbitol reagent solution.**

This reagent solution was prepared by dissolving 57 g of D-sorbitol in about 200 ml of water, adding 25 ml of the prepared 2.0 x 10^{-3} mol.dm^{-3} solution of methyl orange (MO) and diluting to 250 ml with de-ionized water.

6.5.1.4. **Carrier.**

Distilled water was used as a carrier.

6.5.2. **Sample preparation.**

The samples were diluted 2500-fold with water. This was done because of the high content of boric acid in the eye lotions [17].

6.5.3. **Apparatus.**

The sequential injection system was constructed from the following components : a Gilson Minipuls peristaltic pump ; a 10-port selection valve (Model E10-230, VICI Valco Instruments, USA) ; and a Unicam 8625 UV-visible spectrophotometer equipped with a 10 mm Hellma type flow through cell for absorbance measurements. For device control and data acquisition a PC 30-B interface board (Eagle Electric, Cape Town, South Africa) and FlowTEK software package (obtained from MINTEK) for computer aided flow analysis were used throughout.

6.5.4. **Manifold.**

The components of the SIA system were arranged as illustrated in Figure 6.1. A 350 cm holding coil (Tygon tubing) with 1.14 mm internal diameter was used. the reaction coil (Tygon tubing) was 200 cm long with an internal diameter of 0.64 mm.

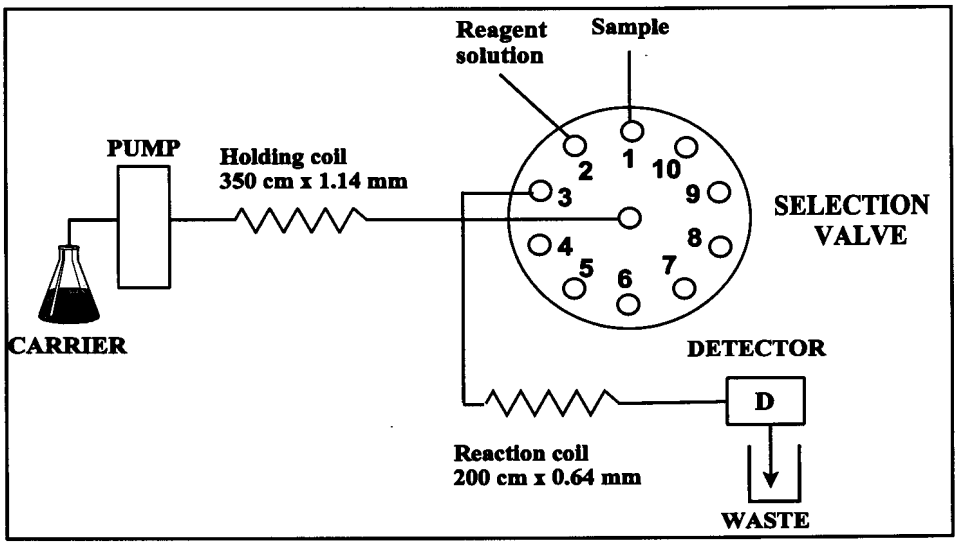

Figure 6.1. A schematic diagram of the SIA system used for the determination of boron as boric acid.

6.5.5. **Procedure.**

Illustration of the device sequence or the determination of boron is given in Table 6.1. The sample and the reagent solution were aspirated sequentially through the selection valve into the holding coil (HC). The carrier solution propelled by the peristaltic pump pushes the stack of well defined zones from the holding coil into the reaction coil (RC). The zones penetrate each other as they pass through the reaction coil to the detector. The absorbance of the complex is measured at 520 nm.

TABLE 6.1. Device sequence for one cycle of the SIA system for the determination of boron as boric acid.

Time (s)	Pump	Valve	Description
0	Off	Sample	Pump off, valve select sample stream. Valve position 1.
1	Reverse		Draw up the sample solution
7	Off		Pump stops
8		Reagent	Select the reagent stream. Valve position 2.
9	Reverse		Draw up the reagent solution.
11	Off		Pump stops.
12		Detector	Select the detector stream. Valve position 3.
13	Forward		Pump stack of zones to the detector.
120	Off	Home	Pump stops. Valve returns to position 1.

6.6. **Results and discussion.**

The determination of boron in eye lotions is based on the change in the methyl orange indicator as a results of the change in acidity of a sorbitol solution in the presence of boric acid. The concentration of the complex is proportional to the change in reaction colour formed when the sorbitol-boron complex is dissociated to a large extent in the presence of a pH indicator.

6.6.1. **Method optimization.**

Various factors that influence the performance of the proposed method were optimized.

6.6.1.1. **Flow rate.**

The effect of the flow rate on the peak height (Figure 6.2) was evaluated in order to establish the optimum working conditions. This was inspected because the flow rate plays an important role in the dispersion of the reaction zones. The best precision was obtained at a flow rate of 1.65 $mL.min^{-1}$.

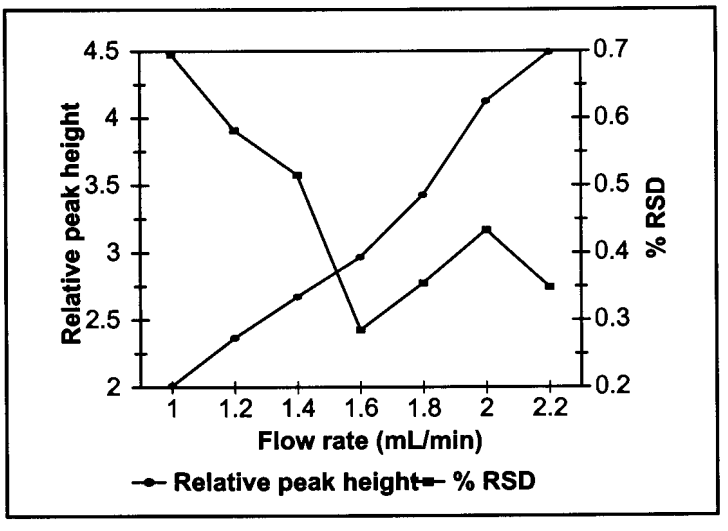

Figure 6.2. Influence of the carrier flow rate on the relative peak height.

6.6.1.2. **Sample and reagent volume.**

The sample and reagent's volume selection is one of the main tools in optimizing a sequential injection analysis system. This parameter plays a vital role in the degree of zones dispersion and zones penetration. Figure 6.3 illustrates the effect of the sample volume on the peak height. The peak height increases with an increase in the sample volume. A drastic increase is shown from 73 μL to 220 μL. The sensitivity of the method changes slightly with a volume of above 220 μL. This volume (220 μL) was regarded as the best volume because it was the one with good

sensitivity and precision. This volume was used to optimize the reagent volume (Figure 6.4). The sensitivity of the method increases with an increase in the reagent volume and the best precision was found with a volume of 73 μL.

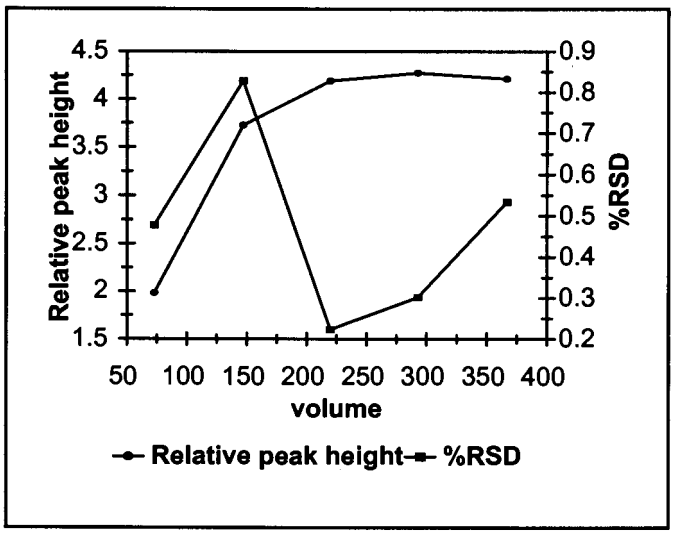

Figure 6.3. Influence of the sample volume (μL) on the relative peak height.

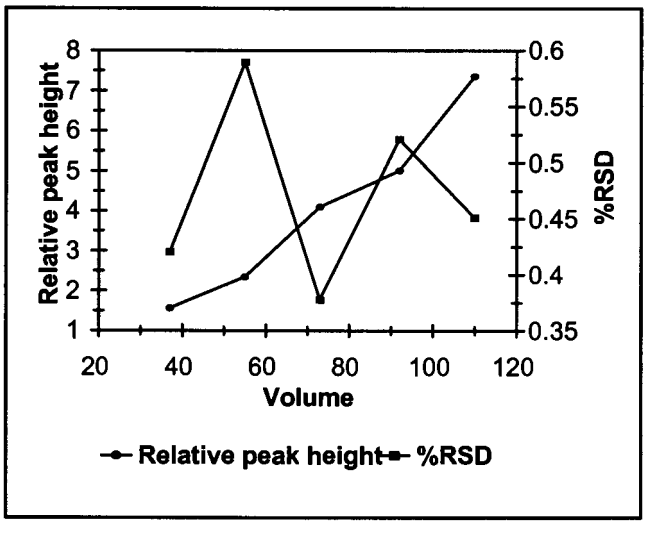

Figure 6.4. Influence of the D-sorbitol reagent volume (μL) on the relative peak height.

6.6.1.3. **Holding and reaction coil.**

A holding coil of 350 cm with an internal diameter of 1.1.4 mm was used. The length of the reaction coil has a significant effect on the sensitivity of the method and was evaluated from 100 cm to 500 cm. The sensitivity decreased when the length of the reaction coil increases. The best precision was found with a reaction coil of 200 cm which has an internal diameter of 0.64 mm.

6.6.2. **Method evaluation.**

The proposed SIA system was critically evaluated with regard to the linearity, accuracy, precision, detection limit and sample frequency.

6.3.2.1. **Linearity.**

Standard solutions of boron were used to evaluate the linearity of the method. The calibration graph is linear up to 12 $mg.L^{-1}$. The relationship between the peak height and the boron concentration is given by the equation below.

$$Y = 0.0328\ x + 2.0522 \qquad r = 0.999$$

where Y is the relative peak height and x is the concentration of boron in $mg.L^{-1}$.

6.6.2.2. **Accuracy.**

The accuracy of the proposed method was evaluated by comparing results obtained with the SIA system with those obtained with a standard procedure (spectrophotometric method) [17]. The

results given in Table 6.2 revealed a good correlation between the two methods.

6.6.2.3. **Precision.**

The precision of the proposed SIA method was evaluated by 10 repetitive analysis of a number of samples and standard solutions within the linear range of the method. In all cases a relative standard solution of less than 0.6 % was obtained as illustrated in Table 6.3.

Table 6.2. Comparison of the results between the proposed SIA method and a standard spectrophotometric method for the determination of boron as boric acid in eye lotions.

Sample	SIA method	spectrophotometric method
SA	3.08	3.12
SB	4.04	4.01
SC	2.23	2.19
SD	2.09	1.99
SE	2.03	1.97
SF	3.2	3.18

*n = 10

6.6.2.4. **Detection limit.**

The detection limit of the proposed method was determined using the following equation.

$$\text{Detection limit} = \frac{(3\delta + K)(K - c)}{m}$$

where δ is the relative standard deviation of the baseline (0.0.5911), K is the average signal value of the baseline (2.0525), c is the y-intercept (2.0521) and m is the slope of the calibration curve (0.0328). The value of the detection limit was found to be 0.06 $mg.L^{-1}$.

Table 6.3. Precision of the proposed SIA method (*n = 10).

Standards and Samples	Concentration in mg/L	% RSD
Standard 1	1 mg/L	0.03
Standard 2	3 mg/L	0.1
Standard 3	6 mg/L	0.06
Standard 4	9 mg/L	0.09
Sample 1	3.09 mg/L	0.43
Sample 2	4.01 mg/L	0.36

6.6.2.5. **Sample frequency**.

It took 130s to complete one cycle, resulting in a sample frequency of 27 samples per hour.

6.6.2.6. **Interferences.**

The determination of boron with the proposed method is not affected by common cations and anions since the method is based on the acid-base reaction of methyl orange. Fluoride and molybdenum can only interfere when present at the same concentration as boric acid.

6.6.2.7. **Statistical comparison.**

The comparison was done between the proposed SIA method and the standard spectrophotometric method. The aim of this comparison was to find out whether the proposed SIA method can be accepted as giving reliable results for the determination of boron in eye lotions. The null hypothesis which assumes that the quantities of the two different methods are the same was used in order to check if the results will agree.

The t-test with multiple samples (paired by difference) was applied to examine whether the two methods differ significantly at 95% confidence interval. The null hypothesis is H_0: $\zeta = 0$, against the alternative hypothesis H_t: $\zeta \neq 0$, where ζ is the population paired difference [18]. The test is two tailed as we are interested in both $\bar{x}_d < 0$ and $\bar{x}_d > 0$, where $\bar{x}_d$ is the mean difference of the two methods. The t-data paired test analysis was performance using the formula:

$t_{calc} = \left|\bar{x}_d\right| \times \sqrt{n}/s_d$, where $\bar{x}_d$ was -0.035 , standard deviation (Sx) was 0.0464 and n was 6.

Substituting for the calculated value of t (t_{calc}) we found 1.848.

For six determinations, the degree of freedom was 5 and at 95 % confidence interval $t_{0.05,5}$ = 2,571 with the critical t-values being ± 2.571. Since the value of t_{calc} = 1.848 lies between the critical values, the proposed SIA method can be accepted as giving reliable results.

6.7. **Conclusion.**

The proposed SIA method is a simple, inexpensive and reliable method for the determination of

boron as boric acid in eye lotions. This method has an advantage of being able to analyze 27 samples per hour with a good precision of less than 0.6 %. The results given by the proposed method were in good agreement with those obtained with a standard spectrophotometric method. The statistical comparison between the proposed SIA and the reference method shows no significant differences at 95 % probability level.

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CHAPTER 7.

Conclusions.

Sequential injection analysis has established itself nowadays as the method of choice in various fields as an analytical technique which is suitable for automation and increasing sample output in most analytical laboratories. While the 'classical' or continuos flow injection systems can operate well without the aid of a computer, they are uneconomical in terms of reagent consumption and waste generation, because all solutions are pumped continuously. In contrast, the recently designed sequential injection analysis, is based on discontinuous flow and consumes reagents only when the sample is being treated by exploiting a combination of stopped, reversal as well as forward flow in the microliter scale.

SIA is a technique that has great potential for on-line measurements due to the simplicity and convenience with which sample manipulations can be automated. The versatility of the sequential injection analysis system is centered around a selection valve where each port of the valve allows a different operation to be performed.

This technique can be recognized not only as a tool for serial assays, but, in fact, a tool for study of kinetics of chemical interactions and for process control. Its scope has broadened into environmental research, pharmaceutical, clinical, agricultural and most importantly into a tool of biotechnology and a tool for the study of the chemistry of life.

The aim of this research was to determine selected substances of biological importance from pharmaceutical industry (zinc, boron and paracetamol). The understanding of the basic operation of SIA made it possible to investigate the chemistry involved around each analysis. The developed SIA methods are simpler, rapid, economical viable and environmental friendly.

ADDENDUM.

ADDENDUM A.

METHOD CONSTRUCTION.

All experimental work done in this project was fully computerized. The software package used was the FlowTEK program, developed by Marshall [1, 2] specifically for the control of SIA and FIA. To explain the use of the FlowTEK program, as it was used in the experimental work, experimental setup for the determination of paracetamol in pharmaceutical products (described in Chapter 5) was chosen. In all the experimental work done, the runs were separated from each other by a rinsing period of 30 seconds to allow the carrier reagent to wash the system. The devices operated from the FlowTEK program were a peristaltic pump, a selection valve and a UV/VIS spectrophotometer. Following are the steps taken for the setup of the FlowTEK program before use.

A1. Setup of the FlowTEK program for device control and data collection.

A 1.1. **Detector**.

For all the experimental work done in this project, the UNICAM 5625 UV/VIS Spectrophotometer was used. When the computer is switched **on**, the main menu appears on the screen. From this menu select **FlowTEK** submenu. The FlowTEK page then appears and upon pressing **enter** the FlowTEK main menu appears. Press **S** for **setup** and the **D** for **detectors**. The questions and demands which follows were answered in the following way:

Enter number of detectors:	1
Enter signal transformation for detectors:	NON
Enter analog input for detector:	1 (position at which the detector is connected to the distribution box).

The base line was set by adjusting the relevant screw within the PC-30 distribution box.

A 1.2. **Pump and selection valve.**

The method with which the pump and the selection valve were operated was constructed in the following way.

From the main menu select **method (M)**. To identify the devices used in the construction of the method, select **Type device (T)** on the method menu. The following questions and demands were answered in the following way:

Enter number of devices:	2 (The pump and the selection valve)
Enter type device 1:	GP (Gilson pump)
Enter digital output point for GP:	1 (Indicates the position of the GP on the distribution box)
Enter type device 2:	SV (Selection valve)
Enter digital output point for SV:	3 (position of the SV on the distribution box)

The actions of the devices can be specified by making use of the arrow keys which moves the

curser from one device to another. The actions of the Gilson pump are specified as follows after selecting **insert** (**I**) on the method menu.

Enter event (FRO):	F (for forward)
	R (or reverse)
	O (for off)

The actions of the selection valve are specified as follows:

Enter event (AH):	A (Moving to the following port)
	H (Home)

An event that was introduced previously can be deleted in the same way. Instead of selecting *Insert* from the menu, one has to select *Delete*.

The experimental time has to be specified by selecting **EXP** time (**E**) from the method menu and answer the questions following:

Enter time to start data collection:	30
Enter experimental time	130

The method can be saved after construction by selecting **File** (**F**) on the method menu. To save the method, choose **Save** (**S**) on the following sequence of instructions: **Save** (**S**), **Retrieve** (**R**), **Delete** (**D**). Then the method file menu must be entered.

For the determination of paracetamol (Chapter 5), the **File** on the **Method** menu was selected and the questions were answered as follows:

Save, Retrieve or Erase (S R E):	S
Enter method file name:	C:\FlowTEK\mutshu\paracet\.met

The device descriptions can be viewed under the *notepad* menu (Figure A.1). The first six device definitions are supplied with the software package. The are six more devices that can be user specified.

Next Page Hard Copy RED Print MET Print PDR Print Quit

Board : PC30-B
Experiment time : 130.0
Zoom min time : 0.0
Zoom max time : 130.0
Start acquisition : 0.0
I/O port for GP : 1
I/O port for SV : 3
Save profile : Yes
Abridged profile : No
Regression on Height
Detector displ : Paged
Inject mode : Auto
Startup : (0)
Rescale Y-axis : Auto

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

Detector	1	2	3	4
A/D channel	1			
Transformation	Inverse			
Auto Zero	None			
AZ time	0.0			
AZ offset	0.000			
Min Integ Lim	0.0			
Max Integ Lim	130.0			
Width Height	0.000			
Peak Time	@ Pk max			

Path : c:\floutek\MUTSHUTSHU\
Main Procedure file : CALIB.PDR
Method file : PARACETA.MET
Reduced data file : CAL.RED
Experiment Profile Root : CAL
Calibration file : DEFAULT.CAL

Name	CP	CS	CV	AP	GP	IV
Action	PICK	PICK	IN	FWD	FWD	INJ
	DISP	DISP	OUT	REV	REV	LOAD
	STOP	STOP		OFF	OFF	
Hotkey	P	P	I	F	F	I
	D	D	O	R	R	L
	T	T		O	O	
Output	01 (1)	01 (1)	1 (1)	01 (1)	10 (2)	01 (1)
	10 (2)	10 (2)	0 (0)	10 (2)	11 (3)	10 (2)
	00 (0)	00 (0)		00 (0)	00 (0)	
Pulse	0.00	0.00	0.00	0.00	0.00	0.00

Figure A.1. Schematic representation of the FlowTEK notepad screen (page 1 for device description).

The letter **N** must be typed to view the second page of the device configurations (Figure A.2.).

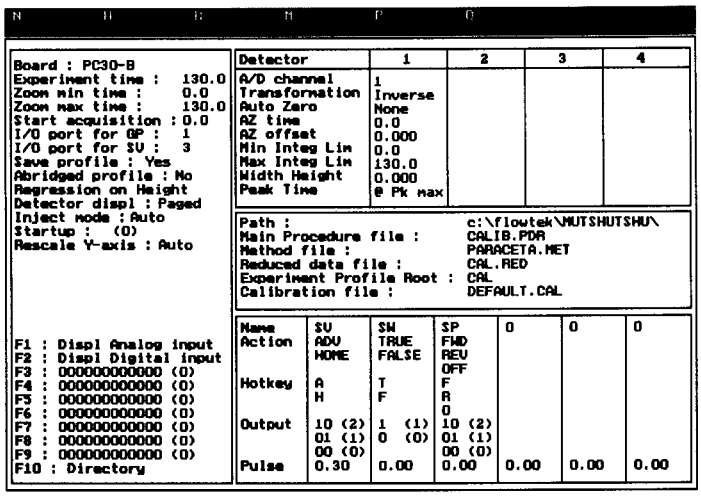

Figure A.2. Schematic representation of the FlowTEK notepad screen (page 2 for device descriptions).

The final method is represented schematically in Figure A.3.

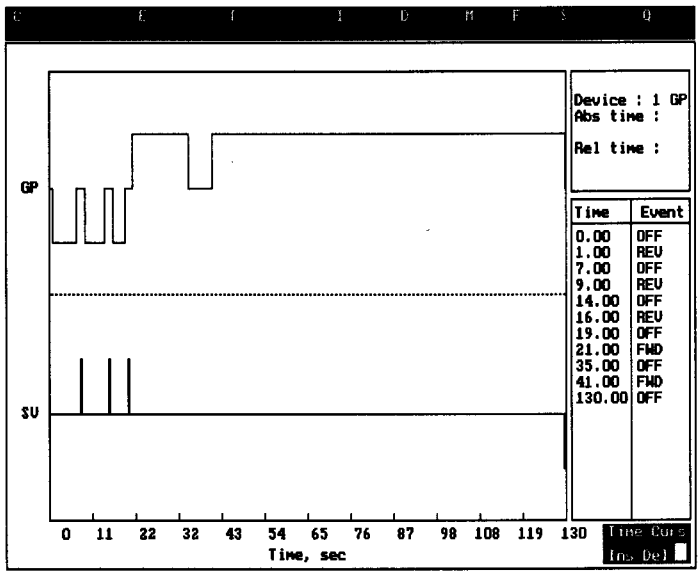

Figure A.3. Schematic representation of the FlowTEK method screen.

Exit the menu until the main menu appears and choose **Once (O)** to initiate a single run.

A 2. **References.**

1. G. D. Marshall, J. F. van Staden *Anal. Instrum.* 1992, **20**, 79.

2. FlowTEK Reference Manual, Device Control and data Acquisition software, ver 1.1, Mintek, 1993.

ADDENDUM B.

PUBLICATIONS AND PRESENTATIONS.

B 1. **Publications.**

1. M. Tsanwani and J. F. van Staden, Determination of zinc in pharmaceutical products by use of sequential injection analysis system, *Fresenius J. Anal. Chem.*, 2001, **371**, 376.

2. M. Tsanwani and J. F. van Staden. Determination of paracetamol in pharmaceutical samples using a sequential injection analysis system. (Submitted).

3. M. Tsanwani and J. F. van Staden. Determination of boron in eye lotions using a sequential injection analysis system. (Submitted).

B 2. **Presentations.**

1. Determination of zinc in pharmaceutical products by use of a sequential injection analysis system.

Instrumental Methods of Analysis 2001, University of Ioannina, Greece, 5-8 September 2001.

M. Tsanwani, **J. F. van Staden and R. I. Stefan.** (Poster)

2. Determination of zinc in pharmaceutical products by use of a sequential injection analysis system..

SACI Young Chemist Symposium, Vaal Triangle Technikon, South Africa, 24 October 2001.

M. Tsanwani. J. F. van Staden. (Oral).

3. Determination of paracetamol in pharmaceutical samples using an SIA system.

11th international conference on flow injection analysis, including related techniques. Chiang Mai 50200, Thailand. 16-20 December 2001.

M. Tsanwani, **J. F. van Staden**. (Poster).

4. Determination of boron as boric acid in eye lotions using an SIA system.

11th international conference on flow injection analysis, including related techniques. Chiang Mai 50200, Thailand. 16-20 December 2001.

M. Tsanwani, **J. F. van Staden**. (Poster).

ADDENDUM C.

LIST OF SYMBOLS.

Sample/reagents:	C	= concentration (mol/L)
	C^o	= initial concentration (mol/L)
	C^{max}	= concentration at signal maximum (mol/L)
	V_i	= injected volume (μL)
Reactor geometry:	L	= overall tubes length (cm)
	l	= partial tube length (cm)
	R	= tubes radius (mm)
	r	= partial tubes radius (mm)
	d	= tube diameter (mm)
	V_r	= volume of reactor/system (mL)
Hydrodynamic aspects:	q	= flow rate (mL. min^{-1})
	u	= linear velocity (cm. sec^{-1})
	u_o	= maximum linear velocity (cm. sec^{-1})
FIA/SIA:	D	= dispersion coefficient
	t_a	= travel time (sec)
	Δt	= baseline-to-baseline time (sec)
	T	= residence time.