CHAPTER 6

Determination of iron as Fe(II) in multi-vitamins, haematinics and natural waters using a sequential injection analysis (SIA) system.

6.1 Introduction

Iron is the second most abundant metal, after aluminium, and fourth most abundant element in the earth's crust. The core of the earth is believed to consist mainly of iron and nickel, and the occurrence of many iron meteorites suggests that it is abundant throughout the solar system. The major iron ores are hematite (Fe_2O_3), Magnetite (Fe_3O_4), Limonite [FeO(OH)], and Siderite ($FeCO_3$) [1].

However, from a biological viewpoint iron falls into the category of trace elements, which are most conveniently classified as essential, non-essential and toxic. The trace elements classified as essential for plants are those elements which cannot be substituted by others in their specific biochemical roles and that have a direct influence on the organism so that it can neither grow nor complete some metabolic cycle. In human and animals systems, trace elements are defined as being essential if depletion consistently results in a deficiency syndrome and repletion specifically reverses the abnormalities. Deficiency of iron causes anaemia and excess causes

liver cirrhosis and haemochromatosis.

The iron containing proteins in a normal adult are haemoglobin, myoglobin, transferrin, ferritin, hemosiderin, catalase, cytochrome *C*, peroxidase, cytochrome and oxidase, flavoprotein dehydrogenase, oxidases and oxygenases.

A wide range of human activities contributes to iron pollution of the aquatic environments. The major activities including mining, industrial processing, agricultural and domestic effluents of sewage, the impact of iron input, duration of input, physical and chemical form and associated ligands or chemicals [2].

Iron occurs primarily in two oxidation states, Fe(II) and Fe(III). In deep waters, especially in lakes where there is no oxygen, iron is in the Fe(II) state and in surface water it is in the Fe(III) state.

The maximum admissible concentration in water recognised by the European Economic Community (EEC) Directive on Quality of water for Human Consumption is 0.2 mg/ℓ while the guide value which is desirable is given as 0.05 mg/ℓ. The Russian standards, the World Health Organisation (WHO) and the EEC Surface Water Directive allows values up to 0.3, 0.1 and 2 mg/ℓ respectively. However, in most potable water supplies a concentration of 0.05 mg/ℓ would appear to be a satisfactory upper limit [3]. It is however, important to understand the specific range of iron concentration the body allows; which will in turn dictate the daily requirement. The daily requirement for men is 10 mg and 15 mg for women [6]. But, Heinrich *et al.* [7] has shown that the amount of average requirement is 1.5 mg/day in males and non-

menstruating females and 14.8 mg/day in menstruating females.

The high presence of iron can give rise to an astrigent taste, discolouration, deposits of rusts and could promote iron bacteria growth.

Finally, iron is vital and required for various biological features in the human body. Deficiency are known to occur in vulnerable populations such as pregnant women, infants and children as well as mal-nutritioned individuals. In order to avoid such deficiencies, an adequate supply of iron that can be utilized for biological functions is needed. Individual components of the diet and iron status of each individual will affect the bio-availability [6]. However, besides diet iron requirements may be supplied by administering multivitamins and haematinics.

6.2 The biochemistry and biological uses of iron

Trace amounts of iron is truly ubiquitous in living systems. It is versatile and unique. It is important for the prevention of anaemia. It is at the active centre of molecules responsible for oxygen transport and electron transport and it is found in, or with, such diverse metalo-enzymes as nitrogenases and dehydrases.

The iron containing proteins in a normal adult perform a specific function as is given by the following proteins:

Haemoglobin - oxygen transport in plasma

Myoglobin - oxygen storage in muscle

Transferrin - iron transport via plasma

Hemosideria - iron storage in cells

Catalase - metabolism of H_2O_2

Cytochrome - terminal oxidation

Cytochrome and oxidase - terminal oxidation

Peroxidase - metabolism of H_2O_2

Flavo protein dehydrogenase,

oxidases and oxygenase - oxidation reactions, incorporation of molecular oxygen [1].

Iron is used or administered to iron deficiency subjects to prevent anaemia. However, if not monitored on individual with excess it may cause liver cirrhosis and haemochromatosis.

6.3 Choice of analytical method

The determination of iron in its various oxidation states in a variety of matrices has been studied and described by numerous researchers [7-12]. Methods used include kinetic spectrometry [8-11], Polarography [13] Graphite Furnace Atomic Absorption Spectrometry (GFAAS) [13] and Flame Atomic Absorption Spectrometry (AAS) [14]. Most of these classical technique have been modified for use in-flow system by making use of the flexibility and ease offered by flow injection analysis (FIA) [9, 15-17].

Speciation of Fe(III) and Fe(II) [18-20] was also described. Faizullah and Townshed [21] determined Fe(II) after complexing with 1,10 Phenanthroline, then reducing the Fe(III) present

with a reducing column. Lynch *et al.* [22] described the use of different complexing agents in the same manifold for determining Fe(II) and Fe(III).

Simultaneously, Masatoshi and Shigeki [23] developed a method for the sequential spectrophotometric determination of Fe(III) and Fe(II) by a copper(II) catalysed reaction with Tiron in a double-injection flow injection system. Oliveira *et al.* [24], proposed an asynchronous merging zones method with simultaneous introduction of the sample and modifier (ascorbic acid) for sequential determination of Fe(II) and Fe(III) in pharmaceutical products. Luque-Perez *et al.* [25] indirectly determined ascorbic acid by reducing Fe(III) to Fe(II) with ascorbic acid and monitoring the Ferroin complex spectrophotometrically. Van Staden and Kluever [26, 27] modified an existing FIA homogeneous system to a heterogeneous systems by incorporating solid-phase reactors into the FIA manifolds.

The homogeneous SIA technique was also used for the determination of iron [28-30]. This technique launched in 1990 [31, 32], is a technique that has great potential for on-line measurement in many routine laboratories due to its simplicity and the convenience with which sample manipulation can be automated. The pre-requisite needed for the determination of iron, in pharmaceutical products and natural waters, was an enhanced technique, which is robust and versatile, reliable with a low frequency of maintenance. The system should be simplified with fewer junctions for reagents and less sample preparations and carrier streams. SIA seemed the ideal technique for this analysis.

6.4 Total iron determination

An iron(III) sample was reduced on-line to iron(II) by a cadmium reductor incorporated into SIA manifold, complexed with 1,10 Phenantroline to a red stable complex and detected at 515 nm with a UV/VIS spectrophotometer[33]. The choice of this wavelength was made after a scan of the standard solution over a range of 200 - 1100 nm.

6.4.1 Experimental

6.4.1.1 Reagents and solutions

All solutions are prepared from analytical grade reagents unless specified otherwise. De-ionised water from a Molulab system (Continental Water Systems, San Antonio, TX, USA) was used to prepare all aqueous solutions and dilutions.

6.4.1.1.1 Stock iron (II) solution

A stock iron(II) solution containing 1000mg/l iron(II) was prepared by dissolving $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ (Kanto Chemical Co., extra pure) and diluting to 1 litre with water. Working standards in the range 1 to 100 mg/ℓ were prepared by appropriate dilution of the stock solution with 0.01 mol/ℓ perchloric acid.

6.4.1.1.2 Perchloric acid solution

A 0.01 mol/ℓ perchloric acid was prepared by diluting 4.4 mℓ of perchloric acid (Merck, GPR, 70%) to 5 ℓ with deionised water.

6.4.1.1.3 1.10 Phenanthroline solution

.A 0.25% 1,10 Phenanthroline solution (Aldrich, 99+%) was prepared by dissolving 0.625 g of 1,10 Phenanthroline in 50 ml 0.01 mol/ℓ perchloric acid and diluting to 250 mℓ with water.

6.4.1.1.4 Acetic acid solution

A 0.1 mol/ℓ acetic acid solution was prepared by diluting 1.45 mℓ of acetic acid (Chemical suppliers, 99.9%) to 250 mℓ with deionised water.

6.4.1.1.5 Hydroxyl ammonium chloride solution

A 10% hydroxyl ammonium chloride (Searle, GPR, 97%) was prepared by dissolving 10 g in water and making up to 100 mℓ with deionised water.

6.4.1.1.6 Sodium acetate solution

A 0.1 mol/ℓ sodium acetate solution was prepared by dissolving 1.36 g of sodium acetate (Merck, extra pure) in water and making up to 100 mℓ.

6.4.1.1.7 Buffer solution

A buffer solution in the pH range range 3 to 5 was prepared by mixing 65 mℓ of 0.1 mol/ℓ acetic acid solution with 35 mℓ 0.1 mol/ℓ sodium acetate solution and adding 1 ml of 10% hydroxyl ammonium chloride to the resulting solution.

6.4.1.1.8 Hydrochloric acid solution

A 1 mol/ℓ HCl solution was prepared by diluting 100 mℓ of concentration HCl (Merck, 32%) and making up to a litre. chloroform (Merck, pro analysis) was used to extract the unwanted organic material from the samples.

6.4.1.2 Instrumentation

A sequential injection system is depicted in Fig. 6.1. It was constructed from the following components:

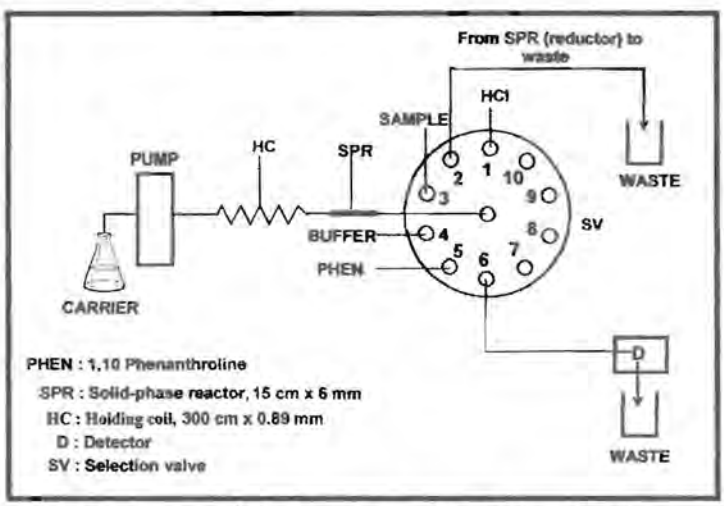

Fig. 6.1 A diagram of a SIA system used in this investigation

a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-Le Bel, France); a 10-port electrically actuated selection valve (Model ECSDIOP, Valco Instruments, Houston, Texas) and a Unicam 8625 UV-Visible spectrophotometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mulheim/Baden, Germany) flow- through cell (volume 80 $\mu\ell$) for absorbance measurements.

Data acquisition and device control was achieved by using a PC30-B interface board (Eagle Electric, Cape Town) and an assembled distribution board (Mintek,Randburg). The flowTEK [34] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition.

6.4.1.3 Operation of the system

The whole SIA procedure involved designing a method which allows a single cycle of the experiment to be run. Fig. 6.2 and Table 6.1 shows the device sequence for one cycle.

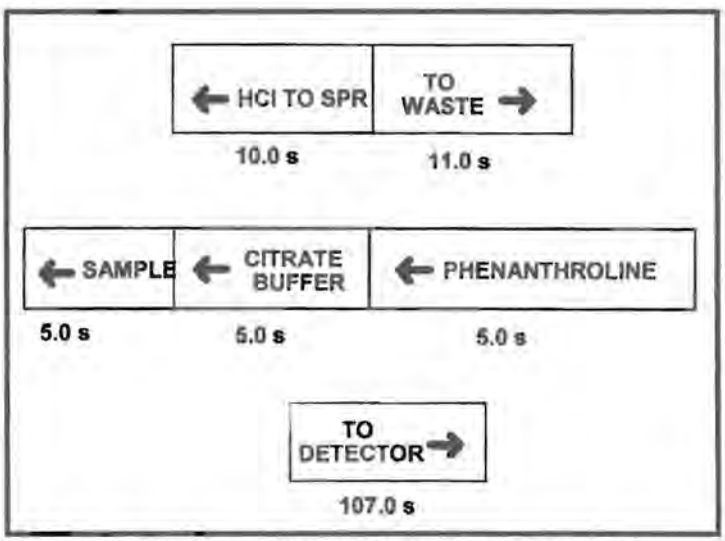

Fig. 6.2 Device sequence diagram for one cycle of the SIA system

The whole procedure, from sample injection to data processing and storage was computer controlled via flowTEK program.

TABLE 6.1 Device sequence for one cycle of the SIA system

Time (s)	Pump	Valve	Description
0	Off	Position 1	Pump off. Select HCl stream.
1	Reverse		Draw HCl solution through SPR for regeneration.
10	Off		Pump stop.
11	Off	Position 2	Select waste stream.
12	Forward		Pump solution from SPR to waste
23	Off		Pump stop
24	Off	Position 3	Select sample stream
25	Reverse		Draw sample
29	Off		Pump stop
30	Off	Position 4	Select buffer stream
31	Reverse		Draw buffer solution
35	Off		Pump stop
36	Off	Position 5	Select 1.10 Phenanthroline stream
37	Reverse		Draw 1.10 Phenanthroline
41	Off		Pump stop
42	Off	Position 6	Select detector stream
43	Forward		Pump zones through reductor to detector
150	Off	Position 1	Valve return home

The zones were stacked in the holding coil and then transported by the carrier stream (0.01 mol/ℓ perchloric acid) through the reactor where all the iron(III) that may be present is reduced to iron(II). The iron(II) then complexes with 1,10 Phenanthroline and is detected at 515 nm with

a spectrophotometer. The working wavelength at 515 nm was established by scanning the standard solution from 200 to 1100 nm.

The complex formation can be described by the equation:

$$Fe^{2+} + 3\ pheH^{+} \rightarrow Fe(phe)_3^{2+} + 3\ H^{+}$$

The data obtained is then converted to a response time graph on the computer screen as a peak profile. The maximum relative peak height was then automatically processed and stored on a computer via the FlowTEK program.

6.4.1.4 The solid-phase reactor preparation

The reactors were made of glass with varying lengths (12, 15, 17, 19 and 21 cm) but with the same internal diameter of 6 mm. Fig. 6.3 shows such a reactor. The columns were then filled with cadmium granules (Merck, 0.3-1.5 mm).

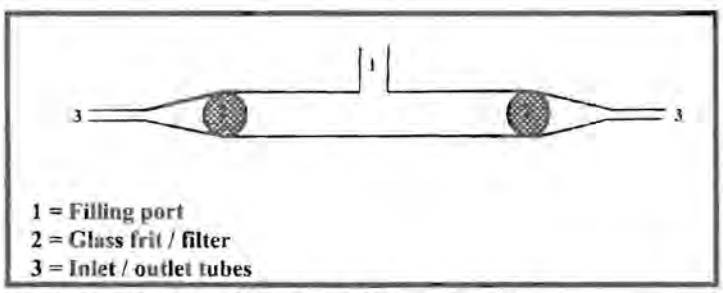

Fig. 6.3 A diagram of the cylindrical reactor used

The particles were held by a glass frit at each end so that they did not block the SIA system. A vibrator was used for close packing of the columns. The cadmium granules used for packing the glass column were prepared by washing with acetone for 10 minutes, adding 2 mol/ℓ hydrochloric acid, de-ionised water and methanol and dried in a desiccator. An acidified cadmium reactor was chosen over a copperised one because copper has a tendency of interfering in the determination of iron. The cadmium reactor was regenerated by passing approximately 470 $\mu\ell$ of 1 mol/ℓ hydrochloric acid at the beginning of every cycle. This was to ensure consistency in the reduction efficiency and capacity of the reactor.

6.4.1.5 Sample preparation

The multivitamin and haematinic samples were digested in 50 mℓ (6% V/V) hydrochloric acid on a hot plate. When a fifth of the solution was remaining a further 30 mℓ of the (6% V/V) hydrochloric acid was added and the digestion continued until approximately 10 mℓ was remaining. Three 50 mℓ portions of chloroform (Merck, pro analysis) were added to the samples with vigorous shaking to separate the organic material from the inorganic. The separation at each instance was allowed two hours. A final 50 mℓ portion was added and left overnight for final separation.

The aqueous layer was collected into a 100 mℓ standard flask and made to volume with a 0.01 mol/ℓ perchloric acid solution. Further dilutions were made from the prepared samples to bring their concentrations within detectable range in the SIA system.

6.4.2 Method optimization

The method was optimised with regard to the following parameters: iron(II) concentration, flow rate, sample and reagent volume, reactor length, carrier type and hydrochloric acid concentration for reactor regeneration (reactor efficiency). Both the relative peak height and % RSD were used as criteria for establishing the most appropriate parameter value in each case.

6.4.2.1 Solid-phase reactor

The cadmium reactor forms the heart of the manifold of the proposed system. The performance of the SIA system depends on the efficiency of the reactor at the interface between the solid and the liquid phases of the cadmium reactor. In addition the reactor packing had to be thorough and the reactor length and efficiency had to be optimised.

6.4.2.1.1 Reactor length

The response and precision of the system were studied by varying the reactor length between 12 and 21cm with the internal diameter fixed at 6 mm. The five reactors (12, 15, 17, 19 and 21 cm) were compared for reduction efficiency. It was found from the results obtained (Table 6.2) that the first three cadmium reactors did not show a significant difference in response; there was, however for the longer lengths. The 15 cm reactor length was chosen as the optimum length because of its good precision as seen in Table 6.2. Fig.6.4 shows the effect of reactor length on response and precision.

TABLE 6.2 Effect of reactor length on response and precision

Length (cm)	12	15	17	19	21
Relative peak heights	5.262	5.281	5.31	5.872	6.521
% RSD	2	1.1	5.8	5.34	4.38

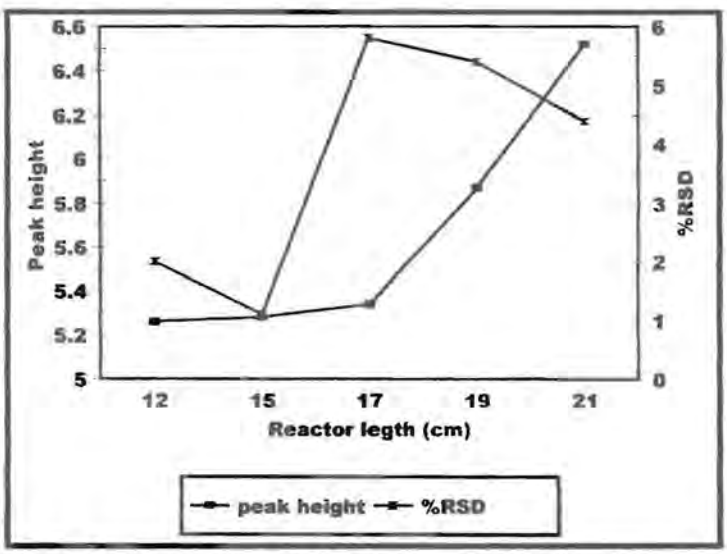

Fig. 6.4 Influence of reactor length on response and precision

6.4.2.2 Chemical parameters

6.4.2.2.1 Fe(II) concentration

The Fe(II) concentration was evaluated between 1 to 100 mg/ℓ. The effect of concentration is presented in Table 6.3 and Fig. 6.5. It is clear from Figure 6.4 that the response steadily increases with an increase in concentration. The 50 mg/ℓ concentration gave the best precision and was chosen as the optimum concentration.

TABLE 6.3 Effect of Fe(II) concentration on response and precision

Concentration mg/ℓ	Peak height	% RSD
1	0.304	1.43
10	1.145	0.68
20	2.166	1.7
30	3.206	1.57
40	4.337	1.28
50	5.329	0.42
60	6.230	1.92
70	7.458	1.5
80	8.260	1.9
90	8.834	0.8
100	8.841	1.0

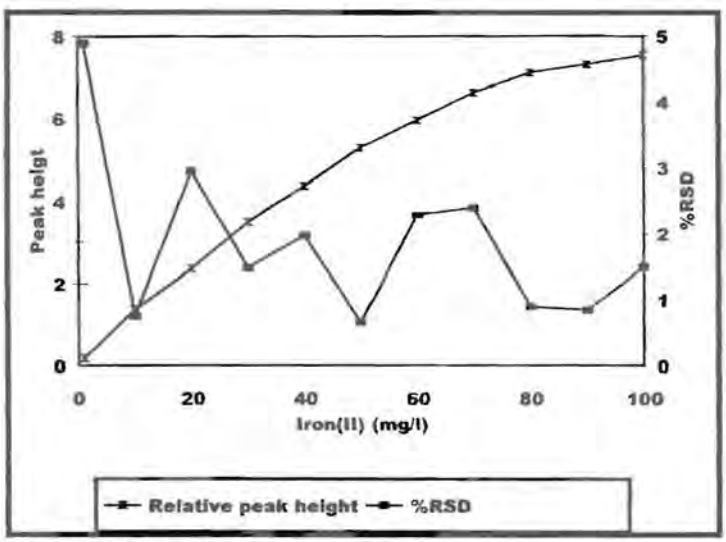

Fig. 6.5 Effect of iron (II) concentration on response and precision

6.4.2.2.2 Carrier concentration

The use of 1 mol/ℓ hydrochloric acid as both reactor regeneration and carrier could not work because bubbles were given off now and then. However, the use of 0.1 mol/ℓ perchloric acid resulted in a better consistency in response and there were no bubbles. The perchloric acid concentration was then studied between 0.005 and 0.1 mol/ℓ and the results given in Fig. 6.6 and Table 6.4. The response increases up to a concentration of 0.05 mol/ℓ. The best precision was however given by a concentration of 0.01 ml/ℓ which was chosen as the optimum.

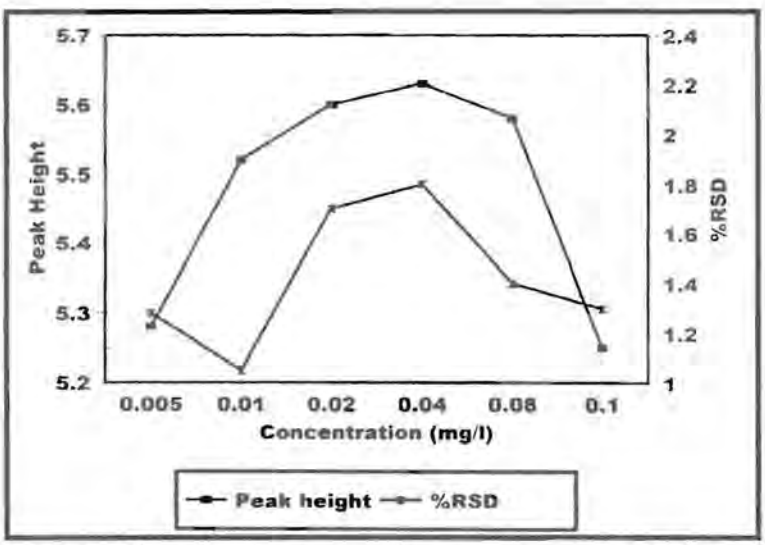

Fig. 6.6 Effect of carrier concentration on response and precision

TABLE 6.4 Effect of carrier concentration on response and precision

Concentration mg/ℓ	Peak height	% RSD
0.005	5.28	1.28
0.01	5.52	1.05
0.02	5.6	1.7
0.04	5.63	1.8
0.08	5.58	1.4
0.1	5.25	1.3

6.4.2.3 Physical parameters

The contact time between the iron and the cadmium reactor is of utmost importance. It was however, found that most of the iron in the pharmaceutical preparation is in the Fe(II) state, with very little in the Fe(III) state. Although the amount of iron present in the water samples analysed was lower, all the iron was in the Fe(III) state and had to be reduced. The 15 cm reactor was found to be optimum and effective with 470 $\mu\ell$ 1 mol/ℓ HCl passed through the reactor for every SIA cycle.

6.4.2.3.1 Flow rate

The flow rate was evaluated between 1.13 and 3.96 mℓ/min. The results and effect of this are illustrated in Table 6.5. Fig. 6.7 shows the response and precision of this optimization. The response increases with an increase in flow rate, due to less dispersion and better zone overlapping. The 2.83 mℓ/min flow rate however gave the best precision and was chosen as

optimum.

TABLE 6.5 Effect of flow rate on response and precision

Rate (mℓ/min)	1.13	1.71	2.26	2.83	3.29	3.96
Relative peak heights	0.557	1.929	2.544	3.612	3.951	4.033
% RSD	5.6	4.6	3.3	2.1	2.4	7.5

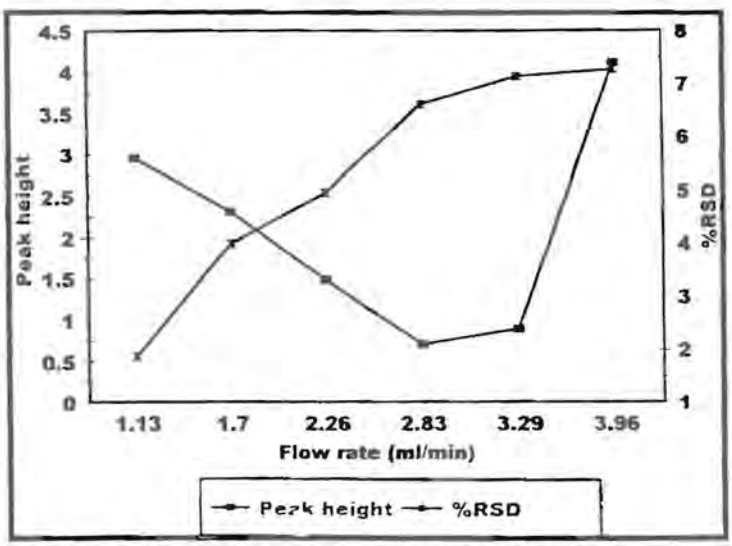

Fig. 6.7 Influence of flow rate on response and precision

6.4.2.3.2 Sample volume

Sample volume was evaluated from 142 to 424 $\mu\ell$ and the results are given in Table 6.6. Fig. 6.8 gives the response and precision of this optimisation. Although the sensitivity increases with an increase in sample volume, the best precision was obtained with a sample volume of 236 $\mu\ell$ which was chosen as optimum sample volume.

TABLE 6.6 Effect of sample volume on response and precision

Volume (mℓ)	142	236	330	424
Relative peak heights	2.847	3.951	4.404	4.376
% RSD	3.3	2.4	3.2	3.1

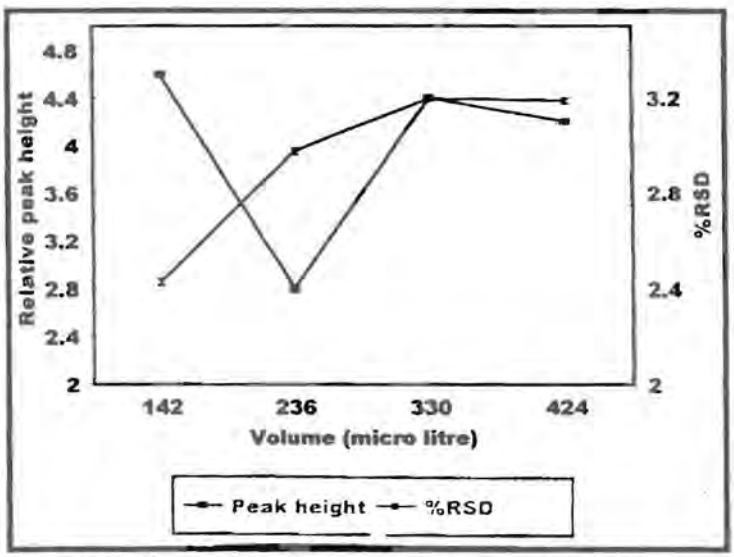

Fig. 6.8 Effect of sample volume on response and precision

6.4.2.3.3 Reagent volume

The reagent volume was evaluated from 94 to 283 $\mu\ell$ (Table 6.7). A volume, 236 $\mu\ell$ was chosen as optimum reagent volume due to the best precision. Fig. 6.9 gives the response and precision for this optimization.

TABLE 6.7 Effect of reagent volume on response and precision

Volume (mℓ)	94	142	189	236	283
Relative peak heights	1.766	4.464	6.847	8.62	8.72
% RSD	2.9	3.2	1	0.8	4.2

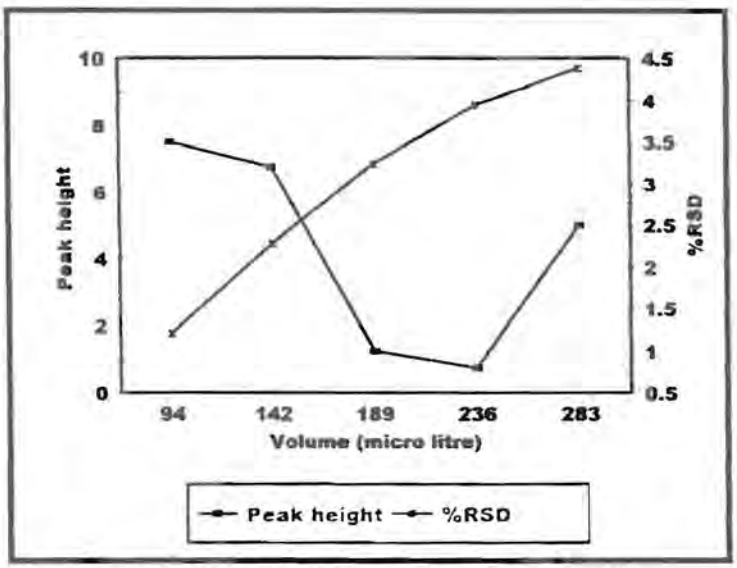

Fig. 6.9 Effect of reagent volume on response and precision

6.4.3 Method evaluation

6.4.3.1 Linearity

The linearity of the system was evaluated for the analyte concentration between 1 and 100 mg/ℓ. The response was found to be linear in the range 1 to 60 mg/ℓ (Fig. 6.9). The relationship between the response and the concentration is given by the equation:

$$H = 0.149x + 0.1722, (r = 99.99\%, n = 10),$$

where H is the relative peak height and x the analyte concentration in parts per million mg/ℓ(ppm).

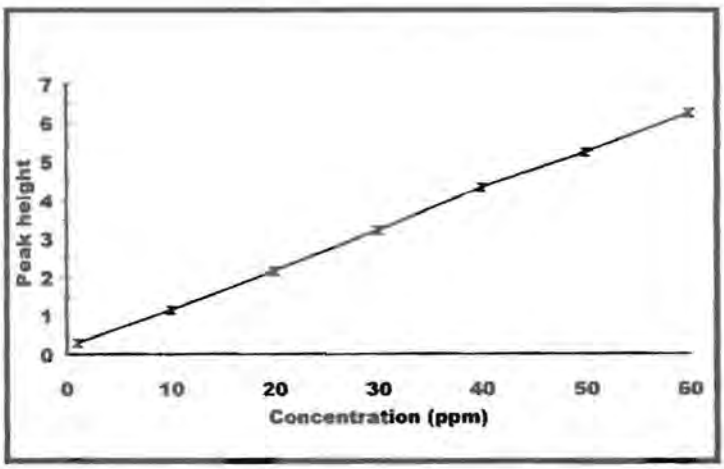

Fig. 6.10 A calibration graph using optimum values

All these were carried out and under optimum conditions (Table 6.8).

TABLE 6.8 Optimum values for the SIA system.

Parameter	**Optimum value**
Reactor length	15 cm
Reactor diameter	6 mm
Carrier concentration	0.01 mol/ℓ
Flow rate	2.83 mℓ/min
Sample volume	236 $\mu\ell$
Reagent volume	236 $\mu\ell$

Real samples (multivitamins and haematinics) and water samples were analysed with the proposed system. The results obtained are a mean of 10 repetitive analyses of each sample. The accuracy was compared to certified values and the standard method (Table 6.9 and 6.10).

TABLE 6.9 Iron in multivitamin and haematinics using SIA and spectrophotometric methods as well as certified values in mg/tablet (capsule) and % RSD's in brackets.

Sample	Certified values	Proposed SIA method	Standard spectrophotometric method
Filibon	15	16.04 (2.3%)	15.3 (4.2%)
Ferrimed	50	47.75 (1.6%)	51.52 (4.3%)
Pregamal	56	52.65 (1.8%)	51.02 (4.5%)
Ferrous C	28	27.19 (1.2%)	27.16 (2.7%)

TABLE 6.10 Iron in effluent streams using SIA and ICP methods

Sample	Concentration in mg/ℓ		Relative standard deviation (%)	
	SIA	ICP-AES	SIA	ICP-AES
A	0.759	0.861	2.3	2.2
B	0.331	0.296	1.3	2.8
C	0.266	0.241	0.3	3.9

6.4.3.2 Accuracy

The accuracy of the proposed SIA analyser was evaluated by comparing results obtained with those from the certified results, standard ICP-AES and spectrophotometer. Tables 6.9 and 6.10 shows that the results compared well, especially between the certified results and SIA results.

6.4.3.3 Recovery

The recovery of the system was evaluated by comparing results obtained with the proposed SIA system and rthe cerified results (Table 6.9). The percentage recovery after analysing the samples with the SIA system was determined according to this equation:

$$\%\,\mathrm{Recovery} = \frac{obtained}{\mathrm{expected}} x100$$

The percentage recovery between certified results and SIA results ranged from 103.1% to 106.9%.

6.4.3.4 Precision

The precision of the method was determined by 10 repetitive analyses of the standard solutions as well as 10 repetitive analyses of the real samples. All these were carried out under optimum conditions. The relative standard deviation for the standard was 1.5% and for the real samples was less than 2.3% (Tables 6.9 and 6.10).

6.4.3.5 Detection limit

The detection limit was calculated using the formula:

$$DL = \frac{[(3\sigma + k) - c]}{m}$$

where σ (0.00529) is the standard deviation of the baseline, k is the average response of the baseline (0.175) and c (0.1722) the intercept and m (0.1049) the slope of the calibration graph. The detection limit was found to be 0.178 mg/ℓ.

6.4.3.6 Sample interaction

The sample interaction carryover effect between consecutive samples was determined by analysing sample with low analyte concentration followed with a high analyte concentration which was again followed by the sample with a low analyte concentration. The sample interaction was then calculated using the following formula:

$$\text{Sample interaction} = \frac{(A_3 - A_1)}{A_2} x100\%$$

where A_1 is the peak height (1.35) of a sample containing to 10 mg/ℓ Fe(II), A_2 is a peak height (5.52) containing 50 mg/ℓ Fe(II) and A_3 is a peak height (1.42) containing 10 mg/ℓ Fe(II). The sample interaction was ± 1.3% which may be considered negligible.

6.4.3.7 Interferences

The only possible interferences that may disturb this analysis are Ag, Bi, Ni, Cu and Co. Fortunately of al these, only Cu was found to be present, but in very low levels which may not affect the results. Table 6.11 gives the elements present in samples analysed and their amounts. In the work done by van Staden and Kluever [27] these levels of cations did not interfere with the analysis of Fe (II) as shown by the recoveries obtained.

TABLE 6.11 Elements present in samples analysed as mg/tablet or capsule

Element	Amount/tablet (capsule)	Element	Amount/tablet (capsule)
Ca	< 5 mg	Na	< 1 mg
K	< 0.83 mg	Mg	< 0.15 mg
Mn	< 0.05 mg	Zn	< 0.085 mg
Mo	< 0.025 mg	Cu	< 0.15 mg

6.5 Statistical comparison of techniques used

The comparison was done between the SIA results and the certified values (Table 6.9) and between the SIA results and the standard spectrophotometric method results (Table 6.10), for pharmaceutical products.

TABLE 6.12 Differences between SIA and certified results for pharmaceutical products

Sample	x_d	x_d^2
Filibon	-1.04	1.082
Ferrimed	2.25	5.063
Preganal	3.35	0.656
Ferrous C	0.81	11.225

TABLE 6.13 Differences between SIA and spectrophotometric results for pharmaceutical products

Sample	x_d	x_d^2
Filibon	0.74	0.55
Ferrimed	-3.77	14.21
Preganal	1.63	2.66
Ferrous C	0.03	0.0009

A further comparison was done between SIA and the standard ICP method for the water samples and Table 6.14 gives the mean differences between the SIA and ICP-AES.

TABLE 6.14 Difference between SIA and ICP-AES results for water samples

Sample	x_d	x_d^2
A	0.103	0.0104
B	-0.049	0.0024
C	-0.039	0.0015

The above comparisons were done to establish whether the SIA system can be accepted as giving reliable results in the iron determination or not. The null hypothesis was used [35, 36]. For the null hypothesis the two methods should agree ideally when the population, H_o mean difference is zero; $H_o: \mu_d = 0$. The alternative hypothesis, $H_1: \mu_d \neq 0$, implies that the two methods failed the test. The t-test with multiple samples (paired by difference) was applied to examine whether the two methods differed significantly at 95% confidence level.

The mean, $\bar{x}_d$ and the standard deviation, s_d are calculated from the following equations:

$$\bar{x}_d = \frac{\sum x_d}{N}$$

and

$$s_d = \sqrt{\frac{\sum (x_d - \bar{x}_d)^2}{N-1}}$$

From Table 6.12 between certified and SIA results the following is deduced:

$$\sum x_d = 5.37 \qquad \text{and} \qquad \sum x_d^2 = 18.02$$

Substituting the above where $N = 4$, we find the mean and standard deviations as:

$$\bar{x}_{d1} = 1.34 \qquad \text{and} \qquad S_{d1} = 1.34$$

From Table 6.13, for SIA and standard method, the following is deduced:

$$\sum x_d^2 = 17.42 \qquad \text{and} \qquad \sum x_d = 1.396$$

Substituting the above for the mean and standard deviation with N = 4, we find:

$$\bar{x}_{d2} = 0.349 \qquad \text{and} \qquad S_{d2} = 2.350$$

From Table 6.14 between SIA and the ICP-AES the following id deduced:

$$\sum x_d = 0.014 \qquad \text{and} \qquad \sum x_d^2 = 0.0143$$

Substituting the above for mean and standard deviation with N = 3, we find:

$$\bar{x}_{d3} = 0.0047 \qquad \text{and} \qquad S_{d3} = 0.0842$$

Now, in the determination of iron in pharmaceutical products there are four determinations (n=4), therefore υ=3 and at 95% confidence level $t_{0.05,3} = 3.18$. The critical values are therefore ± 3.18. The $t_{calculated}$ values are in accordance with the following equation:

$$t_{calculated} = \frac{|\bar{x}_d|}{s_d} \times \sqrt{n}$$

Now between SIA and certified results $t_{calculated.}$ ($t_{calc.}$) is given as:

$$t_{calc.} = 2.00$$

and between SIA and spectrophotometry as:

$$t_{calc.} = 0.297$$

Finally, $t_{calc.}$ is at 1.15 between SIA and certified results. The results indicates that there is no significant difference between the methods at 95% confidence level. Between SIA and spectrometry the t_{calc} is 0.297 which indicates that the two techniques gives the same results, as such there is no statistical significant difference between the techniques

In the determination of iron in water samples there are three determinations (n=3), therefore υ=2 at 95% confidence level $t_{0.05,2} = 4.30$. The $t_{calculated}$ value is obtained as:

$$t_{calc.} = 0.097$$

Therefore the $t_{calc.}$ value, 0.097 implies that there is no significant difference between the two methods at 95% confidence level.

It can therefore, be concluded that, in the determination of iron in pharmaceutical products, the SIA and standard method (spectrophotometry) at 95% confidence level give the same results. It can in the same breath be conducted that SIA and the ICP method in the determination of iron in water samples gives the same results at 95% confidence level. The null hypothesis can therefore be accepted at 95% confidence level.

6.6 Conclusions

The total iron determination by SIA with a cadmium reductor incorporated into the SIA manifold is an improvement on the homogeneous methods applied in FIA and SIA systems. In contrast to the FIA system, the cadmium reductor in SIA was regenerated on-line without having to disconnect the system and replace with a new reductor. Thus, once designed it does not have to be physically reconfigured. The SIA system is easier to use and was found suitable for determination of total iron as Fe(II) in pharmaceutical products and water samples within a wide range as shown by the detection limit.

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CHAPTER 7

Determination of nitrate and nitrite in water using a solid-phase reactor in a SIA system

7.1 Introduction

Nitrate and nitrite play an important role in the nitrogen cycle which involves the complex interaction of different ecosystems of the biosphere. Both nitrate and nitrite are present in food and water, and it is from these sources that humans are exposed to these ions.

Excessive amounts in water supplies indicate pollution from sewage or agricultural effluents. In many ways the analytical chemistry of nitrate is linked to that of nitrite. The presence of nitrite in drinking water would indicate recent pollution. Although it is rare to find appreciable levels in waters in the United Kingdom (UK), there are reports that nitrite may occur to an appreciable extent in some continental waters, in distribution. It may well be that, this is because waters contain no residual disinfectants when put into supply and that there is no bacteriological action taking place in the distribution systems oxidising ammonia or reducing nitrate.

Nitrate is chemically stable throughout the relevant range of pH. It can be reduced to nitrite when in contact with metals, such as occurs during cooking of food in aluminium utensils [1].

Nitrite is very unstable, particularly at acidic pH values [2] at which it can disproportionate to yield nitrate and nitrogen oxide and/or react with many components of foods including amines, phenols and thiols.

The occurrence of waters high in nitrate content seemed to be more common in the UK than in Europe, although, of recent times the nitrate content of some European sources does seem to have increased to similar levels to those found in the UK. This is one of the reasons for high degree of purification of sewage effluents before they are discharged into river water with a corresponding low level of ammonia content.

It has been assumed that the increasing use of fertilisers in agriculture has been coincident with the increase in nitrate content of ground-waters. However, the situation is more complex and the high nitrate in ground-waters may be due to the intensity of agriculture, to better land drainage which results in less de-nitrification in waterlogged sods to increasing use of ground-water.

The World Health Organisation (WHO) Standards include nitrate among those constituents which if present in excessive amounts may give rise to trouble. In the European Standards it is recommended that less than 50 mg/ℓ as nitrate should be present in drinking water, but between 50 and 100 mg/ℓ of nitrate is acceptable and that more than 100 mg/ℓ as nitrate is recommended. The International Standards suggested that a maximum of 45 mg/ℓ be expressed as nitrate because of the risk to infants. The European Economic Community (EEC) Directives suggest a maximum admissible concentration of 50 mg/ℓ and the United States National Interim Primary Water Quality Standards give a maximum content of nitrate (as N) of 10 mg/ℓ (equivalent to 45

mg/ℓ as nitrate). The same value is quoted in the Russian Drinking Water Standards. However, the value given as maximum admissible concentration in the Directive on Water for Human Consumption is 0.1 mg/ℓ.

In the UK, water authorities follow WHO standards laid down in 1970. These recommended that NO^-_3 - N in drinking water should be less than 11.3 mg/ℓ. Values of 11.3 - 22.6 mg/ℓ are acceptable, those in excess of 22.6 mg/ℓ are unacceptable [3, 4].

Nitrate is necessary for plant growth. According to Christy *et al.* [5], probably more than 90% of the nitrogen absorbed by plants is in the form of nitrate. Nitrite gives cured meat its characteristic colour and flavour and it is important in the control of bacteria, particularly *Clostridium botulimum* [6]. It is also used for pigment and other colourants [7].

Nitrate in water could in the presence of some bacteria react with the secondary or tertiary amines present in foodstuffs to produce the carcinogenic materials, nitrosoamines. It has been suspected for some years that the effect of exposure to nitrate and nitrite may cause human cancer [8]. There is evidence that high intra-gastric nitrite concentrations correlate with an increased risk of stomach cancer [9]. The reduction of nitrate to nitrite in the gastric lumen is an important source of nitrite for the formation of N-nitroso compounds. High nitrate levels in domestic water causes cyanosis in young babies and infant methaemoglobinaemia [10, 11] has been traced to high nitrite content.

7.2 Choice of analytical technique

The determination of total oxidized nitrogen is a subject of interest in the routine laboratory analysis of potentially polluted waters. However, the determination of nitrate is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be high and the limited concentration ranges of the various techniques. Consequently the determination of total oxidized nitrogen as nitrate is not recommended for waters, but rather as nitrite.

Several methods are commonly used at present for these, but impose restrictions that make the analysis time-consuming and tedious. These methods include gravimetry, titrimetry, spectroscopy and electro-analytical techniques. It falls into one of the five main categories:

- the reduction of nitrate to ammonia [12, 13]
- photo-induced reduction of nitrate to nitrite [14]
- direct spectrophotometry [15, 16]
- potentiometric methods using ion-selective electrodes [17]
- reduction of nitrate to nitrite [18, 19, 20]

Many colorimetric methods have been proposed for the determination of micro-amounts of nitrite [21-28] which is a modified version of the Shinn [29]. These methods involved a reaction of nitrite with a primary aromatic amine to form a diazonium salt which is coupled with another aromatic compound to form the azo dye of which the absorbance is measured. The strategy commonly adopted is based on the reduction of nitrate to nitrite which is then

spectrophotometrically determined after diazotation and coupling reaction.

A number of flow injection analysis (FIA) methods[30-35] have been developed using this modification. Sequential injection analysis (SIA) methods have also been developed for determination of nitrate and nitrite in waste waters and aqueous extracts of atmospheric aerosols [36]; for the determination of nitrite in fertilizers process streams, natural and waste water effluents [37] and the simultaneous determination of nitrate and nitrite in water samples [38].

In the above methods employed, either a homogeneous or heterogeneous reactor is used. However, good the FIA and SIA systems already in use maybe, a system more advanced where as automated pump in the place of a semi automated burette and an acidified cadmium reactor in the place of a copperised one was developed. Furthermore this reactor is regenerated on line, these are some of the features which makes this new technique so unique. Hence, a SIA system incorporating an acidified cadmium reactor into its manifold was used for the determination of nitrate and nitrite.

The SIA technique launched in 1990 [39] is a technique that has tremendous potential especially for on-line process measurements and in monitoring of the environment. It is simple and convenient to operate. The technique considerably decreases sample and reagent consumption and thus the waste generated. In addition, devices based on SIA yield robust and stable systems that are suitable for routine monitoring.

The SIA system described herein allows for the determination of oxidized nitrogen in water samples from different sources as nitrite.

7.3 Determination of total oxidized nitrogen

A water sample containing nitrate was reduced on-line with a solid-phase reactor, diazotised and coupled to produce a reddish azo dye which is detected a 540 nm with a UV/VIS spectrophotometer. The wavelength at 540 nm was chosen from a scan of the standard nitrite solution over a range of 200-1100 nm.

7.3.1. Experimental

7.3.1.1 Reagents and solutions

All reagents were prepared from analytical grade chemicals unless specified otherwise. All aqueous solutions were prepares from doubly distilled, de-ionised water. A De-ioniser from Modulab system (Continental Water System, Sant Antonio, TX, USA) was used throughout. The solutions were all degassed before introduction into the system and stored in an oxygen free environment.

7.3.1.1.1 Stock nitrate solution

A 0.6070 g of oven dried sodium nitrate (Merck, pro analysis) was dissolve and diluted to 1ℓ with double de-ionised water. A 2 mℓ solution of chloroform (Merck, pro analysis) was added to this solution to maintain stability and stored in a cool place. The solution was then standardised with potassium permanganate (Protea Laboratories service (Pty) Ltd.). Working

standards in the range of 0.25 to 50 mg/ℓ were prepared by appropriate dilution of the solution with deionised water.

7.3.1.1.2 Stock nitrite solution

A 0.5057 g of oven dried sodium nitrite (Riedel-De Haën AG, Seelze-Hannover) was dissolved and diluted to 1ℓ with double deionised water. A 2 mℓ solution of chloroform (Merck, pro analysis) was added to this solution to maintain stability and stored in a cool place. The solution was standardised with potassium permanganate (Protea Laboratories service). Working standards in the range of 0.25 to 50 mg/ℓ were prepared by appropriate dilution of the stock solution with deionised water.

7.3.1.1.3 Buffer solution

The buffer solution was prepared by dissolving 30 g of ammonium chloride (Merck, Darmstadt) and 0.2 g EDTA-disodium salt (GPR, Essex, England) in de-ionised water and diluting to 500 mℓ. The pH was adjusted to 6.5 using ammonia (25% NH_3, SAARCHEM). The carrier solution was prepared by dissolving 13 g ammonium chloride (Merck, Darmstadt) and 2.0 g EDTA. The pH of the solution was 4.75 and was not adjusted to any particular value. The appropriate dilutions were made from this solution during optimisation of the carrier concentration.

7.3.1.1.4 Chromogenic reagent

The chromogenic reagent was prepared by dissolving 5 g sulphanilamide (BDH, Poole, England)

and 0.5 g of N–(1-naphthyl) ethylenediammonium dichloride in a mixture of 50 mℓ hydrochloric acid (32% HCl, Chemical suppliers) and 300 mℓ de-ionised water and diluting to 1ℓ. The solution was stored in an amber bottle.

7.3.1.2 Instrumentation

The sequential injection system depicted in Fig. 7.1A was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-Le Bel, France); a 10-port electrically actuated selection valve (Model ECSDIOP, Valco Instruments, Houston, Texas) and a Unicam 8625 UV-Visible spectrophotometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mülheim/Baden, Germany) flow-through cell (volume 80 $\mu\ell$) for absorbance measurements. .

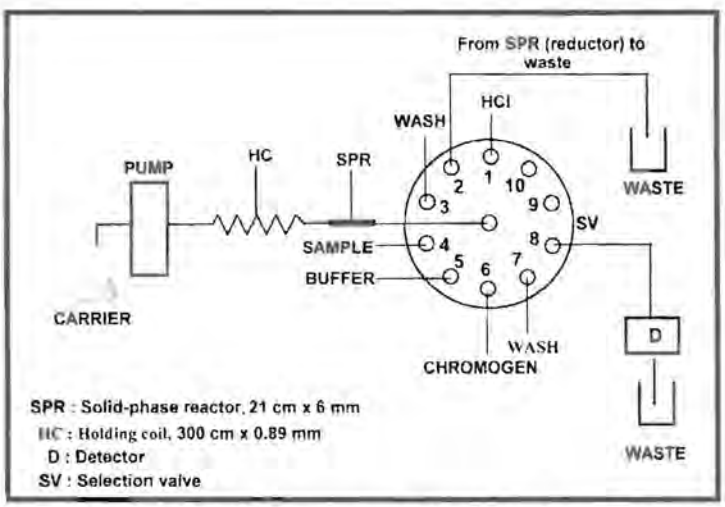

Fig. 7.1A A SIA system diagram used in nitrate determination

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town) and an assembled distribution board (Mintek, Randburg). The flowTEK

[40] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All data given (mean peak height values) are the average of 10 replicates.

7.3.1.3 Operation of the system

A schematic diagram for the SIA system is depicted in Fig. 7.1A. The whole procedure, from sample injection to data processing and storage was computer controlled via the flowTEK program. The whole SIA procedure involved designing a method which allows a single cycle of the experiment to be run. This procedure is well illustrated in Fig. 7.1B and Table 7.1.

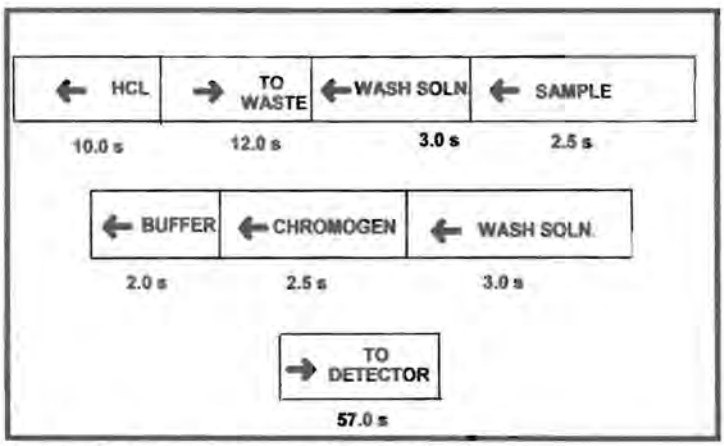

Fig. 7.1B A device sequence for one SIA cycle

When all the zones were placed in the holding coil (HC), they were then flushed with the carrier stream through the reductor to reduce the nitrate to nitrite. The oxidised nitrogen as nitrite was diazotised in the system with sulphanilamide and coupled with N-(1-napthyl) ethylenediammonium dichloride to form a highly coloured azo dye which was detected at 540 nm with a spectrophotometer. The data obtained is converted to a response time graph on the

computer screen as a peak profile. The maximum peak height was then automatically processed and stored on a computer via the FlowTEK program.

TABLE 7.1 Device sequence for one cycle of the SIA system

Time (s)	Pump	Valve	Description
0	Off	Position 1	Pump off. Select HCl stream.
1	Reverse		Draw HCl solution for regeneration.
10	Off		Pump stop.
11	Off	Position 2	Select waste stream.
12	Forward		Pump solution to waste.
23	Off		Pump stop.
24	Off	Position 3	Select wash stream.
25	Reverse		Draw wash solution.
27	Off		Pump stop.
28	Off	Position 4	Select sample stream.
29	Reverse		Draw sample solution.
30.5	Off		Pump stop.
31.5	Off	Position 5	Select buffer stream.
32.5	Reverse		Draw buffer solution.
33.5	Off		Pump stop.
34.5	Off	Position 6	Select chromogen stream.
35.5	Reverse		Draw chromogen solution.
37	Off		Pump stop.
38	Off	Position 7	Select wah stream.
39	Reverse		Draw wash solution.
41	Off		Pump stop.
42	Off	Position 8	Select detector stream.
43	Forward		Pump zones through reductor to detector.
100	Off	Position 1	Pump stop. Valve return home.

7.3.1.4 The solid-phase reactor

The reactor columns were made of glass with varying length (12 cm, 15 cm, 17 cm, 19 cm and 21 cm) but with the same internal diameter of 6 mm. The reactor is shown in Fig. 7.2.

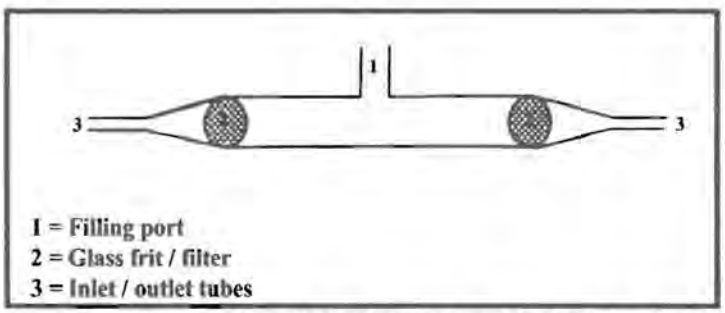

Fig. 7.2 A diagram of a cylindical reactor used in the reduction nitrate to nitrite

The columns were then filled with cadmium granules (Merck, 0.3 - 1.5 mm). The particles were held by a glass frit at each end so that they did not block the SIA system. A vibrator was used to effect close packing of the columns. The cadmium granules prior to being packed in the glass column were prepared by washing with acetone for 10 minutes, adding 20 mℓ of 2 mol/ℓ HCl solution, de-ionised water and methanol then drying in a dessicator. An acidified cadmium reactor was chosen over copperised one because copper has a tendency of interfering in the determination of nitrite in water. Furthermore van Staden and Makhafola [35], have shown that the life span of the acidified cadmium reactor was longer than the copperised one. The cadmium reactor was regenerated by passing approximately 270 $\mu\ell$ of 2 mol/ℓ HCl solution at the beginning of every cycle. This was to ensure consistency in the reduction efficiency and capacity of the reactor.

7.3.1.5 Sample preparation

The samples were obtained from the Institute for Water Quality Studies (Department of Water Affairs and Forestry). The samples were all collected from different localities (streams, rivers, dams, hydro plants, tunnels and effluent streams) at a depth of half a metre. The sample were then preserved in mercury (II) chloride. The samples received were ready for direct analysis. Samples selected were in the pH range 6.8 to 8.2. The buffer solution allowed the adjustment of the pH of the samples.

7.3.2 Method optimization

The method was optimized with regard to the following parameters: nitrite concentration, carrier concentration, flow rate, sample, reagent and buffer volume, reactor length and reduction efficiency. Both the relative peak height and %RSD were used as criteria for establishing the most appropriate optimum value in each case.

7.3.2.1 Solid-phase reactor parameters

The solid phase cadmium reactor forms the heart of the manifold of the proposed system. The performance of the SIA system depends on the reduction efficiency of the reactor at interface between the solid and liquid phases of the reactor. In addition the reactor packing had to be thorough and its length and efficiency had to be optimised.

7.3.2.1.1 Reactor length

The response and precision of the system were studied by varying the reactor length between 12 and 21 cm with the internal diameter fixed at 6 mm. The five reactors (12 cm, 15 cm, 17 cm, 19 cm and 21 cm) were compared for reductor efficiency. From results obtained it was found that the last three reactors did not show a significant difference in response, there was however, for the shorter reactor lengths. The 21 cm reactor length was chosen as optimum because of its good precision. This is illustrated by Fig. 7.3 and given in Table 7.2.

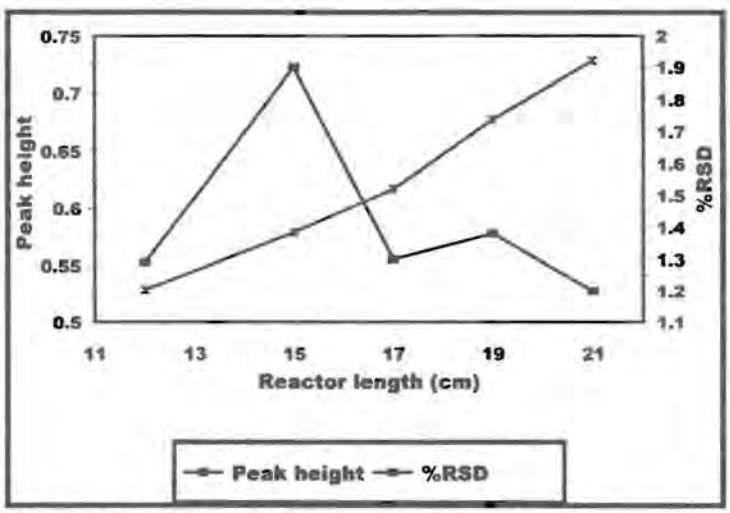

Fig. 7.3 Effect of reactor length on response and precision

TABLE 7.2 Effect of reactor length on response and precision

Length (cm)	12	15	17	19	21
RPh	0.529	0.579	0.617	0.677	0.728
%RSD	1.3	1.9	1.3	1.4	0.7

7.3.2.2 Chemical parameters

7.3.2.2.1 Nitrate concentration

The nitrate concentration was evaluated between 0.5 and 50 mg/ℓ and the 2.5 mg/ℓ concentration was chosen as the optimum concentration for optimising the remaining parameters. The results of this optimisation is presented in Table 7.3 and Fig. 7.4.

TABLE 7.3 Effect of nitrate concentration on response and precision

Conc (mg/ℓ)	0.5	1.0	2.5	5	10	25	50
RPh	0.402	0.412	0.618	0.736	1.708	2.325	5.367
%RSD	1.5	1.0	0.8	1.2	1.8	2.5	5.0

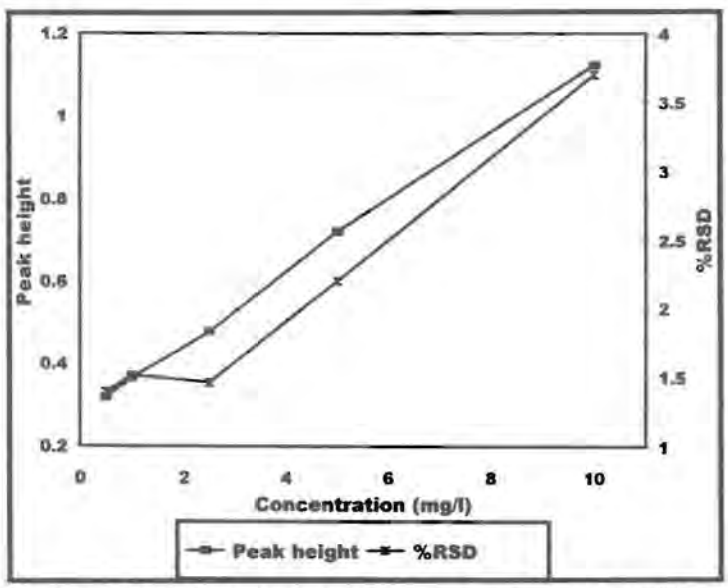

Fig. 7.4 Effect of nitrate concentration on response and precision

7.3.2.2.2 Carrier Concentration

The carrier was evaluated between pure de-ionised water and solution of 13 g ammonium chloride and 2 g EDTA per litre. However, it was a concentration corresponding to 1.3 g ammonium chloride and 0.2 g EDTA per litre of solution that was chosen as the optimum, because it gave the best response and precision as shown in Table 7.4 and by Fig. 7.5.

TABLE 7.4 Effect of carrier concentration on response and precision

Conc (mg/ℓ)	0	0.7	1.3	2.6	6.5	1.3
RPh	0.683	0.731	0.730	0.715	0.692	0.677
%RSD	2.1	1.3	1.0	1.4	1.9	1.4

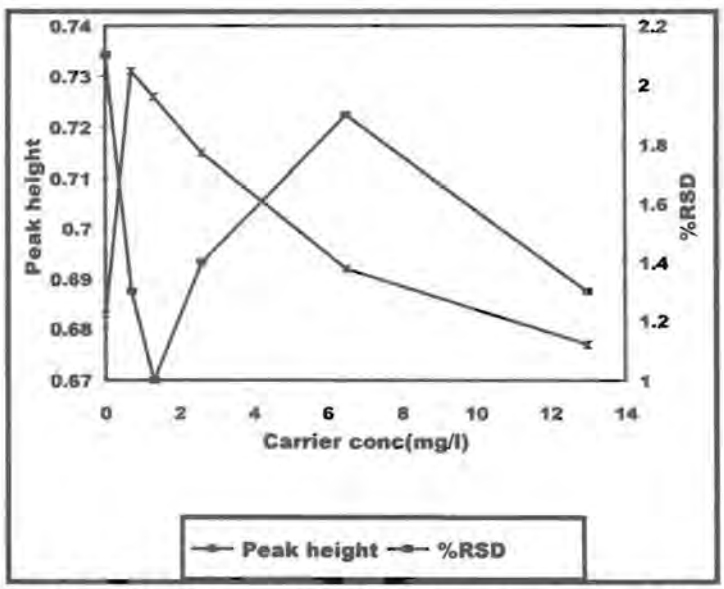

Fig. 7.5 Effect of carrier concentration on response and precision

7.3.2.3 Physical parameters

The contact time between the sample and the reactor is of utmost importance. The 21 cm reactor length was found to be optimum and effective with 270 $\mu\ell$, 2 mol/ℓ HCl solution passing through the reactor for every SIA cycle.

7.3.2.3.1 Flow rate

The flow rate was evaluated between 1.86 and 3.71 mℓ/min. The 3.25 mℓ/min flow rate was chosen as optimum as given by Fig. 7.6 and in Table 7.5.

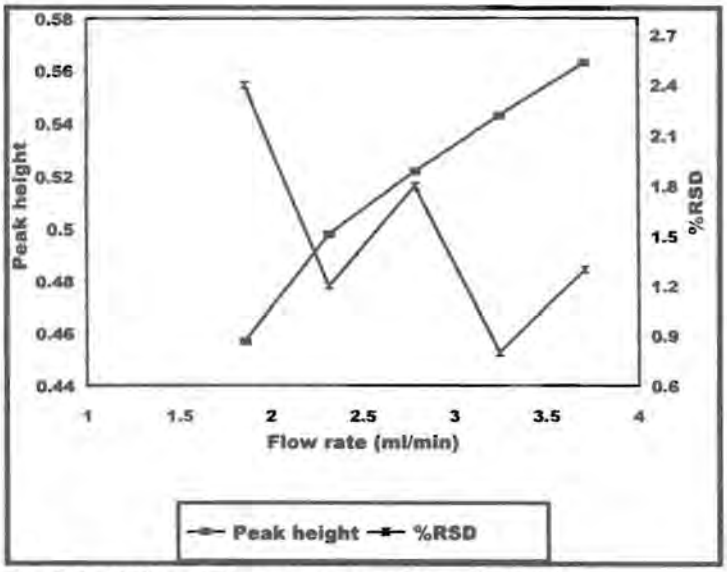

Fig. 7.6 Effect of flow rate on response and precision

TABLE 7.5 Effect of flow rate on response and precision

Rate (mℓ/min)	1.86	2.32	2.79	3.25	3.71
RPh	0.457	0.498	0.522	0.543	0.566
%RSD	2.4	1.2	1.8	1.0	1.8

7.3.2.3.2 Sample volume

The sample volume was evaluated between 25.0 and 140.0 $\mu\ell$. The sample volume was found to be optimum at 82.5 $\mu\ell$. The results are well presented in Table 7.6 and Fig. 7.7.

TABLE 7.6 Effect of sample volume on response and precision

Volume ($\mu\ell$)	27.5	55	82.5	110	137.5
RPh	0.377	0.486	0.629	0.765	0.877
%RSD	1.2	1.7	0.5	1.2	0.8

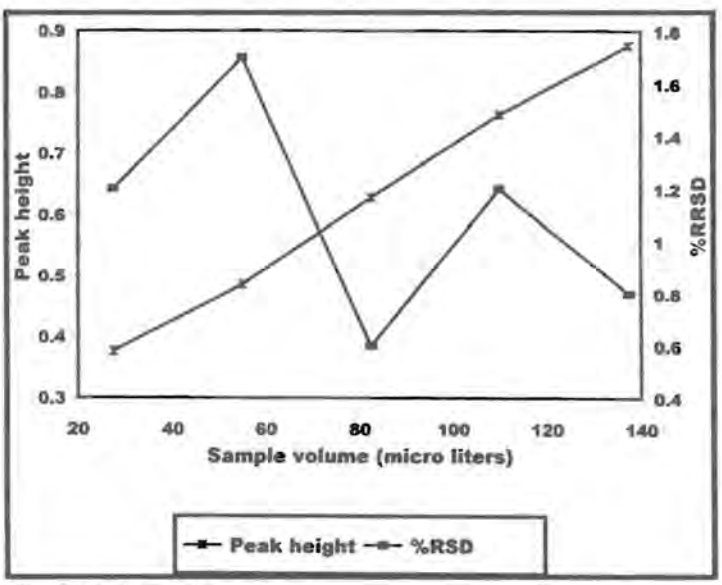

Fig. 7.7 Effect of sample volume on response and precision

7.3.2.3.3 Reagent volume

The reagent volume was evaluated between 25.0 and 140.0 $\mu\ell$. The reagent volume was found to be optimum at 82.5 $\mu\ell$. The results are shown in Table 7.7 and illustrated in Fig. 7.8.

TABLE 7.7 Effect of chromogen volume on response and precision

Volume ($\mu\ell$)	27.5	55	82.5	110	137.5
RPh	0.504	0.507	0.513	0.528	0.558
%RSD	3.6	1.5	0.8	1.6	1.0

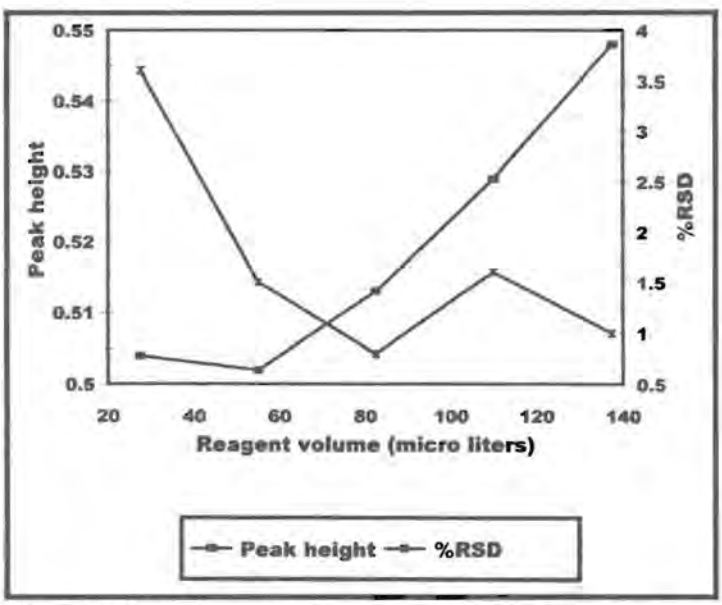

Fig. 7.8 Effect of reagent volume on response and precision

7.3.2.3.4 Buffer volume

The buffer volume was evaluated between 25.0 and 140.0 $\mu\ell$. The buffer volume was found to be optimum at 55 $\mu\ell$. The results are well presented in Table 7.8 and illustrated in Fig. 7.9.

TABLE 7.8 Effect of buffer volume on response and precision

Volume ($\mu\ell$)	27.5	55	82.5	110	137.5
RPh	0.662	0.608	0.574	0.589	0.596
%RSD	1.4	0.8	1.7	1.7	0.8

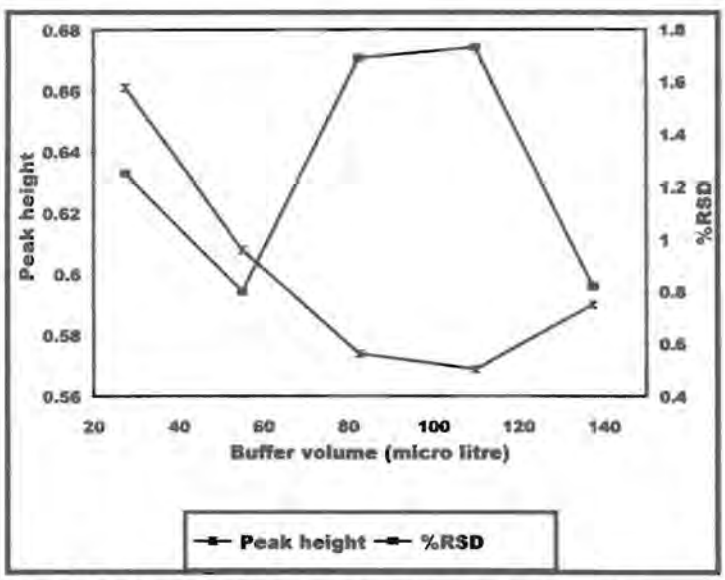

Fig. 7.9 Effect of buffer volume on response and precision

7.3.3 Method evaluation

7.3.3.1 Linearity

The linearity of the system was evaluated for analyte concentration between 0.25 and 50 mg/ℓ. The response was however, found to be linear in the range 0.25 to 5 mg/ℓ. The relationship between the response and the concentration is given by te equation : $H = 0.0877x + 0.2060$ (r = 99.94%), where H is the peak height and x the analyte concentration in mg/ℓ. The calibration graph is illustrated in Fig. 7.10.

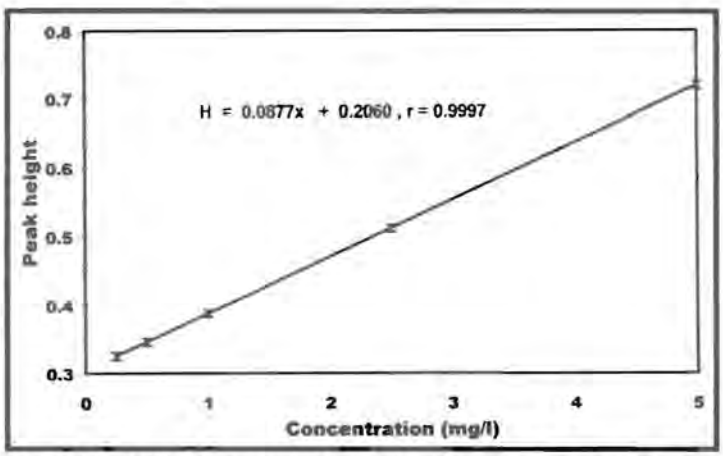

Fig. 7.10 Calibration graph obtained under optimum conditions

7.3.3.2 Accuracy

Water samples from different sources were analysed with the proposed system. The results obtained are a mean of 10 repetitive analysis of each sample (Table 7.9). The accuracy was compared to the standard method results (Table 7.9).

TABLE 7.9 SIA results for water samples analysed

Sample ID	pH	Peak height	mg/ℓ (NO_3^- - N + NO_2^--N)
Unknown	8.2	0.257 (1.2%)	0.65
Mtafufu-Ntafufu	7.8	0.277 (1.2%)	0.88
Tshinane	7.7	0.294 (1.4%)	1.07
Vink	8.3	0.284 (1.5%)	0.96
Wolwekloof tunnel	6.9	0.306 (0.9%)	1.02
Van Rhyreveldspas dam	7.5	0.347 (1.4%)	1.68
Mgwale-clackburg	7.8	0.248 (1.5%)	0.48
Tsitsa-Tsitsa bridge	7.3	0.242 (1.2%)	0.47
Duiwe river	7.0	0.276 (1.1%)	0.80
Mutshedi dam	7.4	0.366 (1.0%)	1.13

TABLE 7.10 Comparison of SIA and Standard method (AAS) results and their paired differences.

Sample Id	SIA method	Standard method	x_d x 10^{-2}	x_d^2 x 10^{-3}
unknown	0.65	0.63	2.0	0.4
Mtafufu-Ntafufu	0.88	0.78	10.0	10.0
Tshinane	1.07	0.97	10.0	10.0
Vink	0.96	1.05	-9.0	8.1
Wolweklof tunnel	1.20	1.16	4.0	1.6
Van Rhyreveldspas dam	1.68	1.77	-9.0	8.1
Mgwale-Clackburg	0.48	0.45	3.0	0.9
Mutshedzi dam	1.13	0.99	14.0	19.6
Tsitsa-Tsitsa bridge	0.47	0.46	1.0	0.1
Duiwe river	0.80	0.72	7.9	6.2

7.3.3.3 Precision

The precision of the method was determined by 10 repetitive analysis of the standard solution as well as that of the water samples. All these were carried out under optimum conditions. The standard deviation for the standards was <1.0% and the samples <1.5%.

7.3.3.4 Detection limit

The detection limit was calculated using the formula:

$$\text{Detection limit} = \frac{[(3\sigma + k) - c]}{m}$$

where σ (0.00455) is the standard deviation of the baseline, k (0.1932) is the average response of the baseline and c (0.0877) is the slope of the calibration graph. The detection limit was found to be 0.01 mg/ℓ.

7.3.3.5 Recovery

The recovery of the proposed system was determined by comparing the expected results with those obtained with the proposed system as follows:

$$\text{Recovery (\%)} = \frac{\text{obtained}}{\text{expected}} \times 100$$

Real samples were spiked with 5 mg/ℓ nitrate solution and analysed with the SIA method. The results ranged between 96.4% and 106.8%.

7.3.3.6 Sample interaction

The sample interaction carryover between consecutive samples was determined by analysing samples with low analyte concentration followed with a high analyte concentration which was again followed by the sample with a low analyte concentration. The sample interaction was then calculated using the following formula:

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

where A_1 is the peak height (0.2937) of a sample containing 1 mg/ℓ, A_2 is a peak height (0.7361) of a sample containing 5 mg/ℓ and A_3, is a peak height (0.3033) of a sample containing 1 mg/ℓ. The sample interaction was 1.3 % which may be considered negligible.

7.3.3.7 Interferences

The only possible interferences that may disturb the accuracy of this analysis are: iron, copper, phosphates, chromium (VI), magnesium, manganese and chloro-amines. Fortunately all these ions were found to be at acceptable levels and did not affect the results. Table 7.11 gives some of the ions, their ranges and tolerance levels in the water samples analysed. Oms *et al.* (1995) found that copper (II) interfered in the reduction step and for it not to interfere, the copper

sulphate used for the copperised cadmium reductor should be less than 25 mg/ℓ, however, in this work the tolerance level is 20 mg/ℓ. Besides, in this work an acidified reactor was used in the place of the copperised one, thus minimising any interference that may arisedue to copper.

TABLE 7.11 Some of the ions present in the water samples, their ranges and tolerance levels in mg/ℓ when 2 mg/ℓ standard (nitrate + nitrite) was added.

Ion	Tolerance level	Range in samples
Phosphate as P	1	0.008 - 0.080
Chloride	800	10 - 400
Sulphate	300	0.018 - 66
Sodium	750	4 - 67
Potassium	700	0.3 - 8.3
Calcium	350	3 - 19
Magnesium	300	2 - 19
Copper	20	0.0011 - 0.057
Nickel	40	0.0043 - 0.79
Chromium	5	0.0084 - 0.14
Iron	20	11.4 - 14.2

7.4 Statistical comparison of techniques used

The comparison was done between the SIA and the standard method (Institute for Water Quality Studies) (Table 7.10). The comparison was done to establish whether the SIA system can be accepted as giving reliable results in the determination of oxidised nitrogen. The null hypothesis was used [41,42]. The t-test with multiple samples (paired by differences) was applied to

examined whether the two methods differed significantly at 95% confidence level. The null hypothesis is $H_o : \mu_d = 0$, against the alternative $H_t: \mu_d \neq 0$, where μ_d is the population paired difference.. The test is two tailed, as we are interested in both $\mu_d < 0$ and $\mu_d > 0$.

The mean, $\bar{x}_d$ standard deviation, s_d and $t_{calculated}$, $t_{calc.}$ can be determined from the following equations:

$$\bar{x}_d = \frac{\sum x_d}{N}$$

$$s_d = \sqrt{\frac{\sum (x_d - \bar{x}_d)^2}{N-1}}$$

and

$$t_{calc.} = \left|\bar{x}_d\right| x \frac{\sqrt{n}}{s_d}$$

From Table 7.10 we can deduce the following:

$$\sum x_d = 0.0339 \qquad \text{and} \qquad \sum x_d^2 = 0.065.$$

Substituting for the mean, standard deviation with N =10,. we get:

$$\bar{x}_d = 0.0034$$

$$S_d = 0.0848$$

and substituting for $t_{calculated}$ with n = 10 we get:

$$t_{calc.} = 0.1267$$

In the determinations we have 10 determinants (n=10), therefore $\upsilon = 9$ and at 95% confidence level $t_{0.05,9} = 2.36$. The critical t-values are therefore ± 2.36. Since the calculated value is less than the critical value, H_o cannot be rejected and it follows that there is no statistically significant difference between the two techniques.

7.5 Conclusions

The determination of the oxidised nitrogen (nitrate + nitrite as N) by SIA using a solid-phase reactor incorporated into the SIA manifold is an improvement on similar techniques which used FIA and semi-automated burettes. Furthermore, in the work an acidified cadmium reactor was

used which eliminated all possible interferences that may have been caused by copper or/and phosphate ions. In contrast to work already done, in this work the cadmium reactor was regenerated on-line without having to disconnect the system or replace it after regeneration. Thus, once more the SIA system was found to be time and reagent saving and suitable for the determination of oxidised nitrogen in water samples to a very low level.

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CHAPTER 8

Determination of total chromium as chromate in electroplating and natural waters with a sequential injection analysis (SIA) system

8.1 Introduction

Chromium is a naturally occurring trace element found in rocks, plants, animals, soil, water and in volcanic dust and gases. It is very hard, is resistant to corrosion and takes a bright polish. The main ore of chromium is chromite, $FeO.Cr_2O_3$. Chromium is a white, hard, lustrous and brittle metal (mp. 1903 ± 10°C). It is extremely resistant to ordinary corrosive agents which accounts for its extensive use as an electroplated protective coating [1- 3].

The world industrial growth has brought in environmental pollution and has also increased the exposure of workers to several toxic substances. Toxicological studies have shown that some essential and non-essential elements become toxic at a certain level of concentration. It has been demonstrated that the degree of toxicity and negative effect of an element on health, depends on the chemical form on which the toxic agent is present and the oxidation state of the element [4, 5].

People are exposed when eating, drinking water, and inhaling air that may contain chromium. Dermal exposure may occur when using products that contain chromium. Occupational exposure occurs from chromate production, stainless steel production, chrome plating and the tanning industry [3].

Chromium is an essential element and is necessary for the maintenance of normal glucose, proteins and fat metabolism. Hexavalent chromium is believed to be toxic and it has been fed to animals in tests at a concentration of 25 mg/ℓ for over a year with no toxic effect in these animals. Studies have shown that hexavalent chromium can cause cancer of the respiratory tract when inhaled as a dust.

It is because of this association with a known hazard to health that a limit of 0.05 mg/ℓ as total chromium has been imposed in the US Interim Primary Drinking Water Standards. The same value appears in the WHO European Drinking Water Standards and in Japanese Standards, but relating to hexavalent chromium only.

The European Economic Community (EEC) Directives for Surface Water and for Drinking Water for Human Consumption both follow the American Standards and suggest 0.05 mg/ℓ as an imperative standard for total chromium. However, the Soviet Standard suggest 0.1 mg/ℓ of hexavalent chromium and 0.5 mg/ℓ for total chromium [7]. The reference dose (RfD) for hexavalent chromium is 0.005 mg/kg per day and the Rfd for trivalent chromium is 1 mg/kg per day. The US Environmental Protection Agency (EPA) estimates that consumption of these doses or less over a lifetime is unlikely to result in the occurrence of chronic non-carrier effects [8].

In water analysis, inorganic chromium is one of the pollutants that needs control. Trivalent and hexavalent chromium compounds are the major species of inorganic chromium in water. Their contamination sources are mainly waste-waters of metallic smelting, electroplating, mining, leather tanning, cement industry, dye stuff industry and corrosive paints [8, 9]. The chromium species most frequently found in water are chromates (CrO_4^{2-}), cations hydroxo complexes, $Cr(OH)^{2+}$ and $Cr(OH)_2^{+}$ and organically bound [10, 11] or colloidally sorbed Cr(III) [12]. The chemistry of hexavalent chromium will be briefly discussed as it will form the basis of our studies.

8.2 The chemistry of chromium (VI), d°

In basic solutions above pH 6, CrO_3 forms the tetrahedral yellow chromate ion, CrO_4^{2-}. Between pH 2 and pH 6, $HCrO_4^-$ and the orange-red dichromate ion $Cr_2O_7^{2-}$ are in equilibrium and at pH values below 1, the main species is H_2CrO_4. The equilibria are the following:

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} + H^+$$

$$H_2CrO_4 \rightleftharpoons HCrO_4^- + H^+$$

$$Cr_2O_7^{2-} + H_2O \rightleftharpoons 2HCrO_4^-$$

In addition there are the base-hydrolysis equilibria :

$$Cr_2O_7^{2-} + OH^- \rightleftharpoons HCrO_4^- + CrO_4^{2-}$$

$$HCrO_4^- + OH^- \rightleftharpoons CrO_4^{2-} + H_2O$$

which have been studied kinetically for a variety of bases [1, 2]. The pH-dependent equilibria are quite labile, and on addition of cations that form insoluble chromates, (e.g. Ba^{2+}, Pb^{2+}, Ag^+) the chromates and not the dichromates are precipitated [1, 2].

8.3 Choice of analytical technique

From an analytical point of view, the determination of chromium is difficult since trace levels analyses are strongly affected by contamination. There are however, only a few analytical techniques available that have sufficient sensitivity and selectivity for the direct determination and speciation of trace levels of chromium in water [13]. The different pre-concentration methods used to determine low levels of individual chromium species are liquid-liquid extraction [14, 15], co-precipitation [11, 12, 16, 17], electroplating [18] and absorption [19].

Kingston *et al.* [20] developed a speciated isotope dilution mass spectrometry (SIDM). They described the applicability of their method to chromium. Bağ *et al.* [21] described the pre-concentration of Cr(III) from Cr(VI) and the determination of that chromium using AAS as detector.

Chromium occurs in the environment on two major valence states, trivalent chromium {Cr(III)} and hexavalent {Cr(VI)}.

Veilon [22] has shown that, so far graphite furnace atomic absorption spectrometry (GFAAS) is still one of the best alternative to determine trace levels of chromium. Even modern multi-element technique such as inductively coupled plasma mass spectrometry (ICPMS) do present limitations [23].

There are several flow injection analysis (FIA) methods used for chromium determination [24-30] with different detectors. But, very little using the sequential injection analysis (SIA) has been done. Luo *et al.* [31] demonstrated the use of organic wetting film extraction to enhance the sensitivity and selectivity in the SIA analysis of Cr(III) and Cr(VI) in natural waters. Oliviera and Masini [32] proposed an SIA method for the determination of Cr(VI) in residual waters from electroplating baths and steels.

From the above discussion, it is evident enough that much has to be done in the analysis of chromium. Therefore, some of the pre-requisites needed for an analyser in the determination of chromium, is that the system should be simple and robust, reliable with minimal needs for maintenance and re-calibration, low consumption of reagents and enhanced sensitivity. The SIA system seem to meet all these requirements. It is thus an ideal technique to use in the determination of chromium.

In this work, owing to the significance of Cr(III) monitoring in industrial electroplating processes and natural waters, the chemistry of chromium had to be revisited. A solid phase lead(IV) oxide is incorporated in the SIA manifold to enhance the selectivity and sensitivity of the chromate ion which is detected with a UV/VIS spectrophotometer in a basic medium. The proposed final reaction [33] is,

$$PbCrO_4 + 4OH^- \rightarrow [Pb(OH)_4]^{2-} + CrO_4^{2-}$$

SIA, launched in 1990 [34, 35] is a technique that has tremendous potential for on-line process measurements, due to its simplicity and convenience with witch sample manipulation can be automated. The versatility of the technique is centred around a selection valve, where each part of the valve allows a different operation to be performed [34-36].

8.4 Chromium determination

A modified version [24, 25], where Cr(III) is oxidised by Ce(VI) to Cr(VI) is investigated. A solid-phase lead(IV) oxide is incorporated into the SIA manifold to precipitate the chromate ion as lead chromate which subsequently releases the chromate ion in excess base to be detected with a UV/VIS spectrophotometer.

The following set of reactions are proposed from sample oxidation to detection:

$$6\ Ce\ (IV) + 2\ Cr\ (III) + 7\ H_2O \rightarrow 6\ Ce\ (III) + Cr_2O_7^{2-} + 14H^+ \text{----------(1)}$$

Reaction (1) occurs in hot acid medium. The Cr (VI) is expected to exist either as the chromate or dichromate[33]. After the pH was adjusted between 6.5 and 7.5, the Cr (VI) may be written as:

$$2CrO_4^{2-} + 2H^+ \Leftrightarrow 2HCrO_4^- \Leftrightarrow Cr_2O_7^{2-} + H_2O \text{----------(2)}$$

OR

$$2CrO_4^{2-} + 2H^+ \Leftrightarrow Cr_2O_7^{2-} + H_2O \text{ ----------(3)}$$

When the sample is propelled through the solid lead (IV) oxide the following occurs:

$$Pb^{2+} + CrO_4^{2-} \Rightarrow PbCrO_4 \text{ ----------(4)}$$

OR/AND

$$Pb^{2+} + CrO_4^{2-} + H_2O \Rightarrow PbCrO_4 + 2H^+ \text{ ----------(5)}$$

When the base is introduced the following reaction occurs:

$$PbCrO_4 + 2H^+ + OH^- \Rightarrow PbCrO_4 + H_2O\text{----------(6)}$$

An excess of the base (ammonium hydroxide) forms the soluble complex tetrahydroxoplumbate (II) ion. This suppresses the Pb^{2+} ion concentration to such an extent that the solubility product (1.8×10^{-14}) of lead chromate is no longer exceeded, and consequently the latter dissolves releasing chromate according to the following reaction:

$$PbCrO_4 + 4OH^- \Rightarrow [Pb(OH)_4]^{2-} + CrO_4^{2-} \text{ ----------(7)}$$

The chromate released in (7) is propelled towards the detector where it detected at 360 nm.

8.4.1 Experimental

8.4.1.1 Reagents and solutions

All reagents were prepared from analytical-reagent grade chemicals unless specified otherwise. All aqueous solutions were prepared from double de-ionised water. De-ionised water from Modulab system (Continental Water System, San Antonio, TX, USA) was used throughout.

8.4.1.1.1 Stock chromium (III) solution

A 5.1234 g of chromium (III)-chloride-6-hydrate (96% pure, Riedelhaën) was dissolved in 100 mℓ hydrochloric acid (1 mol/ℓ) solution and diluted to 1ℓ with water. Working standards in the range 0.01 to 10 mg/ℓ were prepared by appropriate dilutions of the stock solution with water.

8.4.1.1.2 Stock dichromate solution

A 2.8292 g of potassium dichromate (chemically pure, Protea Laboratory) was dissolved and diluted to 1ℓ with double de-ionised water. Working standards in the range of 0.01 to 10 mg/ℓ were prepared by appropriate dilution of the stock solution with water.

8.4.1.1.3 Stock chromate solution

A 3.7355 g of potassium chromate (pure analysis, SAARCHEM) was dissolved and diluted to 1ℓ with double de-ionised water. Working standards in the range 0.01 to 10 mg/ℓ were prepared by appropriate dilution of the stock solution with water.

8.4.1.1.4 Ammonium cerium (VI) sulphate solution

A 5.9635 g of ammonium cerium (IV) sulphate (BDH, Poole, England) was dissolved in 50 mℓ 2 mol/ℓ sulphuric acid (98%, H_2SO_4, Holpro analytics) solution and made up to 500mℓ.

8.4.1.1.5 Sodium nitrite solution

A 5.0076 g of sodium nitrite (pure analysis, Riedel de haën) was dissolved and diluted to 250 mℓ with water.

8.4.1.1.6 Ammonium hydroxide solution

A 18.7 mℓ of ammonia (25% NH_3, SAARCHEM) solution was diluted to 250 mℓ with water.

8.4.1.1.7 Sulphuric acid solution

A 55.6 mℓ of sulphuric acid (98%, H_2SO_4, chemically pure, Holpro analytics) solution was diluted to 500 mℓ with water.

8.4.1.2 Instrumentation

The sequential injection system depicted in Fig. 8.1 was constructed from the following components: a Gilson minipuls peristaltic pump (Model M312, Gilson, Villiers-Le Bel, France); a 10-port electrically actuated selection valve (Model ECSDIOP, Valco Instruments, Houston, Texas) and a Unicam 8625 UV-Visible spectrophotometer equipped with a 10-mm Hellma-type (Hellma GmbH and Co., Mulheim/Baden, Germany) flow-through cell (volume 80 $\mu\ell$) for absorbance measurements.

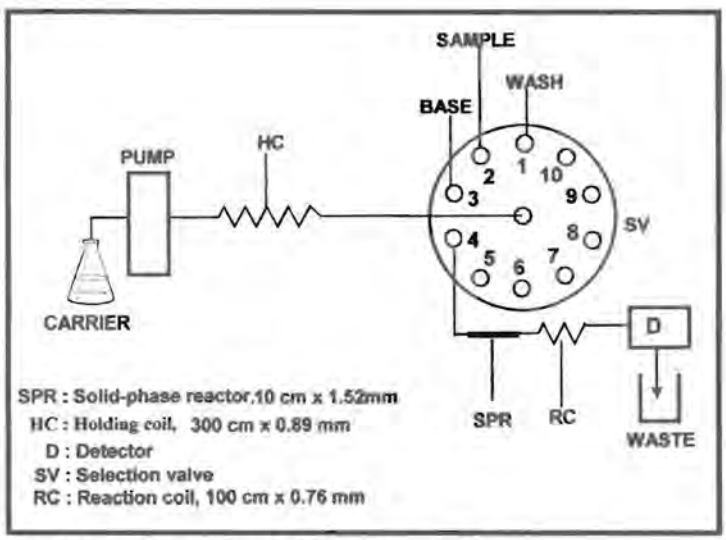

Fig. 8.1 A diagram for the SIA system used.

Data acquisition and device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town) and an asssembled distribution board (Mintek, Randburg). The flowTEK [37] software package (obtainable from Mintek) for computer-aided flow analysis was used throughout for device control and data acquisition. All data given (mean peak height values) are the average of 10 replicates.

8.4.1.3 Solid-phase reactors

The solid-phase reactor (SPR) was constructed using PTFE tubing with an internal diameter of 1.52 mm (Fig.8.2). The reactor consisted of lead(IV)dioxide suspended on silica gel beads (35-70 mesh, 40 Å; Aldrich-Chemical Co. Gillingham-Dorset). The packing was prepared as described by Rüter and Neidhart [41], the only difference being the use of commercial sodium hypochlorite (3.5% m/V, sodium hypochlorite when packed) for supplying the sodium hypochlorite. The sodium hypochlorite oxidises the divalent lead acetate according to the following equation:

$$Pb(CH_3COO)_2 + NaOCl \rightarrow PbO_2 + 2CH_3COOH + NaCl$$

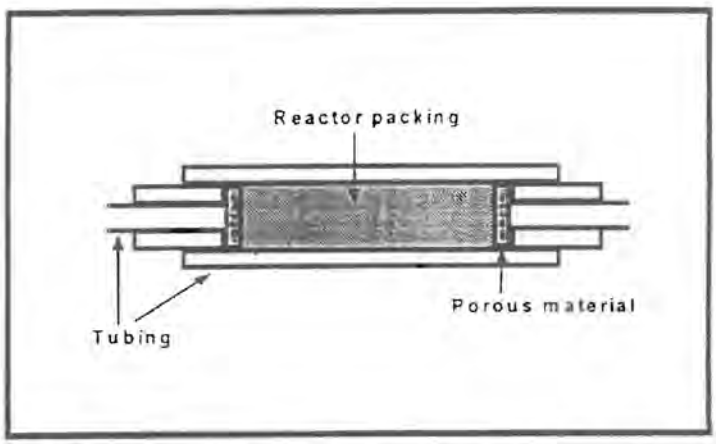

Fig. 8.2 Design of a tubular packed lead(IV) dioxide reactor

After packing each reactor had be conditioned (run in) for at least 60 minutes before use. Conditioning involved pumping de-ionised water through the reactor at a flow rate of 2.2 mℓ

/min for 45 minutes, then the carrier for 15 minutes at the same rate. This was to ensure that there were no air pockets and to ensure close packing of the beads. The lifetime of each reactor was established by comparing peak heights for the same standards from day to day. When the peak heights started to decrease systematically and drastically, the reactor had to be replaced. Another indication that the reactor was losing its conversion capacity was the colour of the packing itself. At the beginning of a new conditioned reactor the colour of the packing was dark brown which gave a greyish appearance inside the PTFE tubing. After the reactor was in use for several samples (400-500 experiments) and depending on the concentration of the chromate in the samples the colour of the packing at the front end of the reactor started to disappear. This meant that al of the led(IV)dioxide had stripped off the beads.

8.4.1.4 Sample preparation

The samples were obtained from the Institute for Water Quality Studies (Department of Water Affairs and Forestry) and Electroplating industries. The samples were collected from different localities (streams, rivers, dams, hydro plants, tunnels and effluent streams) at half a metre depth.

A 50 mℓ aliquot of each sample was transferred into a 100 mℓ Erlenmeyer flask. A 10 mℓ portion of a 2 mol/ℓ sulphuric acid solution was added into the sample. The Erlenmeyer flask and its contents were placed in a boiling water in a water bath. After 15 minutes a 10 mℓ aliquot of ammonium cerium (IV) sulphate solution was added into the hot solution to oxidise chromium(III) to chromium(VI). An excess amount of the cerium(IV) solution was added to ensure complete oxidation (having assumed that the amount of chromium may not exceed 5 mg/ℓ). The oxidised sample was removed from the boiling water after 45 minutes, the solution

was cooled to zero degree Celsius. A 5 mℓ portion of a 2% sodium nitrite solution was added to destroy any excess cerium (VI) solution that may be remaining. (Cerium(IV) may form the yellow cerium(IV)hydroxide which may interfere with the results).

The pH of the solution was adjusted to between 6.5 and 7.5 with ammonium hydroxide solution. The resulting solution was diluted to 100 mℓ with water and was now ready for chromium analyses with the proposed SIA system.

8.4.1.5 Operation of the system

A schematic diagram for the SIA system is depicted in Fig.8.1. The whole procedure, from sample injection to data processing and storage was computer controlled via the FlowTEK program. The whole SIA procedure involved designing a method which allows a single cycle of the experiment to be run. Fig. 8.3 and Table 8.1 shows the device sequence for one cycle.

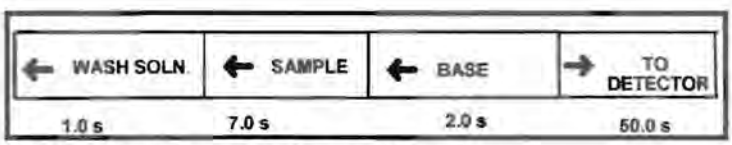

Fig. 8.3 Device sequence for one SIA cycle

The basic components of the system are a peristaltic pump with only one carrier stream, a single channel and a detector. The concept is based on the sequential injection of a wash solution, sample zone and reaction zone(s) into a channel [37-40]. In this way a stack of well defined zones adjacent to each other is obtained in a holding coil.

TABLE 8.1 Device sequence for one cycle of the SIA system

Time (s)	Pump	Valve	Description
0	Off	Position 1	Pump off. Select wash stream.
1	Reverse		Draw wash solution.
2	Off		Pump stop.
3	Off	Position 2	Select sample stream.
4	Reverse		Draw sample solution.
11	Off		Pump stop.
12	Off	Position 3	Select base stream.
13	Reverse		Draw base solution.
15	Off		Pump stop.
16	Off	Position 4	Select detector line.
17	Forward		Pump zones through SPR to detector.
60	Off	Position 1	Pump stop. Valve return home.

After the valve has been selected to the detector position, the flow in the carrier stream is reversed and the zones mutually disperse and penetrate each other as they passed through the reaction coil to the detector. The reversed flow, therefore creates a composite zone in which the sample and reagent zone penetrate each other due to combined axial and radial dispersion.

The sample and reagent zones were pumped through the solid lead(IV) oxide by the carrier stream (de-ionised water) to convert the dichromate to stable chromate. The total chromium as chromate was directed to the detector for measurement at 360 nm. The absorbance of the chromate at 360 nm was chosen after a scan of stock solutions (each diluted to a concentration of 2 mg/ℓ) for maximum absorbance over the 200 to 1100 nm range with a UV/VIS

spectrophotometer. The maximum absorbance was detected at 420, 430 and 360 nm for chromium (III), dichromate and chromate ions respectively. The data obtained was converted to a response time graph and was viewed on the monitor as a peak profile. The maximum peak height was automatically processed and stored on a computer via the FlowTEK program.

8.4.2 Method optimization

8.4.2.1 Chemical parameters

8.4.2.1.1 Chromium(III) concentration

The chromium(III) solution was treated exactly like the samples and its concentration was evaluated between 0.2 and 20 mg/ℓ and the 0.4 mg/ℓ was chosen as the optimum value for optimising the remaining parameters. The results of this optimisation is presented in Table 8.2 and Fig. 8.4. To verify efficiency of the oxidation by the cerium (IV) solution, a similar concentration of dichromate and chromate solutions, were analysed. The results were in agreement within 97% which can be considered acceptable. Table 8.3 gives these results.

TABLE 8.2 Effect of chromium concentration on response and precision

Conc. (mg/ℓ)	0.2	0.4	0.6	0.8	1	1.2
RPh	0.2	0.271	0.368	0.441	0.575	0.743
%RSD	0.81	0.37	1.07	1.02	1.1	0.66

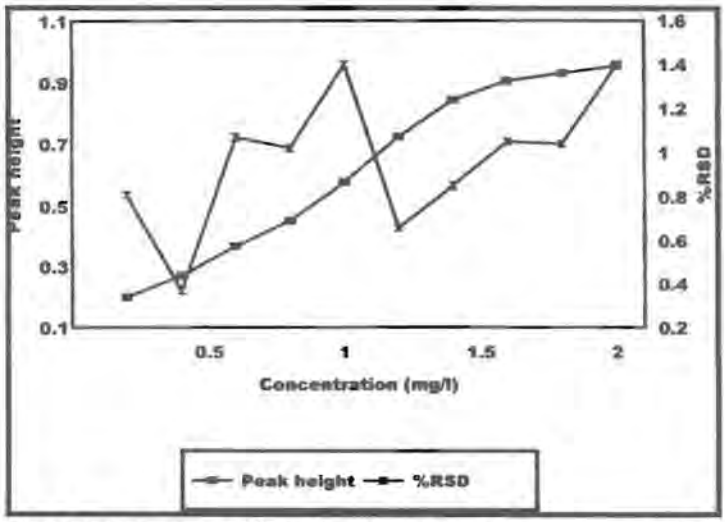

Fig. 8.4 Influence of chromium concentration on response and precision

TABLE 8.3 Comparison of response of oxidised Cr(III) to that of dichromate and chromate

Concentration (mg/ℓ)		0.2	0.4	0.6	0.8	1
Relative peak height	**Chromium (III)**	0.200	0.271	0.368	0.441	0.575
	Dichromate	0.206	0.28	0.379	0.456	0.59
	Chromate	0.208	0.287	0.383	0.461	0.594

8.4.2.2 Physical parameters

The contact time between the sample zone containing the oxidised chromium (III) solution and the solid phase lead (IV) oxide reactor is of utmost importance for total conversion of dichromate/chromate to chromate. This conversion is influenced by reactor length, flow rate, sample volume and base volume.

The performance of the SIA system depends on the efficiency of the solid lead (IV) oxide reactor to convert the chromate to the form in which it has to be detected. It thus form the heart of the manifold in the proposed SIA system.

8.4.2.2.1 Reactor length

The response and precision were studied by varying the length of the solid phase reactor between 6 and 14 cm with internal diameter fixed at 1.52 mm. The five reactors were compared for their conversion efficiency in Table 8.4a and Fig. 8.5.

TABLE 8.4a Effect of solid phase reactor length on response and precision

Length (cm)	6	8	10	12	14
RPh	0.38	0.389	0.421	0.422	0.421
%RSD	1.2	0.92	0.71	0.84	0.73

Furthermore, in order to verify whether the incorporation of the SPR reactor is justified at all, a stock chromium (III) solution was analysed (10 runs) with and without the SPR and the response compared. The incorporation of the SPR gave the best results (Table 8.4b).

TABLE 8.4b Influence of SPR on response and precision

Condition	**Relative peak height**	**%Relative standard deviation**
Without the SPR	0.325	1.46
With SPR	0.421	0.71

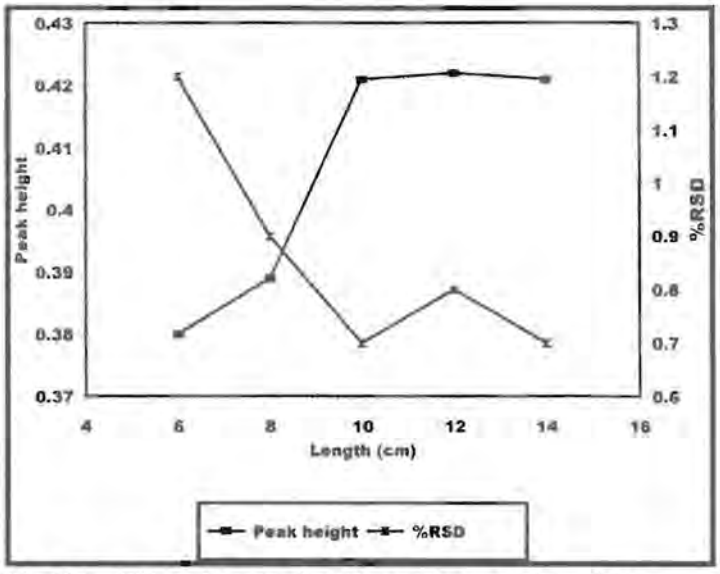

Fig. 8.5 Effect of reactor length on response and precision

From the results obtained it can be seen that there is a significant improvement in response as well as the precision with the incorporation of the SPR into the SIA manifold. This can only mean that although cerium oxidises Cr(III) to Cr(VI) the Cr(VI) is present both as the dichromate and chromate ions. The SPR reactor therefore ensures that all the Cr(VI) is present as chromate prior to detection (see proposed reactions 4, 5 and 6 page 237). Hence the presence of the SPR is important in the determination of chromium as chromate.

8.4.2.2.2 Flow rate

The conversion is also influenced by flow rate. Flow rates between 2.26 and 4.53 mℓ/min were evaluated. The optimum flow rate was found to be 3.96 mℓ/min. This is well presented in Fig. 8.6 and Table 8.5.

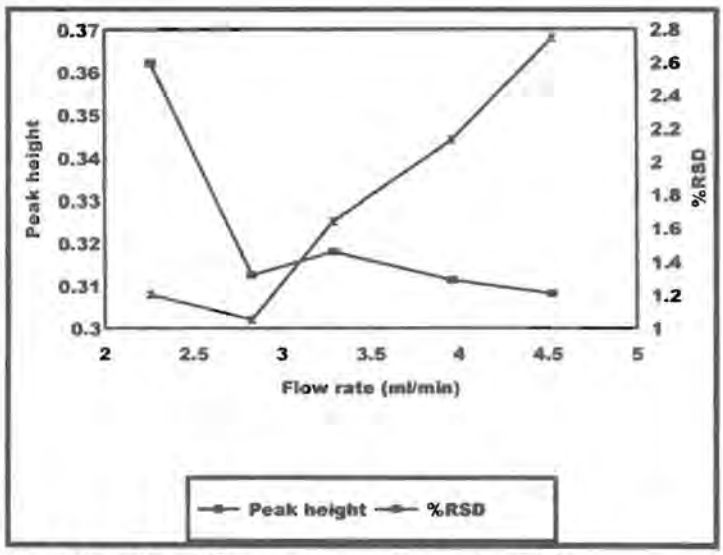

Fig. 8.6 Effect of flow rate on response and precision

TABLE 8.5 Effect of flow rate on response and precision

Rate (ml/min)	2.26	2.83	3.29	3.96	4.53
RPh	0.308	0.302	0.325	0.344	0.368
%RSD	2.6	1.3	1.46	1.29	1.2

8.4.2.2.3 Sample volume

The effect of sample and base were studied as well, and they were found to play a significant role. The sample volume was evaluated between 27.5 and 247.5 $\mu\ell$ and 137.5 $\mu\ell$ volume was found to be the best as shown in Table 8.6 and Fig. 8.7.

TABLE 8.6 Effect of sample volume on response and precision

Volume ($\mu\ell$)	27.5	82.5	137.5	192.5	247.5
RPh	0.232	0.312	0.393	0.454	0.505
%RSD	2.2	1.46	0.78	0.75	0.8

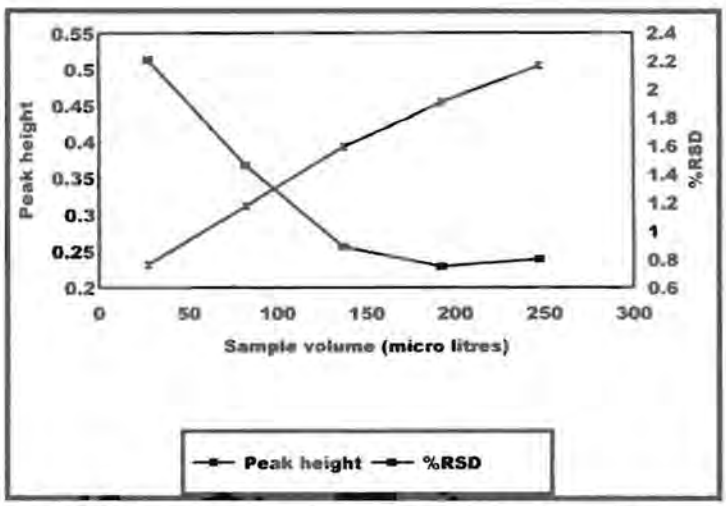

Fig. 8.7 Effect of sample volume on response and precision

8.4.2.2.4 Base volume

The base volume was evaluated between 27.5 and 137.5 $\mu\ell$. The 55 $\mu\ell$ gave the best results. Tables 8.7 and Fig. 8.8 gives the results.

TABLE 8.7 Effect of ammonium hydroxide on response and precision

Volume ($\mu\ell$)	27.5	55	82.5	110	137.5
RPh	0.421	0.393	0.418	0.423	0.416
%RSD	1.7	0.75	1	0.87	0.8

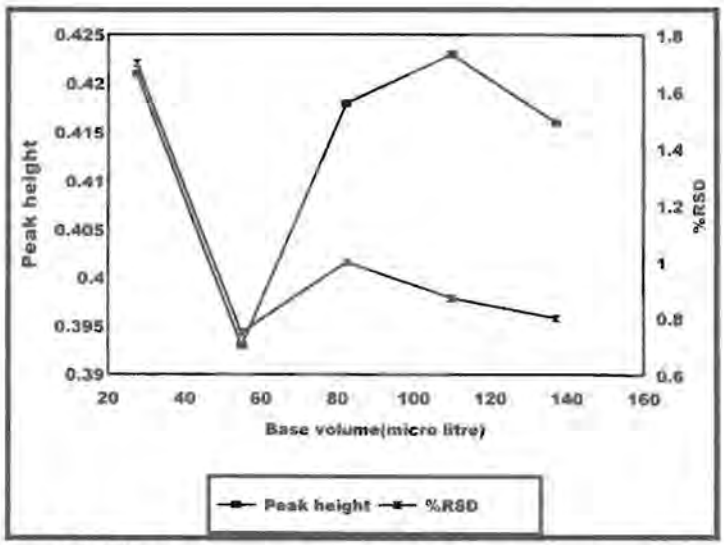

Fig. 8.8 Effect of ammonium hydroxide volume on response and precision

8.4.3 Method evaluation

8.4.3.1 Linearity

The linearity of the system was evaluated for analyte concentration between 0.01 and 5 mg/ℓ under optimum conditions Table 8.8. The system was found to be linear in the range of 0.01 to 1 mg/ℓ (Figure 8.9). The relationship obtained between response and concentration is given by the equation :

$$H = 4.8628x - 0.0495,\ r = 0.999,\ n = 8$$

where H is the relative peak height and x is the analyte concentration in mg/ℓ.

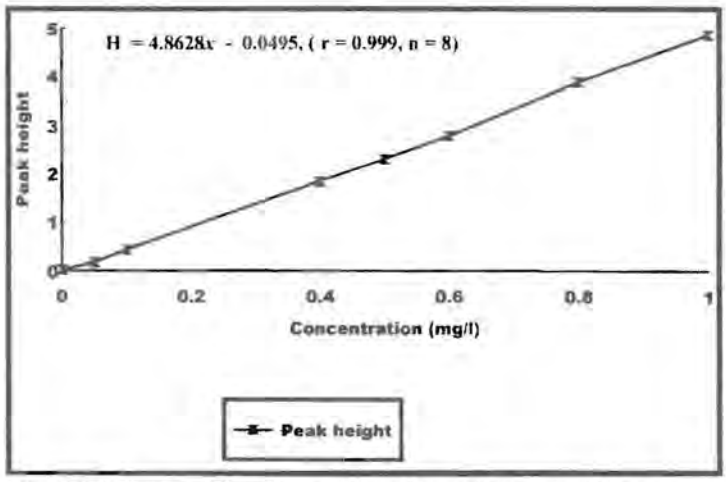

Fig. 8.9 A linear calibration graph under optimum conditions

TABLE 8.8 Optimum conditions

Parameter	Optimum value
Chromium concentration	0.4 mg/ℓ
Reactor length	10 cm
Flow rate	3.96 mℓ/min
Sample volume	137.5 $\mu\ell$
Base volume	55 $\mu\ell$

8.4.3.2 Accuracy

The accuracy was evaluated by comparing results obtained with the SIA system (Table 8.9) and the standard method (AAS) Table (8.10). The results obtained are a mean of 10 repetitive analysis of each sample.

TABLE 8.9 SIA results for samples analysed

Sample Id	Relative peak height	mg/ℓ Chromium
SE 1	3.395 (0.5%)	1.4164
SE 2	3.375 (0.5%)	1.4082
SE 3	0.148 (0.9%)	0.0812
SE 4	0.145 (0.9%)	0.0676
SE 5	6.959 (0.4%)	2.8821
SE 6	1.116 (0.8%)	0.4792
SE 7	3.802 (0.5%)	1.5838
Demiskanaal	0.57 (0.8%)	0.2546
GBR-mond	0.015 (1.0%)	0.0263
Hartebeeskuil dam	0.016 (1.0%)	0.0268
Chechasseur	0.099 (1.0%)	0.0611
Bree rivier	0.194 (0.6%)	0.1101
Mutshedzi dam	0.34 (0.9%)	0.1599
Driel barrage	0.57 (0.8%)	0.2546

TABLE 8.10 Standard AAS results for samples analysed

Sample	mg/ℓ Chromium
SE 1	1.4956
SE 2	1.4456
SE 3	0.0795
SE 4	0.0662
SE 5	2.8161
SE 6	0.4782
SE 7	1.4877
Demiskraal kanaal	0.2257

GBR-mond	0.0264
Hartebeeskuil dam	0.0264
Chechasseur	0.053
Bree Rivier	0.0928
Mutshedzi dam	0.146
Driel barrage	0.2257

8.4.3.3 Recovery

The recovery of the proposed system was determined by comparing results obtained, with the expected ones after addition of 0.05 mg/ℓ standard chromium solution to selected samples (Table 8.11). The recovery was calculated using the equation :

$$\% \text{ Recovery} = \frac{\text{obtained}}{\text{expected}} \times 100\%$$

The recovery ranged between 98.87 and 104.76%.

TABLE 8.11 Recovery results after addition of 0.5 mg of Cr(III) to selected samples

Sample	Expected	Recovered	% Recovery
SE 1	1.9164	1.9021	99.25
S6E	0.9792	0.9683	98.89
Mutshedzi dam	0.2099	0.2199	104.76
Demiskanaal dam	0.7546	0.7746	102.65
Bree rivier	0.6105	0.6305	103.27

8.4.3.4 Precision

The precision of the method was determined by 10 repetitive analysis of the standard solution (Fig. 8.9) as well as 10 repetitive analysis of the real samples (Table 8.9). All these were carried out under optimum conditions. The %RSD for the standard was 0.5% and for the real samples is less than 1.0%.

8.4.3.5 Detection limit

The detection limit was calculated using the formula:

$$\text{Detection limit} = \frac{[(3\sigma + k) - c]}{m}$$

where σ (0.002) is the standard deviation of the base line k (0.0415) and c(-0.04945) is the intercept and m(4.8628) is the slope of the calibration graph. The calculated limit of detection was found to be 0.02 mg/ℓ.

8.4.3.6 General problems

The problem encountered with this system was the reactor. It was discovered that when the packed reactor was left exposed to the atmosphere its effectiveness deteriorated compared to a freshly prepared reactor. This problem was overcome by storing the freshly prepared reactors in a dessicator until it was needed for use.

8.4.3.7 Interferences

The effect of the presence of other ions on the determination of chromium was investigated. For this purpose Ag^{+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}, SO_4^{2-}, Cl^{-}, NO_3^{-}, NO_2^{-} and PO_4^{3-} were added individually to a 100 mℓ solution containing 0.5 mg/ℓ chromium and the general procedure applied. As can be seen in Table 8.12, the tolerance levels and the ranges of the ions found in the samples are given. These ions did not significantly affect the determination of chromium in the various samples. The only ion that may interfere was the Ag^{+}, which was likely to form Ag_2CrO_4 precipitate, but this dissolves easily in the presence of ammonium hydroxide to release the chromate ion [33]. Furthermore, cations found were at very low concentrations to affect the results (Table 8.12). Anions were also found to be below the tolerance levels.

TABLE 8.12 Some of the ions present in the water samples, their ranges and tolerance levels in mg/ℓ when added to 0.5 mg/ℓ of chromium.

Ion	Tolerance level	Range in samples
Phosphate	4	0.008-0.080
Nitrate + Nitrite	10	0.47-1.68
Chloride	800	10-400
Sulphate	350	0.018-66
Sodium	800	4-67
Potassium	800	0.3-8.3
Calcium	450	3-19
Magnesium	350	2-19
Copper	30	0.0011-0.057
Nickel	35	0.0043-0.79
Zinc	50	0.005-14.8
Iron	40	11.4-14.2

Silver	50	0.005-15.9

8.5 Statistical comparison of techniques used

The comparison was done between the SIA and the AAS (Table 8.13) results to establish whether the SIA system can be accepted as giving reliable results in chromium determination. The null hypothesis was used [42, 43].

TABLE 8.13 Comparison of SIA and the standard AAS method results in mg/ℓ and paired differences

Sample	SIA method	Standard AAS	$x_d \times 10^{-4}$	$(x_d)^2 \times 10^{-8}$
SE 1	1.4164	1.4956	-792	627260
SE 2	1.4082	1.4456	-337	113569
SE 3	0.0812	0.0795	17	289
SE 4	0.0676	0.0662	13.6	185
SE 5	2.8821	2.8161	659	434300
SE 6	0.4792	0.4782	10	100
SE 7	1.5838	1.4877	961	923500
Demiskraal kanaal	0.2546	0.2257	289.5	83800
GBR-mond	0.0263	0.0264	-0.7	0.4
Hartebeeskuil dam	0.0268	0.0264	-0.7	0.4
Chechasseur	0.0611	0.053	-423.5	179350
Bree Rivier	0.1101	0.0928	173	29930
Mutshedzi dam	0.1599	0.146	139.5	19460
Driel barrage	0.2546	0.2257	289.5	83810

For the null hypothesis we assert that the two methods agree, that is the population mean

difference is zero, $H_0 : \mu_d = 0$. For the alternative hypothesis $\mu_d \neq 0$, where μ_d is the population (H_0) paired by difference. The t-test with multiple samples (paired by difference) was applied to examine whether two methods differed significantly at 95% and 99.9% level. The test is two tailed, as we are interested in both $\mu_d < 0$ and $\mu_d > 0$. From Table 8.13 the following is deduced:

$$\sum x_d = 0.396 \qquad \text{and} \qquad \sum x_d^2 = 0.02496$$

The mean $\bar{x}_d$, standard deviation, S_d and $t_{calculated}$, $t_{calc.}$ are determined from the following equations:

$$\bar{x}_d = \frac{\sum x_d}{N}$$

$$s_d = \sqrt{\frac{\sum (x_d - \bar{x}_d)^2}{N-1}}$$

and

$$t_{calc.} = \left|\bar{x}_d\right| \times \frac{\sqrt{n}}{s_d}$$

Substituting in the above equations with N = n =14, we get:

$$\bar{x}_d = 0.0283$$

$$S_d = 0.0846$$

and

$$t_{calc.} = 3.74$$

At 95% confidence level $t_{0.05,13} = 1.77$ and at 99.9% confidence level $t_{0.01,13} = 4.22$. Since the calculated value lies within the critical value at 99.9% level, it would imply that H_0 cannot be rejected and it follows that there is no significant difference between the two techniques at this level. However, at 95% level it may be rejected.

8.6 Conclusions

The proposed SIA method have shown that 97% of the chromium (III) can be detected. However, in the work already done [44, 45], the on-line oxidation of chromium (III) to chromium (VI) by Ce (IV) was reported incomplete. The incompleteness could have been caused by the reconversion of chromium (VI) back to chromium (III). The incorporation of the solid phase lead (VI) oxide and the basic media created when the chromium (VI) passed through the lead (IV) oxide, prevented the reconversion of chromium (VI) back to chromium (III). It has instead enhanced the production of chromium (VI) as chromate. The proposed SIA system was thus suitable for the determination of chromium in electroplating waters and natural waters. It is simple and robust, saving in reagents, reliable and sensitive as well as cost effective with a detection limit of 0.02 mg/ℓ. The standard AAS method has a detection limit of 0.03 mg/ℓ.

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CHAPTER 9

Conclusions

The importance of manifold design in developing process analysis which are capable of on-line monitoring with high sample through put, minimum sample and reagent consumption, robust, reliable and requiring low frequency of maintenance has become very evident through the course of this work.

The introduction of super Serpentine reactors and solid - phase reactors into the SIA manifold was an attempt to make injection techniques more rugged for process control application. The SIA system contributes considerably to reducing reagent and sample as well as enhancing sensitivity which is one of its greatest attributes.

The simplicity by which the change from a homogeneous to a heterogeneous system was easily reached, is further evidence of the versatility of the SIA technique. Its simplicity is further attributed to the multiport selection valve by which the samples, reagents reactions lines and detectors are accessed. The system is computer controlled and can be configured to perform most operations of conventional flow injection analyses with no or minimal reconfiguration of the manifold.

It has become evident, that there are various parameters that influences the results that flow

systems deliver. The importance of controlling these parameters when developing a method is achieved by optimizing this parameters to obtain maximum response with the best precision.

In SIA high sensitivity is obtained by minimizing the dispersion in the flow manifold, however it is also important to achieve an adequate amount of dispersion so that, the sample can react effectively with the surrounding reagent. It has, though, been realised that for SIA, zone penetration plays an important role in the sample and reagent interaction.

The introduction of solid - phase reactors into the SIA manifold was to accommodate reagents and samples that are expensive , insoluble or partially soluble. The development of the SIA system is aimed at providing the industrial , agricultural, clinical and pharmaceutical fields with reliable , precise and cost effective instrumentation for performing, analysis they require.

The increasing awareness regarding environmental pollution and the regulation of the effluents that are released into the environment, particularly those affecting potable water sources , places an enormous responsibility on analysts to develop methods that can be effectively applied to determining the actual amount of polluting elements that are discharged.

In its study , theoretical aspects of SIA was done which was followed by a brief discussion of the reactor types used both in FIA and SIA this acted as a precursor to the introduction and evaluation of super Serpentine reactors.

A critical review of solid - phase reactors in FIA was made and its various applications to real systems. The SIA system incorporating solid-phase reactors was adapted from the existing FIA

system. Thus the application of the SIA system to real system further exposed its usefulness as a process analyser.

From the discussion of the reactor types, super Serpentine reactors were not previously studied and subsequently its study was undertaken. Super Serpentine reactors were evaluated with regard to its influence on response and precision. It was evaluated to give an in depth information regarding its effect on dispersion in the SIA system.

A comparative study achieved by overlaying peaks of different super Serpentine reactor types and length revealed that the simultaneous choice of reactor type and length play an important role with regard to response and precision and hence dispersion. The response and precision obtained for the various super Serpentine reactors may with proper choice of the reactor be of use in the analyses of trace elements. Dispersion in addition to zone penetration have an important effect on the amount of zone penetration attained.

For the application of solid-phase reactors to real systems, four elements were identified for analyses, namely manganese, iron, nitrogen and chromium. These elements were identified in relation to their potential toxicity to plants, animals as well as to human beings and how essential they are if present or taken in correct dosages in both water and supplements. The determination of these elements using solid-phase reactors in SIA had not been attempted previously and studies on these were subsequently undertaken.

A number of methods requiring laborious sample preparations, using large sample volumes and involving complicated procedures and expensive instruments have been used for the

determination of manganese. The determination of manganese in domestic waters and effluent streams using a solid lead (IV) dioxide reactor is an improvement on these methods as well as on FIA methods used. The manganese is oxidised to permanganate ion in which form it is determined. The proposed SIA system is found to be suitable for manganese determination in tap, domestic and effluent streams with a relative standard deviation of better than 3%.

In the determination of total iron as iron(II), an in depth research has been conducted using different techniques, however these techniques involved expensive instruments and are time consuming. The use of cadmium granules as solid-phase reactor is used in the determination of iron in pharmaceutical products and natural waters. The iron(III) in the samples is reduced to iron(II) and complexed with a colouring reagent to give a dye in which form the iron was determined. The cadmium reactor is regenerated on-line and this places the SIA system ahead in sample frequency and saving of samples and reagents. This developed system is thus suitable for the determination of total iron in pharmaceutical products and water samples within a wide range.

Many colorimetric methods have been used for the determination of nitrogen as nitrate or nitrite, however, most have been determined as nitrite. These determination are all based on the Griess' reaction. The determination of nitrogen involves the oxidation of nitrogen in water samples to nitrite which is diazotised to a red dye and determined as such. The reactor is regenerated on-line for consistency. Thus, the proposed system is found to be suitable for determination of oxidised nitrogen as nitrite in water samples from different sources.

There are several methods which are used for the determination of chromium, there are however,

a few that have sufficient sensitivity and selectivity for the direct determination of trace levels of chromium in water. Even multi-element techniques such as ICPMS do present limitations.

A method with a proposed reaction was developed for monitoring chromium in industrial electroplating processes and natural waters. This involves a solid-phase reactor incorporated into the SIA manifold. The reactor precipitates all the chromium present as lead chromate which is then released in excess base as the chromate ion in which form it is determined. The propose SIA system is suitable for monitoring chromium in industrial electroplating processes and natural waters.

All the above elements determinations were detected by means of a UV/VIS spectrophotometer as stated in the relevant Chapters. The SIA system in all the above is found to be easy to operate, versatile, robust and have the advantage of material saving.

A statistical comparison using the null hypothesis was used to assert that there is no statistical significant differences at 95% confidence level between the proposed SIA techniques and the different standard methods used. Finally, it may be concluded that the proposed SIA technique is suitable for on-line determination, is accurate, reagent saving and easily used even when reagents are expensive, partially soluble or insoluble.