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DEANSHIP OF GRADUATE STUDIES

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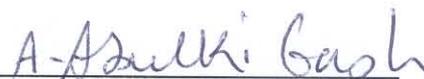
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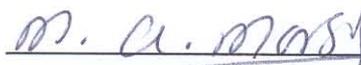
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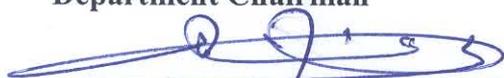
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## **THESIS ABSTRACT**

**NAME:** MAGNUS .U. LEGEMAH  
**TITLE OF STUDY:** SEQUENTIAL INJECTION ANALYSIS  
TECHNIQUE FOR INDUSTRIAL WATER  
QUALITY CONTROL  
**MAJOR FIELD:** CHEMISTRY  
**DATE OF DEGREE:** MARCH 2006

This dissertation describes the adaptation of Sequential Injection Analysis (SIA) for the determination of silica, iron, ammonia, nitrates/ nitrites and phosphates in water as a method suitable for the determination of water quality parameters in industrial water. The results obtained from the adopted SIA methods were found to be in agreement with results obtained from batch spectrophotometric APHA Standard methods (American Public Health Association). SIA has the additional advantage of reducing cost by minimizing time, amount of reagent consumed, man power required and improved accuracy of analysis due to computer aided analysis procedure.

## U خلاصة الرسالة

الاسم: ماجنس لاجيما

**عنوان الرسالة:** تقنية التحليل بالحقن المتتابع في ضبط جودة الماء المستخدم في الصناعة

**التخصص:** كيمياء تحليلية

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إن ملخص هذه الدراسة يتناول استخدام وسيلة الـ (Sequential Injection Analysis) لمعرفة كمية وجود الأملاح مثل (Si, Nitrates/Nitrites, NH<sub>3</sub>, Fe<sup>2+</sup>) في المياه. النتائج التي ظهرت من خلال استخدام وسيلة الـ (SIA) كانت مطابقة للنتائج التي ظهرت عن طريق استخدام وسيلة (APHA). بالإضافة فإن استخدام (SIA) كان لديه إيجابية إضافية ألا وهي اختصار الوقت، والمواد الكيميائية، والطاقة البشرية للقيام بالعمل.



# CHAPTER 1

## INTRODUCTION

### 1.1. PRESENT STATE OF PROBLEM

Most water quality laboratory uses the conventional batch spectrophotometric method for the analysis of silica, iron, ammonia, phosphate, nitrates/nitrites. The batch methods used for analysis of these water quality parameters have the disadvantage of being tedious, reagent consuming, time wasting and highly prone to errors because of the difference in time absorption measurements are taken for each samples. These methods are usually not suitable for analysis of a large number of samples (i.e.200 samples) due to the inconvenience involved in washing glass wares, errors associated with accurate addition of reagents to samples and difference in time of absorption measurement. Analysis using batch method requires a lot of manpower which generally add to overall laboratory operating cost. The batch spectrophotometric methods normally require that high concentration of analyte be diluted to low concentration in order for Beer's law to be obeyed; this can introduce dilution error into the analysis.

Mixing of reagents and analyte in holding coil is usually a big challenge in sequential injection analysis, this is usually circumvented by delay of sample and reagents in holding coil or using a reaction coil to promote radial

dispersion (more effective mixing) while minimizing axial dispersion (dilution of sample). Sequential injection analysis techniques are often considered to be very difficult and technical because the sensitivity and accuracy of the developed Sequential / Flow injection analysis methods depends on a lot of parameters such as length of holding coil, diameter of holding coil, chemistry of method, volume of sample and reagents aspirated, suitability of the computer flow program, manifold design and flow rate. All these parameters need to be optimized. The chemistry involved between the analyte and the reagent can be very slow, this can subsequently make the optimization of the analytical method challenging and decrease analysis through put (number of samples analyzed in a given time). Heating of holding coil in a thermo stated water bath has been used to speed up the chemistry of the slow reactions. The optimum temperature of the water bath has to be determined because air bubbles which can seriously interfere with the analytical method are generated above the optimum condition which is usually in the range of 50-60 degree centigrade.

## 1.2 **OBJECTIVE**

- To automate the wet chemical analysis method for the determination of iron, silica, nitrate/nitrite, ammonia and phosphate in water by sequential injection analysis.
- Optimization and development of a computer flow program for the adapted SIA methods.
- Enhancement of sensitivity of the proposed SIA methods by heating the holding coil in a thermostated water bath, use of reagent as carrier and delaying the reagents and sample in holding coil to promote chemistry.
- Validating the proposed SIA methods by comparing the results with results obtained using standard batch methods of the American Public Health Association.

## 1.2.1 INDUSTRIAL WATER ANALYSIS

The manual handling of solutions (known as "beaker chemistry") remains the Achilles Heel of modern analytical instrumentation. It is currently being replaced by flow injection analysis (FIA), which is computer compatible and allows automated handling of sample and reagent solutions with a strict control of reaction conditions.

Sequential injection analysis (SIA) is a second generation FIA technique for automating wet chemical processes with the aid of a computer flow program.

The determination of silica, ammonia, phosphates, iron, and nitrates/nitrites in water samples is very important because water is used for a variety of purposes which include industrial use such as feed water for boilers, manufacturing processes, water treatment for both domestic use, disposal purposes and agricultural purposes.

Phosphate and silica in water are very important water quality parameters for boiler feed water determination in the industry. Silica in boiler feed water is known to deposit on steam turbine blades at high pressure and temperature, this lowers the efficiency of heat transfer, leading to costly down time for cleaning and may result to total failure of the boiler system. Phosphates is usually added to the boiler feed water to ensure that it's in the alkaline range, this serves as an anticorrosion measure [2]. Nitrates, ammonia and phosphates in water bodies are due to the decomposition of animal remains and agricultural run off. High concentration of phosphate

and nitrates in water can result to nutrient enrichment which results to eutrophication and subsequent depletion of dissolved oxygen in water bodies. Determination of ammonia, iron, nitrates and nitrite in water is very important because these ions can induce corrosion of pipes and other metal structures. Hydrated iron is known to dissociate with the loss of an  $H^+$  (proton) which increases acidity of the water inducing corrosion. Determination of water quality parameters is very important for water treatment, depending on the intended use of the water. Water for drinking should have ammonia, nitrates/nitrites, Iron, phosphates below the maximum permissible limits by the water regulatory bodies. Its accurate determination is very imperative in order to ascertain if the treated water conforms to standards and hence suitable for consumption or intended use. Accurate and reproducible determination of phosphates, ammonia, silica, iron and nitrates/nitrite in water is highly imperative. Adopting SIA for determination of these water quality parameters ensured their accurate determination.

Several flow injection / sequential injection method for the determination of these parameters have been reported [2-62].

### **1.3. WATER QUALITY MANAGEMENT**

A good water quality management system usually incorporates

- Environmental Management (EM)
- Quality Management (QM)

World wide emphasis and focus on environmental management began with the various regulations setup by various Governments to curb pollution problems. This was very important because most pollutants eventually end up in water bodies, deteriorating quality and increasing cost of water treatment especially at the public water treatment plants. It is now recognized that integrating environmental management into the production processes in the industry can help the industries minimize cost of water and waste treatment, Promote compliance to regulations discharge limit of pollutants. These regulations by the Government are written in command and control mode for permitting, monitoring, inspection (auditing), enforcement and required regulatory compliance.

The aim of environmental management is to be proactive in approach to pollution control by:

- Elimination or reduction of contaminants at the source
- Increasing process operating efficiencies
- Recycling and reusing both water and waterborne contaminants
- Treatment through the use of pollution control device.

Quality management (QM) is customer driven and deals with customers' satisfaction and service with regards to quality of goods (treated water) and services produced by the Company. EM and QM focus on different stakeholders, however both are the responsibility of every employee within the organization regardless of the function assigned. Both are designed to prevent potential problems, both tangible and intangible, that have unknown and potential serious cost effects for the organization. Total quality management (TQM) has evolved as the more widely used concept for quality management.

## 1.4 FLOW INJECTION ANALYSIS

Flow injection Analysis (FIA) was first defined by Ruzicka , Hansen in Denmark and Stewart and co-workers in the United States in the mid 1970's [31]. It is a simple technique for automating wet chemical processes with the aid of a computer flow program. Flow injection methods are an outgrowth of segmental flow procedures, which were widely used in clinical laboratories in the 1960s and 1970s for automatic routine determination of a variety of species in blood and urine samples. In Segmental flow systems, samples were carried through the system to a detector by a flowing aqueous solution that contained loosely spaced bubbles. The purpose of the air bubbles was to prevent excess sample dispersion, to promote turbulent mixing of samples

and reagents, and to scrub the walls of the conduit, thus preventing cross contamination between successive samples. The discoverers of flow injection analysis found, however, that excess dispersion and cross contamination are nearly completely avoided in a properly designed system without air bubbles and that mixing of samples and reagent could be easily realized.

The absence of air bubbles imparts several advantages to flow injection measurement, including (1) higher analysis rate (2) enhanced response times (3) Much more rapid start up and shut down times (4) except for the injection system, simpler and more flexible equipment.

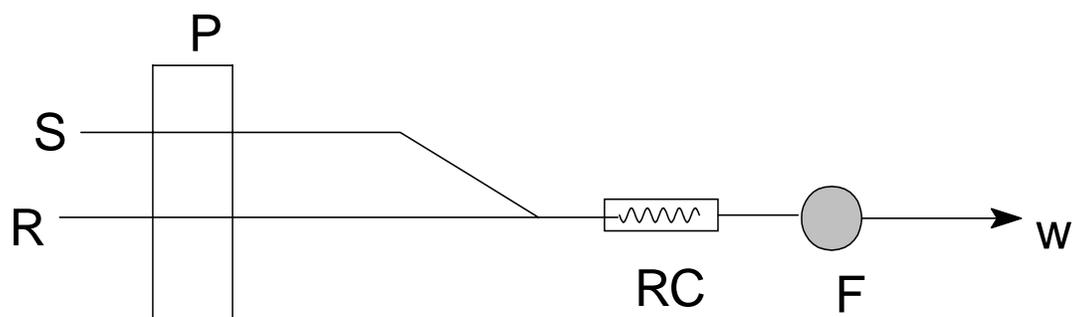


Fig 1. A typical two channel flow injection manifold with; S = sample stream, R = reagent stream, RC = reaction coil, F = flow cell with detector, P = peristaltic pump and W = waste

### **1.4.1 SEQUENTIAL INJECTION ANALYSIS**

Sequential injection analysis (SIA) is a second generation of FIA, and was defined by Ruzicka and Marshall in 1991. This approach to automated sample manipulation arose from a need to simplify manifolds and address the unique requirements of the field and process analysis. In SIA, a selection valve and bidirectional pump is used to draw up small volumes of samples and reagents, and then propel them through a coil to a detector. The major differences in the instrumentation of SIA and FIA are briefly highlighted in table 1.

TABLE I: **Major differences in the instrumentation of FIA and SIA**

SIA	FIA
It consist of a single channel, high precision bi-directional pump called the syringe pump ,a holding coil, multi-selection valve, reaction coil and a detector	It consists of a high quality multichannel peristaltic pump, an injection valve, a coiled reactor and a detector.

The main advantages of SIA over FIA are

- Simpler hardware than FIA
- Minimization of waste and cost saving due to consumption of less reagent
- Simple and Universal manifold and the ease with which different chemistries can be implemented in one manifold.

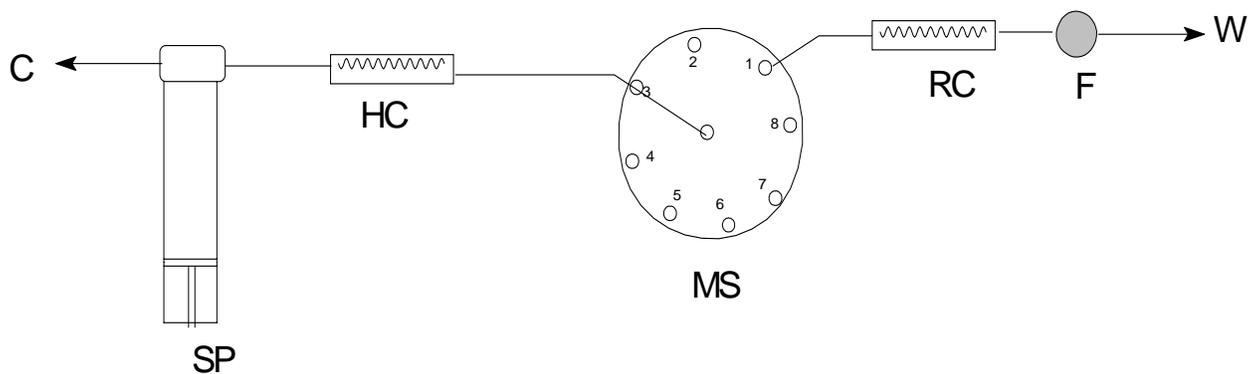
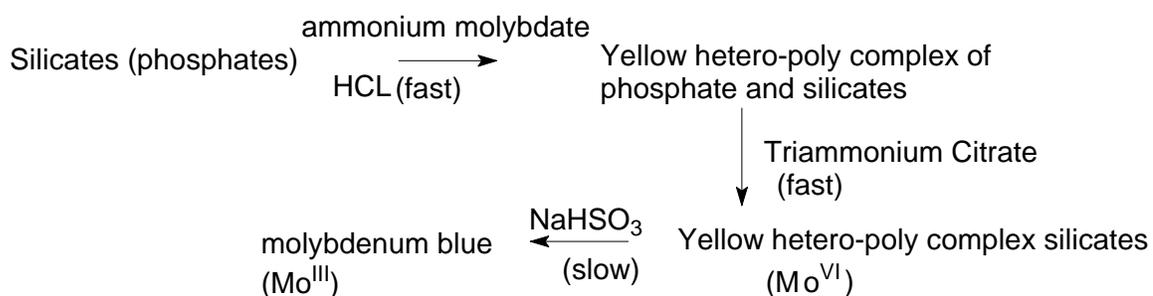


Fig 2: A typical sequential injection manifold with; C = Carrier, SP = syringe pump, HC = holding coil, MS = multi-selection valve, RC = reaction coil, F = flow cell with detector, W = waste

## 1.5 PRINCIPLES AND LITERATURE REVIEW OF ADAPTED SIA METHODS

### 1.5.1 .....DETERMINATION OF SILICA



Scheme 1: Reaction scheme for the proposed SIA method for the determination of silica in the presence of phosphate

The proposed SIA method for silica determination reaction protocol will be briefly described. Silicates and phosphates react selectively with ammonium molybdate and the reaction is catalyzed by acid to give the yellow hetero-poly complex. Triammonium citrate is added to make the reaction specific for silicates and eliminate interference from phosphate. Triammonium citrate complexes phosphates and removes it from the complex formed with molybdate (VI) ions with subsequent loss in yellow coloration. The  $\text{Mo}^{\text{VI}}$  complex is reduced to  $\text{Mo}^{\text{III}}$  on addition of sodium bisulphate and this blue complex can be monitored spectrophotometrically at 813 nm.

A lot of flow injection / sequential injection analysis researchers have also suggested several methods for the determination of silica in water samples. Galhardo and Masini<sup>4</sup> suggested that if low concentration of silicate and phosphates are to be monitored, a further reduction of  $\text{Mo}^{\text{VI}}$  to  $\text{Mo}^{\text{V}}$  needed to be performed to produce intensely coloured molybdenum blue compounds which enhances the sensitivity of this method.

Galhardo and masini<sup>4</sup> determined phosphate and silicate in environmental samples and cell cultivation using the molybdenum blue reaction; the interference of silicate in the determination of phosphate was eliminated by using a reagent composed of  $5\text{mmol}^{-1}$  ammonium molybdate in  $0.2\text{mol l}^{-1}$  nitric acid, containing 0.25% (w/v) oxalic acid to avoid the formation of molybdosilicic acid while the interference of phosphate in the determination of silicate was avoided by adding a 10% (w/v) oxalic acid solution to the reaction to destroy the molybdophosphoric acid complex formed. Grundpan et al<sup>7</sup> determined phosphate and silicate simultaneously by stopped flow injection using the molybdenum blue chemistry. The method was demonstrated to be suitable for determining silicate and phosphate simultaneously in water samples. Y.-S. Li et al<sup>2</sup> also determined simultaneously phosphate and silicates in boiler water at power plants based

on series flow cell by using flow injection spectrophotometry. The principle of the method they proposed is that the total concentration of silicate plus phosphate is determined when an injected sample is passed through the first flow cell and then the concentration of silica is determined at the second flow cell after masking the yellow molybdophosphate in the sample zone. The concentration of phosphate is obtained by difference.

Yaqoob et al<sup>31</sup> determined silicate in freshwater using flow injection with luminal chemiluminescence detection. The molybdosilicic heteropoly acid formed by silicate and ammonium silicate in acidic condition generated chemiluminescence response via oxidation of luminol. Potential interference in freshwaters were removed by passing the sample stream through in line iminodiacetate chelating (to remove cations) and anion exchange (to remove) phosphate micro-columns

Ohashi et al<sup>24</sup> carried out simultaneous kinetic determination of phosphate and silicate based on hetero poly blue formation. They suggested that small amount of phosphate ( $0.08-1.16 \mu\text{g ml}^{-1}$ ) and larger amounts of silicate ( $12-60 \mu\text{g ml}^{-1}$ ) can be determined simultaneously by a kinetic method based on difference in the rates of hetero blue formation with molybdenum (V)- molybdenum (VI) mixtures in 0.28M perchloric acid. The interference of large amounts of iron (III) on the determination of phosphate can be eliminated by masking with sodium hydrogen sulfite.

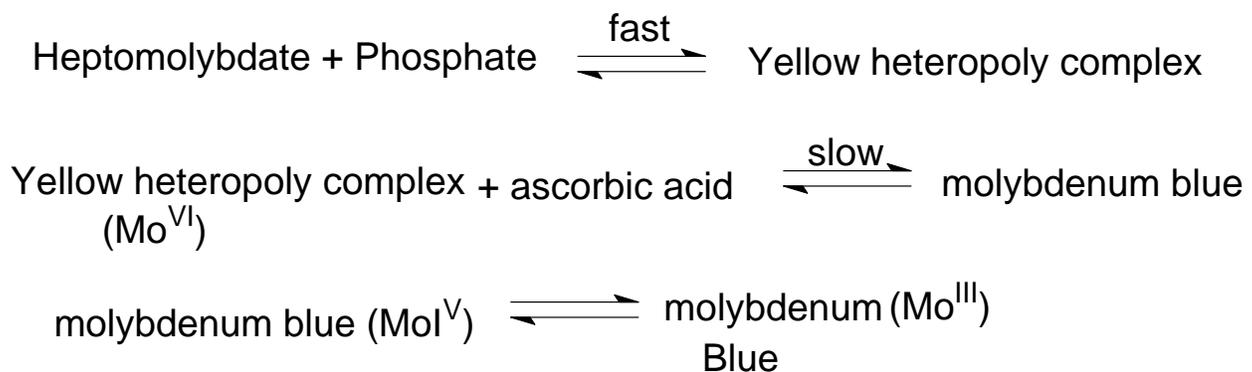
Linares et al<sup>21</sup> determined silicate and phosphate in waters by fluorimetric differential kinetic flow injection method. The method involves monitoring the fluorimetrically monitored product thiochrome which is formed by the oxidation of thiamine by the hetero poly acid. The method permitted the determination of silicates and phosphates in the range of  $30- 600 \text{ ng ml}^{-1}$  in the ratios of 1:10 to 10:1 and can be applied to samples of running and bottled water with good results.

Mas et al<sup>19</sup> proposed a spectrophotometric flow injection method for the determination of silicate based on the formation of an ion pair between molybdosilicic acid and Rhodamine B. This method allows silicate to be determined over the concentration range of 0.17 – 2.0mg l<sup>-1</sup> at a sampling rate of 40 h<sup>-1</sup>. This method was successfully applied for silicate determination in various types of water.

Kuroda et al<sup>17</sup> proposed a flow injection spectrophotometric method for the accurate, continuous determination of silicon in silicate rocks. Silicic acid polymerizes in acid solution, silicic acid was depolymerized in alkaline medium after a simple cation exchange column filtration of the rock sample solution and then determined by a static or FIA spectrophotometric method.

Floch et al<sup>15</sup> developed a method for the determination of silicic acid in sea water with submersible chemical analyzer. The method is based on direct FIA for fast and discrete measurements, and dual wavelength treatment of the signal to correct the refractive index interference. The colorimetric method is based on the formation of beta silicomolybdic acid reduced in intense colored molybdenum blue. Rapid and non temperature dependent reduction was accomplished by using tin (II) chloride as reductor.

## 1.5.2 DETERMINATION OF PHOSPHATE



Scheme 2: Reaction scheme for the proposed SIA method for the determination of phosphate

The reaction protocol for the SIA method proposed for the determination of phosphate involves the reaction of phosphate with ammonium molybdate (heptomolybdate) to give a yellow heteropoly complex which is reduced by ascorbic acid to molybdenum blue which is monitored spectrophotometrically using dual wavelength of 760 nm and a reference wavelength of 420nm.

Several other researchers have developed SIA / FIA methods for the determination of phosphates in water samples. Grudpan et al<sup>7</sup> suggested that the reaction to form the molybdenum blue are reversible and will be in equilibrium; they also suggested that a lower molybdate concentration (below 0.1%w/v) results in the distortion of the  $\text{Mo}^{\text{VI}} / \text{Mo}^{\text{III}}$  equilibrium with resultant formation of  $\text{Mo}^{\text{V}}$ . (Scheme 2) which reduces the intensity of the blue complex formed and the sensitivity of the method.

Worsfold and Clinch<sup>22</sup> proposed a flow injection manifold based on reagent injection into the sample stream for the determination of phosphate in natural water. A double beam photometer detector incorporating light emitting diodes and photodiodes was enclosed in a 20 cm<sup>3</sup> box. The response was linear over the range of 0-2000  $\mu\text{g l}^{-1}$  phosphate phosphorus

( $r = 0.9992$ ) and the limit of detection was 12  $\mu\text{g l}^{-1}$  phosphorus.

Narusawa<sup>18</sup> described a flow injection method for the simultaneous determination of silicate, phosphate and arsenate with on-line column separation. Determinations were based on measurement of absorbance at 810nm of the heteropoly blue formed with ascorbic acid as reducing reagent. Effects of flow rates, temperature of the reaction coil and sample injection volumes were reported.

Motomizu et al<sup>23</sup> described a method for the determination of phosphorus in river water based on the reaction of vanadomolybdophosphate with malachite green. They suggested that the formation of an ion associate between vanadomolybdophosphate and malachite green in aqueous acidic solution (0.5 M Sulphuric acid) enables trace amount of phosphate to be determined.

### 1.5.3 DETERMINATION OF NITRITE / NITRATES



Chromogenic reagent: sulfanilamide/phosphoric acid/ N-1-naphthylethylene dihydrochloride

Scheme 3: Reaction scheme for the proposed SIA method for the determination of nitrate /nitrite

The chemistry of the proposed SIA method involves reduction of nitrates to nitrites by passing it through cadmium column and then reaction of the nitrite formed with the chromogenic reagent to give you a purple colored dye compound

Other researchers have come up with several FIA / SIA methods for the determination of Nitrates / Nitrites. Haghighi and Kurd<sup>12</sup> described a flow injection method on the basis of gas phase molecular absorption for the determination of ammonium and nitrate. For ammonia determination the sample was directed to a line which reacts with NaOH (13M) and produce ammonia

while for nitrate determination the sample zone is passed through the on-line copperised zinc (Zn/Cu) reduction column and produces ammonium ion. The

produced ammonia in both cases was purged into the stream of nitrogen gas carrier gas.

The gas phase was separated from the liquid phase using a gas-liquid separator and then swept into a flow through cell, which has been positioned in the cell compartment of an UV-Visible spectrophotometer. The absorbance of the gas phase is measured at 194nm.

Andrade et al<sup>30</sup> described a novel flow injection method for the spectrophotometric detection of nitrate and nitrite in foodstuffs. The method was based on the reduction of nitrite and nitrate to nitric oxide with subsequent reaction with iron (II) and thiocyanate in an acid medium, forming  $\text{FeSCNNO}^+$ . The absorbance of the complex, with a maximum at 460nm is proportional to the nitrite and nitrate concentrations. The NO was generated in two stages (1) reduction of nitrate to nitrite in a cadmium copper reduction column and (2) reduction of the nitrite to NO in sulfuric acid medium. The precision and accuracy of the proposed method was comparable to those of the reference spectrophotometric official method.

Monser et al<sup>29</sup> proposed a direct spectrophotometric flow injection method for the simultaneous determination of nitrite and nitrate. The method was based on the oxidation of phosphomolybdenum blue complex by the addition of nitrite and the decrease in absorbance of the blue complex was monitored at 820nm. The injected sample was split into two segments, one of the streams was directly reacted with the above reagent and detected as nitrite. The other stream was passed through a copperised cadmium column reductor column where reduction of nitrate to nitrite occurs, and then the sample was then mixed with the reagent and passed through the cell of the spectrophotometer to be detected as nitrite plus nitrate.

Oms and Cerda<sup>28</sup> described an automatic method for the determination of total nitrogen in waste water by sequential injection analysis and mineralization of the samples with sodium persulphate in basic medium under UV radiation. The method was based on the mineralization of samples with sodium persulphate in basic medium under UV radiation. The organic and inorganic nitrogen compounds are oxidized to nitrate that is then measured at 226 nm. The method was compared with the Kjeldahl digestion method by analyzing 15 samples with both methods.

Legnerova et al<sup>26</sup> described a fully automated procedure based on sequential injection analysis (SIA) methodology for the simultaneous monitoring of nitrate and nitrite in surface water. Nitrite was determined directly using the Griess diazo coupling reaction and then the formed azo dye formed was measured at 540 nm in the flow cell of the fiber optic spectrophotometer. The nitrate zone was passed through a reducing mini column containing copperised cadmium. After reduction of nitrate into nitrite the sample was aspirated by flow reversal to the holding coil, treated with the reagent and finally passed through the

flow cell. Nitrates and nitrites were determined in the real samples of surface water and the results were compared with those obtained by using two other flow injection methods. Kazemzahd and Ensafi<sup>27</sup> proposed a direct spectrophotometric method for the sequential determination of nitrate and nitrite by flow injection analysis (FIA). The method was based on the reaction of nitrite with sufranine O to form a diazonium salt which causes the reddish orange dye color of the solution to be changed to blue in acidic media and which absorbs at 520nm.

The injected sample in the flow injection system is split in two streams. One of the streams is transported through a reduction micro column containing copperised cadmium where nitrate is reduced to nitrite. The two streams are mixed and treated with appropriate reagents. Nitrates and nitrites were determined in food samples by the proposed method with satisfactory results.

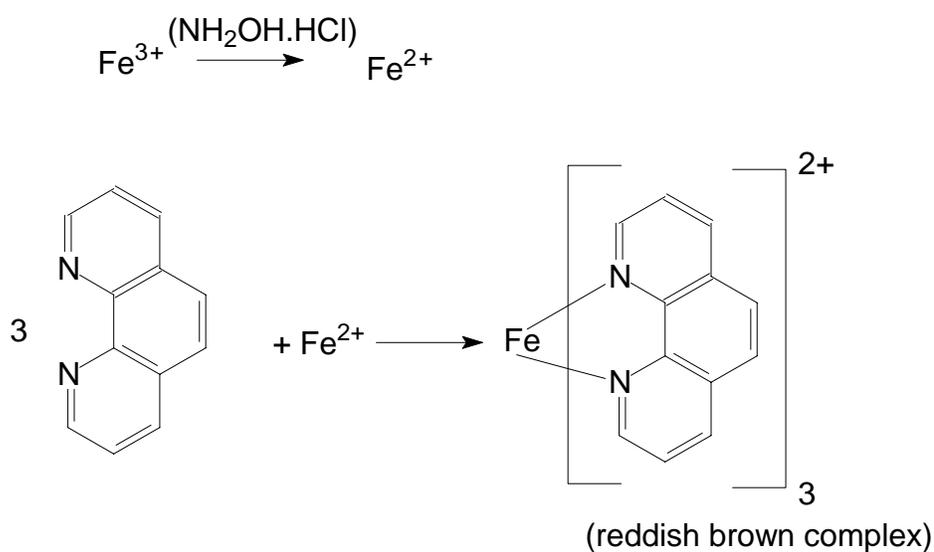
Pinto et al<sup>25</sup> described a sequential injection analysis of nitrites and nitrates in human serum using nitrate reductase. For nitrite determination, 150  $\mu\text{L}$  of sample and 50  $\mu\text{L}$  of Griess reagent were sequentially aspirated to the system and sent to the detector. Nitrates were determined as nitrites after reduction through 0.09 U of nitrate reductase and 75  $\mu\text{L}$  of NADPH. Statistical evaluation showed good agreement between the results obtained for 15 deproteinised serum samples, with both SIA system and comparison batch procedure. The automatic developed method seems to be a good alternative for routine implementation since its four times faster, and it requires one third of sample and one half of nitrate reductase than the comparison batch procedure.

Galhardo and Masini<sup>8</sup> described a sequential injection analysis (SIA) system for sequential monitoring of Fe (II) and Fe (III),  $\text{NO}_3^-$  and  $\text{NO}_2^-$  with typical concentrations of

natural water and waste waters. Determination of nitrite was based on Griess-Ilosvay reaction, while the Fe (II) determination was based on the reaction with 1,10-phenanthroline. Determination of Fe(III) and  $\text{NO}_3^-$  were performed after their reduction respectively in Jones and copperised cadmium column using forward and reversal flow directions.

Santos et al<sup>32</sup> achieved an accurate simultaneous analysis of different anionic species using ion selective electrodes (ISEs) which were non specific sensors by resorting to an ordinary least square multiple regression in the vicinity of the predicted concentrations. An AgCl/  $\text{Ag}_2\text{S}$  electrode based on homogenous crystalline membrane together with a PVC electrode based on tert-octylammonium bromide dissolved in dibuylphthalate were used as potentiometric detectors for chloride and nitrate respectively. The results obtained by the standard addition method were biased for low concentrations of nitrates and were dependent the relative proportions of  $\text{NO}_3^- / \text{Cl}^-$ . The results obtained by the proposed methodology for chloride determination were slightly better when compared to those obtained by standard addition method.

## 1.5.4 PROPOSED SIA METHOD FOR THE DETERMINATION OF TOTAL IRON AS IRON (II)



Schematic 4: Reaction scheme for the determination of iron (II) using the proposed SIA method

The chemistry of the proposed SIA method for the determination of total iron as iron (II) involves the reduction of iron (III) to iron (II) with hydroxylamine hydrochloride and then subsequent reaction of iron (II) with 1,10

Phenanthroline to give a reddish brown complex which can be monitored spectrophotometrically at 510 nm.

Several other SIA / FIA methods have been described by several researchers. Telsfadet et al<sup>5</sup> described a sequential injection analysis (SIA) system for the determination of iron (II) based on the reaction between 1,10-Phenanthroline and iron (II). The proposed technique was found to be accurate, reproducible and sensitive and it has been applied for the determination of total iron as iron (II) in pharmaceuticals products (multivitamins).

Mulaudzi et al<sup>33</sup> described the application of sequential injection analysis to metal speciation. The proposed method was used for the quantitative discrimination of two iron species, Fe (II) and Fe (III). Tiron was used as the chromogenic reagent for Fe (III) and total iron after Fe (II) was oxidized by H<sub>2</sub>O<sub>2</sub>.

Kass and Ivaska<sup>9</sup> outlined a procedure for the determination of iron (III) and total iron by sequential injection analysis. The method is based on the strong blue complex formed between iron (III) and tiron. The absorbance of the complexes is measured spectrophotometrically at 635nm.

Rubi et al<sup>16</sup> described a sequential injection analysis (SIA) assembly for the atomic absorption determination of Fe (III) in natural waters. Iron is preconcentrated on a micro column packed with chelatin resin (chelex 100) that was inserted into the manifold. The sample is passed

though the column and the iron retained by the resin is sequentially eluted with 2M HNO<sub>3</sub>. The proposed SIA system affords automatic preconcentration, elution, detection of Fe(III), data acquisition and treatment.

Sultan et al<sup>35</sup> described a sequential injection spectrophotometric method for the assay of bromazepam anxiolytic drug. The method is based on the complexation reaction of bromazepam with iron (II) in hydrochloric acid media and spectrophotometrically measuring the product at  $\lambda_{\text{max}}=585$  nm. A comprehensive chemometrical optimization treatment was successfully utilized for determining the proper optimum operating conditions for both the system and the chemical variables. The experimental design approach was employed and a 2<sup>k</sup> factorial design was run for studying the interaction effects of four factors namely, hydrochloric acid concentration, iron(II) concentration, delay time and flow rate. The super modified simplex algorithm was utilized for optimizing the three highly interacting factors which were, hydrochloric acid, iron(II), and delay time. The conditions obtained were 150  $\mu\text{l}$  0.110 mol l<sup>-3</sup> hydrochloric acid, 75  $\mu\text{l}$  0.328 mol l<sup>-3</sup> iron(II), 1200 s delay time and 40  $\mu\text{l}$  s<sup>-1</sup> flow rate.

Eldin et al<sup>36</sup> described a sequential injection spectrophotometric method for stoichiometric studies, optimization and quantitative determination of ciprofloxacin and norfloxacin. The work was based on the complexation reaction of ciprofloxacin and norfloxacin with iron(III) in sulfuric acid media and a spectrophotometric measurement of absorbances of the corresponding complexes at 447 and 430 nm respectively. The stoichiometries and formation constants were determined. A 1:2 iron (III) to drug mole ratio was found to give the most predominant complexes for both drugs with  $5.00 \times 10^{-3}$  M H<sub>2</sub>SO<sub>4</sub> and at 0.20 M ionic strength utilizing Job's method and the molar ratio method. A numerical method was utilized for the calculation of the

formation constants, the logarithms of which were found to be  $7.756 \pm 0.121$  and  $7.839 \pm 0.056$ , for ciprofloxacin and norfloxacin respectively. A factorial design together with the all-model-search method was utilized for the optimization of the concentration and aspiration volume of iron(III) as these were the variables which most affected peak absorbance. Working dynamic ranges of 50–500 ppm and 50–400 ppm were obtained for ciprofloxacin and norfloxacin respectively.

Sultan et al<sup>37</sup> described a simple, fast and accurate colorimetric flow injection (FI) method suitable for the assay of vitamin C in drug

formulations was proposed. In the method, vitamin C was injected into a flowing stream of iron(III) and then mixed with 1,10-phenanthroline in

0.05M sulphuric acid media. The mixture was allowed to react in a 45-cm long coil and the resulting solution of tris, 1-10-phenanthroline-iron(II) complex was monitored at 510 nm. The method was adopted by fully investigating the kinetics of the reaction and proposing a suitable mechanism. A throughput of 100 samples per hour was achieved with a relative standard deviation of 0.88% for vitamin C concentration range of 100–400 ppm.

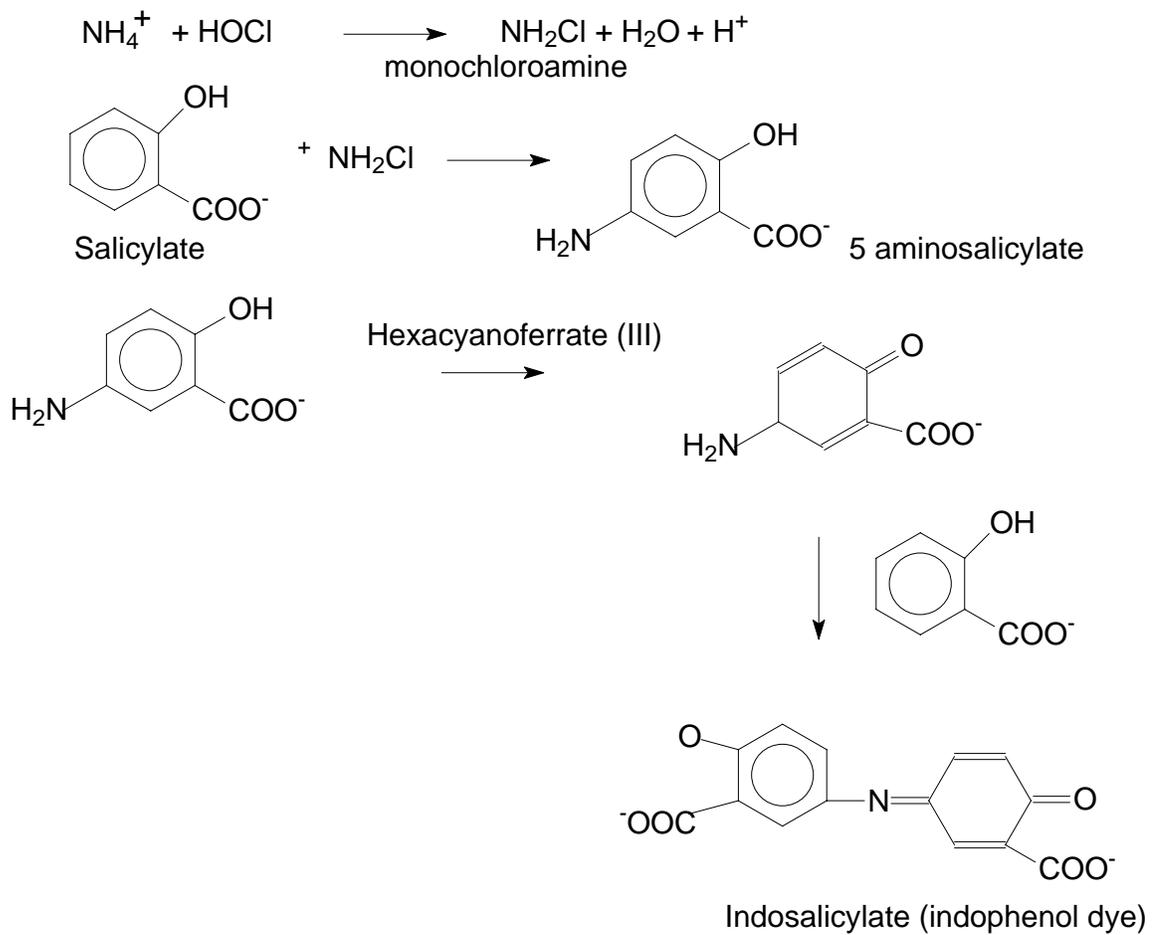
Sultan and Desai<sup>38</sup> described a sequential method employed for full kinetic investigation of the oxidation reaction of vitamin C. Iron(III) in sulphuric acid media was used as an oxidant and 1,10-phenanthroline as an indicator and the resulting solution of tris 1,10-phenanthroline-iron(II) complex was monitored spectrophotometrically at 510 nm. The reaction orders with respect to each reagent were determined by the SI-technique and were found to be 1, 1, and  $-1$  for vitamin C, iron(III) and hydrogen ions respectively. On the basis of these values a rate law was developed and a plausible mechanism was

established. A kinetic method for the analysis of vitamin C in drug formulations based on the results obtained above was thus validated.

The drug in the range 20–300 ppm was determined by the kinetic method using  $1.6 \times 10^{-3}$  mol dm<sup>-3</sup> ammonium ferric sulphate in 0.02 mol dm<sup>-3</sup> sulphuric acid with the aspiration volume of 944 µl and the fixed-time of 180 s. The results thus obtained by the SI-kinetic method were statistically compared with those obtained by the British Pharmacopoeia standard method and found to be accurate, precise and fast.

Sultan et al<sup>39</sup> described an accurate, rapid and very simple spectrophotometric method for the assay of tetracyclines (tetracycline.HCl, chlorotetracycline.HCl, demeclocycline, oxytetracycline.HCl and doxycycline). The method was based on the complexation of iron (III) with tetracyclines in 0.001M sulphuric acid. It has been successfully applied to the assay of tetracyclines in drug formulations, and the interferences of excipients have been examined. The results have been statistically compared with those obtained by two standard methods and found to be very satisfactory.

### 1.5.5 DETERMINATION OF AMMONIA



Scheme 5: Reaction scheme for the proposed SIA method for the determination of ammonia

The chemistry for the proposed method for the determination of ammonia involves the reaction of ammonium ion with hypochlorite to form the monochloroamine which reacts with salicylate ion to give an intermediate

5-aminosalicylate. The intermediate reacts with hexacyanoferrate (III) to give indophenol dye which can be monitored spectrophotometrically at 540 nm. This reaction is usually a very slow reaction and heating is required to speed up the reaction.

Other research work that has been carried out on the determination of ammonia using FIA / SIA methods will be discussed briefly.

Cosano et al<sup>34</sup> described a simple flow injection manifold for the determination of a variety of species in industrial water. The chemical system involved in the determination of ammonia (formation of indophenol blue), sulfate (precipitation with Ba (II) and iron (complexation with 1,10-phenanthroline with the help of a prior redox reaction (for speciation) were selected so that a common manifold could be used for the sequential determination of batches of each analyte. A micro column of a suitable exchange material was used for on-line preconcentration of each analyte prior to injection; linear ranges for the determination of the analytes at the ng/ml levels were obtained with good reproducibility.

## CHAPTER 2

### METHODOLOGY

The ultimate objective of this research is to develop automated SIA methods that can be applied directly in the industry for monitoring water quality control parameters. Therefore, it's highly imperative that the chemistry of the proposed methods be aligned with already existing chemistry for water quality control to promote acceptability of the proposed SIA method.

The proposed SIA method chemistries used for the determination of silica, iron, nitrate / nitrites were adapted from the <sup>1</sup>standards methods of water and waster water examination (American Public Health association.)

The computer flow programs for these SIA methods were written, optimized using the univariate method of approach, empirical approach and chemometrics optimization using the super modified simplex program by Elsevier Scientific Company.

## 2.1 CHEMICALS AND REAGENT PREPARATION

The reagents and solution for the adapted SIA methods were prepared using analar grade chemicals according to the method procedure. Distilled water used was double distilled and demonized. Silica determination was carried out using plastic wares.

## 2.2 INSTRUMENTATION

The manifold used in this method consists of SIA combined with a fiber optic spectrometer. The SIA system is the *FIALab 3500* (Medina, WA USA). It is composed of a syringe pump, a multi-position valve, a Z-flow cell with SMA fiber optic connectors as well as pump tubing and PC. The *Syringe Pump* is 24,000 steps with an optical encoder feedback and 1.5 seconds to 20 minutes per stroke of 2.5 ml size. It is > 99% accuracy at full stroke. The volume capacity of syringe is 2500  $\mu$ l. The *Multi-Position Valve*

has eight ports with a standard pressure of 250 psi (gas)/600 psi (liquid); zero dead volume; chemically inert; port selection is usually done using the software program. The *Z-Flow Cell* is 10 mm path length plexiglass compatible with standard SMA terminated fiber optics was used. *Pump Tubing* of 0.30" ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used for connecting the different units; and making both the holding coil (190 cm long) and the reaction coil (190 cm long).

The fiber optic spectrometer is composed of a light source, 200 micron fiber optic connectors and a detector. The light source is *LS-1 Tungsten Halogen* (Ocean Optics, USA) optimized for VIS-NIR (360nm-2 $\mu$ m) wavelength range. The detector is *USB2000 Spectrometer* (Ocean Optics, USA) adapted to 200-1100nm wavelength range. The SIA manifold is illustrated below.

### **2.2.1 AUTOSAMPLER**

The AIM3200 auto sampler was used for silica, phosphates and ammonia determination by the proposed SIA methods. It guarantees maximum reproducibility as the same manifold is used for samples and reagents. With the aid of a software and sample definition files analysis of about 180 samples in sample tubes, of various volumes, and with any combination of calibration check standards can easily carried out with high accuracy. The probe has access to any standard or sample at any time facilitating the re-analyses, re-standardization or auto dilution. The AIM3200 was connected to FIALab instruments and controlled with the fully-integrated FIALab for windows software.

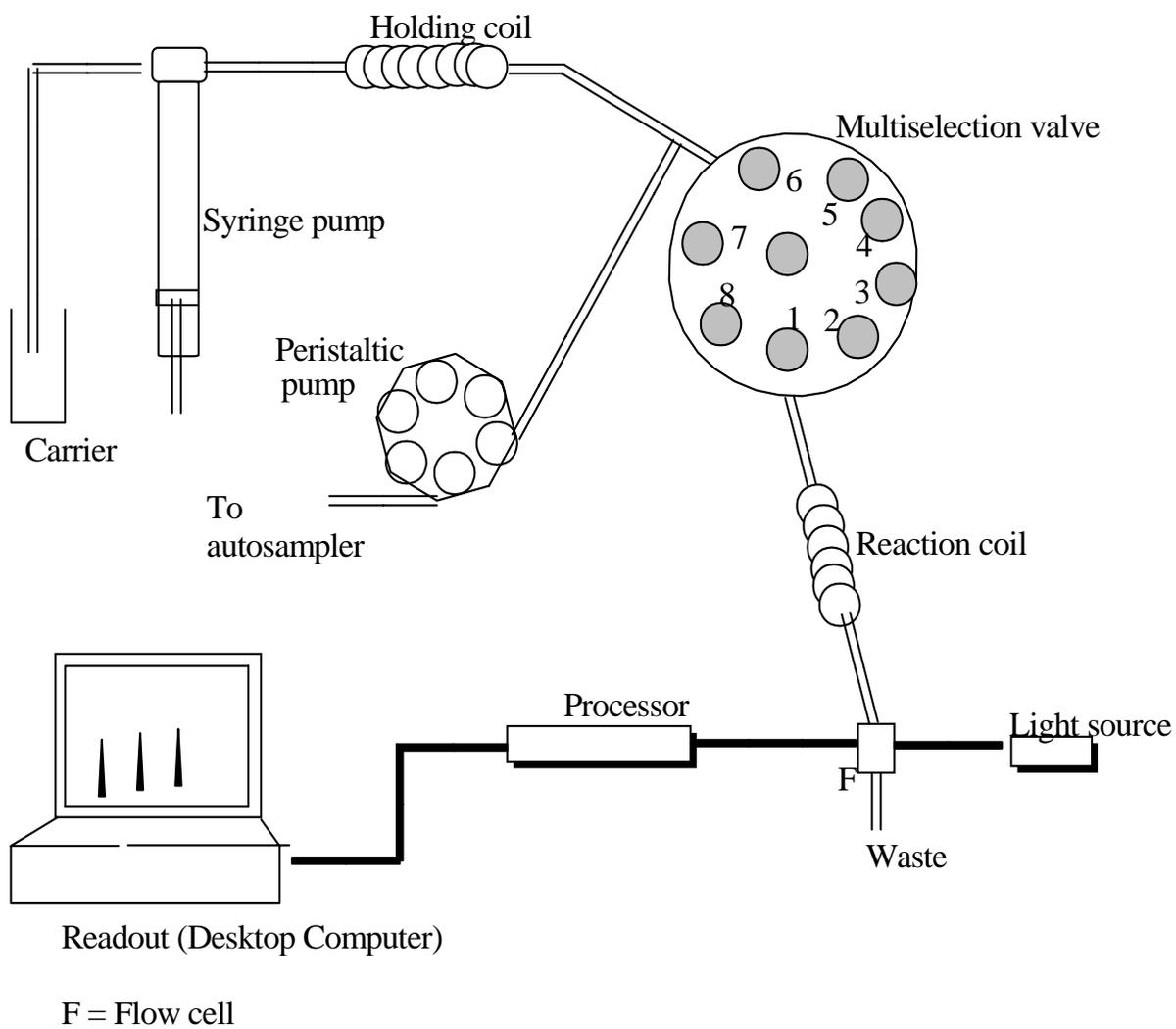


Fig 3. Schematic of SIA manifold used for the proposed SIA methods for phosphates, ammonia and silica determination.

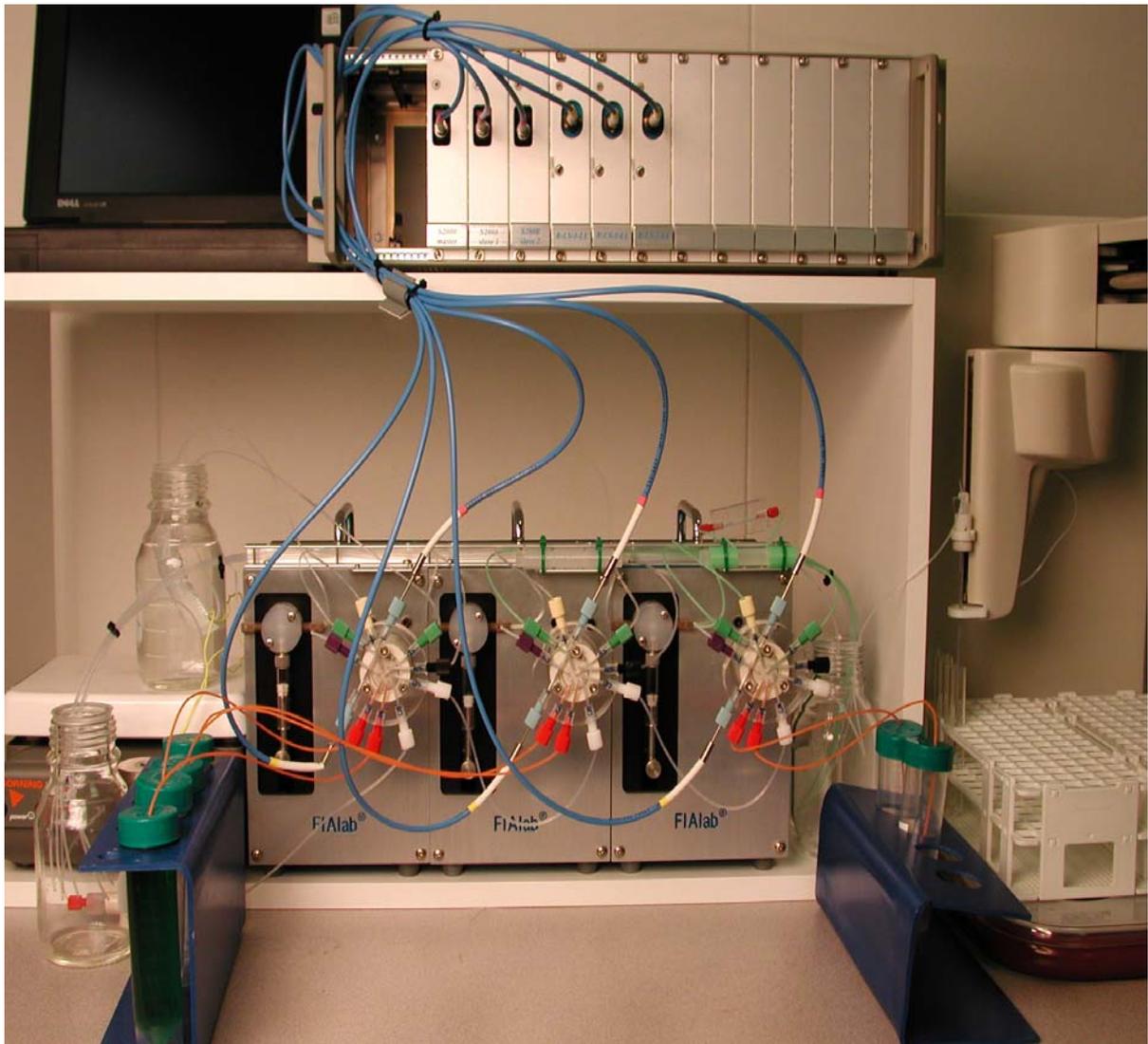


Fig 4: microSIA instrumentation showing the three channeled multiselection valve, fiber optic cables for light source and computer processor. On the right-hand side is an AIM 3200, the instrumentation is capable of analyzing 180 samples.

## 2.2.2 SOFTWARE PACKAGES

*FIALab for Windows* version 5.0 from FIALab® (Medina, WA USA) was utilized for programming, controlling the SIA manifold and data acquisition.

## **2.3 DETERMINATION OF SILICA**

### **2.3.1 PREPARATION OF REAGENTS AND STANDARD SAMPLE SOLUTIONS**

1. Silica stock solution: 0.3530g of  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$  was dissolved in distilled water and diluted to one Liter to give 100ppm of  $\text{SiO}_2$ . Series of standards for the experiments were prepared from the stock solution.
2. Ammonium molybdate solution: 10 g ammonium molybdate tetra hydrate was dissolved in a 100 ml of distilled water. Heated slightly in water a bath until clear solution was obtained
3. Hydrochloric acid (6M), 1+1, 25 ml of concentrated hydrochloric acid was taken and diluted to 50 ml standard flask
4. Ammonium molybdate reagent: 100 ml of ammonium molybdate solution was mixed with 50 ml of 1+1 HCl solution.
5. Triammonium citrate reagent: 10 g of triammonium citrate was dissolved in distilled water and diluted to 100 ml in a standard flask
6. Reducing Agents  
Solution A: 10 g sodium bisulphate ( $\text{NaHSO}_3$ ) was dissolved in 70 ml water.  
Solution B: 0.8 g of  $\text{NaHSO}_3$  and 0.16g 1-amino-2-naphthol-4-sulphonic acid were dissolved in 20 ml distilled water.

Solutions A and solution B were mixed and the total was made up to 100 ml in a volumetric flask, filtered and stored in air tight polyethylene container.

### **2.3.2 METHOD AND PROCEDURE**

The full text of the computer program used for the determination of silica can be found in the appendice I (page 139-143). The following steps were the protocol applied for analysis of Silica in water:

- i. Working solutions of ammonium molybdate/HCl solution, triammonium citrate, sodium sulphite/ 1-amino-2-naphthol-4-sulphonic acid and samples containing silica were linked to the selector valve through ports 3, 4, 6, and 5, respectively and also water as a carrier was linked to the syringe at the in-position valve.
- ii. The auto sampler probe was directed to sample vial, the peristaltic pump switched on, rotated clockwise at 70% for 40 s to propel sample and flush sample tubing.
- iii. The syringe was filled with 2500  $\mu\text{l}$  of the carrier by directing the two-way valve to the (in-position) mode with flow rate of 100  $\mu\text{l s}^{-1}$ .
- iv. Tubes were loaded with their respective reagents by performing aspiration runs and directing the two-way valve to the (out-position) mode with flow rate of 50  $\mu\text{l s}^{-1}$ .

- v. With a  $100 \mu\text{l s}^{-1}$  flow rate, the syringe was emptied and step II was repeated.
- vi. The following volumes were sequentially aspirated and delayed in the holding coil:  $100 \mu\text{L}$  volume of sample,  $40 \mu\text{L}$  ammonium molybdate/ HCl solution delayed for 60 s,  $40 \mu\text{L}$  of triammonium citrate delayed for 30 s,  $40 \mu\text{L}$  sulphite/1-amino-2-naphthol-4-acid solution delayed for 60 s and another  $100 \mu\text{L}$  of sample delayed finally for 120s.
- vii. With a flow rate adjusted at  $10 \mu\text{l s}^{-1}$ , a  $2000 \mu\text{l}$  volume was dispensed to the Z-flow cell passing through the reaction coil and, simultaneously, the reference and absorbance scan were performed by the spectrometer at 813 nm wavelength and the maximum value of the monitored peak was recorded as absorbance. The manifold for this determination is illustrated below.

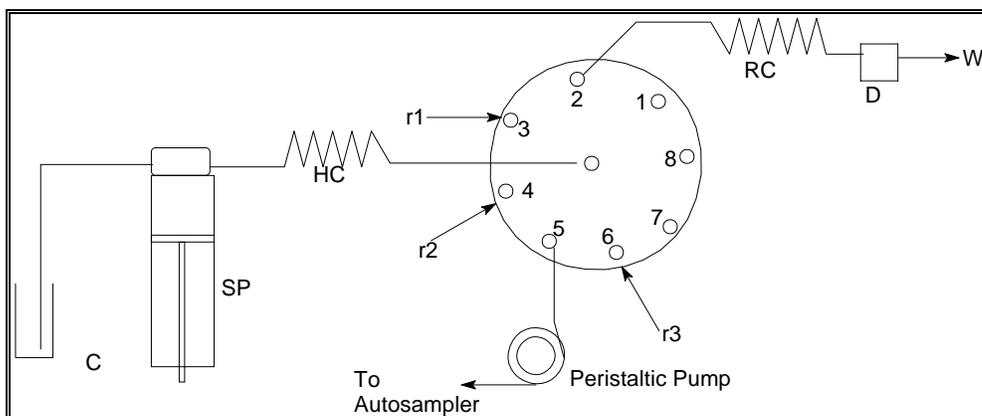


Fig 5. SIA manifold for silica determination

C = carrier (distilled water), SP = syringe pump, HC = Holding coil,

r1 = Ammonium molybdate reagent, r2 = triammonium citrate r3 = sodium sulphite/aminonaphthol reducing agent,

P = peristaltic pump, RC = reaction coil, D = detector, W = waste

## **2.4 DETERMINATION OF PHOSPHATE**

### **2.4.1 PREPARATION OF REAGENTS FOR THE DETERMINATION OF PHOSPHATE**

The chemistry of this procedure was adapted from the standard methods for the examination of water.

Reagent r1: 8 g of ammonium molybdate tetra-hydrate, 0.2 g antimony potassium tartrate half hydrate were dissolved in a one liter volumetric flask.

Reagent r2: 2 ml of acetone and 60 g ascorbic acid were dissolved in a one liter volumetric flask.

Carrier: Reagent r2

### **2.4.2 METHOD AND PROCEDURE**

The full text of the computer program used for the determination of phosphate can be found in **appendice II (page 144-148)**. The following steps were the protocol applied for analysis of phosphates in water samples:

- I. Working solutions of ammonium molybdate/antimony potassium tartrate half hydrate reagent (r1), ascorbic acid/acetone (r2) and samples containing phosphates were linked to the selector valve through ports 3, 4, and 5, respectively and also reagent r2 as a carrier was linked to the syringe at the in-position valve.
- II. The auto sampler probe was directed to sample vial, the peristaltic pump switched on, rotated clockwise at 70% for 40 s to propel sample and flush sample tubing.
- III. The syringe was filled with 2500  $\mu\text{l}$  of the carrier by directing the two-way valve to the (in-position) mode with flow rate of  $100 \mu\text{l s}^{-1}$ .
- IV. Tubes were loaded with their respective reagents by performing aspiration runs and directing the two-way valve to the (out-position) mode with flow rate of  $100 \mu\text{l s}^{-1}$ . First plug of sample from sample tubing was aspirated at a flow rate of  $50 \mu\text{l s}^{-1}$  to eliminate carryover from previous experiment.
- V. With a  $100 \mu\text{l s}^{-1}$  flow rate, the syringe was emptied and steps II, III, IV were repeated.
- VI. The following volumes were sequentially aspirated and delayed in the holding coil: 100  $\mu\text{L}$  volume of sample, 100  $\mu\text{L}$  molybdate/antimony potassium tartrate half hydrate reagent (r1), 120  $\mu\text{L}$  of ascorbic acid/acetone (r2), 10  $\mu\text{L}$  of distilled water was also aspirated to increase flow reversals and finally sample delayed for 100s.
- VII. With a flow rate adjusted to  $10 \mu\text{l s}^{-1}$ , the syringe pump volume was dispensed (emptied) to the Z-flow cell passing through the reaction coil and, simultaneously, the reference and absorbance scan were performed by the spectrometer using a dual wavelengths of 760nm and a reference of 550nm.

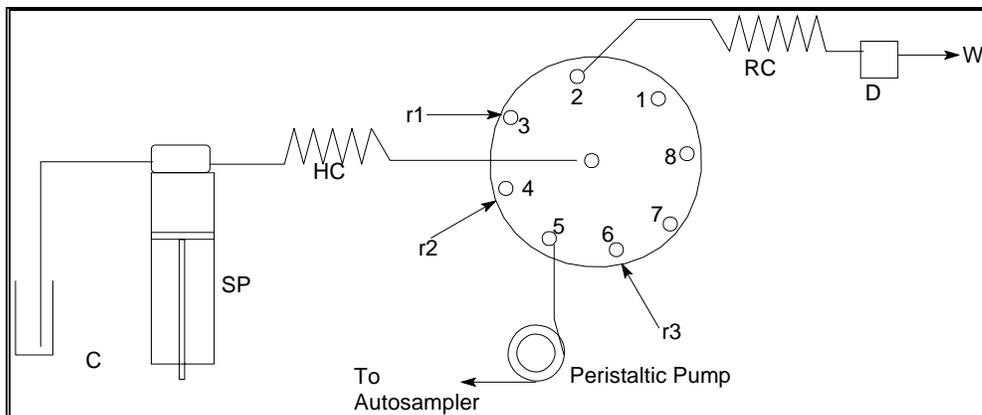


Fig 6: SIA manifold for phosphate determination

C = carrier (reagent r2), SP = syringe pump, HC = Holding coil,  
 r1 = Ammonium molybdate/ antimony potassium tartrate reagent,  
 r2 = ascorbic acid / acetone reagent

## **2.5 DETERMINATION OF TOTAL IRON**

### **2.5.1 PREPARATION OF REAGENTS FOR THE DETERMINATION OF IRON**

#### Reagent r2

10% solution of Hydroxylamine Hydrochloride was prepared by dissolving 10g in 100ml distilled deionised water.

#### Reagent r3

The chromogenic reagent was prepared by dissolving 0.5g of 1,10 Phenanthroline in 100ml of 0.05M HCl in a 100ml standard volumetric flask, 1M sodium acetate is prepared and then mixed with 1,10 phenanthroline in the ratio of 4:1 by volume; this is to ensure that the pH of the iron II/phenanthroline stacked zones in the holding coil was maintained at 3.5 to 4.5 in order to ensure rapid color development.

Carrier was prepared from 0.1M sodium acetate/ 0.1M acetic acid solution.

Stock solution of iron (III) were prepared by dissolving 0.702g of ferrous ammonium Sulphate hexahydrate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ) with 0.05M HCl solution in a 1000ml standard flask.

## 2.5.2 METHOD AND PROCEDURE

The full text of the program used for the determination of total iron can be found in the appendice III (page 148-157). The following steps were the protocol applied for analysis of total iron in water samples:

- i. Working solutions of standards ( $S_1$  to  $S_4$ ), sample ( $S_5$ ), 10% solution of Hydroxylamine Hydrochloride reagent (r2), 1,10 Phenanthroline ,HCl, sodium acetate reagents (r3) were nested around the multiselection valve ports 3 - 6, 7,8 and 1 respectively. Sodium acetate/ acetic acid solution was used as carrier.
- ii. The syringe was filled with the carrier by directing the two-way valve to the (in-position) mode with flow rate of  $100 \mu\text{l s}^{-1}$ .The syringe was directed to the out position and syringe pump volume emptied.
- iii. The Syringe pump was again (in mode) filled and about (out mode)  $500 \mu\text{l}$  dispensed .The following volumes were sequentially aspirated and
- iv. delayed in the holding coil:  $100 \mu\text{L}$  volume of sample/standard,  $20 \mu\text{L}$  Hydroxylamine Hydrochloride reagent (r1) delayed for 10 s,  $100 \mu\text{L}$  of 1,10 phenanthroline (r2) delayed for 60 s,  $100 \mu\text{L}$  of sample/standard delayed for 240 s. With a flow rate adjusted to  $10 \mu\text{l s}^{-1}$ , the syringe pump volume was dispensed (emptied) to the Z-flow cell passing through the reaction coil and, simultaneously, the reference and absorbance scan were performed by the spectrometer at wavelength of 513nm.

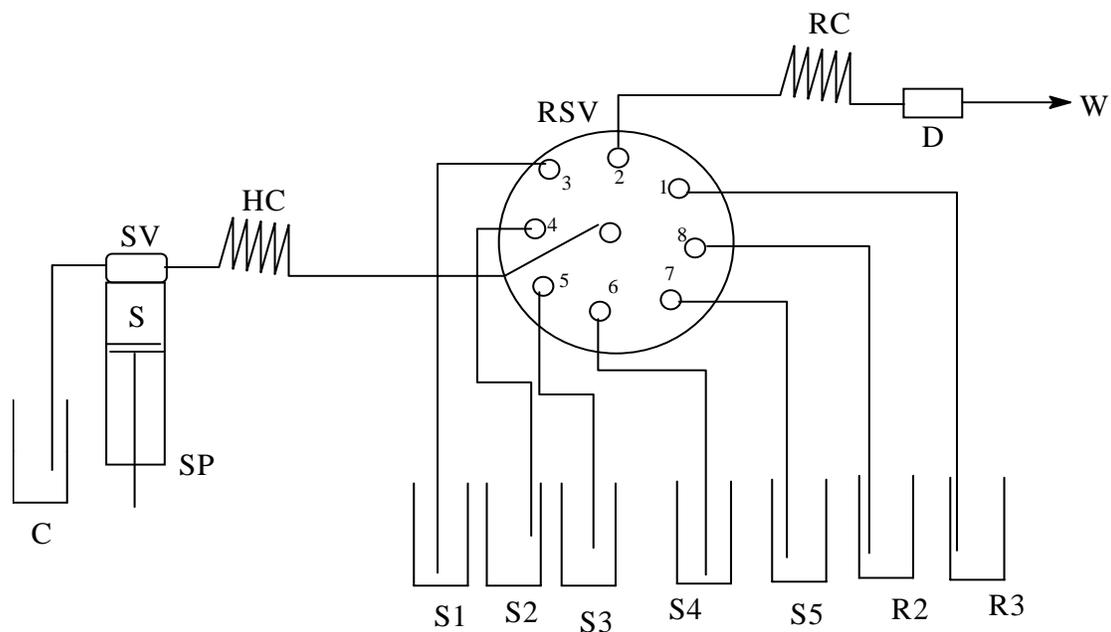


Fig 7: Manifold for the proposed SIA method for the determination of total iron as iron (II)

SV = Syringe Valve, HC = Holding Coil, S = Syringe, SP = Syringe pump, C = Carrier, RSV = Rotatory Selection Valve, RC = Reaction Coil, D = Detector, W = Waste; S<sub>1</sub> to S<sub>4</sub> = standards, S<sub>5</sub> = Sample or standard, R<sub>2</sub> = Hydroxylamine Hydrochloride, R<sub>3</sub> = 1,10-Phenanthroline / sodium acetate reagent.

## **2.6 DETERMINATION OF AMMONIA**

### **2.6.1 PREPARATION OF REAGENTS FOR AMMONIA DETERMINATION**

The procedure was adapted from the FIALab method for the determination of ammonia.

Reagent r1: Sample carrier stream Hypochloride was prepared by pipetting 10 ml of 5.25% sodium hypochloride solution into a one Liter volumetric flask (common household bleach), 4.8 g of sodium hydroxide pellets was weighed, dissolved in the same volumetric flask and made up to mark with distilled water.

Reagent r2: 75g of sodium salicylate, 0.4 g sodium nitroferricyanide (III) dihydrate, 4.8 g of sodium hydroxide pellets, 0.4 ml Brij 35 surfactant were all weighed and dissolved in one liter volumetric flask.

Carrier: distilled deionised water

## 2.6.2 METHOD AND PROCEDURE

The full text of the program used for the determination of ammonia can be found in the appendices IV (page 157-158) The following steps were the protocol applied for analysis of ammonia in water samples:

- I. Working solutions of sodium hypochloride solution, sodium hydroxide (r1), sodium salicylate, sodium nitroferricyanide (III) dihydrate, sodium hydroxide pellets (r2) and samples containing phosphates were linked to the selector valve through ports 3, 4, and 5, respectively
- II. Wash and flushing of tubings were carried out by filling syringe, and aspirating from all ports and dispensing to flow cell with the syringe pump back ( in position), the syringe pump was filled.
- III. The following volumes were sequentially aspirated and delayed in the holding coil: 100  $\mu\text{L}$  volume of hypochloride solution, sodium hydroxide (r1), 120  $\mu\text{L}$  of sample, 100 sodium salicylate, sodium nitroferricyanide (III) (r2), 350  $\mu\text{L}$  of distilled water was also aspirated and finally sample delayed for 100s.
- IV. With a flow rate adjusted, the syringe pump volume was dispensed (emptied) to the Z-flow cell passing through the reaction coil and, simultaneously, the reference and absorbance scan were performed by the spectrometer at a wavelenghts of 770 nm .

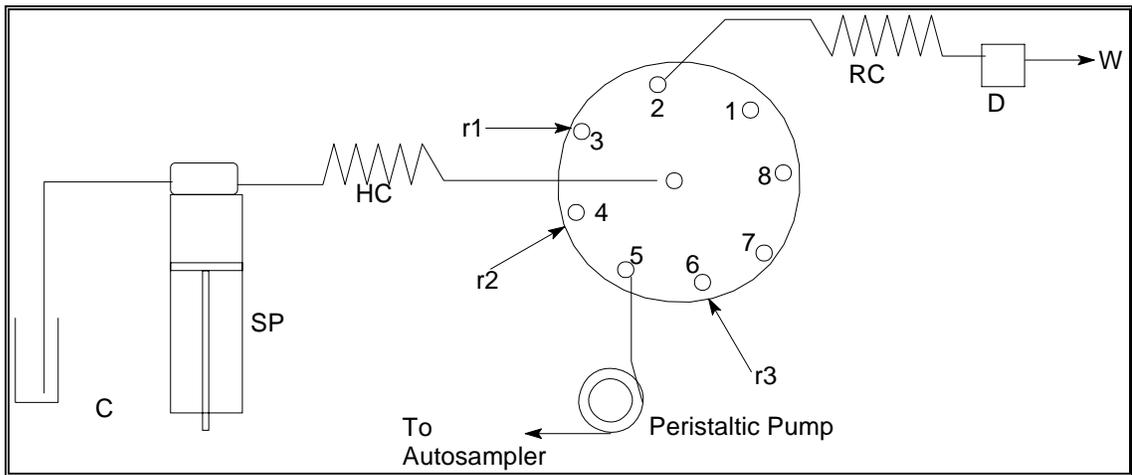


Fig 8: SIA manifold for ammonia determination

C = carrier (distilled water), SP = syringe pump,

HC = Holding coil, r1 = sodium hypochloride reagent, r2 = sodium salicylate  
/nitroferricyanide reagent

## **2.7 DETERMINATION OF NITRATES / NITRITES**

### **2.7.1 PREPARATION OF REAGENTS FOR NITRITES/ NITRATES DETERMINATION**

Reagent R2: weigh 43 grams ammonium chloride, 0.50 grams disodium ethylenediamine tetraacetic acid (EDTA) and approximately 1 gram of sodium hydroxide pellets. The ammonium chloride and EDTA were dissolved in about 400 mL of distilled degassed water in a 500 mL standard volumetric, the pH of the solution was adjusted to 9 using the sodium hydroxide pellets and then made up to mark.

Reagent R3: About 20 grams sulfanilamide, 0.5 grams of N-1- naphthyethylenediamine dihydrochloride were weighed and dissolved in about 400 ml of degassed distilled deionised water in a 500 mL standard flask. About 50 mL of 85% concentrated phosphoric acid was measured and added to the standard flask and made up to 500 mL mark.

## **CHAPTER 3**

### **RESULT AND DISCUSSION**

#### **3.1 A KINETIC SEQUENTIAL INJECTION ANALYSIS METHOD FOR SILICATE DETERMINATION IN WATER SAMPLES CONTAINING PHOSPHATES**

##### **3.1.1 INTRODUCTION**

The determination of silica in water samples is very important in the industry because silica deposits on steam turbine blades at high pressure and temperature. This lowers the efficiency of heat transfer, leading to costly down time for cleaning and may result to total failure of the boiler system. Phosphates is usually added to the boiler feed water to ensure that it's in the alkaline range, this serves as an anticorrosion measure [2]. Phosphates react similarly with reagents used for silica determination to give the same colored product; this poses a serious interference problem for the colorimetric determination of silica in the presence of phosphate. This interference problem is especially challenging when analyzing very low concentration of

silica. For the proposed SIA method, triammonium citrate was used to discriminate silica from phosphates by chelating phosphates from the molybdate complex. Oxalic acid has also been used to eliminate phosphate interference, it was reported that the concentration of oxalic acid greatly determines its selectivity for phosphates or silica. Oxalate concentration of the order of 10% (w/v) prevents phosphate interference, while lower concentration of 0.25% (w/v) avoids the formation of molybdosilicic acid [6]. Mixing of sample and three different reagents in the holding coil of the SIA manifold is usually challenging as four distinct zones are formed in the holding coil. Galhardo and Masini used an auxiliary reaction coil for effective mixing of silicates samples and reagents for the determination of phosphates and silicates by SIA; they suggested that 3 holding coils are necessary for SIA with 3 reagents [15]. For this proposed SIA method two holding coil, delay time, sandwiching of reagents with silica samples and flow reversal were used to promote mixing of silica samples with reagents in the holding coil. The correlation coefficient obtained for silica determination without sandwich of samples and reagents was less than 0.900, this value increased to 0.999 on applying sandwich of sample and reagent, suggesting improved accuracy of the SIA method. Delay time has the advantage of promoting zone penetration while minimizing dispersion and hence increasing the sensitivity of the method.

### **3.1.2 OPTIMIZATION OF PARAMETERS**

The method for the determination of silica in the presence of phosphate by Sequential injection was optimized using the uni-variate method approach. The parameters optimized were delay time, flow rate to detector, volume of sample, volume of reagents and temperature. A standard solution containing 10 ppm silica was used for all optimization procedure.

#### **3.1.2.1 DELAY TIME**

This method makes use of the delay time to ensure zone penetration in the holding coil while at the same time promoting chemistry between samples and reagents. Higher Delay time ensures more intensely colored products formation; this subsequently enhances the sensitivity of the method. The delay time was varied between 1.83 minutes to 6.50 minutes. The optimum delay time was observed at 3.53 minutes. One would expect the absorbance to keep increasing after optimum time is reached. This was found not to be the case due to dispersion taking place in the holding coil which increases with time.

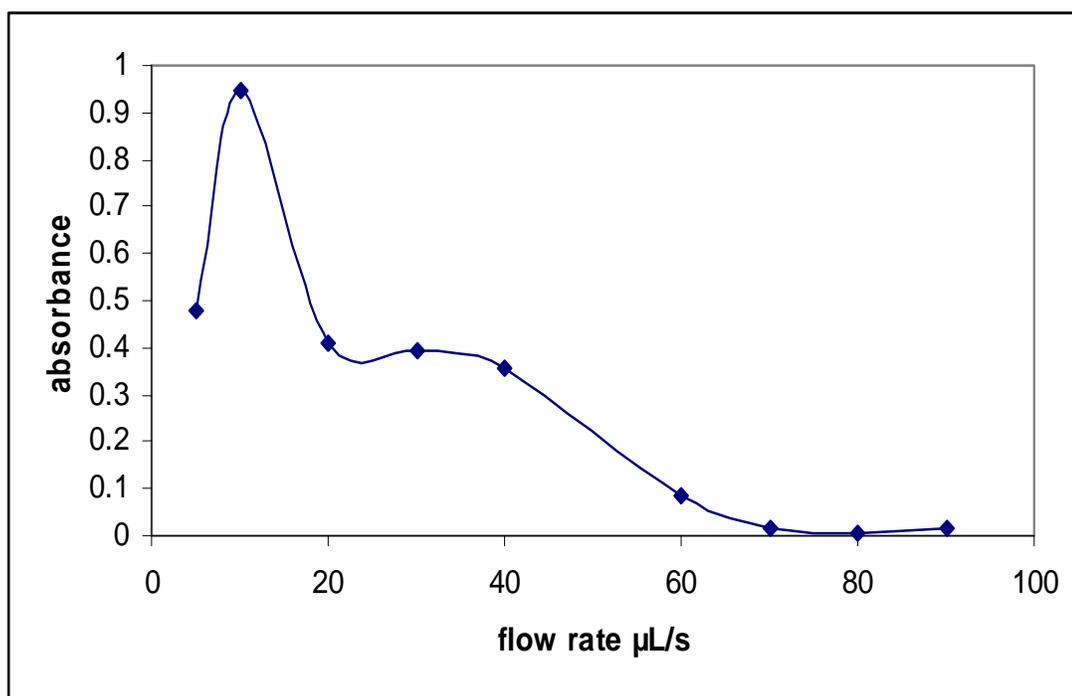
**TABLE II** Effect of delay time on the absorbance of molybdosilicate

<i>Delay time (min)</i>	<i>Absorbance*</i>
1.83	0.528
2.17	0.542
2.50	0.770
2.83	0.823
3.53	0.826
4.17	0.759
4.83	0.783
5.50	0.799
6.50	0.615

\*Average of three determinations

### **3.1.2.2 FLOW RATE**

The flow rate to detector is very important. Low flow rate ensures that the colored products spends more time in the flow cell and absorbance is well measured, It also provides more time for the colored complex to be formed en route to the detector. The optimum flow rate for the proposed SIA method was observed to be 10  $\mu\text{L/s}$ . There was a drop in absorbance at higher flow rate because of high speed of the colored molybdosilicates product through the flow cell and hence very low sensitivity.



\* Average of three determinations.

**Fig 9** Plot of absorbance versus flow rate

### **3.1.2.3 SAMPLE VOLUME / COMBINED REAGENT VOLUME**

The effect of sample volume and combined reagent volume was studied. The sample volume was varied between 40 to 320  $\mu\text{L}$  while the combined reagent volume was varied 30 to 210  $\mu\text{L}$ . Increasing the volume of sample from 40  $\mu\text{L}$  to 200  $\mu\text{L}$  increases the sensitivity of this method. More sample volume means more analyte and hence a higher absorbance reading. Above 200  $\mu\text{L}$ , absorbance decreases due to high sample to reagent volume ratio and increased dispersion. Above 120  $\mu\text{L}$  of combined reagent volume, absorbance started to decrease due to the low sample to reagent volume ratio and increased dispersion. The optimum sample volume and combine reagent volume for the proposed SIA method were 200  $\mu\text{L}$  and 120  $\mu\text{L}$

**TABLE III** Effect of sample volume on the absorbance of molybdsilicates

<i>Sample volume (<math>\mu\text{L}</math>)</i>	<i>Absorbance*</i>
40.00	0.4840
80.00	0.6790
120.0	0.6380
160.0	1.540
200.0	1.600
240.0	1.450
280.0	1.220
320.0	1.020

\*Average of three determinations

**TABLE IV**                    **Effect of combined reagents volume on the absorbance of molybdosilicates**

<i>Reagent volume (<math>\mu\text{L}</math>)</i>	<i>Absorbance*</i>
30.00	0.6440
60.00	0.6740
90.00	1.500
120.0	1.600
150.0	1.340
180.0	1.180
210.0	0.4780

\*Average of three determinations

#### **3.1.2.4 EFFECT OF TEMPERATURE ON ABSORBANCE OF MOLYBDOSILICATES**

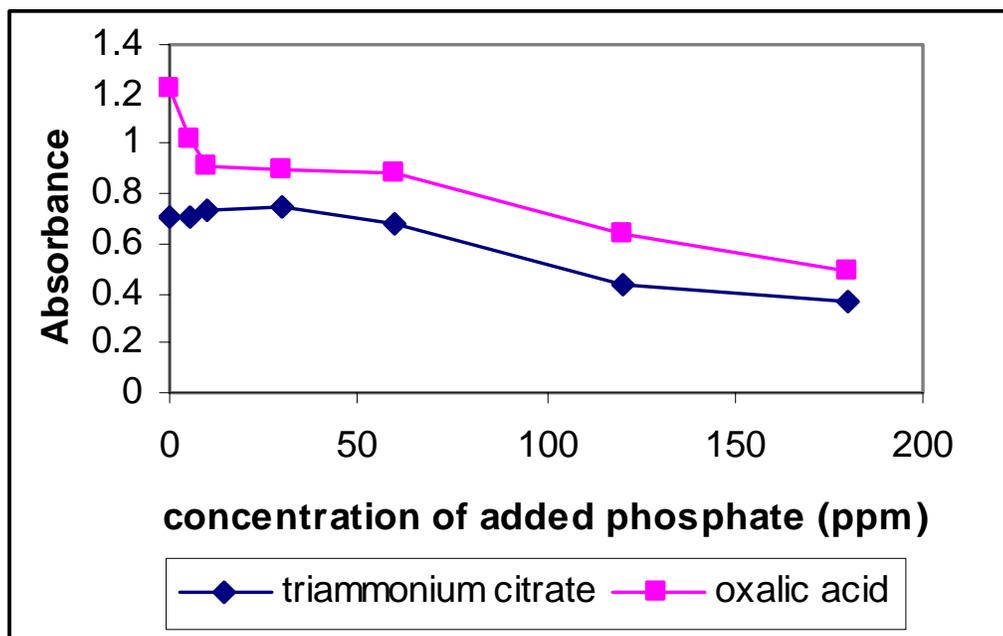
The effect of temperature on the proposed method was studied. This study was carried out by placing the holding coil in a thermostated water bath. It was observed that absorbance values increased with increase in temperature. Higher temperature speeded up the formation of the blue molybdosilicates complex and hence increases the intensity of the blue colored complex formed. The optimum temperature was 60 C. Above the optimum value, there was a decreased absorbance value probably due to dissociation of the blue colored molybdosilicate blue complex.

**TABLE V** Effect of temperature on absorbance of molybdosilicates

<i>Temperature (°C)</i>	<i>Absorbance*</i>
20.0	0.567
40.0	0.570
50.0	0.628
60.0	0.675
70.0	0.630

\*Average of three determinations

### 3.1.2.5 INTERFERENCE STUDY OF PHOSPHATE ON SILICA DETERMINATION AND COMPARISON OF EFFECTIVENESS OF TRIAMMONIUM CITRATE AND OXALIC ACID TO MINIMIZE PHOSPHATE INTERFERENCE



\*average of 3 determinations

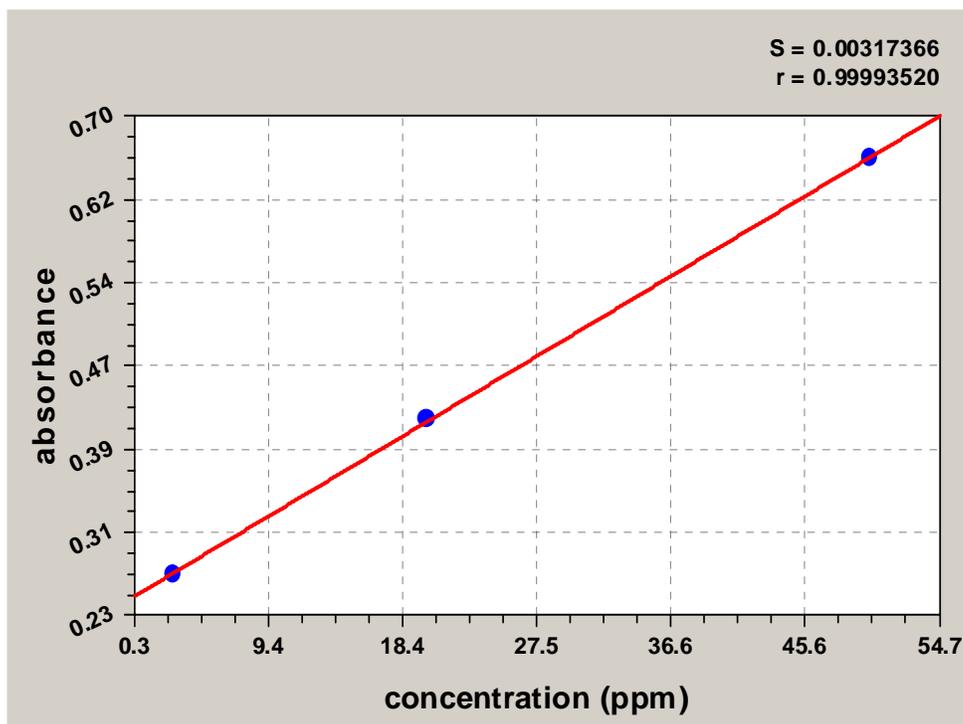
**Fig. 10** Comparative study of effectiveness of oxalic and triammonium citrate to minimize phosphate interference

The interference of added phosphate on this method was studied; a comparison was also made between oxalic acid and triammonium citrate ability to destroy phosphate interference. The concentration of added phosphate was varied between 0, 5, 10, 30, 60, 120, to 180 ppm. It was observed that phosphate interference becomes significant at concentrations higher than 60 ppm of phosphates for both oxalic acid and triammonium citrate phosphate destroying reagents. This indicates that either reagent can be used for destroying phosphate interference at a concentration lower than 60 ppm of phosphates. There was a sharp decrease in absorbance values for the first 2 points for oxalic acid plot indicating that at concentration less than 10 ppm of phosphate, triammonium citrate is a more stable phosphate destroying agent than oxalic acid. The concentration of phosphate usually present in the water samples are in the range of 2 to 21 ppm. The higher absorbance values of oxalic acid plot over triammonium citrate plot suggests higher sensitivity with use of oxalic acid as phosphate destroying reagent because the formation of molybdosilicates blue complex is favored in an acidic medium. However, triammonium citrate has been used for the proposed SIA method because it was more stable to changes in the acidity of the medium compared to oxalic acid.

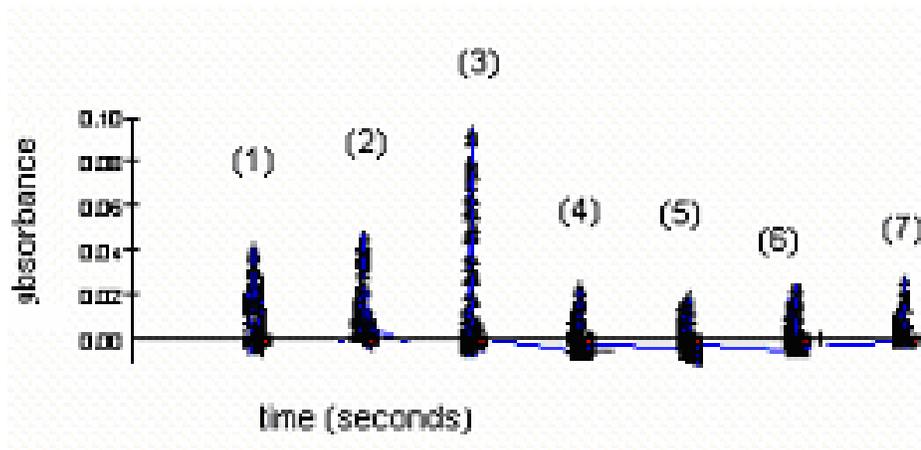
## 3.2 **METHOD EVALUATION**

### 3.2.1 **CALIBRATION CURVE**

The linearity of the proposed SIA method for silica determination was studied under optimum conditions described above. The correlation coefficient was 0.9999. The method is linear between 0.5 to 50 ppm. The detection limit was 0.5 ppm. The equation for absorbance measurement was  $A = 0.220 + 0.009x$



**Fig 11** Calibration Curve obtained for silica determination



**Fig. 12** Absorbance versus run time for silica determination with (1) 2 ppm (2) 5 ppm (3) 10 ppm standards, (4) (5) and (6) (7) were obtained from water samples. Results are in duplicates. The concentration of phosphate present in water samples (4) (5) (6) (7) are usually in the range of 2 to 21 ppm.

### **3.2.2 COMPARISONS OF RESULTS WITH APHA STANDARD METHOD**

The results obtained using the proposed method was compared with standard method of water analysis for silica by American public health association (APHA).The results were found to be in agreement. The calculated t-value was 0.1799 which is less than 2.776 (95% confidence level). The proposed SIA method was found to be more accurate than APHA method at higher concentrations of silica in water.

TABLE VI Comparisons of results with APHA standard method

Added (ppm)	Found (SIA method) (ppm)	Found (APHA) (ppm)
3.00	2.91 ± 0.15	2.55 ± 0.07
20.00	20.14 ± 0.24	17.80 ± 0.14
50.00	49.95 ± 0.09	42.90 ± 0.42

\*Average of three determinations

## CONCLUSION

The proposed SIA method is simple, sensitive, accurate, cost saving, reproducible and avoids the need for dilution of high concentration of silica samples. The sensitivity and linear range of the proposed method is greatly enhanced by delay time and use of 190 cm long tubing for each holding and reaction coil. Sensitivity of this method was further enhanced by placing holding coil in a thermostated water bath.

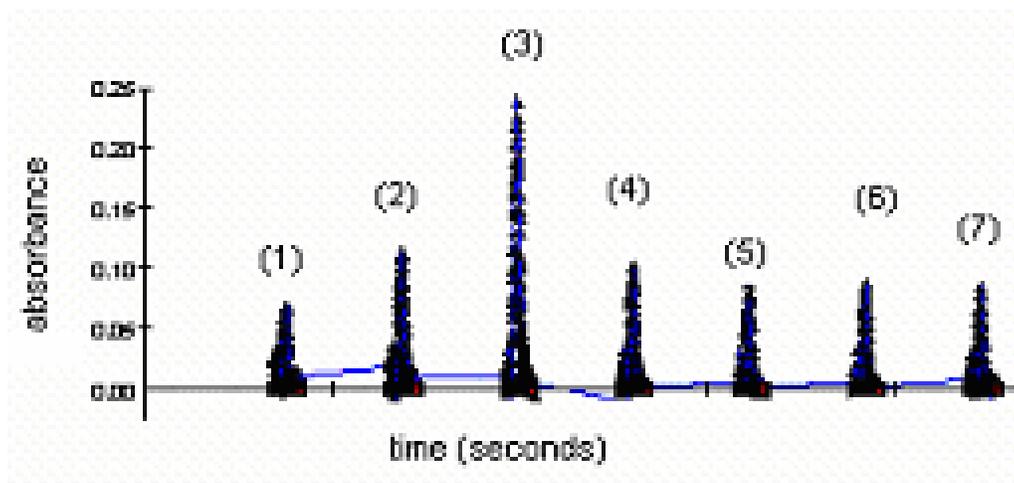
Triammonium citrate phosphate destroying agent was observed to be more stable to changes in pH and more effective at destroying phosphate interference at low concentration of phosphate.

The proposed SIA method was found to be in good agreement with APHA batch spectrophotometric method (t-value 0.1799). This method is far more precise and accurate than the batch APHA standard method in recording absorbance measurements at fixed time as long as the reaction chemistry involved is time dependent, thus rendering the present methods more favorable for routine analysis.

### 3.3 DETERMINATION OF PHOSPHATE

The first step of the reaction protocol involves the formation of molybdophosphate which is reduced by ascorbic acid catalyzed by antimony (Sb) to form a blue complex. This method was adapted from the FIALab flow injection method for the determination of phosphate. A computer flow program was used for carrying out the analysis. 100  $\mu\text{L}$  of sample, 100  $\mu\text{L}$  of reagent r1, 120  $\mu\text{L}$  of reagent r2 and 10  $\mu\text{L}$  of distilled water were aspirated into the holding coil, delayed for 100 s and then propelled by flow reversal to the reaction coil. The colored complex formed in the holding coil was dispensed to the detector with a flow rate of 10  $\mu\text{L}/\text{s}$ . An additional flow reversal (10  $\mu\text{L}$  of distilled water) was incorporated into the flow program to increase the flow reversals and hence maximize zone penetration in the holding coil. A peristaltic pump was used to propel sample from auto sampler into the sample tubing. A reference spectrophotometer scan time of 3 seconds was incorporated into the Program to zero the baseline. Analysis was done in duplicates. The correlation coefficient obtained using water as

Carrier was 0.955, this was greatly improved to 0.999 on using ascorbic acid (r2) as carrier. Suggesting improved sensitivity of the method with use of ascorbic acid as the carrier. Absorbance versus time run for phosphate is shown in Fig, 3. Indicating excellent linearity for 2, 7 and 20 ppm with the following calibration equation:  $A = 0.052 + 0.0085x$ ;  $r = 0.999$  with a sample throughput of 12 samples per hour.



**Fig. 13** Absorbance versus run time for phosphate determination with (1) 2 ppm (2) 7 ppm (3) 20 ppm standards,(4) (5) and (6) (7) were obtained from water samples, results are in duplicates.

**TABLE VII:** Comparison of results obtained with SIA method and batch spectrophotometric method (APHA) for the determination of phosphates in water.

<i>Sample code</i>	<i>Concentration (ppm) Determined by SIA method</i>	<i>Concentration (ppm) Determined by APHA Method</i>
BW1	9.181 ± 0.484	11.604 ± 0.419
BW2	5.739 ± 0.185	5.453 ± 0.146
BW3	3.106 ± 1.007	2.363± 0.136
BW4	6.078 ± 0.434	7.461 ± 0.064
BW12	6.277 ± 0.126	7.190 ± 0.153
BW13	6.198 ± 0.002	4.291 ± 0.029
BW12T	21.730 ± 0.011	21.557 ± 0.104
BW13T	9.860 ± 0.059	10.048 ± 0.362
*Average of two determinations		
BW stand for boiler water		

### **3.4 DETERMINATION OF TOTAL IRON IN WATER**

This method involves the filtration of sample with Whatman filter paper (no 42) to eliminate interference from solids particles present in water samples which can block the tubing and damage the instrument. This was followed by reduction of iron (III) to iron (II) with hydroxylamine hydrochloride. The iron (II) solution is reacted with 1, 10 phenanthroline and the red complex formed determined spectrophotometrically at 510 nm. A computer flow program was used for carrying out the analysis procedure, the following reagents and sample volume were aspirated into the holding coil, 100  $\mu\text{L}$  of sample, 20  $\mu\text{L}$  of hydroxylamine hydrochloride solution delayed for 10 s, 100  $\mu\text{L}$  1,10 phenanthroline solution delayed for 60 s and finally another 100  $\mu\text{L}$  of sample aspirated and delayed for a total time of 240 s. The iron complex formed in holding coil was dispensed to the detector at a flow rate of 10  $\mu\text{L}/\text{s}$ . Although the reaction for the complexation of iron(II) is slow (Fig. 4), nevertheless the precision of determination was very high due to the fact that SIA measurements are always taken at a fixed time.

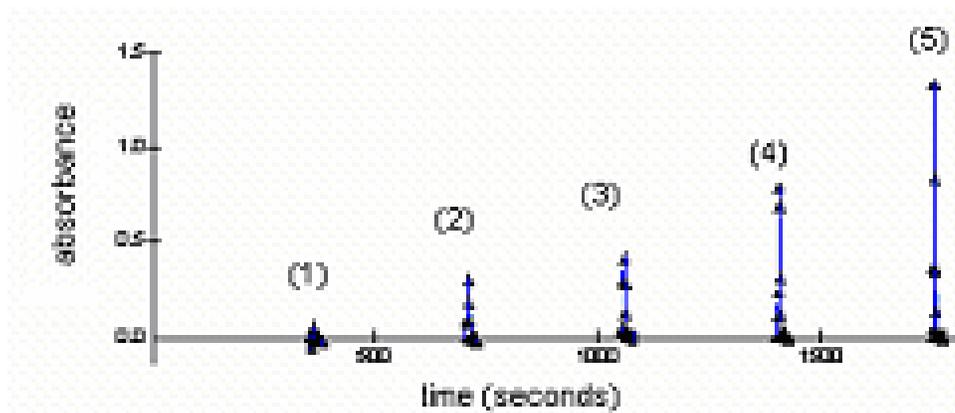


Fig.14 Absorbance versus run time for iron standards with concentration of (1) 0.5 ppm (2) 5 ppm (3)10 ppm (4)30 ppm (5) 50 ppm.

This gives an accuracy that would not be attained with ordinary conventional methods thus rendering the present SIA method much more precise and accurate. The sensitivity of iron determination was greatly enhanced by delay of sample reagents in holding coil and use of 0.1M sodium acetate/ 0.1M acetic acid solution as carrier to ensure that the pH of the iron II/phenanthroline stacked zones in the holding coil was maintained at 3.5 to 4.5 to ensure rapid color development. The sampling frequency is 10 samples/hour; the linear range is 0.5 ppm to 50 ppm, with a detection limit of 0.4ppm. The correlation coefficient of calibration curve obtained for the adapted SIA method for determination of iron was 0.9949. The linear equation obtained for the calibration was  $A = 0.128 + 0.024x$ .

### **3.5 DETERMINATION OF AMMONIA IN WATER**

This method involves reaction of hypochlorite with ammonium ion in water to give the monochloroamine which reacts with salicylate to give a blue colored complex (indophenol) which is monitored spectrophotometrically at 540 nm. Absorbance versus time run for ammonia is shown in Fig. 5 indicating excellent linearity for 0.5, 10, 20 and 30 ppm with the following calibration equation:  $A = 0.0303 + 0.0336x$  with  $r = 0.9998$ .

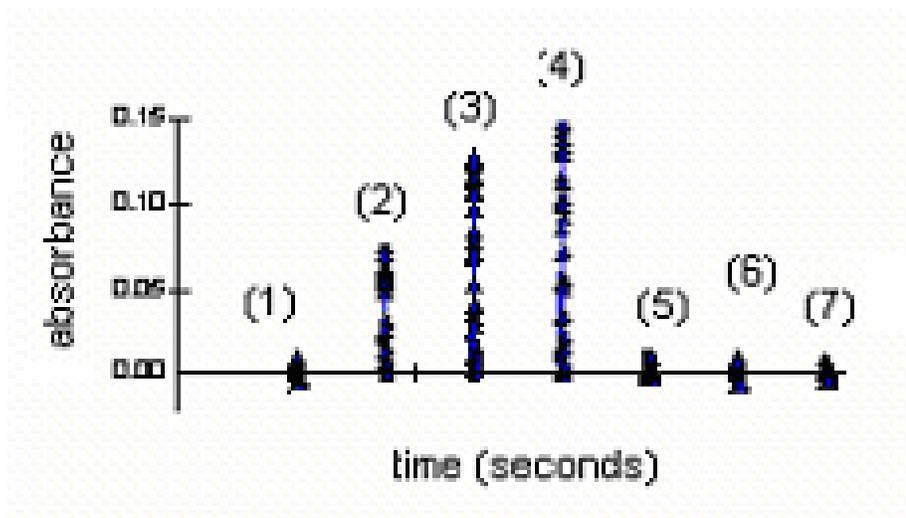


Fig 15 Absorbance versus run time for ammonia determination with (1) 0.5 ppm (2)10 ppm (3) 20 ppm (4)30 ppm standards, (5) (6) (7) were obtained from water samples (triplicates).

### **3.6 DETERMINATION OF NITRATE / NITRITE IN WATER**

The chemistry of the proposed SIA method involves reduction of nitrates to nitrites by passing it through cadmium column and then reaction of the nitrite formed with the chromogenic reagent sulfanilamide / N-1- naphthyethylenediamine dihydrochloride to give you a purple colored dye compound which is determined at 545 nm..

Reduction of nitrate to nitrite poses a serious challenge for SIA determination of nitrate (as  $\text{NO}_3^-$ -N) due to the frequent breakdown in the efficiency of the copperised cadmium column. For the proposed SIA method a 10 cm long column with 2 mm internal diameter was used for reduction of nitrate to nitrite.

The calculated T test value was 0.0365 which is less than the T-Test value of 2.31 (95 % confidence level) suggesting that both methods are in agreement.

TABLE VIII Validation of SIA method with APHA method

Actual (ppm)	Concentration for NO <sub>2</sub> <sup>-</sup> N determination	Standard method (ppm) as NO <sub>2</sub> <sup>-</sup> N	SIA METHOD (ppm) as for NO <sub>2</sub> <sup>-</sup> N
	0.080	0.113 ± 0.003	0.105 ± 0.056
	0.800	0.641 ± 0.003	0.770 ± 0.069
	4.000	3.975 ± 0.003	4.006 ± 0.009

\*Result an average of two determinations

## CHAPTER 4

# 4 A RAPID BATCH AND AUTOMATED SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF NITRITE /NITRATE BASED ON DIAZOTISATION AND COUPLING REACTIONS OF 4-AMINOANTIPYRINE AND 1, 3 DIHYDROXYBENZENE

### ABSTRACT

This manuscript describes a rapid batch and sequential injection analysis (SIA) spectrophotometric method for the determination of nitrite and nitrate in water samples based on diazotization and coupling reactions of 4-aminoantipyrine 1,3 dihydroxy benzene. Nitrate was reduced to nitrite in a copperised cadmium column and subsequently determined as the nitrite. The results obtained using the developed methods were found to be in agreement with that obtained using standard method for the determination of nitrite/ nitrate in water. The proposed batch method was observed to have a wider applicable range of 0.02 ppm to 5 ppm  $\text{NO}_2^-$ -N at 442 nm ( $\lambda_{\text{max}}$ ) when compared with the standard batch method. This proposed method has the advantage of not requiring strict control of reaction conditions such as pH and temperature and the color formation step is rapid. The relative standard deviation of the proposed SIA method was < 2.90% (20 injections of 2 ppm  $\text{NO}_2^-$ -N) with a sample throughput of 14

samples per hour for  $\text{NO}_2^-$ -N SIA method .The detection limit (defined as 10 times standard deviation of ten blank reading) for the  $\text{NO}_2^-$ -N SIA method was 0.2 ppm .While detection limit of batch method for  $\text{NO}_2^-$ -N was 0.03 ppm. The concentrations of four highly interacting parameters namely; HCl, 4-aminoantipyrine, 1, 3 dihydroxy benzene and sodium hydroxide concentrations were optimized using chemometrics optimization by Simplex program (COPS). While flow rate, sample volume and reagent volume on the SIA method were optimized using the univariate method of approach. The proposed batch and SIA methods were applied for the determination of nitrite/nitrate in food and water samples.

**Keywords** 4-aminoantipyrine, 1,3 dihydroxy benzene, diazotisation, coupling, Batch method, SIA

## **4.1 INTRODUCTION**

The meat processing industries usually add sodium or potassium nitrite/nitrate to processed meat to improve on its visual to make it more colorful, attractive and as a preservative. Sodium nitrite has been proven by health researchers to promote the formation of nitrosamine in the body which is known to be carcinogenic and so detrimental to public health. The food and water regulatory bodies rely on standard wet analytical procedure to accurately evaluate the concentrations of nitrites in processed meat and water to see if the critical concentrations are exceeded. Unfortunately, the standard method has the limitation of a lower applicable range and cannot be used for

analysis of high concentrations of nitrite/nitrate except the samples are diluted. Dilution of samples can introduce dilution errors into the analytical procedure.

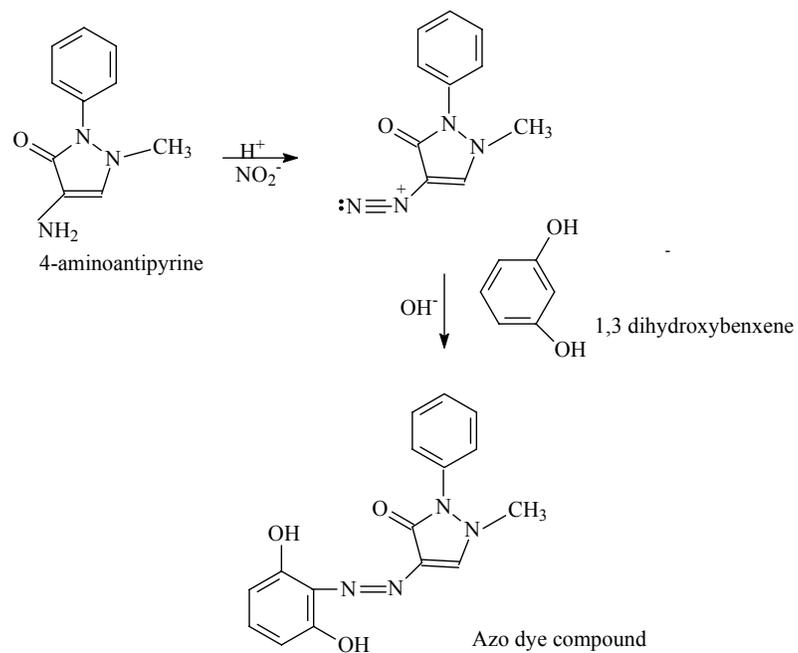
Nitrates levels in water bodies is usually used as indicators of fecal or gross organic pollution and its determination in treated water is particularly important to Public Water treatment plants because high concentrations of nitrates in water have been known to cause methaemoglobinemia (blue baby disease) in the stomach of infants due to the binding action of nitrite with haemoglobin and subsequent diminishing of the transfer of oxygen to the body cells [52].

Astakhina et al determined aminoantipyrine in analgin product mixture by titration with sodium nitrite after the stages of boiling and HCl reduction. They reported that the titration result were not affected by the presence of activated carbon and were close to the theoretical amount than the results obtained using either the benzaldehyde or standard analytical method. The experimental conditions were optimized using the controlled and weighted centroid simplex method [53]. Fan et al developed a flow injection spectrophotometric method based on the coupling reaction of phenols with diazotisation products between sulphanilic acid and sodium nitrite in HCl medium.[54] Fiamegos et al described a spectrophotometric batch method for the determination of phenolic compounds using aminopyrazolone derivatives as chromogenic agent. The method was reported to be considerable more improved than the 4-aminoantipyrine method for the determination of phenol [55]. Alwehaid proposed a flow injection spectrophotometric determination of 4-aminophenazone based on diazotisation of p-nitroaniline with nitrite and coupling with 4-aminoantipyrine[56]. Burakham et al proposed a novel spectrophotometric

reaction for the determination of nitrite as well as nitrate in water samples. The detection was based on the nitrosation reaction between nitrite ion and phloroglucinol (1,3,5-trihydroxybenzene)[57]. Toropov and Mamaeva proposed a photometric determination of nitrate and nitrite ions using antipyrine in sulphuric acid solutions[58].

Zanardi et al carried out a comparative test by 3 different methods (different laboratories) used for reduction of nitrate to nitrite using cadmium column and reductase enzyme and found discrepancies in the results. They suggested that the reproducibility of the results were a function of the efficiency of the cadmium column [59].

For the first time in this study, 4-amino antipyrine/ nitrite in acidic media was diazotized and coupled with 1,3 dihydroxy benzene in alkaline media resulting to the formation of intensely colored compound (azo dye chemistry) having a stability of up to a 24 hours. The intensely colored azo dye compound formed is as a result of increased conjugation on formation of the azo dye compound with subsequent decrease in energy level difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) resulting in a shift of wavelength to higher region (visible region). The reaction scheme is illustrated below.



Reaction scheme for the proposed batch/SIA method for the determination of nitrite/nitrate

It was observed that absorbance increases linearly with nitrite concentration while keeping the other variables (concentration of 4-aminoantipyrine, 1, 3 dihydroxy benzene) constant; suggesting that 4-aminoantipyrine /1,3 dihydroxybenzene can be used for the quantitative determination of nitrite in water or food samples.

There has been a rise in automation of wet analytical chemical processes with the aid of flow injection and sequential injection analysis. However automation suffers the draw back of general acceptability due to high technicality in operating, maintaining instrument and also wide spread unavailability, hence the description of a batch method was also incorporated into this study. The proposed batch method was observed to have a wider applicable range of 0.02 ppm to 5 ppm of nitrite at 442 nm when compared to the standard cadmium reduction method suggesting that dilution of high concentration of nitrite can be avoided using this method. The proposed batch method also enjoy the advantage of not requiring strict control of reaction conditions (pH and Temperature) because the conditions of the reactions were optimized using chemometrics by super modified Simplex optimization program and the univariate method of approach. This proposed method also has the advantage of involving rapid color development chemistry and hence saves time when compared to the standard batch method.

## **4.2 EXPERIMENTAL**

### **CHEMICALS AND REAGENT PREPARATION**

The reagents and solution for the proposed batch and SIA methods were prepared using analar grade chemicals. Distilled water used was double distilled and deionized.

#### **REAGENTS**

1M HCl solution was prepared by diluting concentrated 41 ml HCl (vol % 37.7, density at 20°C 1.191g/ml) to 500ml mark in a 500ml volumetric standard and standardized by titrating with standard Na<sub>2</sub>CO<sub>3</sub> solution.

Chromogenic reagent (SIA) of 4-aminoantipyrine/1,3 dihydroxybenzene was prepared by weighing 1.25g of 4-aminoantipyrine and 0.0825g of 1,3 dihydroxybenzene and dissolving with 1M HCl solution and made up to the 250ml mark of a standard flask with 1M HCl solution. The chromogenic reagent for batch method was prepared by dissolving 2.348g of 4-aminoantipyrine and 0.385g of 1,3 dihydroxybenzene in 250 ml of 0.28M HCl solution. The chromogenic reagent was stored in an air tight container. The stored solution is stable for 3 days.

NaOH/NH<sub>4</sub>Cl/EDTA solution for SIA method was prepared by weighing 10g of NaOH pellets, 0.175 g of disodium EDTA dehydrate, 0.75g ammonium chloride and dissolving in 250 ml standard flask. While for Batch method 1.5g of ammonium

chloride and 0.35g disodium EDTA were weighed and dissolved in the 500ml of 0.28M NaOH solution in a standard flask.

The cadmium reduction column was prepared and used for Nitrate reduction according to APHA standard method procedure[13]. For reduction of nitrates to nitrites, 25 ml of sample was made up to 100 ml mark with ammonium chloride/disodium /NaOH solution for batch /SIA method and then reduced according to APHA/ SIA procedure. A drop of Brij 35 surfactant was added to distill deionised water to be used as carrier (SIA) to reduce surface tension in the SIA tubings and eliminate air bubbles during analysis. Standard solution of nitrite and nitrate were prepared from sodium nitrite and potassium nitrate respectively. The respective working solutions were prepared from these.

## 4.3 INSTRUMENTATION

### SIA INSTRUMENT

The manifold used in this method consists of SIA combined with a fiber optic spectrometer. The SIA system is the *FIALab 3500* (Medina, WA USA). It is composed of a syringe pump, a multi-position valve, a Z-flow cell with SMA fiber optic connectors as well as pump tubing and PC. The *Syringe Pump* is 24,000 steps with an optical encoder feedback and 1.5 seconds to 20 minutes per stroke of 2.5 ml size. It is > 99% accuracy at full stroke. The volume capacity of syringe is 5000  $\mu$ l. The *Multi-Position Valve* has eight ports with a standard pressure of 250 psi (gas)/600 psi (liquid); zero dead volume; chemically inert; port selection is usually done using the software program. The *Z-Flow Cell* is 10 mm path length plexiglass compatible with standard SMA terminated fiber optics was used. *Pump Tubing* of 0.30" ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used for connecting the different units; and making both the holding coil (190 cm long) and the reaction coil (190 cm long).

The fiber optic spectrometer is composed of a light source, 200 micron fiber optic connectors and a detector. The light source is *LS-1 Tungsten Halogen* (Ocean Optics, USA) optimized for VIS-NIR (360nm-2 $\mu$ m) wavelength range. The detector is *USB2000 Spectrometer* (Ocean Optics, USA) adapted to 200-1100nm wavelength range. A 10 cm long copperised cadmium reduction column with 2 mm internal

diameter was used for reduction of nitrates to nitrite for SIA method. The SIA manifold is described below.

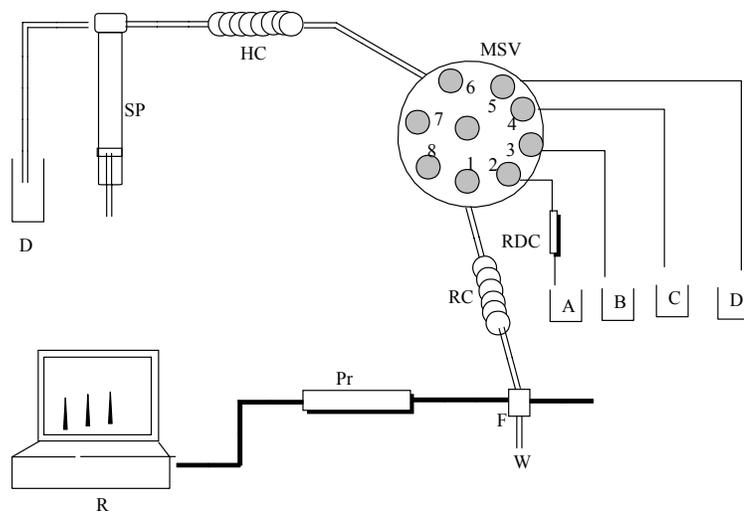


Fig 16 SIA manifold for nitrite/nitrate determination

Where SP = syringe pump, D = carrier, HC = Holding coil, MSV = multiselection valve, RDC = reduction column, LS = light source, Pr = processor, R = computer readout, A = sample for  $\text{NO}_3^-$ -N, B = sample for  $\text{NO}_2^-$ -N, C = chromogenic reagent, D = NaOH solution.

## **SOFTWARE PACKAGES**

*FIALab for Windows* version 5.0 from FIALab® (Medina, WA USA) was utilized for programming, controlling the SIA manifold and data acquisition.

The chemometric optimization by simplex Program (COPS) was obtained from Elsevier Scientific Company, the Netherlands, and utilized for the optimization of variables using a computer compatible IBM desktop.

## **UV-VISIBLE SPECTROPHOTOMETER**

A perkin Elmer UV/Visible Lambda EZ210 spectrophotometer was used for the proposed batch method. The spectrophotometer is equipped with a high resolution concave diffraction grating and Seya-Mamioka mount; Stray light transmittance of 0.05% and wavelength accuracy of  $\pm 0.3$  nm. The wavelength accuracy is  $\pm 0.3$  nm; wavelength reproducibility setting is  $\pm 0.1$  nm; photometric accuracy (measured with NIST 930D filter) is  $\pm 0.002$  Abs (0 to 0.5 Abs) and  $\pm 0.004$  (0.5 to 1.0 Abs). The baseline flatness is  $\pm 0.002$  Abs (200 to 950 nm); Baseline stability (2 hours after power up) is within  $\pm 0.0003$  Abs/hr at 500 nm; Response speed ranges from medium, fast to slow. The light source is a deuterium (D2) lamp, tungsten iodide (WI) lamp and the detector is silicon photodiode and readout is a desktop personal computer. The instrument consists of a 2 nm spectral band pass slit and a wavelength range from

190nm -1100nm. A 10 mm rectangular shaped cell was used for taking spectrophotometric reading.

#### **4.4 METHOD AND PROCEDURE**

##### **METHOD AND PROCEDURE FOR THE SIA METHOD**

The following steps are the protocol applied for analysis of Nitrates/nitrite

- I. Working solutions of aminoantipyrine/1,3 dihydroxybenzene, NaOH solution and samples containing nitrite/nitrate were linked to the selector valve through ports 4, 5, 3 and 2 respectively and also water as a carrier was linked to the syringe at the in-position valve.
- II. The syringe was filled with 5000  $\mu\text{l}$  of the carrier by directing the two-way valve to the (in-position) mode with flow rate of  $100 \mu\text{l s}^{-1}$ .
- III. With a  $100 \mu\text{l s}^{-1}$  flow rate, the syringe pump dispensed about 1400  $\mu\text{l}$  of the carrier to clear out flow cell and flush tubing.
- IV. 350  $\mu\text{l}$  of sample was aspirated through port 3 into the holding coil and dispense to the flow cell to flush sample tubing for  $\text{NO}_2^-$ -N determination.

- V. 150  $\mu\text{l}$  of sample was aspirated into holding coil through port 3 for  $\text{NO}_2^-$ -N determination. This was followed by aspiration of 40  $\mu\text{l}$  of chromogenic reagent delayed in holding coil for 10 s, 100  $\mu\text{l}$  of NaOH delayed for 20 s.
- VI. With a flow rate adjusted at  $10 \mu\text{l s}^{-1}$ , a 2000  $\mu\text{l}$  volume was dispensed to the Z-flow cell passing through the reaction coil and, simultaneously, the reference and absorbance scan were performed by the spectrometer at 442 nm wavelengths and the maximum value of the monitored peak was recorded as absorbance.
- VII. For  $\text{NO}_3^-$ -N determination steps i, ii, iii were repeated followed by a flush step for clearing out the reduction column by aspiration of a 300  $\mu\text{l}$  of sample through port 2 at a flow rate of 5  $\mu\text{l/s}$ . The flush step was repeated before aspiration of 150  $\mu\text{l}$  of sample followed by aspiration of 40  $\mu\text{l}$  of chromogenic reagent delayed for 10 s, aspiration of 100  $\mu\text{l}$  of NaOH solution delayed for 20 s. This was followed by step vi.

## **METHOD AND PROCEDURE FOR THE BATCH METHOD**

25 ml of sample was measured into a 50 ml flask; 5 ml of Chromogenic batch reagent was added followed by 5 mL of NaOH/NH<sub>4</sub>Cl solution. Color change was instantaneous. Absorbance was measured after 5 minutes using a 1cm path length cell in a UV-Visible spectrometer at a wavelength of 442 nm.

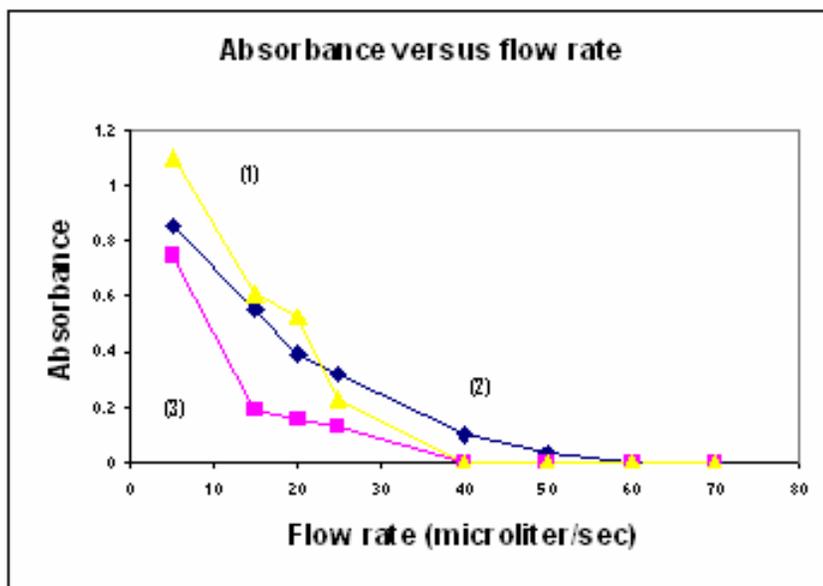
## **4.5 RESULT AND DISCUSSION**

### **4.5.1 OPTIMIZATION OF EXPERIMENTAL VARIABLES FOR SIA METHOD**

Optimization process for SIA method was carried out using the univariate method of approach. For the experiments three different concentrations of  $\text{NO}_2^-$ -N were used for the optimization processes, the concentration are 0.5 ppm, 2.0 ppm and 10 ppm of  $\text{NO}_2^-$ -N.

#### **4.5.2 EFFECT OF FLOW RATE ON SIA METHOD.**

The flow rate to the spectrophotometric detector was varied between 5, 10, 15, 20, 25, 40, 60, and 80  $\mu\text{l s}^{-1}$  respectively. The other parameters were fixed at value of 100  $\mu\text{l}$  sample volume, 50  $\mu\text{l}$  each for chromogenic reagent and sodium hydroxide reagent volume. The optimum flow rate was 5  $\mu\text{l s}^{-1}$ .



\*\*Absorbance reading an average of 3 determinations.

Fig. 17 Absorbance versus flow rate (microliter/sec) at concentrations: (1), 0.5 ppm; (2), 2 ppm; (3), 10 ppm nitrite: Analytical conditions; Sample volume, 100  $\mu$ l; chromogenic reagent, 50  $\mu$ l; sodium hydroxide reagent volume, 50  $\mu$ l

Low flow rate through the detector ensures enough time for absorbance to be taken and hence higher absorbance reading when compared with absorbance taken at higher flow rate. For the proposed SIA method  $10 \mu\text{l s}^{-1}$  was adopted because of the low sample throughput at flow rate of  $5 \mu\text{l s}^{-1}$ .

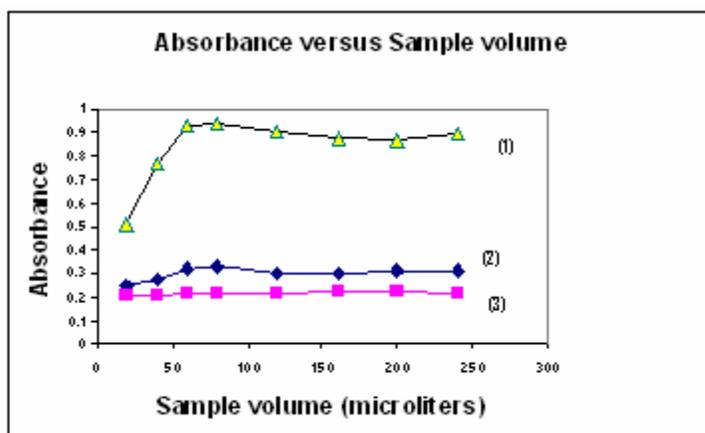
#### **4.5.3 EFFECT OF SAMPLE VOLUME, CHROMOGENIC REAGENT VOLUME AND SODIUM HYDROXIDE VOLUME ON SIA METHOD**

The sample volume for sample volume optimization was varied between 20,40,60,80,100,120,160,200 and 240  $\mu\text{l}$ . The other parameters were fixed at  $10 \mu\text{l/s}$  for flow rate, 50  $\mu\text{l}$  each for chromogenic reagent and NaOH volume respectively. Absorbance values increased from 20 to 60  $\mu\text{l}$  (fig 18) as a result of an increase in sample volume / chromogenic reagent ratio. There was no net increase in absorbance from 60  $\mu\text{l}$  due to increased axial dispersion with increase in sample volume. This suggests that any sample volume more than 60  $\mu\text{l}$  was suitable and for the proposed SIA method 150  $\mu\text{l}$  of sample was adopted for both  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N determination.

The chromogenic reagent volume was varied between 20,40,60,80,100,120,140,160 and 200  $\mu\text{l}$  while keeping other parameters at constant value of 10  $\mu\text{l/s}$  for flow rate, 150  $\mu\text{l}$  for sample volume and 50  $\mu\text{l}$  for NaOH. There was a steady decrease in absorbance values (Fig 19) due to increasing acidity of the reaction medium in the holding coil. Coupling reactions are usually favored in an alkaline medium. The optimum chromogenic reagent volume adopted for both  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N determination was 40  $\mu\text{l}$ .

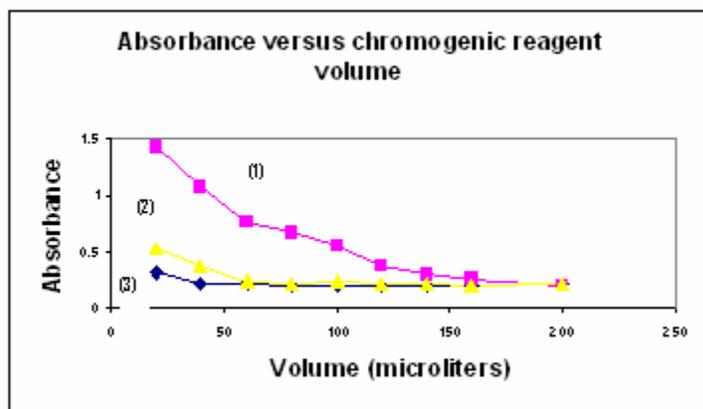
The sodium hydroxide volume was varied between 20,40,60,80,100,120,140,160 and 200  $\mu\text{l}$  while keeping other variables at constant value of 150  $\mu\text{l}$  for sample, 10  $\mu\text{l/s}$  flow rate, 40  $\mu\text{l}$  chromogenic reagent (fig 20). The absorbance value increased steadily due to the more favorable alkaline conditions for coupling reactions. A steady state volume was reached at 160  $\mu\text{l}$ . It was observed that reproducibility of analysis result at 160  $\mu\text{l}$  was less compared to that at 100  $\mu\text{l}$ . Therefore 100  $\mu\text{l}$  was adopted for the proposed SIA method for both  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N determination.

In conclusion, the optimum conditions adapted for the proposed SIA method were flow rate 10  $\mu\text{l/s}$ , sample volume 150  $\mu\text{l}$ , chromogenic reagent volume 40  $\mu\text{l}$ , NaOH volume 100  $\mu\text{l}$  respectively.



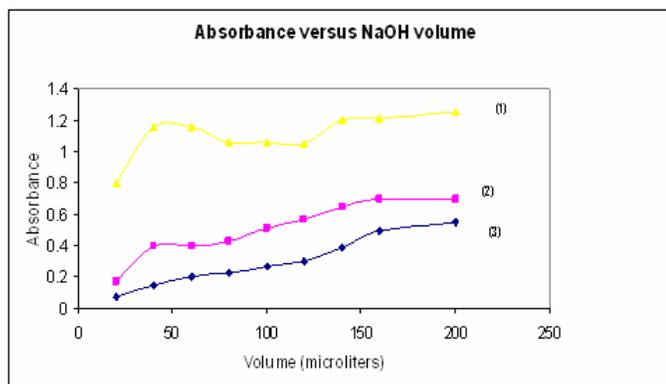
\*\*Absorbance reading an average of 3 determinations

Fig. 18 Absorbance versus sample volume ( $\mu\text{l}$ ) plot at concentrations: (1), 0.5 ppm; (2), 2 ppm; (3), 10 ppm nitrite: Analytical conditions; flow rate, 10  $\mu\text{l/s}$ ; chromogenic reagent, 50  $\mu\text{l}$ ; sodium hydroxide reagent volume, 50  $\mu\text{l}$ .



\*\*Absorbance reading an average of 3 determinations.

Fig. 19 Absorbance versus chromogenic reagent volume ( $\mu\text{l}$ ) plot at concentrations: (1), 0.5 ppm; (2), 2 ppm; (3), 10 ppm nitrite: Analytical conditions; flow rate, 10  $\mu\text{l/s}$ ; sample volume, 150  $\mu\text{l}$ ; NaOH volume, 50  $\mu\text{l}$



\*\*Absorbance reading an average of 3 determinations.

Fig. 20 Absorbance versus NaOH reagent volume ( $\mu\text{l}$ ) plot at concentrations: (1), 0.5 ppm; (2), 2 ppm; (3), 10 ppm nitrite: Analytical conditions; flow rate, 10  $\mu\text{l/s}$ ; sample volume, 150  $\mu\text{l}$ ; chromogenic reagent volume, 40  $\mu\text{l}$

#### 4.5.4 OPTIMIZATION OF BATCH METHOD USING THE SUPER MODIFIED SIMPLEX PROGRAM

TABLE IX Concentration values and corresponding absorbance

Simplex Vertex no	[HCl] M	[NaOH] M	[AM] $10^{-3}$ M	[DH] $10^{-3}$ M	Absorbance*
1	0.0002	0.0400	6.60	2.00	0.0208
2	0.0094	0.0618	7.43	3.00	0.151
3	0.0022	0.133	7.43	3.00	0.0110
4	0.0022	0.0618	10.0	3.00	0.0119
5	0.0022	0.0618	7.43	6.60	0.0128
6	0.0042	0.0100	8.18	4.00	0.0200
7	0.020	0.010	6.60	2.00	0.068
8	0.040	0.0400	6.60	2.00	0.242
9	0.133	0.0618	7.43	3.20	0.052
10	0.0618	0.133	7.43	3.20	0.129
11	0.0618	0.0618	10.0	3.20	0.108
12	0.0618	0.0618	7.43	6.80	0.115
13	0.0002	0.0830	8.24	4.20	0.048
14	0.0792	0.0704	7.76	3.60	0.048
15	0.113	0.0704	7.55	3.40	0.069
16	0.0732	0.0714	7.79	3.60	0.065
17	0.0002	0.0830	8.24	4.20	0.050
18	0.0396	0.0768	8.00	4.20	0.104
19	0.0222	0.0952	5.00	4.80	0.079
20	0.0472	0.0742	8.23	3.80	0.102
21	0.0348	0.0846	6.62	4.40	0.102
22	0.0428	0.0778	7.67	4.00	0.105

\*Absorbance reading an average of 3 determinations

where [AM] is 4 aminoantipyrine and [DH] is 1,3 dihydroxylbenzene concentration in mol/L respectively

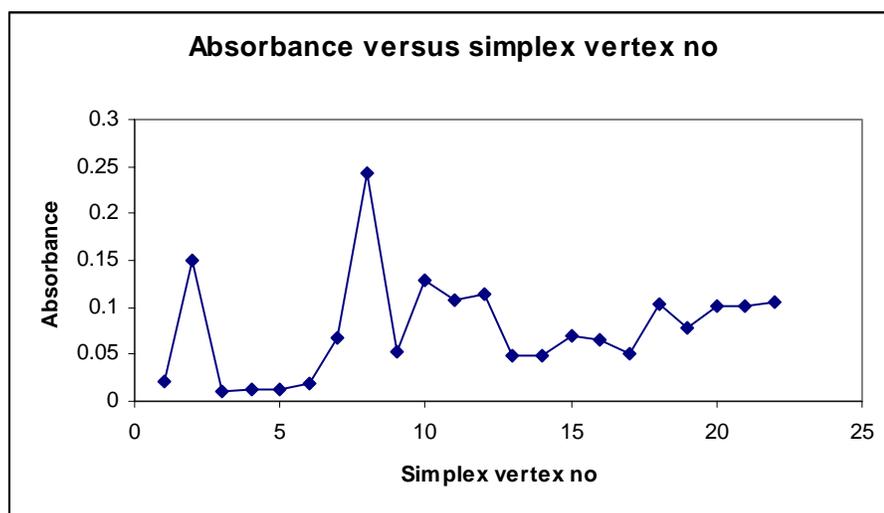


Fig 21 Absorbance versus simplex vertex number

Optimization of the parameters for these proposed methods is highly imperative as diazotization and coupling reactions are highly sensitive to changes in pH and concentration of reagents. The super modified simplex program was used for simultaneous chemometrics optimization of four parameters critical to the stability of the colored azo dye compound formed. The parameters optimized are HCl, NaOH, 4 aminoantipyrine, 1,3 dihydroxybenzene concentration respectively. The higher and lower limits of the variables were obtained from experimental studies of these reactions were defined into the simplex program. The following are the variables defined: The lower limit for HCl concentration  $1.00 \times 10^{-3}$  M and upper limit 2.00 M; lower limit for NaOH concentration was  $5.00 \times 10^{-2}$  M and upper limit 2.00 M; lower limit for 1,3 dihydroxybenzene was  $1.00 \times 10^{-3}$  M and upper limit 0.10M; lower limit of 4 aminoantipyrine 0.025M and upper limit 0.10M. The optimization experiments were carried out by using 1 ppm of  $\text{NO}_2^-$ -N and preparing the concentrations of reagents suggested by the simplex software, mixing equal volumes of sample and reagents and keying in the response into the simplex program which suggest new concentrations for another run of experiment. The optimum concentrations were obtained from the simplex vertex no 8. It was observed that absorbance value are generally low for low concentration of HCl and NaOH (simplex vertex no 1,3,4,5, and 6) thus emphasizing the importance of acid and base concentrations in the diazotization reactions. The simplex experiment was terminated after 22 experiments because there was no considerable increase in the absorbance readings.

#### 4.5.5 ANALYTICAL APPRAISALS

Calibration using the integrated area plot versus concentration for the SIA  $\text{NO}_2^-$ -N/ $\text{NO}_3^-$ -N method was observed to give a better correlation coefficient

( $R^2 = 0.9936$ , linear equation,

$$\text{response} = 3.513431 + 3.486549x$$

for  $\text{NO}_2^-$ -N, where response is the integrated area and x is the concentration in ppm)

when compared to the local maximum (absorbance) concentration plot ( $R^2 = 0.987$ ,

$\text{response} = 0.3118242 + 6.323569E-02x$  for  $\text{NO}_2^-$ -N). Where response is absorbance

and x is concentration in ppm. For this SIA method Integrated area (response) defined

according to FIALab® as

$$n$$

$$\text{area} = \sum_{I=0}^n \text{data}_i \cdot \text{deltatime}$$

$$I=0$$

(deltatime = delta time between two consecutive measurements)

was used for  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N SIA calibration. The relative standard deviation of the proposed SIA method was < 2.90% (20 injections of 2 ppm  $\text{NO}_2^-$ -N) with a sample throughput of 14 samples per hour for  $\text{NO}_2^-$ -N SIA method. The detection limit (defined as 10 times standard deviation of ten blank reading) for the  $\text{NO}_2^-$ -N SIA

method was 0.2 ppm. The linear range for  $\text{NO}_2^-$ -N SIA method was 0.3 ppm to 18 ppm  $\text{NO}_2^-$ -N while The linear range for  $\text{NO}_3^-$ -N SIA method was 0.05 to 1.00 ppm due to the tedious, slow and rate determining reduction of  $\text{NO}_3^-$ -N to  $\text{NO}_2^-$ -N. The linearity and  $R^2$  of  $\text{NO}_3^-$ -N SIA calibration curve (integrated area versus concentration) was response =  $7.47164+2.272655*x$  and 0.999 respectively. Where response is integrated area and x is concentration in ppm.

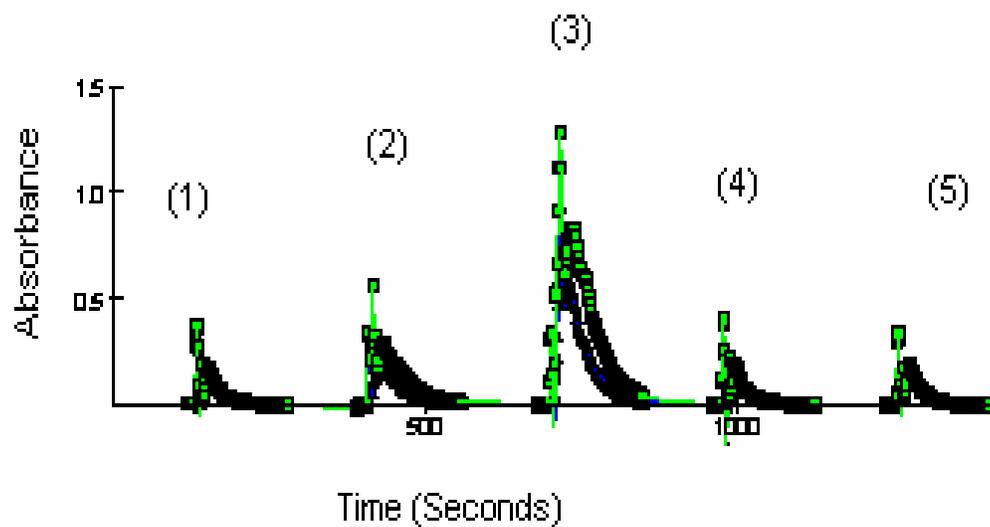


Fig 22 Absorbance versus time run for  $\text{NO}_2^-$  -N SIA determination. (1) is 0.5 ppm (2) 5 ppm (3) 15 ppm. (4) & (5) are duplicates of 0.5 ppm

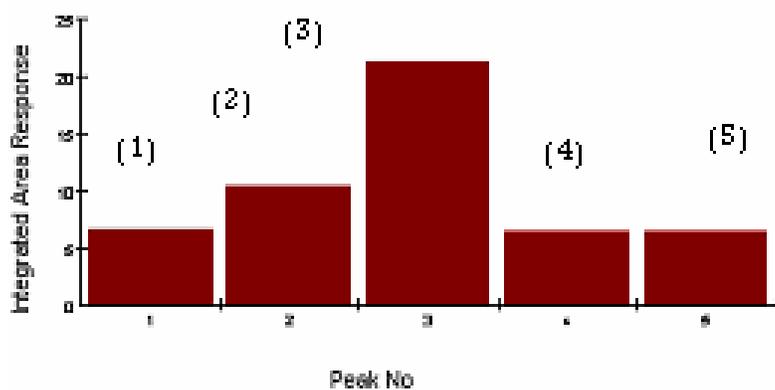


Fig 23 Integrated Area versus no of peaks derived from fig 5 above

The linear range, correlation coefficient and linear equation of absorbance measurement for batch  $\text{NO}_2^-$ -N method were 0.02 to 5.00 ppm  $\text{NO}_2^-$ -N,  $R^2 = 0.999$ ,  
absorbance =  $0.00561 + 0.211\text{Concentration}$ .

The correlation coefficient for batch  $\text{NO}_3^-$ -N was 0.996.

## 4.5.6 METHOD VALIDATION

TABLE X Comparison of results for NO<sub>3</sub><sup>-</sup>-N determination.

Added NO <sub>3</sub> <sup>-</sup> -N (ppm)	Found SIA (ppm)	Found Batch (ppm)	Standard Method (ppm)	Percentage Recovered SIA	Percentage Recovered Batch	Percentage Recovered Std. Method
0.050	0.053	0.070	0.052	106	140	104
0.100	0.097	0.077	0.098	97.0	77	98
0.500	0.500	0.503	0.501	100	101	100

\*\*Each determination an average of two readings

The t test value for SIA  $\text{NO}_3^-$ -N method and batch method were found to be 0.333 and 0.490 (95% confidence level) respectively. While for  $\text{NO}_2^-$ -N determination the t test values were 0.303 for SIA and 0.346 for Batch method. This t values suggested full agreement with the results obtained using APHA method.

TABLE XI Comparison of results for NO<sub>2</sub><sup>-</sup>-N determination

Added NO <sub>2</sub> <sup>-</sup> - N (ppm)	Found	SIA (ppm)	Found Batch (ppm)	Standard Method (ppm)	Percentage Recovered		Percentage Recovered	
					SIA	SIA	Batch	Batch. Method
0.500		0.461	0.450	0.502	92.2		90.0	100.4
5.00		4.45	4.87	5.72	92.2		97.4	114
15.0		15.2	15.2	14.8	101		101	98.0

\*\*Each determination an average of two readings

TABLE XII Tolerance limit of some interfering cations on proposed method

Cation	Added as	Tolerance limit (ppm)
Na <sup>+</sup>	NaCl	8.00
Fe <sup>2+</sup>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .FeSO <sub>4</sub> .6H <sub>2</sub> O	6.00
Fe <sup>3+</sup>	FeCl <sub>3</sub> .6H <sub>2</sub> O	7.00
Cd <sup>2+</sup>	CdSO <sub>4</sub> .8H <sub>2</sub> O	3.00
Cu <sup>2+</sup>	CuSO <sub>4</sub> .5H <sub>2</sub> O	10.0

\*\*Each determination an average of two readings

#### **4.5.7 INTERFERENCE STUDY**

0.2 ppm  $\text{NO}_2^-$ -N samples were used for interference study of some selected cations on the proposed method. The concentration of added cations was varied from 0 to 50 ppm for each cation. The observed tolerance limit for the cations studied are listed in the table XII.

TABLE XIII: Results obtained from the analysis of some food and water samples for  $\text{NO}_2^-$ -N .

Sample	Batch	SIA method	APHA
	Method	( $\text{NO}_2^-$ -N)	( $\text{NO}_2^-$ -N)
	( $\text{NO}_2^-$ -N)		
bottled water	n.d	n.d	n.d
Desalinated water	0.0150 ppm	n.d	0.0172 ppm
Treated salty water	0.0290 ppm	n.d	0.0200 ppm
Corned beef extract	3.03 mg/Kg	2.18 mg/Kg	2.94 mg/Kg
Sardine extract	3.20 mg/Kg	3.39 mg/Kg	2.98 mg/Kg

\*\*Each determination an average of two readings

n.d = not detectable within the limit of analytical method

#### **4.5.8 ANALYSIS OF WATER AND FOOD SAMPLES**

Some water and food samples were analyzed for  $\text{NO}_2^-$ -N . Nitrite was extracted from food samples using the procedure according to Umah et al [63].The results obtained using the three methods corroborated each other.

#### **4.6 CONCLUSION**

The proposed batch method and SIA method were found to have a wider applicable range when compared to the APHA method, thus minimizing the dilution of high concentration of analyte which normally introduces dilution errors. The concentration of four highly interacting parameters were optimized using the super modifies simplex program, hence strict control of color formation reaction for the proposed batch method were minimized and sensitivity greatly improved. The color development step for this proposed method was more rapid and thus does not require long development time like the APHA method.

The proposed methods also offers the advantage of providing automation for analysis of a large number of samples and the option of providing an alternative of either batch or automated method.

## **GENERAL CONCLUSION FOR ALL SIA METHODS**

The adapted SIA methods are simple, sensitive, accurate, cost saving, reproducible time saving and avoid the need for dilution of high concentration of samples. The sensitivity and linear range of the adapted methods have been greatly enhanced by delay time, placing holding coil in a thermostated water bath for silica and ammonia determination and use of reagent as carrier for phosphate determination. The methods were all in agreement with standard methods by APHA.

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## APPENDICES

### (I) OPTIMISED COMPUTER FLOW PROGRAM USED FOR SILICA DETERMINATION

'Select Wavelengths

Hardware Settings Wavelength 1 (nm) 815

' Define Variable

Variable Define New numreagents

Variable Define New Holdtime

numreagents = 3     ' number of reagents in chemistry

Holdtime = 120     ' delay time in holding coil

Global Logon

' Read in sample definition file (set path here or on autosampler page)

' If set on autosampler page, leave blank (no path) after "Sample Description"

Sample Description

“ Configure Hardware”

Hardware Settings portname (number, string) 1, Waste

Hardware Settings portname (number, string) 2, Flowcell

Hardware Settings portname (number, string) 3, ReagentA

Hardware Settings portname (number, string) 4, ReagentB

Hardware Settings portname (number, string) 5, Sample

Hardware Settings portname (number, string) 6, Carrier  
Hardware Settings portname (number, string) 7, ReagentC  
Hardware Settings End Settings  
“ Put autosampler in first sample (usually a blank)”  
Next Sample  
Delay (sec) 5  
Peristaltic Pump Clockwise(%) 70  
Delay (sec) 40  
Peristaltic Pump Off  
“ Wash and prime system”  
SyringePump Flowrate (microliter/sec) 100  
SyringePump Valve In  
SyringePump Fill  
SyringePump Delay Until Done  
SyringePump Valve Out  
Multiposition Valve Flowcell  
SyringePump Empty  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 150  
Multiposition Valve Carrier  
SyringePump Aspirate ( $\mu$ L) 150  
SyringePump Delay Until Done  
Multiposition Valve ReagentA  
SyringePump Aspirate ( $\mu$ L) 100

SyringePump Delay Until Done  
Multiposition Valve ReagentB  
SyringePump Aspirate ( $\mu\text{L}$ ) 100  
SyringePump Delay Until Done  
Multiposition Valve ReagentC  
SyringePump Aspirate ( $\mu\text{L}$ ) 100  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 100  
SyringePump Valve In  
SyringePump Fill  
SyringePump Delay Until Done  
SyringePump Valve Out  
Multiposition Valve Flowcell  
SyringePump Empty  
SyringePump Delay Until Done  
' Start Analysis  
Loop Start (#) 5000  
    Analyte New Sample  
    ' Wash out flow cell  
    Multiposition Valve Flowcell  
    SyringePump Valve Out  
    SyringePump Flowrate (microliter/sec) 100  
    SyringePump Empty  
    SyringePump Delay Until Done

```

' Aspirate Sample (send first plug to waste to prevent carry over)
Multiposition Valve Sample
SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 50
SyringePump Valve Out
Delay (sec) .1
SyringePump Aspirate ( $\mu\text{L}$ ) 100
SyringePump Delay Until Done

' Clear Out Flowcell
SyringePump Flowrate (microliter/sec) 100
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done
SyringePump Valve Out
Multiposition Valve Flowcell
SyringePump Dispense (microliter) 500
SyringePump Delay Until Done
if SampleID < 0 then    ' if complete with last sample then stop
' autosampler wash    'uncomment if using wash with AIM1250 or AIM3200
    Delay (sec) 2
    Stop Program
end if

```

```

Multiposition Valve Sample
SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100
SyringePump Valve Out
SyringePump Aspirate ( $\mu\text{L}$ ) 100
SyringePump Delay Until Done
“ Aspirate ReagentA ”
    if numreagents > 0 then
        Multiposition Valve ReagentA
        SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100
        SyringePump Valve Out
        SyringePump Aspirate ( $\mu\text{L}$ ) 40
        Delay (sec) 60
    End if
” Aspirate ReagentB”
    if numreagents > 1 then
        Multiposition Valve ReagentB
        SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 20
        SyringePump Valve Out
        SyringePump Aspirate ( $\mu\text{L}$ ) 40
        Delay (sec) 30
    End if

```

“ Aspirate ReagentC ”

if numreagents > 1 then

    Multiposition Valve ReagentC

    SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 50

    SyringePump Valve Out

    SyringePump Aspirate ( $\mu\text{L}$ ) 40

    Delay (sec) 60

Multiposition Valve Sample

    SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

    SyringePump Valve Out

    SyringePump Aspirate ( $\mu\text{L}$ ) 100

Delay (sec) Holdtime

autosampler wash

” Send To Flow Cell and Measure Absorbance ”

    Multiposition Valve Flowcell

    SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 30

    SyringePump Valve Out

    SyringePump Empty

    Delay (sec) .5

    Spectrometer Reference Scan

    Delay (sec) 5

    Spectrometer Absorbance Scanning

    SyringePump Delay Until Done

Spectrometer Stop Scanning

' Put probe into next sample vial

Next Sample

Peristaltic Pump Clockwise (%) 70

Delay (sec) 40

Peristaltic Pump Off

## **(II). OPTIMISED COMPUTER FLOW PROGRAM USED FOR THE DETERMINATION OF PHOSPHATE**

‘Select Wavelengths’

Hardware Settings Wavelength 1 (nm) 760

Hardware Settings Wavelength 4 (nm) 550

Hardware Settings Use Wavelength 4 as Reference

' Define Variable

Variable Define New numreagents

Variable Define New Holdtime

numreagents = 2      ' number of reagents in chemistry

Holdtime = 100      ' delay time in holding coil

Global Logon

' Read in sample definition file (set path here or on autosampler page)

' If set on autosampler page, leave blank (no path) after "Sample Description"

Sample Description

' *Configure Hardware*

Hardware Settings portname (number, string) 1, Waste

Hardware Settings portname (number, string) 2, Flowcell

Hardware Settings portname (number, string) 3, ReagentA

Hardware Settings portname (number, string) 4, ReagentB

Hardware Settings portname (number, string) 5, Sample

Hardware Settings portname (number, string) 6, Carrier

Hardware Settings End Settings

' Put autosampler in first sample (usually a blank)

Next Sample

Delay (sec) 5

Peristaltic Pump Clockwise(%) 70

Delay (sec) 40

Peristaltic Pump Off

' Wash and prime system

SyringePump Flowrate (microliter/sec) 200

SyringePump Valve In

SyringePump Fill

SyringePump Delay Until Done

SyringePump Valve Out

Multiposition Valve Flowcell

SyringePump Empty

SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 50

Multiposition Valve Carrier

SyringePump Aspirate ( $\mu$ L) 250

SyringePump Delay Until Done

Multiposition Valve ReagentA

SyringePump Aspirate ( $\mu$ L) 250

SyringePump Delay Until Done

Multiposition Valve ReagentB  
SyringePump Aspirate ( $\mu\text{L}$ ) 250  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 200  
SyringePump Valve In  
SyringePump Fill  
SyringePump Delay Until Done  
SyringePump Valve Out  
Multiposition Valve Flowcell  
SyringePump Empty  
SyringePump Delay Until Done  
' Start Analysis  
Loop Start (#) 5000  
    Analyte New Sample  
    ' Wash out flow cell  
Multiposition Valve Flowcell  
SyringePump Valve Out  
SyringePump Flowrate (microliter/sec) 100  
SyringePump Empty  
SyringePump Delay Until Done  
    ' Aspirate Sample (send first plug to waste to prevent carry over)  
Multiposition Valve Sample  
SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 50

```

SyringePump Valve Out
Delay (sec) .1
SyringePump Aspirate (µL) 100
SyringePump Delay Until Done
' Clear Out Flowcell''
SyringePump Flowrate (microliter/sec) 100
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done
SyringePump Valve Out
Multiposition Valve Flowcell
SyringePump Dispense (microliter) 500
SyringePump Delay Until Done
if SampleID < 0 then    ' if complete with last sample then stop
    autosampler wash    'uncomment if using wash with AIM1250 or AIM3200
    Delay (sec) 2
    Stop Program
end if
Multiposition Valve Sample
SyringePump Flowrate (µL/sec) 20
SyringePump Valve Out
SyringePump Aspirate (µL) 100
SyringePump Delay Until Done
autosampler wash

```

” Aspirate ReagentA”

if numreagents > 0 then

    Multiposition Valve ReagentA

    SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

    SyringePump Valve Out

    SyringePump Aspirate ( $\mu\text{L}$ ) 100

    SyringePump Delay Until Done

End if

” Aspirate ReagentB “

if numreagents > 1 then

    Multiposition Valve ReagentB

    SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

    SyringePump Valve Out

    SyringePump Aspirate ( $\mu\text{L}$ ) 120

    SyringePump Delay Until Done

End if

” Aspirate into holding coil”

    Multiposition Valve Carrier

    SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 50

    SyringePump Valve Out

    SyringePump Aspirate ( $\mu\text{L}$ ) 10

    SyringePump Delay Until Done

    SyringePump Valve Out

Delay (sec) Holdtime  
" Send To Flow Cell and Measure Absorbance"  
Multiposition Valve Flowcell  
SyringePump Flowrate ( $\mu\text{L}/\text{sec}$ ) 10  
SyringePump Valve Out  
SyringePump Empty  
Delay (sec) 0.5  
Spectrometer Reference Scan  
Delay (sec) 5  
Spectrometer Absorbance Scanning  
SyringePump Delay Until Done  
Spectrometer Stop Scanning  
" Put probe into next sample vial  
Next Sample"  
Peristaltic Pump Clockwise (%) 70  
Delay (sec) 40  
Peristaltic Pump Off  
Loop End

### **(III). OPTIMIZED COMPUTER FLOW PROGRAM FOR IRON DETERMINATION**

"Washing and flushing SIA analytical system"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

syringe pump Valve In

syringe pump fill

syringe pump Delay Until Done

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

multiposition valve port 2

syringe pump Valve Out

syringe pump empty

syringe pump Delay Until Done

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

syringe pump Valve In

syringe pump fill

syringe pump Delay Until Done

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

multiposition valve port 2

syringe pump Valve Out

syringe pump dispense ( $\mu\text{L}$ ) 500

syringe pump Delay Until Done

"Aspirating samples and reagents into the coil "

"Aspirate sample from port 3"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 3

syringe pump Aspirate ( $\mu\text{L}$ ) 100

syringe pump Delay Until Done

"Aspirate hydroxylamine reagent from port 8"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 8

syringe pump Aspirate ( $\mu\text{L}$ ) 20

Delay (sec) 10

"Aspirate 1,10 phenanthroline reagent from port 1"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 1

syringe pump Aspirate ( $\mu\text{L}$ ) 100

Delay (sec) 60

"Aspirate sample again from port 3" to make sandwich of sample and reagents"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 3

syringe pump Aspirate ( $\mu\text{L}$ ) 100

Delay (sec) 240

"Chemical measurement "

chemical new sample

chemical name iron complex

chemical Quantity 0.5 ppm

multiposition valve port 2

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 10

syringe pump Dispense ( $\mu\text{L}$ ) 2000

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 4

Spectrometer Absorbance Scanning

syringe pump Delay Until Done

Spectrometer Stop Scanning

"Start of another cycle of analysis for second sample in port 3"

"Washing and flushing SIA analytical system"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

syringe pump Valve In

syringe pump Fill

syringe pump Delay Until Done

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

multiposition valve port 1

syringe pump Valve Out

syringe pump Dispense ( $\mu\text{L}$ ) 500  
syringe pump Delay Until Done  
"Aspirating samples and reagents into the coil "  
"Aspirate sample from port 4"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 4  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
syringe pump Delay Until Done  
"Aspirate hydroxylamine reagent from port 8"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 8  
syringe pump Aspirate ( $\mu\text{L}$ ) 20  
Delay (sec) 10  
"Aspirate 1,10 phenanthroline reagent from port 1"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 1  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
Delay (sec) 60  
"Aspirate sample again from port 4 ' to make sandwich of sample and reagents"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 4  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
Delay (sec) 60

"Chemical measurement "  
chemical new sample  
chemical name iron complex  
chemical Quantity 5 ppm  
multiposition valve port 2  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 10  
syringe pump Dispense ( $\mu\text{L}$ ) 2000  
Delay (sec) 1  
Spectrometer Reference Scan  
Delay (sec) 4  
Spectrometer Absorbance Scanning  
syringe pump Delay Until Done  
Spectrometer Stop Scanning  
'Start of another cycle of analysis for second sample in port 5"  
'Washing and flushing SIA analytical system"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100  
syringe pump Valve In  
syringe pump Fill  
syringe pump Delay Until Done  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100  
multiposition valve port 2  
syringe pump Valve Out

syringe pump Dispense ( $\mu\text{L}$ ) 500  
syringe pump Delay Until Done  
"Aspirating samples and reagents into the coil "  
"Aspirate sample from port 5"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 5  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
syringe pump Delay Until Done  
"Aspirate hydroxylamine reagent from port 8"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 8  
syringe pump Aspirate ( $\mu\text{L}$ ) 20  
Delay (sec) 10  
"Aspirate 1,10 phenanthroline reagent from port 1"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 1  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
Delay (sec) 60  
"Aspirate sample again from port 5" to make sandwich of sample and reagents"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 5  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
Delay (sec) 60

"Chemical measurement "  
chemical new sample  
chemical name iron complex  
chemical Quantity 10  
multiposition valve port 2  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 10 ppm  
syringe pump Dispense ( $\mu\text{L}$ ) 2000  
Delay (sec) 1  
Spectrometer Reference Scan  
Delay (sec) 4  
Spectrometer Absorbance Scanning  
syringe pump Delay Until Done  
Spectrometer Stop Scanning  
'Start of another cycle of analysis for second sample in port 6"  
'Washing and flushing SIA analytical system"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100  
syringe pump Valve In  
syringe pump Fill  
syringe pump Delay Until Done  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100  
multiposition valve port 2

syringe pump Valve Out

syringe pump Dispense ( $\mu\text{L}$ ) 500

syringe pump Delay Until Done

"Aspirating samples and reagents into the coil "

"Aspirate sample from port 6"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 6

syringe pump Aspirate ( $\mu\text{L}$ ) 100

syringe pump Delay Until Done

"Aspirate hydroxylamine reagent from port 8"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 8

syringe pump Aspirate ( $\mu\text{L}$ ) 20

Delay (sec) 10

"Aspirate 1,10 phenanthroline reagent from port 1"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 1

syringe pump Aspirate ( $\mu\text{L}$ ) 100

Delay (sec) 60

'Aspirate sample again from port 6" to make sandwich of sample and reagents"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150

multiposition valve port 6

syringe pump Aspirate ( $\mu\text{L}$ ) 100

Delay (sec) 60

"Chemical measurement "

chemical new sample

chemical name iron complex

chemical Quantity 10

multiposition valve port 2

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 10 ppm

syringe pump Dispense ( $\mu\text{L}$ ) 2000

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 4

Spectrometer Absorbance Scanning

syringe pump Delay Until Done

Spectrometer Stop Scanning

"Start of another cycle of analysis for second sample in port 7"

"Washing and flushing SIA analytical system"

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

syringe pump Valve In

syringe pump Fill

syringe pump Delay Until Done

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 100

multiposition valve port 2

syringe pump Valve Out

syringe pump Dispense ( $\mu\text{L}$ ) 1500  
syringe pump Delay Until Done  
"Aspirating samples and reagents into the coil "  
"Aspirate sample from port 7"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 7  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
syringe pump Delay Until Done  
"Aspirate hydroxylamine reagent from port 8"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 7  
syringe pump Aspirate ( $\mu\text{L}$ ) 20  
Delay (sec) 10  
"Aspirate 1,10 phenanthroline reagent from port 1"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 1  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
Delay (sec) 60  
'Aspirate sample again from port 7" to make sandwich of sample and reagents"  
syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 150  
multiposition valve port 5  
syringe pump Aspirate ( $\mu\text{L}$ ) 100  
Delay (sec) 60

"Chemical measurement "

chemical new sample

chemical name iron complex

chemical Quantity 10

multiposition valve port 2

syringe pump Flowrate ( $\mu\text{L}/\text{sec}$ ) 10 ppm

syringe pump Dispense ( $\mu\text{L}$ ) 2000

Delay (sec) 1

Spectrometer Reference Scan

Delay (sec) 4

Spectrometer Absorbance Scanning

syringe pump Delay Until Done

Spectrometer Stop Scanning

## **(IV) OPTIMIZED FLOW PROGRAM FOR AMMONIA DETERMINATION**

' Read in sample definition file (set path here or on autosampler page)

' If set on autosampler page, leave blank (no path) after "Sample Description"

Sample Description

' *Configure Hardware*

Configure LOV

' *Selected wavelengths to monitor*

Hardware Settings Wavelength 1 (nm) 770 ' ammonia

Hardware Settings End Settings

' Wash and prime system

Syringe Pump All Stop

Prime LOV Carrier

Prime LOV ReagentA

Prime LOV ReagentB

Wash LOV

Loop Start (#) 5000

' get sample

Next Sample ' command autosampler to next sample

Analyte New Sample ' tell software to label next sample  
Delay (sec) 5  
' pump sample to all LOVs if new vial is accessed  
Syringe Pump Ammonia Stop  
SamplerPump Number of pulses 12  
' Prime Sample Line and Clear Out Flowcell  
Aspirate LOV Sample, 50  
Wash LOV  
'Aspirate reagentA/Sample/reagentB/carrier  
Aspirate LOV ReagentA, 100  
Aspirate LOV Sample, 120  
Aspirate LOV ReagentB, 100  
Aspirate LOV Carrier, 350  
Delay (sec) 100  
' Send To Flow Cell and Measure Absorbance  
collectdata LOV  
Loop End

## **Vita**

Magnus .U. Legemah graduated from the Chemistry Department, University of Benin Nigeria with a second class upper division in December 1999. He worked as a scientific officer in Federal Capital Territory Water Board Nigeria and then proceeded to the University of Ibadan, Nigeria for a Masters degree in Analytical Chemistry. He was awarded a Masters degree in Analytical Chemistry in July, 2003 from the University of Ibadan Nigeria. He worked briefly in Nigerian Distilleries Limited as a management trainee. He is presently a Research Assistant at the King Fahd University of Petroleum & Minerals.