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Determination of Trace Iron in Aqueous Samples by Its Catalytic Effect in Trinder's Reaction in a Flow Injection System

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Advisor: Dr. Negussie Wodajo

By: Balkew Zewge

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LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

4-AP	4-Aminophenazone
CL	Chemiluminescence
CSV	Cathodic Stripping Voltammetry
DCBS	Dichlorobenzene Sulphonated
DCPS	Dichlorophenol Sulphonated
DPD	N, N-dimethyl-p-phenylenediamine Dihydrochloride
FAAS	Flame Atomic Absorption Spectrometry
FIA	Flow Injection Analysis
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HRP	Horseradish Peroxidase
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
KHP	Potassium Hydrogen Phthalate
LC	Liquid Chromatography

ABSTRACT

A kinetic catalytic flow injection spectrophotometric method for the determination of trace iron in aqueous samples has been proposed. It is based on the catalytic effect of iron on the oxidative coupling of 4-AP and DCPS in the presence of hydrogen peroxide and in slightly acidic medium to form quinine imine dye that has a maximum peak height at λ_{max} of 510 nm. The peak height observed at the flow through detector was linearly proportional to iron (II, III) concentration over the range 1-20 μM.

In the proposed method, iron acts as a catalyst by changing its oxidation state from Fe^{2+} to Fe^{3+} and recycle Fe^{3+} to Fe^{2+}. This recycling action of iron can magnify its effect for the trace analysis in aqueous samples. Thus small amount of iron can be determined by measuring the rate of the formation of the product.

After optimization of flow rate, pH of the carrier solution and concentration of each reagents attempt was made to utilize the developed method for the determination of iron content in commercially available bottled mineral waters (Aqua Addis, Aqua safe, Abyssinia springs, and Highland). However, the method was not sensitive enough for detection of traces of iron. Different activators were tested for their enhancement of sensitivity but no increment in signal was observed.

1.INTRODUCTION

Metals are a natural part of our environment. Life has evolved in this natural environment and requires that metals be present in appropriate levels and combinations. Concentrations of metals that are too low can lead to health problems as a result of nutrient deficiencies, whereas at high concentrations, trace metals can become toxic for living organisms and behaves as conservative pollutants and are frequently released in large quantities during different processes derived from human activities and may lead to major distraction of aquatic ecosystems. Moreover, human beings located at places contaminated by heavy metals could be especially sensitive to these contaminants due to bioaccumulation [1].

Iron is the fourth most abundant element in the earth's crust (5.6% by mass). It is a shiny, bright white metal which is present in a variety of rocks and soil minerals both as Fe (II) and Fe (III). Its surface is usually discolored by corrosion due to its reaction with the oxygen of the air in the presence of moisture but in absolutely dry air, it does not rust. The oxide that is produced is brittle and soft, giving no protection to the base metal, which finally rusts away [2-4].

Iron is obtained from its most important ores, hematite (Fe_2O_3) and magnetite (Fe_3O_4), by reduction with coke in a blast furnace. Since it is relatively soft and easily corrodes, it is combined with carbon and other metals, such as vanadium, chromium, and manganese, to make alloys (steels) that are harder and less reactive than pure iron [5].

In recent years accumulation of iron in the natural environment where it may persist and exert toxic effects on local ecosystems has received much attention. Several studies showed that industrial and other pollution of water sources were associated with increased levels of metals such as iron, lead, nickel, copper, cadmium, manganese and zinc. Iron concentrations are usually not very high in surface waters (in the order of 0.01–

5.00 mg/L), but accumulation of the metal occurs to very high levels in their sediments (80–164000 mg/kg), aquatic vegetation, and zooplankton [6].

At low concentrations, iron plays an important role in metabolic and fermentation processes, as an enzyme activator, stabilizer and functional component of proteins. Above trace levels, however, iron is a moderately toxic element when compared with other transition metals. However, the toxic doses of iron and its compounds can lead to serious problems, including coma, depression, rapid and shallow respiration. On the other side iron and its compounds have widespread industrial applications (constructional material for drinking-water pipes, food colorant, coagulants in water treatment, pigments in paints and plastics); hence, large quantities of iron are discharged into the environment [1]. Thus, appropriate measurement of the iron content in the environment is necessary to control its effect on the living systems.

1.1. Biochemistry of Iron

Iron appears to be an essential element for all organisms, both plant and animals. Iron deficiency is common for plant grown on soils deficient in iron or with high alkalinity, where the iron is strongly bound as hydroxides. In animals, iron is found in many important proteins that have the major function in oxygen transport, storage and electron transfer processes [7, 8].

In the human body, iron is an essential trace element and has wide physiological function [5, 9]. It complexes with heme protein, that carries oxygen from the lungs to the cells of the body, and plays an equally essential role in respiratory enzymes such as cytochromes, which allows us to use oxygen. Iron deficiency is probably the most prevalent deficiency state affecting the human population. It is much more common in women than in men because of the additional loses in women through menstrual flow and pregnancy. The average requirement of iron is 1.3 mg/day in males and non-menstruating females and 1.8 mg/day in menstruating females [10, 11].

The elemental iron also plays an important role in plant metabolism where it is essential for photosynthetic and respiratory electron transport, nitrate reduction, chlorophyll synthesis and detoxification of reactive oxygen species [12]. The very low concentration of iron measured in some areas of the oceans has been suspected to limit phytoplankton production [13-15].

1.2. Iron in Aqueous Environment

Iron is a nutritional requirement for most organisms, and plays an important role in natural processes. It has been reported to demonstrate a nutrient like profile in many regions of the open ocean, with dissolved Fe typically existing at < 0.2 nM in surface waters and converging to 0.7–0.8 nM in deep waters [16].

Even though its occurrence in the ocean in both form of iron(II) and iron(III) is very low concentrations, it has importance in biogeochemistry. These oxidation states are involved in the formation of soluble inorganic and organic complexes, colloids and particulate phases. The iron(III) oxidation state predominates in oxygenated waters and is highly insoluble through the formation of oxyhydroxides. Iron(II) is thermodynamically unstable in oxygenated seawater and is rapidly oxidized to iron(III). Several workers have detected iron (II) in surface waters and upwelling regions, reporting contributions of as much as 50% of total dissolved iron [12, 17].

Only a small fraction of dissolved iron(III) occurs in a free hydrated (Fe^{3+}) or inorganically complexed form, and 80–99% is strongly complexed by organic ligands, possibly produced by iron limited phytoplankton or bacteria. This organic complexation prevents iron(III) from forming insoluble oxyhydroxides, thereby maintaining enhanced dissolved iron concentrations in seawater [11].

2. METHODS FOR THE DETERMINATION OF IRON

Different non catalytic analytical methods for the determination of iron in aqueous sample have been reported by several researchers. Method like inductively coupled plasma atomic emission spectrometry (ICP-AES) [18, 19], adsorptive cathodic striping voltametry (AdCSV) [12], chemiluminescence (CL) [12, 14], graphite furnace atomic absorption spectrometry (GFAAS) [10-12], flame atomic absorption spectrometry (FAAS) [12], fluorometric analysis , cathodic stripping voltammetry (CSV) [17, 20], and inductively coupled plasma mass spectrometry (ICP-MS) and spectrophotometry have been used.

Most of the methods suffer from drawbacks. Adsorptive cathodic striping voltametry fail to detect iron in pico-molar amounts in region where the concentration of iron is limited [12] and chemiluminescence method is sensitive, but suffers from selectivity problem as the chemiluminescent reaction can be catalyzed by several metals that could also be present in water samples. GFAAS, FAAS, and ICP-MS are expensive and there large size, weight and fragility restricts their use to land-based laboratories [12]. In general most of the methods lack sufficient sensitivity for determining iron in water samples at very low concentration levels. Pre-concentration and separation techniques are usually required prior to analysis which consumes large volume of samples.

As compared with the other techniques, spectrophotometry is very simple, rapid and less expensive for determination of iron in a variety of samples [10].

The majority of methods described up to now for the determination of iron (II),made use of spectrophotometric detection based on the formation of colored products when iron is complexed with chromogenic reagents such as ferrozine (sodium 3-(2-pyridyl)1,2,4-triazine 4,4-disulphonate) (Fz) [4, 21, 22], 1,10-phenanthroline [23, 24-27], batho-phenanthroline (4,7-diphenyl-1,10-phenanthroline) [28], and 2,4,6-tripyridyl-1,3,5-triazine (TPTZ). The reagent bathophenanthroline and TPTZ are very selective reagents

for iron but they have low sensitivity while ferrozine is very sensitive [4]. It allows lower detection limits (100 pM) and selective reagent for iron. However, ferrozine is relatively expensive. The determination of iron(III) can be possible by using tiron (1,2-dihydroxybenzene-3,5-disulphonic acid) and thiocyanate ion $(SCN)^-$ as complexing agents but both the two methods involve an oxidation step in the process, i.e., oxidation of Fe(II) to Fe(III) and require sample pretreatment [12].

2.1 Catalytic - Kinetic Methods

Kinetic–catalytic analysis is based on catalyzed reactions and applied to trace analyses for various catalysts (elements) because of their extremely high sensitivity, low detection limit and selectivity. Several reactions such as redox, ligand substitution and complexation reactions have been utilized as indicator reactions for the development of kinetic-catalytic methods of trace analyses. Most of the catalysts in redox reactions are metal ions such as iron, copper, chromium, manganese, vanadium and selenium [29].

Kinetic-catalytic methods allow the determination of various elements at very small levels with simple and reasonable equipment. The detection limit of the kinetic-catalytic methods is of the order of pictograms per milliliter with good accuracy and precision [29, 30]. Since, kinetic–catalytic method includes time as an experimental variable; care is needed to insure that mixing of reagents takes place at regular time intervals to obtain highly accurate results using a manual procedure. Such disadvantages in the manual system can be overcome by using flow injection analysis (FIA) in which the reaction time can easily be controlled by varying the flow rate of the solutions and the length of the reaction coil.

For kinetic-based methods, Flow-injection analysis (FIA) is well suited because it can easily control the reagent addition and the reaction time [21, 31], ease of automation, accurate reproducible mixing of sample and reagent solutions can be achieved in the closed flow system without any contamination from the environment, and rapid analytical technique that makes it possible to carry out a large number of determinations with a minimum consumption of sample and reagents [28, 29, 31-33]. Flow injection analysis

allows the analytical method to be used in conjunction with a variety of detection techniques. Spectrophotometry in the visible range is by far the most widely used detection principle in FIA, owing to the numerous selective reactions available for almost every type of compound and element [34, 35].

Measures *et. al.* reported that spectrophotometric detection of the iron (II) achieved through its catalytic effect on the oxidation of N,N-dimethyl-p-phenylenediamine dihydrochloride (DPD) by hydrogen peroxide. The catalytic nature of the reaction increases the sensitivity of the method since the amount of oxidized DPD is proportional to the amount of iron and the length of the reaction time [36]. The time dependence of the sensitivity of the catalytic reaction is ideally suited to FIA since this methodology is capable of providing reproducible reaction times [28, 37].

Kiranas *et. al*. have developed the kinetics and mechanism of the reactions between 4-aminoantipyrine (4-AP) and 3,5-dichloro-2-hydroxybenzenesulphonic acid (DCBS) with Fe^{2+} in the presence of H_2O_2 have been investigated. A differential spectrophotometric flow injection (FI) method for the determination of H_2O_2 has been developed; by substituting horseradish peroxidase (HRP) in Trinder's reagent with Fe^{2+}. They used the method for the determination of glucose in human serum by the enzymatic oxidation of glucose to produce hydrogen peroxide that undergoes the subsequent color reaction [38]. The reaction mechanism is shown bellow.

$$\text{Glucose} + O_2 + \text{Glucose oxidase} + H_2O \longrightarrow \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{AAP} + \text{DCBS} + Fe^{2+} \longrightarrow \text{red product}$$

The same reaction developed by Kiranas *et. al.* will be used for the determination of traces of iron in aqueous samples. By catalyzing the coupling of 4-AP with DCPS iron(II)-iron (III) recycling is anticipated and thus signal amplification via catalytic cascade is expected. Thus, one may expect traces of iron to be detected through this reaction. Therefore, this project tries to prove this concept in a flow injection (FIA) system.

3. OBJECTIVES

3.1 General Objective

To prove weather traces of iron can be detected or not through the catalytic action of Fe (II)-Fe(III) recycling in the coupling of 4-AP with DCPS in the presence of H_2O_2 by applying flow injection analysis system.

3.2. Specific Objectives

I. To optimize the parameters such as pH, flow rate, and reagents concentration for the determination of iron.

II. To evaluate the catalytic performance of Fe (II) in Trinder's reaction and its sensitivity.

III. To determine traces of iron in different bottled mineral waters.

4. EXPERIMENTAL PART

4.1. Chemicals and Reagents

Deionized water (conductivity < 1.5 μS cm^{-1}) was used throughout the experiment for preparation of reagents and rinsing glassware.

A stock (0.1M) potassium hydrogen phthalate (KHP) (Hopkins and Williams, England) buffer solution was prepared by dissolving 10.21 g of KHP with deionized water in 500 mL of volumetric flask. And 0.05 M of (KHP) buffer solutions at different pH were prepared by appropriate addition of 0.1M HCl and 0.1M NaOH and diluting the stock solution with deionized water to the required volume in volumetric flask.

Ammonium iron(II) sulfate hexahydrate {Fe $(NH_4)_2(SO_4)_2.6H_2O$}, Ferric chloride anhydrous ($FeCl_3$) (BDH, England), 2,4-dichlorophenol (BDH, England), 4-Amino-antipyrine (4-AP), 37% concentrated HCl, NaOH (pellet), 95% concentrated H_2SO_4 and potassium hydrogen phthalate (KHP) were used as received.

A stock solution of iron(II) (10 mM) was prepared by dissolving 0.0196 g of ammonium iron(II) sulfate hexahydrate {$Fe(NH_4)_2(SO_4)_2.6H_2O$} with 0.05 M of potassium hydrogen phthalate buffer solution (KHP) and diluting to 50 mL with the same buffer solution. A stock solution of iron(III) (10 mM) was prepared by dissolving 0.0401 g of ferric anhydrous ($FeCl_3$) with KHP buffer in 25 mL volumetric flask. A stock solution of 4-aminoantipyrine (4-AAP) 20 mM was prepared by dissolving 0.1015 g of 4-AP with in 0.05 mM KHP buffer solution and diluting to 25 mL.

Dichlorophenol sulphonated (DCPS) was prepared from 2,4-dichlorophenol and concentrated H_2SO_4 according to the methods of Barham and Trinder [39]. Hydrogen peroxide was standardized by titration using potassium permanganate (Wagtech International, UK) [40].

Working solutions of Fe(II) and Fe(III) were prepared by appropriate dilution of the stock solutions with KHP buffer. Working solutions of DCPS were prepared by appropriate dilution of a stock solution (122.7 mM) with KHP buffer solution. Working hydrogen peroxide solutions were prepared by dilution of a 2.37 % standardized solution (766.3 mM) with KHP buffer solution.

4.2. Apparatus

Kinetic measurements were made on a flow injection set-up instrument. The assembly of the system consisted of two-channel propulsion unit (Gilson Minipuls 3, France), P, an injection port (Rheodyne injection valve with a 20 μL sample loop), IV, Teflon flow line tube, a coiled reaction tube for effective mixing, RC, and a flow through detector LKB 2151,UV-Vis spectrophotometer model (KNAUER, GmbH, Germany), D. The pH of the KHP buffer solution was controlled (off-line) with the help of HANNA pH 301 pH meter (Portugal).

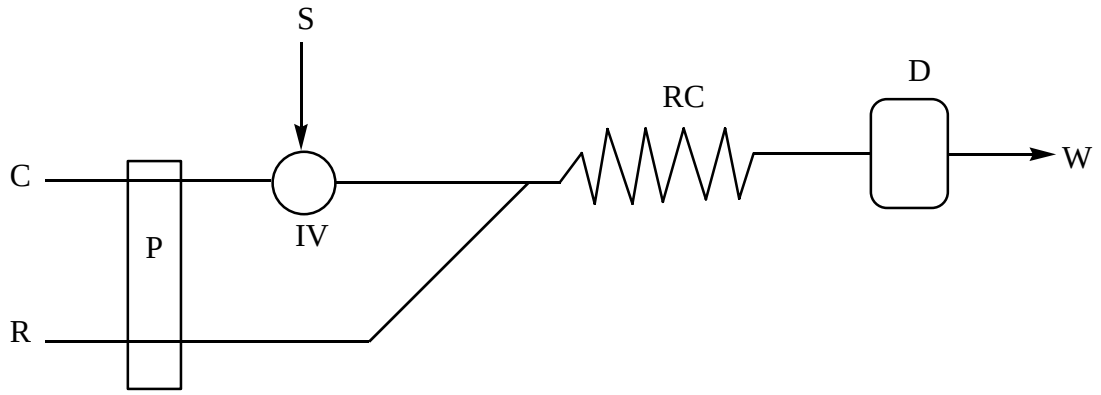


Figure 1. Schematic representation of the FIA system used for the determination of iron.

Where: C is the carrier, R is reagents, P is the pump, IV is the injection valve, S is sample RC is reaction coil, D is the detector and W is waste.

4.3. Procedure

Each reagent mixture was prepared by using KHP buffer solution with the final concentration of 0.05 M (pH 6) and the buffer it self used as a carrier.
In the flow injection system (Fig. 1), a carrier solution (C, 0.05 M potassium hydrogen phthalate, KHP) and a mixture of reagent solutions (4-AP, DCPS, and H_2O_2 with the final concentration of 1.5 mM, 3 mM and 20 mM respectively) were pumped in to the analytical line at a total flow rate of 0.38 mL/min.

A 20 μL aliquot of sample solutions were introduced in to the carrier flow line by a loop-valve injector and then merged in the reagent mixture solution. The color forming reaction of 4-AP with DCPS in the presence of H_2O_2 as oxidant and iron(II) as a catalyst takes place in the reaction coil. Then the absorbance of the colored product corresponding to the concentration of total iron was measured at 510 nm using the flow-through spectrophotometeric detector.

4.4. Sulphonation of 2, 4-Dichlorophenol

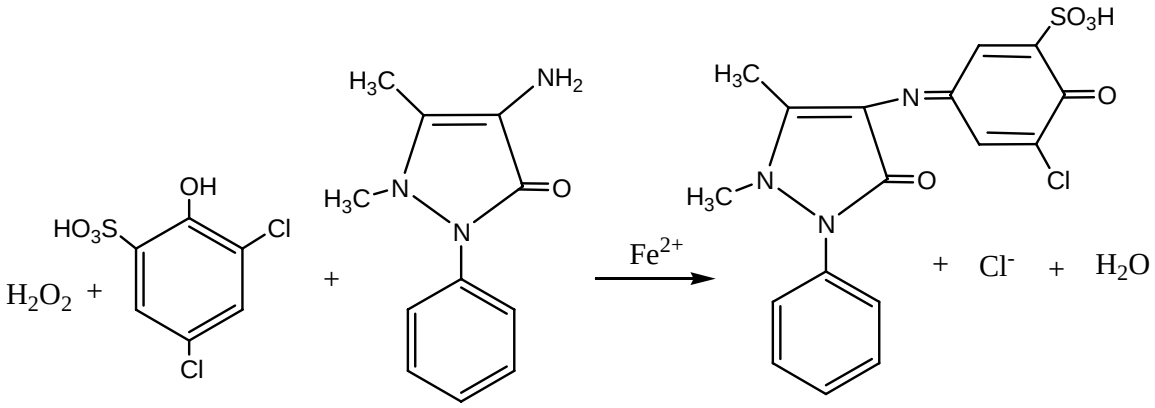
To 5 gram of 2, 4-dichlorophenol 10 mL concentrated H_2SO_4 was added and the mixture was heated on a water bath at 100 ^{0}C for 5 hours. After 5 hours, the flask was put aside from the water bath in order to cool the liquid in the flask. After a while a white solid substance was formed. When this white solid treated with deionized water, the whole portion of it was dissolved to form a solution. To the resulting mixture 200 mL of deionized water was added and neutralized by 10 M NaOH (BDH England). Litmus paper was used to check the pH. Then 2 M H_2SO_4 added and the final volume was made to 250 mL. The solution was then transferred to a brown bottle and kept in a dark place.

5. RESULTS AND DISCUSSION

5.1. Conjugation of 4-AP and DCPS in the presence of H_2O_2 catalyzed by Fe(II)

Iron forms several coordination compounds, in which many of them are colored. The amount of color generated can be used to measure the concentration of iron present in the sample. In this report, the compounds used to form a colored dye in the presence of iron as a catalyst are 4-AP and DCPS in the presence of the oxidant H_2O_2. This mixture results in the formation of a red colored dye that absorbs at 510 nm with a molar absorptive of 22000 $M^{-1}cm^{-1.}$

The maximum absorption wavelength (510 nm) of the resulting red colored dye was the same as that obtained by Barham and Trinder [39, 41]. The product of the reaction mixture (4-AP, DCPS, H_2O_2) in the presence of iron both as catalyst and analyte is a quinine imine dye. The possible chromogenic reaction is as follows [42].



DCPS 4-AP Quinone imine

Figure 2. Reaction of DCPS and 4-AP in the Presence of H_2O_2

5.2. Optimization of Parameters

The conditions for the determination of iron were optimized by studying the influence of different parameters such as pH, flow rate, and reagents concentration. A 4 mM iron(II) solution was used for the optimization of the above parameters. In all cases, the peak height was recorded manually.

5.2.1. Optimization of pH of Carrier Solution

The effect of pH was studied by preparing potassium hydrogen phthalate (KHP) buffer solutions of various pH values ranging from 3 to 6 from 0.1 M potassium hydrogen phthalate (KHP) final concentration 0.05 M, 0.1 M HCl and 0.1 M NaOH. To see the effect of pH on absorbance, 3 mL solution of 4-AP (final concentration 1 mM), 6 mL solution of DCPS (final concentration 4 mM) and 6 mL solution of H_2O_2 (final concentration 50 mM) were mixed and 20 μL of 4 mM solution of Fe(II) was injected through the injection valve. Both the mixture of the reagents and the carrier buffer solution were pumped with the total flow rate of 0.84 mL min^{-1}.

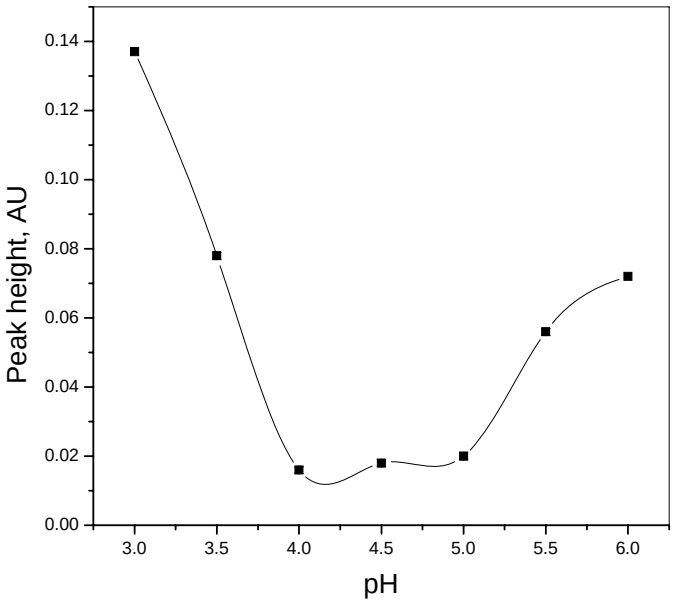


Figure 3. Plot of absorbance versus pH.

Fig. 3 shows the influence of pH on the absorbance. There is a sharp decrease in response from pH 3 to pH 4. Beyond pH 4 there is an increase in responses to about pH 6. Although the absorbance peak height at pH 3 is maximum value, the mixture of the reagents developed color before the injection of the sample which may give high back ground absorbance value. Therefore pH 6 was chosen as the optimum working pH.

5.2.2. Flow Rate Optimization

The flow rate is evaluated between 0.26 mL min^{-1} and 0.90 mL min^{-1} at pH 6 by changing the speed of the rotation of the pump. The final concentrations of H_2O_2, DCPS, and Fe(II) were 50, 4, and 4 mM respectively. The results in Fig. 4 revealed that there is a sharp decrease in response from 0.26 mL min^{-1} to about 0.38 mL min^{-1}. Beyond 0.38 mL min^{-1} there is a slight decrease in response. The reason for the higher signal obtained at lower flow rate is that the residence time at lower flow rate is higher and therefore resulted in better mixing of the reaction component giving better signal.

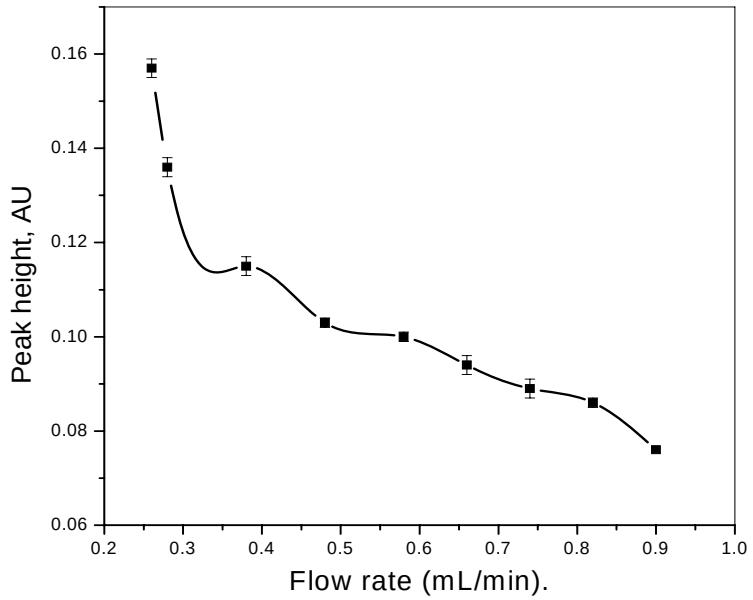


Figure 4. Plot of absorbance versus flow rate.

Although the response at 0.26 mL min^{-1} and 0.28 mL min^{-1} are higher than 0.38 mL min^{-1} the latter gave a better sample throughput and chosen as the optimum working flow rate.

5.2.3. Optimization of Concentration of Reagents

5.2.3.1. Optimization of Concentration of 4-AP

The 4- AP concentration was evaluated between 0.5 mM to 4 mM at pH 6, flow rate 0.38 mL/min, and final concentrations 50, 4, and 4 mM of H_2O_2, DCPS, and Fe(II) respectively. The effect of concentration of 4-AP is presented in figure 5. It is clear from figure 4 that the absorbance steadily increases with an increase in concentration up to 1.5 mM final concentration then gradually decreases up to 4 mM. Therefore 1.5 mM final concentration gave the best absorbance and was chosen as optimum concentration.

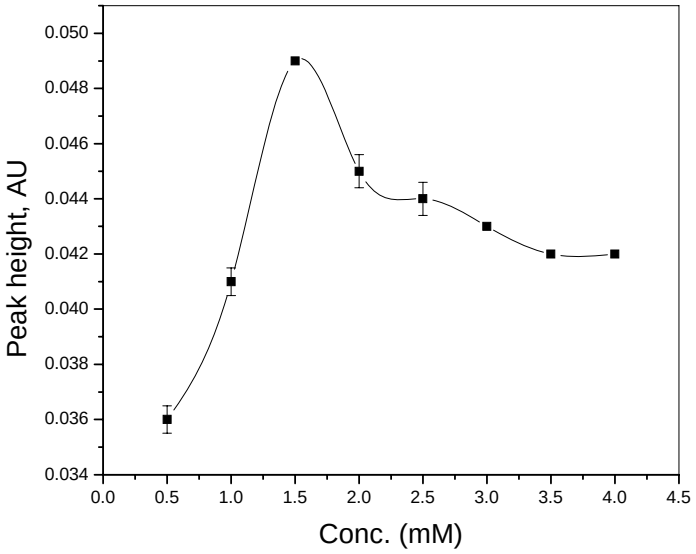


Figure 5. Plot of absorbance versus concentration of 4-AP.

5.2.3.2. Optimization of Concentration of DCPS

The influence of concentration of DCPS on the flow injection system was investigated by changing the concentration of DCPS in the range of 1 mM to 8 mM by using the same

KHP buffer, flow rate 0.38 mL/min, and final concentrations 50, 1.5, and 4 mM of H_2O_2, 4-AP, and Fe(II) respectively. The influence of concentration of DCPS is shown in figure 6 below. The maximum absorbance was obtained at 3 mM final concentration of DCPS and beyond 3 mM the responses were decreasing and reached minimum for 8 mM. Thus 3 mM final concentration of DCPS was chosen as optimum concentration.

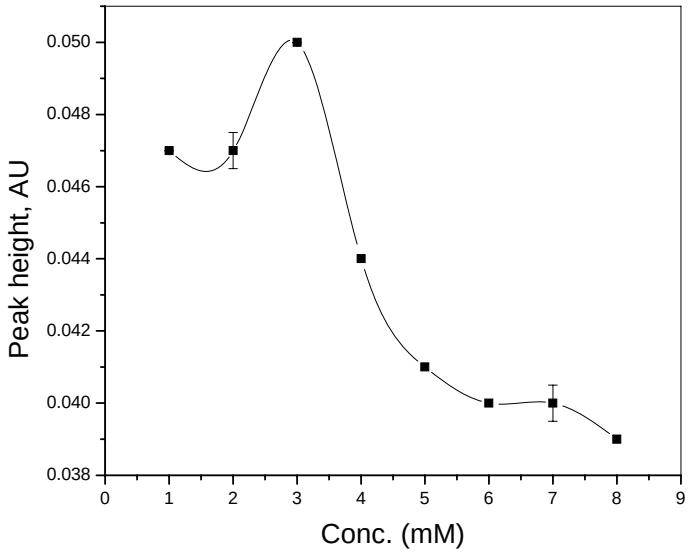


Figure 6. Plot of absorbance versus concentration of DCPS.

5.2.3.3. Optimization of Concentration of H_2O_2

The effect of concentration of H_2O_2 was studied in the range of 10 mM to 50 mM by using all the above optimized conditions and 4 mM of iron(II). The results in Fig. 7 revealed that there is an increase in response from 10 mM and reached maximum response at 20 mM, and then the response decreases gradually to 50 mM. Therefore 20 mM was chosen as the optimum concentration of H_2O_2.

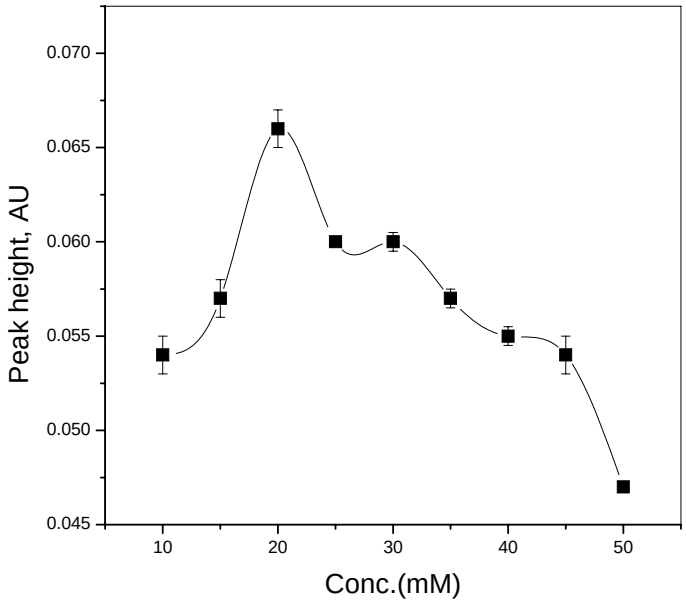


Figure 7. Plot of absorbance versus concentration of H_2O_2.

5.3. Calibration curve for iron(II) and iron(III)

A calibration curve for iron(II) and Fe(III) are shown below in Figure 8 and 9 respectively. For both cases 3 mL of 4.5 mM 4-AP (final concentration 1.5 mM), 3 mL of 9 mM DCPS (final concentration 3 mM) and 3 mL of 60 mM H_2O_2 (final concentration 20 mM) were mixed in to a 50 mL beaker and different concentrations of freshly prepared iron(II) (1, 5, 10, 15, 20, 25 μM) were injected in to the carrier streams and triplicate measurements were recorded manually from the flow-through spectro-photometer.

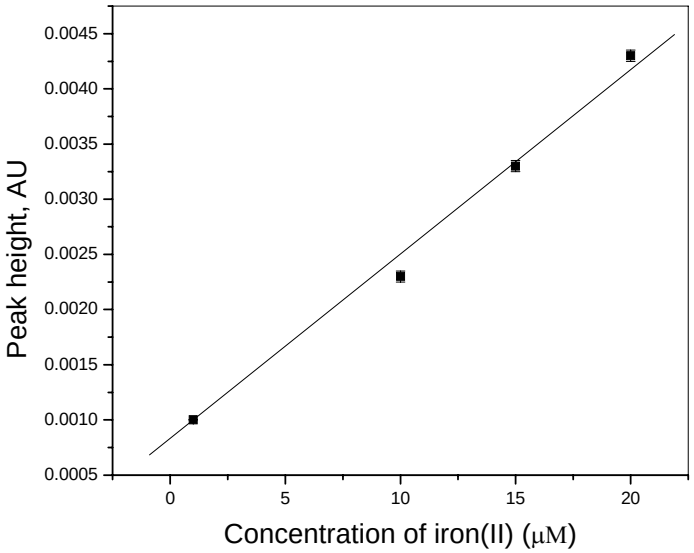


Figure 8. Calibration curve for iron(II) using optimized conditions.

The equation for the calibration curve of Fe(II) was $A = 1.734 \times 10^{-4}C + 7.315 \times 10^{-4}$. Where A is absorbance at 510 nm and C is the concentration of iron in μM. The linear regression coefficient obtained was r = 0.996. The calibration graph was linear in the range between 1 μM and 20 μM. Below 1 μM, the response was alternating with the background value and above 20 μM the response was increasing but not linearly.

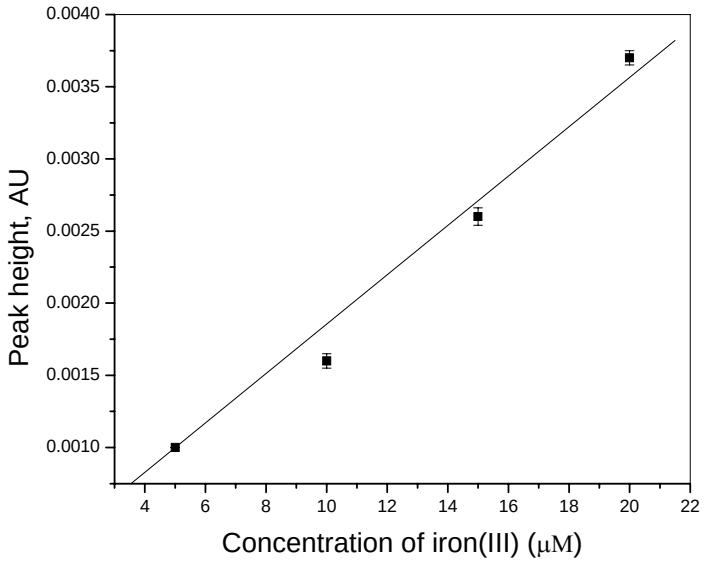


Figure 9. Calibration curve for iron(III) using optimized conditions

The equation for the calibration curve of Fe(II) was $A = 1.82 \times 10^{-4} C - 5 \times 10^{-4}$. Where A is absorbance at 510 nm and C is the concentration of iron in μM. The linear regression coefficient obtained was r = 0.992. The calibration curve was linear in the range between 5 μM and 20 μM above 20 μM the response was deviating from linearity and below 5 μM the response was the same as with the background absorbance.

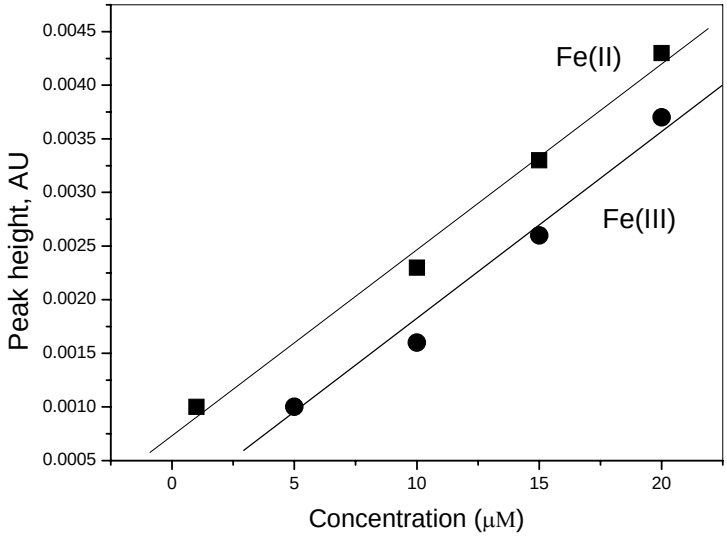


Figure 10. Calibration curve of iron(II) and iron(III).

5.4. Kinetic Study for Iron(II) and Iron(III)

For kinetic studies, 3 mL of 4-AP (6 mM), 3 mL of DCPS (12 mM), 3 mL of H_2O_2 (80 mM) and 3 mL of Fe(II) were mixed in to a 50 mL volumetric flask and KHP buffer solution (pH 6) was used as a carrier and applied on a single line flow injection system with flow rat of 0.66 mL/min. Absorbance readings were taken manually from spectrophotometric detector in five minutes intervals starting from the time of mixing of the reagents to about 50 minutes and the same experiment was repeated for Fe(III). The results obtained for both iron(II) and Fe(III) presented in the figure 10 below. As shown in the figure 10, there is an increase in absorbance with increase in time to about 30 minutes for both iron(II) and iron(III). Beyond 30 minutes the increment of the absorbance decreases with in increase in time. This indicates that the intensity of the colored dye complex increases to about 30 min. and then the intensity of the color dye complex become stable as the time increase.

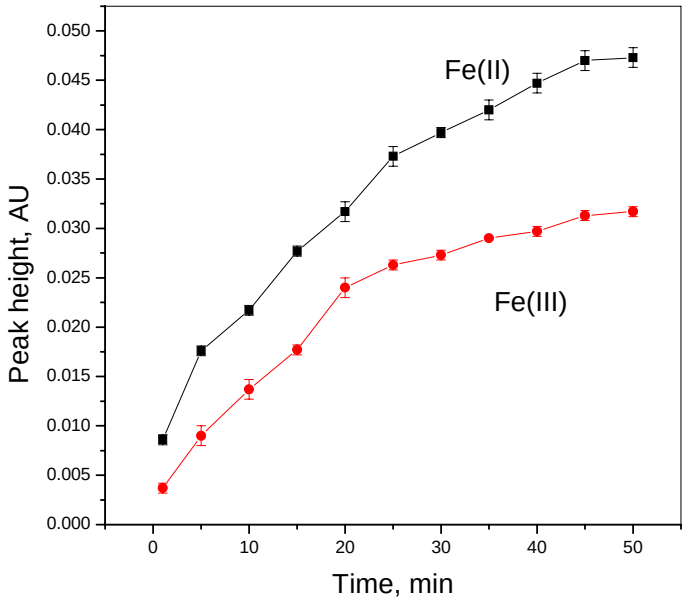


Figure 11. Plot of absorbance versus time for iron(II) and iron(III).

Researchers have reported that FIA assays with spectrophotometric detector have a lower sensitivity and poorer limit of detection than many manual methods. Two reasons for this lower sensitivity and poorer limit of detection are short reaction times in FIA systems (typically less than a minute) which is due to the short residence time that result in a relatively low yield of reaction product and an excessive dispersion of the sample zone which result in an unwanted dilution of the species to be measured [33, 35, 43, 44].

Stopped-flow FIA eliminates the loss of sensitivity due to short reaction times. In the stop-flow mode the pump is stopped for a certain period at a predetermined time after injection of the sample to allow further reaction between sample and reagents to occur. This approach used to enhance the sensitivity of analyses based on reactions with slow kinetics and to discriminate analyte signal from background signal in samples having a large blank [43, 44]. For the present study the stopped-flow technique have been tried so as to increase the sensitivity of the proposed method as others did. However it was not successful. From the stopped-flow principle, after the pump is stopped, the detector should show constant absorbance value obtained just before stopped-flow technique is applied i.e. the absorbance value of the detector reading should be stable until the flow

system is allowed to proceed to flow. But in our case we could not obtain this stable absorbance reading, rather the absorbance reading increased gradually during the prescribed stop flow time. Therefore it was not possible to increase the sensitivity of the method with a stop-flow technique.

As mentioned before one of the drawback of FIA technique is unnecessary dilution (dispersion) of the sample analyte. But many authors tried to overcome this problem by injecting large sample volume [33]. To do this the injection loop should be large so as to accommodate the large sample volume injected. For the present study our injection loop was small which can accommodate only 20 μl. However, due to time and material constraints we could not attempt the large sample volume principle.

Beside the above two techniques researchers tried to use other techniques such as use of activators and pre-concentration technique to increase the sensitivity of different methods to determine iron in aqueous sample [3]. The use of activators has been reported to increase the sensitivity of kinetic-catalytic determination of iron. Nakano *et. al.* reported photometric-catalytic method for the determination of iron (II, III) based on the oxidative coupling of 4-Aminoantipyrine with *N,N*- Dimethylaniline in the presence of hydrogen peroxide as an oxidant and acetate as an activator [3, 45]. Having this as a basis, this activator and others such as tartarate and citrate were checked for their efficiency on the increment of the sensitivity of the proposed method. However the result was not promising i.e. there was no significant change in response before and after the addition of these activators. The possible reason for the discrepancy of the present result with the reported one may be attributed to the difference in the reagent components used.

Some authors indicated that the reaction rate is better at around 50 ^{0}C [45] and therefore it is important to optimize the reaction temperature. However, due to time and material constraints we could not attempt the optimization of reaction temperature. The last option is using online/off line pre-concentration procedure. Thus, attempting the steps suggested above one may improve the sensitivity of the kinetic-catalytic determination of iron described in this project.

6. CONCLUSION

The kinetic catalytic spectrophotometric flow injection analysis (FIA) method developed for the determination of total iron in aqueous sample is simple and inexpensive. To apply the proposed method some parameters such as pH, flow rate and reagent concentration were optimized where as optimization of reaction temperature and sample injection volume are not attempt due to time and material constraints. Depending upon the optimized conditions iron can be determined in the concentration range 1 μM- 20 μ M.

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