INVESTIGATION
OF
IRON-N-PHENYLCINNAMOHYDROXAMIC ACID SYSTEMS
AND
THEIR ANALYTICAL POTENTIALITIES

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Investigation of Iron-N-Phenylcinnamohydroxamic Acid Systems and Their Analytical Potentialities

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TO MY FATHER, MOTHER,
SISTERS AND BROTHERS

CONTENTS

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LIST OF TABLES

LIST OF FIGURES

Page

LIST OF SYMBOLS

A	absorbance
a	activity
α	side reaction coefficient
B	pH-meter reading
$(\beta)_a$	overall thermodynamic stability constant
β	overall concentration stability constant
β'	overall conditional stability constant
β_{HL}	overall protonation constant
C_L	total concentration of the ligand
C_M	total concentration of the metal ion
$[L']$	apparent concentration of the ligand, i.e the concentration of the ligand that has not reacted in the main complex formation reaction
$[M']$	apparent concentration of the metal ion, i.e the concentration of the metal not involved in the main complex formation reaction
D_M	distribution ratio (extraction)
ε	molar absorptivity
f	activity coefficient
I	ionic strength
K_a	dissociation constant of an acid
K	stepwise stability constant

K'	composite stability constant
K_D	distribution constant
λ	wavelength
L	ligand
M	metal; metal ion
$pH_{1/2} = pH_{n-1=n}$	pH of the solution at which two consecutive complex species have equal concentrations
pH_n	pH of the solution at which a partial mole fraction of a complex species would attain a maximum value
U_H	the conversion factor for obtaining the hydrogen ion concentration from the pH-meter reading B
X	partial mole fraction of a complex species

ABSTRACT

INVESTIGATION OF IRON-N-PHENYLCINNAMOHYDROXAMIC ACID SYSTEMS AND THEIR ANALYTICAL POTENTIALITIES

by

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R. Advisor Dr. B.S. Chandravanshi

This work includes the study of the reactions of N-phenylcinnamohydroxamic acid(PCHA) with both bivalent and trivalent iron. The first part of the work deals with the complex equilibria of iron(III)-PCHA system in a mixed aqueous-organic solvent, and the second part deals with the development of a new method for the determination of iron(II) with PCHA.

N-phenylcinnamohydroxamic acid (PCHA), a bidentate chelating agent was found to react with iron(III) to form complex species of different colour depending upon the reaction environment. The reaction conditions for the formation of the complex species were studied in aqueous-alcoholic medium. A mixed solvent of 75% (v/v) ethanol by composition has been found to be a suitable medium for the study of homogenous equilibria of the coexisting complex species. The general spectral properties of the species were investigated. The absorption curves were found to have two isosbestic points. The number and composition of the complexes were determined and found to have composition 1:1, 1:2, 1:3 (Fe:PCHA). The wavelengths of the maximum absorbances were 535, 495, and 445 nm for the I, II, and III complex species, respectively. It was verified that the Beer-Lambert Law holds for these complexes at all wavelengths, and for the mixtures at the wavelengths of the isosbestic points in a wide pH range. The existence of two isosbestic points which are equal in number to those of the complexes, except one, made iron-(III)-PCHA system amenable to the Varielle method for the determination of stepwise stability constants. These constants have been found to be log $K_1 = 11.547$, log $K_2 = 10.106$ and log $K_3 = 7.438$ for the I, II, and III stepwise stability constants, respectively. The distribution diagrams (nomograms) of the complex species as a function of pH were constructed for some concentrations of the metal ion and various concentration ratios of the ligand to metal ion. By using the nomogram, extinction coefficients of the three consecutive complexes have also been determined.

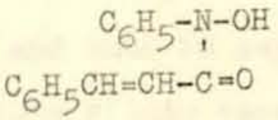
PCHA was also found to react with iron(II) to produce an orange colored, extractable electroneutral complex having a composition of 1:3 (Fe:PCHA). The complex is stable and quantitatively extracted into toluene from aqueous solution having a pH range of 4.5 to 9.0 allowing reproducible and reliable spectrophotometric determination of iron. The complex has maximum absorption at 448 nm and a molar absorptivity of 8.4×10^3 l $mol^{-1}cm^{-1}$. Beer's law is obeyed in the range of 1.00 to 9.00 ppm of iron(II). The overall conditional stability constant of the complex has been also determined. From the study of the effect of several variables, it was found that the method is largely free from rigid control of experimental variables. The effect of foreign ions on the method has also been studied and the method was found to be fairly selective. The method was found to be effective by applying it for the determination of iron in blood and steel samples. Thus a new rapid, precise, sensitive, and selective method for the determination of Fe(II) with N-phenylcinnamohydroxamic acid is developed.

1. INTRODUCTION

The application of organic reagents in analytical chemistry for the detection, separation and determination of trace quantities of nearly all common and most rare elements in a large variety of natural materials and industrial products is becoming increasingly important (1-7). Organic reagents react with inorganic ions in solution to yield products of various properties which can exhibit a change in color, luminescence, solubility and volatility. These properties led to the development of various methods that are sensitive, selective and rapid for the analysis of a variety of materials.

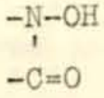
One of the powerful organic reagents that have been employed for the analysis of metal ions in recent years is N-phenylcinnamohydroxamic acid(PCHA), which forms the basis of the present investigation.

N-phenylcinnamohydroxamic acid(I) contains an acid group with a replaceable hydrogen atom and a basic co-ordinating donor group(II) in such a position so as to form five-membered chelate complexes on reaction with metal ions.



$$\begin{array}{r} C_6H_5\text{-}N\text{-}OH \\ | \quad\quad \\ C_6H_5CH{=}CH\text{-}C{=}O \end{array}$$

(I)



$$\begin{array}{l} \text{-}N\text{-}OH \\ \;\; | \\ \text{-}C{=}O \end{array}$$

(II)

PCHA is a highly sensitive and selective spectrophotometric reagent for metals because of the presence of double bond in conjugation with carbonyl group in the molecule. It absorbs very weakly in the region where its colorimetric reaction product with several metal ions exhibit characteristic intense absorption band. Thus spectrophotometric method can particularly be used to advantage for studying complexation equilibria, and for the determination of the metal ions with which it forms complexes.

PCHA is a very weak acid and its dissociation constant is 1.6×10^{-9} (8). It is a crystalline solid and pale green by colour and has a melting point of 159-160°C. It is stable towards air, light and heat and can be stored for a long time without deterioration. It is stable towards the action of dilute alkalies and concentrated hydrochloric acid but decomposes in concentrated sulfuric acid and nitric acid (> 5 N) (9). It is therefore found to be a suitable extraction reagent for use in acidic solution. Thus some metal ions such as vanadium(V) (10), zirconium(IV) (11), niobium(V) and titanium(IV) (12) can be extracted into chloroform from hydrochloric acid solution greater than 2M, and thus be separated from others which need more dilute acidic condition for extraction. It is slightly soluble in water but soluble in common organic solvents. Its solution in common organic solvents can be stored for a long time without deterioration and this quality is very important from an analytical stand point.

N-phenylcinnamohydroxamic acid was first synthesized and used as analytical reagent for the gravimetric determination of niobium and tantalum in 1960 (13). Its analytical applications was further extended for the extraction and photometric determination of vanadium(V) (10), zirconium(IV) and hafnium(IV) (11), titanium(IV) and niobium(V) (12), and for the successive extraction and spectrophotometric determination of iron(III), vanadium(V), and uranium(VI) (14). It was also used for the gravimetric determination of niobium and tantalum (15), uranium (16), and zirconium (17). Recent reports show that PCHA has been used for the extraction and spectrophotometric determination of vanadium(V) as a mixed ligand complex with thiocyanate (18) and P-chlorophenol (19), for the successive extraction and spectrophotometric determination of iron(III) and vanadium(V) (20) and simultaneous determination of iron(III) and vanadium(V) by solvent extraction and spectrophotometric methods (21).

The foregoing discussion reveals that PCHA has been used as a reagent for the extraction and spectrophotometric determination of iron(III). However, no attempt has been made to study its complexation equilibria with tervalent iron and its reaction with bivalent iron. Hence, the detailed studies of PCHA-iron system and their analytical potentialities have been the task of the present investigation.

Iron is the most abundant metal in nature, apart from aluminum (22-24). It is very seldom found as the free metal, but metallic iron occurs with nickel and cobalt in some type of meteorite, and in very finely distributed form in some basalt. Its most important ores and minerals are haematite (Fe_2O_3), magnetite (Fe_3O_4), goethite (FeOOH), limonite (hydrated iron oxide), siderite ($FeCO_3$) and pyrites (FeS_2). Mixed oxides, silicates and aluminates with a number of metals are also encountered. Natural waters sometimes contain considerable amount of iron. Iron can also be found in a number of natural substances, plants, and animals including, human organs. Iron was the first trace or minor element recognized as being necessary for both plants and animals. Manufactured iron always contains carbide, phosphide, silicide, manganese and graphite or amorphous carbon. Samples to be analyzed often contain iron as its sulphates, chlorides, and double or complex salts. The ferrous alloys (e.g ferrochrome, ferrosilicon, ferromanganese, etc.) are also frequently encountered.

The oxidation state encountered in compounds of iron range from 0 to +6; however, the most common compounds are those of Fe(II) and Fe(III). In complex compounds iron(II) may be tetra-coordinate (e.g., $FeCl_4^{2-}$ with a tetrahedral configuration), though more often it has coordination number six, e.g., in the octahedral complexes $Fe(H_2O)_6^{2+}$, $Fe(CN)_6^{4-}$, $Fe(phen)_3^{2+}$, and sometimes even eight (oxyhaemoglobin, carbonyl haemoglobin). The complexes of iron(III} are also more often octahedral, as exemplified by $Fe(acac)_3$, $Fe(CN)_6^{3-}$, $Fe(H_2O)_6^{3+}$, and $FeCl_4(H_2O)_2^-$; the tetrahedral complex $FeCl_4^-$ exists in the medium of concentrated hydrochloric acid (25,26). The coordination number of iron(III) in the complex $Fe(EDTA)(H_2O)^-$ is seven (27).

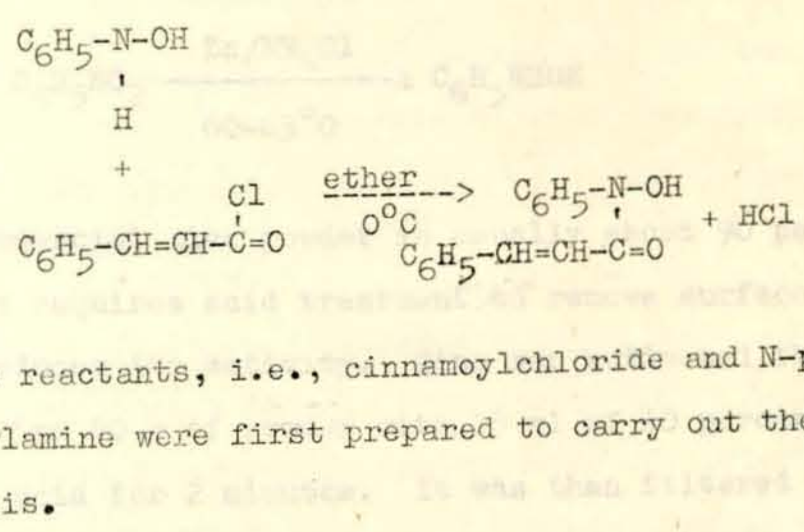
A number of organic reagents are recommended for the detection, separation, and determination of iron. Some of the common organic reagents that are used particularly for spectrophotometric determination of iron are ammonium thiocyanate (28), 2,2'-bipyridyl (29), cupeferron (4), 4,7-diphenyl-1,10-phenanthroline (30), 4,7-diphenyl-1,10-phenanthroline disulphonic acid disodium salt (31), 5-methoxy-2-nitrosophenol (4), 1,10-phenanthroline (32), 2,4,6-tri-2-pyridyl-s-triazine (4), 2-(2-pyridyl)-imidazoline (33), acetylacetone (34), isonitrosodimethyldihydroresorcinol (35), 4-hydroxybiphenyl-3-carboxylic acid (36), etc.

Of these reagents comparatively few are well suited for determination of traces of iron. Therefore, from the point of view of its importance, abundance and occurence, the development of new method for the determination of iron needs no comment.

2. EXPERIMENTAL

2.1 Synthesis of N-Phenylcinnamohydroxamic Acid (PCHA)

PCHA was prepared by following the method used by Priyadarshini and Tandon (37). This involves the condensation of N-phenylhydroxylamine with cinnamoylchloride at low temperature in diethylether medium made alkaline with an aqueous suspension of sodium bicarbonate.



$$C_6H_5\text{-}\underset{H}{N}\text{-}OH + C_6H_5\text{-}CH{=}CH\text{-}\overset{Cl}{C}{=}O \xrightarrow[0^{o}C]{ether} C_6H_5\text{-}\underset{C_6H_5\text{-}CH=CH\text{-}C=O}{N}\text{-}OH + HCl$$

The two reactants, i.e., cinnamoylchloride and N-phenylhydroxylamine were first prepared to carry out the synthesis.

Preparation of cinnamoylchloride: This was prepared by refluxing cinnamic acid with excess thionylchloride.

$$C_6H_5\text{-}CH{=}CH\text{-}COOH + SOCl_2 \xrightarrow[100^{o}C]{reflux} C_6H_5\text{-}CH{=}CH\text{-}COCl + HCl + SO_2$$

Cinnamic acid (14.8 g, 0.1 mol) and thionychloride (12 ml, 0.15 mol) were mixed in a 100-ml round bottom flask and refluxed over boiling water bath for about two hours. The excess thionylchloride was driven off from the clear solution of cinnamoylchloride by fractional distillation under reduced pressure. The yield of acid chloride was about 90%.

Preparation of N-phenylhydroxylamine: The preparation was done by the reduction of nitrobenzene with zinc dust and ammonium chloride in aqueous solution at 60-65°C.

$$C_6H_5NO_2 \xrightarrow[60\text{-}65^\circ C]{Zn/NH_4Cl} C_6H_5NHOH$$

Commercial zinc powder is usually about 90 percent pure and requires acid treatment to remove surface oxide which reduces its activity. Zinc was activated therefore, by stirring 40 g of powder with 15 ml of 10 percent hydrochloric acid for 2 minutes. It was then filtered and washed with 30 ml of water followed by 10 ml of acetone.

To prepare N-phenylhydroxylamine 12.5 g of ammonium chloride, 400 ml of water and 25 g (20.8 ml, 0.205 mol) of nitrobenzene were placed in a 2-litre beaker, equipped with a thermometer and mechanical stirrer. The mixture was stirred vigorously and 29.5 g (0.415 mol) of zinc powder of high purity was added during about 15 minutes. The rate of addition was such that the temperature rapidly rises to 60-65°C and remains in this range until all the zinc has been added. The stirring was continued for further 15 minutes, by which time the reduction was complete as was shown by the fact that the temperature commenced to fall. The warm reaction mixture was filtered by suction to remove the zinc oxide, and washed with 50 ml of hot water. The filtrate was placed in a conical flask, saturated with common salt (about 150 g) and cooled in an ice bath for about one hour to ensure maximum crystallization of the product. The pale yellow crystals of phenylhydroxylamine were filtered with suction. It was then recrystallized from benzene and petroleum ether. The substance deteriorates upon storage and was therefore used immediately for the synthesis of N-phenylcinnamohydroxamic acid.

Preparation of N-phenylcinnamohydroxamic acid: A freshly crystallized N-phenylhydroxylamine (10.9 g, 0.1 mol) was dissolved in 100 ml of diethylether and transferred to a 500-ml conical flask. A fine suspension of sodiumbicarbonate (12.6 g, 0.15 mol) in 20 ml of water was added to it. The flask was kept in a freezing mixture and the contents were stirred. To this, a solution of cinnamoylchloride (16.65 g, 0.10 mol) in 100 ml of diethylether was added dropwise during the course of one hour. Stirring was continued for an additional 15 minutes and the temperature was maintained throughout at 0°C. The yellowish white precipitate of hydroxamic acid was then separated, from the saturated solution of sodium bicarbonate in a mortar for about 15 minutes to remove acidic impurities. It was then filtered under suction, washed with cold water and dried in air. The solid N-phenylcinnamohydroxamic acid was then crystallized from ethanol. Yield, 12 g (50%), m.p. 159-160°C.

2.2 Equipment and Reagents

Apparatus: A beckman CHEM MATE pH meter was used for the measurement of pH and a Beckman Model 24 UV-visible spectrophotometer was used for absorbance measurements. The pH meter was standardized against aqueous buffers prepared from NBS standard samples.

Standard Fe(III) solution: A stock solution of iron(III) was prepared by dissolving 4.0402 g of ferric nitrate nonahydrate (BDH, AnalaR) in 100 ml of 1:5 (v/v) nitric acid and diluted to 1.0 litre with distilled water. The solution was standardized spectrophotometrically using bipyridyl and PCHA (20). A working solution was prepared by diluting a suitable aliquot of the standard solution to a known volume with distilled water.

Standard Fe(II) solution: A stock solution of iron(II) was prepared by dissolving 3.9214 g ammonium iron(II) sulphate hexahydrate (BDH, AnalaR) with distilled water, acidified with about 30 ml concentrated hydrochloric acid and diluted to 1 litre with distilled water. The stock solution was standardized spectrophotometrically by using bipyridyl as analytical reagent.

Electrolyte solutions: A 1.0 M aqueous solution of potassium nitrate (BDH, AnalaR) and potassium chloride (BDH, AnalaR) were used.

Reducing agents: A 0.1 M aqueous solution of hydroquinone and ascorbic acid, and a 10 percent (w/v) solution of hydroxylamine hydrochloride were used for the reduction of iron(III) to iron(II).

Hydrochloric acid and ammonia: Dilute solutions of hydrochloric acid (BDH, AnalaR) and ammonia (Spectrosol) were used for adjusting the pH of the solution.

Reagent solution: A 0.05 M solution of PCHA was prepared in ethanol. The working solutions were prepared by diluting a suitable aliquot of the standard solution to a known volume with ethanol.

2,2'-Bipyridyl solution: A 0.01 M solution of the reagent was prepared in distilled water.

Solutions of foreign ions: They were prepared by dissolving known quantities of reagent grade salts in distilled water to give 10 mg of the ion of interest per millilitre of solution following the procedure of West (38). The solutions were acidified wherever necessary to prevent hydrolysis and to increase the solubilities of certain salts. In general nitrate salts were used for the cations and sodium or ammonium salts were used for the anions.

2.3 Preparation of Sample Solutions

Steel sample: A weighed quantity (approximately 0.1 g) of the steel sample was transferred into a 400-ml beaker and decomposed with 10 ml concentrated nitric acid. The mixture was heated to remove the oxides of nitrogen and about 10-15 ml concentrated hydrochloric acid was added to it. The solution was evaporated to almost dryness. About 50 ml of distilled water was added and the solution was boiled. Tungsten was precipitated as tungstic acid. The undissolved silica and tungstic acid were filtered off and washed several times with hot distilled water. The filtrate and washings were collected into a 500-ml volumetric flask and diluted to volume with distilled water. A suitable aliquot was taken for the analysis.

Blood sample: A 4 ml aliquot of blood sample was transferred to a Kjeldahl flask and decomposed with about 20 ml of concentrated sulfuric acid by heating over the bunsen burner for about two hours. The heating was continued after addition of about 20 ml of perchloric acid until the volume of the content was reduced to about 5 ml. It was then cooled and the residue was reboiled with dilute hydrochloric acid for about one hour. After cooling the residue was transferred quantitatively to 100-ml volumetric flask and diluted to volume with distilled water. A suitable aliquot was taken for the analysis.

2.4 General Procedure for the Investigation of Iron(III)-PCHA Complex Equilibria

A suitable aliquot of the standard iron(III) solution (0.005 M) was transferred into a 50-ml beaker to give a final concentration of 2×10^{-5} - 4×10^{-4} molar solution. Potassium nitrate solution was added to adjust the ionic strength of the solution to 0.1 M. The required amount of PCHA solution in ethanol was added and the solution was diluted to about 20 ml with ethanol. The pH of the solution was adjusted to the required value with dilute hydrochloric acid and ammonia. The solution was transferred quantitatively into a 25-ml volumetric flask. It was then diluted to volume with ethanol and distilled water to give 75% of ethanol in the final solution. The absorption spectrum was recorded against ethanol. The pH of the solution was measured once again after the absorbance measurement. The temperature was kept at $20.0 \pm 0.2^{\circ}C$.

2.5 General Procedure for Extraction and Spectrophotometric Determination of Fe(II).

An aliquot of the solution containing 25-200 µg of iron(II) was transferred into a 50-ml beaker and 1 ml of 0.1 M hydroquinone was added to it. A 5 ml aliquot of 0.005 M solution of the reagent (PCHA) in ethanol was added to the solution and the solution was diluted to 25 ml with distilled water. The pH of the solution was adjusted to 5-8 using dilute solutions of hydrochloric acid or ammonia. The solution was transferredquantitatively into a 100-ml separatory funnel. The electrode of the pH meter and the beaker were washed with about 15 ml of toluene. The washings were added to the funnel and the mixture was shaken vigorously for two minutes. The funnel was allowed to stand to separate the two phases. The organic phase was collected into 50-ml beaker containing a few grams of anhydrous sodium sulphate. The coloured extract was transferred to a 25-ml volumetric flask and the beaker was washed several times with a few ml of toluene. The washings were added to the flask and the solution was diluted to volume with toluene. The absorbance was measured at 448 nm against the reagent blank.

3. INVESTIGATION OF IRON(III)-PCHA SYSTEM IN WATER-ETHANOL MEDIUM

3.1 General Aspects of Complex Equilibria

The formation of metal complexes by the reaction of a metal ion (central ion) and a reagent (ligand) can be represented by the following general equilibrium reaction (5-7, 39):

$$mM + nL \rightleftharpoons M_mL_n \qquad (3.1$$

where M denotes the metal ion and L the ligand. The complex-formation equilibrium is characterized by the thermodynamic equilibrium constant.

$$(\beta_{nm})_a = \frac{a_{M_mL_n}}{a_M^m \; a_L^n} \qquad (3.2$$

where a_i denotes the activity of the ith species. For mononuclear complexes (where $m = 1$) the thermodynamic equilibrium constants is given by:

$$(\beta_n)_a = \frac{a_{ML_n}}{a_M a_L^n} = \frac{[ML_n]}{[M][L]^n} \cdot \frac{f_{ML_n}}{f_M f_L{}^n} \qquad (3.3$$

where f_i denotes the activity coefficient of ith species.

To a first approximation the activity coefficient in dilute solution depend only on the ionic strength. It may often be possible to keep the activity coefficient of each species constant throughout a series of measurements, e.g., by the use of a suitable ionic medium. In such cases, the equilibrium constant may be expressed in terms of the corresponding concentration quotients, or stoichiometric stability constants, defined by

$$\beta_n = \frac{[ML_n]}{[M][L]^n} \qquad (3.4$$

which holds for a given temperature, solvent composition and ionic strength of the solution. Because the activity of a species is equal to the product of its concentration and activity coefficient, there is a simple relationship between the stoichiometric equilibrium constants (involving concentrations) and the thermodynamic equilibrium constants (involving activities) as shown by Equation, 3.3.

The different experimental methods provide information on either concentrations or activities. In general it can be said that electrochemical methods give activities, while optical methods give concentrations.

Generally, it is difficult to determine accurate values of thermodynamic stability constants, especially for systems in which several complexes coexist. Since it is seldom possible to measure the activities or concentrations of more than one or two of the species present, stoichiometric stability constants can usually be determined more conveniently than thermodynamic constants. The latter requires the knowledge of the activity coefficients of all the species under consideration, reliable values of which is often difficult to obtain.

Only seldom does a complexation reaction occur in solution as an isolated process. Simultaneously with the main complex formation equilibrium, the constituents of the complex may also take part in equilibrium reactions with other species present, including ions produced by the solvent. The complex itself may undergo side-reaction. For these conditions the stability constants will be conditional stability constants and will have the following general form:

$$\beta'_n = \frac{[ML'_n]}{[M'][L']^n} \qquad (3.$$

where the concentration terms refer to the apparent free concentration of the species not involved in the main reaction.

One can relate Eq. 3.4 and Eq. 3.5 by the following general expression:

$$\beta'_{ML_n} = \beta_{ML_n} \times \frac{\alpha_{ML_n}(Z)}{\alpha_{M(X)}\alpha^n_{L(Y)}} \qquad (3.6$$

The function $\alpha_{M(X)}$, $\alpha_{L(Y)}$ and $\alpha_{ML_n}(Z)$ are the side-reaction coefficients of the metal M with the ligand X, of the ligand L with side-reaction reactant Y and of the complex with side reaction reactant Z. By use of the overall stability constants, $\beta_p^{(X)}$, $\beta_q^{(Y)}$, $\beta_g^{(Z)}$ of the products formed in the side-reactions,the side-reaction coefficients, are given by:

$$\alpha_{M(X)} = 1 + \beta_1^{(X)}[X] + \beta_2^{(X)}[X]^2 + .. + \beta_p^{(X)}[X]^p = \frac{[M']}{[X]} \qquad (3.7$$

$$\alpha_{L(Y)} = 1 + \beta_1^{(Y)}[Y] + \beta_2^{(Y)}[Y]^2 + .. + \beta_q^{(Y)}[Y]^q = \frac{[L']}{[L]} \qquad (3.8$$

$$\alpha_{ML_n}(Z) = 1 + \beta_1^{(Z)}[Z] + \beta_2^{(Z)}[Z]^2 + .. + \beta_g^{(Z)}[Z]^g = \frac{[ML'_n]}{[ML_n]} \qquad (3.$$

The equilibrium given in Eq. 3.1 may not be attained directly, since the reaction between M and L may occur by stepwise formation of consecutive complexes $ML - ML_N$. The contribution of a particular complex depends on the concentration of the free ligand and on the stepwise stability constants:

$$M + L \rightleftharpoons ML ; \; K_1 = \frac{[ML]}{[M][L]} \tag{3.10}$$

$$ML + L \rightleftharpoons ML_2 ; \; K_2 = \frac{[ML_2]}{[ML][L]} \tag{3.11}$$

$$ML_{n-1} + L \rightleftharpoons ML_n ; \; K_n = \frac{[ML_n]}{[ML_{n-1}][L]} \tag{3.12}$$

The stepwise stability constants are related to the overall stability constants by the following expression.

$$\beta_n = K_1 \cdot K_2 \cdots K_n = \prod_{n=1}^{n=N} K_n \tag{3.13}$$

In general, the value of the first stability constant is the largest and the values of the successive stability constants decrease, i.e. $K_1 > K_2 > K_3 > \cdots K_n$. However, there are exceptions to this generalization. It may also happen that one or more intermediate species practically do not exist, their stability constants being too small to be determined. The fact that a stability constant β_n has been found to be appreciably greater than zero is perhaps one of the most convincing pieces of evidence for the existence of the complex ML_n in solution.

For a system of stepwise complexes of the type ML_n

the mole fraction (X_n) of the nth complex species in the solution, which gives the fraction of the total metal ion C_M is given by:

$$X_n = \frac{[ML_n]}{C_M} = \frac{[ML_n]}{[M]+[ML]+[ML_2]+..+[ML_n]} = \frac{\beta_n[L]^n}{1+\sum_{n=1}^{n=N}\beta_n[L]^n} \qquad (3.14)$$

Thus, if all the stability constants for a given system have been determined, it is possible, in principle, to calculate the equilibrium concentration or activity of each of the species present under a known set of experimental conditions. Such knowledge provide fundamental information necessary for theoretical consideration of the efficiency of an analytical separation or determination based on formation of complexes.

The methods available for determining stability constants are mainly based on the preparation of a series of solutions containing known amounts of the complex-forming components, in which the concentration of one component is gradually varied and the concentration of one of the reactants or products is followed directly or indirectly by a suitable analytical method. The most suitable analytical methods are those which do not distrub the equilibria existing in solution and by which low concentrations can be selectively determined with high accuracy.

Some of the most generally used methods are potentiometric (5,6,39-43), spectrophotometric (5,6,39,44-51) and polarographic methods (5,6,39,52-56).

In the present investigation stepwise stability constants of Fe(III)-PCHA system were determined in partially non-aqueous solvent by spectrophotometric method. The spectrophotometric investigation also includes the study of the nature and reaction condition of the complex formation processes and determination of the number of the successively formed complex species in 75% (v/v) ethanol.

3.2 Results and Discussions

3.2.1 Reaction Condition

It was observed that only in a high percentage of ethanol in water, would the homogenous equilibria in solution be established between the successive complexes of Fe(III)-PCHA. At lower percentage of alcohol or pure aqueous media, the complex with maximum coordination number and the reagent precipitate out. It was also observed that there is a limit as to the highest percentage of ethanol one can use, if an electrolyte is to be added to keep the ionic strength constant. This is because most salts are insoluble in pure ethanol or in a solution with high percentage of ethanol.

It was found that the total concentration of iron(III), C_{Fe}, in the solution can be increased upto 2.4×10^{-4} M with the upper limit of other variables, beyond which the precipitation of the highest complex and hydrolysis of the metal ion occur.

The upper limit of the total concentration of the ligand, C_L, that can be used depends upon the total concentration of the metal in the solution and extent of solubility of the reagent itself in 75% (v/v) ethanol. A concentration as high as 4.8×10^{-3} M can be used, beyond which the precipitation of the highest complex and the reagent take place.

Finally, the range of pH at which the consecutive complex species coexist depends upon the total concentration of iron, ligand and the ratio of the ligand concentration to that of the metal ion.

3.2.2 pH-Meter Measurements in 75% (v/v) Ethanol

The glass electrode has been found to respond reversibly to changes in hydrogen ion activity, in agreement with the Nernst equation in ethanol-water mixtures containing less than 90 percent (w/w) of ethanol (57-59). However, the measurement of hydrogen ion activity by means of a pH-meter which has been standardized using an aqueous buffer is restricted to the aqueous solution, since any variation in the composition of the solvent causes large changes in the junction potential. Therefore the pH-meter readings in the aqueous solution and partially aqueous solution are not the same. Fortunately, it has been possible to bring these scales to the same common denominator, i.e., to express them quantitatively on a single aqueous scale.

One such approach is introduced by Van Uitert and Haas (42,60,61). They measured the 'pH' in dioxan-water mixture with the glass electrode, assuming that the scale-reading is strictly proportional to the pH determined with the hydrogen electrode and established an empirical relationship between the scale reading (B) and the pH, as given by the following equation:

$$pH = B - \log U_H \qquad (3.15)$$

U_H is a conversion factor for obtaining the hydrogen ion concentration from the meter reading B and was found to be in general a function of the percentage of the dioxan and ionic strength of the solution. This same equation was also found to be valid for ethanol (62).

In the present investigation an empirical calibration by means of which the pH-meter reading B could be converted to hydrogen ion concentration was made as follows. Readings were made on a series of solutions containing hydrochloric acid and 0.1 M potassium nitrate in 75% (v/v) ethanol. From a known stoichiometric hydrogen ion concentration $[H^+]$, the value of $\log U_H$ was calculated to be 0.51 ± 0.05. However, it is to be noted that the hydrogen ion concentration obtained from measurement with a glass electrode using this procedure from Eq. 3.15 is the stoichiometric concentration: the actual free hydrogen ion concentration will be less than this because of ion association.

3.2.3 Spectral Properties of Fe(III)-PCHA System

When the absorption curves of the system were recorded by keeping the total ligand to iron concentration ratio constant but increasing the pH of the solution gradually, the concomitant shift of the maximum absorption wavelengths towards shorter wavelengths was observed. (Fig. 3.1). The same result was observed by recording the absorption curves of the complexes at definite pH but varying the ratio of the ligand to metal ion concentration (Fig. 3.2) indicating the existence of more than one complex species in the solution. Thus it was found that with an increase in the ratio of the ligand to metal ion concentration and the pH of the solution more and more of the strongly absorbing species is formed at the expense of the precceding ones.

Moreover, the close inspection of the spectral bands of the complex species in Fig. 3.1 and 3.2 reveals the existence of two common crossing points at two characteristic wavelengths, 612 and 510 nm. These are the isosbestic points or the points of equal absorption for solutions of the same total concentrations of the species under study. Because it is very unlikely that a third species has the very same molar absorptivity at this wavelength, the occurrence of isosbestic point (s) in the formation of a series of complexes gives strong evidence for the simultaneous existence of two absorbing species in the solution.

In Figure 3.3, it can be seen that the ligand and metal ion in 75% (v/v) ethanol do not absorb appreciably in the region greater than 440 nm. It should be particularly noted that at lower pH values the metal ion does not absorb practically beyond 440 nm. This is particularly important from the practical point of view since it is in this range of pH values that free uncomplexed metal ion exists.

3.2.4 Number and Composition of Complexes of Fe(III)-PCHA System

The number and composition of the complex species formed were determined by the continuous varations and mole ratio methods.

Application of the continuous variations method: Method of continuous variations is simple and rapid for determining the formula and also the stability of the complex provided there is assurance that only a single complex is formed from the components in question. However, its use for the determination of the composition and stability values is not as simple when two or more complex species coexist as when a single compound is formed (63-64). Katzin and Gebert (64) derived general relationships for the case of as many as three complexes present simultaneously and showed that eventhough only one complex species absorbs, the position of the maximum is dependent upon the formation constants and may or may not appear at the position corresponding to its stoichiometric composition. A discussion of the various shapes and positions that the maxima in a continuous variations plot might attain, dependent upon the number of species formed and the number absorbing, is given in the article of Katzin and Gebert.

With this caution in mind, continuous variations experiments for the Fe(III)-PCHA were attempted under a variety of conditions most favourable for ascertaining the number and composition of individual complexes in the present investigation. This needs to take measurements at several different wavelengths. It was also necessary to take absorbance measurements of solutions

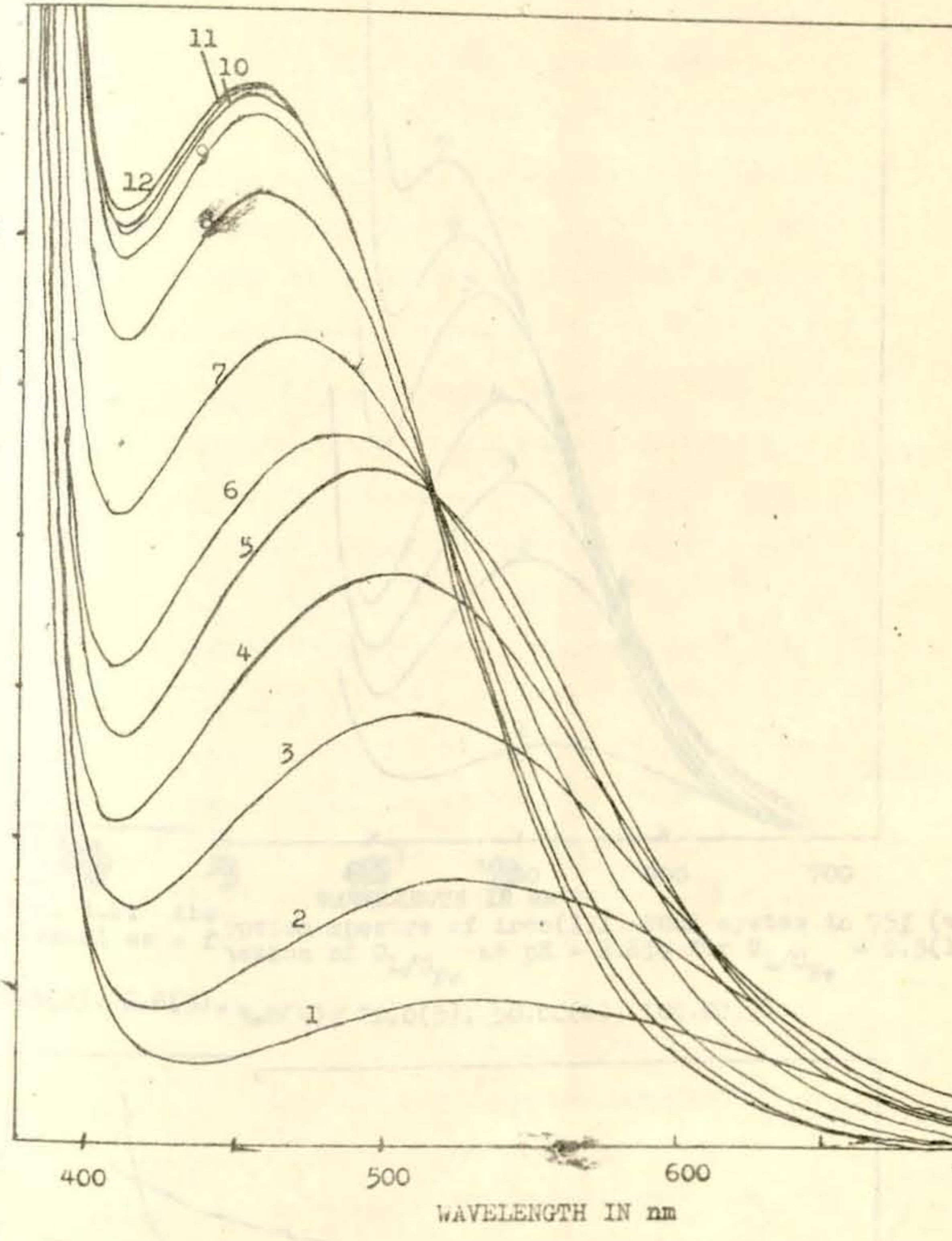


Fig. 3.1. Absorption spectra of iron(III)-PCHA system in 75% (ethanol as a function of pH; $C_{Fe} = 2.0 \times 10^{-4}$; $C_{L/C_{Fe}} = 10$; for values = 0.15(1), 0.65(2), 1.15(3), 1.65(4), 2.25(5), 2.65(6), 2.65(8), 3.15(9), 4.65(10), 5.15(11), 6.65(12).

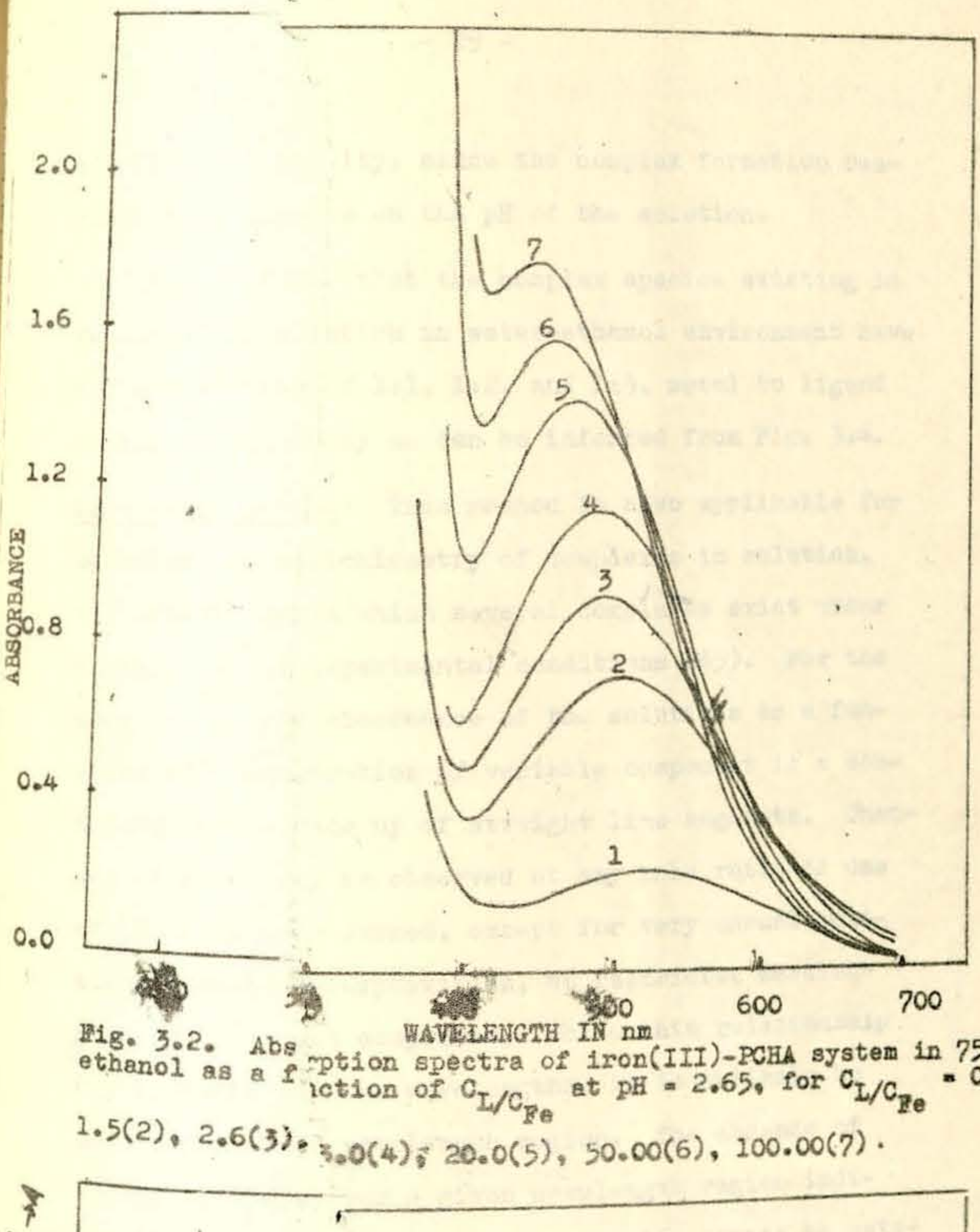


Fig. 3.2. Absorption spectra of iron(III)-PCHA system in 75% (v
ethanol as a function of $C_{L/C_{Fe}}$ at pH = 2.65, for $C_{L/C_{Fe}}$ = 0.5(1
1.5(2), 2.6(3), 3.0(4), 20.0(5), 50.00(6), 100.00(7).

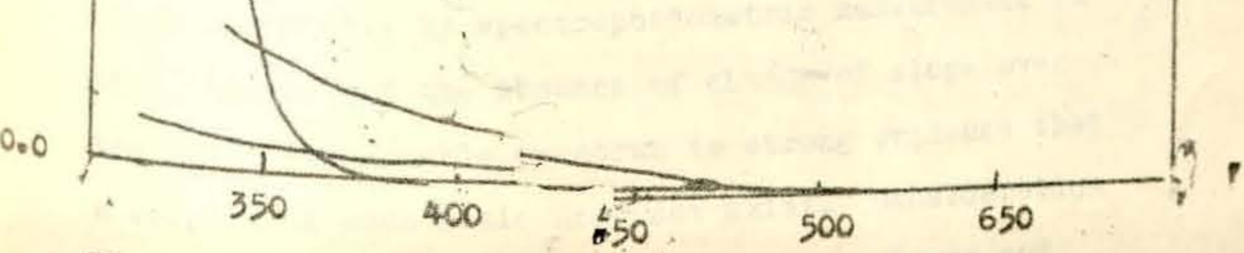


Fig. 3.3. Absorption spectra of PCHA(1) and ferric salts at pH
5.0(3) in 75% (v/v) ethanol

of different acidity, since the complex formation reaction also depends on the pH of the solution.

It was found that the complex species existing in Fe(III)-PCHA solution in water-ethanol environment have the composition of 1:1, 1:2, and 1:3, metal to ligand ratio, respectively as can be inferred from Fig. 3.4.

Mole ratio method: This method is also applicable for deducing the stoichiometry of complexes in solution, for situations in which several complexes exist under a given set of experimental conditions (65). For the ideal case, the absorbance of the solutions as a function of concentration of variable component is a continuous curve made up of straight line segments. Changes of slope may be observed at any mole ratio of one of the complexes formed, except for very unsual relations between absorptivities, at restricted wavelengths, of adjacent complexes. While this relationship may be found at some wavelengths, it is unlikely to hold over a wide wavelength region. The absence of change of slope over a given wavelength region indicates that the existence of the complex cannot be established directly by spectrophotometric measurement in this region, and the absence of change of slope over the entire measurable spectrum is strong evidence that a complex of such ratio does not exist. Consideration of the effects of dissociation of the complexes and

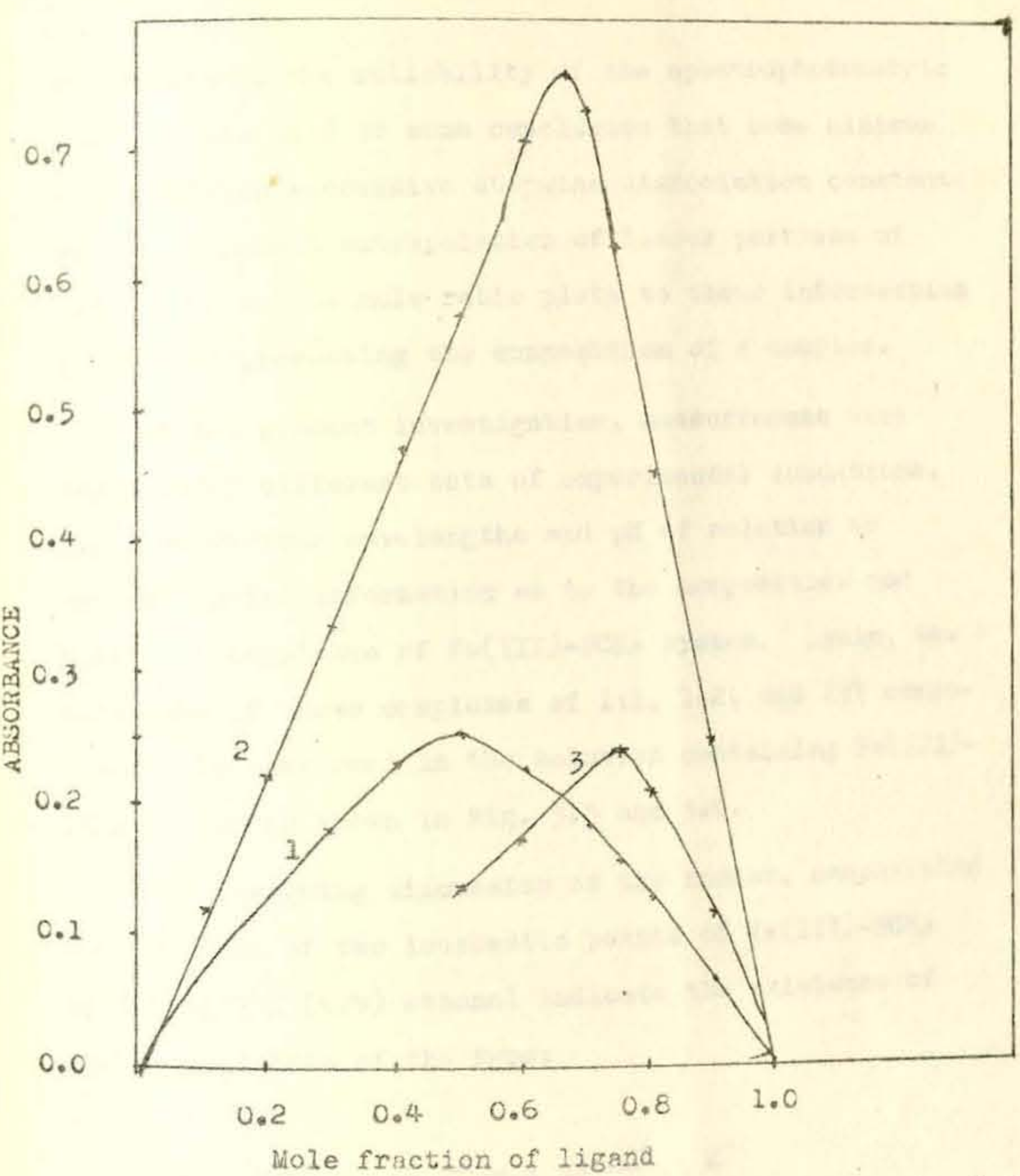


Fig. 3.4. Continuous variations curve for iron(III)-PCHA system in 75% ethanol.

curve 1 at pH = 1.25, λ= 650 nm ; $C_M + C_L = 8 \times 10^{-4}$ M
curve 2 at pH = 3.15, λ= 550 nm , $C_M + C_L = 8 \times 10^{-4}$ M
curve 3 at pH = 4.65, λ= 460 nm , $C_M + C_L = 8 \times 10^{-5}$ M

tolerances in the reliability of the spectrophotometric measurements lead to some conclusion that some minimum ratio between successive stepwise dissociation constants exists to permit extrapolation of linear portions of absorbance versus mole ratio plots to their intersection point as representing the composition of a complex.

In the present investigation, measurements were taken under different sets of experimental conditions, i.e., at various wavelengths and pH of solution to extract useful information as to the composition and number of complexes of Fe(III)-PCHA system. Again, the existence of three complexes of 1:1, 1:2, and 1:3 compositions is confirmed in the solution containing Fe(III)-PCHA system as shown in Fig. 3.5 and 3.6.

The foregoing discussion of the number, composition and presence of two isosbestic points of Fe(III)-PCHA system in 75% (v/v) ethanol indicate the existence of three equilibria of the type:

$$FeL_{(n-1)}^{(4-n)+} + HL \rightleftharpoons FeL_n^{(3-n)+} + H^+ \qquad (3.16)$$

where L is deprotonated PCHA.

The variation of maximum absorption wavelengths with pH and C_L/C_{Fe} show also the presence of three complex species, FeL^{2+}, FeL_2^+, FeL_3 exhibiting absorption maxima at 535 nm, 495 nm, and 445 nm, respectively (Fig. 3.7, 3.8). The absorption spectra of the three complexes are shown in Fig. 3.9.

3.2.5 Beer's Law and Calculation of Molar Absorptivity at the Isosbestic Point

It was possible to verify that the Beer-Lambert's law is obeyed by the colored complexes of Fe(III)-PCHA system at all wavelengths, and for the mixture at the isobestic point wavelengths in a wide pH range. The common molar absorptivity of the complexes FeL_2^+ and FeL_3 at the isobestic point wavelength, λ = 510 nm and that of FeL^{2+} and FeL_2^+ at the second isosbestic point wavelength, λ = 612 nm were calculated to be 4420 and 1343 $l\ mol^{-1}\ cm^{-1}$, respectively. (Table 3.1).

Table 3.1. Molar Absorptivities of Complexes FeL_n (n=1-3) at the Isosbestic Point Wavelengths

C_{Fe}(M)	C_L/C_{Fe}	$\varepsilon_{1=2}$[a] ($l\ mol^{-1}cm^{-1}$)	$\varepsilon_{2=3}$[b] ($l\ mol^{-1}cm^{-1}$)
1.6×10^{-5}	125	1350	4437
3.2×10^{-5}	62.5	1338	4407
6.4×10^{-5}	31.25	1343	4406
9.6×10^{-5}	20.83	1343	4458
1.28×10^{-4}	15.625	1336	4414
1.6×10^{-4}	12.5	1346	4438
3.2×10^{-4}	6.25	1345	4380
		mean = 1343	mean = 4420

[a] At 612 nm and pH = 1.8

[b] At 510 nm and pH = 3.6

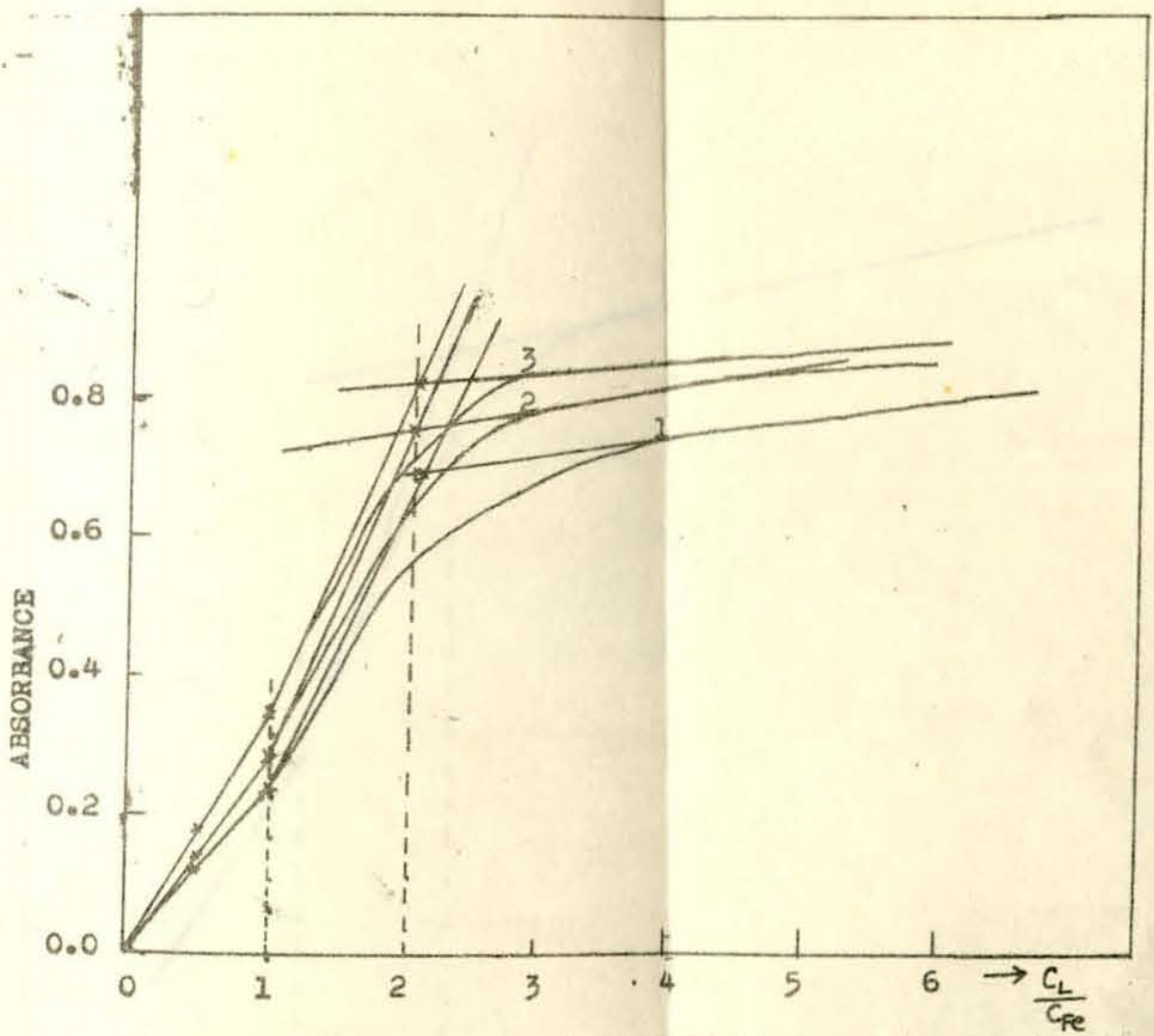


Fig. 3.5. Absorbance of iron(III)-PCHA system as a function of ligand concentration at constant metal ion concentration in 75% ethanol. $C_{Fe} = 2.0 \times 10^{-4}$ M, pH = 2.65; λ = 500 nm(1), λ = 470 nm(2), λ = 450 nm(3).

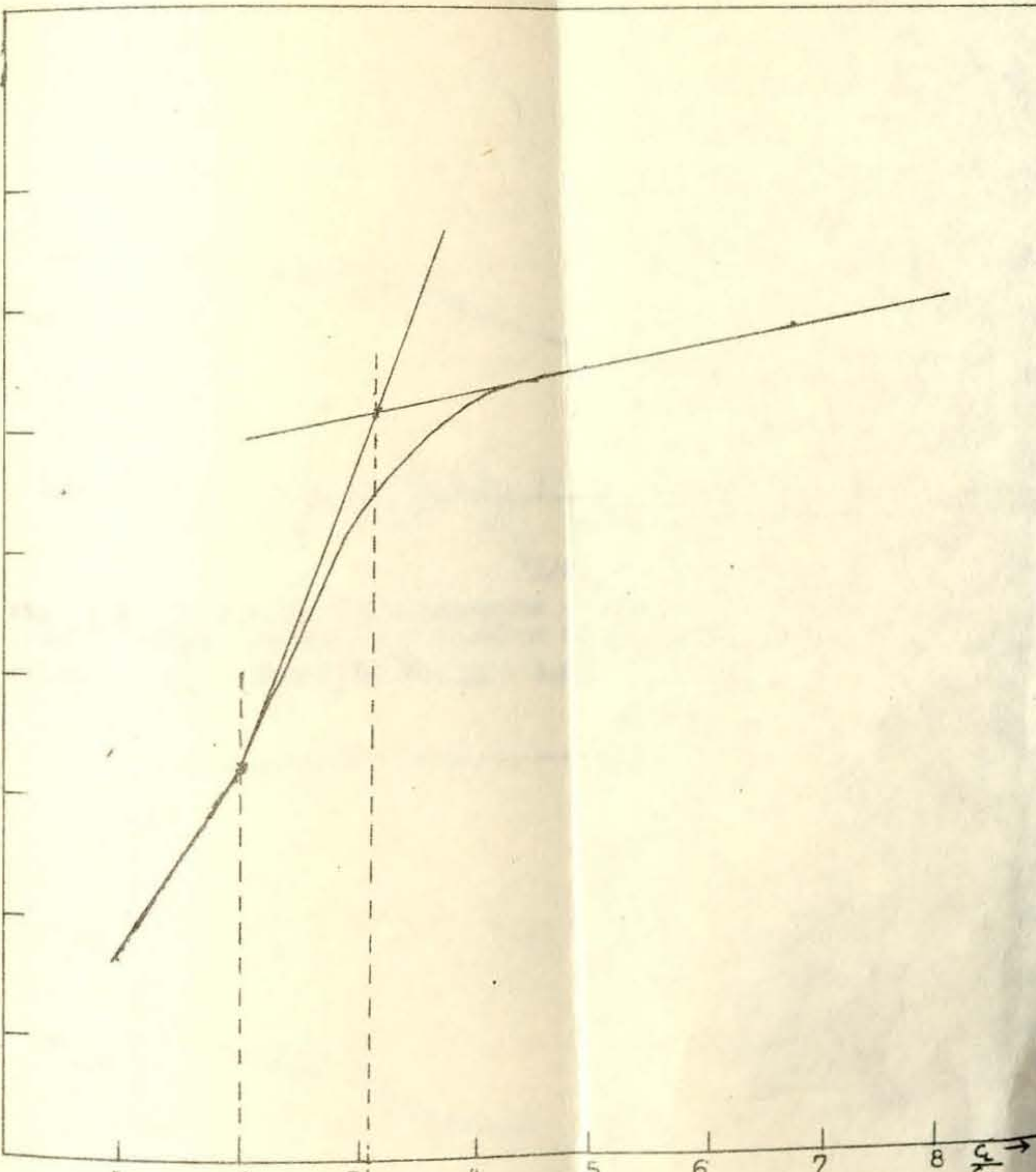


Fig. 3.6. Absorbance of iron(III)-PCHA system as a function of ligand concentration at constant metal ion concentraion in 75% (v/v) ethanol. $C_{Fe} = 2.0 \times 10^{-4}$ M, pH = 5.0, λ = 445 nm

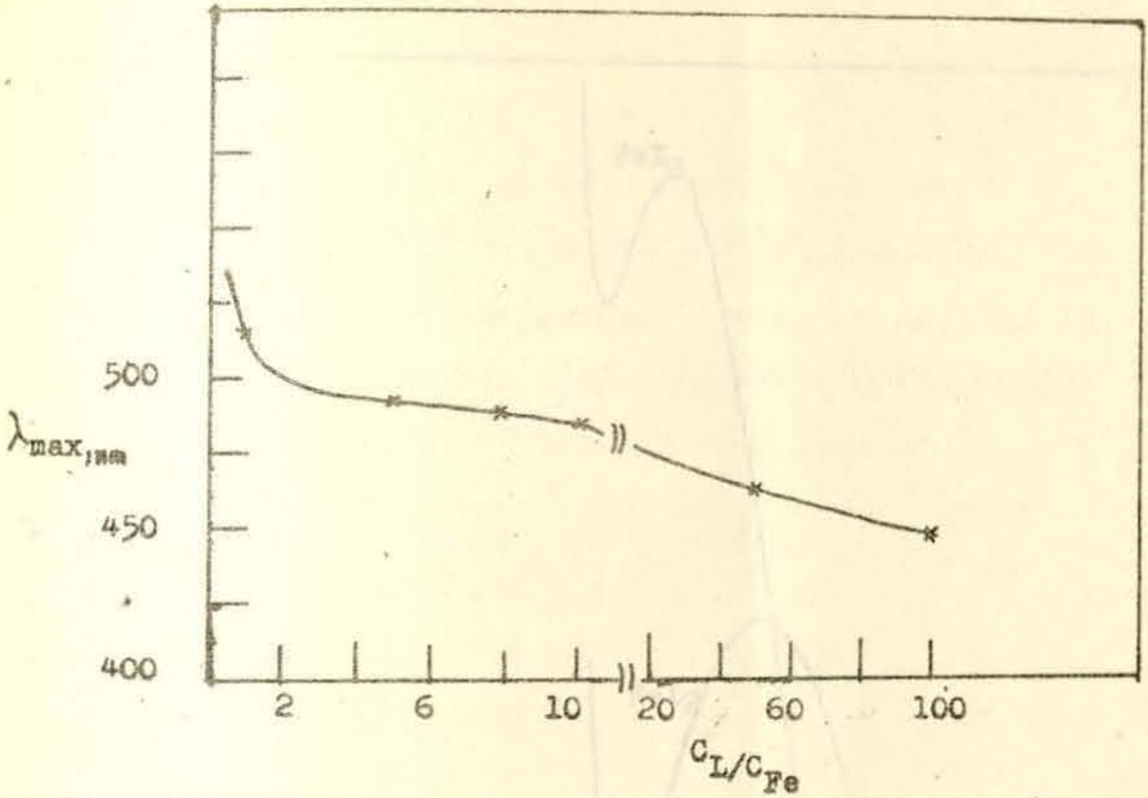


Fig. 3.8. Variation of wavelengths of maximum absorbance of iron(III)-PCHA system as a function of C_{L}/C_{Fe} in 75% (v/v) ethanol. $C_{Fe} = 2.0 \times 10^{-4}$M, pH = 2.65.

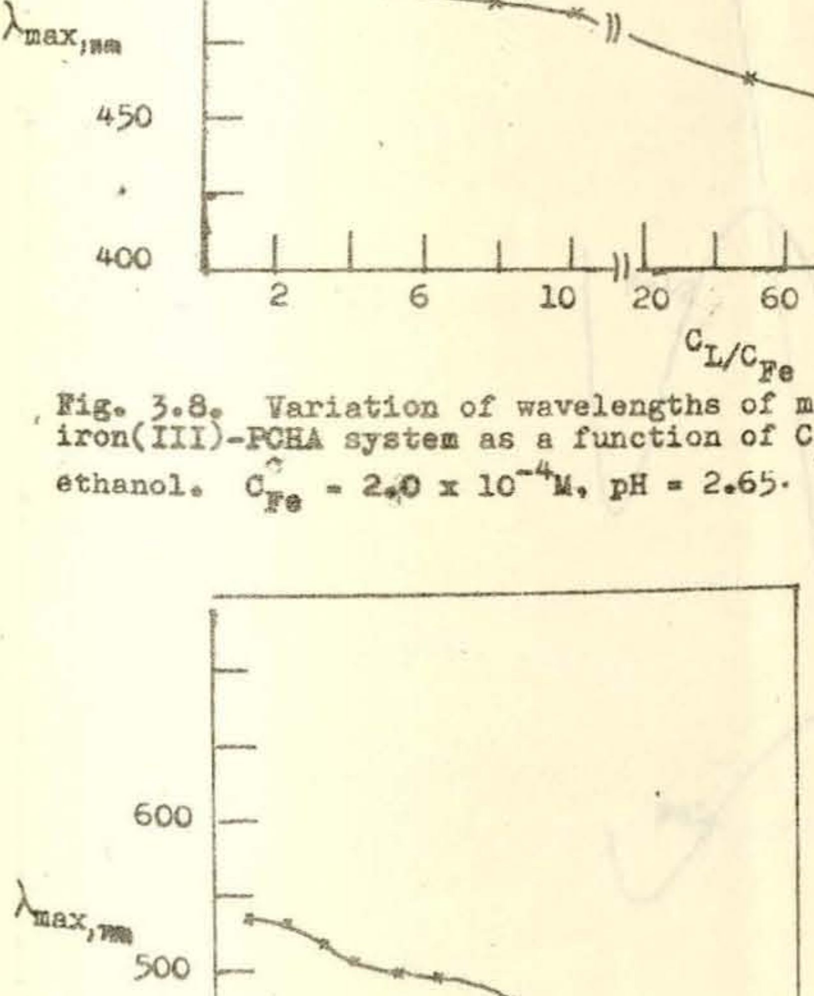


Fig. 3.7. Variation of wavelengths of maximum absorbance of iron(III)-PCHA system as a function of pH in 75% (v/v) ethanol.

$C_{Fe} = 2.0 \times 10^{-4}$M; $C_{L}/C_{Fe} = 3$.

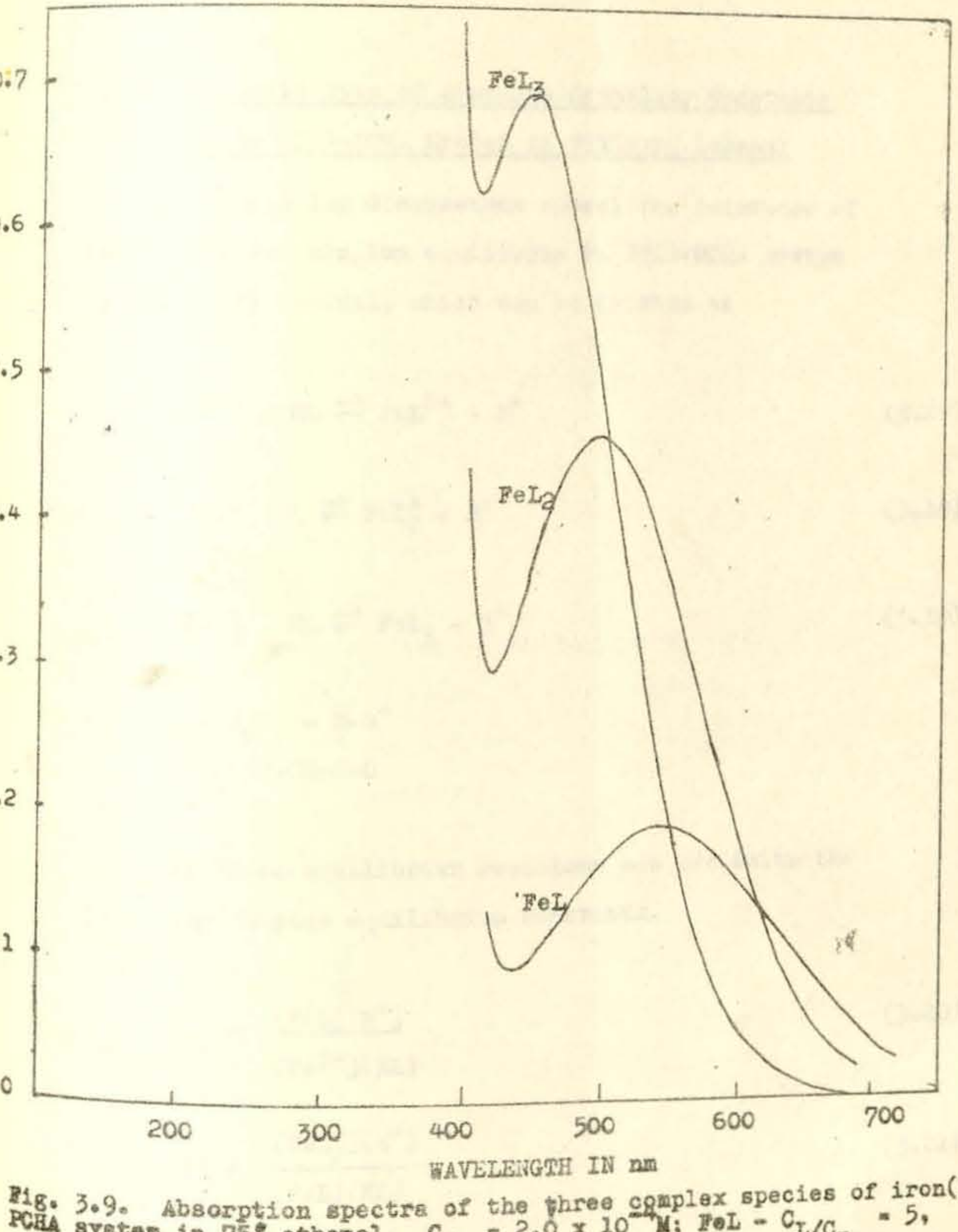


Fig. 3.9. Absorption spectra of the three complex species of iron(II PCHA system in 75% ethanol. $C_{Fe} = 2.0 \times 10^{-4}$ M; FeL - $C_{L}/C_{Fe} = 5$, pH = 1.15, FeL$_2$ - $C_{L}/C_{Fe} = 5$, pH = 2.95; FeL$_3$ - $C_{L}/C_{Fe} = 20$, pH = 6.5

3.2.6 Determination of Stepwise Stability Constants of Fe(III)-PCHA System in 75%(v/v) Ethanol

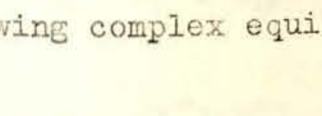
The foregoing discussions reveal the existence of the successive complex equilibria Fe(III)-PCHA system in 75% (v/v) ethanol, which can be written as

$$Fe^{+3} + HL \rightleftharpoons FeL^{2+} + H^{+} \quad (3.17)$$

$$FeL^{2+}\ HL \rightleftharpoons FeL_2^{+} + H^{+} \quad (3.18)$$

$$FeL_2^{+} + HL \rightleftharpoons FeL_3 + H^{+} \quad (3.19)$$

where L =



$$\begin{array}{l} C_6H_5 - \underset{|}{N}-O^{-} \\ C_6H_5-CH=CH-C=O \end{array}$$

For these equilibrium reactions one can write the following complex equilibrium constants.

$$K_1' = \frac{[FeL][H^{+}]}{[Fe^{3+}][HL]} \quad (3.20)$$

$$K_2' = \frac{[FeL_2][H^{+}]}{[FeL][HL]} \quad (3.21)$$

$$K_3' = \frac{[FeL_3][H^{+}]}{[FeL_2][HL]} \quad (3.22)$$

where the concentration of the uncomplexed free iron is written as

$$[Fe^{+3}] = C_{Fe} - \sum_{n=1}^{n=3} [FeL_n] \qquad (3.23)$$

and the concentration of the uncomplexed ligand is given by

$$[HL] = C_L - \sum_{n=1}^{n=3} n[FeL_n] \qquad (3.24)$$

These constants are composite ones K_n' being the product of the stability constant of the type expressed by Eqns 3.10-3.12 and the dissociation constant (acidity constant) of the ligand, K_a:

$$K_n' = K_n \cdot K_a \qquad (3.25)$$

$$\text{where } K_a = \frac{[L^-][H^+]}{[HL]} \qquad (3.26)$$

If one could determine the pH of the solution ($pH_{n-1=n}$) at which the two successive complexes FeL_{n-1} and FeL_n have equal concentrations and all the metal ion is in the form of the complex, the stability constants in Eq. 3.21 and Eq. 3.22 would be written as

$$K_2' = \frac{[H^+]_{1=2}}{[HL]} \qquad (3.27)$$

$$K_3' = \frac{[H^+]_{2=3}}{[HL]} \qquad (3.28)$$

Eq. 3.27 can be written in logarithmic form as:

$$\log K_2' = -(pH_{1=2} + \log [HL]) \qquad (3.29)$$

Under these conditions:

$$C_{Fe} = [FeL] + [FeL_2] = 2[FeL] = 2[FeL_2] \qquad (3.30)$$

and

$$[HL] = C_L - ([FeL] + 2[FeL_2]) \qquad (3.31)$$

Substituting Eq. 3.30 into Eq. 3.31 we get:

$$[HL] = C_{Fe}\left(\frac{C_L}{C_{Fe}} - 1.5\right) \qquad (3.32)$$

and combining Eqns. 3.29 and 3.32 we obtain the relationship:

$$\log K_2' = -\left(pH_{1=2} + \log C_{Fe}\left(\frac{C_L}{C_{Fe}} - 1.5\right)\right) \qquad (3.33)$$

The same arguments hold for Eq. 3.28, which can be written as:

$$\log K_3' = -\,(\mathrm{pH}_{2=3} + \log\,[\mathrm{HL}]) \qquad (3.34)$$

Under these conditions:

$$C_{\mathrm{Fe}} = [\mathrm{FeL}_2] + [\mathrm{FeL}_3] = 2[\mathrm{FeL}_2] = 2[\mathrm{FeL}_3] \qquad (3.35)$$

and

$$[\mathrm{HL}] = C_{\mathrm{L}} - (2[\mathrm{FeL}_2] + 3[\mathrm{FeL}_3]) \qquad (3.36)$$

Substituting Eq. 3.35 into Eq. 3.36, we get:

$$[\mathrm{HL}] = C_{\mathrm{Fe}}\,(\frac{C_{\mathrm{L}}}{C_{\mathrm{Fe}}} - 2.5) \qquad (3.37)$$

Introducing Eq. 3.37 into Eq. 3.34 we obtain the relationship:

$$\log K_3' = -\,(\mathrm{pH}_{2=3} + \log C_{\mathrm{Fe}}(\frac{C_{\mathrm{L}}}{C_{\mathrm{Fe}}} - 2.5)) \qquad (3.38)$$

It has been already shown in Fig. 3.1 and Fig. 3.2, that absorption spectra of Fe(III)-PCHA complex species contain two isobestic points and that the existence of an isosbestic point is a strong evidence for the simultaneous presence in solution of two absorbing forms, and of two only, the sum of whose concentration is constant. Under this condition, all of the Fe(III) is in the form of complexes, which are in equilibrium with each other.

For such system there exists a method called isobestic point method for the determination of stepwise stability constants. It was first introduced by Vareille in 1955 (49).

The Vareille method is based upon graphical determination of $pH_{n-1=n}$ ($pH_{1/2}$) where the two successive complex species have equal concentrations from the curve of absorbance versus pH at the isosbestic points.

The determination of the second stability constant (Eq. 3.21 or 3.27) was done at the wavelength of the isosbestic point(II) ($\lambda = 510$ nm). Since visible absorptions are additive, the solution with the required pH will be that with an absorption equal to the arithmetic mean of the absorptions of the two complexes, FeL^{2+} and FeL_2^+ and this was determined from the curve of absorbance versus pH of the solutions.

The calculation of the third stability constant (Eq. 3.22 or 3.28) was carried out in the same fashion, and in this case the curve used to determine the solution of equal concentrations of complexes II (FeL_2^+) and

III (FeL_3) is that obtained when absorbance values were measured at the wavelength of the first isobestic point ($\lambda = 612$ nm) where one knows the absorption of complex II (given by a step or tangent in the graph) and that of complex III, which corresponds to another graph step at higher pH's, where it is more stable.

The general diagram used to find $pH_{1=2}$ and $pH_{2=3}$ in the calculation of the stepwise formation constant for Fe(III)-PCHA system is shown in Fig. 3.10.

Some values of $pH_{1=2}$ and $pH_{2=3}$ determined at the wavelengths of the isobestic points for a few values of C_{Fe} and C_{L}/C_{Fe} are shown in Table 3.2. The calculated stability constants corresponding to these pH values are also presented in the same Table.

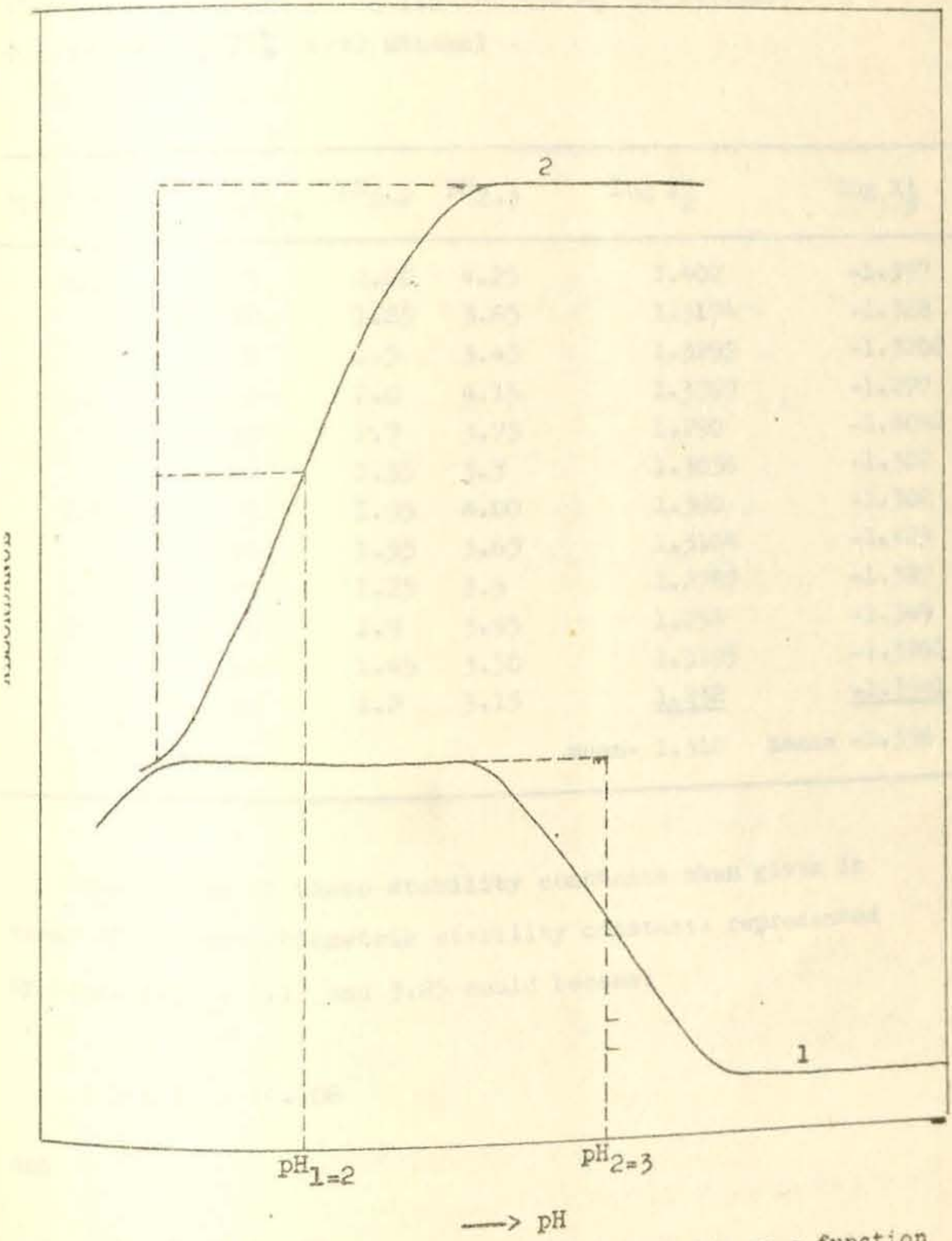


Fig. 3.10. Absorbance of iron(III)-PCHA system as a function of pH at the wavelength of isosbestic points, λ= 612(1), λ= 510(2) in 75% ethanol (v/v)

Table 3.2. Values of $pH_{n-1=n}$ and $\log K'_n$ for Fe(III)-PCHA System in 75% (v/v) Ethanol

$C_{Fe} \times 10^{-4}$ M	C_L/C_{Fe}	$pH_{1=2}$	$pH_{2=3}$	$\log K'_2$	$\log K'_3$
0.8	5	2.20	4.25	1.402	-1.397
	10	1.85	3.85	1.3174	-1.328
	20	1.5	3.45	1.3297	-1.3202
1.2	5	2.0	4.15	1.3767	-1.277
	10	1.7	3.75	1.290	-1.4042
	20	1.35	3.3	1.3036	-1.322
1.6	5	1.95	4.00	1.300	-1.302
	10	1.55	3.65	1.3164	-1.429
	20	1.25	3.3	1.2787	-1.397
20	5	1.9	3.95	1.254	-1.349
	10	1.45	3.50	1.3195	-1.3761
	20	1.2	3.15	1.232	-1.3941
				mean= 1.310	mean= -1.358

The values of these stability constants when given in terms of the stoichiometric stability constants represented by Eqns. 3.10 - 3.12 and 3.25 would become:

$$\log K_2 = 10.106$$

and

$$\log K_3 = 7.438$$

since K_a of the ligand is 1.6×10^{-9} (8)

The solutions at lower pH's contain uncomplexed free iron. The total iron for these series of solutions can be written as

$$C_{Fe} = [Fe] + [FeL] + [FeL_2] \tag{3.39}$$

Since the two complexes are the only absorbing species under these conditions, the concentration of iron in the complexes and in the free form can be calculated at the wavelength of isobestic point (I) (λ= 612 nm), where FeL^{2+} and FeL_2^+ have equal molar absorptivities ($\varepsilon_{1=2}$). At this isosbestic point wavelength:

$$A = \varepsilon_{1=2}([FeL] + [FeL_2]) \tag{3.40}$$

where A denotes absorbance values.

Introducing Eq. 3.40 into Eq. 3.39, the uncomplexed metal ion is given as

$$[Fe] = C_{Fe} - A/\varepsilon_{1=2} \tag{3.41}$$

The individual concentrations of FeL^{2+} and FeL_2^+ were calculated from the mole fraction distribution curve of the type given in Fig. 3.11. This distribution diagram gives the fraction of the complexed metal ion in the form of nth complex. From the composition of the solutions and their absorption at the isobestic point wavelength it was also possible to calculate the concentration of uncomplexed PCHA:

$$[HL] = C_L - \left(\left(\frac{A}{\varepsilon_{1=2}}\right) + [FeL_2]\right) \qquad (3.42)$$

By calculating the values of the above variables at low pH values, it was possible to calculate the first stability constant given by Eq. 3.20 which can be written in the following convenient form:

$$\log K_1' = \log [FeL] - pH - \log [Fe] - \log [HL] \qquad (3.43)$$

Some values of $\log K_1'$ calculated for different concentration of C_{Fe} and $C_{L/C_{Fe}} = 5$ are presented in Table 3.

The value of this composite stability constant when given in terms of stoichimetric stability constant would be

$$\log K_1 = 11.547$$

Table 3.3. Values of log K_1' Calculated for Different Concentration of Iron and pH of the Solution in 75% (v/v) Ethanol

$C_{Fe} \times 10^{-4}$ (M)	pH	log K_1'
0.8	0.50	2.68
	0.75	2.68
	0.85	2.70
1.2	0.55	2.74
	0.65	2.73
	0.85	2.84
1.6	0.45	2.71
	0.65	2.76
	0.85	2.84
2.0	0.5	2.71
	0.65	2.72
	0.85	2.84
		mean= 2.75

The application of the isosbestic point method to Fe(III)-PCHA system required the selection of optimum ranges of concentration of iron (C_{Fe}) and that of PCHA (C_L) for the calculation of stability constants. The optimum ranges used in the study Fe(III)-PCHA equilibria are presented in Table 3.4.

Table 3.4 Optimum Range of C_{Fe} and C_{L}/C_{Fe} used for the Calculation of K_{FeL_n} of Fe(III)-PCHA Systems in 75% (v/v) Ethanol.

K_n	C_{Fe}(M)	C_L/C_{Fe}
K_1	$(0.8 - 2.4)\ 10^{-4}$	4 - 6
K_2	$(0.8 - 2.4)\ 10^{-4}$	5 - 20
K_3	$(0.8 - 2.4)\ 10^{-4}$	5 - 20

3.2.7 Construction of Mole-Fraction Distribution Diagram for the Consecutive Complexes of Fe(III)-PCHA Systems

It was possible to construct distribution diagram (nomogram) that can render the fraction of iron(III) in the complex by using the knowledge of $pH_{1=2}$ and $pH_{2=3}$. For this particular case, the mole fraction equation would assume the following form:

$$[FeL_n]\% = \frac{[FeL_n] \times 100}{[FeL] + [FeL_2] + [FeL_3]} \qquad (3.44)$$

To solve for the percentage distribution of FeL_n, one proceeds as follows. Consider Eqns. 3.20, 3.21 and 3.22 which can be written in the following forms

$$[FeL] = \frac{[H^+][FeL_2]}{[HL]K'_2} \qquad (3.45)$$

$$[FeL_2] = \frac{K'_2[FeL][HL]}{[H^+]} \qquad (3.46)$$

and

$$[FeL_3] = \frac{K'[FeL_2][HL]}{[H^+]} \qquad (3.47)$$

Introducing Eq. 3.46 and 3.47 into Eq. e.44, we obtain, for n = 1

$$[FeL]\% = \frac{1}{1 \times \frac{K'_2[HL]}{[H^+]} + \frac{K'_2K'_3[HL]^2}{[H^+]^2}} \times 100 \qquad (3.48)$$

but $$\frac{K'_2[HL]}{[H^+]} = 10^{pH} - pH_{1=2} \qquad (3.49)$$

and $$\frac{K'_2K'_3[HL]^2}{[H^+]^2} = 10^{(2pH)} - (pH_{2=3} + pH_{1=2}) \qquad (3.50)$$

which can be obtained by considering Eqns. 3.27 and 3.28, respectively.

By combining Eqns. 3.48, 3.49 and 3.50 we obtain the relationship

$$[FeL]\% = \frac{100}{1+10^{pH-pH_{1=2}}+10^{(2pH)-(pH_{2=3}+pH_{1=2})}} \tag{3.51}$$

For n=2, by combining Eqns. 3.44, 3.45, and 3.47, we obtain:

$$[FeL_2]\% = \frac{1}{\frac{[H^+]}{[HL]K'_2}+1+\frac{K'_3[HL]}{[H^+]}} \times 100 \tag{3.52}$$

which upon consideration of Eqns. 3.49 and 3.50 would assume the following form:

$$[FeL_2]\% = \frac{1}{10^{pH_{1=2}-pH}+1+10^{pH-pH_{2=3}}} \times 100 \tag{3.53}$$

By the same argument the mole fraction of the third complex would be given by:

$$[FeL_3]\% = \frac{1}{10^{pH_{1=2}+pH_{2=3}-2pH}+10^{pH_{2=3}-pH}+1} \times 100 \tag{3.54}$$

One example of a nomogram that is constructed by the use of the foregoing calculations is presented in Fig. 3.11. Fig. 3.11 shows that the individual concentrations of two successive complexes would add upto 100% strictly for the reaction environment with pH = $pH_{1=2}$ and pH = $pH_{2=3}$. For the reaction environment with $pH_{1=2} < pH < pH_{2=3}$, all the three consecutive complexes exist simultaneously.

The nomogram of the type given in Fig. 3.11 gives us the knowledge of the composition and hence the individual concentrations of each complexes with reference to the total complexed metal ion corresponding to particular pH. However, it does not give us the mole fraction of the complexes with respect to the total concentration of iron, particularly at low pH's, where iron also exists in uncomplexed form. In this general case, the total concentration of iron, C_{Fe}, will be written as

$$C_{Fe} = [Fe] + [FeL] + [FeL_2] + [FeL_3] \tag{3.55}$$

and the mole fraction is given by

$$[FeL_n]\% = \frac{[FeL_n]}{[Fe] + [FeL] + [FeL_2] + [FeL_3]} \times 100 \tag{3.56}$$

where n = 0 - 3.

For the reaction environment having $pH < pH_{1=2}$, only the first three species exist in solution and the mole fraction equation (Eq. 3.56) would assume the following form:

$$\%[FeL_n] = \frac{[FeL_n]}{[Fe] + [FeL] + [FeL_2]} \times 100 \qquad (3.57)$$

where n = 0 - 2.

Since the concentrations of FeL^{2+} and FeL_2^+ can be determined from the absorbance values at the wavelength of the first isosbestic point (λ= 612) and the nomogram of the type given in Fig. 3.11 corresponding to particular pH value, the concentration of the free iron can also be known. Thus the mole fraction of each species can be calculated by using Eq. 3.57.

For the reaction environment having $pH > pH_{1=2}$, only the last three species (the complexes) exist in solution. Therefore the argument is exactly similar to the one considered in Eq. 3.44.

From the foregoing arguments, it was, therefore, possible to construct a 'complete' nomogram of the type given in Fig. 3.12. These type of nomograms are very important to figure out easily the pH of the solution at which each individual complex relatively has maximum concentration. This is particularly useful to identify the pH of the solution at which the first complex would have maximum concentration, as this is totally impossible from the nomograms of the type given in Fig. 3.11. Few of these pH values for two different concentration of iron(III) with different C_L/C_{Fe} values are presented in Table 3.5. pH_n denotes the pH values at which we have maximum concentration of FeL_n in the solution.

Table 3.5. Values of pH_n (n = 1 - 3) for some Values of C_{Fe} and $C_{L/C_{Fe}}$ for the Complexes of FeL_n (n = 1 - 3) in 75% (v/v) Ethanol

C_{Fe}(M)	$C_{L/C_{Fe}}$	pH_1	pH_2	pH_3
1.2×10^{-4}	5	1.05	3.05	6.65
	10	0.85	2.75	6.15
	20	0.55	2.45	5.65
2.0×10^{-4}	5	1.15	2.95	6.15
	10	0.75	2.45	5.65
	20	0.45	2.15	5.45

Finally the molar absorptivities of FeL^{2+}, FeL_2^+, FeL_3 complexes at their absorption maxima were calculated to be

$$\varepsilon_1 = 2.06 \times 10^3 \text{ l mol}^{-1}\text{cm}^{-1}$$

$$\varepsilon_2 = 4.67 \times 10^3 \text{ l mol}^{-1}\text{cm}^{-1}$$

and

$$\varepsilon_3 = 6.67 \times 10^3 \text{ l mol}^{-1}\text{cm}^{-1}$$, respectively.

These values are calculated by considering the percent contribution of each species existing in solution obtained from the nomogram of the type given in Fig. 3.12.

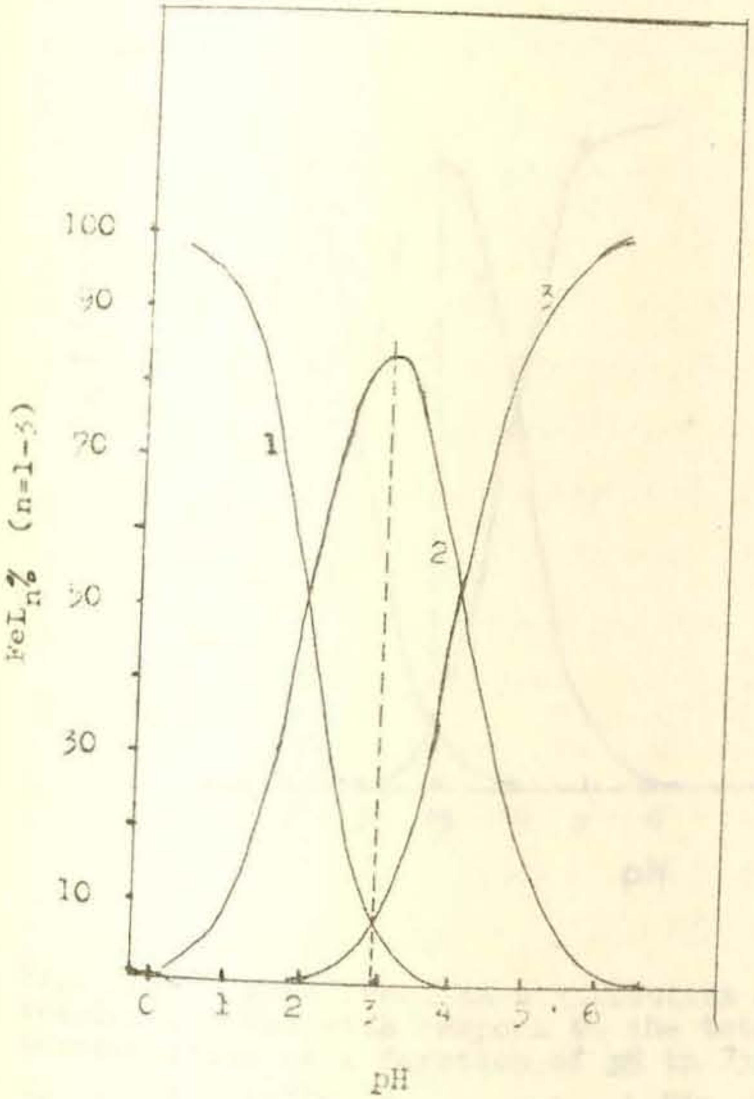


Fig. 3.11. Mole fraction distribution of iron(III)-PCHA complex species with respect to the total complexed metal ion as a function of pH in 75% (v/v) ethanol. $C_{Fe} = 2.0 \times 10^{-4}$ M, $C_{L}/C_{Fe} = 5$, 1-% FeL, 2-% FeL_2, 3-% FeL_3.

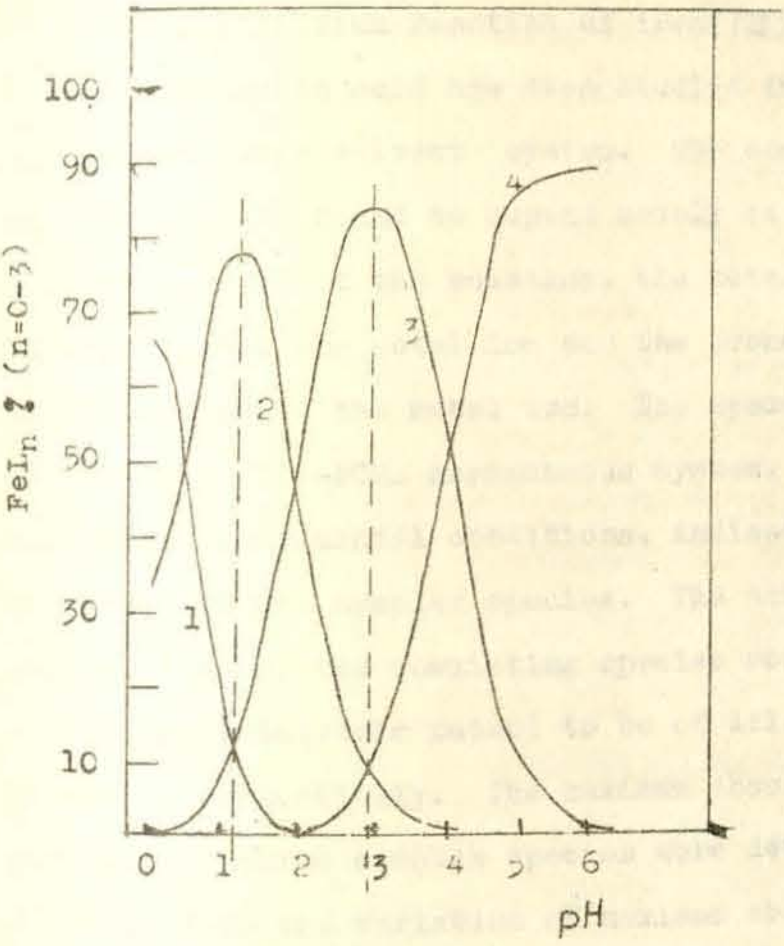


Fig. 3.12. Mole fraction distribution of iron(III)-PCHA complex species with respect to the total iron(III) concentration as a function of pH in 75% (v/v) ethanol. $C_{Fe} = 2.0 \times 10^{-4}$M, $C_{L/C_{Fe}} = 5$, 1-% Fe, 2-% FeL, 3-% FeL_2, 4-% FeL_3.

3.3 Conclusion

The equilibrium reaction of iron(III) with N-phenylcinnamohydroxamic acid has been studied in 75% (v/v) ethanol in water solvent system. The complex formation equilibria were found to depend mainly on the solvent composition, pH of the solution, the total concentration of the ligand, the metal ion and the concentration ratio of the ligand to the metal ion. The spectral properties of the iron(III)-PCHA homogeneous system, observed under different experimental conditions, indicate the presence of more than one complex species. The actual number and compositions of the coexisting species were determined by spectrophotometric method to be of 1:1, 1:2, and 1:3 (Fe:PCHA) respectively. The maximum absorption wavelengths of the three complex species were determined graphically from the variation of maximum absorption wavelength as a function of pH and also from the variation of the maximum absorption wavelength versus the concentration ratio of the ligand to the metal ion. These wavelengths are 535, 495, and 445 nm for FeL^{2+}, FeL^{+}, and FeL_3 species respectively. The fact that Beer's law is obeyed by the mixture of the complexes at the isosbestic point wavelengths is very important for quantitative determination, since measurement of absorbance at the wavelength of isobestic point where the molar absorptivity has a constant value over a wide pH range gives more reliable results than those obtained at another wavelength at which the molar absorptivity is strongly affected by the pH value.

The stepwise stability constants were detemined and found to be log K_1 = 11.547, log K_2 = 10.106 and log K_3 = 7.438 for the I, II, and III stepwise stability constants respectively by using isosbestic point method. The method involves the determination of pH of the solution at which two consecutive complex species would have equal molar concentrations. To detemine these constants, use of proper range of concentration of the metal ion and that of the ligand was important.

Finally mole fraction distribution curves were constructed that would give information regarding the range of pH in which one of the complex species is existing and the knowledge of the percent contribution of each species to the total metal ion in the solution at certain pH. By taking into account the percent contribution of each species at certain pH, calculations of the molar absorptivity values of the complexes at their maximum absorption wavelengths were done.

4. EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF IRON(II) WITH N-PHENYLCINNAMOHYDROXAMIC ACID

4.1 General Aspects of Extraction and Spectrophotometric Methods of Analysis

It was discussed in section 1 that, of the organic reagents available for the determination of iron, comparatively few are well suited. Some reagents react with ferric iron, others with ferrous. The former generally has the disadvantage that they cannot be used in the presence of appreciable concentrations of such ions as fluoride and pyrophosphate which form stable complexes with trivalent iron in acid medium, in which the colour reaction is carried out. In such cases better results can frequently be obtained by resorting to a reagent reacting with ferrous iron, which does not form such complexes or at least forms less stable ones. Thus commonly, for the most accurate determination of minute amounts of iron, the use of 1,10-phenanthroline and 2,2'-bipyridyl are recommended. These reagents react with ferrous iron. However, in their use an account must be taken of the possible interference of copper, nickel, and cobalt, which are normally associated with iron in ores and alloys. Both reagents form cationic complexes and therefore, the use of the extraction method is out of the question. Many derivates of both reagents (30,31) have been prepared, but most of these are little superior to the parent compounds and are also expensive. Thus, the development of a new method for the determination of iron that can overcome some of these shortcomings is necessary.

Chief among the methods for the determination of iron may be mentioned, gravimetric, volumetric and spectrophotometric methods. Presently spectrophotometric methods are widely used.

Spectrophotometry is based on the measurements of the absorbance for monochromatic light passing through the solution containing the substance to be determined. The basic condition for application of spectrophotometric method, as is true for all analytical methods based on the measurement of light absorption is that the Beer-Lambert law is obeyed by the constituents to be determined.

Spectrophotometric methods are commonly used for the determination of inorganic substance with organic reagents (1-7). The reliability of the method heavily depends on the nature of the organic reagent.

Sensitivity is one of the obvious demands in applications of analytical methods. In order to comply with this requirement for reactions which involve a change in color, the molecule of the complex formed or the organic reagent itself must contain chromophoric groups which are characterized by transitions with molar absorption coefficients in the range 10^3 - 10^5 $1\ mol^{-1}cm^{-1}$. Such chromophoric groups are found among d-π and π-chromophores. The complexes with d-π-chromophores are restricted in use to the transition elements, in particular those which can exist in two oxidation states differing by one electron.

The reagents with π-electron groups are most frequently employed in analytical applications. The main reason can be found in the intensity of the transitions (with molar absorption coefficients up to $10^5\ 1\ mol^{-1}cm^{-1}$) which characterize these chromophoric groups. By introducing suitable substituents into the molecules of the organic reagents one can increase the sensitivity and selectivity of spectrophotometric determinations.

If adequate selectivity in the spectrophotometric determination of a given substance is to be achieved some practical considerations must also be taken into account. For example, it is necessary to choose a suitable wavelength at which to make the measurement. The absorption coefficient of the substance being determined should be high, and those of other substances present be negligible. Selectivity can also be increased by pH adjustment or by masking. Ofcourse the masking reagent (and the complexes formed) should be colorless and should not form any complexes with the metal being determined or react with the colorimetric reagent.

The selectivity of spectrophotometric determination can often be increased by extraction of the reaction product; thus it is possible to separate the component to be determined from other interfering substances. Usually a lower limit of determination can also be obtained, because the measured compound can be concentrated in the organic phase by use of a suitable phase volume ratio. Therefore, extraction methods are very important in inorganic analysis, and are superior for the most part to other methods of separation.

Solvent extraction (66-75) involves the distribution of a solute between two immiscible liquid phases. It is extremely useful for very rapid and clean separations of trace and major components of both organic and inorganic substances. Extraction methods are used in many laboratories, and opened up many new applications, especially in the field of natural substances. Currently the following fields of application are active; petroleum chemistry, the chemistry of natural substances, and the chemistry of inorganic complexes.

Metal salts do not have the property to dissolve in organic solvents. In view of their ionic character and the tendency of the ion to form solvates they are, in general, not extractable from aqueous solutions. In order to take up the ions in organic solvents it is necessary to substitute the solvent by means of organic ligands or, at least, by inorganic ligands which are soluble in organic solvents. In this regard, organic reagents play a very important role, because they can react with metal ions to give products having the basic pre-requisite for the extractability. They form complexes which are electrically neutral, essentially covalent and therefore can be taken up readily by organic solvents. This is actually achieved principally by formation of ion association complexes and by formation of metal chelates. Since the most widely used method of extracting metal ion is the formation of chelate, due emphasis is given to it here.

In the extraction of chelates formed from an organic solution of chelating agent, HL, and the metal ion, M^{n+}, the following equilibria can be considered.

$$\begin{tikzcd}
(HL)_{org} \arrow[d, shift left] & & (ML_n)_{org} \arrow[d, shift left] \\
(HL)_{aq} \arrow[u, shift left, "K_{D,L}"] \arrow[r, shift left, "\beta_{HL}"] & H^+ + L^-,\ nL^- + M^{n+} \arrow[l, shift left] \arrow[r, shift left, "\beta_n"] & (ML_n)_{aq} \arrow[l, shift left] \arrow[u, shift left, "K_{D,M}"']
\end{tikzcd} \tag{4.1}$$

where $K_{D,L} = \dfrac{[HL]_{org}}{[HL]_{aq}}$ (distribution of the chelating agent) (4.2)

$$\beta_{HL} = \frac{[HL]_{aq}}{[H^+]_{aq}[L^-]_{aq}} \quad \text{(dissociation of the chelating agent)} \tag{4.3}$$

$$\beta_n = \frac{[ML_n]_{aq}}{[M]_{aq}[L]^n_{aq}} \quad \text{(stability of the chelate)} \qquad (4.4)$$

$$K_{D,M} = \frac{[ML_n]_{org}}{[ML_n]_{aq}} \quad \text{(distribution of the chelate)} \qquad (4.5)$$

On the assumption that the concentration of the chelate in the aqueous phase (aq) is very small, i.e., that any chelate formed in the aqueous phase is at once taken up by the organic phase (org) a distribution relation of the total concentration of the metal in the two solvent phases is given by D_M:

$$D_M = \frac{C_{M(org)}}{C_{M(aq)}} = \frac{K_{D,M}\beta_n}{(K_{D,L}\beta_{HL})^n} \left(\frac{[HL]_{org}}{[H^+]_{aq}}\right)^n \qquad (4.6)$$

These arguments are true only if there are no side reactions in both phases.

On examining the terms in the above equation, it can be seen that the distribution ratio (D_M), i.e., the extraction efficiency can be affected by changing the reagent concentration or by changing the pH. A tenfold increase in the reagent concentration will increase the extraction efficiency by the same amount as an increase in the pH of one unit (tenfold decrease in the hydrogen ion concentration). Each effect is greater as the oxidation state of the metal ion becomes larger. The value of D_M increases with pH upto a certain maximum value beyond which the electroneutral chelate prevails in the equilibrium system. By using a high reagent concentrations, extraction can be performed in more acid solutions. Its concentration cannot be increased arbitrarily, however, due to limited solubility and interference in the subsequent determination as for instance the cause of undesirably high reagent blanks by high concentration of reagent in spectrophotometric determinations.

The more stable the chelate (the larger the β_n) the greater the extraction efficiency, and this principle serves as the basis for the separation of many metals. An acidic reagent (small β_{HL}) that is relatively soluble in water favors good extraction, but chelate stability generally decreases as the reagent acidity increases and the effects of β_{HL} and β_n must be considered together for a series of different chelating agents.

To increase extraction efficiency the value of $K_{D,M}$ should be as high as possible, and the value of $K_{D,L}$ should be low.

Other factors such as temperature (82,83) and ionic strength of the solution, nature of solvent may also affect the value of D_M. For the improvements in selectivity of extractions the following considerations may be taken into account.

1. The separation efficiency depends on the relative formation constants and on the relative solubilities of the chelates (76,77).
2. The selectivity of an extraction can often be controlled by proper pH adjustment.
3. The separation efficiency can be altered by change in reagent concentration.
4. The selectivity of a separation can be increased by stripping technique (i.e by back-extraction).
5. The efficiency of separation can be increased by the scrubbing technique.
6. The selectivity can be influenced by any masking reagent added to bind interfering metals.
7. A selective separation can also be based on the difference in the kinetics of extraction process (78,79).
8. For some combinations of metal ions the extractive separation of complexes can be achieved by choosing a suitable solvent (80,81).
9. A change in the oxidation state of a metal ion offers another possibility for achieving selective separation.
10. A considerable selectivity of separation can be

obtained by means of displacement reactions.

11. If the separation efficiency remains poor even when all these ways of increasing it have failed there always remains the technique of successive batch extraction.

As indicated in the foregoing discussions, the availability of an organic reagent that can be employed as a suitable spectrophotometric and extraction reagent is a very important step in inorganic analysis. It has already been explained that, one of the powerful group of chelating agents used for the solvent extraction and spectrophotometric determination of metal ions in recent years is, N-phenylcinnamohydroxamic acid. This popular reagent is used for the determination of bivalent iron in the present investigation.

4.2 Results and Discussion

4.2.1 Colourimetric Reaction

Iron(II) combines with PCHA giving an orange-red, stable complex that is insoluble in aqueous solution but readily soluble in most organic solvent. This sensitive colour reaction underlies the basis for the development of new extraction and spectrophotometric method for the determination of bivalent iron.

4.2.2 Choice of Solvent and Absorption Spectra

Several organic solvents were examined for the choice of proper solvent for extraction. The complex is readily soluble in all the solvents tried and exhibits essentially similar spectral properties except the slight differences in intensities. Toluene and benzene were found to be the most suitable ones since the complex is readily and quantitatively extracted into them. However, toluene was chosen as a solvent for extraction due to its low toxicity.

The absorption spectra of the reagent (PCHA) and the iron(II)-PCHA complex in toluene are shown in Figs. 4.1 and 4.2. The reagent shows negligible absorption in the region 700-500 nm, slight absorption between 500-400 nm, and strong absorption beyond 400 nm. Hence, a reagent blank is necessary for the precise measurements of absorbance at wavelengths shorter than 500 nm.

The iron(II)-PCHA complex showed two strong absorption band maxima at 448 and 375 nm associated with the electronic transitions from the metal to the ligand (the charge transfer M --> L transitions) and $\pi \rightarrow \pi^*$ transition of the ligand, respectively.

Table 4.1. Effect of Solvent on Extraction of Fe(II)-PCHA Complex; Iron(II) = 111.70 µg/25 ml

pH of the aqueous phase = 5.00

Solvent	Absorbance at λ_{max}	Molar absorbitivity (ε) $l\ mol^{-1}cm^{-1}$
Benzene	0.675	8437.5
Toluene	0.672	8400.0
Xylene	0.665	8312.5
o-dichlorobenzene	0.659	8237.5
Chlorobenzene	0.650	8125.0
Ethylacetate	0.622	7775.0
Carbontetrachloride	0.611	7637.5
Chloroform	0.598	7475.0
Amylacetate	0.515	6437.5
Amylalcohol	0.495	6187.5

4.2.3 Mode of Introduction of the Reagent into the Reaction Mixture

When an organic solution (PCHA in extracting solvent, toluene) of the extractant was used, complete extraction was obtained after several batch extractions and thus the amount of the reagent needed was found to be very high. When an aqueous alcoholic solution of the extractant (PCHA) was used instead, complete extraction was essentially possible with pure solvent in a single extraction. Because of this advantage, the later mode

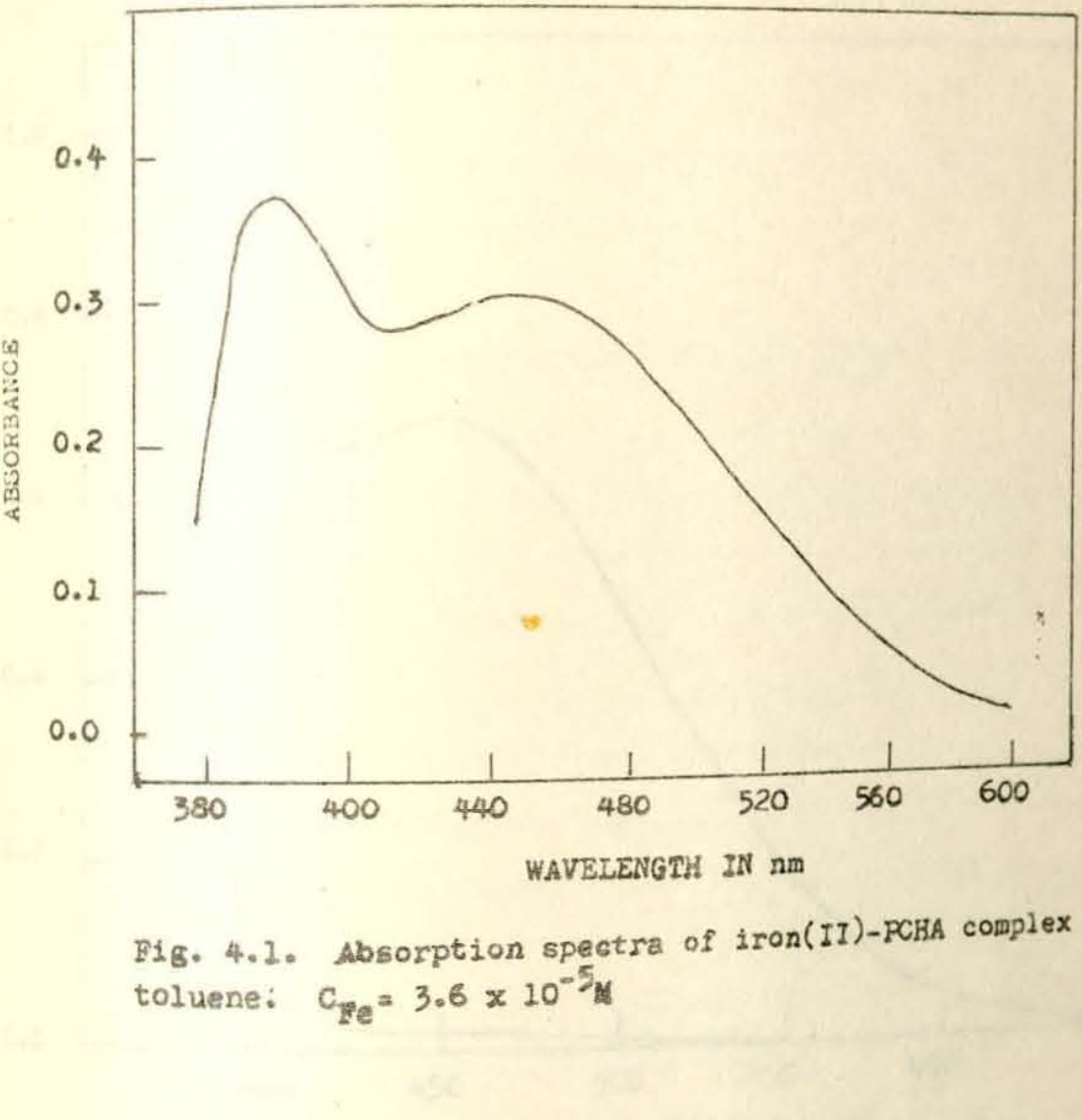


Fig. 4.1. Absorption spectra of iron(II)-PCHA complex in toluene: $C_{Fe} = 3.6 \times 10^{-5}$M

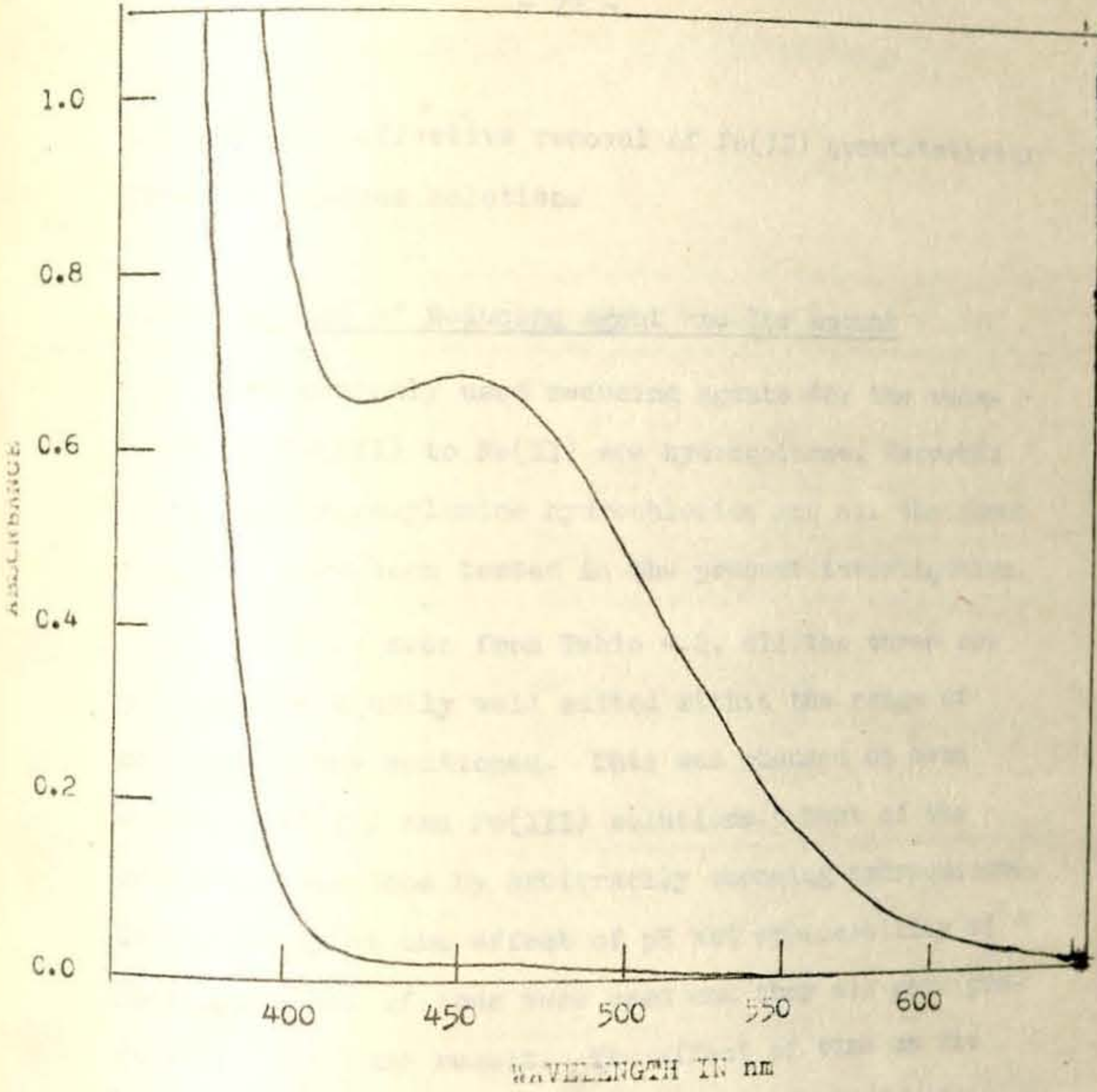


Fig. 4.2. Absorption spectra of iron(II)-PCHA complex(1) and that of PCHA(2) in tolune, for $C_{Fe} = 8 \times 10^{-5}$M and $C_L = 2 \times 10^{-3}$M, respectively.

was used for effective removal of Fe(II) quantitatively from the aqueous solution.

4.2.4 Choice of Reducing Agent and Its Amount

Most commonly used reducing agents for the reduction of Fe(III) to Fe(II) are hydroquinone, ascorbic acid, and hydroxylamine hydrochloride and all the three reagents have been tested in the present investigation.

As can be seen from Table 4.2, all the three are practically equally well suited within the range of concentrations mentioned. This was checked on both standard Fe(II) and Fe(III) solutions. Most of the work has been done by arbitrarily choosing hydroquinone. In the study of the effect of pH and applicability of Beer's law all of them were used and they all gave practically the same result. The effect of time on the action of the reducing agents was also studied before the adjustment of the pH of the aqueous solution. It was observed that there was no time effect on the reducing abilities of the reductants, i.e., all the three led to immediate reduction.

Table 4.2 . Choice of the Proper Amount and Kind of Reducing Agent

Iron = $8.0 \times 10^{-5}M$

Reducing agent	Molar concentration	Absorbance	Remark
Hydroquinone	0.004	0.691	
	0.01	0.685	
	0.05	0.690	pH = 5.0
	0.10	0.690	
	0.15	0.689	
Ascorbic acid	0.004	0.687	
	0.01	0.692	
	0.05	0.687	pH = 6.0
	0.10	0.699	
	0.15	0.694	
Hydroxylamine hydrochloride	0.0144	0.665	
	0.0288	0.660	
	0.0576	0.677	pH = 6.0
	0.1152	0.675	
	0.2880	0.675	

4.2.5 Effect of Variables

The effect of the experimental variables on the extraction and determination of iron(II) has been studied. The effect of particular variable was studied by measuring the absorbance following the general procedure described earlier, keeping all experimental parameters constant, except the one under investigation. This is very important since both accuracy and precision of photometric determination depend on the completeness of a given reaction which in turn depends on the proper adjustment of experimental variables.

Effect of pH: The extent of extraction increases with pH and for a pH range of 4.5-9.00 it becomes independent of pH. For pH more than 9.00 phase separation was difficult. Thus the optimum pH range of the aqueous phase for complete extraction of iron(II) was found to be 4.5-9.00. It was also found that the colour and the wavelength of maximum absorption of the extract remain the same for all pH examined. It is therefore clear, that in the pH range where absorbance does not depend on the pH, the extractable electroneutral chelate prevails in the equilibrium system or practically the only species existing.

As hown in the Table 4.3, the pH values at which one can attain complete extraction decrease with increasing concentration of the reagent. Moreover, once the complexation reaction is complete (i.e once the complex is formed), it cannot easily be dissociated. The extent of complete extraction remains the same even when the pH of the aqueous phase was decreased from higher values to about pH 2.5. This stability of the complex is very important to minimize the interference of hydroxide formation since the precipitation of iron as hydroxide is highly discouraged in the presence of very stable complex.

Table 4.3. Effect of pH

Iron = 111.70 µg/25 ml

pH	Absorbance	Remark
2.0	0.106	
3.0	0.440	
4.0	0.639	
4.5	0.681	
5.0	0.670	
6.0	0.688	0.00 5 M x 5 ml PCHA in ethanol was used
7.0	0.668	
8.0	0.674	
8.5	0.687	
9.0	0.685	
3.0	0.545	
3.6	0.611	0.01 M x 5 ml PCHA in ethanol was used
4.0	0.650	
2.0	0.630	0.005 M x 5 ml PCHA in ethanol was used. First the pH was increased to higher (≥ 7) and then brought down to the desired pH values
2.5	0.664	
3.0	0.669	
3.5	0.669	

Effect of amount of ethanol: Since the reagent was introduced as an ethanolic solution, the effect of the amount of ethanol on the complex to be formed has been studied. It has been found that upto 50% (v/v) of ethanol in the aqueous phase can be added with no adverse effect in the amount and extractability of the complex. Beyond this, phase separation was difficult.

Table 4.4. Effect of Amount of Ethanol
Iron = 111.70 μg/25 ml
pH = 5.0

Ethanol in the aqueous phase % (v/v)	Absorbance at 448 nm
10	0.670
20	0.677
30	0.672
40	0.668
50	0.668

Effect of amount of reagent: The optimum molar ratio necessary for complete extraction of Fe(II) was found to be 1:4. The extractablity of the metal ion remains essentially constant upto 50-fold molar excess of the reagent. Nevertheless, the extent of absorption of the reagent increases with its increasing concentration, and therefore one needs the reagent blank for the measurement of the absorbance of the complex. Since large excess of the reagent has no adverse effect, one can increase the selectivity of the method by using high concentration of the reagent.

Table 4.5. Effect of Amount of Reagent

Iron = 8 x 10^{-5}M

pH = 5.0

Mole ratio of iron to PCHA	Absorbance at 448 nm
1:1	0.210
1:2	0.416
1:3	0.621
1:4	0.672
1:5	0.686
1:6	0.670
1:8	0.671
1:10	0.676
1:50	0.675

Ionic strength, temperature, and volume of aqueous phase: There was no change in the spectral properties and in the extent of extractablity of the complex, with variation of the ionic strength of the aqueous phase from 0.10 M to 1.00 M with respect to potassium chloride and potassium nitrate. An increase of temperature from 20° - 60°C has no effect in the extractability and spectral behaviour of the complex. It has also been found that the variation of volume ratio of the organic to aqueous phase from 1:1 to 1:4 has no change in the spectral behaviour and extraction efficiency of the complex.

Table 4.6. Effect of Temperature of the Aqueous Phase
Iron = 8×10^{-5}M
pH = 5.0

Temperature, °C	Absorbance
20	0.673
30	0.672
40	0.670
50	0.673
60	0.675

Table 4.7. Effect of Volume Ratio of the Organic Phase to Aqueous Phase
Iron = 8×10^{-5}M
pH = 5.0

Volume ratio of organic phase to aqueous phase	Absorbance
1:1	0.676
1:2	0.675
1:3	0.674
1:4	0.677

Table 4.8. Effect of the Ionic Strength of the Aqueous Phase

Iron = 8×10^{-5}M

pH = 5.0

Electrolyte	Concentration (M)	Absorbance
KCl	0.1	0.672
	0.2	0.675
	0.5	0.665
	1.0	0.666
KNO_3	0.1	0.660
	0.5	0.671
	1.0	0.668

Extraction time and stability of the complex: The complete extraction of iron(II)-PCHA complex from the aqueous phase into toluene was achieved within about two minutes. The toluene extract of the complex was found to be stable for at least six months at 20°C ± 2°C.

Table 4.9. Stability of the Complex with Time

Iron = 8.0×10^{-5}M

pH = 5.0

Time of measurement after extraction	Absorbance at 448 nm
5.0 min	0.672
30.0 min	0.672
2.0 hrs	0.672
6.0 hrs	0.671
2.0 days	0.673
7.0 days	0.674
15 days	0.673
1 month	0.674
1½ months	0.673
2 months	0.675
3 months	0.675
4 months	0.676
5 months	0.676
6 months	0.677

4.2.6 Beer's Law, Optimum Concentration Range, Sensitivity and Molar Absorptivity

The Fe(II)-PCHA complex obeys Beer's law in the concentration range of 1.00 to 9.00 ppm. Varying amounts of Fe(II) ranging from 28 µg to 223.4 µg were extracted following the recommended procedure. A plot of absorbance against iron concentration shows linearity in the range mentioned above (Table 4.10 and Fig. 4.3).

The molar absorptivity of Fe(II)-PCHA complex in toluene was found to be 8400 ± 50 $1\ mol^{-1}cm^{-1}$ and the corresponding Sandell's (3) photometric sensitivity was 0.00665 µg of iron per cm^2. Moreover, the optimum concentration range for the determination of iron as evaluated from the Ringbom's plot (Table 4.10 and Fig. 4.4) was found to be 1.5-5.0 ppm of iron.

Table 4.10. Calibration Curve Data for the Determination of Fe(II) with PCHA

pH = 5.0

Molar concentration of iron M ([Fe])	log[Fe]	Absorbance at 448 nm	Percent transmittance
2.0×10^{-5}	-4.69897	0.168	67.920
4.0×10^{-5}	-4.39794	0.336	46.132
8.0×10^{-5}	-4.09691	0.671	21.330
1.2×10^{-4}	-3.9208	1.009	9.795
1.6×10^{-4}	-3.79588	1.344	0.656

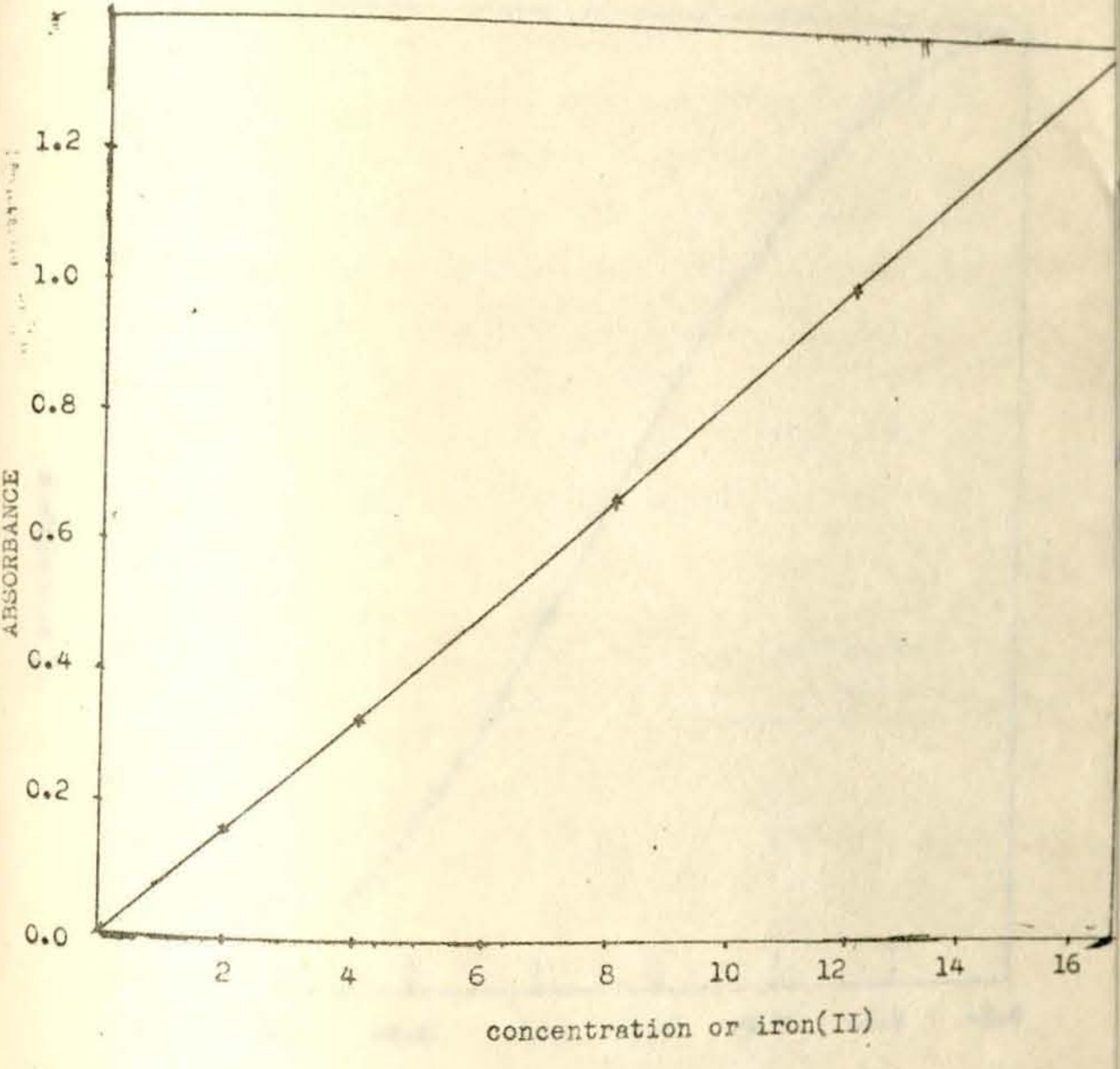


Fig. 4.3. Calibration curve for the determination of iron(II) wi
PCHA in toluene.

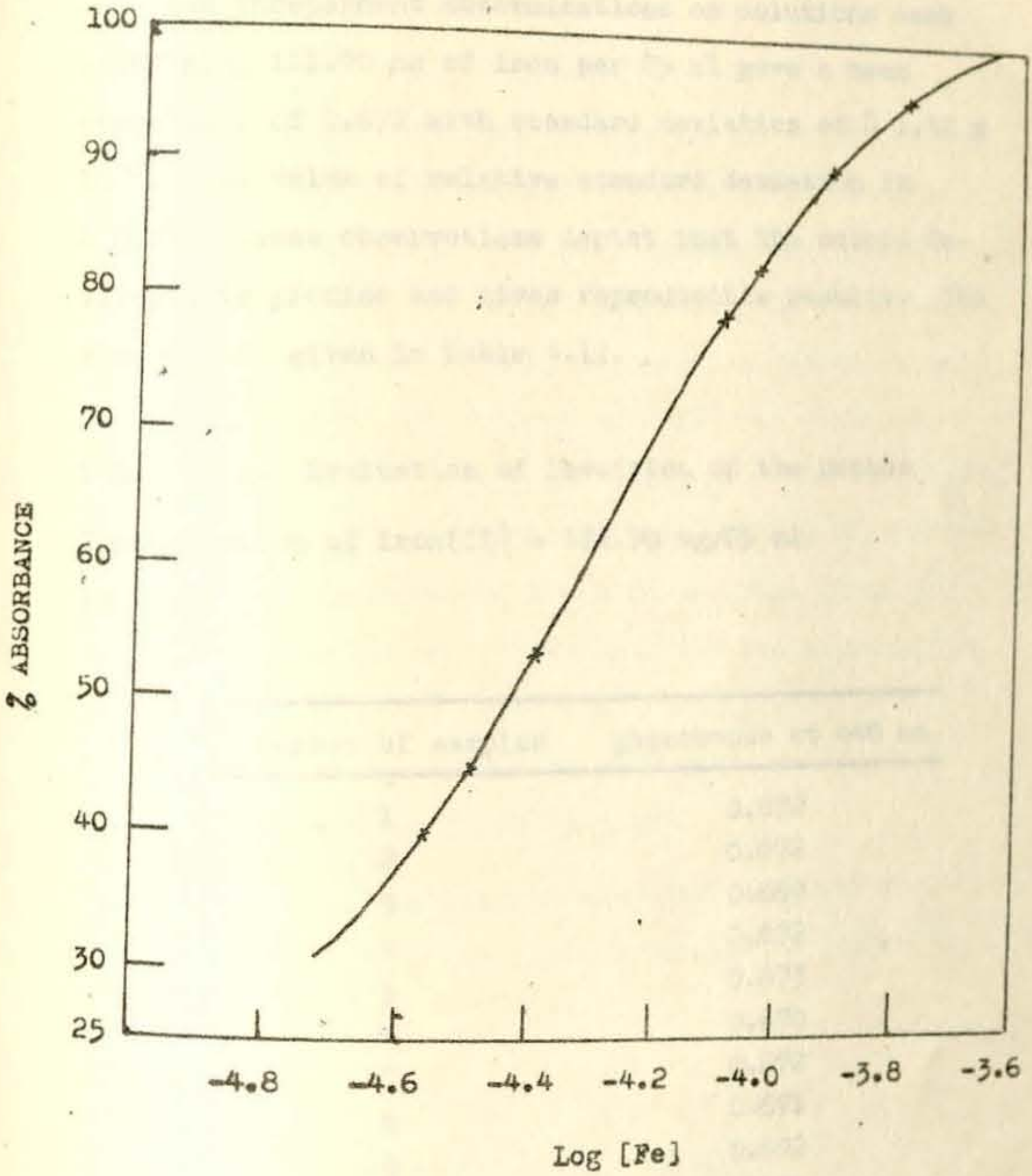


Fig. 4.4. Ringbom's plot for the evaluation of optimum concentration range for the determination of iron(II) with PCHA.

4.2.7 Precision

Ten independent determinations on solutions each containing 111.70 µg of iron per 25 ml gave a mean absorbance of 0.672 with standard deviation of $\pm 1.42 \times 10^{-3}$. The value of relative standard deviation is ± 0.2%. These observations depict that the method developed is precise and gives reproducible results. The results are given in Table 4.11.

Table 4.11. Evaluation of Precision of the Method

Concentration of iron(II) = 111.70 µg/25 ml

pH = 5.0

Number of samples	Absorbance at 448 nm
1	0.672
2	0.672
3	0.669
4	0.672
5	0.673
6	0.670
7	0.672
8	0.671
9	0.672
10	0.674

4.2.8 Composition and Stability Constant of Iron(II) PCHA Complex

The composition and conditional stability constant of iron(II)-PCHA complex have been determined by using spectrophotometric methods; the mole ratio and Job's continuous variations methods.

In the mole ratio method a series of solutions was prepared in which the concentration of iron, the pH, and the ionic strength were kept constant and the concentration of PCHA was varied under similar conditions. The complex was extracted by the general procedure and, the absorbance of the colored extracts was measured at λ_{max} (Table 4.12). After correcting for the absorbance of the ligand, the absorbance of the complex was plotted against the mole ratio of the metal to PCHA (Fig. 4.5). Two straight lines were drawn from the two parts of the curve which intersect each other at a mole ratio of 1:3 of the metal to the ligand respectively, showing the formation a 1:3 Fe(II)-PCHA complex.

The overall conditional stability constant was also calculated from the curve by using the following expression (5):

$$\beta_n = \frac{\frac{A}{A_{ex}} \cdot C_M}{(C_M - \frac{A}{A_{ex}} \cdot C_M)\,(C_L - \frac{A}{A_{ex}} \cdot \frac{C_L}{n})^n} \qquad (4.7)$$

where β_n = overall stability constant

n = number of ligand

A = absorbance on the curve

A_{ex} = extrapolated absorbance (on the straight lines)

C_M = total concentration of metal

C_L = total concentration of ligand

Therefore for n= 3, $A = 0.621$, $A_{ex} = 0.675$, $C_M = 8 \times 10^{-5}$ and $C_L = 2.4 \times 10^{-4}$M

$$\beta'_3 = \frac{\frac{0.621}{0.675} \times 8 \times 10^{-5}}{8 \times 10^{-5} \left(1 - \left(\frac{0.621}{0.675}\right)\right) \left(1 - \frac{0.621}{6.675 \times 3}\right)^3 (2.4 \times 10^{-4})^3}$$

$\beta'_3 = 2.5 \times 10^{12}$ or $\log \beta'_3 = 12.4$

Table 4.12. Result of Mole Ratio Method for Iron(II)-Complex
Concentration of iron(II)= 8.0×10^{-5}M
Concentration of KCl = 0.1 M
pH = 5.0

Mole ratio of Fe(II) to PCHA	Absorbance at 448 nm
1:1	0.210
1:2	0.416
1:3	0.621
1:4	0.672
1:5	0.686
1:6	0.670
1:8	0.671
1:10	0.676
1:50	0.675

In the continuous variations method a series of solution was prepared for each system in which the mole fraction of the metal ion, i.e. Fe(II), and the ligand, i.e., PCHA, was varied between 0 and 1 at constant total concentration under exactly similar conditions. The complex was extracted by following the general procedure and the absorbance of the colored extract taken at λ_{max} (Table 4.13). After correcting for the absorbance of the reagent the absorbance was plotted against mole fraction of the metal ion. (Fig. 4.6).

The maximum absorbance was observed at the mole fraction of 0.25 of Fe(II), indicating the ratio of Fe(II) to PCHA to be 1:3 in the extracted complex.

From the curve it was also possible to calculate the overall conditional stablity constant from the same type of expression mentioned above (Eq. 4.7).

For $A = 0.781$ $\quad C_M = 1 \times 10^{-4}M$

$A_{ex} = 0.840$ $\quad C_L = 3 \times 10^{-4}M$, , pH = 5.0

$$\beta'_3 = \frac{\frac{0.781}{0.840} \times 10^{-4}}{(1 - \frac{0.781}{0.840})\, 10^{-4}\, (1 - \frac{0.781}{0.840 \times 3})^3\, (3 \times 10^{-4})^3}$$

$\beta'_3 = 4.5 \times 10^{12}$; $\log \beta_3 = 12.65$

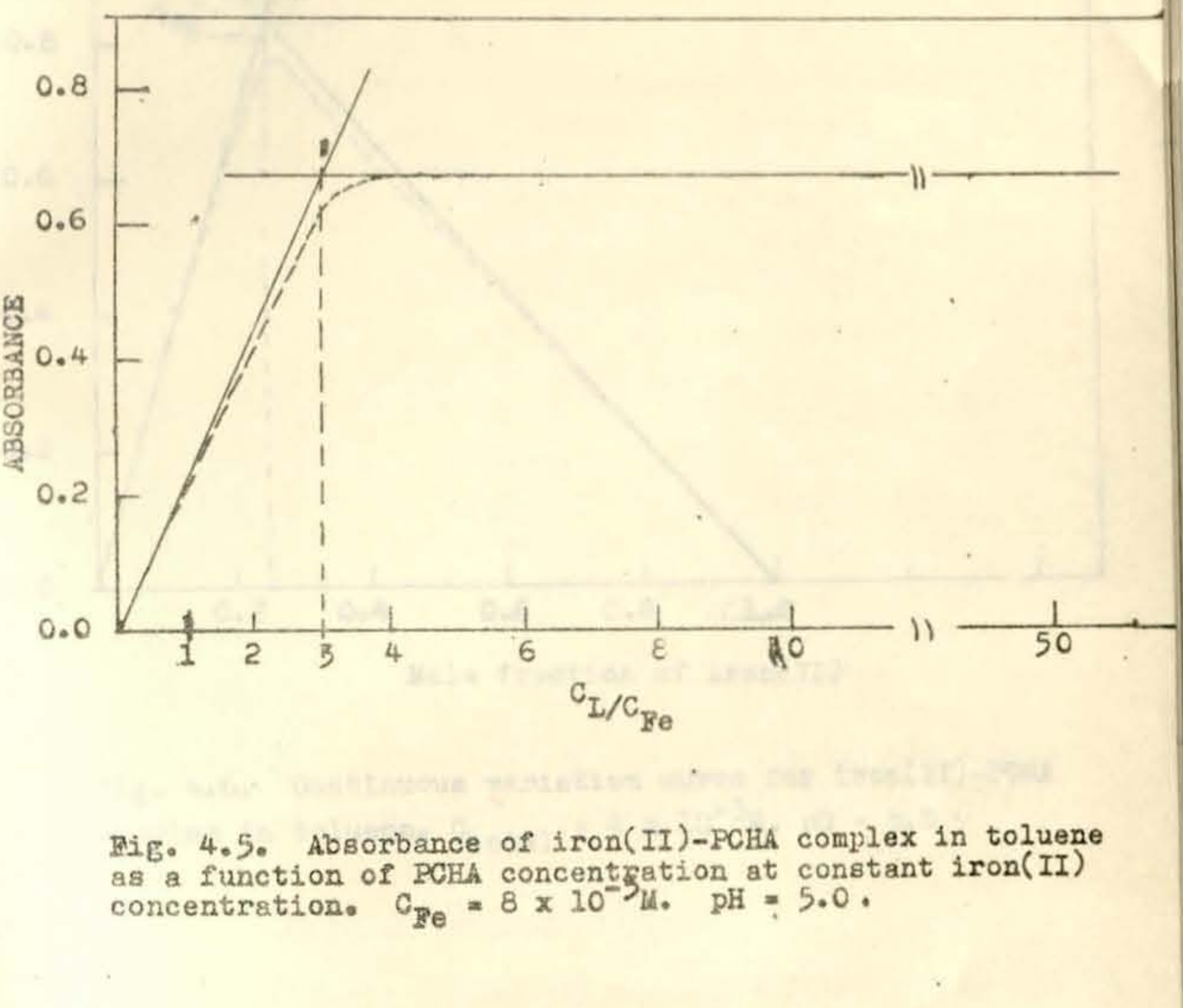


Fig. 4.5. Absorbance of iron(II)-PCHA complex in toluene as a function of PCHA concentration at constant iron(II) concentration. $C_{Fe} = 8 \times 10^{-5}$ M. pH = 5.0.

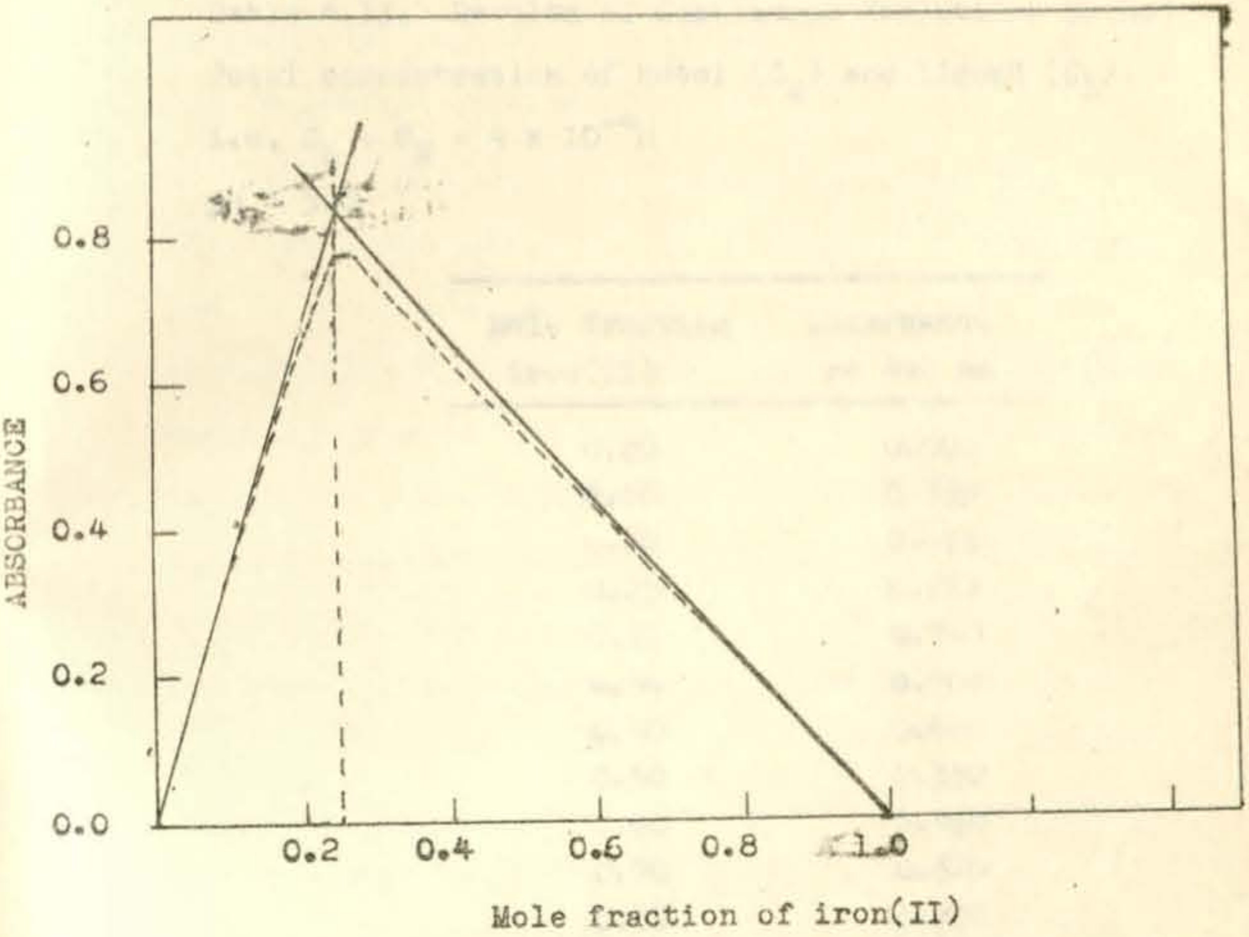


Fig. 4.6. Continuous variation curve for iron(II)-PCHA complex in toluene. $C_{total} = 4 \times 10^{-3}$M, pH = 5.0 ·

Table 4.13. Results of Continuous Variations Method
Total concentration of metal (C_M) and ligand (C_L),
i.e, $C_L + C_M = 4 \times 10^{-4}M$
pH = 5.0

Mole fraction iron(II)	Absorbance at 448 nm
0.00	0.000
0.10	0.335
0.20	0.650
0.25	0.781
0.30	0.740
0.34	0.700
0.40	0.620
0.50	0.530
0.60	0.430
0.70	0.320
0.80	0.220
0.90	0.090
1.00	0.000

4.2.9 Effect of Foreign Ions

In order to evaluate the selectivity of the method, the effect of several diverse ions on the extraction and determination of Fe(II) with PCHA has been investigated by adding a known amount of the foreign ion under question to a solution containing 111.70 µg of iron(II). Unless specifically mentioned, the pH of the working solution was kept in the range of 4.5-5.0 pH. The extractions and determinations were done by following the general procedure described earlier.

As is shown in Table 4.14, alkalis and most of the alkaline earth metals do not interfere with the determination of iron by the method developed. The maximum permissible concentration of ions such as uranium(VI), cobalt(II), zinc(II), bismuth(III) and tin(II) was relatively low. It was possible to increase the permissible limit of the ions, beryllium(II), lanthanum(III), molybdenum(VI), tungsten(VI), chromium(III), to the values mentioned in the Table, by using excess reagent, since they interfere by reacting with reagent forming colorless complexes of their own. It has been also studied that by varying the pH of the aqueous solution, it was possible to diminish or avoid the interference due to cerium(III), zirconium(IV), titanium(IV), vanadium(V) and aluminium(III). Thus, the interferences due to zirconium(IV), vanadium(IV), and titanium(IV) were eliminated by prior extraction of the metal ions with 0.1% (w/v) reagent solution in chloroform from 2 M, 4 M and 8 M hydrochloric acid solutions, respectively. Iron(III), which was taken for this purpose, remains unextracted in the aqueous phase under these relatively high acidic solutions. It was then extracted and determined after increasing the pH to optimum value and treating it with a suitable amount of hydroquinone.

The interference due to cerium(III) was diminished or avoided by first increasing the pH of the solution to somewhat greater than pH 5 and then decreasing to about pH 3. It was thought that once Fe(II)-PCHA complex is formed, it is difficult to dissociate by decreasing the pH, but this may not be true for cerium(III)-PCHA complex.

Aluminum forms hydroxide ion below pH 8, making the phase separation difficult during extraction. This undesirable phenomenon was circumvented by increasing the pH of the aqueous solution to somewhat greater than pH 8.

The shortcoming of the method is the interference of copper(II) that forms extractable colored complex with the reagent at all levels in the pH range required for complete extraction of Fe(II). However, it was possible to diminish greatly or eliminate the interference by employing excess ammonium thiocyanate solution as masking agent for copper(II).

Regarding the anions, the tolerance limit for oxalate was relatively low compared to other anions. The interference due to acetate, citrate, tartarate, borate and phthalate was shown to diminish to a great extent in the presence of excess reagent (PCHA). For instance, it has been seen that acetate can be used as a buffer in the presence of excess reagent and by increasing the extraction time to about 10 minutes. But it was also observed that, if lower concentration of the reagent has to be used as is the case for example, during the study of the composition and thermodynamic properties of the complex, etc., acetate buffer cannot be used, as it interferes seriously under this condition. Infact, ammonia buffer has been found to be effective for this newly developed method of determination of iron.

From Table 4.14, it can be observed that all common ions that normally occur with iron in living and nonliving things do not interfere or their interference can be eliminated by suitable treatment, showing the selectivity and versatility of the newly developed method.

Table 4.14. Tolerance Limit of Diverse Ions in the Determination of Iron(II) with PCHA (with an Acceptable Error Less Than ± 2%)

Iron(II) = 111.70 µg per 25 ml = 4.468 ppm

Ion added	Tolerance limit (ppm)
Li^+	800
Na^+	800
K^+	800
Mg^{2+}	800

Table contd.

Ion added	Tolerance limit (ppm)
Ca^{2+}	800
Sr^{2+}	800
Ba^{2+}	800
Tl^{+}	800
NH_4^{+}	800
Pb^{2+}	400
Ca^{2+}	400
Hg_2^{2+}	400
Be^{2+}	400
Cr^{+3}	400
Ni^{2+}	200
Zr^{4+}	100
Ti^{4+}	100
VO_2^{+}	100
UO_2^{2+}	80
Sn^{2+}	80
Ce^{3+}	80
La^{3+}	80
MoO_4^{2-}	80
WO_4^{2-}	80
Bi^{3+}	60
Co^{2+}	40
Al^{+3}	40

Table contd.

Ion added	Tolerance limit (ppm)
Zn^{2+}	30
Chloride	2000
Nitrate	2000
Sulfate	2000
Thiocyanate	2000
Tartarate	300
Fluoride	200
Acetate	200
Borate	200
Citrate	120
Phathalate	80
Phosphate	80
Oxalate	20
EDTA	-

4.2.10 Application of the Method

The newly developed method was employed for the determination of iron in blood sample and two different British Chemical Standard Steel samples, to assess its analytical potentiality.

Blood sample: The amount of iron in blood sample, prepared by following the procedure described earlier was determined both by the newly developed method and by

2,2'-bipyridyl method. The latter one does not ofcourse involve extraction procedure. The results obtained were 58.9 mg/100 ml and 58.6 mg/100 ml for PCHA and 2,2'-bipyridyl method, respectively. In both cases the results are essentially the same, and fall with the range of content of iron in whole blood reported for an adult person (man). The reported range is from 45 mg/100 ml to 145 mg/100 ml (84). Thus iron(II)-PCHA method is reliable and applicable for the determination of iron in biomaterial.

<u>Steel samples:</u> Since the standard samples were not available, synthetic samples having the same constituents with identical proportion as that of the British Chemical Standard Steel No. 64a and 241/1 were prepared. Extraction and determination of the samples were undertaken following the same procedure described earlier. The experimental results given on Table 4.15 are average of five determinations for each sample.

The results given in the Table indicate that the newly developed method is reliable.

Table 4.15. Determination of Iron in Synthetic Samples

Composition of Samples

Samples No. 241/1: W, 19.61; Cr, 5.03; Mo, 0.52; V, 1.57;
Co, 5.67; C, 0.85; Si, 0.33; S, 0.033;
P, 0.021; Mn, 0.295; Ni, 0.075; Cu, 0.1;
Sn, 0.025; Fe, 65.87%.

Sample No. 64a: C, 0.80; Cr, 4.40; V, 1.57; Mo, 4.11;
W, 5.66; Fe, 83.45%

Sample No	Iron content (%)	Iron found (%)	Error (%)
241/1	65.87	65.32	0.83
64 a	83.45	83.92	0.56

4.3 Conclusion

The colorimetric reaction of iron(II) with N-phenylcinnamohydroxamic acid forms the basis for the development of a new simple, rapid, reliable and versatile method for the extraction and spectrophotometric determination of iron(II). The extractable, neutral complex is insoluble in aqueous solution but soluble in several organic solvents. Among these toluene was found to be a suitable extracting solvent. The complex exhibits two maximum absorption bands, out of which the one at the wavelength of 448 nm was used for the quantitative determination, because the contribution of the ligand to the measured absorbance values is less than what it is at the other wavelength. The effect of several experimental variables on the newly developed method was studied and the method was found to be largely free from rigid control of experimental variables.

Consequently, it is possible to eliminate the interferences due to some ions, and thus maintain its selectivity by making some slight changes in the general procedure. The method was also found to be sensitive and precise.

The results obtained by applying the method for the determination of iron in blood and steel samples are strong evidence for the sensitivity, selectivity, and wide application of the newly developed method.

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DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

Aberra Fura

Signature Aberra Fura

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