Kinetic Studies and Methods of Analysis of some Compounds of Analytical Interest Utilizing Sequential Injection Analysis Technique

by

Nabeel Ibrahim Desai

A Thesis Presented to the

FACULTY OF THE COLLEGE OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

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September, 1996

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KINETIC STUDIES AND METHODS OF ANALYSIS OF SOME COMPOUNDS OF ANALYTICAL INTEREST UTILIZING SEQUENTIAL INJECTION ANALYSIS TECHNIQUE

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This thesis, written by Nabeel Ibrahim Desai under the direction of his Thesis Advisor and approved by his Thesis Committee, has been presented to and accepted by the Dean of the College of Graduate Studies, in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in CHEMISTRY.

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CHEMISTRY

September 1996

To my beloved parents

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ABSTRACT

NAME : Nabeel Ibrahim Desai

TITLE : KINETIC STUDIES AND METHODS OF ANALYSIS OF SOME COMPOUNDS OF ANALYTICAL INTEREST UTILIZING SIA TECHNIQUE

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For the first time, Sequential Injection (SI) technique has been employed for full kinetic investigation of the oxidation reaction of vitamin C with iron(III) in sulfuric acid media using 1,10-phenanthroline as an indicator. The resulting solution of tris 1,10-phenanthroline-iron(II) complex was monitored at 510 nm. Kinetic parameters such as order of reaction with respect to each reactant, the activation energy were all determined and hence the mechanism of its reaction is postulated thus validating a method for determination of vitamin C. In addition to that palladium(II) in hydrochloric acid was used for the complexation reaction with promethazine .The complex was found to be absorbing at 504 nm. The Sequential Injection technique has been utilized for the determination of some physicochemical parameters such as mole ratio and formation constant at different pH values. Both methods have been successfully applied for the determination of these drugs in their pharmaceutical preparations.

MASTER OF SCIENCE DEGREE

KING FAHD UNIVERSITY OF PETROLEUM AND MINERALS

DHAHRAN, SAUDI ARABIA

September, 1996

الخلاصة

إسم الطالب : نبيل إبراهيم ديساى

عنوان الرسالة : دراسة الحركية وطرق التحليل لبعض المركبات ذات الأهمية التحليلية بإستخدام طريقة الحقن التتابعي .

التخصص : كيمياء

تاريخ الشهادة : سبتمبر ١٩٩٦م

تم إستخدام طريقة الحقن التتابعي ، ولأول مرة ، لدراسة حركية شاملة لأكسدة فيتامين ـ ج بواسطة الحديد الثلاثي في وسط حامض الكبريتيك وباستخدام ١٠.١ فينانثرولين كمادة كاشفة .تم في هذه الدراسة تحديد المتغيرات الحركية ، مثل رتبة التفاعل بالنسبة لكل متفاعل ، وطاقة التنشيط للتفاعل وذلك من أجل إقتراح آلية مناسبة للتفاعل ومن ثم إثبات مشروعية طريقة حركية لتحديد فيتامين ـ ج . أيضاً أستخدم البلاديوم الثنائى في وسط حامض الهيدروكلوريك للتفاعل تراكبياً مع البروميثازين ووجد أن المتراكب المتكون يمتص الضوء عند ٥٠٤ نانوميتر كما تم إستخدام طريقة الحقن التتابعي لتحديد بعض المتغيرات الفيزيوكيميائية ، كدرجة الإتحاد العنصري . كلتا الطريقتين المذكورتين أعلاه أستخدمتا بنجاح تام لتحديد هذه الأودية في المستحضرات الصيدلانية .

درجة الماجستير في العلوم

جامعة الملك فهد للبترول والمعادن

الظهران ، المملكة العربية السعودية

سبتمبر ١٩٩٦م

Chapter 1

Introduction

1.1 Objective

The research work comprises the following objectives

- The use of sequential injection (SI) analysis as an innovative technique for the kinetic studies of vitamin C in pharmaceutical products.
- Postulation of reaction mechanism to know whether the reaction is elementary or composite by finding out order of reaction and reaction rate constant wherever possible using kinetic parameters for the purpose of validating a new method using SI technique.
- Devise a better method of analysis or propose to improve the already existing method.
- Applicaton of sequential injection analysis to some fundamental studies, for example, to suggest the stoichiometry of the reaction, equilibrium constants of promethazine drug by its oxidation with palladium(II).

1.2 Automatic instruments and Automation

Any measurements in a chemical laboratory involving liquid materials consists of the following operations: (a) solution handling, (b) Analyte detection, (c) Data collection, and computation of results. There is no shortage of computers and sophisticated detectors to aid chemists in performing the latter two tasks, but solution handling requires an arsenal of skills, which a chemist has to master [1,2].

Sequential injection analysis (SIA) is an automatic analysis system which provides analytical data by handling solutions with a minimum of operation intervention [3]. It is based on continuous flow analysis, in which the concentration of analyte is measured uninterruptedly by injecting it in a stream of liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the absorbance, electrode potential, or other physical parameter.

1.3 Instrumentation

1.3.1 Principles of SIA

Whereas flow injection analysis has undergone a remarkable transformation from a tool for serial assay into a widely accepted means of enhancement of instrumental analysis [4,5]. The comments of users of flow injection analysis (FIA) in the environment led to the development of sequential injection analysis (SIA). Although some would argue that SIA is just a variation of FIA, there are certain fundamental differences in the use and control of the operational parameters used in SIA. Some of the design principles for SIA are

contrasted with those for FIA and can be found elsewhere [6]. The major difference among them is that SIA uses a selector valve rather than an injection port present in FIA. SIA like its predecessor Flow injection analysis (FIA) is based on three principles;

(a) sample injection ; in this the chemical reactions are taking place while the sample material is dispersing within the reagent.

(b) controlled dispersion of the injected sample zone ; as the concentration gradient of the sample zone is being formed by the dispersion process, therefore the concept of dispersion, controlled within space and time is the central issue of SIA.

(c) reproducible timing of its movements from the injection toward and into the detector; so whatever happens to one injected sample happens in exactly the same way to all other subsequently injected samples.

Therefore the design of the sequential injection system also follows the established rules for providing limited, medium or high dispersion of the injected sample zones [7].

1.3.2 Construction

SI uses a single-channel pump to move the column of liquid in reverse and forward steps through a channel, and which consists of a holding coil, multiposition valve and detector (Fig 1.1).

The pump is capable of performing multiple operations. It propels carrier through a holding coil into a multiposition valve and finally toward detector then waste. When the

pump is set to reverse mode in the counter clockwise direction (CCW), it can be used to draw reagents and sample from their respective valves outlets through the multiposition valve back into the holding coil where the sample is dispersed into the reagent and/or carrier. Then again this analyte is sent in the forward clockwise (CW) direction through multiposition valve into the reactor and finally it passes from a flow-through detector where it is sensed and recorded to give a fiagram [5].

The multiposition valve serves as a central distributer through which appropriate volumes of liquid segments are sequenced by aspiration into the holding coil (HC) and then propelled by a flow reversal into the detector [7].

Pump tubes are available in different materials, according to use, they are required to introduce reagents and to transport the specimen from one module to another.

Reactor is a certain arrangement of geometry in the form of tube or chamber. Its function is to increase the intensity of radial mixing by reducing parabolic velocity profile in the axial direction. There are several types of channel geometries, but for our research work we are using mixing chamber.

Depending on the selection of liquid drives (pumps) and the type of reactor used the instrument can be setup in different arrangements.

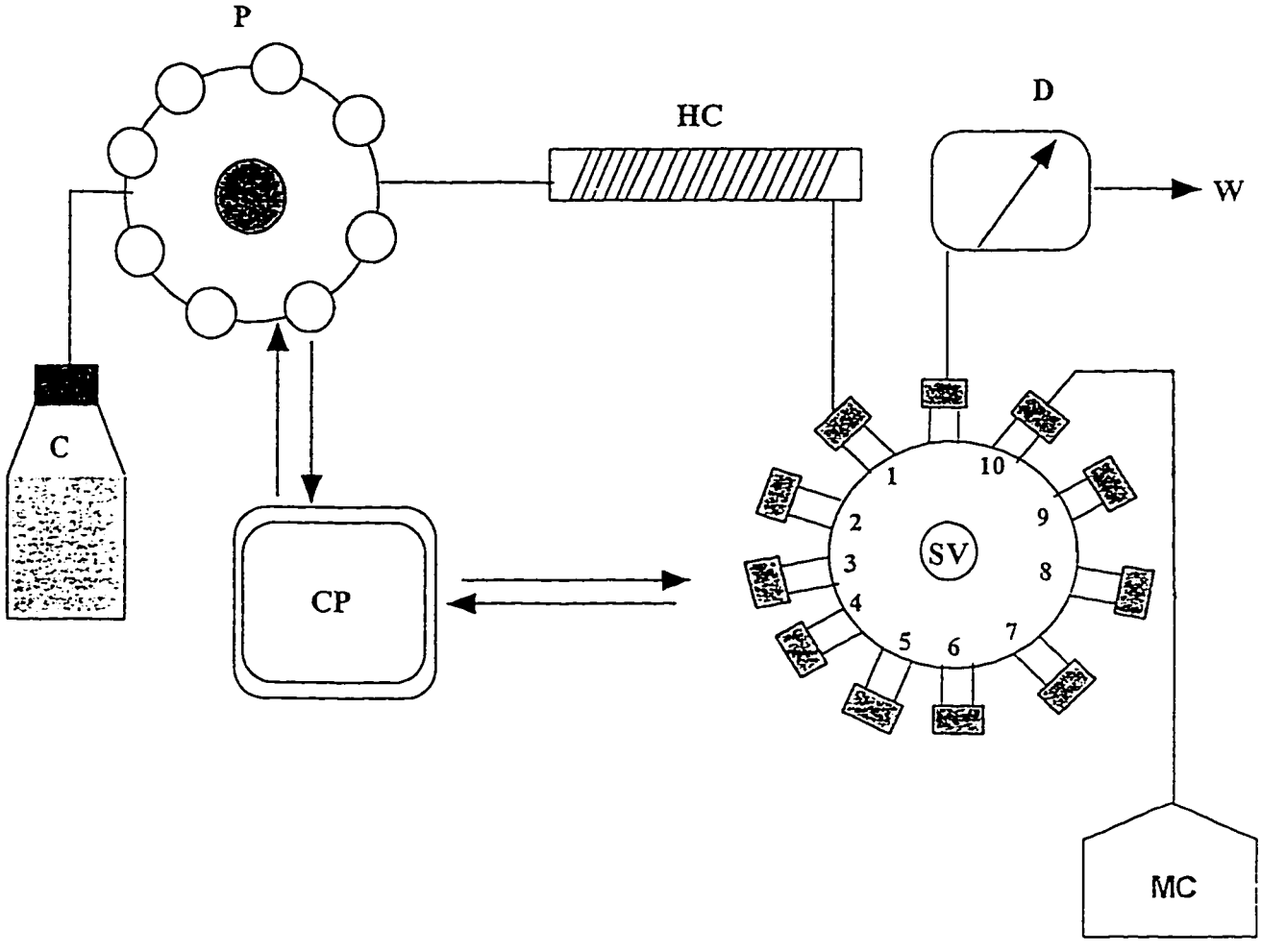


Fig 1.1 Typical SIA manifold : Carrier solution (C), Peristaltic Pump (P), Holding coil (HC), Selector valve (SV), Reagent-1, 2, 3 etc., Computer (CP), Mixing chamber or Reactor (MC), Detector (D) and waste (W).

1.4 Overview of Chemical Kinetics

Chemical kinetics is the branch of chemistry that deals with rate of chemical reactions and of the factors on which the rates depend, whereas *rate of reaction* is the change of concentration of a reactant or product as a function of time.

The common methods for measuring rates are *initial rates* and *integral time method.* The measured rate of reaction is often found to be proportional to the concentrations of the reactants raised to some power e.g. it may be found that the rate is proportional to the concentration of two reactants A and B ,

and that

$$v = k\ [A]^{\alpha}\,[B]^{\beta}$$

The coefficient k is called the rate constant and is independent of the concentration (but dependent on the temperature, so temperature should be kept constant during rate measurements). The exponent α is *order of reaction with respect to A* or *partial order,* similarly, the *partial order* β is the *order with respect to B* . These orders are purely experimental quantities and may not be necessary integral. The sum of all the partial orders, $\alpha + \beta + \gamma + \ldots$, is referred to as the *overall order* and usually denoted by n . The units of rate constant vary with the order of the reaction. There is no simple connection between the stoichiometric equation for the reaction and the order of the reaction. An experimentally determined equation of the above kind is called *rate law* of the reaction.

The rate law have two main applications,

- A practical application is that once we know the rate law and rate constant we can predict the rate of reactions from the composition of the mixture.
- The theoritical application of rate law is that it is a guide to the mechanism of the reaction.

After measuring the rates of reaction, the order of reaction of simple reactions, can be evaluated by : (a) Integration, (b) Half life, (c) Differential or (d) Isolation method.

A useful indication of the rate of chemical reaction is half the *Half-life* $t_{1/2}$ of a substance. The time it takes for its concentration to fall to half the initial value. Half-lives depend on the initial concentration of the substance in a characteristic way for reactions of different orders, so their measurement is a guide to reaction order.

The method of *Integration* can be used for complex reactions where the products themselves might affect the rate. In this case the rate law is fitted to the data throughout the reaction. But this procedure is seldomly entirely satisfactory, owing to the difficulty of distinguishing between various possibilities.

In such cases the best method is usually the *differential* method, in this the rates of change of concentrations dC/dt are measured accurately in the initial stages of the reaction, and runs are carried out at the series of initial concentrations. Values of dC/dt are obtained from a plot of C against t by taking slopes, and these are directly compared with the rate equation. So a plot of rate against concentration is prepared and the dependence of rate on concentration can then be determined by various methods.

The theory of differential method is " the instantaneous rate of a reaction of the nth order involving only one reacting substance is proportional to the nth power of its concentration."

$$v = -\frac{da}{dt} = ka^n$$

therefore $$\log_{10} v = \log_{10} k + n \log_{10} a$$

A plot of ' $\log_{10} v$ ' against ' $\log_{10} a$' therefore will give a straight line if the reaction is of simple order; the slope is the order n . The main difficulty with this method is that slopes cannot always be obtained very accurately. In spite of this drawback the method is on the whole a more reliable one.

The determination of rate law is simplified by the *isolation method* in which the concentration of all the reactants except one are in the large excess. If B is in large excess, for example, it is a good approximation to take its concentration constant throughout the reaction. Then the relation

$$v = k\ [A][B]$$

may be second order overall, we can approximate [B] by $[B]_o$ and write

$$v = k'[A] \quad \text{where } k' = k\,[B]_o$$

which has the form of the first-order rate law, and it is called pseudo first-order rate-law. Rate law may be complicated as

$$v = \frac{k_1 [A]^2 [B]^{1/2}}{k_2 + k_3 [B]}$$

Isolating A by taking B in excess gives

$$v = k\,[A]^2 \qquad\qquad k = \frac{k_1 [B]^{1/2}}{k_2 + k_3\,[B]_o}.$$

This is a pseudo second-order rate law, and much easier to analyse. The dependence of the rate on all the reactants is found by isolating them in turn (by having all the other substances present in large excess), and by doing so constructing an overall rate law. The isolation method may be applied to all the other methods described above. In the present work isolation method is applied to differential method in conjugation with initial rates method.

1.5 Kinetic developments

There have been tremendous advances in the past years in advance instrument technology and measurement techniques. Slowness of some reactions investigated deemed necessary to study them by making use of kinetic methods of analysis. Kinetic method of analysis are based on kinetics of chemical reactions and they make use of the response of dynamic systems, which has several advantages [10,11], to assay the analytes in their respestive samples.

The stop-flow approach is more economical. The carrier stream is not pumped when the assay is not in progress and the reaction time is controlled by the length of the stop-flow intervals, rather than by adjusting the reaction coil as necessitated by continuous flow [7].

Most of the criticism that is usually claimed about kinetic methods is due to the lack of accurate reproduction of the reaction conditions in each experimental determination.

Reproducibility in the experimental conditions is therefore a major concern in kinetic methods compared to equilibrium methods specially regarding the time which is more crucial in the former [11]. It can be concluded that the requirements of a successful kinetic method of analysis are : accurate timing, careful adjustment of experimental conditions, precise sample and reagent measurements and accurate measurement of the response signal.

1.6 Kinetic studies with SIA

Sequential injection analysis (SIA) [2,13] introduced by Ruzicka and Marshall [12] as a second generation of gradient flow technique is based on the sequential aspiration of a sample and reagent through a selector valve into holding coil to form a stack of well defined zones. When the direction of the flow is reversed a composite zone, in which the sample and reagent zone penetrate each other, is formed. Delibrate increase of axial dispersion obtained by flow reversal is required to achieve maximum zone penetration, because in kinetic studies an increase in zone broadening increases the range of useful delay time 't_d' values.

For the first time the SIA technique has been very recently utilized and used by Sultan and Suliman for a full kinetic study of bromazepam by its complexation with Iron(II) in Hydrochloric acid.[1]

The technique is very promising for kinetic determination because injection volumes, reaction times and zone dispersion can all be changed readily and precisely by varying

sequenced volumes, flow rate, stopped-flow times and reversals via computer control of the pump.

In SI only one pump is used to propel the composite zone, therefore the complexity of the system increases and the sampling frequency of SI is always lower than that of the corresponding FI method. Also, although the SI system always has fewer moving components than a comparable FI system, and uses at least an order of magnitude less of reagents (as reagents are injected not pumped continuosly), it will require more complex software.

The most important feature of SI are its versatility and computer compatibility. Hence , once designed, the SI system, in contrast to the FI system, does not need to be reconfigured, even if the essential parameters such as flow rates, sample and reagent volumes, reactant ratios and reaction times are to be altered. All these changes can be made from a computer keyboard or even with the aid of simplex optimization, interactively and automatically. Therefore, SI may well become the preferred tool for exploratory computer-optimized research into reagent based chemistries [7].

1.7 Spectrophotometric technique of analysis

The practice of determining the concentration of a substance in solution by comparing its colour with known solutions has become well established in all branches of chemistry ; especially analytical chemistry.

Certain instruments employ light covering a small wavelength band provided by the use of selective filters. The instruments which use monochromatic light are known as *spectrophotometers* and the process of measurement is known as *spectrophotometry*. The spectrophotometer is an instrument used for measuring the relative amounts of radiant energy or radiant flux as a function of wavelength. It may measure the radiant energy or flux passing through, or reflected from a medium relative to that of a standard solution.

The spectrophotometer, is really two instruments in one cabinet; a *spectrometer* and *photometer*. The spectrometer is a device for producing light of any selected colour (or wavelength) and it is generally calibrated in wavelength units (nm). A photometer is a device for measuring the intensity of light, and when incorporated in a spectrophotometer is used to measure the intensity of a monochromatic beam produced by the associated spectrometer. Photometric measurement indicates directly the relative concentration of a coloured solution if the instrument is arranged to read directly in terms of absorbance measured with monochromatic light.

The portion of electromagnetic spectrum used in spectrophotometric measurements is divided into the *ultraviolet*, extending from 100 to 400 nm, *visible* which extends from 400 to about 800 nm *infrared* which extends above 800 nm. Ultraviolet and visible spectrophotometry has been applied in this research work.

1.7.1 Laws of Absorption

Quantitative analysis by spectrometric methods is based on laws of absorption. The law is called Beer-Lambert's law or simply *Beers's law*, which states that the absorption of light is directly proportional to the length of solution and concentration of solute.

$$-\log \frac{P_o}{P} = abc = A$$

Where Po / P = Transmittance, A = Absorbance, a = absorptivity.

This law of absorption is the law of "Additive absorbances". If the solution contains a mixture of absorbing species, 1, 2, 3, etc., all of which obey Beer's law, the absorbances are additive :

$$A\,(total) = A_1 + A_2 + A_3 ..... = b\,(a_1 c_1 + a_2 c_2 + a_3 c_3 .....)$$

Being in the same cell the path length b is constant, so to correct unwanted absorption "blank" solutions are used. If the concentration of absorbing solution is expressed in *moles per liter* and the length in *centimeters*, " a " becomes the *molar absorptivity* ($\in$). which is expressed in liter mol^{-1} cm^{-1} for a defined wavelength. Molar absorptivity values are useful as an indication of light absorbing property of a system, the higher the value the more sensitive the analytical process based on a particular system.

Molar absorptivity ($\in$) varies from less than one for a very weak absorption to almost 10^5 lit mol^{-1} cm^{-1}, and its order of magnitude is a useful guide in identifying different types of absorption. For example if the electronic absorption band for an octahedral

complex is $1 < \in < 200$, the chances are that it is a *spin-allowed band*, if $\in < 1$ band is spin forbidden. Finally $\in > 200$ the band may be a *charge transfer band.*

In some cases it has been shown that absorbance is not proportional to concentration, i.e. Beer's law is not valid. In such cases spectrophotometry is used but also involves *calibration curve* by plotting absorbance against concentration. For best precision the absorbance of both the known and the unknown should be between 0.2 to 0.8 absorbance units.

1.7.2 Deviations from Beer's law

Deviations from Beer's law fall into three catagories : real, instrumental, and chemical.

1.7.2.1 Real deviation

Real deviation arise from changes in refractive index of a system. Beer's law should be applied at low concentration, although molar absorptivity is constant and independent of concentration, but the refractive index η , which is essentially constant at 10^{-3} M or less concentration, may vary at high concentrations and so will absorptivity. Still, quantitative analysis is possible at high concentrations by bracketing standard solution and forming a calibration curve.

1.7.2.2 Instrumental deviation

Monochromatic light used in experiments require specialized line emission sources to obtain a truly monochromatic light. All monochromators have finite resolving power and

therefore minimum instrumental band width. If absorptivity is constant over the instrumental bandwidth, then Beer's law is followed within close limits. Departure from Beer's law is most serious for wide bandpasses and narrow absorption bands, and is less significant for the broad bands and narrow slits.

1.7.2.3 Chemical deviations

Chemical deviations from Beer's law are caused by shifts in the position of a chemical or physical equilibrium involving the absorbing species. Consider the following equilibria :

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

The dichromate(VI) ion absorbs in the visible region at 450 nm. Upon diluting a dichromate solution the equilibrium shifts to the left. The equilibrium can be controlled by converting all the (VI) species to $Cr_2O_7^{2-}$ by making the solution 0.1 M in sulfuric acid, or coverting all the chromium(VI) species to CrO_4^{2-} by making the solution 0.05 M in potassium hydroxide. Beer's law will then be followed. If the acsorbing species involved is an acid-base equilibrium, Beer's law will fail unless the pH and the ionic strength are kept constant.

1.7.3 Spectrophotometric studies of complex ions

Application of spectrophotometric methods for elucidating the composition of complex ions in solution and determining their formation constants can be carried out without disturbing the equilibria under consideration. Although most spectrophotometric studies of complexes involve a system in which one of the reactants or he products absorbs, this condition is not a requisite provided that one of the components can be caused to participate in a competing equlibrium that does not produce absorbing species.

The following techniques have commonly been used for the study of complex-formation equlibria.

(i) Method of continuous variation

(ii) Mole ratio method

(iii) Slope ratio method

(iv) Method of matching absorbances

(v) Method of isosbestic point plot.

For the present work the Method of continuous variation and Mole ratio method have been applied which are the generally applicable and widely used techniques for elucidating the formula of a complex ion. These methods have the same constraints as of Beer's law (see section 1.7.2). Detailed explanation for these two methods is in chapter 2.

1.8 Drugs Investigated

1.8.1 Vitamin C

Vitamin C (1-keto-1-threo-hexono-g-lactone-2,3-enediol) is commonly known as l-ascorbic acid (Fig 1.1). It is the enediol-group [- C (OH) = C (OH) -] which is responsible for the molecule's acidic and reducing properties. It has received the attention of various researchers in different fields and many analytical methods have been described. [14,15,16,17].

Determination of vitamin C using Flow Injection Analysis (FIA) has been applied, but they are complex and subject to numerous interferences. [18]

In the British Pharmacopoeia (BP) monograph [19] a titrimetric method involving the use of Cerium (IV) as a titrant and ferroin sulphate as an indicator in sulfuric acid media, is the one described for the determination of vitamin C in drug formulations.

In the present work , method for the determination of vitamin C will be investigated for the first time using Sequential Injection (SI) Spectroscopic technique. SI is employed in a full kinetic investigation of the redox reaction of vitamin C with Iron (III) in sulfuric acid leading to the direct postulation of a reaction mechanism, thus validating a comprehensive method of analysis. The oxidation of vitamin C in sulfuric acid media by Iron (III) results in a red-brown product that can be monitored spectrophotometrically at 510 nm. The influence of the most critical variables on the rate of the reaction will be studied and the optimum conditions of these variables will be derived.

The remarkable development of SI technique allows mechanistic scientists to use simple, computer controlled instrument that has been lacking for decades, which will

replace the time consuming manual dilution procedures that are currently being performed. In SIA, reaction times and mutual zone dispersion can be altered directly from the computer keyboard. Therefore an attempt will be made to exploit the capabilities of this technique in studying the chemical kinetics of the system.

Reaction rate constants as well as other kinetic parameters will be determined using sequential flow techniques and a plausible mechanism for the reactions involved will be proposed.

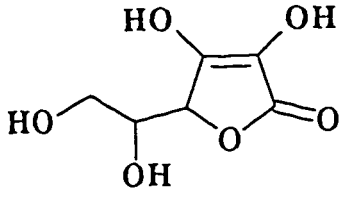
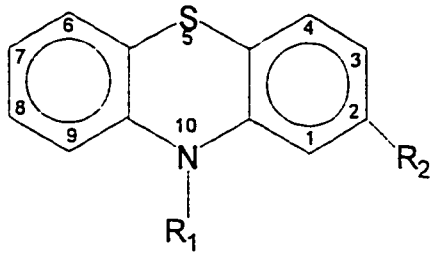


Fig 1.2 Chemical structure of ascorbic acid

1.8.2 Phenothiazines

Phenothiazines which are commonly known as neuroleptic tranquilizers are used as sedatives, antihistamines, antiemetics and anaesthetics. Phenothiazine is a three-ring structure in which two benzene rings are attached to each other by a sulfur and a nitrogen atom in the C-5 and the C-10 positions, respectively (Fig 1.3). Substitutions usually occur at the 2 and 10 position. Substitution of a chlorine, methoxy, thiomethyl, acetyl or trifluoromethyl group in the 2 position increases the



Phenothiazine nucleus

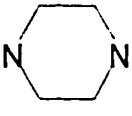
(1) Chlorpromazine

$R_1 = CH_2\text{-}CH_2\text{-}CH_2\text{-}N(CH_3)_2$ $\qquad R_2 = Cl$

(2) Promethazine

$R_1 = CH_2\text{-}CH(N(CH_3)_2)\text{-}CH_3$ $\qquad R_2 = Cl$

(3) Perphenazine

$R_1 = CH_2\text{-}CH_2\text{-}CH_2\text{-}N$  $N\text{-}CH_2\text{-}CH_2\text{-}OH$ $\qquad R_2 = Cl$

(4) Trimeprazine

$R_1 = CH_2\text{-}CH(CH_3)\text{-}CH_2N(CH_3)_2$ $\qquad R_2 = H$

Fig 1.3 Chemical structure of phenothiazines.

antipsychotic potency of these drugs. The nature of the side chain substituent at the 10 position influences both the potency and the pharmacological activity of the compound. Antipsycotic properties are only present if there are three carbon atoms between the nitrogen in the 10 position of the phenothiazine nucleus and the amine group of the side chain. The presence of a fourth carbon atom in the chain causes a loss of antipsychotic effect of the compound. Reduction of the side chain to two carbon atoms can change the antipsychotic activity to antihistaminic or antiparksonian, depending on the nature of the substituents on the basic amino group.

Antipsychotic phenothiazines are divided into three groups based on the nature of the side chain attached to the 10-position: dimethylaminoalkyls, piperazinyl-alkyls and piperidyl-alkyls. The dimethyl amino derivatives, of which chlorpromazine is the best known example, are characterized by significant sedative properties making them useful in the treatment of psychotic patients experiencing sleep disturbances. The piperazine derivatives are the most potent antipsychotic compounds and possess less sedative action than the aminoalkyl phenothiazines an example of this group is perphenazine.[20]

A simple and accurate method for the determination of promethazine by complexation with palladium(II) in hydrochloric acid using automated method will be developed. SIA technique will be fully applied for the determination of some fundamental physicochemical parameters usually encountered in chemical reactions. Palladium(II) is complexed with the promethazine drug to form a colored species found to be absorbing at 504 nm[21]. A method of assay will be suggested.

Chapter 2

Theoretical Background

Apart from the original papers describing the principles of Sequential injection analysis (SIA) few publications have appeared in the literature so far. It is apparent that the sequential injection SI technique is still an infant and its full versality and capacity is yet to be explored. The research in the exploration of the capability of this technique led to publish a first ever paper in kinetic studies by Sultan entitled

" *Use of a Sequential Injection Technique for Mechanistic Studies and Kinetic Determination of Bromazepam Complexed with Iron (II) in Hydrochloric Acid* ".[1]

However, the mechanism of oxidation of vitamin C is still remaining unexplored. The utilization of SI technique for the first time in the full kinetic studies of vitamin C is expected to be a new addition to literature and definitely a positive contribution not only to the analytical chemist and FIA world but also to the kineticists and mechanistic people.

In addition, promethazine, a member of the phenothiazine family, was discovered in preliminary investigation by Sultan to have been reacting by palladium(II) thus

developing a colored product that could be spectrophotometrically measurable for the quantitative determination of this compound using SIA[21]. The development of a new method for this compound by SIA will be a positive introduction since very few methods of analysis of this compound have been reported so far. Two other types of Phenothiazines namely trimeprazine and perphenazine have been analysed using SI technique. [22]

Determination of the optimum operating condition in SIA is considered the corner stone in the development and characterization of an analytical method. Result of studies of the influence of experimental variables on the analytical signal (S) are usually used to choose the optimum operating conditions. Optimization is meant to compromise dispersion of the sample zone, its mixing with a reagent, and the time required to achieve the desired chemical conversion of an analyte into a detectable species, in order to minimize undesired zone broadening and to maximize sensitivity and sampling frequency.

The choice of a specific analytical method for a given analyte in a particular sample depends on the relative importance of the evaluation criteria and figures of merit to the specific situation expected [8,9]. This choice for analytes in drug formulations rely on a number of requirements including the accuracy, precision, analysis time, as well as the range of concentrations of analyte and concominants in the sample matrix.

2.1 Brief Overview of FIA

The kinetic methods employed in Flow Injection Analysis (FIA) which is a predecessor of Sequential Injection (SI) technique is explained below.

In stopped-flow injection the sample is injected into a carrier stream, reagent is added, and the mixture transported into the flow cell where the flow is stopped.

Advantages of this method over the continuous flow method are [2,13]

1. Increase of sensitivity because reactants and products are no longer being diluted, and background signals can be eliminated as they remain unchanged during the stopped-flow interval.
2. Accuracy can be improved as the measurements are less sensitive to perturbations such as lag phases and deviation from linearity.
3. Provide kinetic discrimination and therefore enhance selectivity because kinetic measurements are less subject to interferences.
4. Consumption of reagent solutions is greatly reduced, because reagents are used only when needed rather than continuously.

Gradient stopped-flow FIA [5] is ideally suited for measurement of reaction rates and rate laws of chemical reactions because the FIA peak profile provides as infinite number of reactant ratios, so that stopping the flow at suitable positions on the peak allows the adjustment of conditions to first order, second order, etc. It was found that single line FIA systems provide data that is difficult to interpret[23], because the initial reagent concentrations are not known, because mixing of sample and reagent by mutual penetration begins at the moment of injection and proceeds continuously to the flow cell detector [13,24]. Conversely, a two-line system was used with ease to measure the rate constants for reactions of permanganate with benzaldehyde and crotonic acid [24,25,26].

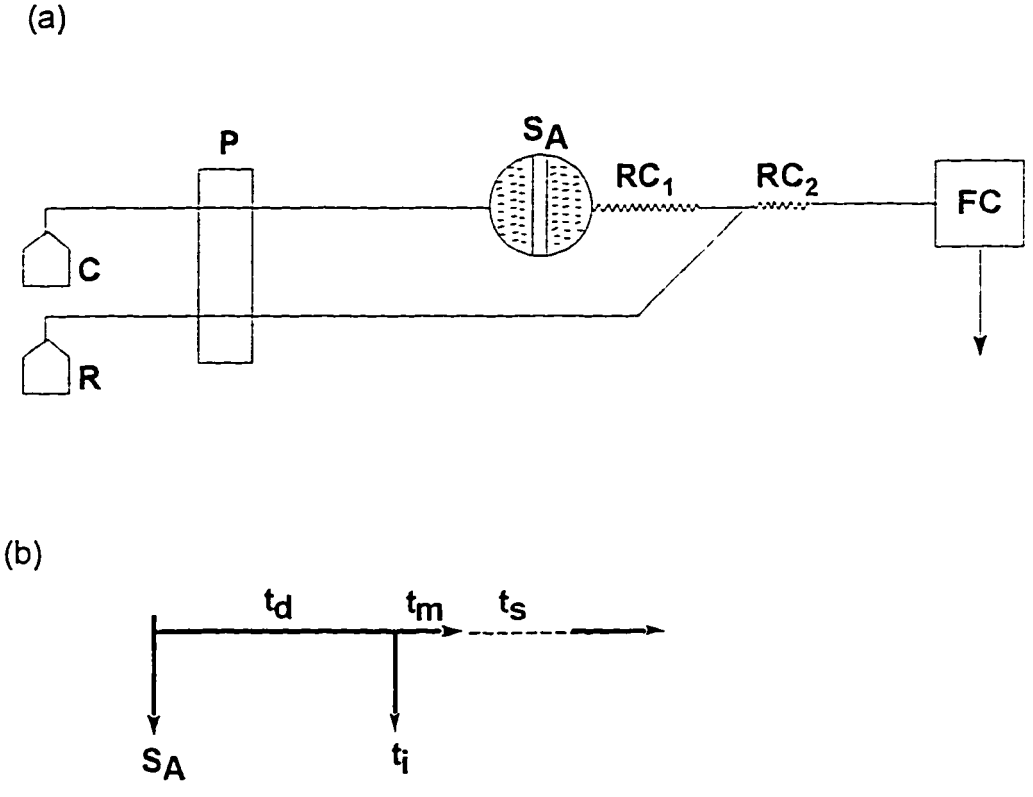


Figure 2.1 (a) Double-line FIA manifold for kinetic studies. R, reagent line; C, carrier solution line; P, peristaltic pump; SA, sample injection valve; RC_1 and RC_2 , reaction coils; FC, flow cell. (b) Timing sequences for stopped- flow injection analysis; t_d , delay time; t_i , initial reaction time; tm , mixing time and t_s , is the stopped flow period.

FIA kinetic conversion techniques are defined as procedures by which a non-detectable species is converted into a detectable species via a kinetically controlled chemical reaction [27,28]. Conversion techniques incorporate most of the chemical assays performed under FIA condition, except the cases when the chemical reaction is very rapid and therefore goes to completion before it reaches the detector. A method for the determination of phenolic and hydrazino drugs with 1-fluoro-2,4 dinitrobenzene using a Fluoride-selective electrode was described by Apostolakis et al. [29]. Taking the advantage of the controllable kinetic conditions when executed in FIA the analytes in pharmaceutical products were determined in a micellar medium. Chung and Ingle [32] determined total ascorbic acid using a kinetic fluorimetric method based on a FIA system. In this method, ascorbic acid solutions were injected into a carrier stream of mercury (II) chloride and 1,2-diaminobenzene to form a fluorescent product on line. A fixed-time method was proposed by Sultan for the determination of bromazepam in pharmaceutical preparations [30,31]. The method was based on stopping the flow for a given time just after sample injection using single line flow injection manifold.

A general treatment for the determination of rate laws and rate constants with the aid of a two-line manifold (Fig. 2.1) will be presented here.

Consider the reaction,

$$nA + B \rightarrow \text{Products} \qquad (2.1)$$

When analyte 'A' is injected into the carrier stream 'C', a well defined zone is formed which disperses on its way through a reactor RC_1 . The concentration of analyte 'A' can be obtained from the knowledge of the dispersion coefficient as follows ,

$$C_A = C_A^o \ / \ D_A \qquad (2.2)$$

where C_A^o is the analyte 'A' concentration before injection (steady-state response), C_A is the maximum response of gradient concentration of the zone element of the aliquot withdrawn from the mixing chamber for a given set of volume V_S , V_T , V_A ; and D_A is the dispersion coefficient describing the concentration of C_A within the sample zone gradient element at delay time 'td'. The dispersion coefficients of sample and reagent(s) have to be carefully considered when developing an assay [14]. In this way the ratio of the reactant is selected by choosing a delay time 'td' that yields optimum conditions for reaction-rate measurements [7]. The reproducibility of the delay time td is critical for repeatable capturing of reacting components as controlled by mutual zone merging [15].

When the analyte 'A' is injected into an inert carrier 'C' and these are confluenced with reagent 'B', each element of the dispersed analyte is mixed with same volume of reagent. The initial reagent concentration C_{Bi} will well defined as

$$C_{Bi} = C_B^o \, Y \, (X + Y)^{-1} \qquad (2.3)$$

where X and Y are the pumping rates of the carrier 'C' and reagent 'B' respectively. The initial concentrations of analyte C_{Ai} for each delay time are obtained by dispersion experiments in which the horizontal portions of the stopped-flow curves correspond to C_A in equation '2.2'. Since in the mixing chamber RC_2 both chemical

reaction and dispersion takes place simultaneously, it is necessary to consider the error caused by the reaction of 'A' with 'R' during the time 't_m' allowed for mixing. If C_{AC} is defined as the concentration of 'A' consumed by reaction in mixing chamber RC_2, the apparent initial concentration of 'A' detected in the flow cell is

$$C_{Ai} = \frac{C_A}{D} - C_{AC} \qquad (2.4)$$

where D is the dispersion calulated at a given delay time, t_d, and C_{AC} is the concentration of 'A' consumed by reaction in mixing chamber (RC_1).

When tm << $t_{1/2}$ of the reaction $C_{AC} << C_{Ai}$ then equation '1.4' will reduce to

$$C_{Ai} = C_A^{\,o} \;/\; D \qquad (2.5)$$

the rate law for equation '2.1' is given by

$$-\frac{dC_A}{dt} = k_2\, C_{At}\, C_{Bt} \qquad (2.6)$$

where k_2 is the second order rate constant and C_{At} and C_{Bt} are the analyte and reagent concentration respectively at time t initiated at t_i.

Integration of this equation will result into

$$k_2 t = \frac{1}{(aC_{Ai} - C_{Bi})} \ln \left[\frac{C_{Bi}(C_{Ai} - X_t)}{C_{Ai}(C_{Bi} - aX_t)} \right] \qquad (2.7)$$

$$Xt = C_{Ai} - C_{At} \qquad (2.8)$$

Substituting equation '2.5' and '2.8' into equation '2.7' and rearranging, we obtain equation '2.9'

$$k_2 t = \frac{1}{\left(\frac{a C_A^{o}}{D} - C_{Bi}\right)} \ln \frac{[C_{Bi} \; C_{At}]}{[C_A^{o} \; / \; D_A(C_{Bi} - aX_t)]} \qquad (2.9)$$

When $C_{Bi} >> C_{Ai}$ then equation '2.9' will simplify to eqution '2.10'

$$\ln \frac{C_A^{o}}{D} - \ln C_{At} = C_{Bi} k_2 t = k't \qquad (2.10)$$

where k' is the pseudo-first order rate constant at a given C_{Bi} . k' can be obtained safely without prior knowledge of C_A^{o} / D_A , but it has little value if C_{Bi} is known. By suitable selection of C_{Bi} / C_{Ai} ratio, pseudo-first order and second order rate constants can be obtained.

2.2 Sequential Injection Analysis (SIA)

Sequential injection (SI) [12,13] is the second generation of gradient flow techniques, based on the sequential aspiration of sample and reagent zones through a selector (rather than injection valve) in a channel or a holding coil (figure 1.3). In this manner a stack of a well defined zones is obtained which is then allowed to penetrate each other by flow reversal. The flow reversal creates a composite zone a section of which can be trapped inside the observation zone of the detector, by stopping the flow, when stop-flow technique is used for reaction rate measurements. Advantages of SIA [14] include mechanical simplicity, since only a single valve and a single pump is needed, and reliability, because once configured the components and associated flow channel do not need physical

restructuring. The degree of zone dispersion can be influenced by tuning the length and the number of flow reversals, and the reaction time can be adjusted through the duration of a stopped flow period.

2.3 SI methodology [6]

The typical measuring cycle of the SI technique comprises the following steps (when a peristaltic pump is used):

(1) Forward pumping of carrier solution with the selector valve in position 1, until the holding coil, reactor and detector have been washed out.

(2) Aspiration of sample solution with the selector valve in position 2 by flow reversal into the holding coil.

(3) Aspiration of reagent solution with the valve in position 3 by another flow reversal.

(4) Forward pumping of carrier solution with the valve in position 1, to propel the composite sample reagent zone through the reactor into the detector.

(5)* A stop of the flow when the selected part of the composite zone reach the detector for reaction rate monitoring.

(6) Resumption of the forward flow until all of the reagent and products are washed out of the detector.

Zone sequencing and mutual interdispersion are the key operations of sequential injection analysis (SIA). The dimensions of the holding coil, and of the reactor, the number and duration of flow reversals the volumes of the aspirated zones and the flow rates used, all together affect the degree of zone overlap within an adequate residence time [15].

**Step (5) is applied when stop-flow technique is used*

In analogy with conventional FIA [2] , the dispersion of the sample zone is to be adjusted in order to fulfill the requirements of an intended assay. It was found that this is easily achieved through selection of injection volumes and the delay time at which the forward movement of the zone through the detector is being stopped [15]. For stopped-flow reaction-rate measurement, the choice of delay time (t_d) will allow optimization of the analyte to reagent ratio. Deliberate increase of axial dispersion obtained by flow reversal is required to achieve maximum zone penetration, because in kinetic studies an increase in zone broadening increases the range of useful t_d values.

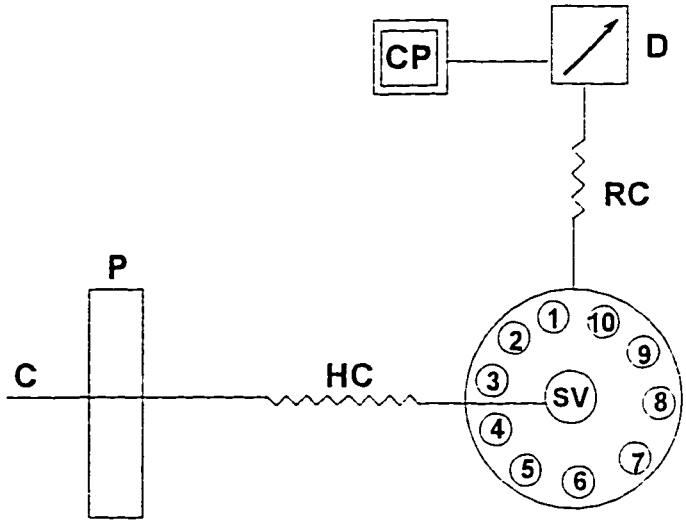


Figure 2.2 Typical SI-manifold; C, carrier; P; pump; HC, Holding coil; SV, selector valve; RC, reaction coil; D, detector; CP, computer

2.4 Determination of Formation Constants

Most of the methods available for determining formation constants are mainly based on preparing a series of solutions containing known proportions of the complex-forming species, in which the concentration of one of the reactants or products is followed directly or indirectly by a suitable analytical technique.[35]

Many factors, e.g. ionic strength, pH, etc., affect the formation constants. Much more important is that the ligand and the metal ions are the only species involved in complexation. Schwarzenbach [36,37] introduced the concept of the 'apparent formation constant' in connection with the theory of complexometric titration. Ringbom [38] developed the theory further, and extended the concept to other fields of analytical chemistry. The name 'conditional constant' was introduced by Ringbom is now widely used and recommended, since it well expresses the notion of a term which can be assumed as constant only under given condition.

Introduction of computers has revolutionized the calculation of formation constants from equilibrium data, utilizing modern numerical methods of analysis, making it an easy task for even complicated systems. Many developments in the mathematical algorithms, computer programs and experimental techniques are continuing rapidly to determine formation constants.[39,40]

2.4.1 The Continuous Variation Method

This method was first introduced by Ostromisslensky [41] and later developed by Job [43] to determine stoichiometries and formation constants of complexes. In the method a series of solutions is prepared by mixing different volumes of equimolar solutions of the ligand and the metal ion and diluting to a constant volume to give solutions of the same total molar concentration but different mole fractions. If a single, stable complex is formed, a plot of absorbance versus mole fraction of one of the reacting species gives a characteristic triangular plot. The mole fraction of the maximum of this plot gives the stoichiometry of the complex. Likussar [48] has developed a theoretical approach to the continuous variation method in which no approximations are necessary. The general equations he developed to calculate the formation constant do not contain molar absorptivities.

Let us consider the general equation for the formation of complex M_mL_n, at equilibrium

$$mM + nL \text{ ----------> } M_mL_n \qquad (2.11)$$

where M represents a metal ion, L a ligand, M_mL_n the complex formed in a molar ratio of " m : n " for M and L.

The formation constant K_f for the complexation reaction is then given by the following equation:

$$K_f = \frac{[M_m L_n]}{[M]^m [L]^n} \tag{2.12}$$

$$= \frac{[M_m L_n]}{\left[\left(C_m - m[M_m L_n]\right)^m \left(C_L - n[M_m L_n]\right)^n\right]} \tag{2.13}$$

where C_M and C_L are the total metal and total ligand concentrations respectively.

The mole fraction (x) is defined by

$$x = \frac{C_M}{(C_M + C_L)} = \frac{C_M}{k} \tag{2.14}$$

$$k = (C_M + C_L)$$

utilizing the normalized absorbance concept [49] together with the foregoing equations an expression for calculating the formation constant can be obtained. This expression does not contain molar absorptivity terms and is given by:

$$K_f = [(m+n)/k]^{(m+n-1)} y[(m+n)x - my]^{-m} . [(m+n)(1-x) - ny]^{-n} \tag{2.15}$$

where y is the normalized absorbance.

2.4.2 The Molar Ratio Method

This method was introduced by Yoe and Jones [52] and since then it has been extensively used for the determination of stoichiometry and formation constants of complexation reactions. The molar ratio method is based on graphical representation of the observed absorbances versus the molar ratios of the two components of the complex when the concentration of one component is held constant while that of the other component is varied. Momoki et.al [49] developed the theoretical and mathematical algorithm that describe this method. Equations similar to those described in the continuous variation method are used to develop these mathematical equations which are also free from molar absorptivity terms. The molar ratio curve is described by the following equation

$$\kappa_M = (m/n).y + (nK)^{-1/m} C_L^{-(m+n-1)/m} .y^{1/m} (1-y)^{-n/m} \qquad (2.16)$$

where κ_M is the mole ratio of the metal ion, other symbols are of the same meanings as above.

Chapter 3

Experimental

3.1 Experimental setup

3.1.1 Sequential Injection Analyzer

The sequential injection analyzer was constructed from the following components:

(1) A high quality **peristaltic pump** (C4V, Alitea, Medina, WA, USA) is used. It features eight stainless steel rollers on individual bearings.

(2) A Valco - **10 port selector valve** (cheminert, Valco instruments, Houston, TX) is used to select the flows. Upchurch fittings (Upchurch, Oak Harbor, WA, USA) were used to lock unused ports.

(3) The **holding coil** and the reaction coil tubings as well as the tubings connecting the different units were made of PTFE (0.8 mm i.d.). Teflon nuts and ferrules (Upchurch, Oak Harbor, WA, USA) were used to fit these tubes into the different parts of the apparatus. **Pump tubings** were Phar Med™ 1.02 mm i.d. (Upchurch Oak Harbor, WA, USA) held

on the pump rollers by FIA peristaltic pump tubing adapters (Upchurch, Oak Harbor, WA, USA).

(4) **Reactor module** consisting of 0.5 mm i.d. PTFE tubing (Thermoplastic Scientific, NY, USA) of different length was used. The length of this reaction coil is chosen as appropriate and coiled in a way to enhance radial mixing.

(5) **Mixing chamber** of 20 mm i.d. is connected at position 10 of the multi-port selector valve, which is used for kinetic studies.

(6) A Spectronic Mini-20 **spectrophotometer** (Milton Roy, Rochester, NY, USA), with a grating monochromator detector, a Unovic ultra-micro-flow-through cell (Unovic instruments, NY, USA) of 20 μl size and with a pathlength of 1.0 mm is used.

(7) A **personal computer** (Austin computers system, Austin, TX, USA) working at 33 MHz, and equipped with a 120 MB hard disk, 4 MB RAM and VGA Graphics, is used to monitor digitally the pump and the valve. The communication between the computer and the external devices was expanded by a general purpose I/O board (Model ADA-110, Real Time Devices (RTD), State Collage, PA, USA). The computer is also used to collect the data, alternatively the data is recorded by Model 0555 single channel strip-chart recorder (Cole Parmer, instrument Co., Chicago).

(8) In addition to the personal computer a **Recorder** Model 0555 single channel-strip-chart recorder (Cole Parmer, Chicago, USA) was used for peak absorbance-time recording.

Perkin Elmer Lambda 5 UV/Visible Spectrophotometer equipped with 10.00 mm cells was used for preliminary investigations.

3.1.2 Software Packages

Microsoft windows 3.10 with DOS 6.20 was utilized to run the following softwares for research work.

FIA Lab *2.0* (*beta release*), Alitea USA, Inc. is used to program the sequential injection system for analysis under predefined specific conditions.

Sigmaplot, *version 1.02*, Jandel Scientific Corporation., for data handling calculations and forming graphs.

Microsoft Word, *version 6.0*, Microsoft Corportion, for building tables and text writing.

3.2 Reagents

Iron(III) Solution: An equimolar **stock** solution of 0.05 M iron(III) solution was prepared in 0.05 M sulfuric acid by dissolving exactly about 6.0412 g of dried ammonium ferric sulfate $NH_4Fe(SO_4)_2.12H_2O$ (99% BDH, Poole, UK) in 0.05 M sulfuric acid in a 250ml calibrated flask. The solution was heated for one hour with frequent stirring, then kept in dark and used after 24 hours to guarantee complete dissolution into a clear green transparent solution.

Amount = Molarity x Molecular weight x Volume / 1000

Amount = 0.05 x 482.18 x 250 / 1000 = 6.02725 g

Solutions of ammonium ferric sulphate (AFS) in which the concentration of acid is lower than the concentration of AFS could not be prepared as in such solutions partial dissolution takes place due to hydrolisation. Therefore equimolar stock solution was prepared.

The **working** solutions were 0.01 M ammonium ferric sulphate (AFS) in 0.01 M sulfuric acid, and 2.5×10^{-3} M (AFS) in 2.5×10^{-3} M sulfuric acid. The former was used to determine *order of reaction with respect to sulfuric acid and vitamin C* also this was used to find the *activation energy* at different temperatures and *calibration curve*, and the latter was used to determine the *order of reaction with respect to* AFS.

Solutions of AFS having concentrations more than 0.01 M were not used in the experiments as AFS solutions have their own color and interferes with the color of tris-1,10-phenanthroline Iron(II) complex. Moreover very dilute equimolar solutions that is

5.0×10^{-4} M AFS in 5.0×10^{-4} M H_2SO_4 were not used either since the acid concentration is very low and such AFS solutions hydrolize or degrade into a turbid solution.

1,10-Phenanthroline Monohydrate Solution: 0.01 M solution of this indicator (Fisher Scientific Company, ACS reagent) $C_{12}H_8N_2.H_2O$ was prepared by adding the required amount in 100 ml flask. The solution was made up to the volume and then heated in a water bath for one hour with shaking at different times. It was used only when freshly prepared.

$$\text{Amount} = 0.01 \times 198.23 \times 100 / 1000 = 0.19823 \text{ g}$$

Sulfuric acid Solution: A **stock** solution of 10 M H_2SO_4 (95-98%, Specific gravity 1.84 kg / l , Merck, UK) was prepared. The concentration of the extra pure 95-98% sulfuric acid solution was determined as follows :

$$\text{Molarity} = \frac{\% \times \text{Density (Kg / l)}}{\text{Molecular weight} \times \text{Vol}} \quad \text{or}$$

$$\text{Molarity} = \frac{\% \times \text{Density (g / l)} \times 1000}{\text{Molecular weight} \times \text{Vol}} = \frac{*96.5 \times 1.84 \text{ g / l} \times 1000}{100 \times 98.08 \text{ g / mol}} = 18.1 \text{ M}$$

*Average % = (95+98) / 2 = 96.5% , also Amount = % x Density

Normality can be found by substituting Equivalent weight instead of Molecular weight in the same equation, which gives 36.2 N sulfuric acid solution. For 10 M acid solution,

$$V_1 = C_2V_2 / C_1 = 10 \times 1000 / 18.1 = 552 \text{ ml}$$

Where V denotes the volume and C represents concentration.

A series of **working** solutions were prepared from 0.001 M to 4.0 M. Among these 1.0 M solution was used to determine the *order of reaction with respect to acid, activation energy* and the *calibration curve*, 0.5 M was used for *order of reaction with respect to ammonium ferric sulphate*. For the *order of reaction with respect to vitamin C* the same acid present in 0.01 M ammonium ferric sulphate was utilized which was 0.01 M, so acid was not introduced from a separate port through the selector valve in the method.

Vitamin C Solution: This was always freshly prepared $C_6H_8O_6$ (99.5% Fluka AG, Switzerland). Therefore after repeated experimental trials the **working** solution proved to be 4.0×10^{-2} M because at this concentration the change of colour with respect to time was slow enough to be measured spectrophotometrically, and this concentration was used for determining *order of reaction with respect to ammonim ferric sulphate*. Similarly **working** solutions of 1.13×10^{-3} M (200ppm) was used when determining *order of reaction with respect to vitamin C* , and 8×10^{-3} M when determining *order of reaction with respect to sulfuric acid* and the *activation energy*. The *calibration curve* was determined by exactly about 5.68×10^{-3} M (1000ppm) **stock** solution and from this 1.1355×10^{-4} , 2.8388×10^{-4}, 5.678×10^{-4} , 8.5164×10^{-4} , 1.1355×10^{-3} , 1.4194×10^{-3} , 1.7033×10^{-3} M, or respectively 20, 50, 100, 150, 200, 250, 300 ppm solutions were prepared.

$$\text{Amount} = 4.0 \times 10^{-2} \times 176.13 \times 500 / 1000 = 3.5226 \text{ g}$$

Tablets formulations of proprietary drugs: The **stock** solutions of 1000 ppm for each proprietary drug were prepared by crushing five to ten tablets priorly weighed out, and dissolving an amount of the powder equivalent to a certain mass of the tested drug in water or dilute acid solution. Beminal C capsules were not crushed, only the casing of the capsules were removed to obtain the active ingredients.

The effervescent tablets (Redoxon, Upsa and Cal-C-Vita) were weighed as mentioned above and dissolved directly to give a **stock** solution of 1000 ppm without further treatment. While **stock** solution of 1000 ppm for Octovit and Beminal C was prepared by heating the mixture at 50°C for 15-30 minutes in a water bath, shaken for five minutes, filtered through an ordinary filter paper, washed with the hot water or hot acid solution several times and the filtrate plus washings were completed to the mark in a calibrated flask. The temperature was not raised above 50°C when preparing stock solutions of Octovit and Beminal C as vitamin C dissociates at higher temperatures. Appropriate dilutions were made from these stock solutions. For calculations see section 3.2.2. Details of the proprietary drugs analyzed are given in table 3.2.

Pd (II) Solution: A **stock** solution of 0.025 M in 0.02 M hydrocholoric acid was prepared by dissolving 0.4440 g of anhydrous palladium chloride $PdCl_2$ (60% Pd, Fluka AG, Switzerland) in 50 ml hydrochloric acid solution of 0.04 M. The mixture was warmed at 80°C in a water bath until all the palladium chloride solid is dissolved, then cooled to room temperature and made up to 100 ml with water to form a clear brownish

yellow transparent solution.

$$\text{Amount} = 0.025 \times 177.31 \times 100 / 1000 = 0.4433 \text{ g}$$

Stock solution containing sulfuric acid concentration lower than 0.02 M could not be prepared as Pd(II) does not dissolve in less acid solutions even after prolonged heating in the water bath.

Pd(II) solutions have their own color and could interfere with the color of palladium(II)-promethazine complex so experiments were done at optimum concentrations. Solutions of Pd(II) having concentrations more than 1.0×10^{-3} M were not used in the experiments as at high concentrations the colour of the complex is intense purple which is an indicative of the possibility that the results may deviate from the Beer's law and Job's method also at higher concentration there is higher acidity so the oxidized product predominates. Moreover very dilute solutions that is 5.0×10^{-5} M Pd(II) in 4.0×10^{-5} M HCl (pH= 4.3979) were not used because at this concentration color is not seen in the solution so there is no complexation.

Whereas solutions with 1.0×10^{-4} M Pd(II) in 8.0×10^{-5} M (pH = 4.0969) HCl were used as they form a light purple color solution which is unstable and diminishes after 24 hours so there is weak complexation at this concentration. Therefore, behaviour of the complex and stability at different pH values was observed at this concentration.

The **working** solution of 1.0×10^{-3} M Pd (II) in 8.0×10^{-4} M HCl and 1500 ppm (4.674×10^{-3} M) drug were used to prepare 1.0×10^{-4} M Pd(II) in 8.0×10^{-5} M HCl and

150 ppm (4.674×10^{-4} M) drug in 10 ml flasks with the addition of appropriate volume of a certain concentration of HCl to form a series of solutions with different pH values, the method of preparing solutions with different pH values is described in section 3.2.1. These were used to find maximum absorbance (λ_{max}) at pH values ranging from 2.0000 to 4.0929 given in table 3.1 and 4.9, also the **working** solution of 1.0×10^{-3} M Pd (II) in 8.0×10^{-4} M HCl was used directly in Job's plot and Molar ratio methods.

Hydrochloric acid solution (0.1-2.0 M): A **stock** solution of 10 M HCl (35.4% , 11.4569 M, sp. gr. 1.18, BDH, UK) was prepared by diluting AnalaR concentrated acid.

$$V_1 = C_2V_2 / C_1 = 10 \times 500 / 11.4569 = 436 \text{ ml}$$

A series of **working** solutions were prepared from 0.001 M to 1.0 M.

Promethazine Solution: The **stock** solution was 1500 ppm (4.674×10^{-3} M) in 100 ml flask $C_{17}H_{20}N_2S.HCl$ (Rhone-Poulenc, France). It is prepared by dissolving 0.15 g of drug in deionized water, **working** solutions of 150 ppm were used to determine the maximum absorbance (λ_{max}) at different pH values as mentioned above (see table 4.9). It is prepared fresh when required.

$$\text{Amount} = 1500 \text{ mg} / 1000 \text{ ml} = 1500 \ \mu\text{g} / \text{ml} \ ; \ \text{or}$$

$$\text{Amount} = 4.674 \times 10^{-3} \times 320.9 \times 100 / 1000 = 0.1499 \text{ g}$$

The **working** solution of 1.0×10^{-3} M drug in 8.0×10^{-4} M HCl was utilized to run in SIA for applying Job's plot and Molar ratio methods. The *calibration curve* was determined by exactly about 3.1162×10^{-3} M (1000ppm) **stock** solution and from this 6.2325×10^{-5}, 1.5581×10^{-4}, 3.1162×10^{-4}, 4.6744×10^{-4}, 6.2325×10^{-4}, 7.7906×10^{-4}, 9.3487×10^{-4}, 1.2465×10^{-3}, 1.5581×10^{-3} M or respectively 20, 50, 100, 150, 200, 250, 300, 400, 500 ppm solutions were prepared.

Pure drug solutions. The drugs included in this study together with the supplier name and other relevant information are given in table 3.2. Stock solutions of 1000 ppm of pure analytic generic form of each of these drugs, were prepared by dissolving the drug in water or dilute acid solution at room temperature from which working solutions were prepared by appropriate dilution. Details for preparing drug samples is given in section 3.2.2.

Syrups and Elixir: Tested solutions were prepared by diluting a pipetted amount of the syrup or elixir in a calibrated flask with water or dilute acidic solution without any treatment. Working solutions are prepared from these solutions by further dilution, and the concentration of working solution was not made more than 200 ppm because syrups and elixir are viscous, so in order to keep the flow-rate unaltered non-viscous solutions were used. For calculations see section 3.2.2. Details of the proprietary drugs analyzed are given in table 3.2.

3.2.1 Preparation of Palladium(II) and Promethazine in solutions of different pH

The *working solution* is 1.0×10^{-3} M Pd(II) in 8.0×10^{-4} M sulfuric acid, and 1500 ppm promethazine.

To prepare solutions of different pH in 10 ml flasks we do the following :

Volume of Pd (II) taken in 10 ml flask = 1 ml ; since $V_1 = 1 \times 10^{-4} \times 10 / 1 \times 10^{-3} = 1$ ml

the palladium(II) solution contains acid so the initial concentration of sulfuric acid in 10 ml flask when *one* ml of Pd(II) solution is added will be,

$$C_1 = 8 \times 10^{-4} \times 1 / 10 = 8 \times 10^{-5} \text{ M}$$

Volume of drug taken in 10 ml flask = 1 ml ; since $V_2 = 4.674 \times 10^{-4} \times 10 / 4.674 \times 10^{-3}$

$= 1$ ml

Total volume of flask = 10 ml, which is distributed as ;

Volume final = (8 ml acid and deionized water) + (1 ml Pd + 1 ml drug) = 10 ml

So, concentration of Pd in 10 ml flask will be $= C_1 = 1 \times 10^{-4}$ M

and concentration of drug in 10 ml flask will be = $C_2 = 4.674 \times 10^{-4}$ M (150 ppm)

Now, from the *working solution*, to prepare solution containing 1×10^{-4} M Pd(II), 150 ppm promethazine in 8.0×10^{-5} M acid (pH= 4.0969) we just take 1 ml of each Pd and drug solutions in 10 ml flask and dilute to mark.

For preparing 1×10^{-4} M acid (pH = 4.0) solution :

# of mmol = volume (ml) x concentration (mmol / ml)

Total number of mmol *required* for 1×10^{-4} M acid solution = $10 \times 1 \times 10^{-4}$ M

$= 1 \times 10^{-3}$ mmol

# of mmol of acid *from Pd(II)* = 1 ml x 8×10^{-4} mmol / ml = 8×10^{-4} mmol

# of mmol of acid *remaining* = 1×10^{-3} mmol - 8×10^{-4} mmol = 2×10^{-4} mmol

We know, volume (ml) = # of mmoles / M ,

and if 0.001 M is utilized then, the required volume of this 0.001 M acid in the 10 ml flask will be

volume = $2 \times 10^{-4} / 1 \times 10^{-3} = 0.2$ ml , thus,

In a 10 ml flask 0.2 ml of 0.001 M HCl , 1 ml of 1×10^{-3} M Pd(II) in 8×10^{-4} M, and 1 ml of 1500 ppm promethazine is added and made up to the mark with deionized water (7.8 ml) to give 1×10^{-4} M Pd(II) with 150 ppm promethazine in 1×10^{-4} M acid (pH = 4.0) solution.

Similarly a wide range of solutions with different acid concentrations were prepared shown in table 3.1.

Table 3.1 Different pH values of hydrochloric acid used to determine maximum absorbance (λ_{max}).

No.	HCl / M	pH	No.	HCl / M	pH
1	8.0×10^{-5}	4.0969	15	7.5×10^{-4}	3.1249
2	1.0×10^{-4}	4.0000	16	8.0×10^{-4}	3.0969
3	1.5×10^{-4}	3.8239	17	9.0×10^{-4}	3.0458
4	2.0×10^{-4}	3.6989	18	9.5×10^{-4}	3.0223
5	2.5×10^{-4}	3.6021	19	1.0×10^{-3}	3.0000
6	3.0×10^{-4}	3.5228	20	2.0×10^{-3}	2.6989
7	3.5×10^{-4}	3.4559	21	3.0×10^{-3}	2.5228
8	4.0×10^{-4}	3.3979	22	4.0×10^{-3}	2.3979
9	4.5×10^{-4}	3.3468	23	5.0×10^{-3}	2.3010
10	5.0×10^{-4}	3.3010	24	6.0×10^{-3}	2.2218
11	5.5×10^{-4}	3.2596	25	7.0×10^{-3}	2.1549
12	6.0×10^{-4}	3.2218	26	8.0×10^{-3}	2.0969
13	6.5×10^{-4}	3.1871	27	9.0×10^{-3}	2.0469
14	7.0×10^{-4}	3.1549	28	0.01	2.0000

3.2.2 Preparation of drug samples

Effervescent tablets

1) *Redoxon: 1g vitamin C per tablet* . To prepare 1000ppm in 100ml flask.

1000mg vitamin C is present in 4.3997g tablet

100mg vitamin C is present in 0.43997g tablet

Therefore exactly about 0.4400g was weighed, and working solution of 100ppm was prepared.

2) *Upsa: 1g vitamin C per tablet.* To prepare 1000ppm in 100ml flask.

1000mg vitamin C is present in 3.6022g tablet

100mg vitamin C is present in 0.36022g tablet

Therefore exactly about 0.3604g was weighed, and working solution of 150ppm was prepared.

3) *Cal-C Vita: 1g vitamin C per tablet.* To prepare 1000ppm in 100ml flask.

1000mg vitamin C is present in 4.5545g tablet

100mg vitamin C is present in 0.45545g tablet

So exactly about 0.4597 g was weighed, and working solution of 200ppm was prepared.

Tablet

4) *Octovit: 30mg vitamin C per tablet.* To prepare 1000ppm in 100ml flask.

30mg vitamin C is present in 0.8170g tablet

100mg vitamin C is present in (0.8170g / 30mg) x 100mg tablet

= 2.7233g tablet powder

0.8170g is one tablet (Note : 0.8170 is the average weight of five tablets)

2.7233g is 3.33 tablet

Therefore exactly about 2.7274g was weighed, and working solution of 200ppm was prepared.

Capsule

5) *Beminal C: 300mg vitamin C per capsule.* To prepare 1000ppm in 50ml flask.

300mg vitamin C is present in one capsule powder of 0.4404g

50mg vitamin C is present in (0.4404g / 300mg) x 50mg tablet

= 0.0734g tablet powder

0.4404g is within one capsule powder

0.0734 is within 0.1666 capsule powder

Thus exactly about 0.7357g was weighed, and working solution of 200ppm was prepared.

Syrup

6) *Phenergen Expectorant: 0.1g promethazine in 150ml syrup.* To prepare 100ppm working solution in 50ml flask.

0.1g promethazine is present in 150ml syrup

150ml syrup contains 100 mg promethazine

1000 ml syrup contains (100mg / 150ml) x 1000ml = 666.67mg ,

666.67mg / 1000ml = 666.67ppm

$V_1 = C_2 \times V_2 / C_1 = 100 \times 50 / 666.67 = 7.5ml$

7) *Phenergen: 0.1g promethazine in 150ml syrup.* To prepare 150ppm working solution in 50ml flask.

0.1g promethazine is present in 150ml syrup

0.1×10^6g promethazine is present in 150ml syrup

0.1×10^6µg / 150ml = 666.67 µg / ml , or 666.67ppm

$V_1 = C_2 \times V_2 / C_1 = 150 \times 50 / 666.67 = 11.25$ml

Elixir

8) *Cigan: 5.0mg promethazine hydrochloride in 5.0ml elixir.* To prepare 200ppm working solution in 50ml flask.

5.0mg promethazine is present in 5.0ml elixir

5.0ml elixir contains 5.0mg promethazine

1000 ml syrup contains (5.0mg / 5.0ml) x 1000ml = 1000mg ,

1000mg / 1000ml = 1000ppm

So the drug contains 1000ppm promethazine.

$V_1 = C_2 \times V_2 / C_1 = 200 \times 50 / 1000 = 10$ml

Table 3.2 List of drugs used and their suppliers.

Generic name	Supplier name	Batch number
Vitamin C	Fluka AG, Switzerland	238304 883
Promethazine-HCl	Rhone-Poulenc, France	W3021

Proprietary name	Supplier name	Batch number
Redoxon	F.Hoffmann-Roche, Switzerland	B374
Upsa	Upsa laboratories, France	UPSC19 9312 178102
Cal-C-Vita	F.Hoffmann-Roche, Switzerland	B3525
Octovit	SK&F, England	379400
Beminal with C Fortis	Wyeth-Ayerst, Canada	(L) 1 FXE-G5
Phenergan Expectorant	Rhone-Poulenc, France	--
Phenergan	Rhone-Poulenc, France	--
Cigan Elixir	Cimabrex, Denmark	061989D

3.3 Procedure

3.3.1 SIA-Procedure

Figure 3.1 shows the SI-manifold used in this study, the components of which has been outlined above. The procedure starts by nesting reagents, samples and carrier (wash) solutions around the selector valve (SV). A certain amount of each of these solutions was first aspirated into the lines from ports by selecting each port at a time. This step was aimed to fill these lines with appropriate solutions. The excess solution introduced into the holding coil (HC), one at a time, was expelled through port 9 to auxiliary waste.

The steps of the analysis procedure using SIA are then fed into the computer as follows:

1. The carrier solution is pumped through port 1, by setting the pump in the forward direction for a 25-45 seconds in order to flush the system (holding coil, reactor and detector) with the carrier solution.
2. A predetermined volume of the reagent solution was aspirated through port 2 into the holding coil (HC) in the reverse direction. The remaining reagents if any were aspirated into the holding coil via ports 3-8. The number of ports were utilized according to the number of reagents.
3. Drug solutions were aspirated into the holding coil from ports 9, one at a time. Both of the last two steps were achieved by setting the pump in the reverse mode.

Finally the composite zone is propelled by the carrier solution through port (1) to the reaction coil and then into the detector. When the mixing chamber was used instead of

a reactor coil the reagents, one or more at a time (according to the time of withdrawal and length of the tube), and the sample are initially injected into the mixing chamber. At same intervals of time aliquots of the solution in the mixing chamber were withdrawn into the holding coil then analyte is pushed to the detector using the carrier by forward pumping.

The data was then acquired by the computer and transferred to a plotting software for further calculations.

In each of the above steps the volume of the solutions aspirated was determined from the time of aspiration and the volumetric flow rate of the pump (using the relation, " Volume = Flow-rate x time ") which was 29.5 $\mu l\ s^{-1}$ in this study.

3.3.2 Determination of Flow-rate

The pump speed can be altered by changing the rpm (revolution per minute) meter on the instrument. The rate of flow of the reagent inside the pump tubes was found out by withdrawing liquid (distilled water) at one end of the tube and collecting at the other end in a measuring cylinder. The volume of the liquid collected at a certain time gives the flow-rate in milliliter per minute (ml/min) at that particular rpm value.This process was repeated for different rpm values giving different amount of flow-rate, thus a graph between flow-rate and rpm is formed to obtain a straight line giving slope and intercept as follows :

$$\text{Flow-rate} = 0.73644 + 0.05726 \times \text{rpm}$$

Therefore, for pump speed of 500 rpm, which was used throughout this research work, flow-rate will be 1.77 ml / min or 29.5 μl / s.

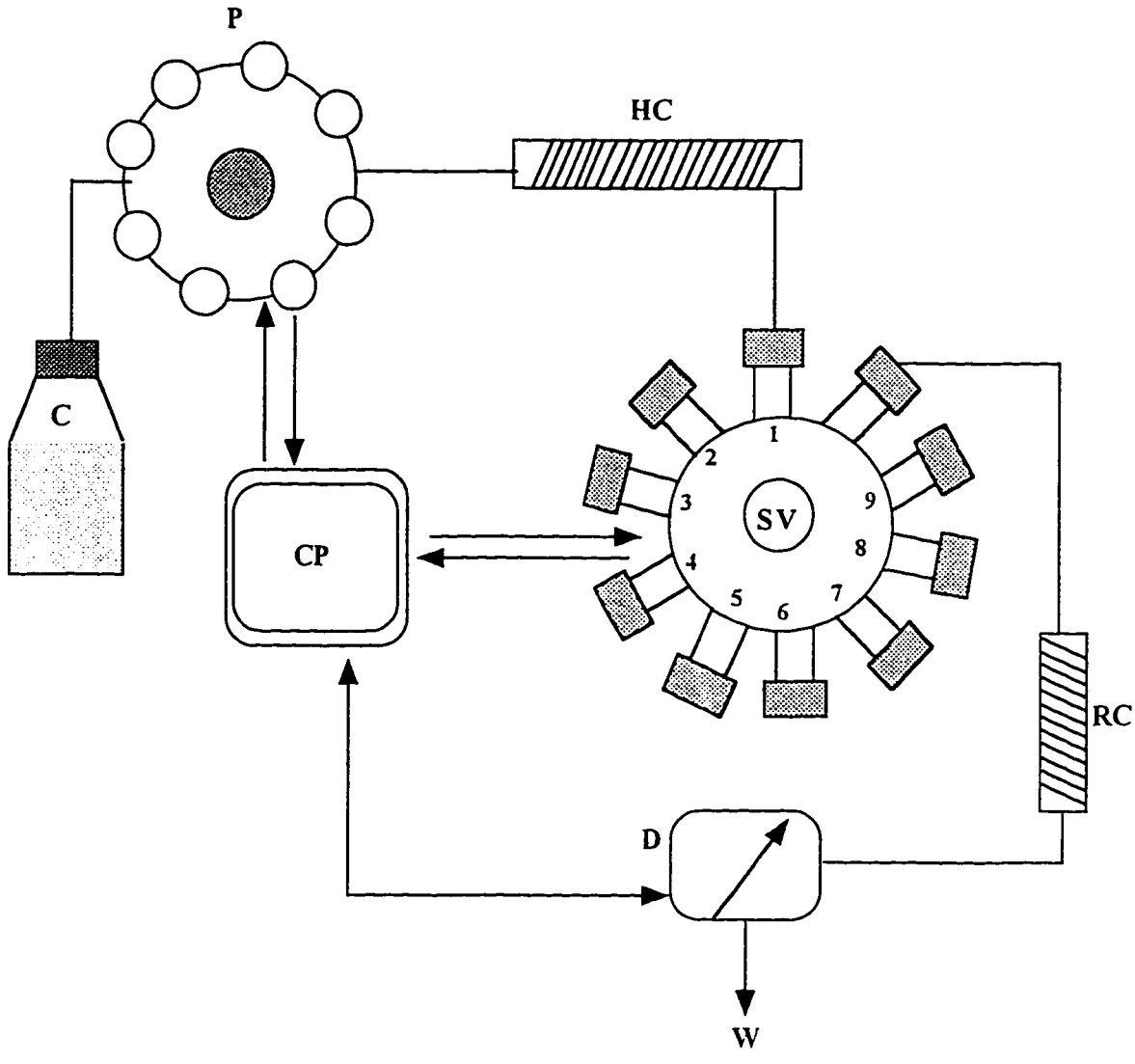


Figure 3.1 SIA-manifold; C, carrier; P, peristaltic pump; HC, holding coil; SV; selector valve; RC, reaction coil; D, detector, CP, computer; W, waste.

3.3.3 SIA-Kinetics

The SIA manifold as represented by Figure (1.1) with the pump operable at a flow rate of 29.5μl s^{-1}, volumes from standard solutions could be withdrawn for certain time precisely adjustable by the pump stroke through the computer board and the following steps were performed :

1. Iron(III), sulfuric acid, 1,10-phenanthroline and the drug (vitamin C) were connected to the selector valve through ports 2, 3, 4 and 9 respectively. Whereas the mixing chamber was connected at port 10. Water (deionized) is pumped as a carrier solution through valve 1 in the forward mode to the detector then waste for 25.0 s.
2. About 80 μl each of the above reagents around the selector valve were introduced sequentially into the holding coil in the reverse mode for 3.0 s, then transferring the excess together with some carrier to auxilliary waste through valve 9 in the forward mode for 8.0 s.
3. In the analysis method for vitamin C, Iron(III) and sulfuric acid concentrations are kept constant by transferring fixed volumes by fixing the time of withdrawal of reagents with the help of a computer program. The fixed volume being transferred is previously calculated from known concentrations of these reagents. Iron(III) is aspirated into the holding coil in a reverse mode for 8.0 s and transferred to the mixing chamber by forward motion of the pump for 10 s. Sulfuric acid is taken in the holding coil for 1.0 s which is then pushed into the mixing chamber together with the carrier for 12.0 s.

1,10-phenanthroline is also withdrawn for 8.0 s then pushed to the mixing chamber for 12.0 s. This phase is referred to as the transfer volume (V_T).

4. A certain known volume of the test solution is withdrawn into the holding coil then pushed into the mixing chamber together with some carrier solution for 16.0 s in a separate step referred to as the sample volume (V_S). Note that both (V_T) + (V_S) were always pushed to the mixing chamber for 50.0 s to keep its volume constant throughout the experiment. Moreover the concentration of test solution was kept at least ten times diluted as compared to the other reagents in the mixing chamber.
5. A known volume of the aliquot solution is withdrawn from the mixing chamber and transferred to the holding coil in a reverse mode for 3.0 s, then pushed in the forward direction for 28.0 s to the detector for signal monitoring. This phase is referred to as the analysis volume (V_A).
6. For kinetic measurements at variable times, reagents and drug solutions are arrested for the predetermined time in the mixing chamber prior to the analysis volume is withdrawn.
7. For determining reaction orders with respect to one reactant, all other components are kept constant and treated as V_T. The one varied is considered the V_S and the process is repeated by taking different volumes for V_S from a single standard solution so that the kinetics monitored corresponds to different required solutions i.e. having different concentrations.

3.3.4 SIA-Stoichiometry

Before using SI technique, preliminary investigations were made where the maximum absorbance (λ_{max}) at a wide range of pH values from 8.0 x 10^{-5} to 0.01 M (table 3.1), were determined using Perkin Elmer Lambda 5 UV-VIS spectrophotometer.

In hydrochloric acid concentration ranging from 8.0 x 10^{-5} to 8.0 x 10^{-1} M, a purple color solution is formed instantaneously when promethazine is added to palladium(II) solutions, indicative of complex formation. It was found that the absorbance of the purple color complex increases with the decrease in pH value giving each time λ_{max} at 504 nm , but at a certain concentration of the acid not only the λ_{max} starts decreasing , as color changes to pale red, with the increasing acid concentration but the color intensity (absorbance) also decreases, till a λ_{max} of 496 nm is reached which is the maximum absorbance of oxidized product formed by the metal. The pH value of 3.0969 was found to be suitable for the work.

The SIA manifold used for stoichiometric studies is shown in Figure (3.1), as before the system was flushed, including all the tubes attached to the selecter valve, with deionized water (carrier) flowing at the rate of 29.5 μl s^{-1}. The following operation were conducted :

1. Equimolar solutions of palladium(II) and the drug (promethazine) were connected to the selector valve through ports 2 and 3 respectively.
2. About 80 μl of the above reagents around the selector valve were introduced sequentially into the holding coil in the reverse mode for 3.0 s, then transferring the

excess together with some carrier to the auxilliary waste through valve 7 in the forward mode for 8.0 s.

3. In the analysis method, using the Job's method of continuous variation, different aliquots of equimolar solutions of Pd(II) and drug were drawn and mixed in holding coil such to give solutions of identical total concentration (Pd(II) + drug) but different mole fractions, then the solution was pushed for 40.0 s to the detector for signal monitoring. The volume of each reagent aspirated was varied between 14.8 μl (0.5 s) and 147.5 μl (5.0 s). Therefore, a total volume of aspiration of 162.0 μl was maintained constant by adjusting the aspiration times. This step was repeated by changing the aspiration time by varying it between 14.8 μl (0.5 s) and 280.3 μl (9.5 s).
4. In the analysis method, using the Mole ratio method, the total concentration of the ligand (drug) was maintained constant by aspirating 147.5 μl (5.0 s) into the holding coil by flow reversal, whereas Pd(II) solution volume was varied between 14.8 μl (0.5 s) and 192.0 μl (6.5 s). This step was repeated but this time the total concentration of the metal was held constant by fixing the flow reversal for 147.5 μl (5.0 s) while the volume of drug solution was changed between 14.8 μl (0.5 s) and 192.0 μl (6.5 s).

   Ideally, in the molar ratio method two straight lines of different slopes are obtained when the absorbance is plotted verses the Pd(II) to drug ratio, and the point of intersection of these two lines corresponds to the stoichiometric ratio upon interpolation to the mole ratio axis.

Steps 3 and 4 were performed again but using 8.0×10^{-4} M sulfuric acid as a carrier instead of water. It was found that there is no significant difference in the absorbance values by either using acid or water as carrier.

3.3.5 Programming of (SI) Instrument with Optimum Reagent concentrations for kinetic studies

Table 3.3 To determine the order of reaction with respect to acid, the following concentrations were used

		Duration
I.	0.01 M ammonium ferric sulphate in 0.01 M sulfuric acid	8.0 s
II.	0.01 M 1,10-phenanthroline	8.0 s
III.	8×10^{-3} M vitamin C	4.0 s
IV.	1.0 M sulfuric acid	0.25 to 1.75 s

The SI instrument was programmed as follows

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	8.000
3	33.000	100	off	10	-	off	10.00
4	43.000	-100	off	3	-	off	1.000
5	44.000	100	off	10	-	off	12.00
6	56.000	-100	off	4	-	off	8.000
7	64.000	100	off	10	-	off	12.00
8	76.000	-100	off	9	-	off	4.000
9	80.000	100	off	10	-	off	16.00
10	96.000	off	off	10	-	off	2.000
11	98.000	-100	off	10	-	off	3.000
12	101.00	100	off	home	-	on	28.00
13	129.00	-100	off	10	-	off	3.000
14	132.00	100	off	home	-	on	28.00
15	160.00	-100	off	10	-	off	3.000
16	163.00	100	off	home	-	on	28.00
17	191.00	-100	off	10	-	off	3.000
18	194.00	100	off	home	-	on	28.00

3.3.6 Calculation of concentration in the mixing chamber:

Following is the method of calculating the concentration of ammonium ferric sulphate, sulfuric acid, 1,10-phenanthroline and vitamin C from the program described in table 3.3

Valve # 2 , Iron(II) :

mmol Iron(III) = M x Duration x flow-rate

$$0.01 \frac{\text{mmol}}{\text{ml}} \times 8\ \text{s} \times 29.5 \frac{\mu\text{l}}{\text{s}} \times 10^{-3} \frac{\text{ml}}{\mu\text{l}} = 2.36 \times 10^{-3}\ \text{mmol}$$

Total volume = Volume of mixture x flow-rate

$$= 50\ \text{s} \times 29.5 \frac{\mu\text{l}}{\text{s}} \times 10^{-3} \frac{\text{ml}}{\mu\text{l}} = 1475 \times 10^{-3}\ \text{ml}$$

(Since volume of mixture = 10+12+12+16 = 50 s)

Therefore concentration of Fe^{+3} in mixing chamber (m.c.) = $\frac{2.36 \times 10^{-3}\ \text{mmol}}{1475 \times 10^{-3}\ \text{ml}}$

$$[Fe^{+3}]_{m.c.} = 1.6 \times 10^{-3}\ \text{M}$$

Valve # 4 , 1,10-Phenanthroline :

mmol 1,10-Phenanthroline = $0.01 \times 8 \times 29.5 \times 10^{-3}$ = 2.36×10^{-3} mmol

$$[1,10\text{-Phen}]_{m.c.} = 2.36 \times 10^{-3}\ \text{mmol} / 1475 \times 10^{-3}\ \text{ml} = 1.6 \times 10^{-3}\ \text{M}$$

Valve # 9 , Vitamin C :

mmol vitamin C = $8 \times 10^{-3} \times 4 \times 29.5 \times 10^{-3}$ = 9.44×10^{-4} mmol

$$[\text{vitamin C}]_{m.c.} = 9.44 \times 10^{-4}\ \text{mmol} / 1475 \times 10^{-3}\ \text{ml} = 6.4 \times 10^{-4}\ \text{M}$$

Reagent	Time / s	Concentration in mixing chamber / M
Iron(II)	8.0	1.6×10^{-3}
Vitamin C	4.0	6.4×10^{-3}
1,10-Phen	8.0	1.6×10^{-3}

Valve # 2 , Sulfuric acid:

mmol sulfuric acid from AFS $= 0.01 \times 8 \times 29.5 \times 10^{-3} = 2.36 \times 10^{-3}$ mmol

$[H_2SO_4] = 2.36 \times 10^{-3}$ mmol / 1475×10^{-3} ml $= 1.6 \times 10^{-3}$ M

Valve # 3 :

Sulfuric acid was injected for 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 s. The method of calculation is given only for 0.25 s below.

mmol sulfuric acid $= 1 \times 0.25 \times 29.5 \times 10^{-3} = 7.375 \times 10^{-3}$ mmol

$[H_2SO_4] = 7.375 \times 10^{-3}$ mmol / 1475×10^{-3} ml $= 5 \times 10^{-3}$ M

Total acid entering mixing chamber (m.c.) = $[H_2SO_4]_{m.c.} = 2.360 \times 10^{-3}$ mmol
$+ \underline{7.375 \times 10^{-3}}$ mmol
9.735×10^{-3} mmol

$$[H_2SO_4]_{m.c.} = \frac{9.735 \times 10^{-3} \text{ mmol}}{1475 \times 10^{-3} \text{ ml}} = 6.6 \times 10^{-3} \text{ M}$$

The calculated acid concentrations of the remaining aliquots drawn from *valve # 3* are :

Time / s	Concentration in mixing chamber / M
0.25	0.0066
0.50	0.0116
0.75	0.0166
1.00	0.0216
1.25	0.0266
1.50	0.0316
1.75	0.0366

Similar method is adopted for calculating the concentration of other reagents.

Chart speed of single channel-strip-chart recorder 0555 (Cole Parmer, Chicago, USA) was 30 cm / hr when the order of reaction with respect to Ammonium ferric sulphate, sulfuric acid and vitamin C was determined, also when determining activation energy.

Table 3.4 To determine the order of reaction with respect to ammonium ferric sulphate (Fe^{3+}), the following concentrations were used

		Duration
I.	2.5×10^{-3} M ammonium ferric sulphate in 2.5×10^{-3} M sulfuric acid	7.5 s to 5.5 s
II.	0.01 M 1,10-phenanthroline	8.0 s
III.	4×10^{-2} M vitamin C	7.0 s
IV.	0.5 M sulfuric acid	1.7525 to 1.7625s

The SI instrument was programmed as follows

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	8.000
3	33.000	100	off	10	-	off	10.00
4	43.000	-100	off	3	-	off	1.752
5	44.750	100	off	10	-	off	12.00
6	56.750	-100	off	4	-	off	8.000
7	64.750	100	off	10	-	off	12.00
8	76.750	-100	off	9	-	off	7.000
9	83.750	100	off	10	-	off	16.00
10	99.750	off	off	10	-	off	2.000
11	101.75	-100	off	10	-	off	3.000
12	104.75	100	off	home	-	on	28.00
13	132.75	-100	off	10	-	off	3.000
14	135.75	100	off	home	-	on	28.00
15	163.75	-100	off	10	-	off	3.000
16	166.75	100	off	home	-	on	28.00
17	194.75	-100	off	10	-	off	3.000
18	197.75	100	off	home	-	on	28.00

Calculations of the concentration in the mixing chamber were done similarly as given in section 3.3.6, therefore

$[\text{Vitamin C}]_{m.c.} = 5.6 \times 10^{-3}$ M ; $[\text{1,10-Phen}]_{m.c.} = 1.6 \times 10^{-3}$ M

$[H_2SO_4]_{m.c.} = [H_2SO_4]_{AFS.} + [H_2SO_4]_{\text{valve \# 3}} = 0.0179$ M

The AFS was kept at least 15 times diluted than vitamin C and sulfuric acid. Details for solution preparations with constraints are given in section 3.3.

The stock solution of ammonium ferric sulphate (AFS) was prepared in equimolar acid. Therefore when AFS is injected into the mixing chamber, same concentration of sulfuric acid also enters the mixing chamber.which is withdrawn alongwith the AFS for a certain time.

The calculated concentration of the ammonium ferric sulphate drawn from *valve # 2* are given below

Time / s	[Fe^{+3}]m.c,
7.5	3.75×10^{-4} M
7.0	3.50×10^{-4} M
6.5	3.25×10^{-4} M
6.0	3.00×10^{-4} M
5.5	2.75×10^{-4} M

The stock solution of ammonium ferric sulphate (AFS) contains equimolar acid and when AFS is injected into the mixing chamber, same concentration of sulfuric acid also enters the mixing chamber, therefore, in order to maintain a constant concentration of acid in the mixing chamber, the acid volume drawn at *valve 3* was also changed as follows

Time / s	[H_2SO_4]m.c./ M
1.7525	0.017525
1.7550	0.017550
1.7575	0.017575
1.7600	0.017600
1.7625	0.017625

Hence the actual concentration of acid in the mixing chamber is the sum of mmol of sulfuric acid coming from their respective *valve # 2* (AFS) and *valve # 3* (sulfuric acid) divided by the total volume of the mixing chamber.

Table 3.5 To determine the order of reaction with respect to vitamin C, the following solutions were prepared

		Duration
I.	0.01 M ammonium ferric sulphate in 0.1 M sulfuric acid	8.0 s
II.	0.01 M 1,10-phenanthroline	8.0 s
III.	1.13552×10^{-3} M (200ppm) vitamin C	7.0 s to 4.5 s

The SI instrument was programmed as given below

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	8.000
3	33.000	100	off	10	-	off	10.00
4	43.000	-100	off	3	-	off	8.000
5	51.000	100	off	10	-	off	12.00
6	63.000	-100	off	9	-	off	7.000
7	70.000	100	off	10	-	off	15.00
8	85.000	off	off	10	-	off	2.000
9	87.000	-100	off	10	-	off	3.000
10	90.000	100	off	home	-	on	28.00
11	118.00	-100	off	10	-	off	3.000
12	121.00	100	off	home	-	on	28.00
13	149.00	-100	off	10	-	off	3.000
14	152.00	100	off	home	-	on	28.00
15	180.00	-100	off	10	-	off	3.000
16	183.00	100	off	home	-	on	28.00

Detail method of calculation of concentrations is given in section 3.3.6

$[Fe^{+3}]_{m.c.} = 2.162 \times 10^{-3}$ M ; $[1,10\text{-Phen}]_{m.c.} = 1.6 \times 10^{-3}$ M
$[H_2SO_4]_{m.c.} = 2.162 \times 10^{-3}$ M

Vitamin C was kept at least 11 times diluted than ammonium ferric sulphate and sulfuric acid.

The calculated concentration of the vitamin C drawn from *valve # 9* are given below

Time / s	[Vitamin C]m.c./ M
4.5	1.3810×10^{-4}
5.0	1.5345×10^{-4}
5.5	1.6879×10^{-4}
6.0	1.8414×10^{-4}
6.5	1.9948×10^{-4}
7.0	2.1483×10^{-4}

Table 3.6 To determine the activation energy the solutions used were

		Duration
I.	0.01 M ammonium ferric sulphate in 0.01 M sulfuric acid	8.0 s
II.	1.0 M sulfuric acid	1.5 s
III.	0.01 M 1,10-phenanthroline	8.0 s
IV.	8×10^{-3} M vitamin C	2.0 s

Method entries for SI instrument were

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	8.000
3	33.000	100	off	10	-	off	10.00
4	43.000	-100	off	3	-	off	1.500
5	44.500	100	off	10	-	off	12.00
6	56.500	-100	off	4	-	off	8.000
7	64.500	100	off	10	-	off	12.00
8	76.500	-100	off	9	-	off	2.000
9	78.500	100	off	10	-	off	16.00
10	94.500	off	off	10	-	off	2.000
11	96.500	-100	off	10	-	off	3.000
12	99.500	100	off	home	-	on	28.00
13	127.50	-100	off	10	-	off	3.000
14	130.50	100	off	home	-	on	28.00
15	158.50	-100	off	10	-	off	3.000
16	161.50	100	off	home	-	on	28.00
17	189.50	-100	off	10	-	off	3.000
18	192.50	100	off	home	-	on	28.00

Detail method of calculation of concentrations is given in section 3.3.6

$[Fe^{+3}]_{m.c.} = 1.6 \times 10^{-3}$ M ; $[1,10\text{-Phen}]_{m.c.} = 1.6 \times 10^{-3}$ M
$[H_2SO_4]_{m.c.} = 0.03$ M ; $[\text{Vitamin C}]_{m.c.} = 3.2 \times 10^{-4}$ M

The temperature at which the activation energy was measured are
20.0, 25.0, 35.0, 40.0, 45.0 °C.

Table 3.7 To determine the calibration curve (plot of absorbance vs concentration of vitamin C in the range of 20-300ppm), the solutions used were

		Duration
I.	0.01 M AFS in 0.01 M H_2SO_4	8.0 s
II.	0.01 M 1,10-phenanthroline	8.0 s
III.	1 M H_2SO_4	1.0 s
IV.	20 to 300 ppm, vitamin C	8.0 s

The SI instrument was programmed as follows

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	8.000
3	33.000	100	off	10	-	off	10.00
4	43.000	-100	off	3	-	off	1.000
5	44.000	100	off	10	-	off	12.00
6	56.000	-100	off	4	-	off	8.000
7	64.000	100	off	10	-	off	12.00
8	76.000	-100	off	9	-	off	8.000
9	84.000	100	off	10	-	off	16.00
10	100.00	off	off	10	-	off	60.00
11	160.00	-100	off	10	-	off	3.000
12	163.00	100	off	home	-	on	27.00
13	190.00	off	off	10	-	off	90.00
14	280.00	-100	off	10	-	off	3.000
15	283.00	100	off	home	-	on	27.00
16	310.00	off	off	10	-	off	90.00
17	400.00	-100	off	10	-	off	3.000
18	403.00	100	off	home	-	on	27.00
19	430.00	off	off	10	-	off	150.0
20	580.00	-100	off	10	-	off	3.000
21	583.00	100	off	home	-	on	27.00

Chart speed of single channel-strip-chart recorder 0555 (Cole Parmer, Chicago, USA) was 15 cm / hr for the determination of calibration curve and its application on proprietary drugs (table 3.8).

Table 3.8 To determine the concentration of vitamin C in proprietary drug samples from the calibration curve, the solutions used were

		Duration
I.	0.01 M AFS in 0.01 M H_2SO_4	8.0 s
II.	0.01 M 1,10-phenanthroline	8.0 s
III.	1 M H_2SO_4	1.0 s
IV.	vitamin C (in proprietary drugs)	8.0 s

Drugs :

(i)	Redoxon	100 ppm
(ii)	Upsa	150 ppm
(iii)	Cal-C-Vita	200 ppm
(iv)	Octovit	250 ppm
(v)	Beminal C	200 ppm

Method entries :

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	8.000
3	33.000	100	off	10	-	off	10.00
4	43.000	-100	off	3	-	off	1.000
5	44.000	100	off	10	-	off	12.00
6	56.000	-100	off	4	-	off	8.000
7	64.000	100	off	10	-	off	12.00
8	76.000	-100	off	9	-	off	8.000
9	84.000	100	off	10	-	off	16.00
10	100.00	off	off	10	-	off	60.00
11	160.00	-100	off	10	-	off	3.000
12	163.00	100	off	home	-	on	27.00
13	190.00	off	off	10	-	off	90.00
14	280.00	-100	off	10	-	off	3.000
15	283.00	100	off	home	-	on	27.00
16	310.00	off	off	10	-	off	90.00
17	400.00	-100	off	10	-	off	3.000
18	403.00	100	off	home	-	on	27.00
19	430.00	off	off	10	-	off	150.0
20	580.00	-100	off	10	-	off	3.000
21	583.00	100	off	home	-	on	27.00

3.3.7 Programming of (SI) Instrument with Optimum Reagent concentrations for stoichiometric studies

Table 3.9 Determination of stoichiometry for palladium(II) and promethazine complex was carried out using the following concentrations of analyte and the SI method.

		Duration
I.	1×10^{-3} M palladium in 8×10^{-4} M HCl	10.0 s to 0.0 s
II.	1×10^{-3} M promethazine in 8×10^{-4} M HCl	0.0 s to 10.0 s
III.	Water (deionized)	Carrier

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	0.000
3	25.000	-100	off	3	-	off	10.00
4	35.000	100	off	home	-	on	40.00
5	75.000	-100	off	2	-	off	1.000
6	76.000	-100	off	3	-	off	9.000
7	85.000	100	off	home	-	on	40.00
8	125.00	-100	off	2	-	off	2.000
9	127.00	-100	off	3	-	off	8.000
10	135.00	100	off	home	-	on	40.00
11	175.00	-100	off	2	-	off	3.000
12	178.00	-100	off	3	-	off	7.000
13	185.00	100	off	home	-	on	40.00
14	225.00	-100	off	2	-	off	4.000
15	229.00	-100	off	3	-	off	6.000
16	235.00	100	off	home	-	on	40.00
17	275.00	-100	off	2	-	off	5.000
18	280.00	-100	off	3	-	off	5.000
19	285.00	100	off	home	-	on	40.00
20	325.00	-100	off	2	-	off	6.000
21	331.00	-100	off	3	-	off	4.000
22	335.00	100	off	home	-	on	40.00
23	375.00	-100	off	2	-	off	7.000
24	382.00	-100	off	3	-	off	3.000
25	385.00	100	off	home	-	on	40.00
26	425.00	-100	off	2	-	off	8.000
27	433.00	-100	off	3	-	off	2.000
28	435.00	100	off	home	-	on	40.00

29	475.00	-100	off	2	-	off	9.000
30	484.00	-100	off	3	-	off	1.000
31	485.00	100	off	home	-	on	40.00
32	525.00	-100	off	2	-	off	0.000
33	525.00	-100	off	3	-	off	10.00
34	535.00	100	off	home	-	on	40.00

Chart speed of single channel-strip-chart recorder 0555 (Cole Parmer, Chicago, USA) was 30 cm / when the stoichiomertric ratio was determined.

Table 3.10 Determination of stoichiometry for palladium(II) and promethazine complex was carried out using the following concentrations of analyte and the SI method.

		Duration
I.	1×10^{-3} M palladium in 8×10^{-4} M HCl	10.0 s to 0.0 s
II.	1×10^{-3} M promethazine in 8×10^{-4} M HCl	0.0 s to 10.0 s
III.	Water (deionized)	Carrier

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	0.000
3	25.000	-100	off	3	-	off	10.00
4	35.000	100	off	home	-	on	40.00
5	75.000	-100	off	2	-	off	0.500
6	75.500	-100	off	3	-	off	9.500
7	85.000	100	off	home	-	on	40.00
8	125.00	-100	off	2	-	off	1.000
9	126.00	-100	off	3	-	off	9.000
10	135.00	100	off	home	-	on	40.00
11	145.00	-100	off	2	-	off	2.000
12	147.00	-100	off	3	-	off	8.000
13	155.00	100	off	home	-	on	40.00
14	195.00	-100	off	2	-	off	3.000
15	198.00	-100	off	3	-	off	7.000
16	205.00	100	off	home	-	on	40.00
17	245.00	-100	off	2	-	off	4.000
18	249.00	-100	off	3	-	off	6.000
19	255.00	100	off	home	-	on	40.00
20	295.00	-100	off	2	-	off	5.000
21	300.00	-100	off	3	-	off	5.000
22	305.00	100	off	home	-	on	40.00
23	345.00	-100	off	2	-	off	6.000
24	351.00	-100	off	3	-	off	4.000
25	355.00	100	off	home	-	on	40.00
26	395.00	-100	off	2	-	off	7.000
27	402.00	-100	off	3	-	off	3.000
28	405.00	100	off	home	-	on	40.00

29	445.00	-100	off	2	-	off	8.000
30	453.00	-100	off	3	-	off	2.000
31	455.00	100	off	home	-	on	40.00
32	495.00	-100	off	2	-	off	9.000
33	504.00	-100	off	3	-	off	1.000
34	505.00	100	off	home	-	on	40.00
35	545.00	-100	off	2	-	off	9.500
36	554.50	-100	off	3	-	off	0.500
37	555.00	100	off	home	-	on	40.00
38	595.00	-100	off	2	-	off	9.750
39	604.75	-100	off	3	-	off	0.250
40	605.00	100	off	home	-	on	40.00
41	645.00	-100	off	2	-	off	0.000
42	645.00	-100	off	3	-	off	10.00
43	655.00	100	off	home	-	on	40.00

Table 3.11 Determination of stoichiometry for palladium(II) and promethazine complex was carried out using the following concentrations of analyte and the SI method.

		Duration
I.	1×10^{-3} M palladium in 8×10^{-4} M HCl	0.0 s to 5.0 s
II.	1×10^{-3} M promethazine in 8×10^{-4} M HCl	4.9 s to 0.1 s
III.	Water (deionized)	Carrier

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	0.000
3	25.000	-100	off	3	-	off	5.000
4	30.000	100	off	home	-	on	40.00
5	70.000	-100	off	2	-	off	0.500
6	70.500	-100	off	3	-	off	4.500
7	75.000	100	off	home	-	on	40.00
8	115.00	-100	off	2	-	off	1.000
9	116.00	-100	off	3	-	off	4.000
10	120.00	100	off	home	-	on	40.00
11	160.00	-100	off	2	-	off	1.500
12	161.50	-100	off	3	-	off	3.500
13	165.00	100	off	home	-	on	40.00
14	205.00	-100	off	2	-	off	2.000
15	207.00	-100	off	3	-	off	3.000
16	210.00	100	off	home	-	on	40.00
17	250.00	-100	off	2	-	off	2.500
18	252.50	-100	off	3	-	off	2.500
19	255.00	100	off	home	-	on	40.00
20	295.00	-100	off	2	-	off	3.000
21	298.00	-100	off	3	-	off	2.000
22	300.00	100	off	home	-	on	40.00
23	340.00	-100	off	2	-	off	3.500
24	343.50	-100	off	3	-	off	1.500
25	345.00	100	off	home	-	on	40.00
26	385.00	-100	off	2	-	off	4.000
27	389.00	-100	off	3	-	off	1.000
28	390.00	100	off	home	-	on	40.00
29	430.00	-100	off	2	-	off	4.500
30	434.50	-100	off	3	-	off	0.500
31	435.00	100	off	home	-	on	40.00

32	475.00	-100	off	2	-	off	4.700
33	479.70	-100	off	3	-	off	0.300
34	480.00	100	off	home	-	on	40.00
35	520.00	-100	off	2	-	off	4.900
36	524.90	-100	off	3	-	off	0.100
37	525.00	100	off	home	-	on	40.00

Table 3.12 To determine the calibration curve (plot of absorbance vs concentration of promethazine in the range of 50-400 ppm), the solutions used were

		Duration
I.	1×10^{-3} M palladium in 8×10^{-4} M HCl	7.0 s
II.	20 to 300 ppm, promethazine in 8×10^{-4} M HCl	8.0 s
III.	Water (deionized)	Carrier

The SI instrument was programmed as follows

Method entries :

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	7.000
3	32.000	-100	off	3	-	off	7.000
4	39.000	100	off	home	-	off	1.000
5	40.000	-100	off	home	-	off	1.000
6	41.000	100	off	home	-	on	45.00

Table 3.13 To determine the concentration of promethazine•HCl in proprietary drug samples from the calibration curve, the solutions used were

		Duration
I.	1×10^{-3} M palladium in 8×10^{-4} M HCl	7.0 s
II.	promethazine from proprietary drugs in 8×10^{-4} M HCl	7.0 s
III.	Water (deionized)	Carrier

Drugs :

(i)	Phenergen Expectorant	100 ppm
(ii)	Phenergen	150 ppm
(iii)	Cigan Elixir	200 ppm

Method entries :

ID	Time	Pump 1	Pump 2	Valve 1	Valve 2	Data Acquisition	Duration
1	0.0	100	off	home	-	off	25.00
2	25.000	-100	off	2	-	off	7.000
3	32.000	-100	off	3	-	off	7.000
4	39.000	100	off	home	-	off	1.000
5	40.000	-100	off	home	-	off	1.000
6	41.000	100	off	home	-	on	45.00

Chapter 4

Experimental results

The results for all the experimental work performed in this research is compiled in this chapter.

4.1 Kinetic determination of Vitamin C

The present SIA method was based on the oxidation of vitamin C with iron(III), using 1,10-phenanthroline indicator in sulfuric acid media. The kinetics of this reaction was throughly investigated by monitoring the increase of absorbance of tris-1,10-phenanthroline iron(II) red complex at the wavelength of maximum absorbance which is 510 nm. Intense absorption of tris-1,10-phenanthroline iron(II) complex is due to charge transfer from iron(II) to 1,10-phenanthroline ligand[53]. The reaction order with respect to each reactant, the activation energy were determined and the mechanism was postulated. Finally a kinetic method for the determination of vitamin C was adopted.

The partial orders with respect to the different variables assumed to have an influence on the rate equation was carried out by considering the differential form of the rate equation involving the pseudo zero order reactions; when the rate of formation of the products and other reactants are virtually negligible. In this method all reactants, except the one under investigation [A], are kept constant at higher concentrations and the rates are measured for the reactant being investigated by varying its concentration. Details for this method are given in section 1.4. From the plot between absorbance and time, rates were calculated by applying the fixed-time method which involves measuring the absorbance as "ΔA" of the product at a predetermined time "Δt" from the start of the reaction.

4.1.1 Reaction order with respect to [H^+]

The complexation reaction was found to be reasonably slow in sulfuric acid concentration range of 0.0100 to 0.0400 mol dm^{-3} allowing for possible kinetic investigations to be followed spectrophotometrically. It was observed that the reaction rate decreases as the acid concentration is increased as represented in fig 4.1. The change in the absorbance with respect to time for each aliquot drawn from the mixing chamber was monitored to find the order of reaction with respect to sulfuric acid (table 4.1). The concentration of ammonium ferric sulphate and ascorbic acid (table 3.3) were kept constant while the concentration of acid in the mixing chamber was varied by withdrawing the acid through valve # 3 at different times, which gave the following readings :

Table 4.1

Time	A1	A2	A3	A4	A5	A6	A7
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6.2600	0.0326	0.0261	0.0206	0.0170	0.0150	0.0131	0.0118
45.420	0.0747	0.0670	0.0595	0.0521	0.0472	0.0426	0.0365
84.590	0.0838	0.0771	0.0709	0.0647	0.0609	0.0561	0.0504
123.75	0.0877	0.0820	0.0757	0.0702	0.0668	0.0630	0.0576

Where A1 to A7 are the absorbances when the acid was taken at time 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 s respectively from valve # 3.

Absorbance-time curves are given in figure 4.1. The curves were obtioned by collecting the maximum value of absorbances for each aliquots obtained from mixing chamber after a time interval of 31 s using the SIA-Lab Program shown in table 3.3.

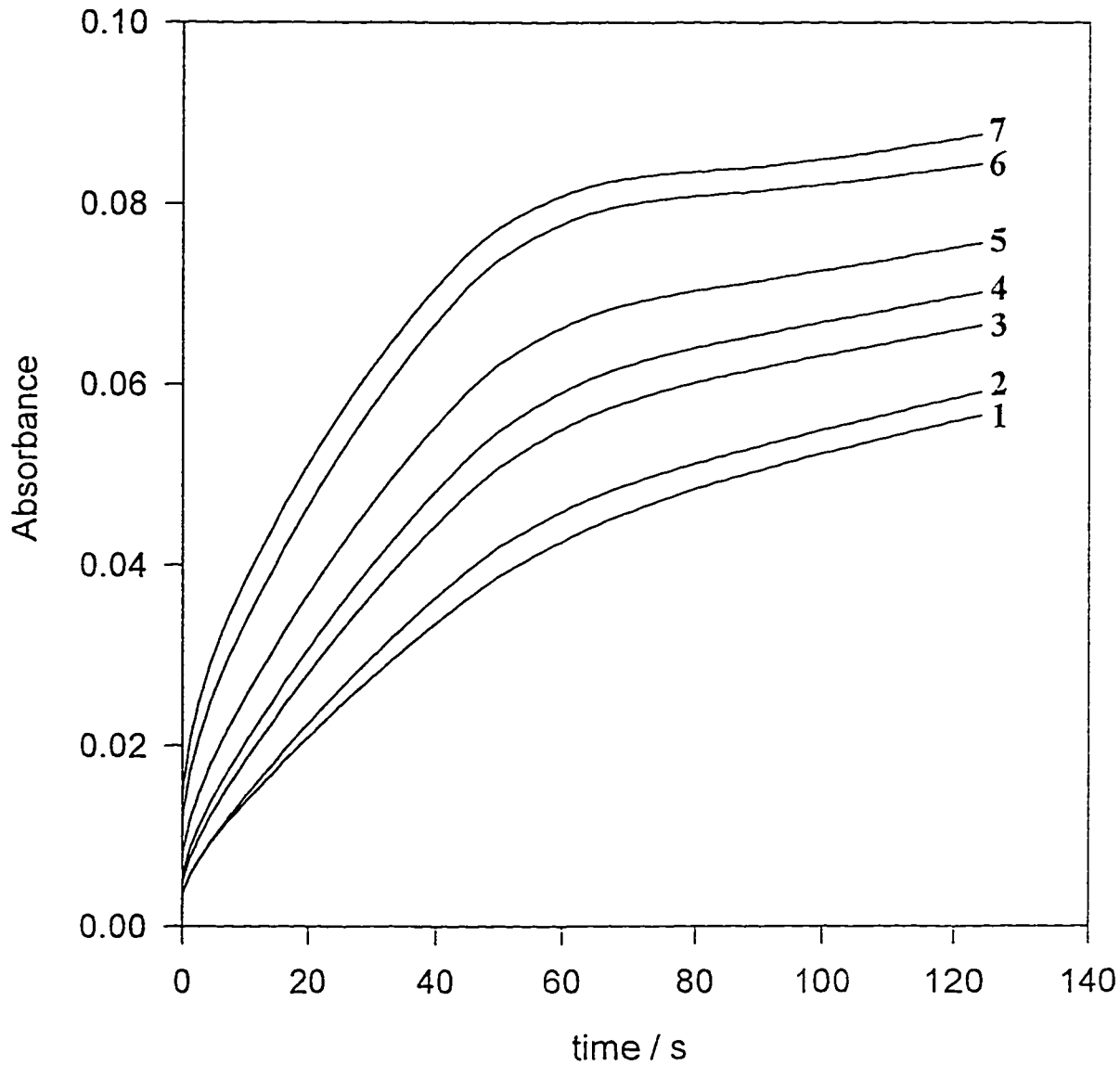


Fig. 4.1 Absorbance-time curves for the determination of the rate of reaction with respect to hydrogen ions. [Fe^{3+}] = 1.6 x 10^{-3} mol dm^{-3} ; [vitamin C] = 6.4 x 10^{-4} mol dm^{-3} ; [H_2SO_4] = (1) 0.0366, (2) 0.0316, (3) 0.0266, (4) 0.0216, (5) 0.0166, (6) 0.0116, (7) 0.0066 mol dm^{-3}; delay time (t_d) = 12.0 s.

As mentioned before rates were calculated from the plot by applying the fixed-time method involving measuring the absorbance as "ΔA" of the product at a predetermined time "Δt" from the start of the reaction. The values of ΔA is given in table 4.2.

Table 4.2 Calculated rates of the reaction at 25°C for different sulfuric acid concentrations at constant concentrations of ammonium ferric sulphate (1.6×10^{-3} M) and ascorbic acid (6.4×10^{-4} M).

No.	$[H^+]$ in m.c.	$\log[H^+]$	ΔA	$\frac{\Delta A}{\Delta t}$ (10^{-3})	$\log\frac{\Delta A}{\Delta t}$
1	0.0066	-2.1805	0.01530	4.8920	-2.3105
2	0.0116	-1.9355	0.01370	4.3638	-2.3601
3	0.0166	-1.7799	0.01120	3.5687	-2.4475
4	0.0216	-1.6655	0.00714	2.2812	-2.6418
5	0.0266	-1.5751	0.00644	2.0585	-2.6864
6	0.0316	-1.5003	0.00443	1.4151	-2.8492
7	0.0366	-1.4365	0.00427	1.3642	-2.8651

The plot of log (ΔA/Δt) versus log $[H_2SO_4]$ for the determination of order of reaction and rate constant with respect to hydrogen ions resulted in a straight line (Fig 4.2) the slope of which was -1.03676 with a correlation coefficient (r^2) of 0.977. The order of reaction with respect to hydrogen ion concentration is therefore equal to inverse one (-1), indicating that hydrogen ions are generated as a product. The slope of figure 4.2 is equal to log k which gave the value of the pseudo rate constant k' equal to 4.33696×10^{-5}.

Therefore, $\text{Rate} = k'[H^+]^n$ where n = -1.

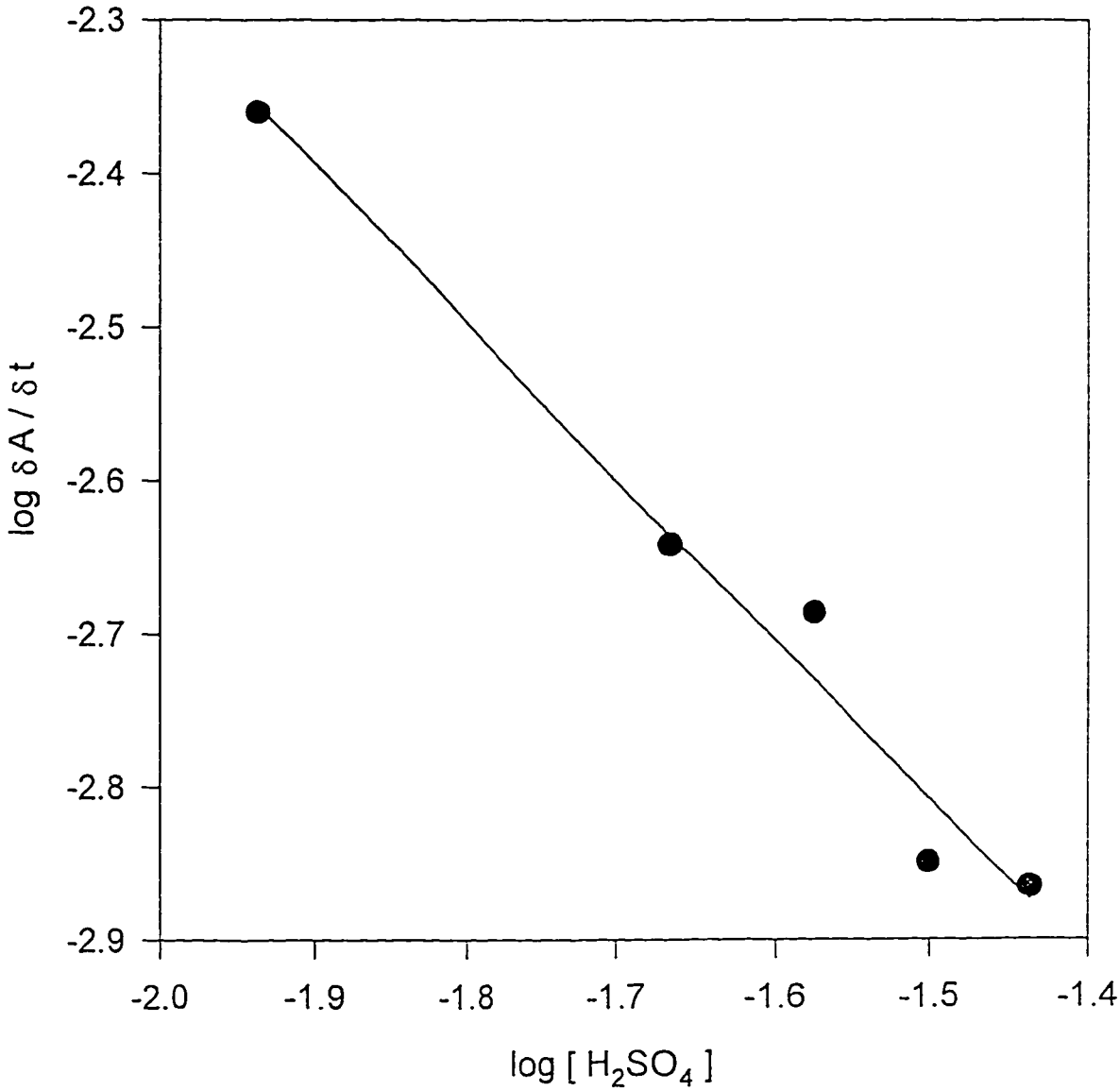


Fig 4.2 Plot of log δA/δt versus log [H_2SO_4] for the determination of order of reaction and rate constant with respect to sulfuric acid

4.1.2 Reaction order with respect to Ammonium ferric sulphate

The order of reaction with respect to ammonium ferric sulphate was determined by observing the change in the absorbance with respect to time for each aliquot drawn from the mixing chamber (table 4.3). The concentration of sulfuric acid and ascorbic acid (table 3.4) were kept constant while the concentration of ammonium ferric sulphate (AFS) in the mixing chamber was varied by withdrawing the AFS from valve # 2 at different times, which gave the following readings :

Table 4.3

Time	A1	A2	A3	A4	A5
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6.2600	0.0206	0.0191	0.0175	0.0164	0.0156
45.430	0.0548	0.0527	0.0507	0.0483	0.0457
84.590	0.0644	0.0623	0.0600	0.0578	0.0554
123.75	0.0696	0.0675	0.0654	0.0627	0.0605

Where A1 to A5 are the absorbances when the ammonium ferric sulphate was taken at times 7.5, 7.0, 6.5, 6.0, 5.5 s respectively from valve # 2. Fig 4.3 shows the absorbance-time curves for ammonium ferric sulphate. The curves were obtained by collecting the maximum value of absorbances for each aliquots obtained from mixing chamber after a time interval of 31 s using the SIA-Lab Program shown in table 3.4.

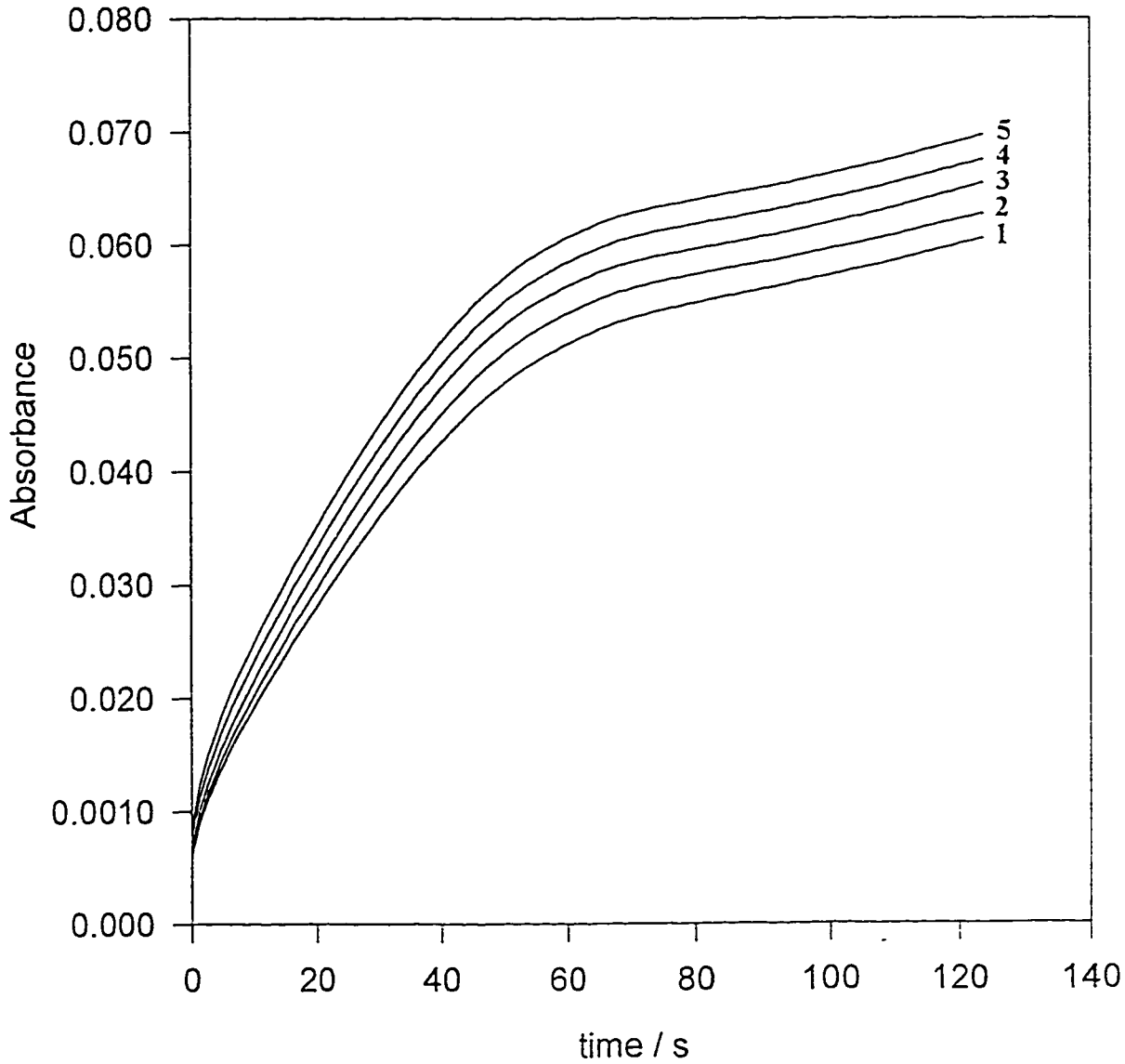


Fig. 4.3 Absorbance-time curves for the determination of the rate of reaction with respect to ammonium ferric sulphate (Fe^{3+}). [Vitamin C] = 5.6 x 10^{-3} mol dm^{-3}; [H_2SO_4] = 0.0179 mol dm^{-3} ; [ammonium ferric sulphate] = (1) 2.75 x 10^{-4}, (2) 3.00 x 10^{-4}, (3) 3.25 x 10^{-4}, (4) 3.50 x 10^{-4}, (5) 3.75 x 10^{-4} mol dm^{-3}; delay time (t_d) = 12.0 s.

Rates were calculated from the plot by applying the fixed-time method involving measuring the absorbance as "ΔA" of the product at a predetermined time "Δt" from the start of the reaction.

Table 4.4 Calculated rates of the reaction at 25°C for different ammonium ferric sulphate concentrations at constant concentrations of sulfuric acid (0.0179 M) and ascorbic acid (5.6×10^{-3} M).

No.	$[Fe^{+3}]$ in m.c.	$\log[Fe^{+3}]$	ΔA	$\frac{\Delta A}{\Delta t}$	$\log\frac{\Delta A}{\Delta t}$
1	3.75×10^{-4}	-3.4260	0.0136	2.598×10^{-3}	-2.5853
2	3.50×10^{-4}	-3.4559	0.0134	2.564×10^{-3}	-2.5911
3	3.25×10^{-4}	-3.4881	0.0118	2.266×10^{-3}	-2.6448
4	3.00×10^{-4}	-3.5229	0.0114	2.175×10^{-3}	-2.6626
5	2.75×10^{-4}	-3.5607	0.0103	1.965×10^{-3}	-2.7067

The plot of log (ΔA/Δt) versus log $[Fe^{3+}]$ for the determination of order of reaction and rate constant with respect to ammonium feric sulphate resulted in a straight line (Fig 4.4) the slope of which was 0.93701 with a correlation coefficient (r^2) of 0.962. The order of reaction with respect to ammonium ferric sulphate concentration is therefore equal to one (1), indicating that ammonium ferric sulphate is consumed as a reactant. The slope is equal to log k so the value of the rate constant k' equals to 4.29288.

Therfore,

$$\text{Rate} = k'[Fe^{3+}]^n \qquad \text{where } n = 1.$$

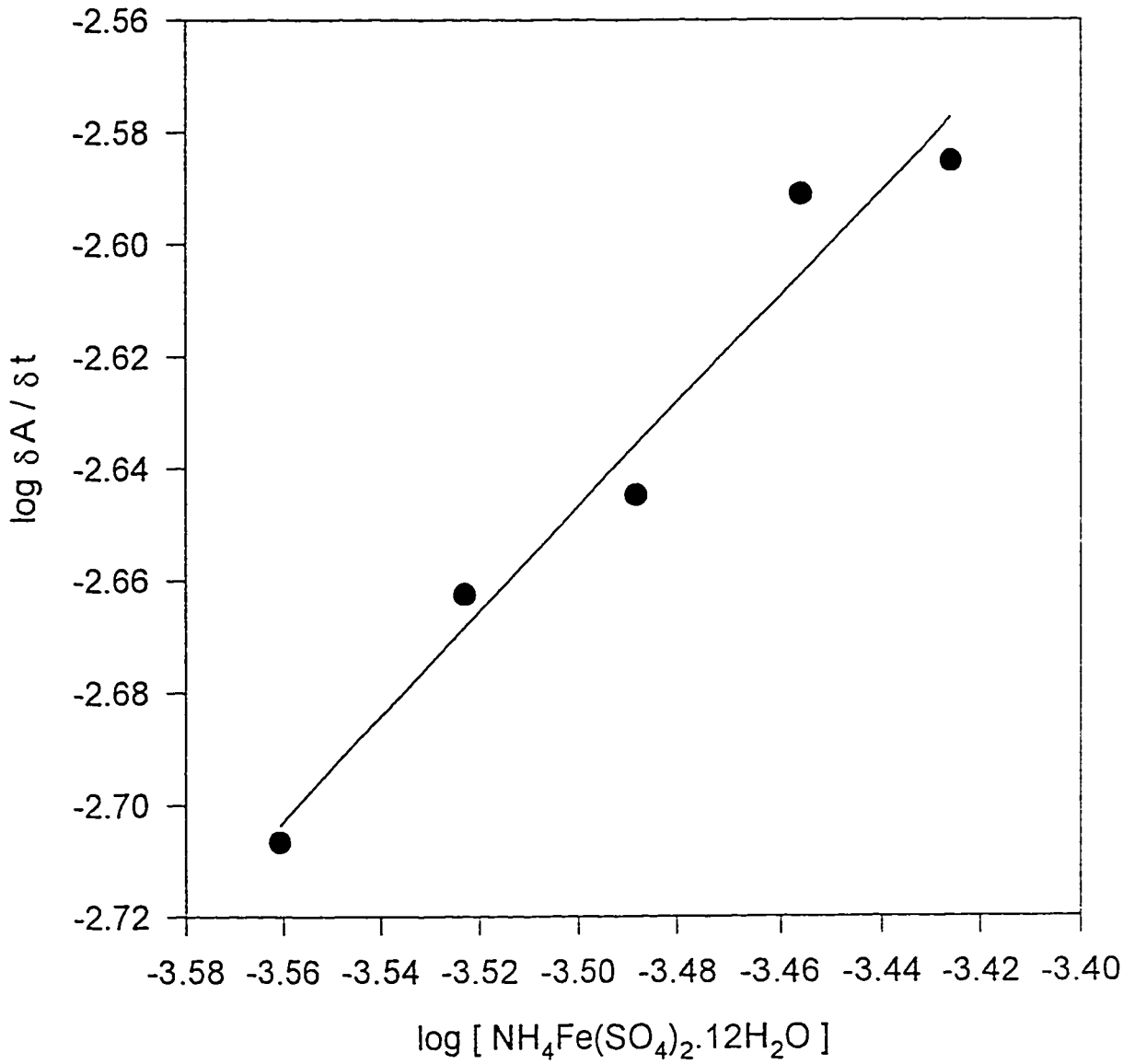


Fig 4.4 Plot of log δA/δt versus log [$NH_4Fe(SO_4)_2.12H_2O$] for the determination of order of reaction and rate constant with respect to iron(III)

4.1.3 Reaction order with respect to Ascorbic acid (vitamin C)

Change in the absorbance with respect to time for each aliquot drawn from the mixing chamber was observed and the order of reaction with respect to vitamin C was determined (table 4.5). The concentration of sulfuric acid and ammonium ferric sulphate (table 3.5) was kept constant while the concentration of vitamin C in the mixing chamber was varied by withdrawing the vitamin C from valve # 9 at different times, giving the following values

Table 4.5

Time	A1	A2	A3	A4	A5	A6
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6.2600	0.0198	0.0177	0.0168	0.0154	0.0150	0.0135
45.420	0.0489	0.0450	0.0430	0.0399	0.0381	0.0351
84.590	0.0576	0.0535	0.0515	0.0482	0.0454	0.0428
123.75	0.0626	0.0585	0.0559	0.0532	0.0506	0.0471

Where A1 to A6 are the absorbances when the vitamin C was taken at times 7.0, 6.5, 6.0, 5.5, 5.0, 4.5 s respectively from valve # 9. The curves were obtioned by collecting the maximum value of absorbances for each aliquots obtained from mixing chamber after a time interval of 31 s using the SIA-Lab Program shown in table 3.5.

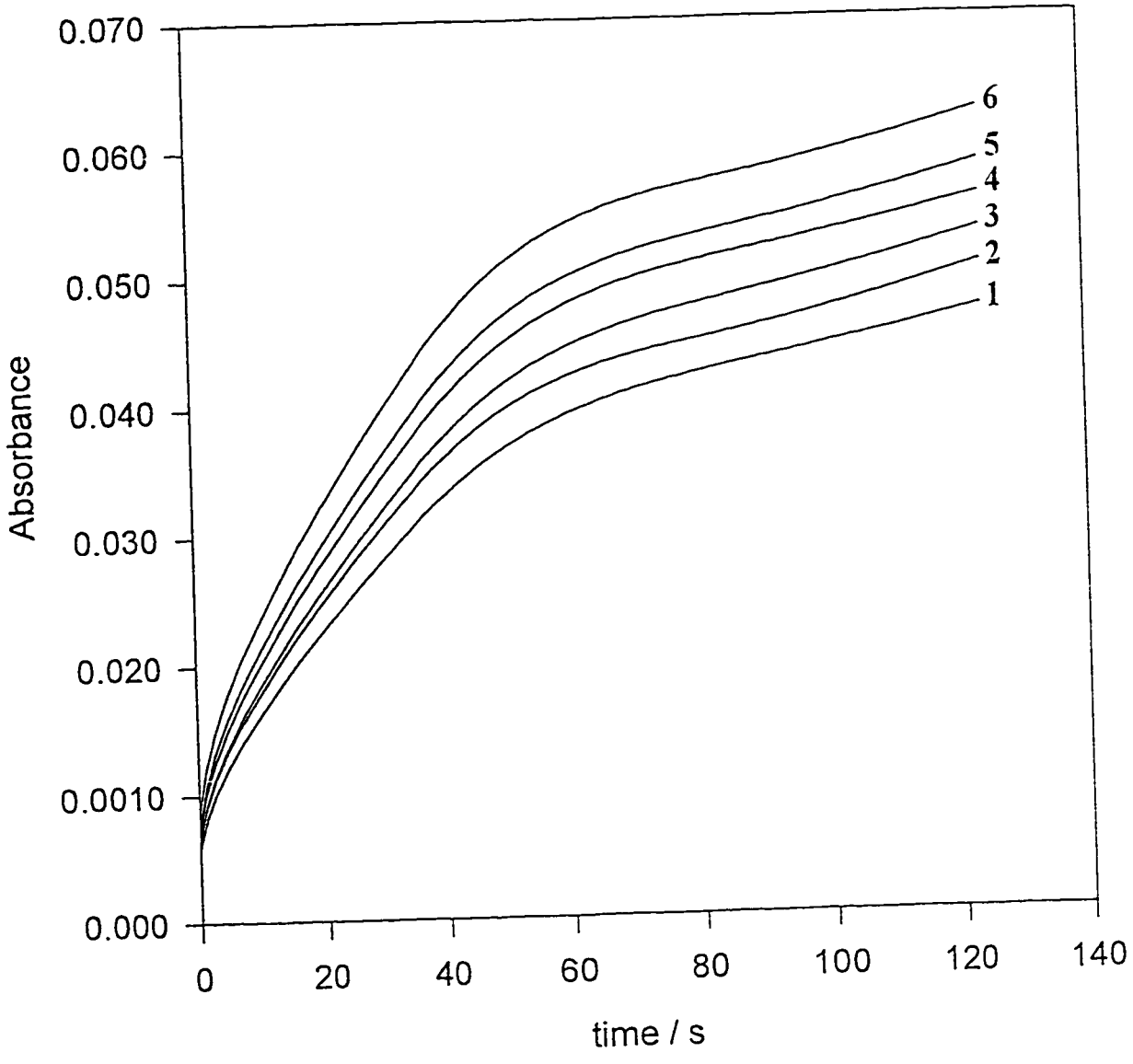


Fig. 4.5 Absorbance-time curves for the determination of the rate of reaction with respect to vitamin C. [Fe^{3+}] = [H_2SO_4] = 2.162 x 10^{-3} mol dm^{-3} ; [vitamin C] = (1) 1.3810 x 10^{-4}, (2) 1.5345 x 10^{-4}, (3) 1.6879 x 10^{-4}, (4) 1.8414 x 10^{-4}, (5) 1.9948 x 10^{-4}, (6) 2.1483 x 10^{-4} mol dm^{-3} ; delay time (t_d) = 12.0 s.

Again by applying the fixed-time method rates were calculated from the plot Fig 4.5 by measuring the absorbance "ΔA" of the product at a predetermined time "Δt" from the start of the reaction.

Table 4.6 Calculated rates of the reaction at 25°C for different ascorbic acid concentrations at constant concentrations of sulfuric acid (0.0179 M) and ammonium ferric sulphate (5.6×10^{-3} M).

No.	[Vitamin C] in m.c.	log[Vit C]	ΔA	$\frac{\Delta A}{\Delta t}$	$\log\frac{\Delta A}{\Delta t}$
1	2.148×10^{-4}	-3.6679	0.0153	7.328×10^{-3}	-2.1350
2	1.995×10^{-4}	-3.7001	0.0137	6.558×10^{-3}	-2.1833
3	1.841×10^{-4}	-3.7349	0.0126	6.041×10^{-3}	-2.2189
4	1.688×10^{-4}	-3.7727	0.0118	5.643×10^{-3}	-2.2485
5	1.535×10^{-4}	-3.8140	0.0103	4.924×10^{-3}	-2.3077
6	1.381×10^{-4}	-3.8598	0.00965	4.617×10^{-3}	-2.3356

The plot of log (ΔA/Δt) versus log [Vitamin C] for the determination of order of reaction and rate constant with respect to vitamin C resulted in a straight line (Fig 4.6) the slope of which was 1.04523 with a correlation coefficient (r^2) of 0.987. The order of reaction with respect to vitamin C concentration is therefore equal to one (1), indicating that vitamin C is consumed as a reactant. The slope gave the value of the rate constant k' which equals to 48.9822.

Therfore,

$$\text{Rate} = k' [\text{ ascorbic acid }]^n \qquad \text{where } n = 1.$$

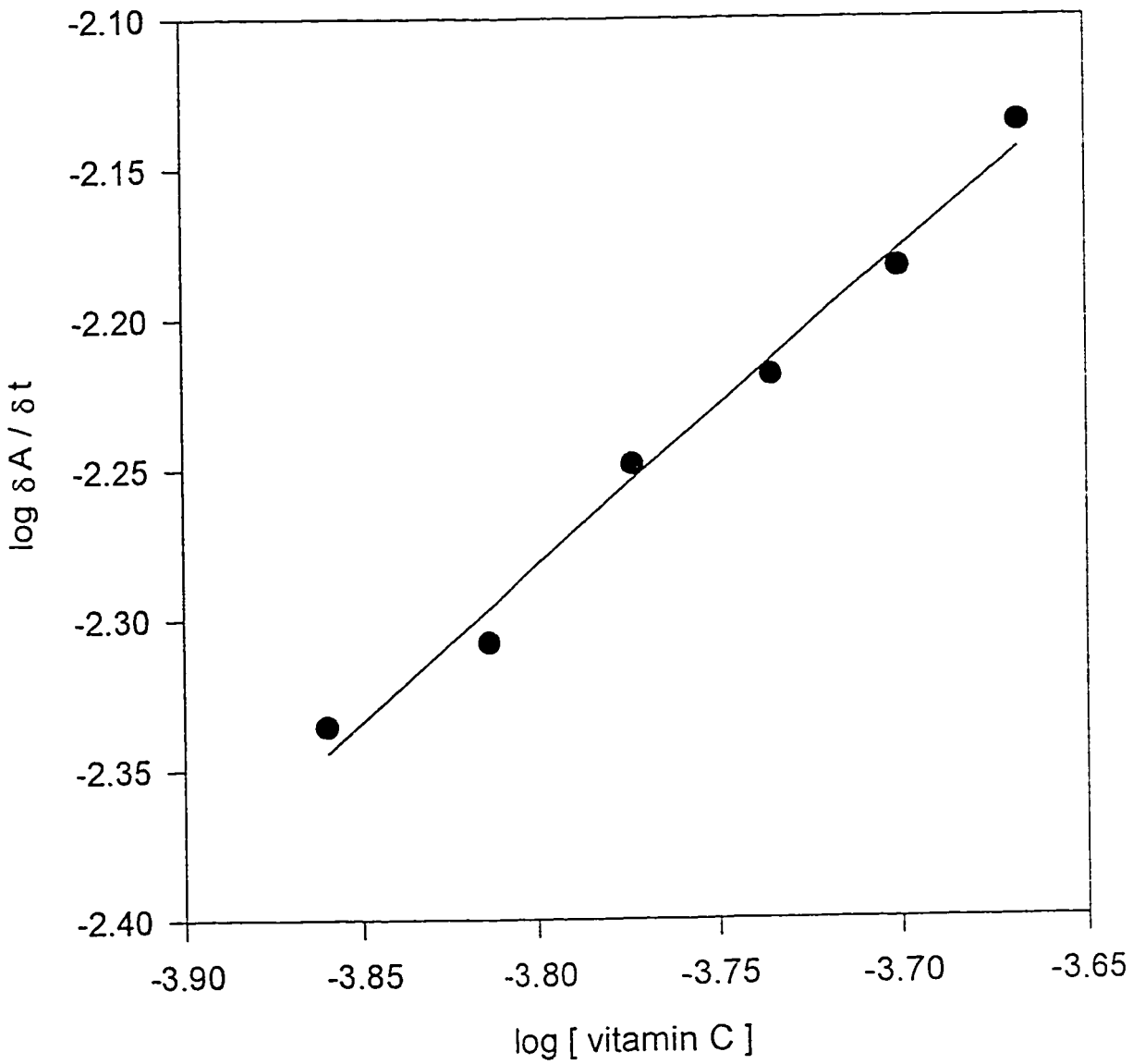


Fig 4.6 Plot of log δA/δt versus log [vitamin C] for the determination of order of reaction and rate constant with respect to vitamin C

4.1.4 Activation energy

Experiments were conducted at varied temperatures between 20.0 - 45.0°C using the program described in table 3.6. The concentration of the solutions in the mixing chamber (m.c.) were kept constant that is 1.6×10^{-3} M iron(III), 0.03 M sulfuric acid, 3.2×10^{-4} M vitamin C and 1.6×10^{-3} M 1,10-phenanthroline.

These concentrations in the mixing chamber were kept constant by fixing the time of withdrawal of each reagent from their respective ports, only the temperature was varied for each run. Data in table 4.7 shows the effect on absorbance with the increase of temperature.

Table 4.7 Temperature effect on absorbance

Time	20°C	25°C	30°C	35°C	40°C	45°C
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6.2600	9.04×10^{-3}	9.60×10^{-3}	0.0104	0.0112	0.0121	0.0133
45.420	0.0309	0.0324	0.0340	0.0360	0.0381	0.0404
84.580	0.0422	0.0438	0.0455	0.0475	0.0498	0.0519
123.75	0.0494	0.0511	0.0530	0.0553	0.0575	0.0597

The pseudo-first order rate constants were calculated for each kinetic curve at different temperatures and are given in table 4.8.

Table 4.8 Calculated values of rate constants for the reaction mixture containing 1.6×10^{-3} M ammonium ferric sulphate, 0.0316 M sulfuric acid and 3.2×10^{-4} M vitamin C at variable temperatures.

No.	T (°C)	$1 / T \times 10^{+3}$ (K)	k' (10^{-3})	$\log k'$
1	20.0	3.41297	0.058631	-4.23187
2	25.0	3.35570	0.187253	-3.72757
3	30.0	3.30033	1.199140	-2.92113
4	35.0	3.24675	1.774557	-2.75091
5	40.0	3.19489	3.823448	-2.41754
6	45.0	3.14465	6.503393	-2.18686

Regression analysis of the plot of log k' verses 1 / T was carried out using the following Arrhenius (R) equation

$$\log k' = \frac{-Ea}{2.303R} \cdot \frac{1}{T} + \log [A]$$

where Ea is the activation energy; R is the gas constant ($R = 8.314\ J\ K^{-1}\ mol^{-1}$); T is the

temperature in Kelvin and *A* is the pre-exponential factor. The activation energy is calculated from the slope of the plot given in fig 4.7 and was found to be

$$Ea = -2.303 \times R \times slope \qquad , \text{where slope} = -\frac{Ea}{2.303R}$$

$$= -2.303 \times 8.314 \times -7.663 \times 10^{3} \times 10^{-3}$$

$$= 146.72 \text{ kJ / mol}$$

The plot has a correlation coefficient (r^2) value of 0.953.

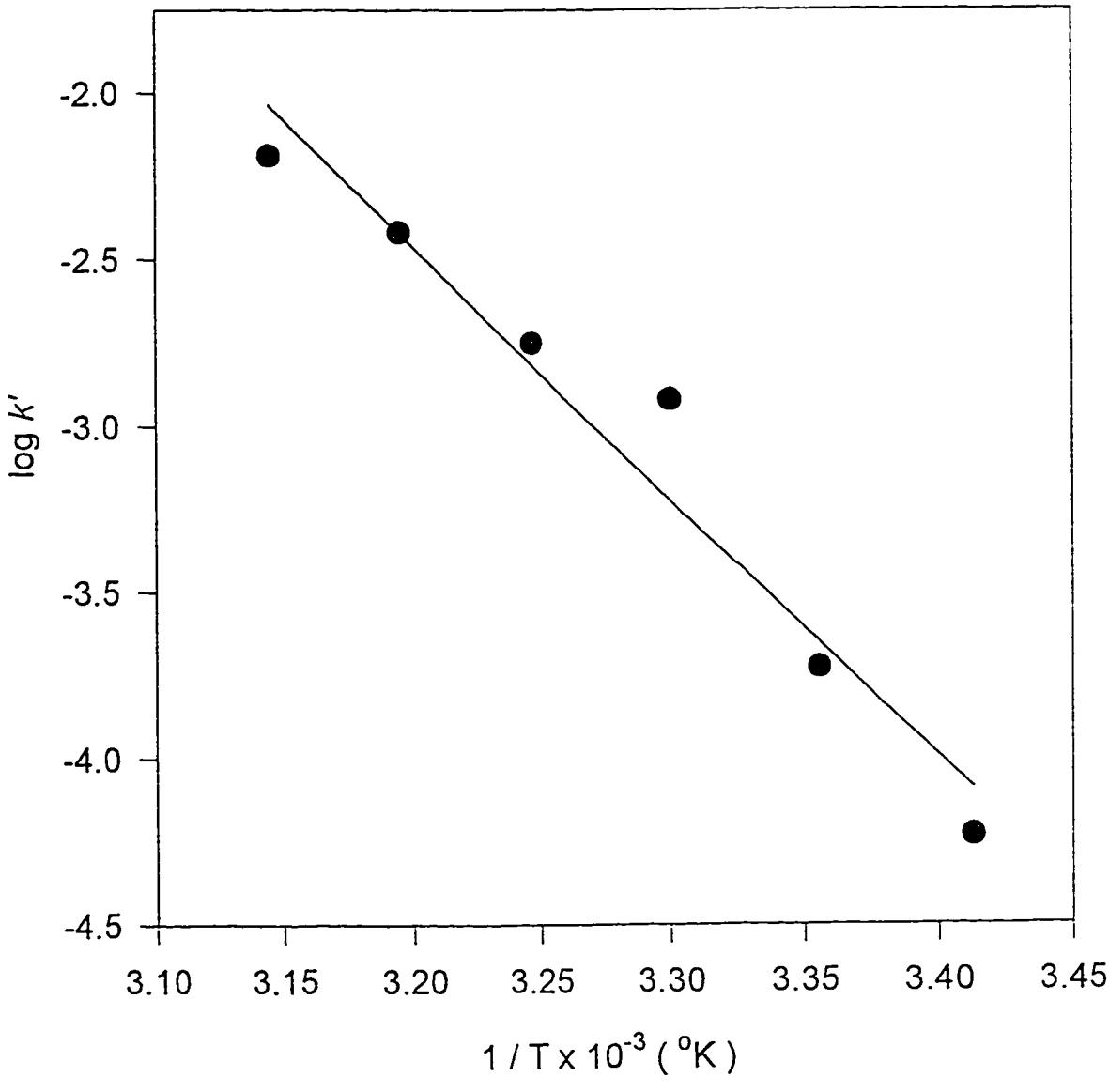


Fig 4.7 Plot between log k' and inverse of temperature in kelvin at fixed concentrations of acid, ammonium ferric sulphate and vitamin C for the determination of activation energy (*Ea*)

4.1.5 Rate law

The rate law can be formed from the order of reactions of each species derived in section 4.1, that is the order of the reaction with respect to ammonium ferric sulphate or iron(III) is *one*, with respect to ascorbic acid is *one* and with respect to hydrogen ions is *minus one*. The rate law thus has the following form

$$\text{Rate} = k \frac{[\,Fe^{+3}\,]\,[\,\text{ascorbic acid}\,]}{[\,H^{+}\,]}$$

4.1.6 Reaction mechanism

The reaction proceeds in two steps as can be seen in figures 4.1, 4.3, or 4.5. To find the mechanism of the reaction we first have to determine the " *active reacting species* " in the oxidation reaction. We know that the protons shifting between enol- and keto- forms are the ones which initiate the reaction as these are susceptible to electrophilic species. Now there can be three possibilities ; either the active reactive species is ascorbic acid (H_2Asc) itself in its original form or the ascorbate anion ($HAsc^-$) or the ascorbate anion radical ($HAsc^{\div}$).

From figure 4.1 it is obvious that the rate of reaction decreases when the acid concentration increases, also we know that ascorbic acid is a weak acid and it dissociates into its respective ions, we can write as

$$H_2Asc \Leftrightarrow 2\,H^{+} + Asc^{2-}$$

So, by increasing the acid concentration we actually shift the above reaction equilibrium in the reverse direction which means more ascorbic acid molecular form will be formed so in this case presence of an acid in molecular form ascorbic acid will not let the reaction go through proving that ascorbic acid is not the active reacting species.

To determine which is the active reacting species among the ascorbate anion ($HAsc^-$) or the ascorbate anion radical ($HAsc^{\div}$) we initially devise a possible reaction mechanism in the form of chain reactions and find out the rate law from the various possibilities. A unique equation from the chain reactions which gives the rate law that is consistent with our experimentally obtained rate law is the equation proved to be the *rate determining step*. The procedure adopted to obtain the rate determing step is given below.

On the light of the fact that the stoichiometry experimentally determined was 1:2 ascorbic acid : iron respectfully, we can write a possible intermediate in such way that the overall equation has ascorbic acid : iron ratio of 1 : 2.

$$H_2Asc \Leftrightarrow H^+ + HAsc^- \qquad (4.1)$$

$$HAsc^- + Fe^{3+} \Leftrightarrow Fe^{2+} + HAsc^{\div} \qquad (4.2)$$

$$\underline{HAsc^{\div} + Fe^{3+} \Leftrightarrow Fe^{2+} + Asc + H^+} \qquad (4.3)$$

$$H_2Asc + 2Fe^{3+} \Leftrightarrow 2Fe^{2+} + 2H^+$$

The rate constant for equation 4.1, 4.2 and 4.3 are k_1, k_2 and k_3 respectively.

To begin with we supposed that equation 4.3 is the rate determining step. The rate law for this equation is written as

$$\text{Rate} = k_3 \, [\, HAsc^{\div} \,] \, [\, Fe^{3+} \,] \qquad (4.4)$$

In the above equation $HAsc^{\div}$ is the intermediate, its value can be found by the equilibrium constant K of equation 4.2 which is

$$K = \frac{[\, Fe^{2+} \,][\, HAsc^{\div} \,]}{[\, HAsc^{-} \,][\, Fe^{3+} \,]}$$

or

$$[\, HAsc^{\div} \,] = \frac{[\, HAsc^{-} \,][\, Fe^{3+} \,]}{[\, Fe^{2+} \,]}$$

Substituting this value in equation 4.4 , we get

$$\text{Rate} = k_3 k_2 \, \frac{[\, HAsc^{-} \,][\, Fe^{3+} \,]^2}{[\, Fe^{2+} \,]} \qquad (4.5)$$

Again $HAsc^{-}$ is an intermediate in the above equation, it can be substituted by the equilibrium constant K′ of equation 4.1, that is

$$K' = \frac{[\, H^{+} \,][\, HAsc^{-} \,]}{[\, H_2Asc \,]}$$

or

$$[\, HAsc^{-} \,] = K' \frac{[\, H_2Asc \,]}{[\, H^{+} \,]} \qquad (4.6)$$

Substituting the value of $HAsc^-$ in equation 4.5 we get.

$$\text{Rate} = k_3 k_2 \frac{[H_2Asc][Fe^{3+}]^2}{[H^+][Fe^{2+}]} \qquad (4.7)$$

Equation 4.7 is not the rate equation that we have found experimentally, therefore equation 4.3 is not the rate determining step.

Considering equation 4.2 , the rate equation for this is given as

$$\text{Rate} = k_2 [HAsc^-][Fe^{3+}] \qquad (4.8)$$

In equation 4.8 , $HAsc^-$ is an intermediate, this intermediate is substituted using equation 4.6 to give

$$\text{Rate} = k_2 K' \frac{[H_2Asc][Fe^{3+}]}{[H^+]} \qquad (4.9)$$

This rate equation is consistent with the experimental results, which has proved that equation 4.2 is the rate determining step, and thus the mechanism postulated is correct.

$$HAsc^- + Fe^{3+} \longrightarrow Fe^{2+} + HAsc^{\div}$$

4.1.7 Application

The sequential injection fixed-time method was applied to the determination of vitamin C in the proprietary drugs. The analysis was repeated six times and the results obtained were found to be highly precise with the standard deviation of less than 1.2% and a recovery of not less than 99.89% of the claimed content in the drugs. The method suffered no interferences from excipients usually added to the drug formulations such as calcium, citric acid, carbohydrates and vitamins B, and D. The accuracy was judged by comparing the results obtained for the same batch of samples with those obtained by the British Pharmacopeia method.[19]

It has been found that sequential injection fixed-time method is simple, accurate, more sensitive than the spectrophotometric methods and the electrochemical methods of this compound. The method is not elaborate and time consuming as the chromatographic methods.

The calibration curve was determined for the standard vitamin C samples in a wide range but found to obey Beer's law in the range of 20 - 300 ppm, which was used to derive the calibration equation. The program made for this purpose is given in table 3.7, in which the time interval for the withdrawal of each aliquot from the mixing chamber is 60, 180, 300, 480 s. Analysis of the proprietary drugs were performed by applying the same program. The calibration plot is shown in figure 4.8, 4.9, 4.10 and 4.11 for 60, 180, 300, 480 s respectively for the vitamin C concentration ranging from 20 - 300 ppm.

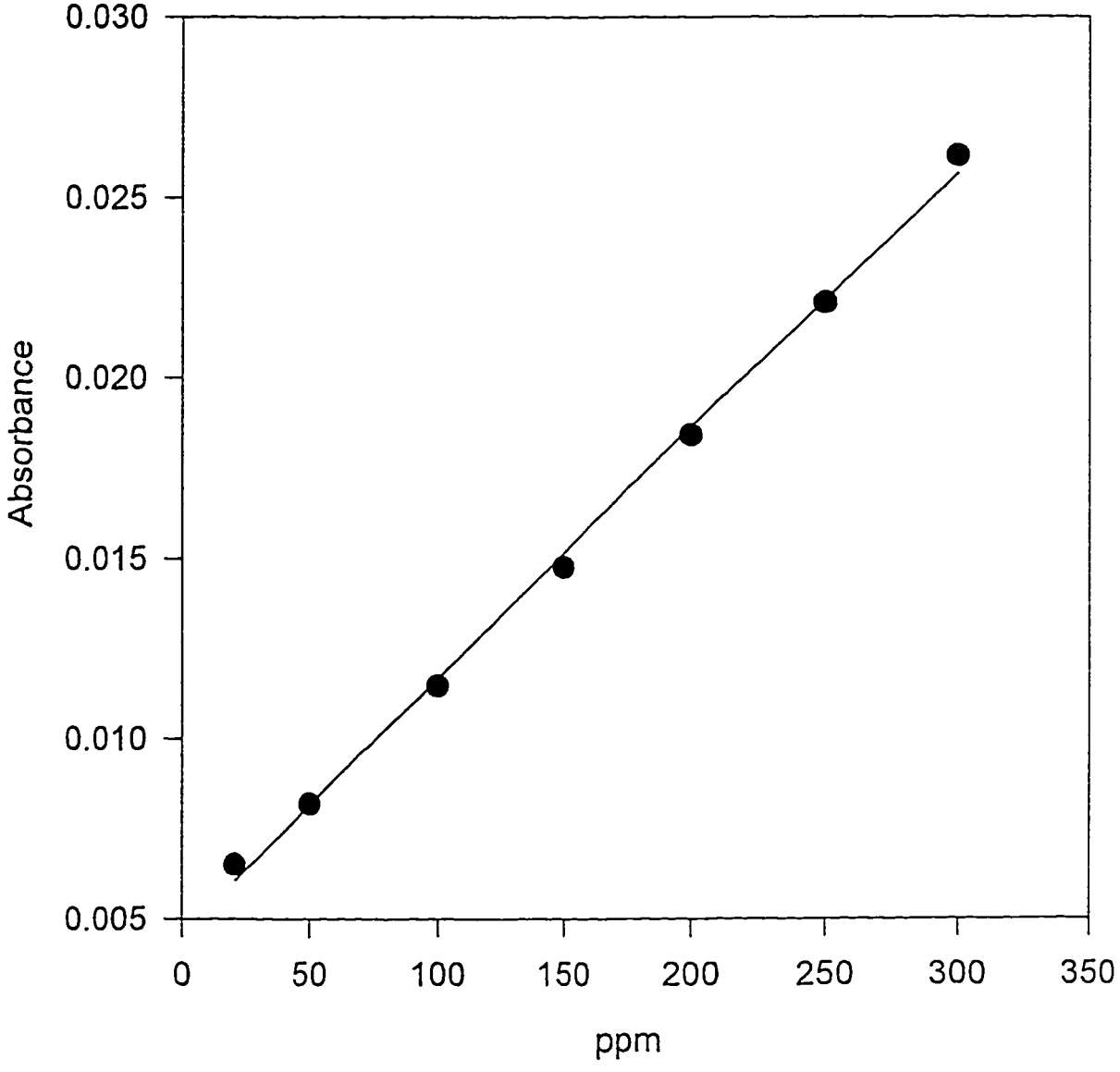


Fig 4.8 Calibration curve using standard vitamin C samples in the range of 20 - 300 ppm for the time interval of 60 s.

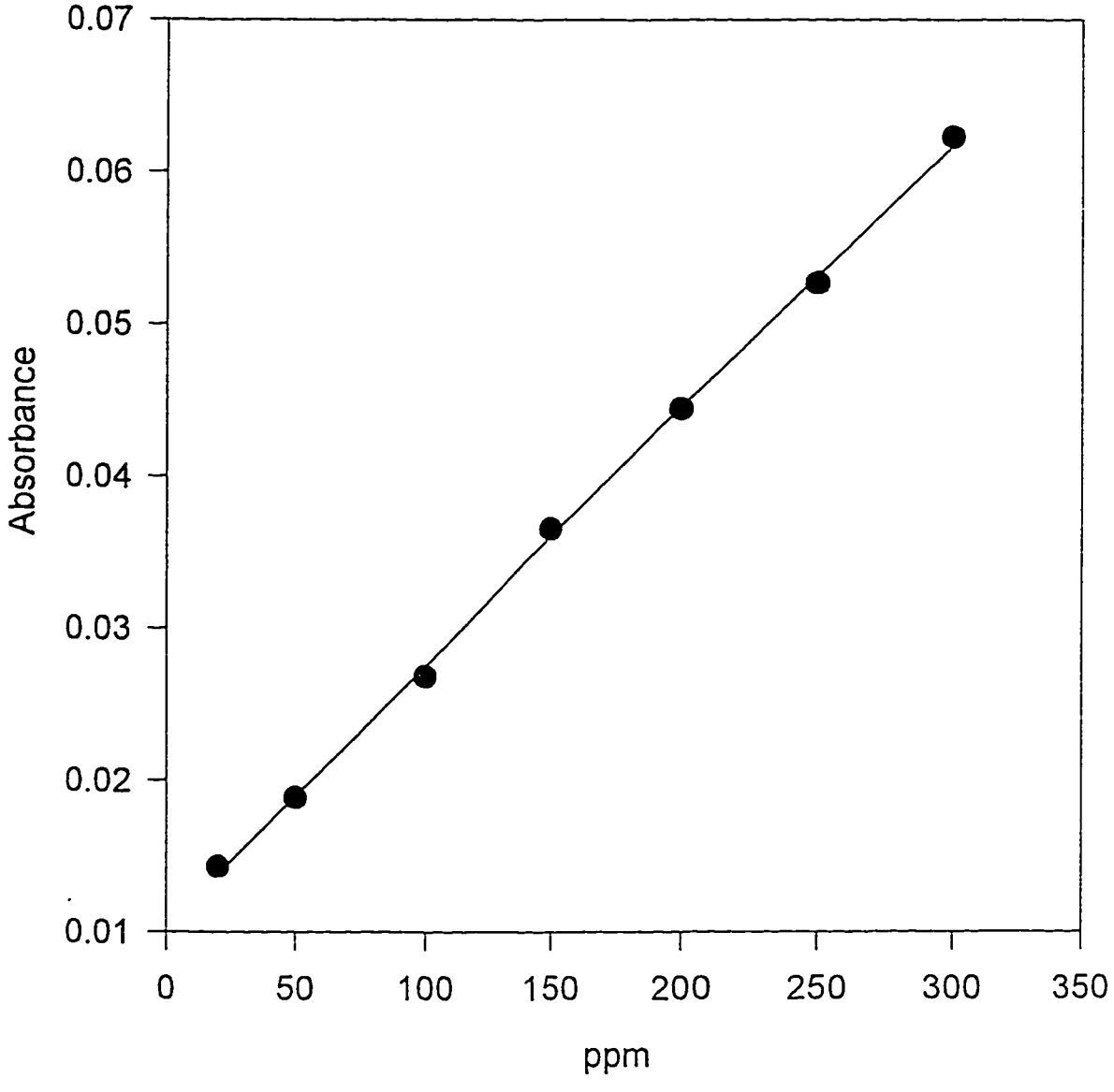


Fig 4.9 Calibration curve using standard vitamin C samples in the range of 20 - 300 ppm for the time interval of 180 s.

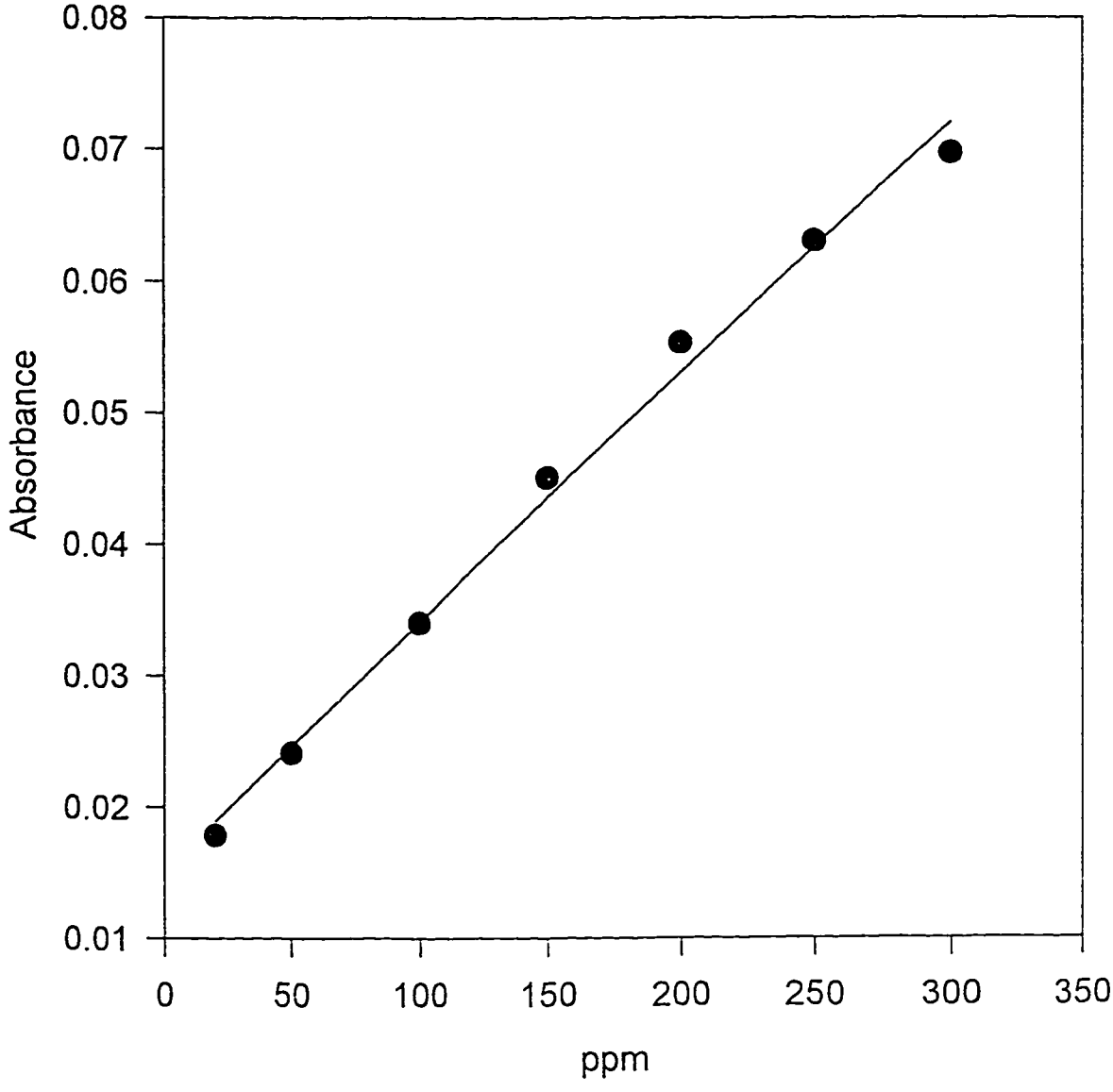


Fig 4.10 Calibration curve using standard vitamin C samples in the range of 20 - 300 ppm for the time interval of 300 s

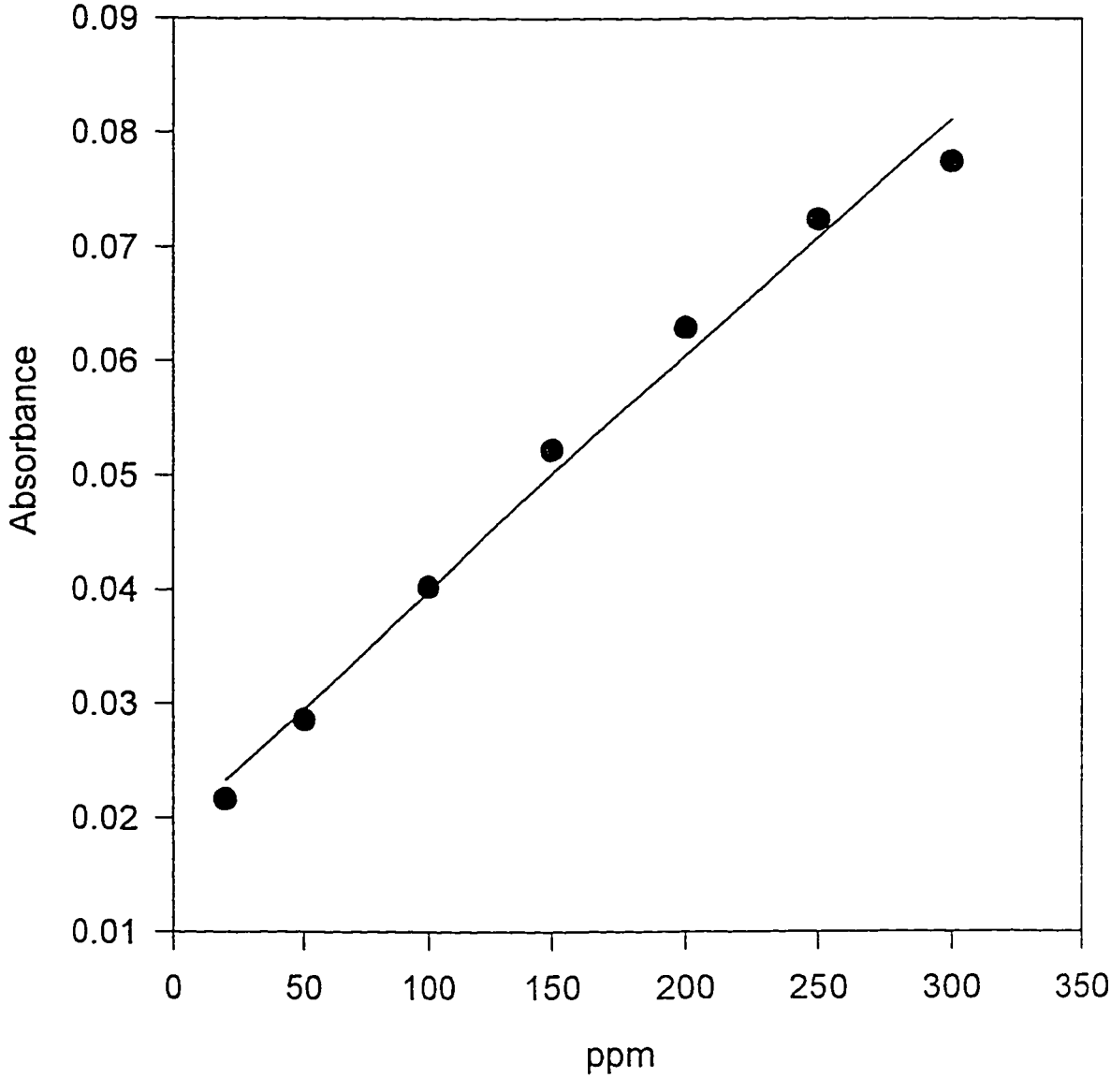


Fig 4.11 Calibration curve using standard vitamin C samples in the range of 20 - 300 ppm for the time interval of 480 s.

Table 4.9 shows the calibration equations for the different times, while the results for the analysis of proprietary drugs is given in tables 4.10 which shows the percentage recovery, and table 4.11 which shows the mean recovery, the relative standard deviation and the student's t test values.

Table 4.9 Calibration equations at different times using 1.6×10^{-3} M ammonium ferric sulphate 0.02 M sulfuric acid.

Time (s)	Calibration equation	Correlation coefficient (r^2)
60	A = 0.0047 + 0.00007 C	0.9978
180	A = 0.0104 + 0.000171 C	0.9992
300	A = 0.0151 + 0.000190 C	0.9938
480	A = 0.0191 + 0.000207 C	0.9892

In the determination of the calibration equation, it has been found that the time interval of 180 s gives the best correlation coefficient. Hence for the determination of vitamin C in proprietary drugs the best calibration equation A = 0.0104 + 0.000171 C was utilized and the fixed time of 180 s was chosen for analysis. The proprietary drugs were withdrawn from the mixing chamber after 180 s and sent to the detector for monitoring which gave the absorbance values given in table 4.10.

Tables 4.10 Absorbance values and the percentage recovery of proprietary drugs.

Redoxon, 100 ppm

Absorbance	Recovery	% recovery
0.0272046	98.23	98.23
0.0275508	100.25	100.25
0.027508	100.00	100.00
0.0272543	98.52	98.52
0.0273537	99.10	99.10
	Mean recovery	99.22

The standard deviation is 0.79 and the relative std. dev. is 0.8 %.

Upsa, 150 ppm

Absorbance	Recovery	% recovery
0.0360420	149.79	99.86
0.0356957	147.77	98.51
0.0361654	150.51	100.34
0.0359734	149.39	99.59
0.0359631	149.33	99.55
	Mean recovery	99.57

The standard deviation is 0.6 and the relative std. dev. is also 0.6 %.

Cal-C-Vita, 200 ppm

Absorbance	Recovery	% recovery
0.0449359	201.68	100.84
0.0441989	197.38	98.69
0.0445486	199.42	99.71
0.0447919	200.84	100.42
0.04435319	198.28	99.14
	Mean recovery	99.76

The standard deviation is 0.79 and the relative std. dev. is 0.8 %.

Octovit, 250 ppm

Absorbance	Recovery	% recovery
0.0531546	249.63	99.85
0.0533859	250.98	100.39
0.0535951	252.20	100.88
0.0532522	250.20	100.08
0.0529180	248.25	99.30
	Mean recovery	100.10

The standard deviation is 0.528 and the relative std. dev. is also 0.528 %.

Beminal C, 200 ppm

Absorbance	Recovery	% recovery
0.0443429	198.22	99.11
0.0439213	195.76	97.88
0.0441715	197.22	98.61
0.0448845	201.38	100.69
0.0440858	196.72	98.36
	Mean recovery	98.93

The standard deviation is 0.965 and the relative std. dev. is 0.975 %.

Table 4.11 Results obtained by the SIA method and the BP method for the analysis of vitamin C in proprietary drugs.

Drug	Supplier	Contents (*mg*)	Mean recovery + SD %*		t ⊗
			SIA method	BP method	
Redoxon	Roche, Switzerland	Vitamin C (1000)	99.22 ± 0.8	99.53 ± 0.9	0.87
Upsa	Lab. Upsa, France	Vitamin C (1000)	99.57 ± 0.6	99.41 ± 0.3	0.60
Cal-C-Vita	Roche, Swizerland	Vitamin C (1000) Calcium (250) Vitamin D (300 i.u.) Citric acid (1,350) Vitamin B6 (15) Carbohydrates (881) Sodium (170)	99.76 ± 0.8	100.2 ± 0.8	1.23
Octovit	SK&F, England	Vitamin C (30) Vitamin A (2,500 i.u.) Vitamin B1 (1) Vitamin B2 (1.5) Vitamin B6 (2) Vitamin B12 (2μg) Nicotinamide (20) Vitamin D (100 i.u.) Vitamin E (10) Calcium (100) Iron (10)	100.1 ± 0.5	99.86 ± 0.7	1.07
Beminal with C Fortis	Wyeth-Ayerst, Canada	Vitamin C (300) Vitamin B12 (mcg) Thiamine (35) Riboflavin (15) Niaciamide (50) Pyridoxine (5) Pantothenic Acid (18.4)	98.93 ± 1.0	98.77 ± 0.9	0.36

* Standard deviation (SD) for five determinations based on label claim, $n = 5$.

⊗ Theoretical value at 95% confidence level = 2.13.

4.2 SI-method for the determination of Promethazine•HCl

Promethazine•HCl is dimethyl- [1-methyl-2-(phenothiazine-1-yl) ethyl] amine HCl . It is commonly prescribed for its antihistamine action. Now-a-days, it is often used after minor operations as an emetic, analgesic, sedative and hypnotic drug. Various methods for its determination have been reported in the literature; mainly, spectrophotometric, chromatographic and electrochemical methods have been recently reviewed [31]. However no method has been reported on the determination of promethazine by sequential injection (SI) technique. Recently two methods for the determination of promethazine by Flow injection technique have been reported including the metavanadate method[31] and the cerium(IV) method[21]. The metavanadate method was found to be suitable for other phenothiazines also this method suffers interferences from excipients when applied to the determination of drug in pharmaceutical preparations. The oxidation of promethazine using metavanadate showed maximum absorbance at 515 nm and the product was proposed to be a dication radical. The chemical system of cerium(IV) which is used to oxidize promethazine in sulfuric acid media is complicated with respect to the formation and stability of the oxidized form of the drug and requires computerized simplex optimization procedures for obtaining generous and reasonable conditions. The cerium(IV) method yields a reddish colored product that gave the same maximum absorbance at 515 nm believed to be due to the same product.

Stoichiometric determination of other phenothiazines with palladium(II) had been reported by Sultan[22] for the first time using SI-technique. Besides the SIA method, other phenothiazines drugs were reported to form complexes with palladium(II) of unusual stoichiometry resulting in doubtful rationalization of the complex structures[54-56]. The official BP method[57] for the assay of promethazine·HCl in tablets and syrups is carried out by titrating the sample in hydrochloric acid followed by reacting with palladium(II) reagent, and measuring the absorbance of the resulting solution at 472 nm.

The present work describes a sequential injection spectrophotometric method for the determination of promethazine•HCl. The method involves the use of palladium(II) to react with promethazine in hydrochloric acid media to yield instantaneously a purple colored complex that shows maximum absorbance at 504 nm. The SI-manifold used here is described in figure 3.1.

4.2.1 Chemical system

Initially the behaviour of the complex was extensively investigated and found to be feasible in a specific pH range. Is was found that the complex was stable from a pH of 4.00 to 3.10 and the maximum absorbance in this range is 504 nm (table 4.12). When the concentration of the acid is further increased the absorbance value decreases, since the color of the solution changes to pale orange, and finally reaches to a maximum absorbance of 496 nm which is the λ_{max} of the oxidized product meaning that there is dissociation of the complex

at higher acidities. This behaviour can be seen in figure 4.12 and 4.13. Below pH value of 4.00 the color of the complex diminishes.

The stability of the complex was also determined and it was found that the complex is stable for more than 2 days, therefore the pH value of 3.10 was chosen for stoichiometric determination.

Table 4.12 Effect of pH on complexation containing 1 x 10^{-4} M palladium(II) and 150 ppm promethazine drug.

No	pH	λ_{max}	Abs	No	pH	λ_{max}	Abs
1	4.10	502.2	0.279	15	3.12	503.9	0.369
2	4.00	503.9	0.284	16	3.10	503.9	0.369
3	3.82	503.9	0.299	17	3.05	503.5	0.353
4	3.70	503.9	0.304	18	3.02	503.4	0.348
5	3.60	503.9	0.318	19	2.90	500.2	0.337
6	3.52	503.9	0.323	20	2.70	497.1	0.323
7	3.46	503.9	0.328	21	2.52	496.3	0.317
8	3.40	503.9	0.334	22	2.40	496.2	0.312
9	3.35	503.9	0.338	23	2.30	496.2	0.312
10	3.30	503.9	0.346	24	2.22	496.2	0.310
11	3.26	503.9	0.350	25	2.15	496.2	0.308
12	3.22	503.9	0.354	26	2.10	496.2	0.305
13	3.19	503.9	0.359	27	2.05	496.2	0.305
14	3.15	503.9	0.364	28	2.00	496.2	0.303

The effect of pH on absorbance and the λ_{max} was determined using Perkin-Elmer Lambda 5 UV/VIS spectrophotometer in the range of 300 nm to 700 nm with a scan speed of 60 nm/min and lamp wavelength of 332.8 nm.

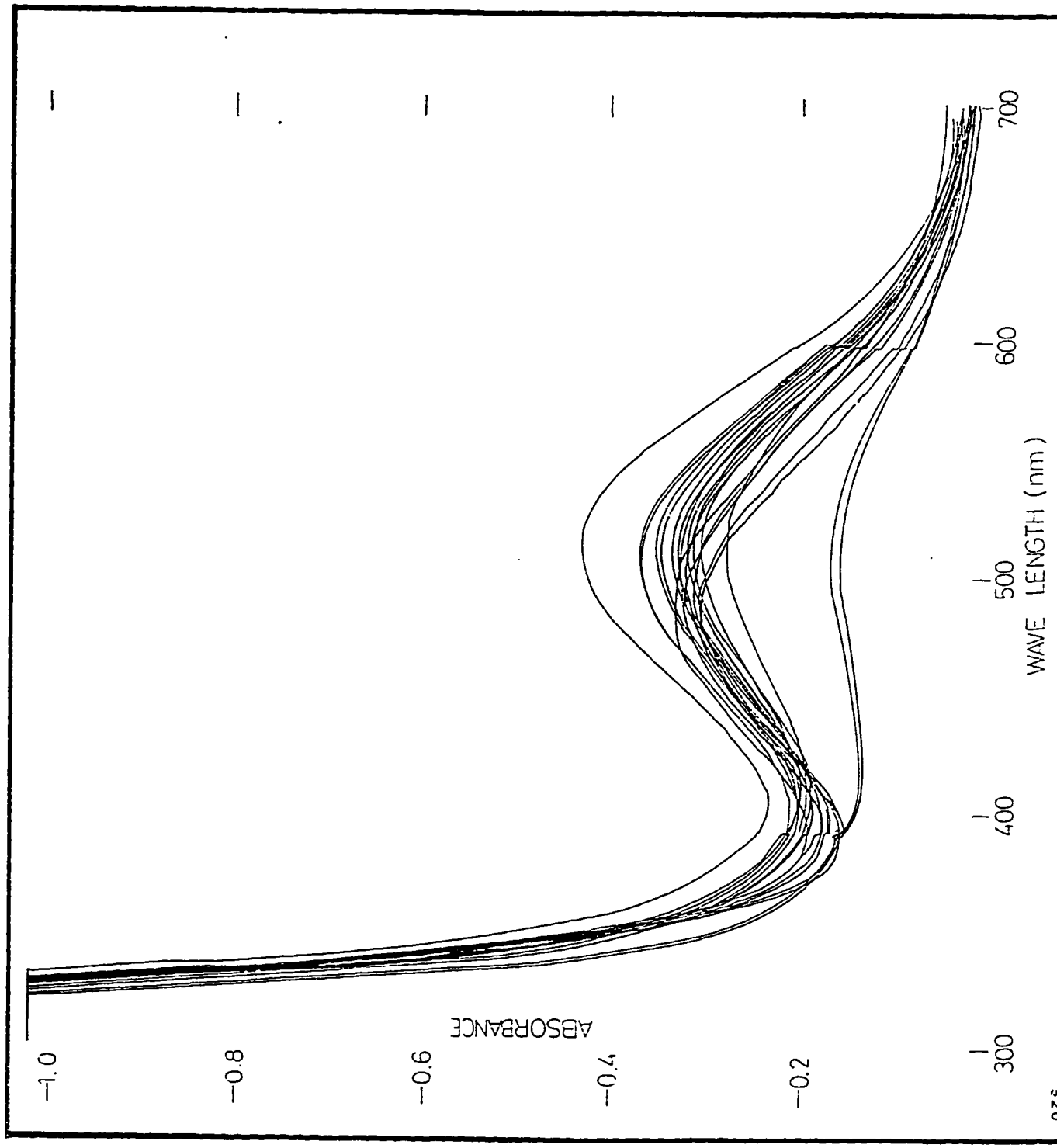


Figure 4.12 UV / VIS Spectrum

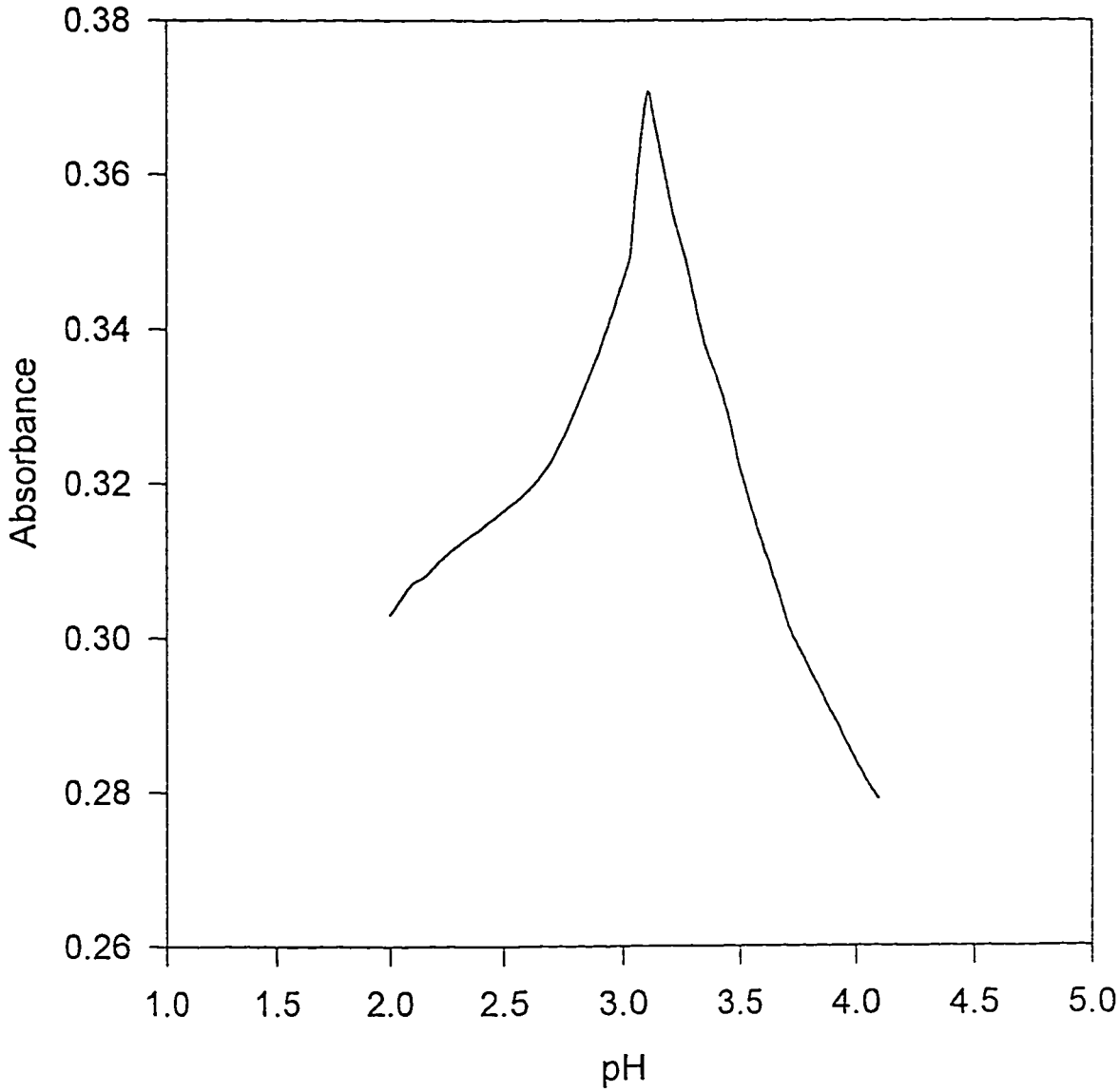


Fig 4.13 Effect of pH on complexation, which contains 1 x 10^{-4} M palladium(II) and 150 ppm promethazine

The effect of palladium(II) concentration on peak absorbance was also determined at pH 3.10 using palladium concentration in the range 1.0×10^{-4} to 4.5×10^{-3} M. It was observed that the increase in absorbance was not significant beyond 3.0×10^{-3} M. This is shown in table 4.13 and figure 4.14.

Table 4.13 Effect of palladium(II) concentration on absorbance.

Palladium(II) / M	Absorbance
1.0×10^{-4}	0.212
2.5×10^{-4}	0.301
5.0×10^{-4}	0.358
7.5×10^{-4}	0.430
1.0×10^{-3}	0.460
2.5×10^{-3}	0.550
4.5×10^{-3}	0.593

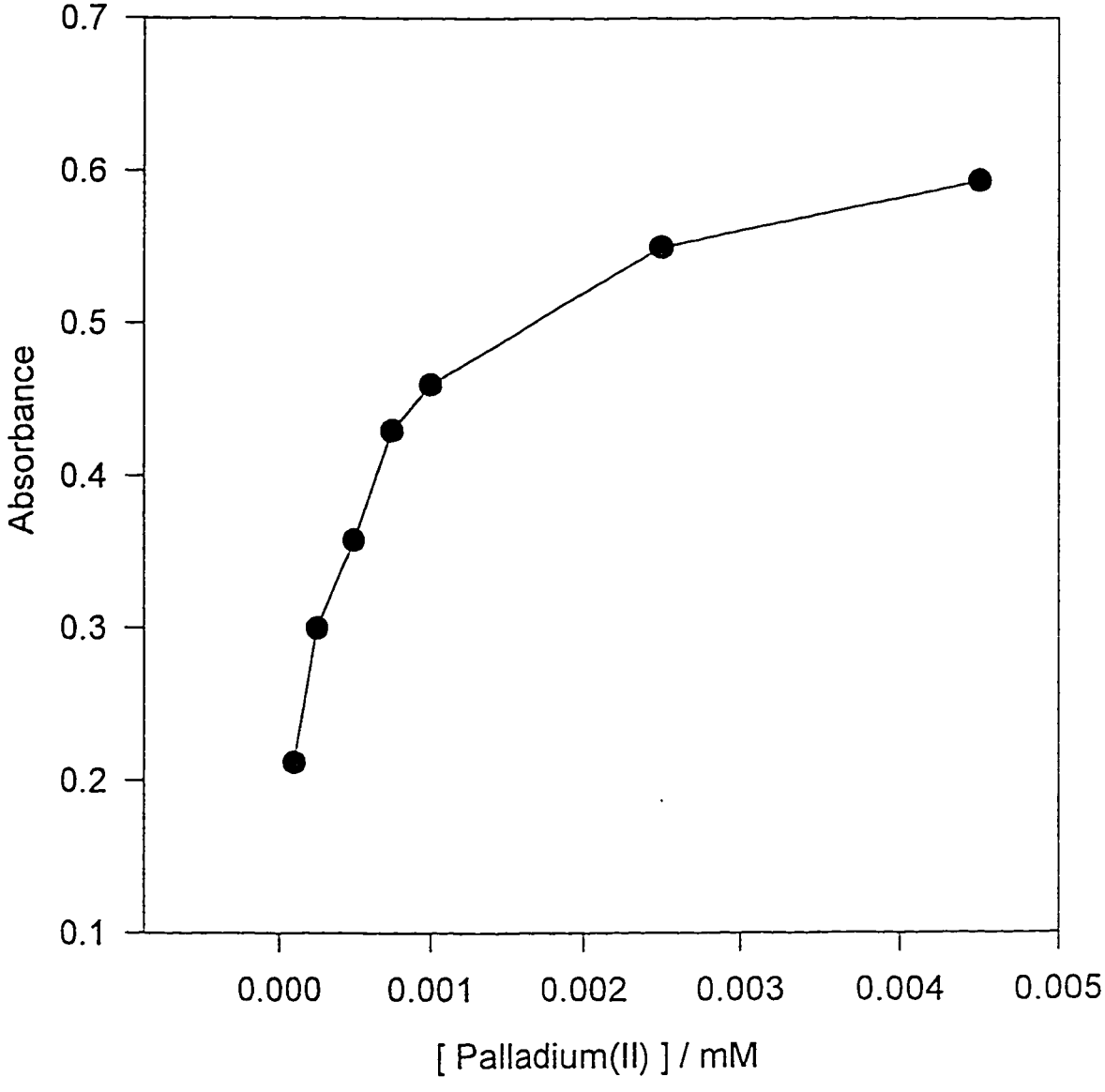


Fig 4.14 Effect on palladium(II) concentration on peak absorbance, which contains 150 ppm promethazine at pH = 3.0969

4.2.2. General consideration for using equilibrium method

In hydrochloric acid concentration range of 1.0×10^{-4} to 9.0×10^{-4} M , a purple color complex is formed instantaneously when palladium(II) solution is added to promethazine solutions, indicative of complex formation. The spectrum of the complex shows an absorption band at 504 nm. It has been observed that the absorbance of the colored complex increases with the increase in acid concentration to a certain limit then starts decreasing which may be dissociation of the complex at high acid concentrations. Preliminary investigations revealed that in acidities higher than 4.0×10^{-3} M, another absorbance maximum at a longer wavelength (496 nm) than that of the complex is observed. This could be attributed to dissociation of the complex followed by the appearance of the oxidized form of the phenothiazine. It is therefore, necessary to optimize the chemical conditions prior to the quantitative measurements.

The stability of the complexes formed favored this method over the previous methods involving oxidation and in which a highly unstable radical product is monitored for quantification[21,31,58,59].

The nature of the interaction between the palladium and the drug investigated was believed to involve protonation of the drug followed by hydrogen bonding between the protonated nitrogen and one of the chlorine atoms on the palladium(II) [54,55,56,60]. Complexes of the type ML_2X_2 and MLX_3 have also been reported [60], where M = Pd, L = phenothiazine ligand, X = Cl)

4.2.3 Job's plot

In the Job's method of continuous variation, different aliquots of equimolar solutions of palladium(II) and of drug were mixed to give solutions of identical total concentration (palladium(II) + drug) but different mole fractions. A volume of carrier solution was then aspirated, the carrier volume was selected to allow a optimal mixing upon flow reversal towards the detector. The Job's plot analysis was used to determine the stoichiometry of the complexation of palladium(II) with promethazine in hydrochloric acid media.

The SI Methods described in table 3.9, 3.10 and 3.11 were used to give figure 4.15, 4.16, 4.17 respectively. All these plots were produced using 8×10^{-4} M hydrochloric acid and an ionic strength of 0.20 M which was maintained by using lithium perchlorate. Water (deionized) was used as a carrier for analysis, but the system was also repeated several times with hydrochloric acid as carrier and even with mixing of the reactants in the holding coil, and it was found that there is no significant difference when water or acid was used as a carrier and even if the mixing steps are added in the SI-program. The total volume of the two reagents for each run was held to a constant value of 295.1μl by using SI-program given in table 3.9 and 3.10 and 162.3 μl using the program given in table 3.11.

It is interesting to note that both reagents, palladium(II) and the sample (drug) were injected, thus consuming minimum reagent. The ability of the SI apparatus to vary the volume of a reagent conveniently with millisecond precision is considered a great advantage and a revolution in FI methodology, having the potential to solve many research problems.

Figure 4.15, 4.16, 4.17 are typical Job's plots generated by using equimolar solutions of palladium and promethazine of 1×10^{-3} M present in 8×10^{-4} M hydrochloric acid. It is clear that the curves $A = f (X_{drug})$ exhibit a maximum for the mole fractions equal to 0.5, indicating that the ratio of palladium(II) : drug in the complex is 1 : 1. It can also be observed that only ten minutes are needed to generate the Job's plot using SIA and less than 10 ml are enough to repeat the procedure as many times as possible.

It is suggested that at higher acidities the oxidation reaction proceeds via formation of the monocation radical in one step thus leading to unstable products as shown in figure 4.18. This reaction is earlier suggested with chlorpromazine, which belongs to the same phenothiazine family, using cerium(IV) as an inorganic oxidant[46]. The mechanism offered here is in agreement with, that the complex dissociates at higher acidity as a result of the reaction favouring the backward direction.

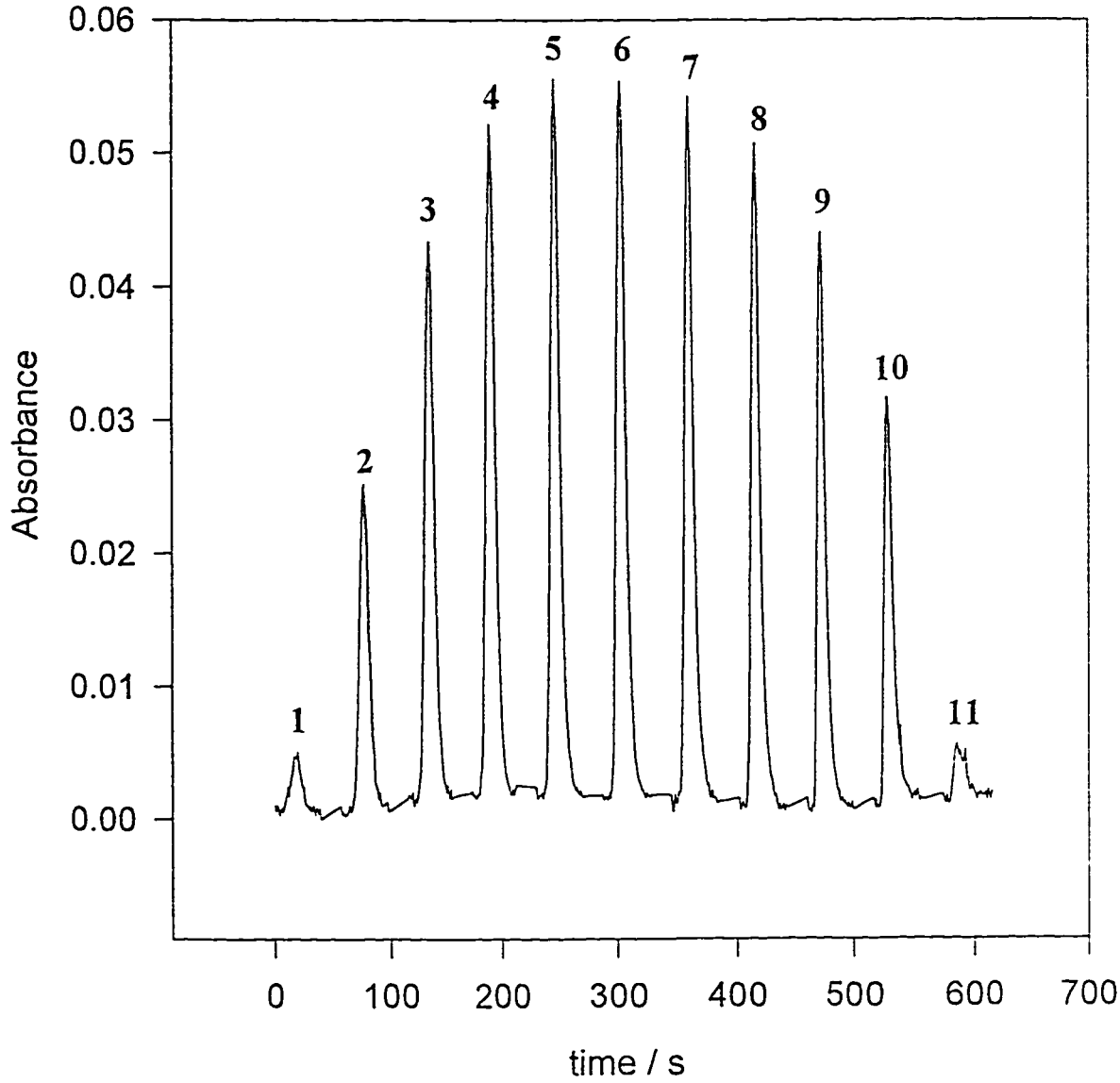


Fig 4.15 Siagram representing Job's plot for the determination of stoichiometry of palladium(II)-promethazine system ; [Pd(II)] = [promethazine] = 1 x 10^{-3} M in 8 x 10^{-4} M HCl; ionic strength 0.20 M; Total aspiration volume is equivalent to 295 µl and aspiration volumes were varied between 14.8 µl and 280.2 µl; mole fractions of the drug were (1) 0.0, (2) 0.1, (3) 0.2, (4) 0.3, (5) 0.4, (6) 0.5, (7) 0.6, (8) 0.7, (9) 0.8, (10) 0.9, (11) 1.0.

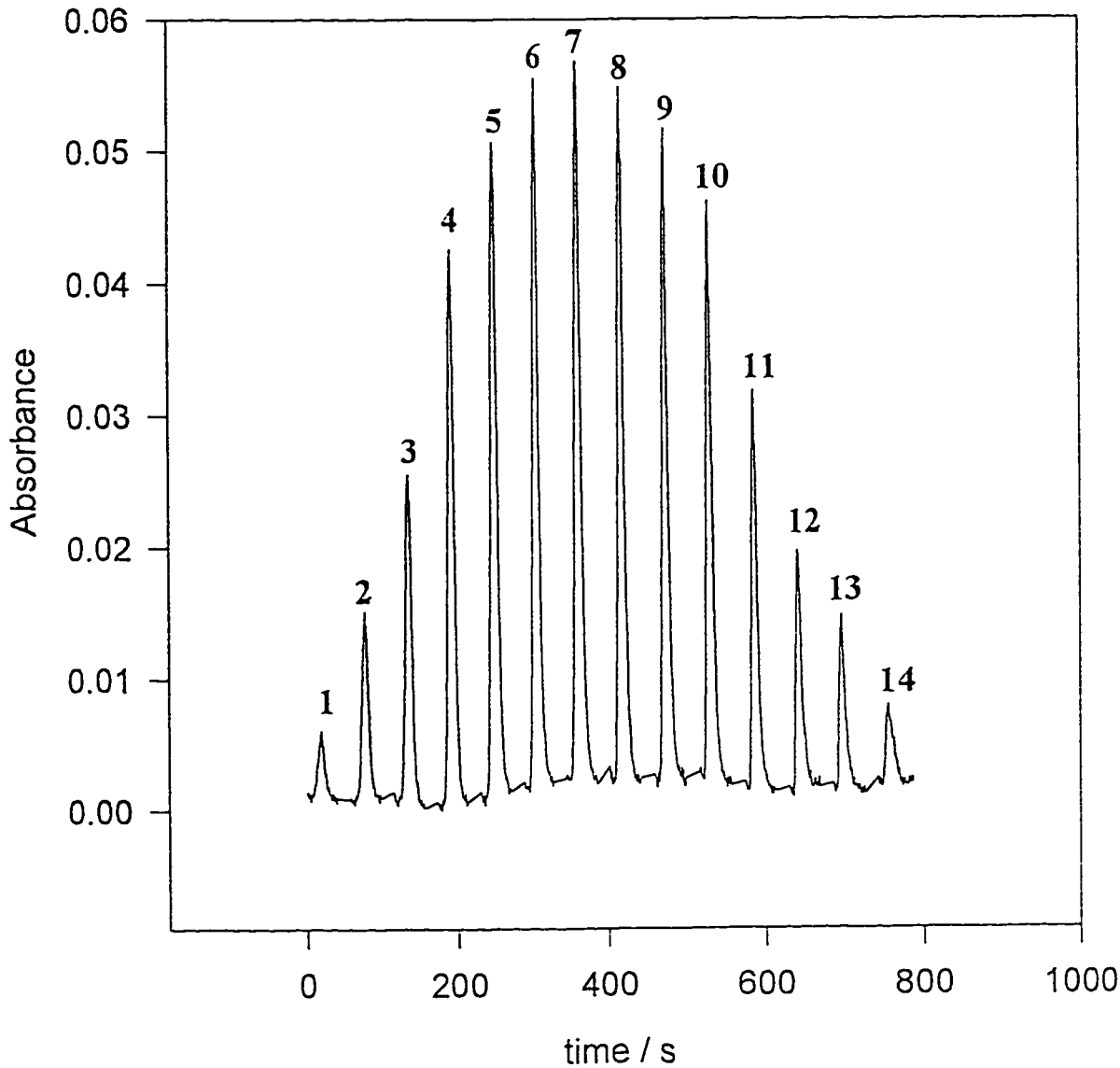


Fig 4.16 Siagram representing Job's plot for the determination of stoichiometry of palladium(II)-promethazine system ; [Pd(II)] = [promethazine] = 1 x 10^{-3} M in 8 x 10^{-4} M HCl; ionic strength 0.20 M; Total aspiration volume is equivalent to 295 µl and aspiration volumes were varied between 14.8 µl and 280.3 µl; mole fractions of the drug were (1) 0.0, (2) 0.05, (3) 0.1, (4) 0.2, (5) 0.3, (6) 0.4, (7) 0.5, (8) 0.6, (9) 0.7, (10) 0.8, (11) 0.9, (12) 0.95, (13) 0.975, (14) 1.0.

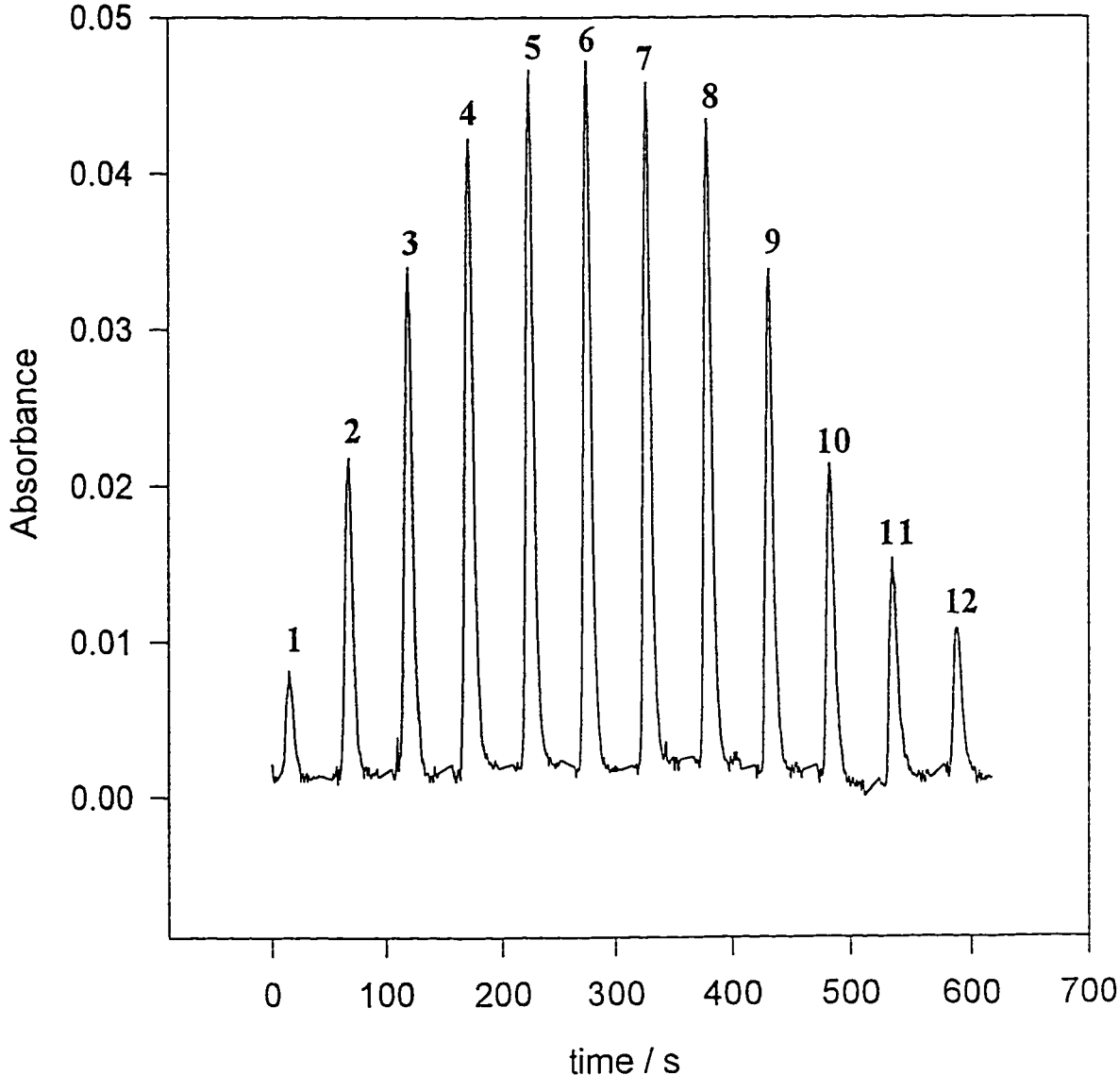


Fig 4.17 Siagram representing Job's plot for the determination of stoichiometry of palladium(II)-promethazine system ; [Pd(II)] = [promethazine] = 1 x 10^{-3} M in 8 x 10^{-4} M HCl; ionic strength 0.20 M; Total aspiration volume is equivalent to 162.3 µl and aspiration volumes were varied between 14.8 µl and 147.5 µl; mole fractions of the drug were (1) 0.0, (2) 0.1, (3) 0.2, (4) 0.3, (5) 0.4, (6) 0.5, (7) 0.6, (8) 0.7, (9) 0.8, (10) 0.9, (11) 0.94, (12) 0.98.

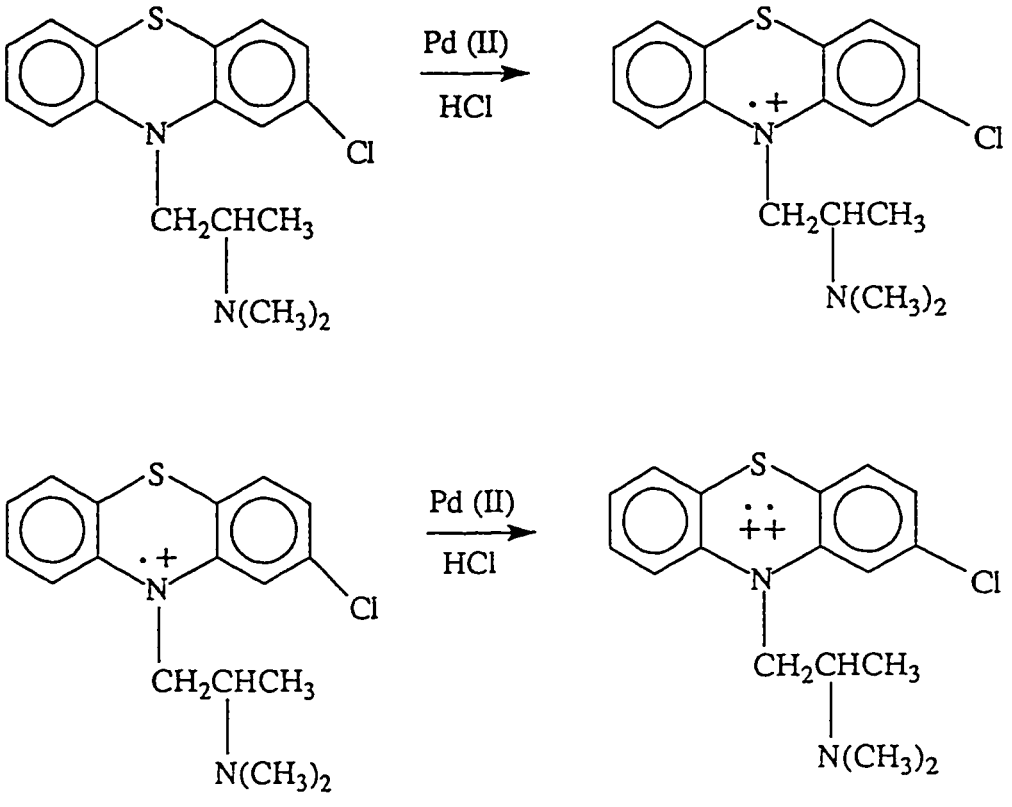


Fig 4.18 Suggested reaction mechanism for the oxidation of promethazine by palladium(II)

4.2.4 Molar ratio plots

In the molar ratio method ideally two straight lines are obtained when the absorbance is plotted verses the palladium(II) to drug ratio, and the point of intersection of these two lines corresponds to the stoichiometric ratio upon interpolation to the mole ratio axis. Therefore, initially, the total volume of the ligand was kept constant by aspirating 147.5 μl into the holding coil by flow reversal whereas palladium(II) solution was varied between 14.8 μl (0.5 s) and 192.0 μl (6.5 s). The ionic strength of all solutions was adjusted to 0.02 M with lithium per chlorate and the hydrochloric acid concentration was 8×10^{-4} M. The method did not give satisfactory results as the palladium(II) concentration was always in excess in the system which cause erronous measurements. The same was repeated by maintaining the palladium(II) volume constant by aspirating 147.5 μl into the holding coil by flow reversal whereas drug solution was varied between 14.8 μl (0.5 s) and 192.0 μl (6.5 s). Still there was no improvement. Finally the 8×10^{-4} M acid was taken as a carrier solution but results were the same.

The lack of application of the Molar ratio method, as described above, is due to the fact that palladium(II) concentration has a greater effect on the system resulting in a mixed complex formation together with the oxidized form of the drug at higher concentrations of palladium(II) which makes the method unsatisfactory.

On the whole the Job's plot method is the more reliable one therefore it has already been proved that the molar ratio in the complex is of 1 : 1, palladium(II) : drug.

4.2.5 Formation constants

The formation constant and the composition of the complexation of palladium(II) with promethazine were also investigated by numerical methods. The JOBCON [61,62] program was used to analyze the continuous variation data. This program was modified by us and were re-written in C-language and applied on a PC/AT computer. The calculations are based on fitting a function $f(x, \beta)$ to a set of experimental data, using a least square method. Unknown parameters are estimated by minimizing U, the sum of squares of residuals defined by the equation given below

$$U = \sum_{i=1}^{n} (A_{expi} - A_{calci})^2$$

where n represents the number of experimental points, A_{exp} the experimental absorbance, and Acalc = $f(x, \beta)$, the absorbance calculated by the program from formation constants and the stoichiometric ratios. Therefore various experimental models could be fitted to the experimental data iteratively by varying the values of formation constants and those of the stoichiometric ratios.

Table 4.14 shows part of the computer output for the palladium(II)-promethazine system using the Jobcon program for the calculation of the formation constants from the continuous variation data. It is clear from this table that the 1 : 1 , metal to ligand ratio is the dominant one with the relative error of 14.99 % and resulting in a value of logarithm of

formation constant (log K_f) of 4.349. Other metal to ligand ratios (m : n) deemed to be improbable with high relative errors, especially those where m : n > 2.

Table 4.14 Computer output from the Jobcon program for the promethazine system #.

m : n *	log K_f'	RE %
1 : 1	4.349	15.00
1 : 2	8.621	36.71
1 : 3	13.51	54.12
1 : 4	17.12	23.74
2 : 1	7.365	75.16
3 : 1	11.75	82.33
4 : 1	14.66	78.95

[Palladium(II)] = [promethazine] = 2.00×10^{-3} M

* metal to ligand mole ratio.

The value of log K_f reported earlier for the promethazine was 5.52. It is apparent from these results, that the value of the formation constant for the 1 : 1, palladium(II)-promethazine is close to the value reported earlier. Is was also observed that the stoichiometry and the value of the formation constant are independent of the total concentration of the metal ion and the ligand.

4.2.6 Calibration graph

Figure 4.19 was generated by running a series of standard solutions of Promethazine•HCl in triplicate over a wide range from which absorbance versus concentration was found to be linear in the range of 50 - 400 ppm, with the following calibration equation :

$$A = 0.008654 + 0.000110\ C$$

Where A is the absorbance, and C is concentration in ppm, with a correlation coefficient (r^2) of 0.997.

The peak width at base line was measured to be 18 seconds thus defining the sample frequency of 200 samples per hour. Peak width was also determined at 60% peak height and found to be 2.4 seconds indicating minimal dispersion. Average relative standard deviation (RSD) of 0.92% was obtained for repeated six determinations of 250 ppm indicating excellent reproducibility.

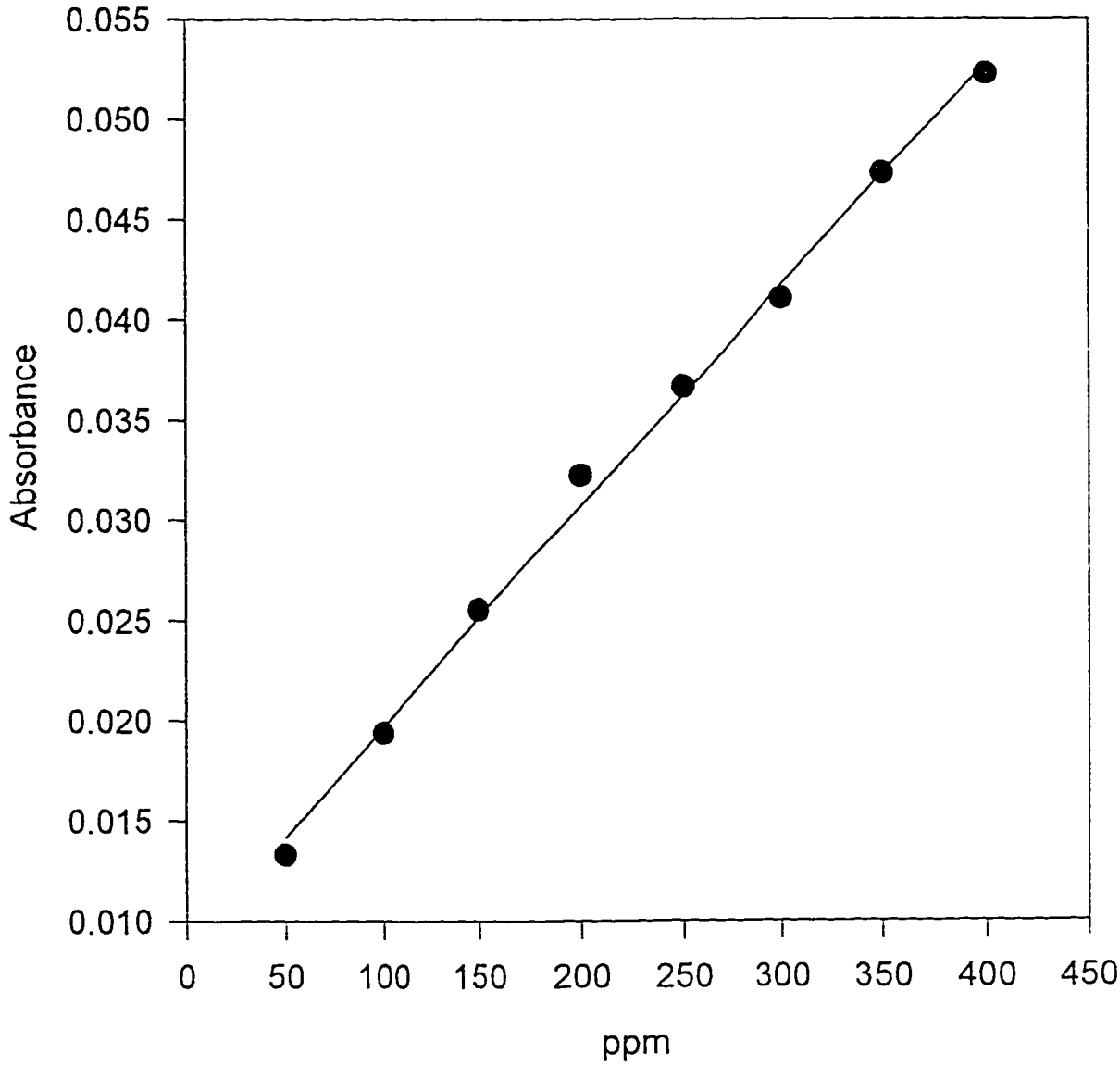


Fig 4.19 Calibration curve using standard promethazine•HCl samples in the range of 50 - 400 ppm

4.2.7 Application

This method is applied to the determination of Promethazine•HCl in the proprietary drugs namely, (i) Phenergen tablets, (ii) Phenergen promethazine, (iii) Phenergen Expectorant and (iv) Cigan Elixir

All these drugs formulations contains starch and glucose excipients. The same batch were analyzed by the BP method [57] and the results were statistically compared by calculating the percent recoveries, standard deviations and the student t-test values as in table 4.15.

The present method showed the same degree of accuracy as of the BP method. Results also show that the effect of the common drug fillers do not interfere with the determination.

The present method has the advantage of selectivity and simplicity over the BP and previous methods. The utilization of palladium(II) reagent removed the complication of the drug being oxidized during the analysis.

Table 4.15 A statistical comparison of the results for the determination of Promethazine•HCl in proprietary drugs by the present method with those obtained by the official BP method.[57]

Proprietary drug	Active material	Recovery + SD %*		t ⊗
		SIA	BP	
Phenergen tablets (Specia, France)	Promethazine•HCl 25 mg	99.72 ± 0.6	99.31 ± 0.5	1.53
Phenergen syrup (Specia, France)	Promethazine•HCl 666.67 ppm	99.9 ± 0.7	99.8 ± 0.3	0.32
Phenergen syrup Expectorant (Specia, France)	Promethazine•HCl 666.67 ppm	99.4 ± 1.0	99.9 ± 0.6	1.12
Cigan Elixir (Cimabrex,Denmark)	Promethazine•HCl 1000 ppm	100.1 ± 0.8	99.8 ± 0.4	0.84

* Standard deviation (SD) for five determinations based on label claim, $n = 5$.

⊗ t, theoretical value = 2.78 ($p = 0.05$).

Chapter 5

Conclusion

In this study the recently developed SI-technique , which is a newly born sister of FIA technique, was undertaken, and the full utilization of this technique was a prime goal to be achieved and demonstrated during this work.

A systematic application of Kinetic methods was performed in an attempt to develop and validate analytical methods suitable for the assay of vitamin C to be applied in their pharmaceutical preparations. The methods developed, were aimed to satisfy some desirable features required for routine analysis. These features include, simplicity, short time of analysis where no sacrifice should be made with respect to precision and accuracy. Tolerance of the methods to uncontrolled factors, known as ruggedness, is also considered. Moreover this method is environmentally safe and the only factor which requires caution in handling is the acid but that too is used here in mmol concentrations.

Also this system was successfully used for the optimization of four independent variables; flow-rate, mixing chamber diameter (reaction coil length), ammonium ferric sulphate concentration and sulfuric acid concentration. The optimum operating conditions were reached faster with few number of experiments.

In the determination of promethazine the SI technique was far more efficient and promising than its predecesssor FI-technique. In the promethazine system the sensitivity was selected as the primary performance criterion whereas the time of analysis was the secondary criterion. The secondary criterion was maintained by setting the lower threshold of the flow-rate to 4.10 ml/min to obtain a sampling frequency of not less than 120 h^{-1}. The method developed was applied for the determination of promethazine in drug formulations. This method was found to be superior to official methods with a wide dynamic range (50-400 ppm), an excellent reproducibility and with a relative standard deviation of less than 0.96%.

The versatility of the Sequential Injection (SI) technique was examined for optimization as well as for the kinetic studies involving the development of a kinetic method of analysis. In the second part of this study SI-technique was applied to the determination of concentrations, stoichiometries, and formation constants of complexation reactions.

The SI-technique was first utilized for fully investigating the kinetics of the oxidation of vitamin C with iron(III) in sulphuric acid media. The reaction orders with respect to each reagent were determined by SI-technique and were found to be 1, 1, and -1 for vitamin C, iron(III) and hydrogen ions respectively. On the basis of these values a rate law was developed and a plausible mechanism was established. A kinetic method for the analysis of vitamin C in drug formulations was also developed based on the results obtained above. This full investigation of the kinetics of the reaction using SI-technique was the first of its kind in the literature.

As was observed from the literature, most of the kinetic methods of analysis reported lack a full investigation of the kinetics and mechanism of the chemical reactions involved. Therefore, it can be seen that with the emergence of robust techniques such as the SI-technique there is a excellent chance to reshape the kinetic methods of analysis in order to include a full investigation of the kinetics and mechanism of the chemical reaction. In addition the computer controlled SI-technique could lead to a vast resurgence in the activity in this field and other related ones.

The capacity of the SI-technique was demonstrated by applying the technique, for the determination of stoichiometries of complexation reactions. The system studied was

the complexation of promethazine with palladium(II) in dilute hydrochloric acid media. The Job's method of continuous variation was utilized and the drug : palladium(II) mole ratio was found to be 1 : 1 for both systems. It is interesting to note that both reagents, the drug and palladium(II), were aspirated in ml amounts, thus consuming very small amount of the reagents. This is a great advantage achieved, making feasible the full investigation of physico-chemical parameters which are usually ignored or studied superficially to avoid consuming a valuable and expensive reagent. This study opens the way for scientists from discipline other than those involved in the chemical analysis to use such a technique. A large number of experimental trials were performed utilizing a relatively small amount of reagents. The values obtained for the formation constants were found to be closely related to literature values.

Optimization studies were found to be easier when SI-technique is used compared to the conventional flow injection (FI) technique, owing to the simple mechanical assembly of the former.

References

[1] Sultan, S.M., and Suliman, F.E.O., Analyst, 1996, 121, 617.

[2] Ruzicka, J., and Hansen, E.H., Flow Injection Analysis, 2nd ed., Wiley, New York, 1988.

[3] Skoog, D.A., and Leary, J.J., Principles of Instrumental Analysis, 4th ed., Saunders HBJ, Florida, 1992.

[4] Flow Analysis, Proc., 5th Intl.Conf., Kamamoto, Japan, Anal. Chim. Acta., 1992, 261, 1-577.

[5] Ruzicka, J., Anal. Chim. Acta., 1992, 261, 3.

[6] Marshall, G. D., van Staden, Jacobus F., Process Control and Quality 1992, v 3, n 1- 4, 251-261.

[7] Avi Ivaska and Jaromir Ruzicka, Analyst, 1993, 118, 885.

[8] Ingle, Jr J. D., Crouch, S.R., Spectrochemical Analysis, Prentice-Hall, 1988.

[9] Mathews, R.J. , Goode, S.R., and Morgen, S.L., Anal. Chim. Acta., 1981, 133, 169.

[10] Mottola, H.A., Kinetics aspects of Analytical Chemistry, Wiley, New York, 1988.

[11] Perez-Bendito, D., and Silva, M., Kinetic Methods in Analytical Chemistry; Ellis Horwood,Chichester, 1988.

[12] Ruzicka, J., and Marshall, G. D., Anal. Chim. Acta., 1990, 237, 329.

[13] Christian, G.D., and Ruzicka, J., Anal. Chim. Acta., 1992, 261, 11.

[14] Ruzicka, J., and Gubeli, T., Anal. Chem., 1991, 63, 1680.

[15] Gubeli, T., Christian, G.D., and Ruzicka, J., Anal. Chem., 1991, 63, 2407.

[16] Baron, A., Guzman, M., Ruzicka, J., and Christian, G.D., Analyst, 1992, 117, 1839.

[17] Sultan, S. M., and Bishop E., Journal of Pharmaceutical & Biomedical Analysis, 1990, 8, No. 4, 345.

[18] Sultan, S. M., Abdennabi, A. M., and Suliman, F. O., Talanta, 1994, 41, No.1, 125.

[19] British Pharmacopeia, 5th Ed., vol II, p.47-48. HM Stationary Office, London, 1988.

[20] Bevan, J. A., "Essentials of Pharmacology", 2nd ed., Harper and Row, 1976.

[21] Sultan S.M., and Suliman, F. O., Analytical Sciences [Japan], 1992, 8, 841.

[22] Sultan S. M., Suliman, F.O., and Bahruddin, B. S., Analyst, 1995, 120, 561.

[23] Hungerford, J.M., and Christian, G.D., Anal. Chim. Acta., 1987, 200, 1.

[24] Hungerford, J.M., Christian, G.D., Ruzicka, J., and Giddings, J. C., Anal. Chem., 2457,1794.

[25] Mahvous M. S., and Abdel-Khalek, M. M.,Talanta, 1984, 31, 289.

[26] Morgan S.L., Deming S.N., Anal. Chem., 1974, 46, 1170.

[27] Hausen, E.H., Anal. Chim. Acta., 1992, 261, 125.

[28] Sultan, S.M., Al-Zamil, I.Z., and Al-Arfaj N.A., Talanta, 1988, 35, 375.

[29] Apostolakis, J.C., Georgiou, C.A., and Koupparis, M.A., Analyst, 1991, 116, 233.

[30] Sultan, S.M., Analyst, 1992, 117,773.

[31] Sultan, S.M., Analyst, 1991, 116,177.

[32] Chung, H. K., Ingke Jr. J. D., Talanta, 1991, 38, 355

[33] Meloun, M., Javurek M., Talanta, . 1984, 31, 1083

[34] Sultan, S.M., Suliman F.O., Duffuaa S.O., and Abu-Abdoun, I. I., Analyst, 1992, 117, 1179.

[35] Inczedy, J., "Analytical Applications of Complex Equilibria", Wiley, New York, 1976.

[36] Vander Heyden Y., Khots, M.S., and Massart D.L., Anal. Chim. Acta., 1993, 276, 189.

[37] Schwarzenbach, G., " Die Komplexometrische Titration", Enke Verlag, Stuttgart, 1957.

[38] Ringbom, A., "Complexation in Analytical Chemistry", Wiley, New York, 1963.

[39] Sabatini, A., and Vacca A., and Gans, P., coord. chem., Rev., 1992, 120, 289.

[40] Sultan, S.M., Anal Lett, 1991, 24, 1785.

[41] Ostromisslensky, I., Ber. Deut. Chem. Ges., 1911, 44, 268.

[42] Ryan, J. A., J. Am. Pharm. Assoc., 1959, 48, 240.

[43] Job, P., Ann. Chim. (Paris), 1928, 113.

[44] Guzman, M., Ruzicka, J., and Christian, G. D., Talanta, 1993, 40, 81.

[45] Ramappa, P. G., Gowda, H. S., and Nayak, A. N., Analyst, 1980, 105, 663.

[46] Suliman, F. O., Sultan, S. M., Talanta, 1994, 41, 1865.

[47] Wade, A. P., Shiundue, P. M., and Wentzell, P. D., Anal. Chim. Acta, 1990, 237, 361.

[48] Likussar, W., and Boltz, F. D., Anal. Chem., 1971, 43, 1265.

[49] Momoki, K., Sekino, J., Sato, H., and Yamaguchi, N., Anal. Chem., 1969, 41, 1286.

[50] Hull, R. D., Malick, R.E., and Dorsey, J.G., Anal. Chim. Acta, 1992, 276, 1.

[51] Martindale, The Extra Pharmacopoeia, 28th ed., 1982.

[52] Yoe, J. H., and Jones, A. L., Ind. Eng. Chem. Anal. Ed., 1944, 64, 111.

[53] Bishop E., ' Indicators ' Oxford, 1973.

[54] E. Pellizzetti, J. Chem. Soc., Dalton Trans., 1980, 484.

[55] Geary, W. J., Mason, N. J., Nowell, I. W., and Nixon, L. A., J. Chem. Soc., Chem. Commun., 1984, 1064.

[56] Duddell, D. A., Goggin, P. L., Goodfellow, R. J., Norton, M. G., and Smith, J.G., J. Chem. Soc. A, 1970, 546.

[57] Brirish Pharmacopeia, H. M. Stationary Office, London, 5th ed., pp 749, and 995, 1988.

[58] Sultan, S. M., Microchem. J., 1991, 44, 304.

[59] Sultan, S. M., and Abdennabi, A. M., Microchem. J., 1993, 48, 343.

[60] Geary, W. J., Mason, N. J., Nowell, I. W., and Nixon, L. A., J. Chem. Soc., Dalton Trans., 1982.

[61] Likussar W., Anal. Chem., 1973, 45, 1926.

[62] Meloun, M., Javurek, M., Talanta, 1984, 31, 1083.