Chemical Analysis of Some Penicillin And Cephalosporin Drugs

by

Sheikheldin Sami Yassin

A Thesis Presented to the

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This thesis, written by SHEIKHELDIN SAMI YASSIN 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 fulfillment of the requirements for the degree of MASTER OF SCIENCE in CHEMISTRY.

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TO ALL MEMBERS OF MY FAMILY

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I would like to offer my indebtedness and sincere appreciation to my committee chairman Dr.S.J.Lyle who has been a constant source of help and encouragement during this research. I greatly appreciate the invaluable co-operation and support extended by Dr. A.Abdennabi and Dr. C.Tsonis who served as members of thesis committee. I express my thanks to Messrs Martin,Baig,M.Nasser, Arab,and M.Antar for their help . I also thank A/Aleem, Salah, Limalia, and Khaled, for their extensive help during the time of this thesis.

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THESIS ABSTRACT

NAME OF STUDENT: SHEIKHELDIN SAMI YASSIN

TITLE OF STUDY : CHEMICAL ANALYSIS OF SOME PENICILLIN AND CEPHALOSPORIN DRUGS

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This thesis describes work on the polarographic analysis of pencillin and cephalosporin antibiotics. Methods for the quantitative analysis of Ampicillin,Amoxycillin and Cephalexin were developed.These methods are based on the formation of Ni(II) or Co(II) complexes of the antibiotics or on degradation products namely, 2,5-diketopiperazine derivitives.

The optimum conditions of buffering,support electrolyte,pH, temperature and the effect of time were studied for the Ni(II) complexes of Ampicillin and Amoxycillin . Under these optimum conditions, linear calibration ranges were 5.00×10^{-7} to $1.00 \times 10^{-5}M$ and 4.00×10^{-7} to $2.00 \times 10^{-5}M$ for Ampicillin and Amoxycillin respectively. Reversibility tests suggest that the electrode reactions are probably reversible and they are one electron transfer processes. The Co(II) complex of Cephalexin was compared with that of Ni(II) for the analysis and found to be inferior. A satisfactory method based on the formation of the 2,5-diketopiperazine derivatives of Amoxycillin was developed.

The methods devised were tested on pharmaceutical products (capsules) and the results compared with pharmacopoeia methods. Good agreement was obtained.

MASTER OF SCIENCE DEGREE

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خلاصة الرسالة

إسم الطالب : سامي يس شيخ الدين

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تاريخ الشهادة : يناير ١٩٩١م .

تصف هذه الأطروحة التحليل البولاروجرافي لكل من البنسلين والسيفالوسبورين .ولقد تم تطوير الطرق الكمية لتحليل كل من الأمبسلين ، الأموكسسلين و السيفاليكسين، وتعتمد هذه الطرق على تكوين معقدات كل من النيكل الثنائي والكوبلت الثنائي مع المضادات الحيوية أو نواتج التفكك وبالأخص مشتقات داي كيتوبايبرازين.

ولقد تم دراسة الظروف المثالية لأنظمة معقدات نيكل ثنائي الأمبسلين والأموكسيسلين من حيث درجة الحموضة ، ومثبت الحموضة ، والمحلول الإلكتروليتي المساعد ودرجة الحرارة وتأثير الزمن .

وقد كان مدي المنحنيات القياسية تحت الظروف المثالية كما يلي : ـ

٥,٠٠ X ١٠ $^{-٧}$ - ١,٠٠ X ١٠ $^{-٥}$ م للأمبسلين

٤,٠٠ X ١٠ $^{-٧}$ - ٢,٠٠ X ١٠ $^{-٥}$ م للأموكسسلين

تجارب إنعكاسية التفاعل القطبي أوضحت أن تفاعلات الأقطاب يمكن أن تكون إنعكاسية وتحتوي على إنتقال إلكترون واحد .

كما تم مقارنة كل من معقدات الكوبلت ومقدات النيكل ووجد الأخير هو الأفضل للتحليل . كما أنه تم تطوير طريقة مناسبة للتحليل بواسطة تكوين مشتقات ٢ . ٥ ثنائي كيتوبايبرازين للأموكسيسلين .

لقد تم تجربة الطرق على نواتج بعض المواد الصيدلية وقورنت النتائج مع طرق الدلائل الصيدلية ، ووجد تطابق جيد بينهما .

درجة الماجستير في العلوم

جامعة الملك فهد للبترول والمعادن

الظهران ، المملكه العربية السعودية

التاريخ يناير ١٩٩١م

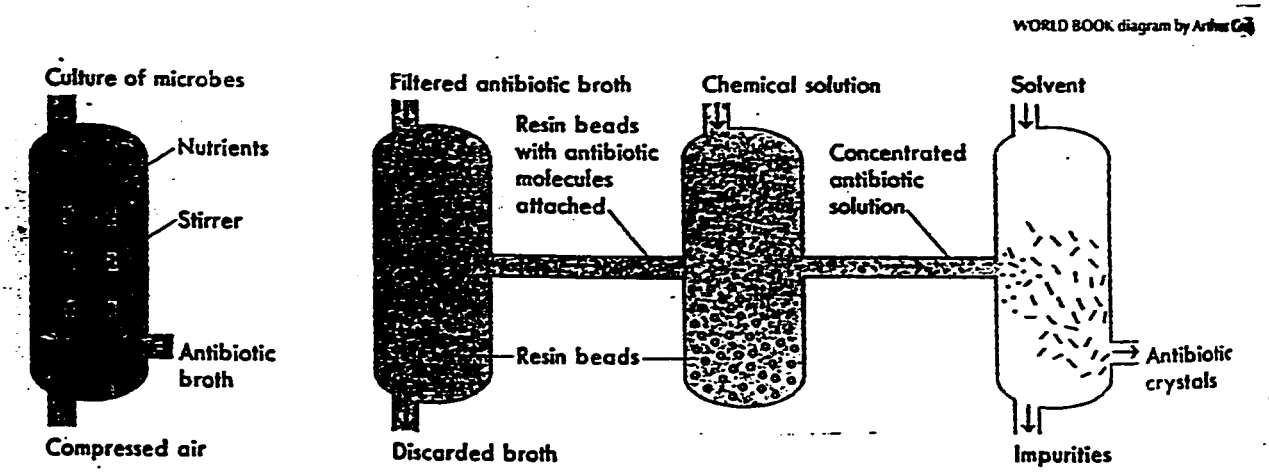
CHAPTER ONE

INTRODUCTION

I) General

According to the original definition by S.A. Wakman [1], antibiotics are substances which are produced by microorganisms exhibiting either an inhibitory or destructive effect on other microorganisms, usually bacteria. Antibiotics which are growth inhibiting are called bacteriostatic, example, tetracyclines, while those which are cell destroying are called bactercidal, Example penicillins and Cephalosporins.The bacterial effects result from (1) inhibition of cell wall synthesis, e.g. pencilins and cephalosponins, (2) inhibition of protein synthesis; e.g., by chloromphenicol and streptomycin; or (3) interference with the function of the cytoplasmic membrance, e.g., by polymyxins. The inhibitory action on a particular biosynthetic reaction by an antibiotic takes place by specific, covalent or non-covalent binding and results in the inactivation of substrate enzyme or factor. Usually antibiotics are classified also as being Gram positive, Gram negative or as broad - spectrum i.e. showing both Gram positive and Gram negative effects. This classification is based on whether a certain species of bacteria retain, the Gram stain after treatment with decolorizing agent. The Gram stain is one of the methyl violet stains, e.g., pararosaniline stain.

Antibiotics today are among the most efficient weapons in the armoury of the physician in his fight against infections. Therefore they are used widely and constitute the largest class of medicaments with respect to turnover value.



Fermentation causes a culture of microbes to grow rapidly and produce antibiotic broth. Stirring, air, and nutrients help stimulate growth.

Purification. Antibiotic broth is filtered into a tank containing substances called *resin beads*. Antibiotic molecules separate from impure elements in the broth and attach onto the resin beads. The remaining broth is flushed from the tank. A chemical solution is added to force the beads to the bottom of the tank, and the concentrated antibiotic solution flows into another tank. There, a solvent causes the solution to form pure antibiotic crystals.

(The world book encyclopedia,"A",1989,page-552.)

Fig.1: The process used mostly in the manufacturing of Antibiotics

Today, antibiotics are also used in veterinary medicine and as additives to animal feeds. In the past they were used in addition as plant protection agents and as food preservatives. Though antibiotics is the name generally given to all the compounds used to treat human infections, there is a distinction between compounds which are laboratory synthetized (e.g. sulfonomides) and are known as antibacterials, and those compounds which are derived from or produced by living organisms. The latter are called antibiotics.

Antibiotics are produced using different processes. The process used by most manufacturers involves fermentation and is shown schematically in (Fig. 1). Fermentation is followed by collection of antibiotic molecules on resin beads to separate them from fermentation broth. The antibiotic thus separated is released from the resin, concentrated and precipitated as explained in the caption to the figure.

II) ANALYSIS OF ANTIBIOTICS.

The two groups of methods used in quality control of antibiotics are (1) biological analysis and (2) chemical analysis.

In biological analysis, tests are devised to demonstrate the level of effectiveness of added antimicrobial agent. This is done by running series of standards with the substances under test on agar test plates. Thus the effectiveness of the antibiotic is tested in relation to a selected specified organism. Such tests are semi quantitative at best.

SEPARATION	IDENTIFICATION	MEASUREMENT
Instrumental separation by Discriminating detection;	**Physical Methods;**	**Dependent on Physical properties;**
NMR (by chemical shift dispersion), selective potentiometry MS (by single or multiple ion detection.	NMR, IR/Raman MS and GC-MS,UV comparison with properties of a standard.	Fluorescence Thermal analysis, Microscopy, UV absorbance Atomic absorption Nmr.
Physical Separation;	**Chemical methods;**	**Dependent on chemical properties;**
phase extraction, chemical separation chromatography (lc,tlc,gc).	functional group analysis spot tests elemental analysis, atomic absorption.	polarography, potentiometric titration, Radio-Chemistry, GC-MS.

Table 1.1 Summary of the methods of chemical analysis.

The chemical analysis of antibiotics is based on one or more of the operations (1) Separation (2) identification, and (3) measurement. These are summarized in table (1.1).

Penicillins -

Historically, Penicillin is synonymous with antibiotic.

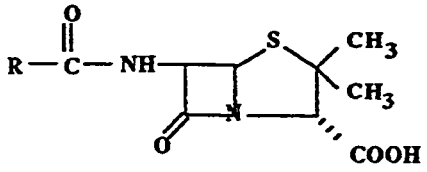


Fig. 2

Penicillin has the structure shown in Fig.2. It acts on bacteria by blocking the final step in the assembling of the nucopeptide of the cell wall; consequently, pencillin compounds (with the exception of ampicillin) are more effective against Gram-positive than Gram-negative bacteria.

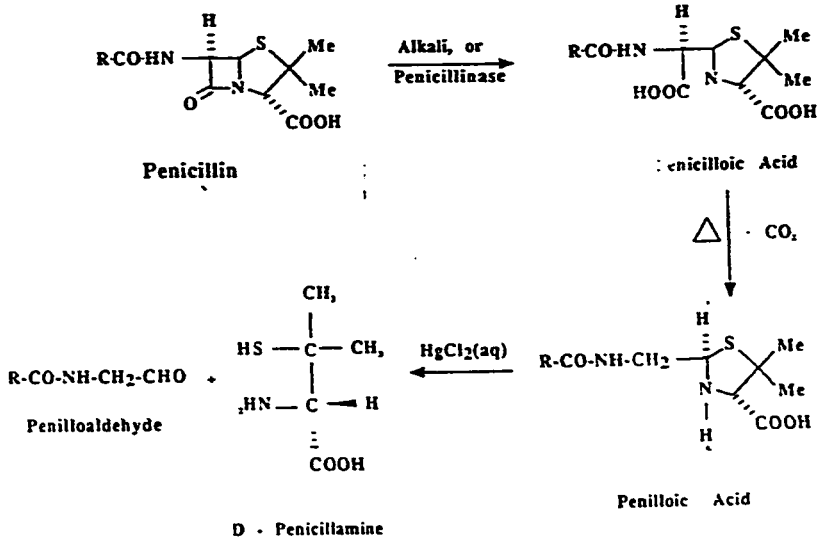
All members of the pencillin family of antibiotics contain a common nucleus consisting of a four-membered β - lactam ring fused to a five-membered sulfur - containing thiazolidine ring. A substituted benzomide bonded to the β - lactam ring completes the common features of the structure. It is this substituted benzamide group that differentiates membranes of the penicillin family.

The following reaction schemes below indicate the main degradation

reactions and rearrangements undergone by penicillins [1].

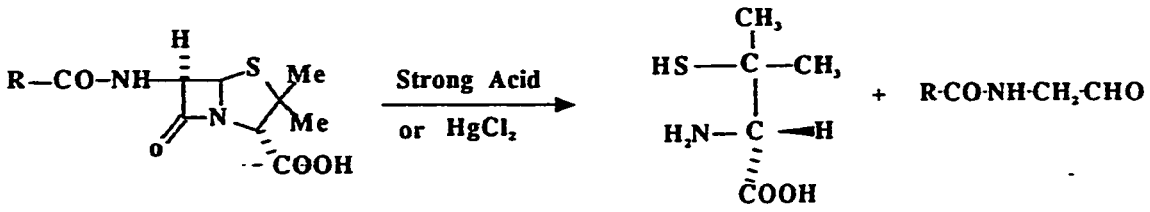
(1) Effect of alkali, Pencillinase and Mercury (II) chloride.

Treatment of pencillins with alkali like sodium hydroxide (excess) or the enzyme penicillinase cause opening of the β - lactam ring with the formation of penicilloic acids which undergo decarboxylation on heating to give the corresponding penicilloic acids. The latter are converted into D-penicillamine and the corresponding penilloaldehydes on treatment with equeous mercuric chloride.



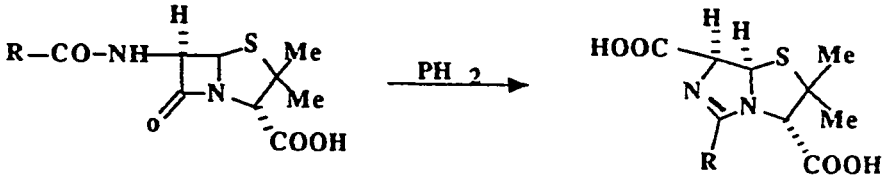
(2) Effect of Acids, Mercury (II) Chloride and Diazomethane.

Pencillins are split on treatment with hot strong acids or with aqueous merrcuric chloride to afford D-pencillamine (D- *beta-* mercaptovaline) and unstable penaldic acids, which readily decarboxylate to the corresponding penilloaldehydes,



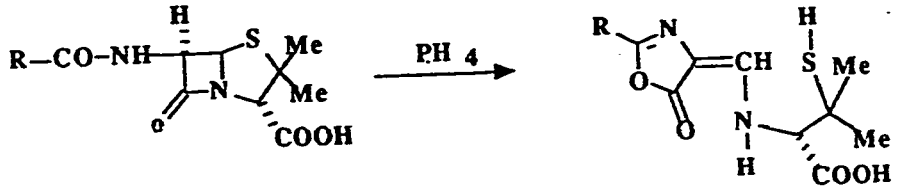
Penicillin **D-Penicillamine** **Penilloaldehyde**

Upon treatment with dilute acid (pH2) at room temperature, Penicillins undergo rearrangement to the well-crystallized penillic acids.



Penicillin **Penillic Acid**

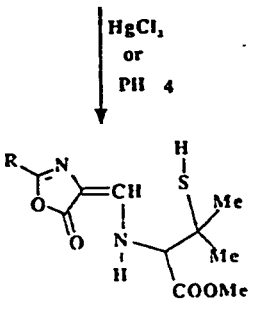
Under slightly acid conditions the carbonyl group of the side-chain of pencillins reacts with the β -lactam to give a pencillenic acid



Penicillin **Penicillenic Acid**

The mild esterification of penicillins with diazomethane in ether gives the corresponding methyl esters which rearrange in ether in the presence of mercuric chloride or hydrogen ion to methyl pencillenate.

Methyl penicillin

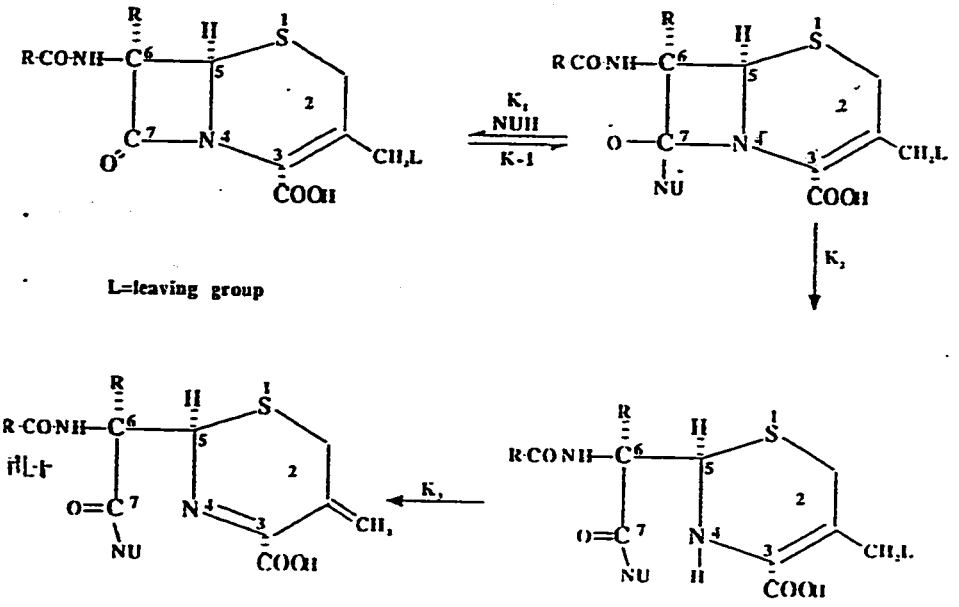


Methyl penicillenate

CEPHLOSPORINS

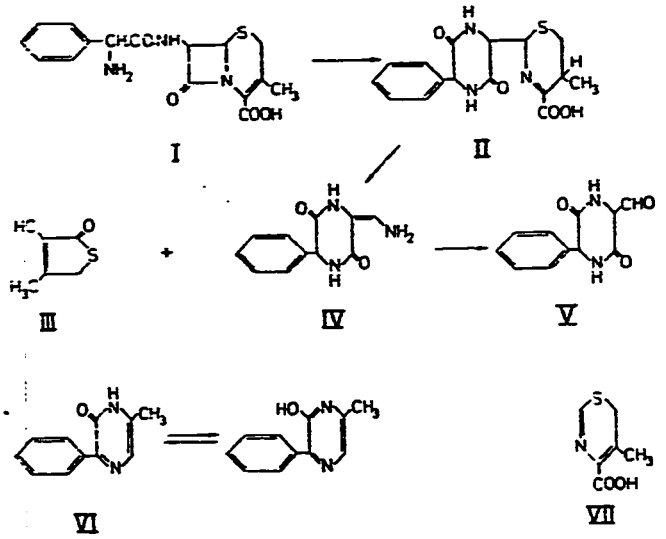
Cephalosporins can formaly be regarded as derivatives of 7-aminocephalosporanic acid (7-ACA) [2].

Cephalosporins differ from penicillins in having a thiazine ring replacing the thiazolidine ring, and also by characteristically having a potential leaving group at "C-3". For example, acetate or pyridine which are expelled during reactions of nucleophiles with β -lactams. The reaction sequence is as follows:



Studies have been made of the degradation reactions of cephalosporins with an unsubstituted 3-methyl group .For example cephalexin (I) at pH 7 and 35 °C, undergoes a 98% loss of the primary amine indicating essentially complete degradation of the cephalosporin via the diketopiperazine derivative (II) [63]. Also, cephalosporins like cephalexin, when first treated with 1M NaOH solution

at room temperature and then the pH adjusted to 5.0 by buffer and the resulting solution taken to 100 °C yields a fluorescent product (VI). [63,64,65]. This has been shown to be 2-hydroxy-3-phenylpyrazine [63,64,65].



(III) NEED OF THE STUDY

In spite of the discovery of various other antibiotics, pencillin and cephalosporins still continue to be the most commonly used drugs against bacterial infection.

In recent years fake pharmaceuticals have started showing up in hospitals and pharmacies all around the world, especially in poor third word countries [3].

It is said that drug piracy has hit the poorest nations like a plague. This is because they are ill-equipped to fight back, lacking the means of quality control and regulation. Antibiotics in general are among these fake pharmaceuticals. Though there are many methods available in the pharmacopocias and the chemical literature for the analysis of penicillins and cephalosporins , most of these methods are generally non stoichiometric and / or of very limited selectivity. Both characteristics make the methods vulberable to changes in the matrix (diluents) of the antibiotics and therefore are undesirable characteristics. In addition many pharmacopoeia methods are wasteful of material, based on ill-defined chemical reactions, and cumbersome, hence the need to improve procedures in chemical analysis.

In this thesis, some polarographic methods for the determination of selected penicillins and cephalosporins in pharmaceutical formulations are examined in detail.One assay is based on the differential pulse polarographic technique using prewaves of Ni(II) and Co(II) complexes formed with the penicillin and cephalosporin antibiotics.The drugs studied using this technique are Ampicillin trihydrate,Amoxycillin trihydrate and cephalexin. Other studies were conducted using Cu(II) and Zn(II) complexes. Another assay is based on the conversion to the appropriate diketopiperazine derivative; this procedure was developed only for Amoxycillin as it had already been applied to the other two antibiotics .

CHAPTER TWO

LITERATURE REVIEW

2.1. General Introduction

The area of antibiotic research has experienced an explosive like world wide development. Indeed, this area has become the most important in the whole field of pharmaceutical research. As a consequence, an almost incredible amount of new knowledge on antibiotics involving their Chemistry, Biochemistry, Pharmacy and medical usage has been published.

Quality control during the manufacturing and subsequent storage of drugs is currently considered to be of great medical importance. There are two broad approaches to quality control. One involves biological techniques and the other chemical techniques.

Since the work described in this thesis is chemical in nature, only chemical analysis will be surveyed. This can be done conveniently under the heading set out below.

1. Titrimetry (including all forms of end-point detection).
2. Spectrophotometry
3. Electrochemistry
4. Chromatography
5. Other qualitative and quantitative techniques.

2.2. Titrimetry

Several titrimetric procedures has been described, some incorporated in the pharmacopolia methods. Generally end-point detection is achieved using chemical indicators but occassionally potentiometric end-point detection is suggested and can have advantages where chemical indication would be obscured by sample matrix or in the presence of precipitates suspended in solution.

Penicillin is inert to iodine solution while penicilloic acid produced by the action of alkali from the parent, consumes 6-9 equivalent of iodine depending on the reaction conditions used. This observation was first reported [8] in 1946. Wild, [9], improved the reliability of the procedure of Alicino [8] and this conditions were incorporated in the pharmacopolia method which were used in the present work. An alternative to this procedure involves treatment of the penicillin with ICL in concentrated HCL titration of the I_2 produced with KIO_3 has been recomended [10,11,12], since publication of the pharmacopoeia procedure. An alternative oxidant, $K_2Cr_2O_7$ has been suggested [13] ; The finish involved titration of I_2 with $Na_2S_2O_3$. A variant of this previously described procedure has recently been published for cephalosporins [14] and an iodometric titration using electro-generated iodine under flow through condition has been described recently for the penicillins [15].

Mercury(II), has been used as titrant with success in the analysis of the penicillins and cephalosporins. The N-acylated thiazolidene carboxylic acid in $\beta-$ lactam binding formed in the course of alkaline hydrolysis of penicillins,

reacts selectively with Hg(II) under controlled conditions while some penicillins react directly with Hg(II) [16,17]. This enables degradation products to be determined in certain situations [17]. For penicillins the corresponding penicilloic acid can be titrated directly with Hg(II) but for amplicillin and amoxycillin it is desirable to acytilate the amino group group before titration [18]. Various modifications such as the replacement of pyridine by acetate-buffer [19] and potentiometric end point detection [21] has been described. In addition minor modification to the European pharmacopoeia method based on Hg(II) titration has been described [20]. The Hg(II) method has been discussed recently in relation to potentiometric end point detection [22]. Modifications to enable cephalosporin to be identified potentiometrically with Hg(II) has recently been described [23].

In addition to the major classical methods described above with their modification and improvement several other methods have been suggested. An interesting method for penicillins involves treatment of the sample with potassium plumbite in excess. The plumbite reacts with the thiazine ring to produce "Pbs" and the excess "Pb" is titrated with EDTA to a potetiometric end-point using a "Pb" selective electrode [24]. Selected cephalosporins [25] and penicillins [26] have been determined by titration of their solution in acetonitrile with NaOH. 1,3-dibromo-5-5 dimethylhydantion has been recomended as a brominating reagent in the determination of penicillins. Methyl red in ethanol can be used as a visual indicator or for increased sensitivity spectrophotometric titration may be used [28]. Selected penicillins such as Ampicillin and Amoxycillin can be titrated with $CuSO_4$ solution after hydrolysis of the penicillin

with *NaOH*. The titration is conducted potentiometrically in a solution buffered at pH 6.2 or 11.3 using Cu(II) sensitive electrode for potentiometric end-point [27].

2.3. Spectrophotometry

Several spectrometric procedures have been described for the determination of penicillin and cephalosporia drugs; some of these are incorporated in the pharmacopoeia methods.

2.3.1. Hydroxylomine based assay.

Penicillin reacts rapidly with hydroxylamine to give a hydroxamic acid, which forms a purple complex with ferric ion, and the concentration of this can be determined colorimetrically. Such a procedure was first reported [29], in 1946. Ford [30], modified the procedure of Staab et al [29], to include the use of penicillinase-inactivated blanks to eliminate the effect of interfering substances, and this modified procedure was incorporated in the pharmacopoeia method which was used in the present work. Details of the hydroxylamine assay for penicillin and its derivatives have been reported [31], For certifaction under the FDA. Also an automated dual channel hydroxylamine assay for formulated penicillin products has been reported for use as an official method for the Food and Drug Administration [*FDA*] ,[32]: Comparable results have been obtained for the analysis of Ampicillin dosage forms from a hydroxylamine colorimetric method and a microbiological method with former being reported to be

significantly less complex [33]. The colorimetric determination of penicillins and related compounds using a nickle(II)-catalysed hydroxamic acid formation also has been described [34].

2.3.2. Copper(II) Based Assay

Penicillins can be converted into their corresponding penicillenic acids. The penicillinic acids reacts with copper(II) to form a complex that can be determined spectrophotometrically. This is the basis of the copper(II) assay and was first reported by stock [35]. This method is incorporated in the pharmacopoeia and was used as one of the standard methods in this work.

Several spectrophotometric procedures for selected penicillins and cephalosporins have been described [36,37,38,39], since the publication of the pharmacopoeia procedure. One of these procedures was a simple spectrophometric method for Amoxycillin based on $CuSO_4$ in $CO_3^{2-} - HCO_3^-$ buffer pH 10.0 [37]. Another method was based on Cu(II) catalyzed convertion of Ampicillin to the corresponding penicillenic acid derivative at pH 5.2 and 75°C [38]. Two spectrophotometric methods were compared for Amoxycillin based on (I) formation of copper complex and measurement of absorbance at 320 nm and (II) reaction with imidazole and $HgCl_2$ and absorbance measurement at 325 nm [6]. Recently, Issopoulos et al [39], described a spectrophotometric method for the determination of Cephalexine, Ampicillin and Amoxycillin using copper(II) acetate as a complexing agent and reported that the results obtained were slightly more accurate than those of BP and USP methods.

2.3.3. Imidazole-Mercury(II) Based Assay

Few spectrophotometric methods based on imidazole-mercury(II) reaction with cephalosporins and penicillins have been reported. Sengun et al [49], described a spectrophotometric method for the determination of cephalosporines based on the reaction with imidazole and $HgCl_2$ at $37°C$ and absorbance measurement at 325 or 345nm. Another paper described a modified mercury(II)-imidazole method for some seleted cephalosporins, (cephalexin & cephradine) [50]. An imidazole-mercury(II) based spectrophotometric method for Amoxycillin has been compared with a copper(II) based spectrophotometric method [36]. It was reported that the former give better results. Recently(1988) the imidazole-mercury(II) spectrophotometric assay has been incorporated in the pharmacopoeia [45].

2.3.4. Assay of Penicillins & Cephalosporins Based on a Molybdenum Blue formation

This assay is based on the formation of a blue solution of Mo(VI) or by the oxidation of an acidified solution of Mo(V). Several spectrophotometric methods have been described based on molybdate ion. Some of these involve reaction of cephalosporins [42] and penicillins [46] with Ammonium-Molybdate. Morelli et al, [46] improved the spectrophotometric method for Amoxycillin by working with concentrated sulphuric-acid. Spectrophotometric methods using Molybdophosphoric acid as an oxidizing agent [47] and ParaMolybdate anion in 1 M H_2SO_4[48] have also been reported.

2.3.5. Ammonium-Vanadate Based Assay

This assay is based on the formation of a blue solution due the reduction of Vanadium(V) to vanadium(IV) by the acid degradation products of penicillins and cephalosporins. Few papers have been reported. El-Sebai et al [41] used Ammonium Vanadate in concentrated H_2SO_4 medium for the spectrophotometric determination of penicillins. Morelli et al [43], also described a procedure for penicillin using Ammonium Vanadate under strong acidic condition. The solution mixture was heated at 180^0C for 15 minutes and absorbance measured at 760 nm.

2.3.6. Miscellaneous-Spectrophotometric Methods

Several other spectrophotometric methods for the quantitative determination of penicillins and/or cephalosporins have been reported in the literature.

Andras et al [40], reported that cephalosporins (cephalexin) and penicillins (Ampicillin and Amoxycillin) containing the $\alpha-$ Amino-acetamido group condense with dicabonyl compounds (glyoxal, methylglyoxal, etc) to form ketopyrazines which can be determined spectrophotometrically at 355nm in aqueous solutions. Two spectrophotometric methods was based on the condensation with 4- amino antipyrine in the presence of an alkaline oxidizing agent and measuring the absorbance of the red colored antipyrine dye at 500 nm. The second method involved the use of Folin's reagent and measuring the absorbance of the resulting blue color at 670 nm. A rapid method for the

determination of cephalosporins has been reported. This method was based on an immobilized enzyme reactor containing highly purified cephalosporinase covalently bonded to activated glass beads. Detection was made sequentially by subtractive spectrophotometry in an automated flow injection system [44]. Penicillins were determined by spectrophotometry based on reaction with 1, 2, 4 - triazole and $HgCl_2$ at pH 9.0 and the absorbance measurement at 246-329nm [51]. However this method was reported to be inconvenient for Ampicillin and Amoxycillin. Ninhydrin in citrate buffer (pH 5.0) containing $SnCl_2$ was proposed as a new reagent for the spectrophotometric determination of penicillins. [52]. Sastry et al, [53], described spectrophotometric method for the determination of penicillins and cephalosporins based on their reactions with chloranillic acid, forming colored complex in dioxane and dioxane-DMF media; the absorbance was measured at 520 nm. Spectrophotometric procedures for cephalexin in its dosage form based on its reaction with 1,4 - dinitrobenzene in alkaline medium and absorbance measurement at 390 nm [54], and 1- flouro-2, 4-dinitroaniline and measured of absorbance of the resulting N-substituted 2, 4-dinitoaniline derivatives at 385nm [55], have been reported. Ampicillin was estimated in dosage forms spectrophotometrically with the use of Na 3, 4-naphthaquinone -1-sulphonate. The pH of the solution was adjusted to 8.5; heated at 60°C and absorbance measured at 475nm [56]. Recently the use of derivative spectrophotometry for the determination of penicillins [61] and cephalosporins [57,59,60] has been reported.

2.4. Electrochemistry Methods.

Several electrochemical methods have been used in quantitative analysis of penicillins,cephalosporin and their degradation products. One of the most used techniques appears to be polarography. This is because polarography is a very selective electrochemical technique and offers a means of direct investigation of drugs and their degradation products in biological and pharmaceutical formulations without separation.

2.4.1. Polarographic Methods.

Several methods based on polarographic techniques for the analysis of penicillins and cephalosporins has been reported in the literature. Squella et al, [62] ,described a D.C. polarographic technique for the determination of Ampicillin after acidic hydrolysis and looking at the half wave potential at -0.91V vs SCE. Several differential pulse polarographic (D.P.P.) techniques for the determination of some cephalosporins [63,65,68,70] and some penicillins [64,72] have been reported. Fogg and Fayad, [63] ,studied several cephalosporins using D.P.P.. Cephalosporins with unsubstituted 3-methyl groups (E.g cephalexin) which donot give D.P.P. peaks were studied using only degradation products , while those with the 3-methyl group substituted were studied directly. They also reported a D.P.P. study of the degradation products of cephalexin based on the determination of hydrogen sulphide liberated. [65]. A D.P.P. determination of some cephalosporins based on the study of a distinctive wave at -1.25 to 1.30V vs Ag/AgCL at pH(1-2) has been described [69]. Hernandez et

al, [70] ,described a quantitative D.P.P. assay for the determination of cephalexin ; it was based on the catalytic pre-wave of Nickel(II) at -0.72V vs Ag/AgCL. The degradation products of Ampicillin in pH 2.5 buffer at 37°C or 80°C have been studied using D.P.P. [64]. Derivative polarography has also been used for the determination of Amoxycillin . The technique was based on the diffusion current of the free Ni(II) ions. [72].

Two polarographic methods for the study of penicilloic acid have been reported. In one, the D.P.P. and direct current polarographic behaviour of penicilloic acid at pH 9.2 borate buffer and half-wave potential of -0.25V was discussed [66]. In the other ,a flow injection mehtod was described in which the penicilloate was detected at a dropping mercury flow-through detector mounted on a conventional polarographic capillary and applying a constant potential of + 0.04 vs SCE [67].

The polarographic behaviour and determination of some penicillins after bromometric oxidation has been reported [69]. Cathode-ray polarographic techniques for the study of degradation of selected penicillins, were described [71]. Solutions were heated in boiling water with buffer pH 1.0 to 10.0. The sweep amplitude and sweep rate were 1000 mV and $400mVS^{-1}$ respectively. Recently two direct current polarographic techniques for the the study of ampicillin complexes and their stability constants with Co(II) and Ni(II) ions [73] and Cu(II) ion [74] have been described.

2.4.2. Miscellaneous Electrochemical Methods.

Few electrochemical techniques other than polarography have been reported for the determination of penicillins and cephalosporins. Olof et al, [75] ,presented principles for the construction of an autoclave enzyme electrode and gave some characterstics of penicillin electrodes constructed according to the principle.

A cathodic stripping voltammetric study of penicillins after conversion to penicilloic acid by alkaline hydrolysis has been described [76]. Analysis was carried out at a hanging mercury electrode at - 0.10V vs SCE in a pH 4.6 buffer containing excess Cu(II).Some cephalosporins were determined using linear scan voltammetry in which the anodic wave at +0.80V vs Ag/AgCL at pH 7.3 was studied [68]. Recently a PVC ion selective electrode employing Benzylacetylammoniumpenicillin as a sensor was prepared, characterized and applied to analysis of penicillins and cephalosporins [77].

2.5. CHROMATOGRAPHY

Chromatography is essentially a series of techniques for the separation of mixtures of chemical substances so that they can be identified. Thus it becomes a branchof qualitative analysis . However, the most versatile forms, Gas chromatography (GLC and GSC) and Liquid-Liquid chromatography (HPLC) are provided with detectors which give signals proportional to the concentrations of separated components and therefore they can be used for quantitative purposes. In the summary which follows ,the two kinds of chromatography are concedered separately.

2.5.1. Separation methods (Qualitative).

Thin-layer chromatography (TLC) is one of the most useful techniques in the qualitative analysis of penicillins and sephalosporins. Wang et al [92], described a simple and rapid thin-layer polyamide chramatographic method for the separation of eight penicillins. Their method showed good sensitivity, separability and reproducibility. Results were reported of a TLC study of eighteen penicillins on silica gel and silanized silica gel, using thirty-five mobile phases. The silanized silica gel allowed better separations than silica gel [97]. The use of $\pi-$ acceptors as spray reagents for the detection of penicillins on thin layer plates has been described [98].

TLC continues to be an important technique in the analysis of cephalasporins. Several TLC visualization reagents have been characterized [95]. Cephalosporins have been detected with chloroplatinic acid on thin-layer chromotoplates [95]. Chemically modified TLC plates have been used for separation of cephalosporin C from its Cofator [96]. TLC has been shown to be quite useful in determining initial HPLC operating conditions [94].

2.5.2.Separation & Determination (Qualitative & Quantitative Analysis)

High performance liquid chromatography (HPLC) has increasingly been applied to the analysis of penicillins and cephalosporins. A reversed phase HPLC method was used for the analysis of some penicillins and cephalosporins of closely related structures, [78]. The influence of eluent, pH and NaCl concentration on the resolution of Ampicillin was discussed. The method was

reported to be highly sensitive. Cephalexin determination using reversed phase HPLC has also been reported [79]. Another HPLC method described the determination of penicillins based on the separation of the drugs by using methanol as an organic mobile phase-modifier and a reversed phase C_{18} column followed by post column alkaline degradation with NaOH, $HgCl_2$ and Na_2EDTA at room temprature [80]. The resulting products of each penicillin were detected at 290 nm. A review with 45 references for HPLC analysis of penicillins in biological fluids and pharmaceutical preparations has been reported [81]. Haginaka et al, [82] developed a HPLC technique for the determination of some penicillins by post column degeradation with sodium hypochlorite. The degradation products of each penicillin was detected at 280 - 284 nm. The proposed method permitted detection of 100ng/ml of penicillins from a 50 *micro* l injection. Penicillins were also analysed by HPLC-photolysis-electrochemical detection in which the penicillins were first separated by conventional reversed-phase HPLC. Then each separated penicillin was photolytically degraded to stable anionic species which were subsequently conevyed to an on-line electochemical detector for qualitative and quantitative determination [83]. Cephalosporins and their decomposition products after HPLC separation were detected by (I) Flourescence detection at 485 nm following derivatization with flourescamine, (II) Direct electrochemical detection at +1.1 V (vs AglAgCl) at a vitreous-carbon electrode and (III) indirect electrochemical detection of generated Bromine, [84]. A method for the quantitative determination and study of stability of Cephalexin using HPLC has been described [85]. HPLC technique for the separation and identification of

penicillins with C_4 to C_{10} aliphatic side chains has been reported [86]. Marinal and Nada, [87] , compared methods for cephalexin based on HPLC with microbiological techniques. They reported that HPLC has some advantages in being more specific, faster, simpler and more accurate and therefore they recommended it for qualitative and quantitative determination of cephalexin in samples. Recently penicillins and cephalosporins were determined by an HPLC method based on N-pyrrolyl derivatives [88]. Also penicillins have been determined by liquid chromatography by post-column alkaline degradations using a hollow fibre membrane reactor [89].

Two papers have been found in the litature describing the use of gas chromatography (GC) in the detection and the determination of penicillins and cephalosporins [90,91]

The first one describes the gas chromatographic and GC/MS analysis of ampicillin [91], while the second one describes the determination of some penicillins and cephalosporins by GC in which flame photometric detector (FPD) has been used. It has been showed that the detector was selective and sensitive to S-containing compounds [90].

2.6. Other Qualitative And Quantitative Techniques.

Penicillins and Cephalosporins have been idetified using UV, IR, H^1NMR

spectroscopy . Wilson et al [106] , reported the NMR spectra of various common available Penicillins and Cephalosporins . They discussed the general spectral features, as well as specific resonances and their application in the identificaiton of these antibiotics. IR spectroscopy have been used for the identification of selected Penicillins . The difference in absorption spectra in the region of 3500-3200 and 1500-650 cm^{-1} were used in the identification [107]. A review has been presented on the UV, IR, H^1NMR of 16 commercial drugs including Cephalosporins [108]. Pyrolsis Mass spectroscopy has also been used in the characterization of Penicillins and Cephalosporins [109].

Two other quantitative techniques , namely Electrophoresis [99,100,101] and Flourimetry [102,103,104,105] have been used in the determination of Penicillins and Cephalosporins.

CHAPTER 3

PHARMACOPOEIA METHODS

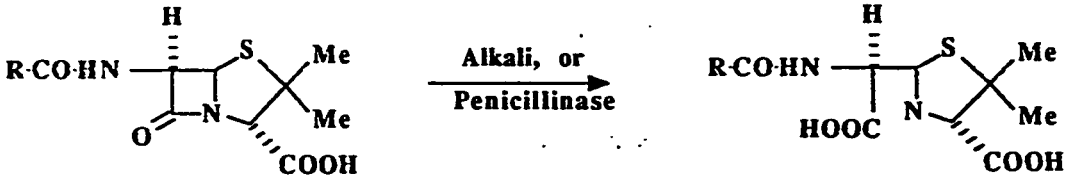
Pharmacopoeia is a combination of two words: Pharmaco and Poeein a German word meaning to make. The Pharmacopoeia is an authoritative treatise on drugs and their preparations; a book containing a list of products used in medicine, with descriptions, chemical tests for determining identity and purity, and formulas for certain mixtures of these substances. It generally contains also a statement of average dosage. There are several pharmacopeia. The best known are the British Pharmacopeia (BP) and the United States Pharmacopeia (USP). The British Pharmacopeia is a publication of the General Medical Council, describing and establishing standards for medicines, preparations, materials and articles used in the practice of medicine, surgery, or midwifery. The United States Pharmacopeia is a legally recognized compendium of standards for drugs published by the United States Pharmacopeial Convention Inc., and it includes assays and tests for the determination of strength, quality and purity.

In this thesis, selected assays taken from the above mentioned pharmacopoeia are used as reference or bench mark methods against which assays based on the polarographic technique is compared. Of particular interest are the methods based (1) on iodometry, (2) hydroxylamine iron(III) and (3) copper (II) complex formation. Method (1) is a titrimetric based procedure while (2) and (3) are spectrophotometric determinations.

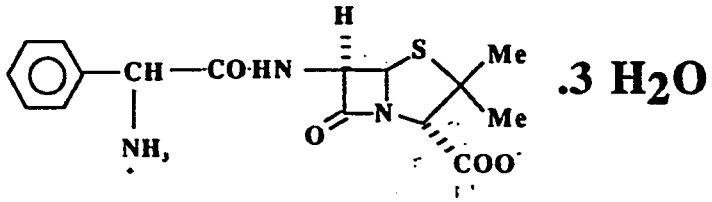
Experimental

Iodometric Assay [5].

In this assay the penicillin (I) is converted to penicilloic acid (II) by the action of an alkali. The latter absorbs iodine as a result of a complex oxidation reaction. These reactions are not clearly defined as will be discussed later in chapter five.



I II



III

Name of penicillin drug used - Ampicillin trihydrate (III).

Reagents Used

(1) Sodium thiosulphate (1.57570 gms of hydrous sodium thiosulphate Na2S2O3, 0.12483 g of sodium carbonate (Na2CO3) and 3 drops of chloroform per liter in water.

(2) 1.0N Sodium hydroxide (4 gm dissolved in 100 ml of water)

(3) 1.2 N Hydrochloric acid (10.68 ml of conc.HCL in 100 ml aqueous solution.

(4) 0.01 N Iodine solution was prepared from aqueous 1.0 N iodine in potassium iodide.

(5) Starch iodide solution - freshly prepared by pouring a paste(made up of 1g of soluble starch in a little of water) in 100 ml of boiling water with constant stirring and then allowed to boil for 1 minute. After cooling 2.3 g of Potassium iodide were added.

Standardization of Sodium Thiosulphate

Procedure: 25.00 ml of 0.01004 N KIO_3 are transferred by pipet to a 250 ml titration flask and then 1 gm of KI is added and dissolved completely. This is followed by the addition of 20 ml of 1.4 v/v H_2SO_4 and the solution is then titrated with sodium thiosulphate. Near the end point 5 ml of freshly prepared starch solution is added as an indicator. The color change is dark blue to colorless at the end point. The results of the titration are shown in Table 3.1.

No. Trial	Vol. of $Na_2S_2O_3$
1	25.70 ml
2	25.65 ml
3	25.70 ml
Average	25.69 ml.

Table 3.1. Results for the standardization of $Na_2S_2O_3$.

Calculations:

$m_1V_1 = m_2V_2$

$m_2 = (m_1V_{1)} - V_2$

$m_2 = (0.01004 \times 25)/25.69$

m_2 = Normality of sodium thiosulphate used = 0.009770N ±0.00000539

Preparation of Working Standard and Sample Solutions

(1) Wt of pure Ampicillin trihydrate taken in 200 ml of water = 0.24817g.

(2) Weight of Ampicillin trihydrate capsules taken in 200 mls of water = 0.25000 g. (Note the solution was then filtered to remove insoluble fillers).

Procedure for the Iodometric Assay

(*a*) *For the Standard*

1. 2.00 ml of the working standard were transferred to a glass stoppered erlenmeyer flask.

2. 2.00 ml of 1.0 N Sodium hydroxide were then added and allowed to stand at room temperature for 15 minutes.

3. 2.0 ml of 1.2N hydrochloric acid were then added and this was followed by

4. Addition of 10.00 ml of 0.01 N Iodine solution. The Erlenmeyer flask was then stopped and allowed to stand at room temperature for 15 minutes. Then the excess iodine was titrated using 0.00976N sodium thiosulphate.

Towards the end of the titration, one drop of starch solution (freshly prepared) was added and the titration was continued till the blue color of the starch iodine complex is discharged. The same procedure was repeated three times. Results are given in table (3.2 a).

(*b*) *Sample Solution: Ampicillin trihydrate capsules* .

1. 2 ml of the sample solution were transferred to the glass stoppered erlenmeyer flask.

2. Then this solution was treated in the same way as the standard solution proceeding through steps 2 to 4 in (a). Table (3.2 b).

(*c*) *Blank Determination*:

2.00 ml of the sample and the working standard solutions were transferred to separate glass stoppered erlenmeyer flasks. 10.00 ml of 0.01 N iodine solution were then added to each flask and the contents immediately titrated with thiosulphate to the starch end point. (See Table 3.2 a and b).

Calculations:

Vol.of $S_2O_3^{-2}$ consumed by standard = 10.08 ml - 4.20 ml

= 5.88 ml

(a).

Pure Ampicillin trihydrate (Standard)		Blank (standard).	
No. of trial	Vol. of $Na_2S_2O_3(ml)$.	No. trial	vol. of $Na_2S_2O_3(ml)$.
1 2 3	4.15 4.20 4.25	1 2 3	10.10 10.05 10.10
Average SD	4.20 ml 0.04	Average SD	10.08 ml 0.02

(b).

Ampicillin trihydrate (capsules)		Blank capsules.	
No. of trial	Vol. of $Na_2S_2O_3(ml)$.	No. trial	vol. of $Na_2S_2O_3(ml)$.
1 2 3	4.25 4.20 4.25	1 2 3	10.10 10.10 10.10
Average SD	4.23 ml 0.02	Average SD	10.10 ml 0.00

Table 3.2. Titres for Ampicillin trihydrate (a) standard (b) capsules ,Iodometric assay.

Vol.of $S_2O_3^{-2}$ consumed by sample = 10.10 ml - 4.23 ml

= 5.87 ml

therefore

No. of m moles of $S_2O_3^{-2}$ consumed by standard = 0.00977 N × 5.90

= 0.05745 m moles and

No. of m moles of $S_2O_3^{-2}$ consumed by sample = 0.00977 N × 5.87*ml*

= 0.05734 m moles.

Then

# of m moles of I_2 consumed according to the equation:

$2S_2O_3^{-2} + I_2 \text{ -------> } S_4O_6^{-2} + I_2^-$

For standard = $(\frac{0.05745}{2})$ = 0.02873 m moles.

For sample i.e. Ampicillin trihydrate capsules

$= \frac{0.057096}{2}$ = 0.02867 m moles.

Also

# of m. moles of standard used

$= (\frac{0.24817}{403.46}) \times (\frac{1}{200}) \times 10^3 = 3.076 \times 10^{-3}$

# of moles of sample used

$= (\frac{0.2500}{403.46}) \times (\frac{1}{200}) \times 10^3 = 3.098 \times 10^{-3}$

therefore

$$\text{Molar ratio of } \frac{I_2}{STD} = \frac{0.02873}{3.0755 \times 10^{-3}} = 9.34$$

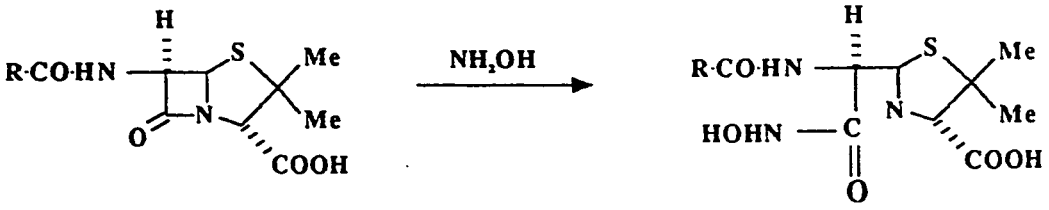
$$\text{Molar ratio of } \frac{I_2}{Sample} = \frac{0.02867}{3.098 \times 10^{-3}} = 9.25$$

therefore

$$\% \text{ of active substance} = \frac{9.25}{9.34} \times 100 = 99.0\ \% \pm 1.0$$

Hydroxylamine Assay [5].

The addition of hydroxylamine to an aqueous solution of the penicillin results in the formation of a hydroxamic acid derivative according to the equation [30].



Penicillin **Hydroxamic Acid**

The hydroxamic acid reacts with iron (III) to give a complex which has a maximum absorbance at 480 mm in water.

Name of drug used: Ampicillin Trihydrate (M-wt 403.46)

Reagents used:

1. Hydroxylamine hydrochloride solution (350 g/litre).

2. Buffer - 17 g of NaOH and 20.6 g of sodium acetate dissolved in water to 1 litre.

3. Neutral hydroxylamine - (1 vol each of hydroxylamine hydrochloride solution and the buffer were mixed and the pH was checked and adjusted to pH 7.0 0.1 by adding an additional amount of one of the components. To 1 vol. of the neutral solution, 8 volumes of water and 2 vol. of 95% ethanol were added. Note: This solution was prepared fresh each day.

4. Ferric Ammonium Sulphate 27.2 g of ferric ammonium sulphate dissolved in a mixture of 2.6 ml of concentrated- H_2SO_4 and sufficient distilled water to make 100 ml. This reagent could be used for a week when kept in a brown bottle.

Preparation of Working Standard and Sample

(a) Pure ampicillin trihydrate: 0.024895 gm were dissolved in distilled deionized water to make 200 ml.

(b) Ampicillin trihydrate capsules: 0.25023 g. were dissolved in a distilled deionized water to make 200 ml and filtered.

Procedure

(a) To 2.00 ml. of standard and sample solutions in separate container, and equal volumes of water were added and mixed.

(b) Then to each solution, 2.5 mls of neutral hydroxylamine reagent were added and allowed to reach for 5 minutes.

(c) 2.5 ml of Ferric Ammonium sulphate were added to each solution and mixed. After 3 minutes, the absorbances were determined using a spectronic 20 spectrophotometer set at the wave length of 480 mm, (Refer to TABLE 3.3).

(d) Note: A reagent blank was prepared by treating a volume of water in the same manner as the standard and sample.

Calculations

$$\frac{A_{sample}}{A_{std}} \times C_{std} = C_{sample}$$

Where

A_{sample} = Absorbance for sample i.e. Ampicillin trihydrate capsules

A_{std} = Absorbance for standard or pure Ampicillin trihydrate

Pure Ampicillin trihydrate.		Ampicillin trihydrate Capsules	
No. of trial	Absorbance	No. of trial	Absorbance
1	0.350	1	0.340
2	0.340	2	0.345
3	0.340	3	0.342
Average	0.343	Average	0.342
SD	0.004	SD	0.002

TABLE 3.3: Results for the Hydroxylamine assay for Ampicillin trihydrate.

C_{std} = Concentration of pure Ampicillin trihydrate

C_{sample} = Concentration of Ampicillin trihydrate capsules.

Therefore ,

$$C_{sample} = \frac{0.342}{0.343} \times 0.24895g/200ml. = 0.248224g/200ml.$$

And

$$\% \text{ of active subtance} = \frac{0.248224g/200ml}{0.25023g/200ml} \times 100 = 99.1\%$$

Using Copper(II) Sulphate Assay [4].

The conditions described here are such that the appropriate penicillenic acid is stabilized by the formation of a Cu(II) complex. This complex is determined spectrophotometrically, [35].

Name of drug - Ampicillin Trihydrate (M-wt = 403.46)

(a) Reagents Used.

Buffered Copper(II)Sulphate solution pH 5.2.

Preparation of the buffer-

1.52184 g of unhydrous disodium hydrogen phosphate were dissolved in 53.6 ml distilled deionized water. The pH of the solution was adjusted to 5.2, by

adding 2.1% W/V solution of citric acid. The solution was then transfered to a 100 ml volumetric flask and the level of the solution was brought to the mark using distilled deionized water. To 98.5 ml of the resulting solution , 1.5 ml of 0.393% W/V solution of Copper(II) Sulphate was added and the two solutions mixed.

(*b*) *Preparation of the working standard and sample solutions.*

Mass of Ampicillin Trihydrate pure taken = 0.2520 g/200 ml. Mass of Ampicillin trihydrate capsules taken = 0.2490 g/ 200 ml.

(*c*) *Procedure for the assay*

2 ml of the pure (standard) Ampicillin Trihydrate were diluted to a 100 ml with the buffered Copper(II)Sulphate (pH 5.2). 10 ml of the resulting solution were transfered to a stoppered test tube and heated in a water_bath at 75 degrees for 30 minutes. The solution was rapidly cooled to room temperature and volume adjusted to 10 ml with distilled deionized water. Then the extinction of the solution at 320 nm was measured using Varian Cary UV spectrophotometer model 2390 (FIG.3.1). and / or Lambda 5 UV spectrophotometer. The unheated buffered solution of the substance being examined was used in the reference cell. The assay was simultaneously carried out for the sample solution . According to Fig 3.1 , the results obtained using Varian Cary Model 2390 at 320 nm are as follows: Max. absorbance observed at 320 mm.

For standard ampicillin trihydrate = 0.6626

For Ampicillin trihydrate capsules = 0.7352

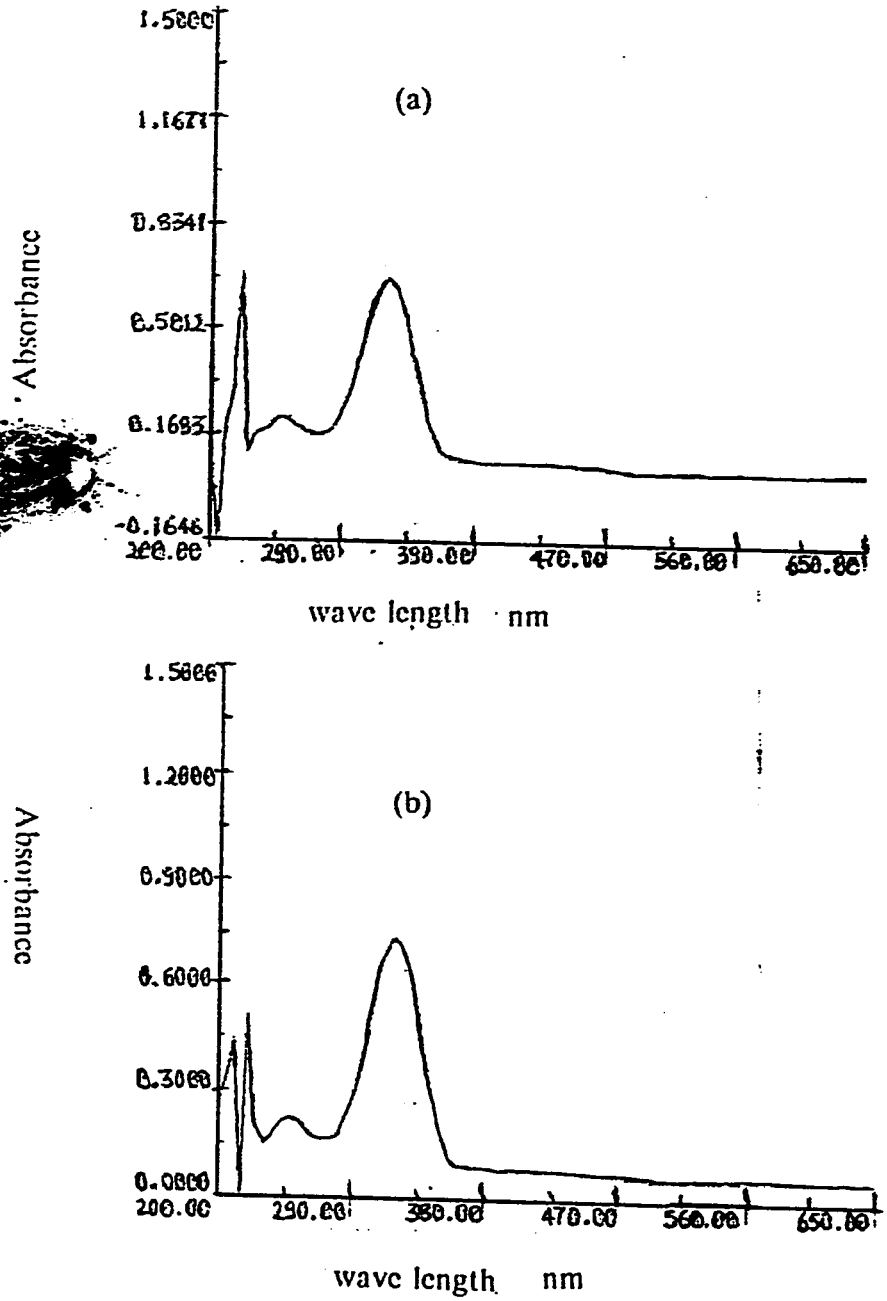


Fig. 3.1. Absorbance as a function of wavelength for (a) freshly prepared solutions of pure Ampicillin ; (b) Ampicillin capsules in the assay using Copper(II)sulphate.(Varian Cary Model 2390).

Calculations:

$$\frac{A_{sample}}{A_{std}} = \frac{C_{sample}}{C_{std}}$$

$$C_{sample} = \frac{A_{sample}}{A_{std}} \times \frac{C_{std} \times MM\ of\ unhydrous}{MM\ of\ hydrate}$$

therefore

$$C_{sample} = \frac{0.7352}{0.6626} \times 0.2520 \times \frac{349.40}{403.46} = 0.242g/200ml.$$

therefore

% of active substance = (0.242–0.2490) × 100 g / 200 ml.

= 97.26%

The copper (II) sulphate assay was repeated using the Lamda-5 UV-visible spectrophotometer.

Weights and corresponding absorbances for standard ampicillin trihydrate and ampicillin trihydrate capsules are presented in table 3.4. A calibration curve was produced from these results (Fig 3.2) to calculate the % of active substance.

From Fig 3.2 ,

Weight of active substance = 0.1030 g/100ml.

(a) Standard Ampicillin trihydrate.		
Solution #	Weight I g/100 ml	Absorbance
1 2 3	0.04860 0.09869 0.19271	0.196 0.488 1.020
SD	0.03071	SD 0.385

(b) Ampicillin trihydrate capsules.		
Solution #	Weight I g/100 ml	Absorbance
1	0.10338	0.515

TABLE 3.4: Masses and corresponding Absorbances for standards and capsules containing Ampicillin trihydrate for the Cu(II)-assay.

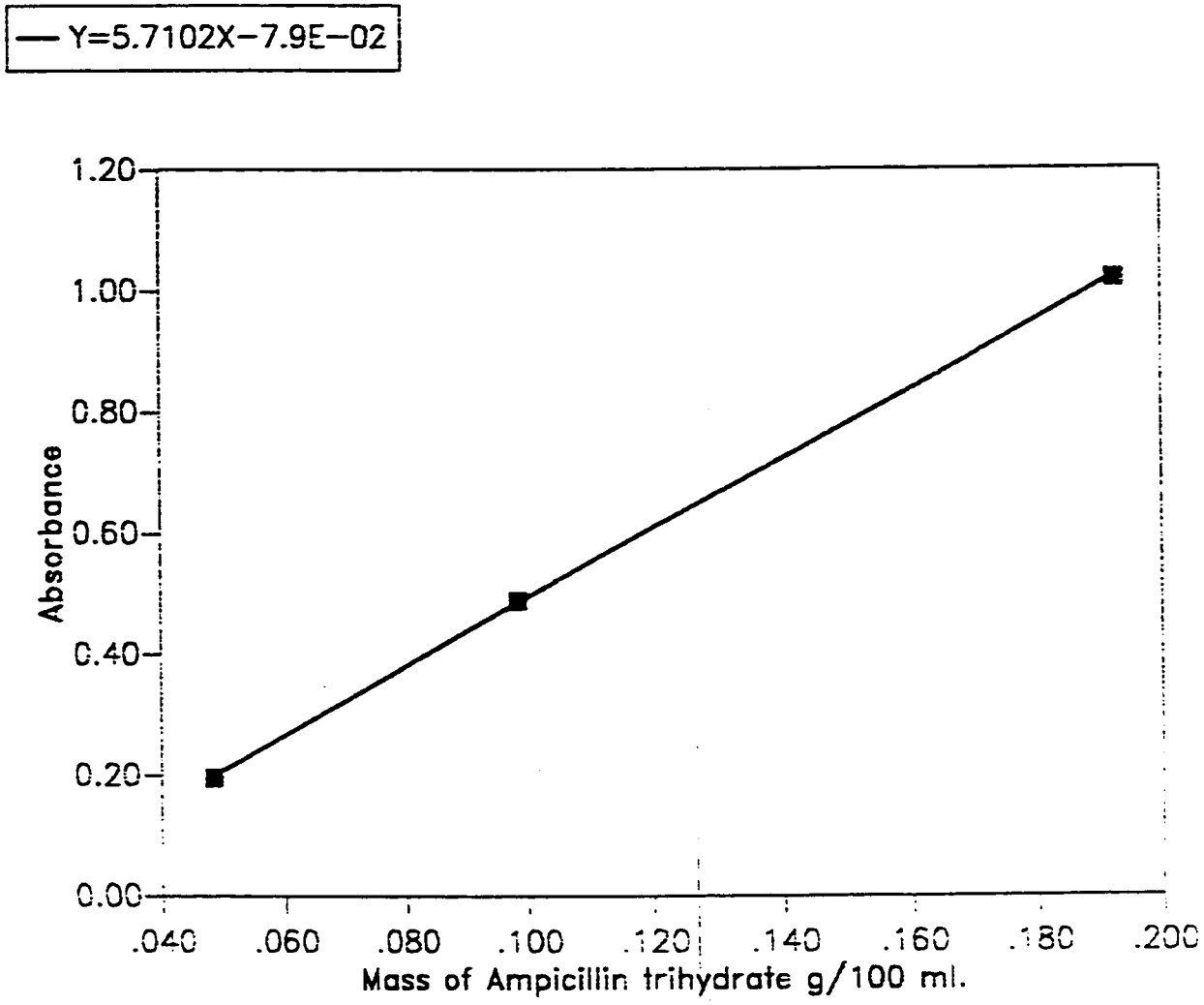


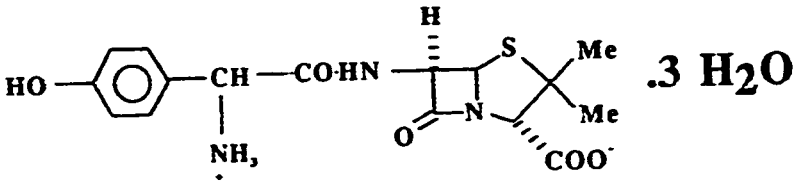
Fig. 3.2. Absorbance vs mass of Ampicillin for Cu(II) assay.

therefore

% of active substance = 0.1030/0.1034) × 100

= 99.60% ±0.4

Pharmacopoeia Methods for Amoxycillin Trihydrate:



M.WT. 419.4 g/mole

Iodometric Assay: (USP) [5].

(*a*) *Reagents used*: The reagents used for the assay are the same as those used for Ampicillin trihydrate.

(a).

Pure Ampicillin trihydrate (Standard)		Blank (standard).	
No. of trial	Vol. of $Na_2S_2O_3(ml)$.	No. trial	vol. of $Na_2S_2O_3(ml)$.
1	5.40	1	8.95
2	5.40	2	9.00
3	5.40	3	9.00
4	5.40	4	9.05
Average	5.40 ml	Average	9.00 ml

(b).

Ampicillin trihydrate (capsules)		Blank capsules.	
No. of trial	Vol. of $Na_2S_2O_3(ml)$.	No. trial	vol. of $Na_2S_2O_3(ml)$.
1	5.50	1	9.25
2	5.60	2	9.20
3	5.55	3	9.25
4	5.55	4	9.30
Average	5.55 ml	Average	9.25 ml

Table 3.5. Titres for Amoxycillin trihydrate (a) standard (b) capsules .Iodometric assay.

(*b*) *Preparation of working standard and sample solutions*:

(i) Wt. of pure Amoxycillin trihydrate taken in 200 mls = 0.19940 g.

(II) Wt. of Amoxycillin Trihydrate capsules taken in 200 mls = 0.19919g. (Note: The solution was filtered)

(*c*) *Procedure for the Assay*: The same procedure used for the Ampicillin trihydrate was carried out for the amoxycillin trihydrate. Titration values in TABLE 3.5 a and b, are used for the calculations.

Calculations:

Vol.of $S_2O_3^{-2}$ consumed by standard = 8.95 ml - 5.40 ml

= 3.60 ml

Vol.of $S_2O_3^{-2}$ consumed by sample = 9.25 ml - 5.55 ml

= 3.70 ml

therefore

No. of m moles of $S_2O_3^{-2}$ consumed by standard = 0.00977 N × 3.60

= 0.09514 m moles and

No. of m moles of $S_2O_3^{-2}$ consumed by sample = 0.00977 N × 3.70*ml*

= 0.03611 m moles.

Then

# of m moles of I_2 consumed according to the equation:

$$2S_2O_3^{-2} + I_2 \text{ -------> } S_4O_6^{-2} + I_2^-$$

For standard $= (\frac{0.09514}{2}) = 0.01757$ m moles.

For sample i.e. Amoxycillin trihydrate capsules

$= \frac{0.036112}{2} = 0.01806$ m moles.

Also

# of m. moles of standard used

$$= (\frac{0.1994}{419.40}) \times (\frac{1}{200}) \times 10^3 = 2.370 \times 10^{-3}$$

# of moles of sample used

$$= (\frac{0.1995}{419.40}) \times (\frac{1}{200}) \times 10^3 = 2.375 \times 10^{-3}$$

therefore

$$\text{Molar ratio of } \frac{I_2}{STD} = \frac{0.01757}{2.3772 \times 10^{-3}} = 7.39$$

$$\text{Molar ratio of } \frac{I_2}{Sample} = \frac{0.01805}{2.375 \times 10^{-3}} = 7.60$$

therefore, % of active substance $= \frac{7.60}{7.39} \times 100 = 103\ \% \pm 1.0$

Hydroxylamine Assay (USP)

Name Of Drug: Amoxycillin trihydrate

(*A*) *Reagents used*: The reagents used for the assay are the same as those used for Ampicillin trihydrate.

(*B*) *Preparations of working standard and sample* solutions:

(I) Wt. of pure Amoxycillin Trihydrate taken in 200 ml = 0.19940

(ii) Wt. of Amoxycillin trihydrate capsules taken in 200 ml = 0.19919 g.

(*c*) *Procedure for the Assay*: The procedure used for the Ampicillin trihydrate was carried out for the Amoxycillin trihydrate. The results are given in table 3.6.

Calculations

$$\frac{A_{sample}}{A_{std}} \times C_{std} = C_{sample}$$

Therefore ,

$$C_{sample} = \frac{0.400}{0.385} \times 0.19940 g/200 ml. = 0.2070 g/200 ml.$$

therefore,

$$\% \text{ of active substance} = \frac{(0.2070}{0.19919)} \times 100.$$

$$= 102\%$$

Pure Ampicillin trihydrate.		Ampicillin trihydrate Capsules	
No. of trial	Absorbance	No. of trial	Absorbance
1	0.385	1	0.400
2	0.390	2	0.405
3	0.395	3	0.395
4	0.390	4	0.400
Average	0.390	Average	0.400
SD	0.004	SD	0.004

TABLE 3.6: Results for the Hydroxylamine assay for Ampicillin trihydrate.

Using Copper Sulphate Assay

Name of drug - Amoxycillin Trihydrate

Reagents

The reagents used for the assay are the same as those used for Ampicillin Trihydrate. The results using the Lambda 5 Spectrophotometer are recorded in table 3.7 a and b . Fig 3.3 shows the calibration curve obtained for the Copper (II) Sulphate assay using Lambda 5 spectrophotometer.

From FIG 3.3,the mass of active substance were read and recorded as in table 3.8.

Iodometric Assay For Cephalexin

(*a*) *Reagents* Reagents used for this assay are the same as those used for the Ampicillin trihydrate assay.

(*b*) *Preparation of Working Standard and Sample Solutions*:

i) Wt. of standard cephalexin (92.4% w/w taken in 200 ml = 0.39682g

ii) Wt. of Cephalexin capsules taken in 200 ml = 0.39932g.

(Note: The Solution was then filtered to remove insoluble fillers).

(*c*) *Procedure for the Assay*

The procedure used for Ampicillin trihydrate was also used for

(a) Standard Amoxycillin trihydrate.		
Solution #	Weight I g/100 ml	Absorbance
1 2 3 4	0.05620 0.11300 0.20150 0.21420	0.270 0.571 0.982 1.054
SD	0.03071	SD 0.385

(b) Amoxycillin trihydrate capsules.		
Solution #	Weight I g/100 ml	Absorbance
1 2 3	0.06150 0.09970 0.10750	0.320 0.480 0.540

TABLE 3.7: Masses and corresponding Absorbances for standards and capsules containing Amoxycillin trihydrate for the Cu(II)-assay.

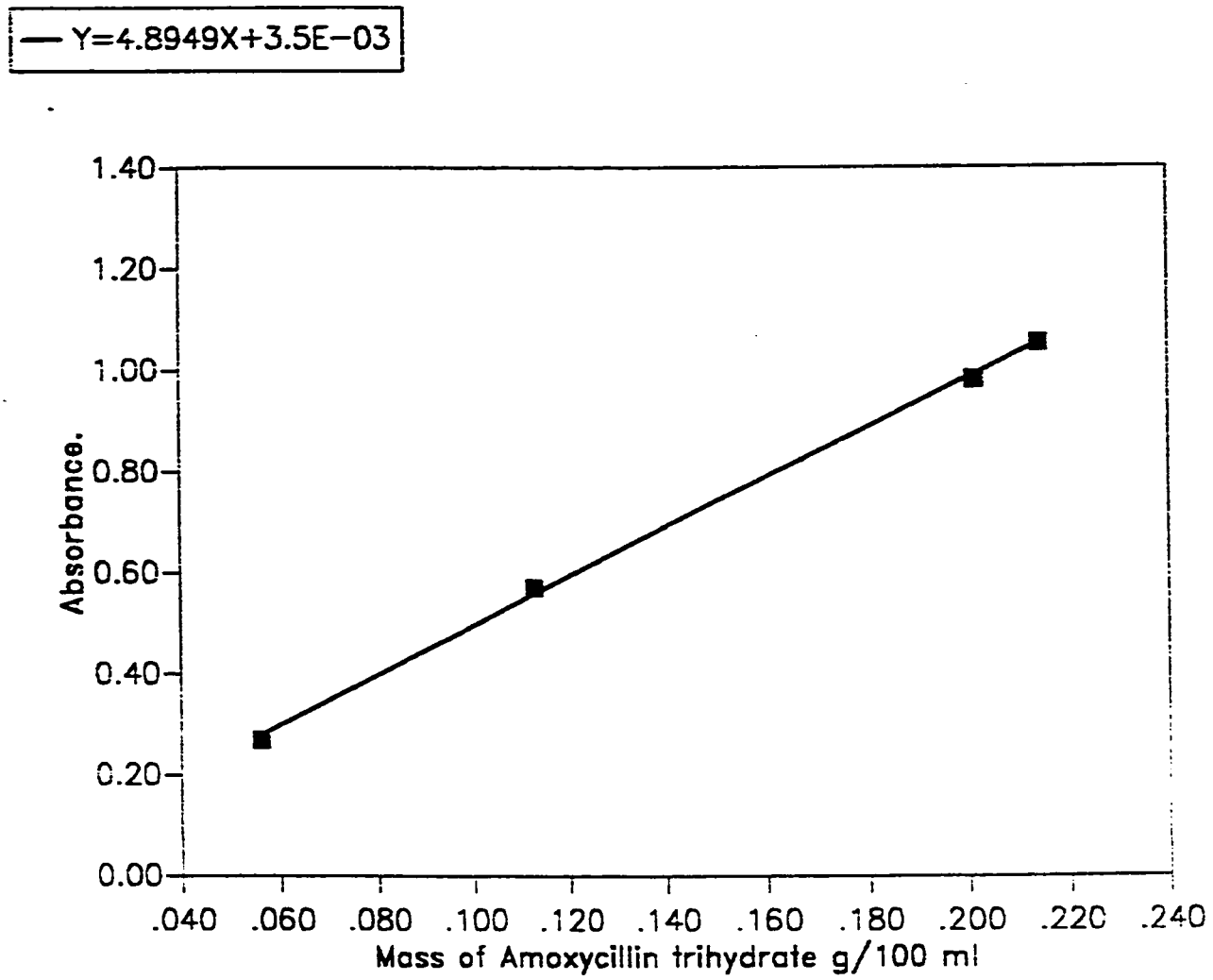


Fig. 3.3. Absorbance vs Mass of Amoxycillin trihydrate using Cu(II)-sulphate assay.

Solution #	Weight g/100 ml	% Of active substance.
1	0.0630	101.9
2	0.0980	98.20
3	1.1000	102.3
Average % of Active substance		100.8% ± 3.

TABLE 3.8: Data relating the masses of Capsules taken and the corresponding % Of Amoxycillin by the copper(II) method.

(a).

Pure Cephalexin (Standard)		Blank (standard).	
No. of trial	Vol. of $Na_2S_2O_3(ml)$.	No. trial	vol. of $Na_2S_2O_3(ml)$.
1	6.00	1	15.00
2	6.10	2	15.10
3	6.10	3	15.00
Average	6.07 ml	Average	15.03 ml

(b).

Cephalexin (capsules)		Blank capsules.	
No. of trial	Vol. of $Na_2S_2O_3(ml)$.	No. trial	vol. of $Na_2S_2O_3(ml)$.
1	4.50	1	15.00
2	4.60	2	15.10
3	4.10	3	15.20
Average	4.50 ml	Average	15.10 ml

Table 3.9. Titres for Cephalexin (a) standard (b) capsules ,Iodometric assay.

Cephalexin. The results for the titrations are

given in table 3.9 a and b .

Calculations

Vol.of $S_2O_3^{-2}$ consumed by standard = 15.00 ml - 6.07 ml

= 8.93 ml

Vol.of $S_2O_3^{-2}$ consumed by sample = 15.10 ml - 4.50 ml

= 10.60 ml

therefore

No. of m moles of $S_2O_3^{-2}$ consumed by standard = 0.00977 N × 8.93

= 0.08725 m moles and

No. of m moles of $S_2O_3^{-2}$ consumed by sample = 0.00977 N × 10.60*ml*

= 0.10356 m moles.

Then

# of m moles of I_2 consumed according to the equation:

$2S_2O_3^{-2} + I_2 \text{ -------} > S_4O_6^{-2} + I_2$

For standard = $(\frac{0.08725}{2})$ = 0.04363 m moles.

For sample i.e. Cephalexin capsules

$$= \frac{0.10356}{2} = 0.005178 \text{ m moles.}$$

Also

# of m. moles of standard used

$$= (\frac{0.39682}{347.40}) \times (\frac{1}{200}) \times 10^3 = 5.7113 \times 10^{-3}$$

# of moles of sample used

$$= (\frac{0.39932}{347.40}) \times (\frac{1}{200}) \times 10^3 = 5.7473 \times 10^{-3}$$

therefore

$$\text{Molar ratio of } \frac{I_2}{STD} = \frac{0.04360}{5.7113 \times 10^{-3}} = 7.64$$

$$\text{Molar ratio of } \frac{I_2}{Sample} = \frac{0.05178}{5.7473 \times 10^{-3}} = 9.00$$

therefore

$$\% \text{ of active substance} = \frac{9.00}{7.64} \times 100 = 117\ \%$$

But since the purity of the standard is 92.4%, then % of Active substance = $117 \times 0.924 = 108 \pm 1.0$

The cephalexin is known to be hygroscopic. The standard used was analyzed by the Glaxo Pharmaceuticals in June 1990. Although care was

taken in the storage to keep the container, sealed storage was used in a refrigerator for several months before use. And during this time, some water was tekan up by the samples. Drying to constant weight at room temperature in a good vaccum in a container containing $Mg(ClO_4)_2$ dessicant to constant weight, revealed a loss of 6.9%.

The data obtained from the analysis were, therefore, corrected by applying the factor.

Corrected % for active substance in capsule

$= 108 \times 0.93$

$= 100 \pm 1$

Cephalexin Hydroxylamine Assay

a) *Reagents* The reagents used for the assay are the same as that used for Ampicillin trihydrate.

b) *Preparation of working standard and sample solutions.*

i) Mass of Standard cephalex taken = 0.39682 g / 200 ml

ii) Mass of cephalexin capsules taken = 0.39932 g / 200 ml.

c) *Procedure for the assay* The same procedure carried out for the Ampicillin trihydrate was carried out for Cephalexin. Results are recorded in table 3.10.

Pure Cephalexin		Cephalexin Capsules.	
No. of trial	Absorbance	No. of trial	Absorbance
1	0.034	1	0.039
2	0.033	2	0.038
3	0.033	3	0.040
Average	0.033	Average	0.039
SD	0.001	SD	0.001

TABLE 3.10 : Results for the Hydroxylamine assay of Cephalexin.

Calculations

$\frac{A_{sample}}{A_{std}} \times C_{std} = C_{sample}$ Therefore ,

$C_{sample} = \frac{0.039}{0.033} \times 0.39682g/200ml. = 0.46897g/200ml.$

therefore

% of active substance = (0.46897–0.39932) × 100

= 117%

but the % purity of the standard = 92.4%

Then,

% Active substance = 117.4 × 0.924 = 108 ± 1%.

Corrected % of active substance = 108 × 0.93 = 101 ± 1%.

(See Iodometric assay of cephalexin).

The data set out in tables 3.11, 3.12 and 3.13 summarize the results obtained by the Pharmacopoeia methods.

Assay	% of Active substance	SD
Iodometric	99	1
Hydroxylamine	99	1
Copper(II)Sulphate	98	1

TABLE 3.11:Summary of data for Ampicillin trihydrate determined by pharmacopoeia methods.

Assay	% of Active substance	SD
Iodometric	103	1
Hydroxylamine	102	1
Copper(II)Sulphate	101	1

TABLE 3.12: Summary of data for Amoxycillin trihydrate determined by pharmacopoeia methods.

Assay	% of Active substance	SD
Iodometric	100	1
Hydroxylamine	101	2

TABLE 3.13: Summary of data for Cephalexin determined by Pharmacopeia methods.

CHAPTER FOUR

POLAROGRAPHIC METHODS

4.1. Principles

Polarography is a type of voltammetry, first introduced by J. Heyrovsky in 1922. In this technique, current-versus-voltage is applied to a cell containing (a) the solution of interest, (b) a stable reference electrode, and (c) a small area working or indicator electrode . In Polorography, the solution of interest is prepared in such a way that a diffusion current is measured. In polarography. the working electrode is the dropping mercury electrode (DME), consisting of a glass capillary attached to a mercury reservoir, (Fig 4.2) .

Drops of mercury fall from the orifice of this capillary at a constant rate. Each drop serves as the indicator electrode while attached to the column of mercury in the capillary. To distinguish this method from modern variants, it is sometimes called conventional or DC (Direct Current) Polarography.

Improvements and simplifications in the design of instrumentation with the growth of solid state electronics and operational amplifiers led to versatile commercial electrochemical instruments for such techniques as pulse and stripping methods.

Modern polarography is generally carried out with three electrodes; a dropping mercury electrode, a reference electrode and a counter electrode. The

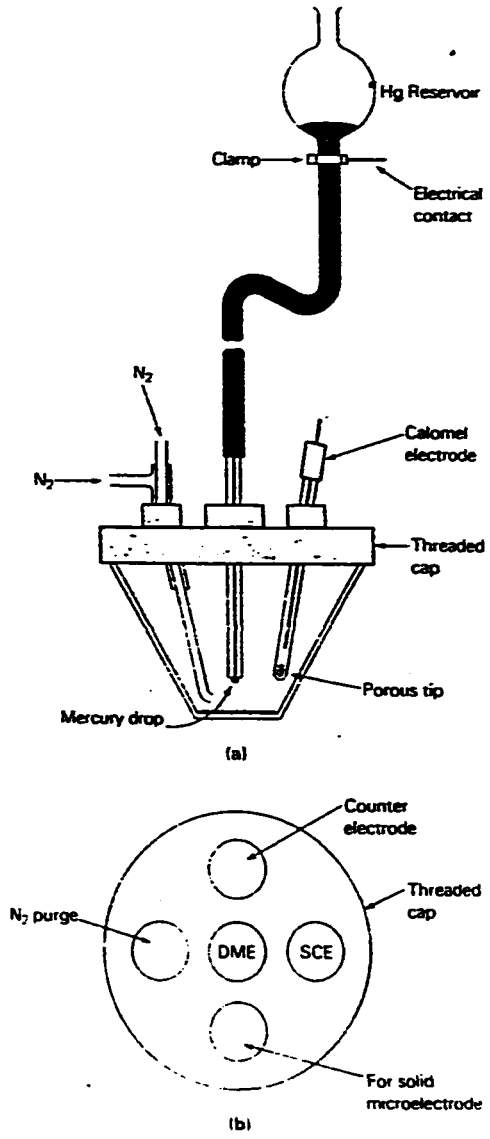


Figure 4.2. A dropping mercury electrode and cell: (a)cross-section view; (b) top view of cap.

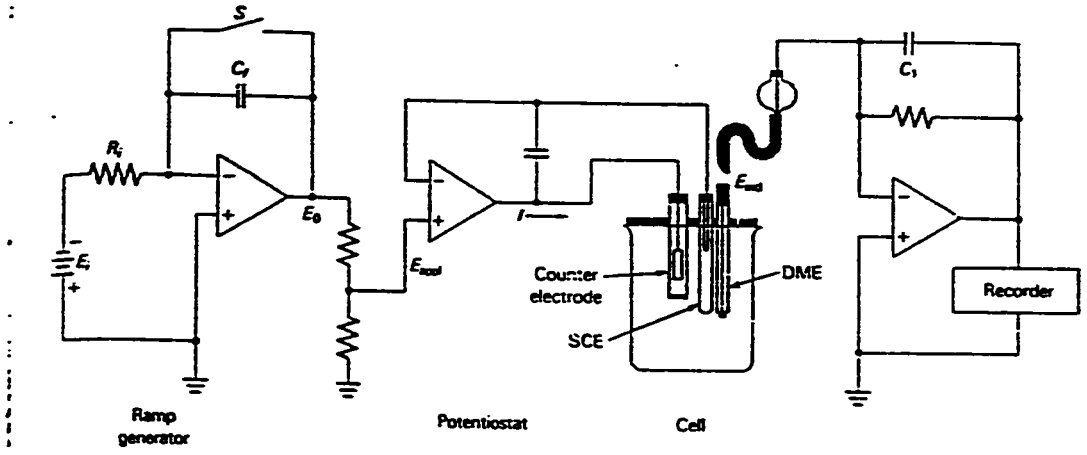


Figure 4.3. Schematic diagram showing the components of a modern, three-electrode polarograph and cell.

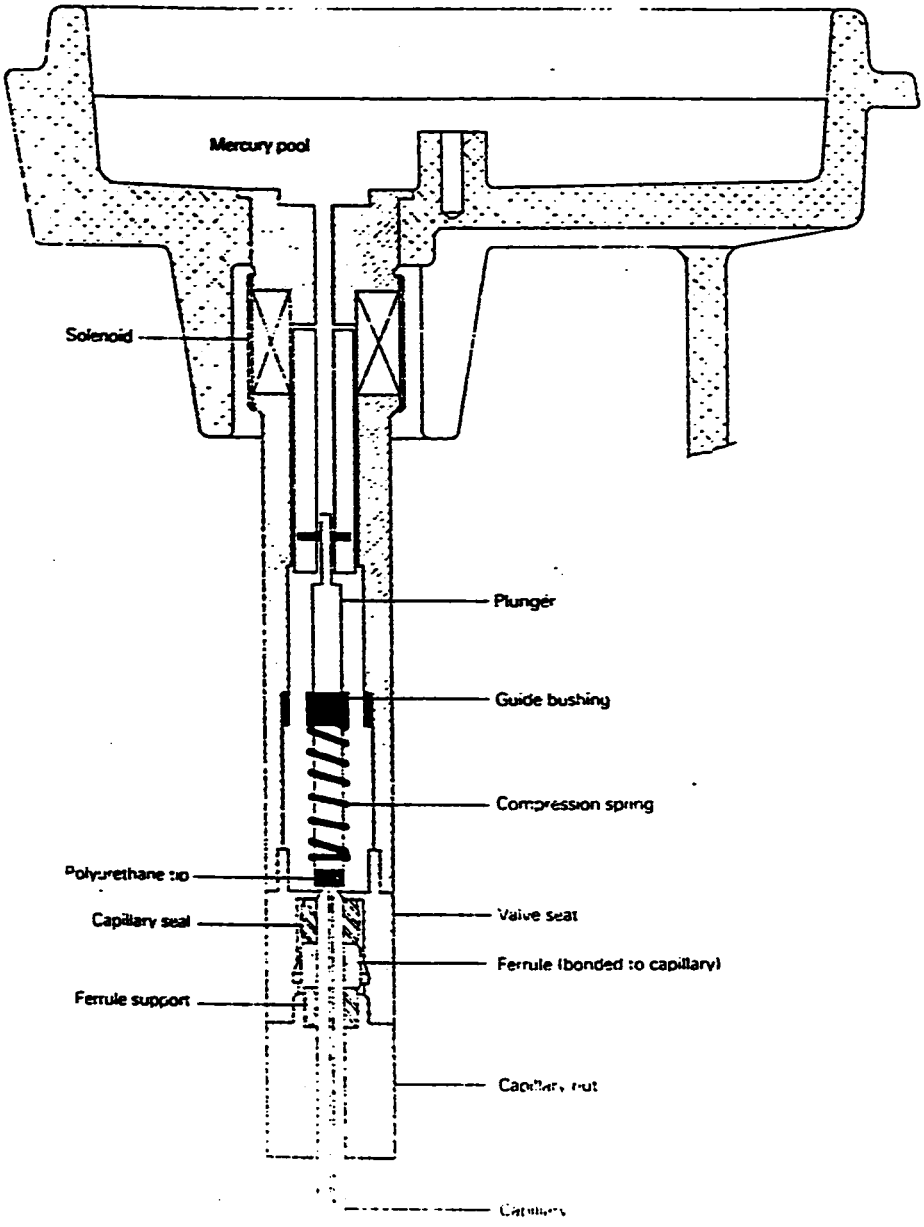


Figure 4.4. A modern dropping mercury electrode with mechanical control of drop size and time.(Courtesy of EG&G Princeton Applied Research , Princeton,Nj.)

schematic diagram on Fig. 4.3 shows the components of a modern, three-electrode polarograph and a cell.

Also, the dropping mercury electrode (DME) is mechanically controlled (Fig.4.4).

Quantitative analysis by DC polarography using the DME is limited to solutions with concentrations greater than about $10^{-5}M$. This limitation, results from the non-faradiac current associated with the charging of each mercury drop as it forms. Thus, when the ratio of the faradaic current (from the reduction of the analyte) to nonforadaic approaches unity, large uncertainties in determining diffusion currents are inevitable. One of the major goals of recent modifications of the classical method has been that of increasing the ratio between the faradaic and nonfaradaic currents by suppressing the latter, thus permitting the quantitative determination of species at lower concentrations. Some of these modern adaptations of the classical method are summarized in fig 4.5.

Polarography has been successfully applied to both basic research and practical analysis. One of the most important applications is its use in quality control in the pharmaceutical industry [7].

4.2. Quantitative Analysis in Organic Polarography

The methods of polarographic analysis of organic substances can be divided into two main groups: Direct and Indirect Methods [7].

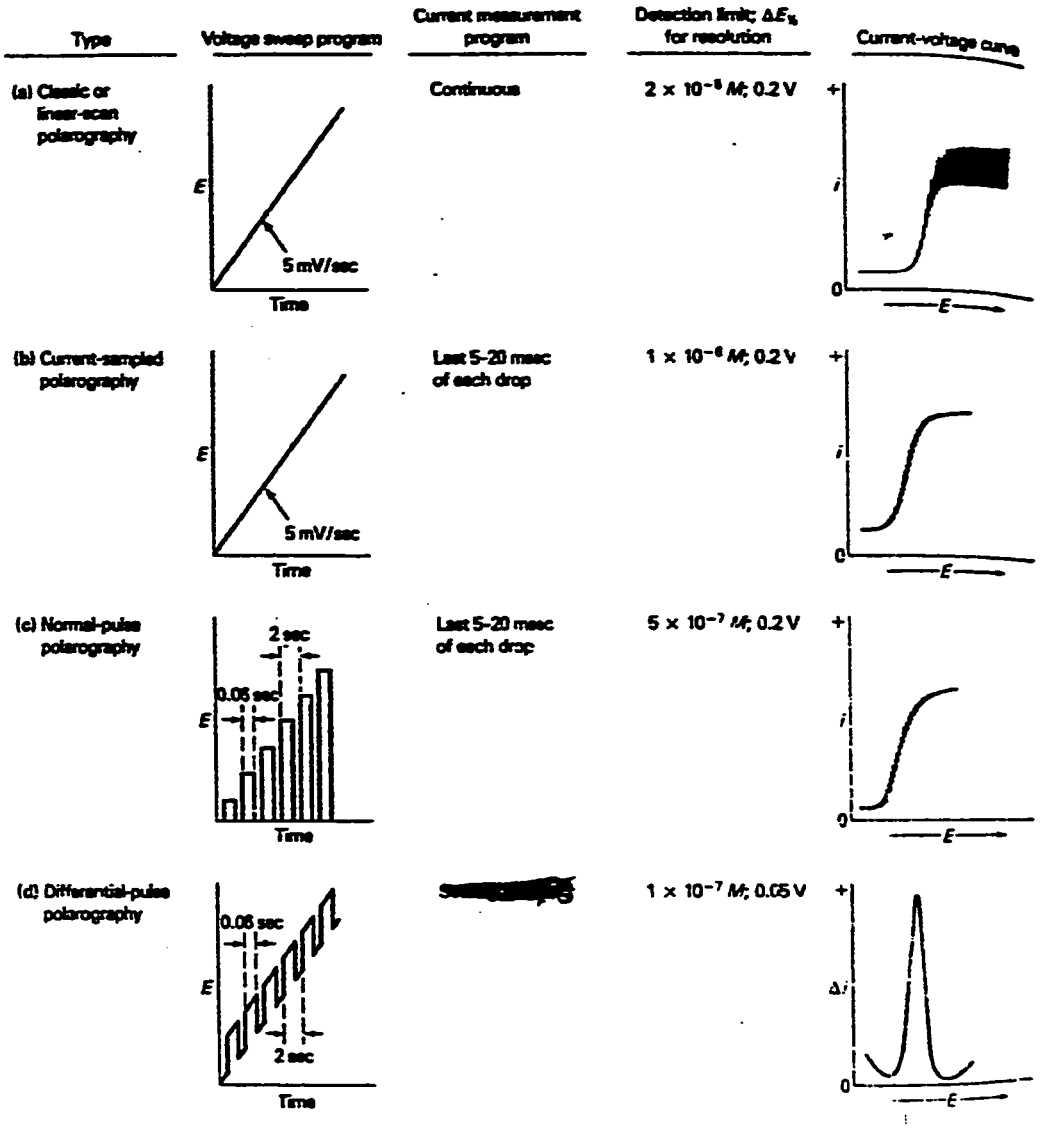


Fig. 4.5. Some types of polarography :(a) Linear-scan; (b) Current sampled; (c) Normal-pulse; (d) Differential-pulse.

(*a*) ***Direct methods*** ;

Direct methods involve dissolution of the sample in an appropriate supporting electrolyte, recording of the current voltage curve, and evaluation. These methods anticipate that the substance under study is soluble in the chosen supporting electrolyte and that it is electroactive under conditions employed, i.e. that it gives a measurable wave on the current voltage curve. These methods, of course, can be used only in those instances where the sample analyzed contains interfering substances.

(*b*) ***Indirect methods*** ;

Indirect methods permit the determination of substances which do not exhibit a polarographic wave of practical applicability. Such polarographically inactive substances may sometimes be transformed by a chemical reaction into compounds that do exhibit useful polarographic waves in an appropriate supporting electrolyte. The most frequently used methods are, nitration, nitrosation, condensation, addition, substitution, oxidation, and complex formation [7]. Here the method of complex formation will be described. This method can be divided into two groups. In the first group, the analyzed substance is a strong complex forming agent (usually with possibility of formation of chelate compounds) and it reacts stoichiometrically with the inorganic ion. The wave of the complex ion is then measured under the given conditions (i.e. the kind of metal ion and the pH value of solution). The formation of complexes of this type is rather specific, each complex being characterized by a given half-wave potential. This method can also be rather

selective , and even allows the determination of complex forming reagents in mixtures. For such analysis, the use of several metal ions and / or several pH values can be recommended for increasing the selectivity and reliability of these methods. In the second group of methods, the complex-forming reagents is added to a suspension of a slightly soluble salt of a heavy metal (Ni^{2+},Cu^{2+}, etc.) When the equilibrium between the solid phase and solution is established owing to complex formation, the concentration of the metal ion in the liquid phase is measured polarographically.

The remaining part of this chapter is devoted to the experimental work carried out using the indirect method in quantitative organic polarography as an assay for the determination of penicillin and cephalosporin drugs, in particular, Ampicillin trihydrate and Amoxycillin trihydrate and cephalexin in pharmaceutical formulations.

Experimental

4.3. Differential Pulse Polarographic Determinations Based on the pre-wave of a nickel (II) or cobalt (II) complex.

4.3.1. Apparatus

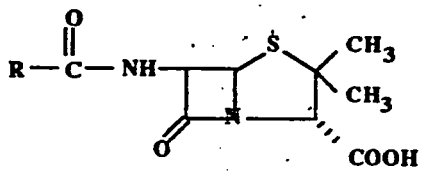
A Princeton Applied Research Polarographic Analyzer Model 174A with an X-Y recorder, Model RE 0074 was used with three electrodes, namely a dropping mercury electrode (DME) with mechanical control of drop size and time, a Ag/AgCL reference electrode and platinum counter electrode. The pH values were measured with a pH meter. Where controlling of time was needed and a stop-watch was used.

4.3.2. Reagents -

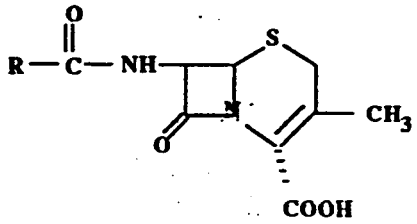
Drugs-Ampicillin trihydrate (pure) was obtained from and Amoxycillin trihydrate pure was obtained as a gift from SmithKline Beecham Pharmaceutivals, Worthing, England. The Cephalexin was also a gift from Glaxo Group Research Ltd, Greenford, England. Ampicillin trihydrate capsusles ( ) and Amoxycillin trihydrate capsules were obtained from the King Fahd University of Petroleum and Minerals Clinic. All other chemicals used were of analytical reagent grades.

4.3.3. Study of the Optimum Conditions for the Quantitative Assay of Ampicillin and Amoxycillin.

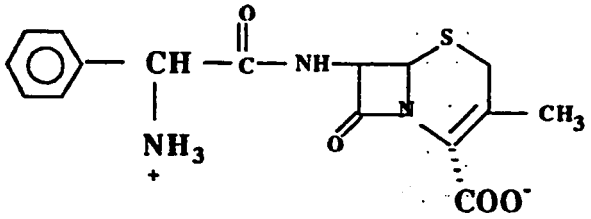
The work done in the study of the optimum conditions was based on work done previously on a cephalosporin drug [70], namely cephalexin. This was because cephalosporins and pencillins have the same carbon skeleton. The only difference is that the former have thiazine rings while the latter have thiazolidine rings attached to the β -lactam group ,(Fig. 4.7).



Penicillin



Cephalosporin



Cephalexin

Fig 4.7. Comparative structures of Penicillins and Cephalosporins.

4.3.3. (a) Effect of pH-

Organic electrode processes ordinarily involve hydrogen ions, the typical reaction being represented by,

$$R + nH + ne = RH_n$$

Where R and RH_n are the oxidized and reduced form of the organic molecule. Half wave potentials for organic compounds are therefore, markedly pH dependant. Furthermore, alteration of the pH may result in a change in reaction product.

In the study of the pH effect of Ampicillin trihydrate and Amoxycillin Trihydrate, solutions were prepared in such a way that the final concentration of Ampicillin trihydrate was $3.50 \times 10^{-6} M$ and that of Amoxycillin trihydrate was $2.64 \times 10^{-6} M$ in the Polarographic cell. Sodium Acetate (final concentration 1 M) was selected to be the buffer and the supporting electrolyte [70]. Nickel sulphate salt (final concentration of $1 \times 10^{-2} M$) was used as the complexing reagent [70]. Then 4.00 ml of Ampicillin trihydrate solution or Ampicillin trihydrate solution, 5 ml of sodium acetate, 1 ml. of Nickel (II) solution were transferred to the polarographic cell to give the required final concentrations. The pH of solution was adjusted to be in the range of 5.50 to 8.40. This was done by adding dilute acetic acid or very dilute sodium hydroxide..

After 6 minutes, the solution was purged with nitrogen for 4 minutes. Then the solution was scanned from -0.4 to -1.0V with a modulation amplitude of 25

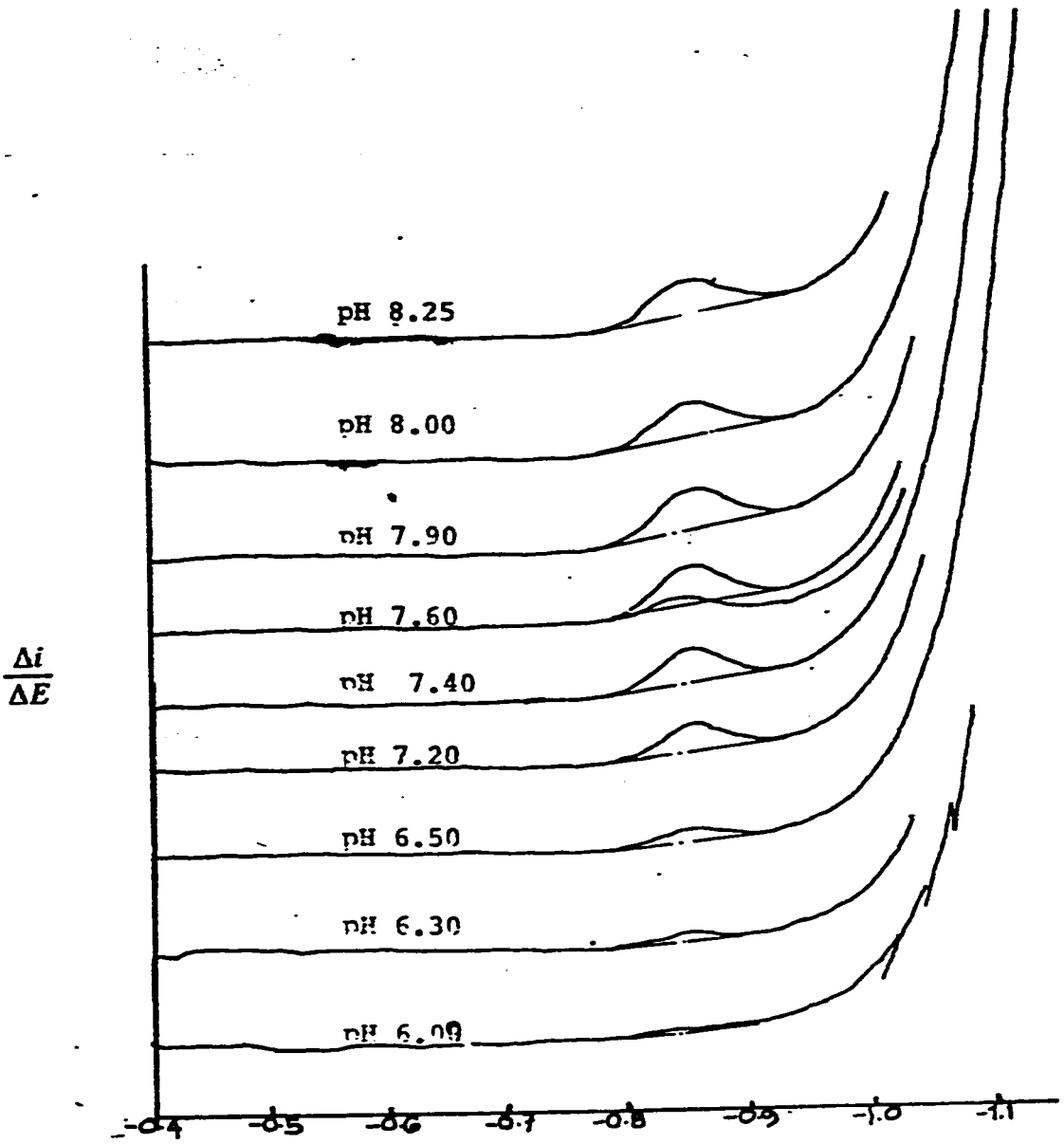


Fig. 4.8. Differential polarogram of Amoxycillin trihydrate at a concentation of 2.64×10^{-6} *M in 1 M Sodium acetate and* 1×10^{-2} *M Ni(II) showing the effect of pH.*

No.	pH	Peak height (h) cm.
1	5.50	0.00
2	5.80	0.00
3	6.00	0.00
4	6.30	0.10
5	6.50	0.20
6	6.95	0.35
7	7.50	0.50
8	7.60	0.55
9	7.90	0.60
10	8.00	0.62
11	8.10	0.62
12	8.40	0.61

Table 4.1: pH and corresponding peak height for Ampicillin Trihydrate Ni (II) complex pre-wave.

No.	pH	Peak height (h) cm.
1	6.00	0.10
2	6.30	0.15
3	6.50	0.30
4	7.20	0.60
5	7.40	0.80
6	7.60	0.90
7	7.90	0.95
8	8.00	0.95
9	8.25	0.95

Table 4.2: pH and corresponding peak height for Amoxycillin Trihydrate Ni(II) complex pre-wave.

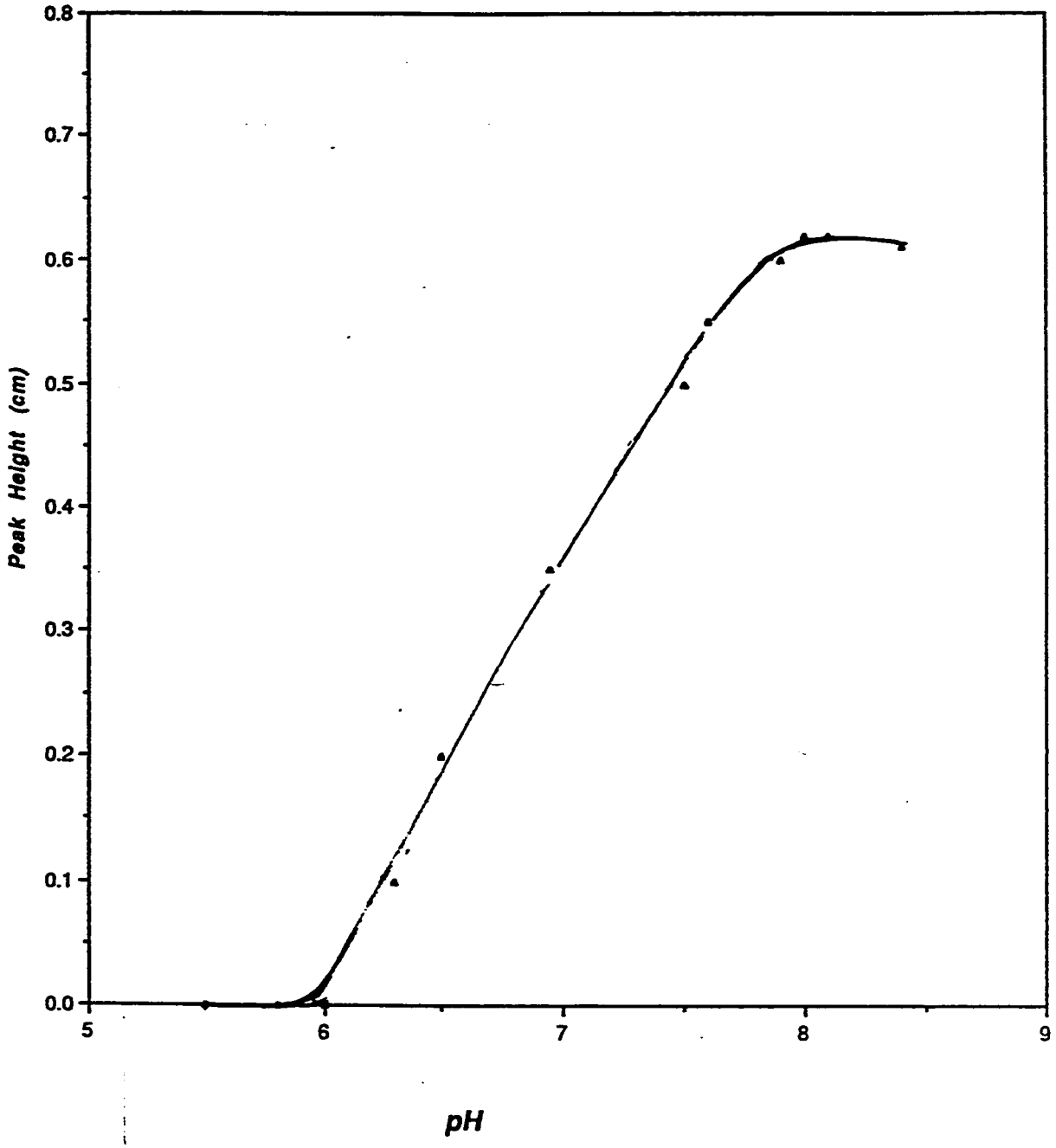


Fig. 4.9. pH vs peak height for Ampicillin trihydrate-Ni(II) complex pre-wave.

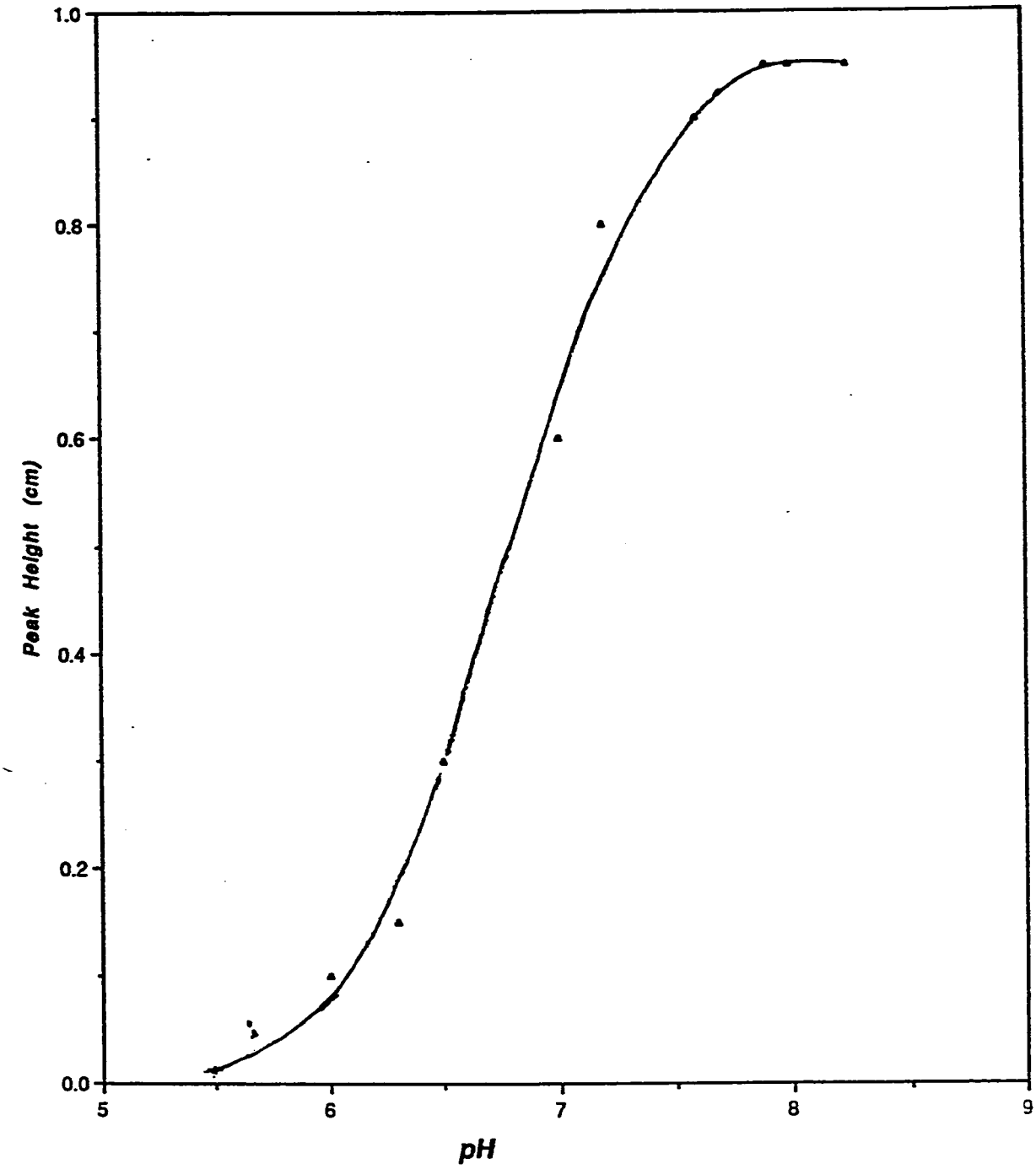


Fig. 4.10. pH vs peak height for Amoxycillin trihydrate-Ni(II) complex pre-wave.

mV,drop time 0.5s, scan rate 2 mVs^{-1} and sensitivity of 20 *micro* A. Fig (4.8) shows the differential polarogram for Amoxycillin trihydrate-Ni(II) complex. The differential polarogram of Ampicillin trihydrate-Ni(II) complex ,is similar to that of Amoxycillin trihydrate-Ni(II) complex. Then the effect of pH on peak heights were recorded for Ampicillin (Table 4.1), and Amoxycillin (Table 4.2) and graghs plotted ,Figures (4.9 ,4.10) for Ampicillin and Amoxycillin respectively.

4.3.3. (b) Effect of Sodium Acetate Concentration

In this experiment, sodium acetate is acting as a buffer as well as a supporting electrolyte. A series of sodium acetate solutions (from 0.20 - 1.5 M for an Ampicillin trihydrate final concentration of $6.00 \times 10^{-6} M$ were prepared and transferred separately to a polarographic cell together with the drug solution and $1.00x10^{-2}M$ Ni(II) solution. The pH of the drug was then adjusted to pH 8.00. After 6 minutes, the solution was purged with Nitrogen for 4 minutes. Then it was scanned from -0.4 - 1.0 with modulation amplitude of 25 mV,drop time 0.5s, scan rate 2 mVs^{-1} and sensitivity of 20 *micro* A. Here only the differential polarogram for Ampicillin showing the effect of sodium acetate on the peak height is give, (Fig 4.11). Tables (4.3 . 4.4), show the results obtained for Ampicillin and Amoxycillin respectively. These results were then plotted as shown in figures (4.12) for Ampicillin and (4.13) for Amoxycillin.

4.3.3. (c) Effect of Temperature and Time

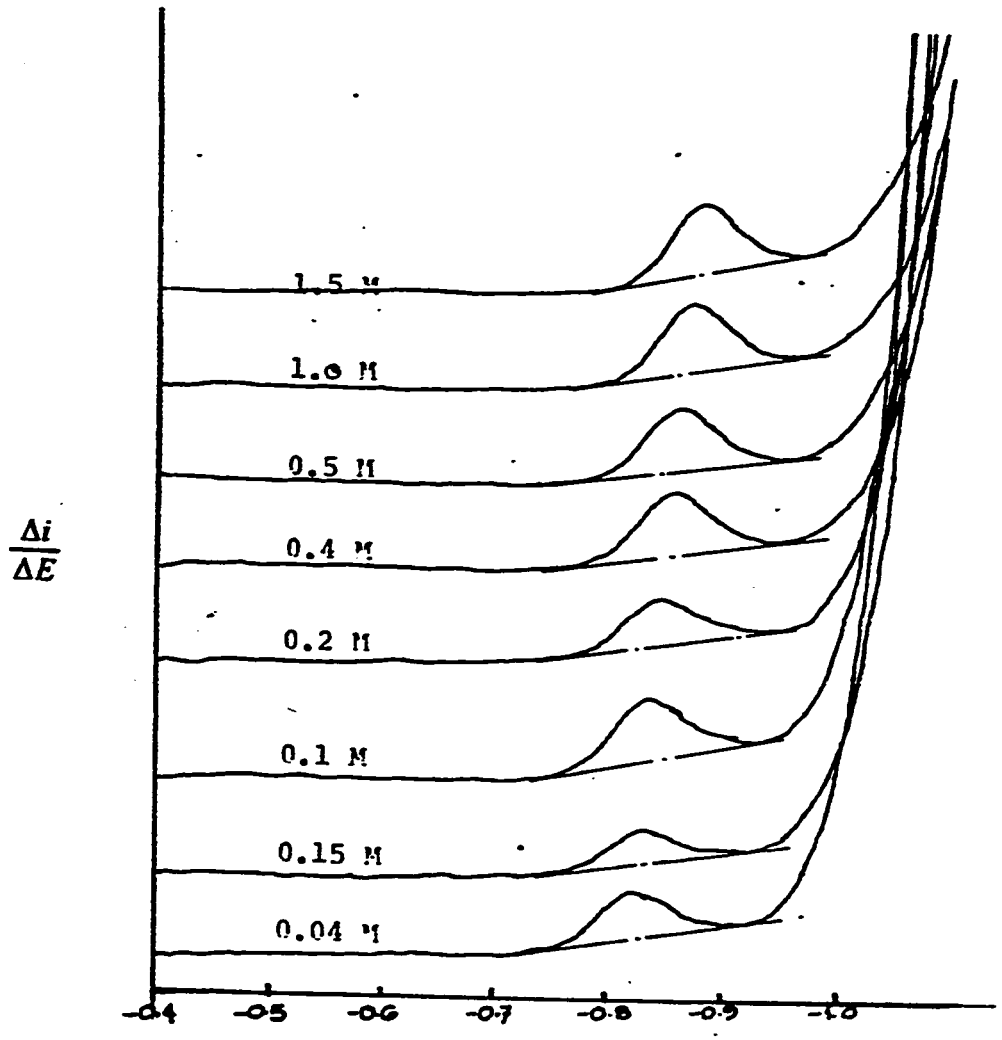


Fig. 4.11. Differential polarogram of Ampicillin trihydrate at a concentration of $6.00 \times 10^{-6}M$, *pH (8.00), and Ni(II),* $1 \times 10^{-2}M$ *showing the effect of Sodium acetate solution.*

No.	Sodium Acetate Concentration (M)	Peak height (h) Cm.
1	0.10	1.40
2	0.15	1.20
3	0.20	1.10
4	0.30	1.20
5	0.40	1.30
6	0.50	1.35
7	1.00	1.50
8	1.50	1. 50

Table 4.3. Sodium Acetate Concentration Vs. peak height for Ampicillin trihydrate Ni(II) complex.

No.	Sodium Acetate Concentration (M)	Peak height (h) Cm.
1	0.10	0.70
2	0.15	0.85
3	0.30	0.87
4	0.35	0.88
5	0.40	0.89
6	0.50	0.90
7	1.00	0.91
8	1.50	0.89

Table 4.4. Sodium Acetate Concentration Vs. peak height for Amoxycillin trihydrate Ni(II) complex.

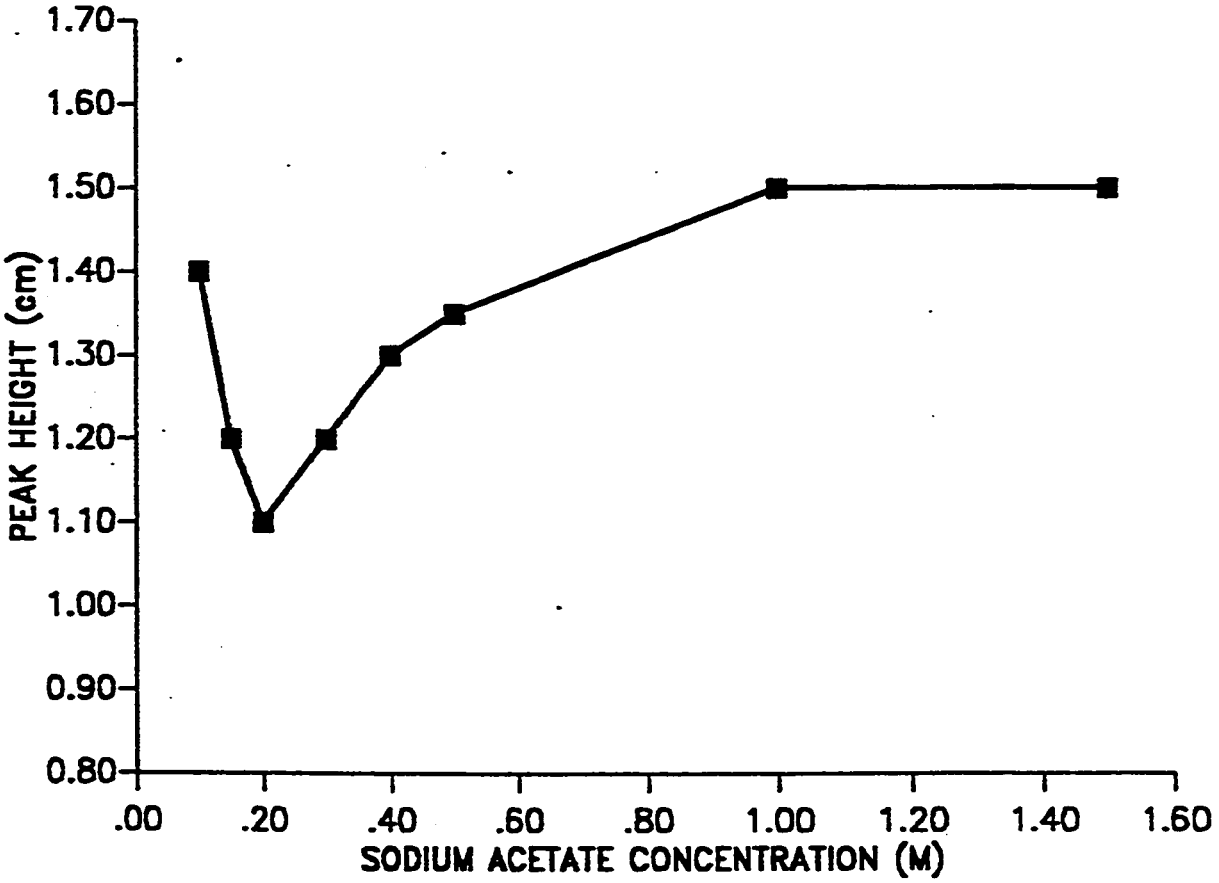


Fig. 4.12. The effect of Sodium acetate concentration on peak height for Ampicillin trihydrate-Ni(II) complex.

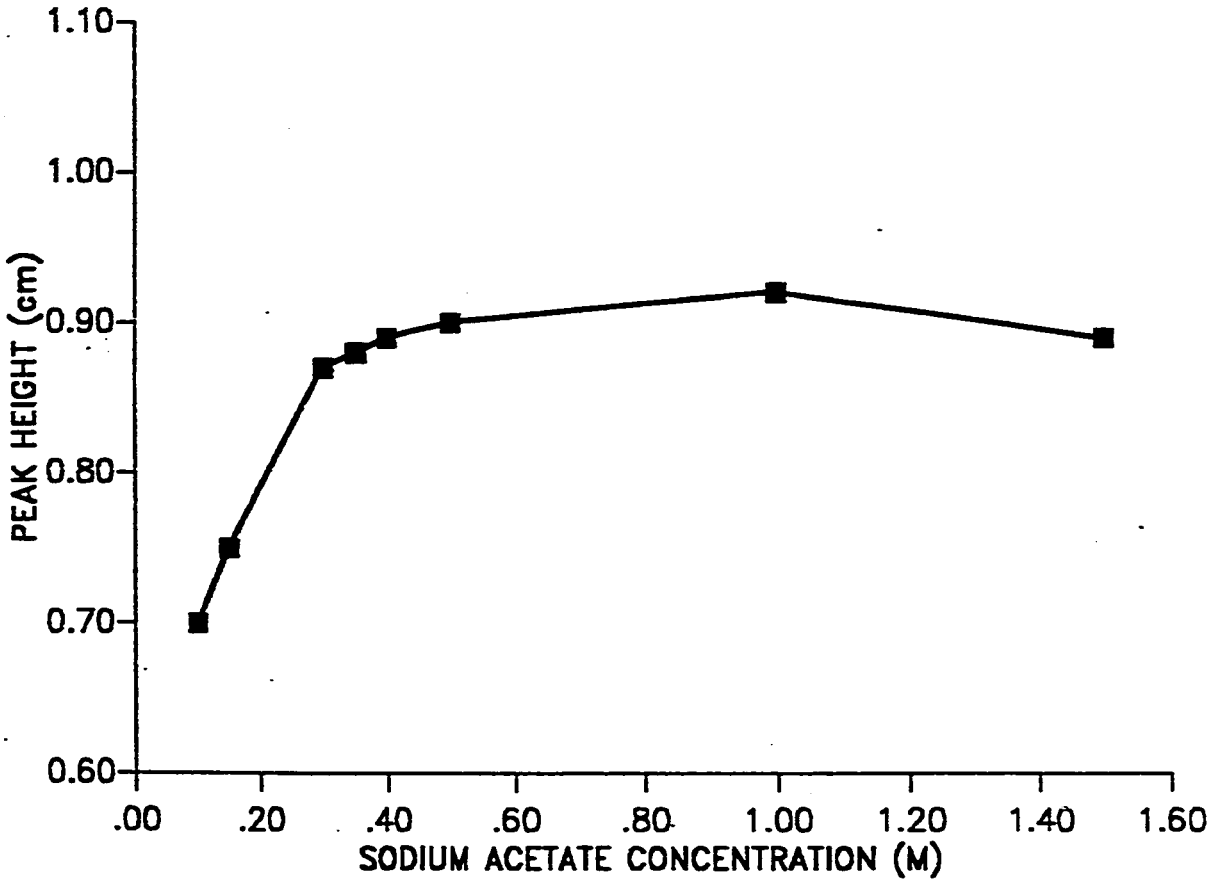


Fig. 4.13. The effect of Sodium acetate concentration on peak height for Amoxycillin trihydrate-Ni(II) complex.

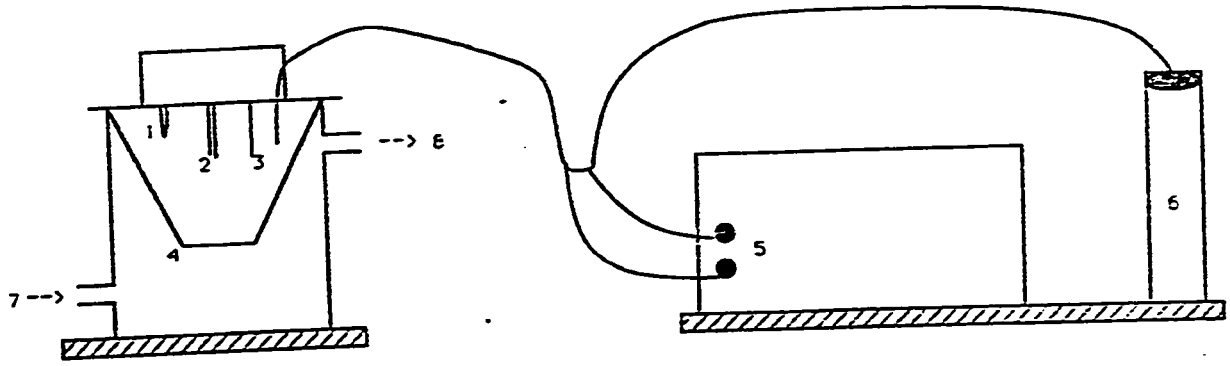
Temperature has a remarkable effect on the Ni (II) complex wave of Ampicillin trihydrate and Amoxycillin trihydrate when measured as a function of time.

In this study, the instrumental set up shown in fig. (4.34) was used. Temperatures were measured using a chromel-alumel thermocouple.

For Ampicillin trihydrate, this study was carried out for solutions at a concentration of $5.60 \times 10^{-5} M$ containing 1 M sodium acetate and $1.00 \times 10^{-2} M$ Ni(II). For Amoxycillin trihydrate the concentration was $1.32 \times 10^{-4} M$ containing 1 M sodium acetate and $1.00 \times 10^{-2} M$ Ni(II). The pH in all instances was adjusted to 8.00. After 15 minutes, the solutions were purged with nitrogen for 4 minutes. Then each solution was canned from -0.4 to 1.00 at (vs Ag/AgCL) with a modulation amplitude of 25 mV, drop time 0.5s, scan rate 2 mVs^{-1} and sensitivity of 20 *micro* A. The scanning was repeated at intervals of time. The temperature range studied was 10-40°C as shown in figures (4.14) for Ampicillin and (4.15) for Amoxycillin. Tables (4.5,4.6) give the results obtained for Ampicillin and Amoxycillin respectively . These results were then plotted as shown in figures (4.16) for Ampicillin and (4.17) for Amoxycillin.

The scan at 25 degrees was repeated and in this case the solutions were purged with nitrogen after 5 minutes,to see the effect of time ,fig (4.18).

4.3.3. (d) Reproducibility Test



Footnotes;

1. Reference electrode (Ag/AgCL).
2. Dropping mercury electrode (DME).
3. Counter electrode (Platinum-wire).
4. Polarographic cell.
5. Thermocouple.
6. Vacuum flask containing mixture of ice and distilled water.
7. Water inlet from thermostat.
8. Water outlet to thermostat.

Fig. 4.34. Instrumental set up used in the study of temperature effect.

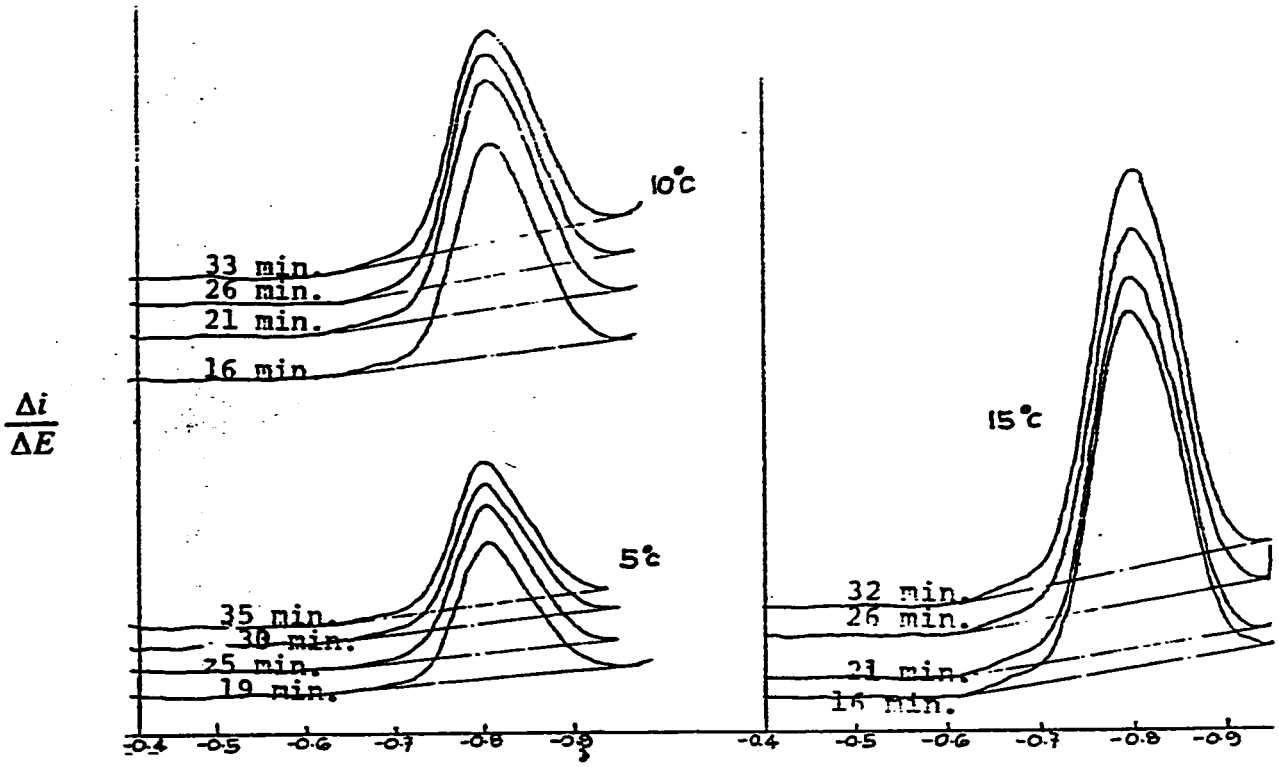


Fig. 4.14. Differential polarogram of Ampicillin trihydrate-Ni(II) complex showing the effect of temperature with time at 5,10,and 15°C.

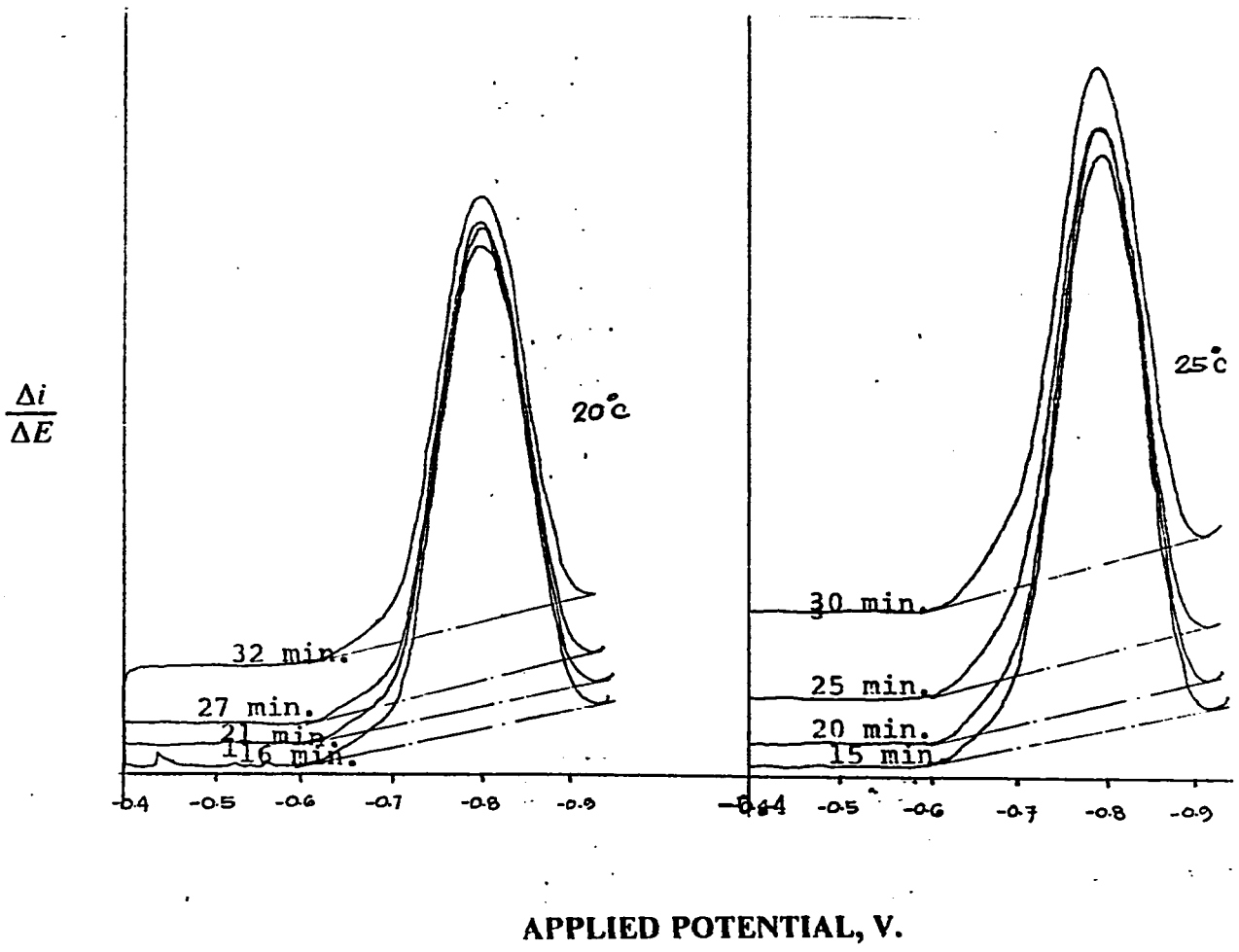


Fig. 4.14. Differential polarogram of Ampicillin trihydrate-Ni(II) complex showing the effect of temperature with time at 20,and 25°C.

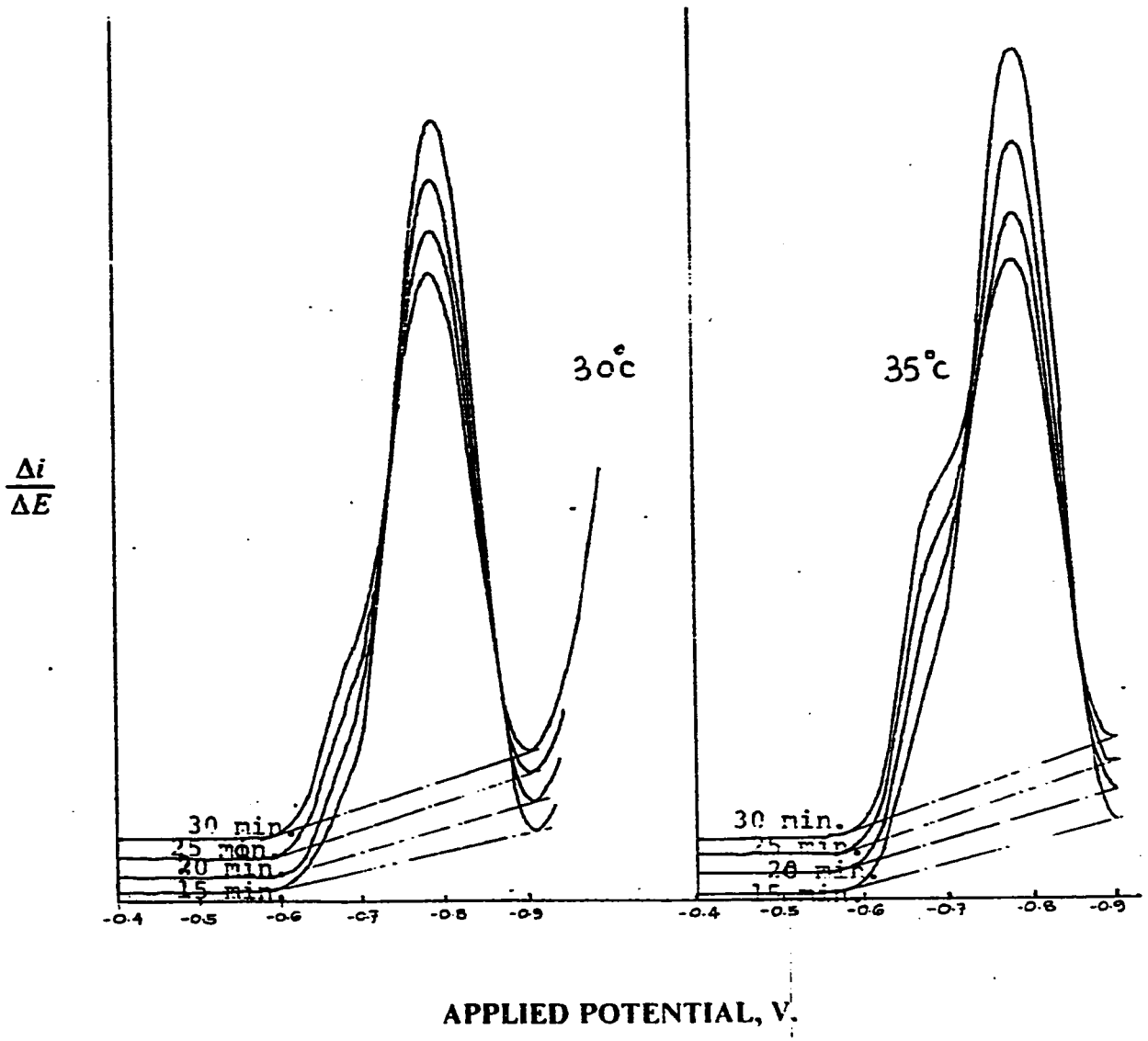


Fig. 4.14. Differential polarogram of Ampicillin trihydrate-Ni(II) complex showing the effect of temperature with time at 30 and 35°C.

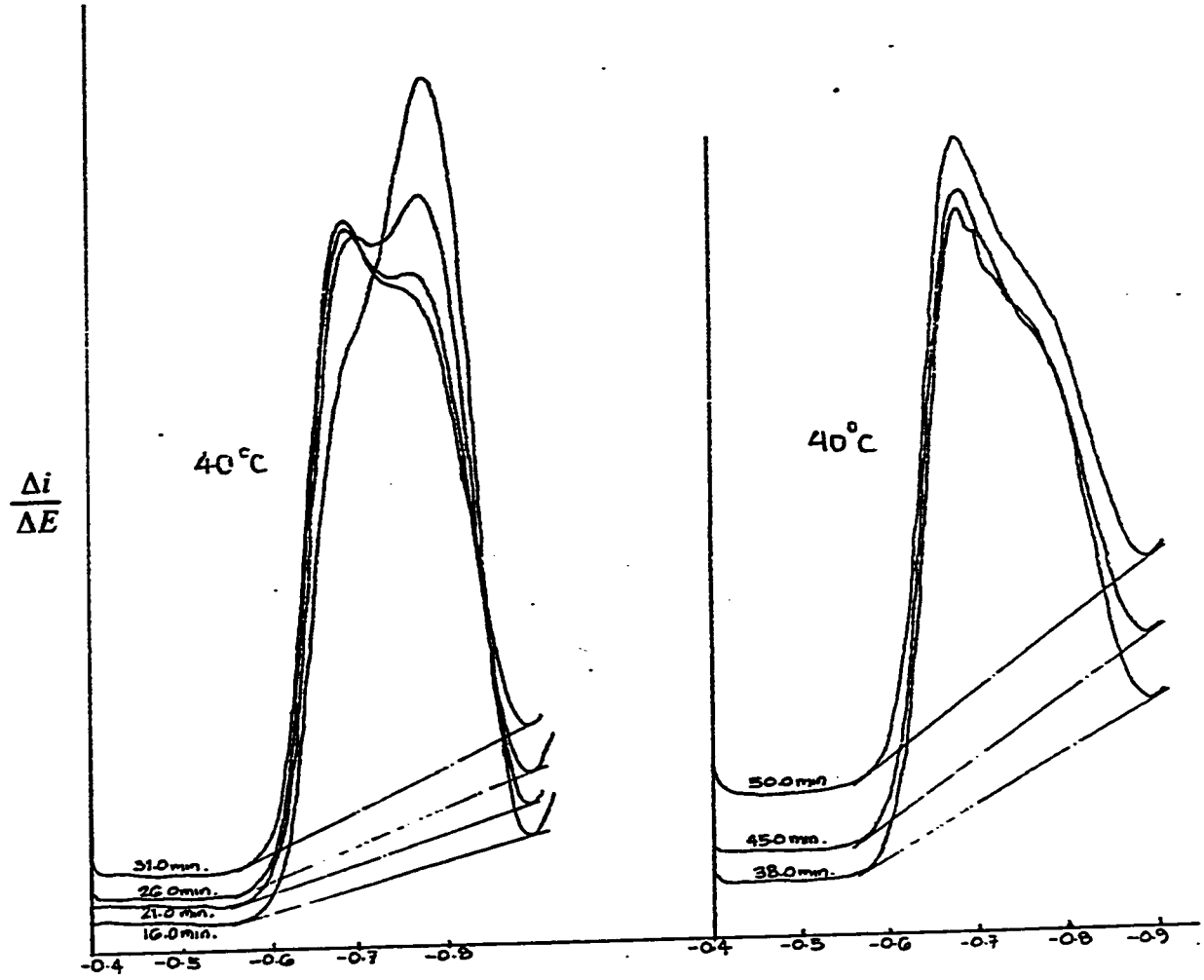


Fig. 4.14. Differential polarogram of Ampicillin trihydrate-Ni(II) complex showing the effect of temperature with time at 40°C.

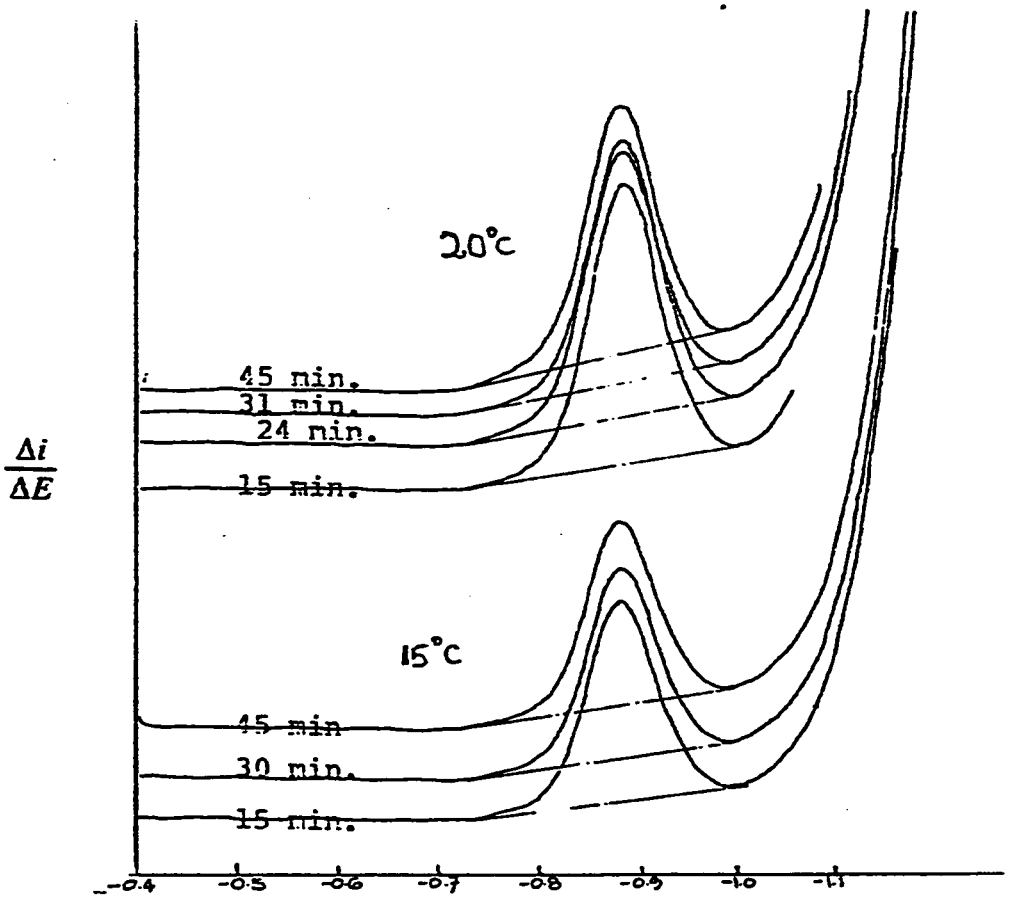


Fig. 4.15. Differential polarogram of Amoxycillin trihydrate-Ni(II) complex showing the effect of temperature with time at 15 and 20°C.

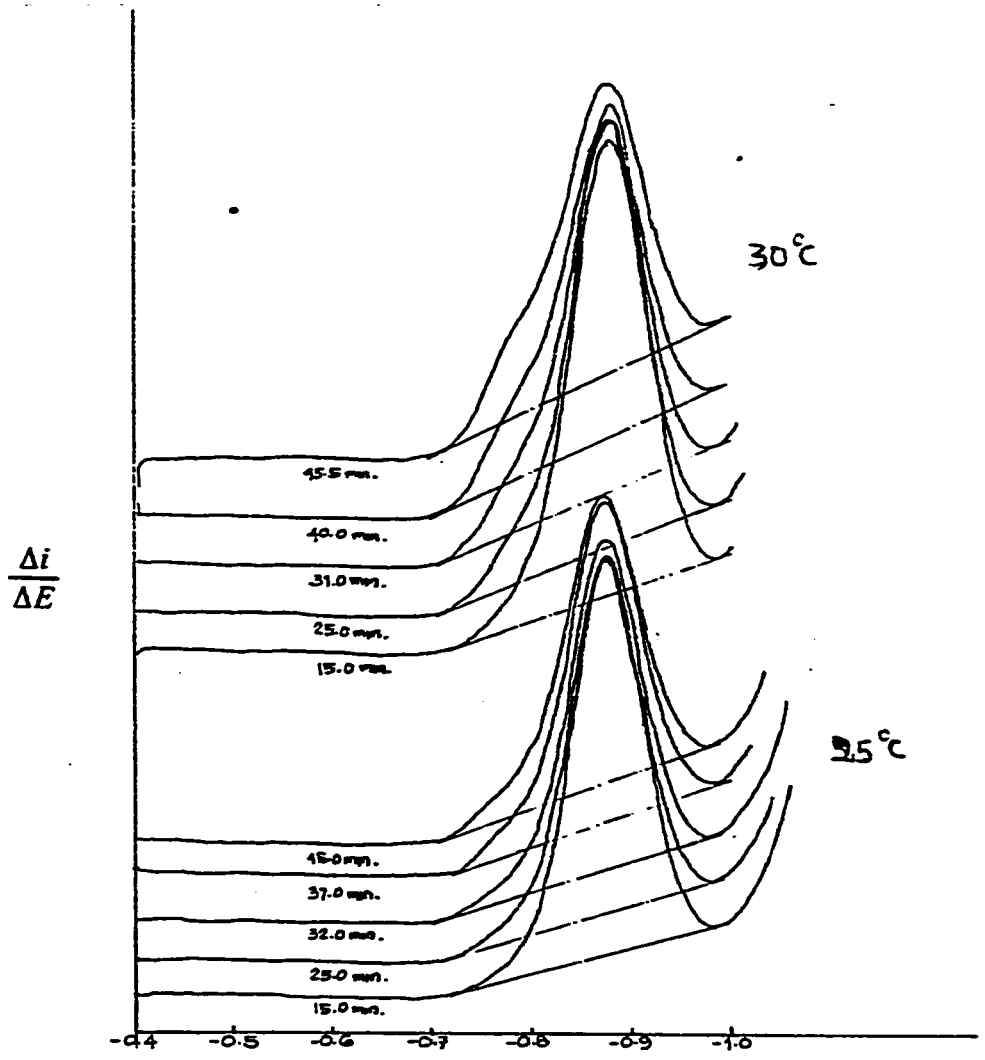


Fig. 4.15. Differential polarogram of Amoxycillin trihydrate-Ni(II) complex showing the effect of temperature with time at 25 and 30°C.

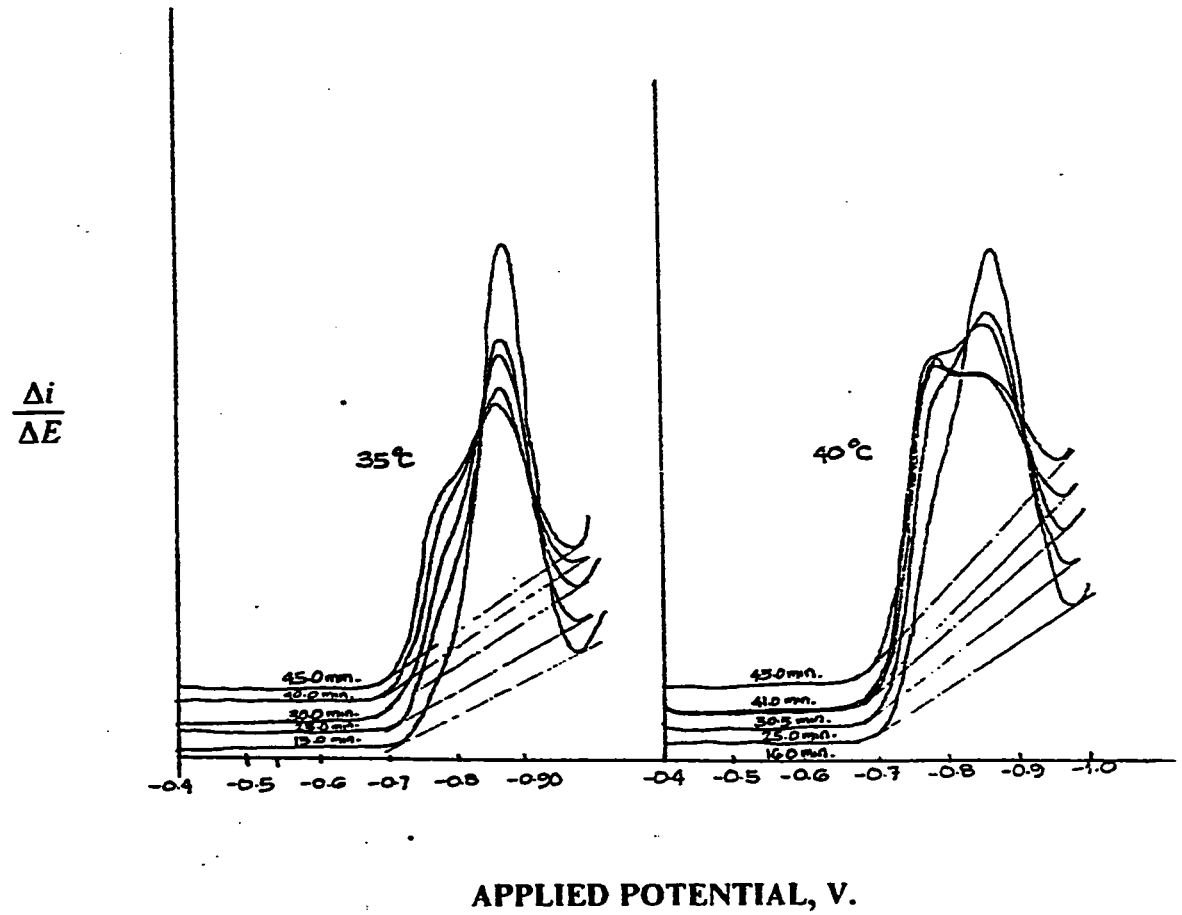


Fig. 4.15. Differential polarogram of Amoxycillin trihydrate-Ni(II) complex showing the effect of temperature with time at 35 and 40°C.

Temp. Degree Celsius	Time (t) min.	Peak height cm.	Temp. Degree Celsius	time (t) min.	Peak height cm.
5.0	19.0 5.0 30.0 35.0	3.80 4.05 3.75 3.90	10.0	16.0 21.0 26.0 30.0	5.80 6.30 6.10 5.80
15.0	16.0 21.0 26.0 32.0	10.00 10.10 10.30 10.80	20.0	16.0 21.0 27.0 32.0	14.10 13.80 12.40 12.10
25.0	15.0 20.0 25.0 30.0	17.30 15.80 24.95 14.25	30.0	15.0 20.0 25.0 30.0	22.00 19.60 17.20 15.25
35.0	15.0 20.0 25.0 30.0	24.00 20.30 17.40 15.40	40.0	16.0 21.0 26.0 31.0 38.0 45.0 50.0	22.20 18.10 15.50 13.50 11.60 9.90 9.40

Table 4.5:Results of the effect of time on peak height for Ampicillin trihydrate-Ni(II)Complex at different temperatures.

Temp. Degree Celsius	Time (t) min.	Peak height cm.	Temp. Degree Celsius	time (t) min.	Peak height cm.
15.0	15.0 30.0 45.0	4.95 4.80 4.70	20.0	15.0 24.0 31.0 45.0	7.00 6.60 6.10 6.10
25.0	15.0 25.0 30.0 37.0 45.0	10.85 8.90 8.25 8.00 7.30	30.0	15.0 25.0 31.0 40.0 45.5	12.35 10.25 9.30 8.00 7.30
35.0	15.0 25.0 30.0 40.0 45.0	15.90 11.60 10.00 8.10 7.10	40.0	16.0 25.0 30.5 41.0 45.0	14.55 11.15 9.60 6.80 5.80

Table 4.6:Results of the effect of time on peak height for Amoxycillin trihydrate-Ni(II) Complex at different temperatures.

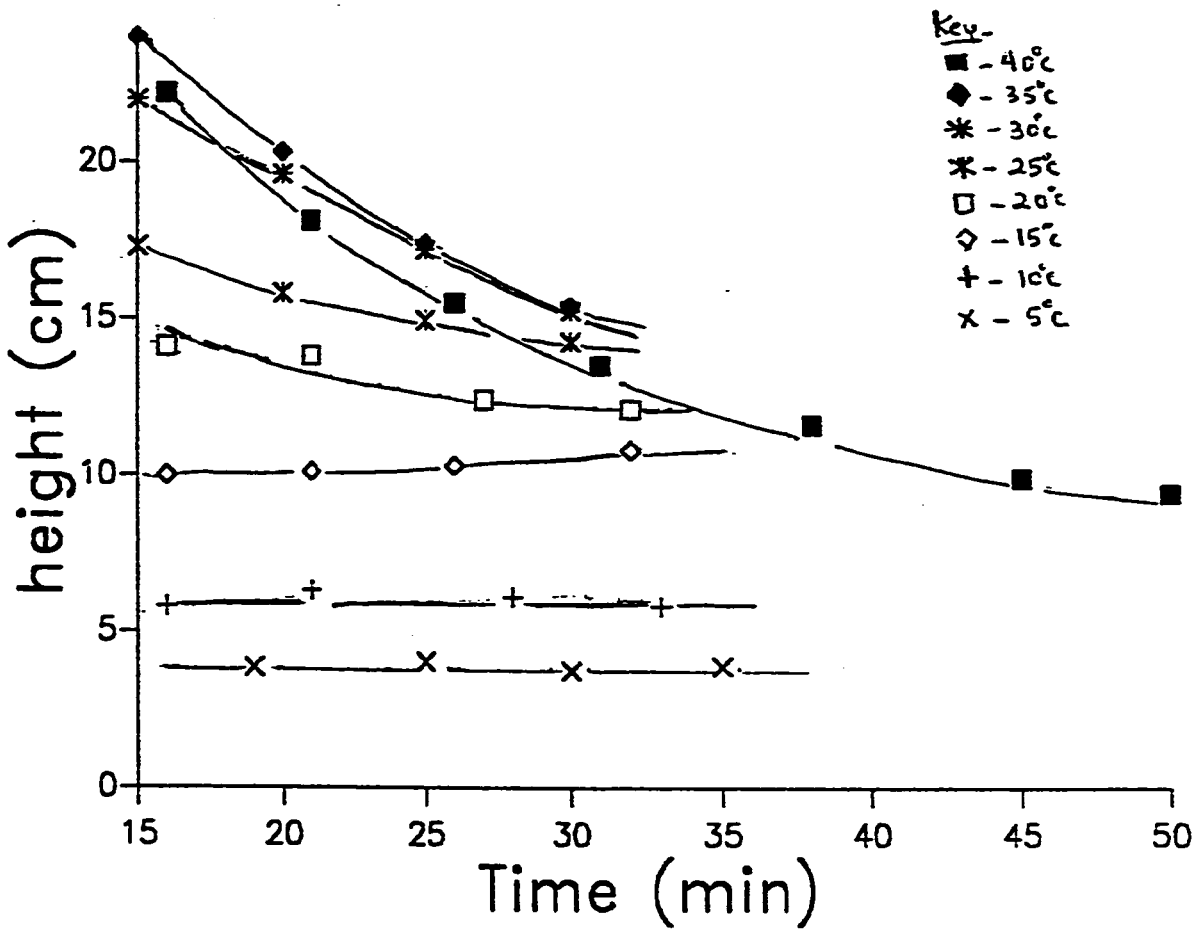


Fig. 4.16. The effect of temperature as a function of time on peak height for Ampicillin trihydrate.

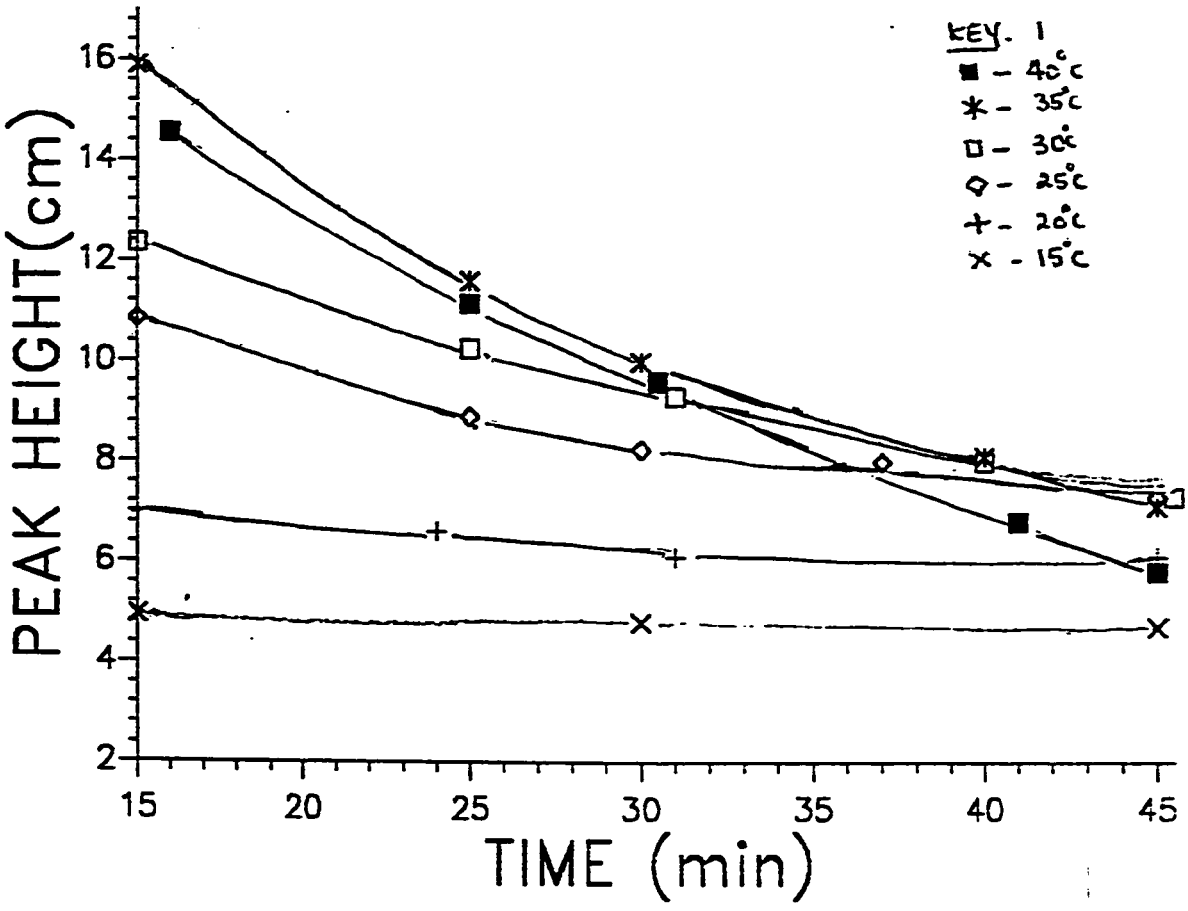


Fig. 4.17. The effect of temperature as a function of time on peak height for Amoxycillin trihydrate.

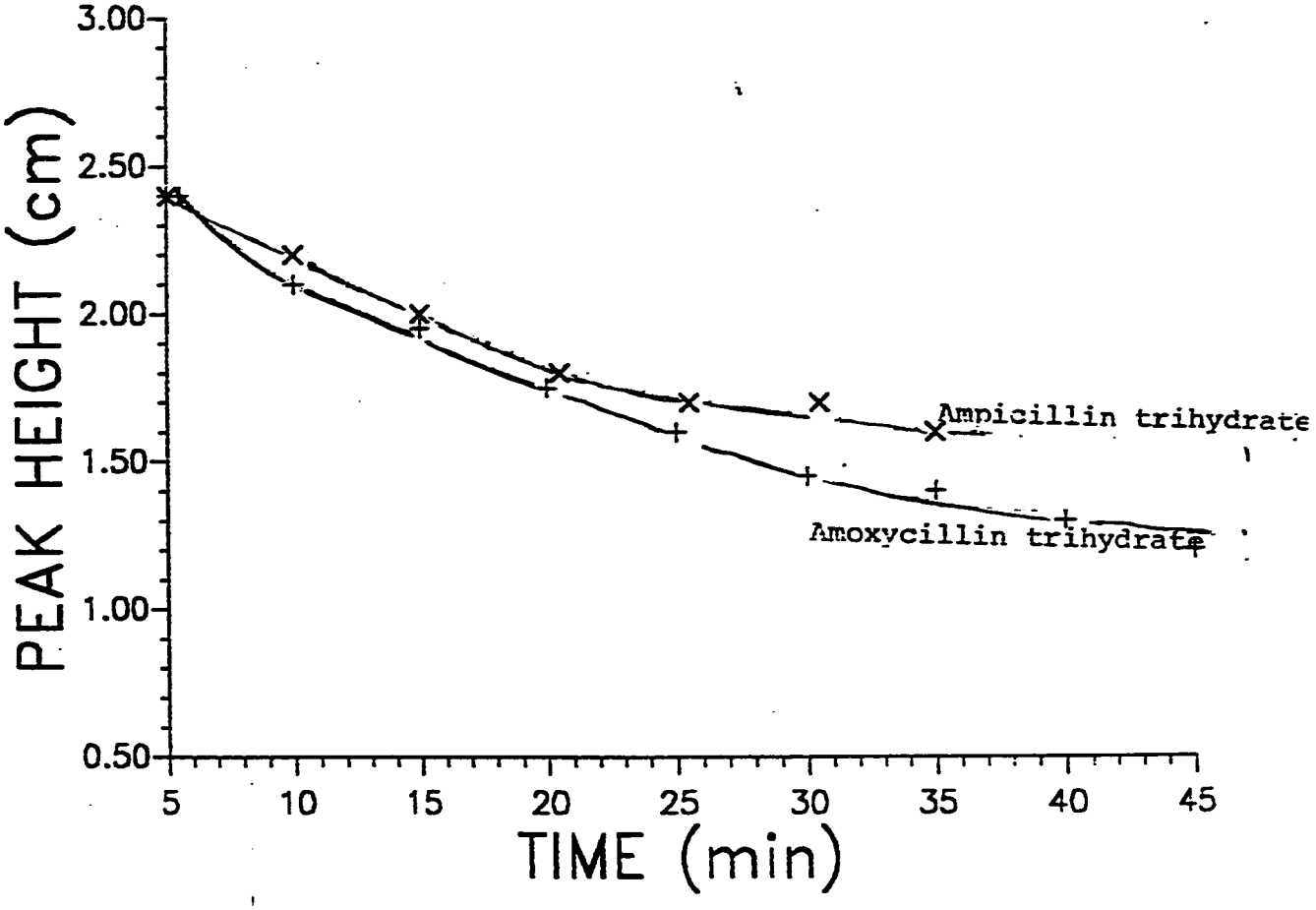


Fig. 4.18. The effect of time on peak height for Amoxycillin and Ampicillin trihydrate at room temperature (25°C).

(a)		(b)	
Trial #	Peak height cm	Trial #	Peak height cm
1	7.00	1	1.30
2	7.10	2	1.30
3	6.80	3	1.35
4	7.10	4	1.40
5	7.20	5	1.40
6	7.20	6	1.40
Mean =	7.07	Mean =	1.36
SD =	0.14	SD =	0.04

Table 4.7. Results for the reproducibility tests of (a) Ampicillin trihydrate and (b) Amoxycillin trihydrate, Ni(II) complexes.

In this test, solutions of Ampicillin trihydrate at a concentration of $2.18 \times 10^{-6} M$ containing 1 M sodium acetate, $1.00 \times 10^{-2} M$ and Amoxycillin trihydrate at a concentration of $6.20 \times 10^{-6} M$ were used. The solutions were purged after waiting 6 minutes with nitrogen for 4 minutes. Then they were scanned from -0.4 to -1.0V, with a modulation amplitude of 25 mv , drop time 0.5s, scan rate 2 mVs^{-1} and sensitivity of 20 *micro* A. These results are tabulated in table (4.7).

4.3.3. (e) Reversibility Test

The reversibility of the system may be evaluated from the ratio between the cathodic and anodic peak currents and the difference between the pulse potential applied and the measured peak potentials [110]. For reversible polarographic processes, the ratio should be unity, and the potential difference should be the same as the pulse amplitude dE. More over the $E_{1/2}$ value obtained by DC polarography for the cathodic and anodic waves should be the same. Figures (4.19 ,4.20) show the differential and D.C. polarograms for Ampicillin trihydrate-Ni(II) complex as a test for reversibility . The results of the reversibility test are given in tables (4.8,4.10,4.11) for Ampicillin and (4.9,4.10,4.11) for Amoxycillin.

4.4.3 (f): Calculation of Number of Electrons involved in the electrode reactions. for ampicillin and Amoxycillin Ni(II) complex.

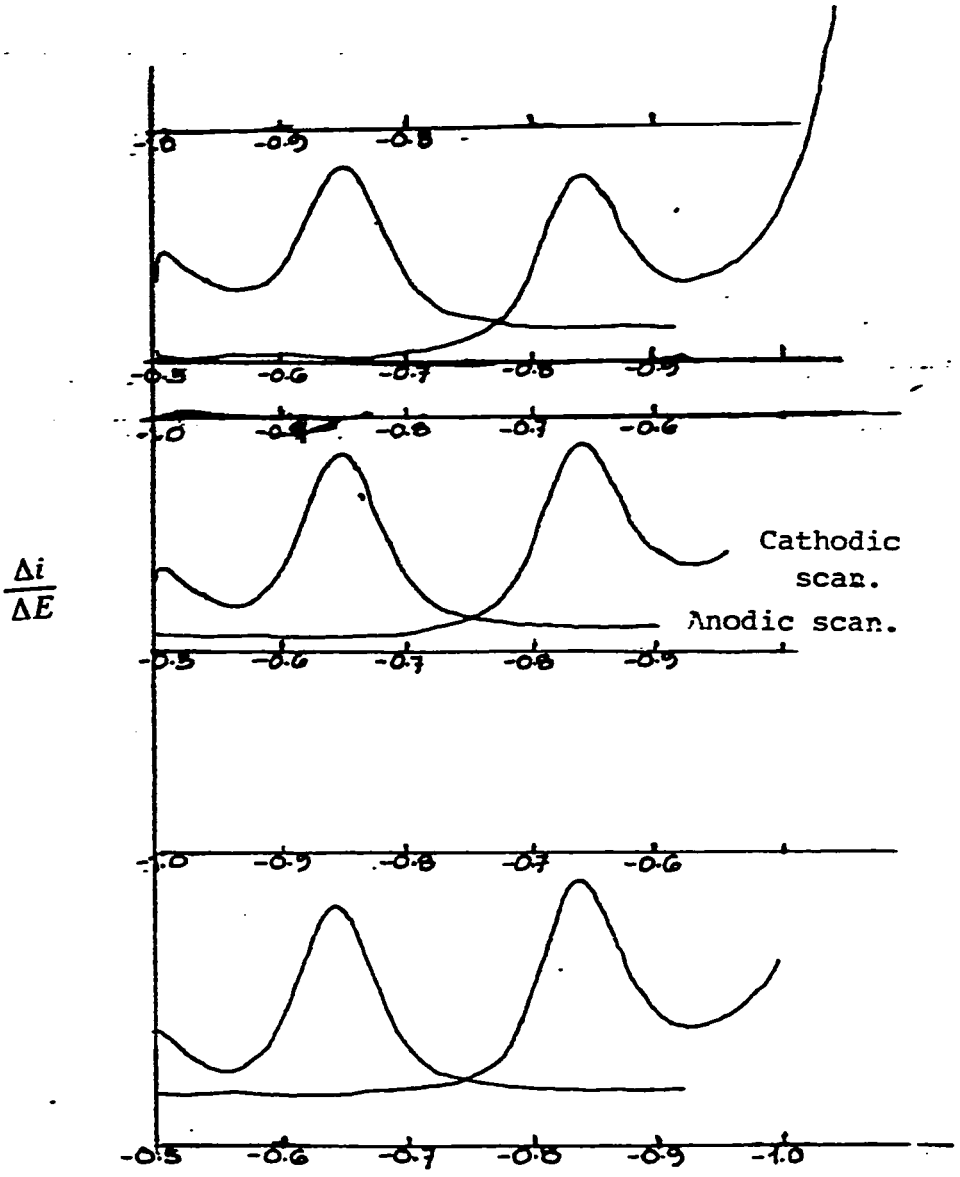


Fig. 4.19. Differential polarogram for Ampicillin trihydrate-Ni(II) complex showing cathodic and anodic scans as a test for reversibility.

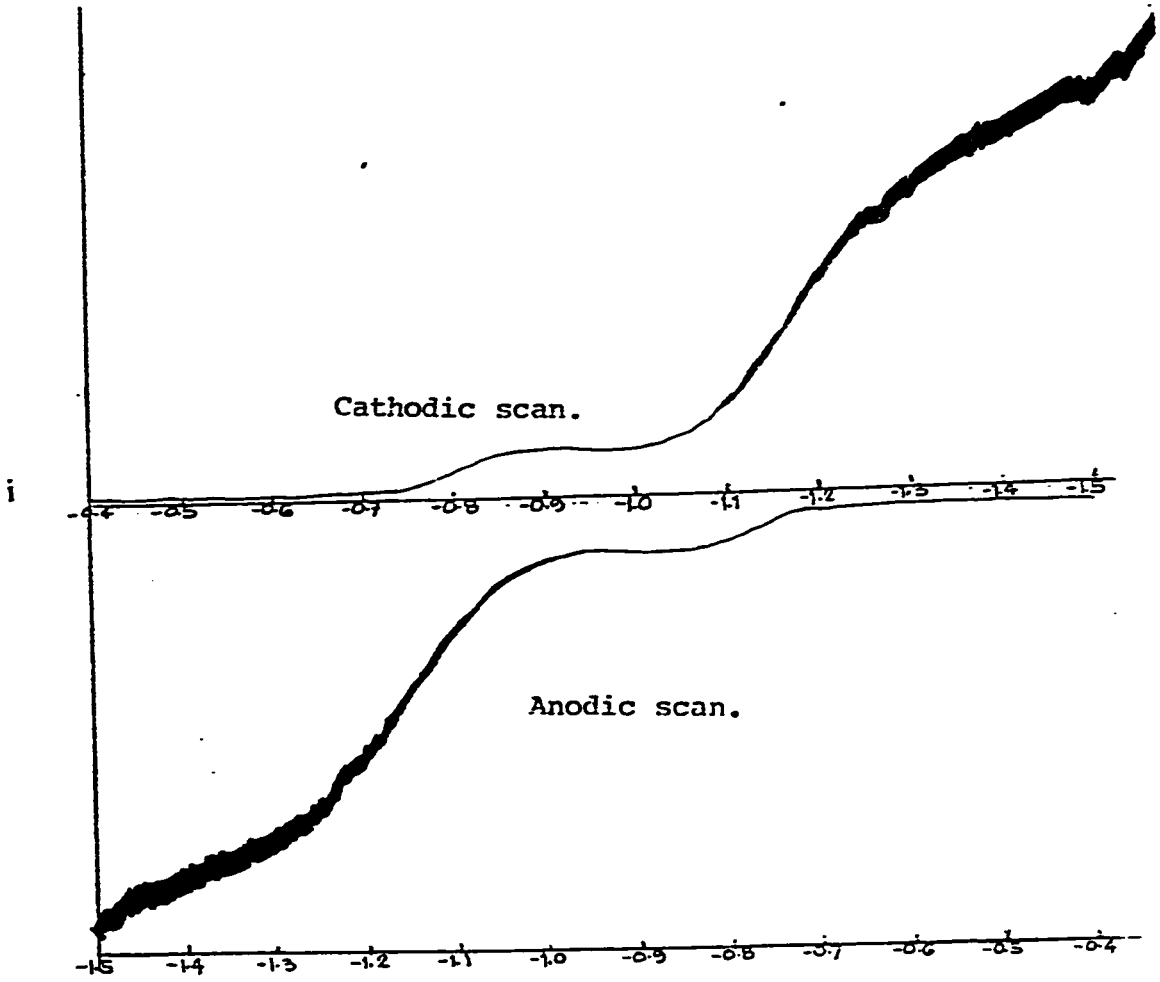


Fig. 4.20. Direct current polarogram for Ampicillin trihydrate-Ni(II) complex showing cathodic and anodic scans as a test for reversibility.

No.	Cathodic peak ht.cm	Anodic peak ht.cm	Cathodic/Anodic
1	3.40	3.30	1.03
2	3.00	3.10	0.97
3	2.70	2.65	1.02
4	2.30	2.40	0.96
5	2.10	2.15	0.98
		mean =	0.99
		SD =	0.03

Table 4.8 Data for the reversibility test of Ampicillin trihydrate Ni(II) complex, showing the ratio of peak height of the cathodic and anodic differential scans.

No.	Cathodic peak ht.cm	Anodic peak ht.cm	Cathodic/Anodic
1	1.55	1.55	1.00
2	1.90	1.90	1.00
3	1.65	1.70	0.97
4	1.45	1.60	0.91
5	1.40	1.40	1.00
6	1.20	1.30	0.92
		mean =	0.97
		SD =	0.04

Table 4.9.Data for the reversibility test of Amoxycillin trihydrate Ni(II) complex, showing the ratio of peak height of the cathodic and anodic differential scans.

(*a*)

Trial #	$E_c - E_a = (dE) mV$
1	20
2	10
3	10
4	23
5	20

(*b*)

Trial #	$E_c - E_a = (dE) mV$
1	10
2	10
3	10
4	14
5	13
6	15
7	17

Table 4.10: Data for the reversibility test of (a) Ampicillin trihydrate (b) Amoxycillin trihydrate Ni(II) complex , showing the difference in peak potentials between the cathodic and anodic differential scans using 25 mV modulation or pulse amplitude.

(*a*)

type of scan	$E_{1/2}$ (V)
Cathodic scan Anodic Scan	- 0.806 - 0.755

(*b*)

type of scan	$E_{1/2}$ (V)
Cathodic scan Anodic Scan	- 0.932 - 0.900

Table 4.11: Data for the reversibility test of (a) Ampicillin trihydrate (b) Amoxycillin trihydrate Ni(II) complex , showing the $E_{1/2}$ values for the cathodic and anodic Dc-scans.

According to the nearest equation for a totally reversible system,

$E_{applied} = E_{1/2} - (0.0591/n) \log(i/(i_d - i))$.

where,

Eapplied = potential applied

$E_{1/2}$ = Potential at half the diffusion current

i = current at the applied potential

eq i sub d = diffusion current

n = number of electrons involved in the electrode reaction.

Then a plot of $E_{applied}$ vs $\log(i/(i_d - i))$ gives a slope of - 0.0591 / n.

In this test solutions of Ampicillin and Amoxycillin containing 1 M sodium acetate and 1.00×10^{-2} *M* Ni(II), were prepared. These solutions were scanned in the DC-Scan mode cathodically from -0.4 to 1.1V at scan rate of 2 mV^{-1} modulation amplitude 10 mV drop time 0.5s and sensitivity of 20 *micro* A. Here only the D.C. scan for Amoxycillin is shown ,figure (4.21). The results obtained from these tests are given in tables (4.12 , 4.13) for Ampicillin and Amoxycillin respectively. From the plots of the results obtained (Refer to tables 4.12 and 4.13), the slopes for Ampicillin and Amoxycillin - NI(II) complexes were found to be -0.0443 and -0.0600 respectively.

CALCULATIONS

Slope n = - 0.0591 / slope.

Therefore,

n for Ampicillin = - 0.0591 / - 0.0565 = 1.05

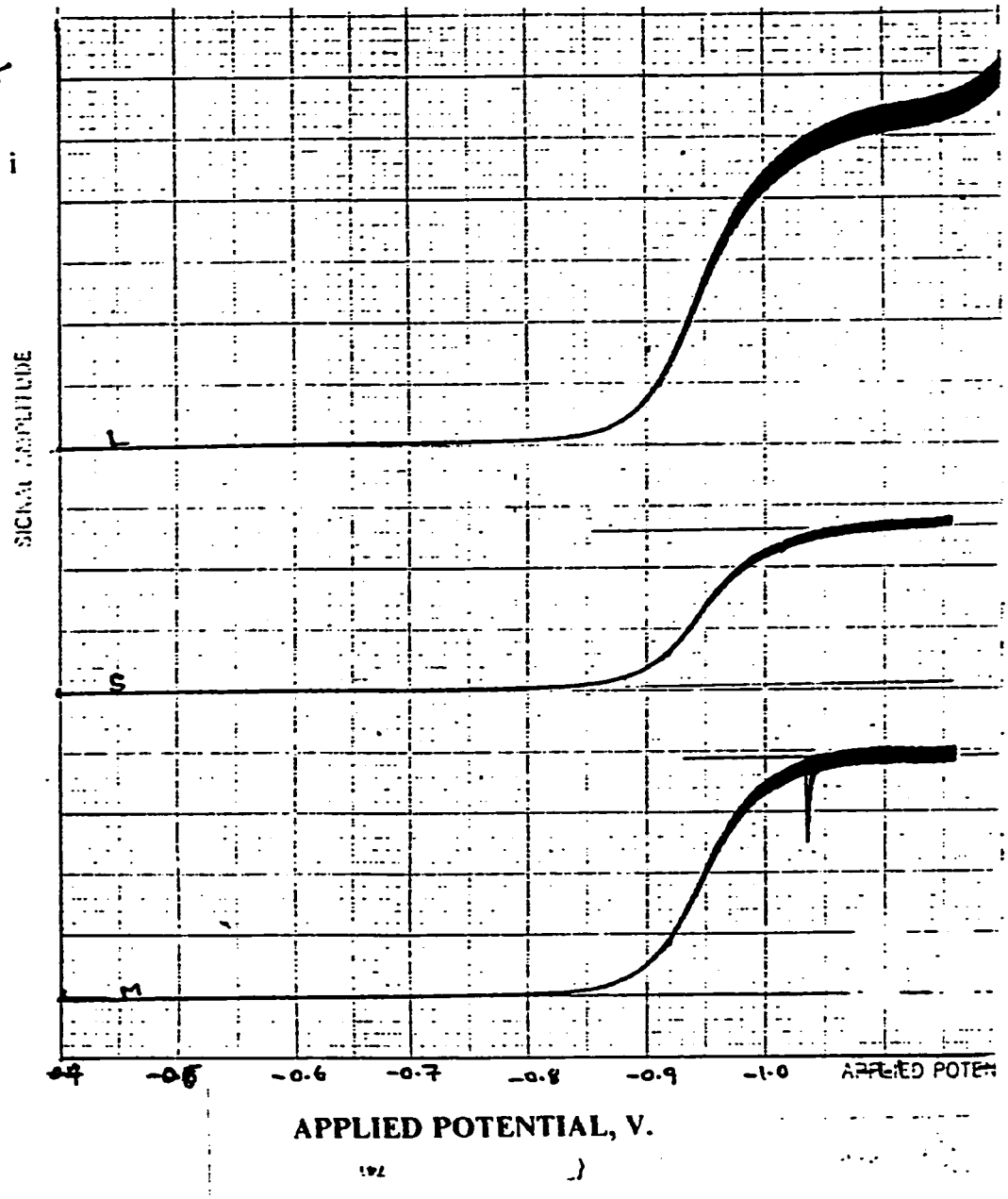


Fig. 4.21. DC polarogram for Amoxycillin trihydrate-Ni(II) complex showing scans with different drop sizes for the calculation of the number of electrons involved in the electrode reaction.

i_d = 6.40 *mm*

No	$E_{applied}$	i (mm)	$i_d - i$ (*mm*)	log ($\frac{i}{(i_d - i)}$)
1	-0.74	12.80	51.20	-0.60
2	-0.77	26.50	37.50	-0.15
3	-0.79	38.50	25.50	0.18
4	-0.82	55.00	9.00	0.78
5	-0.84	62.00	2.00	1.49

Table 4.12: Data for the plot of log ($\frac{i}{(i_{d-i})}$) *vs* $E_{applied}$ (V) **for Ampicillin Ni(II) complex.**

$i_d = 6.40\ mm$

No	$E_{applied}$	i (mm)	$i_d - i\ (mm)$	$\log\left(\frac{i}{(i_d - i)}\right)$
1	-0.90	0.65	4.25	-0.82
2	-0.92	1.10	3.80	-0.54
3	-0.95	2.50	2.40	0.08
4	-0.97	3.80	1.10	0.54
5	-1.00	4.30	0.60	0.86
6	-1.20	4.60	0.30	1.18

Table 4.13: Data for the plot of $\log\left(\frac{i}{(i_d - i)}\right)$ *vs* $E_{applied}$ (V) **for Amoxycillic Ni(II) complex.**

n for Amoxycillin = -0.0591 / -0.0600 = 0.99

4.3.3. (g) Interference test for Ampicillin trihydrate and Amoxycillin trihydrate.

In this test, solutions containing different ratios of concentrations of the standard form and capsules were prepared. The first solutions containing the standard form and capsules separately were scanned. This was then followed by scanning solutions containing different ratios of standard form to capsules. Then the effect of the fillers in the capsules on the standard form solutions were seen. The test was carried out in this manner because of the lack of knowledge of the fillers present in the capsules. The results are tabulated in tables (4.14, 4.15) for Ampicillin and table (4.16) for Amoxycillin.

CALCULATIONS:

h_s = Peak height of standard alone

h_c = Peak height of capsule alone

h_{cs} = Calculated height for standard

h_{cc} = calculated peak height for capsules.

Total volume = 25.0 ml in all the cases.

Then for solutions containing 15.0 ml standard and 10 ml capsule.

h_{cs} = (15.0 / 25.0) x 132.2 = 79.3 mm

h_{cc} = (10.0 / 25.0) x 145.0 = 58.0 mm.

Total height calculated = 79.3 + 58.0 = 137.3 mm

Peak ht. (mm) for Standard alone	Peak ht. (mm) for capsules alone	Peak ht. (mm) for 15ml.std. & 10 ml capsules	Peak ht. (mm) for 10ml.std & 15 ml capsules	Peak ht. (mm) for 5ml std. & 20 ml capsules.
132	145	139	139	141

Table 4.14 Data for the interference test of Ampicillin Trihydrate.

	Solution containing 15ml std & 10ml caps.	Solution containing 10ml std & 15 ml. caps.	Solution containing 5ml std & 20 ml.caps.
Calculated value of peak ht(mm)	137	140	142
Found value (mm)	139	139	141

Table 4.15 Summary of data of interference test of Ampicillin trihydrate Ni(II) complex.

	Solution containing 15ml std & 10ml caps.	Solution containing 10ml std & 15 ml. caps.	Solution containing 20ml std & 5 ml.caps.
Calculated value of peak ht(mm)	123	119	127
Found value (mm)	124	119	124

Table 4.16 Summary of data of interference test of Amoxycillin trihydrate Ni(II) complex.

Total height found = 139.0 mm

The calculations for the interference test of Amoxycillin trihydrate was carried out in the same way as that of Ampicillin trihydrate.

4.3.4. Procedure for Quantitative Assay

All glass-ware (volumetric flasks, funnels, pipets, burets, etc.) and spatulas were cleaned thoroughly and if necessary with chromic or nitric acids and dried before they were used. Where water is mentioned, distilled deionized water is implied. Solutions of standard drugs and capsules were prepared fresh every time before use.

a) Ampicillin trihydrate.

Solutions of standard Ampicillin trihydrate, Nickel sulphate and sodium acetate were prepared in such a way that in the final volume (10ml) in the polarographic cell, the solutions had concentrations of Ampicillin trihydrate in the range $10^{-7} - 10^{-4}$ Nickel Sulphate 1.00×10^{-2} *M* and sodium acetate 1 M. For Ampicillin trihydrate, this was done by first preparing a solution in the 10^{-1} *M* range by direct weighing of the solid on a sensitive balance and transferring it to a 100 ml volumetric flask using a funnel and filling the flask with water to the mark. Mixed the contents thoroughly to dissolve all of the solid. Then solutions to give the required range were prepared by dilution. For Nickel sulphate and sodium acetate, appropriate masses were weighed to give 0.1M and 2 M in 100 ml respectively.

Once that had been done, 4 ml of Ampicillin trihydrate, 5 ml of 2M sodium acetate and 1 ml of Nickel sulphate solutions were transferred using pipets to the polarographic cell. Then the pH was recorded to make sure that it was that required (pH8). After 6 minutes, nitrogen was bubbled through the solution for 4 minutes. Then the voltage was scanned from -0.4 to -1.0V (vs Ag/AgCL) with a modulation amplitude of 25 mV, drop time 0.5s,scan rate 2 mVs^{-1} and sensitivity of 20 *micro* A,fig.(4.23). The differential polarogram in fig (4.22) show the nature of the Ampicillin-Ni(II) complex wave and the free Ni(II) ion wave.

The sample solutions were treated in the same way as the standards. The results obtained are tabulated in table (4.17) for the standard (pure) Ampicillin and table (4.18) for Ampicillin capsules. Then a graph of log concentration vs log peak heights was plotted to calculate the % of active substance in the samples, fig.(4.24) and the results thus obtained are summarized in table (4.19).

CALCULATIONS

From table 4.18, Actual concentration of capsule solutions and the obtained were:

Sol # 1 - prepared $= 2.870 \times 10^{-6} M$

obtained $= 2.880 \times 10^{-6} M$

% of active substance =

$(2.890 \times 10^{-6}/2.870 \times 10^{-6}) \times 100$

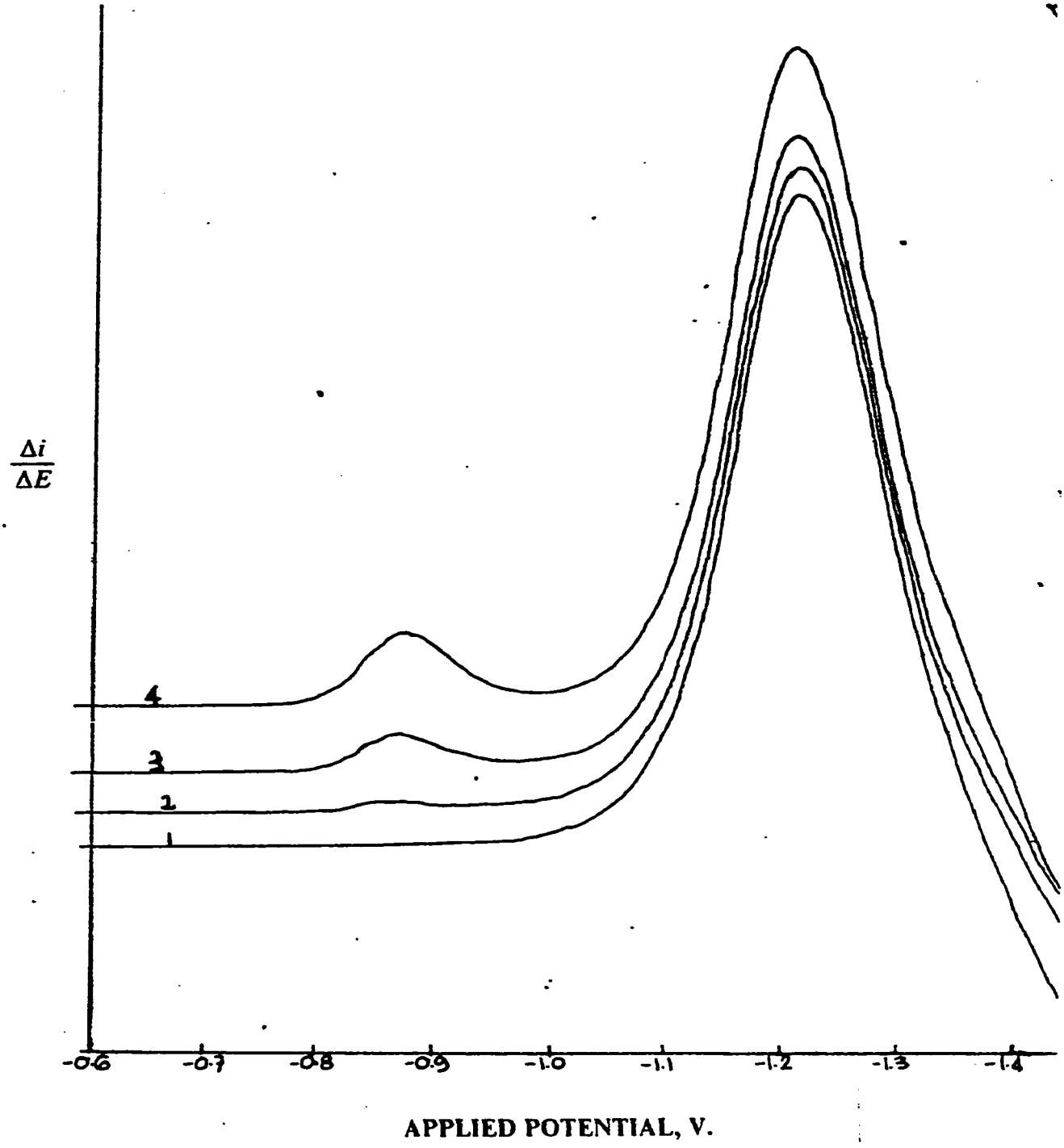


Fig. 4.22. Nature of Ampicillin trihydrate-Ni(II) complex and free Ni(II) ions differential peak at conc.: Ni(II) 10^{-2}*M, sodium acetate 1 M,Ampicillin (1) 0 (2)* 4.00 × 10^{-6} *(3)* 1.50 × 10^{-5} *(4)* 3.20 × 10^{-5}.

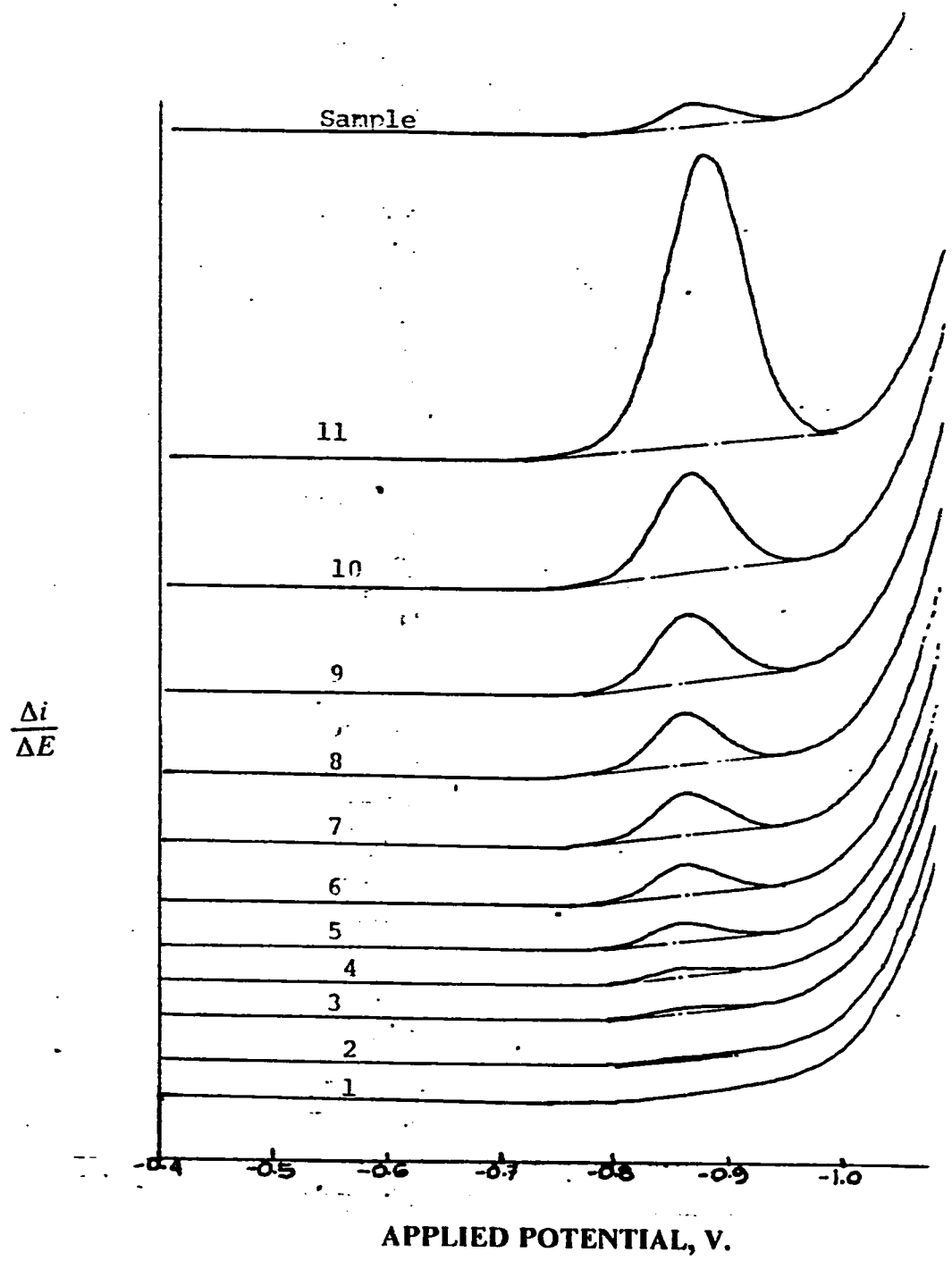


Fig. 4.23. Differential polarogram of, $10^{-2}M$ *Ni(II) in 1.0 M Sodium acetate. Ampicillin trihydrate concentrations in the range* $1.39 \times 10^{-7} - 3.47 \times 10^{-5}M$.

No.	Concentrations(M) $\times 10^{-7}$	Log Conc.	Peak height (h) cm.	log h.
1	1.39	-6.86	0.01	-2.00
2	2.77	-6.56	0.05	-1.30
3	6.94	-6.16	0.10	-1.00
4	13.90	-5.86	0.22	-0.66
5	27.70	-5.56	0.45	-0.35
6	41.60	-5.38	0.70	-0.15
7	55.50	-5.26	0.95	-0.02
8	69.40	-5.16	1.19	0.08
9	83.20	-5.08	1.49	0.17
10	139.0	-4.86	2.20	0.34
11	347.0	-4.46	6.35	0.80
12	1460.0	-3.33	13.20	1.12

Table 4.17. Concentrations (M) Vs Peak heights (h) cm for Standard Ampicillin Trihydrate Concentrations in the range of $1.39 \times 10^{-7} - 4.65 \times 10^{-7}$ **M.**

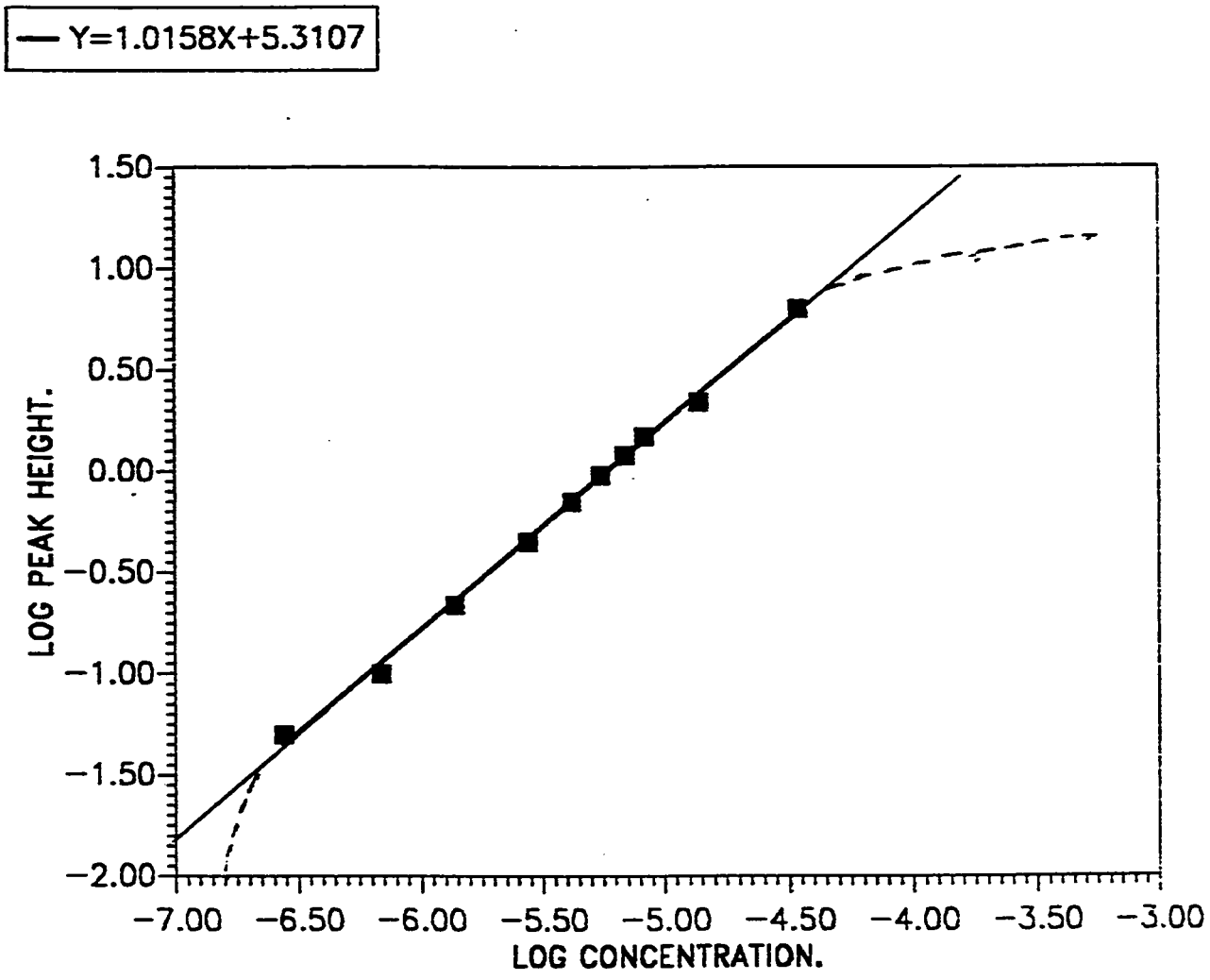


Fig. 4.24. Calibration curve for log Ampicillin vs log peak height.

No.	Actual Concen. (M) prepared. $\times 10^{-7}$	Peak height (cm)	Conc. obtained from fig (4.24) $\times 10^{-7}$
1	28.70	0.48	28.8
2	42.00	0.71	41.6
3	32.00	0.54	32.1

Table 4.18 Concentrations of Ampicillin Trihydrate capsules vs. Peak heights cm.

Solution No.	% of Active Substance
1	100.3
2	99.0
3	100.4
	Mean = 99.9%
	SD = 0.7

Table 4.19 Summary for the results obtained for calculations of % of active substance in Ampicillin trihydrate capsules.

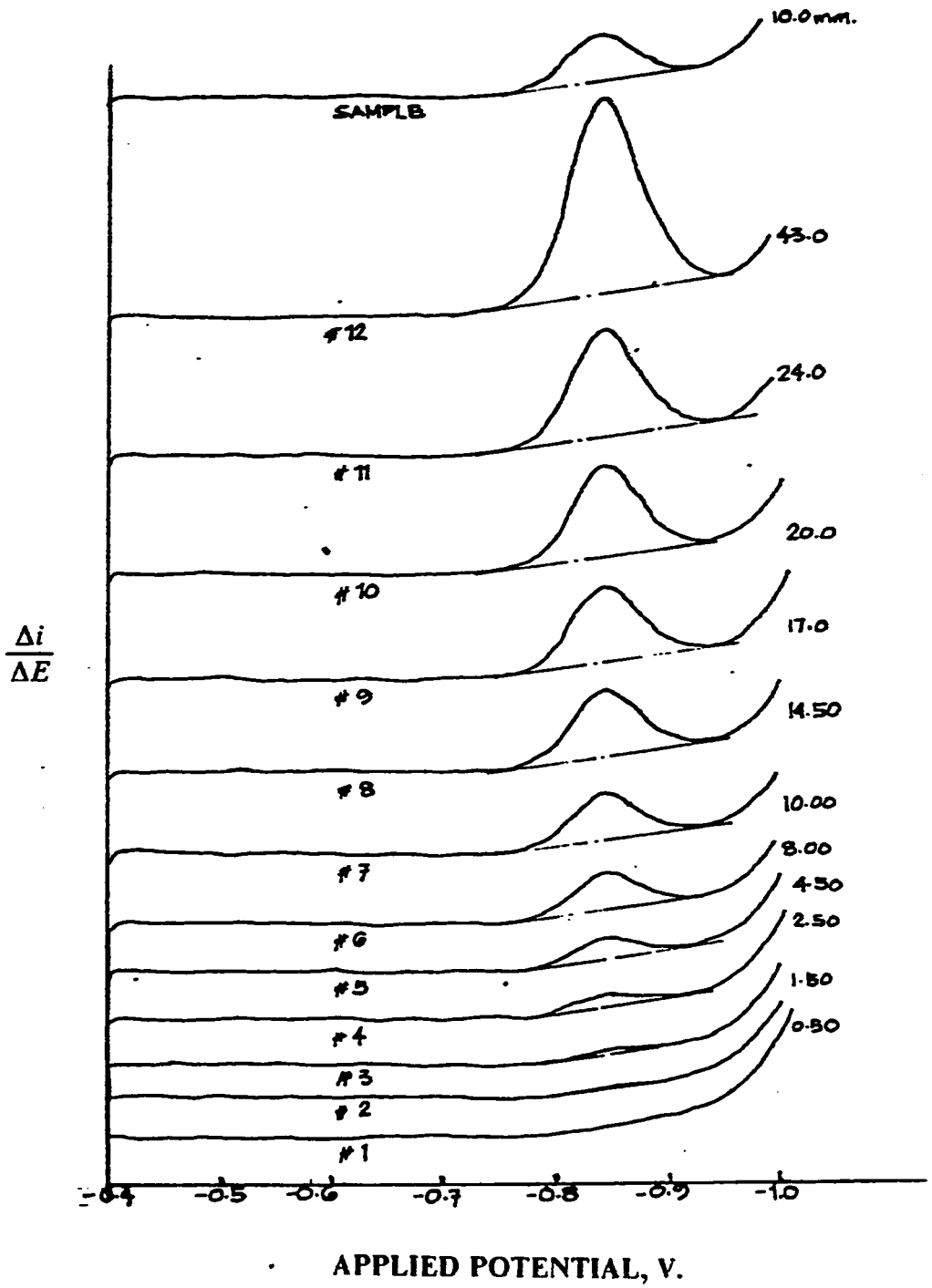


Fig. 4.25. Differential polarogram of, $10^{-3}M$ Ni(II) in 1.0 M Sodium acetate. Amoxycillin trihydrate concentrations in the range $2.47 \times 10^{-7} - 1.55 \times 10^{-5}M$.

No.	Concentrations(M) $\times 10^{-7}$	Log Conc.	Peak height (h) cm.	log h.
1	3.09	-6.51	0.05	-0.30
2	6.18	-6.21	0.15	0.18
3	12.4	-5.91	0.28	0.45
4	18.5	-5.73	0.45	0.65
5	30.9	-5.51	0.85	0.90
6	43.2	-5.36	1.10	1.04
7	55.6	-5.26	1.45	1.16
8	61.8	-5.21	1.70	1.23
9	74.1	-5.13	2.00	1.30
10	92.6	-5.03	2.40	1.38
11	155.0	-4.81	4.30	1.63
12	5560.0	-3.38	14.10	2.15

Table 4.20. Concentrations (M) vs Peak heights (h) cm for Standard Amoxycillin Trihydrate Concentrations in the range of $3.09 \times 10^{-7} - 5.66 \times 10^{-3}$ *M*.

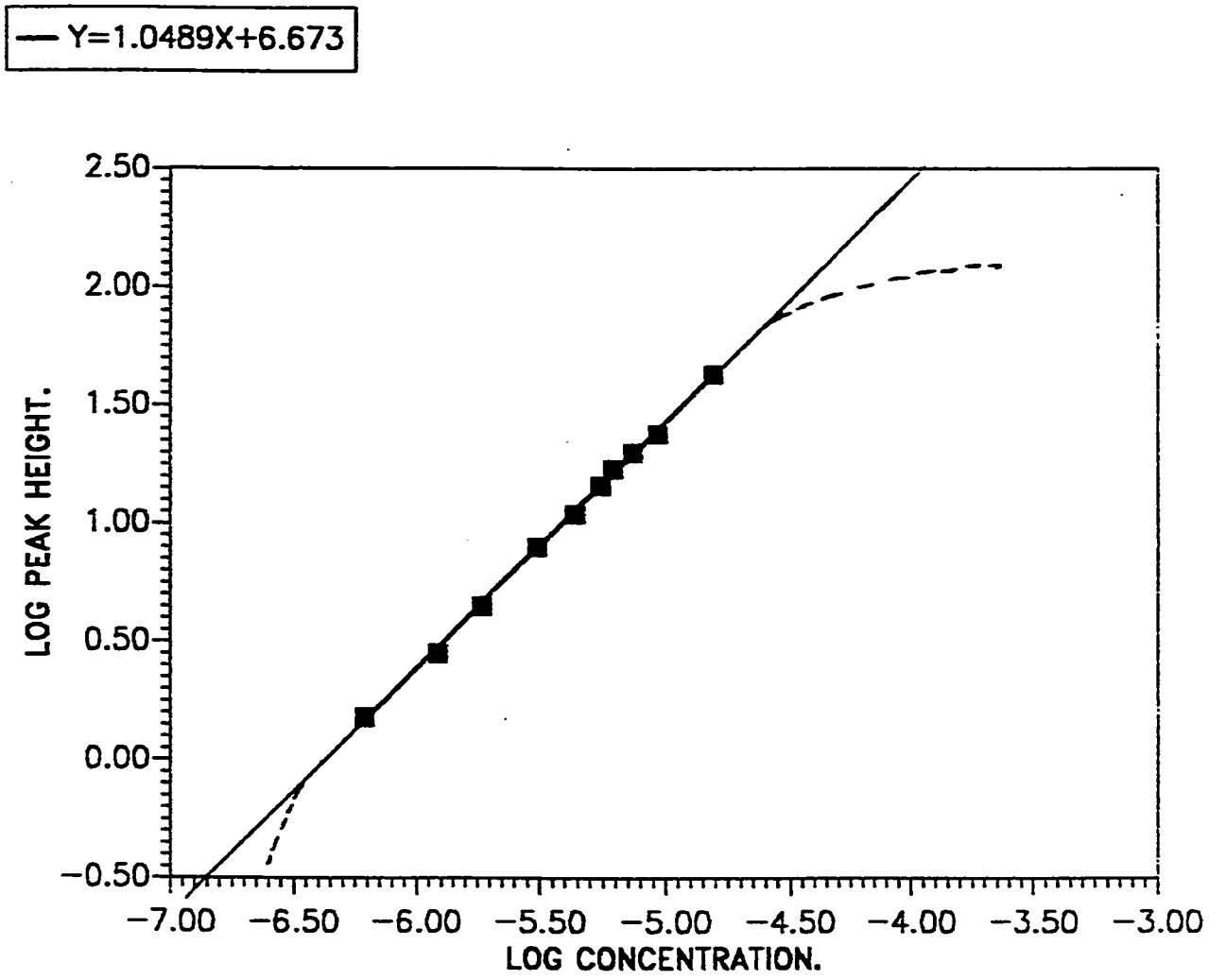


Fig. 4.26. Calibration curve for log Amoxycillin vs log peak height.

No.	Actual Concen. (M) prepared. $\times 10^{-7}$	Peak height (cm)	Conc. obtained from fig (4.24) $\times 10^{-7}$
1	36.20	0.97	37.30
2	16.50	0.42	16.85
3	44.20	1.18	45.12

Table 4.21 Concentrations of Amoxycillin Trihydrate Capsules Vs. Peak heights in cm.

Solution No.	% of Active Substance
1	103.0
2	102.1
3	102.1
	Mean = 102.6 %
	SD = 0.5

Table 4.22 Summary for the results obtained for calculations of % of active substance in Amoxy. capsules.

$= 100.0 \pm 0.5.$

Similarly for solutions 2 and 3, % of active substance was found to be 99.0%, 100.4% respectively.

b) Amoxycillin Trihydrate

The same procedure was carried out as that for the Ampicillin Trihydrate, i.e., the same range of concentrations of standard Amoxycillin trihydrate, the same Nickel, sulphate and sodium acetate concentrations, and other conditions like pH and instrumental settings, fig (4.25). The results obtained are given in tables (4.20) for the standard Amoxycillin and (4.21) for the sample solution (capsules). The plot of the results to obtain the % of active substance in the capsules is shown in fig (4.26).Table (4.22) summarizes the results of % of active substance in Amoxycillin capsules.

4.4.2 Quantitative Assay for Amoxycillin based on the 2, 5 diketo piperazine derivative polarographic peak.

In this assay, 15.0 ml portion of aqueous solution containing between 0.100 and 0.150 g of standard Amoxycillin was pipetted into a 50 ml standard flask containing 2.5 ml of 1 M sodium hydroxide. Ten minutes later 2.5 ml of 1 M hydrochloric acid were added, followed by dilution to volume with pH 5 citrate buffer containing 1% formaldehyde.

Samples (capsules) were treated in the same way as the standards. Then the standard and sample solutions were heated at 100°C for 30 minutes, allowed to

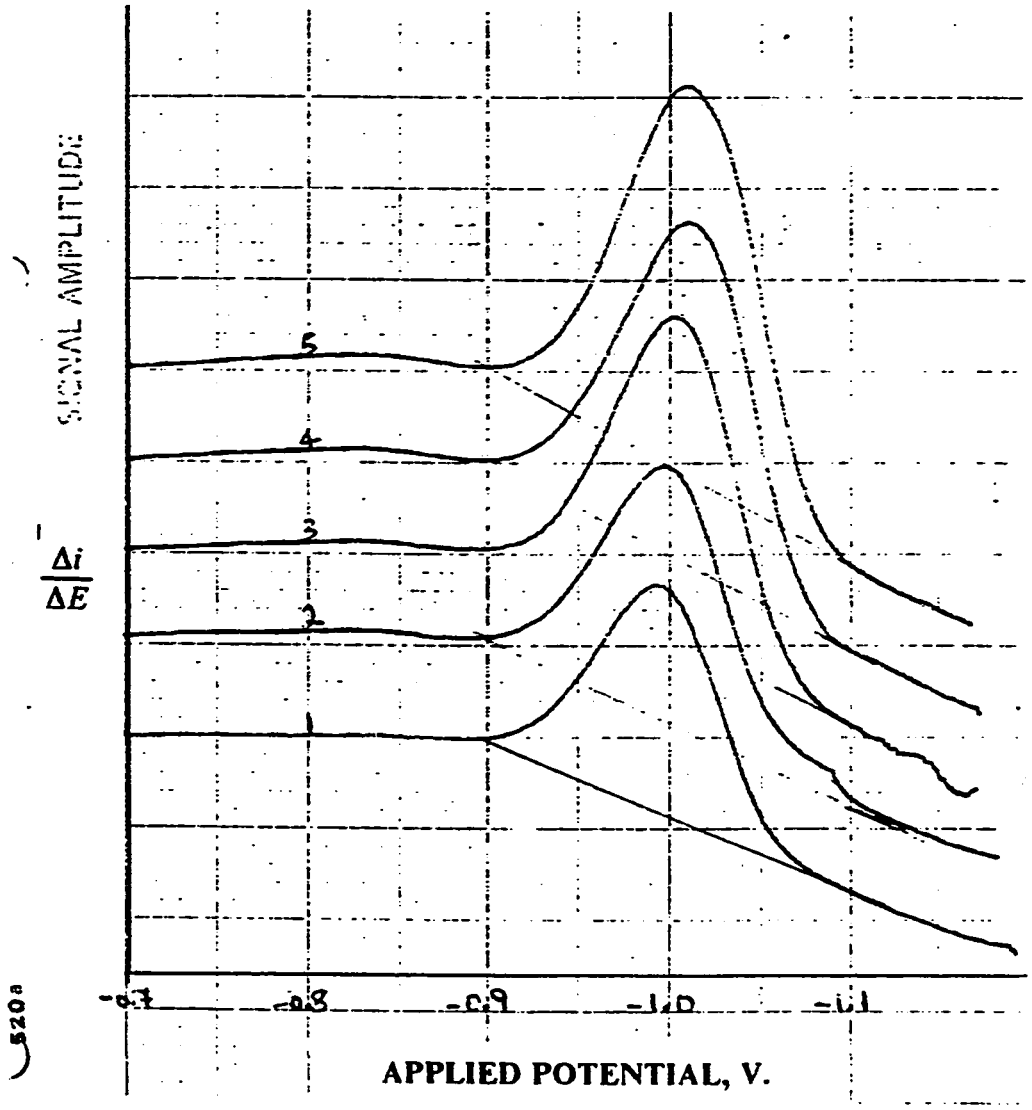


Fig. 4.27. Differential polarogram of Amoxycillin for the polarographic assay. baesd on 2,5-diketopiprazine derivative wave.

No.	Conc. g/50ml.	Peak Height Cm.
1	0.104	3.10
2	0.110	3.50
3	0.123	4.40
4	0.129	4.70
5	0.143	5.50

Table 4.23: Data for standard Amoxycillin concentrations and their corresponding peak heights for the assay based on 2,5 dikitopiperazine derivative peak.

Sample peak ht. cm	Prepared conc. g/50 ml.	Obtained conc. g/50 ml from fig 4.28	% of Active substance
5.60	0.140	0.144	103
4.20	0.113	0.118	104
4.30	0.128	0.129	101
			Mean = 103%
			SD = 1

Table 4.24: Data Summarizing the results obtained for the 2.5 disketo piperazine derivative, peak based, Amoxycillin assay.

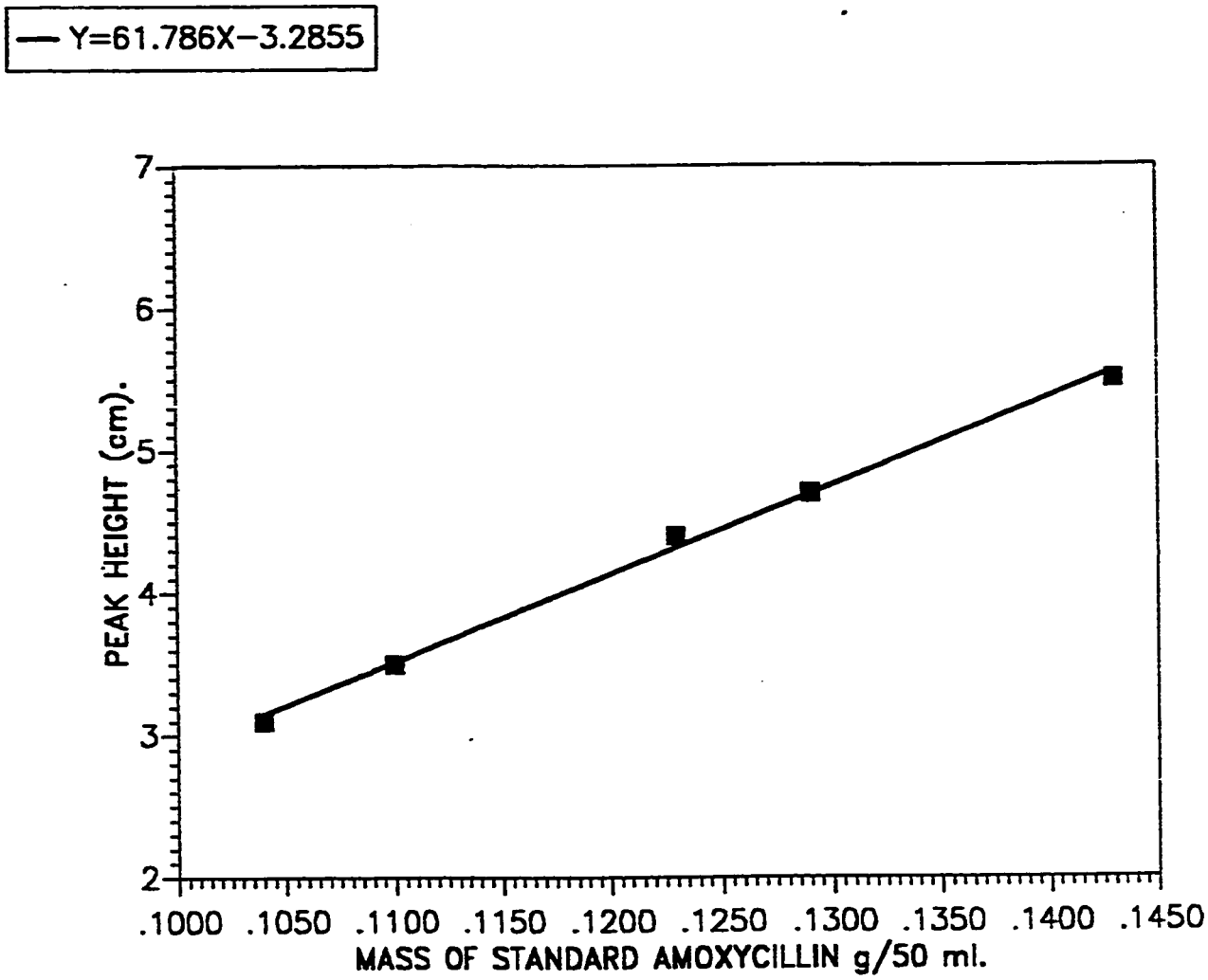


Fig. 4.28. Calibration curve for Amoxycillin : Assay based on 2,5-diketopiperazine derivatives.

cool and then the strongly fluorescent yellow product thus formed was analysed polarogrphically. This was done by purging the sample for 10 minutes. Then scanned from -0.7 to -1.1V (vs Ag/AgCL) at scan rate of 2 mVs^{-1}, drop time 0.5 s , modulation amplitude of 10 mV and sensitivity 100 *micro* A, fig (4.27). The results obtained are given in tables (4.23,4.24). Figure 4.28 shows the calibration curve used in calculating the % of active substance in the sample solutions.

4.5.6: *Quantitative Assay of Cephalexin based on Ni(II) and Co(II) Complex.*

This assay was based on work done on cephalexin [70], in which two sets of solutions of cephalexin at a concentration of $6.75 \times 10^{-7} - 6.17 \times 10^{-6}$ *M* (a) containing 1 M sodium acetate and 1.00×10^{-2} *M* Ni(II) (b) containing 1 M sodium acetate and 1.00×10^{-2} *M* Co(II), were prepared. These solutions were then analyzed polarographically. This was done by purging them after 6 minutes for 4 minutes, then scanning them from -0.5 to -1.1V for Ni(II) system and -0.8 to -1.3V for the Co(II) system at scan rate of 2 mVs^{-1}, drop time 0.5 sec and modulation amplitude of 25 mV. The differential polarograms for the Cephalexin-NI(II) wave and the Cephalexin-Co(II) wave are shown in figures (4.29,4.30) respectively. The results obtained are given in tables (4.25, 4.26). Figures (4.31, 4.32) show calibration curves for the calculations of the % of active substances in the sample solutions. But since the purity of standard is 92.4%, then the mean % 0f the Cephalexin in the capsules is = 107% ±1 ,and the Corrected % of active substance = 100% ±1 for the Ni (II) based assay

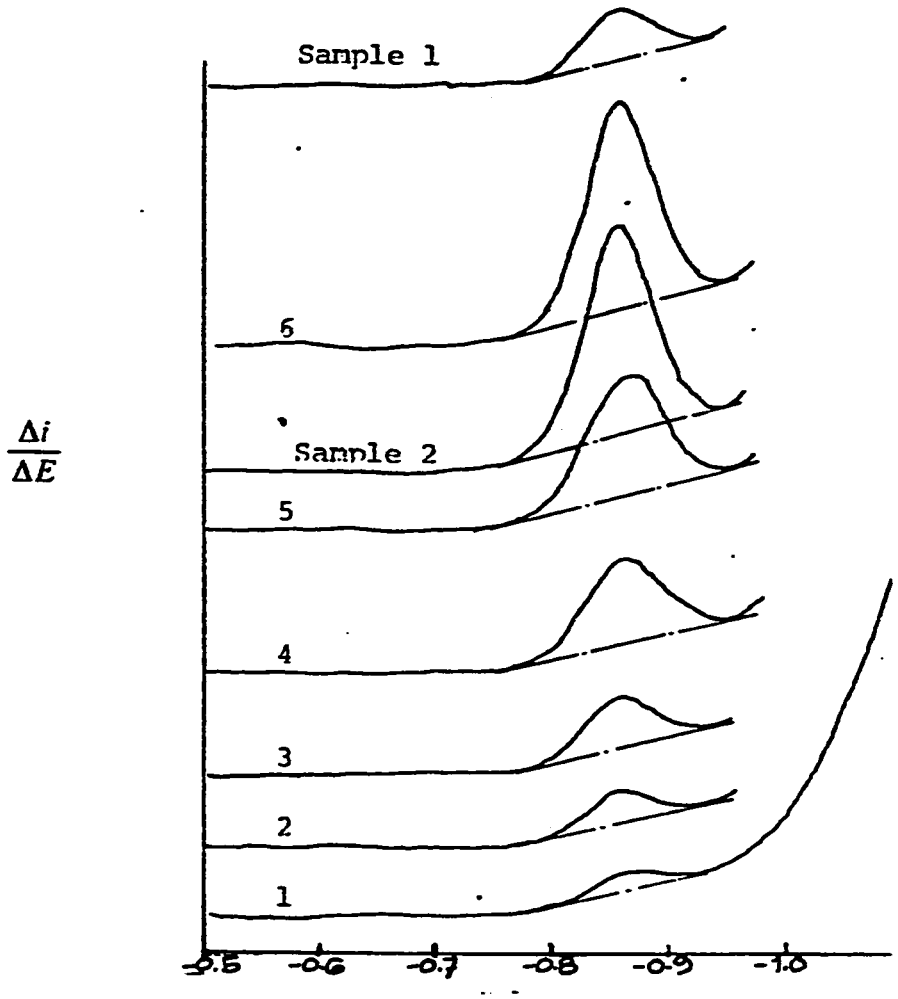


Fig. 4.29. Differential polarogram of Ni(II) Cephalexin system at different concentraitons of Cephalexin.

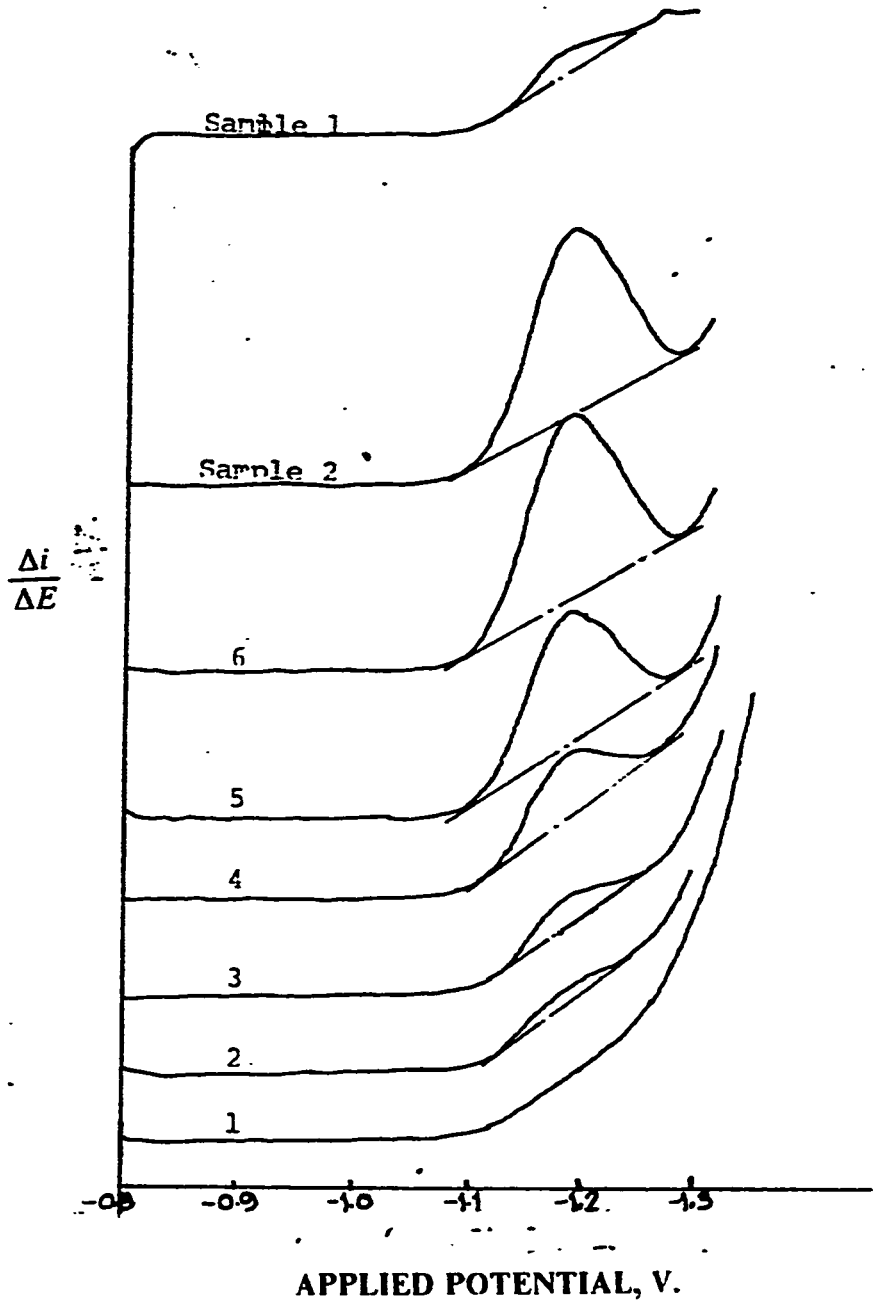


Fig. 4.30. Differential polarogram of Co(II) Cephalexin system at different concentraitons of Cephalexin.

(a)

No.	Conc. (M) $\times 10^{-7}$	Peak height (cm).
1	61.70	4.49
2	48.00	2.58
3	34.30	2.35
4	20.60	1.12
5	13.70	0.71
6	6.85	0.40

(b)

No.	Conc. (M) $\times 10^{-7}$	Peak height (cm).
1	61.70	3.85
2	48.00	2.75
3	34.30	1.41
4	20.60	0.68
5	13.70	0.35
6	6.85	0.08

Table 4.25: Data for quantitative assay of cephalexin (a) Ni(II) (b) Co(II) complex systems showing concentrations and corresponding peak heights.

(a)

No.	Sample peak ht. Cm	Prepared conc. × 10*sup*-7 (*M*).	Obtained conc. from fig.4.31 × 10^{-7} (*M*).	% of Active substance
1	4.49	57.60	67.00	116
2	1.58	21.90	25.70	117
3	3.50	46.00	52.90	115
			Mean	= 116%
			SD	= ± 1

(b)

No.	Sample peak ht. Cm	Prepared conc. × 10*sup*-7 (*M*).	Obtained conc. from fig.4.32 × 10^{-7} (*M*).	% of Active substance
1	3.95	57.60	65.70	114
2	1.16	21.90	25.70	117
3	3.00	46.00	52.00	113
			Mean	= 115%
			SD	= 2

Table 4.26: Data for the summary fo cephalex capsules (a) Ni(II) complex system (b) Co(II) complex system.

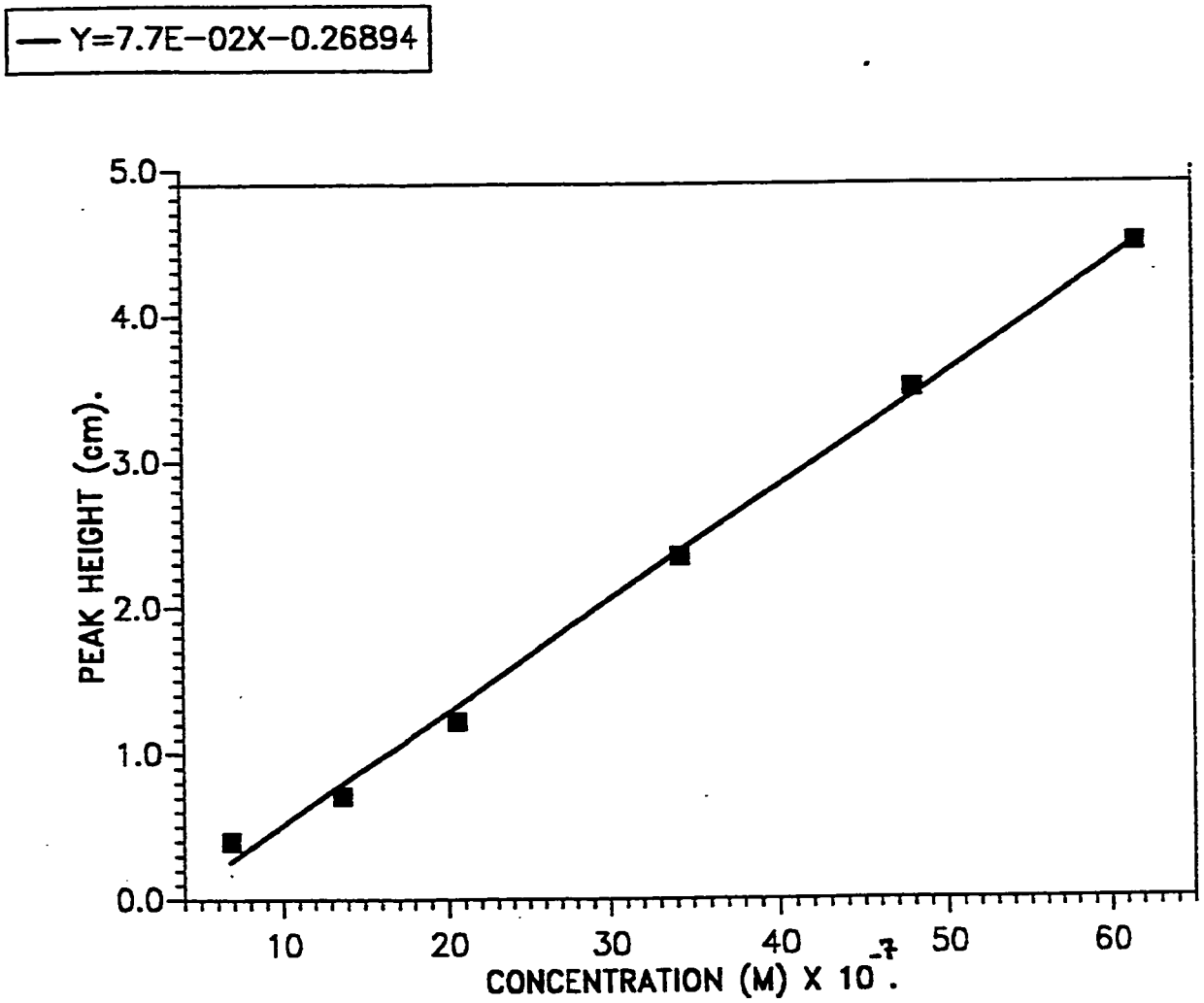


Fig. 4.31. Calibration curve of , the concentraiton of Ni(II)-Cephalexin complex vs peak height.

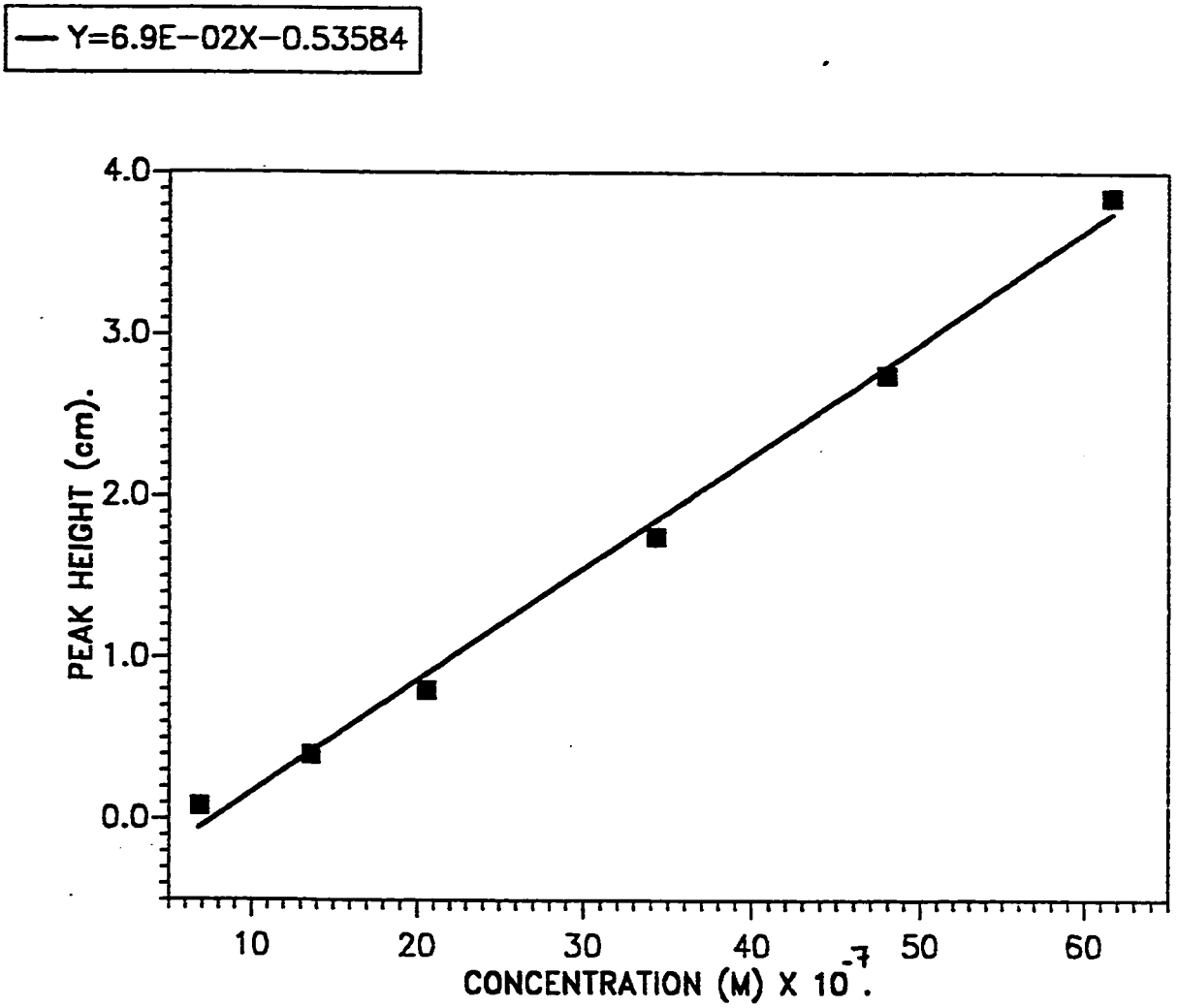


Fig. 4.32. Calibration curve of , the concentraiton of Co(II)-Cephalexin complex vs peak height.

and for the Co(II) based assay the mean % will be, 106% ±2 , and the Corrected % of active substance = 99 % ±2. (See iodometric Assay for Cephalexin - Chapter 3).

4.4.7. Reversibility test for Co(II) - Cephalexin Complex System.

The test was carried out in the same manner as for the Ampicillin Ni(II) systems. Figure (4.33) shows the differential polarogram used in carrying out the reversibility test. The results obtained are given in table (4.27).

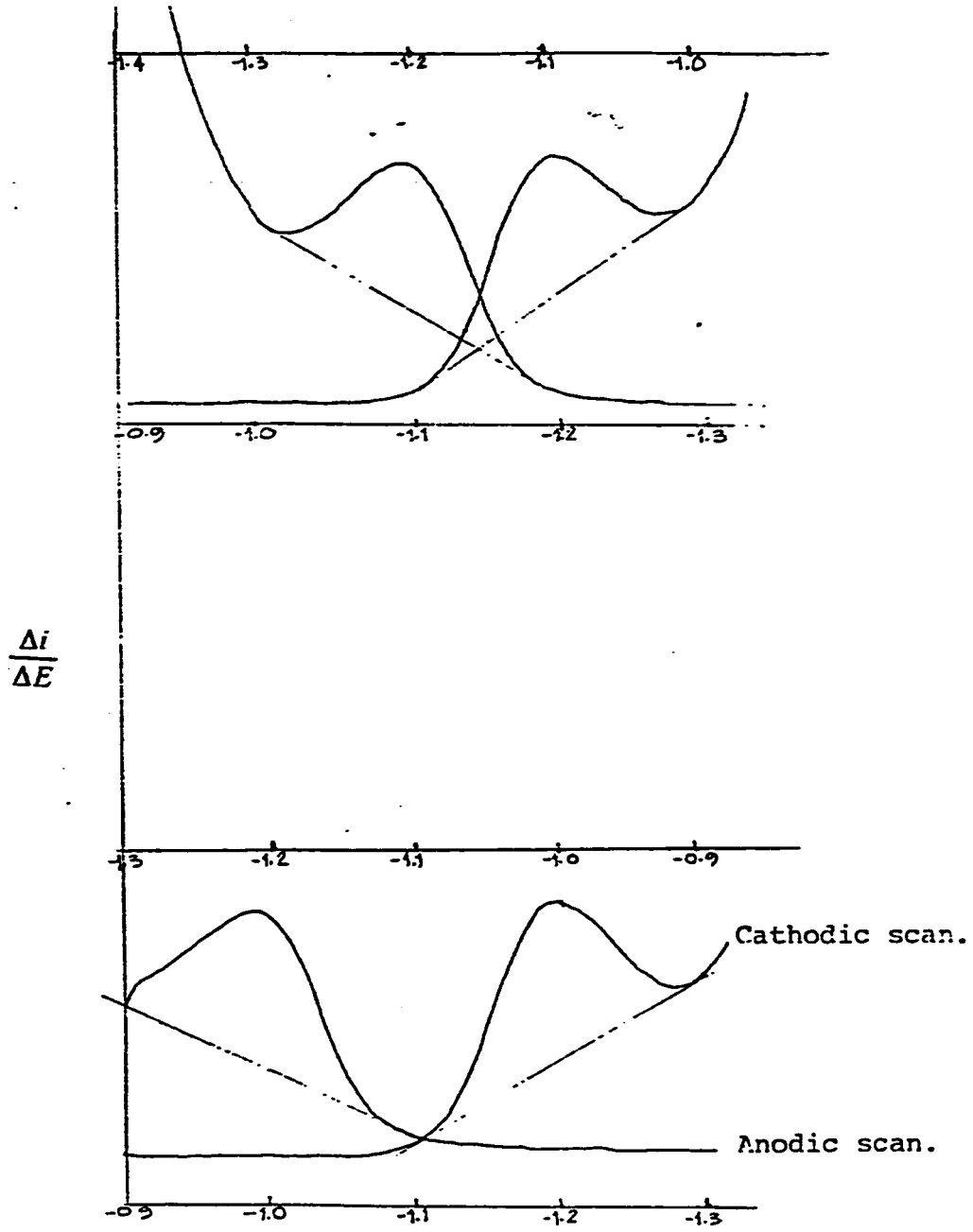


Fig. 4.33. Differential polarogram of Cephalexin Co(II) system as a reversibility test.

No.	Pulse amplitude applied (mV).	$E_c - E_a = dE(mV)$.	$\frac{I_a}{I_c}$
1	25	10	0.93
2	25	10	1.04
3	10	20	0.80
4	50	41	0.90
			Mean = 0.92 SD = 0.09

Table 4.27. Summary of the results for the Co(II) Cephalexin System:reversibility test.

CHAPTER 5

DISCUSSION

The polarographic studies on penicillin and cephalosporin antibiotics have been summarized in chapter two. The present work is based on the study of cephalexin described by Hernandez Mendez et al [70] In it an, excess of Nickel (II) is added to a buffered solution of the drug and the polarographic wave associated with a nickel (II) complex is made the basis of quantitative analysis. Another study describes the measurement of the equilibrium for the formation of the nickel (II) complex with amoxycellin and mentions that the complex can be made the basis of a quantitative method fo analysis without giving details of the procedure used. A 1:1 complex with nickel (II) was formed with nickel (II) with K = 3.3 x103 (log K = 3.52). In these studies differential pulse methods were used [73].

Optimum conditions with respect to pH, temperature buffer (and supporting electrolyte), reaction time were studied. When satisfactory working conditions were established, measurements were made to determine linearity of calibration curves, reproducibility, reversibility, number of electrons involved in the reduction process and interferences from other constituents in the capsules.

5.1. pH EFFECT

Below pH 6.0 in 1 M sodium acetate and 1 x 10-2M Ni(II), no wave was observed from a nickel complex with antibiotic present at 10-6M. Maximum wave height for the nickel complex was observed at pH 8.0 for Ampicillin and 7.9 for Amoxycillin. These results suggest that the Amoxycillin complex is slightly more stable than that of Ampicillin. The pH study could not be extended beyond (pH 8.25) because of the hydrolysis of Ni(II). The cephalexin was not studied because the data was already published. The highest sensitivity was observed at pH of 8.0 at 1 M sodium acetate [70].

In all of these three antibiotics, there is a primary amino group which can form the Betaine structure.

This amine group only becomes available for complex formation with nickel when the proton is released or otherwise displaced by the metal ion. From studies with amino acids and transition metal ions, this usually appears to take place at high pH, providing evidence that the amino group in the antibiotics is involved in complex formation.

5.2. Effect of Sodium Acetate Concentration.

In addition to acting as buffer, the sodium acetate is also the supporting eletrolyte. Acetate ion forms weak complexes with Ni(II) and therefore will be in competition with antibiotic in metal complex formation. Hence there is a need to examine the effect of acetate concentration on sensitivity. The results are

recorded in chapter four tables (4.3,4.40) and they show clearly that maximum sensitivity was obtained at 1 M sodium acetate concentration (pH 8.0 for Ampicillin and pH 7.9 for Amoxycillin.)

5.3. Effect of Time and Temperature

In a differential pulse polarogram at room temperature, a peak is observed for the Ni (II) antibiotic complex at about (-0.84V). It is followed by a large peak due to the excess Nickel (II). Obviously in dealing with thermally unstable antibiotic the time allowed for reaction and purging of the samples will have to be taken into account. The data for these studies are summarized in tables (4.5,4.6) for Ampicillin and Amoxycillin.

In all the studies, the reaction mixtures were left 15 minutes before purging to remove oxygen and starting the polarographic scans. The data shows that upto 15°C, the peak heights did not change with time. As the temperature were taken higher the change in peak height with time becomes more marked. The peak heights appear to decrease exponentially with time. This is what would be expected if the nickel (II) complex is undergoing degradation. Direct evidence for Chemical transformation is observed from the appearance of a new polarographic wave approximately (-0.68V). This wave becomes evident above 35°C.

Tests were conducted to establish the origin of the new wave. This was done by taking a sample of Ampicillin, treating it with sodium hydroxide under

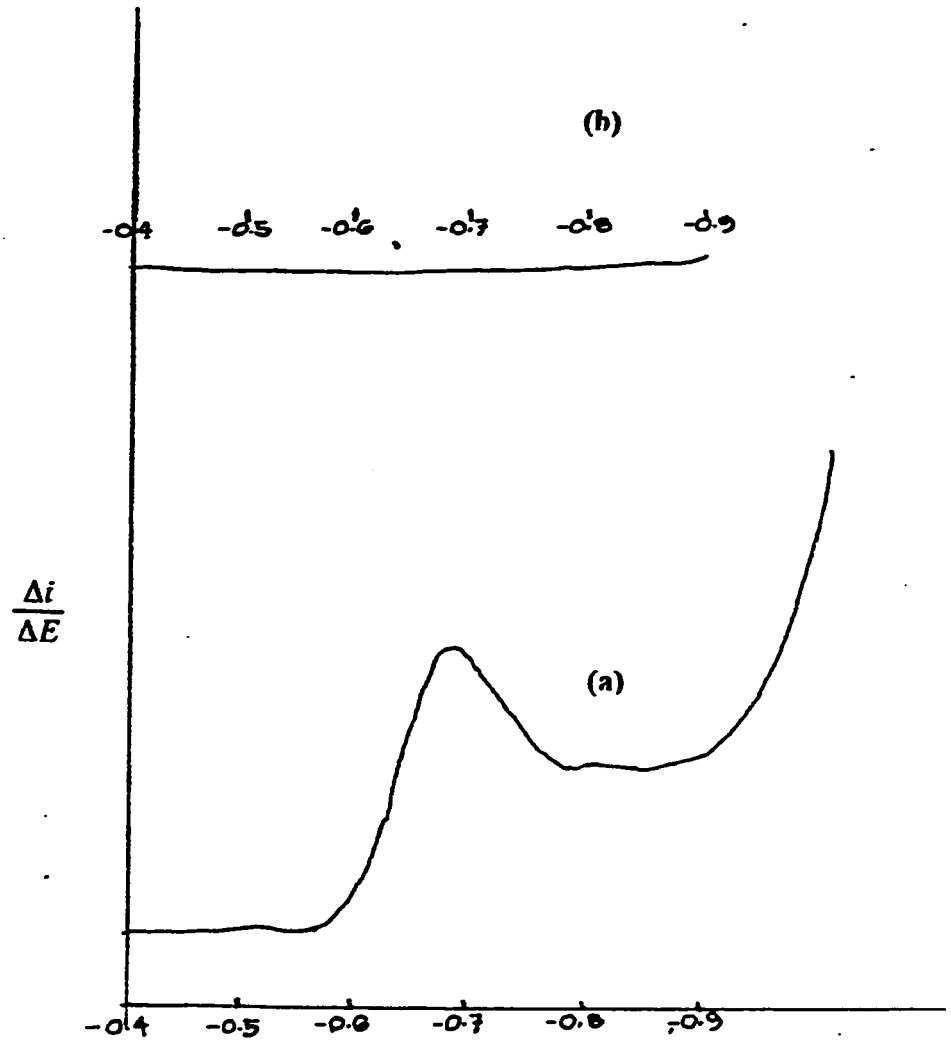


Fig.5.1: Test for the nature of the new peak appearing at temperatures above 35° C. (a) treated Antibiotic complexed with Ni(II). (b) uncomplexed Antibiotic.

conditions leading to penicilloic or penilloic acid. The pH was adjusted by addition of acetic acid to 7.0, Nickel (II) added and polarograms obtained in the usual way,fig. (5.1). As is seen from figure 5.1, a new wave peak maximum at -0.68V was obtained. This wave occurs at the same position as the waves developing in the scans at higher temperatures following the standard procedure. This results confirm that the nickel antibiotic complex is transormed to a Ni(II) penicilloic acid or Ni(II) penilloic acid complex. The nickel (II) is involved in the formation of the peak because in its absence no peak is observed. (See fig. 5.1).

The ambient temperature around (25°C) is the most convenient for routine analysis. Therefore, some additional studies were performed at this temperature. The reaction was allowed to proceed for only five minutes before purging and scanning. The results,(figure 4.18) show that the peak height decreases steadily under these conditions particularly in the case of Amoxycillin. Therefore, in order to obtain good reproducibility and minimize the time of analysis, it is desirable to follow a routine in whcih timing within 1minute for each of the steps (1) reaction time (2) purging (4 minutes) and (3) scanning.

5.4. Reproducibility Test

Reproducibility test were performed using the penicillin drugs complexed with Ni(II). The results of these studies are summarized in table 5.1

Drug	Concentration.(M)	Coefficient of variation	No. of measurements
Ampicillin	2.18×10^{-6}	2.0	6
Amoxycillin	6.20×10^{-6}	2.9	6

Table 5.1 Summary of Reproducibility Tests.

It is seen that the coefficient of variation in each case is between 2-3 % and this is highly satisfactory for polarographic analysis.

5.5. Number of electrons transferred and checks on the reversibility of electrode reactions:

According to Hernandez Mendez etal [70], from their study of the Ni(II) complex of cephalexin, the electrode process involves the exchange of two electrons per mole of Ni(II) complex in the electrode process. In the work described here, the studies on the Ni(II) compexes of Ampicillin and Amoxycillin both point to the transfer of one electron per mole. The tests were performed 4 times for each system using different drop sizes and the results are all consistent with this conclusion .

5.6. Reversibility Studies:

The reversibility studies were perfromed as discussed by Birke et al [110] using the differential pulse technique both for cathodic and anodic sweeps. The ratios of peak heights obtained in these sweeps are close to unity for the Ampicillin and Amoxycillin Ni(II) systems (Refer to tables 4.8 and 4.9). The test when applied to the Co(II) cephalexin system also gave a value close to unity.(See table 4.27). The other test for reversible charge transfer namely that of the difference in the peak potentials for cathodic and anodic sweeps should be equal to the modulation potential amplitude (25mV) for the Ni(II) studies). As it is seen from the table (4.10),the values obtained for Ampicillin and Amoxycillin

vary considerably and are generally less than 25 mV. This is particularly so for Amoxycillin. The errors in making this measurements are considerable as is seen from the standard deviations. Hence the results are considered to be ambiguous and less reliable than the current ratio test. It is concluded that the electrode processes is probably reversible or at worst quazireversible for these Nickel complexes. The one electron processes deduced from the current measurements is at variance from the study of Hernandez Mendez et tal for the cephalexin complex. The reasons are not obvious from existing data. The test for reversibility involving differences in the potentials of the peak maxima (DE) in relation to voltage amplitude are recorded in table (4.10). In this case, the modulation amplitude was varied from 10 to 50 mV and (δE) measured. Again the values are lower than expected and therefore, don't give a clear result.

DC-Polarographic scans were obtained for Ampicillin and Amoxycillin complexed with Ni(II). The cathodic scan in each case tended to give a more negative value for $E_{1/2}$ than the corresponding anodic scan. While there is some uncertainity in locating the $E_{1/2}$ From the experimental traces, these differnce would appear to persist. Thus, this test suggest that the electrode processes while being close to reversibility are not truely reversible. This test could not be applied usefully for the Co(II) cephalexin system because the waves for the complex and the excess Co(II) overlap.

5.7. Quantitative Studies

Drugs	Linear ranges (M)
Ampicillin	$5.00 \times 10^{-7} - 1.00 \times 10^{-5}$
Amoxycillin	$3.98 \times 10^{-7} - 2.00 \times 10^{-5}$

Table 5.2. Linear concentration ranges for Ampicillin and Amoxycillin .

Using the procedures established in chapter 4, Calibretion curves were prepared for each of the two penicillins. Because the concentration ranges extended over several orders of magnitude a log-log plot of peak height versus concentration was made in each case. From these plots the linear ranges were readily established.

The table 5.2, lists the ranges for each antibiotic. For the cephalexin - Ni(II) system, it was considered unnecessary to establish the data since a detailed study already exists [70]. Beyond the lower limit, the error in measuring the peak height is exaggerated by uncertainity in the position of the base line.

At the upper end, the calibration,curves down words (concave downwards). This is probably due to secondary reactions occuring at the electrode surface. The linear portions of the calibration curve for these two antibiotics complexed with Ni(II) are plotted separately and correlation coefficients etc. established over these ranges.

It has been recorded [73] that the Co(II) complex of Penicillin and Cephalosporin can be used for the assay of these families of drugs. Some measurements were carried out in the course of the present work on the cobalt complex of cephalexin,(Fig.4.30) showing scans for the cobalt(II) complex as a function of cephalexin concentration, shows that the polarographic peak for the drug complex is close to the peak for the excess cobalt (II) than is the case when Ni(II) is the complexing agent. Thus the establishment of the base line for quantitative work is less satisfactory. This can be seen by comparing fig.4.29 and fig. 4.30.

The range of measurement is narrower than for the corresponding Ni(II) complex system. (See tables 4.25 a and b). Nevertheless, within the specified ranges, the values obtained using both complexes are comparable as is evident from these tables.

When penicillin or cephalosporin drug is treated with 1.0 M sodium hydroxide at 100°C, it is converted to the corresponding 2,5_diketopiperazine (see chapter one). If the reaction mixture is buffered with citrate buffer pH 5, it has been shown [62], that the stated reaction product can be made the basis of polarographic methods of analysis of Ampicillin and Cephalexin. This analytical method was extended in the present work to Amoxycillin. Within the range established in the literature [62], for the above mentioned drugs, it was shown that satisfactory results were obtainable for Amoxycillin. Compared to the Ni(II) method, large quantitatives (0.2 - 0.3 g/100 ml of measuring solution) samples are required. Further more the number of steps is greater and the procedure, more time consuming for the preparation of the measurement solution than for the Ni(II) system.

Quantitative Analysis of Capsules.

Table 5.3 summarizes the results of measurements made by the pharmacopoeia and polarographic methods developed at the course of the present work.

NO.	Method (Assay)	Technique	% of Amp. Trihyd.	% of Amoxy. Trihyd.	% of Cepha-lexin
1	Iodometry	Titrimetric	99 ± 1	103 ± 1	100 ± 1
2	Hydroxylamine Iron(III)	Spectropho tometric	99 ± 1	102 ± 1	101 ± 1
3	Copper (II) Sulphate	Spectrophoto metric	99 ± 1	101 ± 1	---
4	Differential pulse Ni(II) Complex system	Polarography	100 ± 1	103 ± 1	100 ± 1
5	Differential pulse Co(II) complex system	Polarography	---	---	99 ± 2
6	Differential pulse 2.5 diketo pipe-razine derivative system.	Polarography	---	103 ± 1	---

Table 5.3 Summary of results obtained in chapters 3 and 4 for quantitative measurements in capsules.

It is seen that agreement between the different methods is very good. The pharmacopoeia methods are slow, for example, an iodometric assay takes about 45 minutes assuming the reagents are already prepared.

Furthermore, the iodometric method results in a variable consumption of iodine (From 6 to 9 moles of I_2 per mole of antibiotic). This is a considerable weakness of this method.

Preparation of Iron (III) solution for the hydroxylamine method is very slow requiring more than one day to obtain. The actual analysis with hydroxylamine Iron (III) is quicker than the iodometric method taking about 15 minutes. However, it is essential to time the absorbance measurement within 10 second for reproducibility after mixing the reagents. Thus, it is a highly emperical procedure. The copper (II) method requires the reaction mixture to be maintained at 75°C for 30 minutes and then quenched to room temperature for absorbance measurement. Thus, it is not particularly convenient.

The polarographic method based on the Ni(II) complex is more convenient than the pharmacopoeia methods for repeatitive analysis.Indeed, it could well lend itself to automation. Such development has been demonstrated by the publication of FIA procedure [67]. It would appear from the measurements that the electrode is reversible or close to reversibility. The study with Co(II) complex gave similar results with cephalexin as the corresponding Ni(II) complex. Preliminary measurements not reported on table (5.3) suggest that it could also be used for the determination of Ampicillin and Amoxycillin. However, the Ni(II) complex with cephalexin is more sensitive. A further disadvantage of the

Co(II) cephalexin system is that the peak for the cobalt complex overlaps appreciably with the peak for excess cobalt,(See fig.4.30). This restricts the calibration ranges possible. Therefore, the nickel system is preferred over that of the cobalt system. However,this does not seem to be a problem for Ampicillin for which the Co(II) complex and the excess Co(II) peaks are well separated.

The differential pulse analysis based on the 2, 5 diketopiperazine derivative has been reported for the analysis of Ampicillin and cephalexin [6263]. This method was extended to Amoxycillin in the present work. Although, it appears to be reliable, the number of steps involved make it slower than the Ni(II) method. Moreover, a problem with precipitation can occur on cooling the alkaline hydrolysis mixture after reaction at 100°C. This occurs at the higher concentration levels which it may have only a small effect on the analytical result. The presence of the precipitate results in contamination of the polarographic capillary.

5.7. Interference Test:

Pharmaceutical preparations such as capsules may contain impurities or diluents deliberately added to create bulk. Since the impurities are unknown except for the manufacturers, some method of checking for interference in an analytical procedure is desirable. In the prepsent work, this was done by a dilution procedure in which standard and unknown were mixed in known proportions and the analysis performed polarographically using the Ni(II) complex. The results of such test for Ampicillin and Amoxycillin are

summarized in tables 4.15 & 4.16. They show that no interference was experienced in mixing the standard and sample materials. In the present study, this is to be expected since the capsules would appear to be pure penicillin drugs, (See table 5.3). However, if the capsules contained impurities or additives that interfered in the analysis, the interference would be expected to show up in the results.

Conclusions and some suggestions for further work.

The polarographic methods have been shown to be considerably more convinient and informative than the pharmacopoeia methods. They are better suited for repeatitive analysis and to the study of degradation products and instability of the solutions of the antibiotics. This is particularly true with respect to spectrophotometric methods based on electronic transitions in the near UV and vissible spectral ranges in solutions. There is potential for automation using polarographic detection in measurments.

The polarographic techniques are considerably more economic in their use of pure antibiotics used as standards and as pharmaceutical formulations.

Of the polarographic methods studied , that using Ni(II) complex of the antibiotic was prefered as it gives a better range than the corresponding Co(II) complex. However as the preliminary studies showed , it is subject to change

with temperature and with time. Therefore further studies could be conducted on metal complexes using other metal ions with the aim of increasing the stability of the resulting complex.

The work showing that the Ni(II) complexes of Ampicillin and Amoxycillin degrade at higher temperatureS either to Ni(II) penicilloic or Ni(II) penilloic acid complexes can be extended. It could be useful to establish which of these two acids is producde in the form of the Ni(II) complex. This can be done by preparing Ni(II) penicilloic and Ni(II) penilloic acid complexes and studying the polarographic behavior.

The conditions for the degradation of these antibiotics to the corresponding 2,5_diketopiperazine derivatives could be re_examined for the time envolved and improving the reproducibility of the process.

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