ENHANCEMENT OF BIOAVAILABILITY AND COMPATIBILITY STUDIES OF RIFAMPICIN

THESIS
Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

By
KOUR CHAND JINDAL
M. Pharm.

Under the Supervision of
DR. SUSHIL KHANNA
M. Pharm., Ph.D.

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN) INDIA

1994

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE
PILANI RAJASTHAN

CERTIFICATE

This is to certify that the thesis entitled **ENHANCEMENT OF BIOAVAILABILITY AND COMPATIBILITY STUDIED OF RIFAMPICIN** *and submitted by* **KOUR CHAND JINDAL** *ID. No.* **91 PZXF 803** *for award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.*

S. Khanna

Date: 1994

(Dr. SHUSHIL KHANNA)
GENERAL MANAGER
(TECHNICAL - PHARMA)

Lupin Laboratories Limited,
A - 28 / 1, M I D C, Chikalthana,
Aurangabad - 431 210

C O N T E N T S

1. INTRODUCTION 1

1.1 Bioavailability enhancment 2

1.2 Analytical methods for stability and biofluid samples 34

1.3 Absorption experiments in animals 39

1.4 In vitro - in vivo correlation 40

1.5 Drug characteristics and compatibility studies 42

2. DRUG PROFILE OF RIFAMPICIN 51

3. OBJECTIVE AND EXPERIMENTAL DESIGN 58

4. EXPERIMENTAL-I : DEVELOPMENT OF ANALYTICAL METHODS 66

4.1 Development of stability indicating HPLC method for rifampicin estimation 67

4.2 Development of HPTLC method for monitoring degradation products of rifampicin in drug-excipient interaction studies. 68

4.3 Development of a suitable dissolution rate test method for rifampicin-isoniazid fixed dose combination products 73

4.4 HPLC method for analysis of rifampicin in biofluids. 74

RESULTS AND DISCUSSION 84

5. EXPERIMENTAL-II : STUDY OF PHYSICOCHEMICAL DRUG CHARACTERISTICS IMPORTANT FOR DRUG COMPATIBILITY AND BIOAVAILABILITY 89

5.1 The characterization of rifampicin and excipients used 90

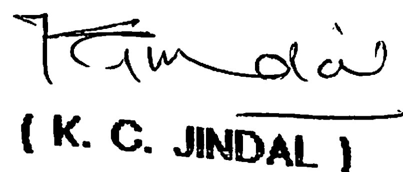
ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to my guide Dr. S. Khanna, General Manager (Technical-Pharma), Lupin Laboratories Limited, Aurangabad for his invaluable guidance, consistent encouragement and unstinted help in the accomplishment of this work.

I am grateful to Mr. P. V. Bhandarkar, Sr. General Manager (Manufacturing and Logistics) and Lupin management for permitting me to carry out this work in the laboratories and providing the necessary facilities. I sincerely thank Professor J. K. Lalla, Principal, P K M K College of Pharmacy, Bombay for his guidance and timely help. My sincere thanks to Mr. R. S. Chaudhary, Mr. S. S. Gangwal, Mr. A. K. Singla, Mr. T. Vijan and other colleagues for their valuable support.

My sincere thanks to Dr. L. K. Maheshwari, Dean R&C Division and Dr. R. N. Saha, In-charge, Ph. D. Programme, B I T S, Pilani for their prompt response and excellent co-ordination from the institute.

I also wish to express my sincere thanks to Mr. S. M. Dailkar for typing the manuscript.



(K. C. JINDAL)

AURANGABAD
JULY 1994

C O N T E N T S

1. INTRODUCTION 1

1.1 Bioavailability enhancement 2

1.2 Analytical methods for stability and biofluid samples 34

1.3 Absorption experiments in animals 39

1.4 In vitro - in vivo correlation 40

1.5 Drug characteristics and compatibility studies 42

2. DRUG PROFILE OF RIFAMPICIN 51

3. OBJECTIVE AND EXPERIMENTAL DESIGN 58

4. EXPERIMENTAL-I : DEVELOPMENT OF ANALYTICAL METHODS 66

4.1 Development of stability indicating HPLC method for rifampicin estimation 67

4.2 Development of HPTLC method for monitoring degradation products of rifampicin in drug-excipient interaction studies. 68

4.3 Development of a suitable dissolution rate test method for rifampicin-isoniazid fixed dose combination products 73

4.4 HPLC method for analysis of rifampicin in biofluids. 74

RESULTS AND DISCUSSION 84

5. EXPERIMENTAL-II : STUDY OF PHYSICOCHEMICAL DRUG CHARACTERISTICS IMPORTANT FOR DRUG COMPATIBILITY AND BIOAVAILABILITY 89

5.1 The characterization of rifampicin and excipients used 90

5.2 Preparation and characterization of amorphous and polymorphic form of rifampicin 90

5.3 Effect of pH on solubility of rifampicin 91

5.4 Particle size and size distribution 94

5.5 Density and flowability 95

5.6 Study of moisture sorption by rifampicin at various relative humidities 96

5.7 Dielectric constant 98

5.8 Lipohilicity 98

5.9 Intrinsic dissolution rate 99

5.10 UV/visible scans of rifampicin in neutral, acidic and alkaline solutions 100

RESULTS AND DISCUSSION 119

6 EXPERIMENTAL-III : DRUG STABILITY AND PREFORMULATIONS STUDY 126

6.1 Drug stability and preformulation study 127

6.2 Effect of excipients on rifampicin solubility 130

6.3 Effect of UV/visible light on rifampicin stability 130

6.4 Effect of oxygen on rifampicin stability 131

6.5 Effect of pH on stability of rifampicin in aqueous solutions 132

6.6 Effect of buffering agents on stability of rifampicin 133

6.7 Effect of rifampicin-isoniazid incompatibility on their bioavailability 134

RESULTS AND DISCUSSION 223

7 EXPERIMENTAL-IV : BIOAVAILABILITY ENHANCEMENT 231

7.1 Effect of particle size on dissolution rate and bioavailability of drug 232

7.2 Effect of various concentrations of commonly used surfactants on dissolution rate of rifampicin 233

7.3 Effect of surfactants on the partition of rifampicin 234

7.4 Effect of surfactants on bioavailability of rifampicin in rats 235

7.5 Effect of different concentrations of polysorbate 80 on bioavailability of rifampicin 237

7.6 Effect of amorphous and polymorphic forms of rifampicin on its dissolution rate and bioavailability 238

7.7 Effect of cyclodextrins on rifampicin dissolution 240

7.8 Effect of povidones on rifampicin dissolution 240

7.9 Preparation and study of solid surface dispersions of rifampicin 241

7.10 Preparation and study of solid dispersions 243

RESULTS AND DISCUSSION 295

8. CONCLUSIONS 306

9. REFERENCES 309

10. LIST OF PUBLICATIONS 341

INTRODUCTION

CHAPTER–1

A. BIOAVAILABILITY ENHANCEMENT

The oral route is the route of first choice for drug administration. However on oral administration various drugs exhibit relatively low bioavailability.

The absorption of drugs administered orally as solids involves two consecutive processes : the process of dissolution followed by the transport of the dissolved material across gastrointestinal membranes into the systemic circulation. The rate determining step in the overall absorption process for relatively insoluble compounds is generally the rate of dissolution white relatively soluble compounds the rate of permeation across biological membranes is the limiting factor. The rate of dissolution can be improved by physical means whereas the rate of permeation which is dependent on size, relative aqueous and lipid solubilities and ionic charge of the drug molecules can be altered in majority of cases only through molecular modification [1].

In order to lower the physical barrier function of intestinal mucosa towards poorly absorbed drugs the potential of co-administration of absorption enhancing agents have been investigated extensively in recent years. These agents belong to widely different chemical entities. Poor bioavailability may also be caused by precipitation or binding of the drug in the gastro-intestinal lumen or during absorption by poor penetration of intestinal mucosa or by extensive first pass metabolism [2-4]. The improvement in bioavailability of proteins and peptides have been tried with peptidase inhibitors. The three main reasons for poor bioavailability

include : low water solubility (< 1mg/ml at pH 7), low lipophilicity (log P < 2) and high first pass liver or gut metabolism [5].

1.1A Physicochemical properties

Typical physicochemical properties of drug that either characterize or may exert significant influence on its bioavailability, stability or compatibility with other drugs or pharmaceutical excipients are discussed below :

1. Solubility

Any drug administered orally for systemic activity must dissolve in the gastrointestinal fluids prior to their absorption. Aqueous solubility is a useful biopharmaceutical parameter. A minimum aqueous solubility of 1 % is required to avoid solubility limited absorption problems [6]. Solubility of acidic and basic drugs is pH dependent, hence solubility of a drug over the entire physiological pH range of 1 to 8 is important.

Knowledge of solubility of a drug not only helps in making some judgement about its bioavailability but also is useful in the development of appropriate media for dissolution testing and choosing the right solvent for the purposes of granulation and coating.

The inherent solubility can be enhanced by various means, like : pH adjustment, salt formation, prodrug design, complexation, particle size reduction and use of a cosolvent/surfactants.

2. Dissolution rate

Since dissolution precedes absorption, any change in the process of dissolution would influence the absorption. Investigation of dissolution behaviour of a drug especially the one having moderate and poor solubility is essential. The comparative dissolution rates of different physical (polymorphic, amorphous, solvates etc) and chemical (ester, salt, prodrug etc) forms is also equally important for selection of optimum form.

Intrinsic Dissolution Rate (IDR) :

The dissolution rate (dc/dt) of a solid in its own solution is described by Noyes-Nernst equation :

$$dc/dt = AD\,(Cs - C)\,/\,h\,V$$

During the early phases of dissolution the solute concentration in diffusion layer (Cs) is much greater than solute concentration in the bulk medium (C) and is essentially equal to saturation solubility S. When surface area exposed (A), volume of solvent (V), temperature and agitation rate are held constant.

$$dc/dt = KiS,$$

Where Ki = AD/hV = Intrinsic dissolution rate constant.
D = Diffusion coefficient
h = Diffusion layer thickness (influenced by agitation)

IDR is defined as the dissolution rate of pure drug substance under the conditions of constant surface area and is characteristic of a each solid under a given set of hydrodynamic conditions and solvent system. IDR helps in predicting dissolution rate limited absorption. Kaplan [7] determined that the compounds with intrinsic dissolution

rates below 0.1 mg/min.sq.cm usually exhibit dissolution rate limited absorption and with rates between 0.1-1.0 mg/min.sq. cm may show this problem and need confirmation with more data.

Out of the various methods reported for checking dissolution rate, the rotating disk method has been found to be superior [8]. Constant surface rotating disk method of Wood *et al* [9] is useful as this method eliminates the effect of surface area and surface electric charges as dissolution variables. In this method the drug is compressed into a disk of known area and constant surface is exposed to the dissolution media.

Amount of drug released is plotted against time and slope of the line divided by the exposed surface area gives IDR. The IDR is also applicable for the determination of solubility of metastable forms which transform to more stable forms when exposed to solvents [10]. These metastable forms when exposed to solvents are sufficiently stable to permit measurement of initial dissolution rate which according to Noyes-Nernst equation are proportional to the respective solubility of the polymorphic form.

Particulate Dissolution :

This is another method to study the dissolution of solids. Weighed amount of powder sample from a particular sieve fraction is introduced into the sample and agitated at constant speed. This method is useful to study the influence of particle size, surface area and excipients on dissolution rate. Dissolution rate is influenced by effective or available rather than absolute surface area. Particulate

dissolution can be enhanced by reduction in particle size and hydrophobicity. Particle size can be reduced by mechanical means or adsoption on inert excipient with a large surface area e.g. colloidal silicon dioxide [11] or preparation of solid dispersions.

Factors affecting solubility and dissolution rate

I. Crystal properties and polymorphism

Many drugs can be crystallized in more than one polymorphic form via appropriate manipulation of conditions of crystallization including nature of the solvent, temperature, rate of cooling and other factors. These drugs may also be prepared in amorphous form e.g. by shock cooling, sudden evaporation of solvent or lyophilization[1].

Factors that may affect crystal habits include, supersaturation, rate of cooling, degree of solution agitation, crystallizing solvent, presence of cosolutes, cosolvents and adsorbable foreign ions [13].

These forms may have distinct physical properties such as melting point, solubility, dissolution rate, true density, compaction behaviour, flow properties, solid state stability and optical activity [12].

Polymorphism is particularly common within certain structural groups e.g. 63 % of barbiturates, 67 % steroids and 40 % sulphonamides exhibit polymorphism [14]. It is conventional to number the polymorphs in order of stability at room temperature starting with form I using Roman numerals. Form I usually has the lowest solubility and highest melting point [5].

The absorption of an otherwise poorly absorbed drug may be enhanced by use of a more soluble and fast dissolving polymorphic form [6]. When one polymorph is more effective clinically than its counterparts, it is undoubtly due to difference in water solubility [15]. Generally, two fold increase in solubility and dissolution rate can be achieved through polymorphic modifications [16].

Polymorph B of chloramphenicol palmitate was more soluble and most bioavailable whereas polymorph A was less soluble and least bioavailable [17 - 19]. Similarly beta form of chlortetracycline hydrochloride was found to be more soluble and more bioavailable than alpha form [20, 21]. The other compounds that have shown significant difference in bioavailability due to polymorphism include amobarbital [22], fluprednisolone [23], glutathione [24], 6-mercaptopurine [25], cimetidine [26], methylprednisolone [27], furosemide [28], chlorpropamide, phenylbutazone, ampicillin and griseofulvin [29].

Amorphous Drug :

Since the amorphous form is the highest energy form of a pure drug, it should produce faster dissolution and absorption rates than the crystalline form. The successful use of an appropriate polymorph may provide in some instances, therapeutic blood levels from otherwise inactive drugs [30]. Crystalline novobiocin acid is subtherapeutically absorbed orally. Amorphous novobiocin has 10-fold higher solubility, faster dissolution and higher blood levels and is therapeutically active [31]. In the coevaporation method, the drug may precipitate out as an amorphous form in crystalline carrier and become primary

contributing factor in increasing its oral absorption e.g. sulfathiazole in urea [32]. The drugs where absorption of amorphous form was found to be superior than crystalline forms include chloramphenicol palmitate [33], indomethacin and cephalexin [34]. Mechanical processes such as size reduction and compression have been reported to bring about polymorphic changes in drugs [35 - 40]. Compression transformed needle shaped polymorph of piroxicam to cubic polymorph [41].

Different polymorphs may differ significantly in their compression characteristics e.g. sulphathiazole, barbitone, aspirin [42] and indomethacin [43]. Physical forms also may influence the rate of degradation of labile drugs e.g. Aztreonam; in the presence of high humidity, its beta form is much stable than alpha form [44].

The physical stability of any crystalline form is dictated by its free energy : the most stable form is one with lowest free energy. All other forms transform to most stable form at a rate depending on environment factors and molecular structure. Many drugs are reported to undergo phase transitions in suspensions [45 - 53]. Such changes have been prevented by use of structurally related compounds [54, 55], viscosity imparting agents [56, 57] and other additives [58].

Different polymorphic forms may be characterized by X-ray, hot stage microscope, scanning electron microscope, differential thermal analysis, optical crystallography, IR and NMR spectroscopy [59-65]. Single crystal X-ray although provides the most complete information but is tedious and time consuming and it is difficult sometimes to get

a bold crystal of desired size. Powder X-ray diffraction is simple, rapid and preferred method. DTA and DSC provide pertinent thermodynamic data and are vary useful for polymorphism study. For characterizing crystal forms, the heat of fusion, can be obtained from the area under the DSC curve for the melting endotherm [66].

II. Particle size and surface area :

Particle size reduction is a method of choice for improving dissolution. The effect of particle size could be very important when the solubility of drug is 1 mg/ml or less [67].

Particle size not only affects drug dissolution and bioavailability but also solid state stability, flow, mixing efficiency, compaction and disintegration. Reduction in particle size of poorly soluble drugs generally improves dissolution rate [68] and bioavailability. Bioavailability of griseofulvin and phenacetin is directly related to particle size distribution of these drugs [69,70]. Particle size has been found to significantly affect the bioavailability of sulfadimethoxine [71], benaxoprofen [72], griseofulvin [73,74], norfloxacin [75], nitrofurantoin [76] digoxin [77 - 79], sulfisoxazole [80] and spironolactone [81]. Increase in drug absorption with reduction in particle size may reach a limit when the blood concentration time curve is similar as that of aqueous solution. The particle size at this stage is called critical particle size (CPS) in absorption [82].

The dissolution rate of a drug regardless of dissolution mechanisms is always directly proportional to the effective surface area of the drug i.e. the surface area of drug available to the dissolution fluids. The effective surface area of a drug is usually much smaller than the specific surface area which is an idealized *in vitro* measurement [83].

Reduction in particle size beyond a point often leads to aggregation and an apparent increase in hydrophobicity, possibly lowering the dissolution rate due to reduced liquid penetration [84] and making handling more difficult. Many drugs whose dissolution characteristics could be improved by particle size reduction are extremely hydrophobic and may resist wetting by gastrointestinal fluids. Therefore the gastrointestinal fluids may come in intimate contact with only fraction of potentially available surface area.

Hydrophobicity can be reduced by granulation with hydrophilic polymer e.g. hydroxyethyl cellulose [85], gelatin [86-88] and sorbitol [89]. Granulation with low molecular gelatin enhanced absorption of ibuprofen [90].

Wettability of a drug is important for its dissolution and for penetration of liquid into the powder bed. It is reported that 0.02 % w/v polysorbate 80 solution lowers the surface tension of the media to 45 dynes/cm which is equivalent to the interfacial tension in the intestine (achieved due to presence of bile salts). Reducing the surface tension of the media should naturally improve its IDR due to improved wettability. The contact angle between the solid and liquid

drop gives a precise and quantitative idea of wetting [91]. The effective surface area of hydrophobic drug particles can be increased by the addition of a surfactant to the formulation which functions to reduce the contact angle between the solid and gastrointestinal fluids. There are many reports where drug solubilization and absorption has been increased by surface active agents e.g. marked increase in absorption of spironolactone by polysorbate 80 [92], increase in rectal absorption of sulfisoxazole by polysorbate 80 [93], increase in dissolution rate of nalidixic acid by Myrj [94].

Particle size reduction may adversely affect drug stability because fine particles are relatively more open to attack from atmospheric oxygen, heat, light, humidity and interacting excipients.

Determination of particle size and size distribution

For a drug having heterogenous particles, the size, shape, surface area and size distribution are important. For frequency distribution curve either number or weight of particles is plotted against the size range of mean particle size. Alternately cumulative percentage over size or under size can be plotted versus particle size.

To get the log distribution curve, the frequency or cumulative percent frequency is plotted versus log of particle diameter. The slope of this plot gives geometric standard deviation and 50 % size gives Geometric Mean Diameter. Assuming the shape and density factors to be constant throughout the size range, the relative number in each

size interval are obtained by dividing the weight by cube of mean particle diameter. The cumulative distribution by weight are calculated similarly to the number distribution.

Microscopy and sieve analysis are convenient and commonly used methods for particle size analysis of pharmaceutical powders. Other instrumental techniques are based on lasers, light scattering, light blockage, electric current blockage, centrifugation and air suspension. Surface area is related to size and shape of particles.

Particle size of coarse drug can be determined by sieve analysis. The utility of fine-mesh sieves for dry powders is limited to about 75 um (200 mesh), because the gravitational force on small particles is insufficient to overcome surface forces that cause the particles to adhere to each other and the wire mesh. Hence for particle size analysis of fine drug, microscopic method is useful. Optical, polarizing or scanning electron microscope can be used for this purpose. Polarizing microscope is also useful to distinguish between amorphous and crystalline materials.

III. pH

The pH of product can be adjusted where solubility of drug is relatively higher, however the stability of drug at that pH should be considered. Compounds show differences in solubility characteristics with changes in solution pH, depending on ionization constant of their acidic or basic functionality. The pH-solubility profile is determined within the range of 3 to 4 pH units on both sides of ionization constant [95].

IV. Soluble salts

Salt formation is one of the most common approaches used to increase drug solubility and dissolution rate [6]. As a rule, a pharmaceutical salt exhibits a higher dissolution rate than the parent drug at an equal pH even though they may have the same equilibrium solubility [96]. Sodium and potassium salts of weak acids dissolve much more rapidly than the parent drug, independently of the dissolution media pH e.g. solubility of sodium sulphathiazole is over 5500 fold at pH 1.5 and over 1600 fold at pH 6.8 than sulphathiazole. Sodium salicylate is 1100 times more soluble at pH 1.5 and 92 times at pH 6.8, than salicylic acid [5].

The water soluble salts of poorly water soluble acidic or basic drugs have been shown to have better absorption than parent compounds. Forming salts of a drug rarely changes its pharmacology [97] but they do affect the intensity of response [98].

V. Complexation

Complex formation between drug and potential ligand can be studied using the phase solubility technique [99]. Drug solubility is checked in increasing concentration of complexing agent. Concentration of drug is plotted verus concentration of complexing agent. Gentisate ions enhance solubility of hexamethylmelamine 90 fold [100]. Xanthines enhance the apparent solubility of ribavirin [101-103]. Egg albumin formes complex and increases bioavailability of vitamin E [104].

Cyclodextrins form inclusion complexes leading to stabilization and /or solubilization of many insoluble drugs. Solubility of some drugs is increased manifold.

An inclusion compound is a unique form of chemical complex in which one molecule is enclosed within another molecule or structure of molecules [105]. The stereochemistry and possibly the polarity of both host and guest molecules determine whether inclusion can occur. This combination is characterized by the absence of ordinary chemical bonds. The guest molecule should be of a suitable size and shape to fit into a cavity within a solid structure formed by host molecule. The hollow space formed by the host may be in the form of a channel (urea, thiourea), cage (hydroquinone, water) or layer like spaces. Cyclodextrins form monomolecular inclusion complexes. Cyclodextrins are crystalline, nonhygroscopic water soluble macrocyclic polymers containing six, (alpha-CD), seven (beta-CD) and eight (gamma-CD) glucose units enclosing a cavity of about 6, 8 and 10 A in diameter. Their outer surface is hydrophilic and internal cavity is apolar. Beta -cyclodextrin is the most promising drug complexing agent [106-108]. Molecules of a suitable size and shape can be held within the cavity of a particular cyclodextrin by Van der Waals forces. The interior of the cavity behaves as a Lewis base due to its high electron density. A stable complex is formed when molecules tightly fit into the cavity.

Cohen and Lach [109, 110] were the first to report that beta-Cyclodextrin can form inclusion complexes with various drugs in solution. Increase in solubility, bioavailability and stability of

drugs by cyclodextrins has been reviewed [111]. The dissolution rate and absorption may decrease for highly soluble drugs but may increase for pooly soluble drugs when complexed with cyclodextrins [112]. These compounds were found to increase drug solubility and improve dissolution rate e.g. cinnamic acid derivatives [113] benzodiazepines [114,115], spironolactone [116], flurbiprofen [117,118], prostaglandins [119]. famotidine [120], cinnarizine [121], bendrofluthiazide [122], phenobarbitone [123,124], paracetamol [125], digoxin [126], estradiol [127], steroids [128], 6-benzoyl benzoxazolidinone [129], naproxyn [130, 131], amobarbital [132], proscillaridin [133], chloramphenicol [134], warfarin [135], glibenclamide [136], griseofulvin [137], caromofur [138], phenylbutazone [139], indomethacin [140], nalidixic acid [141], ibuprofen [142], 4-biphenylacetic acid [143], NSAIDs [144], prednisolone [145], metronidazole benzoate [146] and betamethasone [147]. Relatively higher bioavailability or pharmacological activities has been reported for flurazepam [148], methicillin, ampicillin, phenytoin [149], spironolactone [150], flurbiprofen [151-153], digoxin [154], beclomethasone dipropionate [155], acetohexamide [156], ibuprofen, indomethacin [157], ketoprofen [158] and dicoumarol [159]. Solubility of drugs increases with increase in cyclodextrin concentration [160, 161]. Complexation may be formed in ratio of 1:1 [162, 163], 1:2 [164] or 1:3 [165]. Inclusion complexation with cyclodextrins may also adversely affect drug solubility e.g. nitroglycerin [166,167]. Complex formation depends on type of

cyclodextrin [168], ionization and pH of medium [169,170]. The formation of inclusion complex formation by sparingly soluble drugs has been studied by solubility method [171], IR [172], NMR [173], X-ray [174], partition coefficient [175], membrane permeation [176], conductometry [177], potentiometry [178], spectroscopy [179] and fluorescence measurements [180].

The solubility curves of drugs with cyclodextrins may be Bs type [181] or Al type [182]. Drugs with small formation constant form unstable complexes [183]. Stability constants are calculated from initial straight line portion of phase solubility diagram by the equation [184]

$$K = \text{slope} / \text{intercept} [1 - \text{slope}]$$

Methods of cyclodextrin-drug complex formation include freeze drying [185], spray embedding [186,187], precipitation [188] and granulation [189]. The tabletting and other physicochemical properties of cyclodextrin complexes has been studied [190-192].

VI. Cosolvents

When a drug has low solubility in common pharmaceutical solvents and dose is high, it becomes necessary to use mixture of solvents (cosolvents) to get desired solubility e.g. use of alcohol, benzyl alcohol and propylene glycol mixture for diazepam [193] and water, glycerin and alcohol mixture for phenobarbital [194]. Water miscible solvents can be used as cosolvents in formulations to improve solubility or stability and in analysis to facilitate extraction and separation.

Most acceptable pharmaceutical solvents are glycerin, propylene glycol and ethanol [5]. Miscibility of a solvent in water is determined by its polarity which in turn is referred to in terms of dielectric constants (DE) or solubility parameter (SP).

According to dielectric theory every solute shows a maximum solubility in any given solvent system at one or more specific dielectric constants. The solubility profile obtained as a function of dielectric constant appears to be similar for a solute in a wide variety of solvent systems. Thus, adjusting the dielectric constant of a system to its dielectric requirement (DR) the solubility of the drug can be improved. The dielectric requirement of the drug is independent of the nature of the solvent or solvent blends. Although, the absolute solubility of the solute may vary in two different solvents of same dielectric constant, the solubility profile remains the same. The equation :

$$E\ mixture = f\ [(E\ solvent) + 1 - f\ (E\ water)]$$

can be used to manipulate the solvent blends so as to obtain dielectric constant of a cosolvent blend in required range, while the equation :

$$\text{Log } Cs = f(\log Csolvent - \log Cwater) / 100 + \log Cwater$$

will give the solubility of the drug in the particular cosolvent system ; where Emix, Esolvent and E water are dielectric constants of solvent mixture, solvent and water respectively, f is the fraction of solvent used and Cs, Csolvent and Cwater are solubilities of the drug in solvent mix, pure solvent and water respectively.

Log linear solubility equation (Extended Hildebrand solubility approach) proposed by Yalkowsky *el al* [195] can be applied to drugs which are significantly less polar than either of the solvents in binary mixture [196].

$$\text{Log } S = \text{Log } Sw + sf$$

Where S = solubility in cosolvent consuming a fraction f of non aqueous solvent

Sw = solubility in water

s = constant and is characteristic of system under study, represented by solubilizing power.

This equation has been applied to study cosolvent effect on solubilities of phenytoin [197] and metronidazole [198].

A solubility profile can be obtained, by plotting equilibrium solubility of solute in different solvents (dioxane-water mixtures) with known dielectric constants, as a function of dielectric constant. A plot of solubility vs dielectric constant for a solute in a given solvent system shows a typical curve specific for the solute in that system. The solute may show one or more solubility peaks in the given system. Sometimes, addition of solvents like alcohol to a buffered aqueous solution of a weak electrolyte,increases the solubility of the unionized species by adjusting the polarity of the solvent to a more favoured value. This combined effect of pH and cosolvents can also be made use of in improving the aqueous solubility of various drug solutes.

The solubility parameter (SP) of a binary solvent mixture for a specific drug can be calculated from the solubility of drug in pure dioxan (Sd), pure water (Sw) and volume fractions of dioxan (fd) and water (fw) [582]

$$SP = fd.Sw + fw.Sd / fd + fw$$

VII. Prodrug design

Prodrug is a compound that undergoes biotransformation prior to eliciting a pharmacological response e.g. with the use of metronidazole phosphate the solubility of metronidazole is increased by 5 times [199]. The bioavailability of beta-estradiol increases 17 and 5 fold when given as prodrugs 3-salicylate or 3-acetyl-beta-estradiol respectively [200]. Bioavailability of methyl dopa increases when administered as pivaloyloxy-ether and of naltrexone when administered as anthranilate and acetyl salicylate [201]. Bioavailability of cefpodoxime improved when used as prodrug cefpodoxime proxetil [202].

3. Partition coefficient

Partition coefficient (p) reflects the relative aqueous and lipid solubilities of a material. The gastrointestinal membranes are largely lipoidal in character, hence the lipid solubility of a drug is an important factor in the assessment of its absorption potential. Schanker [203] in a study on a series of barbituric acids with comparable ionization characteristics found a good rank-order

correlation between the amounts absorbed from the rat colon and the chloroform/water partition coefficient of the unionized forms. Solubility behaviour can be related to lipophilicity (log p), since more hydrophobic the drug, greater the effect of water miscible solvents. Lipohilicity is best quantified by using the oil-water partition coefficient (Kw o/w = p). Lipophilicity is measured by log p [5]. Too low lipohilicity (log p < 2) results in poor oral bioavailability. There exists a optimum log p and a parabolic relationship between lipohilicity and biological activity.

4. Ionization constant

The ionization constant provides information about the solubility dependence of drug on pH of the formulation. It is determined by potentiometric pH titration or by pH solubility analysis.

Weakly acidic or basic compounds exist as ionized or unionized form in solution depending on the pH value. The unionized species are more lipid soluble and hence more readily absorbed.Thus the absorption of weakly acidic and basic drugs is influenced by the pH at the site of absorption[1], the ionization constant and the lipid solubility of the unionized species. Schanker [203] observed that weakly acidic compounds (pka > 4.3) were absorbed relatively rapidly; those with pka 2.0 - 4.3 were absorbed slowly and strong acids (pka < 2.4) were hardly absorbed. For bases, those with pka values < 8.5 were absorbed relatively rapidly ; those with a pka 9-12 were absorbed more slowly ; and completely ionized quaternary ammonium compounds were not absorbed.

1.1B. Absorption enhancers

In order to lower the physical barrier function of structural elements of the intestinal mucosa towards poorly absorbed drugs, the potential of co-administration of absorption enhancing agents have been investigated extensively to increase absorption of drugs like antibiotics, peptides and proteins as model compounds [2]. NSAIDs have been used to promote rectal and oral absorption of many drugs e.g. indomethacin, diclofenac, phenylbutazone and acetyl salicylic acid enhanced the rectal absorption of sulfanilic acid and insulin [204-206]. Diclofenac enhanced rectal absorption of pepleomycin [207], ampicillin, cephalothin [208] and diazepam [209] in rats. Sodium salicylate enhanced rectal absorption of ampicillin [210,218], insulin [211], theophylline [212], gentamicin [213, 214], methionyl human growth hormone [215], penicillins and cephalosporins [216-221]. Diclofenac [222] and sodium salicylate cause epithelial cell loss [220] and damage the intestinal mucosa. Medium chain glycerides enhanced bioavailability of bromothymol blue, phenol red [223, 224], and Ceftizoxime [225]. Phenothiazine increased absorption of gentamicin and cefoxitin sodium [226]. N-acetyl amino acids increased rectal absorption of ampicillin sodium due to their calcium ion sequestration effect and increasing membrane permeability [227].

Surfactants

Polyoxyethylene ethers have been reported to enhance the gastric or rectal absorption of lincomycin [228], penicillins and

cephalosporins [229], sulphonamides [230], sulphanilic acid [231] fosfomycin [232], insulin [233] and ergot alkaloids [234]. Nonionic surfactants increased solubilization of piroxicam [235], tolbutamide [236], thiacetazone, isoniazid [237], benzoic acid [238], sulfonamides [239], temazepam [240], carbamazipine [241], methaqualone chloramphenicol [242] and 4-[4-phenyl-yl]-butanol [243]. Poloxamer 188 increased the absorption of sulfisoxazole and sulfadiazine [244] and sodium lauryl sulphate of glibenclamide [245]. Castor oil derivatives enhanced absorption of heparin and insulin [246] and dissolution rate of phenylbutazone [247] and glibenclamide [248]. In rats the combination of polyethylene 1000 monocetyl ether with polyethylene glycol 400 enhanced rectal bioavailability of phenol red and insulin and oral bioavailability of gentamicin [249,250]. Anionic and nonionic surfactants increased the dissolution and permeation of lorazepam [251]. Dimyristyl phosphatidylcholine increases absorption of griseofulvin [252].

The promoting effect of surfactant monomers on intestinal drug absorption may be mediated by drug : surfactant interaction [253], reduction of the resistance of the unstirred water layer [254] or of mucosal barrier [255]. In general the non-ionic surfactants of polyoxyethylene type exert benign effects on membrane structure in comparison to cationic surfactants (tertiary and quaternary ammonium salts) and anionic surfactants e.g. sodium lauryl sulphate [256,257].

The effects of surfactants on drug absorption appeared to be correlated with the occurence of adverse effects on mucosal integrity

[2] e.g. epithelial cell loss [258,259], release of proteins and lipids [260] and unspecified mucosal damage [261]. Nissim found marked pathological changes on the gastrointestinal tract when the ionic surfactants (cationic and anionic) were fed to mice but no effects when nonionic surfactants were tested [262].

Surfactants may also affect by altering the viscosity of gastrointestinal fluids thereby increasing gastric emptying time e.g. enhancement of gastrointestinal absorption of vitamin B12 by polysorbates [263]. One of the most important groups of surfactants present in man is bile salts.

Bile Salts

The natural occurence of bile salts in the intestinal tract has triggered various studies to investigate the applicability of bile salts as potentially safe absorption promoters. Bile salts enhanced dissolution of diazepam [264] and digoxin [265]. The mixed bile salt system possesses significantly lower CMC than individual salts and have significant role in absorption of water insoluble drugs [266]. Mixed micelle system has been used to enhance solubilization of indomethacin [267, 268], streptomycin and gentamicin [269]. The mixed micelles of sodium oleate and sodium taurocholate were most effective absorption promoters [270]. Bile salts proved to enhance absorption of phenol red [271], heparin [272], indomethacin [273], amphotericin-B [274], glibenclamide [275], and insulin [276, 277]. Bile salts influence gastrointestinal motility and transit time and thus

influence drug absorption e.g. increase in absorption of riboflavin upon co-administration of sodium deoxycholate may be due to reduced gastric emptying [278].

Although bile salts have been demonstrated to enhance drug uptake to a significant extent, applicability of these compounds as safe absorption promoters in man is not without problems because mucosal damage seems to be correlated with their effect on drug uptake [2]. Co-carcinogenic and co-mutagenic effects of secondary bile salts [279, 280] discouraged the development of bile salt containing pharmaceutical formulations.

Absorbable Sugars

Absorbable sugars like dextrose can enhance absorption presumably by solvent drag mechanism [281].

1.1C. Solid dispersions

Solid dispersion is defined as the dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by the melting, solvent or melting solvent method [282]. A unique approach of solid dispersion to reduce particle size and increase rate of dissolution and absorption of poorly soluble drugs was first proposed by Sekiguchi and Obi [32]. The eutectic mixture of poorly water-soluble sulphathiazole and urea possessed higher absorption and excretion after oral administration than drug alone.

carriers e.g. the solid dispersion of griseofulvin with urea [287].

Solid dispersions possess tremendous potential to increase the dissolution rates and bioavailability of drugs whose absorption is limited by solubility or dissolution rate. These provide advantages of maintaining a drug in bioavailable form and enhancing bioavailability [288,289], dosage reduction and cleaner manufacturing conditions [290]. The dose of reserpine is reduced to one-third [291] and of griseofulvin to one-half [292].

Broadly speaking there are only two methods of preparing solid disperson namely, by fusion or solvent processes.

Fusion Method

This method introduced by Sekiguchi and Obi [32], involves melting of a drug carrier mixture followed by rapid cooling. A modification of the process involves spray-congealing from a modified spray-drier onto cold metal surfaces [293,294] thus obtaining pellets of the dispersion without grinding and without altering the crystalline nature of the drug [295]. Melting method is reported to give better dispersions e.g. ibuprofen [296] and griseofulvin [297] in polyethylene glycol. Walker et al filled the liquid melt in hard gelatin capsules thus avoiding grinding-induced change in crystallinity, dusting and cross contamination [290]. The problems associated with fusion method include miscibility gap resulting in irregular crystallization and drug instability at high melt temperature [293] sublimation and polymorphic transformation [298].

increase in solubility of chlorpropamide [310] and paracetamol [311].

The specific hydrogen bond interaction between PVP and nabilone in solid dispersion resulted in stabilization of an active polymorphic form over two years of storage thus enhancing pharmacological response significantly [312].

The problems associated with solid dispersions include -

- Suitable only for low dose drugs : A large amount of carrier is often required (generally over 70 %) to improve the dissolution of the drug thus limiting the use of solid dispersions to low or medium dose drugs.
- Method of preparation may degrade drug
- Poor physicochemical stability
- Poor compression and disintegration properties when used for tabletting.
- Poor handling qualities [313].
- Decrease in dissolution rate on aging [314, 315].

Carriers

CITRIC ACID : The easy release of its water of hydration and very low localised pH may decompose many moisture sensitive or labile drugs. Dissolution rate and solubility of primidone was increased by clear glass dispersion with citric acid [316].

SUGARS : Sugars have the advantages of low toxicity, high aqueous solubility and physiological acceptance. Mannitol melts at 167°C is stable upto 250°C and has good flow and compaction properties [293].

increase in solubility of chlorpropamide [310] and paracetamol [311].

The specific hydrogen bond interaction between PVP and nabilone in solid dispersion resulted in stabilization of an active polymorphic form over two years of storage thus enhancing pharmacological response significantly [312].

The problems associated with solid dispersions include -

- Suitable only for low dose drugs : A large amount of carrier is often required (generally over 70 %) to improve the dissolution of the drug thus limiting the use of solid dispersions to low or medium dose drugs.
- Method of preparation may degrade drug
- Poor physicochemical stability
- Poor compression and disintegration properties when used for tabletting.
- Poor handling qualities [313].
- Decrease in dissolution rate on aging [314, 315].

Carriers

CITRIC ACID : The easy release of its water of hydration and very low localised pH may decompose many moisture sensitive or labile drugs. Dissolution rate and solubility of primidone was increased by clear glass dispersion with citric acid [316].

SUGARS : Sugars have the advantages of low toxicity, high aqueous solubility and physiological acceptance. Mannitol melts at 167°C is stable upto 250°C and has good flow and compaction properties [293].

Other sugars except xylitol decompose at elevated temperatures. Sugar dispersions have enhanced absorption of drugs like trimethoprim [317], nitrofurantoin [318,319] and improved dissolution of hydrochlorothiazide [320,321], corticosteroids [322-325], spironolactone [326], digoxin [327], tolbutamide [328-330], chloramphenicol [331], glibenclamide [332], diazepam [333], carbamazepine [334], sulfamethoxazole [335] and sulfabenzamide [336].

UREA : It is nontoxic, pharmacologically inert, has low melting point of 131°C and high aqueous solubility. It increased the dissolution rate manifold of sulphathiazole (by 700-times) [337,32], chlorpropamide (by 930 times) [310, 338] chlorthalidone (130 times) [339, 340] salicylic acid [308], miconazole [341], chloramphenicol [342, 343], hydrochlorothiazide [344], ibuprofen [345], ketoprofen [346], tolbutamide [347], phenytoin [348,349] and sulfisoxazole [350].

POLYETHYLENE GLYCOLES [PEG] : PEGs are water soluble and non-absorbable. PEGs are excellent universal carriers for improving the dissolution rate and absorption of water insoluble drugs like griseofulvin [351, 352], phenytoin [353], mefenamic acid [354], nifedipine [355]. These have been used to increase aqueous solubility and dissolution rate of many drugs like griseofulvin [307, 356] betamethasone [309], tolbutamide [357, 43], chlorothiazide, flumethiazide and hydrochlorthiazide [358-360], chloramphenicol [361, 362], etoposide [303], hydroflumethiazide [363], spironolactone [326], dicoumarol [364], nalidixic acid [365], benzothiadiazine [358], nitrofurantoin [366], berberine chloride [367], glybornuride [368,315],

propylphenazone [369], methaqualone [370], nortryptyline hydrochloride [371], sulphonamides [372-375], corticosteroids [376-378], nonsteroidal antiinflammatory drugs [379,353,380-384] and benzodiazepines [385-388].

By the use of high energy coprecipitates that are mixtures of solid solutions and dispersions, e.g. dispersions of griseofulvin in polyethylene glycols, the bioavailability of drug was increased to more than twice [352].

PEGs of 4000, 6000 and 20000 molecular weight are crystalline, water soluble polymers with two parallel helixes in a unit cell. Significant amounts of drug can be trapped in the helical interstitial space when PEG-drug melts are solidified. PEG 6000 has the ability to dissolve different drugs [389-391]. PEG may also act as a protective colloid in retarding the aggregation or coarsening of the fine crystallites before solidification.

The increase in solubility from PEG solid dispersions could be due to -

- Reduction in particle size
- Decreased aggregation and agglomeration due to physical separation of particles [282].
- Improved wetting of particles e.g. aspirin [392].
- Increase in drug solubility [247,393].

POLYVINYLPYRROLIDONE [PVP] : PVP melts at high temperature of above 275°C with decomposition. It is only suitable for solvent prepared dispersions. It is freely soluble in a variety of solvents

including water, alcohol and chloroform. The molecular weight of povidones has effect on dissolution characteristics of dispersions. Generally the dissolution rate of drugs decreases with increase in molecular weight of PVP due to decrease in solubility and increase in viscosity of polymer e.g. hydrochlorothiazide [394,359],sulphathiazole [395], frusemide [396], propylthiouracil [397], allopurinol [398] and nifedipine [399]. PVP is an effective carrier since it retards the crystallization of many drugs [306,400]. It is imperative therefore that the molecular fraction of PVP used should retard crystallization and the fraction which retards crystallization to the greatest extent may provide the dispersion with the fastest release characteristics. PVP increased aqueous solubility of hydroflumethiazide by 16 fold [401] and reserpine by 200 fold [402] and spray drying retarded the crystallization better than coevaporation procedure [403]. Nabilone -PVP resulted in complete absorption of drug in humans compared to only 60 % from crystalline drug [289]. PVP results in marked increase in dissolution of griseofulvin [404], digitoxin [405], hydroflumethiazide [406], chloramphenicol [361], sulphonamides [407, 408], nifedipine [409], chlorthalidone [373], nalidixic acid [365], ibuprofen [410], frusemide [411], phenytoin [348], indomethacin [412, 413], nitrofurantoin [414], oxyphenbutazone [415], nitrazepam [416] and glibenclamide [248]. Significant increase in bioavailability was reported for chlorpropamide [417, 418], apazone, ibuprofen, tinidazole, lorazepam [419], nifedipine [117], sulfamethoxazole [420], hydrochlorthiazide, phenytoin [421] tolbutamide [422], and chlorthiazide [423].

1.1D Hydrotropic solubilization

It is the process of increasing the solubility of a solute by the addition of salts e.g. solubility of riboflavin is increased with sodium ascorbate [424]. The increase in solubility by hydrotropes could be due to complex formation or "salting-in" machanism [425]. The solubility of chartreusin was increased by sodium benzoate and postulated that increase in solubility was due to plane to plane orientation between drug and the substrate [426]. Hydrotropes like benzoates and salicylates open the water structure and increase the solubility of poorly soluble drugs e.g. diazepam [427].

1.1E Solid Surface Dispersions

Dissolution rate of poorly soluble drugs can be improved by increasing the surface area exposed to the dissolution medium. The surface area can be increased by decreasing the particle size, but there is a limit to which the particle size can be reduced. Excessive reduction in the particle size causes formation of static charges on the surface of the particle and makes the system thermodynamically unstable. As a result, the particles agglomerate, thus reducing the surface area.

To overcome this problem, a new approach was introduced, by Monkhouse and Lach [11] where the dissolution rates can be increased by solid surface dispersion techniques. The drug is dissolved in organic solvent and then deposited on to excipients (insoluble in

organic solvent) by evaporation of solvent. The significant increase in dissolution rate has been achieved by adsorption on both water soluble and insoluble adsorbents like lactose [428,429], dextrose [430], colloidal silicon dioxide [423,431], colloidal magnesium aluminium silicate [432, 433], starch [434] and others [435]. The precipitation of the drug takes place in very fine form or 'miniscular form' i.e., the drug undergoes molecular micronization. The dissolution rate of the drug increases by increasing the total surface area of the drug available for contact with the dissolution medium [436]. The deposited drug particles are bound to the adsorbents or excipients by hydrogen bonding or Van der Wall's forces. These bonds are weak and reversible and as a result the drug molecules are easily displaced by dissolution media [437].

These differ from the solid dispersions by virtue of the fact that, the drugs get deposited on the surface of the carrier material (which may be water soluble or insoluble), rather than in the matrix of the carrier, as in case of solid dispersions. Deposition of the drug only on the surface of the carrier materials (adsorbates) takes place because, the solvent used for coprecipitation technique has no solubility for the carrier materials, but maximum solubility for the drugs.

1.1F **Miscellaneous methods to improve dissolution and bioavailability**

The inclusion of a water soluble carrier in a formulation is likely to cause interaction with the interparticle attraction forces

between drug particles, thereby hinder aggregation and promote dissolution e.g. use of lactose in metronidazole [438] and sodium chloride in griseofulvin formulations [439]. Dissolution rate of drug increased by crystallization of phenacetin in presence of nonionic surfactant above CMC [440]) and use of ground mixture of phenytoin with microcrystalline cellulose [441]. Diluents having a higher intrinsic dissolution rate permitted faster drug dissolution than the ones having a lower intrinsic dissolution rate [442]. Hydration may affect dissolution rate and bioavailability of drugs e.g. anhydrous theophylline has much higher dissolution rate than hydrate form [443].

1.2 ANALYTICAL METHODS FOR STABILITY AND BIOFLUID SAMPLES

Stability indicating method

In order to follow drug stability both in solution and in the solid phase, it is necessary to have suitable stability indicating methods. In some cases UV spectroscopy can be used but in general chromatography is required to separate the drug from its degradation products and interfering excipients. HPLC is most versatile and the method of choice for the quantitation of the active and degradation products, because HPLC separates and measures besides lending itself well to automation [444]. The lack of specificity of UV generally requires the separation of mixtures by HPLC before UV detection of indivisual components.

The impurity profile is important because an impurity even at very low concentration may degrade the drug (e.g. heavy metals), affect appearance of drug, or may be potentially toxic (e.g. aromatic amines are carcinogenic).

Stability indicating methods may be based for measurement of amount of drug remaining, the amount of drug lost, or both drug as well as all known impurities and its degradation products. The choice of method depends upon the chemical nature of the drug, the complexities of the dosage form and, often on availability of resources and instrumentation.

High pressure liquid chromatography [HPLC], high performance thin layer chromatography (HPTLC) and gas chromatography are excellent tools for characterizing the chemical homogeneity and quantitative estimates of purity. Differential thermal analysis and gravimetric thermal analysis also are useful for quantitative evidence of purity and solvates.

The success of stability testing programme depends largely on the test methods used. The most important aspect of the development of a stability indicating procedure is method validation [445].

Method validation should include evaluation of the following parameters (446) :

1. Specificity: Method should be capable of quantitating the component of interest. There should be no interferrence by impurities, excipients, degradation products, intermediates etc.

2. <u>Linearity</u> : The linearity of the method must be demonstrated over a range in concentrations spanning at least 50 - 150 % of the expected work range.

3. <u>Precision</u> : The precision of the method and the system should be determined by the analysis of sample replicates in the first case and the repetitive measurement of a single sample in the latter.

4. <u>Accuracy</u> : Recovery of all the components of interest is ascertained from the matrix with no bias. Spiked samples of the drug in matrix spanning at least 50-150 % of expected content are prepared and assayed.

5. <u>Sensitivity</u> : The smallest quantity of the component of interest which can be quantitated should be determined.

6. <u>Ruggedness</u> : The stability of analytical solutions and the variability of components of the analytical system, such as chromatographic columns, should be assessed. The test for precision should also be repeated by a second analyst using separate instrumentation, where feasible.

Various analytical techniques useful for analysis of stability samples include :

Chromatography

The drug-excipient mixtures are subjected to accelerated storage conditions and examined periodically for appearance including odour

and analysed for any decomposition using HPLC or TLC. Chromatographic methods provide unequivocal evidence of degradation besides the degradation product can be isolated, identified and quantified. A compatible excipient shows no new peak/spot or does not increase intensity of peaks/spots when compared to peaks/spots in the drug itself when drug-excipient sample is analysed at end of stability programme [447].

Differential Thermal Analysis (DTA)

Conventional method of detecting drug-excipient interactions by accelerated stability studies is time consuming and tedious. Differential thermal calorimetery (DSC) is a fast and often regarded as method of choice to screen drug-excipient compatibility and provide maximum information about the possible interaction. It has been applied in a number of drug excipient interaction studies [448-458].

Stability is often a function of both moisture content and temperature [459]. However, the results of DSC analysis are based on effect of temperature only. Role of humidity is not considered. Similarly, extrapolations cannot be made conclusively to room temperature storage from the data obtained from the DSC scans since, DSC thermograms are obtained due to reactions which usually occur at high temperature. e.g. polyvinylpyrrolidone melts when heated and may dissolve the drug leading to altogether different degradation pattern. Elevated temperatures fail to reflect the degradation pathway occurring under ambient storage. Evaluation of DSC curves is often

difficult and clear conclusions are rarely obtained [460]. Hence, DSC studies are complimented by short term stress studies at accelerated temperature and humidity to assess the drug-excipient interactions.

Thermograms are generated for the pure components and their physical mixtures with excipients. In the absence of any interaction, the thermograms of mixtures show patterns corresponding to those of the indivisual components. In a DSC scan (or a thermogram), difference in energy input required to maintain the sample and the reference material exactly at same temperature is plotted against the temperature of the sample. Interaction is indicated in the thermograms of a mixture by appearance of one or more new peaks or the disappearance of one or more endothermic or exothermic peaks corresponding to individual components.

Diffuse Reflectance Spectroscopy [DRS]

It is a useful tool for detection and monitoring of drug-excipient interactions [461]. Drug, excipients and their physical mixtures are exposed to incident radiation. A portion of the incident radiation is partly absorbed and partly reflected in the diffused manner. DRS not only can detect drug degradation in the presence of excipients but also can detect physical and chemical adsorption of excipients onto drug, the result of which affects the bioavailability of drug [462]. A shift in the diffuse reflectance spectrum of the drug due to the presence of excipients indicates physical adsorption whereas the appearance of a new peak indicates chemisorption or formation of degradation product.

Melting Point : It is a first good indicator of purity.

Absorption Spectra

Ultraviolet spectrophotometery : The absorption spectra of drug is useful for quantitative and qualitative analysis. Spectra is recorded in acidic, alkaline and neutral solutions versus the appropriate solvent blank. Absorption maxima and molar absorptivity are determined.

Infrared spectroscopy : The infrared spectrum [between 2.5 to 15 um] is highly specific for chemical structure. Samples may by prepared as a dispersion in mineral oil or as a potassium bromide pellet.

1.3 ABSORPTION EXPERIMENTS IN ANIMALS

Laboratory animals are widely used for estimating the potential for oral absorption of drugs in humans, although these studies are mildely predictive of bioavailability in humans. These studies provide some initial estimates of how well a compound is absorbed [281].

Many isolated rat or mouse intestine methods are described in literature e.g.test tube method of Kaplan and Cotler [463] and everted rat gut technique of Penzotti and Poole [464]. Such techniques are reported to indicate poor in vitro in vivo correlation [465] and tend to under estimate absorption potential due to lack of blood supply to the tissue. The in situ technique described by Doluisio *et al* [466] gives closely reproducible results and produces absorption rates which

are realistic and comparable to those calculated from human bioavailability data. In this technique rat intestine is exposed and one syringe is connected at each end using L-shaped canulas. The drug is introduced into the intestine after clearing the gut with perfusion fluid. Aliquots of lumen solution are analysed at intervals. Using this technique Chow *et al* [467] showed a good rank-order correlation between in situ and in vivo absorption rates.

1.4 *IN VITRO - IN VIVO* CORRELATION

In vitro-in vivo correlations can be achieved using correlations based on (a) clinical observations. Clinical response however is a very poor tool for accurate measurments (b) the blood levels of drug or urinary excretion data and derived pharmacokinetic parameters [468].

Dissolution analysis when correlated with bioavailability is the single most important factor to ensure the consistency and quality of a pharmaceutical dosage form. To do so the test should be so designed as to closely mimic biological environmental conditions [469]. For simulating *in vivo* conditions, parameters like pH, surface tension, viscosity and sink conditions need to be controlled in dissolution medium. Generally 0.1 N hydrochloric acid or buffered solutions with pH close to 1.2 are used as dissolution medium [470, 471].

Various attempts have been made to correlate *in vitro* dissolution data and some in vivo availability parameter [472-485]. Most commonly the single point type correlative methods have been utilized where the

percentage of drug dissolved in a given time or the time taken for a percentage of the drug to dissolve is correlated to a certain parameter of the bioavailability. However due to the arbitrary selection of these single correlative points the interpretation of results can be misleading [486, 487]. The correlation of the entire *in vivo* response time profile to the complete dissolution rate time curve can more reliably predict the in vivo behaviour of the drug [488,489]. Any established *in vitro-in vivo* correlation is specific to formulation and manufacturing process [490]. There are also many instances where no significant correlation could be established [491-499].

Wagner recommended that correlation of ratio of half absorption times estimated from plasma concentration data with the ratios of the *in vitro* t 50 % values as the best way of correlation [479]. Khan and Rhodes [500] suggested dissolution efficiency (DE) for *in vitro-in vivo* correlation. The dissolution efficiency (DE) is defined as the area under the dissolution curve upto a certain time "t" expressed as percentage of the area of the rectangle described by 100 % dissolution in the same time. Constant time interval is chosen for comparison preferably greater than T 90 % value of formulation.

Lara and Cruz used RDR (relative dissolution rate) for comparison of dissolution rates [501].

$$RDR = kx / ko$$

where Kx, and ko are the dissolution rates of dispersion and pure drug respectively. Arias *et al* used DE 30 (dissolution efficiency over

first 30 minutes) and DP 30 (dissolution percentage over first 30 minutes) for *in vitro-in vivo* correlation [502]. The latter was found to give best correlation.

1.5 DRUG CHARACTERISTICS AND COMPATIBILITY STUDY

The main objective of a preformulation study is to identify compatible, potentially useful pharmaceutical excipients so that a viable formulation is developed. Inherent stability of drug and potential toxicity of degradation products is also important.

By investigating the intrinsic stability of the drug it is possible to advise on formulation approaches and indicate types of excipient, specific protective additives and packaging which can improve the stability of product. Besides its chemical structure the physical properties of the drug such as its solubility, density, pka, crystal form, melting point and equilibrium moisture content also influence its stability [1]. Amorphous materials are less stable than crystalline forms. Relatively dense material may better withstand ambient stresses e.g. aminobenzylpenicillin trihydrate is more dense and more stable than its anhydrous crystalline counter part [503].

The stress conditions recommended for preformulation stability assessment include :

Heat and humidity

Moisture uptake

Physical stress like milling

pH in aqueous solutions

Light : UV (254, 366 nm) and visible at room temperature

Oxygen

For the development of a stable pharmaceutical dosage form, the stability of drug under the following three categories, needs to be investigated.

1. Solid state stability of drug alone
2. Stability in the presence of excipients
3. Stability in solution phase including stability in gastrointestinal fluids.

The basic requisite for the execution of these studies is the availability of a reliable stability indicating analytical method.

1.5A Solid state stability of the drug

The chemical decomposition of drugs in the solid state has been the subject of many papers and reviews and the mechanism of drug degradation in their pure solid forms is still being debated [504].

The most common factors that cause solid-state reactions are heat, light, oxygen and most importantly moisture. Most often there is a considerable interplay among these factors. Long term storage demands resistance to extremes of temperature and moisture.

Solution phase stability

Stability of drug in solution at different pH in buffered and

unbuffered solutions is necessary to estimate the sensitivity of drug when exposed to contents of gastrointestinal tract.

Solid state reactions

In general the pharmaceutical solids degrade as a result of hydrolysis / solvolysis (solvents, moisture, pH, hydrogen and hydroxyl ions), oxidation (oxygen), photolysis (UV, visible light) and pyrolysis (activation energy) [1] and ion catalysis (trace metals and water) [5]. Pyrolysis is considered to occur in the absence of solvent and for oxidation or photolysis solvent may or may not be necessary.

Decomposition in solid dosage form is usually first order e.g. ascorbic acid tablets [505] and acetyl salicylic acid in microscrystalline cellulose [313] but becomes zero order when the products are moist indicating that the level of free water is critical. The pathways either show an initial rapid change with a gradual slowing down (topochemical) or an initial lag phase (nucleation). Some compounds degrade to produce a liquid product which acts as a solvent for the parent and promotes further degradation via a different route e.g. p-amino benzoic acid degrades to produce aniline. Quite often the solid-state degradation curves have a sigmoid shape [506,507] with initial lag phase approaching zero order followed by an acceleration phase approaching first order [508]. The acceleration phase could follow any reaction order kinetics depending on the conditions and mechanism of degradation process. Initial lag

phase is when liquid films are being formed followed by a first order reaction due to solution decomposition. Solid liquid hydrolysis is often zero order whereas in solution it is first order [509].

Solid rate hydrolysis of some water soluble drugs like propantheline bromide followed zero order kinetics below critical relative humidity (CRH) and equation X = kt above CRH ; where X is percent decomposed and k is constant independent of temperature (t) and vapour pressure [510].

The overall degradation of some drugs has been found to follow pseudo zero-order kinetics because only that fraction of the drug in solution, underwent chemical degradation. The source of solvent could be the melt of drug itself or an excipient, moisture from drug, excipient, granulation process or atmosphere.

Solid-state decomposition mechanisms are not as well understood as those involving solutions. Presence of excipients further complicates the mechanisms. The temperature effects are complicated by presence of an excipient having low melting point,loosely bound moisture or hygroscopic nature.

1.5B Stability in the presence of excipients

Knowledge of drug-excipient interactions is very useful in the selection of appropriate excipients to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation. Stability of drug in solution form is different than in solid state and studies on dilute solutions should

The drug excipient ratio is important e.g. stability of captopril in mixtures with lactose, starch and microcrystalline cellulose is inversely proportional to its concentration [511]. Captopril itself was found to be stable to temperature and high humidity but got readily oxidised in prsence of excipients.

Several binary mixtures of a drug and potential excipients are therefore prepared and subjected to accelerated stability studies. The interactions are accentuated for easier detection by granulating drug-excipient mixture with water or another solvent. The techniques commonly employed in drug-excipient compatibility screening include : chromatography (HPLC or HPTLC), differential thermal analysis and diffuse reflectance spectoscopy.

1.5C **Factors affecting drug stability**

1. Light

Oxidation and to some extent hydrolysis are often catalysed by light. When molecules are exposed to electro-magnetic radiation they absorb the light at characteristic wavelength which causes increase in the energy state of the compound leading to drug decomposition. Many drug substances fade or darken on exposure to light. The extent of degradation is usually small and limited to exposed surface area e.g. Cycloprofen becomes intensely yellow after 5 days under 900 fc of light but degradation was less than 2 % only [1]. Photodegradation is dependent on both the intensity and wavelength of light and is usually mediated by free radicals to produce dark-coloured products. For light

effects the samples are kept in laboratory window. Lamps generally produce a lot of heat hence are not true representative to natural sunlight behind glass [509]. The changes in appearance may be recorded visually or quantitated by diffuse refleclance spectroscopy or high performance thin layer chromatography.

2. Oxygen

Evaluation of drug sensitivity to atmospheric oxygen is necessary to establish if the final product should be packed under inert atmospheric conditions or should contain an antioxidant.

Sensitivity to oxidation can be ascertained by investigating its stability in an atmosphere of high oxygen tension. Usually a 40 % oxygen atmosphere allows for a rapid evaluation. Samples may be kept in desiccator and flushed with desired atmosphere [1]. The samples are flushed with oxygen and studied for stability at accelerated temperature alongwith control which is flushed with inert gas, nitrogen or argon.

3. pH

Many drugs are stable between pH 4 and 8 and the degradation of most drugs is catalysed by extremes of pH. Weakly acidic and basic drugs are most soluble when ionized and in that form they are most likely to be unstable since the species are charged. In some cases the inclusion of a water miscible solvent in the formulation will increase stability by suppressing ionization ; reducing the extremes of pH to achieve solubility ; and decreasing the water activity by reducing the polarity of the solvent mixture [5].

4. Moisture

Moisture has its influence on stability of drug especially in presence of other active or inactive ingredients besides its effect on compression characteristics and hardness of tablets. A high degree of hygroscopicity may adversely affect physical and chemical properties of drug making it difficult to work with.

Many drugs has tendency to absorb moisture. The amount of moisture absorbed by a fixed weight of anhydrous sample in equilibrium with the moisture in the air at a given temperature is referred to as equilibrium moisture content [EMC].

Most effective and simple means of assessing drugs sensitivity to moisture is to measure hygroscopicity [509]. Materials unaffected by humidity are termed non-hygroscopic, whereas those in dynamic equilibrium with water from the atmosphere are hygroscopic and this varies in extent.

Moisture absorbing tendency of a drug should be investigated by exposing accurately weighed samples to various humidity conditions. The weight gain or loss at equilibrium is calculated. Hygroscopicity can be classified based on moisture gain in one week at different relative humidities [512].

1.5D Interpretation of solid state stability data

The mechanisms of solid state degradation are complex and difficult to elucidate [513-515]. Most solid-state reactions are not amenable to the Arrhenius treatment. Effect of temperature can be

described by Arrhenius equation but effect of humidity however can not be explained by a general equation because moisture may affect the rate in a variety of ways depending on decomposition mechanism [516]. In solid dosage forms more than one degradation route may be operative, proceeding at different rate. Moreover the thermal degradation of excipients like sugar may catalyse the degradation of other components [517]. Such pyrolytic reactions virtually donot proceed at room temperature. Solid state reactions in general are slow and it is customary to use stress conditions in the investigation of stability. However care should be exercised in extrapolation. Degradation pathways observed at elevated temperature may not be operant at lower temperature e.g. ergot alkaloids degrade completely within a year when stored at temperature above 45°C, however the rate is less than 1 % per year below 35°C [518].

Shelf life calculation in which the concentration change is generally no more than 10 - 20 %, the distinction between reaction orders is relatively unimportant [519]. This can simplify the data treatment. Expiration dates are usually based on assumed zero or first order kinetics. Since statistical methodology is most conveniently applied to linear concentration -time profiles and the error involved in this assumption for decomposition upto 25 % is generally small, most stability protocols make this assumption [519]. The statistical treatment of stability data is based on use of linear regression analysis.

DRUG PROFILE

OF

RIFAMPICIN

CHAPTER-2

Rifampicin (rifampin, rifamycin, rifamycin AMP), 3-(4-methyl-1-piperazinyl-iminomethyl)-rifamycin SV [520] (molecular weight, 822.96) is a semisynthetic rifamycin. Rifampicin was developed in 1965 jointly by Lepetit and Ciba Geigy and introduced in 1968 [521].

Rifampicin is a red-brown crystalline powder. It has high bactericidal activity against *Mycobacteria*, *Staphylococci* *chlamydia* [522], *Neisseria meningitidis* and *Haemophilus influenzae* and is most useful for tuberculosis and leprosy. High concentrations of rifampicin inhibit pox and adeno viruses [523].

A combination of rifampicin and isoniazid produces a more rapid bacterial negativity than any other combination [524]. Rifampicin is lipid soluble and thus can penetrate the cell membrane and kill intracellular organisms. Rifampicin is by far the most potent anti-leprosy drug at present. Minimum inhibitory concentration of rifampicin is 0.5 mcg/ml for *M. tuberculosis* and 0.3 mcg/ml for *M. leprae*. Rifampicin is generally given orally although intravenous preparations are also available.

Ionization constant : It exists as a Zwitterion in slightly acidic conditions, with isoelectric point equal to 4.8. The acidic function of rifampicin is associated with C1, C4 and C8 hydroxyl groups (pka = 1.7) and basic function with piperazine nitrogen group (pka = 7.9) [525, 526].

Optical rotation : + 10.6, of 0.5 % solution in $CDCl_3$.

Crystal properties : Grinding causes the crystallanity of rifampicin to disappear and amorphous form to originate.

Thermal analysis : Rifampicin melts at 183-188°C with decomposition.

Solubility : It is freely soluble in chloroform, soluble in methanol and slightly soluble in water [527]. Solubility is increased by addition of ascorbic acid [528]. Rifampicin dissolves better at low pH at which it is however strongly dissociated and hence its physicochemical properties may cause unsatisfactory absorption [529].

Surface activity : In acidic pH rifampicin has pronounced surface tension lowering activity.

UV spectra : The variation of the UV spectrum of rifampicin with pH indicates the presence of an ionizable function attributed to the acidic 8-OH group.

Bioavailability

The results of various bioavailability studies conducted on brands of rifampicin capsules indicate that variations in particle size, excipients and manufacturing process produced a marked change in bioavailability of rifampicin [530]. One or more of these factors were identified as the cause of poor bioavailability of many products. The absorption of rifampicin was reduced by food and excipients like talc, kaolin and bentonite. Bentonite adsorbs rifampicin from solutions hence is not recommended in formulations. Lower serum levels have been observed in patients after prolonged administration [530]. The absorption of rifampicin is decreased by the administration of barbiturates [531] opiates and anticholinergic drugs [532] and increased by probenecid which depressed hepatic uptake of rifampicin [533]. Female subjects are known to have higher serum concentrations

of rifampicin than males. In another study on rifampicin preparations, the significant differences in bioavailability were attributed to physicochemical properties of drug [529]. Absorption from syrup was found to be twice than that of best capsules [534]. The most important factors that may influence the absorption of rifampicin from the gastrointestinal tract have been known to be the crystal form, particle size, manufacturing formula and process [535-537]. Pande and Mishra also reported significant bioinequivalence among marketed rifampicin products [538].

Pepper extract has been reported to enhance significantly the bioavailability of rifampicin and reduce the dose to mere 200 mg per day. Pepper enhances the absorption of rifampicin and prevents it from getting metabolised. Sesame oil and middle chain glycerides considerably increased bioavailability of rifampicin by depressing its hepatic biliary transport [539].

Stability

Rifampicin is stable when drug is protected from light and air [540]. It is stable in dry state upto 70°C [541]. In mildly alkaline aqueous solutions and in the presence of atmospheric oxygen it oxidises into rifampicin-quinone at room temperature and into 25-desacetyl rifampicin, 25-desacetyl-23-acetyl- rifampicin and 25-desacetyl-21-acetyl-rifampicin at 60-70°C [542]. Ascorbic acid slows down oxidation in alkaline solutions [543]. In acidic aqueous solutions and in absence of oxidative reactions, the main decomposition products are 3- formyl rifamycin SV and 1-amino-4

methyl-piperazine [544] formed by the reversible azomethine bond cleavage. Former has low solubility and detectable only when initial drug concentrations are low (< 21 mcg/ml) and latter is not detectable due to its polarity and UV transparency [545]. Rifampicin hydrolysis is subject to specific acid catalysis. Alkaline pH values result in further degradation to 25-desacetyl rifampicin [546]. In solid state 3-formyl rifampicin SV and rifampicin quinone are two main impurities. Seydel [547] studied pH rate profile for the pH range 0-2. The drug was found to be unstable in this pH range and the activation energy was 19.2 Kcal/mol. Phosphate buffer was found to catalyze the degradation of rifampicin. Frankerd and Co-workers studied stability of rifampicin in aqueous buffered solutions at pH 1 to 5 and reported degradation by reversible hydrolysis of azomethine ring [545]. Effect of buffers on stability was not studied. The concentration time plots initially were concentration dependent and approached equilibrium, consistent with a reversible reaction.

Pharmacokinetics

Rifampicin is well absorbed, widely diffused in tissues and body fluids. Therapeutically active concentrations are attained in tears, saliva [548-550] and cerebrospinal fluid [551, 552]. Clinically significant levels of drug cross plancenta and reach foectus [553]. About 80 % of rifampicin is protein bound *in vivo* [554]. plasma half life is between 2.3-5.1 hours from a single dose [525]. The peak serum concentration is usually reached in 2 hours but may vary from 1-4 hours [555]. Larger doses result in greater than proportional peak

levels because there is a limit to the rate at which liver can transport the drug into the bile. Peak serum levels achieved are 2,4,6,10 and > 30 mcg/ml after dose of 150,300,450,600 and 1200 mg dose respectively. The serum levels change after continuous administration of rifampicin [556]. The half life becomes shorter during first 6 days of treatment due to increased metabolism caused by its own enzyme activity which then stabilizes [555].

After absorption from the intestine, rifampicin is partly metabolised in liver to relatively more water soluble desacetyl rifampicin which is excreted via bile into the intestine. Desacetyl rifampicin is transferred into bile three times faster than rifampicin [555]. Unchanged rifampicin excreted in bile is readily reabsorbed from the gut but desacetyl metabolite is poorly absorbed. Rifampicin levels attained in bile are about 100 times higher than those in serum at the time [557]. Some 15-20 % of desacetyl rifampicin is converted to glucuronide in the liver [574].

In general, urinary concentrations and recovery of drug are related to serum levels[558]. Desacetyl rifampicin accounts for >50% of all antibacterial activity in the urine on day one of administration [555]. Eventually about 60 % of a single dose of the drug is excreted in the faeces [557].

An increase of 4-6 fold in sputum levels and 20-30 % urine levels of rifampicin has been reported when combined with ambroxol-HCl [559]. Elimination is mainly through bile and also through urine. The main metabolite in man is 25-desacetyl rifampicin [560] which is less

lipophilic and is easily excreted in urine and is not reabsorbed. Desacetyl rifampicin is active antibacterially but activity is less than parent drug. Second metabolite reported is 3-formyl rifamycin SV [561]. Rifampicin is one of the most potent inducers of hepatic mixed function oxidase activity in man, increasing the metabolism of other concomittantly administered drugs such as oral contraceptives, oral anticoagulants and hypoglycemics [525, 562].

Rifampicin specifically inhibits bacterial RNA polymerase, thus inhibiting bacterial synthesis [563]. It has no action on mammalian RNA polymerase [564]. Rifampicin is a well-tolerated drug and serious reactions are uncommon. Rifampicin causes a reddish discolouration of the urine and other body fluids such as sputum, sweat and tears [565].

OBJECTIVE

AND

EXPERIMENTAL DESIGN

CHAPTER-3

The main objective of this investigation was to find the ways and means to improve the bioavailability of rifampicin and study its stability and compatibility to generate data for the formulation pharmacist to develop stable, elegant, safe and effective pharmaceutical dosage forms. Rifampicin and excipients were of pharmacopoeial grade and were tested for their identity and purity against their respective compendial standards. The non-pharmacopoeial materials were tested against suppliers specifications. All the reagents and solvents were of analar grade. All powder excipients were sifted through 40 mesh before use to remove any extraneous matter. Total experimental work was conducted in four phases.

Experimental-I

The success of any experimental work lies in the availability of specific, precise accurate and sensitive analytical methods. For compatibility and bioavailability evaluation the methods should be able to discriminate the parent drug from its degradation products and metabolites respectively. In the first phase the stability indicating HPLC and HPTLC analytical methods were developed suitable for analysis of rifampicin and its degradation products in stability samples, and biofluids. The isoniazid being most common therapeutic adjunct of rifampicin, a suitable dissolution method for these fixed dose combination forms was also developed.

A well characterised and validated analytical method for quantitative determination of drug in biological fluids is essential

to satisfactorily evaluate and interpret bioavailability, bioequivalence and pharmacokinetic data. A suitable HPLC method was developed to analyse drug in biofluids.

Experimental-II

In phase two, various physicochemical properties of drug which have influence on the stability, formulation and bioavailability of drug were investigated. The pH of drug and common pharmaceutical excipients was recorded. The effect of pH on solubility and stability of drug was investigated. Effect of drug concentration and common buffering agents on rifampicin stability was studied. Other important properties studied include particle size, size distribution and related parameters, moisture sorption capacity, dielectric constant, solubility parameter and intrinsic dissolution rate of drug. Effect of pH of media on the light absorbance pattern was also studied.

Experimental-III

Phase three of investigation was devoted to the drug stability and compatibility. Stability of drug was studied *per se* and in the presence of common pharmaceutical excipients. Effect of these excipients on drug's solubility was also determined. The photosensitivity of drug was studied in the presence of visible (day light) and UV light (254 and 366 nm wavelengths) on both solid state and aqueous solutions. Effect of atmospheric oxygen on aqueous drug solutions was also investigated. The pH is an important determinant of

drug stability *in vitro* and in gastrointestinal tract. The stability of drug over a wide pH range was investigated. Effect of common buffering agents on drug stability was also studied.

Shah *et al* [566] have reported strong incompatibility between rifampicin and isoniazid in aqueous solutions although the fixed dose combinations of both drugs are commonly available and popularly used. Investigation was undertaken to see the influence of this incompatibility on bioavailability of both drugs.

Experimental-IV

In the last phase, attempts were made to improve the *in vitro* dissolution rate and in vivo availability of drug. The effect of an important physical property of drug i.e. its particle size on dissolution and bioavailability was studied. Bioavailability from different size fractions was compared.

Effect of common pharmaceutical surface active agents like polysorbate 80, sodium lauryl sulphate, docusate sodium, poloxamer 188 and bile salts on drug's dissolution and partition coefficient was evaluated. The influence of these surfactants on drug's bioavailability in rats was investigated. Polysorbate 80 which had maximum beneficial effect on drug availability in rats was further investigated for its effect on bioavailability in man. Effect of three concentrations of polysorbate 80 (0.25 %, 1.0%, 2.5 %) was evaluated, so as to select the appropriate concentration.

The amorphous form and one additional polymorph of rifampicin were prepared in laboratory and compared with regular crystalline rifampicin for their effect on dissolution rate and bioavailability.

Cyclodextrins and povidones of different molecular weight were studied for their influence on drug solubility. The solid dispersions were prepared using povidone, polyethylene glycol 4000, and mannitol and studied for their influence on dissolution rate of drug. Similarly solid surface dispersions were prepared using talc, starch, lactose, colloidal silicon-dioxide and microcrystalline cellulose as adsorbents and studied for their effect on drug dissolution. The selected solid dispersion and solid surface dispersion were investigated for their effect on drug bioavailability.

Bioavailability studies

Animal bioavailability experiments were conducted in albino rats using *in situ* model of Doluisio and coworkers [466] Human bioavailability of drug was studied in healthy male volunteers. Volunteers were selected from the employees of the organisation. A written consent was taken from each selected volunteer.

Selection criteria for volunteers

All volunteers selected for the study were males between the age of 20 and 40 years. Weight range of the volunteers was 50-80 kgs, with individual weight variation not more than $\pm$ 10 % and normal for height and body frame (L.I.C. chart). Each volunteer was given a general

physical examination, which included blood pressure, general observations, history, complete haemogram, urine analysis & biochemistry of blood (blood urea, nitrogen, serum alkaline phosphatase, SGOT, serum bilirubin etc). Volunteers having no clinically significant abnormal findings were considered.

Volunteers with a history of alcohol or drug addiction during the past two years, gastrointestinal, renal, hepatic, cardiovascular disease, tuberculosis, epilepsy, asthama, diabetes, psychosis or glaucoma were also not considered for this study. None of the volunteers had a history of allergic response to Rifampicin.

Restrictions

Volunteers were not allowed to take drug two weeks prior to and during the study period. No food was allowed 12 hours prior to commencement of study.

Dosage forms

The marketed products where ever use were from recently manufactured batch. All preparations/marketed products were coded prior to the commencement of study by an independent person and decoded only after completion of the study.

MATERIALS

Material	Grade	Source
1. Rifampicin	IP	Lupin Laboratories, Bombay
2. Lactose	IP	C. C. F. Holland
3. Maize Starch	IP	Universal Starch, Bombay
4. Sodium Lauryl Sulphate	IP	Suyash Chemicals, Bombay
5. Polysorbate 80	IP	Hico products, Bombay
6. Bile Salts	Fr.P	Dien Chemicals Vadodra
7. Sucrose	IP	J.S.S.K. Jalana
8. Microcrystalline Cellulose	IP	FMC Corporation, USA
9. Mannitol	IP	Jayant Vitamins, Ratlam
10. Gelatin	IP	Shaw Wallace & Gelatins, Calcutta
11. Dibasic Calcium phosphate	IP	Enar Chimie, Vadodra
12. Magnesium Stearate	IP	Parag Fine Organics, Bombay
13. Talc	IP	Deeprag Chemicals, Bombay
14. Colloidal Silicon Dioxide	IP	Aerosil 200 of Degussa, Belgium
15. Sodium Starch Glycolate	IP	AMK Chemical Industries
16. Docusate Sodium	BP	Alpha Biochem., Bombay
17. Povidones	IP	BASF, Germany
18. Urea	IP	S.D. Fine Chemicals,Bombay
19. Isopropyl Alcohol	IP	Ranbaxy, Delhi

20. Polyethylene glycols	IP	Technoproducts, Bombay.
21. Hydroxypropyl Beta-cyclodextrin		Aldrich Chemical Co. INC, USA
22. Alpha, Beta and gamma cyclodextrins		American Maize products, USA
23. Poloxamer 188 (Lutrol F68)	USNF	BASF, Germany

EXPERIMENTAL-I

DEVELOPMENT OF ANALYTICAL METHODS

CHAPTER-4

4.1 DEVELOPMENT OF STABILITY INDICATING HPLC METHOD FOR RIFAMPICIN ESTIMATION

The following HPLC method was developed to analyse rifampicin using Waters (Milford, USA) HPLC system comprising of two 510 dual pump solvent delivery system, a 680 automated gradient controller, a 490E multiwavelength UV-visible detector and a fixed loop of 100 ul with 7125 Rheodyne (Cotati, USA) injector. The chromatograms were recorded on PC/AT integrator using Oracle 2 software.

Column : Microbondapak C18 (3.9 mm x 30 cm)

Mobile phase : 0.01 M disodium hydrogen orthophosphate solution : methanol (30 : 70)

pH : 4.6 $\pm$ 0.1 adjusted with orthophosphoric acid

Detection : 254 nm

Flow rate : 1.0 ml/min.

Purity of the rifampicin peak was confirmed by ratio plot method. Impurities/degradation products such as 25-desacetyl rifampicin, rifampicin quinone, 3-formyl rifamycin SV and rifampicin N-oxide are well separated from the rifampicin peak. The above method was validated for its linearity, precision and accuracy.

Linearity

A stock solution of rifampicin was prepared by dissolving 100 mg of rifampicin in 100 ml of methanol. It was suitably diluted with mobile phase to get the solutions having concentrations 10, 20, 40, 80, 160 and 200 mcg/ml of rifampicin. These solutions were injected into HPLC. The analysis and measurement at each concentration

point of the calibration curve was repeated six times. The plot of average peak area versus concentration of rifampicin was found to be linear in the range of 0-200 mcg/ml.

Precision

Both within-day and between-day variations were checked. A sample having a known quantity of rifampicin was subjected to 6 repeat analysis on the same day. The same sample was also subjected to analysis on four alternate days. The analysis results are summarised in Tables 4.1A and 4.1B.

Accuracy

The method was also subjected to the recovery studies. Two brands of capsules (A & B) were chosen for this purpose. The recovery of the added working standard of rifampicin was studied at four different levels. Known amounts of the drug at concentration levels 40, 80, 120 and 160 mcg/ml were added to the preanalysed samples and analysed by the present method. Each level was repeated six times. The results are given in Tables 4.1C, 4.1D, 4.1E and 4.1F.

4.2 DEVELOPMENT OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR MONITORING DEGRADATION PRODUCTS OF RIFAMPICIN IN DRUG EXCIPIENT INTERACTION STUDIES

This study was undertaken to develop a simple and sensitive TLC method making use of the UV absorbance characteristics of rifampicin and its degradation products.

The following HPTLC method was developed to monitor the degradation products of rifampicin.

Instrument

Camag HPTLC system (Camag, Muttenz, Switzerland) comprising of

1. Linomat IV automatic sample applicator
2. TLC Scanner II coupled to PC/AT with software CATS 3, version 3.15.

Conditions

HPTLC plates	: Silica gel 60 F254 coated, 10 x 20 cm, glass plates (E. Merck)
Mobile phase	: Chloroform : methanol : water (80:20:2.5 v/v/v)
Developing chamber	: Camag twin trough glass tank
Band length	: 6 mm
Interband distance	: 4 mm
Spotting rate	: 15 sec/ul
Lamp	: Deuterium for rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide, 25-desacetyl rifampicin and other unknown impurities. Tungesten lamp for rifampicin quinone.
Wavelength	: 330 nm for rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide, 25-desacetyl rifampicin and other unknown impurities. 490 nm for rifampicin quinone
Scanning speed	: 4 mm/sec.
Scanning slit	: 0.2 x 3 mm.

Preparation of rifampicin, 3-formyl rifamycin SV, rifampicin-N-oxide and 25-desacetyl rifampicin standard solutions

The stock solutions containing 10 mcg/ml rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide and 25-desacetyl rifampicin separately were prepared by dissolving in chloroform. An aliquot of 5, 10, 15, 20, 25 and 30 ul of each standard were applied (6 mm band) on TLC plates (4 mm between bands) by Linomat IV sample applicator to get 50, 100, 150, 200, 250 and 300 ng/spot for the determination of linearity. The spots were dried by nitrogen.

Preparation of rifampicin quinone standard

A stock solution containing 25 mcg/ml rifampicin quinone was prepared by dissolving in chloroform. For the determination of linearity 4, 6, 8, 10, 12 and 14 ul of stock solution were applied (6 mm band) on TLC plate (4 mm between bands) by Linomat IV sample applicator to get 100, 150, 200, 250, 300 and 350 ng per spot. The spots were dried by nitrogen.

Preparation of rifampicin test solution

A stock solution containing rifampicin test sample was prepared in chloroform to get 1000 mcg/ml concentration. A 20 ul of this stock solution was applied (6 mm band) on TLC plate (4 mm between bands) by Linomat IV sample applicator. The spots were dried by nitrogen.

Thin Layer Chromatography

The TLC plates were developed in an unlined glass twin trough

tanks (Camag) with chloroform-methanol-water (80:20:2.5 v/v/v) as mobile phase. The equilibration time to saturate the tank atmosphere was 1 h. TLC plates were developed over a distance of 5 cm and finally air dried.

Densitometric measurement of the chromatograms

The developed plates were quantified by linear scanning at 4 mm/sec with a TLC scanner II (Camag). A monochromator absorbance mode with a deuterium light source at 330 nm and slit dimension of 0.2x3 mm was used for determination of rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide, 25-desacetyl rifampicin and other unknown impurities. Rifampicin quinone was determined at 490 nm using tungsten lamp. The quantification of rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide, 25- desacetyl rifampicin and rifampicin quinone was done by comparing the peak areas of test samples with the peak areas of respective standards. A 1 % equivalent spot of rifampicin was used to quantify the unknown impurities for which reference standards were not available. The procedure adopted is based on the USP recommendation.

Linearity

The calibration curves were linear in the range of 50-300 ng/spot for rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide, 25-desacetyl rifampicin and 100-350 ng/spot for rifampicin quinone. Linearities of rifampicin, 3-formyl rifamycin SV, rifampicin N-oxide and 25-desacetyl rifampicin were tested with sample spots of 50, 100,

150, 200, 250 and 300 ng/spot and of rifampicin quinone with sample spots of 100, 150, 200, 250, 300 and 350 ng/spot.

Recovery

Recovery studies were performed by spiking a test solution with known concentrations of rifampicin, rifampicin quinone, 25-desacetyl rifampicin, rifampicin N-oxide and 3-formyl rifamycin SV. For calculation of recovery yields, the resulting peak areas were compared with those of rifampicin, rifampicin N-oxide, 25-desacetyl rifampicin, 3-formyl rifamycin SV and rifampicin quinone reference standard in chloroform. Samples containing three different concentrations of rifampicin, rifampicin N-oxide, 25-desacetyl rifampicin, 3-formyl rifamycin SV and rifampicin quinone were determined (n=6). The results are summarised in Table 4.2A.

Precision

Reproducibility experiments were carried out for five components for three concentrations (n=6). The results obtained are summarised in Table-4.2A

The applicability of the method was demonstrated with rifampicin samples drawn from preformulation accelerated stability samples blended with various excipients.

The results are discussed under section 6.1 of Experimental III. The representative chromatograms are given in Figure 4.2A.

4.3 THE DEVELOPMENT OF A SUITABLE DISSOLUTION RATE TEST METHOD FOR RIFAMPICIN-ISONIAZID FIXED DOSE COMBINATION PRODUCTS

Isoniazid is most common therapeutic adjunct for rifampicin. Because of known incompatibility of rifampicin and isoniazid in solution, it was decided to develop a suitable methodology for *in vitro* evaluation of rifampicin and isoniazid fixed dose combination formulations. Two marketed capsule dosage forms, each containing rifampicin 450 mg + isoniazid 300 mg and labelled as products A and B were studied.

The experiments were conducted using 900 ml of 0.1 N hydrochloric acid solution (pH 1.2) and 0.4 % w/v sodium lauryl sulphate solution (pH 7.0) as dissolution media maintained at 37 ± 0.5°C in USP type-I apparatus with basket speed set at 50 rpm. Both samples were analysed on HPLC system as the presence of isoniazid in the solution can not be differentiated from rifampicin by UV spectrophotometer. Isoniazid interferes in rifampicin absorption.

10 ml samples were drawn at the intervals of 10, 20, 30, 45, 60 and 120 minutes and suitably diluted with mobile phase. Volume was replaced with 10 ml fresh dissolution fluid. The diluted solution was then chromatographed using HPLC system, mentioned earlier under section 4.1 for rifampicin. Isoniazid also gets resolved under same conditions. The test was performed on two marketed products to confirm the results. The values are mean of 12 unit determinations and the concentration values are corrected for volume withdrawn. The results are given in Tables 4.3A and 4.3B. The dissolution profiles are plotted in Figures 4.3A and 4.3B.

4.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ANALYSIS OF RIFAMPICIN IN BIOFLUIDS

The following HPLC method was developed to estimate the content of rifampicin in biofluids, using Waters HPLC configuration described earlier under section 4.1.

Column	: u Bondapak C18 (3.9 mm x 30 cm)
Mobile phase	: 0.01M disodium hydrogen orthophosphate : methanol: acetonitrile (45 : 55 : 10 v/v/v)
pH	: 4.6 ± 0.1 adjusted with orthophosphoric acid
Detection	: 334 nm
AUFS	: 0.008
Flow rate	: 1.4 ml/min.
Injection volume	: 100 ul

Preparation of stock solution

A stock solution of rifampicin was prepared by dissolving 100 mg of rifampicin in 100 ml methanol. It was suitably further diluted with water to get a stock solution containing 100 mcg/ml of rifampicin. The retention time of rifampicin (6.4 min) was determined by injecting an aliquot of the standard solution into the HPLC system.

Sample preparation

1 ml of serum was taken in a centrifuge tube and 2 ml of acetonitrile was added to it. The sample was then vortexed for 5 minutes on vortex shaker and centrifuged at 5000 rpm for 10 minutes. The supernatent was filtered and injected into HPLC system.

Precision

The within-day reproducibility was checked by repeat analysis of four concentrations on the same day. The between-day reproducibility was assessed by reanalysing two extreme concentration samples (0.1 mcg/ml and 10.0 mcg/ml) on alternate days for one week. Each concentration was repeated six times. The results are given in Table 4.4A.

Construction of the calibration curve

Stock solution was suitably diluted with serum to get 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mcg/ml of rifampicin. These solutions were processed as described in the sample preparation. Under the stated experimental conditions the calibration curve was linear from 100 ng to 10 mcg/ml. The analysis and measurement at each concentration point of the calibration curve was repeated six times.

Recovery

The recovery of rifampicin was assessed at concentrations of 0.1 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mcg/ml in serum. Six samples at each level containing rifampicin were processed by sample preparation procedure and injected. Six injections of same amount of rifampicin in mobile phase were injected directly. The peak areas in both sets of samples were measured. Recovery of each sample was computed using the following equation :

$$\text{Recovery (\%)} : \frac{\text{Peak area of extracted sample}}{\text{Mean peak area of direct injection}} \times 100$$

The results are summarised in Table 4.4B

Table 4.1A : Precision data of within-day study (Intraday precision)

Product	Analysis results (%)						C V (%)
	I	II	III	IV	V	VI	
Rifampicin capsules Brand A (450 mg/cap)	99.23	99.76	98.99	100.60	99.56	99.03	0.607

Table 4.1B : Precision data of bewteen-day study (Interday precision)

Product	% Recovery				C V (%)
	1st day	3rd day	5th day	7th day	
Rifampicin capsules Brand A (450 mg/cap)	100.60	98.99	99.02	99.63	0.76

Table 4.1C : Recovery data of within-day study (Brand A)

Amount added (mcg/ml)	Amount found	% Recovery (mean $\pm$ s.d.,n=6)	Coefficient of variation (%)
40	39.57	98.92 $\pm$ 0.21	0.21
80	81.20	101.50 $\pm$ 0.35	0.34
120	118.92	99.10 $\pm$ 0.82	0.83
160	158.14	98.84 $\pm$ 0.57	0.58

Table 4.1D : Recovery data of bewteen-day study (Brand A)

Amount added (mcg/ml)	Amount found	% Recovery (mean ± s.d.,n=6)	Coefficient of variation (%)
40	39.37	98.43 ± 0.47	0.47
80	80.95	101.18 ± 0.85	0.85
120	118.14	98.45 ± 0.72	0.73
160	159.31	99.57 ± 0.66	0.66

Table 4.1E : Recovery data of within-day study (Brand B)

Amount added (mcg/ml)	Amount found	% Recovery (mean ± s.d.,n=6)	Coefficient of variation (%)
40	39.97	99.93 ± 0.47	0.47
80	81.10	101.37 ± 0.82	0.83
120	120.09	100.07 ± 0.35	0.35
160	158.17	99.23 ± 0.78	0.78

Table 4.1F : Recovery data of between-day study (Brand B)

Amount added (mcg/ml)	Amount found	% Recovery (mean ± s.d.,n=6)	Coefficient of variation (%)
40	39.77	96.42 ± 0.71	0.71
80	80.12	100.00 ± 0.45	0.44
120	119.19	99.34.± 0.97	0.98
160	161.17	100.73 ± 0.47	0.47

Table 4.2A : Recovery and Precision data

Concentration (ng/spot)	n	Recovery (%)	Average recovery %	Precision C V (%)
Rifampicin				
50	6	99.6		1.59
150	6	99.3	99.4	0.86
300	6	99.4		1.01
Rifampicin quinone				
100	6	98.3		2.01
250	6	99.8	99.6	1.93
350	6	100.7		2.58
3-formyl rifamycin SV				
50	6	98.9		1.96
150	6	99.7	98.4	1.32
300	6	96.8		1.68
Rifampicin N-oxide				
50	6	99.3		1.88
150	6	98.9	99.8	1.72
300	6	101.3		1.77
25-Desacetyl rifampicin				
50	6	102.1		1.54
150	6	99.3	100.3	1.23
300	6	99.7		1.86

Table 4.3A : Dissolution of rifampicin and isoniazid fixed-dose combination products in 0.1 N hydrochloric acid solution

Time (min)	Percent dissolved			
	Product A		Product B	
	Rifampicin	Isoniazid	Rifampicin	Isoniazid
0	0	0	0	0
10	10.11 ± 1.80	88.16 ± 1.56	16.78 ± 1.56	27.61 ± 5.31
20	43.63 ± 3.42	99.43 ± 1.34	44.35 ± 4.38	54.61 ± 4.43
30	77.19 ± 3.28	102.95 ± 1.85	62.51 ± 4.40	76.18 ± 5.44
45	87.27 ± 2.71	102.13 ± 1.34	68.34 ± 3.61	90.58 ± 2.65
60	85.21 ± 2.08	105.18 ± 1.34	69.32 ± 1.32	98.02 ± 2.23
120	64.59 ± 4.87	101.64 ± 1.42	57.10 ± 2.93	94.67 ± 2.91

All values are mean of twelve determinations (Mean ± S.E.)

Table 4.3B : Dissoluton of rifampicin and isoniazid fixed-dose combination products in 0.4% w/v sodium lauryl sulphate solution

Time (min)	Percent dissolved			
	Product A		Product B	
	Rifampicin	Isoniazid	Rifampicin	Isoniazid
0	0	0	0	0
10	17.85 ± 1.86	86.11 ± 2.16	12.50 ± 1.11	30.02 ± 6.78
20	45.05 ± 3.18	87.39 ± 5.59	28.54 ± 3.87	47.98 ± 5.76
30	60.31 ± 2.43	96.63 ± 0.63	41.66 ± 4.18	63.39 ± 5.62
45	85.05 ± 1.94	97.07 ± 0.75	62.14 ± 3.39	90.57 ± 3.61
60	90.07 ± 3.03	97.42 ± 0.54	73.52 ± 2.47	99.48 ± 1.99
120	97.16 ± 2.51	99.72 ± 2.09	81.36 ± 1.15	104.18 ± 1.00

All values are mean of twelve determinations (Mean ± S.E.)

Table 4.3C : *In vitro - in vivo* correlation coefficient for rifampicin and isoniazid fixed-dose combination

Dissolution medium	Product A	Product B
0.4 % w/v sodium lauryl sulphate solution	0.9465	0.9478
0.1 N hydrochloric acid solution	0.8021	0.7894

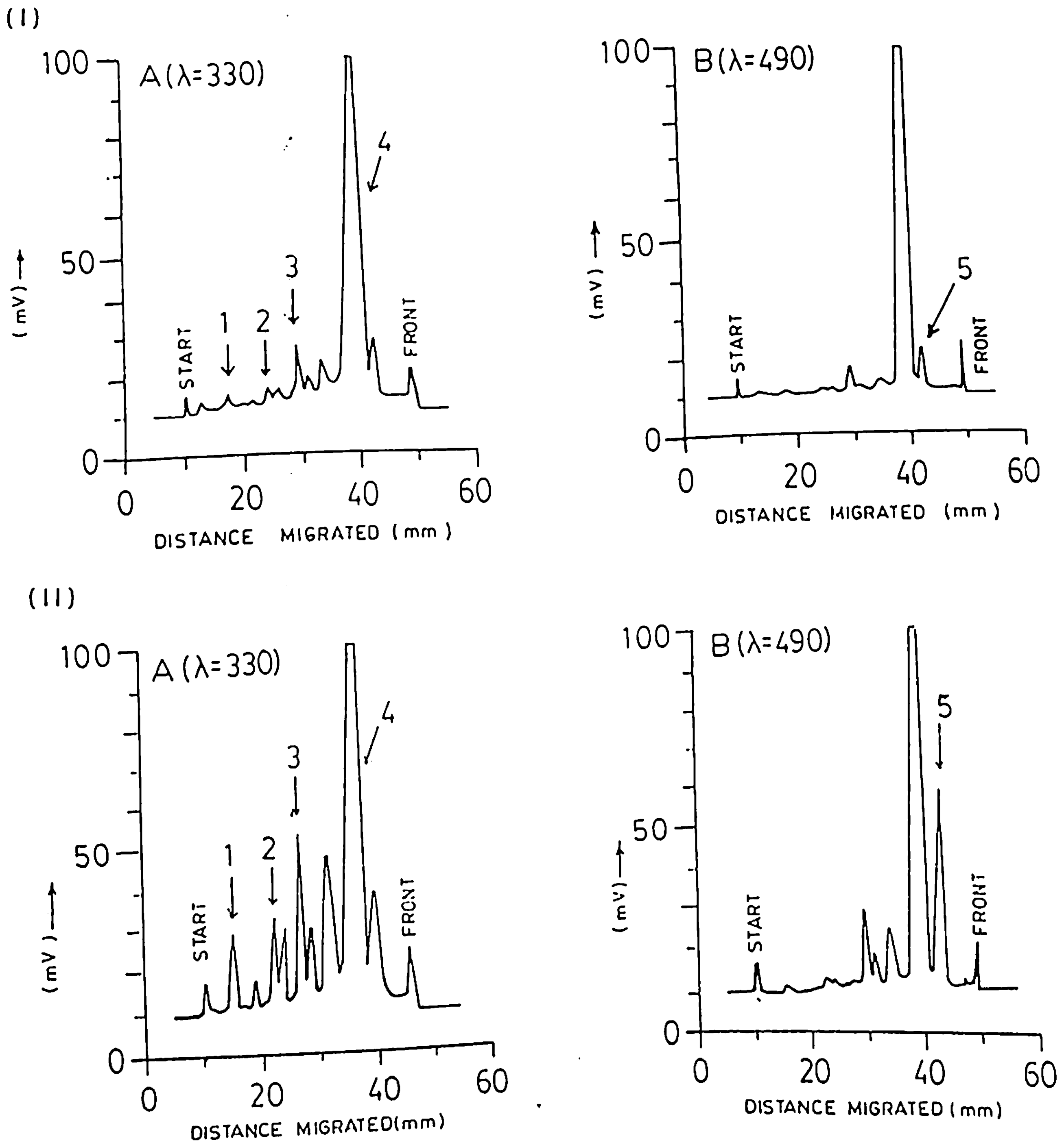
Table 4.4A : Reproducibility and precision study

Amount spiked (mcg / ml)	Amount assayed (Mean ± S.D),n = 6	Coefficient of variation (%)
within - day		
0.1	0.099 ± 0.411	4.91
2.0	1.989 ± 1.102	2.97
4.0	4.015 ± 0.907	1.78
10.0	9.980 ± 1.320	2.53
between-day		
0.1	0.098 ± 0.440	3.41
10.0	9.978 ± 1.230	2.78

Table 4.4B : Recovery of rifampicin

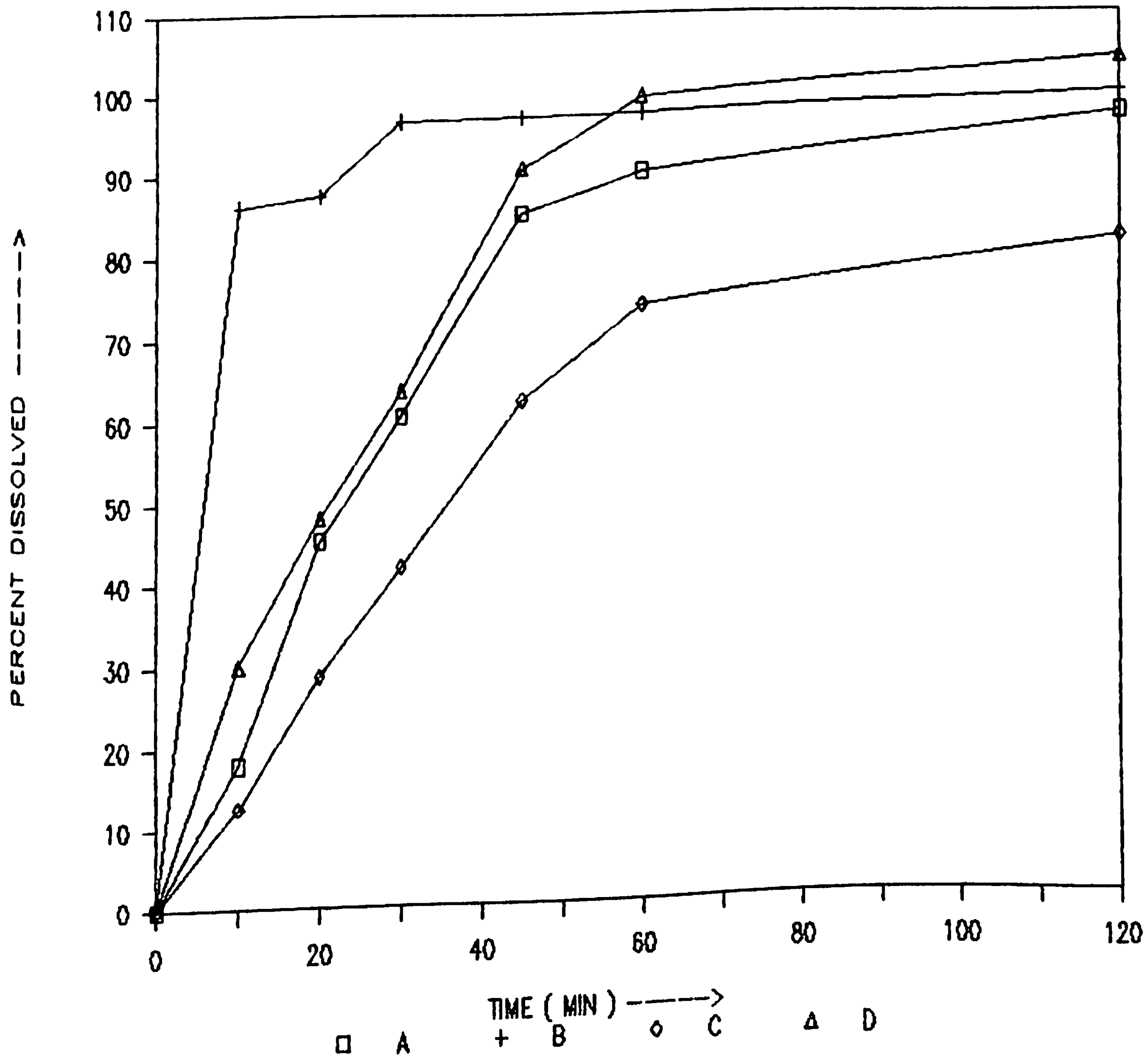
Concentration added (mcg/ml),n=6	Mean conc. found (mcg/ml)	Recovery (%)	Coefficient of variation (%)
0.1	0.101	101.00	4.02
1.0	0.980	98.00	3.21
2.0	1.990	99.50	4.01
4.0	4.050	101.25	2.50
6.0	5.950	99.17	0.80
8.0	8.100	101.25	1.32
10.0	9.920	99.20	0.89

Fig.4.2A : Representative HPTLC chromatograms



I - Detection of (A) RNo(1); 25-DAR(2); 3-FRSV(3); and rifampicin (4), (B) RQ(5) in rifampicin raw material.

II - Detection of (A) RNo(1);25-DAR(2); 3-FRSV(3); and rifampicin (4), (B) RQ(5) in rifampicin-sucrose mixture (1:1).



A = Rifampicin dissolved from product A

B = Isoniazid dissolved from product A

C = Rifampicin dissolved from product B

D = Isoniazid dissolved from product B

RESULTS AND DISCUSSION

4.1 Stability indicating HPLC method for rifampicin estimation

The analytical methods specified for rifampicin by Indian and British Pharmacopoeias are not stability indicating. The degradation products like 25-desacetyl rifampicin are biologically active and absorb in the same spectral region as parent drug and therefore interfere in both spectrophotometric and microbio-assays. For that reason rifampicin can not be isolated and quantified from its degradation components.

The purity of rifampicin peak was checked by ratio plot method which enables the detection of hidden impurities or coeluting compounds under a sample peak. A pure peak shows a constant ratio, while coeluting impurity causes a change in the shape of the ratio plot. The ratio plot of a pure compound appears as a square while an impurity tends to distort the square. In this method a square peak was obtained for rifampicin, indicating purity of peak. Well separation of impurities/degradation products from parent drug indicates the method to be stability indicating.

The linearity of the method from 0-200 mcg/ml was demonstrated by the high correlation coefficient (0.999). The very low coefficient of variation i.e. 0.607 % for intraday and 0.76 % for interday variations indicated a good precision of the method. The coefficient of variation for recovery from four different concentrations of rifampicin and two dosage forms in the within-day and between-day study, varied between 0.21 - 0.98 indicating good accuracy of the method.

This validated stability indicating method can be used for analysis of rifampicin and its finished products including stability samples.

4.2 HPTLC method for monitoring degradation products of rifampicin in drug excipient interaction studies

Under the conditions described, rifampicin showed no interference from 3-formyl rifamycin SV, rifampicin N-oxide, 25-desacetyl rifampicin, rifampicin quinone and other degradation components.

Average recovery yields were 99.4 % for rifampicin, 99.6% for rifampicin quinone, 98.4 % for 3-formyl rifamycin SV, 99.8 % for rifampicin N-oxide and 100.3 % for 25-desacetyl rifampicin indicating good accuracy.

The C.V. values for reproducibility data ranged from 0.86 to 1.59% for rifampicin, 1.93 to 2.58% for rifampicin quinone, 1.32 to 1.96% for 3-formyl rifamycin SV, 1.72 to 1.88 % for rifampicin N-oxide and 1.23 to 1.86 % for 25- desacetyl rifampicin indicating good precision.

The method described allows a simple and rapid determination of rifampicin, rifampicin quinone, 3-formyl rifamycin SV, rifampicin N-oxide and 25-desacetyl rifampicin levels without interference from each other and other degradation components present in the sample. One of the main advantages of this method is the very low limit of detection. The detection limit of each component was upto 10 ng/spot, which was possible because there was no dilution effect at the time of detection by mobile phase. The HPLC methods described in literature

are not suitable to separate all the degradation components simultaneously without any interference [567,568]. The method described here is sensitive, precise and involves a single step sample preparation. The technique may be used for routine raw material purity analysis, stability monitoring studies and dosage form analysis.

4.3 Dissolution rate test method for rifampicin-isoniazid fixed dose combination products

For any method of analysis to be acceptable,the sample should not be affected during analysis process. The dissolution system specified by USP [527] for rifampicin capsules has been reported to be unsuitable as decomposition upto 17 % was observed within 45 min in the medium specified [570]. Water can not be used as dissolution medium because of low aqueous solubility of drug. Supramicellar surfactant concentrations are used for drugs of low solubility [571] and sodium lauryl sulphate in a dissolution medium is considered to have solubilizing activity similar to that of the naturally occuring bile salts, sodium cholate and sodium taurocholate [572]. The dilute solutions of sodium lauryl sulphate have been reported to give acceptable dissolution parameters for rifampicin alone and the optimum concentration was 0.4% w/v. This medium was found to be most appropriate without significant degradation of rifampicin [243].

Many drugs or excipients readily form complexes with drug thereby enhancing or reducing the drug's solubility [282,569] or stability. In the presence of isoniazid, rifampicin degrades very quickly to form a complex [566]. The azomethine bond (-N=CH-) degrades in acidic aqueous solution by reversible azomethine bond cleavage. Reversible addition

of water across the azomethine bond forms a transient carbinolamine, which then rearranges to give 3-formyl rifamycin SV and 1-amino-4-methyl piperazine [545]. The 3-formyl rifamycin SV and isoniazid then probably undergo Schiff's reaction to form soluble complex.

The use of 0.1 N hydrochloric acid and 0.4 % w/v sodium lauryl sulphate solutions as dissolution media were tried for rifampicin and isoniazid fixed dose combination products.

In 0.1 N hydrochloric acid, maximum concentration of rifampicin obtained was 87.27% in 45 minutes for product A and 69.32 % in 60 minutes for product B which started decreasing thereafter indicating degradation. Additional peak of degradation product was also observed. This phenomenon was not observed in 0.4 % w/v sodium lauryl sulphate solution. This medium also shows a very good *in vitro- in vivo* correlation (correlation coefficient is about 0.95 for both products compared to about 0.8 in 0.1 N HCl solution) as given in Table 4.3C (refer : section 6.7 of Experimental III for *in vivo* data). Recoveries of isoniazid are also marginally better in 0.4 % w/v sodium lauryl sulphate solution. This indicates suitability of 0.4 % w/v sodium lauryl sulphate solution as dissolution medium for rifampicin and isoniazid fixed dose combination formulations.

4.4 High performance liquid chromatographic method for analysis of rifampicin in biofluids

The linearity of the method is demonstrated by high correlation coefficient (0.9998) of area versus concentration of rifampicin.

The within-day coefficient of variation (C.V.) were 1.78 - 4.91 % The between-day C.V. for analysis of the same serum samples on three different days over a period of one week were 3.41 % at 0.1 mcg/ml (n=6) and 2.78 % at 10 mcg/ml (n = 6) (Table 4.4A). The recovery values for 0.1 - 10 mcg/ml concentrations of rifampicin ranged from 99.17 to 101.25 % and C.V. values 0.8 to 4.02 % (Table 4.4B). These values are well with in 15 % C.V. limit recommended for precision and accuracy studies [576] indicating good precision and accuracy of the method. This method is suitable for estimation of rifampicin in biological fluids upto very low concentrations (0.1 mcg/ml).

EXPERIMENTAL-II

STUDY OF PHYSICOCHEMICAL DRUG CHARACTERISTICS IMPORTANT FOR DRUG COMPATIBILITY AND BIOAVAILABILITY

CHAPTER 5

EXPERIMENTAL-II

STUDY OF PHYSICOCHEMICAL DRUG CHARACTERISTICS IMPORTANT FOR DRUG COMPATIBILITY AND BIOAVAILABILITY

CHAPTER 5

5.1 THE CHARACTERIZATION OF RIFAMPICIN AND EXCIPIENTS USED

Rifampicin and all excipients used in preformulation study were tested against their respective specifications as given in Table 5.1A. The materials complying with these specifications only were used for further experimental work. The 1 % w/v and 5 % w/v solution or suspension of each ingredient was prepared in distilled water and pH was recorded. The results are given in Table 5.1A.

5.2 PREPARATION AND CHARACTERIZATION OF AMORPHOUS AND POLYMORPHIC FORMS OF RIFAMPICIN

Preparation of samples

Amorphous rifampicin

The saturated solution of rifampicin was prepared in chloroform and filtered through Whatman No 1 filter paper to remove any undissolved drug. The clear solution was then poured dropwise on a glass plate kept hot (80-85°C) on water bath so as to evaporate off the solvent fast. The dried material was then scrapped, ground in dry glass pestle mortar and passed through 100 mesh sieve. The drug was analysed as per IP specifications.

Polymorph A

The regular crystalline rifampicin (finer than 100 mesh) was labelled as polymorph A and used as control.

Polymorph B

Rifampicin was recrystallized from its saturated solution in nonpolar solvent, n-dodecane. The crystals were removed by decanting off the clear liquid followed by centrifugation of the sediment. The crystals were given two washings with petroleum ether to remove traces of n-dodecane followed by drying, first in air and then by keeping in a vacuum desiccator for 48 hours. The drug was analysed as per IP specifications and labelled as polymorph B.

Characterization of samples

Above three samples were characterized using differential scanning calorimetry and IR spectroscopy.

DSC analysis was performed using Mettler DSC system comprising of DSC 25 measuring cell, TC11 TA processor and TA72 graphware. Instrument was calibrated with indium standard. Samples (2-8 mg) were sealed in aluminium sample pans and heated in an atmosphere of nitrogen at a constant rate of 10°C/min upto 250°C. The thermograms were recorded at a chart speed of 1 cm/min. Thermal characterization values are given in Table 5.2A and thermograms in Figure 5.2A. The infrared spectra were obtained in nujol mull using JASCO FT/IR 5300 spectrophotometer. The IR spectra are given in Figures 5.2 B, 5.2 C and 5.2 D.

5.3 EFFECT OF pH ON SOLUBILITY OF RIFAMPICIN

In view of the exposure of drug to varying pH conditions (highly

acidic to mildly alkaline) of gastrointestinal tract especially due to extensive hepatic-biliary recirculation of drug, when administered orally it was decided to examine solubility and stability of rifampicin over a wide range of pH values. Solubility of rifampicin was studied in both buffered and unbuffered solutions over pH range of 1.0 to 9.0

Preparation of buffered pH solutions

Buffered solutions of pH ranging from 1.0 to 9.0 were prepared by mixing two buffer solutions A & B [577].

Solution A

Hydrochloric acid 1 M	-	94.00 ml
Glycocoll (Glycine)	-	0.50 g
Sodium chloride	-	3.68 g
Distilled water, q.s.to	-	1000 ml

Solution B

Sodium phosphate dibasic (anhydrous)	-	16.35 g
Potassium dihydrogen phosphate (anhydrous)	-	2.80 g
Sodium chloride	-	0.15 g
Distilled water, q.s. to	-	1000 ml

pH	Volume of solution A (ml)	Volume of solution B (approximately) (ml)
1.0	100	00 (pH-adjusted with dilute HCl solution)
2.0	70	30
3.0	58	45
4.0	56	48
5.0	55	49
6.0	50	50
7.0	30	83
8.0	-	100 (pH adjusted with dilute NaOH solution)
9.0	-	100 (pH adjusted with dilute NaOH solution)

Preparation of unbuffered pH solutions

Unbuffered aqueous solutions of pH ranging from 1.0-9.0 were prepared by adjusting pH of distilled water with hydrochloric acid or sodium hydroxide solutions.

Dissolution test

The dissolution rate of rifampicin was checked using USP type-II apparatus (paddle speed, 150 rpm) at 37 ± 0.5°C. Samples were drawn after 0.25, 1, 2, 4, 6 and 24 hours and analysed by standard HPLC method as described under section 4.1 of Experimental-I.

The values of rifampicin content and maximum concentration achieved (Cmax) are given in Tables 5.3A and 5.3B.

5.4 PARTICLE SIZE AND SIZE DISTRIBUTION

Particle size distribution was checked by two methods :

Sieve analysis method

Sieves were arranged in the sieve shaker with coarsest sieve at the top. 100 g rifampicin was placed on the top sieve. The sieves were shaken for 1 h. The retained fraction over each sieve was collected and weighed. The results are given in Table 5.4A.

Microscopy

The utility of fine-mesh sieves for dry powders is limited to about 75 um (200 mesh) because the smaller particles adhere to each other and the sieve wire mesh. Hence for particle size analysis of amorphous and polymorph B, microscopic method was used.

For irregular shaped particles the linear scale is preferred over British Standard graticule. On linear scale the Feret's diameter measures the gross external dimensions, whereas, the Martin's diameter bisects the particle area [581]. In this study the measurement was made in a fixed direction from edge to edge, between two parallel tangents to get Feret's diameter.

A small amount of drug was dispersed in a drop of liquid paraffin on a haemocytometer and size of particles was measured with the help of on eye piece micrometer having a transparent linear scale placed on top of an eye piece. The results are given in Table 5.4B.

5.5 DENSITY AND FLOWABILITY

Polymorph A, polymorph B and amorphous rifampicin were checked for these parameters.

A. True Density

True density of rifampicin was checked by solvent displacement method using benzene as solvent since rifampicin is insoluble in this solvent. True density was also checked by preparing hard pellets using IR pelletiser. Pellets were prepared by compressing rifampicin at 170 kg/sq.cm. pressure under vacuum. Density of pellets was computed from the weight and dimensions of pellets. The average densities obtained by two methods is reported in Table 5.5A.

B. Bulk Density

Accurately weighed 10 g rifampicin was transferred to a 50 ml dry glass measuring cylinder and tapped in the densitometer till constant volume. Packed and loose density were calculated from the tapped and untapped volumes of rifampicin.

C. Angle of Repose

The angle of repose was measured by the method of Pilpel [578]. The powder was filled in a container having built-in flat round glass platform supported on a tripod stand. The powder was then allowed to flow out at the base leaving an undisturbed conical heap on the platform. The angle of repose was calculated from the height (average of 3 readings) and diameter of the heap.

D. Carr's Compressibility

Carr defined compressibility by the expression :

(Packed density - loose density) x 100 / packed density

The compressibility of rifampicin was derived from packed and loose density.

E. Intrinsic Flowability

50 g material was weighed and placed in funnel having internal diameter of 2.5 cm. The funnel end was blocked initially. The funnel was placed above a measuring cylinder and the time required to pass the complete material through the orifice was calculated. The volume of powder achieved in the measuring cylinder was also measured. The intrinsic flowability was calculated from the following equation and expressed as cc of powder that flows through funnel per second.

$$\text{Intrinsic Flowability} = \frac{\text{Volume of powder}}{\text{Area of orifice X time}}$$

The results of above tests are given in Table 5.5A.

5.6 STUDY OF MOISTURE SORPTION BY RIFAMPICIN AT VARIOUS RELATIVE HUMIDITIES (HYGROSCOPICITY)

Moisture sorption property was studied for amorphous and crystalline forms of rifampicin by placing samples in desiccators having different relative humidity conditions.

Dry glass weighing bottles were kept open overnight in desiccator containing phosphorous pentoxide. About 1.5 g crystalline and amorphous rifampicin was transferred to two bottles each. All the four bottles were kept in desiccator containing phosphorous pentoxide and weighed every 12 hours till the constant weight was attained. Moisture content was checked by heating the sample at 105°C to a constant weight (30 minutes).

Controlled relative humidity atmosphere was created in set of glass desiccators using saturated solutions of following salts in distilled water. A liberal amount of saturated salt solutions (with excess crystals) was placed in the wells of desiccators and allowed to equilibrate for 48 hours.

Salt	Relative Humidity (%)
Lithium chloride	11
Potassium acetate	23
Magnesium chloride	33
Potassium carbonate	42
Sodium bromide	57
Sodium chloride	75
Potassium bromide	83
Potassium nitrate	93

The bottles were then transferred to next desiccator having higher humidity. Bottles were weighed every 12 hours till constant weight was attained. The average of two readings was used to calculate the increase in weight. Equilibrium moisture content was computed for each relative humidity condition. The results are given in Table 5.6A.

5.7 DIELECTRIC CONSTANT

Solubility of rifampicin was determined in various combinations of dioxan and water. Dioxan-water mixture was chosen for this study, because a range of dielectric constant values (from 2.2 to 80.2) could be obtained.

Excess amount of drug (about 2 g) was added to 50 ml solvent system in stoppered conical flasks. The flasks were agitated on a horizontal shaker operating at 30 strokes per minute and maintained at 37 ± 1°C, till equilibrium was reached. The attainment of equilibrium (about 6 hours) was verified by repeated sampling and analysis at various time intervals. At the end of 6 hours, the flasks were allowed to remain static for a period of 0.5 hour. The supernatant liquid was removed and the contents individually filtered through filter paper, rejecting first few ml of the filtrate. The filtrate was analysed by HPLC as described under section 4.1 of Experimental-I.

All the determinations were performed in duplicate and reported as mean value in a series of dioxan water combinations (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% and 100% v/v solutions of dioxan in water). Maximum solubility was obtained in dioxan : water mixtures containing 60 % and 70 % dioxan. To find exact dielectric constant the solubility was further determined in solutions containing 62.5 %, 65 % and 67.5 % dioxan. Results are given in Table 5.7A.

5.8 LIPOPHILICITY

Lipophilicity (log p) was quantified by measuring partition coefficient of drug in n-octanol and water.

Rifampicin was dissolved in n-octanol previously saturated with water to get 2 mg/ml concentration. Distilled water demineralised through Milli-Q water purification system was saturated overnight with n-octanol. 10 ml rifampicin solution in n-octanol and 10 ml water (saturated with n-octanol) was transferred to each of 21 conical flasks (capacity 50 ml) fitted in a water bath shaker maintained at 37±1°C. Triplicate units were removed after 1, 2, 3, 4, 8, 16 and 24 hours. The contents of flasks were transferred into centrifuge tubes and centrifuged for 10 minutes at 4000 rpm. The clear aqueous layer was analysed by standard HPLC method (refer : Experimental 1, section 4.1). The results are given in Table 5.8A.

5.9 INTRINSIC DISSOLUTION RATE (IDR)

Pellets of polymorph A, amorphous and polymorph B forms of rifampicin were prepared containing 125 mg of drug per pellet on IR pelletiser at 170 kg/sq. cm pressure for 2 minutes. Pellets were then mounted on lower side of basket shaft of dissolution test apparatus with molten mixture of white bees wax and carnauba wax. Before fixing, white soft paraffin was applied on thickness side of pellets, so that the dissolution of rifampicin should take place from one flat surface only.

Boiled and cooled Milli-Q purified distilled water (200 ml) maintained at 37° ± 0.5°C was used as dissolution medium. The speed of shaft was fixed at 50 rpm. Samples were withdrawn at predetermined time intervals of 10, 20, 30, 40, 60, 90, 120, 180 min and analysed by standard HPLC method (refer Experimental-I, section 4.1). The

experiment was repeated. The average of two values are given in Table 5.9A.

5.10 UV/VISIBLE SCANS OF RIFAMPICIN IN NEUTRAL, ACIDIC AND ALKALINE SOLUTIONS

Stock solution of rifampicin was prepared in methanol containing 1 mg/ml. One ml of this solution was diluted to 100 ml using water, 0.1 N hydrochloric acid solution and 0.1 N sodium hydroxide solution and labelled the flasks accordingly. All the three solutions were scanned from 200 nm to 440 nm using respective solvents as blank. The scans were recorded and compared.

UV scans of rifampicin in different solvent systems are shown in Figure 5.10A. The absorption maxima and the molecular absorptivity values obtained from the UV scans are reported in Table 5.10A.

Table 5.1A : The specifications and pH values of rifampicin and excipients

Ingredient	Specification	pH of 1% w/v solution /susp.	pH of 5% w/v solution /susp.
Rifampicin	I.P.	4.98	5.04
Polyethylene Glycol 4000	I.P.	5.43	5.36
Povidone K-30	I.P.	5.65	3.39
Urea	I.P.	6.15	7.80
Lactose	I.P.	5.75	4.58
Maize Starch	I.P.	6.60	5.10
Sodium Lauryl Sulphate	I.P.	9.56	10.26
Polysorbate 80	I.P.	6.25	6.20
Bile Salts	Fr. P.	6.25	6.17
Mannitol	I.P.	5.34	5.36
Microcrystalline Cellulose	I.P.	6.31	6.71
Sucrose	I.P.	5.80	6.17
Gelatin	I.P.	5.40	5.30
Calcium Phosphate Dibasic	I.P.	7.85	7.35
Magnesium Stearate	I.P.	8.10	8.50
Talc	I.P.	6.27	9.20
Colloidal Silicon Dioxide (Aerosil 200)	I.P.	4.94	4.30
Sodium Starch Glycolate	I.P.	5.15	4.68
Docusate Sodium	I.P.	4.27	4.10
Beta-Cyclodextrin	In house	6.44	7.30
Polaxamer 188	USNF	6.49	6.63

Table 5.2A : Thermal characterization of the transitions obtained from DSC scans of polymorphs of rifampicin

Sample	W	Endotherm					Exotherm				
		TO	TM	TR	H	Hf	TO	TM	TR	H	Hc
Polymorph A	6.0	172.0	193.4	198.0	178.0	29.7	198.0	201.7	224.0	161.0	26.9
Amorphous	7.8	159.0	166.2	184.0	31.0	4.0	187.0	200.4	221.4	167.0	21.5
Polymorph B	2.1	115.0	131.0	137.5	12.0	5.6	143.5	170.0	195.4		

W = Weight of samples (mg)
TM = Peak temperature (°C)
Hf = Heat of fusion (J/g)
Tr = Recovery temperature (°C)
TO = Onset temperature (°C)
H = Enthalpy change (MJ)
Hc = Heat of crystallisation (J/g)

Table 5.3A : Dissolution of rifampicin in buffered solutions of different pH values

pH	Rifampicin dissolved (mg/ml) at time (h)						Cmax (mg/ml)
	0.25	1.00	2.00	4.00	6.00	24.00	
1.0	27.663	25.412	20.040	19.430	19.076	14.072	27.663
2.0	9.861	10.787	9.543	8.036	7.028	2.963	10.787
3.0	0.812	0.876	0.766	0.632	0.510	0.198	0.876
4.0	0.382	0.921	0.757	0.600	0.551	0.287	0.921
5.0	1.071	1.233	0.998	0.902	0.846	0.452	1.233
6.0	1.160	1.287	1.062	0.958	0.876	0.469	1.287
7.0	1.170	1.284	1.171	1.073	1.010	0.628	1.284
8.0	2.879	2.813	2.705	2.659	2.506	1.756	2.879
9.0	4.376	4.341	4.283	4.055	3.838	2.539	4.376

Table 5.3B : Dissolution of rifampicin in unbuffered solutions of different pH values

pH	Rifampicin dissolved (mg/ml) at time (h)						Cmax (mg/ml)
	0.25	1.00	2.00	4.00	6.00	24.00	
1.0	19.017	20.530	19.907	19.686	19.535	17.513	20.530
2.0	11.753	12.144	12.132	11.779	11.627	11.993	12.144
3.0	1.153	1.205	1.050	0.903	0.725	0.448	1.205
4.0	1.345	1.772	1.155	1.102	1.043	1.018	1.772
5.0	1.177	1.913	1.660	1.439	1.219	1.192	1.913
6.0	1.286	1.249	1.209	1.173	1.173	1.463	1.463
7.0	1.249	1.211	1.185	1.110	1.173	1.412	1.412
8.0	1.173	1.160	1.135	1.103	1.047	1.412	1.412
9.0	1.286	1.274	1.185	1.212	1.249	1.463	1.463

Table 5.4A : Particle size distribution of rifampicin (polymorph A)

Sieve No (as per IP)	Nominal aperture size	Size range (um)	Mean of size range (um)	% Frequency by weight	% Frequency by number	Cumulative % frequency under size by number
36	425	-	-	0.00	-	-
44	355	355-425	390.0	1.00	0.004	100.000
50	300	300-355	327.5	1.57	0.011	99.996
60	250	250-300	275.0	3.17	0.036	99.985
85	180	180-250	215.0	6.16	0.328	99.949
100	150	150-180	165.0	71.85	3.821	99.621
150	105	105-150	127.5	8.10	93.360	95.790
170	90	90-105	97.5	6.15	1.590	2.440
200	75	75-90	82.5	2.00	0.850	0.850
300	53	53-75	64.0	0.00	-	-

Table 5.4B : Particle size distribution of polymorph B and amorphous rifampicin

Particle size		Polymorph B		Amorphous	
Size range (um)	Mean size range (um)	Frequency (%)	Cumulative % frequency (under size)	Frequency (%)	Cumulative % frequency (under size)
0 - 14	7	61.30	61.30	57.00	57.00
14 - 28	21	25.70	87.00	25.50	85.50
28 - 42	35	8.00	95.00	12.00	97.50
42 - 56	49	5.00	100.00	2.50	100.00

Table 5.5A : Physical parameters of regular rifampicin (polymorph A), polymorph B and amorphous forms

Sr. No.	Parameter	Polymorph A	Polymorph B	Amorphous
1.	Bulk density packed	0.51	0.45	0.45
2.	Bulk density loose	0.33	0.35	0.36
3.	True density	1.38	-	-
4.	Intrinsic flowability (cc/sec.)	2.18	2.10	2.03
5.	Angle of repose (°)	45.00	46.1	50.20
6.	Carr's compressibility index	35.20	22.00	20.00

Table 5.6A : Moisture gain by rifampicin at various relative humidities

Initial moisture conentent : Amorphous rifampicin - 0.41 %
Crystalline rifampicin - 0.28 %

Relative humidity (%)	Amorphous rifampicin				Crystalline rifampicin			
	Wt. of sample (mg)	Gain in weight (mg)	Amount of water in sample (mg)	EMC	Wt. of sample (mg)	Gain in weight (mg)	Amount of water in sample (mg)	EMC
0.0	1600	--	1.60	0.10	1536	--	1.50	0.09
11.0	1600	--	1.60	0.10	1536	0	1.50	0.09
23.0	1600	0	1.60	0.10	1536	0	1.50	0.09
33.0	1600	0	1.60	0.10	1536	0	1.50	0.09
42.0	1614	14	15.60	0.97	1538	02	3.50	0.22
57.0	1628	28	29.60	1.81	1541	05	6.50	0.42
75.0	1659	59	60.60	3.55	1550	14	15.50	1.00
83.0	1682	82	83.60	4.97	1557	21	22.50	1.44
93.0	1732	132	133.60	7.67	1567	31	32.50	2.07

Table 5.7A : Solubility of rifampicin in dioxan : water mixtures having different dielectric constants

Dioxan : water ratio	Dielectric constant	Solubility of rifampicin (mg/ml)
0.0 : 100	80.20	1.14
10.0 : 90.0	72.40	0.78
20.0 : 80.0	64.60	1.46
30.0 : 70.0	56.80	4.22
40.0 : 60.0	49.00	7.50
50.0 : 50.0	41.20	9.54
60.0 : 40.0	33.40	11.21
62.5 : 37.5	31.45	11.70
65.0 : 35.0	29.50	12.10
67.5 : 32.5	27.55	12,50
70.0 : 30.0	25.60	11.49
80.0 : 20.0	17.80	7.56
90.0 : 10.0	10.00	4.75
100.0 : 0.0	2.20	7.19

Table 5.8A : Partition coefficient of rifampicin in n-octanol : water system (in three sets)

Time (h.)	Partition coefficient			
	Set 1	Set 2	Set 3	Mean
1	25.67	26.03	25.04	25.58
2	22.27	21.65	21.72	21.68
3	23.22	24.06	23.41	23.56
4	24.09	24.50	25.19	24.59
8	29.95	28.46	29.66	29.36
16	32.58	33.02	30.58	32.06
24	33.93	34.60	34.27	34.27

Table 5.9A : Dissolution rate profile and intrinsic dissolution rate constant (Ki) of rifampicin polymorphs from pellets

Time (min.)	Polymorph B		Polymorph A		Amorphous form	
	Conc. (mcg/ml)	Amount dissolved (mg)	Conc. (mcg/ml)	Amount dissolved (mg)	Conc. (mcg/ml)	Amount dissolved (mg)
10.0	3.35	0.670	2.41	0.482	0.853	0.171
20.0	6.08	1.216	4.26	0.852	1.372	0.274
30.0	8.39	1.678	6.36	1.272	1.844	0.369
40.0	10.85	2.170	8.53	1.706	2.545	0.509
60.0	16.72	3.344	12.14	2.428	3.547	0.709
90.0	24.48	4.896	18.39	3.678	5.175	1.035
120.0	32.30	6.460	23.50	4.700	6.790	1.358
180.0	48.07	9.614	31.78	6.356	9.765	1.953
Ki(mg.min./cm2)		7.98	5.42		1.66	

Table 5.10A : The absorption maxima and molecular absorptivity of rifampicin in water, 0.1 N HCl and 0.1 N NaOH solutions

Solvent	Blank	Absorption maxima (nm)	Molar absorptivity (litres / mole cm)
Water	Water	235.4	36245.73
0.1 N HCl	0.1 N HCl	231.0	28854.00
0.1 N NaOH	0.1 N NaOH	236.4	32658.12

Fig. 5.2A : DSC thermograms of rifampicin

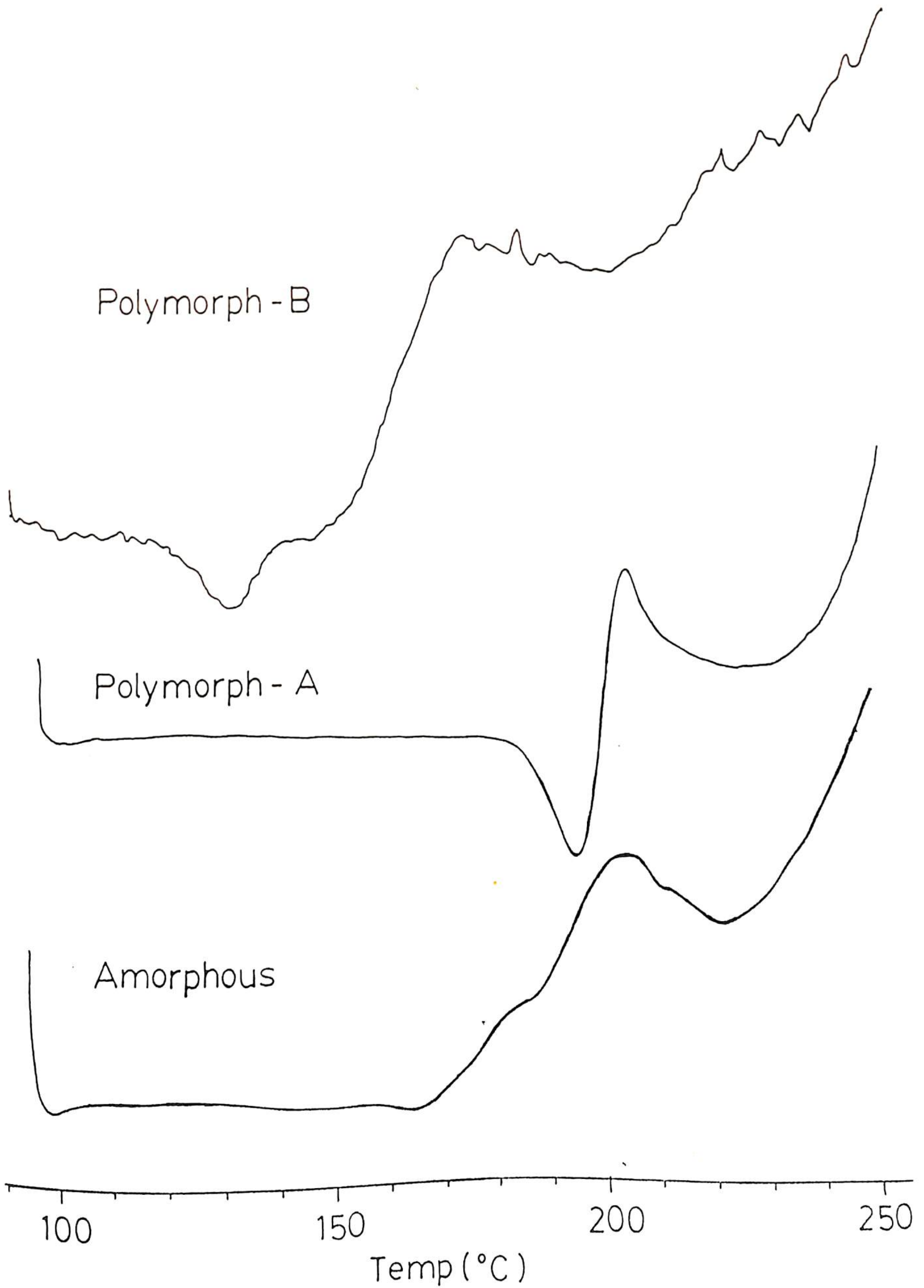


Fig. 5.2B : IR spectrum of rifampicin (Polymorph-B)

Resolution : 4
Scan : 16
Gain : 2

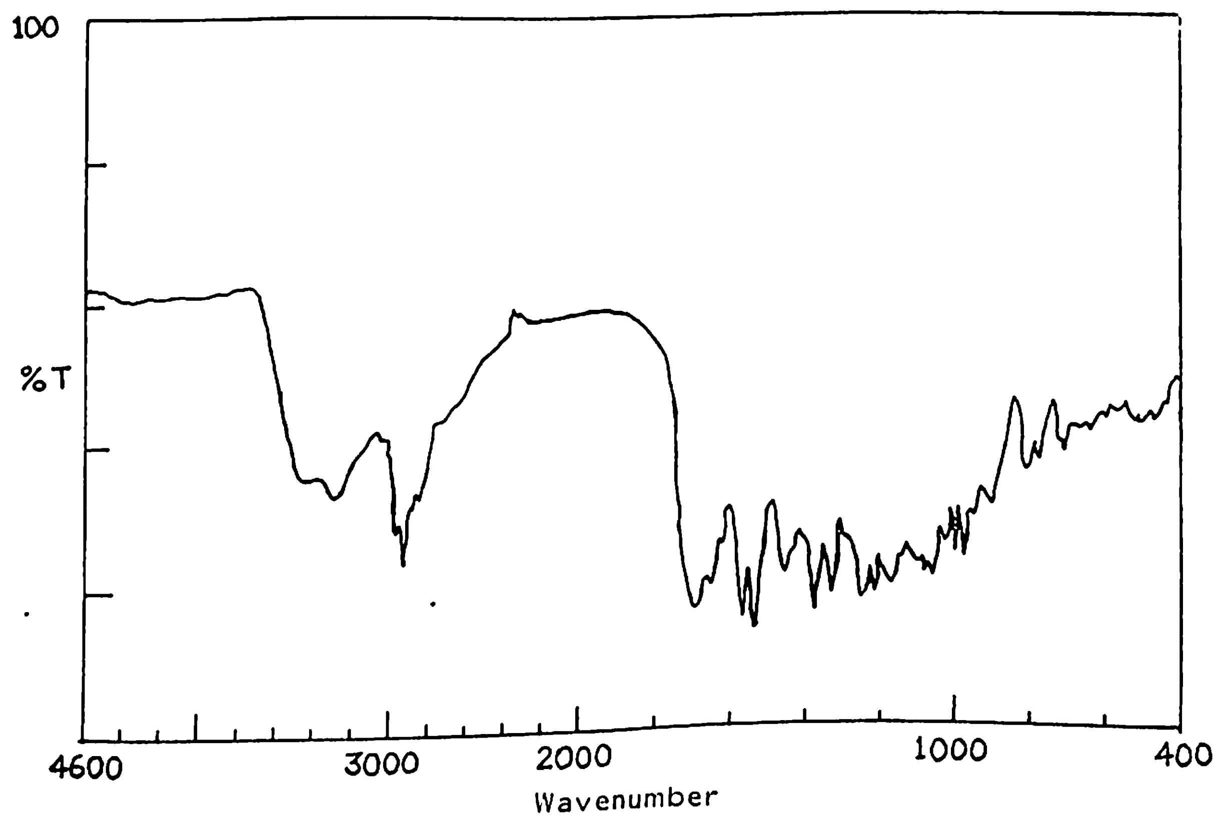


Fig. 5.2C : IR spectrum of rifampicin (Amorphous)

Resolution : 4
Scan : 16
Gain ; 2

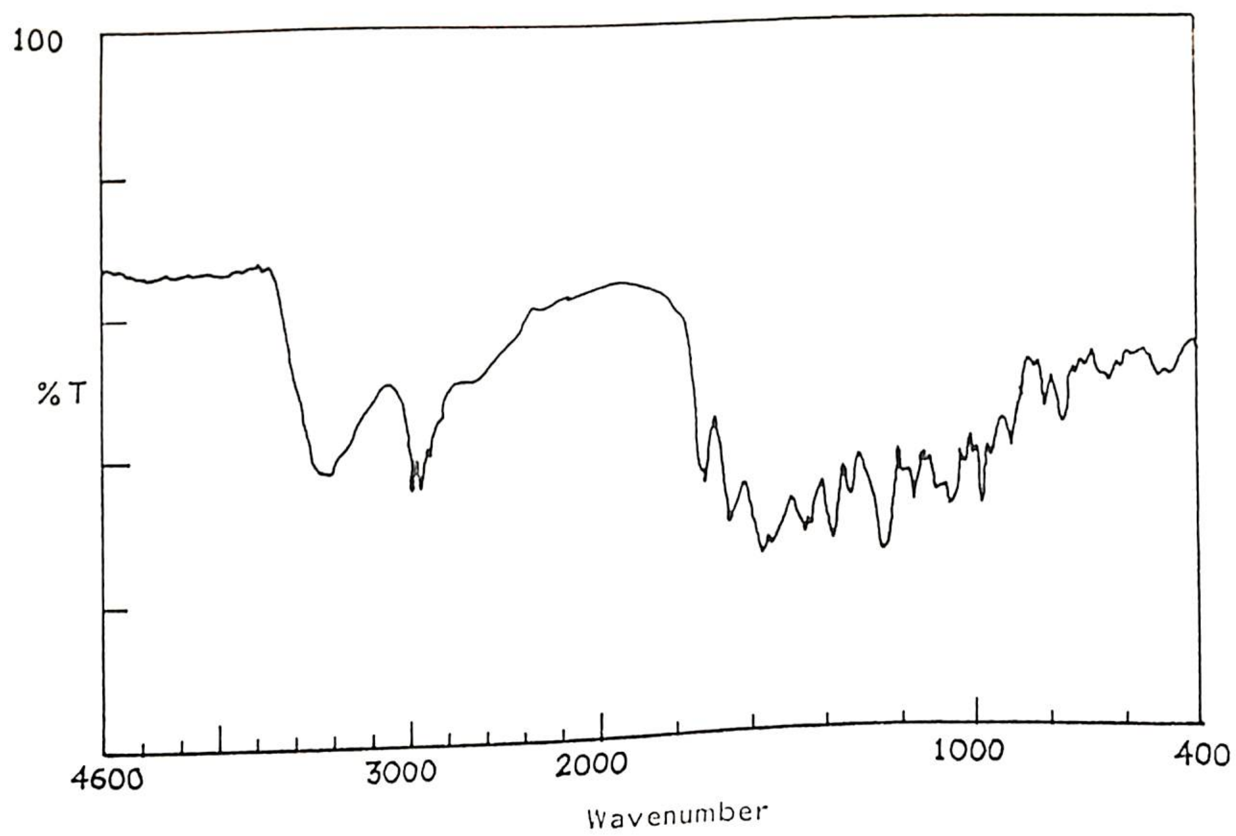
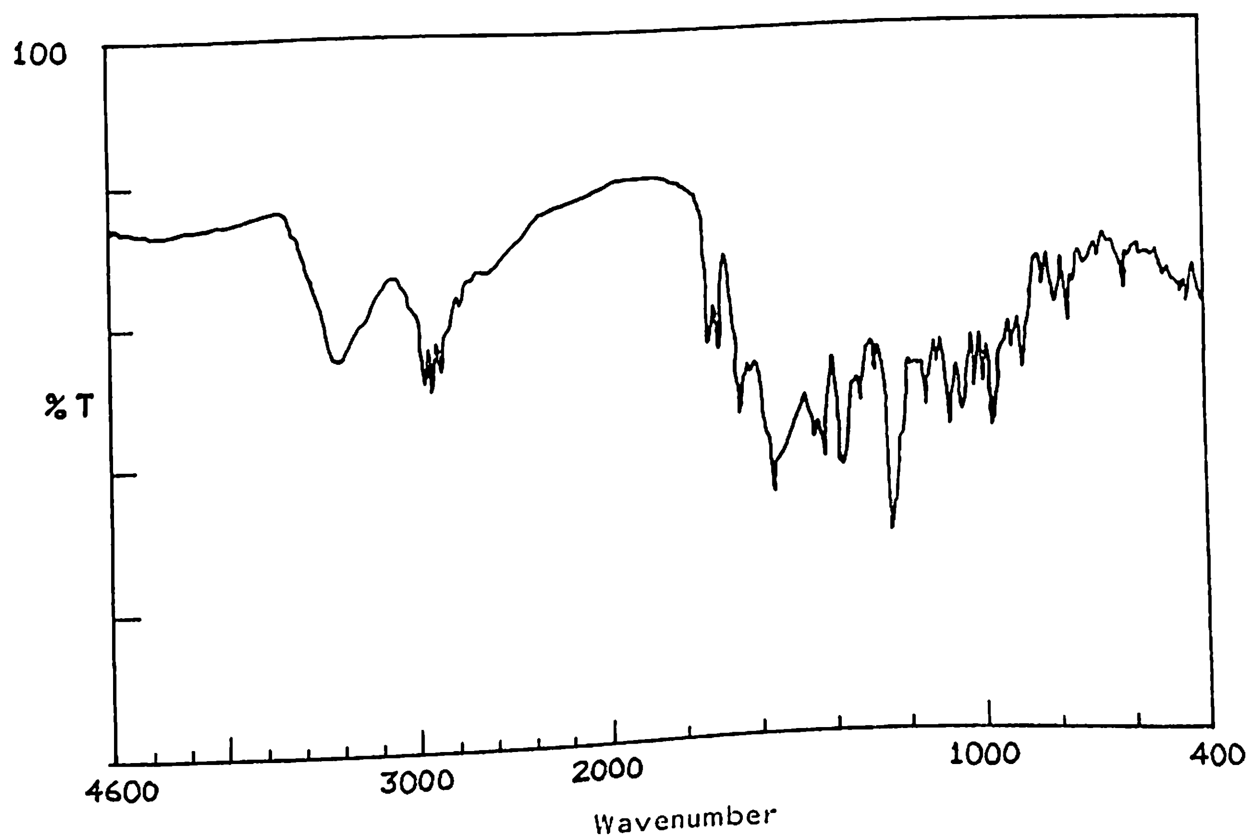
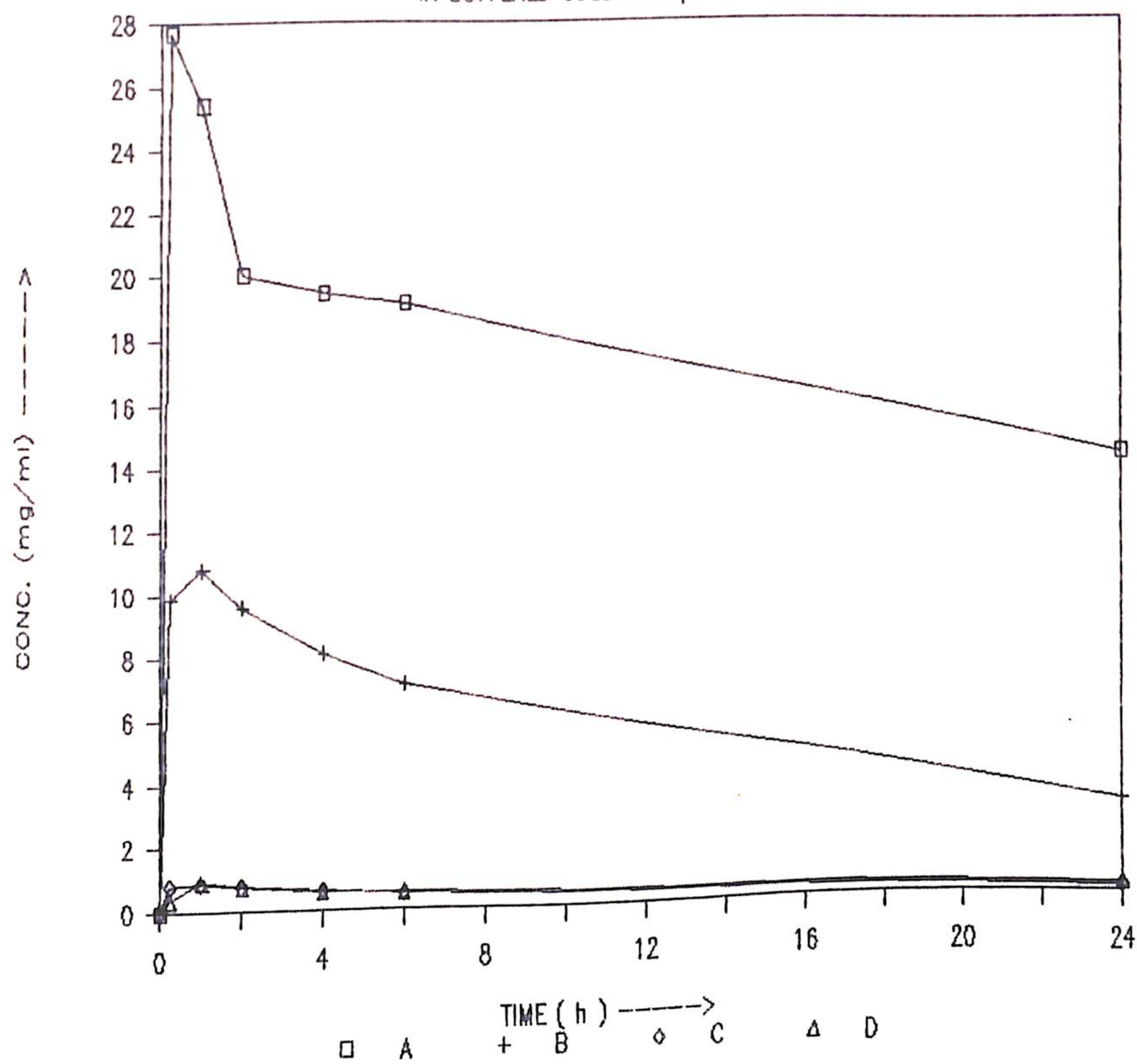


Fig. 5.2D : IR spectrum of rifampicin (Polymorph-A)

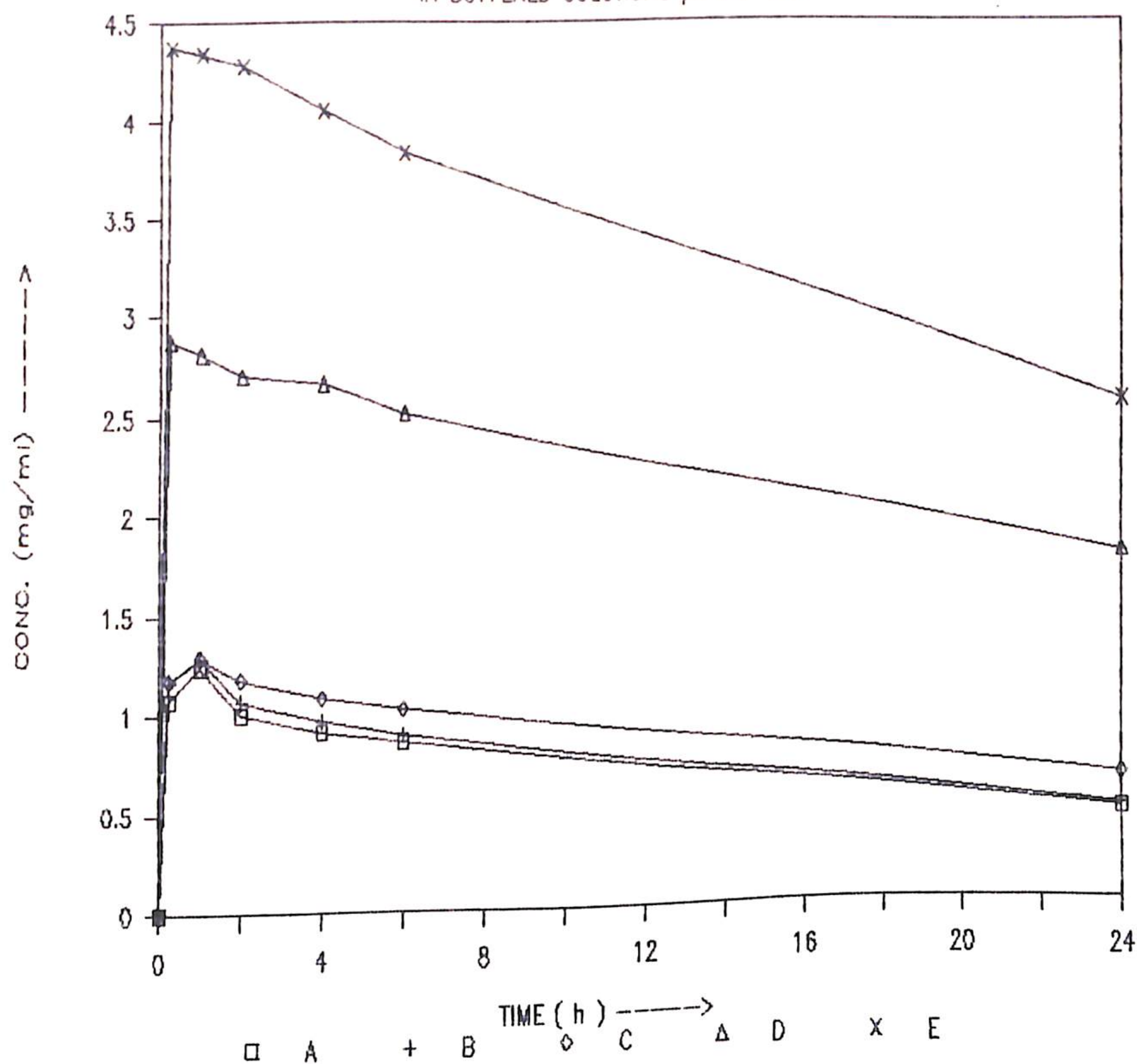
Resolution : 4
Scan : 16
Gain : 2





A = pH 1.0 ; B = pH 2.0

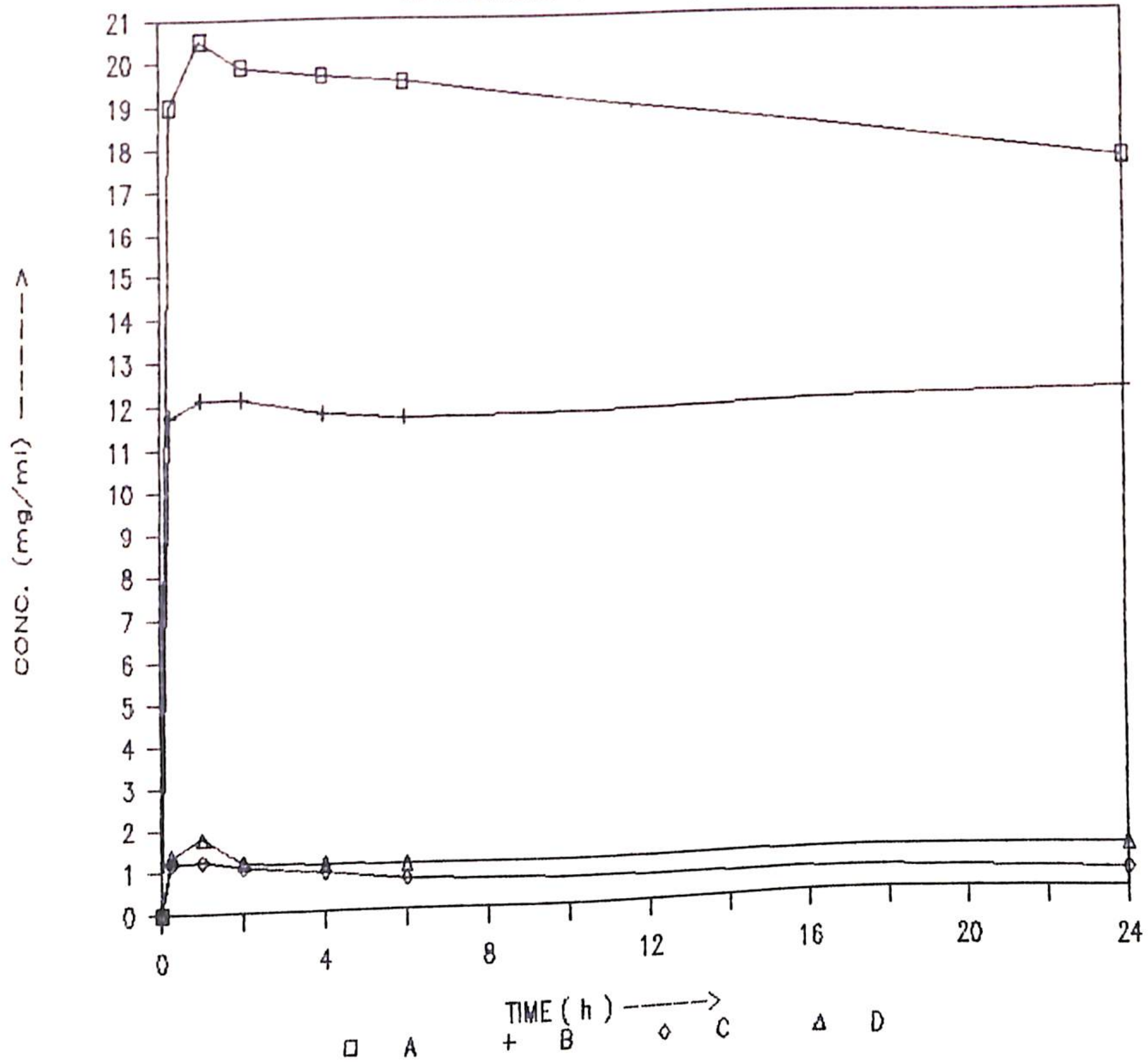
C = pH 3.0 ; D = pH 4.0



A = pH 5.0 ; B = pH 6.0

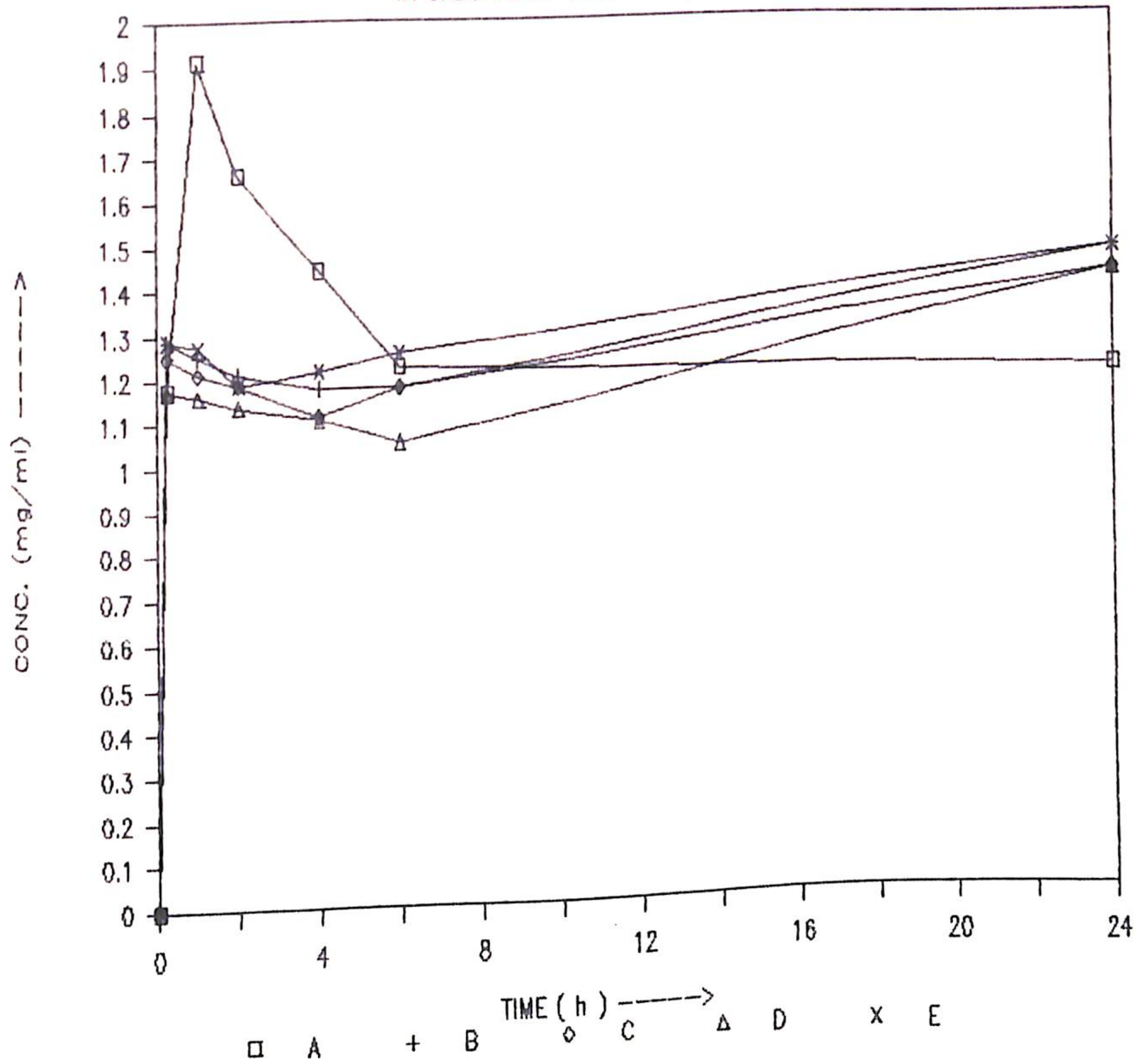
C = pH 7.0 ; D = pH 8.0

E = pH 9.0



A = pH 1.Ø ; B = pH 2.Ø

C = pH 3.Ø ; D = pH 4.Ø



A = pH 5.0 ; B = pH 6.0

C = pH 7.0 ; D = pH 8.0

E = pH 9.0

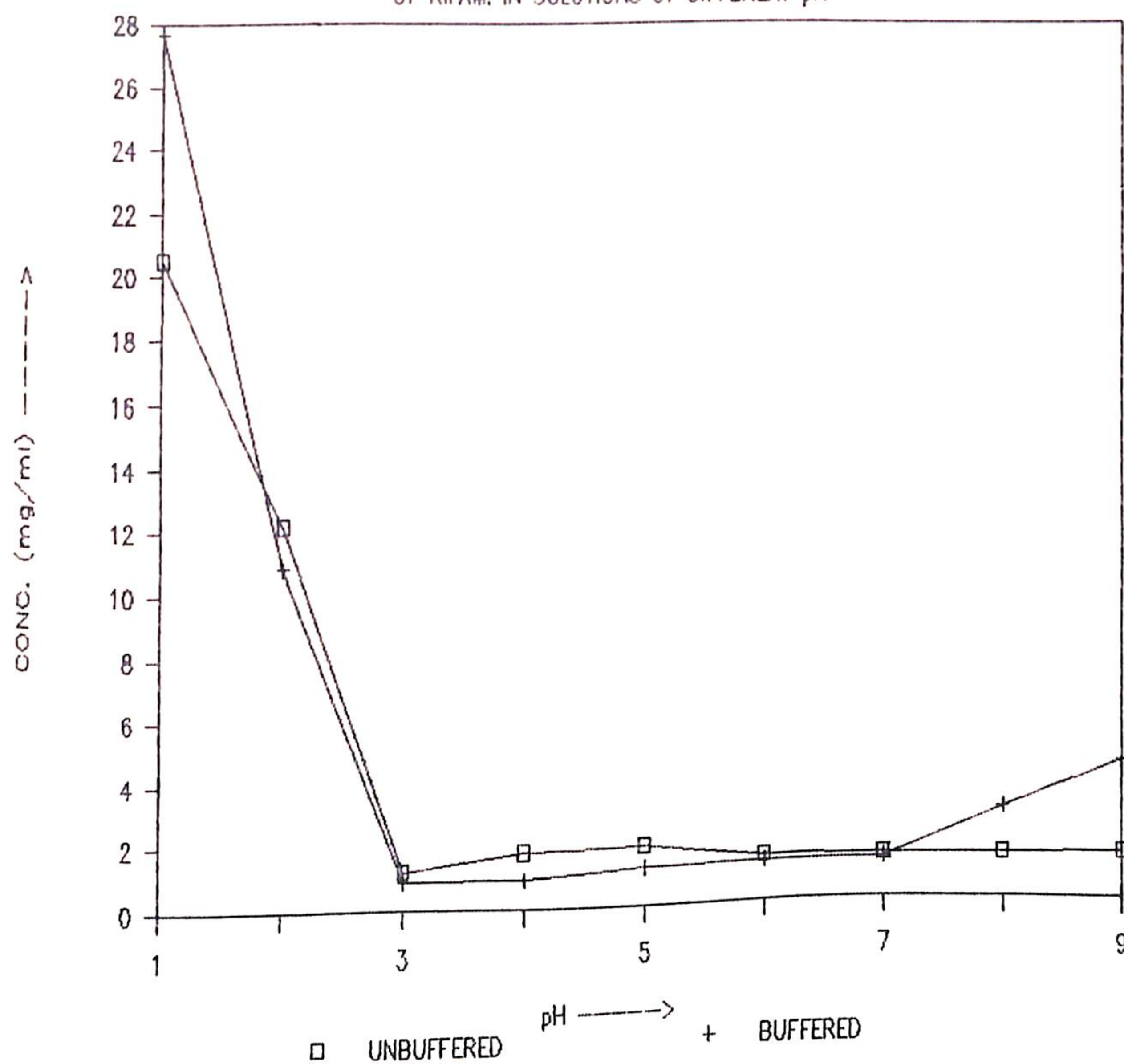
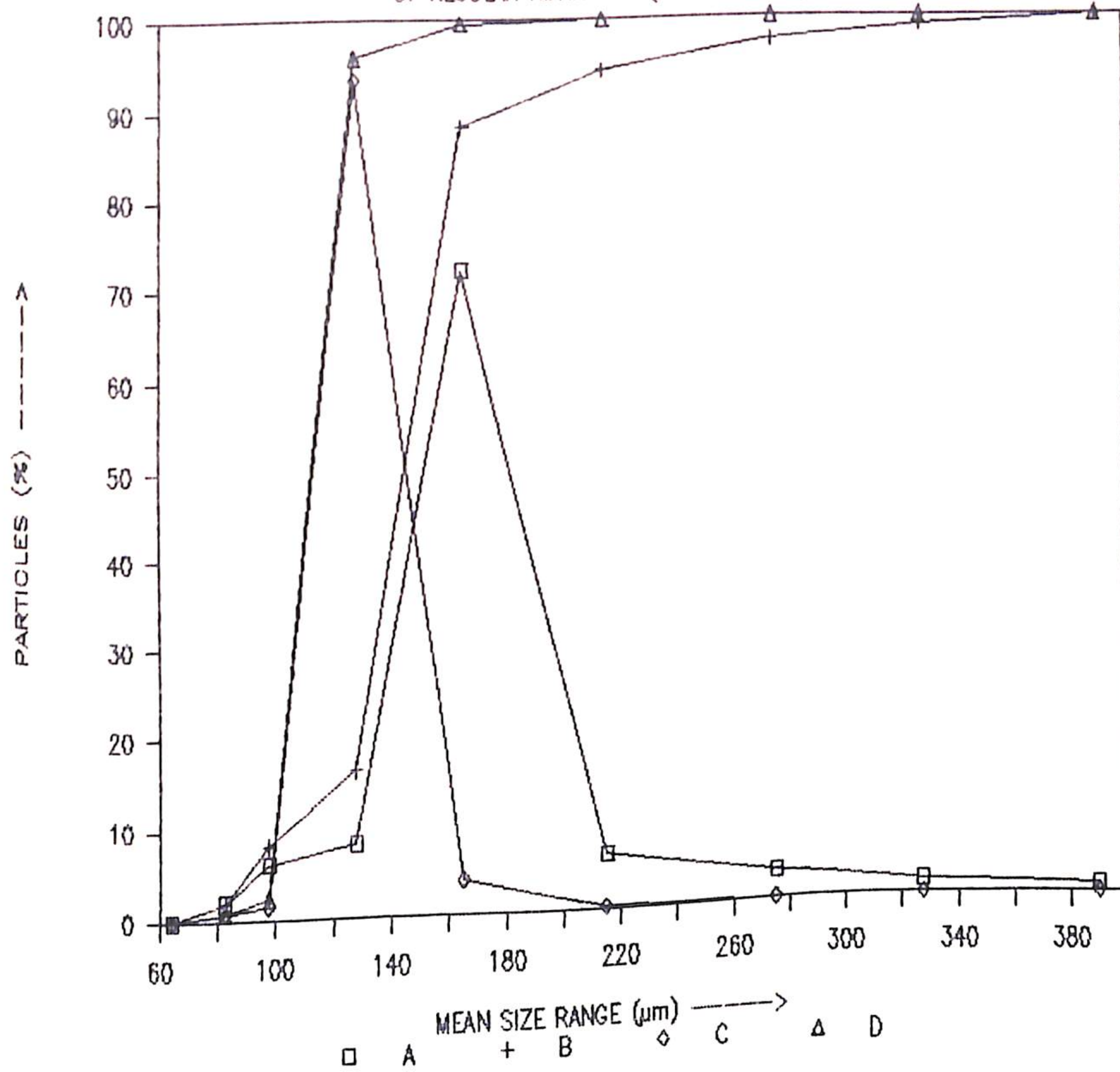


FIG 5.3C : MAX CONCENTRATION DISSOLVED
OF RIFAM. IN SOLUTIONS OF DIFFERENT pH
CONC. (mg/ml) ------>
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1
3
5
7
9
pH ------>
UNBUFFERED
BUFFERED

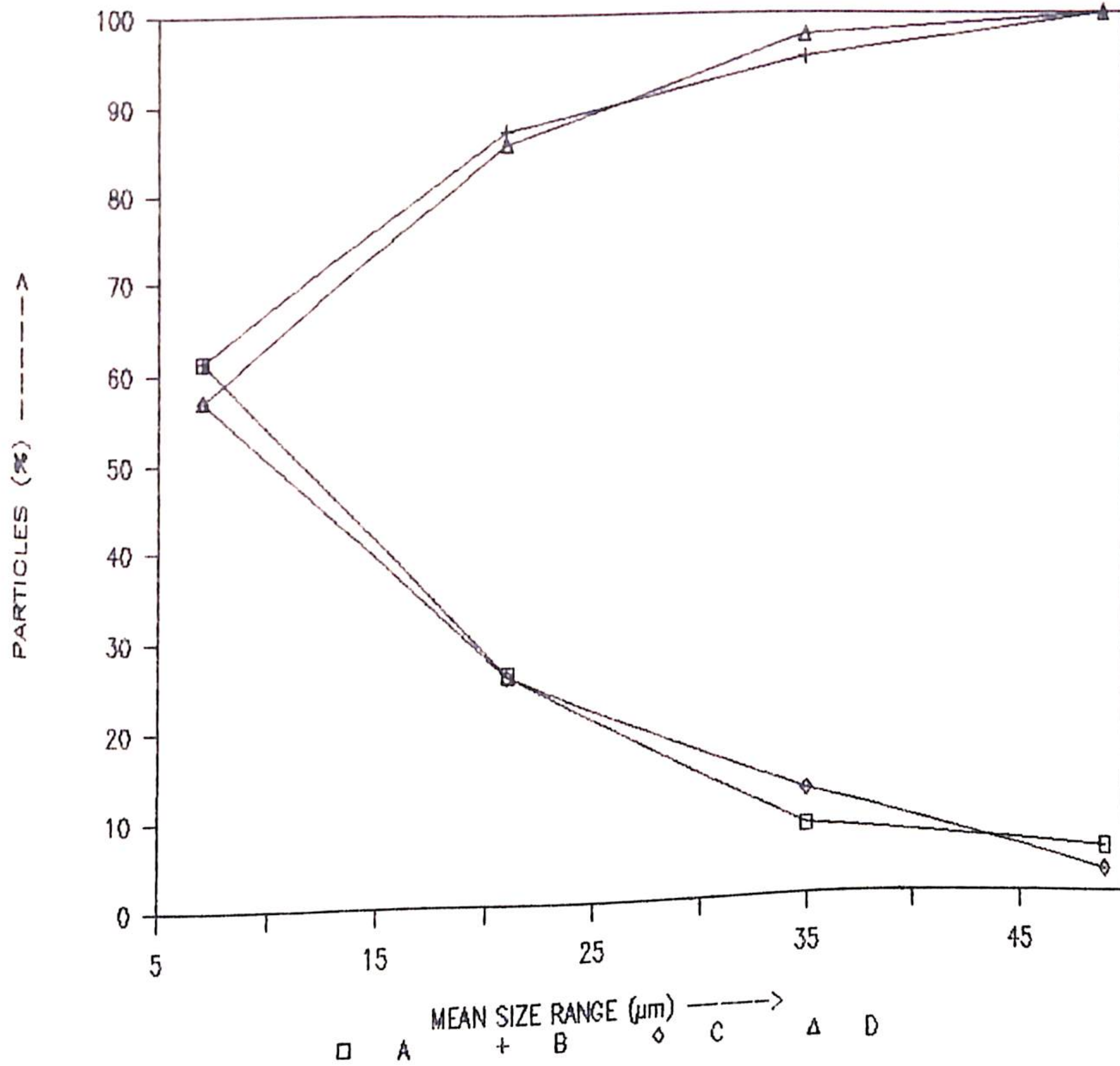


A = Frequency by weight

B = Cumulative frequency by weight

C = Frequency by number

D = Cumulative frequency by number



A = Frequency by number polymorph A

B = Cumulative frequency by number polymorph B

C = Frequency by number amorphous from

D = Cumulative frequency by number amorphous from

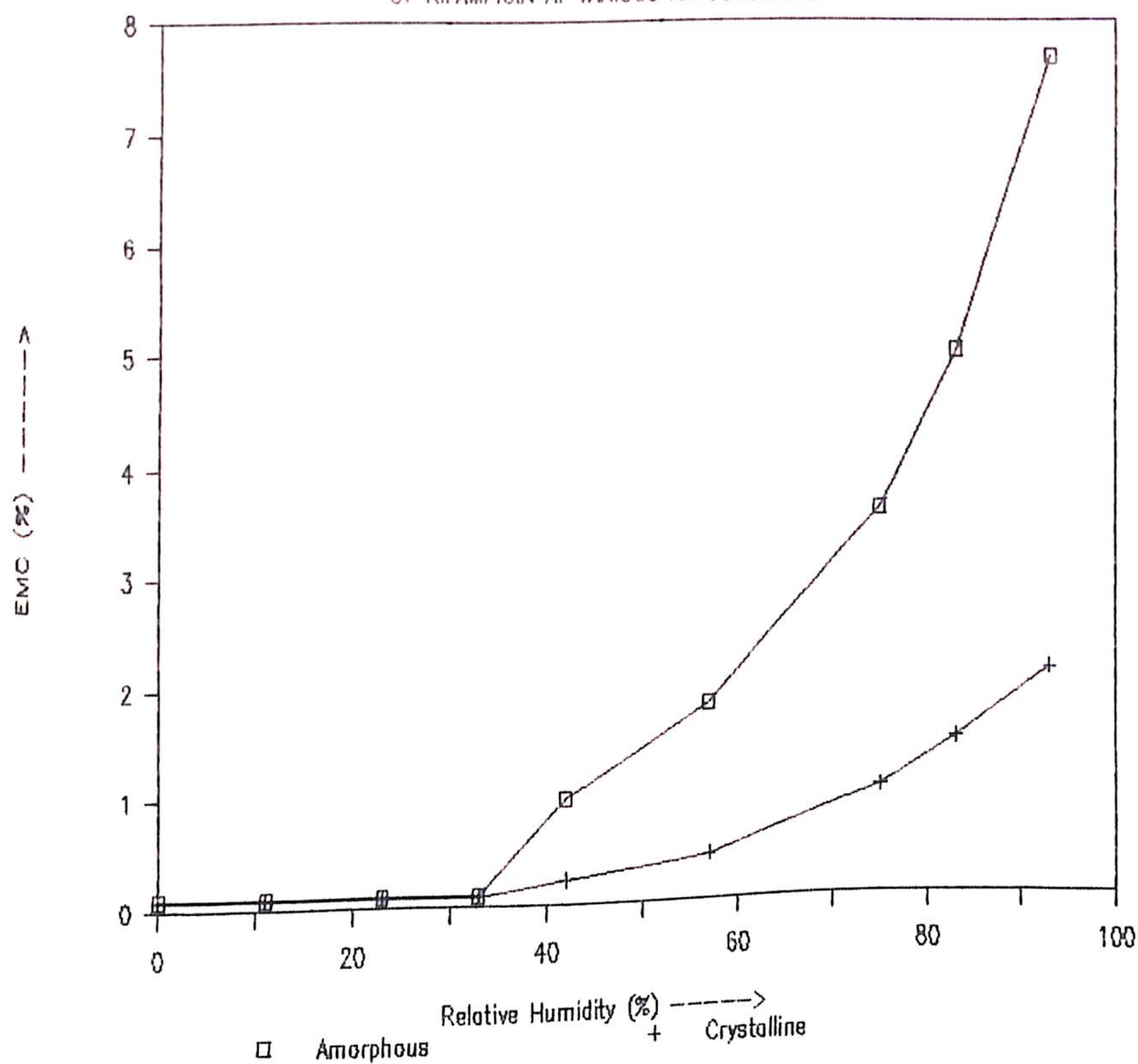


FIG 5.6A :EQUILIBRIUM MOISTURE CONT.
OF RIFAMPICIN AT VARIOUS RH CONDITIONS
EMC (%) ------>
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
Relative Humidity (%) ------>
Amorphous
Crystalline

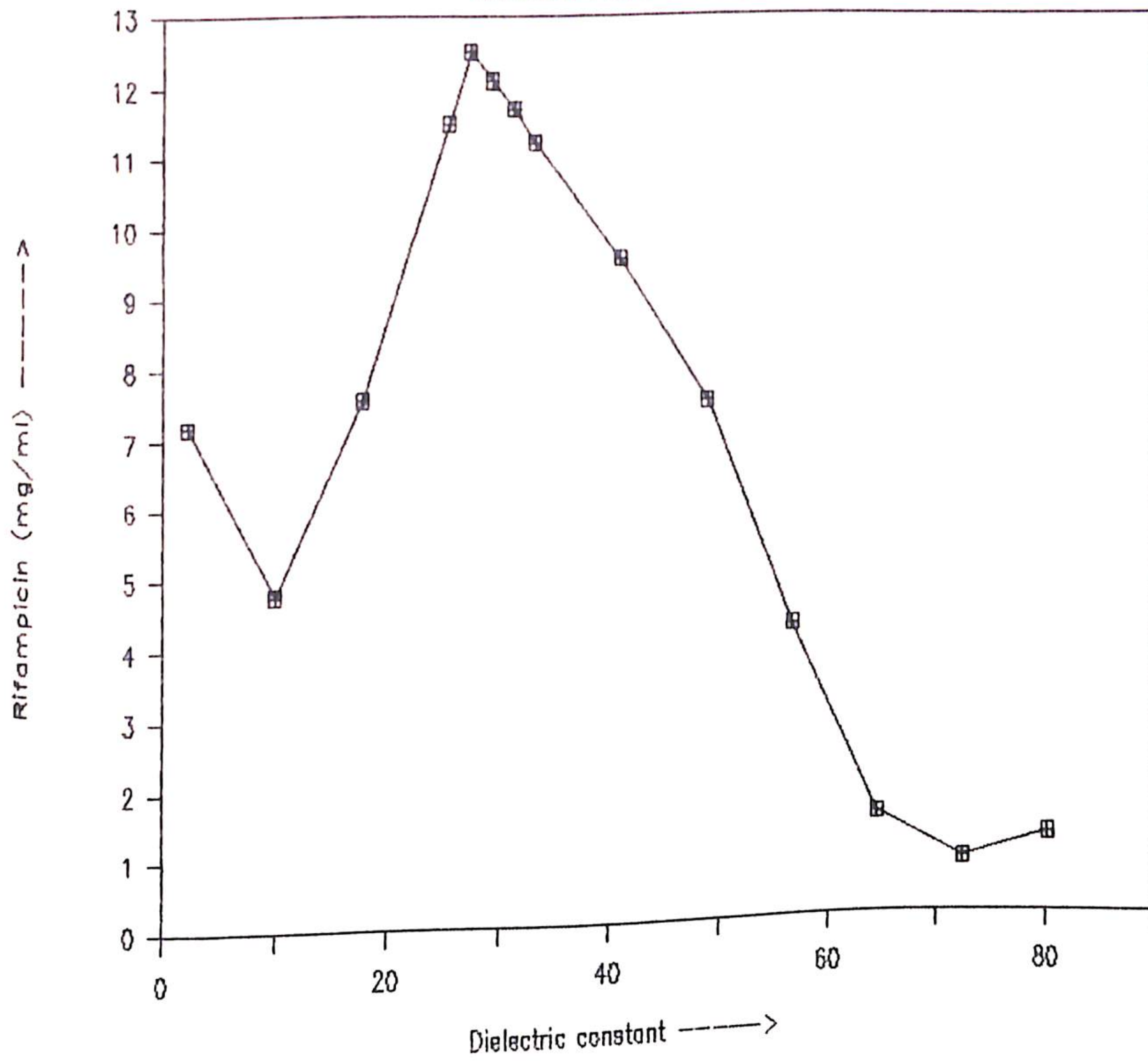


FIG 5.7A : RIFAMPICIN SOLUBILITY
IN DIOXAN : WATER MIXTURES
Rifampicin (mg/ml) ------>
Dielectric constant ------>
0
1
2
3
4
5
6
7
8
9
10
11
12
13
0
20
40
60
80

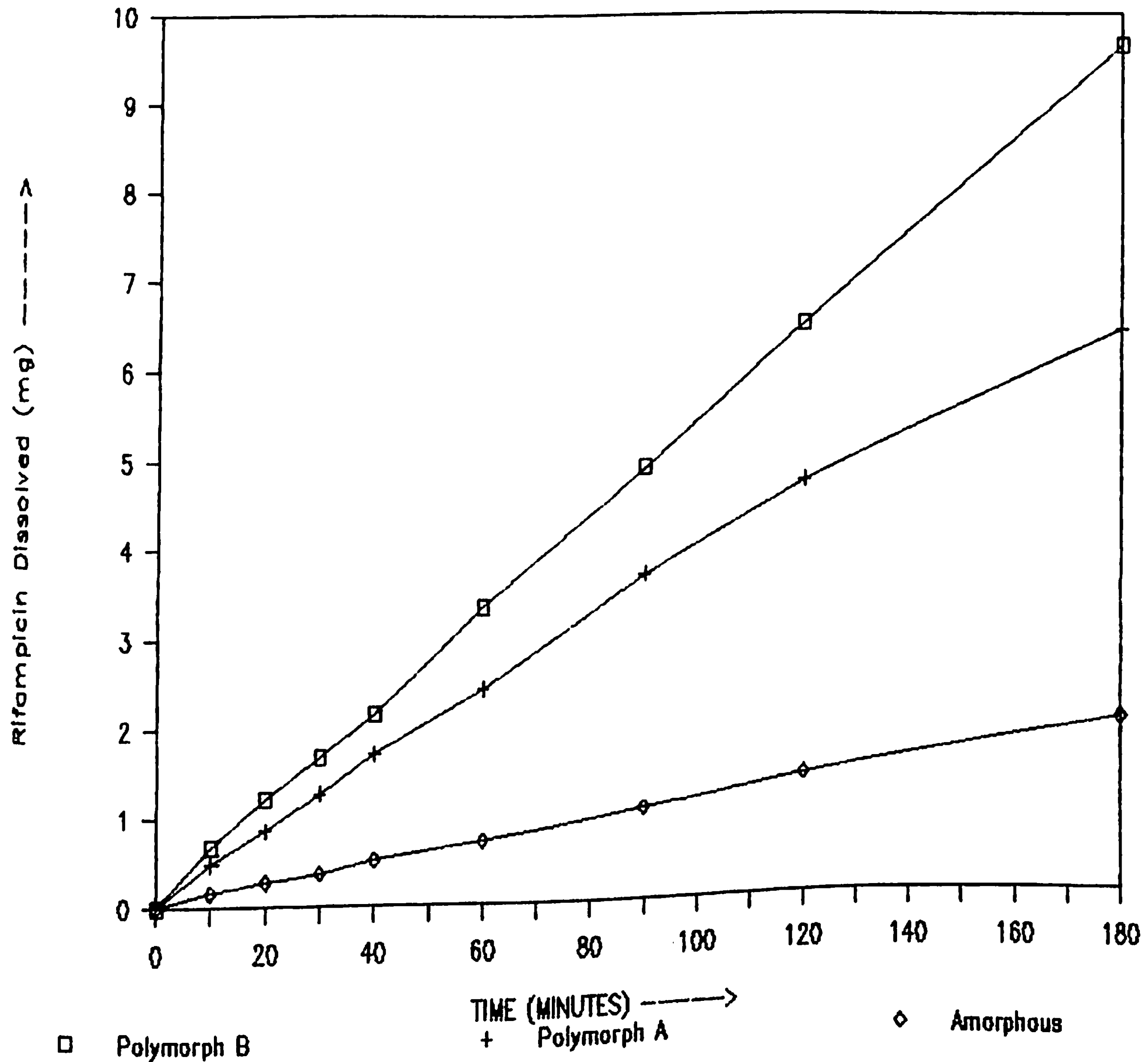
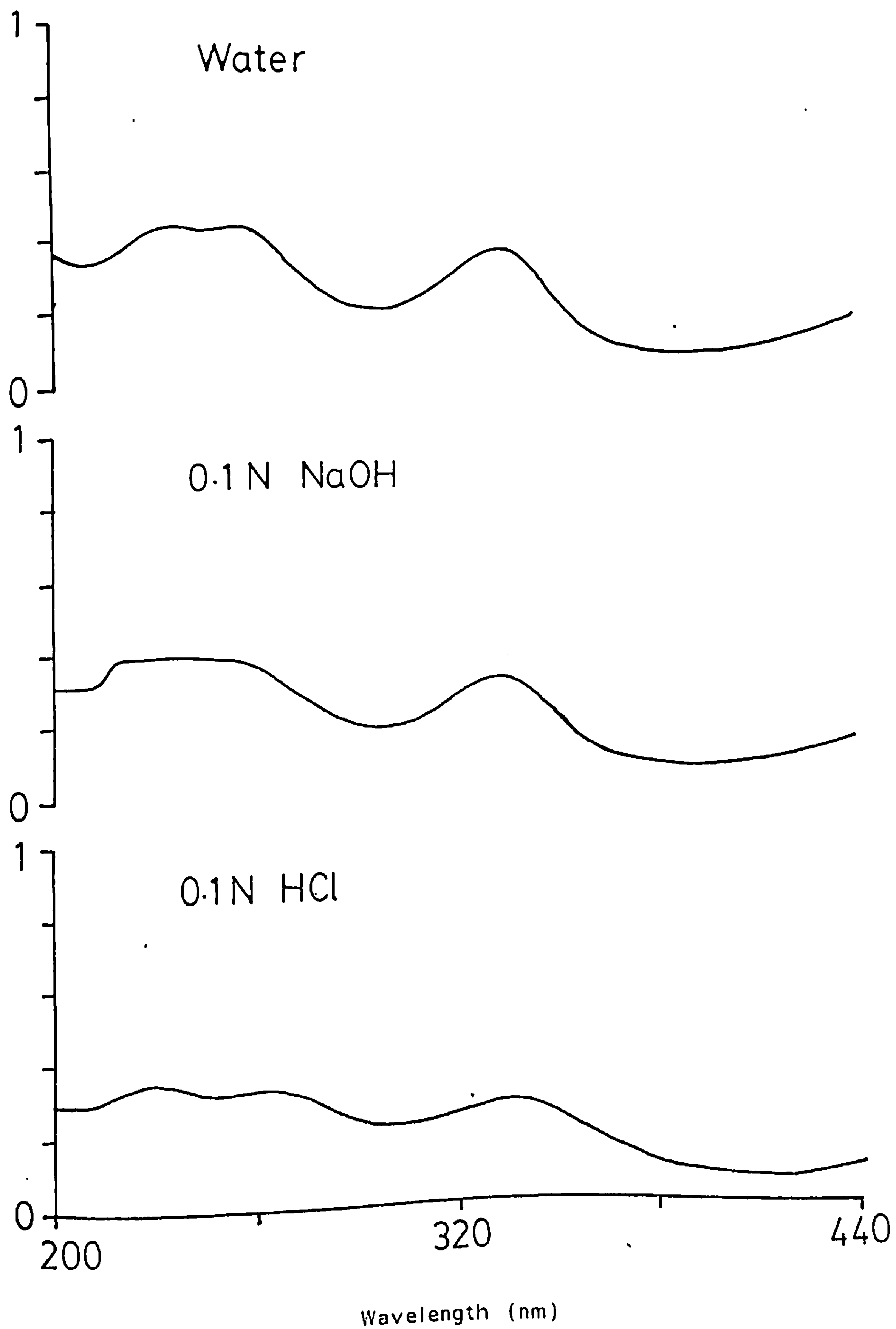


FIG 5.9A :INTRINSIC DISSOLUTION RATE
OF RIFAMPICIN FROM PELLETS
Rifampicin Dissolved (mg) ------->
0
1
2
3
4
5
6
7
8
9
10
0
20
40
60
80
100
120
140
160
180
TIME (MINUTES) ------->
Polymorph B
Polymorph A
Amorphous

Fig. 5.10A : UV scans of rifampicin



RESULTS AND DISCUSSION

5.2 Preparation and characterization of amorphous and polymorphic forms of rifampicin

The DSC scans of polymorph A, polymorph B and amorphous forms are distinctly different. The melting peaks (endothermic) for polymorph A, polymorph B and amorphous forms appear at 172°C, 115°C and 159°C respectively and recrystallization peaks at 201.7°C, 200.4°C and 170.0°C respectively. The IR spectra also exhibit differences in main peaks.

This indicates that all three forms are different. For convenience the existing regular crystalline form was labelled as polymorph A and the other crystalline form prepared in laboratory was labelled as polymorph B.

5.3 Effect of pH on rifampicin solubility

The pH solubility profile of rifampicin is graphically presented for both buffered (Figures 5.3AI and 5.3AII) and unbuffered solutions (Figures 5.3BI and 5.3BII). Figure 5.3C presents maximum concentration of rifampicin (Cmax) achieved at different pH values.

<u>Solubility in buffered solutions</u>

Solubility of rifampicin was found to be maximum in highly acidic solutions (pH < 2.0) and minimum at pH 3.0. Solubility was over 31 times and 12 times at pH 1.0 and 2.0 respectively as compared to pH 3.0. Solubility increased gradually as pH was increased from pH 4.0 to 9.0. Solubility was found to be 3.3 and 5 times at pH 8.0 and 9.0 compared to pH 3.0.

Solubility in unbuffered solutions

In unbuffered solutions, too, the solubility was high in highly acidic solutions and minimum at pH 3.0. Solubility was 17 times at pH 1.0 and 10 times at pH 2.0 as compared to pH 3.0. Solubility increased marginally (0.6 times) as pH was increased to 5.0 and again decreased as pH is further raised. Compared to unbuffered solutions, the solubility of rifampicin in buffered solutions is significantly high at extremes of pH and low at pH 3.0 to 7.0. The solubility in buffered solution at pH 1, 8 and 9 is 1.3, 2 and 3 times respectively and at pH 3, 4, 5, 6 and 7 it is only 0.9, 0.7, 0.5, 0.6, 0.9 and 0.9 times respectively, than that of unbuffered solutions.

For formulation of stable and palatable oral suspensions, minimum quantity of drug should be in solution form. The aqueous medium buffered at pH 3.0 using glycine-phosphate buffer exhibits minimum solubility for rifampicin. However both phosphate buffer and pH 3.0 have adverse effect on rifampicin solubility (refer : section 6.5 of Experimental III). At pH 4.0 the pH of maximum stability, the solubility of drug is still considerably less than in highly acidic medium (pH < 3.0) and can be used for liquid suspension products.

5.4 **Particle size and size distribution**

Sieve analysis provides size distribution by weight and microscopy by number. Assuming the shape and density factors to be constant throughout the size range, the relative number in each size interval was obtained by dividing the weight by cube of mean particle diameter.

Size distribution alongwith cumulative percent frequency by number were plotted against mean of size range (Figures 5.4A, 5.4B). The size distribution curves are bell shaped for regular rifampicin (polymorph A) indicating normal distribution of particles around the mean size range. The size distribution of polymorph B and amorphous is skewed. Most particles were less than 14 um, which was the minimum subdivision on micrometer scale.

The mean size is where 50% of the population have sizes greater than the mean and 50 % less and it can be estimated from the cumulative distribution curve [581]. The mean size was 112 um for polymorph A. The mean size for polymorph B and amorphous forms could not be estimated correctly due to skewed distribution of particles. The effect of particle size on dissolution and bioavailability of drug has been studied and discussed in section 7.1 of Experimental IV.

5.5 Density and flowability

Knowledge of true and bulk density of the drug substance is very useful in forming some idea as to the size of the final dosage form e.g. capsules size. It also affects their flow properties. Significant difference in absolute densities of components could lead to segregation.

The angle of repose is a useful parameter in evaluating the rheological properties of powders because it is not strongly dependent on average particle size but more on surface properties that are also the main factors in controlling the flow. The angle of repose <25° indicates excellent flow, 25°-30° good flow, 30°-40° passable

flow and > 40° poor flow. All the three forms of rifampicin exhibit poor flow.

Flowability is the powder's ability to flow evenly by means of gravity and other forces from tube of certain diameter. Flowability is very important for smooth functioning of high speed tabletting/capsule filling machines, and to achieve consistent parameters like unit weight/content, hardness, friability, disintegration, dissolution rate and blood levels. Data in Table 5.5A indicates intrinsic flowability of polymorph A to be marginally better than other two forms.

Carr's compressibility index of over 23 indicates poor flow, 18-21 as fair to passable flow and < 16 as good flow [579, 580]. Data in Table 5.5A indicates that none of these three forms of rifampicin has adequate flow.

Looking at these physical parameters, it can be deduced that :

- Rifampicin of such low bulk density would have tendency to float on the surface and hence create problems in the formulation of oral suspension dosage forms. High shear mixer with some surfactant would be essential for processing.
- Low density may also affect the filling of drug in normal sized capsules. Poor flow of drug would result in weight variation of capsule and tablet dosage forms.
- The density and flow of drug will have to be improved by additional processing like granulation, compaction, slugging etc.

5.6 Hygroscopicity

From the equilibrium moisture content (EMC) versus RH graph (Figure 5.6A) the relatively higher hygroscopicity of amorphous form compared to crystalline form is evident. EMC at 93 % RH of crystalline form is only 2.07 % and of amorphous form 7.67 % (3.7 times higher than crystalline form). Rifampicin is absolutely nonhygroscopic upto 33 % relative humidity and therefore rifampicin formulations should be processed preferably below this relative humidity.

5.7 Dielectric constant

A plot of solubility versus dielectric constant for a solute in a given solvent system shows a typical curve specific for the solute in that system. The dielectric constant corresponding to the maximum solubility is the dielectric requirement of the drug.

Solubility profile of rifampicin as a function of dielectric constant (Figure 5.7A) in dioxan-water mixtures showed a peak in solution containing 67.5 % dioxan corresponding to the dielectric constant of 27.55. Thus 27.55 is the dielectric requirement of rifampicin. The solubility parameter of 67.5 : 32.5, dioxan : water mixture for rifampicin as calculated from solubility of drug in pure dioxan (7.19 mg/ml) and pure water (1.14 mg/ml) is 3.1 mg/ml.

The mixture of solvents having dielectric constant of 27.55 can be selected for preparation of solution dosage forms of rifampicin e.g. opthalmic or parentral solutions.

5.8 Lipophilicity

The partition coefficient of rifampicin in n-octanol-water system obtained is 21.68 after 2 hours. The concentration of drug decreases thereafter due to its degradation in aqueous medium (refer : section 6.3 of Experimental- III). The corresponding lipophilicity is 1.336 which is below the recommended mininum value of 2.0 for proper absorption. This could be one of the reasons for inadequate absorption of drug.

5.9 Intrinsic dissolution rate

Intrinsic dissolution rate constant (Ki) was calculated using following equations [346] and reported in Table 5.9A.

$$Ki = Ft \cdot V/S.t \quad mg/min.cm2$$

$$Kap = Ki \times S/V \quad mg/ml.\ min$$

Where Ft = Fraction of drug dissolved at time t

S = surface area of the pellet (1.328 cm2)

V = volume of dissolution medium (200 ml)

Kap = Apparent dissolution rate constant (slope of the plot Ft vs t in Figure 5.9A)

The intrinsic dissolution rate constant is higher by 47 % for polymorph B and lower by 70 % for amorphous form when compared to regular rifampicin (polymorph A). This reflects the high potential of polymorph B for enhancing dissolution and bioavailability of rifampicin.

5.10 UV scans of rifampicin in neutral, acidic and alkaline solutions

There was no significant shift in absoption maxima values in all the three media. However, the molar absorptivity values were significantly different (0.1 N HCl < 0.1 N NaOH < H2O).

EXPERIMENTAL-III

DRUG STABILITY AND PREFORMULATION STUDY

CHAPTER 6

6.1 DRUG STABILITY AND PREFORMULATION STUDY

Stability of drug was conducted in the presence of common pharmaceutical excipients and compared with stability of drug in pure form.

Preparation of physical mixtures of drug and excipients

Rifampicin (400 mesh) and excipients (100 mesh) were used in this study so as to maximise their surface area and particle surface to surface contact. The drug : excipient ratios used were, 1:10 for polyethylene glycol 4000, urea, lactose, starch, mannitol, sucrose and dibasic calcium phosphate ; 1:1 for gelatin, magnesium stearate, talc, Aerosil 200 and sodium starch glycolate ; 10 : 1 for povidone K-30, sodium lauryl sulphate, polysorbate 80, bile salts, docusate sodium, beta-cyclodextrin and poloxamer 188 and 1:4 for microcrystalline cellulose. Drug and excipients were thoroughly blended and mix was passed through 100 mesh to ensure uniform blending. The mixture was then filled in tubular glass vials made of USP type I glass. The sealed vials were kept at different temperatures. The open vials were kept at 75 % RH/40°C. Rifampicin filled in same way was used as a control sample.

Storage conditions for stability

Samples were stored at room temperature (RT), 45°C, 60°C, 75% RH/40°C and FT (refrigerator).

For room temperature conditions (RT), the actual temperature and relative humidity was recorded daily using maximum-minimum thermometer and hygrometer for entire storage period. The average temperature and relative humidity were found to be 28 ± 5°C and 55 ± 20 % respectively. The maximum and minimum temperature achieved in 24 hours was recorded daily for refrigerator condition (FT) also and was found to be 5 ± 3°C.

Analysis of samples

Samples were analysed initially and thereafter at 2, 4 and 6 months interval for drug content and its degradation components. Initial samples were also checked for moisture content, pH and UV scan.

I. Drug Content : Rifampicin content was determined by HPLC method, described under section 4.1 of Experimental-I. For preparation of test solution the amount of sample equivalent to 25 mg of rifampicin, was dissolved and volume made upto 50 ml with methanol. 1 ml of this solution was diluted to 25 ml with mobile phase in a volumetric flask. Standard solution was prepared by diluting 1 mg/ml rifampicin reference standard solution in methanol to contain 40 mcg/ml with mobile phase.

II. Moisture Content : Moisture content was determined by Karl-fisher reagent method, using Mettler apparatus (Mettler Instruments Highstown, NJ, USA).

III. pH : Sample equivalent to 100 mg of rifampicin was dispersed in 10 ml water and vortexed for 10 min. The pH of this suspension was checked, using digital pH meter (Control Dynamics, Bombay).

IV. UV Scan : Samples equivalent to 50 mg of rifampicin were vortexed for 5 minutes with 25ml of methanol and volume was made upto 50 ml with methanol. 1 ml of this solution was diluted to 50 ml with methanol and filtered. This solution (containing approximately 20 mcg/ml drug) was scanned from 500nm to 200 nm on Lambda 15 spectrophotometer (Perkin-Elmer Co, USA).

V. Impurities/degradation products : The degradation products / impurities were estimated by HPTLC. The test samples were dissolved in chloroform, filtered and suitably diluted to obtain a final concentration of 1000 mcg/ml. Standard solutions were prepared in chloroform to contain 50 mcg/ml of rifampicin quinone (RQ), and 10 mcg/ml each of rifampicin, 3-formyl rifamycin SV (3FRSV), rifampicin-N-oxide (RNO) and 25-desacetyl rifampicin (25-DAR).

TLC was performed on 20 x 10 cm HPTLC plates precoated with a 0.25mm layer of silica gel 60 F254 (Merck). The plates were prewashed with methanol and then dried in air. Sample and standards were applied to the plates by means of Camag Linomat IV applicator. The spotting, development and scanning of TLC plates and quantification of individual components was done as described under section 4.2 of Experimental-I.

The results of samples analysed initially and after storage for 2, 4 and 6 months are given in Tables 6.1A to 6.1Q.

6.2 EFFECT OF EXCIPIENTS ON RIFAMPICIN SOLUBILITY

The solubility of rifampicin and its preformulation samples was determined in water at 37° ± 0.5°C, using USP Type II apparatus. 200 ml distilled water was taken in beakers of dissolution rate test apparatus. The samples, equivalent to about 2 g of rifampicin, were dispersed in media and stirred at 150 rpm. The 5 ml samples were withdrawn at predetermined intervals upto 24 hours, filtered and analysed using HPLC method, described under section 4.1 of Experimental-I. Data on drug solubility are given in Table 6.2A.

6.3 EFFECT OF UV/VISIBLE LIGHT ON RIFAMPICIN STABILITY

Rifampicin Powder

Rifampicin was spread as thin layer in petriplates (3 g each). Two plates each were kept in UV cabinets adjusted at 254 and 366 nm wavelengths, diffused sunlight, and dark area respectively. Desiccant silica gel was kept alongside the petriplates to keep the atmosphere free from excessive moisture. The samples from each plate were taken at 2 days intervals for 16 days and analysed by HPLC method for rifampicin content and by HPTLC for degradation products/impurities as given in sections 4.1 and 4.2 of Experimental-I. The results are given in Tables 6.3A to 6.3D.

Rifampicin Solution

Rifampicin solution was prepared to contain about 1.4 mg/ml drug in distilled water and analysed for drug content by HPLC and degradation components/impurities by HPTLC. 20 ml solution was filled in each of the eight tranparent polyethylene bags and closed securely. Two bags each were exposed to diffused sunlight, UV light of 254 and 366 nm wavelengths and total darkness.

Samples were analysed after 24, 48 and 72 hours for drug content by HPLC and degradation products/impurities by HPTLC (refer : sections 4.1 and 4.2 of Experimental-I).

The results are given in Tables 6.3E to 6.3H.

6.4 EFFECT OF OXYGEN ON RIFAMPICIN STABILITY

Rifampicin solution was prepared to contain about 1.4 mg/ml drug in distilled water and analysed for rifampicin content by HPLC and for degradation components/impurities by HPTLC. 7.5ml of rifampicin solution each was transferred to cleaned and dried USP type-I clear glass vials. Pure oxygen was bubbled through the solution of 2 vials for 10-15 minutes and immediately closed with clean polyplugs and aluminium seals. Similarly pure nitrogen was bubbled through solution of another 2 vials and sealed. Remaining 2 vials were kept as control (without any gas bubbling). All these vials were transferred to 45°C incubator. After 24, 48 and 72 h, 1 ml samples were withdrawn from each vial and analysed for drug content by HPLC and for impurities/degradation components by HPTLC. The respective gas was

flushed for 2-3 minutes through the solution before putting these back in stability incubator (at 45°C). The results are given in Tables 6.4A to 6.4C.

6.5 EFFECT OF pH ON STABILITY OF RIFAMPICIN IN AQUEOUS SOLUTIONS

The stability of rifampicin was studied in aqueous solutions at pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. Two concentrations of rifampicin were selected (25 mcg/ml and 1000 mcg/ml) to study the effect of drug concentration on stability.

Distilled water deionised with Milli-Q water purification system was boiled, cooled and flushed with nitrogen. Sodium chloride was dissolved in this water, to get 0.1 M ionic strength so as to facilitate pH adjustment. The pH of distilled water was adjusted to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with dilute solutions of hydrochloric acid or sodium hydroxide as the case may be. The calculated quantities of rifampicin were dissolved in these pH solutions to get desired concentration of rifampicin. The solutions were tranferred to volumetric flasks in duplicate. The flasks were immediately transferred to constant temperature water bath maintained at 30 $\pm$ 1°C.

1000 mcg/ml concentration solutions

Samples were withdrawn at 0, 1, 2, 4, 6, 8, 12, 16 and 24 hours intervals and analysed for rifampicin content by standard HPLC method as described under section 4.1 of Experimental-I.

In this study only rifampicin was analysed because its main degradation component 3-formyl rifamycin SV (3FRSV) precipitates out due to limited solubility and high concentration. This precipitation however has no effect on rifampicin estimation. The average values of duplicate analysis are given in Table 6.5A.

25 mcg/ml concentration solutions

1.0 ml samples were withdrawn at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20 and 24 hours. In this set the contents of both rifampicin and 3FRSV were determined because at such low concentration the degradation components do not precipitate. The samples of 25 mcg/ml solutions were used for direct injection. All experiments were performed in duplicate. The average values of duplicate analysis are given in Tables 6.5B and 6.5C.

6.6 EFFECT OF BUFFERING AGENTS ON THE STABILITY OF RIFAMPICIN

Following buffer solutions were prepared using distilled water, deionised through Milli-Q water purification system, boiled, cooled and flushed with nitrogen.

Chloroacetate Buffer (0.01M)-pH 3.0 : 5.824g of sodium chloroacetate was dissolved in 500 ml water and pH adjusted to 3.0 ± 0.1 with acetic acid.

Acetate Buffer (0.01M)-pH 4.0 : 4.1015g of sodium acetate anhydrous was dissolved in 500 ml water and pH adjusted to 4.0 ± 0.1 with acetic acid.

Formate Buffer (0.01M)-pH 5.0 : 3.4g of sodium formate was dissolved in 500 ml water and pH adjusted to 5.0 ± 0.1 with formic acid.

Phosphate Buffer (0.01M)-pH 6.0: 7.098g of disodium hydrogen phosphate was dissolved in 500 ml water and pH adjusted to 6.0 ± 0.1 with orthophosphoric acid.

The calculated quantities of rifampicin were dissolved in these pH solutions to get desired concentration of rifampicin. The flasks were immediately transferred to constant temperature water bath maintained at 30 ± 1°C. Samples were withdrawn at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20 and 24 hours and analysed for rifampicin and its main degradation product, 3-formyl rifamycin SV. The samples withdrawn were used for direct injection to HPLC system (refer : section 4.1 of Experimental-I). The results are given in Table 6.6A.

6.7 EFFECT OF RIFAMPICIN-ISONIAZID INCOMPATIBILITY ON THEIR BIOAVAILABILITY

The dissolution rate and bioavailability of a product containing only rifampicin (product C, each capsule containing rifampicin 450 mg) were compared with two products containing rifampicin and isoniazid (products A & B, each capsule containing rifampicin 450 mg and isoniazid 300 mg).

Dissolution rate

Dissolution rate of products was studied by the method developed and described under section 4.3 of Experimental-I using 900 ml of 0.4 % w/v solution of sodium lauryl sulphate as dissolution medium in each

beaker of USP type-I apparatus (basket type). Basket speed was kept at 50 rpm. 10 ml samples were drawn at intervals of 10, 20, 30, 45, 60 and 120 minutes and suitably diluted with mobile phase. Volume was replenished with 10 ml fresh dissolution fluid. The diluted solution was then chromatographed using standard HPLC system (refer : section 4.1 of Experimental-I). The concentration values were corrected for dilution factor. The mean values of 6 unit determinations for Products A and B have already been reported under Experimental-I, Tables 4.3A and 4.3B. The values for Product C are reported in Table 6.7A.

Bioavailability

A total of six volunteers participated in the study. All volunteers passed the selection criteria described under Experimental Design (chapter 3). In the first set three volunteers were given Product A and other three volunteers were given Product C. The groups were crossovered after seven days of washout period. In the second set same six volunteers were given Product B. The products were administered with 200 ml water on one occasion. A standard meal schedule was given and it started approximately four hours after dosing.

Blood samples (10 ml) were drawn and collected in tubes at 0.0, 0.75, 1.5, 3.0, 6.0, 9.0 and 12.0 hours after the drug administration. Serum was separated immediately by centrifugation and stored at - 15°C till analysed.

Analysis

Rifampicin : Serum samples were analysed for rifampicin content by HPLC technique, described under section 4.4 of Experimental-I.

Isoniazid : 1 ml of serum was extracted with 10 ml chloroform and chloroform layer was passed through anhydrous sodium sulphate. 7 ml of chloroform layer was evaporated to dryness under vacuum. The residue was reconstituted with methanol. The tubes were vortexed for 10 minutes and 100 ul of this solution was injected into HPLC system, set at following chromatographic conditions :

Mobile phase	:	0.01 M disodium hydrogen orthophosphate solution : methanol : triethylamine (60:40:0.1 v/v/v)
Column	:	Micro-Bondapak C18 (30 cm x 3.9 mm)
Wavelength	:	263 nm
AUFS	:	0.008
Attenuation	:	2.
Flow rate	:	1.4 ml/min.

The data obtained after analysis was used to calculate the pharmacokinetic parameters. The results of the study are given in Tables 6.7B to 6.7F.

Table 6.1A : Results of rifampicin : excipient compatibility study at zero time (initial analysis)

Batch code	Excipient (E)	Description	Initial analysis			UV scan	
			Assay (%)	Moisture content (%)	pH	Peaks nm	Relative Absorb. (%)
R	RIFAMPICIN	brick red, free flowing	101.33	1.0000	6.40	471 332 235	20.34 35.74 43.92
A	PEG 4000	brown, free flowing	100.84	0.1100	6.51	472 332 236	20.34 35.74 43.92
B	POVIDONE K-30	orange, free flowing	103.66	1.3200	6.32	472 332 236	20.37 35.66 43.97
C	UREA	brown, free flowing	102.49	0.2000	6.91	472 332 235	20.37 35.74 43.88
D	LACTOSE	orange, free flowing	96.51	4.5500	6.40	471 331 235	20.40 35.87 43.72
E	STARCH	orange, free flowing	105.76	9.1800	6.32	471 332 235	20.93 35.42 43.65
F	SODIUM LAURYL SULPHATE	red, free flowing	105.12	0.0900	7.16	473 332 235	20.51 35.63 43.86
G	POLYSORBATE 80	red, free flowing	104.48	1.4000	6.56	473 332 235	20.39 35.30 44.31
H	BILE SALTS	orange, free flowing	103.79	5.5100	6.45	472 332 235	20.39 35.50 44.10
I	MANNITOL	orange, free flowing	100.95	0.3700	6.29	472 332 235	20.75 35.68 43.57

Table 6.1A : continued

Batch code	Excipient (E)	Description	Initial analysis			UV scan	
			Assay (%)	Moisture content (%)	pH	Peaks nm	Relative Absorb. (%)
J	SUCROSE	brown, free flowing	99.55	0.1900	6.35	471 332 235	20.50 35.80 43.70
K	MICROCRYSTALL-INE CELLULOSE	red, free flowing	102.81	3.8000	6.55	472 332 235	21.15 35.58 43.27
L	GELATIN	brown, free flowing	96.85	6.5000	6.06	472 332 235	20.33 35.50 44.17
M	DIBASIC CALCIUM PHOSPHATE	light brown, free flowing	99.92	0.6400	6.73	472 332 235	21.86 35.51 42.63
N	MAGNESIUM STEARATE	light brown, free flowing	105.52	2.5000	7.05	472 331 235	20.82 34.86 44.32
O	TALC	red, free flowing	103.94	0.6000	6.88	472 331 235	20.28 35.25 44.46
P	AEROSIL-200	scarlet red, free flowing	103.33	1.2000	6.52	472 331 235	20.31 35.27 44.42
Q	SOD STARCH GLYCOLATE	brick red, free flowing	103.03	4.5000	5.92	472 331 235	20.34 35.30 44.36
S	DOCUSATE SODIUM	brick red, free flowing	108.95	1.5000	7.12	472 335 234	21.16 35.29 43.65
T	CYCLODEXTRIN	orange free flowing	101.45	2.0000	6.49	473 334 234	21.37 34.90 43.73
U	POLOXAMER	orange free flowing	102.14	1.1200	6.52	473 333 232	20.27 35.50 43.23

Table 6.1B : Results of rifampicin : excipient compatibility study at zero time (initial analysis)

Batch code	Excipient	Impurities / Degradation products					
		RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	0.45	0.98	1.51	0.53	0.23	3.70
A	PEG 4000	0.44	1.66	1.12	0.52	1.00	4.74
B	POVIDONE K-30	0.47	1.92	2.30	0.17	1.25	6.11
C	UREA	0.57	2.21	2.79	0.35	1.77	7.69
D	LACTOSE	0.46	2.34	2.85	0.31	1.52	7.48
E	STARCH	0.104	1.40	1.09	0.45	0.90	3.94
F	SLS	0.35	1.69	1.64	0.37	0.75	4.80
G	POLYSORBATE 80	0.28	1.52	1.37	0.31	0.64	4.12
H	BILE SALTS	0.28	1.58	1.41	0.29	0.69	4.25
I	MANNITOL	0.35	1.23	1.33	0.58	0.92	4.41
J	SUCROSE	0.52	1.89	1.57	0.64	0.69	5.31
K	MCC	0.45	1.82	1.53	0.51	0.71	5.02
L	GELATIN	0.42	1.71	1.49	0.85	0.73	5.20
M	DCP	0.42	1.91	1.47	0.39	0.61	4.80
N	MAG. STEARATE	0.36	1.88	0.74	0.34	0.98	4.30
O	TALC	0.43	1.71	1.36	0.31	0.71	4.52
P	AEROSIL-200	--	1.45	1.94	0.35	0.32	4.06
Q	SSG	--	1.90	1.43	0.812	0.70	4.84
S	DOCUSATE SOD.	0.47	1.51	1.21	0.48	0.88	4.84
T	CYCLODEXTRIN	0.44	1.45	2.04	1.92	0.71	6.56
U	POLOXAMER	0.47	1.00	1.67	2.28	1.23	6.65

I = Rifampicin N-oxide ; II = 3-formyl rifamycin SV ;
III = 25-desacetyl rifampicin ; IV = Rifampicin quinone

Table 6.1C : Results of rifampicin : excipient compatibility study after 2 months at FT (2-8°C)

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	101.27	0.48	1.04	1.55	0.59	0.25	3.91
A	PEG 4000	99.35	0.49	1.71	1.21	0.61	1.20	5.22
B	PVP K30	103.45	0.49	1.96	2.30	0.20	1.32	6.27
C	UREA	100.15	0.59	2.71	2.88	0.99	2.21	9.38
D	LACTOSE	96.39	0.47	2.39	2.87	0.35	1.52	7.60
E	STARCH	104.00	0.21	1.56	1.20	0.48	0.96	4.41
F	SLS	104.14	0.40	1.77	1.72	0.42	0.82	5.13
G	POLYSORBATE 80	103.82	0.30	1.61	1.37	0.38	0.67	4.33
H	BILE SALTS	103.59	0.30	1.58	1.45	0.32	0.71	4.36
I	MANNITOL	100.36	0.39	1.30	1.40	0.61	0.96	4.66
J	SUCROSE	98.85	0.56	1.95	1.63	0.67	0.82	5.63
K	MCC	102.06	0.50	1.92	1.65	0.58	0.75	5.40
L	GELATIN	96.49	0.45	1.72	1.55	0.94	0.76	5.42
M	DCP	98.83	0.45	2.10	1.61	0.42	0.80	5.38
N	MAG STEARATE	104.55	0.39	1.92	0.85	0.37	1.10	4.63
O	TALC	103.86	0.44	1.73	1.39	0.32	0.72	4.60
P	AEROSIL-200	102.25	0.24	1.52	2.06	0.35	0.47	4.64
Q	SSG	102.49	0.27	1.95	1.46	0.82	0.71	5.21
S	DOCUSATE SOD.	107.76	0.55	1.57	1.34	0.80	0.98	5.24
T	CYCLODEXTRIN	101.30	0.45	1.51	2.05	1.92	0.72	6.65
U	POLOXAMER	101.74	0.51	1.12	1.75	2.33	1.24	6.95

Descritpion : No significant change in any sample.

Table 6.1D : Results of rifampicin : excipient compatibility study after 2 months at RT

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	101.08	0.49	1.03	1.55	0.59	0.28	3.94
A	PEG 4000	97.42	0.67	1.90	1.37	1.18	1.45	6.57
B	PVP K30	102.94	0.52	1.98	2.40	0.21	1.45	6.56
C	UREA	95.79	0.62	2.77	2.88	1.93	2.17	10.37
D	LACTOSE	95.81	0.55	2.41	2.90	0.31	1.59	7.76
E	STARCH	103.74	0.28	1.70	1.25	0.49	0.98	4.70
F	SLS	103.83	0.40	1.76	1.69	0.47	0.79	5.11
G	POLYSORBAT 80	103.63	0.38	1.65	1.47	0.40	0.70	4.60
H	BILE SALTS	103.47	0.32	1.59	1.47	0.34	0.74	4.46
I	MANNITOL	100.24	0.39	1.34	1.46	0.61	1.01	4.81
J	SUCROSE	98.49	0.57	2.01	1.66	0.76	0.85	5.85
K	MCC	101.67	0.54	1.94	1.70	0.61	0.82	5.61
L	GELATIN	96.31	0.47	1.79	1.61	0.91	0.79	5.57
M	DCP	96.73	0.55	2.10	1.87	0.87	0.92	6.31
N	MAG STEARATE	104.25	0.51	1.96	0.87	0.46	1.12	4.92
O	TALC	103.54	0.47	1.75	1.40	0.41	0.75	4.78
P	AEROSIL-200	101.83	0.29	1.57	2.07	0.41	0.50	4.84
Q	SSG	102.36	0.31	1.98	1.49	0.84	0.78	5.40
S	DOCUSATE SOD.	105.00	0.68	1.69	1.51	0.89	1.36	6.13
T	CYCLODEXTRIN	100.19	0.54	1.66	2.30	2.08	0.85	7.43
U	POLOXAMER	101.25	0.54	1.13	1.85	2.54	1.35	7.41

Description : No significant change in any sample

Table 6.1E : Results of rifampicin : excipient compatibility study after 2 Months at 45°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	100.16	0.52	1.15	1.67	0.62	0.42	4.38
A	PEG 4000	94.71	1.65	1.81	1.99	1.87	1.65	8.97
B	PVP K30	102.08	0.59	1.99	2.41	0.29	1.45	6.73
C	UREA	91.68	0.71	2.85	3.23	2.45	2.51	11.75
D	LACTOSE	94.86	0.59	2.57	3.09	0.38	1.59	8.22
E	STARCH	103.26	0.85	1.59	1.21	1.05	1.00	5.70
F	SLS	103.58	0.95	1.79	1.75	0.50	0.80	5.79
G	POLYSORBATE 80	102.43	0.92	1.75	1.51	0.95	0.91	6.04
H	BILE SALTS	103.35	0.41	1.61	1.53	0.41	0.74	4.70
I	MANNITOL	99.86	0.42	1.33	1.49	0.65	0.99	4.93
J	SUCROSE	96.92	0.65	2.51	1.65	0.81	1.28	6.90
K	MCC	101.04	0.52	1.95	1.72	1.02	0.75	5.96
L	GELATIN	94.92	0.51	1.81	1.67	1.01	1.75	6.75
M	DCP	92.82	0.57	2.85	1.97	1.51	1.43	8.33
N	MAG STEARATE	103.81	0.95	1.95	0.85	0.47	1.11	5.33
O	TALC	103.21	0.49	1.79	1.49	0.50	0.79	5.06
P	AEROSIL-200	101.23	0.41	1.49	2.05	0.44	0.48	4.87
Q	SSG	101.58	0.37	2.05	1.55	0.93	0.87	5.77
S	DOCUSATE SOD.	104.70	0.65	2.59	1.88	1.42	0.99	7.53
T	CYCLODEXTRIN	100.02	1.01	1.67	2.17	2.09	0.81	7.15
U	POLOXAMER	100.78	0.65	1.07	1.81	2.57	1.25	7.35

Description : No significant change in any sample

Table 6.1F : Results of rifampicin : excipient compatibility study after 2 Months at 60°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	98.73	0.55	1.21	1.72	0.67	0.55	4.70
A	PEG 4000	89.57	1.07	1.91	1.71	2.90	1.71	9.30
B	PVP K30	101.38	0.69	2.03	2.52	0.32	1.57	7.13
C	UREA	79.98	2.05	4.05	5.09	4.57	3.63	19.39
D	LACTOSE	93.29	0.62	2.80	3.15	0.51	1.67	8.75
E	STARCH	102.81	0.45	1.67	1.37	0.58	1.21	5.28
F	SLS	103.07	1.48	1.89	1.81	0.60	0.97	6.75
G	POLYSORBATE 80	102.06	0.49	1.82	1.59	0.66	0.75	5.31
H	BILE SALTS	102.31	0.49	1.79	1.78	0.52	0.89	5.47
I	MANNITOL	98.54	0.55	1.38	1.58	0.74	1.22	5.47
J	SUCROSE	95.47	0.75	2.09	1.85	1.52	1.67	7.88
K	MCC	100.77	0.59	2.03	1.85	0.79	0.83	6.09
L	GELATIN	90.35	1.59	1.85	1.72	2.03	1.79	8.98
M	DCP	86.04	2.95	3.91	3.01	3.17	2.43	15.47
N	MAG STEARATE	102.16	0.52	2.52	0.89	1.04	1.17	6.14
O	TALC	102.67	0.57	1.81	1.57	0.54	0.87	5.36
P	AEROSIL-200	100.70	0.47	1.53	2.11	0.48	0.55	5.14
Q	SSG	101.09	0.41	2.07	1.57	1.08	1.07	6.20
S	DOCUSATE SOD.	103.94	0.79	1.63	1.45	1.73	1.04	7.14
T	CYCLODEXTRIN	99.13	0.55	1.70	2.91	2.24	1.04	8.44
U	POLOXAMER	100.51	0.79	1.12	1.85	2.78	1.35	7.89

Description : No significant change except PEG 4000 sample which turned to a dark solid mass

Table 6.1G : Results of rifampicin : excipient compatibility study after 2 Months at 75% RH/40°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	99.98	0.54	1.24	1.67	0.60	0.51	4.56
A	PEG 4000	93.04	0.71	2.01	1.71	2.18	1.77	8.38
B	PVP K30	102.52	0.59	2.06	2.62	0.28	1.45	7.00
C	UREA	83.27	0.79	3.04	4.04	2.89	3.91	14.67
D	LACTOSE	95.46	0.60	2.57	2.95	0.59	1.51	8.22
E	STARCH	103.39	0.44	1.58	1.34	0.65	1.19	5.20
F	SLS	103.47	0.49	1.91	1.84	0.57	0.89	5.70
G	POLYSORBATE 80	100.76	0.56	1.76	1.75	0.69	1.29	6.05
H	BILE SALTS	102.76	0.32	1.73	1.72	0.43	0.75	4.95
I	MANNITOL	99.97	0.41	1.34	1.47	0.72	1.05	4.99
J	SUCROSE	98.52	0.57	1.97	1.67	0.94	0.77	5.92
K	MCC	101.37	0.49	2.02	1.79	0.69	0.89	5.88
L	GELATIN	89.16	0.76	1.94	2.04	1.28	2.45	8.45
M	DCP	93.10	0.67	2.91	2.07	1.85	0.87	8.37
N	MAG STEARATE	104.43	0.45	1.85	0.86	0.50	1.12	4.78
O	TALC	103.49	0.47	1.75	1.51	0.43	0.75	4.91
P	AEROSIL-200	101.75	0.42	1.51	2.05	0.49	0.37	4.84
Q	SSG	101.61	0.37	2.01	1.53	0.85	0.87	5.63
S	DOCUSATE SOD.	104.99	0.67	1.67	1.56	1.14	1.36	6.42
T	CYCLODEXTRIN	100.13	0.54	1.69	2.22	2.21	0.89	7.55
U	POLOXAMER	99.76	0.67	1.16	1.91	2.79	1.79	8.32

Description : No significant change in any sample

Table 6.1H : Results of rifampicin : excipient compatibility study after 4 Months at FT (2 - 8°C)

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	101.26	0.49	1.06	1.57	0.59	0.38	4.09
A	PEG 4000	98.12	0.71	1.92	1.71	0.20	1.50	6.04
B	PVP K30	103.41	0.50	2.00	2.32	0.21	1.33	6.36
C	UREA	100.04	0.63	2.74	2.95	1.05	2.94	10.00
D	LACTOSE	96.05	0.49	2.41	2.91	0.38	1.62	7.81
E	STARCH	103.06	0.25	1.59	2.47	0.55	1.41	6.27
F	SLS	103.23	0.45	1.92	1.82	0.84	1.03	6.06
G	POLYSORBATE 80	102.91	0.46	1.75	1.56	0.47	0.96	5.20
H	BILE SALTS	103.29	0.38	1.71	1.47	0.40	0.78	4.74
I	MANNITOL	100.22	0.45	1.36	1.51	0.66	1.10	5.08
J	SUCROSE	98.50	0.61	2.25	1.67	0.74	0.91	6.18
K	MCC	101.44	0.57	2.20	1.74	0.66	0.90	6.07
L	GELATIN	95.94	0.47	1.87	1.61	1.04	0.87	6.49
M	DCP	98.07	0.51	2.40	1.75	0.61	1.22	6.49
N	MAG STEARATE	103.46	0.56	1.82	1.42	0.79	1.50	6.09
O	TALC	103.75	0.45	1.75	1.40	0.37	0.81	4.78
P	AEROSIL-200	101.59	0.35	1.87	2.29	0.41	0.71	5.63
Q	SSG	102.35	0.29	1.97	1.51	0.89	0.80	5.46
S	DOCUSATE SOD.	106.37	0.71	1.86	1.62	1.04	1.30	6.53
T	CYCLODEXTRIN	101.15	0.50	1.54	2.08	1.96	0.78	6.86
U	POLOXAMER	101.12	0.53	1.21	1.89	2.49	1.41	7.53

Description : No significant change in any sample

Table 6.1I : Results of rifampicin : excipient compatibility study after 4 Months at RT

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	100.69	0.52	1.17	1.60	0.67	0.40	4.36
A	PEG 4000	94.30	0.88	2.80	2.21	1.47	2.51	9.87
B	PVP K30	102.54	0.54	2.00	2.43	0.23	1.47	6.67
C	UREA	90.24	0.75	3.21	3.75	2.90	3.85	14.46
D	LACTOSE	95.31	0.59	2.47	2.97	0.51	1.78	8.32
E	STARCH	102.41	0.36	1.94	1.59	0.73	1.35	5.97
F	SLS	102.83	0.65	2.05	1.96	0.60	0.98	6.24
G	POLYSORBATE 80	101.48	0.48	1.87	1.69	0.55	0.95	5.54
H	BILE SALTS	103.04	0.41	1.69	1.60	0.41	0.85	4.96
I	MANNITOL	99.48	0.47	1.45	1.57	0.69	1.07	5.25
J	SUCROSE	97.06	0.69	2.34	1.87	1.01	1.14	7.05
K	MCC	100.95	0.61	2.18	1.89	0.91	0.89	6.48
L	GELATIN	95.70	0.53	1.87	1.75	1.07	0.97	6.19
M	DCP	95.42	0.70	2.39	1.96	2.03	1.21	8.29
N	MAG STEARATE	103.11	0.59	2.21	1.06	0.65	1.63	6.14
O	TALC	103.39	0.49	1.81	1.44	0.44	0.81	4.99
P	AEROSIL-200	101.27	0.33	1.74	2.24	0.48	0.98	5.77
Q	SSG	101.57	0.35	2.16	1.70	0.97	0.88	6.06
S	DOCUSATE SOD.	104.26	0.81	2.07	1.95	1.16	2.06	8.05
T	CYCLODEXTRIN	100.76	0.55	1.72	2.35	2.19	0.87	7.68
U	POLOXAMER	100.26	0.59	1.28	2.09	2.68	1.54	8.18

Description : No significant change in any sample

Table 6.1J : Results of rifampicin : excipient compatibility study after 4 Months at 45°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	99.72	0.64	1.28	1.71	0.77	0.67	5.07
A	PEG 4000	88.43	2.41	2.84	2.47	3.27	3.74	14.73
B	PVP K30	101.01	0.72	2.25	2.71	0.39	1.55	7.62
C	UREA	83.60	0.98	3.51	4.69	3.49	3.81	16.48
D	LACTOSE	94.13	0.68	2.72	3.49	0.51	1.74	9.14
E	STARCH	101.19	1.20	1.86	1.71	1.41	1.42	7.60
F	SLS	102.39	1.16	2.07	2.14	0.61	0.89	6.87
G	POLYSORBATE 80	100.36	1.11	1.91	1.72	1.10	1.26	7.10
H	BILE SALTS	102.67	0.46	1.72	1.62	0.48	0.79	5.07
I	MANNITOL	97.17	0.51	1.44	1.68	0.71	1.26	5.60
J	SUCROSE	94.47	0.72	2.77	1.67	0.96	1.42	7.54
K	MCC	100.52	0.64	2.21	1.97	1.26	2.34	8.42
L	GELATIN	93.57	0.65	2.39	2.02	1.30	2.21	8.57
M	DCP	87.65	0.75	3.30	2.82	2.81	2.93	12.61
N	MAG STEARATE	101.78	1.12	2.24	1.14	0.64	1.41	6.55
O	TALC	102.55	0.56	1.89	1.63	0.64	0.94	5.66
P	AEROSIL-200	100.17	0.49	1.79	2.40	0.57	0.65	5.90
Q	SSG	98.44	0.39	2.90	2.37	1.18	1.46	8.30
S	DOCUSATE SOD.	100.06	0.94	2.90	3.51	1.73	1.71	10.79
T	CYCLODEXTRIN	98.27	1.24	1.94	2.63	2.49	1.19	9.49
U	POLOXAMER	99.64	0.80	1.18	2.04	2.75	1.49	8.26

Description : No significant change in any sample

Table 6.1K : Results of rifampicin : excipient compatibility study after 4 Months at 60°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	98.26	0.59	1.34	1.86	0.94	0.67	5.40
A	PEG 4000	79.77	1.61	2.54	2.49	4.31	2.51	13.46
B	PVP K30	100.38	0.78	2.57	2.96	0.47	2.06	8.84
C	UREA	60.07	2.41	4.67	6.47	5.49	4.65	23.69
D	LACTOSE	92.69	0.77	3.12	3.51	0.65	1.91	9.96
E	STARCH	99.64	0.52	1.91	1.46	0.71	1.87	6.47
F	SLS	101.13	1.61	2.49	2.17	0.74	1.12	8.13
G	POLYSORBATE 80	98.56	0.67	2.22	1.86	0.81	1.34	6.90
H	BILE SALTS	100.74	0.61	1.54	1.89	0.95	1.74	6.73
I	MANNITOL	96.49	0.72	1.55	2.10	1.14	1.54	7.05
J	SUCROSE	92.69	0.99	1.92	1.95	0.96	1.78	7.60
K	MCC	99.82	0.67	2.35	2.27	0.92	1.21	7.42
L	GELATIN	89.17	1.72	2.11	2.03	2.44	2.15	10.45
M	DCP	78.48	3.25	4.69	4.52	3.73	3.01	19.20
N	MAG STEARATE	99.91	0.69	2.87	1.03	1.31	1.37	7.27
O	TALC	101.89	0.67	2.22	1.70	0.57	1.02	6.18
P	AEROSIL-200	99.25	0.65	1.75	2.37	0.64	0.59	6.00
Q	SSG	94.09	0.87	2.97	2.15	1.21	1.35	8.55
S	DOCUSATE SOD.	98.26	0.95	2.04	1.99	2.31	1.61	8.90
T	CYCLODEXTRIN	95.44	0.67	1.92	3.67	2.71	1.38	10.35
U	POLOXAMER	99.14	0.98	1.38	2.16	2.91	1.51	8.94

Description : No significant change except PEG 4000 sample which turned to a dark semisolid

Table 6.1L : Results of rifampicin : excipient compatibility study after 4 Months at 75% RH/40°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	98.21	0.71	1.49	1.95	0.80	0.78	5.73
A	PEG 4000	82.35	0.97	2.68	2.41	2.89	2.75	11.70
B	PVP K30	101.45	0.66	2.29	2.88	0.35	1.65	7.83
C	UREA	62.46	1.48	4.61	6.63	4.15	4.51	21.38
D	LACTOSE	94.63	0.69	2.72	3.30	0.72	1.64	9.07
E	STARCH	98.18	0.75	1.87	1.62	1.16	1.89	7.29
F	SLS	102.04	0.58	2.29	2.02	0.91	1.30	7.10
G	POLYSORBATE 80	96.76	0.68	2.25	2.30	0.87	1.90	8.00
H	BILE SALTS	100.61	0.46	2.04	1.94	0.67	0.95	6.06
I	MANNITOL	98.35	0.52	1.53	1.83	1.06	1.26	6.20
J	SUCROSE	96.01	0.59	2.40	2.11	0.84	1.06	7.00
K	MCC	100.18	0.57	2.21	2.04	0.79	0.96	6.57
L	GELATIN	87.74	0.91	2.40	2.56	1.42	3.16	10.45
M	DCP	90.01	0.88	3.49	2.79	2.81	1.60	11.57
N	MAG STEARATE	102.84	0.53	2.37	1.03	0.67	1.46	6.06
O	TALC	103.32	0.51	1.76	1.54	0.45	0.76	5.02
P	AEROSIL-200	100.75	0.57	1.78	2.47	0.78	0.53	6.13
Q	SSG	96.09	0.49	2.83	1.92	1.10	1.43	7.77
S	DOCUSATE SOD.	100.97	0.84	1.98	1.92	1.64	2.87	9.25
T	CYCLODEXTRIN	97.25	0.71	1.91	2.59	2.81	1.38	9.40
U	POLOXAMER	98.08	0.82	1.32	2.22	3.28	2.30	9.94

Description : No significant change except PEG 4000 sample which turned moist and sticky

Table 6.1M : Results of rifampicin : excipient compatibility study after 6 Months at FT (2 - 8°C)

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	101.12	0.51	1.06	1.60	0.62	0.43	4.22
A	PEG 4000	96.91	0.77	2.12	1.97	0.38	2.21	7.45
B	PVP K30	103.27	0.52	2.05	2.32	0.24	1.34	6.47
C	UREA	99.15	0.70	2.82	3.22	1.13	2.43	10.30
D	LACTOSE	95.76	0.50	2.44	2.92	0.40	1.74	8.00
E	STARCH	101.57	0.29	1.78	2.73	0.69	1.85	7.34
F	SLS	102.42	0.48	2.16	1.88	0.90	1.23	6.65
G	POLYSORBATE 80	102.58	0.50	1.79	1.62	0.50	1.01	5.42
H	BILE SALTS	103.20	0.38	1.71	1.49	0.44	0.82	4.84
I	MANNITOL	99.85	0.48	1.42	1.56	0.71	1.22	5.39
J	SUCROSE	98.23	0.67	2.29	1.71	0.77	1.01	6.45
K	MCC	100.66	0.64	2.24	1.86	0.76	1.14	6.64
L	GELATIN	95.83	0.49	1.87	1.64	1.06	0.89	5.95
M	DCP	97.50	0.54	2.46	1.82	0.66	1.29	6.77
N	MAG STEARATE	102.45	0.63	2.04	1.59	0.91	1.79	5.96
O	TALC	103.63	0.45	1.76	1.42	0.36	0.84	4.83
P	AEROSIL-200	101.04	0.35	1.89	2.29	0.42	0.71	5.66
Q	SSG	102.28	0.30	1.97	1.53	0.89	0.82	5.51
S	DOCUSATE SOD.	105.92	0.75	1.97	1.70	1.22	1.43	7.07
T	CYCLODEXTRIN	101.07	0.52	1.56	2.10	1.95	0.78	6.91
U	POLOXAMER	100.81	0.57	1.24	1.89	2.57	1.56	7.83

Description : No significant change in any sample

Table 6.1N : Results of rifampicin : excipient compatibility study after 6 Months at RT

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	100.52	0.52	1.19	1.60	0.67	0.42	4.40
A	PEG 4000	90.06	0.96	3.56	2.82	1.93	3.99	13.26
B	PVP K30	102.22	0.60	2.10	2.43	0.23	1.53	6.89
C	UREA	85.23	0.80	8.33	3.77	3.12	3.86	19.88
D	LACTOSE	95.05	0.64	2.50	2.96	0.57	1.83	8.50
E	STARCH	100.36	0.48	2.24	1.81	1.13	1.72	7.38
F	SLS	101.99	0.74	2.15	2.13	0.68	1.11	6.81
G	POLYSORBATE 80	99.87	0.59	2.16	1.88	0.87	1.54	7.04
H	BILE SALTS	102.54	0.46	1.78	1.69	0.46	0.98	5.37
I	MANNITOL	98.69	0.53	1.58	1.69	0.80	1.24	5.84
J	SUCROSE	95.76	0.77	2.56	2.06	1.18	1.55	8.12
K	MCC	100.07	0.67	2.33	2.00	0.98	1.18	7.16
L	GELATIN	95.18	0.58	1.96	1.87	1.16	1.01	6.58
M	DCP	93.07	0.78	2.57	2.24	2.12	1.15	8.86
N	MAG STEARATE	101.82	0.65	2.36	1.20	0.81	2.24	7.26
O	TALC	103.27	0.49	1.84	1.50	0.45	0.86	5.14
P	AEROSIL-200	100.73	0.36	1.78	2.29	0.49	1.06	5.98
Q	SSG	96.24	0.55	2.78	2.50	1.72	2.91	10.46
S	DOCUSATE SOD.	102.34	0.87	2.39	2.47	1.34	2.54	9.61
T	CYCLODEXTRIN	100.58	0.56	1.75	2.38	2.19	0.90	7.78
U	POLOXAMER	99.36	0.71	1.39	2.25	2.84	1.67	8.86

Description : No significant change in any sample

Table 6.10 : Results of rifampicin : excipient compatibility study after 6 Months at 45°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	99.46	0.68	1.35	1.74	0.80	0.69	5.26
A	PEG 4000	80.42	2.67	3.92	3.87	4.94	5.51	20.91
B	PVP K30	100.09	0.78	2.41	2.94	0.47	1.76	8.36
C	UREA	77.95	1.68	5.94	6.41	5.14	9.69	28.86
D	LACTOSE	93.21	0.68	2.94	3.81	0.54	1.85	9.82
E	STARCH	99.12	1.38	2.17	1.91	1.68	2.25	9.39
F	SLS	101.63	1.24	2.28	2.34	0.65	1.01	7.52
G	POLYSORBATE 80	97.88	1.22	2.14	1.88	1.18	2.72	9.14
H	BILE SALTS	102.11	0.51	1.86	1.81	0.51	0.86	5.55
I	MANNITOL	95.26	0.57	1.66	1.82	0.76	2.53	7.34
J	SUCROSE	92.45	0.88	3.37	1.94	1.28	1.77	9.24
K	MCC	99.88	0.68	2.38	2.10	1.38	2.37	8.91
L	GELATIN	91.96	0.76	2.66	2.27	1.46	2.58	9.73
M	DCP	81.68	0.93	4.51	3.21	3.19	5.54	17.38
N	MAG STEARATE	100.13	1.16	2.37	1.22	0.71	1.56	7.02
O	TALC	101.98	0.60	2.05	1.78	0.69	0.99	6.08
P	AEROSIL-200	99.44	0.56	1.88	2.66	0.68	0.70	6.48
Q	SSG	90.23	0.45	3.32	2.68	1.39	2.43	10.27
S	DOCUSATE SOD.	97.59	1.21	3.68	4.62	2.23	1.98	13.72
T	CYCLODEXTRIN	96.33	1.38	2.22	2.95	2.80	1.63	10.98
U	POLOXAMER	98.62	0.88	1.29	2.27	2.88	1.78	9.10

Description : No significant change in any sample

Table 6.1P : Results of rifampicin : excipient compatibility study after 6 Months at 60°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNO I	3FRSV II	25-DAR III	RQ IV	Others	Total
R	RIFAMPICIN	98.02	0.62	1.39	1.91	0.98	0.72	5.62
A	PEG 4000	70.30	1.92	3.84	3.76	5.71	4.61	19.84
B	PVP K30	99.80	0.78	2.62	3.14	0.53	2.22	9.29
C	UREA	45.60	2.67	5.84	7.77	6.89	9.67	32.84
D	LACTOSE	92.28	0.79	3.27	3.57	0.71	1.92	10.26
E	STARCH	94.20	0.81	2.82	2.96	1.61	3.04	11.24
F	SLS	100.08	1.74	2.92	2.45	0.95	1.83	9.89
G	POLYSORBATE 80	94.40	0.82	2.96	2.88	1.67	1.59	9.92
H	BILE SALTS	98.90	0.74	1.78	2.56	1.30	1.89	8.27
I	MANNITOL	94.40	0.84	1.80	2.28	1.44	2.52	8.88
J	SUCROSE	90.30	1.22	2.28	2.36	1.30	2.26	9.42
K	MCC	99.10	0.69	2.42	2.40	1.14	1.26	7.91
L	GELATIN	88.20	1.78	2.24	2.18	2.60	2.25	11.05
M	DCP	74.00	3.50	5.12	4.84	4.05	5.33	22.84
N	MAG STEARATE	97.60	0.77	3.20	1.18	1.59	2.20	8.94
O	TALC	101.24	0.74	2.34	1.79	0.62	1.10	6.59
P	AEROSIL-200	98.40	0.70	1.78	2.49	0.68	1.13	6.78
Q	SSG	78.20	1.45	5.29	3.89	3.34	4.51	18.48
S	DOCUSATE SOD.	92.06	1.10	2.38	2.24	3.52	3.51	12.75
T	CYCLODEXTRIN	91.60	0.77	2.24	4.18	3.21	1.85	12.25
U	POLOXAMER	97.72	1.17	1.47	2.29	3.18	2.05	10.16

Description : No significant change except PEG 4000 sample which turned to a dark brown semisolid mass and urea sample which to turned a dark solid

Table 6.1Q : Results of rifampicin : excipient compatibility study after 6 Months at 75 % RH / 40°C

Batch code	Excipient	Drug Assay	Impurities / Degradation products					
			RNo	3FRSV	25-DAR	RQ	Others	Total
R	RIFAMPICIN	96.14	0.86	1.72	2.26	0.88	1.44	7.16
A	PEG 4000	65.28	1.42	3.79	3.18	4.24	5.93	18.56
B	PVP K30	100.33	0.70	2.42	2.94	0.40	1.78	8.24
C	UREA	29.77	1.71	5.82	8.48	5.92	7.53	29.46
D	LACTOSE	93.71	0.72	2.89	3.40	0.76	1.87	9.64
E	STARCH	85.58	0.88	2.41	2.16	1.35	4.06	10.86
F	SLS	96.95	0.74	3.14	2.22	1.16	3.23	10.49
G	POLYSORBATE 80	91.28	0.85	2.89	3.02	1.16	3.50	11.42
H	BILE SALTS	97.93	0.54	2.86	2.38	0.87	1.49	8.14
I	MANNITOL	97.13	0.55	1.62	1.95	1.26	1.80	7.18
J	SUCROSE	93.07	0.71	2.94	2.74	0.98	1.87	9.24
K	MCC	99.57	0.62	2.35	2.10	0.82	0.99	6.88
L	GELATIN	87.25	0.93	2.46	2.66	1.48	3.39	10.92
M	DCP	88.09	0.90	3.62	2.86	2.95	1.74	12.07
N	MAG STEARATE	101.34	0.65	2.58	1.19	0.77	2.02	7.21
O	TALC	103.08	0.52	1.75	1.56	0.43	0.76	5.02
P	AEROSIL-200	100.01	0.58	1.82	2.52	0.84	0.72	6.48
Q	SSG	87.32	0.68	4.19	3.47	1.82	3.66	13.82
S	DOCUSATE SOD.	94.27	0.94	2.62	2.58	2.18	3.89	12.21
T	CYCLODEXTRIN	93.63	0.87	2.24	2.74	3.16	2.71	11.72
U	POLOXAMER	96.52	0.87	1.44	2.37	3.40	3.03	11.11

Description : No significant change except PEG 4000 sample which turned to moist semisolid.

Table 6.1R : Degradation rate constant (Ko), correlation coefficient (r) and shelf life (t90%) of rifampicin ; excipient samples.

Batch Code	Rate Constant (Ko = % / month)					Arrhenius plot data	
	FT (5°C)	RT (28°C)	45°C	60	75 % RH /40°C	r	t 90 % at 30°C (months)
R	0.0320	0.1410	0.3025	0.5200	0.8670	0.9973	72.13
A	0.6510	1.7730	3.3770	5.0710	5.8685	0.9992	5.35
B	0.0605	0.2360	0.5890	0.6290	0.5530	0.9784	42.84
C	0.5065	2.8665	4.0850	9.5290	11.9485	0.9845	4.30
D	0.1295	0.2440	0.5315	0.6945	0.4615	0.9899	33.70
E	0.6755	0.8765	1.0995	1.8925	3.2875	0.9387	10.04
F	0.4505	0.5195	0.5830	0.8530	1.2970	0.9108	17.71
G	0.3305	0.7990	1.0935	1.6870	2.1800	0.9945	13.13
H	0.1035	0.2090	0.2860	0.8120	0.9865	0.9536	41.95
I	0.1720	0.3770	0.9380	1.0850	0.6540	0.9846	22.12
J	0.2155	0.6400	1.1875	1.5265	1.0975	0.9922	15.90
K	0.3535	0.4470	0.4655	0.6040	0.5455	0.9589	22.41
L	0.1805	0.2810	0.8010	1.3565	1.5110	0.9660	23.30
M	0.4010	1.0930	2.9945	4.2660	2.0290	0.9938	7.50
N	0.5150	0.6120	0.9100	1.3005	0.7065	0.9533	13.50
O	0.0520	0.1080	0.3270	0.4440	0.1375	0.9827	67.52
P	0.3765	0.4180	0.6365	0.8120	0.5480	0.9428	19.60
Q	0.1195	1.0580	2.0770	4.0745	2.6325	0.9864	12.06
S	0.5240	1.1285	1.9360	2.8175	2.4030	0.9998	8.24
T	0.0645	0.1470	0.8555	1.6620	1.3170	0.9713	35.78
U	0.2305	0.4665	0.5850	0.7315	0.9270	0.9877	23.08

Table 6.15 : Relative shelf-life of rifampicin excipient samples at 75 % RH / 40°C and 40°C

Batch code	Excipient	90 % t at 40°C/75%RH I	t90°C at 40°C II	Ratio I/II
R	RIFAMPICIN	11.53	43.18	0.87
A	PEG 4000	1.70	3.66	0.46
B	PVP K30	18.10	27.21	0.67
C	UREA	0.84	2.57	0.33
D	LACTOSE	21.66	24.45	0.89
E	STARCH	3.04	8.43	0.36
F	SLS	7.70	15.93	0.48
G	POLYSORBATE 80	4.59	9.81	0.47
H	BILE SALTS	10.14	29.65	0.34
I	MANNITOL	15.29	15.44	0.99
J	SUCROSE	9.11	11.01	0.83
K	MCC	18.33	20.49	0.89
L	GELATIN	6.62	15.92	0.42
M	DCP	4.92	4.78	1.03
N	MAG STEARATE	14.15	11.41	1.24
O	TALC	72.73	44.61	1.63
P	AEROSIL-200	18.25	16.97	1.07
Q	SSG	3.99	6.35	0.63
S	DOCUSATE SOD.	4.16	6.04	0.69
T	CYCLODEXTRIN	7.59	19.19	0.40
U	POLOXAMER-188	10.79	18.71	0.58

Table 6.2A : Rifampicin solubility from rifampicin : excipient compatibility study samples

Batch No	Excipient	Concentration of rifampicin (mg/ml)				Cmax
		2 (h)	4 (h)	6 (h)	24 (h)	
R	RIFAMPICIN	1.228	1.386	1.560	1.713	1.713
A	PEG - 4000	2.188	1.827	1.551	0.842	2.188
B	PVP - K 30	1.431	1.625	1.881	1.494	1.881
C	UREA	2.096	2.262	2.447	2.779	2.779
D	LACTOSE	1.391	1.368	1.152	0.298	1.391
E	STARCH	1.292	1.362	1.481	1.670	1.670
F	SODIUM LAURYL SULPHATE	2.356	2.502	2.610	3.811	3.811
G	POLYSORBATE 80	0.470	0.481	0.499	0.334	0.499
H	BILE SALTS	1.298	1.362	1.447	2.064	2.064
I	MANNITOL	1.336	1.366	1.212	0.419	1.366
J	SUCROSE	1.283	1.421	1.363	0.875	1.421
K	MCC	1.657	1.190	0.838	0.269	1.657
L	GELATIN	1.417	1.110	1.060	1.026	1.417
M	DCP	1.338	1.290	1.232	1.151	1.338
N	MAGNESIUM STEARATE	1.674	1.809	1.573	0.709	1.809
O	TALC	2.104	2.199	2.265	2.041	2.265
P	AEROSIL 200	0.662	0.602	0.582	0.547	0.662
Q	SODIUM STARCH GLYCOLATE	1.118	1.209	1.252	1.206	1.253
S	DOCUSATE SOD.	1.845	1.997	2.071	1.814	2.071

Table 6.3A : Effect of UV light (254 nm) on stability of rifampicin powder

Time (Days)	Drug assay (%)	Impurities / degradation components (%)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	99.15	0.45	1.78	1.32	0.52	1.20	5.27
2	99.14	0.47	1.66	1.41	0.52	1.21	5.27
4	99.21	0.45	1.72	1.35	0.52	1.25	5.29
6	98.71	0.47	1.70	1.40	0.55	1.21	5.33
8	98.69	0.47	1.73	1.41	0.53	1.20	5.34
10	98.82	0.47	1.68	1.40	0.59	1.22	5.36
12	98.35	0.51	1.71	1.40	0.55	1.25	5.42
14	98.72	0.50	1.71	1.38	0.59	1.24	5.43
16	98.97	0.55	1.75	1.42	0.53	1.17	5.42

Table 6.3B : Effect of UV light (366 nm) on stability of rifampicin powder

Time (Days)	Drug assay (%)	Impurities / degradation components (%)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	99.25	0.45	1.78	1.32	0.52	1.20	5.27
2	99.21	0.41	1.70	1.40	0.55	1.21	5.27
4	99.21	0.47	1.67	1.35	0.59	1.21	5.29
6	99.25	0.49	1.68	1.41	0.51	1.21	5.30
8	99.23	0.50	1.61	1.40	0.51	1.20	5.22
10	99.28	0.45	1.51	1.33	0.71	1.25	5.25
12	99.35	0.48	1.51	1.31	0.71	1.25	5.26
14	99.19	0.42	1.53	1.32	0.69	1.31	5.27
16	99.21	0.49	1.55	1.32	0.65	1.29	5.30

Table 6.3C : Effect of Day light on stability of rifampicin powder

Time (Days)	Drug assay (%)	Impurities / degradation components (%)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	99.25	0.45	1.78	1.32	0.52	1.20	5.27
2	99.31	0.45	1.67	1.47	0.35	1.31	5.27
4	99.32	0.48	1.69	1.45	0.42	1.25	5.29
6	99.31	0.47	1.70	1.40	0.55	1.21	5.33
8	99.31	0.51	1.73	1.40	0.50	1.20	5.34
10	99.25	0.45	1.70	1.40	0.51	1.21	5.27
12	98.29	0.41	1.61	1.35	0.40	1.51	5.28
14	99.21	0.45	1.59	1.47	0.47	1.25	5.23
16	98.24	0.45	1.51	1.51	0.51	1.30	5.28

Table 6.3D : Stability of rifampicin powder kept in dark area

Time (Days)	Drug assay (%)	Impurities / degradation components (%)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	99.15	0.45	1.78	1.32	0.52	1.20	5.27
2	99.14	0.47	1.65	1.41	0.52	1.21	5.27
4	99.29	0.51	1.63	1.40	0.50	1.20	5.24
6	99.25	0.45	1.59	1.47	0.47	1.25	5.23
8	98.97	0.42	1.52	1.49	0.45	1.20	5.18
10	98.98	0.51	1.49	1.45	0.51	1.25	5.21
12	99.11	0.59	1.25	1.49	0.55	1.32	5.20
14	99.15	0.58	1.31	1.47	0.52	1.31	5.19
16	98.17	0.51	1.41	1.39	0.57	1.32	5.20

Table 6.3E : Effect of UV light (254 nm) on stability of rifampicin solution

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	0.9240	1.1012	0.1720	0.0271	0.0912	0.0061	0.3976
48 hours	0.6981	0.2131	0.2572	0.0612	0.1210	0.0097	0.6622
72 hours	0.5510	0.2751	0.2979	0.0932	0.1513	0.0121	0.8296

Table 6.3F : Effect of UV light (366 nm) on stability of rifampicin solution

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	1.2665	0.0213	0.0457	0.0175	0.0299	0.0061	0.1205
48 hours	1.1847	0.0217	0.0461	0.0197	0.0310	0.0079	0.1264
72 hours	1.1621	0.0235	0.0491	0.0210	0.0389	0.0097	0.1422

Table 6.3G : Effect of day-light on stability of rifampicin solution

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	1.1983	0.0217	0.0471	0.0210	0.0321	0.0061	0.1280
48 hours	1.1519	0.0240	0.0507	0.0215	0.0409	0.0098	0.1469
72 hours	1.1510	0.0241	0.0509	0.0217	0.0410	0.0098	0.1475

Table 6.3H : Stability of rifampicin solution kept in dark

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	1.3312	0.0129	0.0317	0.0151	0.0310	0.0047	0.0954
48 hours	1.2984	0.0214	0.0510	0.0172	0.0420	0.0072	0.1388
72 hours	1.2790	0.0310	0.0594	0.0191	0.0510	0.0075	0.1680

Table 6.4A : Stability of rifampicin solution at 45°C under nitrogen atmosphere

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	1.2021	0.0340	0.0517	0.0310	0.0375	0.0043	0.1585
48 hours	1.0448	0.0397	0.0817	0.0610	0.0471	0.0061	0.2356
72 hours	0.9140	0.0410	0.0914	0.0617	0.0479	0.0097	0.2517

Table 6.4B : Stability of rifampicin solution at 45°C under oxygen atmosphere

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	0.8691	0.1910	0.1917	0.0614	0.1210	0.0047	0.5698
48 hours	0.7957	0.2071	0.2120	0.0894	0.1510	0.0071	0.6666
72 hours	0.4744	0.3571	0.3109	0.1251	0.1910	0.0082	0.9923

Table 6.4C : Stability of rifampicin solution at 45°C under natural (air) atmosphere

Time (h)	Conc.of rifampicin (mg/ml)	Conc. of Impurities/degradation components (mg/ml)					
		RNO	3FRSV	25-DAR	RQ	Others	Total
Initial	1.3977	0.0123	0.0214	0.0124	0.0275	0.0031	0.0767
24 hours	1.0821	0.0398	0.1251	0.0350	0.0895	0.0051	0.2945
48 hours	1.0327	0.0472	0.1591	0.0970	0.0898	0.0071	0.4002
72 hours	0.8425	0.0971	0.1751	0.1210	0.1009	0.0089	0.5030

Table 6.4D : Shelf-life (t 90%) of rifampicin solutions subjected to different stress conditions

Figure No	Stress condition	K1 (per h)	t 90 % (h)
7.3E	UV light - 254 nm	0.03189	8.20
7.3F	UV light - 366 nm	0.00792	40.71
7.3G	Day light	0.00875	40.71
7.3H	Darkness	0.01136	87.68
7.3I	Nitrogen atmosphere	0.01651	17.88
7.3J	Oxygen atmosphere	0.03266	7.57
7.3K	Air atmosphere	0.02479	16.10

Table 6.5A : Stability of rifampicin in 1000 mcg/ml concentration solution at different pH values

pH	Concentration of rifampicin (mg/ml) at time (h)								
	0	1	2	4	6	8	12	16	24
2.0	0.988	0.945	0.909	0.843	0.787	0.700	0.609	0.527	0.417
3.0	0.984	0.952	0.936	0.892	0.832	0.802	0.766	0.739	0.686
4.0	0.986	0.962	0.952	0.923	0.905	0.862	0.839	0.822	0.781
5.0	0.989	0.970	0.958	0.929	0.904	0.789	0.739	0.703	0.638
6.0	0.998	0.974	0.954	0.913	0.861	0.762	0.655	0.545	0.466
7.0	0.991	0.969	0.941	0.892	0.845	0.748	0.685	0.607	0.505
8.0	0.802	0.789	0.770	0.730	0.719	0.637	0.543	0.458	0.341
9.0	0.805	0.787	0.779	0.746	0.714	0.590	0.495	0.387	0.241

Table 6.5B : Stability of rifampicin in 25 mcg/ml concentration unbuffered solutions at pH 2.0, 3.0, 4.0 and 5.0

Time (h)	Conc. of rifampicin (mcg/ml)				Conc. of 3-formyl rifamycin SV (mcg/ml)			
	pH 2.0	pH 3.0	pH 4.0	pH 5.0	pH 2.0	pH 3.0	pH 4.0	pH 5.0
0	25.20	25.20	26.20	26.40	0.00	0.00	0.00	0.00
1	24.15	24.00	25.74	25.79	0.00	0.00	0.00	0.00
2	21.41	21.95	25.10	24.66	2.18	0.52	0.00	0.00
3	17.95	21.30	24.70	24.08	2.78	0.80	0.00	0.00
4	15.05	19.34	24.74	23.07	3.44	1.07	0.35	0.00
5	13.01	18.86	24.56	22.72	4.14	1.34	0.62	0.00
6	11.18	18.06	24.46	22.39	5.04	1.79	1.05	0.00
8	6.18	11.87	22.68	21.20	6.78	2.48	1.50	0.88
10	5.01	10.94	21.35	20.96	8.22	2.96	1.93	1.45
12	4.16	10.85	20.03	20.91	10.16	4.13	2.22	2.36
14	3.59	9.41	19.40	21.22	13.01	4.71	2.15	2.98
16	3.10	9.04	18.09	20.56	13.80	6.00	2.09	3.56
18	2.60	8.40	18.38	19.92	13.40	6.24	2.03	3.43
20	2.14	7.95	17.58	18.96	13.24	6.45	1.95	3.22
24	1.49	7.21	15.62	18.35	10.03	6.62	1.82	3.04

Table 6.5C : Stability of rifampicin in 25 mcg/ml concentration unbuffered solutions at pH 6.0, 7.0, 8.0 and 9.0

Time (h)	Conc. of rifampicin (mcg/ml)				Conc. of 3-formyl rifamycin SV (mcg/ml)			
	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
0	25.90	26.40	26.40	27.04	0.27	0.48	0.36	0.39
1	25.28	25.52	26.28	26.57	0.42	0.66	0.50	0.60
2	24.48	25.03	24.72	25.40	0.61	0.88	0.59	0.75
3	23.83	23.39	23.88	24.46	0.69	1.00	0.80	1.00
4	23.07	23.43	23.51	23.18	0.81	1.18	0.95	1.31
5	21.32	22.12	21.43	23.03	0.87	1.30	1.10	1.38
6	20.97	22.07	20.89	21.85	0.94	1.50	1.40	1.56
8	20.81	21.54	19.31	20.58	1.23	1.78	1.62	1.90
10	20.86	21.33	17.15	18.42	1.50	2.10	2.04	2.22
12	20.86	20.95	16.56	17.59	1.73	2.42	2.04	2.58
14	19.92	20.25	16.00	17.45	2.11	2.84	2.53	3.07
16	19.88	19.15	15.57	17.05	2.29	3.31	2.62	3.00
18	19.06	19.01	15.16	16.98	2.30	3.70	3.06	3.30
20	18.24	18.05	15.05	16.91	2.39	4.10	3.27	3.56
24	16.58	17.12	14.74	16.74	2.41	4.70	2.82	3.83

Table 6.5D : The degradation rate constant values for degradation of rifampicin in unbuffered solutions

pH	25 mcg/ml		1000 mcg/ml
	K1b First 6 h	K1c 12 - 24 h	K1a First 6 h
2.0	0.14387	0.086224	0.03779
3.0	0.05744	0.03222	0.027014
4.0	0.01122	0.01951	0.013956
5.0	0.02909	0.01276	0.01474
6.0	0.03692	0.018424	0.024135
7.0	0.03176	0.016858	0.026853
8.0	0.04143	0.009558	0.019506
9.0	0.03632	0.004168	0.019806

Table 6.6A : Stability of rifampicin in 25 mcg/ml concentration buffered solutions at pH 3.0, 4.0, 5.0 and 6.0

Time (h)	Conc. of rifampicin (mcg/ml)				Conc. of 3-formyl rifamycin SV (mcg/ml)			
	pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 3.0	pH 4.0	pH 5.0	pH 6.0
0	25.00	26.20	26.40	25.90	0.00	1.20	0.42	2.82
1	20.18	23.20	25.34	23.60	1.00	2.09	0.73	13.58
2	16.90	20.62	25.31	20.85	1.84	3.60	1.06	17.83
3	13.82	17.82	24.00	19.29	2.70	5.34	1.45	22.09
4	11.88	16.03	22.96	17.28	4.04	7.07	1.65	24.94
5	10.03	14.47	22.50	15.29	4.54	8.22	1.87	25.62
6	8.44	13.06	21.70	13.79	5.43	10.15	2.04	26.45
8	7.22	13.11	21.10	12.07	6.73	13.85	2.47	27.27
10	6.42	12.09	20.74	11.00	7.28	15.28	3.22	28.41
12	5.98	11.27	20.24	10.36	9.20	14.40	4.08	30.46
14	5.14	10.82	20.11	10.30	10.63	12.84	5.09	35.10
16	4.80	9.77	19.06	10.02	11.17	12.05	5.20	39.44
18	4.42	9.36	18.83	9.75	11.40	10.62	5.63	43.26
20	4.07	8.75	18.30	8.94	11.61	10.45	6.20	45.23
24	3.22	7.70	16.98	6.93	11.94	9.06	5.95	35.79

Table 6.6B : The degradation rate constant values of rifampicin in buffered solutions

pH	Buffer	K-VALUE	
		K1b First 6 h	K1c 12 - 24 h
3.0	Chloroacetate	0.17890	0.04882
4.0	Acetate	0.11732	0.03227
5.0	Formate	0.03298	0.01658
6.0	Phosphate	0.10525	0.03268

Table 6.7A : Percent of rifampicin released form product C in 0.4 % w/v sodium lauryl sulphate solution as dissolution medium

Time (min)	Rifampicin released (%)
0	0.0
10	18.14 ± 1.49
20	47.78 ± 1.74
30	62.08 ± 2.05
45	87.13 ± 1.92
60	94.21 ± 2.10
120	97.84 ± 2.37

Table 6.7B : Serum concentration and pharmacokinetic parameters of rifampicin from Product A (rifampicin 450 mg and isoniazid 300 mg)

Time (h)	Serum concentration (mcg/ml) in volunteer								
	1	2	3	4	5	6	Mean	Std.Dev	Std.Err
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	13.595	2.640	13.170	4.310	3.960	7.060	7.456	4.813	1.965
1.50	8.970	5.120	11.612	12.410	6.010	11.390	9.252	3.091	1.262
3.00	7.780	4.790	10.270	11.246	5.610	9.790	8.248	2.630	1.074
6.00	6.420	3.390	6.430	5.890	4.530	4.520	5.197	1.238	0.505
9.00	3.570	1.630	4.760	4.660	2.410	2.480	3.252	1.288	0.526
12.00	2.240	0.890	2.640	1.500	1.960	2.280	1.918	0.631	0.258
AUC 0-inf	88.075	39.672	102.292	83.099	62.888	78.638	75.777	21.844	8.918
Cmax	13.595	5.120	13.170	12.410	6.010	11.390	10.283	3.741	1.527
Tmax	0.750	1.500	0.750	1.500	1.500	1.500	1.250	0.387	0.158

Units : AUCo-inf (mcg.h/ml), Cmax (mcg/ml), Tmax (h) and t1/2 (h).

Table 6.7C : Serum concentration and pharmacokinetic parameters of isoniazid from Product A (rifampicin 450 mg and isoniazid 300 mg)

Time (h)	Serum concentration (mcg/ml) in volunteer								
	1	2	3	4	5	6	Mean	Std.Dev	Std.Err
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	8.739	9.068	9.513	8.934	8.893	8.691	8.973	0.298	0.121
1.50	11.581	12.282	10.853	8.746	10.389	10.433	10.714	1.207	0.493
3.00	7.070	8.010	6.133	5.952	7.084	7.013	6.877	0.747	0.305
6.00	3.751	4.718	3.329	2.363	3.068	3.621	3.475	0.783	0.320
9.00	1.953	2.332	2.889	1.887	1.948	1.993	2.167	0.388	0.158
12.00	1.126	1.107	1.689	1.283	0.963	1.302	1.245	0.251	0.102
AUC 0-inf	59.807	66.486	66.119	51.625	55.132	59.789	59.826	5.886	2.403
Cmax	11.581	12.282	10.853	8.934	10.389	10.433	10.745	1.147	0.468
Tmax	1.500	1.500	1.500	0.750	1.500	1.500	1.375	0.306	0.125

Units : AUCo-inf (mcg.h/ml), Cmax (mcg/ml), Tmax (h) and t1/2 (h).

Table 6.7D : Serum concentration and pharmacokinetic parameters of rifampicin from Product B (rifampicin 450 mg and isoniazid 300 mg)

Time (h)	Serum concentration (mcg/ml) in volunteer								
	1	2	3	4	5	6	Mean	Std.Dev	Std.Err
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	9.525	5.611	9.556	4.110	4.960	5.160	6.487	2.415	0.986
1.50	8.810	5.672	11.106	12.220	6.410	10.490	9.118	2.637	1.076
3.00	7.595	5.607	9.085	9.825	5.425	9.605	7.857	1.974	0.806
6.00	6.149	3.119	6.159	5.619	4.259	4.249	4.926	1.238	0.505
9.00	3.296	1.356	4.438	4.436	2.136	2.206	2.978	1.288	0.526
12.00	1.798	1.290	1.870	1.949	1.821	1.940	1.778	0.247	0.101
AUC 0-inf	77.055	46.466	85.346	82.869	60.067	70.473	70.379	14.851	6.063
Cmax	9.525	5.672	11.106	12.220	6.410	10.490	9.237	2.636	1.076
Tmax	0.750	1.500	1.500	1.500	1.500	1.500	1.375	0.306	0.125

Units : AUCo-inf (mcg.h/ml), Cmax (mcg/ml), Tmax (h) and t1/2 (h).

Table 6.7E : Serum concentration and pharmacokinetic parameters of isoniazid from Product B (rifampicin 450 mg and isoniazid 300 mg)

Time (h)	Serum concentration (mcg/ml) in volunteer								
	1	2	3	4	5	6	Mean	Std.Dev	Std.Err
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	7.549	9.321	7.539	7.438	7.389	8.122	7.893	0.748	0.305
1.50	10.532	11.422	9.668	9.659	9.132	9.431	9.974	0.849	0.347
3.00	7.432	8.934	7.889	7.934	7.649	6.164	7.667	0.899	0.367
6.00	4.821	4.928	4.849	3.906	3.984	3.516	4.334	0.605	0.247
9.00	1.993	2.434	2.622	2.061	2.163	2.113	2.231	0.244	0.100
12.00	1.346	1.440	1.686	1.392	1.386	1.486	1.456	0.123	0.050
AUC 0-inf	63.783	71.289	69.056	61.484	60.848	59.087	64.258	4.873	1.990
Cmax	10.532	11.422	9.668	9.659	9.132	9.431	9.974	0.849	0.347
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Units : AUCo-inf (mcg.h/ml), Cmax (mcg/ml), Tmax (h) and t1/2 (h).

Table 6.7F : Serum concentration and pharmacokinetic parameters of rifampicin from Product C (rifampicin 450 mg)

Time (h)	Serum concentration (mcg/ml) in volunteer								
	1	2	3	4	5	6	Mean	Std.Dev	Std.Err
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	11.600	2.770	3.930	10.760	5.710	10.910	7.613	3.932	1.605
1.50	9.760	7.010	7.690	10.410	11.940	13.020	9.972	2.340	0.955
3.00	7.280	5.020	10.750	7.510	6.920	11.530	8.168	2.476	1.011
6.00	4.510	1.850	5.990	5.340	4.960	8.840	5.248	2.264	0.924
9.00	2.370	1.740	5.230	4.800	3.820	4.720	3.780	1.427	0.582
12.00	1.310	0.280	2.250	1.790	2.810	5.280	2.287	1.701	0.694
AUC 0-inf	65.514	33.322	86.609	80.459	91.902	158.216	86.004	41.172	16.808
Cmax	11.600	7.010	10.750	10.760	11.940	13.020	10.847	2.060	0.841
Tmax	0.750	1.500	3.000	0.750	1.500	1.500	1.500	0.822	0.335

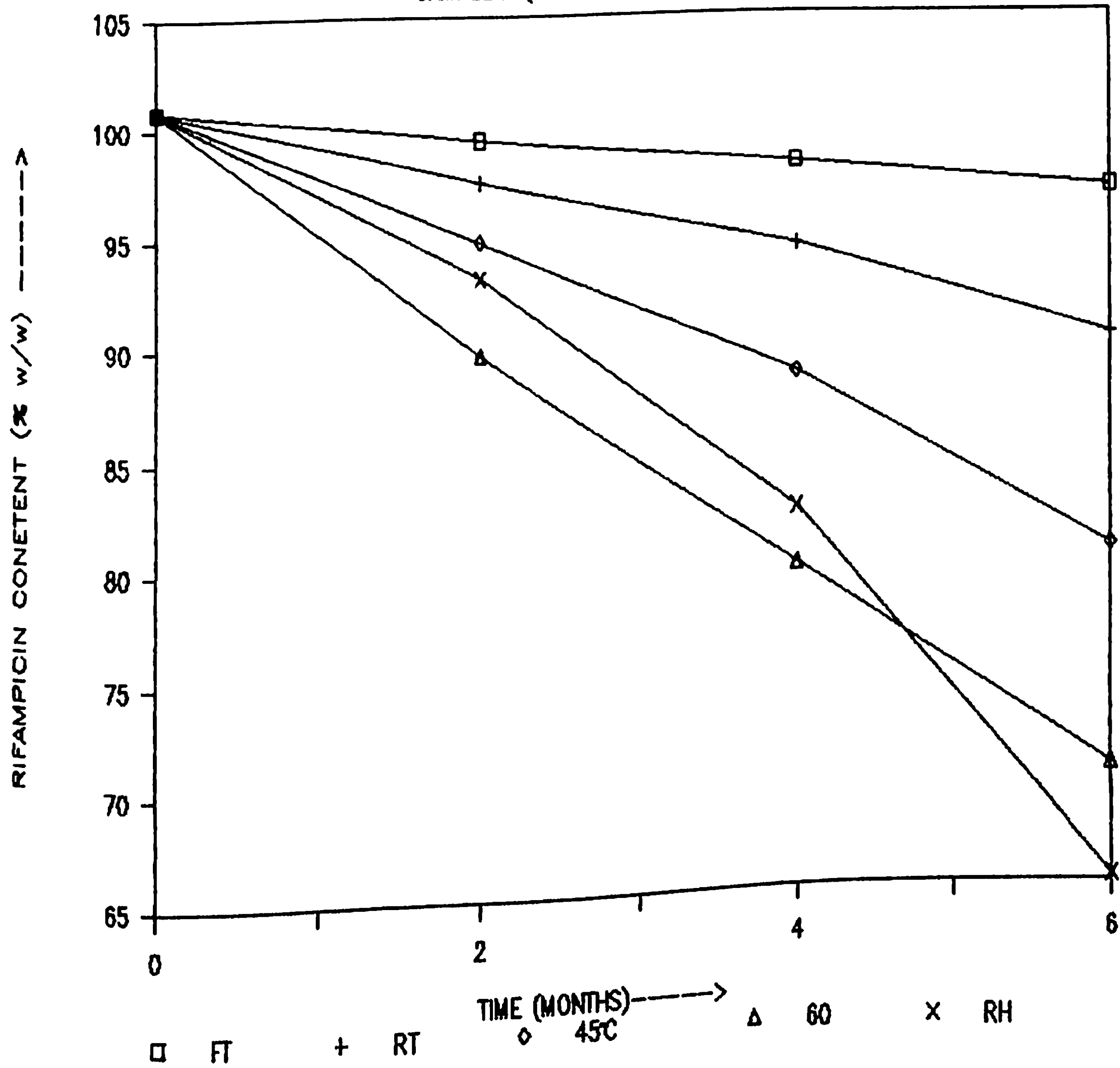
Units : AUCo-inf (mcg.h/ml), Cmax (mcg/ml), Tmax (h) and t1/2 (h).

Table 6.7G : Summary of pharmacokinetic and dissolution test parameters of rifampicin and isoniazid for Products A, B and C

Drug	Parameter	Product A	Product B	Product C
Rifampicin	DP30 (%)	60.31	41.66	62.08
	DE30	31.02	20.62	32.32
	AUC (mcg h/ml)	75.777	70.379	86.004
	Cmax (mcg/ml)	10.283	9.237	10.847
	Tmax (h)	1.250	1.375	1.50
Isoniazid	DP30 (%)	96.63	63.39	-
	DE30	79.68	40.10	-
	AUC (mcg h/ml)	59.826	64.258	-
	Cmax (mcg/ml)	10.745	9.974	-
	Tmax	1.375	1.500	-

DP30 and DE30 are dissolution percentage and dissolution efficiency over first 30 minutes respectively.

FIG. 6.1A : RIFAMPICIN ASSAY



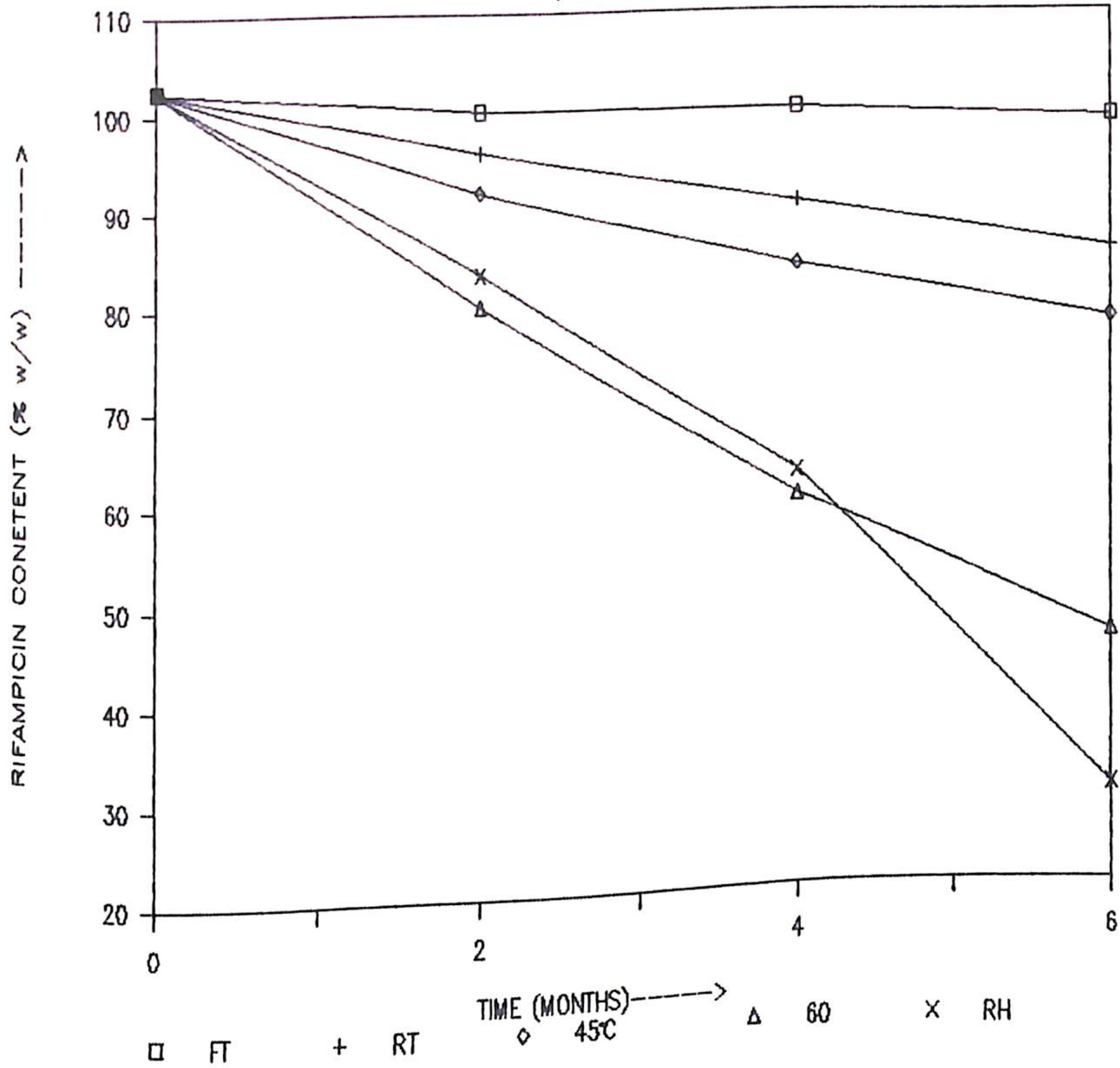


FIG. 6.1C : RIFAMPICIN ASSAY
SAMPLE C (RIFAMPICIN + UREA)
RIFAMPICIN CONETENT (% w/w) ------>
110
100
90
80
70
60
50
40
30
20
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

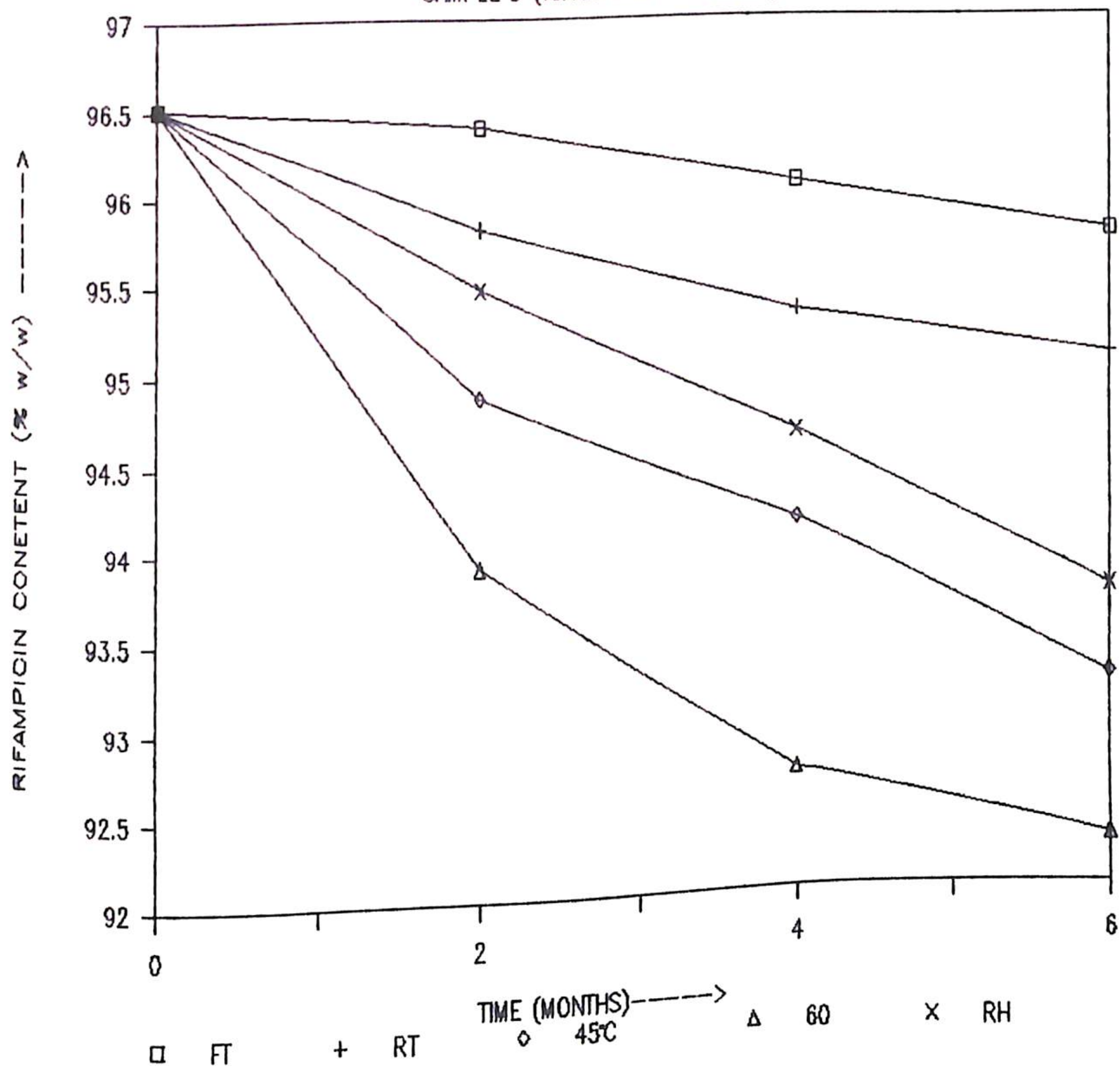


FIG. 6.1D : RIFAMPICIN ASSAY
SAMPLE D (RIFAMPICIN + LACTOSE)
RIFAMPICIN CONETENT (% w/w) ------->
97
96.5
96
95.5
95
94.5
94
93.5
93
92.5
92
0
2
4
6
TIME (MONTHS)------->
FT
RT
45°C
60
RH

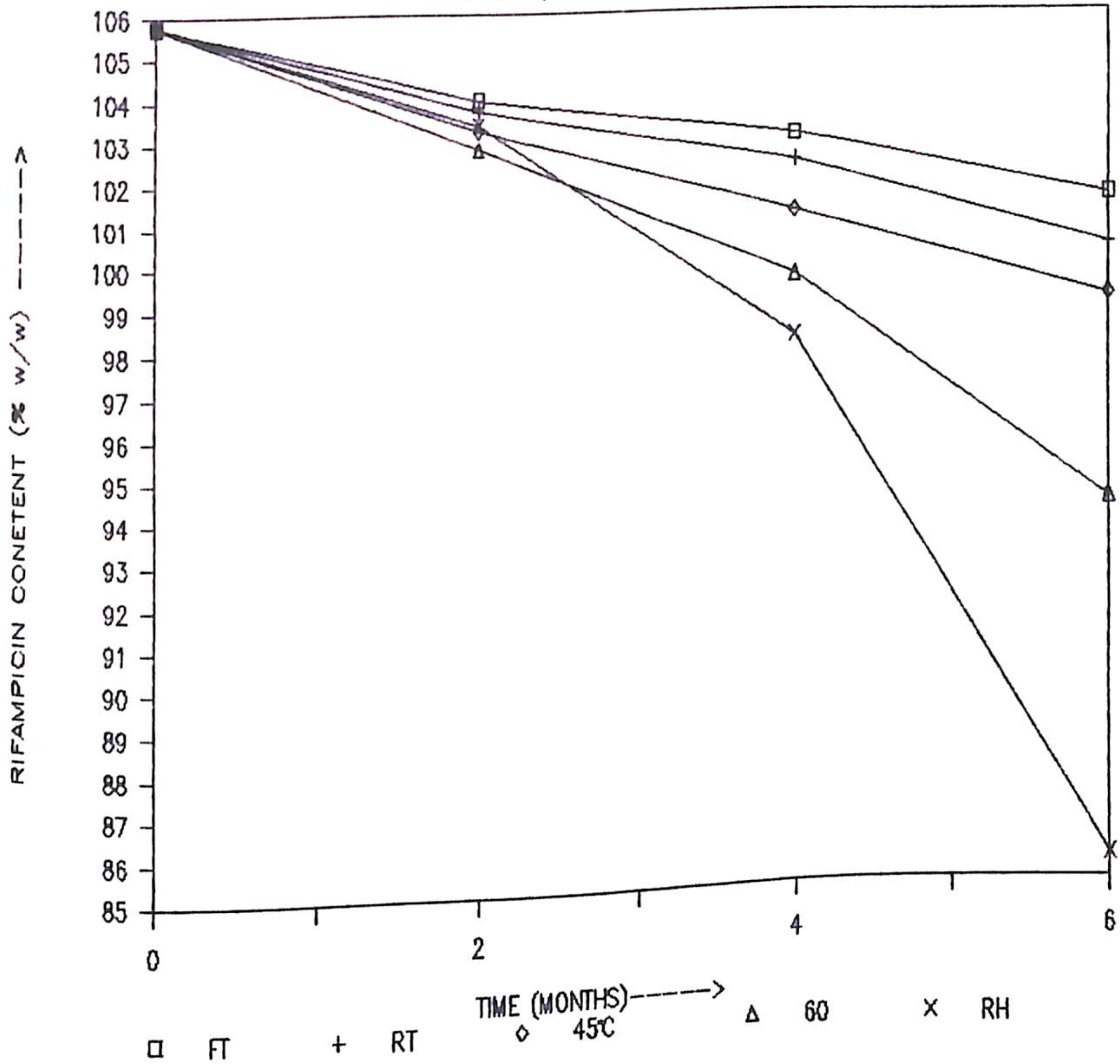


FIG. 6.1E : RIFAMPICIN ASSAY
SAMPLE E (RIFAMPICIN + STARCH)
RIFAMPICIN CONETENT (% w/w) ------>
106
105
104
103
102
101
100
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

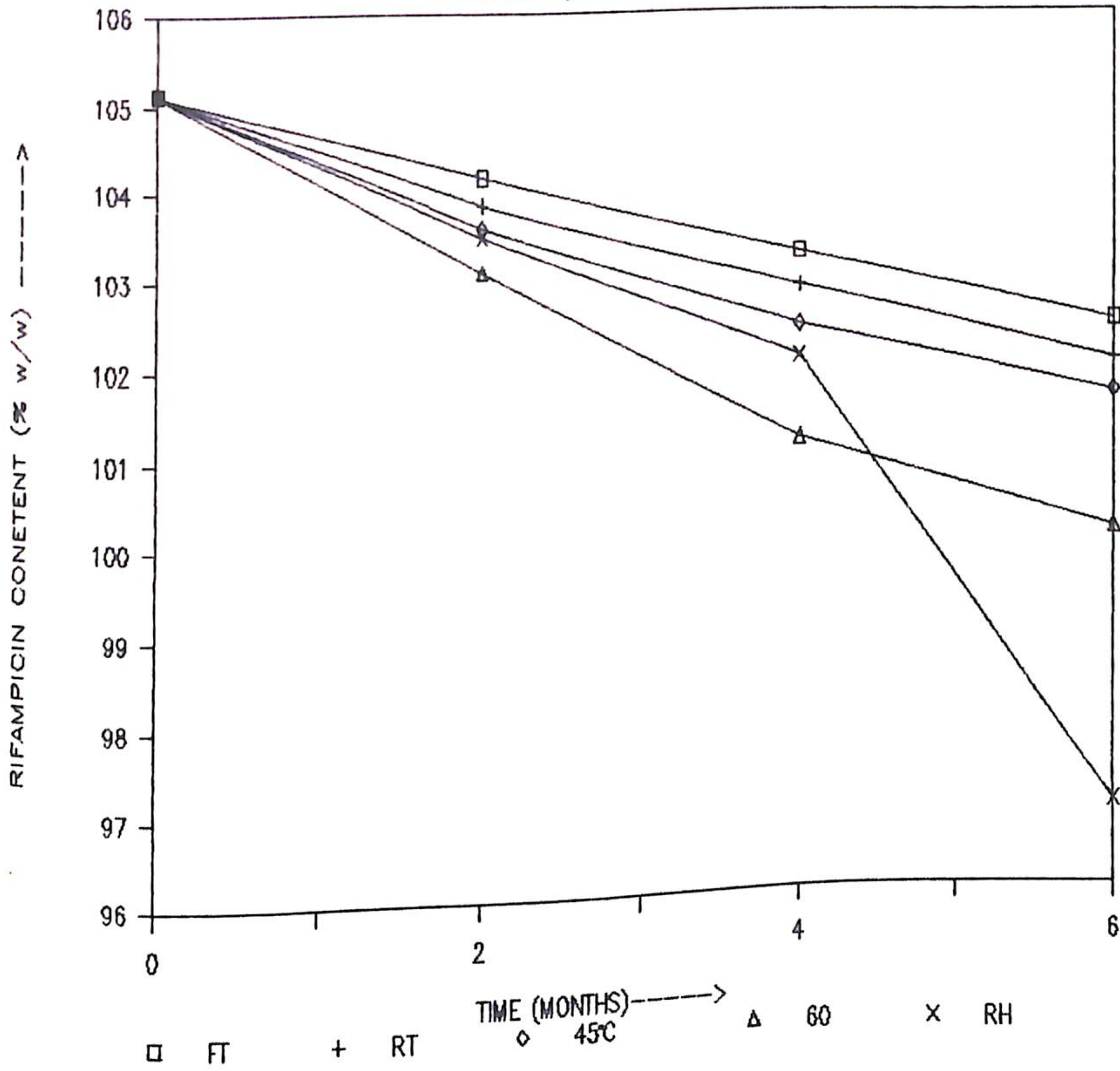


FIG. 6.1F : RIFAMPICIN ASSAY
SAMPLE F (RIFAMPICIN + SLS)
RIFAMPICIN CONETENT (% w/w) ------->
106
105
104
103
102
101
100
99
98
97
96
0
2
4
6
TIME (MONTHS)------->
FT
RT
45°C
60
RH

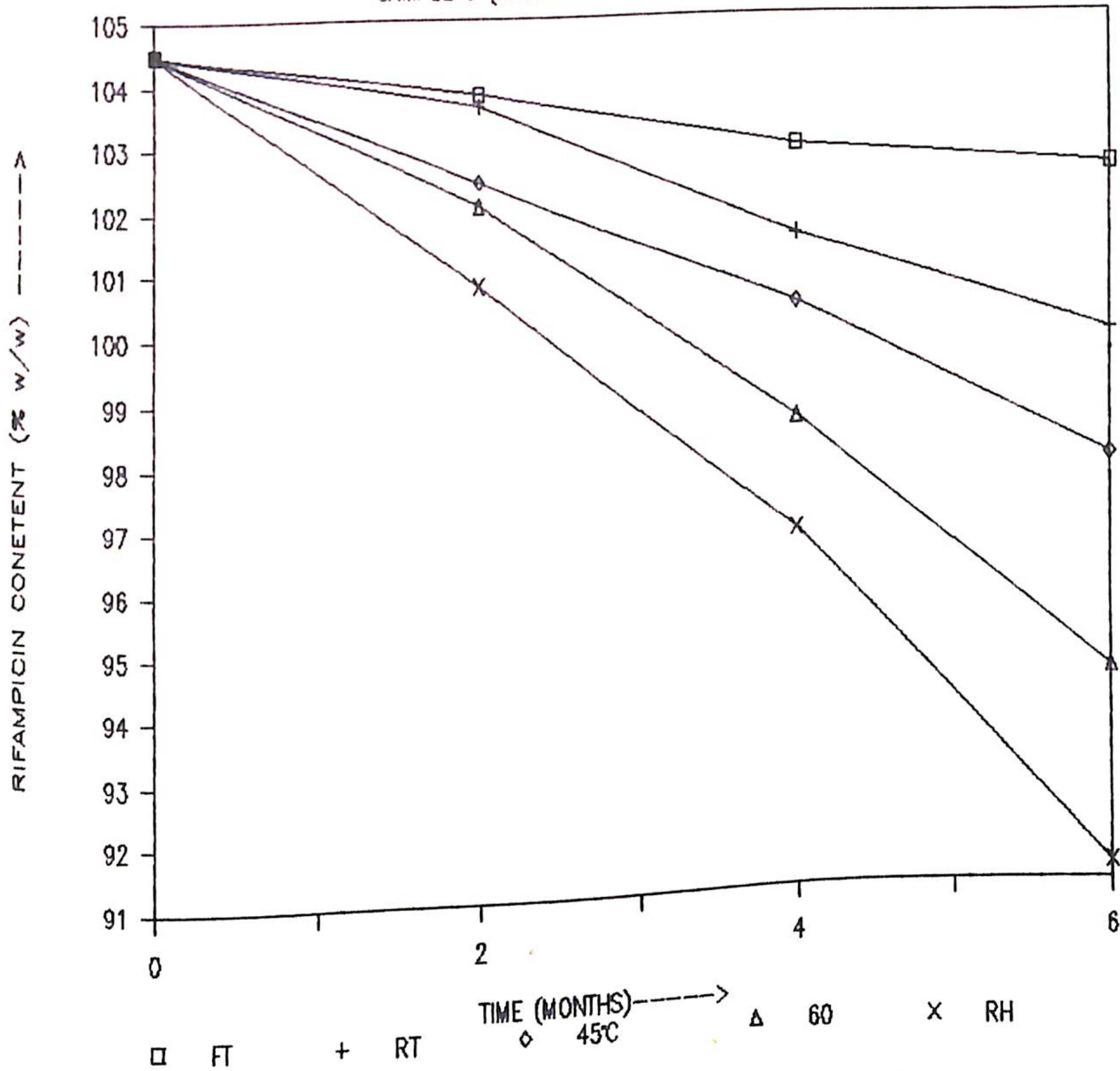


FIG. 6.1G : RIFAMPICIN ASSAY
SAMPLE G (RIFAMPICIN + POLYSORBATE 80)
RIFAMPICIN CONETENT (% w/w) ------>
105
104
103
102
101
100
99
98
97
96
95
94
93
92
91
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

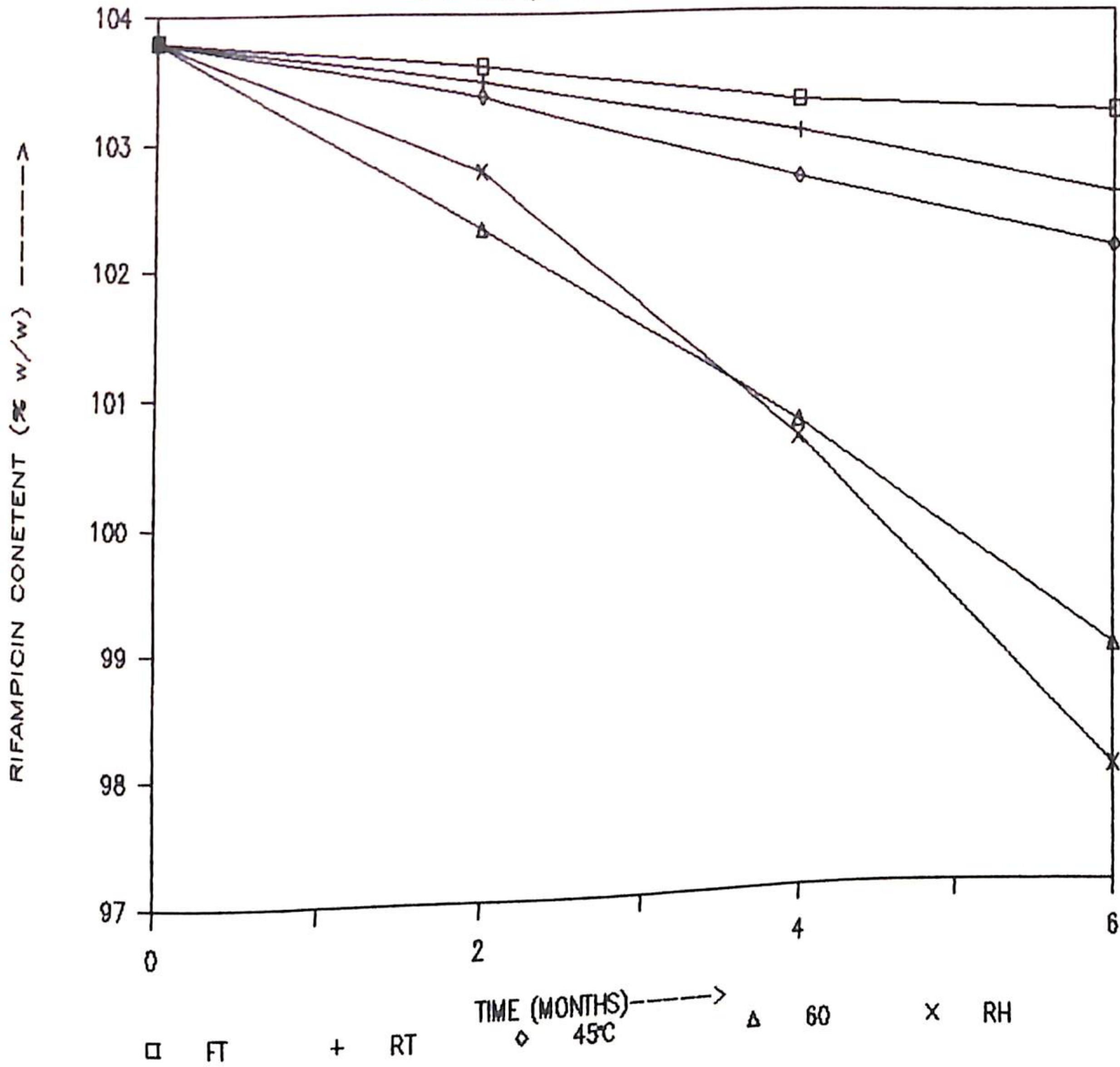


FIG. 6.1H : RIFAMPICIN ASSAY
SAMPLE H (RIFAMPICIN + BILE SALTS)
104
103
102
101
100
99
98
97
RIFAMPICIN CONETENT (% w/w) ------->
0
2
4
6
TIME (MONTHS)------->
□ FT
+ RT
◇ 45°C
Δ 60
X RH

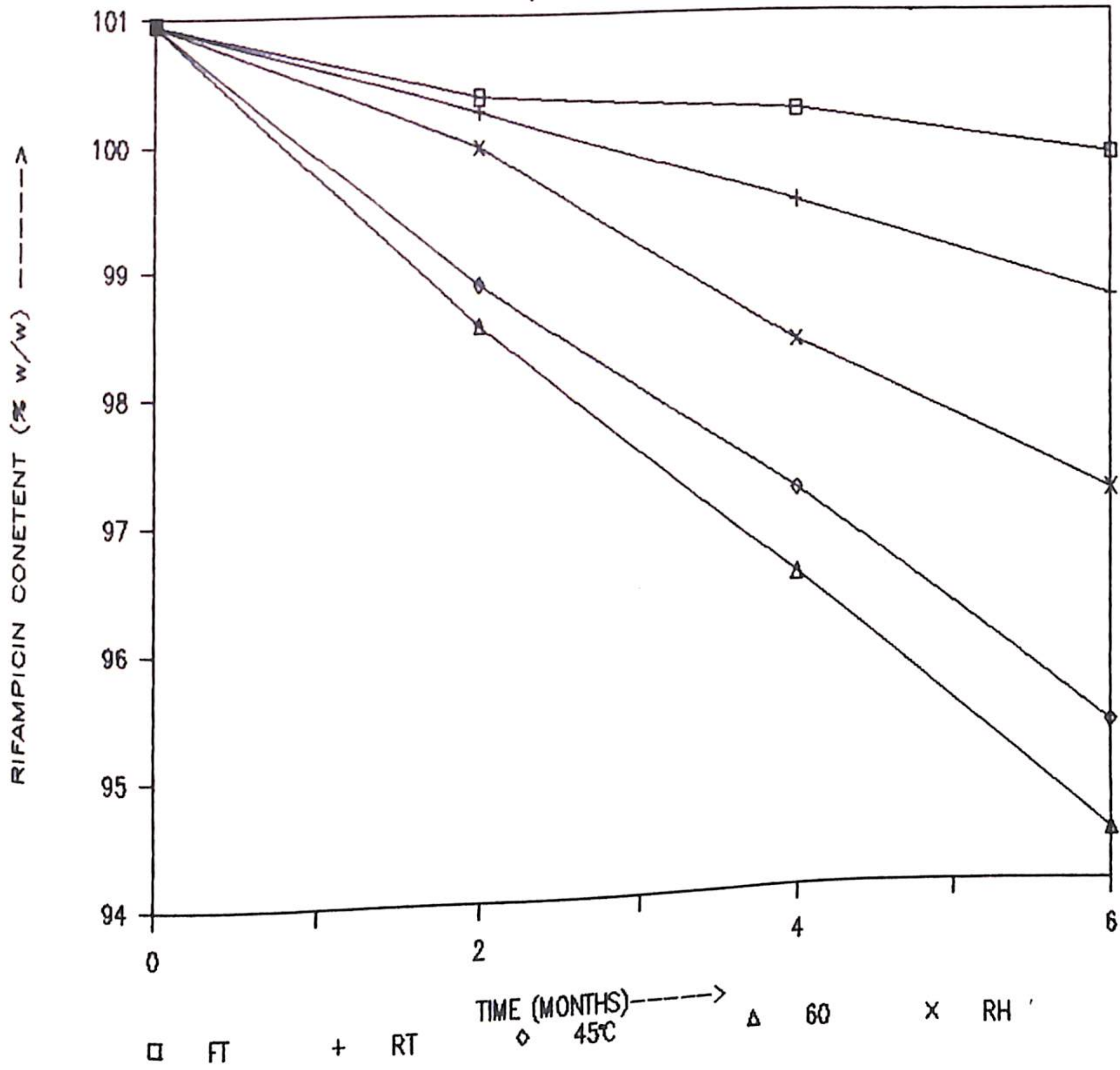


FIG. 6.1I : RIFAMPICIN ASSAY
SAMPLE I (RIFAMPICIN + MANNITOL)
RIFAMPICIN CONETENT (% w/w) ------>
101
100
99
98
97
96
95
94
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

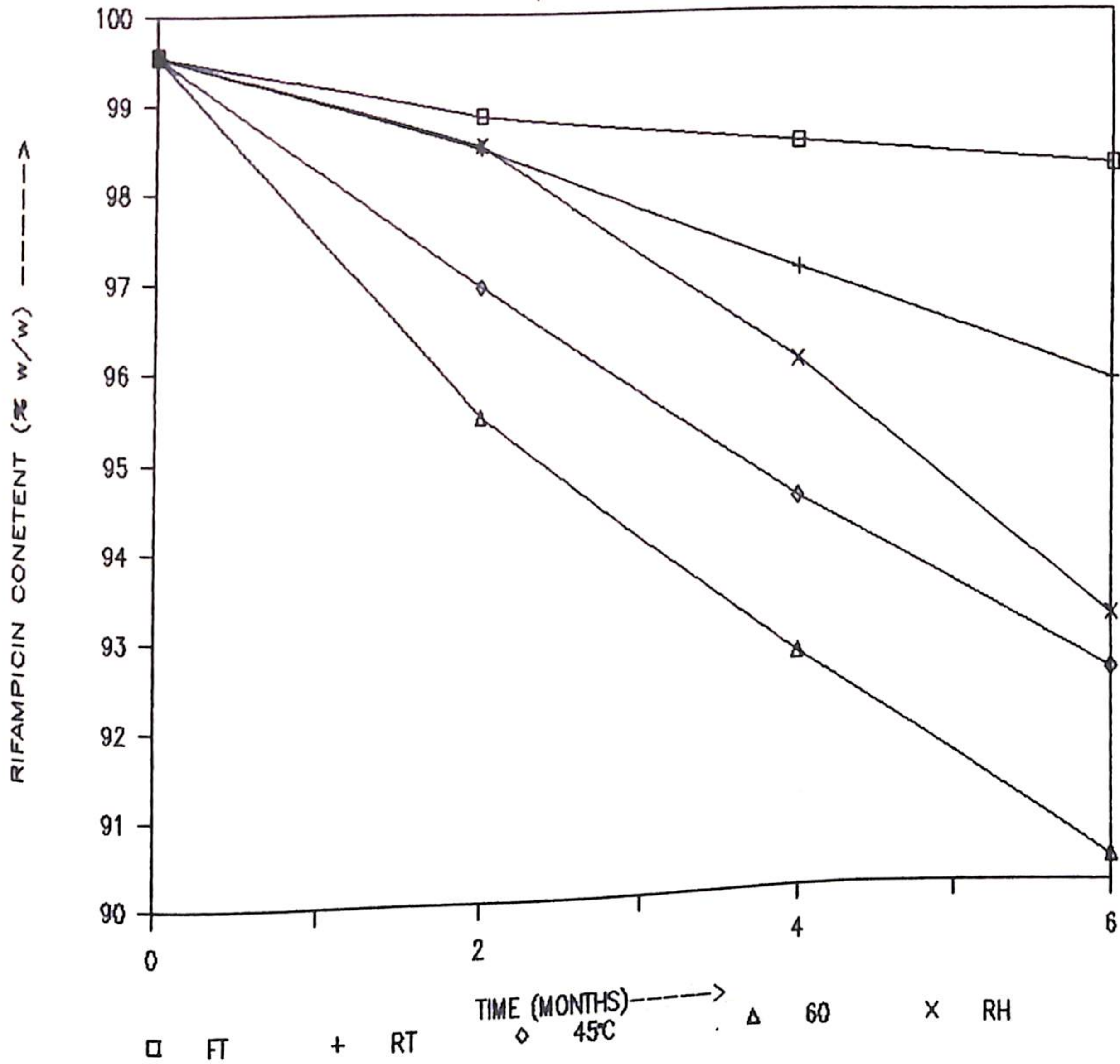


FIG. 6.1J : RIFAMPICIN ASSAY
SAMPLE J (RIFAMPICIN + SUCROSE)
RIFAMPICIN CONETENT (% w/w) ------->
100
99
98
97
96
95
94
93
92
91
90
0
2
4
6
TIME (MONTHS)------->
□ FT
+ RT
◇ 45°C
Δ 60
× RH

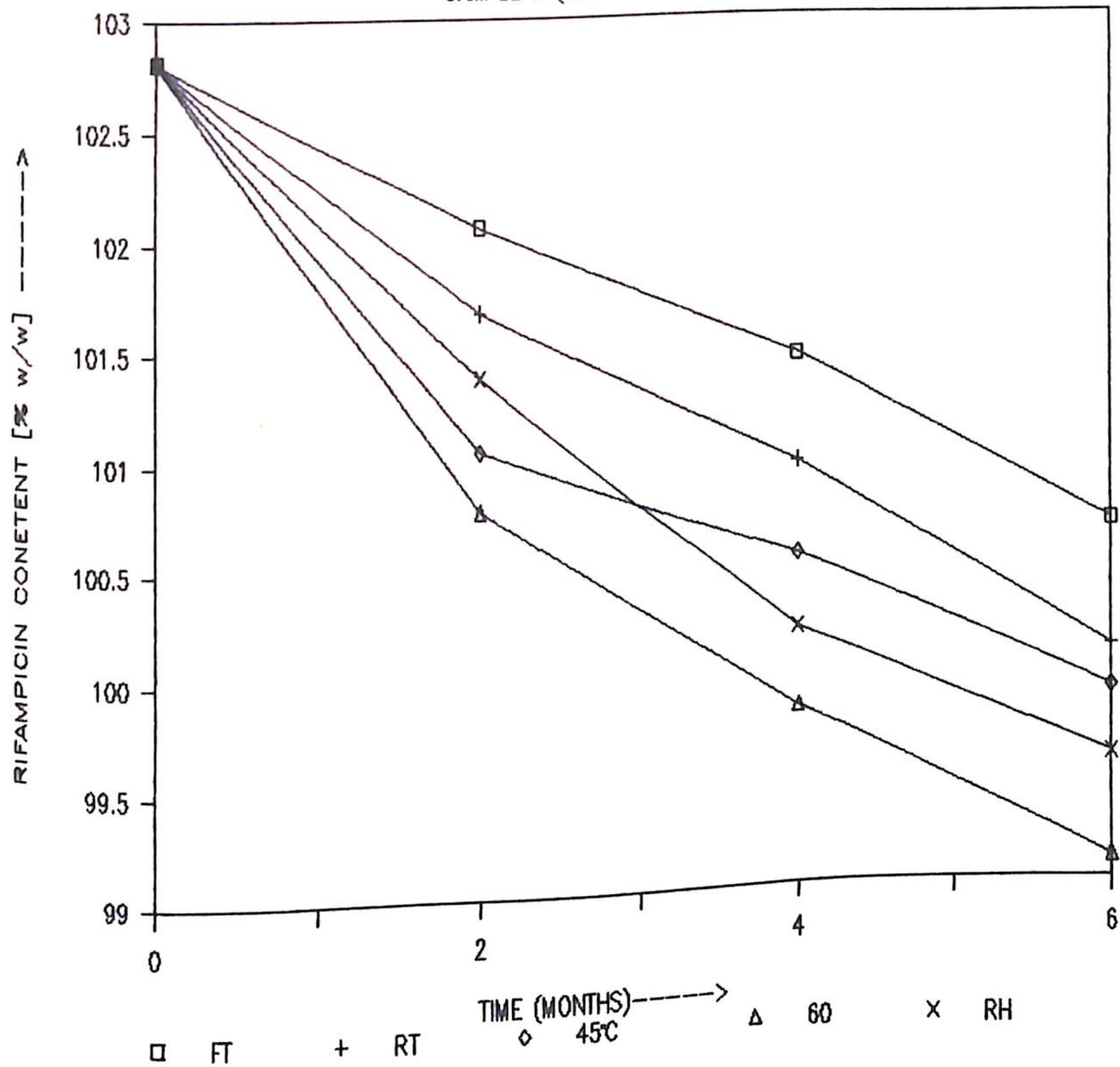


FIG. 6.1K : RIFAMPICIN ASSAY
SAMPLE K (RIFAMPICIN + MCC)
RIFAMPICIN CONETENT [% w/w] ------->
103
102.5
102
101.5
101
100.5
100
99.5
99
0
2
4
6
TIME (MONTHS)------->
FT
RT
45°C
60
RH

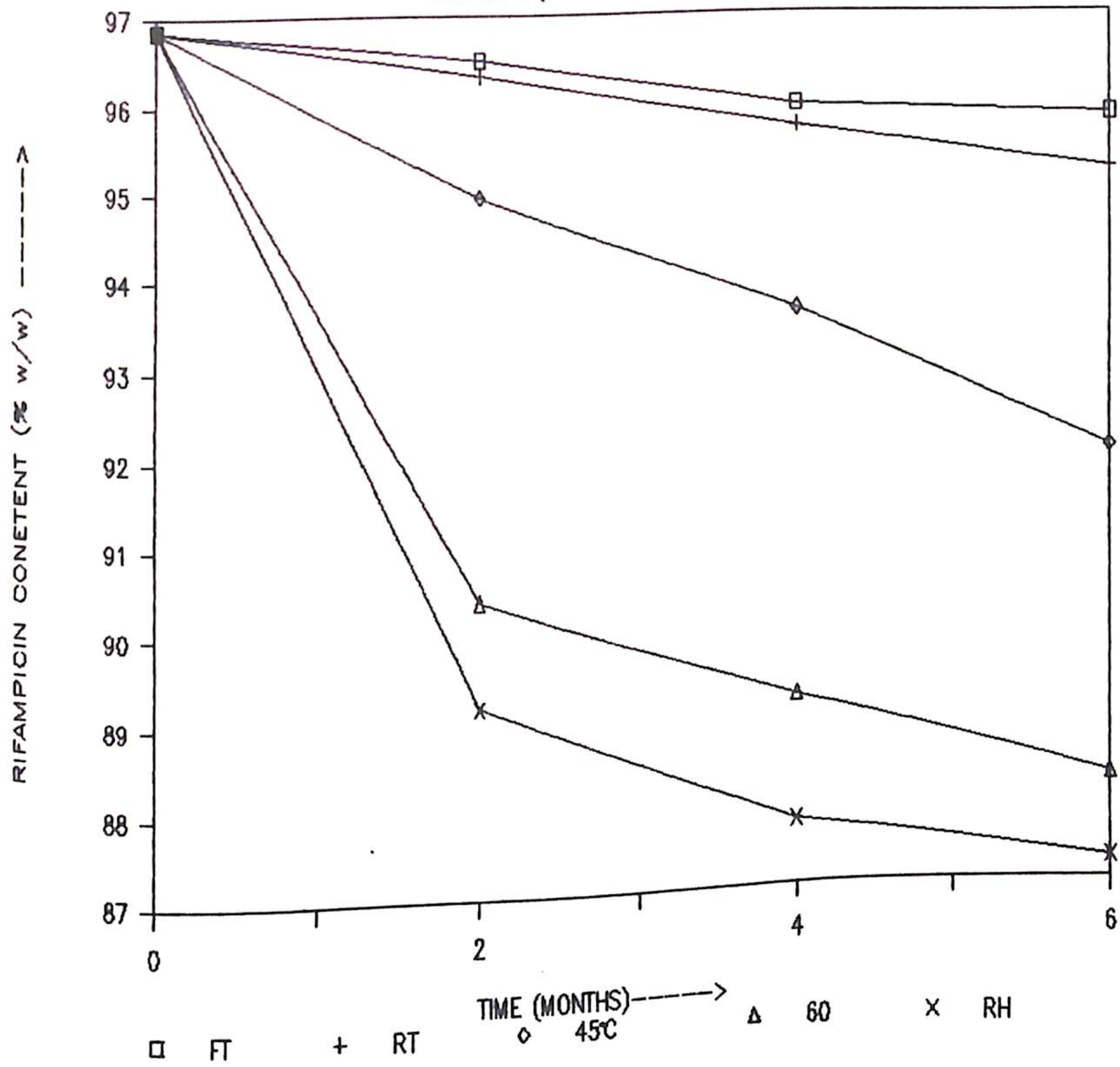


FIG. 6.1L : RIFAMPICIN ASSAY
SAMPLE L (RIFAMPICIN + GELATIN)
RIFAMPICIN CONETENT (% w/w) ------->
97
96
95
94
93
92
91
90
89
88
87
0
2
4
6
TIME (MONTHS)------->
FT
RT
45°C
60
RH

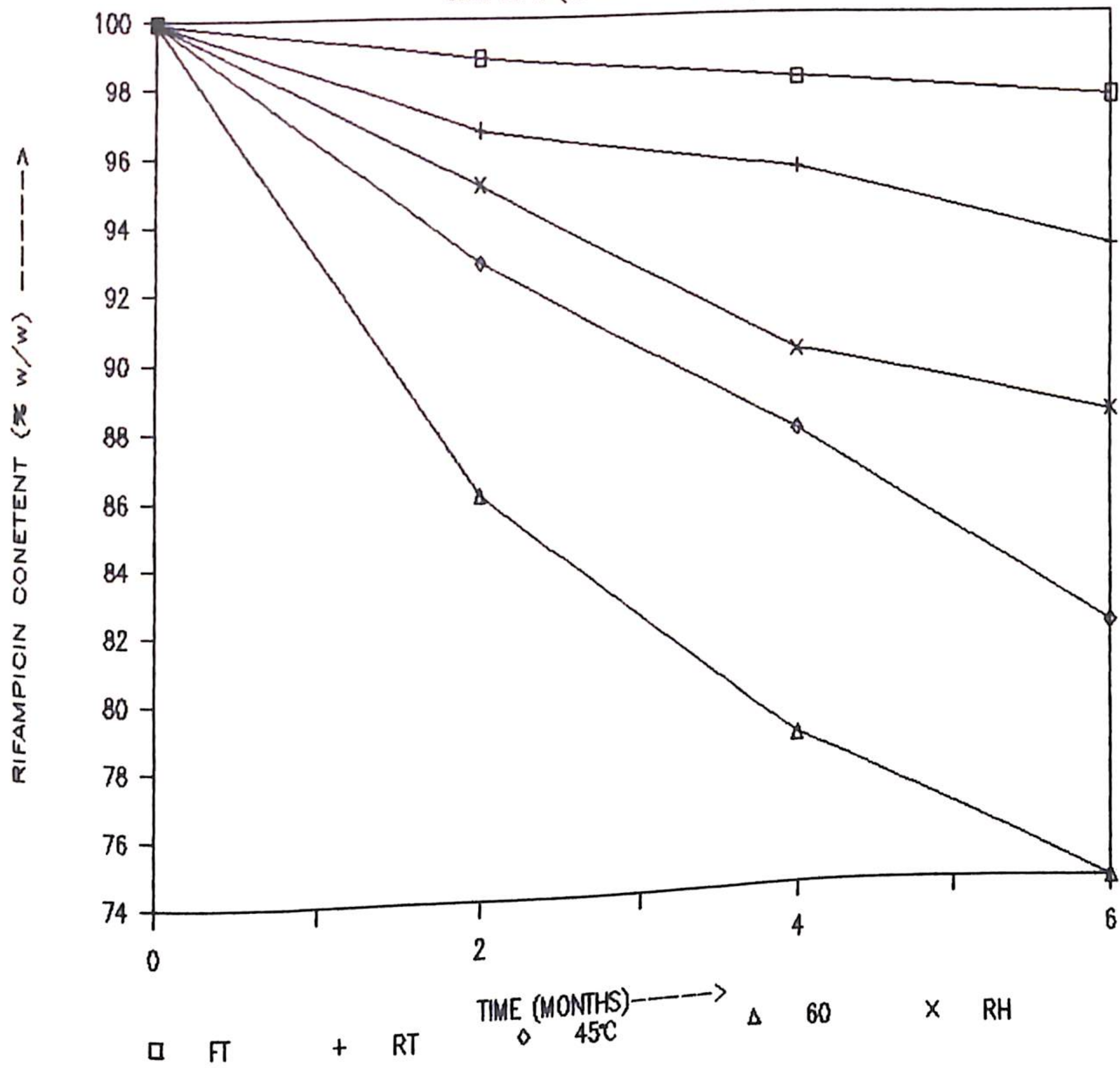


FIG. 6.1M : RIFAMPICIN ASSAY
SAMPLE M (RIFAMPICIN + DCP)
RIFAMPICIN CONETENT (% w/w) ------>
100
98
96
94
92
90
88
86
84
82
80
78
76
74
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

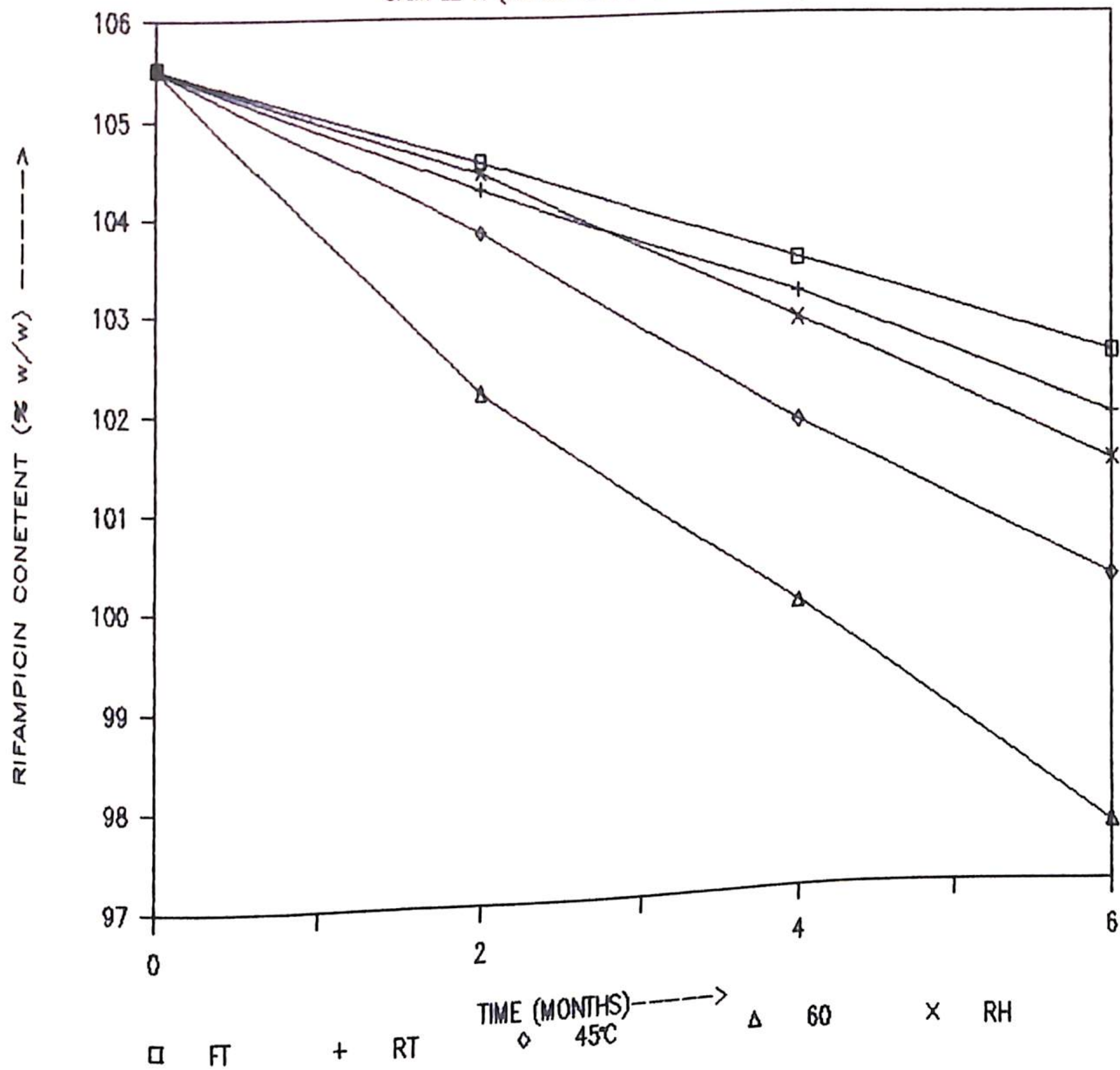


FIG. 6.1N : RIFAMPICIN ASSAY
SAMPLE N (RIFAMPICIN + MAG. STEARATE)
RIFAMPICIN CONETENT (% w/w) ------>
106
105
104
103
102
101
100
99
98
97
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

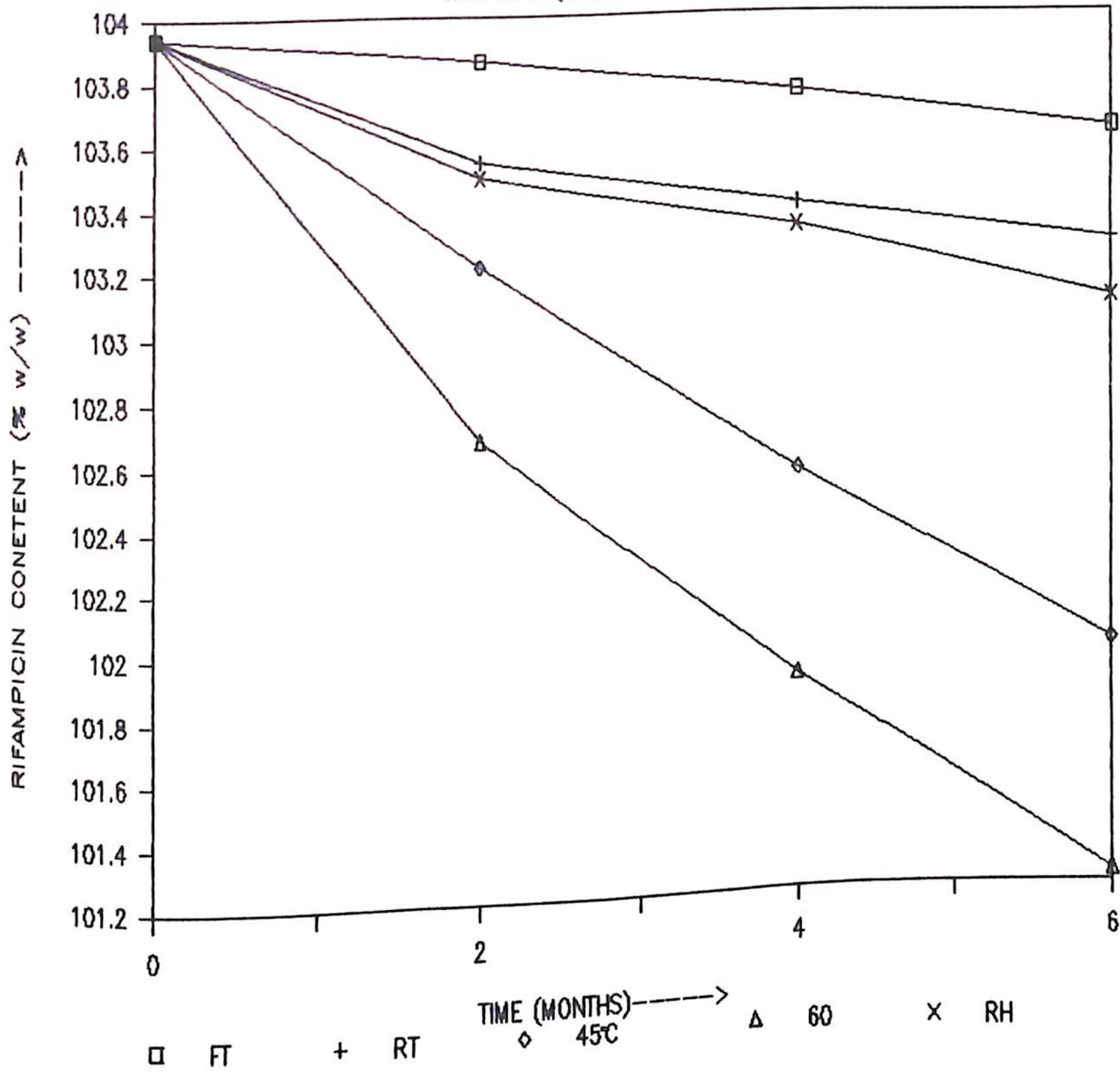


FIG. 6.10 : RIFAMPICIN ASSAY
SAMPLE 0 (RIFAMPICIN + TALC)
RIFAMPICIN CONETENT (% w/w) ------>
104
103.8
103.6
103.4
103.2
103
102.8
102.6
102.4
102.2
102
101.8
101.6
101.4
101.2
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

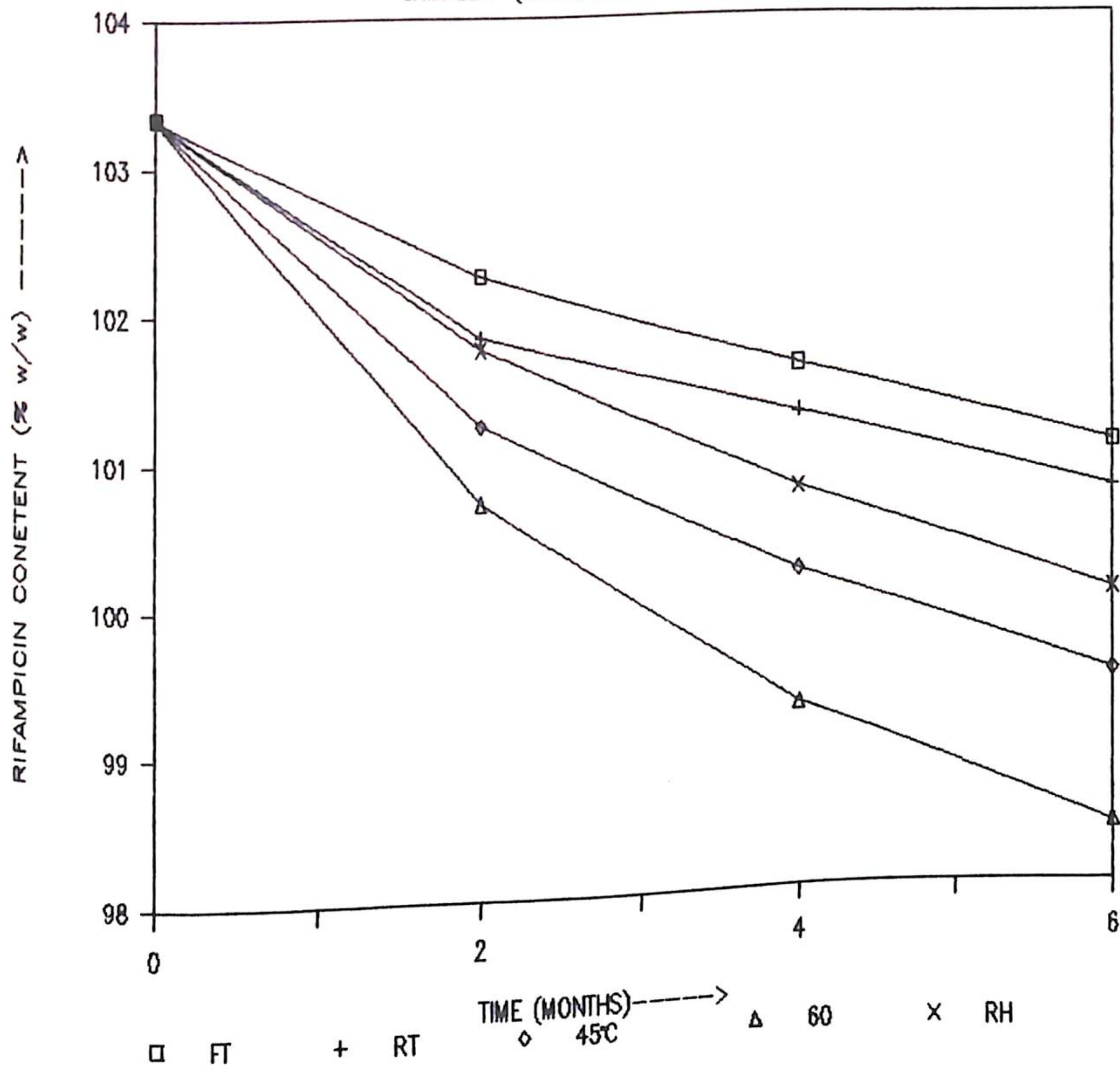


FIG. 6.1P : RIFAMPICIN ASSAY
SAMPLE P (RIFAMPICIN + AEROSIL 200)
RIFAMPICIN CONETENT (% w/w) ------->
104
103
102
101
100
99
98
0
2
4
6
TIME (MONTHS)------->
FT
+ RT
45°C
60
RH

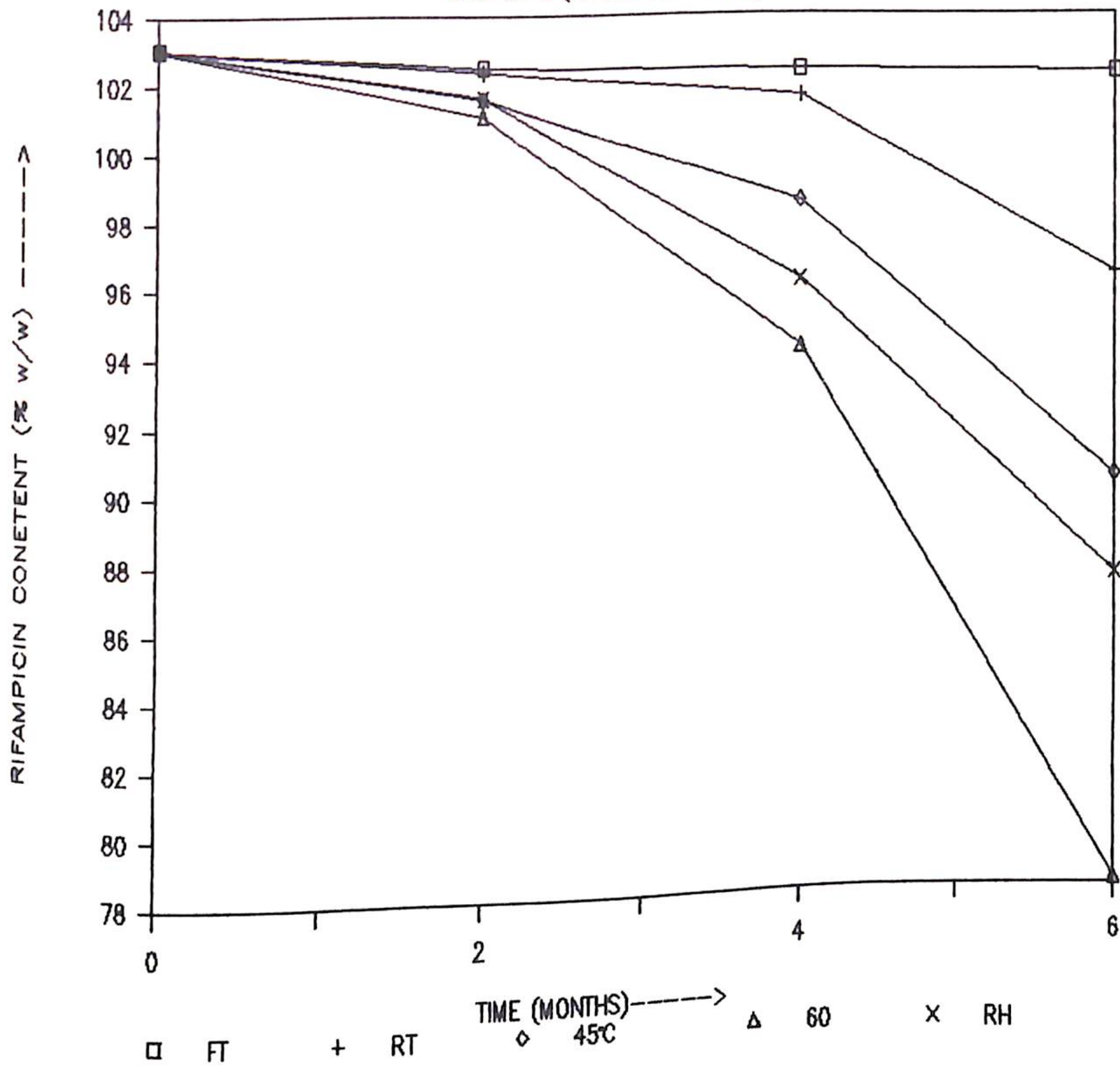


FIG. 6.1Q : RIFAMPICIN ASSAY
SAMPLE Q (RIFAMPICIN + SSG)
RIFAMPICIN CONETENT (% w/w) ------>
104
102
100
98
96
94
92
90
88
86
84
82
80
78
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

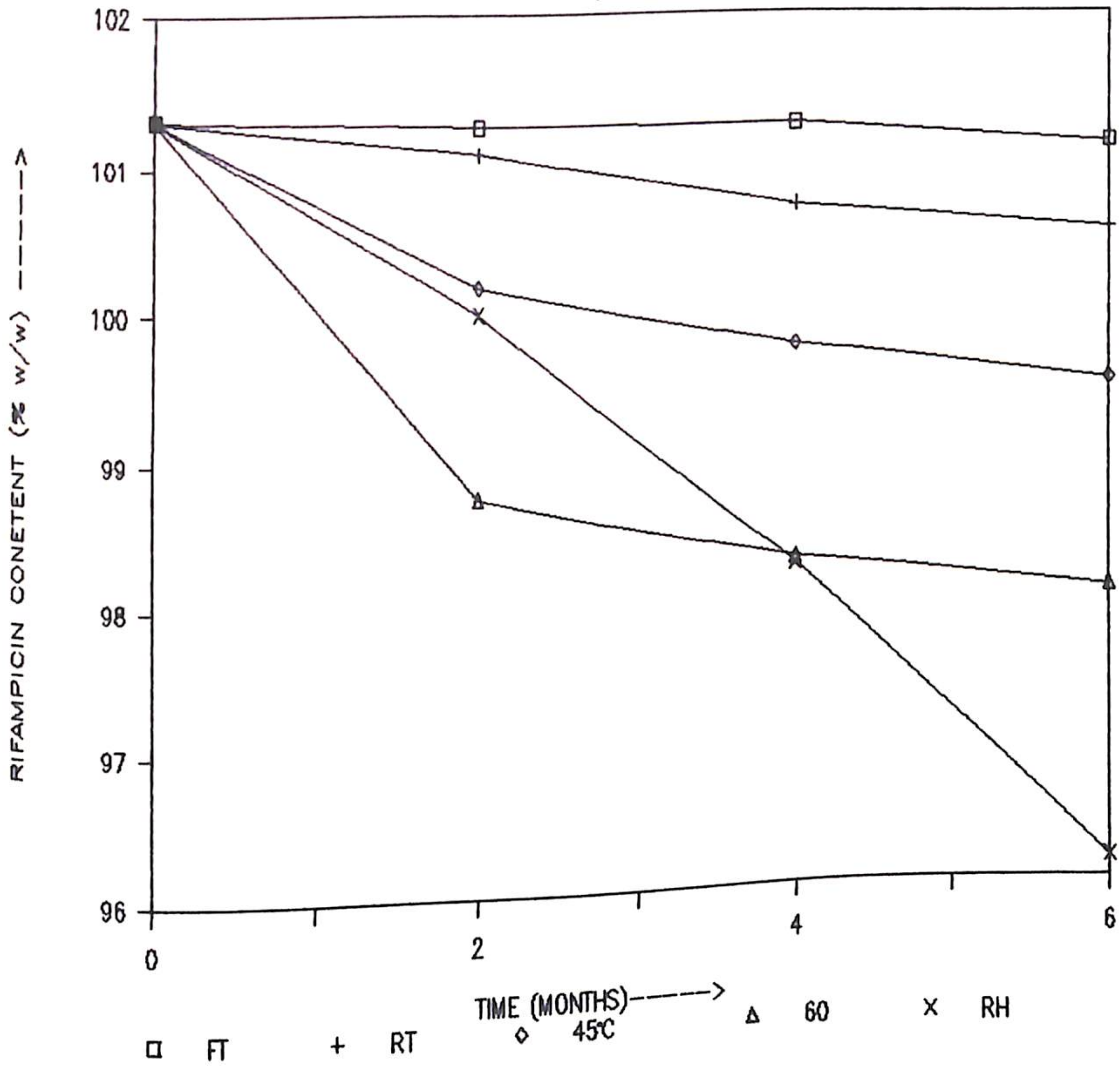


FIG. 6.1R : RIFAMPICIN ASSAY
SAMPLE R (RIFAMPICIN)
RIFAMPICIN CONETENT (% w/w) ------->
102
101
100
99
98
97
96
0
2
4
6
TIME (MONTHS)------->
FT
RT
45°C
60
RH

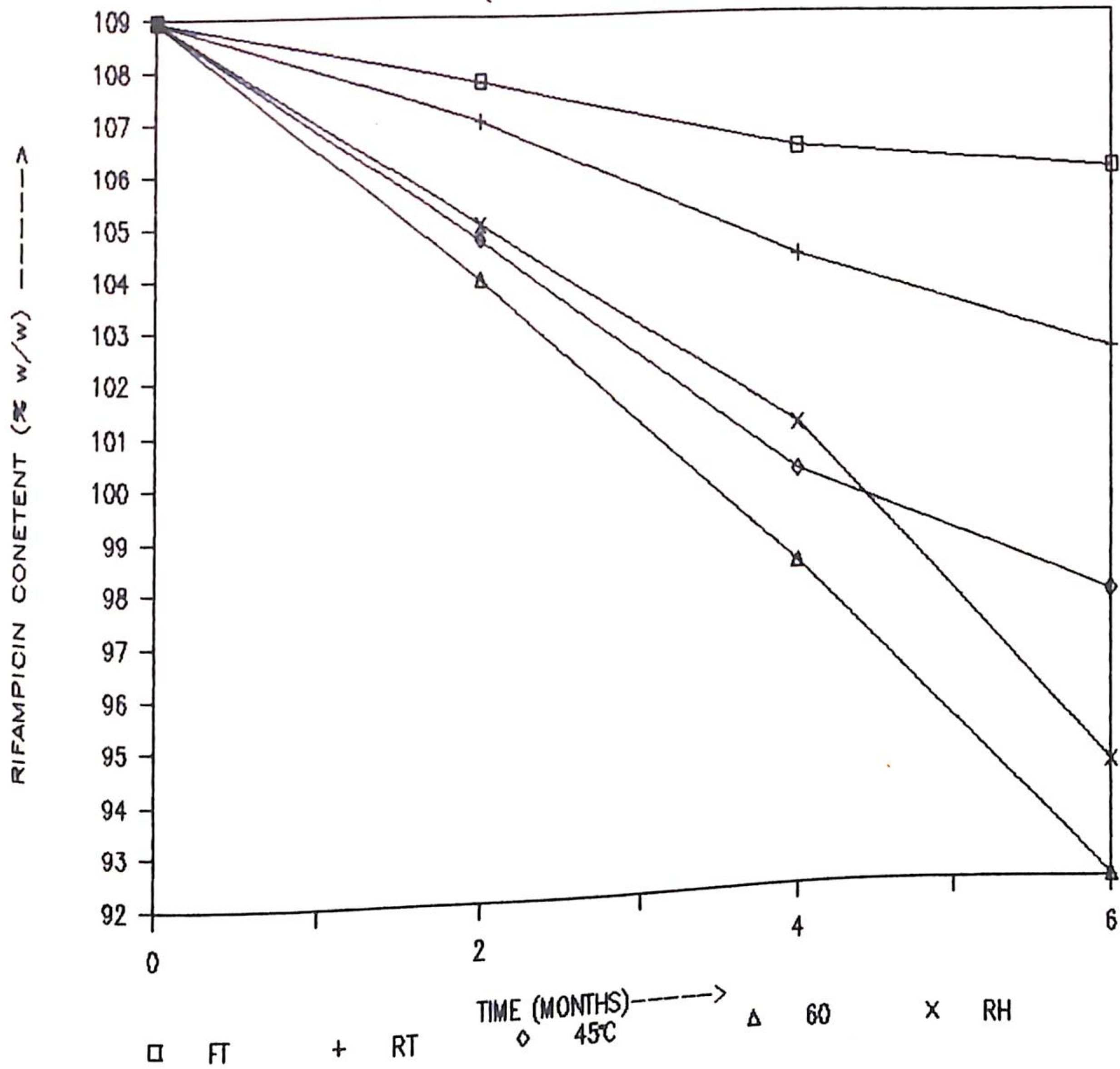


FIG. 6.1S : RIFAMPICIN ASSAY
SAMPLE S (RIFAMPICIN + DOCUSATE SODIUM)
RIFAMPICIN CONETENT (% w/w) ------>
109
108
107
106
105
104
103
102
101
100
99
98
97
96
95
94
93
92
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

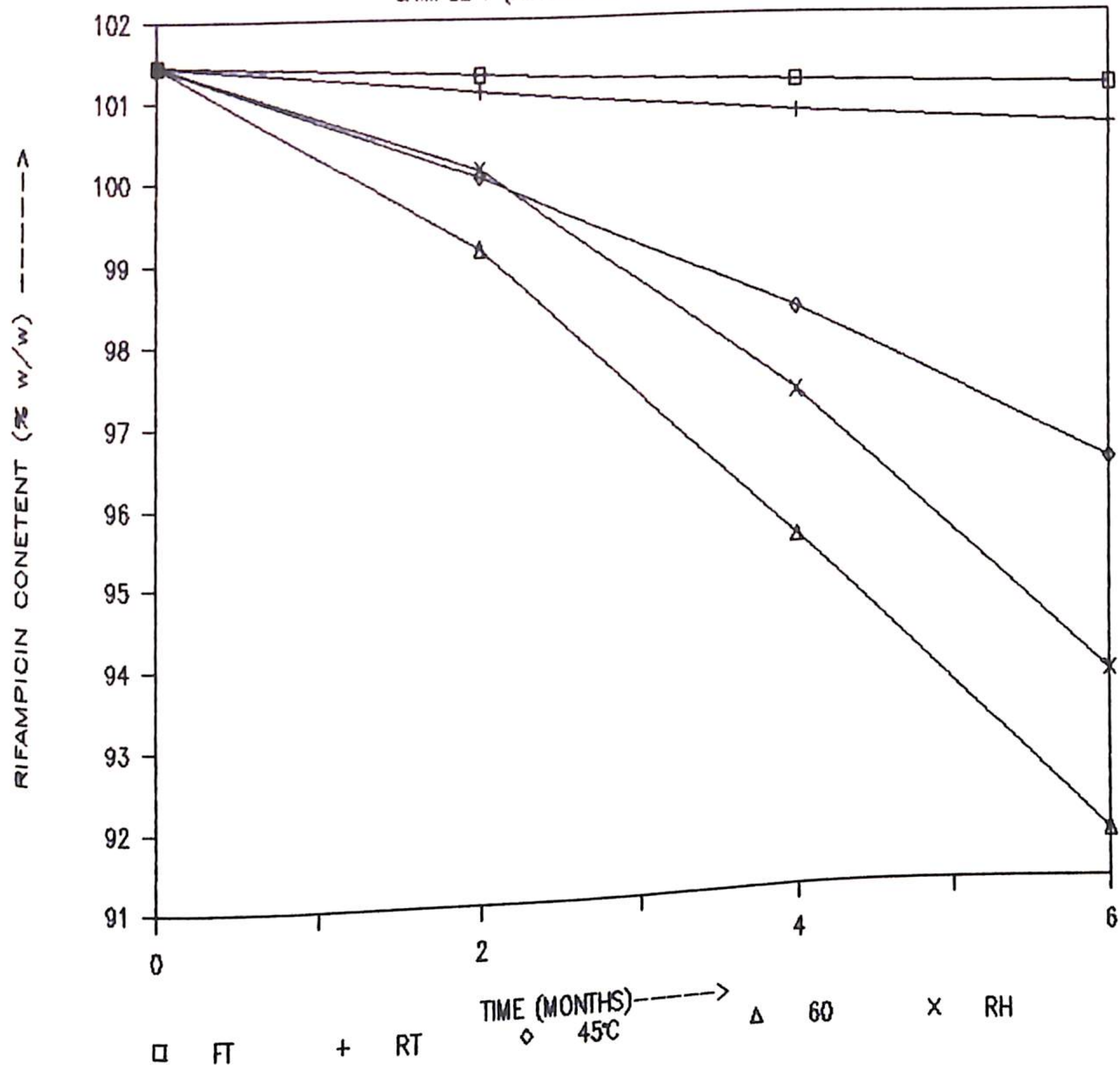


FIG. 6.1T : RIFAMPICIN ASSAY
SAMPLE T (RIFAMPICIN + CYCLODEXTRIN)
RIFAMPICIN CONETENT (% w/w) ------>
102
101
100
99
98
97
96
95
94
93
92
91
0
2
4
6
TIME (MONTHS)------>
□ FT
+ RT
◇ 45°C
Δ 60
X RH

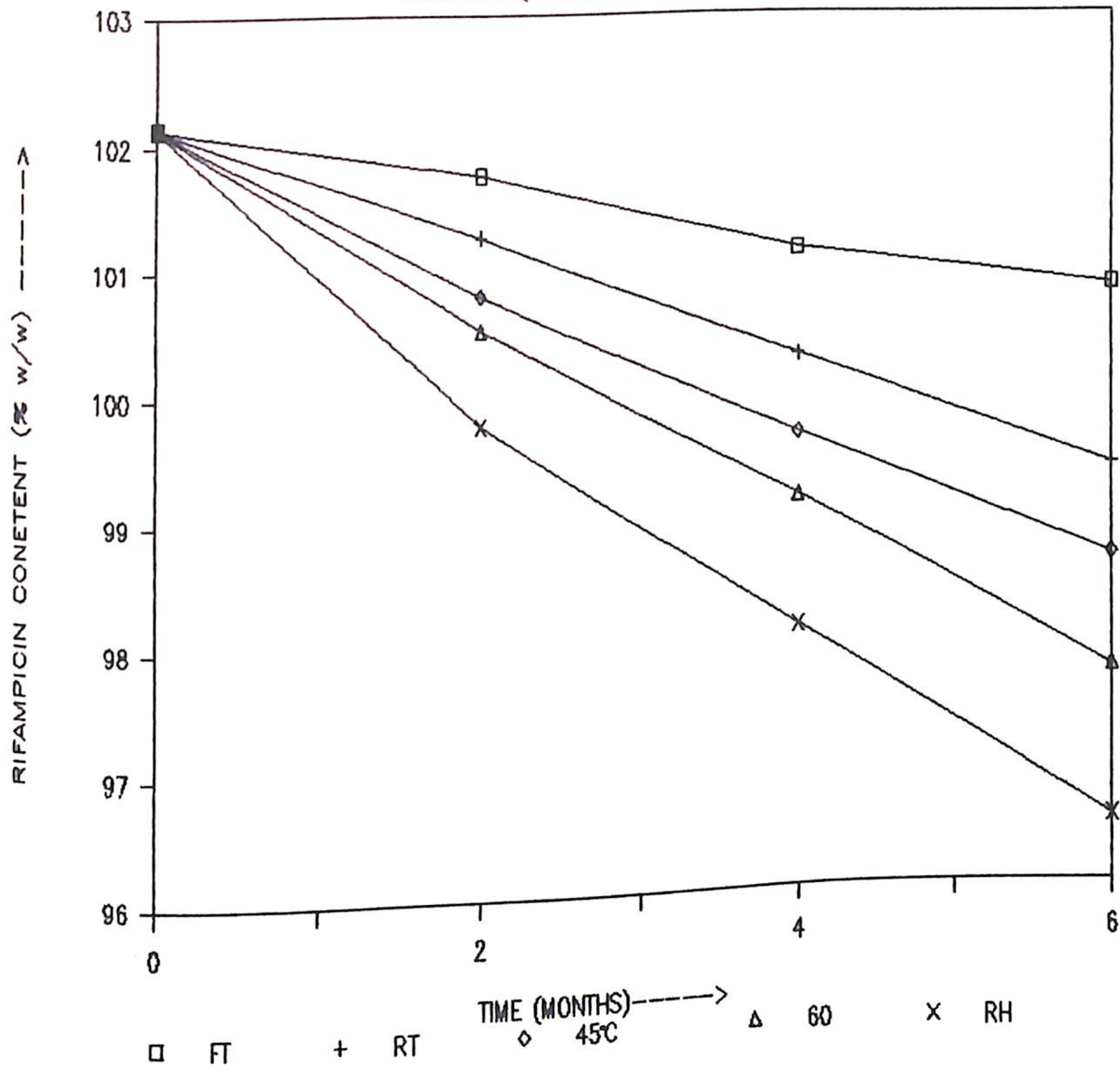


FIG. 6.1U : RIFAMPICIN ASSAY
SAMPLE U (RIFAMPICIN + POLOXAMER)
RIFAMPICIN CONETENT (% w/w) ------>
103
102
101
100
99
98
97
96
0
2
4
6
TIME (MONTHS)------>
FT
RT
45°C
60
RH

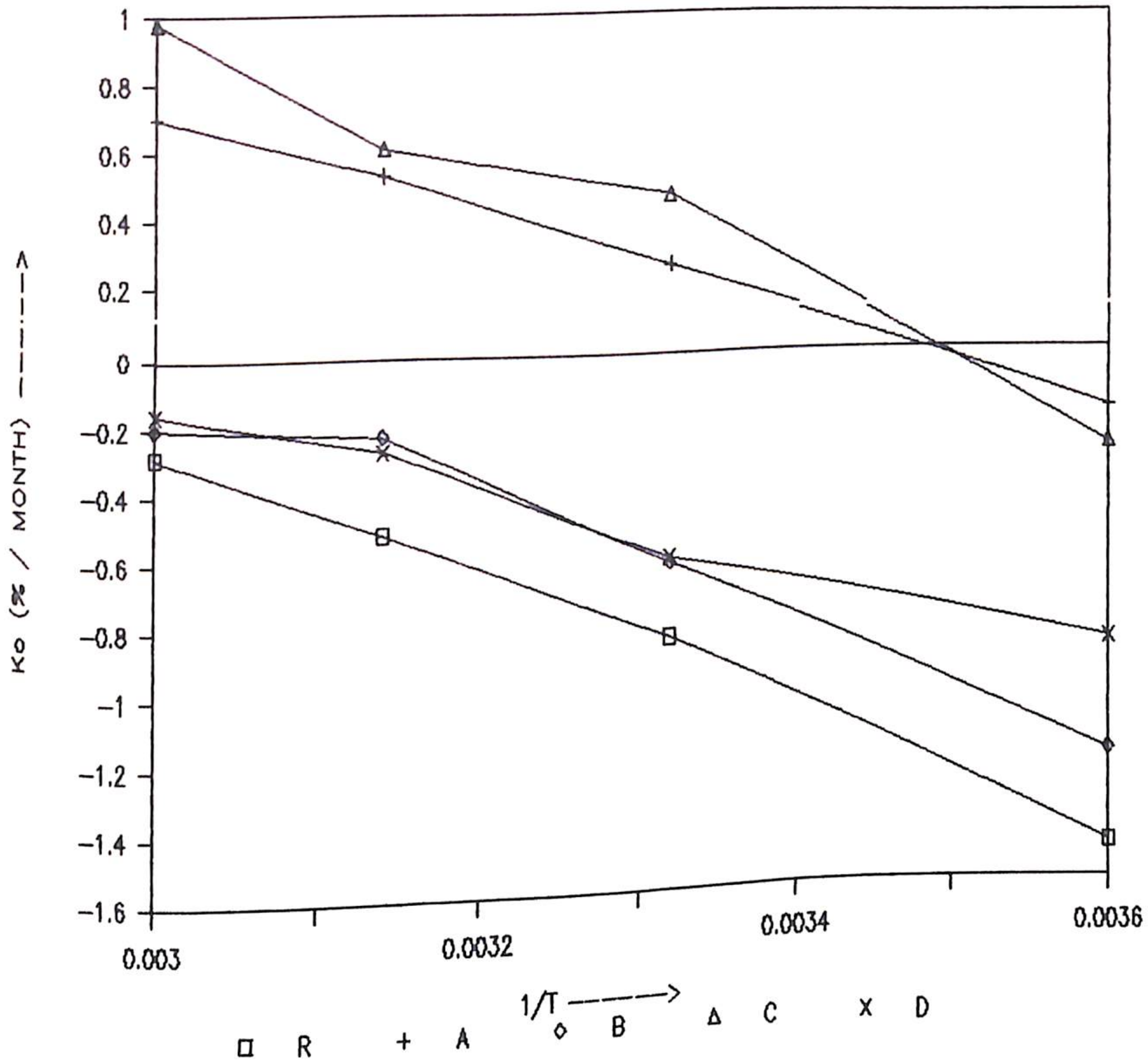


FIG. 6.1RI : ARRHENIUS PLOT
RIFAMPICIN–EXCIPIENT STABILITY SAMPLES
Ko (% / MONTH) ———>
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
0.003
0.0032
0.0034
0.0036
1/T ———>
R
A
B
C
D

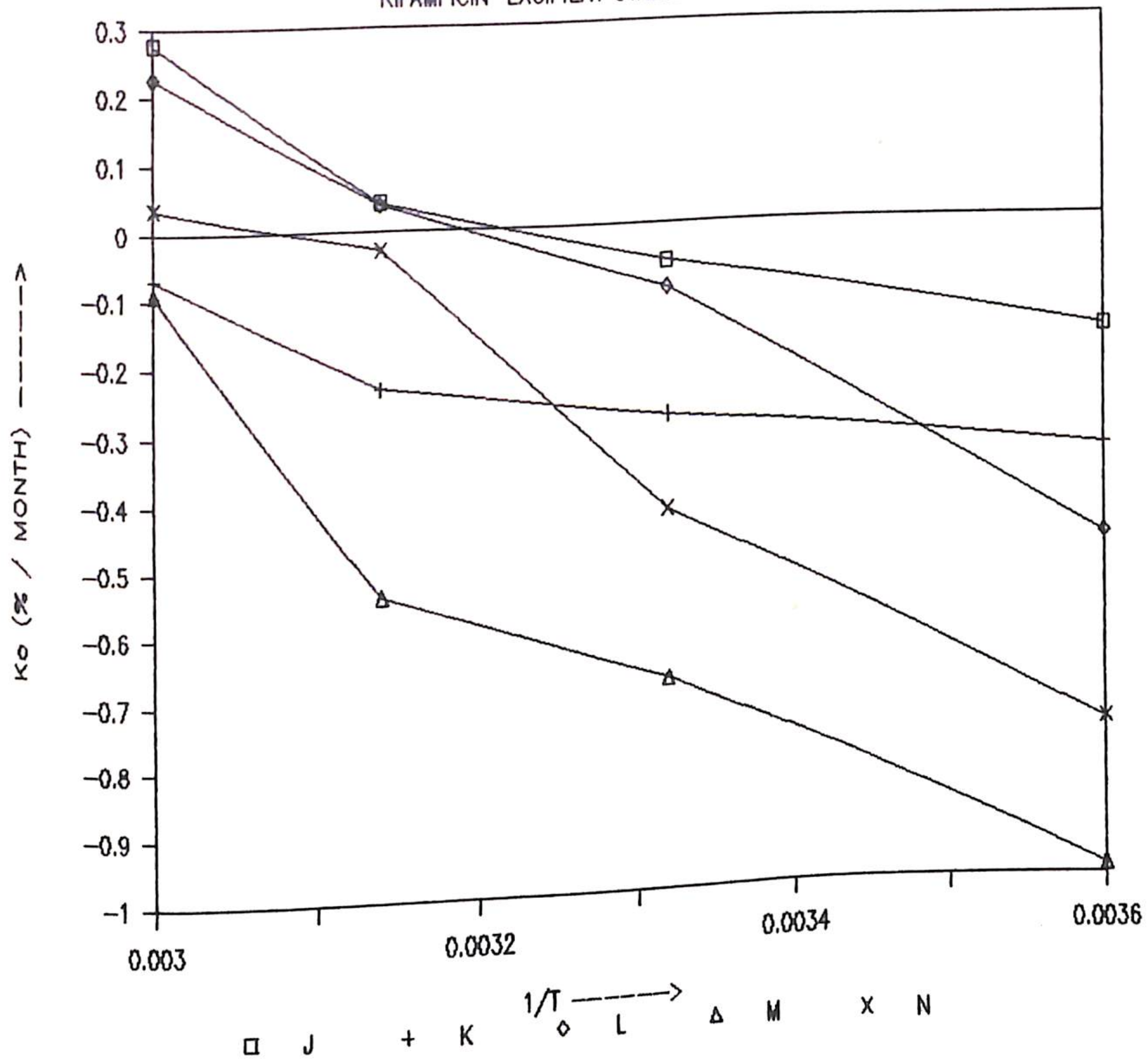


FIG. 6.1RII : ARRHENIUS PLOT
RIFAMPICIN-EXCIPIENT STABILITY SAMPLES
Ko (% / MONTH) ------>
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1
0.003
0.0032
0.0034
0.0036
1/T ------>
J
K
L
M
N

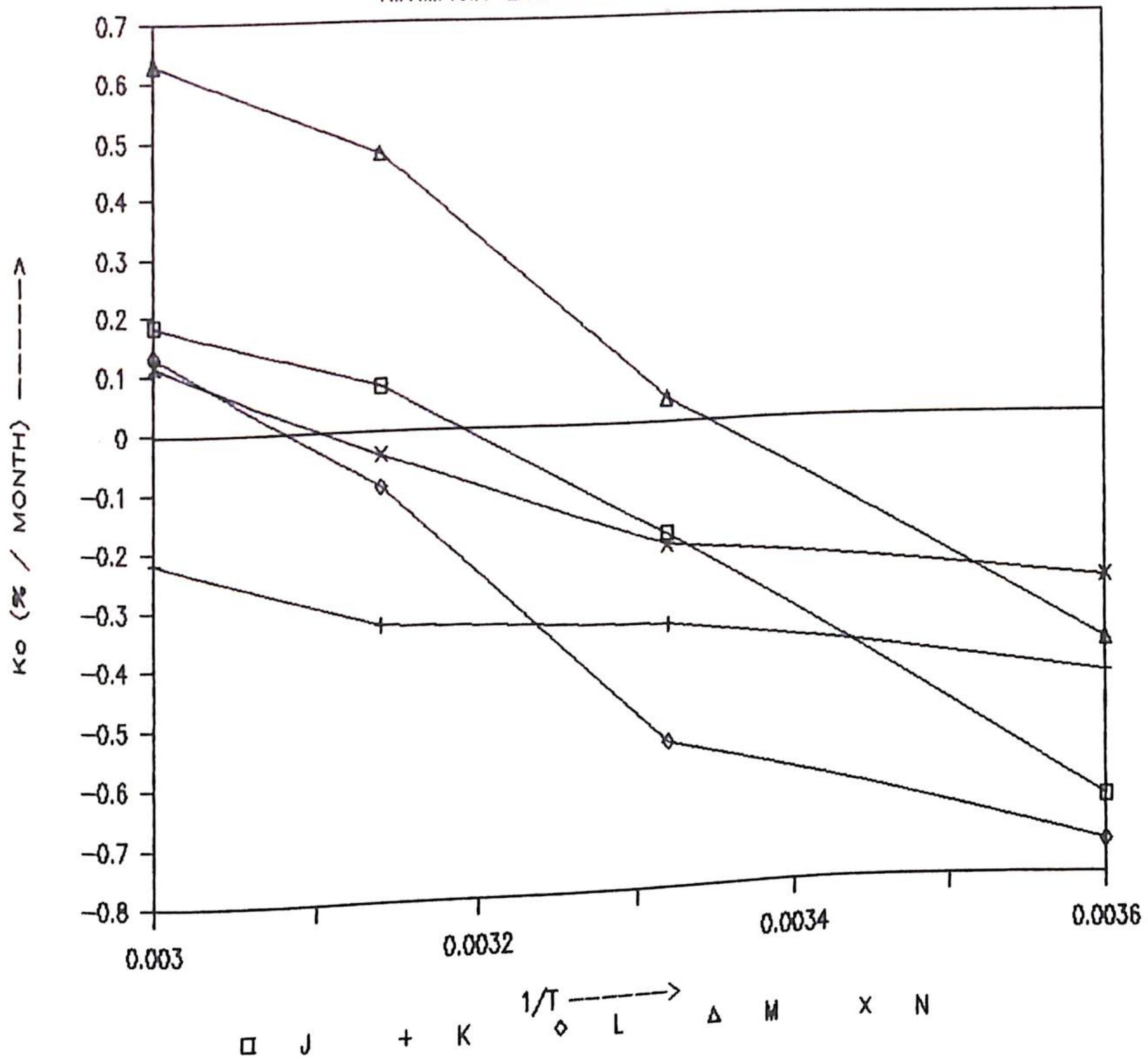


FIG. 6.1RIII : ARRHENIUS PLOT
RIFAMPICIN-EXCIPIENT STABILITY SAMPLES
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
Ko (% / MONTH) ------>
0.003
0.0032
0.0034
0.0036
1/T ------>
J
K
L
M
N

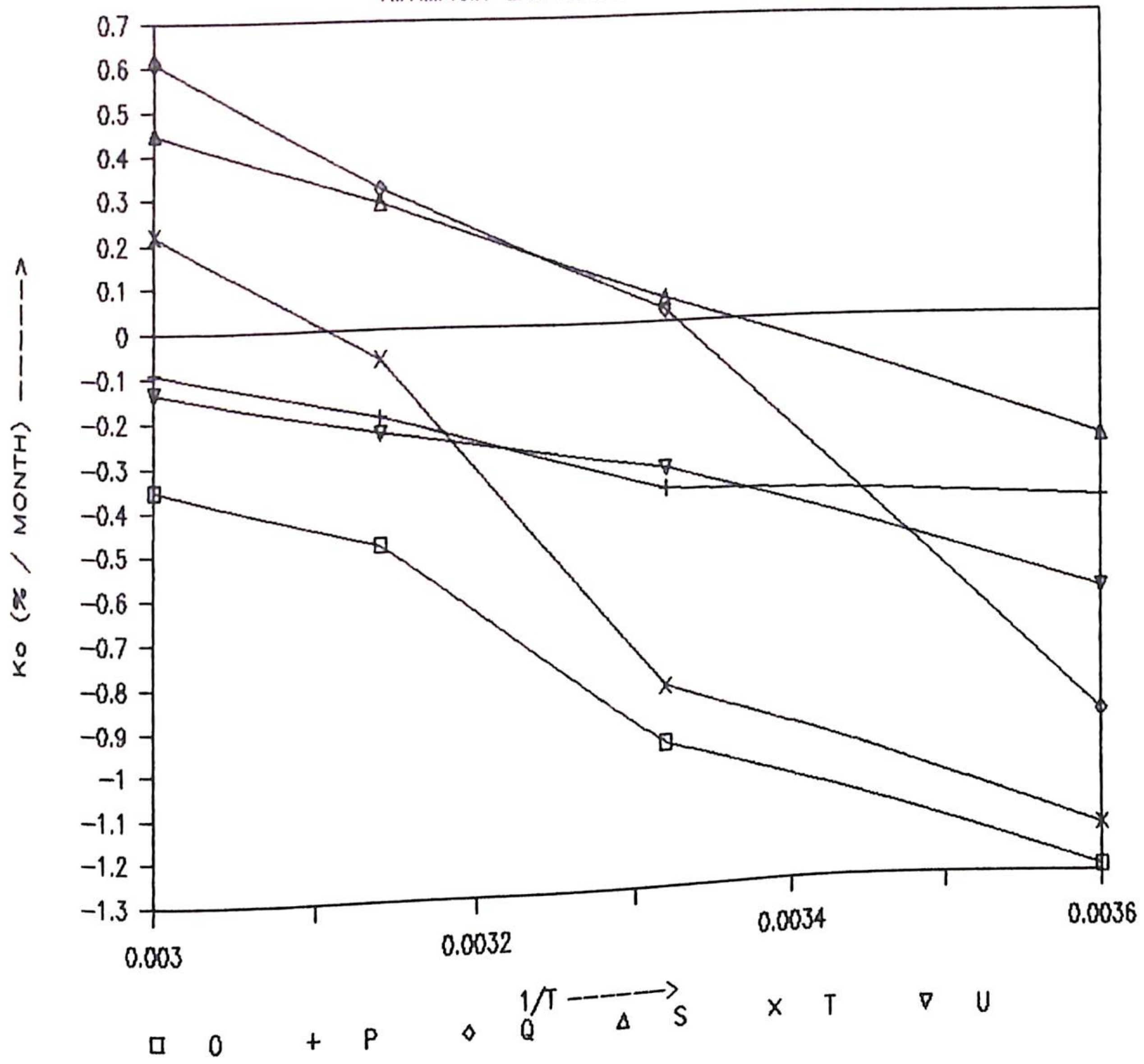


FIG. 6.1RIV : ARRHENIUS PLOT
RIFAMPICIN–EXCIPIENT STABILITY SAMPLES
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−0.1
−0.2
−0.3
−0.4
−0.5
−0.6
−0.7
−0.8
−0.9
−1
−1.1
−1.2
−1.3
Ko (% / MONTH) ------->
0.003
0.0032
0.0034
0.0036
1/T ------->
O
P
Q
S
T
U

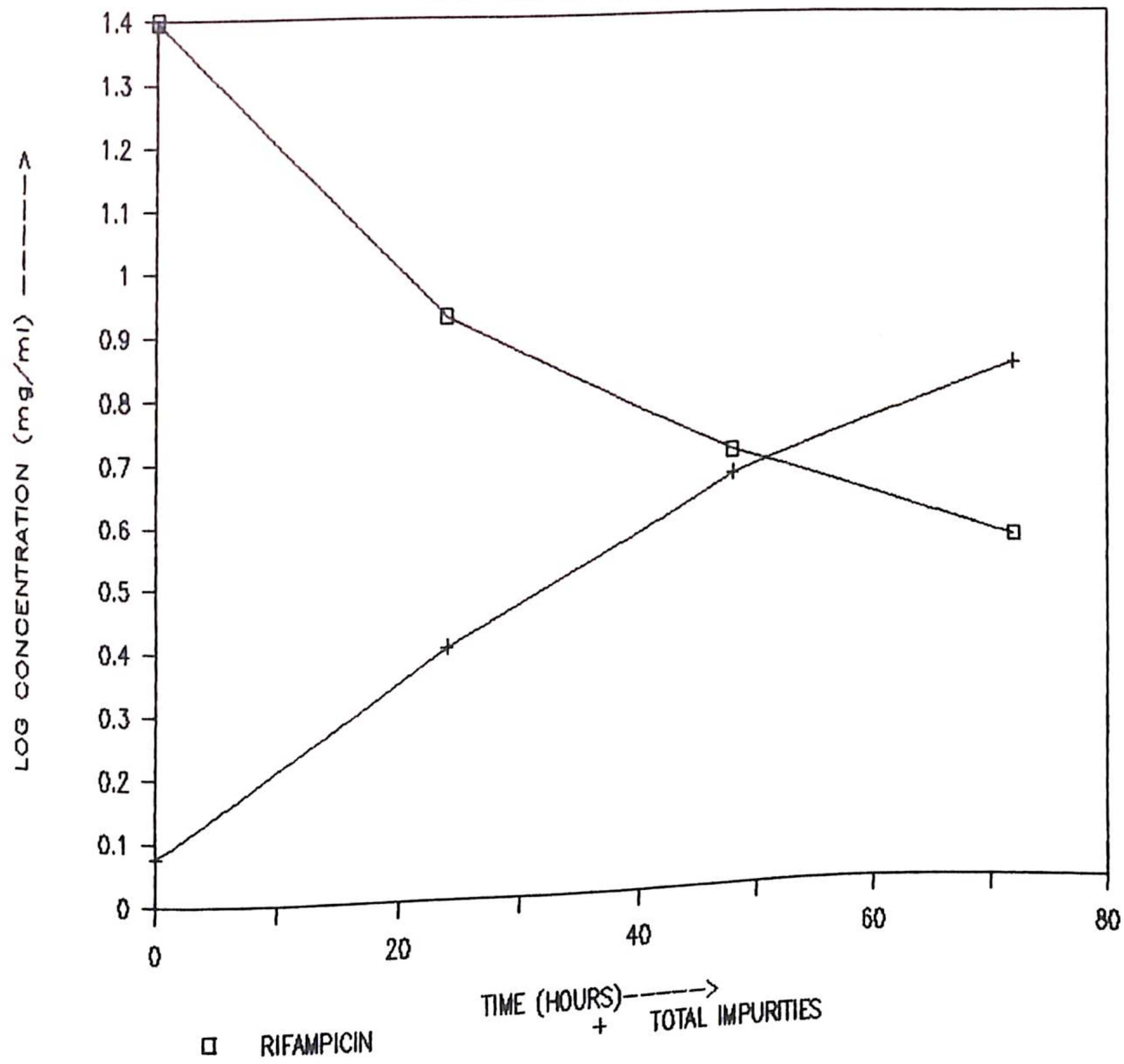


FIG 6.3E :STABILITY OF RIFAMPICIN SOLN.
IN UV LIGHT-254 nm WAVELENGTH
LOG CONCENTRATION (mg/ml) ------>
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
TIME (HOURS)------>
RIFAMPICIN
TOTAL IMPURITIES

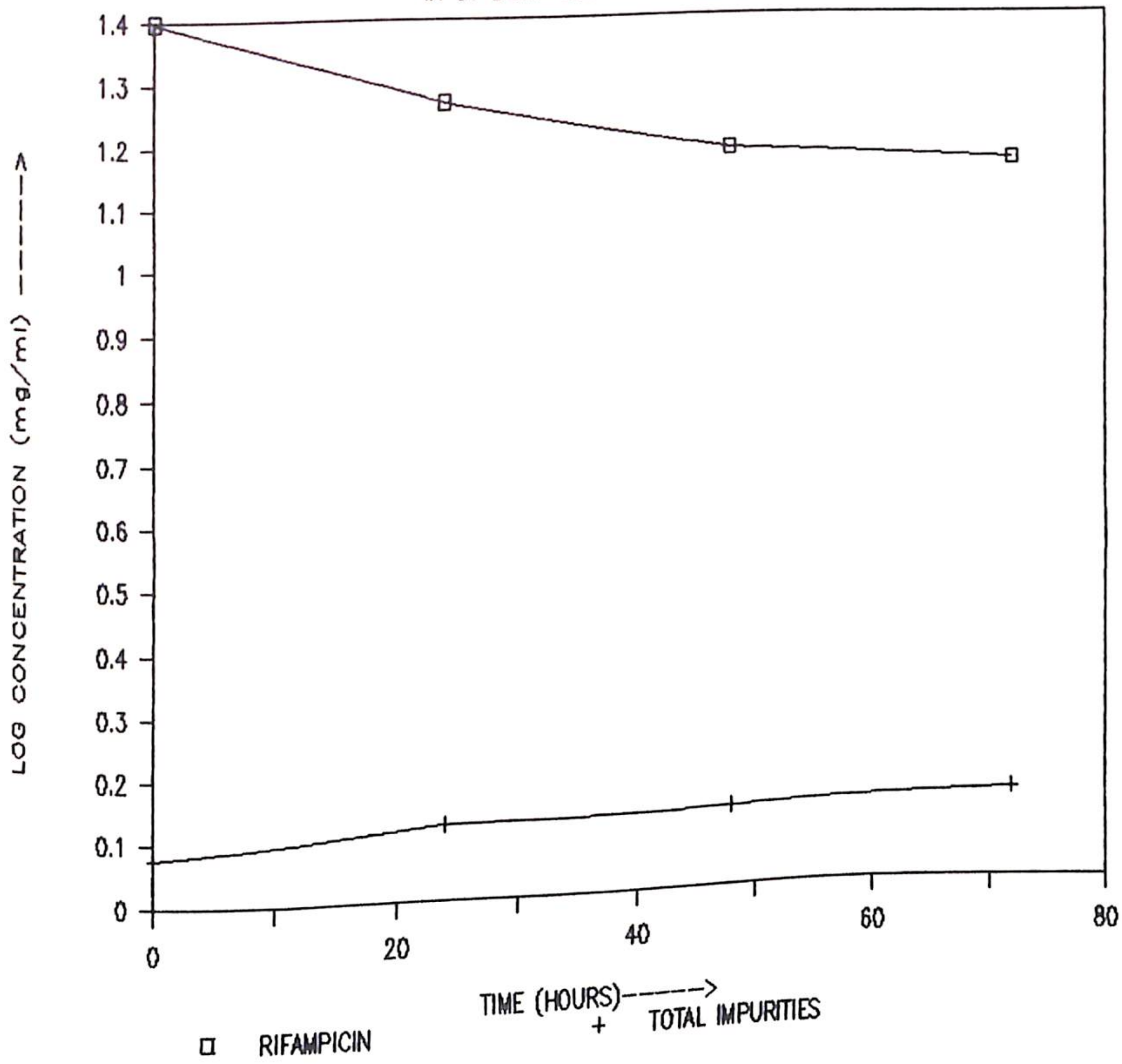


FIG 6.3F :STABILITY OF RIFAMPICIN SOLN
IN UV LIGHT-366 nm WAVELENGTH
LOG CONCENTRATION (mg/ml) ------>
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
TIME (HOURS)------>
RIFAMPICIN
TOTAL IMPURITIES

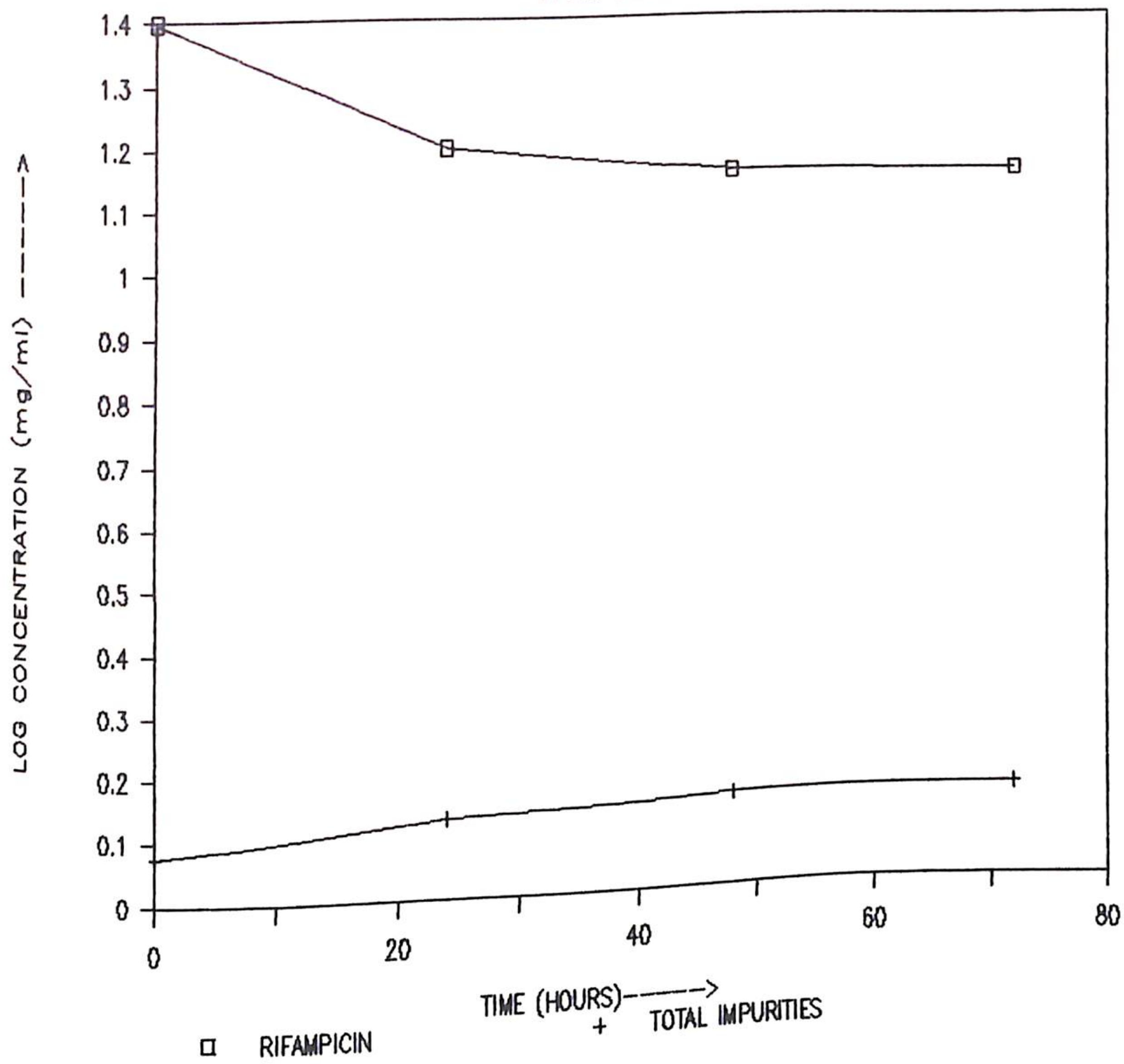


FIG 6.3G:STABILITY OF RIFAMPICIN SOLN
IN DAY LIGHT
LOG CONCENTRATION (mg/ml) ------->
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
TIME (HOURS)------->
RIFAMPICIN
TOTAL IMPURITIES

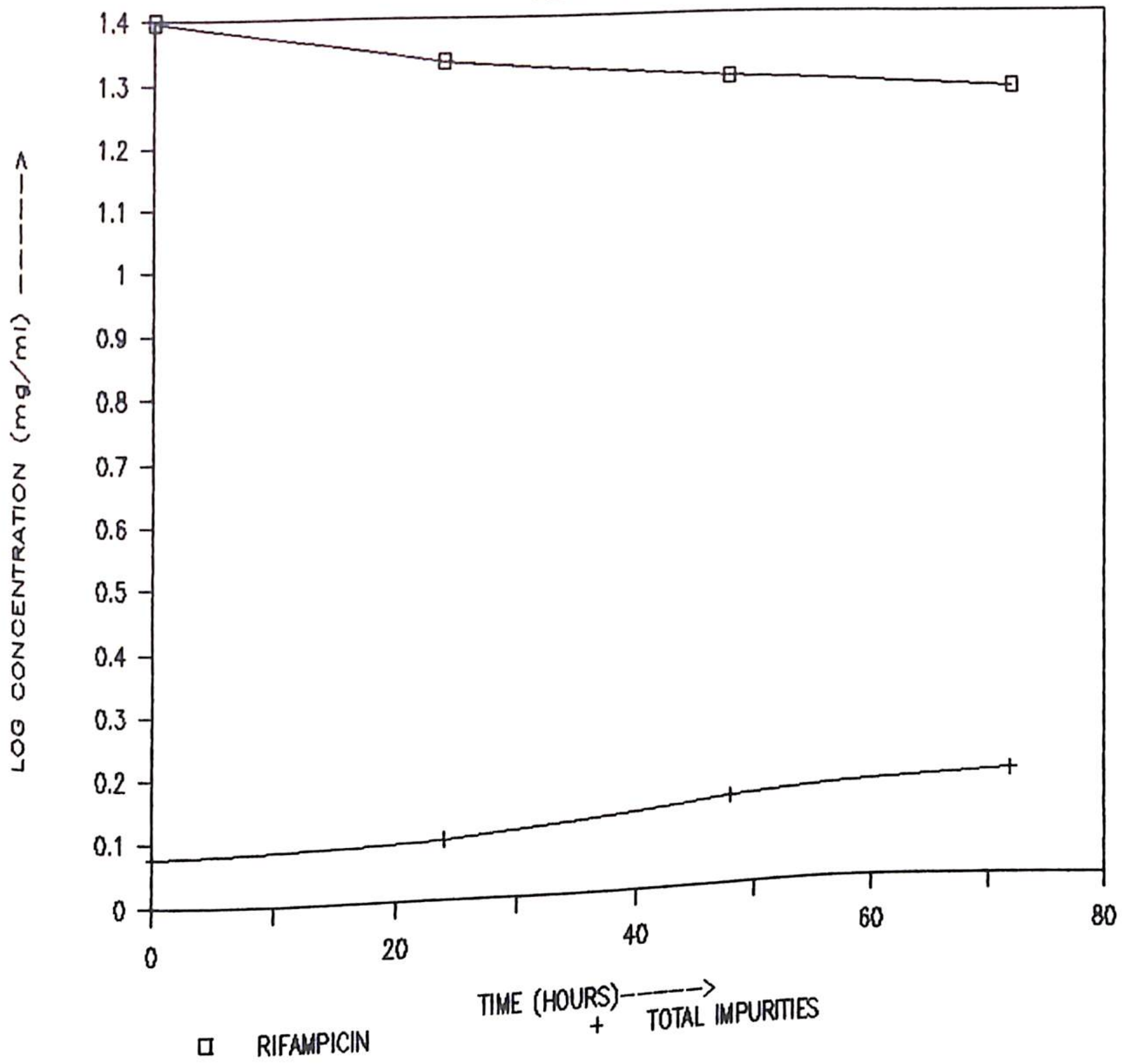


FIG 6.3H :STABILITY OF RIFAMPICIN SOLN
KEPT IN DARK
LOG CONCENTRATION (mg/ml) ------>
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
TIME (HOURS)------->
RIFAMPICIN
TOTAL IMPURITIES

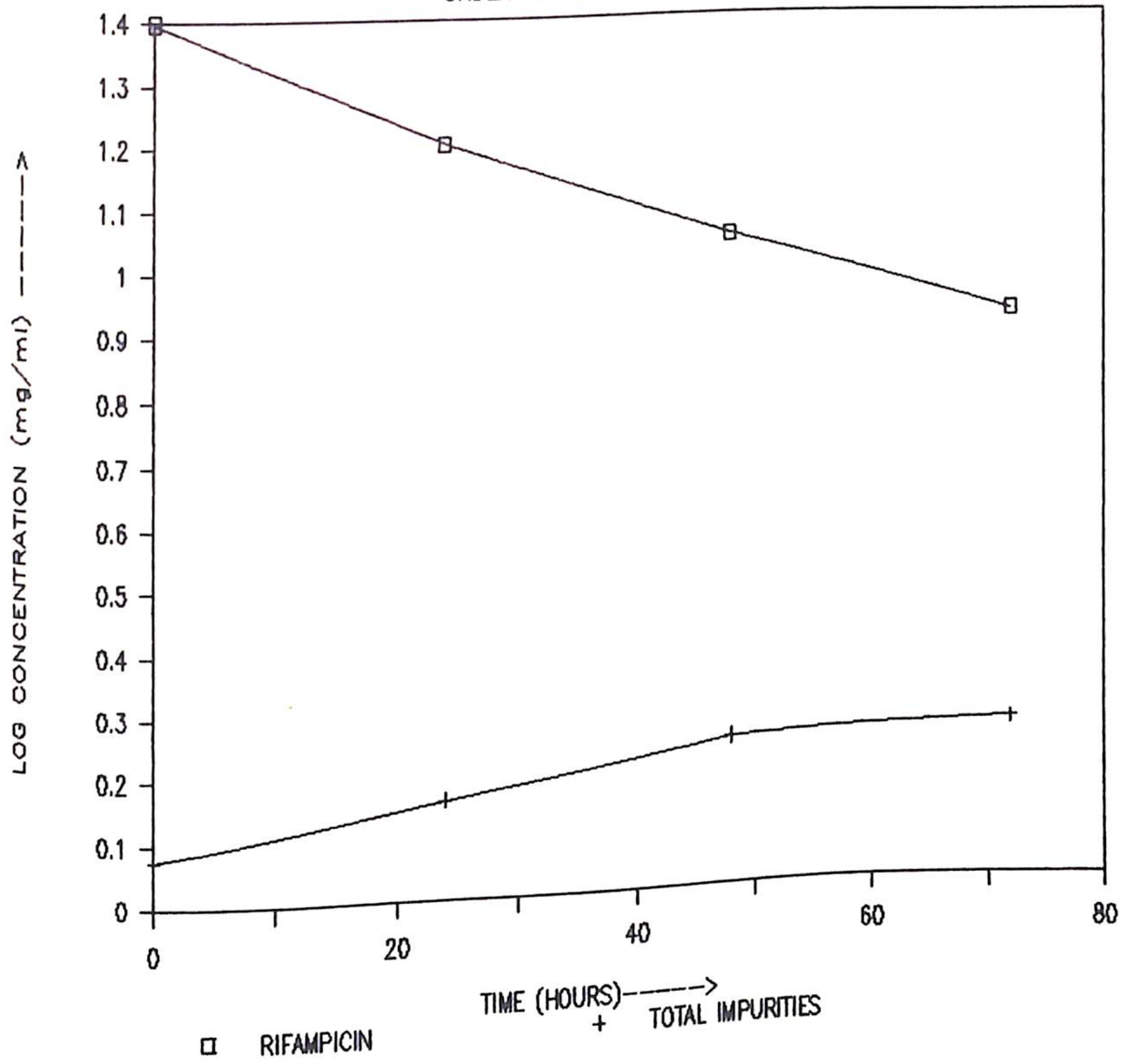


FIG 6.4A:STABILITY OF RIFAMPICIN SOLN
UNDER NITROGEN ATMOSPHERE
LOG CONCENTRATION (mg/ml) ------>
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
TIME (HOURS)------>
RIFAMPICIN
+ TOTAL IMPURITIES

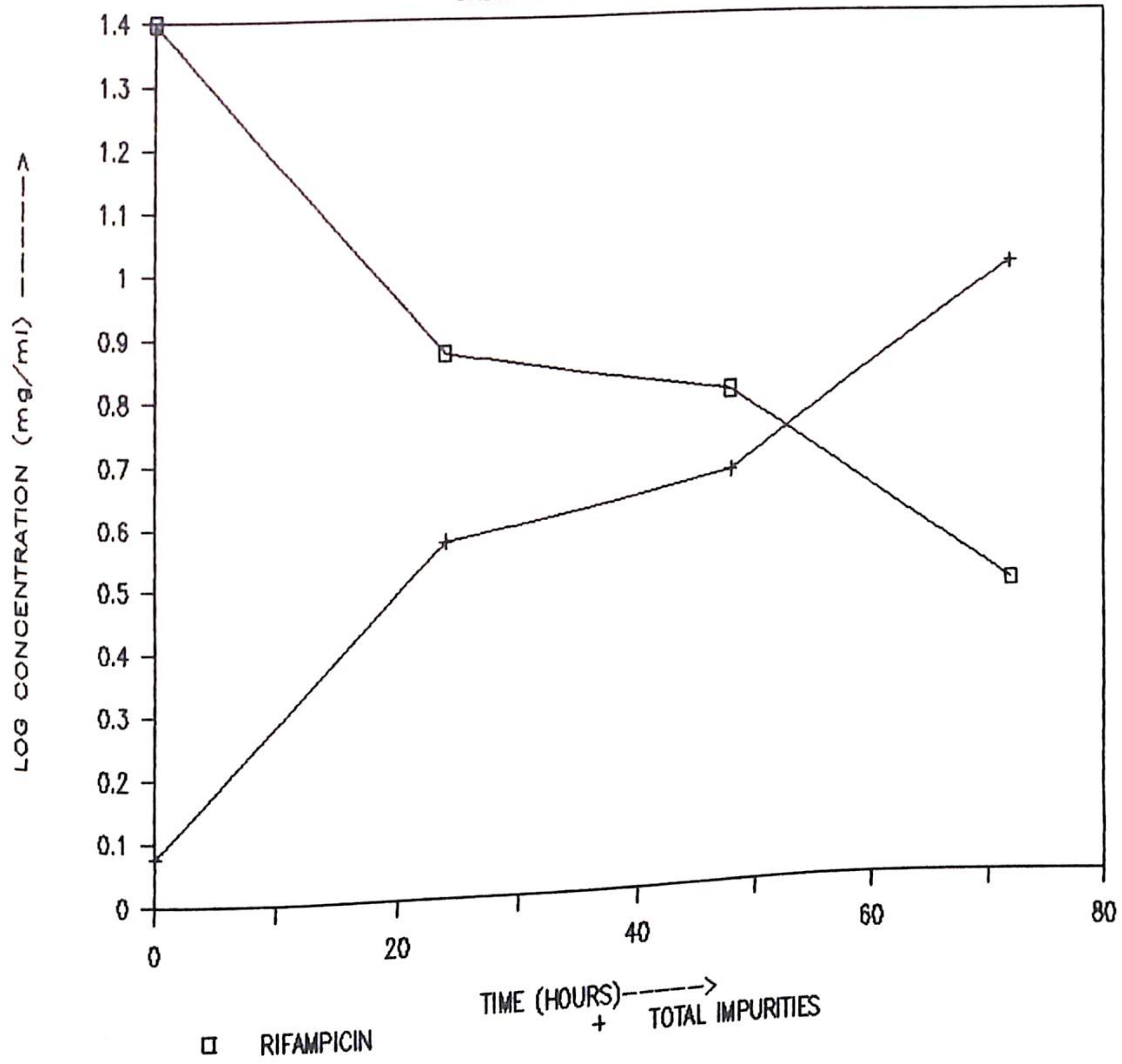


FIG 6.4B:STABILITY OF RIFAMPICIN SOLN
UNDER OXYGEN ATMOSPHERE
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
LOG CONCENTRATION (mg/ml) ------->
0
20
40
60
80
TIME (HOURS)------->
RIFAMPICIN
+ TOTAL IMPURITIES

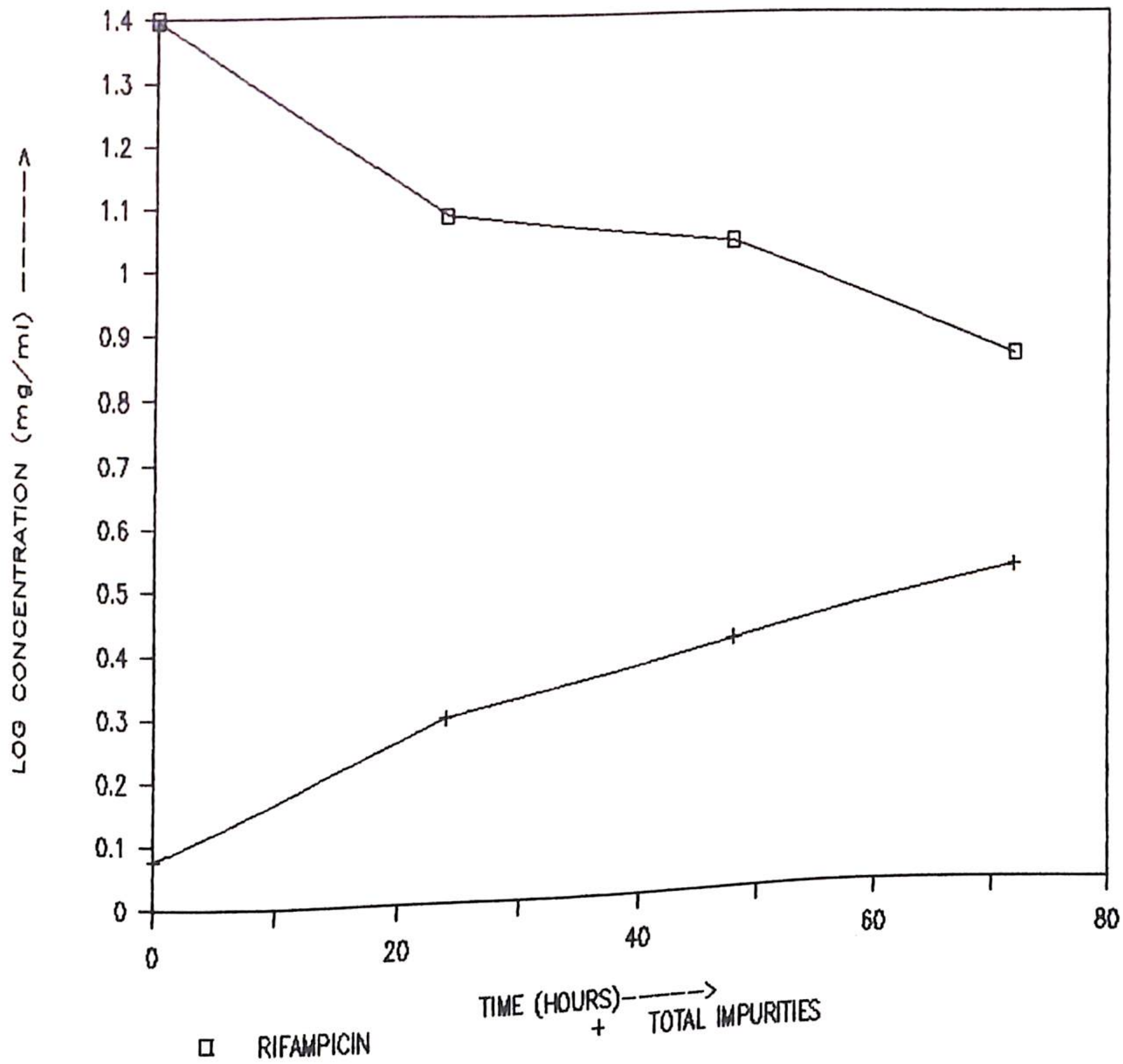


FIG 6.4C:STABILITY OF RIFAMPICIN SOLN
UNDER AMBIENT ATMOSPHERE
LOG CONCENTRATION (mg/ml) ------>
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
TIME (HOURS)------>
RIFAMPICIN
+ TOTAL IMPURITIES

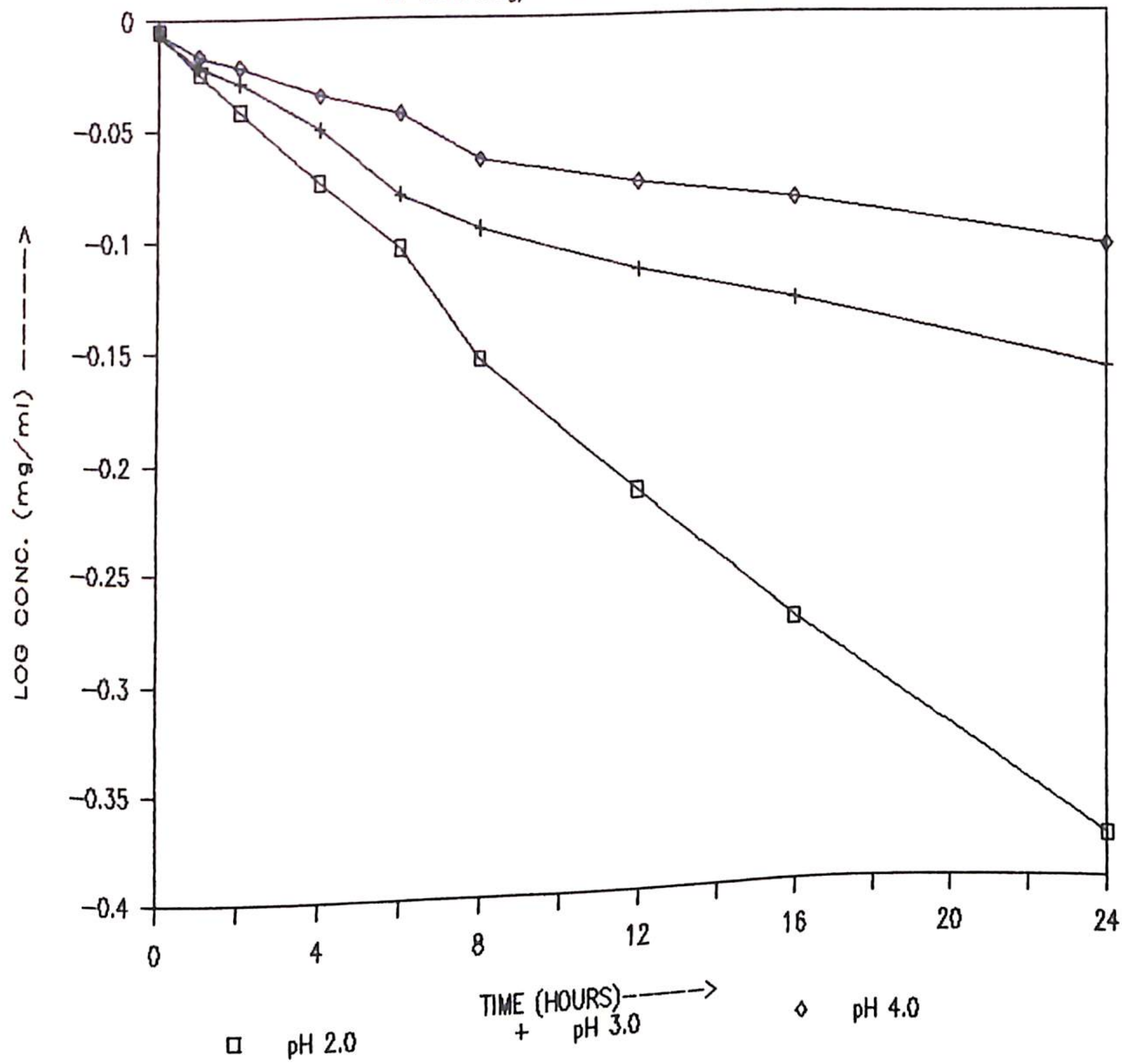


FIG 6.5AI : RIFAMPICIN STABILITY
IN 1000 mcg/ml SOLUTION AT pH 2,3 & 4
LOG CONC. (mg/ml) ------->
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.35
-0.4
0
4
8
12
16
20
24
TIME (HOURS)------->
pH 2.0
pH 3.0
pH 4.0

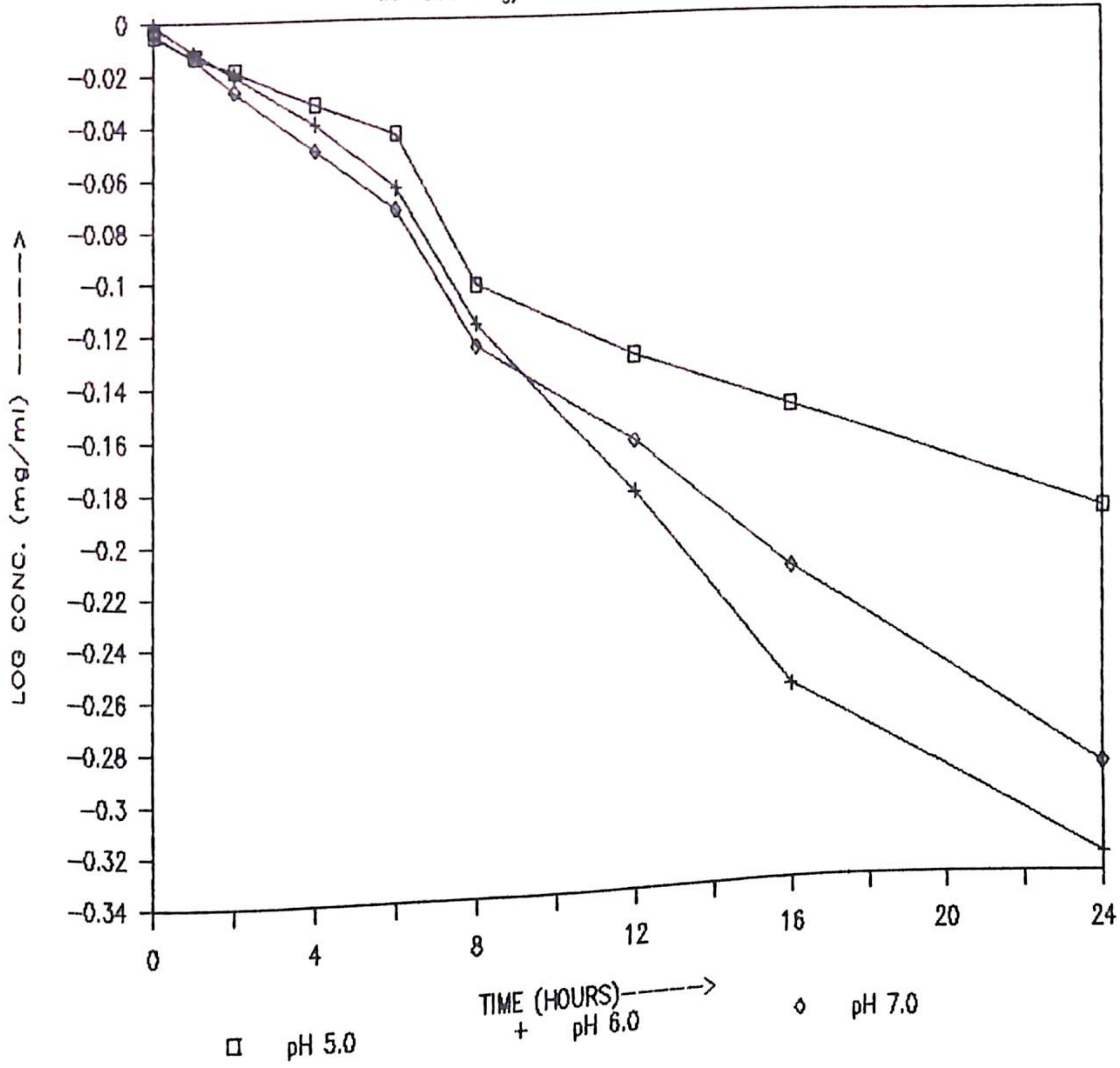


FIG 6.5AII : RIFAMPICIN STABILITY
IN 1000 mcg/ml SOLUTION AT pH 5,6 & 7
LOG CONC. (mg/ml) ------->
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
-0.14
-0.16
-0.18
-0.2
-0.22
-0.24
-0.26
-0.28
-0.3
-0.32
-0.34
0
4
8
12
16
20
24
TIME (HOURS)------->
pH 5.0
pH 6.0
pH 7.0

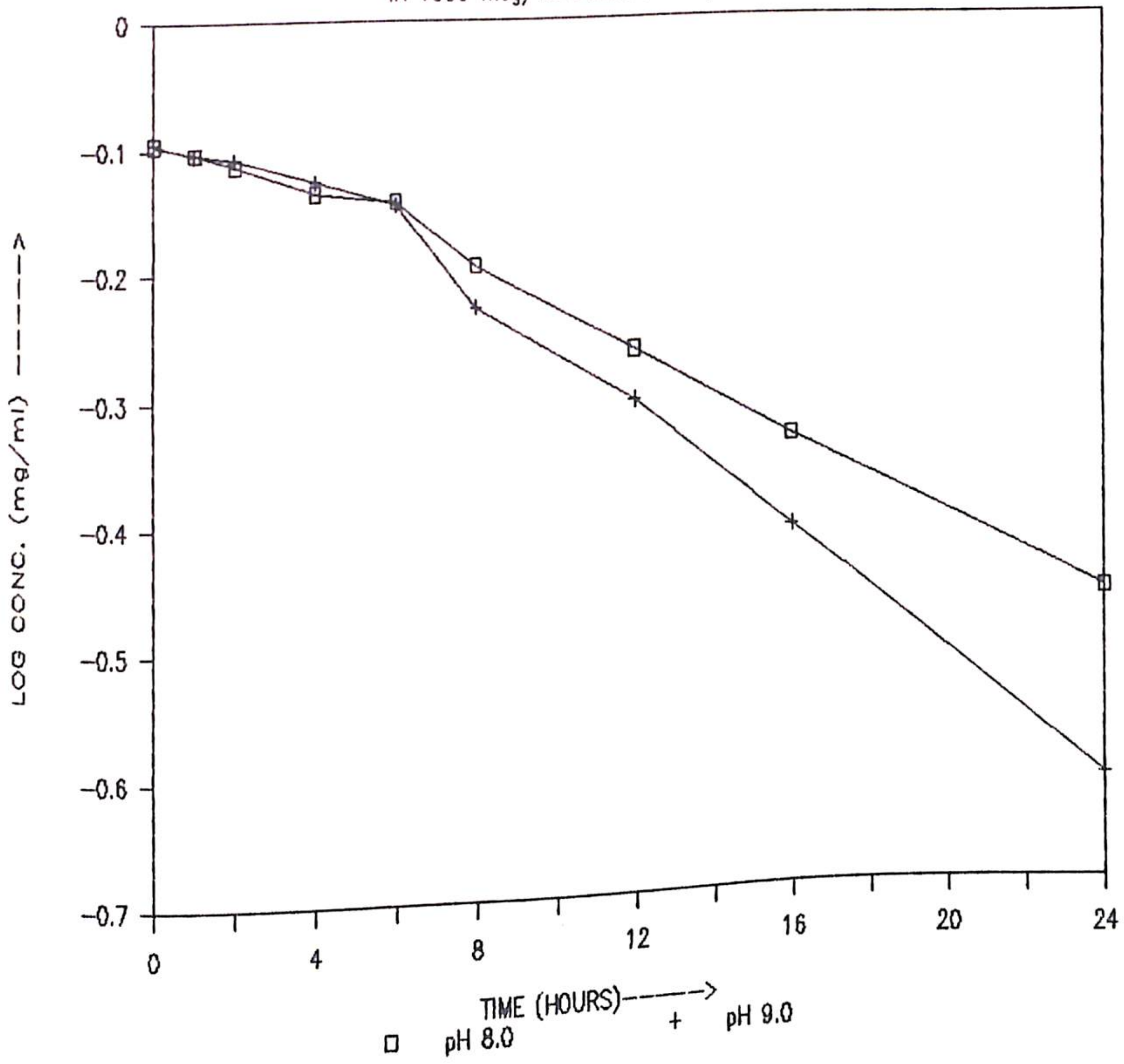


FIG 6.5AIII : RIFAMPICIN STABILITY
IN 1000 mcg/ml SOLUTION AT pH 8 & 9
LOG CONC. (mg/ml) ------>
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
0
4
8
12
16
20
24
TIME (HOURS)------>
pH 8.0
+ pH 9.0

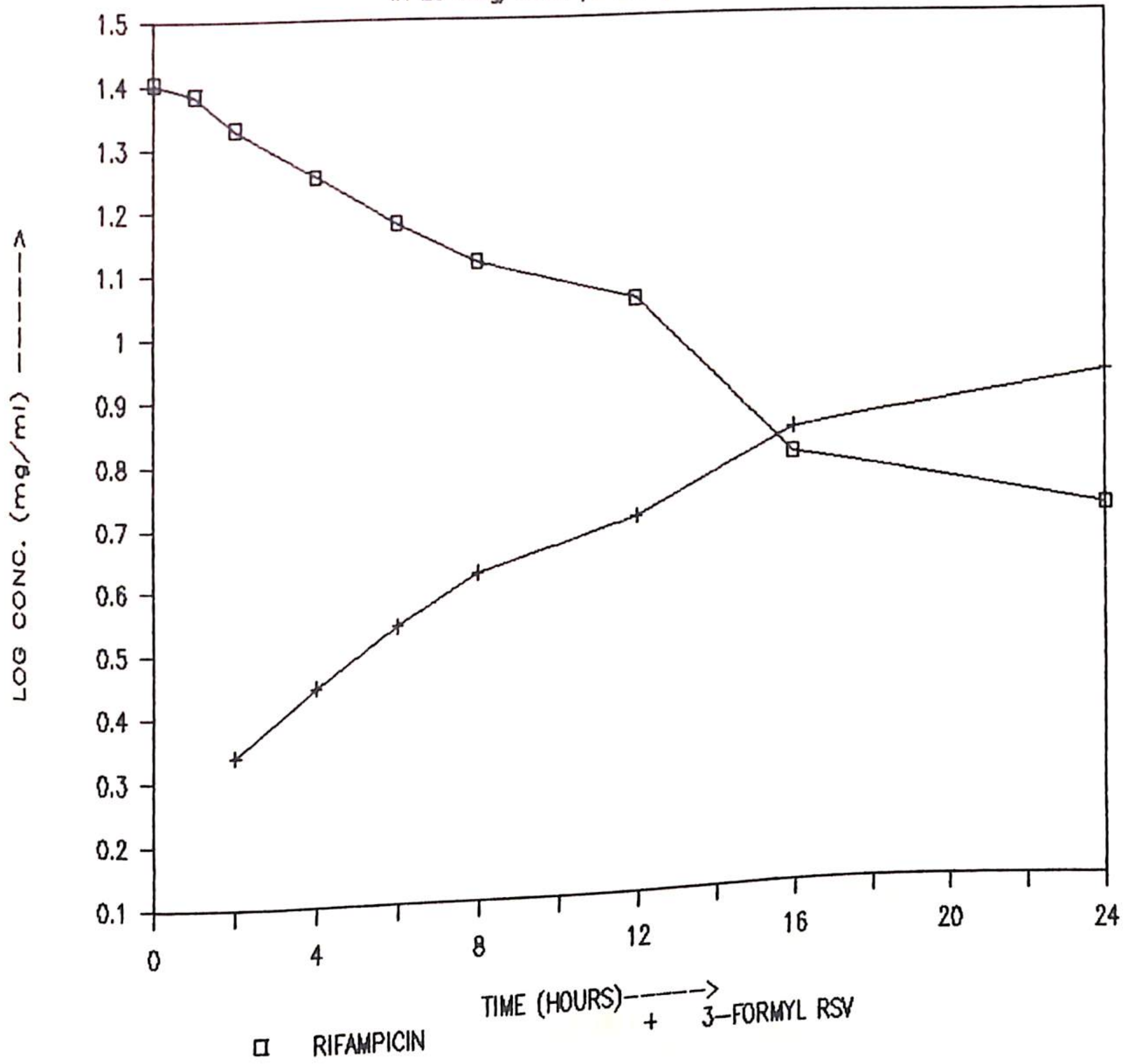


FIG 6.5B : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 2.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------->
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
4
8
12
16
20
24
TIME (HOURS)------->
RIFAMPICIN
+ 3-FORMYL RSV

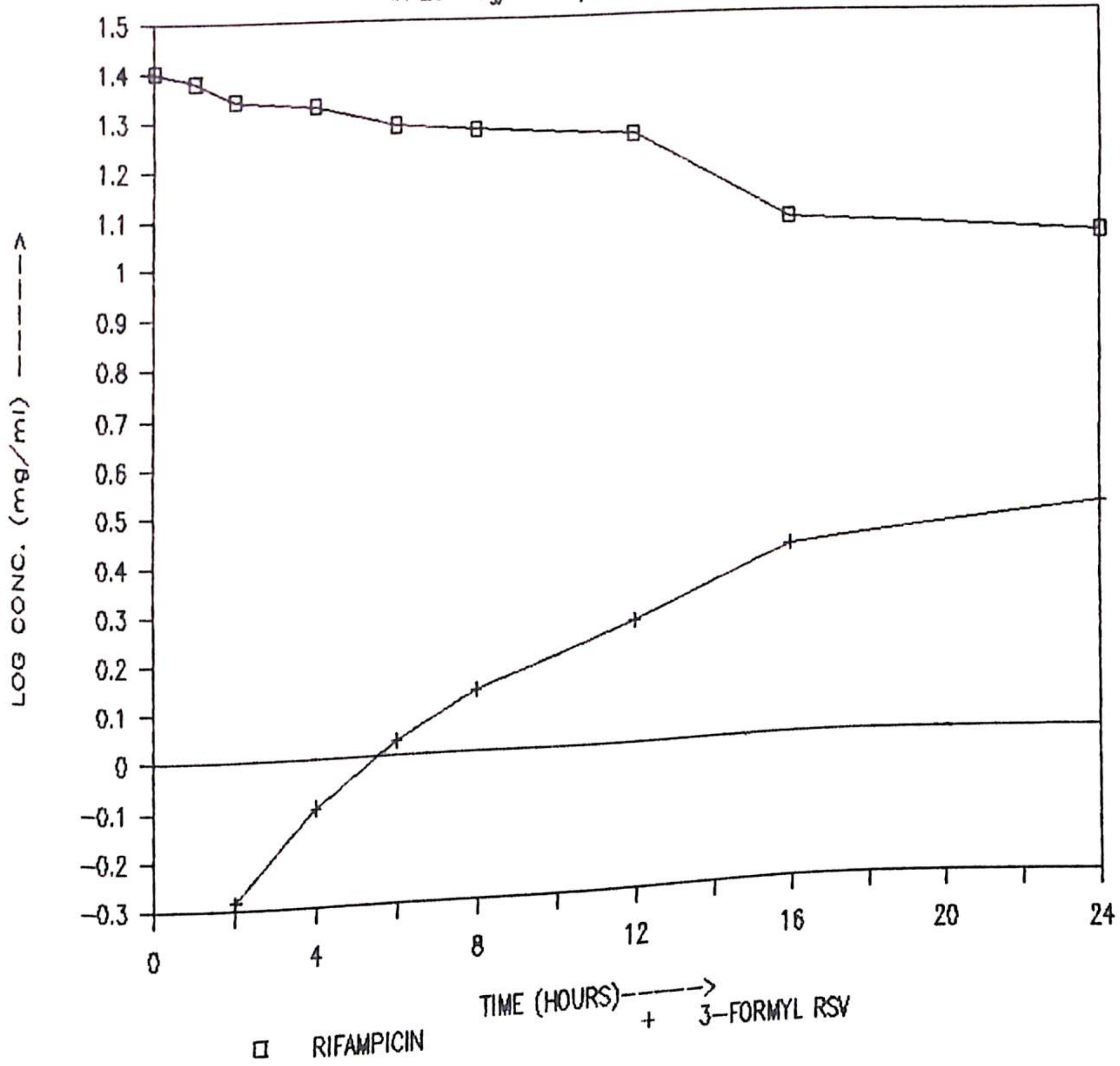


FIG 6.5C : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 3.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------>
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
0
4
8
12
16
20
24
TIME (HOURS)------>
RIFAMPICIN
3-FORMYL RSV

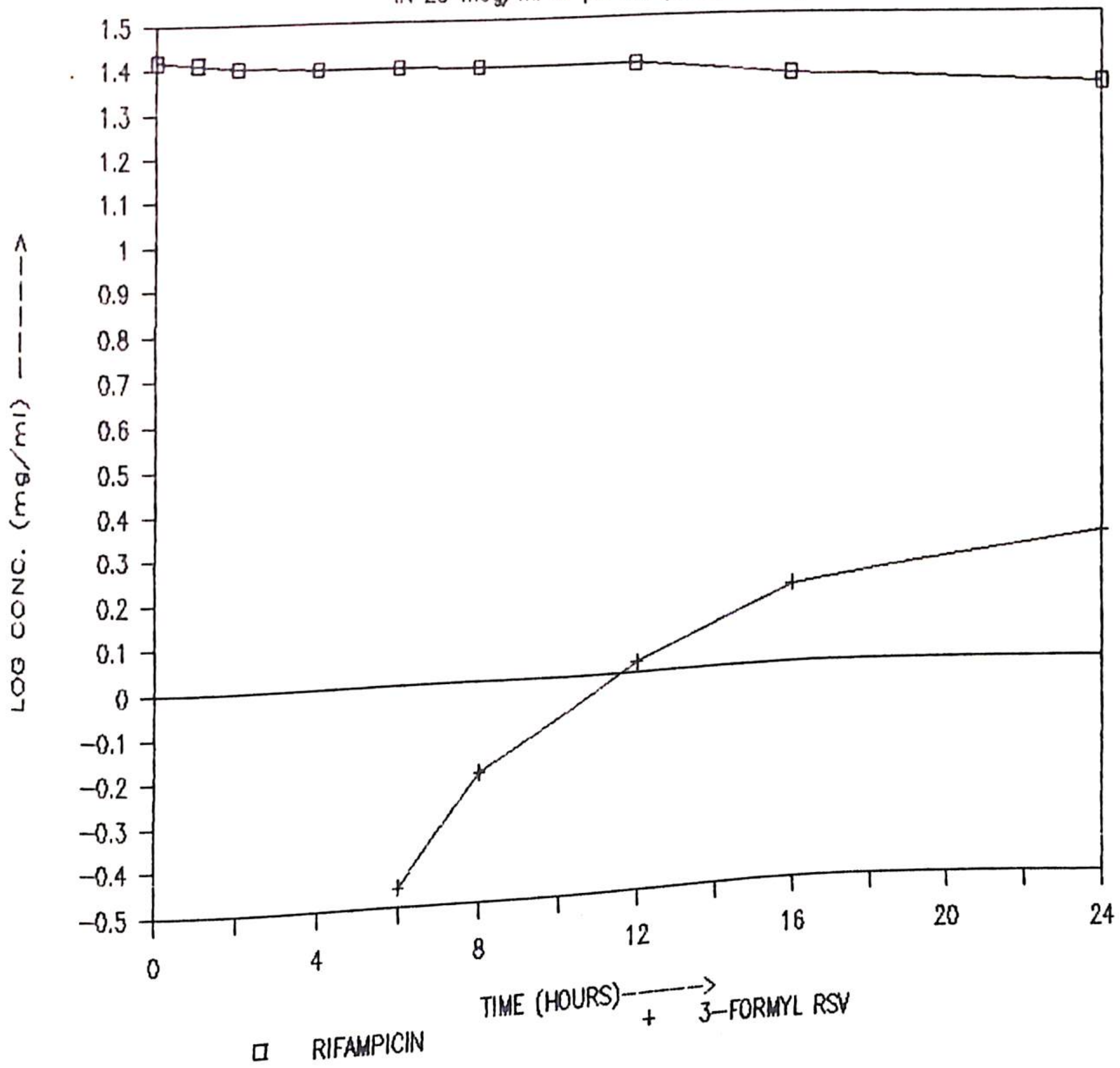


FIG 8.5D : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 4.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------>
TIME (HOURS)------>
RIFAMPICIN
3-FORMYL RSV

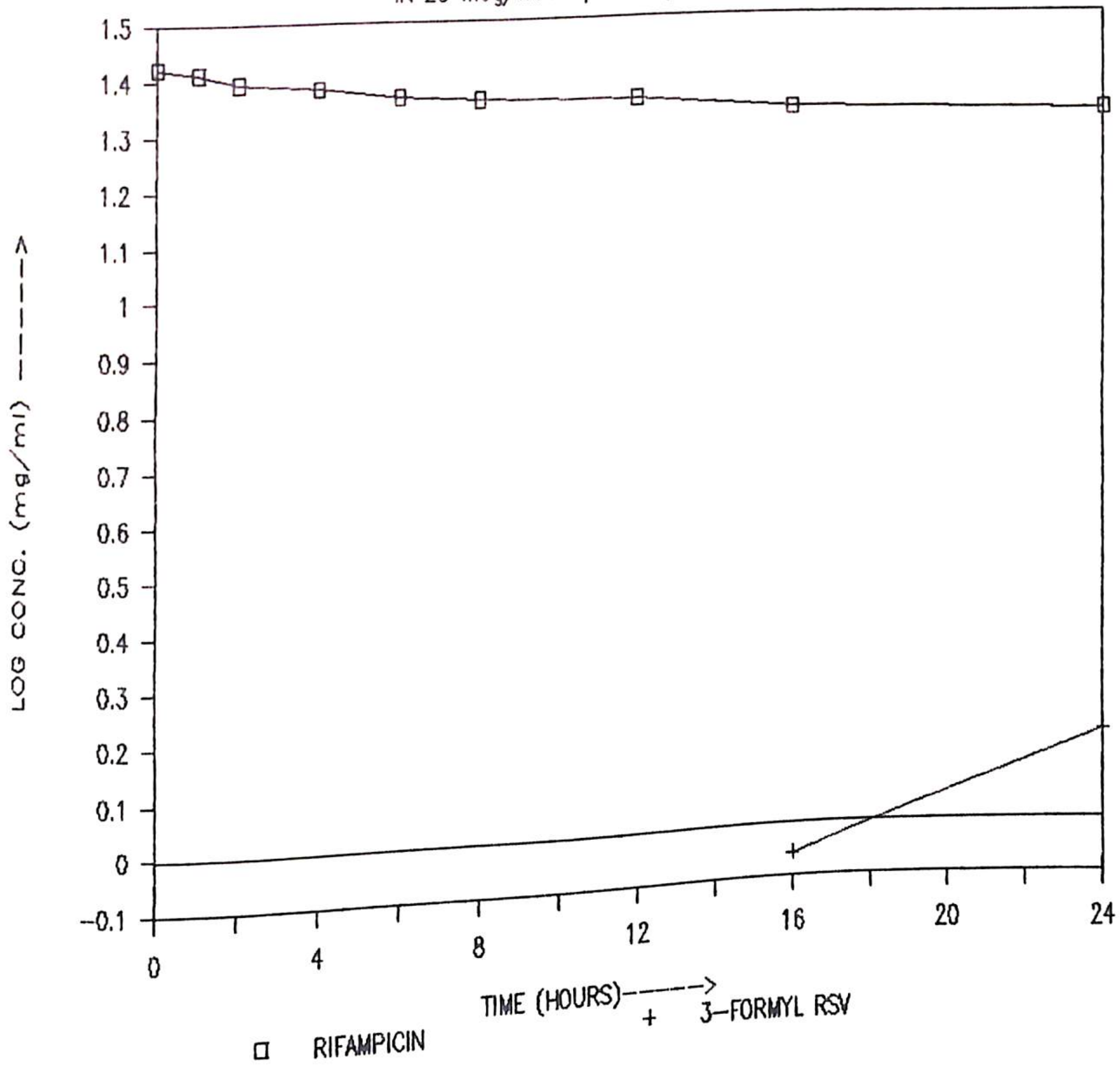


FIG 6.5E : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 5.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------->
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
4
8
12
16
20
24
TIME (HOURS)------->
RIFAMPICIN
3-FORMYL RSV

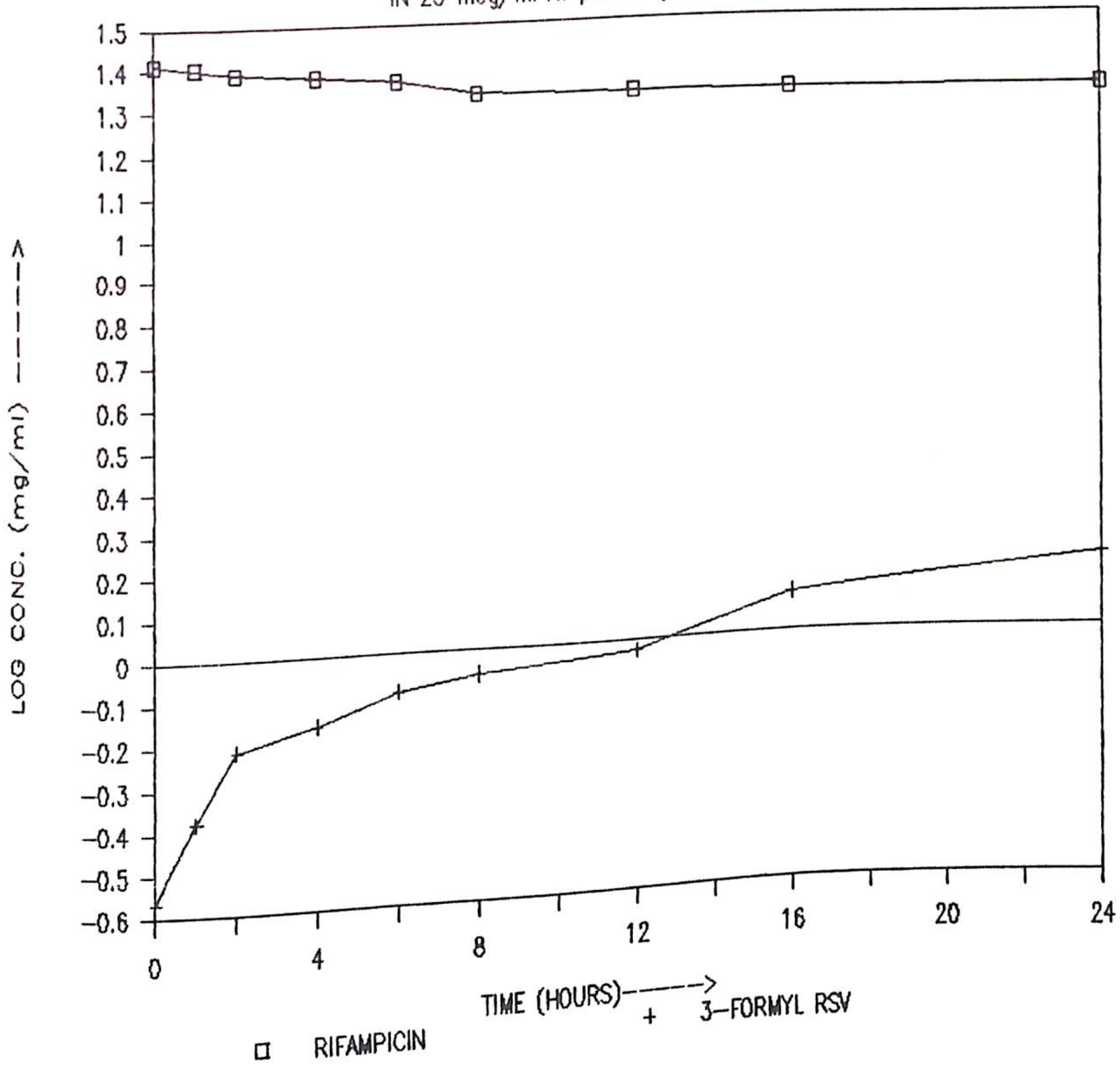


FIG 6.5F : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 6.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------>
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
0
4
8
12
16
20
24
TIME (HOURS)------>
□ RIFAMPICIN
+ 3-FORMYL RSV

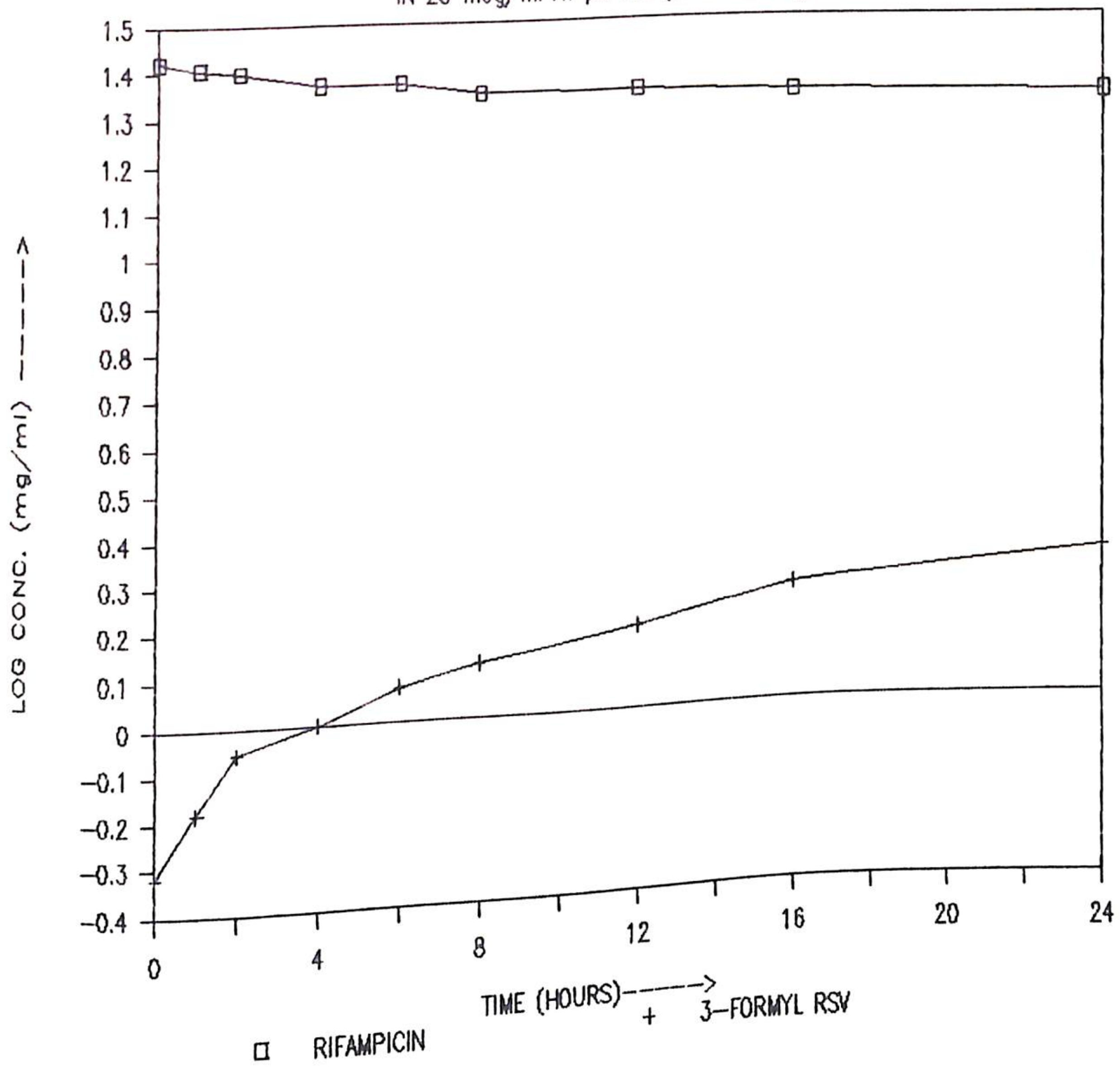


FIG 6.5G : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 7.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------>
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0
4
8
12
16
20
24
TIME (HOURS)------>
RIFAMPICIN
3-FORMYL RSV

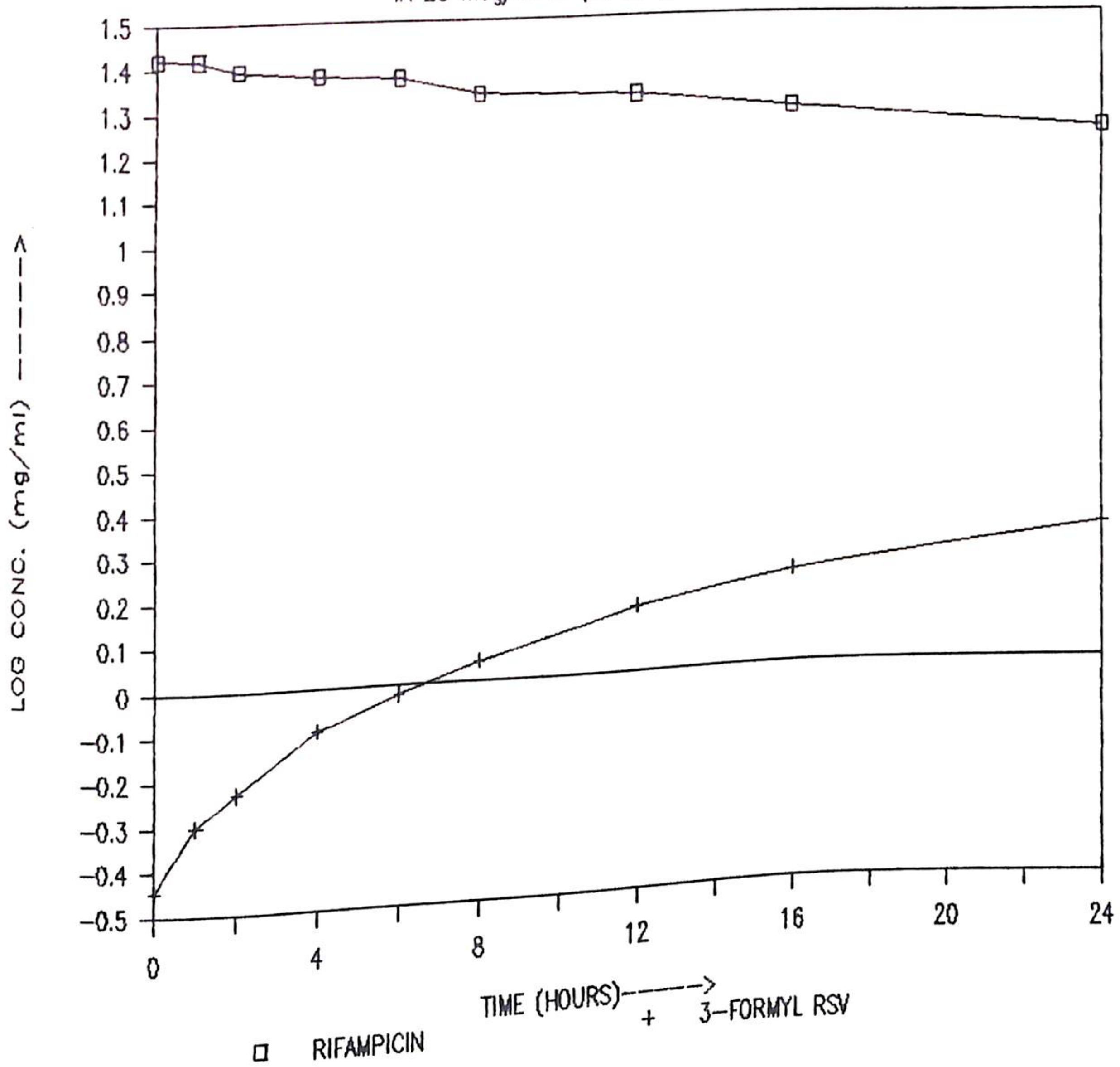


FIG 6.5H : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 8.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------>
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
0
4
8
12
16
20
24
TIME (HOURS)------>
RIFAMPICIN
3-FORMYL RSV

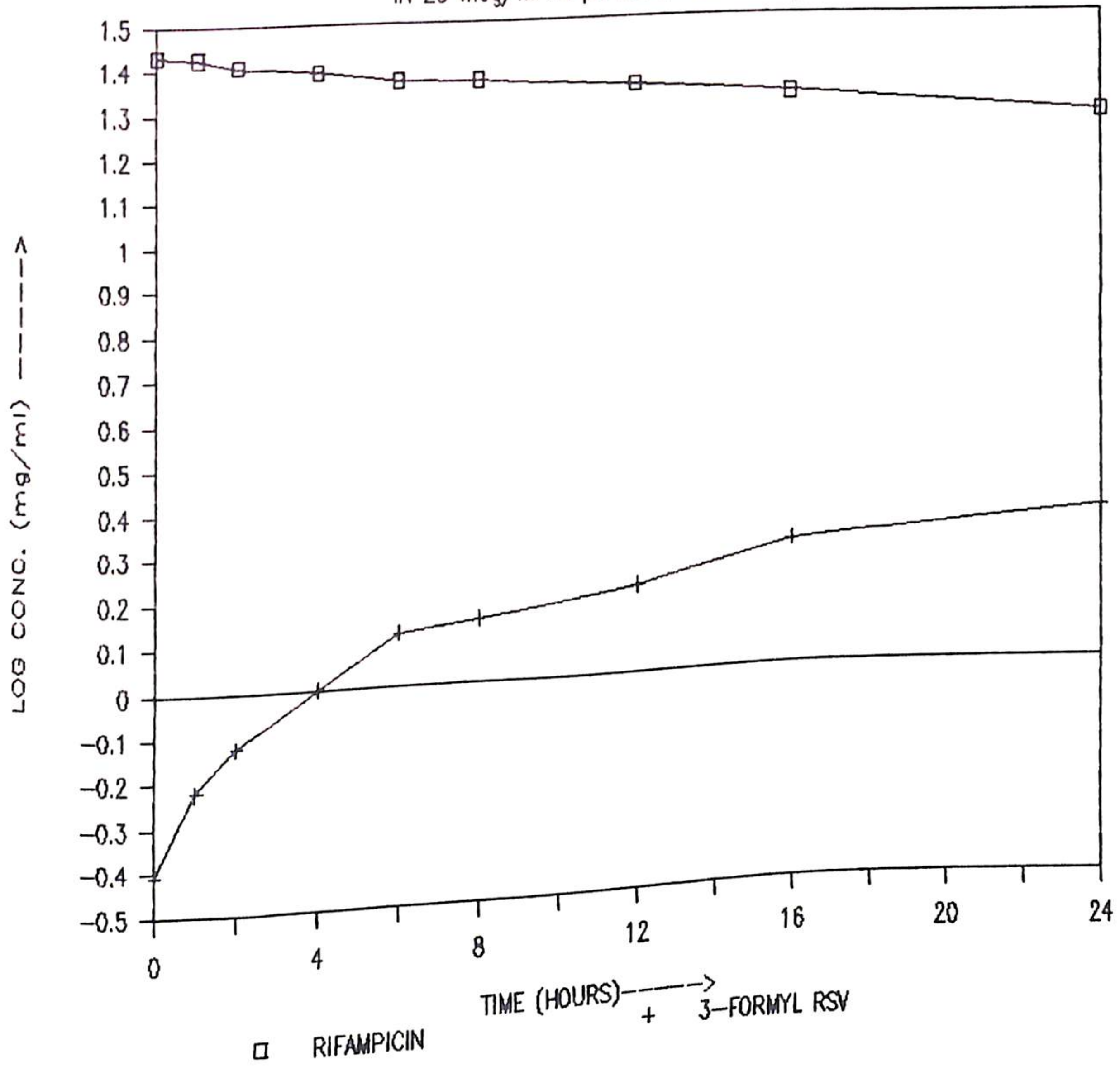


FIG 6.5I : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 9.0 (UNBUFFERED)
LOG CONC. (mg/ml) ------->
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
0
4
8
12
16
20
24
TIME (HOURS)------->
RIFAMPICIN
3-FORMYL RSV

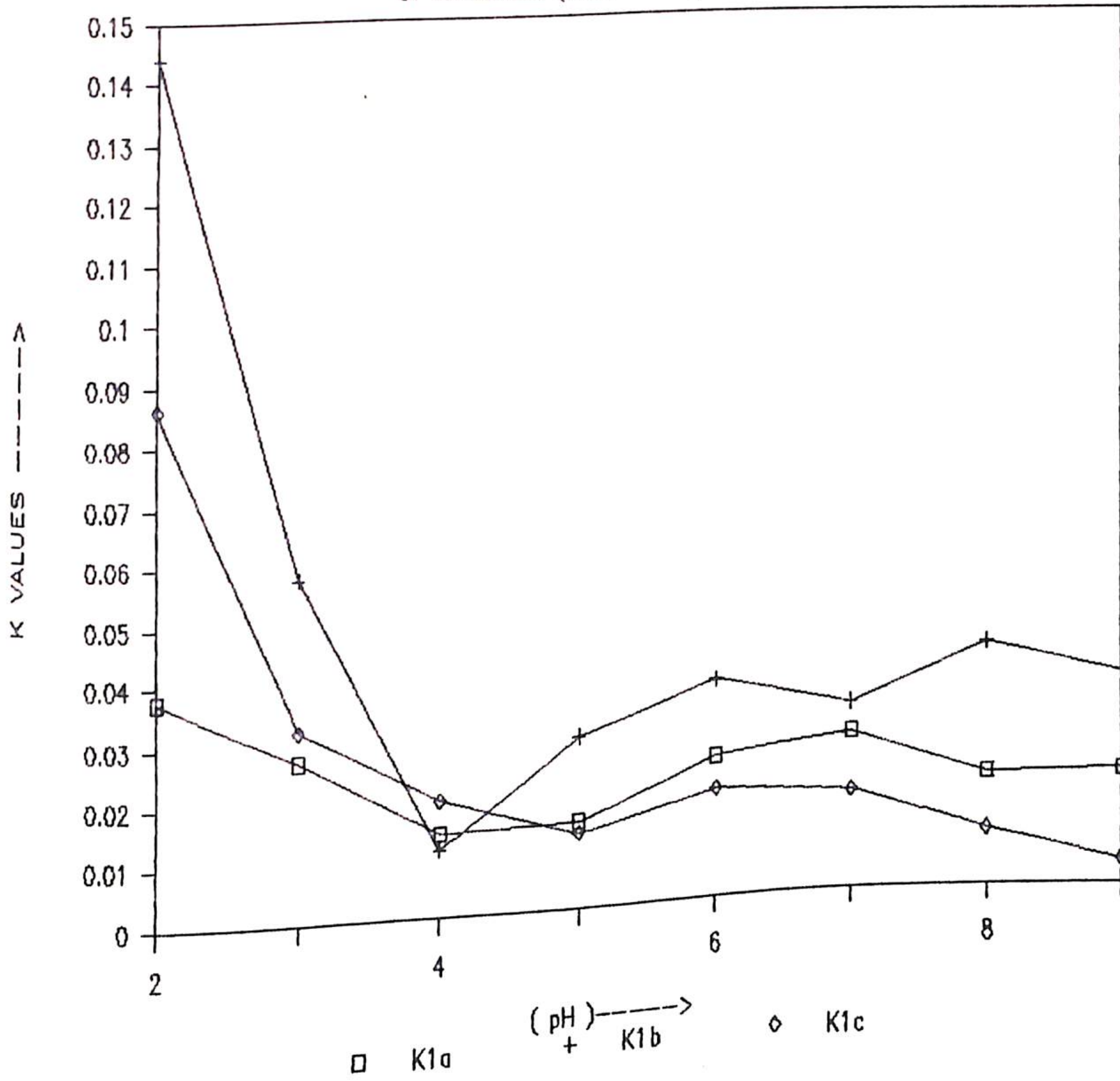


FIG 6.5J :DEGRADATION RATE CONSTANTS
OF RIFAMPICIN (UNBUFFERED) SOLUTIONS
K VALUES ------->
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
2
4
6
8
(pH)------->
□ K1a
+ K1b
◇ K1c

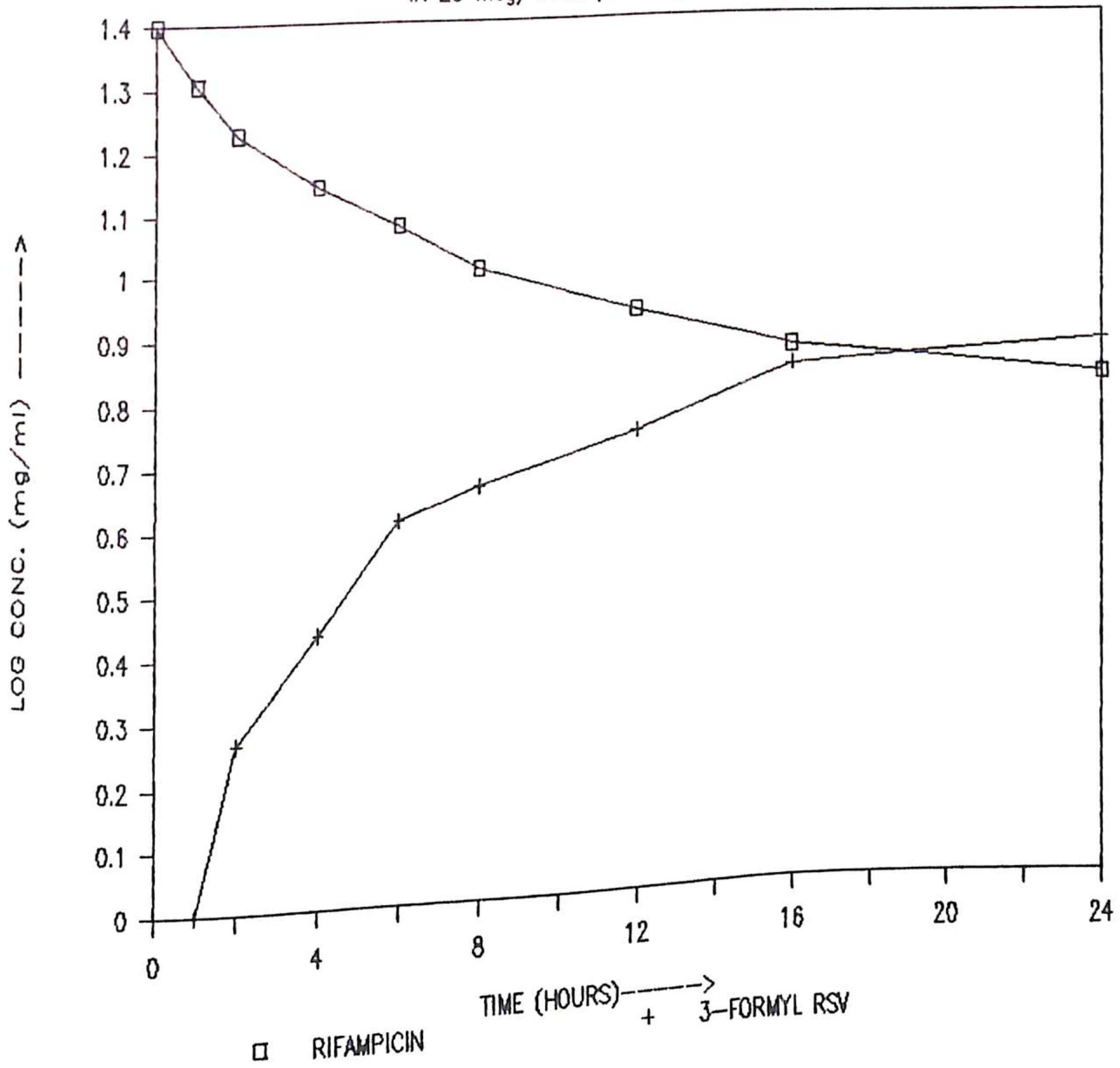


FIG 6.6A : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 3.0 (BUFFERED)
LOG CONC. (mg/ml) ------->
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
4
8
12
16
20
24
TIME (HOURS)------->
RIFAMPICIN
3-FORMYL RSV

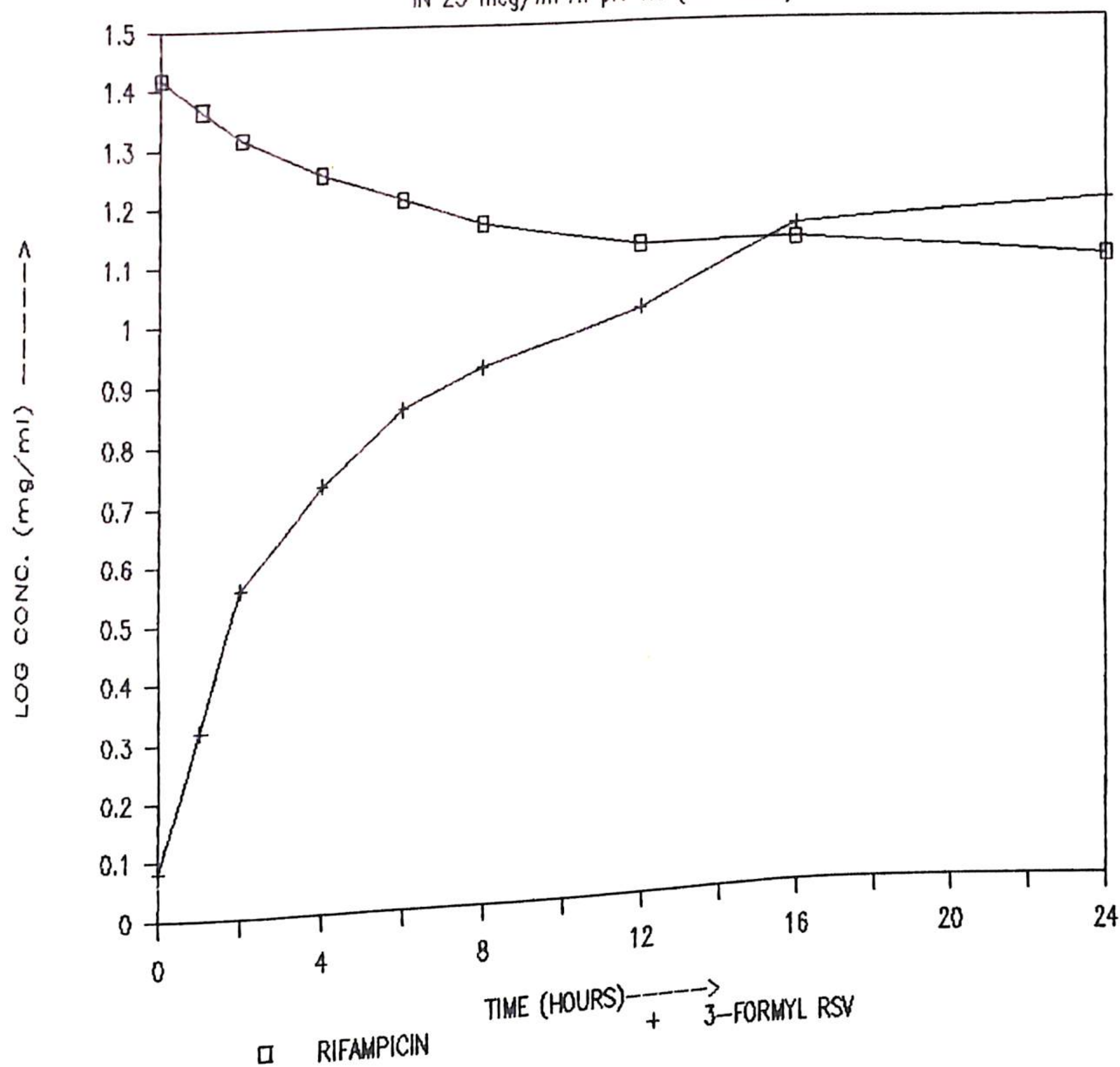


FIG 6.6B : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 4.0 (BUFFERED)
LOG CONC. (mg/ml) ------>
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
4
8
12
16
20
24
TIME (HOURS)------->
RIFAMPICIN
3-FORMYL RSV

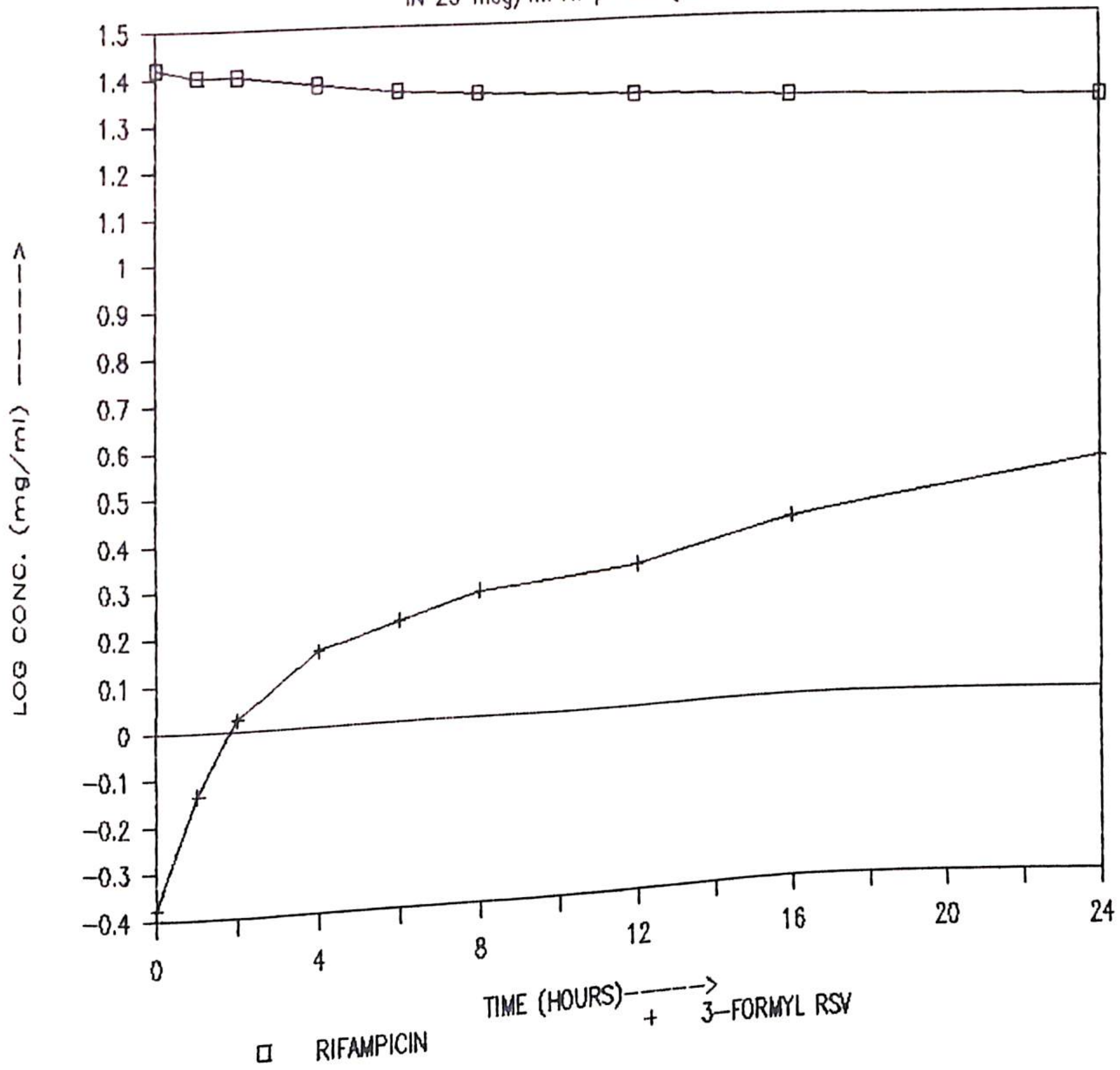


FIG 6.6C : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 5.0 (BUFFERED)
LOG CONC. (mg/ml) ------->
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0
4
8
12
16
20
24
TIME (HOURS)------->
RIFAMPICIN
3-FORMYL RSV

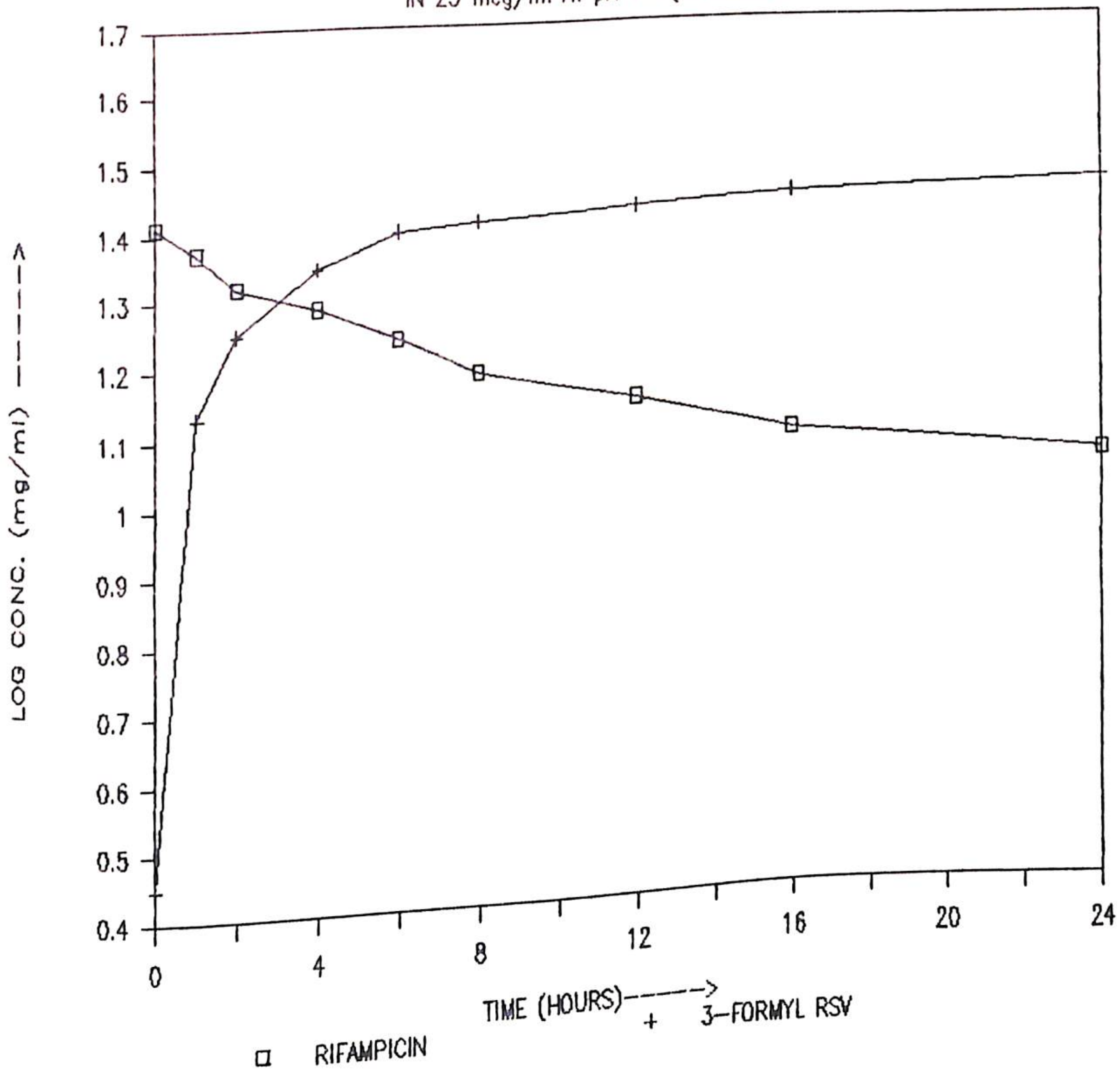
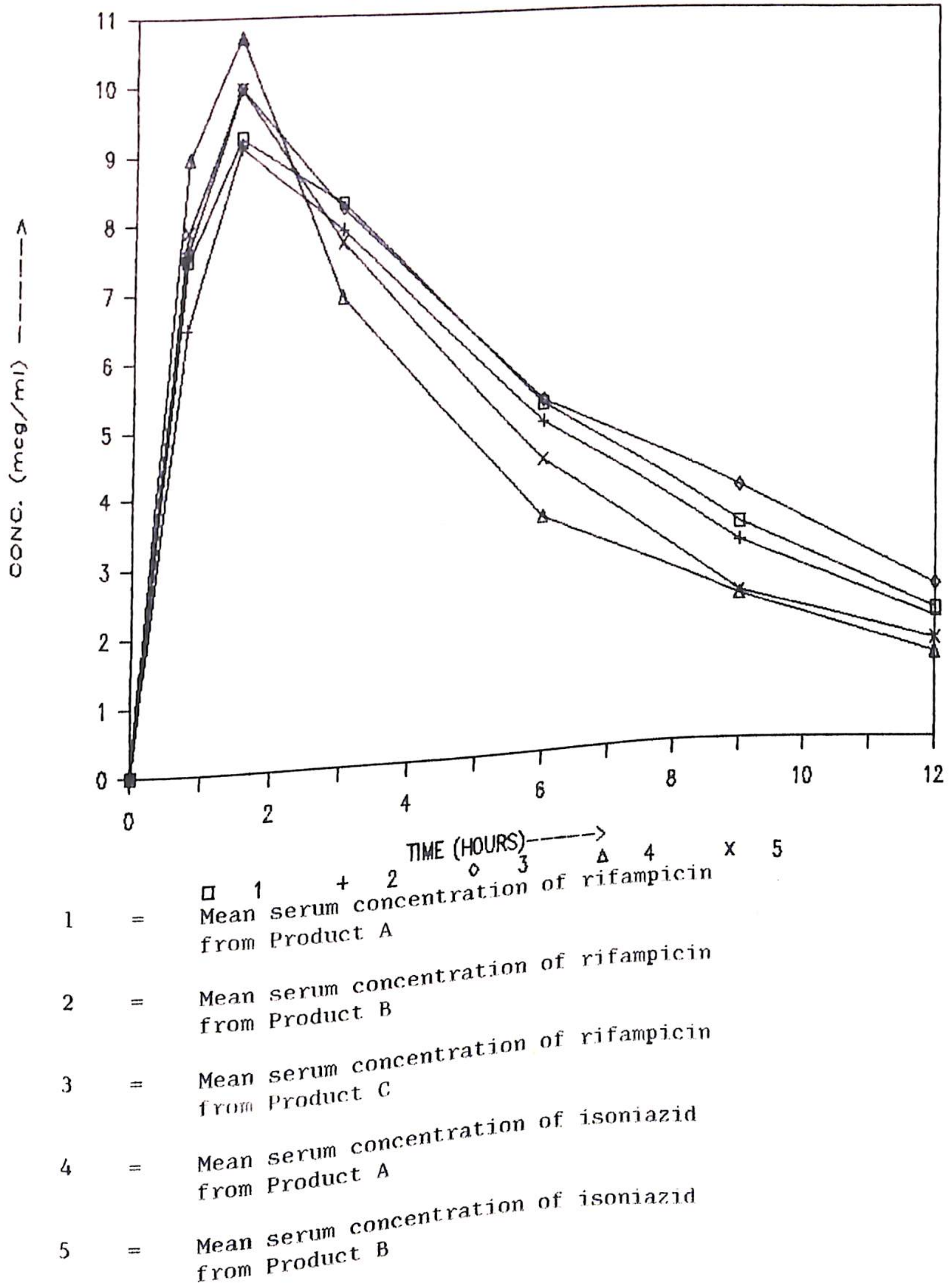


FIG 6.6D : RIFAMPICIN STABILITY
IN 25 mcg/ml AT pH 6.0 (BUFFERED)
LOG CONC. (mg/ml) ------->
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0
4
8
12
16
20
24
TIME (HOURS)------->
□ RIFAMPICIN
+ 3-FORMYL RSV

FIG 6.7A : SERUM CONCENTRATIONS

OF DRUGS FROM PRODUCTS A, B AND C



RESULTS AND DISCUSSION

6.1 Drug stability and preformulation study

The percent of rifampicin remaining was plotted against time (Figures 6.1A to 6.1U) and values of zero order degradation rate constants (Ko) computed from graphs are given in Table 6.1R. The Arrhenius plots were drawn using log Ko values versus 1/T (Figures 6.1RI to 6.1RIV) where T is the absolute temperature. The four temperature points used were 60°C, 45°C, RT (28°C) and FT (5°C). From the plots, Ko value at 30°C was computed and used for calculation of shelf life considering 90 % as the minimum potency at the end of shelf life (t90 %). The correlation coefficient (r), Ko and t90 % values at 30°C are summarised in Table 6.1R.

Rifampicin was found to be stable for over 6 years at 30°C in the absence of any excipient. Polyethylene glycol 4000 and urea had most deleterious effect on rifampicin, reducing its shelf life to less than 6 months while talc had no adverse effect. The excipients could be categorised for their adverse effect on rifampicin shelf life in solid state as given below :

Reduction in shelf life of rifampicin to :	Incompatibility	Excipients
less than 25 % (< 18 months)	Severe	PEG 4000, urea, docusate sodium, sodium starch glycolate, dicalcium phosphate, starch, polysorbate 80, magnesium stearate, sucrose, and sodium lauryl sulphate,
25 - 50 % (18 - 36 months)	Moderately severe	lactose, mannitol, gelatin, Aerosil 200, microcrystalline cellulose, cyclodextrin and poloxamer 188.

50 - 75 % (37 - 55 months)	Moderate	Povidone, bile salts
75 - 100 % (> 55 months)	Insignificant	Talc

The increase in degradation components/impurities is not same as decrease in rifampicin content. Certain degradation products like piperazine side chain are not detectable due to non absorbance in UV range.

To see the effect of high humidity (75 % RH) on samples the shelf life of samples at 75 % RH/40°C (actual) was compared with shelf-life at 40°C (computed from Arrhenius plots) and are given in Table 6.1S.

The high humidity had maximum adverse effect on samples containing polyethyline glycol 4000, urea, starch, sodium lauryl sulphate, polysorbate 80, bile salts and gelatin besides rifampicin as such. The reduction in shelf-life by high humidity was less than 50 % in these cases. All these excipients have tendency to pick up moisture which appears to be the cause of this accelerated degradation effect. Reduction in shelf-life by high humidity was 50-75 % of samples containing docusate sodium, povidone, sodium starch glycolate, cyclodextrin and poloxamer 188. High humidity had less effect on samples containing lactose, mannitol, sucrose and microcrystalline cellulose . Talc, mangesium stearate and dicalcium phosphate appear to protect the drug from humidity. Talc and magnesium stearate had maximum protective effect probably due to their hydrophobic nature.

The above effects have been observed in solid state and may not be necessarily applicable to drug's stability in liquid dosage forms because localised concentration of some excipients like urea, sodium starch glycolate, docusate sodium etc may provide extremely adverse environment. The ingredients like polysorbate 80 and sucrose have been found to be compatible in oral suspensions. The initial high content of moisture (> 4 %) in mixtures containing starch, lactose, gelatin and sodium starch glycolate may also be contributing factor for drug degradation. Drug has been found to be compatible with starch and lactose when used after drying.

The UV scans of drug-excipient physical mixtures do not show shift in absorbance peaks or relative absorbance, indicating absence of any chemical interaction between rifampicin and excipients tested.

No significant change in physical description of samples was observed in samples except the ones containing polyethylene glycol and urea at 40°C/75 % RH and 60°C. This is due to low melting and hygroscopic nature of these excipients.

6.2 Effect of excipients on rifampicin solubility

The solubility data indicates that some of the ingredients (in the concentration used) enhance the solubility of rifampicin while some others retard it. Considering over 20 % difference in Cmax (maximum concentration of drug achieved in 20 h) as significant, the following conclusions could be drawn :

<u>The excipients having significant positive effect on rifampicin solubility</u> : sodium lauryl sulphate > urea > talc > polyethylene glycol 4000 > docusate sodium > bile salts.

<u>The excipients having significant negative effect on rifampicin solubility</u> : polysorbate 80 > Aerosil 200 > sodium starch glycolate > dicalcium phosphate > mannitol.

<u>The excipients having insignificant effect (< 20 %)</u> : starch, microcrystalline cellulose, sucrose, lactose, povidone K30, gelatin and magnesium stearate.

6.3 Effect of UV/Visible light on rifampicin stability

A. <u>Rifampicin powder</u> :

Neither UV light nor visible light had any significant adverse effect on stability of rifampicin in dry state, as the degradation rate constant values were insignificant.

B. <u>Rifampicin solution</u> :

The concentration versus time plots are drawn on semilog scale (Figures 6.3E to 6.3H). The first order degradation rate constant and t90% values are tabulated in Table 6.4D. The following conclusions could be drawn from the data :

- Both UV and visible light had significant degradation effect on rifampicin stability.

- The degradation by UV light of 254 nm wavelength was 5 times compared to UV light of 366 nm wavelength.
- The degradation effect of UV light of 254nm wavelength was same as that of daylight. Both reduced the shelf life to less than half as compared to samples stored in darkness.
- Keeping sensitivity of drug in view the liquid products should be protected from light.

6.4 Effect of oxygen on rifampicin stability

The concentration versus time plots are drawn on semilog scale (Figures 6.4A to 6.4C). The first order degradation rate constant and t90 % values are tabulated in Table 6.4D.

Oxygen had degradation effect on rifampicin solution. Shelf life (t90 %) was reduced by less than half in presence of oxygen. For the same reason flushing with nitrogen had stabilising effect on solutions. Since the drug is sensitive to oxygen, it should be protected by storing under nitrogen atmosphere and flushing nitrogen through its dosage forms.

6.5 Effect of pH on stability of rifampicin in aqueous solutions

<u>1000 mcg/ml solution</u> :

The values of rifampicin concentration remaining vs time were plotted on semi-log scale (Figures 6.5AI to 6.5AIII). The log concentration-time plot was linear for first 6 hours. The slope of

first 6 hours straight part of curve was computed by regression and degradation rates were calculated using pseudo-first order kinetics. The values of apparent degradation rate constant (K1a) are given in Table 6.5D.

Rifampicin was found to be unstable in highly acidic solutions and maximum stable at pH 4.0. The degradation at pH 2.0 and 3.0 was 2.7 and 2 times respectively compared to pH 4.0. The difference in degradation rates at pH 4.0 and 5.0 was insignificant.

25 mcg/ml solutions :

Figures 6.5B to 6.5I present semilog graphs of rifampicin concentration remaining and concentration of its main degradation product 3-formyl rifamycin SV versus time. The rifampicin concentration plots are biphasic. First 6 h and last 12 h (12 - 24 hours period) plots are almost straight following first order kinetics. The degradation rate constants for first 6 h (K1b) and 12 - 24 h (K1c) part of the plots are given in Table 6.5D.

The degradation rate constants (K1a, K1b and K1c) are plotted against pH in Figure 6.5J.

The pH stability profile of rifampicin in 25 mcg/ml solutions is similar to 1000 mg/ml solutions as discussed above. Solutions were maximum stable at pH 4.0 and minimum stable at pH 2.0. Degradation rate was 13 times and 5 times at pH 2.0 and 3.0 respectively compared to pH 4.0.

Effect of concentration :

The degradation in 25 mcg/ml concentration solutions was higher than 1000 mcg/ml solutions except at pH 4.0 where degradation is minimum for both concentrations. The higher degradation in diluted solutions is probably due to sufficient oxygen available to degrade limited amount of drug. From the above data the pH 4.0 - 5.0 could be recommended to get stable liquid products.

6.6 Effect of buffering agents on the stability of rifampicin

The concentrations of rifampicin and 3-formyl rifamycin SV were plotted against time on a semi-log scale (Figures 6.6A to 6.6D).

The first 6 h and last 12 h part of rifampicin concentration versus time plots were found to be linear. From the plots the degradation rate constants for first 6 h curve (K1b) and 12-24 h part of curve (K1c) were computed. The values are given in Table 6.6B.

The following conclusions could be made by comparing initial degradation rate constants of buffered and unbuffered, rifampicin solutions (25 mcg/ml) as given in Table 6.5D and 6.6B.

- Acetate buffer has maximum adverse effect on rifampicin stability. Degradation was over 10 times in acetate buffered solutions.
- Chloroacetate and phosphate buffers degrade the drug 3 times faster.
- Formate buffer has insignificant adverse effect.
- The adverse effect of these buffers is maximum for initial 6-8 hours and then decreases gradually.

6.7 Effect of rifampicin-isoniazid incompatibility on their bioavailability

The mean serum concentrations of rifampicin and isoniazid are plotted against time in Figure 6.7A. The comparative dissolution percent (DP30) and dissolution efficiency (DE30) values and pharmacokinetic parameters for Products A, B and C are summarised in Table 6.7G. Product B is poor in dissolution. The DE30 and DP30 values are significantly less than similar product (Product A). The dissolution behaviour of Products A and C is similar and therefore can be compared. There is no significant difference in values of AUC, Cmax and Tmax of rifampicin when administered alone (Product C) or in presence of isoniazid (Product A). We can conclude that isoniazid has no adverse effect on rifampicin bioavailability.

EXPERIMENTAL-IV

BIOAVAILABILITY ENHANCEMENT

CHAPTER-7

7.1 EFFECT OF PARTICLE SIZE ON THE DISSOLUTION RATE AND BIOAVAILABILITY OF DRUG

Preparation of samples

Rifampicin was size fractioned through sieves 30, 50, 100, 200 and 400 mesh ASTM. The particle size of 400 mesh fraction was determined microscopically and the average particle size was found to be 10 um.

The following fractions were collected.

Fraction	Sieve No.	Sieve opening	Mean Particle Size
A	30 - 50	(300 - 600 um)	450.0 um
B	50 - 100	(300 - 75 um)	187.5 um
C	100 - 200	(75 - 150 um)	112.5 um
D	less than 400	< 1 - 20 um (by microscopy)	10.0 um

Fractions A, B, C and D were filled in hard gelatin capsules (450 mg rifampicin per capsule) and were labelled as samples, A, B, C and D respectively.

Dissolution rate

The dissolution rate of samples was determined using both 0.1 N hydrochloric acid (USP medium) and 0.4 % w/v sodium lauryl sulphate solution in water, so as to determine the methodology which gives better correlation with *in vivo* absorption data. Each beaker contained 900 ml medium at 37 ± 0.5°C and basket speed was 50 rpm. 10 ml samples were withdrawn at intervals of 10, 20, 30, 45, 60 and 120 minutes and replaced with equal volume of dissolution medium. 12 units of each sample were tested for dissolution rate in each medium.

The samples were filtered through Whatman No 1 filter paper. 2 ml of filtrate was diluted to 25 ml with mobile phase and analysed by HPLC method as described under section 4.1 of Experimental-I. The results of dissolution rate test of various size fractions are given in Table 7.1A.

Bioavailability study

A total of twelve volunteers participated in the study. The volunteers were selected as per the selection criteria discussed under Experimental Design (Chapter 3). Four volunteers each were given sample A, C and D in Latin square design, after seven days of washout period each time. The volunteers were randomly assigned to these treatment groups. A single dose of rifampicin 450mg was given orally with 200 ml water on one occasion. A standard meal schedule was given starting approximately four hours after dosing. Blood samples (10 ml each) were drawn and collected in tubes at 0.0, 0.75, 1.5, 3.0, 6.0, 9.0 and 12.0 h after the drug administration. Serum was separated immediately by centrifugation and stored at - 15°C till analysed.

Serum samples were analysed by standard HPLC technique as described under section 4.4 of Experimental-I.

The results are given in Table 7.1B to 7.1D.

7.2 EFFECT OF VARIOUS CONCENTRATIONS OF COMMONLY USED SURFACTANTS ON DISSOLUTION RATE OF RIFAMPICIN

The dissolution rate of rifampicin was determined in the aqueous solutions of different concentrations (0.1, 0.2, 0.5, 1.0, 2.0 % w/v) of polysorbate 80, sodium lauryl sulphate, bile salts, docusate sodium

and poloxamer 188 as dissolution media. The test was performed using 900 ml dissolution medium maintained at 37 ± 0.5°C in each beaker of USP type-II apparatus, operating at 50 rpm. 10 g drug was added to each beaker. 5 ml samples were withdrawn at predetermined intervals of 15, 30, 45 and 60 minutes, and were analysed by standard HPLC method as given under section 4.1 of Experimental-I.

The data is given in Tables 7.2A to 7.2E.

7.3. EFFECT OF SURFACE ACTIVE AGENTS ON THE PARTITION OF RIFAMPICIN

Partition coefficient of rifampicin was checked between water and n-octanol (saturated with distilled water overnight). Distilled water demineralised through Milli-Q water purification system was boiled, cooled and flushed with nitrogen for 5 min. Accurately weighed (200 mg) rifampicin was dissolved in n-octanol and volume was made up to 100 ml with the same. 10 ml of this solution was transferred along with an equal volume of water in a 50 ml flask. 15 such flasks were fitted on a flask shaker maintained at 37°± 1°C. Triplicate units were removed at intervals of 1, 2, 3, 4, 8, 16 and 24 h. The contents of flasks were transferred into a centrifuge tube and centrifuged for 10 min at 4000 rpm. 2 ml of aqueous layer was diluted to 25 ml with mobile phase in a 25ml volumetric flask and analysed by standard HPLC method as given under section 4.1 Experimental-I. The 2 h samples yielded maximum concentration of rifampicin in aqueous layer. The partition coefficient of rifampicin in n-octanol-water system at this stage was 21.68.

$$\text{Partition coefficient } (p\ o/w) = \frac{\text{conc. of rifampicin in n-octanol layer}}{\text{conc. of rifampicin in aqueous layer}}$$

Solutions of sodium lauryl sulphate, polysorbate 80, docusate sodium, bile salts and poloxamer 188 were prepared in concentrations of 0.1, 0.2, 0.5, 1.0, 2.0 %w/v in water. 10 ml of rifampicin solution in n-octanol was shaken with an equal volume of surfactant solution on a bath shaker maintained at 37 ± 1°C. After 2 h the contents of flask were centrifuged to separate clear aqueous layer. The aqueous layer was analysed as discussed earlier. All experiments were conducted in triplicate.

The average of three partition coefficient values for each concentration of surfactants are given in Table 7.3A.

7.4. EFFECT OF SURFACTANTS ON BIOAVAILABILITY OF RIFAMPICIN IN RATS

In situ rat intestine model

Albino rats weighing 130-170 g were fasted overnight. Water was allowed *et libidum*. Animals were anaesthetised by intraperitoneal injection of 40 mg sodium pentobarbitone per kg of body weight. Abdomen was opened from midline and intestine was located. The small intestine was punctured at a place 1 cm further to bile duct entry, since rifampicin is known to enter hepato-biliary recirculation and a plastic cannula was inserted. Similarly another cannula was inserted by puncturing intestine 2-3 cm short of colon end. The thread knots were given over the inserted cannula so as to fix the cannulas and to prevent flow of liquid towards stomach or colon. The intestine was subjected to minimum disturbance so as to minimise disruption of mesentry.

A 10 ml syringe was inserted into open end of cannula (stomach end) and intestine was flushed with normal saline maintained at 37°C till the intestine was free from extraneous matter. The water was removed from intestine as far as possible by injecting air from stomach end. The intestine was put back into abdominal sac and partly closed, to maintain it at body temperature.

Rifampicin recovery study from intestine

5.0 ml aqueous solution of rifampicin (0.5 mg/ml) maintained at 37°C was injected into intestine and flushed out immediately to assess the recovery of rifampicin. The solution was collected in a 50 ml volumetric flask from colon end by injecting air from stomach end. The intestine was washed with about 30 ml water so as to remove the complete drug from the intestine and washings were collected in volumetric flask. The volume was made upto 50 ml with water and analysed for rifampicin content. It was found that rifampicin was almost completely recovered from intestine (98 - 99 %).

Absorption study

Rifampicin solutions (0.5 mg/ml) were prepared in aqueous solution of different concentrations of surface active agents. The solutions were brought to 37°C before injection. 5 ml of solution was injected into the intestine from stomach end. After 30 minutes the solution was collected in a 50 ml volumetric flask from colon end as described above and analysed for drug content.

Analysis of intestinal fluid samples

Above solution was filtered through Whatman No 1 filter paper and centrifuged to remove any colloidal suspended matter. 200 ul of the clear solution was directly injected into the column and analysed by standard HPLC method (refer : section 4.4 of Experimental-I).

Blood sample analysis

Immediately after collection of solution from intestine the abdomen was opened again and blood sample was collected from the portal vein and analysed by HPLC method as described under section 4.4 of Experimental-I.

All experiments were conducted in triplicate and the average values are given in Table 7.4A.

7.5 EFFECT OF DIFFERENT CONCENTRATIONS OF POLYSORBATE 80 ON BIOAVAILABILITY OF RIFAMPICIN

Preparation of samples :

Four lots of rifampicin suspension were prepared in simple syrup IP (100 mg/5ml). Sufficient quantity of polysorbate 80 was dissolved in above suspensions to get 0.25 % w/v (Product B), 1.0 % w/v (Product C) and 2.5 % w/v (Product D) concentration. No polysorbate 80 was added to fourth lot (Product A, control).

Procedure :

Six volunteers selected as per the selection criteria discussed under Experimental Design (chapter 3) participated in the study. In

the first part of study, three volunteers each were given Product A and Product B. After a wash out period of 7 days the products were crossed over. Second part of study was conducted using Products C and D on same volunteers after a gap of 10 days. Three volunteers each were given Product C and Product D and crossed over after a week.

A single dose of each product was given orally with 200 ml water on one occasion. A standard meal schedule was given which started 4 h after administration of drug. Blood samples (10.0 ml) were with drawn and collected in tubes at 0.0, 0.75, 1.5, 3.0, 6.0, 9.0 and 12.0 h after the drug administration. Serum was separated immediately by centrifugation and stored at - 15°C till analysed. Samples were analysed by standard HPLC method (refer: section 4.4 of Experimental-I).

The results of the study are given in Tables 7.5A to 7.5D.

7.6 EFFECT OF AMORPHOUS AND POLYMORPHIC FORMS OF RIFAMPICIN ON ITS DISSOLUTION RATE AND BIOAVAILABILITY

Preparatin of samples

Amorphous and two crystalline forms of drug were filled in hard gelatin capsules (450 mg rifampicin per capsule) and labelled as Product X (polymorph-A), Product Y (amorphous) and Product Z (polymorph B).

Dissolution rate profile

The dissolution rate was checked using 0.4 % w/v sodium lauryl sulphate solution and same test conditions as described under section 4.3 of Experimental-I.

10 ml samples were withdrawn at intervals of 5, 10, 20, 30, 45, 60, 90 and 120 minutes and suitably diluted with mobile phase. The diluted solution was chromatographed using HPLC system mentioned earlier under section 4.1 of Experimental-I.

The results are given in Table 7.6A.

Bioavailability

The bioavailability study was conducted in two sets. In the first set amorphous rifampicin (Product Y) was compared with polymorph A (Product X) and in the second set polymorph B (Product Z) was compared with polymorph A.

A total of six volunteers participated in one study. Three volunteers each were given Products X and Y in first set and Products X and Z in second set. After seven days of washout period the groups were crossedover for samples. A single dose of rifampicin 450 mg (one capsule) was given orally with 200 ml water on one occasion. Blood samples (10 ml) were drawn and collected in tubes at 0.0, 0.5, 1.0, 1.5, 3.0, 6.0, 9.0 and 12.0 h after the drug administration. Serum was separated immediately by centrifugation and stored at -15°C till analysed. Serum samples were analysed by standard HPLC technique described under section 4.4 of Experimental-I.

The data obtained after analysis for each volunteer was used to calculate the pharmacokinetic parameters. The results are given in Tables 7.6B to 7.6E.

7.7 EFFECT OF CYCLODEXTRINS ON RIFAMPICIN DISSOLUTION

The dissolution of rifampicin was studied with beta-cyclodextrin, alpha-cyclodextrin, gamma-cyclodextrin and hydroxypropyl beta-cyclodextrin in 1:1 stoichiometric ratio at pH 4.0 and 7.9.

0.01 M solutions of cyclodextrins were prepared in distilled water having 0.1 M concentration of sodium chloride and adjusted to pH 4.0 and 7.9 with hydrochloric acid or sodium hydroxide. The solutions were transferred to flasks (60 ml solution in each flask) and kept in constant temperature water bath maintained at 45 ± 1°C. Calculated quantity of rifampicin was added to each flask to get 0.01 M concentration. The shaker was put on at 30 rpm.

2 ml samples were withdrawn at 1, 2, 3, 4, 5 and 6 h intervals and analysed by standard HPLC method (refer : section 4.1 of Experimental-I). All experiments were conducted in duplicate and the averages of two sets are given in Table 7.7A.

7.8 EFFECT OF POVIDONES ON RIFAMPICIN DISSOLUTION

The dissolution of rifampicin was studied in the presence of 0.5%, 1.0 %, 2.0 %, 4.0 % and 5.0 % w/v concentrations each of K-30 (mol. weight 40000), K-90 (mol. weight 360000), and K-120 grades of povidone at pH 4.0 and 7.9.

0.01 M solutions of povidones were prepared in distilled water, having 0.1 M concentration of sodium chloride and adjusted to pH 4.0 and 7.9 with hydrochloric acid or sodium hydroxide. The solutions were transferred to flasks (60 ml solution in each flask) and kept in

constant temperature water bath maintained at 45 ± 1°C. Calculated quantity of rifampicin was added to each flask to get 0.01 M concentration. The flasks were shaken at 30 rpm.

2 ml samples were withdrawn at 1, 2, 3, 4, 5 and 6 h intervals. Samples were analysed by HPLC method as described under section 4.1 of Experimental-I.

All experiments were conducted in duplicate and the averages of two sets are reported in Tables 7.8A to 7.8C.

7.9 PREPARATION AND STUDY OF SOLID SURFACE DISPERSIONS OF RIFAMPICIN

In the first part of study the solid surface dispersions were prepared having rifampicin excipient ratio of 1:2 using talc, maize starch, microcrystalline cellulose, lactose and colloidal silicon dioxide as adsorbents.

Rifampicin was dissolved in chloroform and filtered. Adsorbent (microcrystalline cellulose, talc, starch, lactose and Aerosil 200), in required quantity was added to rifampicin solution and stirred to form a slurry. The chloroform was used as solvent as it has good solubility for rifampicin but poor for the carriers. Chloroform was evaporated from rifampicin-adsorbent slurry by pouring it on hot petriplate kept on water bath. Solvent was completely removed by drying to constant weight in vacuum at 45°C. The dried mass was passed through 60 mesh. The fraction passing through 60 mesh and retained over 100 mesh was collected and stored in air tight containers The surface dispersion were analysed for drug content by the HPLC method discussed under section 4.1 of Experimental-I.

Dissolution rate

The dissolution profile of all surface dispersions alongwith that of pure rifampicin was performed using USP type-II dissolution test apparatus with paddle speed of 50 rpm.

Rifampicin - adsorbent mixture equivalent to 4.50 g of rifampicin was added to beakers of dissolution test apparatus containing 900 ml distilled water at 37 ± 0.5°C. Samples of 3 ml were withdrawn with cotton tipped pipette at predetermined time intervals of 10, 20, 30, 40, 60, 90, 120, 180, 240 minutes. The samples were filtered and analysed by standard HPLC method as described under section 4.1 of Experimental-I.

The dissolution rate of all solid dispersions was conducted in duplicate. The averages of two values are given in Table 7.9A.

Highest concentration of rifampicin was achieved from surface dispersion of talc among dispersions having drug : excipient ratio of 1:2.

In the second part of study, surface dispersions of talc were prepared with the drug : excipient ratios of 1:1 and 1:4. The dissolution profiles of all these dispersions are also given in Table 7.9A.

Bioavailability

The surface dispersion exhibiting optimum dissolution rate (drug: talc ratio of 1:2) was selected and studied for bioavailability alongwith best surface dispersion. The study details are discussed in section 7.10 of Experimental-IV.

7.10 PREPARATION AND STUDY OF SOLID DISPERSIONS

Povidone K-30, mannitol and polyethylene glycol 4000 were used as carriers. Mannitol was selected among sugars because unlike other sugars, mannitol does not decompose upto 250°C although it melts at 167°C. Dispersions were prepared using drug : carrier ratio of 1:3 and evaluated for dissolution rate and other tests.

Preparation :

I. Povidone K-30 :

The drug and povidone K30 were dissolved in chloroform. The solvent was evaporated off on a thermostatic plate at 40-45°C.

II. Mannitol :

Mannitol was melted by heating to 170°C in a S.S.container over a silicone oil bath. To the melted mannitol, calculated quantity of rifampicin was added and mixed thoroughly for 20-30 seconds followed by quick cooling.

III. Polyethylene glycol 4000 :

Solid dispersions with polyethylene glycol 4000 were prepared by both solvent and fusion methods. 0.01 % butylated hydroxytoluene (BHT) was used as antioxidant to neutralise any organic peroxides present in polyethylene glycol 4000 which otherwise may degrade oxidation sensitive rifampicin.

Solvent method :

Accurately weighed polyethylene glycol 4000 was first melted on a thermostatic plate at 80°C and drug solution in chloroform was added

under stirring. The solution was stirred till bubbles of solvent ceased to evolve from the solution and then cooled by keeping in cold water for 1 h.

Melt method :

Polyethylene glycol 4000 was melted by heating on a silicone oil bath and butylated hydroxytoluene was dissolved in it. To the melted polyethylene glycol 4000, calculated quantity of rifampicin was added and quickly mixed thoroughly for 20-30 seconds and then quickly cooled.

All the dispersions were passed through 52 mesh sieve. The fraction passing through 52 mesh and retained over 100 mesh (52-100 mesh) were collected and dried in vacuum desiccator for 48 h so as to remove any traces of solvent or moisture. The dispersion was stored in air tight containers. These dispersions were analysed for rifampicin content by the method discussed under section 4.1 of Experimental-I.

Dissolution rate

USP Type II dissolution test apparatus was used with paddle speed of 50 rpm. 900 ml distilled water maintained at 37 $\pm$ 0.5°C was used as dissolution medium. Solid dispersion equivalent to 20 g rifampicin for solid dispersion of povidone K30 and equivalent to 4.50 g of rifampicin for other dispersions and rifampicin (as such) was added in each of 2 beakers containing dissolution test medium. Samples of 3 ml were withdrawn with cotton tipped pippete at intervals of 10, 20, 30, 40, 50, 60, 90, 120 and 180 minutes. The sample was filtered and analysed by standard HPLC method (refer : section 4.1 of Experimental-

I). The sample volume was replaced with distilled water. The data is given in Table 7.10A.

Bioavailability

Among the carriers studied, povidone had best dissolution rate profile and was selected for bioavailability. Rifampicin-povidone K30 solid dispersion was studied alongwith the selected surface dispersion as discussed under section 7.9 of this Experimental-IV.

The calculated quantity of solid dispersion (rifampicin-PVP) and surface dispersion (rifampicin : talc, 1:2 ratio) were filled in hard gelatin capsules and labelled as Product A and Product B respectively. The number of capsules equivalent to 450 mg rifampicin were used as single dose. 450 mg rifampicin filled in capsules and labelled as Product C was used as control.

Two volunteers each were given Products A, B and C and crossed over in Latin square design after seven days washout period each time. The volunteers were randomly assigned to these treatment groups. The dose equivalent to 450 mg rifampicin was given orally with 200 ml water on one occassion. Blood samples (10 ml each) were withdrawn and collected in centrifuge tubes at 0.0, 0.75, 1.5, 3.0, 6.0, 9.0 and 12.0 h after the drug administration. Serum was separated immediately by centrifugation and stored at -15°C till analysed. Samples were analysed by HPLC method described under section 4.4 of Experimental-I.

The results are given in Tables 7.10C to 7.10E.

Table 7.1A : Dissolution rate profile of rifampicin from various size fractions in Ø.1 N HCl and Ø.4 % w/v SLS solutions

Time (min)	Rifampicin dissolution (%) from sample							
	A		B		C		D	
	Ø.1 N HCL	Ø.4 % SLS	Ø.1 N HCL	Ø.4 % SLS	Ø.1 N HCL	Ø.4 % SLS	Ø.1 N HCL	Ø.4 % SLS
Ø	ØØ.ØØ	ØØ.ØØ	ØØ.ØØ	ØØ.ØØ	ØØ.ØØ	ØØ.ØØ	ØØ.ØØ	ØØ.ØØ
1Ø	15.29	18.78	14.63	24.86	15.52	16.64	7.52	2.98
2Ø	62.66	37.32	48.8Ø	44.Ø7	5Ø.1Ø	37.12	6Ø.67	18.76
3Ø	74.73	56.6Ø	7Ø.17	6Ø.36	69.83	62.47	83.46	7Ø.2Ø
45	75.Ø1	7Ø.3Ø	79.73	72.Ø6	82.78	85.47	87.48	9Ø.56
6Ø	71.42	76.37	78.33	8Ø.59	8Ø.46	92.22	85.18	96.14
9Ø	67.5Ø	79.72	74.Ø6	84.11	76.94	94.86	8Ø.71	99.1Ø
12Ø	62.76	81.87	69.1Ø	86.2Ø	73.Ø6	95.32	75.Ø7	99.Ø7

Table 7.1B : Serum concentration and pharmacokinetic parameters of rifampicin from test sample A

Time (h)	Serum concentration (mcg / ml) in volunteer														
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	10.350	3.170	7.050	3.490	7.860	8.240	9.350	4.170	6.050	4.490	7.500	8.600	6.693	2.391	0.690
1.50	10.220	9.370	9.680	11.120	8.930	7.940	10.500	9.090	9.680	10.120	9.930	7.940	9.543	0.960	0.277
3.00	9.020	7.350	9.200	10.090	7.010	5.680	10.090	7.850	7.010	8.520	9.200	5.680	8.058	1.539	0.444
6.00	5.970	4.500	4.810	5.210	4.790	3.150	5.970	4.500	3.150	4.510	5.510	4.790	4.738	0.908	0.262
9.00	4.370	2.090	3.200	1.590	2.840	2.930	3.200	2.840	2.590	3.038	2.880	2.480	2.837	0.672	0.194
12.00	2.660	1.480	1.500	1.230	1.900	2.200	1.560	1.980	2.230	2.600	1.400	1.200	1.828	0.509	0.147
AUC 0-inf	95.315	59.757	70.601	65.335	70.552	67.431	78.898	68.944	69.288	80.182	71.459	58.441	71.350	9.877	2.851
Cmax	10.350	9.370	9.680	11.120	8.930	8.240	10.500	9.090	9.680	10.120	9.930	8.600	9.634	0.835	0.241
Tmax	0.750	1.500	1.500	1.500	1.500	0.750	1.500	1.500	1.500	1.500	1.500	0.750	1.313	0.339	0.098

Remark..: Units of Cmax = mcg/ml, Tmax = h, AUC = mcg.h/ml,

Table 7.1C : Serum concentration and pharmacokinetic parameters of rifampicin from test sample C

Time (h)	Serum concentration (mcg / ml) in volunteer														
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	10.380	4.170	7.280	3.490	7.500	9.280	10.880	3.670	3.280	7.250	9.530	7.490	7.017	2.763	0.798
1.50	10.200	10.370	10.120	11.240	8.940	8.240	10.200	10.120	8.240	11.240	8.940	10.370	9.852	1.029	0.297
3.00	9.210	7.850	9.350	10.310	7.120	6.100	9.120	7.350	10.310	7.210	6.100	9.850	8.323	1.551	0.448
6.00	4.500	4.910	5.310	4.790	3.520	5.001	4.310	4.790	5.520	4.003	3.910	5.500	4.672	0.641	0.185
9.00	2.130	3.150	1.580	2.820	3.000	2.810	2.700	3.150	2.582	2.410	1.630	3.020	2.582	0.546	0.158
12.00	1.530	1.510	1.250	2.000	2.200	1.950	1.250	1.710	2.200	2.100	1.850	1.330	1.740	0.360	0.104
AUC 0-inf	70.636	67.146	66.018	75.266	71.421	70.520	69.312	67.614	76.883	72.016	62.656	72.979	70.206	3.981	1.149
Cmax	10.380	10.370	10.120	11.240	8.940	9.280	10.880	10.120	10.310	11.240	9.530	10.370	10.232	0.714	0.206
Tmax	0.750	1.500	1.500	1.500	1.500	0.750	0.750	1.500	3.000	1.500	0.750	1.500	1.375	0.626	0.181

Remark..: Units of Cmax = mcg/ml, Tmax = h, AUC = mcg.h/ml,

Table 7.1D : Serum concentration and pharmacokinetic parameters of rifampicin from test sample D

Time (h)	Serum concentration (mcg / ml) in volunteer														
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	3.120	9.340	11.760	1.560	5.920	3.320	5.320	7.340	11.760	2.920	4.560	3.120	5.837	3.497	1.009
1.50	5.220	10.630	13.290	5.830	10.860	7.620	7.620	10.220	13.090	6.030	10.860	5.630	8.908	2.930	0.846
3.00	4.370	5.592	5.942	3.750	5.270	5.870	3.738	4.350	6.028	4.390	4.340	3.210	4.738	0.967	0.279
6.00	1.450	4.830	4.420	2.570	4.470	4.880	2.450	3.370	5.530	3.570	4.120	2.984	3.720	1.200	0.346
9.00	0.680	1.490	2.300	1.800	2.020	2.390	0.980	2.190	2.390	1.300	2.220	1.600	1.780	0.575	0.166
12.00	0.260	1.230	1.800	1.620	0.510	1.370	0.410	1.382	1.500	1.570	1.420	0.510	1.132	0.546	0.158
AUC 0-inf	25.654	59.661	73.566	49.077	50.715	56.609	33.548	56.390	72.825	49.558	57.992	32.908	51.542	14.914	4.305
Cmax	5.220	10.630	13.290	5.830	10.860	7.620	7.620	10.220	13.090	6.030	10.860	5.630	8.908	2.930	0.846
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: Units of Cmax = mcg/ml, Tmax = h, AUC = mcg.h/ml,

Table 7.1E : Comparative *in vitro* and *in vivo* parameters from different size fractions of rifampicin

Parameter	Products			r
	A	C	D	
DP30 in 0.1 N HCl solution	74.73	69.83	83.46	0.9154
DE30 in 0.1 N HCl solution	38.43	33.51	36.63	0.1020
DP30 in 0.4 % w/v SLS solution	56.60	62.47	70.20	0.9236
DE30 in 0.4 % w/v SLS solution	28.13	28.33	18.95	0.9975
AUC (mcg . h / ml)	71.350	70.206	51.542	
Cmax (mcg / ml)	9.634	10.232	8.908	
Tmax (h)	1.313	1.375	1.500	

NOTE : i) DP30 and DE30 are dissolution percentage and dissolution efficiency over first 30 minutes respectively.

ii) r is coefficient of correlation of particular parameter with AUC.

Table 7.2A : Amount of rifampicin dissolved (mg/ml) in different concentrations of polysorbate 80

Time (min)	Concentration of polysorbate 80 (% w/v)					
	0.0	0.1	0.2	0.5	1.0	2.0
15	0.13	0.79	1.01	0.94	0.97	1.92
30	0.17	1.09	0.92	0.89	0.85	1.16
45	0.37	1.26	0.54	0.52	0.54	0.70
60	0.38	1.28	0.31	0.37	0.38	0.57

Table 7.2B : Amount of rifampicin dissolved (mg/ml) in different concentrations of sodium lauryl sulphate

Time (min)	Concentration of sodium lauryl sulphate (% w/v)				
	0.1	0.2	0.5	1.0	2.0
15	1.20	1.68	6.13	6.86	8.71
30	0.80	1.15	6.66	7.15	9.34
45	0.34	1.85	7.27	7.69	9.31
60	0.28	2.25	7.84	7.84	9.36

Table 7.2C : Amount of rifampicin dissolved (mg/ml) in different concentrations of docusate sodium

Time (min)	Concentration of docusate sodium (% w/v)				
	0.1	0.2	0.5	1.0	2.0
15	0.23	1.03	1.45	2.09	3.95
30	0.44	1.26	2.29	3.14	4.51
45	0.44	1.34	2.79	3.66	4.97
60	0.67	1.48	2.88	4.04	5.45

Table 7.2D : Amount of rifampicin dissolved (mg/ml) in different concentrations of poloxamer 188

Time (min)	Concentration of poloxamer 188 (Lutrol F68) (% w/v)				
	0.1	0.2	0.5	1.0	2.0
15	0.67	0.70	0.80	0.87	1.02
30	0.83	0.86	0.82	0.88	1.17
45	0.85	0.92	0.82	0.87	1.21
60	0.88	0.98	0.83	0.84	1.20

Table 7.2E : Amount of rifampicin dissolved (mg/ml) in different concentrations of bile salts

Time (min)	Concentration of bile salts (% w/v)				
	0.1	0.2	0.5	1.0	2.0
15	1.02	1.25	1.69	2.65	4.76
30	1.27	1.49	2.08	2.87	4.93
45	1.28	1.56	2.29	3.01	5.31
60	1.33	1.64	2.32	3.14	5.59

Table 7.4A : Absorption of rifampicin from different concentrations of surfactants in albino rats (*in situ model*)

Surfactant	Conc. of surfactant (% w/v)	Amount of rifampicin				Serum conc. of rifampicin (mcg/ml)
		Injected (mg)	Recovered (mg)	Absorbed (mg)	Absorbed (%)	
Control (water)	0.0	2.500	1.762	0.738	29.53	3.54
Polysorbate 80	0.1	2.655	1.839	0.816	30.73	3.61
	0.25	2.610	1.760	0.850	32.57	3.98
	1.0	2.508	1.497	1.011	40.31	4.89
	2.5	2.625	1.190	1.435	54.67	7.11
Sodium lauryl sulphate	0.1	2.380	1.658	0.722	30.35	3.70
	0.25	2.440	1.652	0.788	32.31	3.84
	1.0	2.650	1.726	0.924	34.85	4.42
	2.5	2.400	1.481	0.919	38.30	5.85
Docusate sodium	0.1	2.510	1.764	0.746	29.71	3.52
	0.25	2.650	1.848	0.802	30.25	3.66
	1.0	2.720	1.867	0.853	31.36	3.69
	2.5	2.690	1.803	0.887	32.96	4.02
Poloxamer 188	0.1	2.724	1.908	0.816	29.94	3.60
	0.25	2.580	1.803	0.777	30.13	3.62
	1.0	2.642	1.843	0.799	30.23	3.69
	2.5	2.728	1.864	0.864	31.68	3.83
Bile salts	0.1	2.560	1.800	0.760	29.67	3.64
	0.25	2.654	1.829	0.825	31.10	3.76
	1.0	2.720	1.820	0.900	33.08	4.16
	2.5	2.680	1.728	0.952	35.51	5.29

Table 7.5A : Serum concentration and pharmacokinetic parameters of rifampicin from Product A (Rifampicin, without polysorbate 80)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	0.418	0.348	0.368	0.426	0.383	0.397	0.390	0.030	0.012
1.50	3.228	4.078	3.428	3.278	3.328	3.678	3.503	0.324	0.132
3.00	6.182	6.060	5.333	5.053	5.757	5.556	5.657	0.431	0.176
6.00	4.162	4.142	3.952	3.287	4.057	3.715	3.886	0.336	0.137
9.00	2.095	2.197	1.965	1.825	2.030	2.011	2.021	0.125	0.051
12.00	0.939	0.953	0.766	0.642	0.852	0.797	0.825	0.117	0.048
AUC 0-inf	41.818	42.822	37.833	34.034	39.799	38.417	39.121	3.145	1.284
Cmax	6.182	6.060	5.333	5.053	5.757	5.556	5.657	0.431	0.176
Tmax	3.000	3.000	3.000	3.000	3.000	3.000	3.000	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.5B : Serum concentration and pharmacokinetic parameters of rifampicin from Product B (Rifampicin, with 0.25 % polysorbate 80)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	1.208	1.278	1.368	1.098	1.288	1.188	1.238	0.094	0.038
1.50	5.452	5.592	5.792	5.912	5.622	5.752	5.687	0.164	0.067
3.00	7.718	7.408	7.298	7.158	7.508	7.283	7.396	0.198	0.081
6.00	4.833	5.023	5.303	5.043	5.068	5.033	5.051	0.150	0.061
9.00	2.653	2.573	2.793	2.993	2.723	2.783	2.753	0.144	0.059
12.00	1.315	1.495	1.676	0.375	1.495	0.935	1.215	0.482	0.197
AUC o-inf	54.897	56.350	59.495	49.116	57.147	51.932	54.823	3.755	1.533
Cmax	7.718	7.408	7.298	7.158	7.508	7.283	7.396	0.198	0.081
Tmax	3.000	3.000	3.000	3.000	3.000	3.000	3.000	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.5C : Serum concentration and pharmacokinetic parameters of rifampicin from product C (Rifampicin, with 1.0 % polysorbate 80)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	3.180	2.950	3.080	3.310	3.130	3.130	3.130	0.118	0.048
1.50	8.687	8.837	9.297	9.057	8.992	8.947	8.970	0.207	0.084
3.00	6.465	6.385	5.935	6.015	5.935	6.425	6.193	0.257	0.105
6.00	4.950	4.660	4.860	4.730	4.805	4.795	4.800	0.101	0.041
9.00	4.167	4.067	3.597	3.527	3.882	3.797	3.840	0.253	0.103
12.00	1.517	1.487	1.397	1.317	1.457	1.402	1.430	0.072	0.030
AUC o-inf	65.749	64.116	62.281	60.756	63.400	63.037	63.223	1.686	0.688
Cmax	8.687	8.837	9.297	9.057	8.992	8.947	8.970	0.207	0.084
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.5D : Serum concentration and pharmacokinetic parameters of rifampicin from Product D (Rifampicin, with 2.5 % polysorbate 80)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	2.130	2.460	2.220	2.360	2.175	2.410	2.293	0.136	0.055
1.50	9.980	10.580	10.240	9.960	10.110	10.270	10.190	0.230	0.094
3.00	7.980	9.860	9.980	9.120	8.980	9.490	9.235	0.730	0.298
6.00	5.456	6.123	6.221	5.386	5.838	6.254	5.880	0.385	0.157
9.00	3.987	3.263	3.118	3.031	3.552	3.147	3.350	0.361	0.147
12.00	1.680	1.753	1.946	1.844	1.813	1.798	1.806	0.089	0.036
AUC o-inf	71.333	75.855	77.289	71.894	74.298	75.341	74.335	2.325	0.949
Cmax	9.980	10.580	10.240	9.960	10.110	10.270	10.190	0.230	0.094
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.6A : Dissolution rate profile of amorphous and polymorphic forms of rifampicin

Time [Min]	Rifampicin dissolved (%)		
	Amorphous	(Polymorph A)	(Polymorph B)
0	0.00	0.00	0.00
5	0.00	7.70	17.17
10	0.65	16.45	76.13
20	38.12	46.15	84.94
30	62.89	65.09	93.63
45	86.38	81.05	97.40
60	93.50	91.39	98.44
90	96.10	95.22	99.04
120	98.54	97.89	99.73

Table 7.6B : Serum concentration and pharmacokinetic parameters of rifampicin from product X (Polymorph A) in first set

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.50	0.280	0.450	0.580	0.280	0.390	0.406	0.398	0.113	0.046
1.00	2.070	1.800	2.750	0.470	1.766	1.780	1.773	0.740	0.302
1.50	6.230	9.070	4.780	0.850	5.231	5.235	5.233	2.651	1.082
3.00	4.630	6.110	4.400	3.040	4.510	4.580	4.545	0.974	0.398
6.00	3.670	2.870	1.690	2.490	2.680	2.680	2.680	0.638	0.260
9.00	2.690	2.570	0.450	1.890	1.912	1.888	1.900	0.797	0.325
12.00	1.860	1.760	0.290	1.050	1.213	1.267	1.240	0.565	0.231
AUC 0-inf	58.048	55.629	24.160	30.076	40.365	41.111	41.565	13.462	5.496
Cmax	6.230	9.070	4.780	3.040	5.231	5.235	5.598	1.997	0.815
Tmax	1.500	1.500	1.500	3.000	1.500	1.500	1.750	0.612	0.250

Remark..: UNITS of Cmax = mcg/ml, Tmax = h, AUC = mcg.h/ml.

Table 7.6C : Serum concentration and pharmacokinetic parameters of rifampicin from Product Y (Amorphous)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.50	0.210	0.150	0.170	0.240	0.201	0.185	0.193	0.032	0.013
1.00	4.520	2.920	4.370	1.490	3.125	3.527	3.325	1.107	0.452
1.50	6.060	5.190	5.350	3.560	5.200	4.880	5.040	0.825	0.337
3.00	5.100	3.780	3.260	4.390	4.486	3.780	4.133	0.654	0.267
6.00	2.280	2.720	1.990	3.390	2.395	2.797	2.595	0.489	0.199
9.00	1.020	1.130	1.690	2.210	1.410	1.616	1.513	0.431	0.176
12.00	0.580	0.690	0.490	1.430	0.599	0.997	0.798	0.356	0.145
AUC 0-inf	33.071	31.466	29.057	43.188	31.947	36.708	34.240	5.047	2.060
Cmax	6.060	5.190	5.350	4.390	5.200	4.880	5.178	0.551	0.225
Tmax	1.500	1.500	1.500	3.000	1.500	1.500	1.750	0.612	0.250

Remark..: UNITS of Cmax = mcg/ml, Tmax = h, AUC = mcg.h/ml.

Table 7.6D : Serum concentration and pharmacokinetic parameters of rifampicin from Product X (Polymorph A) in second set

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.50	0.215	0.210	0.170	0.259	0.241	0.159	0.209	0.039	0.016
1.00	4.710	2.920	2.970	0.471	1.801	2.070	2.490	1.419	0.579
1.50	7.210	5.970	4.950	4.210	5.980	6.020	5.723	1.031	0.421
3.00	6.370	3.850	3.750	3.510	4.210	4.630	4.387	1.047	0.428
6.00	3.210	3.010	2.070	2.360	3.570	3.670	2.982	0.647	0.264
9.00	2.010	1.850	1.510	2.150	2.150	2.610	2.047	0.365	0.149
12.00	1.100	0.690	0.550	1.060	1.060	1.350	0.968	0.294	0.120
AUC 0-inf	46.952	35.425	29.101	35.560	42.141	48.275	39.576	7.480	3.054
Cmax	7.210	5.970	4.950	4.210	5.980	6.020	5.723	1.031	0.421
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.6E : Serum concentration and pharmacokinetic parameters of rifampicin from Product Z (Polymorph B)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.50	1.100	0.711	0.725	0.668	0.724	0.601	0.755	0.176	0.072
1.00	5.070	3.520	3.550	3.205	3.464	3.314	3.687	0.690	0.282
1.50	9.190	10.200	9.880	10.340	10.020	10.207	9.973	0.416	0.170
3.00	8.180	8.650	8.824	8.560	8.823	8.046	8.514	0.329	0.135
6.00	3.500	6.010	5.340	5.003	5.640	6.573	5.344	1.054	0.430
9.00	1.722	3.170	3.430	2.977	3.408	3.885	3.099	0.740	0.302
12.00	0.655	1.520	2.041	2.450	2.528	2.086	1.880	0.669	0.285
AUC 0-inf	49.663	69.465	73.790	76.777	81.104	78.678	71.580	11.470	4.683
Cmax	9.190	10.200	9.880	10.340	10.020	10.207	9.973	0.416	0.170
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.6F : Comparative pharmacokinetic and dissolution test parameters derived from amorphous and polymorphs of rifampicin

Parameter	Set I		Set II	
	Product X (Polymorph A))	Product Y (Amorphous)	Product X (Polymorph A)	Productc Z (Polymorph B)
DP30 (%)	65.09	62.89	65.09	93.63
DE 30	65.81	23.35	31.62	65.81
AUC (mcg.h/ml)	41.565	34.24	39.576	71.580
Cmax (mcg/ml)	5.598	5.178	5.723	9.973
Tmax (h)	1.750	1.750	1.50	1.50
Ka (/h)	0.364	0.351	0.360	0.357

Note : 1. DE30 and DE 30 are dissolution percent and dissolution efficiency values over first 30 minutes respectively

2. r is the *in vivo-in vitro* correlation coefficient for AUC versus dissolution parameter.

Table 7.7A : Quantity of rifampicin dissolved (mg/ml) from 0.01 M solutions of cyclodextrins

Time (h)	Control (Without-CD)		Alpha-CD		Beta-CD		Gamma-CD		Beta-HPCD	
	pH		pH		pH		pH		pH	
	4.00	7.90	4.00	7.90	4.00	7.90	4.00	7.90	4.00	7.90
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.00	1.258	1.223	1.246	1.315	1.234	1.469	1.352	1.697	1.664	1.610
2.00	1.261	1.249	1.299	1.445	1.272	1.515	1.512	1.777	1.677	1.633
3.00	1.267	1.252	1.280	1.428	1.240	1.498	1.635	1.790	1.601	1.616
4.00	1.266	1.264	1.260	1.405	1.227	1.479	1.695	1.797	1.510	1.598
5.00	1.267	1.284	1.242	1.381	1.221	1.471	1.648	1.764	1.491	1.564
6.00	1.264	1.300	1.192	1.356	1.200	1.463	1.592	1.712	1.472	1.502

Table 7.8A : Dissolution rate profile of rifampicin in povidone K30 solutions at pH 4.0 and 7.9

Time (min)	Concentration of rifampicin (mg/ml)											
	Povidone concentration at pH 4.0						Povidone concentration at pH 7.9					
	0.0	0.5%	1.0%	2.0%	4.0%	5.0%	0.0%	0.5%	1.0%	2.0%	4.0%	5.0%
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.00	0.71	1.04	1.55	2.12	2.63	2.88	0.90	1.08	1.82	2.06	2.59	3.02
30.00	1.02	1.36	1.99	2.49	3.01	3.26	1.25	1.82	2.44	2.59	3.31	3.86
45.00	1.05	1.49	2.13	2.57	3.19	3.30	1.27	2.08	2.54	2.89	3.86	4.20
60.00	1.10	1.49	2.11	2.60	3.19	3.41	1.29	2.41	2.69	3.01	3.94	4.26
90.00	1.14	1.50	2.12	2.61	3.22	3.44	1.30	2.45	2.71	3.05	4.10	4.38
120.0	1.16	1.51	2.09	2.62	3.25	3.47	1.32	2.44	2.71	3.08	4.16	4.47
180.0	1.16	1.53	2.10	2.61	3.26	3.48	1.34	2.46	2.75	3.05	4.19	4.48

Table 7.8B : Dissolution rate profile of rifampicin in povidone K90 solutions at pH 4.00 and 7.9

Time (min)	Concentration of rifampicin (mg/ml)											
	Povidone concentration at pH 4.0						Povidone concentration at pH 7.9					
	0.0	0.5%	1.0%	2.0%	4.0%	5.0%	0.0%	0.5%	1.0%	2.0%	4.0%	5.0%
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.00	0.75	1.16	1.44	1.58	2.07	2.50	0.96	1.04	1.26	1.55	2.12	2.50
30.00	0.98	1.45	1.87	2.56	3.56	3.82	1.31	1.57	1.91	2.28	3.02	3.34
45.00	1.01	1.55	1.98	2.85	4.09	4.30	1.34	1.77	2.20	2.67	3.45	3.85
60.00	1.07	1.62	2.02	2.93	4.35	4.60	1.37	1.85	2.30	2.79	3.81	4.16
90.00	1.10	1.72	2.06	3.13	4.36	4.64	1.42	1.89	2.31	3.10	4.14	4.48
120.0	1.14	1.74	2.06	3.17	4.44	4.73	1.44	1.98	2.40	3.25	4.42	4.77
180.0	1.15	1.79	2.10	3.18	4.52	4.78	1.44	1.96	2.45	3.26	4.47	4.83

Table 7.8C : Dissolution rate profile of rifampicin in povidone K120 solutions at pH 4.0 and 7.9

	Concentration of rifampicin (mg/ml)											
	Povidone concentration at pH 4.0						Povidone concentration at pH 7.9					
Time (min)	0.0	0.5%	1.0%	2.0%	4.0%	5.0%	0.0%	0.5%	1.0%	2.0%	4.0%	5.0%
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.00	0.68	1.05	1.31	1.46	1.66	2.01	0.86	1.10	1.35	1.51	2.51	3.00
30.00	1.04	1.47	1.97	2.34	2.71	2.96	1.21	1.41	1.99	2.29	3.51	4.20
45.00	1.08	1.68	2.31	2.61	3.18	3.50	1.26	1.42	2.35	2.81	3.73	4.68
60.00	1.13	1.71	2.41	2.77	3.34	3.79	1.30	1.52	2.40	2.98	4.01	5.04
90.00	1.18	1.71	2.60	2.92	3.48	4.26	1.32	1.58	2.41	3.11	4.29	5.25
120.0	1.21	1.72	2.72	3.31	3.73	4.65	1.33	1.60	2.45	3.14	4.43	5.46
180.0	1.20	1.76	2.73	3.27	4.03	5.20	1.35	1.61	2.50	3.15	4.45	5.45

Table 7.9A : Dissolution rate profile of rifampicin surface dispersions using various adsorbents (The ratios of rifampicin : excipient are given within parentheses)

Time (Mins)	Rifampicin Concentration (mg/ml)							
	Rifampicin Pure	Talc (1 : 2)	Starch (1 : 2)	Lactose (1 : 2)	Aerosil (1 : 2)	MCC (1 : 2)	Talc (1 : 1)	Talc (1 : 4)
10.0	1.15	0.62	1.16	0.83	0.38	0.93	0.89	0.57
20.0	1.20	0.89	1.22	1.18	0.60	1.40	1.11	0.90
30.0	1.20	1.11	1.25	1.34	0.76	1.44	1.32	1.10
40.0	1.22	1.29	1.29	1.44	0.87	1.46	1.37	1.30
60.0	1.22	1.49	1.28	1.49	0.95	1.49	1.42	1.54
90.0	1.20	1.60	1.27	1.53	1.04	1.46	1.43	1.66
120.0	1.19	1.75	1.26	1.52	1.10	1.43	1.45	1.83
180.0	1.17	1.84	1.24	1.57	1.11	1.39	1.45	1.92
240.0	1.15	1.84	1.20	1.55	1.10	1.36	1.44	1.91

Table 7.10A : Dissolution rate profile of rifampicin from solid dispersions (drug : carrier ratio 1 : 3)

Time (min)	Rifampicin concentration (mg / ml)				
	Rifampicin (as such)	SD with PEG (solvent method)	SD with PEG (fusion method)	SD with PVP K-30	SD with mannitol
10	0.5	1.87	5.47	20.60	0.97
20	0.7	2.03	5.89	20.75	1.85
30	0.85	2.19	6.17	20.09	2.41
40	1.03	2.33	6.00	21.05	2.60
60	1.40	2.38	6.52	21.10	2.80
90	1.74	2.47	6.63	21.20	3.03
120	2.10	2.56	6.70	21.27	3.24
180	2.19	2.77	6.72	21.27	3.54

Table 7.10B : Relative dissolution test parameters for solid dispersions

Parameter	Rifampicin (as such)	SD with PEG (solvent method)	SD with PEG (fusion method)	SD with PVP K-30	SD with mannitol
A30 (mg/ml)	0.85	2.19	6.17	20.09	2.41
Cmax(mg/ml)	2.19	2.77	6.72	21.27	3.54

Table 7.10C : Serum concentration and pharmacokinetic parameters of rifampicin from Product A (rifampicin-PVP solid dispersion)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	0.670	0.580	0.690	0.650	0.590	0.640	0.637	0.044	0.018
1.50	8.210	8.470	7.810	8.170	8.050	7.510	8.037	0.336	0.137
3.00	7.420	7.310	7.510	6.510	7.210	6.890	7.142	0.376	0.154
6.00	6.510	5.210	5.270	5.870	5.590	5.750	5.700	0.474	0.194
9.00	4.370	3.210	3.500	3.790	3.330	3.780	3.663	0.418	0.171
12.00	1.570	1.040	1.470	1.590	1.250	1.490	1.402	0.215	0.088
AUC 0-inf	70.527	58.031	62.828	65.843	60.776	64.014	63.670	4.307	1.758
Cmax	8.210	8.470	7.810	8.170	8.050	7.510	8.037	0.336	0.137
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.10D : Serum concentration and pharmacokinetic parameters of rifampicin from Product B (rifampicin-talc surface dispesions

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	0.570	0.610	0.690	0.410	0.650	0.530	0.577	0.099	0.041
1.50	8.250	7.950	7.810	8.110	7.810	6.950	7.813	0.457	0.186
3.00	7.510	6.240	7.530	7.200	6.350	5.870	6.783	0.718	0.293
6.00	5.350	5.050	5.240	4.970	5.170	4.230	5.002	0.401	0.164
9.00	2.790	2.170	3.120	2.510	2.790	1.980	2.560	0.427	0.174
12.00	1.210	0.970	1.350	1.010	0.980	0.870	1.065	0.179	0.073
AUC 0-inf	58.806	51.246	60.420	54.214	53.631	45.460	53.963	5.385	2.199
Cmax	8.250	7.950	7.810	8.110	7.810	6.950	7.813	0.457	0.186
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

Table 7.10E : Serum concentration and pharmacokinetic parameters of rifampicin from Product C (rifampicin, control)

Time (h)	Serum concentration (mcg / ml) in volunteer								
	1	2	3	4	5	6	Mean	StdDev	StdErr
0.00	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.75	0.720	0.640	0.690	0.580	0.650	0.660	0.657	0.048	0.019
1.50	6.930	7.470	7.210	7.070	7.100	7.240	7.170	0.184	0.075
3.00	6.070	6.250	6.050	5.900	6.000	6.130	6.067	0.118	0.048
6.00	2.970	4.210	4.370	3.870	3.821	3.890	3.855	0.485	0.198
9.00	2.030	2.150	1.910	2.540	2.038	2.280	2.158	0.225	0.092
12.00	1.510	1.010	0.720	0.930	1.040	1.050	1.043	0.259	0.106
AUC 0-inf	49.027	48.529	45.206	46.819	46.453	47.873	47.318	1.425	0.582
Cmax	6.930	7.470	7.210	7.070	7.100	7.240	7.170	0.184	0.075
Tmax	1.500	1.500	1.500	1.500	1.500	1.500	1.500	0.000	0.000

Remark..: UNITS of Cmax = ug/ml, Tmax = h, AUC = ug.h/ml.

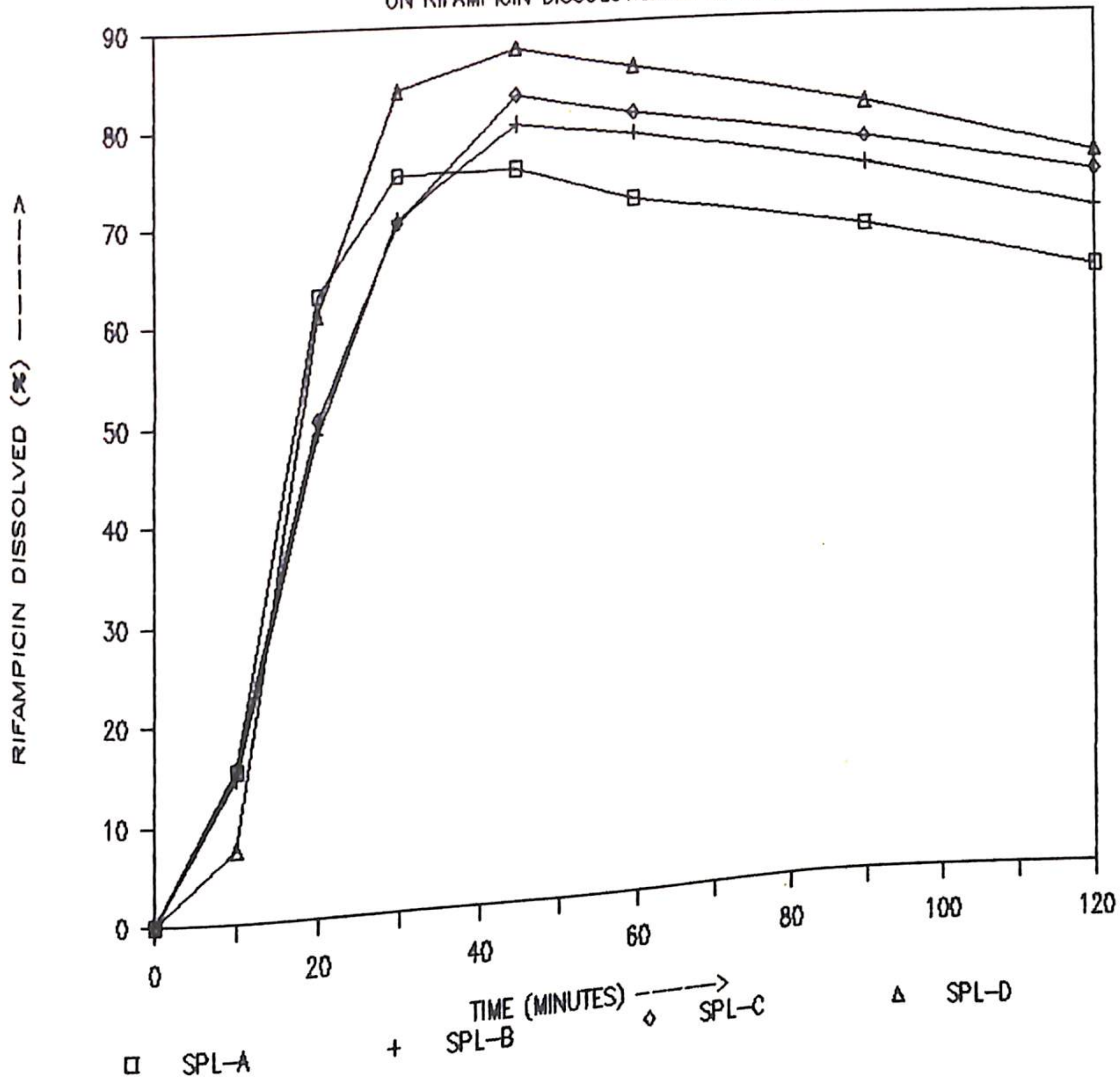


FIG 7.1AI : EFFECT OF PARTICLE SIZE
ON RIFAMPICIN DISSOLUTION IN 0.1 N HCL
RIFAMPICIN DISSOLVED (%) ------>
TIME (MINUTES) ------>
0
10
20
30
40
50
60
70
80
90
100
120
SPL-A
SPL-B
SPL-C
SPL-D

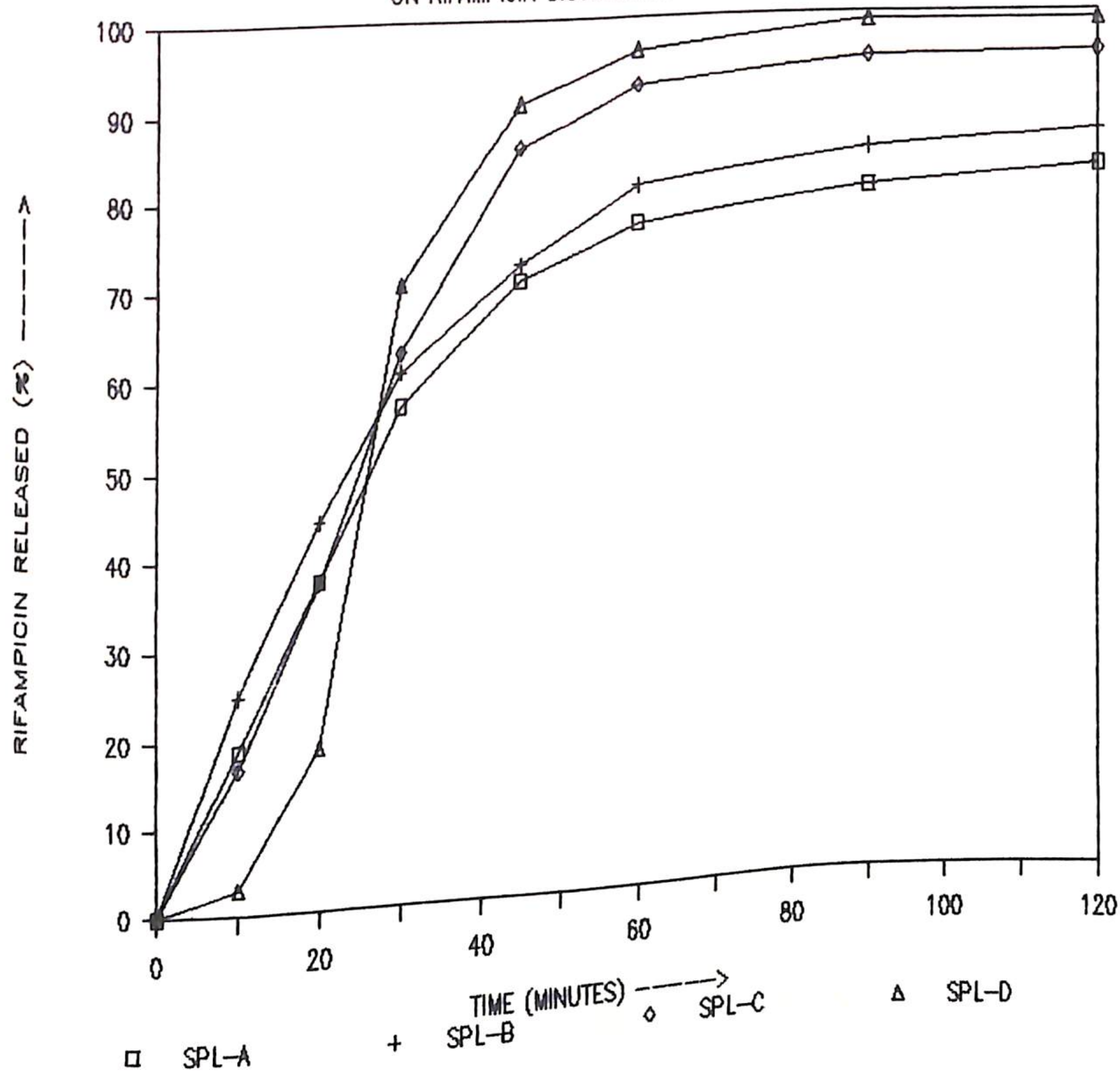


FIG 7.1AII : EFFECT OF PARTICLE SIZE
ON RIFAMPICIN DISSOLUTION IN 0.4 % SLS
RIFAMPICIN RELEASED (%) ------->
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120
TIME (MINUTES) ------->
SPL-A
SPL-B
SPL-C
SPL-D

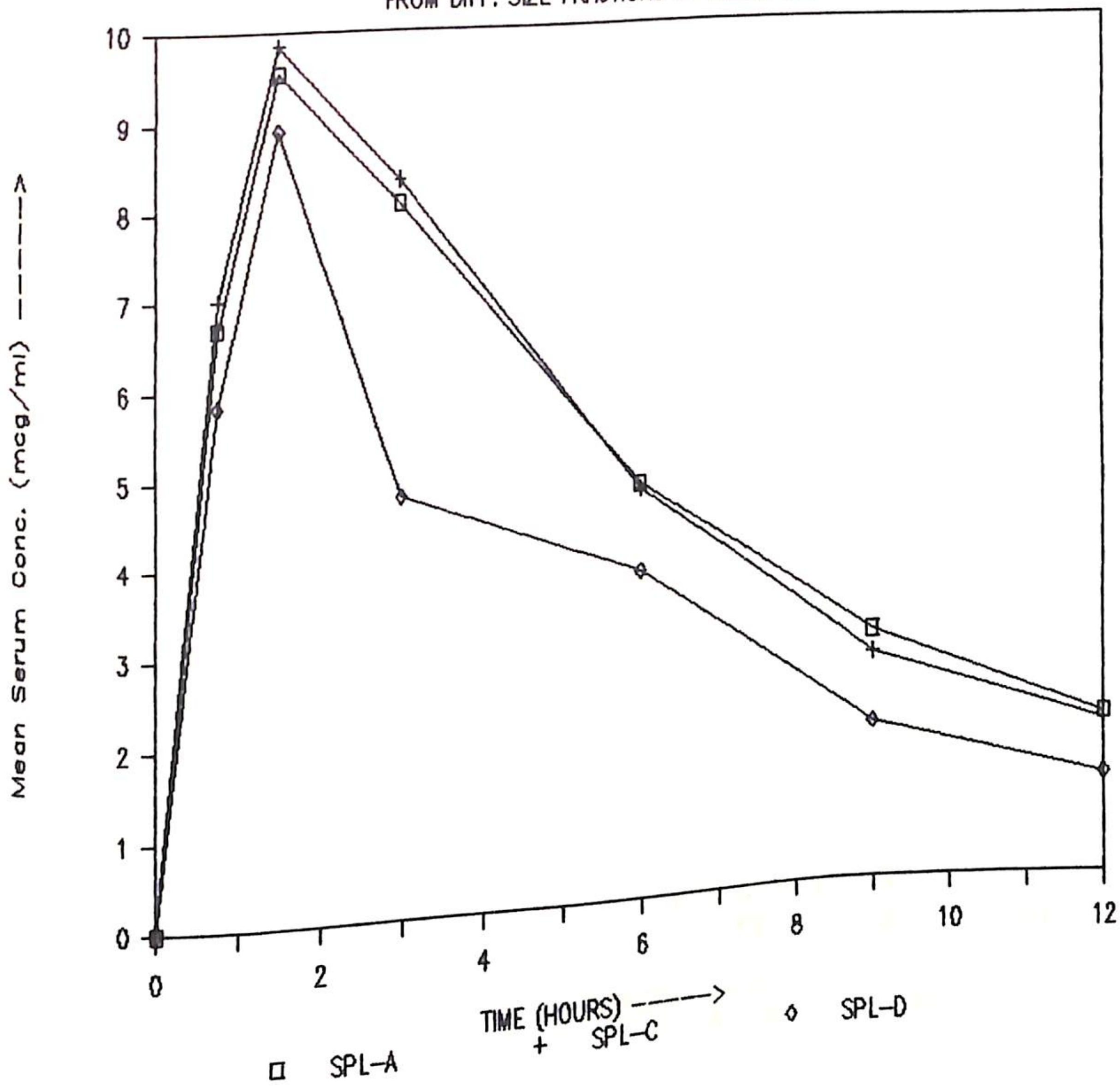
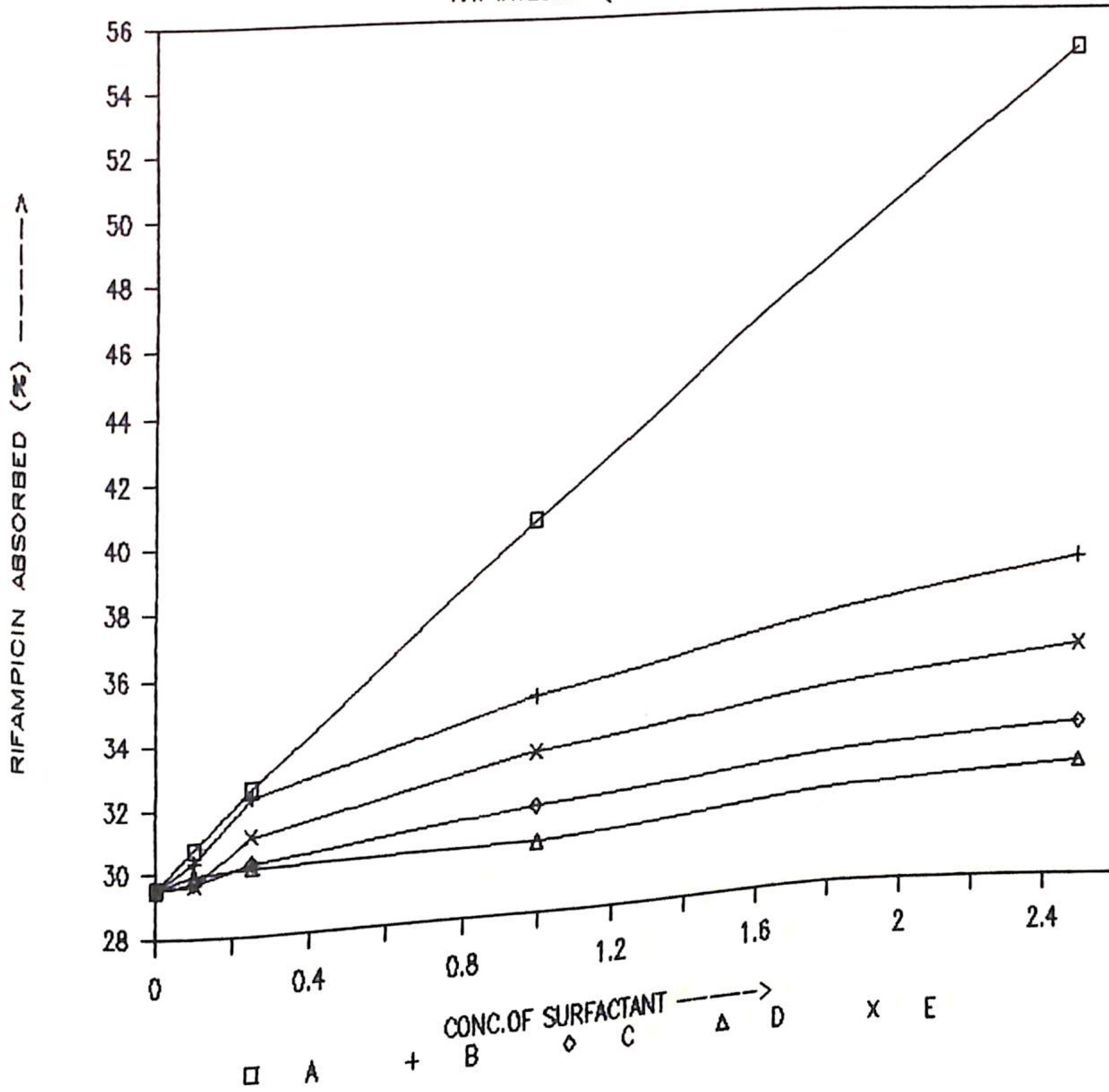


FIG 7.1B : SERUM CONC. OF RIFAMPICIN
FROM DIFF. SIZE FRACTIONS OF RIFAMPICIN
Mean Serum Conc. (mcg/ml) ------>
10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
TIME (HOURS) ------>
SPL-A
SPL-C
SPL-D



SURFACTANT :

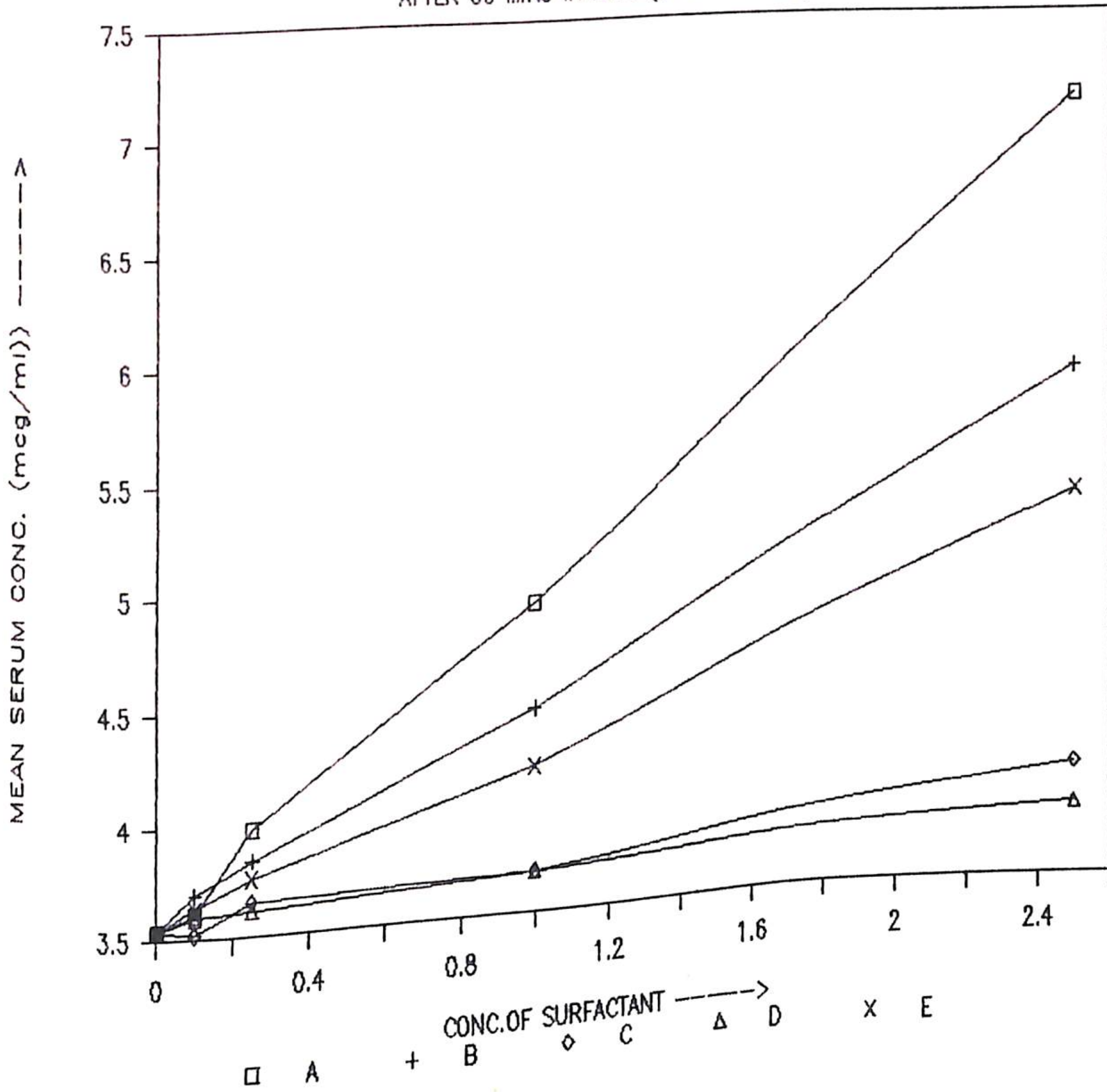
A = Polysorbate 80

B = Sodium lauryl sulphate

C = Docusate sodium

D = Poloxamer 188

E = Bile salts



SURFACTANT :

A = Polysorbate 80

B = Sodium lauryl sulphate

C = Docusate sodium

D = Poloxamer 188

E = Bile salts

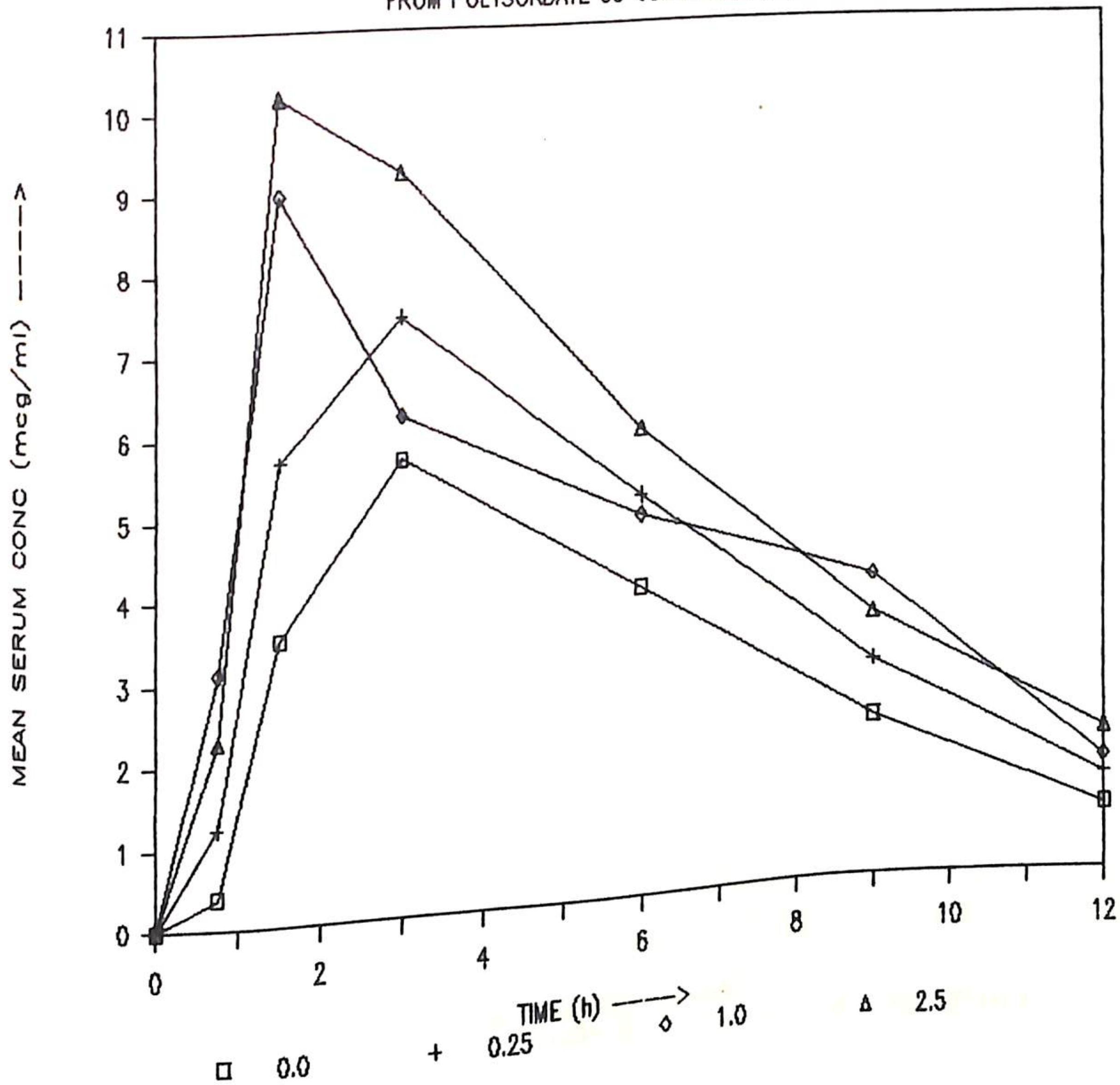


FIG 7.5A : SERUM CONC. OF RIFAMPICIN
FROM POLYSORBATE 80 CONCENTRATIONS
MEAN SERUM CONC (mcg/ml) ----->
TIME (h) ----->
0
2
4
6
8
10
12
1
3
5
7
9
11
□ 0.0
+ 0.25
◇ 1.0
Δ 2.5

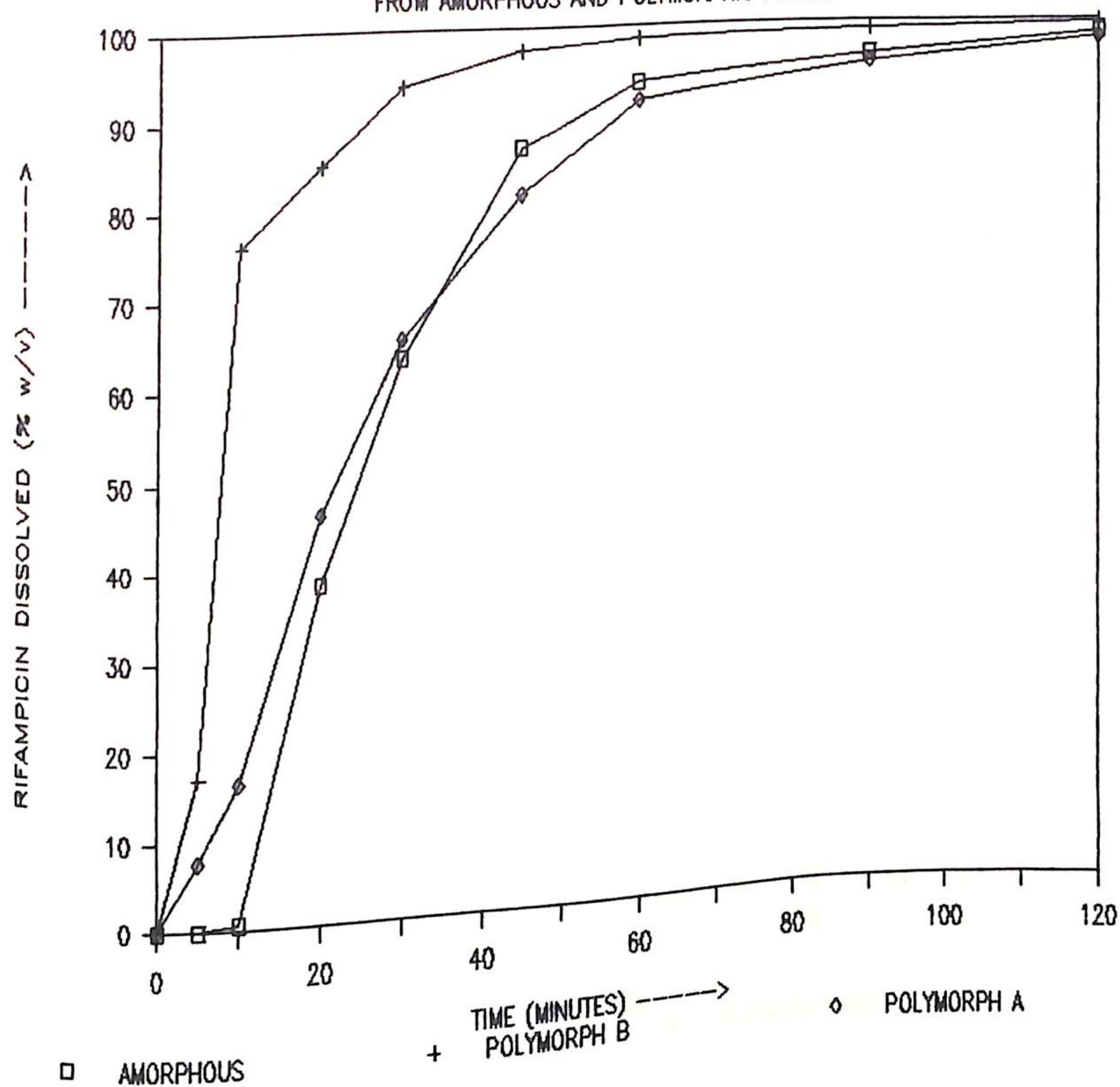


FIG 7.6A :DISS.RATE PROFILE OF RIFA
FROM AMORPHOUS AND POLYMORPHIC FORMS
RIFAMPICIN DISSOLVED (% w/v) ------->
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
TIME (MINUTES) ------>
AMORPHOUS
POLYMORPH B
POLYMORPH A

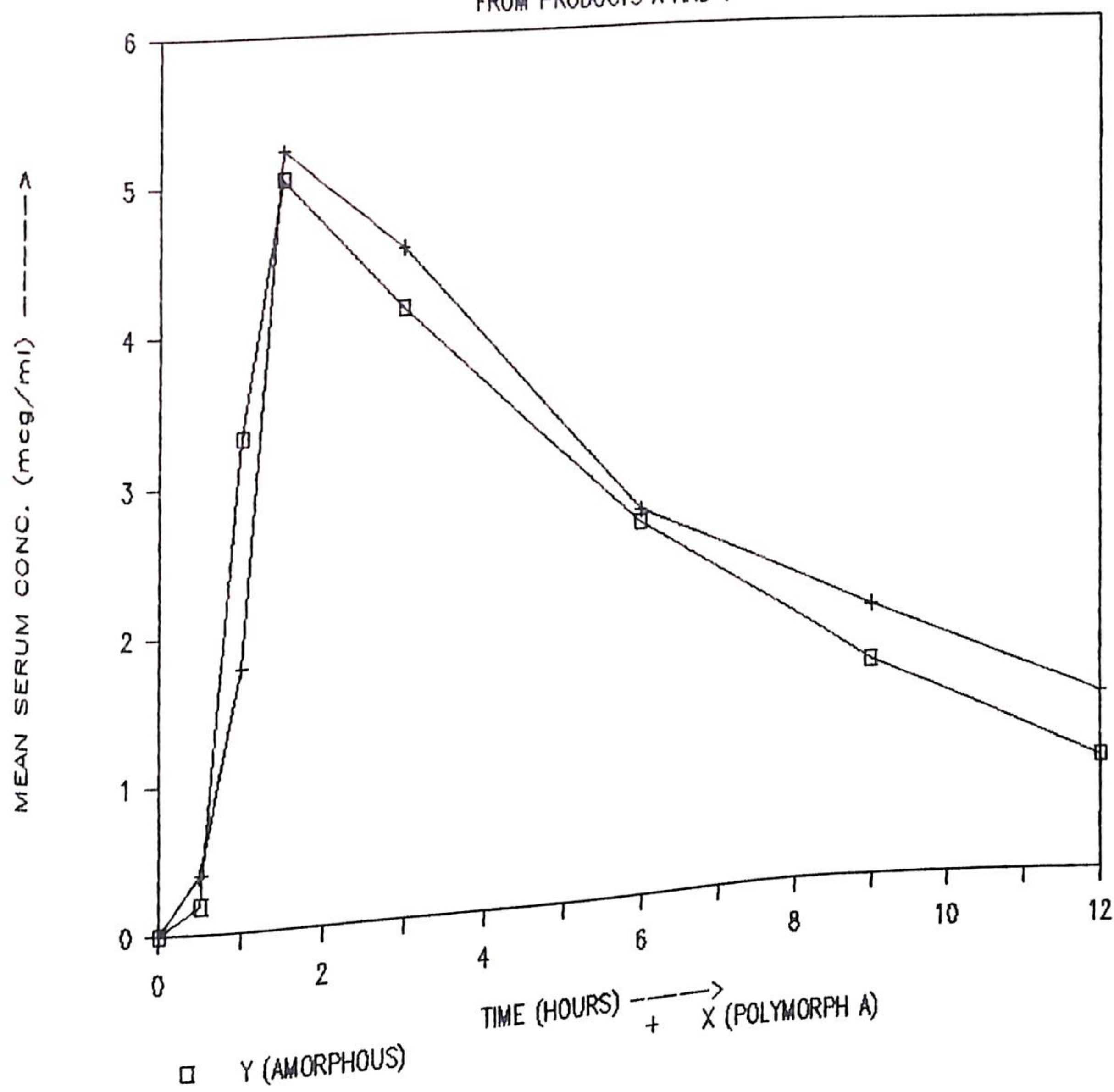


FIG 7.6B :SERUM CONC. OF RIFAMPICIN
FROM PRODUCTS X AND Y
MEAN SERUM CONC. (mcg/ml) ------>
0
1
2
3
4
5
6
0
2
4
6
8
10
12
TIME (HOURS) ------>
+ X (POLYMORPH A)
Y (AMORPHOUS)

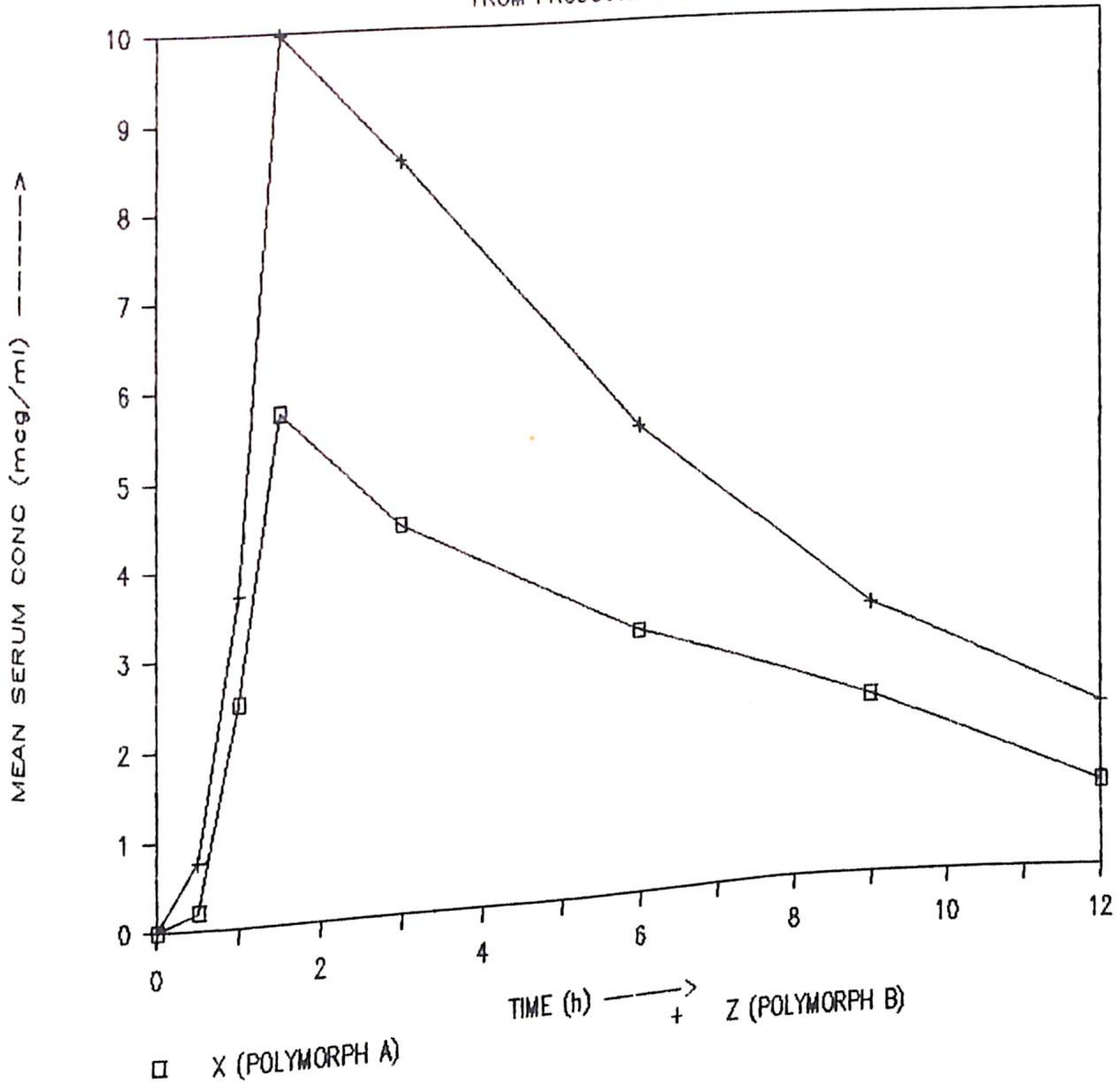
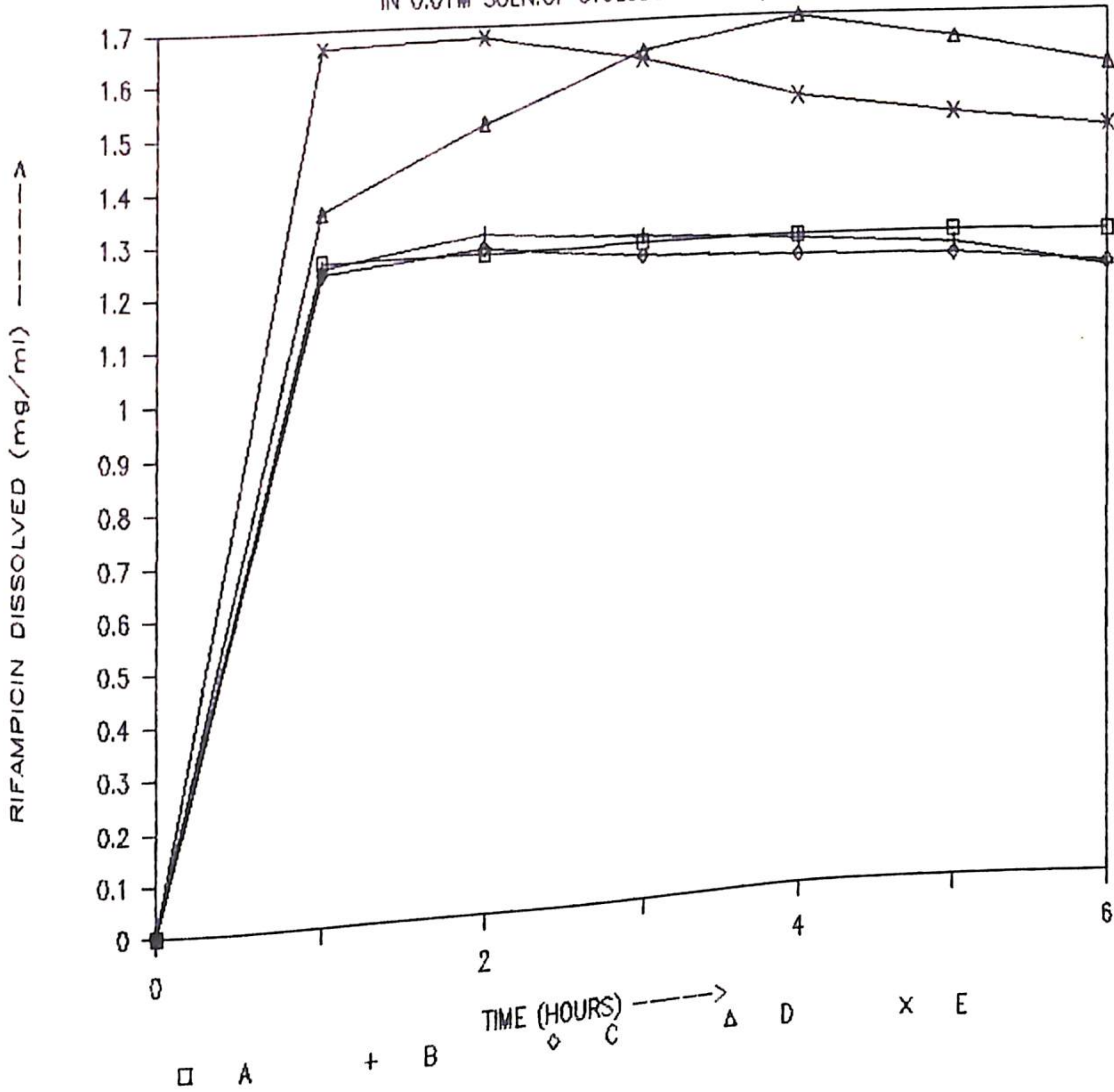


FIG 7.6C :SERUM CONC. OF RIFAMPICIN
FROM PRODUCTS X AND Z
MEAN SERUM CONC (mcg/ml) ------->
10
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
TIME (h) ------>
+ Z (POLYMORPH B)
X (POLYMORPH A)

FIG 7.7A : DISS.RATE PROFILE OF RIFA

IN 0.01M SOLN.OF CYCLODEXTRIN AT pH 4.0



A = Control (No cyclodextrin)

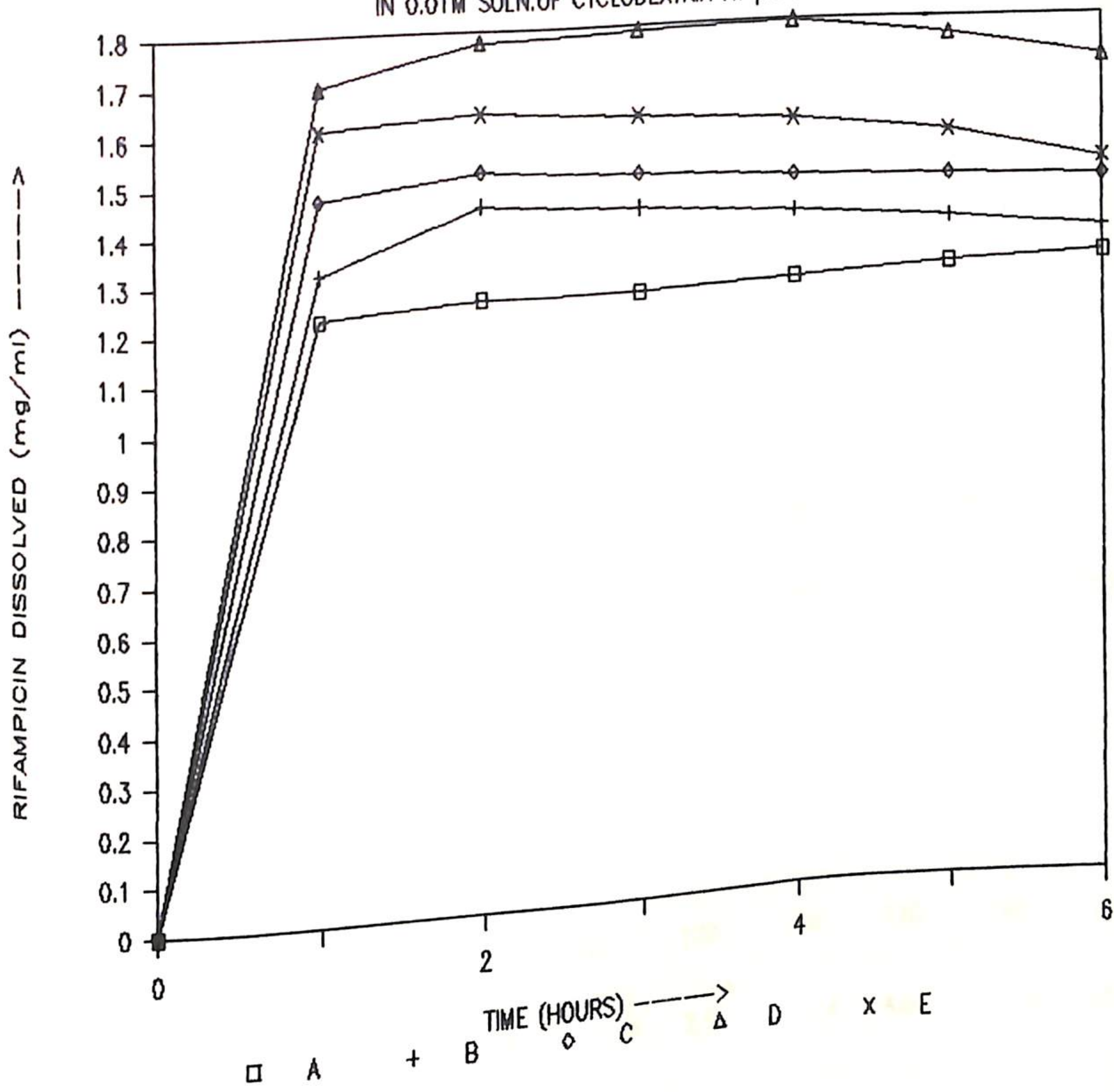
B = Alpha-cyclodextrin

C = Beta-cyclodextrin

D = Gamma-cyclodextrin

E = Hydroxypropyl beta-cyclodextrin

FIG 7.7B :DISS.RATE PROFILE OF RIFA



A = Control (No cyclodextrin)

B = Alpha-cyclodextrin

C = Beta-cyclodextrin

D = Gamma-cyclodextrin

E = Hydroxypropyl beta-cyclodextrin

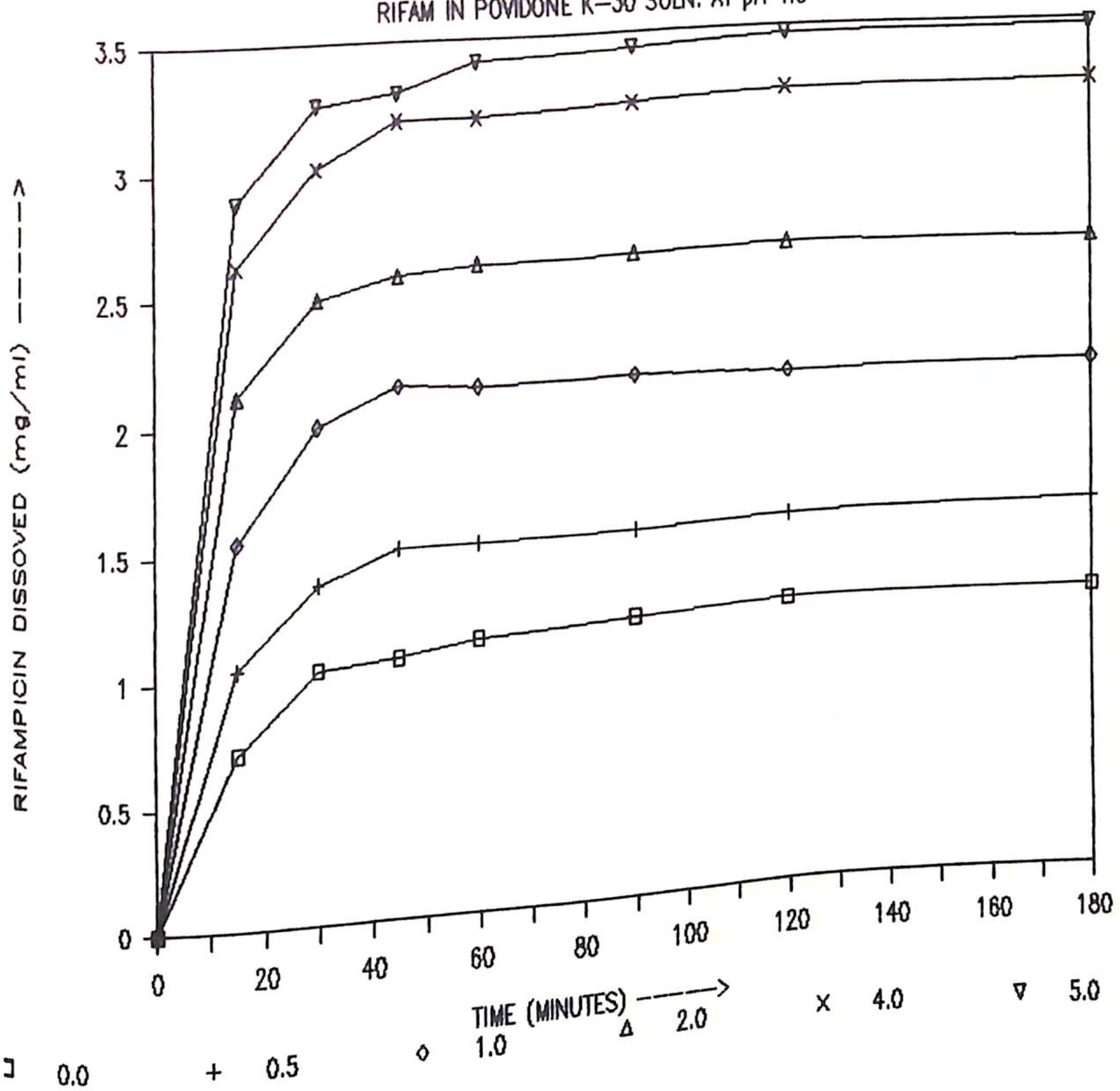


FIG 7.8AI : DISS. RATE PROFILE OF
RIFAM IN POVIDONE K-30 SOLN. AT pH 4.0
RIFAMPICIN DISSOVED (mg/ml) ------>
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
140
160
180
TIME (MINUTES) ------>
0.0
+ 0.5
◇ 1.0
Δ 2.0
x 4.0
∇ 5.0

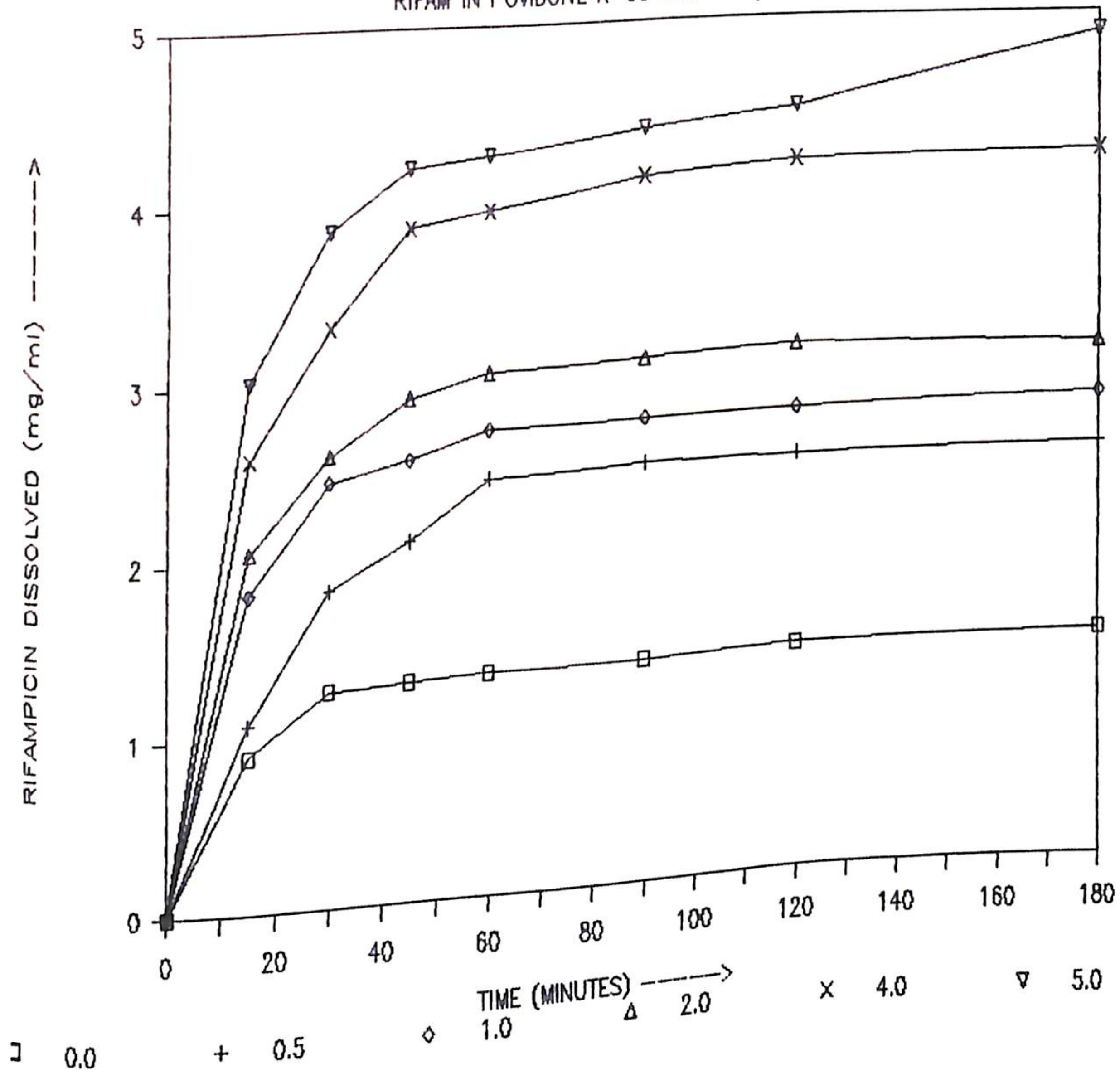


FIG 7.8AII : DISS.RATE PROFILE OF
RIFAM IN POVIDONE K-30 SOLN. AT pH 7.9
RIFAMPICIN DISSOLVED (mg/ml) ------>
0
1
2
3
4
5
0
20
40
60
80
100
120
140
160
180
TIME (MINUTES) ------>
0.0
0.5
1.0
2.0
4.0
5.0

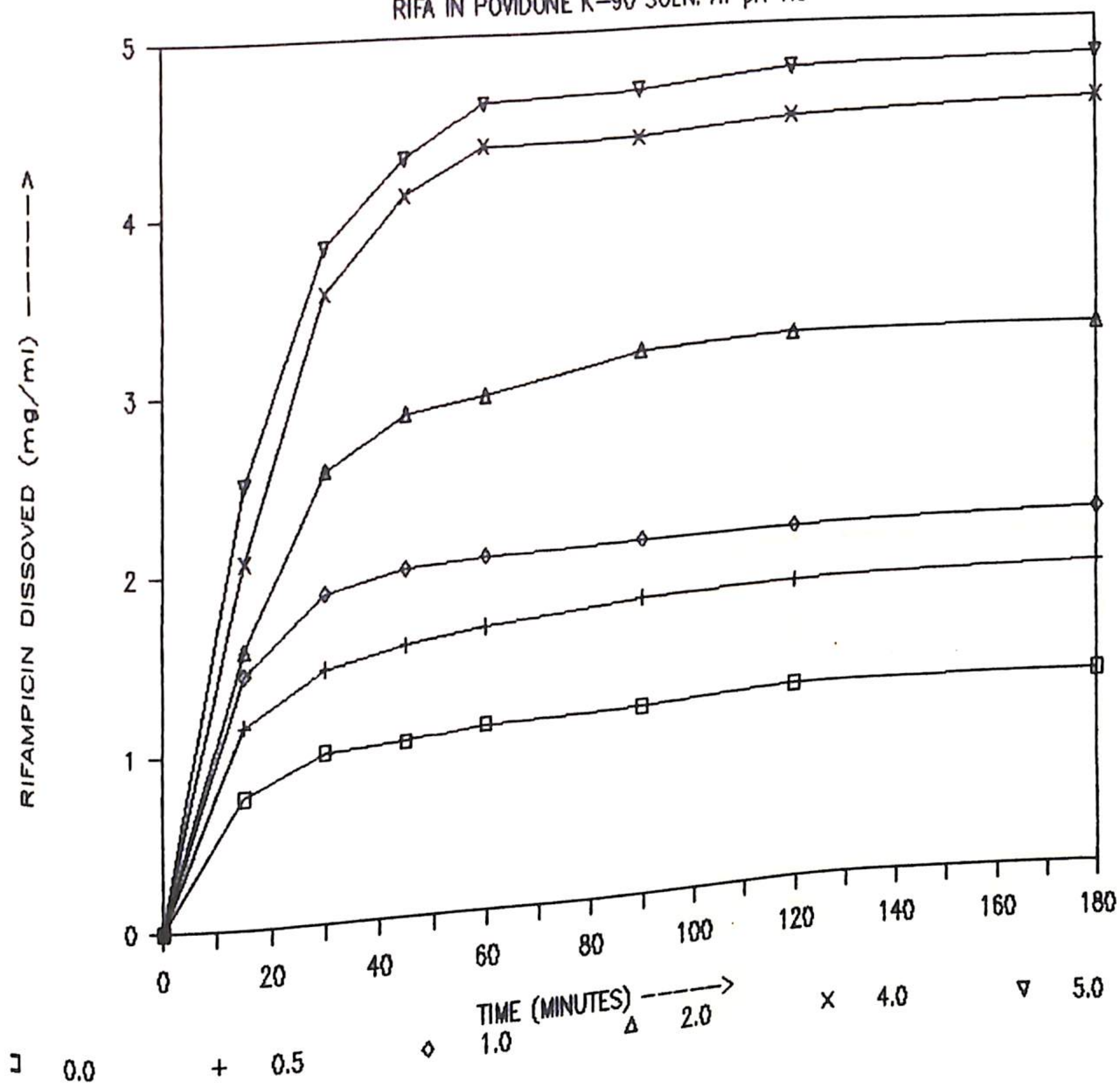


FIG 7.8BI : DISS. RATE PROFILE OF
RIFA IN POVIDONE K-90 SOLN. AT pH 4.0
RIFAMPICIN DISSOVED (mg/ml) ------->
TIME (MINUTES) ------->
0
1
2
3
4
5
20
40
60
80
100
120
140
160
180
0.0
+ 0.5
◇ 1.0
Δ 2.0
X 4.0
▽ 5.0

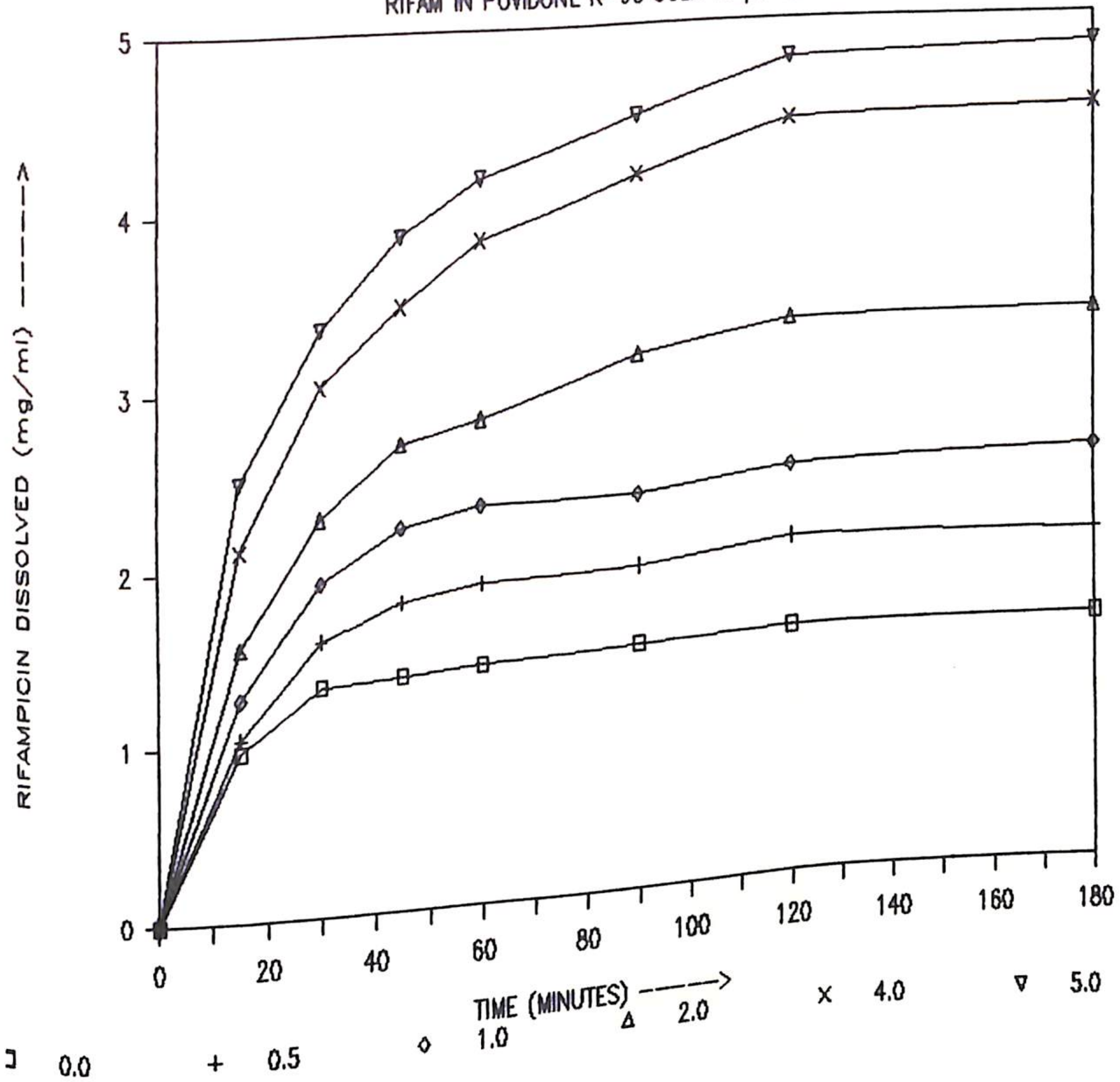


FIG 7.8BII : DISS.RATE PROFILE OF
RIFAM IN POVIDONE K-90 SOLN AT pH 7.9
RIFAMPICIN DISSOLVED (mg/ml) ------>
TIME (MINUTES) ------>
0
1
2
3
4
5
0
20
40
60
80
100
120
140
160
180
0.0
+ 0.5
◇ 1.0
Δ 2.0
× 4.0
▽ 5.0

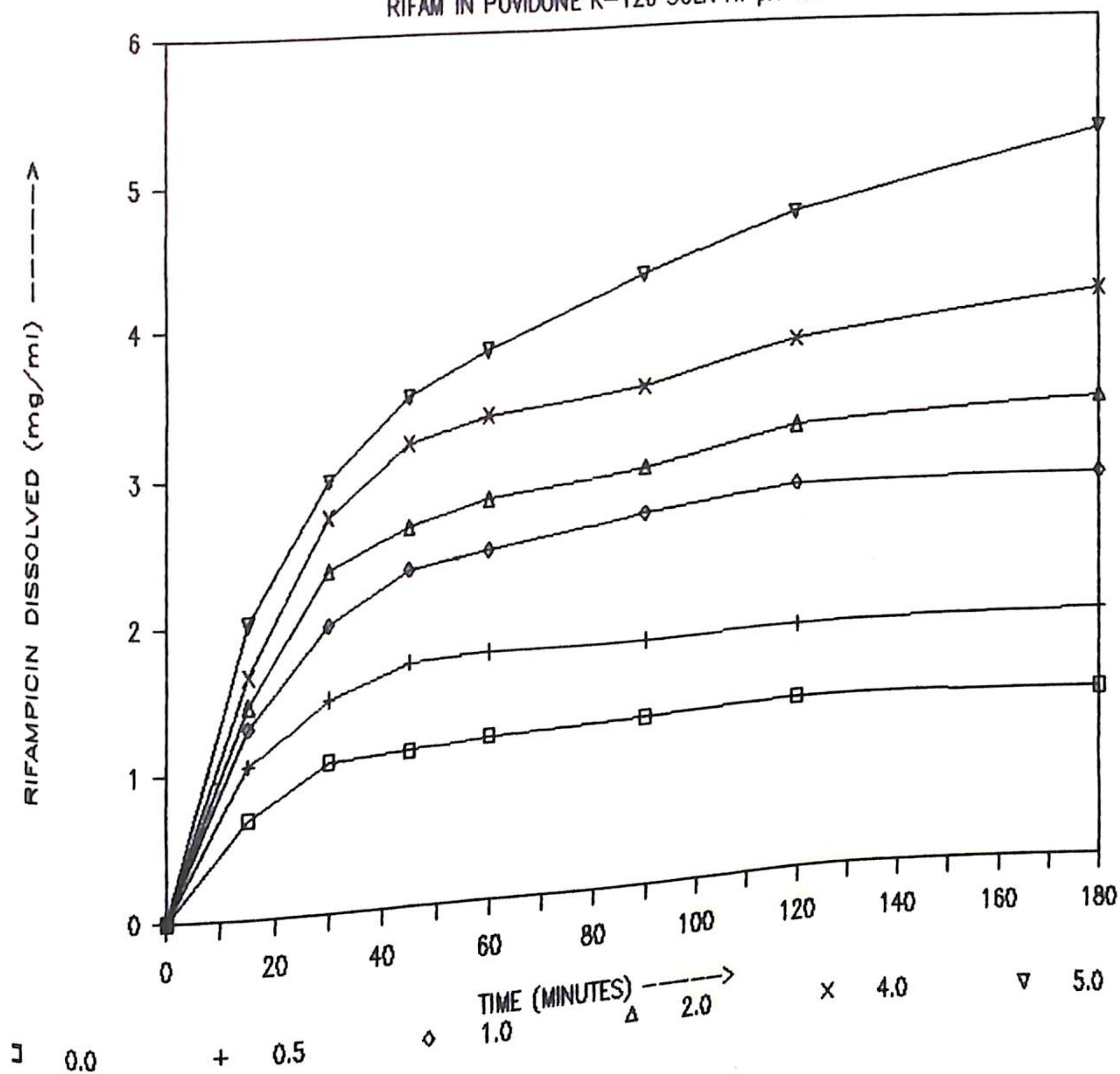


FIG 7.8CI : DISS. RATE PROFILE OF
RIFAM IN POVIDONE K-120 SOLN AT pH 4.0
RIFAMPICIN DISSOLVED (mg/ml) ------->
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
TIME (MINUTES) ------->
0.0
+ 0.5
◇ 1.0
Δ 2.0
× 4.0
∇ 5.0

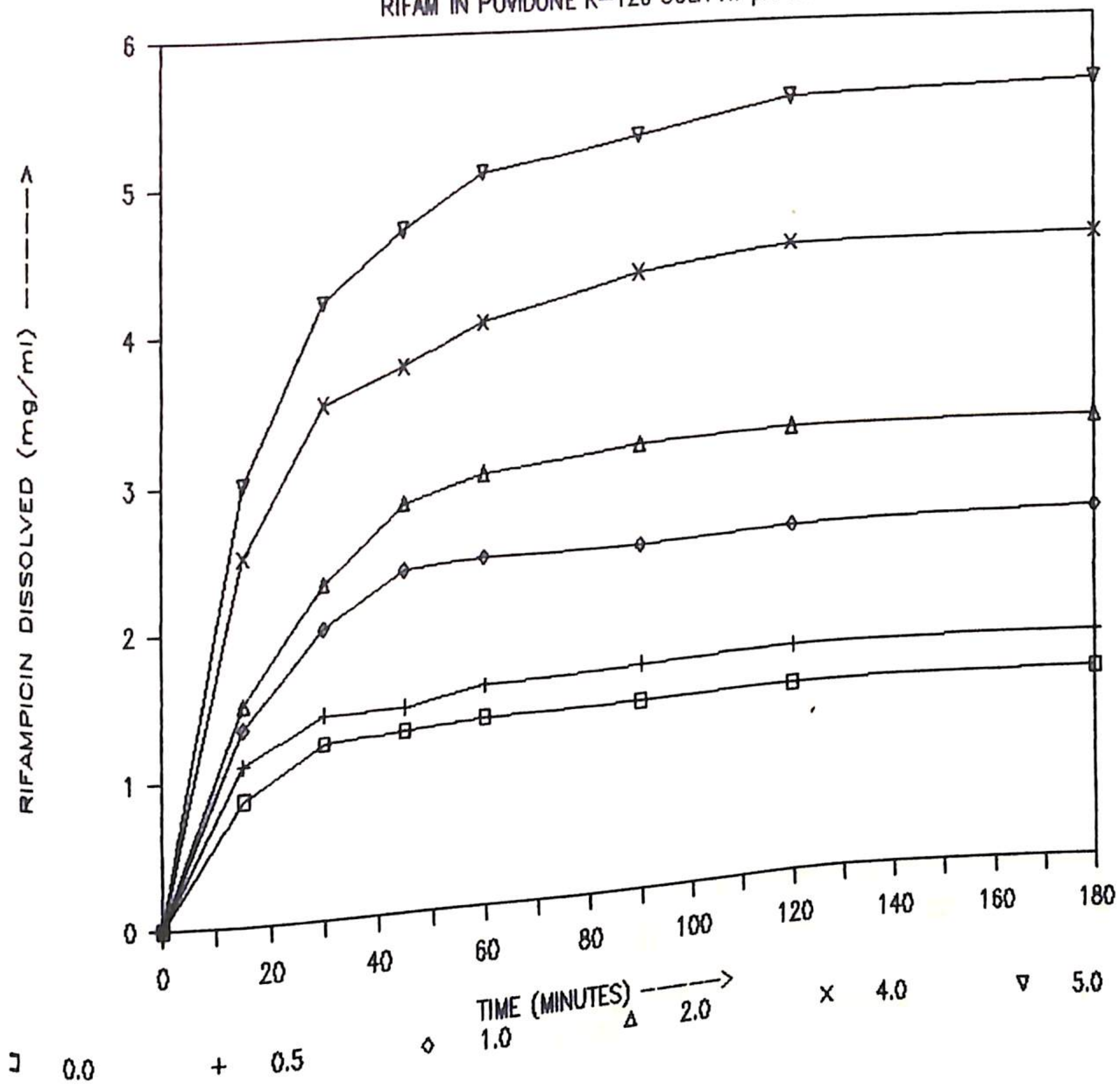


FIG 7.8CII : DISS.RATE PROFILE OF
RIFAM IN POVIDONE K-120 SOLN AT pH 7.9
RIFAMPICIN DISSOLVED (mg/ml) ------->
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
180
TIME (MINUTES) ------->
0.0
+ 0.5
◇ 1.0
Δ 2.0
× 4.0
∇ 5.0

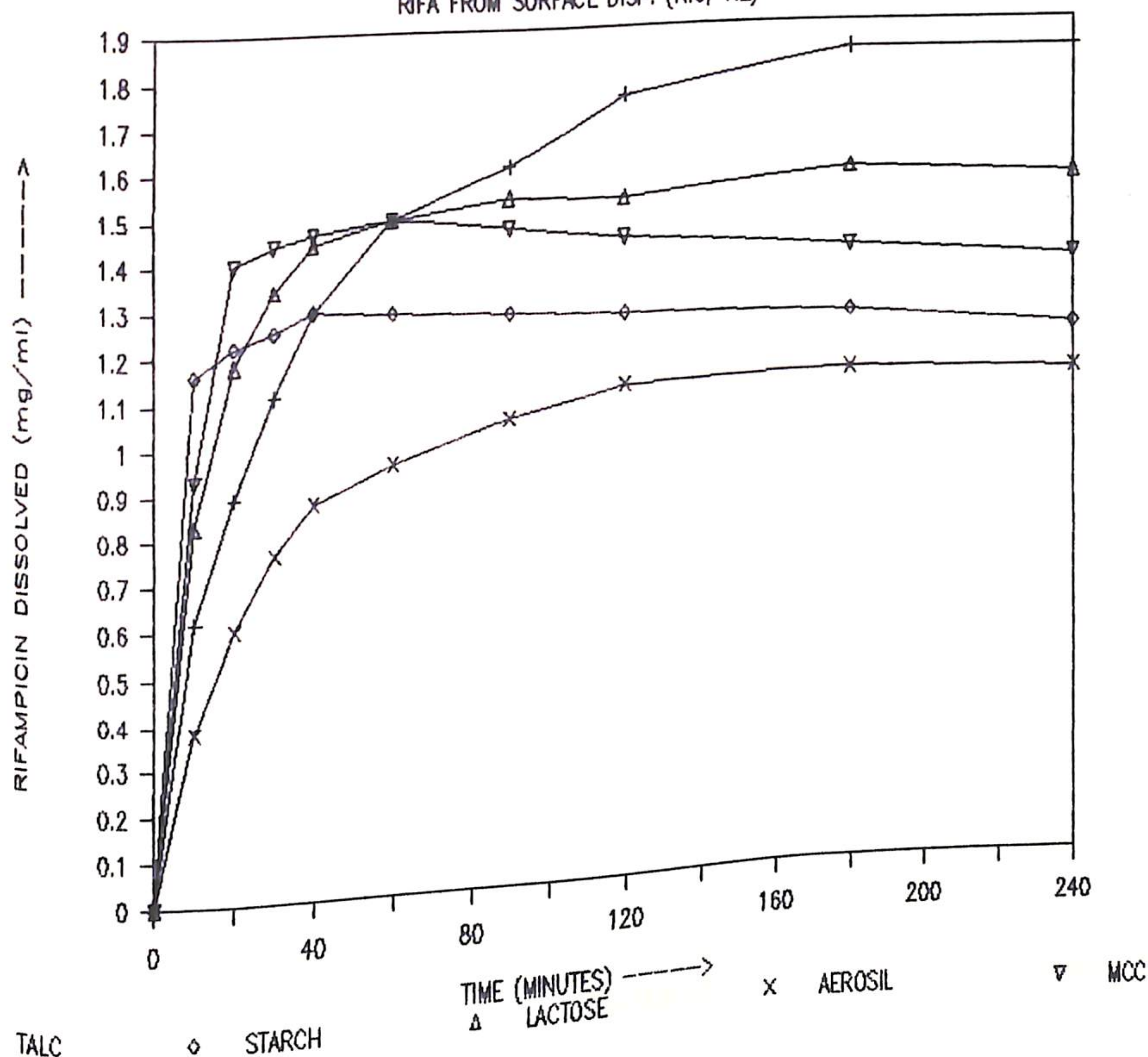


FIG 7.9A : DISS. RATE PROFILE OF
RIFA FROM SURFACE DISP. (R:C, 1:2)
RIFAMPICIN DISSOLVED (mg/ml) ------>
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
40
80
120
160
200
240
TIME (MINUTES) ------>
TALC
STARCH
LACTOSE
AEROSIL
MCC

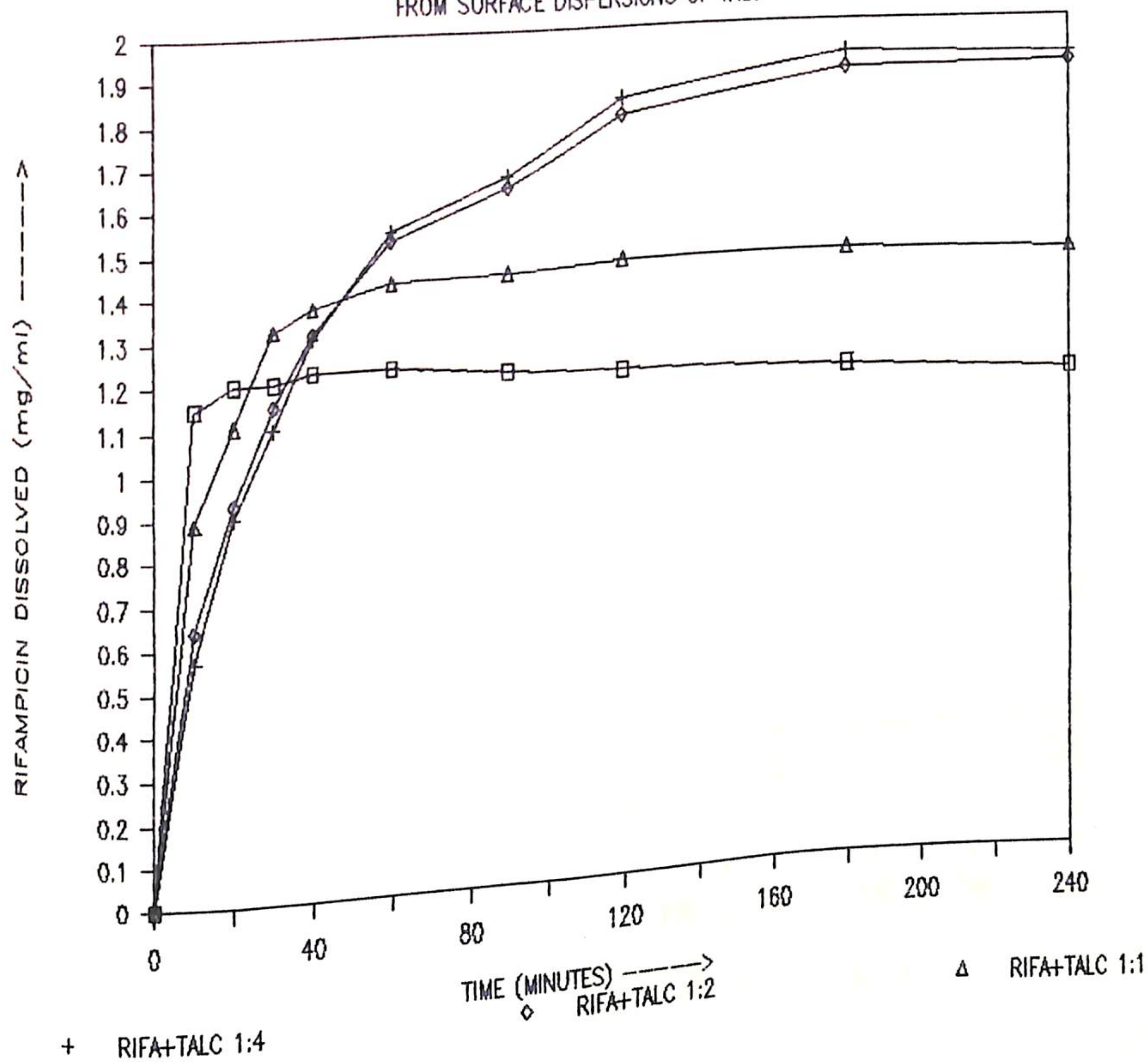
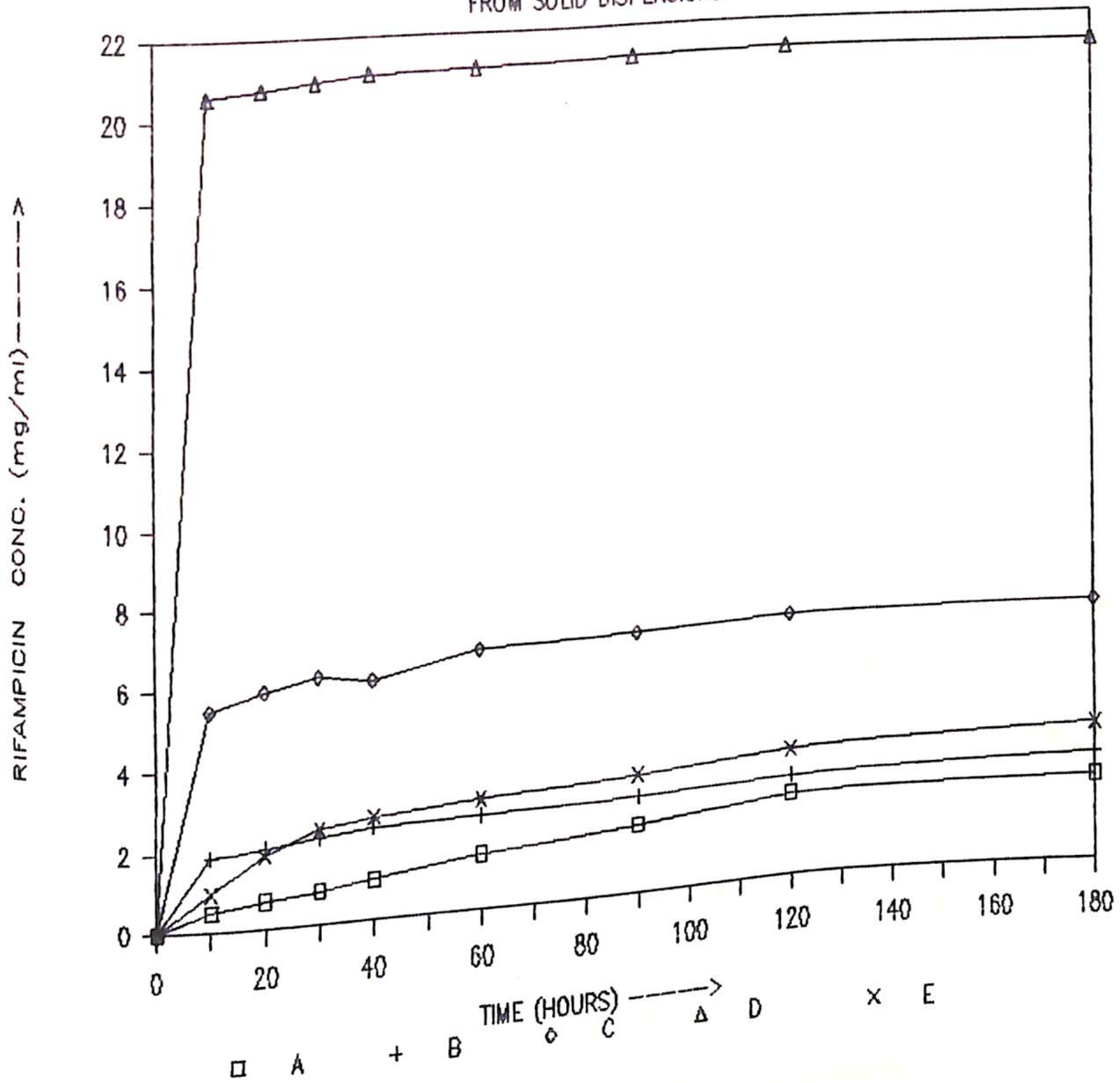


FIG 7.9B : DISS.RATE PROFILE OF RIFA
FROM SURFACE DISPERSIONS OF TALC
RIFAMPICIN DISSOLVED (mg/ml) ------->
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
40
80
120
160
200
240
TIME (MINUTES) ------->
△ RIFA+TALC 1:1
◇ RIFA+TALC 1:2
+ RIFA+TALC 1:4



A = Rifampicin (as such)

B = Solid dispersion of rifampicin with PEG 4000 (solvent method)

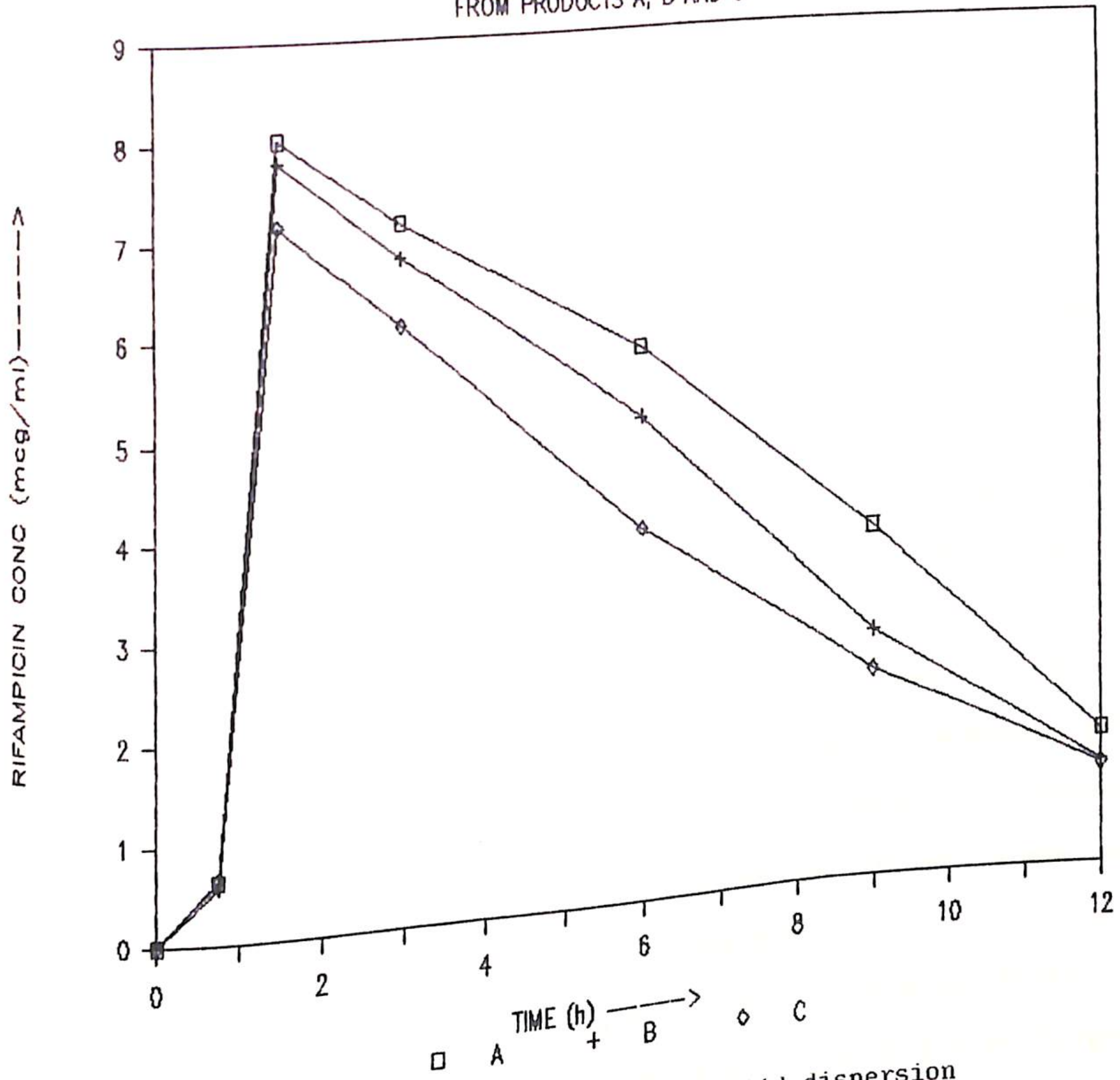
C = Solid dispersion of rifampicin with PEG 4000 (fusion method)

D = Solid dispersion of rifampicin with PVP K 30

E = Solid dispersion of rifampicin with mannitol

FIG 7.10B: SERUM CONC. OF RIFAMPICIN

FROM PRODUCTS A, B AND C



A	=	Product A, containing solid dispersion of rifampicin with PVP K 30
B	=	Product B, containing surface dispersion of rifampicin with talc
C	=	Product C, containing rifampicin as such

RESULTS AND DISCUSSION

7.1 Effect of particle size on the dissolution rate and bioavailability of drugs

USP specifies 0.1 N hydrochloric acid solution as dissolution fluid for dissolution test of rifampicin. However rifampicin has been reported to degrade in acidic medium and alternate medium containing 0.4 % w/v sodium lauryl sulphate (SLS) solution in water has been reported to be suitable for dissolution test of rifampicin products [243]. In this study samples were tested in both dissolution media i.e. 0.1 N hydrochloric acid and 0.4 % w/v SLS solution.

Comparision of parameters derived from dissolution profile (Figures 7.1AI and 7.1AII) and bioavailability study (Figure 7.1B) are summarised in Table 7.1E.

No significant difference both in rate and extent of bioavailability was observed between Products A and C containing drug having mean particle size of 450 um and 112.5 um respectively. However when the mean particle size was reduced to 10 um, there was significant reduction in extent of bioavailability.

The reduction in bioavailability from micronized drug (Product D) can be attributed to delayed wetting of product due to static charges. This is reflected in the dissolution rate study. The amount of drug dissolved in first 10 minutes is significantly less in both 0.1 N hydrochloric acid and sodium lauryl sulphate dissolution fluids. However once the drug is wetted in 10-20 minutes, the rate of dissolution from micronized drug is much faster and the concentration level of drug in dissolution fluid crosses the other two products

AUC values were correlated with DP30 (dissolution percentage in first 30 minutes) and DE30 (dissolution efficiency over first 30 minutes) using 0.1 N hydrochloric acid and 0.4 % w/v sodium lauryl sulphate solutions as dissolution media (Table 7.1E). Both DP30 and DE30 values have better *in vivo-in vitro* correlation when 0.4 % w/v sodium lauryl sulphate solution is used as dissolution medium. Moreover the concentration of drug in 0.1 N hydrochloric acid increases upto 45 minutes and starts decreasing thereafter indicating degradation. Our phenomenon is not observed in 0.4% SLS solution. This confirms are earlier finding about the superiority of 0.4 % sodium lauryl sulphate as dissolution medium for rifampicin.

From this study we could conclude the following : particle size has insignificant effect on bioavailability. Finer drug (less than 400 mesh) may require suitable wetting agent in the formulation suitability of 0.4 % w/v sodium lauryl sulphate as dissolution fluid for rifampicin dosage forms is confirmed.

7.2 Effect of surfactants on rifampicin dissolution

The maximum concentration of rifampicin achieved was 9.36 mg/ml in 2 % w/v sodium lauryl sulphate solution. Considering this as maximum achievable concentration, the relative DE30 values were computed for all surfactants and are summarised in Table 7.2F.

Data indicates the maximum dissolution occurs using sodium lauryl sulphate. Assigning value of 100 % to DE30 for 2 % w/v sodium lauryl sulphate solution, the relative DE30 for bile salts, docusate sodium, polysorbate 80 and poloxamer 188 are 54 %, 46 %, 19 % and 12 %

respectively. Polysorbate 80 shows erratic dissolution behaviour. The concentration of drug gradually increases with time in 0.1 % concentration whereas in 0.2-2.0 % concentrations the maximum concentration of drug is achieved in 15 minutes and decreases there after. To summarise, sodium lauryl sulphate is the best solubilizer among the surfactants tested for rifampicin. Solubility of drug increases by 60 times in 2 % w/v aqueous solution of sodium lauryl sulphate.

7.3 Effect of surfactants on the partition of rifampicin

Sodium lauryl sulphate reduces the partition coefficient significantly at all concentrations tested (considering 20 % change as significant) (Table 7.3A). Bile salts cause significant reduction in partition coefficient above 0.5 % concentrations. Docusate sodium increases partition of drug in oily phase at low concentrations (0.1 %) but has no significant effect at higher concentrations. At concentration above 1 % docusate sodium precipitates out of the solution. Poloxamer 188 reduces partition coefficient significantly above 1 % concentration. Polysorbate 80 favours partition towards n-octanol phase upto 0.2 % concentration. At concentration 0.5 % and above the partition coefficient is significantly reduced.

7.4 Effect of surfactants on bioavailability of rifampicin in rats

The amount of rifampicin absorbed in 30 minutes from the *in situ* model of rat intestine is plotted versus concentration of surfactants used (Figure 7.4AI). The mean serum concentration of rifampicin

achieved after 30 minutes using the above model is plotted versus concentration of surfactants (Figure 7.4AII).

Considering 20 % enhancement in the extent of drug absorbed (Figure 7.4AI) as significant, the results could be interpreted as under :

- Polysorbate 80 at 1 % w/v and 2.5 % w/v concentration and bile salts and sodium lauryl sulphate at 2.5 % w/v concentrations each have significant enhancement effect on absorption of rifampicin.
- Polysorbate 80 is most effective absorption enhancer for rifampicin. The increase in absorption by its 1 % w/v concentration is more than the enhancement by 2.5 % w/v concentration of sodium lauryl sulphate and bile salts.
- Docusate sodium and poloxamer 188 has no influence on drug absorption.
- The enhancement of drug absorption by various surfactants is in the following order : polysorbate 80 > sodium lauryl sulphate> bile salts > docusate sodium, poloxamer 188.

Similar conclusions could be drawn from serum concentration data (Figure 7.4AII) considering 20 % increase in serum concentration as significant. This data shows significant absorption enhancement effect of above 1 % w/v concentration of sodium lauryl sulphate. Other conclusions were same as made from intestinal absorption data. The 2.5 % w/v concentration of polysorbate 80 enhanced serum concentration by 100 % in rats.

7.5 Effect of polysorbate 80 on bioavailability of rifampicin

The pharmacokinetic parameters for Products A (control, without surfactant), B (0.25 % w/v polysorbate 80), C (1.0 % w/v polysorbate 80) and D (2.5 % w/v polysorbate 80) are given in Tables 7.5A to 7.5D respectively. The mean serum concentration values versus time are plotted in Figure 7.5A.

The AUC values for Products B, C and D were enhanced by 40%, 62 % and 90 % respectively as compared with control Product A. This indicates a significant enhancement effect of polysorbate 80 on extent of rifampicin absorption. Similarly the increase in peak plasma concentration (Cmax) values for Products B, C and D was 31 %, 59 % and 80 % respectively. The time to reach peak plasma concentration (Tmax) was reduced by half in case of Products C and D. The taste of suspensions containing upto 2.5 % polysorbate 80 was acceptable. It could thus be concluded that polysorbate 80 enhances bioavailability of rifampicin significantly. Bioavailability could be enhanced to extent of 90 % w/v with a concentration of 2.5 % w/v polysorbate 80 in the product.

7.6 Effect of amorphous and polymorphic forms on dissolution rate and bioavailability of rifampicin

The dissolution efficiency and dissolution percent over first 30 minutes (Figure 7.6A) alongwith pharmacokinetic parameters (Fugures 7.6B and 7.6C) from the serum drug concentration data are summarised

Amorphous (Product Y) versus polymorph A (Product X)

The AUC and Cmax values for amorphous form are less by 18% and 8% respectively as compared to polymorph A which are within acceptable limits of ± 20 %. The time required to achieve peak plasma concentration is same for both forms. Marginally low AUC and Cmax values are due to initial slow dissolution in first 10 minutes (less than 1 % compared to 16.45 % for polymorph A, which could be due to delayed wetting caused by air entrapment and static charges on relatively finer particles of amorphous form. Both forms could be considered as bioequivalent.

Polymorph A (Product X) versus polymorph B (Product Z)

The AUC and Cmax values for polymorph B are higher by 81 % and 74 % respectively as compared to polymorph A. This is a substantial increase in bioavailability.

The high bioavailability of polymorph B is due to its higher dissolution rate. The DP30 and DE30 values for polymorph B are higher by 44 % and 108 % respectively as compared to polymorph A. There is a very good *in vitro-in vivo* correlation between AUC and DP 30 and DE30 values ($r > 0.995$). It could thus be concluded from this study that bioavailability of rifampicin can be enhanced significantly (over 80%) by using polymorph B of rifampicin.

7.7 Effect of cyclodextrins on rifampicin dissolution

Alpha-, beta-, gamma- and hydroxypropyl beta- cyclodextrins were studied for complexation behaviour. The plots of the amount of

rifampicin dissolved versus time in 0.01 M solutions of different cyclodextrins are shown in Figures 7.7A (for pH 4.0) and 7.7B (for pH 7.9).

The increase in maximum amount of rifampicin dissolved (Cmax) in alpha-cyclodextrin and beta-cyclodextrin is less than 3 % at pH 4.0 and less than 16 % at pH 7.9. This is an insignificant increase (< 20 %).

The increase in Cmax at pH 4.0 and 7.9 as 34 % and 38 % respectively for gamma-cyclodextrin and 32 % and 26 % respectively for hydroxypropyl beta-cyclodextrin, appears to be significant. However considering 1:1 stoichiometric ratio for complexation, the concentration of 8.23 mg/ml should have been achieved which is over 600 % than the maximum concentrations achieved without cyclodextrin at both pH values (less than 1.3 mg/ml). The concentration obtained are not near to even 1:2 or 1:3 stoichiometric ratios. Thus it could be concluded that cyclodextrins do not form inclusion complexes with rifampicin.

7.8 Effect of povidones on rifampicin dissolution

Three grades of povidone K30, K90 and K120 were tried to determine the solubilizing effect of povidones on rifampicin. The concentrations of rifampicin achieved in aqueous solutions of povidones at pH 4.0 and 7.9 were plotted versus concentration of povidones in (Figures 7.8AI to 7.8CII). From the above data following conclusions could be made :

Povidones have solubilizing effect on rifampicin at pH 4.0 and 7.9. This effect increases with increase in molecular weight of povidones (povidone K120 > K90 > K30). The solubilizing effect increases with increase in concentration of povidones. At 5 % concentrations the solubility of rifampicin increases 3-4 times depending on the grade of povidone used.

7.9 Solid surface dispersions

When surface dispersions having same drug : excipient ratio of 1:2 were compared (Figure 7.9A), the dispersion with talc showed maximum dissolution enhancement effect. The maximum concentration achieved was 53 % more than from rifampicin alone. Starch and Aerosil 200 had no effect on rifampicin dissolution. The dissolution enhancing effect of talc compared to other absorbents could be due to better reversible adsorption of drug.

Comparison of three surface dispersions of rifampicin-talc having drug : excipient ratios of 1:1, 1:2 and 1:4 (Figure 7.9B) indicates the influence of talc content on dissolution from dispersions. Dissolution of rifampicin increases with increase in talc content in the surface dispersion. The increase in dissolution was 18 %, 53 % and 57 % from dispersions having drug : talc ratio of 1:1 1:2 and 1:4. The 1:1 ratio has insignificant effect on drug dissolution. Dissolution from 1:2 and 1:4 ratios is equally significant. It could be concluded that surface dispersion having rifampicin : talc ratio of 1:2 has optimum effect on drug dissolution.

The serum concentration versus time data and pharmacokinetic parameters of drug from the rifampicin-talc (1:2 ratio) surface dispersion (Product B) and rifampicin as such (Product C) are given in Tables 7.10D and 7.10E. Mean serum concentration versus time are plotted in Figure 7.10B.

The AUC0-inf and Cmax values for surface dispersion are higher by 14 % and 9 % respectively as compared to rifampicin alone. This is within ± 20 % limits for bioequivalency. Results conclude that solid surface dispersion of talc does not enhance bioavailability although it exhibited significant increase in drug dissolution.

7.10 Solid dispersions

The dissolution profile of all solid dispersions is plotted in (Figure 7.10A). The parameters derived from dissolution data/graph are summarised in Table 7.10B. The parameters covered include A30 (amount of drug dissolved in 30 minutes) and Cmax (maximum amount of drug dissolved in 3 h).

Maximum dissolution enhancement effect was observed with povidone solid dispersions. The A30 and Cmax values are 24.6 and 9.7 times as compared to rifampicin as such.

Polyethylene glycol 4000 dispersions were prepared by two alternate methods. The fusion method was found to be more effective than solvent method. A30 and Cmax values for fusion method are 2.8 and 2.5 times respectively compared to solvent method.

The overall dissolution enhancement effect of solid dispersions observed was in following order ; povidone > PEG 4000 (fusion method) > PEG 4000 (solvent method) > mannitol.

The serum concentration of rifampicin-povidone solid dispersion (Product A) and rifampicin as such (Product C) are plotted in Figure 7.10B. The pharmacokinetic parameters are given in Table 7.10C and 7.10E.

The AUC0-inf and Cmax values for solid dispersion are higher by 35 % and 12 % respectively. The 35 % increase in AUC is significant.

It could thus be concluded that bioavailability of rifampicin can be enhanced significantly by using its solid dispersion in povidone K30 (drug : carrier ratio 1:3).

CONCLUSIONS

CHAPTER-8

This investigation was aimed to : determine rifampicin's compatibility with pharmaceutical excipients ; study physicochemical parameters, not reported in literature, which may influence its stability or bioavailability ; and find ways to enhance its bioavailability. The findings are briefed below :

(1) The developed HPLC methods are validated and appropriate for analysis of rifampicin in stability and biofluid samples. The validated HPTLC method is suitable for analysis of rifampicin alongwith its degradation products/impurities in raw material or preformulation samples.

(2) For rifampicin - isoniazid combination products, 0.4 % w/v sodium lauryl sulphate aqueous solution is a suitable dissolution fluid.

(3) Rifampicin has minimum solubility at pH 3.0 in both buffered and unbuffered solutions.

(4) The powder flowability of drug is inadequate.

(5) The drug is nonhygroscopic below 33 % RH.

(6) The dielectric requirement of drug for solvent systems is 27.55.

(7) The lipophilicity of drug is 1.336.

(8) In solid state the following excipients (in the concentration used for preformulation study) reduce the shelf-life of rifampicin to less than half (listed in descending order of adverse effect)- urea, polyethylene glycol, calcium phosphate dibasic, docusate sodium, starch, sodium starch glycolate, polysorbate 80, magnesium

stearate, sucrose, sodium lauryl sulphate, Aerosil 200, mannitol, microcrystalline cellulose, poloxamer 188, gelatin and cyclodextrin.

(9) Rifampicin is stable to UV/visible light in solid state but is sensitive in aqueous solutions. Visible light and UV light of 254 nm wavelength are more harmful than UV light of 366nm wavelength.

(10) Oxygen degrades rifampicin solutions.

(11) Rifampicin is maximum stable at pH 4.0 and unstable in highly acidic solutions (pH < 3.0).

(12) Acetate, chloroacetate and phosphate buffers degrade the drug faster by 10, 3 and 3 times respectively compared to unbuffered solutions. Formate buffer has insignificant effect.

(13) Isoniazid although incompatible with rifampicin in aqueous solutions, has no significant effect on its absorption.

(14) Particle size (from 30-200 mesh) as insignificant effect on bioavailability. However finer drug (< 400 mesh) has poor bioavailability due to its delayed wetting.

(15) Bioavailability of drug can be significantly enhanced by use of

(a) polysorbate 80

(b) polymorph B

(c) solid dispersions with povidone

REFERENCES

CHAPTER 9

1. Wadke, D.A., Serajuddin, A.T.M., Jacobson, H. : Preformulation Testing. In Pharmaceutical Dosage Forms : Tablets.Vol.1,2nd Ed.,Edited by Herbert A. Lieberman, Leon Lachman. Marcel Dekker, Inc., N.Y. 1989, pp. 1 - 73.

2. Hoogdalm, E.J.V., Boel, A.G.D., Briemer, D.D. : Pharmacol. Ther., 44 : 407, 1989.

3. Fujii, S., Yokoyama, T., Ikegawa, K., Sato, F., Yakoo, N. : J. Pharm. Pharmacol, 37 : 545, 1986.

4. Aungst, B.J., Rogers, N.J., Schefter, E. : J. Pharmacol. Exp. Ther., 244 : 23, 1988

5. James I. Wells : Pharmaceutical Preformulation, The Physicochemical Properties of Drug Substances, Ellis Horwood limited, Chichester, England 1988, pp. 21 - 85.

6. Abdou, H.M. : Dissolution, Bioavailability Bioequivalence, Mack Publishing Co, Easton, 1989, pp. 53 - 72.

7. Kaplan, S.A. : Drug Metab. Rev., 1 : 15, 1972.

8. Nogami, H., Nagai, T., Suzuki, A. : Chem. Pharm. Bull., 14 : 329, 1966.

9. Wood, J.H., Syarto, J.E., Letterman, H. : J. Pharm. Sci., 54 : 1068, 1965.

10. Millosovich, G. : J. Pharm. Sci., 53 : 484, 1964.

11. Monkhouse, D.C., Lach, J.L. : J. Pharm. Sci., 61 : 1430, 1972.

12. Halebeian, J.K. : J. Pharm. Sci., 64 : 1269, 1975.

13. Biles, J.A. : J. Pharm. Sci., 51 : 499, 1962.

14. Kunhert-Brstatter, M. : Pure Appl. Chem., 10 : 133, 1965.

15. Frederick, K.J. : J. Pharm. Sci., 50 : 531, 1961.

16. Yalkowsky, S.H. : Techniques of Solubilization of Drugs. Edited by S. H. Yalkowsky, Marcel Dekker, New York, 1981, p. 160.

17. Aguiar, A.J., Zeimer, J.E. : J. Pharm. Sci., 58 : 983, 1969.

18. Aguiar, A.J., Krc, J., Kinkel, A.W., Symyn, J.C. : J. Pharm. Sci., 56 : 847, 1967.

19. Maeda, T., Takenaka, M., Yamahera, Y., Noguchi, T. : Chem. Pharm. Bull., 28 : 431, 1980.

20 Miyazaki, S., Arita, T., Hori, R., Ito, K. : Chem. Pharm. Bull., 22 : 638, 1974.

21 Miyazaki, S., Nakano, M., Arila, T. : Chem. Pharm. Bull., 24 : 2094, 1976.

22 Kato, Y., Kohketsu, M. : Chem. Pharm. Bull., 29 : 268, 1981.

23 Haleblian, J.K., Koda, R.T., Biles, J.A. : J. Pharm. Sci., 60 : 1488, 1971.

24 Kuroda, K., Nomura, T., Koba, J. : Med. Sci., 20 : 47, 1974.

25 Kuroda, T., Yokayama, T., Umeda, T., Matsuzava, A., Kuroda, K., Asada, S. : Chem. Pharm. Bull., 30 : 3728, 1982.

26 Shibata, M., Kokubo, H., Morimoto, K., Morisaka, K., Ishida, T., Inove M. : J. Pharm. Sci., 72 : 1436, 1983.

27 Higuchi, W.I., Lau, P.K., Higuchi, T., Shell, J.W. : J. Pharm. Sci., 52 : 150, 1963.

28 Coherty, C., York, P. : Int. J. Pharm., 47 : 141, (Nov) 1988.

29 Banakar, U.V. : Pharmaceutical Dissolution Testing, Marcel Dekker Inc., New York, 1992, pp. 133-187.

30 Heleblian, J., McCrone, W. : J. Pharm. Sci., 58 : 911, 1969.

31 Mullins, J.D., Macek, T.J. : J. Pharm. Sci., 49 : 245, 1960.

32 Sekiguchi, K., Obi, N. : Chem. Pharm. Bull., 9 : 866, 1961.

33 Banerjee, S., Byopadhyay, A., Bhattacharjee, R., Mukherjee, A., Halder, A. : J. Pharm. Sci., 60 : 153, 1971.

34 Egawa, H., Maeda, S., Yonemochi, E., Oguchi, T., Yamamoto, K., Nakai, Y. : Chem. Pharm. Bull., 40 : 819, 1992.

35 Ibrahim, H., Pisano, F., Bruno, A. : J. Pharm. Sci., 66 : 669, 1977.

36 Chan, H., Doelkar, E. : Drug Dev. Ind. Pharm., 11 : 315, 1985.

37 Florence, A., Salole, E. : J. Pharm. Pharmacol., 28 : 637, 1976.

38 Kanewa, N., Otsuka, M. : Chem. Pharm. Bull., 33 : 1660, 1985.

39 Otsuka, M., Mastumoto, T., Kaneniwa, N. : J. Pharm. Pharmacol., 41 : 665, 1989.

40 Takahashi, Y., Nakashima, K., Ishihara, T., Nakagawa, H., Sugimoto, I. : Drug Dev. Ind. Pharm., 11 : 1543, 1985.

41 Ghan, G.A., Lalla, J.K. : J. Pharm. Pharmacol., 44 : 678, 1992.

42 Summers, M. P., Enever, R.P., Carless, J.E. : J. Pharm. Pharmacol., 28 : 89, 1976.

43 Imaizumi, H., Nambu, N., Nagai, T. : Chem. Pharm. Bull., 28 : 2565, 1980.

44 Floyd, D.M., Kocy, O.R., Monkhouse, D.C., Pipkin, J.D. : U.S. Patent, 28 : 2636, 1981.

45 Callow, H., Kennard, O. : J. Pharm. Pharmacol., 13 : 723, 1961.

46 Carless, J., Moustafa, M., Rapson, H. : J. Pharm. Pharmacol., 18 (Suppl) : 190 S, 1966.

47 Ebian, A., Moustafa, M. and Khalil, S. : J. Pharm. Sci., 64 : 1481, 1975.

48 Graf, E., Beyer. C., Abdallah, O. : Pharm. Ind., 44 : 1071, 1982.

49 Nail, S., White, J., Hem. S. : J. Pharm. Sci., 65 : 1192, 1976.

50 Morefield, E., Peck, G., Feldkamp, J., White, J., Hem, S. : J. Pharm. Sci., 75 : 403, 1986.

51 Rowe, E., Anderson, B. : J. Pharm. Sci., 73 : 1673, 1984.

52 Hoelgaard, A., Moller, N. : Int. J. Pharm. 15 : 213, 1983.

53 Pearson, J., Varney, G. : J. Pharm. Pharmacol., 21 : 60 S, 1969.

54 Saad, N., Higuchi, W. : J. Pharm. Sci., 54 : 1303, 1965.

55 Carless, J., Moustafa, M., Rapson, H. : J. Pharm. Pharmacol., 20 : 639, 1968.

56 Ebian, A., Moustafa, M., Khalil, S., Motawi, M. : J. Pharm. Pharmacol., 25 : 13, 1973.

57 Nail, S., White, J., Hem. S. : J. Pharm. Sci., 65 : 1195, 1976.

58 Higuchi, T. : J. Am. Pharm. Associ., Sci. Ed., 47 : 657, 1958.

59 Biles, J.A. : J. Pharm. Sci., 51 : 601, 1962.

60 Kendall, D.N. : Anal. Chem., 25 : 382, 1953.

61 Cleverley, B., Williams, P.P. : Tetrahedron, 7 : 277, 1959

62 Ebert, A.A. Jr., Gottlieb, H.B. : J. Am. Chem. Soc., 74 : 2806, 1952.

63 Higuchi, W.I., Bernardo, P.D., Mehta, S.C. : J. Pharm. Sci., 56 : 200, 1967.

64 Mesley, R.J., Clements, R.L., Flaherty, B., Goodhead, K. : J. Pharm. Pharmacol., 20 : 329, 1968.

65 Yang, S.S., Guillory, J. : J. Pharm. Sci., 61 : 26, 1972.

66 Fiese, F.E., Hagen, T.A. : Preformulation. In The Theory and Practice of Industrial Pharmacy. 3rd Ed., Edited by Leon Lachman et al. Lea and Febiger, Philadelphia, 1986, pp. 171 - 196.

67 Dare, J.G. : Australian, J. Pharm. Sci., 45 : S-58, 1964.

68 Bates, T.R., Young, J.M., Wu, C.M., Rosenberg, H.A. : J. Pharm. Sci., 63 : 643, 1974.

69 Prescott, L.F., Steel, R.F., Ferrier, W.R. : Clin Pharmacol. Ther., 11 : 496, 1970.

70 Atkinson, R.M., Bedford, C., Child, K.J., Tomich, E.G. : Nature, 193 : 588, 1962.

71 Watari, N., Hanano, M., Kanenuva, N. : Chem. Pharm. Bull., 28 : 2221, 1980.

72 Ridolfo, A. S., Tompkins, L., Bechtol, L. D., Carmicheal, R. H. : J. Pharm. Sci., 66 : 850, 1979.

73 Atkinson, R.M., Bedford, C., Child, K.J., Tomich, E.G. : Antibiot. Chemotherapy, 12 : 232, 1962.

74 Bedford, C., Busfield, D., Child, K.J., Mac, Gregor, I., Sutherl, P., Tomich, EG. : Arch. Dermatol., 81 : 735, 1960.

75 Katdare, A.V., Oddoye, D.D., Bavitz, J.F. : Drug Dev. Ind. Pharm., 13 : 281, 1987.

76 Medes, R.W., Masih, S.Z., Kanumuri, R.R. : J. Pharm. Sci., 67 : 1613, 1978.

77 Shaw, T.R.D., Carless, J.E. : Eur. J. Clin. Pharmacol., 7 : 269, 1974.

78 Dressman, J.B., Fleisher, D. : J. Pharm. Sci., 75 : 109, 1986.

79 Journela, A.J., Pentikainen, P.J., Sothmam A. : Eur. J. Clin. Pharmacol., 8 : 365, 1974.

80 Finchor, J.H., Adams, J.G., Beal, H.M. : J. Pharm. Sci., 54 : 704, 1965.

81 Levy, G. : Lancet, 2 : 723, 1962.

82 Kaneniwa, N., Watari, N., Iuima, H. : Chem. Pharm Bull., 26 : 2603, 1978.

83 Gibaldi, M., Feldman, S. : J. Pharma. Sci., 59 : 579, 1970.

84 Samyn, J.C., Jung, W.Y. : J. Pharm. Sci., 59 : 169, 1970.

85 Lerk, C.F., Lagas, M., Fell, J.T., Nauta, P. : J. Pharm. Sci., 67 : 935, 1978.

86 Finholt, E. : Influence of Formulation on dissolution rates. In Dissolution Technology, Edited by Leeson L.J. Carstenson J.T.,Academy of Pharmaceutical Association, Washington DC, 1974, p. 106.

87 Solvang, S., Pinholt, P. : J. Pharm. Sci., 59 : 49, 1970.

88 Acarturk, F., Kislal, O., Celebi, N. : Int. J. Pharm., 85 : 1, 1992.

89 Walters, V. : J. Pharm. Pharmacol., 20 (Suppl) : 2285, 1968.

90 Kumura, S., Imai, T., Otagiri, M. : Chem. Pharm. Bull., 39 : 1328, 1991.

91 Stamm, A., Gissinger, D., Boymond, C. : Drug Dev Ind. Pharm., 10 : 381, 1984.

92 Gantt, C.L., Gochman, N., Dyniewicz, J.M. : Lancet, 1 : 486, 1960

93 Kakemi, K., Arita, T., Muranishi, S. : Chem. Pharm, Bull., 13 : 976, 1965.

94 El-Gindi, N.A., Shalaby, A.A., Abd, El Khalek, M.M. : Drug Dev. Ind. Pharm., 9 : 363, 1983.

95 Motola, S, Agharkar S.N. : Preformulation, Research of Parentral Medications. In Pharmaceutical Dosage Forms : Parentral Medications. Vol.1 Ed by K.E. Avis, H.A.Liberman, L. Lachman, Marcel Dekker Inc, New York, 1992, p. 115 - 172.

96 Berge, S.M., Bighley, L.D., Monkhouse, D.C. : J. Pharm. Sci., 66 : 1, 1977.

97 Miller, L.C., Holl, A.H. : Mod. Med., 28 : 312, 1960.

98 Wagner, J.G. : J. Pharm. Sci., 50 : 359, 1961.

99 Higuchi, T., Connors, K.A. : In Advances in Analytical Chemistry and Instrumentation Edited by C.N. Reilley, Vol.4, Wiley-Interscience, New York, 1965, pp. 117.

100 Krellard, B., Higuchi, T., Repta, A. J. : J. Pharm. Sci., 64 : 1850, 1975.

101 Guttman, D.E., Athalye, M. : J. Am. Pharm. Associ., Sci. Ed., 49 : 687, 1960.

102 Wadke, D.A., Guttman, D.E. : J. Pharm. Sci., 54 : 1293, 1965.

103 Higuchi, T., Lach, J.L. : J. Am. Pharm. Associ., 43 : 349, 1954.

104 Nohtomi, K., Imai, T., Otagiri, M., Masumoto, H., Satoh, T. : Japan-US congress of Pharmaceutical Sciences, Honululu, 1987.

105 Frank, S.G. : J. Pharm. Sci., 64 : 1585, 1975.

106 Szabo-Revesz, P. : Pharm. Ind., 51 : 94, 1989.

107 Bettinetti G.P. : Farmaco Ed. Prat., 44 : 195, 1989.

108 Backensfeld, T., Muller, B.W. : Pharm. Res., 7 : 484, 1990.

109 Cohen, J., Lach, J. : J. Pharm. Sci., 52 : 132, 1963

110 Lach, J., Cohen. : J. Pharm. Sci., 52 : 137, 1963.

111 Duchene, D. : Pharm. Tech., 14(6) : 26, 1990.

112 Szeitli, J. : Pharm, Technol., 15(8) : 24, 1991.

113 Uekama, K., Hirayama, F., Esaki, K., Inoue, M. : Chem. Pharm. Bull., 27 : 76, 1979.

114 Uekama, K., Narisawa, S., Hirayama, F., Otagiri, M. : Int J. Pharm., 16 : 327, 1983.

115 Bootsma, H.P.R., Frijlink, H.W., Eissens, A., Proost, J.H., Doorne, V.H., Lerk, C.F. : Int. J. Pharm., 51 : 213, 1989.

116 Seo, H., Tsuruoka, M., Hashimoto, T., Fujinaga, T., Otagiri, M., Uekama, K. : Chem. Pharm. Bull., 31 : 286, 1983.

117 Otagiri, M., Imai, T., Uekama, K. : J. Pharm. Dyn., 5 : 1027, 1982.

118 Otagiri, M., Imai, T., Hirayama, F., Uekama, K., Yamasaki, M. : Acta Pharm. Suec., 20 : 11, 1983.

119 Uekama, K., Fujise, A., Hirayama, F., Otagiri, M., Inada, K. : Chem. Pharm. Bull., 32 : 275, 1984.

120 Islam, M.S., Narurkar, M.M. : Drug Dev. Ind. Pharm., 17 : 1229, 1991.

121 Tokumura, T., Ueda H., Tsushima, Y., Kasai, M., Kayano M., Amada, I., Nagai, T. : Chem. Pharm. Bull., 32 : 4179, 1984.

122 Corrigan, O.I., Stanley, C.T. : J. Pharm. Pharmacol., 34 : 621, 1982.

123 Corrigan, O.I., Stanley, C.T. : Pharm. Acta. Helv., 56 : 204, 1981.

124 Koizumi, K., Miki, H., Kubota, Y. : Chem. Pharm. Bull., 28 : 319, 1980.

125 Tasic, L. J. M., Jovanovic, M.D., Djuric, Z.R. : J. Pharm. Pharmacol., 44 : 52, 1992.

126 Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Kurono, Y. : J. Pharm. Pharmacol., 34 : 627, 1982.

127 Brewster, E., Kerry, S. E., Loftsson T., Perchalski, R., Gotelind, D.M., Bodor, N. : J. Pharm. Sci., 77 : 981, 1988.

128 Pitha, J., Pitha, J. : J. Pharm. Sci., 74 : 987, 1985.

129 Nang, L.S., Simond, J., Schiff, V., Trottier, D., Pourrat, A. : Pharm. Acta. Helv., 60 : 112, 1985.

130 Bettinetti, G., Gazzaniga, A., Mura, P., Giordano, F., Setti, M. : Drug Dev. Ind. Pharm., 18 : 39, 1992.

131 Erden, N., Celebi, N. : Int. J. Pharm., 48 : 83, (Dec) 1988.

132 Thakkar, A.L., Kuehn, P.B., Perrin, J.H., Wilham, W.L. : J. Pharm. Sci., 61 : 1841, 1972.

133 Uekama, K. : Acta. Pharm. Suec., 20 : 287, 1983.

134 Aboutaleb, A.E., Abdel, R.A.A., Sayed, I. : Drug Dev. Ind. Pharm., 12 : 2259, 1986.

135 Lin, S.Y., Yang, J.C. : Pharm. Weekbl. Sci., 8 : 223, 1986.

136 Vila-Jato, J.L., Blanco, J., Torres, J.J. : S.T.P. Pharma., 3 : 28, (Jan) 1987.

137 Kata, M. : Pharmazie, 43 : 52, (Jan) 1988.

138 Kikuchi, M., Uekama, K. : Yakugaku Zasshi, : 108 : 156, 1988.

139 Pawlaczyk, J., Hladon, T., Sobczak, H. : Acta. Pol. Pharm., 46(1) : 48, 1989.

140 Uekama, K., Udo, K., Irie, T., Yoshida, A., Tsuruoka, M. : Acta. Pharm. Suec., 24 : 27, 1987.

141 Celebi, N., Nagai, T. : S.T.P. Pharma., 3 : 868, 1987.

142 Menard, F.A., Dedhiya M.G., Rhodes, C.T. : Drug Dev. Ind. Pharm., 14 : 1529, 1988.

143 Puglisi, G., Santagati, N.A., Pignatello, R., Ventura, C., Bottino F.A., Mangiafico S., Mazzone, G. : Drug Dev. Ind. Pharm., 16 : 395, 1990.

144 Hamada, Y., Nambu, N., Nagai, T. : Chem. Pharm. Bull., 23 : 1205, 1975.

145 Fukuda, N., Higuchi, N., Ohno, M., Kenmochi, H., Sekikawa, H., Takada, M. : Chem. Pharm. Bull., 34 : 1366, 1986.

146 Andersen, F.M., Bundgaard, H. : Int. J. Pharm., 19 : 189, (Apr) 1984.

147 Otagiri M., Fujinaga, T., Sakai, A., Uekama, K. : Chem. Pharm. Bull., 32 : 2401, 1984.

148 Yuan, S.L., Shi, T.S., Ruan, J.X. : Clin. J. Hosp. Pharm., 8 : 145, (Apr) 1988.

149 Hsu, P.F., et. al. : Drug Dev. Ind. Pharm., 10 : 601, 1984.

150 Yusuff, N.T., York, P., Chrystyn, H., Swallow, R.D., Bramley, P.N., Losowsky, M.S. : J. Pharm. Pharmacol., 42(Suppl) : 8P. 1990.

151 Imai, T., Otagiri, M., Saito, H., Uekama, K. : Chem. Pharm. Bull., 36 : 354, 1988.

152 Otagiri, M., Imai, T., Hirayama, F., Uekama, K. : Acta. Pharm. Suec., 20 : 1, 1983.

153 Duchene, D., Wouessidjewe, D. : Pharm. Technol., 14(8) : 22, 1990

154 Uekama, K. : J. Pharm. Sci., 72 : 1338, 1983.

155 Uekama, K. : J. Pharm. Pharmacol., 37 : 532, 1985.

156 Uekama, K., Matsuo, N., Hirayama, F., Yamaguchi, T., Imamura, Y., Ichibagase, H. : Chem. Pharm. Bull., 27 : 398, 1979.

157 Nambu, N., Shimoda, M., Takahashi, Y., Ueda, H., Nagai, T. : Chem. Pharm. Bull., 26 : 2952, 1978.

158 Nakai, Y., Yamamoto, K., Terada, K., Horibe, H., Ozawa, K. : Chem. Pharm. Bull., 31 : 3745, 1983.

159 Sekikawa, H., Fukuda, W., Takada, M., Ohtani, K., Arita, T., Nikano, M. : Chem. Pharm. Bull., 31 : 1350, 1983.

160 Andersen, F.M. Bundgaar, H. : Arch. Pharm. Chem., Sci. Ed., 10 : 81, 1982.

161 Kata, M. Papp, L. : Pharmazie., 42 : 65, 1987.

162 Iwaoku, R., Arimori, K., Nakano, M., Uekama, K. : Chem. Pharm. Bull., 30 : 1416, 1982.

163 Uekama K., Oh, K., Otagiri, M., Tsuruoka, S.M. : Pharm. Acta. Helv., 58 : 338, 1983.

164 Andersen, F.M., Bundgaar, H. : Int. J. Pharm., 21 : 51, (Aug) 1984.

165 Andersen, F.M., Bundgaar, H. : Arch. Pharm. Chemi., Sci. Ed., 11 : 7, 1983.

166 Yamaoka, K., Yamagishi, Y., Takayama, K., Nambu, N., Nagai, T. : Yakuzaigaku, 41 : 231, 1981.

167 Andersen, F.M., Bundgaar, H. : Arch. Pharm. Chem., Sci. Ed., 11 : 61, (Feb) 1983.

168 Shan-Yang, Lin. : Drug Dev. Ind. Pharm., 16 : 2221, 1990.

169 Menard, F.A., Dedhiya, M.G., Rhodes, C.T. : Drug Dev. Ind. Pharm., 16 : 91, 1990.

170 Zecchi, V., Orienti, I., Fini, A. : Pharm. Acta. Helv., 63 (11) : 299, 1988.

171 Jones, S.P., Parr, G.D. : J. Pharm. Pharmacol., 35(Suppl) : 5, 1983.

172 Nagai, T. : In Proceedings of the International innovations. In Pharmaceutical Sciences, Technology, Ahmedabad, 1 : 1, 1990,

173 Nakajima, T., Sunagawa, M., Hirohashi, T., Fujioka, K. : Chem. Pharm. Bull., 32 : 383, 1984.

174 Giordano, F., Farmaco Edi., Prat., 43 : 345, 1988.

175 Nakai, Y., Yamamoto, K., Terada, K., Horibe, H. : Chem. Pharm. Bull., 30 : 1796, 1982.

176 Lin, S.Y. : Int. J. Pharm. Technol., 4 : 14, (Apr) 1983.

177 Hirsch, W., Fried, V. Altman, L. : J. Pharm. Sci., 74 : 1123, 1985.

178 Miyaji, T., Kurono, Y., Uekama, K., Ikeda, K. : Chem. Pharm. Bull., 24 : 1155, 1976.

179 Otagiri, M., Fokkens, J.G., Hardee, G.E. Perrin, J.H. : Pharm. Acta. Helv., 53 : 241, 1978.

180 Palmieri G.F., Wehrle, P., Duportail, G., Stamm, A. : Drug Dev. Ind. Pharm., 18 : 2117, 1992.

181 Koizumi, K., Okada, Y., Kubota, Y., Utamura, T. : Chem. Pharm. Bull., 35 : 3413, 1987.

182 Choudhury S., Nelson, K.F. : Int. J. Pharm., 85 : 175, 1992.

183 Nakanishi, K., Masada, M., Nadai, T., Miyajima, K. : Chem. Phram. Bull., 37 : 211, 1989.

184 Uekama, K., Sakai, A., Arimori, K., Otagiri, M., Saito, H. : Pharm. Acta. Helv., 60(4) : 117, 1985.

185 Kurozumi, M., Nambu, N., Nagai, T. : Chem. Pharm. Bull., 23 : 3062, 1975.

186 Kata, M., Kedvessy, G. : Pharm. Ind., 49(1) : 98, 1987.

187 Blanco, J., Jose, L., Vila-Jato, Otero, F. Anguiano, S. : Drug Dev. Ind. Pharm., 17 : 943, 1991.

18... Drug Dev. Ind. Pharm., 14 : 63, 1988.

...ta. Helv., 60 : 53, 1985.

190 Nakai, Y. : Drug Dev. Ind. Pharm., 12 : 1017, 1986.

191 Sekulovic, D., Zajic, L. : Pharmazie, 42 : 556, 1987.

192 Fenyvesi, E., Takayama, K., Szejtli, J., Nagai, T. : Chem. Pharm. Bull., 32 : 670, 1984.

193 Yalkowsky, S.H., Roseman, T.J. : In Techniques of Solubilization of Drugs, Edited by S.H. Yalkowsky, Marcel Dekker, New York, 1981, p. 91.

194 Krause, G.M., Cross. J. M. : J. Am. Pharm. Associ., Sci. Ed., 40 : 137, 1951.

195 Yalkowsky, S.H., Flynn, G.L., Amidon, G.L. : J. Pharm. Sci., 61 : 983, 1972.

196 Martin, A., Wu. P.L., Lindestron, R.E., Elworthy, P.H. : J. Pharm. Sci., 71 : 849, 1982.

197 Rubino, J.T., Blanchard, J., Yalkowsky. S.H. : J. Parent. Sci. Technol., 38(6) : 215, 1984.

198 Chien Y.W. : J. Parent. Sci. Technol., 38(1) : 32, 1984.

199 Cho, M.J., Kurtz, R.R., Lewis, C., Machkovech, S.M., Houser, D. J. : J. Pharm. Sci., 71 : 510, 1982.

200 Hussain, M.A., Aungst, B.J., Shefter, E. : Japan-US congress of Pharmaceutical Sciences. Honululu, 1987.

201 Hussain, M.A., Kovai, C.A., Myers, M.J., Shami, E.G., Shefter, E. : J. Pharm. Sci., 76 : 356, 1987.

202 Hamaura, T., Kusai, A., Nishimura, K. : 13th Pharmaceutical Technology Conference Strasbourg, Vol. 1a, April 1994, p. 763.

203 Schanker, L.S. : J. Pharmacol. Exp. Ther., 126 : 283, 1959.

204 Yamashita, S., Saitoh, H., Nakanishi, K., Masada, M., Nadai, T., Kimura, T. : J. Pharm. Pharmacol., 37 : 512, 1985.

205 Nakanishi, K., Ogata, A., Masada, M., Nadai, T. : Chem. Pharm. Bull., 32 : 1956, 1984.

206 Nakanishi, K., Masada, M., Nadai, T. : Chem. Pharm. Bull., 34 : 2628, 1986.

207 Nishihata, T., Yasui, K., Yamazaki, M., Kamada, A. : J. Pharmacobiodyn., 7 : 278, 1984.

208 Yaginuma, H., Nakata, T., Toya, H., Murakami, T., Yamazaki, M., Kamada, A. : Chem. Pharm. Bull., 29 : 2974, 1981.

209 Yamahira, Y., Noguchi, T., Takenaka, H., Meada, T. : Chem. Pharm. Bull., 27 : 1990, 1979.

210 Nishihata, T., Sakakura. T., Hitomi, M., Yamazaki, M., Kamada, A. : Chem. Pharm. Bull., 32 : 2433, 1984.

211 Caldwell, L., Nishihata, T., Fix, J., Selk, S., Cargill, R., Gardner, C.R., Higuchi, T. : Methods Find. Exp. Clin. Pharmacol., 6 : 503, 1984.

212 Nishihata, Y., Rytting, J.H., Higuchi, T. : J. Pharm. Sci., 70 : 71, 1981.

213 Fix, J.A., Leppert, P.S., Porter, P.A., Cardwell, I.J. : J. Pharm. Sci., 72 : 1134, 1983.

214 Fix, J.A., Porter, P.A., Leppert, P.S. : J. Pharm. Sci., 72 : 698, 1983.

215 Moore, J.A., Pletcher, S.A., Ross, M.J. : Int. J. Pharm., 34 : 35, 1986.

216 Suzuka, T., Furuya, A., Kamada, A., Yamazaki, M., Nishihata, T. : Chem. Pharm. Bull., 35 : 1619, 1987.

217 Nishihata, T., Rytting, J.H., Higuchi, T. : J. Pharm. Sci., 71 : 865, 1982.

218 Nishihata, T., Rytting, J.H., Higuchi, T. : J. Pharm. Sci., 71 : 869, 1982.

219 Nishihata, T., Tomida, H., Frederick, G., Rytting, J.H., Higuchi, T. : J. Pharm. Pharmacol., 37 : 159, 1985.

220 Nishihata, T., Kawabe, S., Miyake, M., Kim, S., Kamada, A. : Int. J. Pharm., 22 : 147, 1984.

221 Nishihata, T., Rytting, J.H., Higuchi, T., Cardwell, L.J., Selk, S.J. : Int. J. Pharm., 21 : 239, 1984.

222 Yaginuma, H., Isoda, Y., Wada, Y., Itoh, S., Yamazaki, M., Kamada, A., Shimazu, H., Makita, I. : Chem. Pharm. Bull., 30 : 1073, 1982.

223 Higaki, K., Takechi, N., Kato, M., Hashiba, M., Sezaki, H. : J. Pharm. Sci., 79 : 334, 1990.

224 Higaki, K., Hashida, M., Sezaki, H. : Japan-US congress of Pharmaceutical Sciences, Honululu, 1987.

225 Ueda, I., Shimojo, F., Kozatani, J. : J. Pharm. Sci., 72 : 454, 1983.

226 Fix, J.A., Leppert, P.S., Porter, P.A., Alexer, J. : J. Pharm. Pharmacol., 36 : 286, 1984.

227 Wu, M.M., Murakami, T., Yamojo, R., Higashi, Y., Yata, N. : J. Pharm. Sci., 78 : 499, 1989

228 Brookes, L.G., Marshal, R.C. : J. Pharm. Pharmacol., 33 : 43, 1981.

229 Davis, W.W., Pfeiffer, R.R., Quay, J.F. : J. Pharm Sci., 59 : 960, 1970.

230 Kaneda, A., Nishimura, K., Muranishi, S., Sezaki, H. : Chem. Pharm. Bull., 22 : 525, 1974.

231 Nakanishi, K., Masada, M., Nadai, T. : Chem. Pharm. Bull., 31 : 3255, 1983.

232 Izhizawa, T., Hayashi, M., Awazu, S. : J. Pharm. Pharmacol., 39 : 892, 1987.

233 Ichikawa, K., Ohata, I., Mitomi, M., Kawamura, S., Maeno, H., Kawata, H. : J. Pharm. Pharmacol., 32 : 314, 1980.

234 Urbancic, S.J., Lenardic, A., Kopitar, Z. : Jugoslav. Physiol. Pharmacol. Acta., 23 : 127, 1987.

235 Chowdhary K.P.R., Madhusudhan P. : East. Pharm., 33(387) : 143, 1990.

236 Sanghavi, N.M., Jivani, N.J. : Indian Drugs, 19(11) : 421, 1982.

237 Ibrahim S.A., Hafez E., El-Faham T.H., Mohamed F.A. : Bull. Pharm. Sci. Assuit. Univ., 11 : 196, 1988.

238 Braun, R.J., Parrott, E.L. : J. Pharm. Sci., 61 : 175, 1972.

239 Heng, P.W.S., Wan, L.S.C., Ang, T.S.H. : Drug Dev. Ind. Pharm., 16 : 951, 1990.

240 Mulley, B.A., Aboutaleb, A.E., Abdel, Rahman, A.A., Ahmed, S.M. : Bull. Pharm. Sci. Assuit. Univ., 9 : 157, 1986.

241 Samaha, M.W., Gadalla, M.A.F. : Drug Dev. Ind. Pharm., 13 : 93, 1987.

242 Sanghavi, N.M., Bailur, B.Y. : Indian Drugs 21(2) : 43, 1983.

243 Pandit, N. K. : Drug Dev. Ind. Pharm., 11 : 1797, 1985.

244 Reddy, R.K., Khalil, S.A., Gouda, W.M. : J. Pharm. Sci., 65 : 115, 1976.

245 Singh, J. : Drug Dev. Ind. Pharm., 12 : 851, 1986.

246 Taniguchi, K., Muranishi, S., Sezaki, H. : Int. J. Pharm., 4 : 219, 1980.

247 El-Banna, H.M., Abdallah, O.Y. : Pharm. Acta Helv., 55 : 256, 1980.

248 Geneidi, A.S., Adel, M.S., Shehata, E. : Can. J. Pharm. Sci., 15(4) : 81, 1980.

249 Touitov, E., Donbrov, M., Azaz, E. : J. Pharm. Pharmacol., 30 : 662, 1978.

250 Rubinstein, A., Rubinstein, E., Touitou, E., Donbrov, M. : Antimicrob. Agents Chemother., 19 : 696, 1981.

251 Singh, J., Singh, S. : Drug Dev. Ind. Pharm., 16 : 1717, 1990.

252 Venkataram, S., Rogers, A. : Japan-US Congress of Pharmaceutical Sciences, Honululu, 1987.

253 Urban, M., Arnaud, P., Zuber, M., Chaumeil, J.C. : Drug Dev. Ind. Pharm., 17 : 1325, 1991

254 Pla-Delfina, J.M., Perez, Buenadia, M.D., Casabo, V.G., Peris-Ribira, J.E., Sanchez-Moiano, E., Mastin-Villadre, A. : Int. J. Pharm., 31 : 49, 1987.

255 Whitmore, D.A., Brooks, L.G., Wheeler, K.P. : J. Pharm. Pharmacol., 31 : 277, 1979.

256 Kakemi, K., Sezaki, H., Muranishi, S., Tsujmura, Y. : Chem. Pharm. Bull., 17 : 1641, 1969.

257 Nakamura, J., Takada, S., Ueda, S., Hamaura, T., Yamamoto, A., Kimura, T., Sezaki, H. : Chem. Pharm. Bull., 33 : 3527, 1985.

258 Walter, K.A., Dugard, P.N., Florence, A.T. : J. Pharm. Pharmacol., 33 : 207, 1981.

259 Yamasaki, Y., Shichiri, M., Kawamori, R., Morishima, T., Hakui, N., Yogi, T., Abe, H. : Can. J. Physiol. Pharmacol., 59 : 1, 1981.

260 Sakai, K., Kutsuma, T.M., Nishino, T., Fujihara, Y., Yata, N. : J. Pharm Sci., 75 : 387, 1986.

261 Siegel, I.A., Gordon, H.P. : Toxicol, Lett., 26 : 153, 1985.

262 Nissim, J.A. : Nature : 187 : 305, 1960.

263 Okuda, K., Duran, E.V., Chow, B.F. : Proc. Soc. Exp. Biol. Med., 103 : 588, 1960.

264 Rosoff, M., Serajuddin, A.T.M. : Int. J. Pharm. 6 : 137, (Aug) 1980.

265 Kassem, M.A., Mattha, A.G., El-Nimn, A.E.M., Omar, S.M. : Int. J. Pharm., 12 : 1, (Sept) 1982.

266 Bates, T. R., Gibaldi, M., Kanig, J.L. : J. Pharm. Sci., 55 : 901, 1966.

267 Miyazaki, S., Yamahira, T., Morimoto, Y., Nadai, T. : Int. J. Pharm., 8 : 303, 1981.

268 Krasowska H. : Int. J. Pharm., 7 : 137, 1980.

269 Muranishi S., Muranishi N. Sezaki H. : Int. J. Pharm., 2 : 101, 1979.

270 Hayashi M., Tomita M., Horie T. Awazu S., Japan-US congress of Pharmaceutical Sciences, Dec 1987, Honolulu.

271 Feldman, S., Salvino, M., Gibaldi, M. : J. Pharm. Sci., 59 : 705, 1970.

272 Guarini, S., Ferrari, W. : Experientia, 41 : 350, 1985.

273 Pandit, J.K., Jagadeesh, G., Nagabooshanam, M., Tripathi, M.K. : Drug Dev. Ind. Pharm., 10 : 85, 1984.

274 Goyal, V.C., Kohli, D.V., Uppadhaya, R.K. : Indian Drugs, 19 : 233, (March) 1982.

275 Singh, J., Singh, S. : Drug Dev. Ind. Pharm., 16 : 2193, 1990.

276 Ziv, E., Kidron, M., Berry, E.M., Bar-on, H. : Life Sci., 29 : 803, 1981.

277 Raz, I., Bar-on, H., Kidron M., Ziv, E. : Int. J. Med. Sci., 20 : 173, 1984.

278 Mayersohn, M., Feldman, S., Gibaldi, M. : J. Nutri., 98 : 288, 1969.

279 Breuer, N., Goebell, H. : Klin Wochenschr., 63 : 97, 1985.

280 Rainey, J.B., Maeda, M., Williamson, R.C.N. : Cell Tissue Kinet., 19 : 485, 1986.

281 Loper, A.E., Gardner, C.R., Gastrointestinal Absorption of Drugs. In Encyclopedia of Pharmaceutical Technology. Vol.6 Editer by James Swarbrick and James, C., Boylan. Marcel Dekker Inc., New York, 1992, pp. 385 - 413.

282 Chiou, W.L., Riegelman, S. : J. Pharm. Sci., 60 : 1281, 1971.

283 Said, S.A., Fatatry, H.M., Geneidi, A.S. : Aust. J. Pharm. Sci., 53 : 42, 1974.

284 Lin, S.L., Menig, J., Lachman, L.: J. Pharm. Sci., 57 : 2143, 1968.

285 Finholt, P., Kristiansen, H., Schmidt, O.G., Word, K. : Med. Norsk. Farm Selskap., 28 : 17, 1966.

286 Udupa, N., Tatwawadi, S.V., Gode, K.D. : East. Pharm., 29(394) : 205, 1986.

287 Goldberg, A., Gibaldi, M., Kanig, J. : J. Pharm. Sci., 55 : 487, 1966.

288 Ford, J.L., Eliott, P.N.C. : Drug Dev. Ind. Pharm., 11 : 523, 1985.

289 Lemberger, L., Rubin, A., Wolen, R. De Sante, K., Rowe, H., Forney, R., Pence, P. : Cancer Treat Rev., 9 : 17, 1982.

290 Walker, S.E., Ganley, J.A., Bedford, K., Eaves, T. : J. Pharm. Pharmacol., 32 : 389, 1980.

291 Stupak, E.F., Bates, T.R. : J. Pharm. Sci., 61 : 400, 1972.

292 Aoyagi, N., Ogata, H., Kaniwa, N., Koibuchi, M., Shibazaki, T., Ejima, A. : J. Pharm. Sci., 71 : 1165, 1982.

293 Kanig, J.L. : J. Pharm. Sci., 53 : 188, 1964.

294 Kreuschner, K., Froemming, K.H., Hosemann, R. : Acta Pharm. Technol., 26 : 159, 1980.

295 Froemming, K.H., Simons, B., Haase, J., Hosemann, R. : Pharm. Ind., 40 : 967, 1978.

296 Mura, P., Liguori, A Bramanti, G., Farmaco Ed. Prat., 42 : 149, (Jun) 1987.

297 Sjokvist, E., Nystron, C. : Int. J. Pharm., 47 : 51, (Nov) 1988.

298 Goldberg, A.H., Gibaldi M., Kanig., J.L. : J. Pharm Sci., 54 : 1145, 1965.

299 Ford, J.L., Stewart, A.F., Rubinstein M.H., J. Pharm. Pharmacol., 31 : 726, 1979.

300 Carcamo, E.C., Gana, I.M., An R. : Acad. Farm., 40 : 487, 1974.

301 Chiou, W.L., Riegelman, S. : J. Pharm. Sci., 58 : 1505, 1969.

302 Malone, M.H., Hochman, H., I, Nieforth, K.A. : J. Pharm Sci., 55 : 972, 1966.

303 Takayama, K., Nambu, N., Nakai, T. : Chem. Pharm. Bull., 30 : 3013, 1982.

304 Simonelli, A.P., Mehta, S.C., Higuchi, W.I. : J. Pharm. Sci., 58 : 538, 1969.

305 Sanghavi, N.M., Kamath, K. : Indian Drugs, 21 : 209, 1984.

306 Sekiguchi, K., Obi, N., Ueda, Y. : Chem. Pharm. Bull., 12 : 134, 1964.

307 Chiou, W.L. : J. Pharm. Sci., 66 : 989, 1977.

308 Colleq, J.H., Flood, B.L., Sale, F.R. : J. Pharm. Pharmacol., 28 : 305, 1976.

309 Hargreaves, B.J., Pearson, J.E., Connor, P. : J. Pharm. Pharmacol., 31 : 47 P., 1979.

310 Ford, J.L., Rubinstein, M.N. : J. Pharm. Pharmacol., 29 : 688, 1977.

311 Goldberg, A.N., Gibaldi, M., Kanig, J.L. : J. Pharm Sci., 55 : 482, 1966.

312 Thakkar, A., Hirsch, C.A., Page, J.G. : J. Pharm. Pharmacol., 29 : 783, 1977.

313 Ford, J.L. : Pharm. Acta Helv., 61 : 69, 1986.

314 Hajratwala, B.R., Ho, D.S.S. : J. Pharm. Sci., 73 : 1539, 1984.

315 Vila-Jato, J.L., Alonso, J., Blanco, J. : Drug Dev. Ind. Pharm., 12 : 1545, 1986.

316 Summers, M.P., Enever, R.P. : J. Pharm. Sci., 65 : 1613, 1976.

317 Meshali, M., Ghanem, A., Ibraheem, Y. : Pharm. Acta. Helv., 58 : 62, 1983.

318 Ali, A.A., Gorashi, A.S. : Int. J. Pharm., 19 : 297, (May) 1984.

319 Madhusudan, V., Nasa, S.L. : East. Pharm., 31 : 129, (Apr) 1988.

320 Deshpande, A.V., Agrawal, D.K. : Drug Dev. Ind. Pharm., 8 : 965, 1982.

321 Bloch, D.W., El Egakey, M.A., Speiser, P.P. : Acta Pharm. Technol., 28 : 177, 1982.

322 Salah, U., Ahmed, Madan, P. L. : Drug Dev. Ind. Pharm., 15 : 1243, 1989.

323 Jachowicz, R. : Int. J. Pharm., 35 : 1, (Feb) 1987.

324 Allen, L.V. Jr., Yanchick, V.A., Maness, D.D. : J. Pharm. Sci., 66 : 494, 1977.

325 Allen, L.V. Jr., Levinson R.S., Martono D.D. : J. Pharm. Sci., 67 : 979, 1978.

326 Stein, M., Schwabe, L., Fromming, K.H. : Pharm. Ind., 53 : 186, 1991.

327 Salah, U., Ahmed, Madan, P.L. : Drug Dev. Ind. Pharm., 17 : 831, 1991.

328 Kaur, R., Grant, D.J.W., Eaves, T. : J. Pharm. Sci., 69 : 1321, 1980.

329 Miralles, M.J., McGinity, J.W., Martin A. : J. Pharm. Sci., 71 : 302, 1982.

330 Miralles, M.J., Mcginity, J.W., Martin, A. : J. Pharm Sci., 72 : 302, 1983.

331 Goundalkar A.G., Nikore R.L., Pratibha, N. : East. Pharm., 33(386) : 125, 1990.

332 Geneidi, A.S. : Can. J. Pharm. Sci., 15 : 78, (Oct) 1980.

333 Geneidi, A.S., Hamacher, H. : Pharm. Ind., 42 : 401, 1980.

334 Attia, M.A., Habib, F.S. : Drug Dev. Ind. Pharm., 11 : 1957, 1985.

335 Ghanem, A.H., Sakr, F.M., Abdel-Ghany, G. : Acta Pharm. Fenn., 95 : 167, 1986.

336 McGinity, J.W., Maness, D.D., Yakatan, G.J. : Drug Dev. Ind. Pharm., 1(369) : 1974 - 1975.

337 Chiou, W.L., Niazi, S. : J. Pharm. Sci., 60 : 1333, 1971.

338 Ford, J.L., Rubinstein, M.H. : J. Pharm Pharmacol., 29 : 209, 1977.

339 Bloch, D.W., El-Egakey, M.A., Speiser, P.P. : Pharm. Acta. Helv., 57 : 231, 1982.

340 Bloch, D.W. : Drugs Made Ger., 25 : 231, 1982.

341 Jafari, M.R., Danti, A.G., Ahmed, I. : Int. J. Pharma., 48 : 207, 1988.

342 Goldberg, A.H., Gibaldi, M., Kanig, J.L., Mayersohn, M. : J. Pharm. Sci., 55 : 581, 1966.

343 Chiou, W.L. : J. Pharm. Sci., 60 : 1406, 1971.

344 El-Banna, H.M., El-Gholmy, Z.A., Hammouda, Y. : Pharm. Acta. Helv., 55 : 244, 1980.

345 Mura, P., Liguori, A., Bramanti, G. : Farmaco Ed. Prat., 41 : 377, 1986.

346 Rogers, J.A., Anderson, A.J. : Pharm. Acta. Helv., 57 : 276, 1982.

347 Wu, Q.F. : Chin. J. Hosp. Pharm., 5 : 147, 1985.

348 Jachowicz, R. : Int. J. Pharm., 35 : 7, (Feb) 1987.

349 Stavchansky, S., Walter, G.G. : J. Pharm. Sci., 73 : 733, 1984.

350 Kassem, M.A., El-Ridy, M.S., Khairy, L.M. : Drug Dev. Ind. Pharm., 13 : 1171, 1987.

351 Chiou, W., Riegelman, S. : J. Pharm. Sci., 59 : 937, 1970.

352 Chiou, W.L., Riegelman, S. : J. Pharm. Sci., 60 : 1377, 1971.

353 Kawashima, Y., Ha, T., Takeuchi, H., Okumura, M., Katau, H., Nagata, O. : Chem. Pharm. Bull., 24 : 3376, 1986.

354 Ramadan, E.M., El-Gawad, A.E., Nouh, A.T. : Pharm. Ind., 49 : 508, 1987.

355 Lahr, W. : Pharm. Zig., 131(51) : 871, 1986.

356 Nishihata, T., Chigawa Y., Kamada A., Sakai, K., Tabata Y. : Drug Dev. Ind. Pharm., 14 : 1137, 1988.

357 Said, S.A., Saad, S.F. : Aust, J. Pharm. Sci., N S 4 : 121, 1975.

358 Deshpande, A., Agrawal, D. : Drug Dev. Ind. Pharm., 8 : 883, 1982.

359 Kassem, M.A., Salama, H.A., Ammar, H.D., El-Ridy, M.S. : Pharm. Ind., 44 : 1186, 1982.

360 Pandit, J.K. Khakurel, B.K. : Drug Dev. Ind. Pharm., 10 : 1709, 1984.

361 Kassem, A.A., Zaki, S.A., Mursi, N.M., Tayel, S.A. : Pharm. Ind., 41 : 390, 1979.

362 Goundalkar, A.G., Nikore, R.L., N, P. : East. Pharm., 33(386) : 125, 1990.

363 Corrigan, O.I., Timoney, R.F. : Pharm. Acta. Helv., 51 : 268, 1976.

364 Ravis, W.R., Chien-Yu : J. Pharm. Sci., 70 : 1353, 1981.

365 El-Gindy, N.A., Shalaby, A.A., El-Khalek, M.M.A. : Drug Dev. Ind. Pharm., 9 : 1031, 1983.

366 Akade, M.A., Agrawal, D.K., Lauwo, J.A.K. : Phramazie, 41 : 849, 1986.

367 Ren, T.C. : Chin. Trad. Herbal Drugs, 16 : 392, 1985.

368 Vila-Jato, J.L., Alonso, M.J. : Drug Dev. Ind. Pharm., 12 : 701, 1986.

369 Bogdanova, S., Gentcheva, P., Shekerdjiiski, R., Piskjulev, B., Minkov, E. : Labo Pharma. Probl. Tech., 32 : 835, 1984.

370 Sanghavi, N.M., Kotwaney, H.N., Shah, V.J. : Indian Drugs, 19 : 112, (Dec) 1981.

371 Craig, D.Q.M., Newton, J.M. : Int. J. Pharm., 78 : 175, 1992.

372 Badawi, A.A., El-Sayed, A.A. : J. Pharm. Sci., 69 : 492, 1980.

373 Kassem, A.A., Zaki, S.A., Mursi, N.M., Tayel, S.A. : Pharm. Ind., 42 : 202, 1980.

374 Jain, N.K., Parikh, R.H. : Indian J. Pharm. Sci., 48 : 64, 1986.

375 Singla, A.K., Vijan, T. : Drug Dev. Ind. Pharm., 16 : 875, 1990.

376 Chiou, W.L., Riegelman, S. : J. Pharm Sci., 60 : 1569, 1971.

377 Doshi, U., Allen, L.V. Jr., Greenwood R., Stiles, M.L. : Japan-US, congress of Pharmaceutical Sciences, Honululu, Dec 1987.

378 Ho, D. S. S., Hajratwala, B. R. : Aust. J. Pharm. Sci., 10 : 65, (Sept) 1981.

379 Ford, J.L., Rubinstein, M.H. : Pharm. Acta. Helv., 53 : 327, 1978.

380 Fernez, M., Rodriguez, I.C., Margarit, M.V., Cerezo, A. : Int. J. Pharm., 84 : 197, 1992.

381 Forni, F., Iannucceli, V., Coppi, G., Bernabei, M.T. : Pharm. Ind., 50 : 1405, 1988.

382 Ford, J.L., Elliott, P.N.C. : Drug Dev. Ind. Pharm., 11 : 537, 1985.

383 Mura, P., Liguori, A., Bramanti, G., Poggi, L. : Farmaco Ed. Prat., 42 : 157, (Jun) 1987.

384 Najib, N.M., Sheikh Salem, M.A. : Drug Dev. Ind. Pharm., 13 : 2263, 1987.

385 Gines, J.M., Sanchez-Soto, P.J., Justo, A., Vela, M.T., Rabasco, A.M. : Drug Dev. Ind. Pharm., 16 : 2283, 1990.

386 Fernez, J., Vila-Jato, J.L., Blanco J. : Drug Dev. Ind. Pharm., 15 : 2491, 1989.

387 Anastasiadou, C., Henry, S., Legendre, B., Souleau, C., Duchene, D. : Drug Dev. Ind. Pharm., 9 : 103, 1983.

388 Dordunoo, S.K., Ford, J.L., Rubinstein, M.H. : Drug Dev. Ind. Pharm., 17 : 1685, 1991.

389 Frances, C., Veiga, M.D., Espansol, O.M., Cadorniga, R. : Int. J. Pharm., 77 : 193, 1991.

390 Vera, N., Veiga, M.D., Cadorniga, R. : S.T.P. Pharm. Sci., 2 : 125, 1991.

391 Gines, J.M., Arias M.J., Moyano, J.R., Novak, C., Fernandez-Hervas M.J., Rabasoco, A.M. : 13th Pharmaceutical Technology Conference, Strasbourg, Vol. 1a. April 1994, p. 688.

392 Asker, A.F., Whitworth, C.W. : Pharmazie, 30 : S 30, 1975.

393 Daabes, N.A., Mostada, L.M. : Sci. Pharm., 48 : 16, 1980.

394 Corrigan, O.I., Timoney, R.F., Whelan, M.J. : J. Pharm. Pharmacol., 28 : 703, 1976.

395 Simonelli, A.P., Mehta, S.C., Higuchi, W.I. : J. Pharm. Sci., 59 : 633, 1970.

396 Doherty, C., York, P. : J. Pharm., 34 : 197, (Jan) 1987.

397 Adbel-Rahman, S.E., El-Sayed, A.M., Aboutaleb, A.E. : Bull. Pharm. Sci., 11 : 261, 1988.

398 Collett, J.H., Kesteven, G. : Drug Dev. Ind. Pharm., 4 : 555, 1978.

399 Sugimoto, I., Kuchiki, A., Nakagawa, H., Tohgo, K., Kondo, S., Iwane, I., Takahashi, K. : Drug Dev. Ind. Pharm., 6 : 137, 1980.

400 Guo, J.L., Lu, X.P. : Yaoxue Tongbao., 21 : 261, 1986.

401 Corrigan, O.I., Timoney, R.F. : J. Pharm. Pharmacol., 27 : 759, 1975.

402 Bates, T.R. : J. Pharm. Pharmacol., 21 : 711, 1969.

403 Corrigan, O.I., Sabra, K., Holohan, E.M. : Drug Dev. Ind. Pharm., 9 : 1, 1983.

404 Mayersohn, M., Gibaldi, M. : J. Pharm. Sci., 55 : 1323, 1966

405 Stupak, E., Rosenberg, H., Bates, T. : J. Pharmcokinet. Biopharm., 2 : 511, 1974.

406 Corrigan, O.I., Holohan, E.M. : J. Pharm. Pharmacol., 36 : 217, 1984.

407 Trivedi, B.M., Gohel, M.C., Patel, M.M., Desai, B.A. : East. Pharm., 35(417) : 113, 1992.

408 Simonelli, A.P., Mehta, S.C., Higuchi, W.I. : J. Pharm. Sci., 65 : 355, 1976.

409 Nozawa, Y., Mizumoto, T., Higashida, F. : Pharm. Acta. Helv., 61 : 337, 1986.

410 Lu, J.F., Wang, M.Z., Chen, G.L., Wang, D.M. : Yaoxue Tongbao 24 : 346, 1989.

411 Doherty, C., York, P. : J. Pharm. Pharmacol., 41 : 73, 1989.

412 Takayama, K., Nambu, N., Nagai, T. : Chem. Pharm. Bull., 28 : 3304, 1980.

413 Imaizumi, H., Nambu, N., Nagai, T. : Chem. Pharm. Bull., 31 : 2510, 1983.

414 Geneidi, A.S., Ali, A.A., Salama, R.B. : J. Pharm. Sci., 67 : 114, 1978.

415 Chowdary, K.P.R., Murty, S.R.A. : East. Pharm., 31(369) : 127, 1988.

416 Shenoy, K.R.P., Thampi, P.P. : Indian Drugs, 22 : 423, 1985.

417 Deshpande, A.V., Agrawal, D.K. : Drug Dev. Ind. Pharm., 10 : 1725, 1984.

418 Deshpande, A.V., Agrawal, D.K. : Pharmazie., 40 : 496, 1985.

419 Udupa, N., Tatwawadi, S.V., Gode, K.D. : Indian J. Hosp. Pharm., 23 : 268, 1986.

420 Sekikawa, H., Yagi, N., Sakuragi, J., Tanaka, K., Sakamoto, N., Itoh, M., Takada, M., Arita, T. : Chem. Pharm. Bull., 30 : 739, 1982.

421 Sekikawa, H., Fujiwara, Jun-El., Naganuma, T., Nakano, M., Arita, T. : Chem. Pharm. Bull., 26 : 3033, 1978.

422 Sekikawa, H., Fujiwara, Jun-El., Naganuma, T., Nakano, M., Arita, T. : Chem. Pharm. Bull., 27 : 31, 1979.

423 Kildsig, D.O., Peck, G.E. : Drug Dev Ind. Pharm., 10 : 1, 1984.

424 Huttenrauch, R. : Pharmazie, 20 : 243, 1965.

425 Jain, N.K., Patel, V.V. : East. Pharm., 29 : 51, 1986.

426 Poochikian, G.K., Crodock, J.C. : J. Pharm Sci., 68 : 728, 1979.

427 Badwan, A.A., El-Khordagui, L.K., Saleh, A.M., Khalil, S.A. : J. Pharm. Phramacol., 32(Suppl) : 74, 1980.

428 Mendes, R.W., Masih, S.Z., Kanumuri, R.R. : J. Pharm. Sci., 67 : 1616, 1978.

429 Kerc, J., Mohar, S., Srcic, S., Kofler, B. : 13th Pharmaceutical Technology Conference, Strasbourg, Vol. 1a. April 1994, p. 775.

430 Sanghavi, N.M., Munot, D.S., Kamath, P. : Indian Drugs, 22 : 84, 1984.

431 Forni, F., Iannuccelli, V., Velli, M.A., Cameroini, R. : S.T.P. Pharma., 3 : 758, 1987.

432 McGinity, J., Harris, M. : Drug Dev. Ind. Pharm., 6 : 35, 1980.

433 Yang, K.Y., Glemza, R., Jarowski, C.I. : J. Pharm. Sci., 68 : 560, 1979.

434 Law, S.L., Chiang, C.H. : Drug Dev. Ind. Pharm., 16 : 137, 1990.

435 Jain, S.C., Agrawal, G.P. : Indian Drugs, 19 : 108, (Dec) 1981.

436 Monkhouse, D.C., Lach, J.L. : J. Pharm. Sci., 61 : 1435, 1972.

437 Jain, N.K., Kothari, R.P. : Indian Drugs, 22 : 51, 1984.

438 Lauwo, J.A.K. : Drug Dev. Ind. Pharm., 11 : 1565, 1985.

439 Nystron, C., Westerberg, M. : J. Pharm. Pharmacol., 38 : 161, 1986.

440 Ismal, S., Shawky, S., Rafez, E. : Drug Dev. Ind. Pharm., 13 : 2147, 1987.

441 Yamamoto, K., Nikano, M., Arita, T., Takayama, Y., Nakai, Y. : J. Pharm. Sci., 65 : 1484, 1976.

442 Koparkar, A.D. : Pharm. Res., 7 : 80, (Jan) 1990.

443 Shefter, E., Higuchi, T. : J. Pharm. Sci., 52 : 781, 1963.

444 Bochlert, J.P. : Drug Dev. Ind. Pharm., 10 : 1343, 1984.

445 Debersis, E., Boelert, J.P., Givand, T.E., Sheridan, J.C. : Pharm. Tech., 6(9) : 120, 1982.

446 Grimm, W. : Drug Dev. Ind. Pharm., 12 : 1259, 1986.

447 Cartensen, J.T. : In Modern Pharmaceuticals. Edited by G.S. Banker, C.T. Rhodes. Marcel Dekker Inc., New York, 1990, pp.239 - 262.

448 Erram, S.V., Tipnis, H.P. : Indian Drugs, 30 : 122, 1993.

449 Botha, S.A., Lotter, A.P. : Drug Dev. Ind. Pharm., 15 : 1843, 1989.

450 Erram, S.V., Tipnis, H.P. : Indian Drugs, 30 : 61, 1993.

451 Erram, S.V., Tipnis, H.P. : Indian Drugs, 30 : 35, 1993.

452 Erram, S.V., Tipnis, H.P. : Indian Drugs, 30 : 264, 1993.

453 Erram, S.V., Tipnis, H.P. : Indian Drugs, 30 : 230, 1993.

454 Ager, D.J., Alexer, K.S., etal : J. Pharm. Sci., 75 : 97, 1986.

455 Jacobson, H., Reiar, G. : J. Pharm. Sci., 58 : 631, 1969.

456 El-Shattawy, H.H. : Drug Dev. Ind. Pharm., 10 : 491, 1984.

457 Botha, S.A., Lotter, A. P. : Drug Dev. Ind. Pharm., 16 : 1945, 1990.

458 Botha, S.A., Du-Preez, J.L., Lotter, A.P. : Drug Dev. Ind. Pharm., 12 : 811, 1986.

459 Yoshioka, S., Carstensen, J.T. : J. Pharm. Sci., 79 : 943, 1990.

460 Van Dooran, A.A. : Drug Dev. Ind. Pharm., 9 : 43, 1983.

461 Pope, D.G., Lach, J.L. : Pharm. Acta. Helv., 50 : 165, 1975.

462 Akers, M.J. : Can. J. Pharm. Sci., 11 : 1, 1976.

463 Kaplan, S.A., Cotler, J. : J. Pharm. Sci., 61 : 1361, 1972.

464 Penzotti, S.C., Poole J.W. : J. Pharm. Sci., 63 : 1803, 1974.

465 Taylor, D.C., Grundy, R.U. : J. Pharm. Pharmacol., 27(Supp.) : 1975.

466 Doluisio J.T., Billups, N.F., Dittert L.W., Sugita, E.T., Swintosky, J.V. : J. Pharm. Sci., 58 : 1196, 1967.

467 Chow, W.S., Rive, S.A., Varia, S.A., Kripalani, K.J. : Abstr. Acad. Pharm. Sci., 14 : 109, 1984.

468 Abdou, H.M. : Dissolution, Bioavailability, Bioequivalence, Mac Publishing Co, Easton, 1989, pp. 491 - 516.

469 Banakar, U.V. : Theory of Dissolution. In pharmaceutical Dissolution Testing. Marcel Dekker, New York 1992, pp. 19 - 51

470 Pinholt, P., Solvang, S. : J. Pharm. Sci., 57 : 1322, 1968.

471 Newton, J.M., Muhammad, N.A.H. : J. Pharm. Pharmacol., 36 : 42, 1984.

472 Banakar, U.V., Block, L.H. : Pharm. Tech., 7 : 107, 1983.

473 Dakhuri, A., Shah, A. : Pharm. Tech., 6 : 67, 1982.

474 Campbell, D.J., Thevagt, J.G. : Drug Stand, 26 : 73, 1958.

475 Wagner, J.G. : Drug Intell. Clin. Pharm., 3 : 198, 1969.

476 Wagner, J.G. : Drug Intell. Clin. Pharm., 4 : 432, 1970.

477 Smolen, V., Erb, R. : J. Pharm. Sci., 66 : 297, 1977.

478 Brown, L., Wei, C., Langer, R. : J. Pharm. Sci., 72 : 1181, 1983.

479 Wagner, J. : Drug Intell. Clin. Pharm., 4 : 160, 1970.

480 Needham, T., Javid, P., Brown, W. : J. Pharm. Sci., 68 : 952, 1979.

481 Melikian, A., Straughn, A., Slywka, G., Whyatt, P., Meyer, M.J. : Pharmacokinet. Biopharm., 5 : 133, 1977.

482 Wagner, J. : J. Pharm. Sci., 60 : 666, 1971.

483 Yau, M., Meyer, M. : J. Pharm. Sci., 72 : 681, 1983.

484 Meyer, M., etal. : J. Clin. Pharmacol., 22 : 131, 1982.

485 Riegelman, S., Collier, P. : J. Pharmacokinet, Biopharm., 8 : 509, 1980.

486 Smolen, V. : Pharm. Tech., 1 : 27, 1977.

487 Smolen, V., Ball, L., Schefier, M. : Pharm. Tech., 3 : 89, 1979.

488 Smolen, V. : Hosp. Pharm., 4 : 14, 1969.

489 Smolen, V. : J. Pharm. Sci., 60 : 878, 1971.

490 Banakar, U.V. : Pharmaceutical Dissolution Testing, Marcel Dekker Inc, New York, 1992, pp. 347 - 390.

491 DiSanto, A., DeSante, K. : J. Pharm. Sci., 64 : 109, 1975.

492 McGilveray, I., Midha, K., Rowe, M., Beoin, N., Chanette, C. : J. Pharm. Sci., 70 : 524, 1981.

493 Flanagan, T., Brosard, R., Rubinstein, M., Longworth, A. : J. Pharm. Pharmacol., 21(Suppl) : 129, 1969.

494 Sullivan, T., Sloll, R., Sakmar, E., Blair, D., Wagner, J.J. : Pharmacokinet. Biopharm., 2 : 29, 1974.

495 Chun, A., Buddenhagen, J. : Indust. Pharm. Tech. Sect. A Ph A Conference, Nov'1978.

496 Strum, J., Ebersole, J., Jaffe, J., Colaizzi, J., Poost, R. : J. Pharm. Sci., 67 : 568, 1978.

497 Patel, R.B., Rogge, M.C., Selen, A., Goehl, T.J., Shah, V.P., Prasad, V.K., Welling, P.G. : J. Pharm. Sci., 73 : 964, 1984.

498 Mattok, G., McGilveray, L., Mainville, C. : J. Pharm. Sci., 60 : 561, 1971.

499 Stavchansky, S., Doluisio, J., Martin, A., Martin, C., Cabana, B., Dighe, S., Loper, A. : J. Pharm. Sci., 69 : 1307, 1980.

500 Khan, K., Rhodes, C.T. : Pharm. Acta. Helv., 47 : 594, 1972.

501 Lara, Q.L.M., Cruz, R.R. : 13th Pharmaceutical Technology Conference, Strasbourg, Vol. 1a. April 1994, p. 718.

502 Arias, M.J., Gines, J.M., Moyano, J.R., Vela, M.T., Rabasco, A.M. : 13th Pharmaceutical Technology Conference, Strasbourg, Vol. 1a. April 1994, p. 743.

503 Wadke, D.A., Reier, G.E. : J. Pharm. Sci., 61 : 868, 1972.

504 K.A. Connors, G.L., Amidon., V.J. Stella : Chemical Stability of Pharmaceuticals, John Wiley Sons, New York, 2nd Edition, 1986, pp. 115 - 134.

505 Enezian, G. : Rev. Prod. Probl. Pharm., 23 : 1, 1968.

506 Preut, E.G., Tompkins, F.C. : Trans. Faraday Soc., 40 : 488, 1944.

507 Bawn, C.E.H. : In Chemistry of Solid State. Edited by W.E. Garner, Butterworths, London, 1955, p. 254.

508 Carstensen, J.T. : Drug Dev. Ind. Pharm., 10 : 1277, 1984.

509 James I. Wells : Pharmaceutical Preformulation, The Physicochemical Properties of Drug Substances, Ellis Horwood Limited, Chichester, England 1988, pp 152 - 191.

510 Yoshioka, S., Uchiyama, M. : J. Pharm. Sci., 75 : 92, 1986.

511 Jain, N.B., Garren, K.W., Patel, M.R. : Abstr. Acad. Pharm. Sci., 12 : 146, 1982.

512 Callahan, J.C., Cleary, G.W., Elefant, M., Kaplan, G., Kensler, T., Nash, R. A. : Drug Dev. Ind. Pharm., 8 : 355, 1982.

513 Carstensen, J.T. : J. Pharm. Sci., 63 : 1, 1974.

514 Byrn, S.R. : J. Pharm. Sci., 65 : 1, 1976.

515 Monkhouse, D.C., Campen, L.V. : Drug Dev. Ind. Pharm., 10 : 1175, 1984.

516 Yoshioka, S., Uchiyama, M. : J. Pharm. Sci., 75 : 459, 1986.

517 Carratt, E.R. : J. Am. Pharm. Associ., Sci. Ed., 45 : 470, 1956.

518 Woolfe, A.J., Worthington, H.E.C. : Drug Dev. Commun., 1(3): 185, 1974 - 1975.

519 K.A. Connors, G.L., Amidon., V.J. Stella : Chemical Stability of Pharmaceuticals, John Wiley sons, New York, 2nd Edition, 1986, pp. 135 - 159.

520 Sensi, P., Maggi, N., Furesz, S., Maffii, G. : Antimicrob. Agents Chemother., 1966 : 699, 1967.

521 Traxler, P., Vincher, W.A., Zak, O. : Drugs Future, 13 : 845, 1988.

522 Dur, D.V., Hampden, C., Boobis, A.R., Park, B.K., Davies, D.S. : Br. J. Clin. Pharmacol., 21 : 1, 1986.

523 Heller, E., Arguman, M., Levy, H., Goldblum, N. : Nature, 222 : 273, 1969.

524 Nitya, N. : Current R & D Highlights, 16 : 3, 1993.

525 Kenny, M.T., Strates, B. : Drug Metab. Rev., 12 : 159, 1981.

526 Maggi, N., Pasqualucci, C.R., Ballagotta, R., Sensi, P. : Chromatographia, 11 : 285, 1968.

527 The United States Pharmacopoeia XXII, United States Pharmacopoeia Convention, Inc, Rockville, 1990, pp. 1840.

528 Bowman, G., Lundgren, P., Stjenstrom, G. : Eur. J. Clin. Pharmacol., 8 : 293, 1975.

529 Mannisto, P. : Clin. Pharmacol, Ther., 21 : 370, 1976.

530 Buniva, G., Pagani, V., Carozzi, A. : Int, J. Clin. Pharmacol. Ther. Toxicol., 21 : 404, 1983.

531 Council on drugs, JAMA, 220 : 414, 1972.

532 Archer, G.L., Armstrong, B.C., Kline, B.J. : Antimicrob. Agents Chemother, 21 : 800, 1982.

533 Kenwright, S., Levi, A.J. : Lancet, 2 : 1401, 1973.

534 Mannisto, P., Helsinki, M.D. : Clin. Pharmacol. Ther., 21 : 370, 1976.

535 Dans, P.E., McGehee R.F. Jr., Wilcox, C., Finl, M. : Am. J. Med. Sci., 259 : 120, 1970.

536 Siegler, D.I., Bryant, M., Burley, D.M., etal. : Lancet, 2 : 197, 1974.

537 Acocella G., : Clin. Pharmacokinet., 3 : 108, 1978.

538 Pande, S., Mishra, A.K. : East. Pharm., 30(354) : 141, 1987.

539 Takada, K., Mikami, H., Asada, S., Tatsuo, K., Muranishi, S. : Chem. Pharm. Bull., 26 : 19, 1978.

540 Osol, A.A., Pratt, R., Gennaro, A.R. : The United States Dispensatory. 27th Ed. J.B. Lippincott Company, Philadelphia, 1973, pp. 1020 - 1030

541 Sano, M., Tsunakawa, N. : Shinryo, 23 : 928, 1970.

542 Maggi, N., Vigevani, A., Gallo, G.G., Pasqualucci. : J. Med. Chem., 11 : 936, 1968.

543 Donovan, M. : Rifampin.In Chemical Stability of Pharmaceuticals. Edited by K.A., Connors, G.L., Amidon, V.J. Stella, 2nd Ed. John Willey Sons, New York, 1986, pp. 728 - 732.

544 Gallo, G.G., Radealli, P. : Rifampicin, in Analytical Profiles of Drug Substances, Vol. 5, Edited by Florey K, 1976, pp 467 - 513.

545 Prankerd, R.J., Walters, J.M., Parnes, J.H. : Int. J. Pharm., 78 : 59, 1992.

546 Ratti, B., Parenti, R.R., Toselli, A., Zerilli, L.F. : J. Chromatogr. Biomed. Appl., 225 : 526, 1981.

547 Seydel, J.K. : Antibiotic et Chemother., 16 : 380, 1970.

548 Hoeprich, P.D. : J. Infect Dis., 123 : 125, 1971.

549 McCracken, G.H. Jr., Ginsburg, C.M., Ziveighaft, T.C. : Pediatrics, 66 : 17, 1980.

550 Cox, F., Trincher, R., Rissing, J.P. et al. : JAMA., 245 : 1043, 1981.

551 Curci, G., Cava, F.D., Vitalo, L. : Minerva Medica, 60 : 2399, 1969.

552 D'oliveira, J.J.G. : Amer. Rev. Resp. Dis., 106 : 432, 1972.

553 Binda, G., Domenichini, E., Gottardi, A. et al : Arzneim Forsch, 21 : 1907, 1971.

554 Boman, G. : J. Resp. Dis., 84 (Suppl) : 40, 1973.

555 Acocella, G. : Rev. Infect. Dis., 5(Suppl 3) : 428, 1983.

556 Acocella, G., Scotti, R. : J. Antimicrob. Chemother., 2 : 271, 1976.

557 Keberle, H., Schid, K., Meyer-Brunot, H.G. : In A Symposium on Rimactane, Ciba, Basle, 1968.

558 Murdoch, J.M.C., Speirs, C.F., Wright, N., Wallace, E.T. : Lancet, 1 : 1094, 1969.

559 Ger Offen DE 3242796 (cl. A61 k 31/135) June 1983.

560 Maggi, N., Furesz, S., Pallanza, Pelizza G. : Arzneimittel-Forsch, 19 : 651, 1969.

561 Sano, K., Hakusui, H. : Japan. J. Antibiotics, 23 : 416, 1970.

562 Zilly, W., Breimer, D.D., Richter, E. : Clin. Pharmcokinet., 2 : 61, 1977.

563 Wehrili, W. : Rev. Infect. Dis., 5(Suppl 3) : 407, 1983.

564 Hartmann, G., Honikel, K.O., Knusel, F., Nuesch J. : Biochim. Biophys. Acta, 145 : 843, 1967.

565 Kucers, A., Bennett, M.N., (Editors) : The use of Antibiotics, 4th Ed. William Heinimann Medical Books, London. 1987, pp. 914 - 970.

566 Shah, Y.N., Khanna, S., Jindal, K.C., Dighe, V.S. : Drug Dev. Ind. Pharm., 18 : 1589, 1992.

567 Schmit, J.A., Henry, R.A., Williams, R.C., Dieckman, J.F. : J. Chromatogr. Sci., 9 : 645, 1971.

568 The United States Pharmacopoeia XXII, United States Pharmacopoeia Convention Inc., Rockville, 1990, p. 1226.

569 Carmichael, G.R., Shah, S.A., Parrott, E.L. : J. Pharm. Sci., 70 : 1331, 1981.

570 Gharbo, S.A., Cognion, M.M., Williamson, M.J. : Drug Dev. Ind. Pharm., 15 : 331, 1989.

571 Gander, B., Ventouras, K., Gurni, R., Doclker, E. : Int. J. Pharm., 27 : 117, 1985.

572 Baukelaer, P.D., Von. Ootegan, M. : Int. J. Pharm., 16 : 345, 1983.

573 Uekama, K., Uemura, Y., Irie, T., Otagiri, M. : Chem. Pharm. Bull., 31 : 3637, 1983.

574 Acocella, G., Conti, R. : Tubercle 61 : 171, 1980.

575 Zutshi, U., Bedi, K.L. : Press release from Reginal Research Laboratory, Jammu in Indian Express, dated 13.12.1993.

576 Shah, V.P., et al. : Int. J. Pharm., 82 : 1, 1992.

577 Herzfeldt,C.D., and Kummel, R. : Drug Dev. Ind. Pharm., 9 : 767, 1983.

578 Pilpel, N. : Chem. Process Engng., 46 : 167, 1965.

579 Carr, R.L. : Chem. Engr., 72, 163, (Jan) 1965.

580 Carr, R.L. : Chem. Engr., 72, 69 (Feb) 1965.

581 James, I. Wells. : Pharmaceutical Preformulation. The physicochemical properties of drug substances, Ellis Horwood Limited, Chichester, England, 1988. pp. 192 - 214.

582 Samaha, M.W., Naggar, V.F. : Drug Dev. Ind. Pharm., 16 : 1135, 1990.

LIST OF PUBLICATIONS

CHAPTER 10

1. High Performance Thin Layer Chromatographic method for monitoring degradation products of rifampicin in drug excipient interaction studies, K. C. Jindal, R. S. Chaudhary, S. S. Gangwal, A. K. Singla, S. Khanna, Presented at *2nd International Symposium on Innovations in Pharmaceutical Sciences and Technology*, Feb. 1994, Ahmedabad.

2. Dissolution test method for rifampicin - isoniazid fixed dose formulations, K. C. Jindal, R. S. Chaudhary, A. K. Singla, S. S. Gangwal, and S. Khanna, under print in *Journal of Pharmaceutical and Biomedical Analysis.*

3. High Performance Thin Layer Chromatography method for monitoring degradation products of rifampicin in drug excipient interaction studies, K. C. Jindal, R. S. Chaudhary, S. S. Gangwal, A. K. Singla, S. Khanna, accepted in *Journal of Chromatography A*

4. Effect of particle size on the bioavailability and dissolution rate of rifampicin, K. C. Jindal, R. S. Chaudhary, A. K. Singla, S. S. Gangwal and S. Khanna, communicated to *Indian Drugs.*

5. Rifampicin stability : effect of buffers and pH, K. C. Jindal, R. S. Chaudhary, A. K. Singla, S. S. Gangwal, and S. Khanna, communicated to *Arzneimittel Forschung Drug Research.*