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PHYSICAL CHARACTERIZATION AND BIOAVAILABILITY OF GRISEOFULVIN:PHOSPHOLIPID COPRECIPITATES

by

© SURESH VENKATARAM

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Date Sept. 4, 1984

DEDICATED TO

THE TWO PEOPLE I LOVE MOST - MY PARENTS

ABSTRACT

Solid dispersions of griseofulvin and phospholipids were prepared as coprecipitates by the solvent method. The coprecipitates were characterized by differential thermal analysis, thermalmicroscopy, x-ray diffractometry, photomicrography and dissolution rate determinations. The dissolution rate of griseofulvin from coprecipitates of low phospholipid fraction (19:1-4:1 griseofulvin:DMPC) was 2-3 fold that of micronized or solvated griseofulvin, or physical mixtures in HCl-KCl buffer at pH 2.0 and 37°C. The phase diagram indicated no eutectic or solid solution formation. X-ray diffraction spectra indicated the presence of griseofulvin crystallites in the coprecipitates and photomicrographs showed that the crystals essentially retained their characteristic shapes and sizes in all systems. A study of dissolution as a function of the coprecipitating solvent showed that the existence of griseofulvin in the chloroform-solvated state was essential for obtaining increased dissolution. The incorporation of various water-soluble agents in coprecipitates of non-solvated griseofulvin and phospholipid did not provide increased dissolution. Incorporation of phospholipids having various phase transition temperatures (T_c) into coprecipitates gave higher dissolution when T_c was below 37°C and vice versa. Cholesterol-phospholipid combinations generally decreased the dissolution rate but increased the total amount dissolved after 60 min, thus controlling the

release of griseofulvin depending on the cholesterol concentration. Evidence of high partition coefficient of griseofulvin in liposomes at low phospholipid concentrations, rapid disintegration of crystals upon contact with water and the formation of myelinic structures at the crystal surfaces as observed under the microscope suggest a mechanism of increased dissolution due to crystal fracture as a result of hydration of the phospholipid molecules within the crystal lattice and the subsequent sequestering of griseofulvin in the myelinic structures. Kinetic analysis of the dissolution under sink conditions demonstrated the applicability of the second-order and the Weibull distribution functions to describe the dissolution behavior of these systems. The performance of the coprecipitates was found to be subject to processing and aging factors. Thus, tablets containing coprecipitates or 1 month aging indicated almost complete loss of the improved dissolution features. Oral administration of coprecipitates to rats yielded a significant increase in the relative bioavailability of griseofulvin and correlation of *in vitro-in vivo* parameters was obtained under sink and non-sink conditions of dissolution.

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AUC	Area under the Plasma Concentration - Time profiles from 0-24 hours.
CHOL	Cholesterol.
C_{max}	Peak Plasma Concentration.
$C_{1\ hr}$	Plasma Concentration one hour after dosing.
DCP	Dicetylphosphate.
DMF	Dimethylformamide.
DMPC	Dimyristoylphosphatidylcholine.
DPPC	Dipalmitoylphosphatidylcholine.
DSPC	Distearylphosphatidylcholine.
DTA	Differential Thermal Analysis.
EPC	Egg Phosphatidylcholine.
HCA	Hydrocortisone acetate.
IDR	Initial Dissolution Rate.
M.P.	Melting Point.
PEG 400	Polyethyleneglycol 400.
PEG 4000	Polyethyleneglycol 4000.
Polysorbate	Polyoxyethylene sorbitan monooleate.
POS	Polyoxyl 40 monostearate.
PVP	Polyvinylpyrrolidone.
SA	Stearylamine.
Solv.Gris.	Griseofulvin-Chloroform solvate.
T_c	Phase Transition Temperature.
TM	Thermal microscopy.

t_{max} - Average time taken for attainment of peak plasma concentration.

1. INTRODUCTION

When a drug is administered perorally in a solid dosage form such as a tablet, capsule, or suspension it must be released from the dosage form and dissolved in the gastrointestinal fluids before it can be absorbed. Hence, two consecutive transport processes can be identified to describe the oral absorption of drugs from solid dosage forms:(i) dissolution of the drug *in vivo* to produce a solution and, (ii) transport of the dissolved drug across the gastrointestinal membrane. Each process can be characterized by a rate constant. If the rate of dissolution of the drug is significantly slower than the rate of absorption, the dissolution of the drug becomes the rate-limiting step in the absorption process.

Different formulation approaches have been used to improve the oral absorption of poorly water soluble drugs either by increasing the dissolution rate or solubility. Examples include solid dispersion, micronization, solvent deposition, and development of prodrug formulations. Solid dispersions of drugs incorporating inert, water-soluble carriers have been extensively studied since their introduction by Sekiguchi and Obi (1). Generally, these occur as eutectic mixtures, solid solutions or glass solutions. Formulations of this type have been shown to provide dramatic increases in the dissolution of a drug. For instance, a 900-fold increase in the dissolution rate of chlorpropamide from dispersions of 30% w/w in urea has been

reported (2). Also several examples of improved dissolution of griseofulvin from solid dispersions with inert adjuvants such as succinic acid (3), polyvinylpyrrolidone (PVP) (4), polyethyleneglycol (PEG) (5), polyoxyethylene 40 monostearate (POS)(6) have appeared in the literature. Similarly, increases in the dissolution of tolbutamide from solid dispersions with PEG-dextrose (7), POS, or PEG (6), and a number of water-soluble polymers (8) have been reported.

An increase in the pharmacological activities of reserpine, nitrofurantoin and digitoxin have been reported following oral administration of cholanic acid coprecipitates (9-11). Recently, Iwaoku *et al.*, (12) reported enhanced absorption of phenobarbital from suppositories containing its coprecipitates with PVP. In spite of many such examples only two solid dispersion products are commercially available, namely, Nabilone-PVP^R and Gris-PEG^R. The problems with commercialization of these types of formulations are primarily related to scale-up in manufacturing.

Lipids have been used, occasionally either in solid dispersions as carriers or as liquid vehicles for poorly water-soluble or lipophilic drugs. For instance, the bioavailability of N'acetyl-sulfisoxazole in rats and humans was greater after oral administration of the drug as a vegetable oil-in-water emulsion (13). The absorption of chlorophenothane was increased above that from aqueous

solution, following its administration in corn oil or olive oil (14). On the other hand, phospholipids have seldom been used in solid dosage forms although it has been known for some time that phospholipids, under appropriate conditions are able to spontaneously form liposomes from a solid film (15) and entrap polar or nonpolar molecules (16). Phospholipids and liposomes have potential use in drug delivery systems to provide sustained release of drugs, localized drug delivery and enhanced uptake of drugs by target cells (17-22).

Dissolution rate testing has become a compendial requirement for a large number of drugs. This is in recognition of the necessity to set standards for the dissolution rate of certain active ingredients of a tablet or capsule dosage form in order to establish batch-to-batch uniformity and as an indicator of bioavilability. This is particularly necessary for those drugs known to have a dissolution rate-limited absorption. For example, a good correlation between dissolution rate and bioavailability has been shown for the potassium and calcium salts of penicillin V administered in powder form (23), aspirin (24) and tolbutamide tablets (25), and three dosage forms of salicylamide (26). However, there are difficulties that may be encountered during the selection of dissolution criteria to predict bioavailability. Certain reports in the literature show instances where no direct correlation between *in vitro* and *in vivo* experiments was found (27).

Furthermore, the situation becomes even more complex since the correlation between dissolution rate and bioavailability is dependent on such factors as the dissolution apparatus employed, and the agitation rate. Hence a thorough standardization of the *in vitro* test procedure has to be carried out before meaningful predictions can be made.

This thesis was aimed at developing a formulation approach using phospholipid to increase the dissolution behavior of griseofulvin and to improve its bioavailability according to the following objectives:

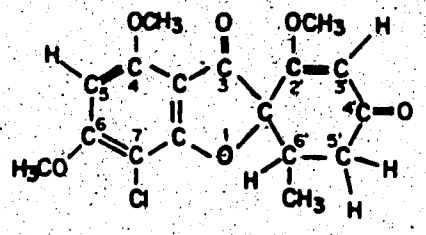
1. Preparation of solid dispersions of griseofulvin : phospholipid as coprecipitates using the solvent method.
2. Physical characterization of griseofulvin formulations including micronized, solvated, physical mixtures and coprecipitates employing DTA, thermalmicroscopy, X-ray diffraction, and photomicrography.
3. Determination of the dissolution behavior of various griseofulvin formulations under sink and non-sink conditions at pH 2.0 and 37°C.
4. Analysis of the dissolution profiles by application of a suitable kinetic model.
5. Determination of the relative bioavailability of selected formulations using the rat model and *in vitro-in vivo* correlations.

2. BACKGROUND

2.1 SELECTION OF A MODEL DRUG

A number of drugs exhibit bioavailability problems for several reasons, including poor dissolution due to low water solubility, high first pass metabolism, high water solubility, and decomposition at physiological sites. Some drugs which in particular are known to show erratic absorption include digoxin (11), ergotamine (28), griseofulvin (29), phenytoin (30), reserpine (9), sulfamethoxazole (31). These drugs can be used as models for studying the effects of formulation on bioavailability.

Griseofulvin is a metabolic by-product of several species of the mould Penicillium; e.g, P. griseofulvum, P. janczewski. It has the following chemical structure and name



7-chloro-2',4,6-trimethoxy-6'β-methyl spiro [benzofuran-2(3H), 1'-[2]-cyclohexene]3,4'-dione.

The importance of griseofulvin was realized when it was discovered that it was active against dermatophyte fungi *in vivo* (32). It has been extensively used in the treatment of superficial dermatomycoses, tinea capitis, and

dermatophytoses in humans as an antifungal agent (33). It is used to treat superficial infections of the scalp, groin, glabrous skin and acute lesions of the feet. Some of the recent studies have shown its effectiveness in the treatment of herpes zoster (34, 35). Cook (36) reported striking results with griseofulvin in children with Raynaud's disease when all other vasodilators had failed. However, Coffman (37) did not observe any beneficial responses to the drug in moderate to severe cases of Raynaud's disease. Griseofulvin has been recommended for the treatment of progressive systemic sclerosis and eosinophilic fascitis (38), and mycosis fungoides (39).

Except for a few side effects such as transitory leukopenia, granulocytopenia, acute intermittent porphyria, gastric intolerance, headache, urticaria (40) it is considered a very safe drug (41). In biological experiments it has been known to induce mitotic arrest, thyroid tumours in rats, and hepatoma in mice (33).

Griseofulvin is a relatively water insoluble, systemic, antifungal antibiotic having dissolution rate-limited oral absorption (42-45). Its absorption is also influenced by gastric emptying rate (46). It is erratically and incompletely absorbed after oral administration. Variations in absorption from 27% to 72% have been reported by Rowland *et al.*, after oral administration of a tablet of micronized griseofulvin in man (44). The average amount absorbed from oral dosage forms administered as tablets is approximately

50% of the dose. These poor absorption characteristics are considered to be responsible for the sub-therapeutic blood levels found in some patients and reports of clinical failure with griseofulvin therapy (47).

2.2 GRISEOFULVIN ABSORPTION

Those factors which mainly influence the absorption of griseofulvin are species variations, dosage regimen, fat intake and the formulation.

2.2.1 Species Variations:

It has been reported that following oral adminstration of micronized griseofulvin tablets, 45% of the dose in man (48) and 33% of the dose in dog (49) was absorbed. After the administration of an aqueous suspension of griseofulvin to rats and mice 60% and 85%, respectively, of the dose of griseofulvin was absorbed (29).

2.2.2 Dosage Regimen:

Kabasakalian (50) has suggested that the absorption of griseofulvin varies with the time of the day of administration; it is the lowest in the morning and highest at noon. However, this conclusion was based on a study in only a single subject. Atkinson *et al.*, (51) found that a divided dosage regimen produces a higher blood level in man than a single dose regimen.

2.2.3 Fat-intake:

Crounse (52) first demonstrated that the simultaneous oral administration of micronized griseofulvin with a high fat meal in man resulted in its increased gastro-intestinal absorption. The extent of increase in absorption was proportional to the amount of fat ingested. Similar observations have been made by others (50, 54).

2.2.4 Formulation Factors:

To improve the bioavailability of griseofulvin a number of formulation approaches have been used and these are discussed in detail in subsequent sections.

2.3 DISSOLUTION METHODOLOGY

2.3.1 Dissolution Theories and Kinetic Models

2.3.1.1 Noyes-Whitney and Nernst-Brunner Equations

Noyes-Whitney in 1897 (55) stated that "the rate at which a solid substance dissolves in its own solution is proportional to the difference between the concentration of that solution and the concentration of the saturated solution". Mathematically it can be expressed as,

$$\frac{dc}{dt} = K(C_s - C_b) \qquad (1)$$

where $\frac{dc}{dt}$ is the dissolution rate, K is a proportionality

constant, C_s is the solubility of the solute, and C_b is the concentration at any time, t.

In the integral form Eq. 1 can be used to calculate the value of the proportionality constant if the solubility is known. Thus

$$K = \frac{1}{t} \ln \frac{C_s}{(C_s - C_b)} \qquad (2)$$

The Noyes-Whitney Equation can be explained by the following theroy: (a) a thin layer of saturated solution is formed at the surface of the solid and the rate of dissolution is governed by the rate of diffusion from this layer to the bulk of the solution, and (b) there is negligible change in the surface area with time during dissolution.

Noyes and Whitney (56), and Brunner and Tolloczko (57) revised the equation assuming, that under well-defined conditions of temperature and agitation, the dissolution rate is proportional to the surface area, S, giving

$$\frac{dc}{dt} = K_1 S(C_s - C_b) \qquad (3)$$

where K_1 is called the intrinsic dissolution rate constant.

Applying Fick's Law of Diffusion, Nernst (58) and Brunner (59) developed a modified form of this equation:

$$\frac{dc}{dt} = \frac{DS}{hV}(C_s - C_b) \qquad (4)$$

where, D is the diffusion coefficient of the solute, h is the thickness of the diffusion layer, and V is the volume of the dissolution medium. This has been referred to as the film theory of Nernst Brunner, which applies to some situations but not others (60).

2.3.1.2 Cube Root Law

Hixon and Crowell (61) introduced the concept of changing surface area during dissolution and derived the "cube root law" given by

$$(W_o)^{1/3} - (W_t)^{1/3} = (\frac{\pi N \rho}{6})^{1/3} \frac{2DC_s t}{h\rho} \qquad (5)$$

where, W_o is the initial weight of solid, W_t is the weight of solid at time t, N is the number of particles, and ρ is the density of the solid. There are a number of assumptions on which this equation is based: (a) dissolution takes place normal to the surface of the dissolving solid particle, (b) no stagnation of liquid occurs in any region, (c) the same effect of agitation is observed on all areas of the solid surface, (d) solid particles remain intact during the dissolution process, and (e) the stagnant or diffusion layer thickness is independent of the particle diameter.

Pedersen derived a general equation for characterizing drug powders based on single particle dissolution according to the cube root or the square root law (62):

$$Q^{1/(m-1)} \approx (mWK_m)^{1/(m-1)} - (mW)^{1/(m-1)} - (K_m)^{m/(m-1)} t \qquad (6)$$

where Q is the dissolution rate, K_m is called specific dissolution rate parameter, W is weight of the powder and t is the time.

For a monodisperse powder having a log normal distribution, the above equation predicts a linear relationship between $\sqrt{Q}$ and t when the particles dissolve according to cube root model (m=3) and between Q and t when the particles dissolve according to the square root law (m=2).

2.3.1.3 Convective-Diffusion Models

(A) The Hixon-Crowell model does not incorporate a quantitative consideration of the hydrodynamic effects. In order to overcome this deficiency, a model for a moving sphere was introduced by Nielsen (63), described by the expression

$$-\frac{da}{dt} = \frac{DC_s F}{a\rho} \qquad (7)$$

where, a is the particle radius, $F \approx (1+A)^{0.285}$ and

$$A = \frac{2a^3 g(\rho - \rho_o)}{9D\eta_o}, \qquad (8)$$

ρ_o is the density of the medium, η_o is the viscosity of the medium and g is the acceleration due to gravity. This model

takes into consideration a combined diffusion, convection-controlled dissolution rate of a particle and is based on the following assumptions: (a) the dissolution rate is diffusion-rate controlled, but convection also contributes to the transport process, (b) the effective diffusion layer thickness is the same for all particles of the same size and is equal to or greater than the particle radius, (c) particles are dissolving under sink conditions, and (d) the particles are spherical.

As the particle radius approaches zero, the quantity A approaches zero, the function F in Eq.(7) approaches unity and the equation reduces to that of a pure diffusional model, i.e., the Higuchi-Hiestand model (64).

$$-\frac{da}{dt} = \frac{DC_s}{a\rho} \qquad (9)$$

(B) Nelson and Shah (65) developed a mathematical model based on convective diffusion to describe the dissolution from the surface of a compressed compact. They evaluated the expression for two shapes - circular and rectangular, given by Eq. (10) and (11), respectively

$$Q = 2.157\ D^{2/3}\ C_s\ a^{1/3}\ r^{5/3} \qquad (10)$$

$$Q = 0.808\ D^{2/3}\ C_s\ a^{1/3}\ b\ L^{2/3} \qquad (11)$$

where Q is the dissolution rate, b is the width of the

tablet perpendicular to flow, L is the length of the tablet parallel to flow, α is the rate of shear in the boundary layer, and r is the radius of the tablet.

2.3.1.4 Dankwerts Model

Dankwerts (66) proposed a model in which macroscopic packets of solvent reach the solid-liquid interface by eddy diffusion. He discarded altogether the possibility of the existence of a stagnant layer. It was assumed that the surface is continually replaced with fresh liquid.

Mathematically, this model is described by:

$$Q = S^{1/2} D^{1/2} A (C_s - C_b) \qquad (12)$$

where, S is the rate of surface renewal, and A is the area of the solid. Dankwerts originally applied this model to the dissolution of a gas in a liquid. However, it has been used by others to describe the dissolution of solids in multiparticulate systems (67).

2.3.1.5 Dissolution Kinetics Using Weibull Plot

Weibull (68) suggested a general function which is applicable to a number of common types of dissolution curve. The Weibull equation,

$$m = 1 - \exp[-(t - T_i)^b (1/a)] \qquad (13)$$

where m is the accumulated fraction of the material in solution at time t, a is the scale parameter which defines the time scale of the process, T_i is the location parameter which represents the time lag before the onset of dissolution, and b is the shape parameter which characterizes the curve as being curved upwards (b>1), or exponential (b=1) or as one with a steeper initial slope than the exponential (b<1). The Weibull distribution functions can be arranged (69) in the form of a more useful equation as follows:

$$(1 - m) = \exp[-(t - T_i)^b(1/a)]$$

$$-\ln(1 - m) = (t - T_i)^b(1/a)$$

$$\log[-\ln(1 - m)] = b \log(t - T_i) - \log a] \qquad (14)$$

Using the linear relationship given by Eq. 14 the shape parameter b and the scale parameter a can be obtained.

The scale parameter a is normally replaced by means of a more informative term, the dissolution time T_d which is defined by

$$T_d = a^{1/b} \qquad (15)$$

Eq. 15 gives the time required to dissolve 63.2% of the drug. It can also be read from the graph directly as the

time value corresponding to the ordinate value of zero. This condition is satisfied when m=0.63212, i.e.

$$\log [-\ln(1-0.63212)] = 0 \qquad (16)$$

2.3.1.c First-Order Kinetic Model

The first-order rate equation to explain dissolution profiles is mathematically given by,

$$\log(W_\infty - W) = \log M - \frac{K}{2.303}(t - t_o) \qquad (17)$$

where, W_∞ is the amount of drug in solution at infinite time, W is the amount of drug in solution at time t, M is an integration constant, t_o is the lag time before the onset of dissolution, and K is the apparent first-order dissolution rate constant (70). The equation may be slightly modified by using percent drug dissolved at time t as W, and using a value of 100 for W_∞. The time lag, t_o for a powder formulation can be taken to be zero for all practical purposes. Thus Eq.(17) becomes

$$\log(100 - \%\text{Dissolved}) = \log M - \frac{K\,t}{2.303} \qquad (18)$$

If the dissolution profile follows first-order kinetics, a straight line should result when log(100-% dissolved) is plotted against t. The rate constant K is then obtained from the slope of the line.

2.3.1.7 Second-Order Kinetic Model

The second-order rate equation is given by,

$$\frac{W}{W_e(W_e - W)} = K_2 t \qquad (19)$$

where, W is the weight of the drug in solution at time t, W_e is the maximum amount of drug available for dissolution, and K_2 is the apparent second-order dissolution rate constant (71). A plot of $W/W_e(W_e-W)$ against t yields a straight line if the dissolution follows second order kinetics. The apparent second order dissolution rate constant K_2 is given by slope of the line.

2.3.2 Dissolution Methods

In the past several years many attempts have been made to develop a reliable *in vitro* dissolution test which can thoroughly characterize the *in vivo* dissolution rate-controlled absorption of drugs. Criticisms in the early 1960's about the conventional tablet disintegration test as an indicator of drug release initiated an intense search for a better method. Researchers attempted to improve the disintegration test apparatus. However, by the mid 1960's it was suggested that the disintegration test be phased out in favour of a dissolution test. This led to the inclusion of dissolution standards for a dozen monographs in the USP XVIII and NF XIII. Since then, the number of monographs required to meet the dissolution standards has increased to

400 in the USP XXI.

The Rotating Basket dissolution method was included as the first official compendial dissolution test (Method 1). The USP XX in 1980 included a modification of this method and also introduced (Method 2) - the Paddle Method as an official compendial test. The disintegration test apparatus for dissolution (Method 3) has been deleted from the USP XXI.

A review of dissolution tests cited over 150 different apparatus designs (72). The various methods differ in hydrodynamic properties, agitating intensities and mechanical destructive forces to which the intact drug is subjected. One of the methods that has received acceptance by the scientific community is the Spin-Filter Stationary Basket apparatus developed by Shah and Nelson (73). This has been recommended for approval by the FDA (74). The apparatus has several advantages over the current USP methods. It consists of a dynamic *in situ* microporous, non-clogging rotating filter which permits continuous and efficient filtration of the dissolution fluid. Furthermore, its large filter permits representative sampling of the bulk dissolution medium. The smooth cylindrical surface of the rotating filter assembly and its agitation of fluid over a long vertical axis provide uniform, mild, laminar, nonturbulent, and reproducible stirring at relatively high speeds. The spin filter apparatus was also found to give more reproducible results than the USP method (the

coefficient of variation being 3% and 42% respectively) (73).

2.4 FACTORS AFFECTING DISSOLUTION RATE

In principle variation of any of the parameters given in the dissolution equations, e.g. Eq.(4) will cause a change in the rate of dissolution. However, in practice only the solubility and the surface area of the drug are actually controllable parameters. The diffusion layer thickness 'h' is a function of the hydrodynamics of the system and, therefore, difficult to control. Also, the diffusion coefficient 'D' cannot be made to vary except perhaps by slight changes in the viscosity of the dissolution medium. Hence, h and D are regarded as non-controllable parameters in dosage form design.

2.4.1 Solubility of the Drug in Diffusion Layer

There are several methods of formulation which can be used to alter the solubility of a drug in the theoretical diffusion layer.

2.4.1.1 Selection of Salt Form of the Drug

The dissolution rate of the salt form of a drug would normally be expected to be greater than its non-salt form, since the salt form is generally more soluble. However, the solubility of the salt depends on the counter-ion; the smaller the counter-ion the more soluble is the salt. The sodium and potassium salts of para-amino-salicylic acid are

approximately 1000 times more soluble than the unionized weak acid. Consequently, when these were administered to patients, higher blood levels of the ionized forms were obtained (75). Similarly, tolbutamide sodium salt gave higher blood levels than the free acid (76). In most of the salt forms studied, increased *in vitro* dissolution rate correlated well with increased decline of blood sugar level.In some cases the use of salt forms has been found to decrease the absorption of the drug, e.g. aluminium acetyl salicylate, sodium warfarin, benzphetamine pamoate (77, 78).

2.4.1.2 Effect of pH

A majority of drugs are either weak acids or weak bases. This makes it convenient to increase the aqueous solubility of such drugs by adjustment of the pH. A slight modification of the Nernst-Brunner equation shows that as the pH increases, the dissolution rate of a weak acid increases and that of a weak base decreases. A classic example is the addition of buffering ingredients such as sodium bicarbonate to aspirin formulations in order to raise the pH of the microenvironment of the dissolving solid particle which enhances its dissolution and thus its oral absorption (79).

2.4.1.3 Change of Crystal Form

(a)Polymorphism describes the existence of a drug in two or more crystalline forms, each of which possesses a different space lattice arrangement, but otherwise they are

chemically identical. Polymorphs exhibit different x-ray diffraction patterns, densities, melting points, stabilities and solubilities (75). A well-known example of the use of a polymorphic form to enhance bioavailability is that of chloramphenicol palmitate which exists in 3 crystalline forms A, B, and C, and one amorphous form (80). The highest mean blood levels following oral dosing of the polymorphs to humans was obtained with Form B. Haleblian and McCrone (81) have reviewed the earlier literature with respect to pharmaceutical applications of polymorphism.

(b)Solvate Formation: Solvates are crystalline forms of drugs combined with one or more molecules of solvent in the crystal lattice (82). There appears to be no general rule concerning the dissolution of solvates formed from non-aqueous solvents as compared to the non-solvated form. Generally the aqueous solubility and dissolution rate of the anhydrous form is greater than those of the hydrated forms (83). For example, it has been shown that the anhydrous form of theophylline and cholesterol dissolve more rapidly than their hydrated forms (75). Similarly, anhydrous ampicillin has a greater aqueous dissolution rate and yields greater area under the serum concentration-time curve than its corresponding trihydrate form (84). Bates *et al.*, (85) obtained an improved dissolution rate and oral absorption from the griseofulvin-chloroform solvate than from the non solvated micronized griseofulvin following oral administration to humans. A thorough review of drug solvates

has been published by Haleblian (82)

(c)Presence of crystal lattice imperfections has been shown to affect dissolution rate due to changes in the thermodynamic properties of crystals. Chiou and Kyle (86) have shown the dissolution rate and the bioavailability of digoxin to be highly dependent on its crystal properties. Similar observations have been reported for adipic acid crystals by Chow *et al.*, (87). A positive correlation between the dissolution rate constant and the dislocation density has been reported for potassium perchlorate crystals (88). A dimensionless term called disruption index has been proposed by York and Grant (89) to thermodynamically quantify the disorder imperfections in crystals induced by an impurity or additive.

2.4.1.4 Complexation

Interaction of a drug with a complexing agent could result in either an increase or a decrease in the solubility and dissolution rate of the drug. A drug may complex with both absorbable and nonabsorbable excipients in a dosage form. The dissolution rates of a complex of digoxin with hydroquinone (90) and benzocaine with caffeine were enhanced as compared to the pure drugs (91).

2.4.2 Surface Area of the Drug Particles

A decrease in particle size increases the specific surface area and, consequently, the dissolution rate and sometimes the solubility (92) of the drug. Traditional

methods of decreasing particle size include fluid energy micronization, trituration and grinding, controlled precipitation by change of solvents or temperature, application of ultrasonic waves, ball milling, spray drying, administration of water-soluble salts of poorly soluble compounds from which the parent neutral forms precipitate in ultrafine form in the gastrointestinal fluids (93), freeze-drying (94), solvation and desolvation in some cases (95), and by the method of solid dispersion discussed in detail in the following sections. The effect of particle size-reduction is a concomittant increase in the absorption of several drugs e.g. proquazone (96), benoxaprofen (97), tolbutamide (98), griseofulvin (99) and tetracycline (100). Kraml *et al.*,(101) found appreciably higher blood levels from micronized griseofulvin as compared to its regular crystalline form when administered orally to humans. However, in another study the administration of either 0.5g of micronized or 1.0 g of regular crystalline griseofulvin to humans produced similar blood levels (102).

2.5 SOLID DISPERSION TECHNOLOGY

2.5.1 Definition

The term solid dispersion refers to the dispersion of one or more active ingredients in an inert carrier or matrix in the solid state prepared by the melting (fusion), solvent or the melting-solvent method. The term coprecipitate refers

to products obtained by the solvent method. Upon exposure of such a solid dispersion system to gastrointestinal fluids the carrier usually dissolves rapidly releasing finely dispersed drug particles. Hence, this approach facilitates drug dissolution and, therefore, the dissolution rate-limited absorption of poorly water-soluble drugs.

2.5.2 Classification of Solid Dispersions

Solid dispersions have been classified mainly into six major categories as suggested by Chiou and Riegelmen (93), (1) simple eutectic mixtures, (2) solid solutions, (3) glass solutions and suspensions, (4) amorphous precipitations of a drug in a crystalline carrier, (5) compound or complex formations between the drug and the carrier, and (6) any combinations among the above groups.

2.5.2.1 Simple Eutectic Mixtures

An eutectic mixture of a sparingly water soluble drug and a highly water-soluble carrier may be regarded thermodynamically as an intimately blended physical mixture of its two crystalline components. These components are assumed to crystallize simultaneously in very small particulate sizes. The increase in specific surface area, therefore, is mainly responsible for the increase in the rate of dissolution of a poorly water-soluble drug.

Differential thermal analysis (DTA) of binary mixtures normally exhibits two endotherms but a binary mixture of eutectic composition normally exhibits a single major

endotherm. In the case of a simple eutectic system, the thaw points of binary mixtures of varying compositions will be equal to the eutectic temperature of the system.

Chiou and Niazi (103) examined griseofulvin-succinic acid solid dispersions prepared by the fusion method. They showed, with the aid of x-ray diffraction and DTA that solid solubility was negligible, contrary to earlier suggestions by Goldberg *et al.*, (3), and classified this system as a simple eutectic mixture. Additional studies (104) of fused compositions of griseofulvin in succinic acid showed that the dissolution of griseofulvin was inversely proportional to the concentration of griseofulvin in the dispersion. This led to the conclusion that the increase in dissolution was mainly due to the decreased particle size obtained, although other factors such as increased wettability, reduction or absence of aggregation, and solubilization of the drug by the carrier at the site of the diffusion layer may have also contributed. On the other hand, Goldberg *et al.*, (105) obtained similar dissolution profiles for both the fused solid dispersion of acetaminophen-urea at the eutectic composition and a physical mixture of the same composition.

2.5.2.2 Solid Solutions

These consist of a solid solute dissolved in a solid solvent. If the carrier is crystalline, a mixed crystal is formed because the two components crystallize together in a homogeneous one phase system (93). Perhaps, as suggested by Goldberg, particle size is reduced in solid solution to the

molecular level, i.e., the dissolution of the drug occurs in the solid state matrix. Hence, this system would be expected to yield much higher rates of dissolution than simple eutectic systems. In practice, the occurrence of solid solubility of less than 2% is considered insignificant. It has been shown that in binary systems, where solid solution formation is evident, the phase diagram is characterized by the disappearence of thaw points at a temperature higher than the eutectic temperature.

Chiou and Riegelman (106) reported a marked increase in dissolution rates of the sparingly water-soluble drugs digitoxin, 17-methyl testosterone, hydrocortisone acetate, and prednisolone acetate when dispersed in PEG 6000. This is believed to be due to formation of colloidal or molecular dispersion of the drug in the carrier. Similarly, Goldberg *et al.*, (107) obtained a large increase in dissolution, from the fused mixtures of chloramphenicol-urea which formed a solid solution.

2.5.2.3 Glass Solution or Suspension

The principle of glass solution formation was first reported by Chiou and Riegelman (5) to enhance drug dissolution and absorption. A glass solution is a homogeneous system in which a glassy or a vitreous form of the carrier solubilizes drug molecules in its matrix. PVP dissolved in organic solvents undergoes a transition to a glassy state upon evaporation of the solvent. Glass solutions of digitoxin with PVP (108), methisazone with PVP

(109), corticosteroids with dextrose or galactose (110), sulfamethoxazole with sugars (111), and primidone with citric acid (112) have been reported.

2.5.2.4 Compound or Complex Formation

This system is characterized by complexation of two components in a binary system during solid dispersion preparation. The availability of a drug from the complex is dependent on the solubility, dissociation constant, and the intrinsic absorption rate of the complex. For example, PVP has been shown to retard the pharmacological actions of penicillin, novocaine, prostigmine, hexobarbital, quinine and hexylresorcinol (93). Similarly, Geneidi *et al.*, (114) reported a decrease in dissolution rate of nitrofurantoin from its coprecipitate or physical mixture with PVP 25000, because of the formation of an insoluble complex.

2.5.2.5 Amorphous Precipitation

This occurs when the drug precipitates as an amorphous form in the inert carrier. The high energy state of the drug in this system generally produces much greater dissolution rates than the corresponding crystalline forms of the drug.

The conversion of a drug to an amorphous form on coprecipitation resulting in increased dissolution has been reported for sulfisoxazole-PVP (115) and chloramphenicol-PVP or -hydroxypropyl cellulose (116) systems. There may be other less clear examples of amorphous precipitation such as the phenytoin-PVP or sulfamethizole-PVP coprecipitate

systems (117, 118) where the incidence of coacervation of the carrier is believed to play a role in the particle size and form of the released drug.

2.5.2.6 Ultramicrosize Griseofulvin

Chiou and Riegelman (49) have shown that the oral absorption of griseofulvin was complete from its aqueous solution, only 88% from a 9:1 PEG 6000:griseofulvin solid dispersion, only 45% from a commercial capsule and 33% from a tablet. It has been suggested that "ultramicrosize" griseofulvin is produced in the PEG 6000 matrix. It has been reported that the efficiency of oral absorption from ultramicrosize systems is approximately twice that of conventional micronized griseofulvin (119) but this is not always the case (120).

2.6 CHARACTERIZATION OF SOLID DISPERSIONS

A number of methods have been used to characterize solid dispersions including, (a) thermal methods of analysis- differential thermal and thermomicroscopical, (b) powder X-ray diffraction, (c) microscopical studies, including the use of polarized light and the scanning electron microscope, (d) spectroscopic methods, especially I.R, (e) dissolution rate determination, (f) thermodynamic investigations involving determinations of the heats of dissolution(ΔH) and the melting points in order to calculate the resulting changes in entropy, and (g) dynamic dialysis to characterize the formation of highly supersaturated

solutions after dissolution of solid dispersions, (93, 121). The most important and frequently used methods among these are thermoanalytical, powder X-ray diffraction and dissolution rate.

2.6.1 Thermoanalytical Methods

2.6.1.1 Thermomicroscopical Analysis

This is a visual method of analysis using a polarized microscope with a hot stage to determine the thaw and melting points of solids. Its advantages are the small amount of sample required (virtually one crystal) and direct observation of the changes taking place in the sample through the thaw and melt stages. However, it does not provide the thermodynamics of the melting process and some instances it is not as sensitive as DTA. The technique has been used by others often to support DTA or DSC measurements (122, 123).

2.6.1.2 Differential Thermal Analysis (DTA)

This is an effective thermal method for studying the phase equilibria of pure substances or solid mixtures. Differential heat changes that accompany physical and chemical changes are recorded as a function of temperature as the substance is heated at a uniform rate. In addition to thawing and melting, polymorphic transitions, evaporation, sublimation, desolvation and other types of changes such as decomposition of the sample can be detected. DTA records

energy changes occuring in the sample as it is being heated as either exothermic or endothermic. However, for the interpretation of DTA thermograms, prior knowledge of the type of reactions which may be occurring is essential. For instance, it is necessary to know whether the sample is undergoing polymorphic change, decomposition, or desolvation. DTA has been used routinely to identify different types of solid dispersions (124, 125).

It has been shown by Borchardt and Daniels (126) that the total heat of reaction, ΔH, is proportional to the area under the DTA peak as described by,

$$\Delta H = K \int_{t1}^{t2} \Delta T \, dT = K \, A \qquad (20)$$

where, K is a proportionality constant, ΔT is the temperature differential, A is the area under the DTA peak, and t is the time.

2.6.2 Powder X-ray Diffraction

X-rays have been used in crystal structure studies in two different ways- a) single crystal X-ray crystallography dealing with the determination of bond angles and inter-atomic distances, and b) powder X-ray diffraction dealing with the study of crystal lattice parameters where, the X-ray diffraction intensity from a sample is measured as a function of the diffraction angles. Thus, changes in the diffraction pattern indicate changes in crystal structure.

The relationship between the wavelength λ of the X-ray, the angle of diffraction, θ, and the distance between each set of atomic planes of a crystal lattice, d, is given by Bragg's equation (127):

$$M \lambda = 2 d \sin\theta \qquad (21)$$

where m represents the order of diffraction.

X-ray diffraction spectra of simple eutectic systems show peaks of each crystalline component. Any change in the crystal lattice parameter will displace the diffraction peaks. Solid solutions exhibit a gradual shift in the positions of the diffraction lines with changes in composition. The lattice parameters of complexes are markedly different from those of pure components. Hence, the x-ray diffraction method can also be used in detecting complex formation. However, its major drawback has been the inability to differentiate between amorphous precipitation and molecular dispersion if the lattice parameter of the solvent component is unchanged. The technique has been frequently used by researchers to characterize solid dispersions (128, 129).

2.7 LIPIDS IN DRUG DELIVERY SYSTEMS

Lipids used in drug delivery systems or coadministered with drugs have yielded a variety of behaviors. Lipids have either increased or decreased the bioavailabilities of

orally administered drugs having low aqueous solubility. These effects on bioavailability have been largely due to changes in chemical or metabolic stability and dissolution rates confered by the use of lipids. Lipids have been incorporated in drug formulations by a variety of means.

2.7.1 Lipids as Vehicles

Wagner *et al.*, (130) reported an increase in the bioavailability of indoxole following its oral administration in a lipid emulsion to humans as compared to corresponding aqueous suspensions. Similar results were obtained when indoxole was administered to rats as a solution in polysorbate 80 and as a solution or suspension in cotton-seed oil (131). Sulfisoxazole acetyl and dicumarol yielded increased absorption in rats following oral administration with polysorbate 80 and triolein in comparison to aqueous suspensions. However, the use of hexadecane and oleyl alcohol had no effect on absorption. It was also found that the bioavailability of griseofulvin was decreased when hexadecane, oleyl alcohol and triolein were used; only polysorbate 80 gave an increase in absorption of griseofulvin (132). Carrigan and Bates (133) used a corn oil-in-water emulsion as a vehicle to improve the bioavailability of micronized griseofulvin in rats and it was found to yield more rapid and uniform absorption of griseofulvin than from an aqueous suspension or commercial tablets when administered to humans (134). It was suggested

that this difference was due to the formation of linoleic and oleic acids during the digestion of corn oil which inhibit gastrointestinal motility and also stimulate gall bladder evacuation. The bile salts then aid in solubilization of griseofulvin. Bates *et al.*, (47) further demonstrated that a 10 gram dose of emulsion equivalent to 4 grams of corn oil is required to obtain the maximum extent and uniformity of absorption and that the emulsion dosage form was as effective as a PEG solid dispersion of griseofulvin and better than the conventional micronized griseofulvin.

Further studies reported the absorption of dissolved griseofulvin from an aqueous phase, a micellar phase, and an oil phase (135). The disappearance of griseofulvin from each of the three phases administered to the intestinal lumen of the rat was expressed in terms of clearance constants with the result that clearance from the aqueous phase> oil phase> micellar phase. Hence, it was concluded that the lipid components augmented dissolution of griseofulvin in the aqueous phase rather than provide increased absorption of drug in solution.

2.7.2 Lipid-Coated Systems

These systems are generally prepared by uniformly dispersing a solution of the lipid in small amounts on the drug particles until the amount of lipid required is added. Chemical inactivation of Penicillin G has been shown to be

the main cause of its poor oral bioavailability. Enteric-coated preparations as a means of overcoming this problem have failed because it is not absorbed beyond the duodenum. However, significant improvement of the *in vitro* stability in gastric fluid has been obtained for potassium salts of penicillin G and V when coated with cholesterol, its acetate ester, or β-sitosterol (136). Solutions of erythromycin are highly unstable at gastric pH. The bioavailability of a series of erythromycin esters was inversely proportional to their dissolution rate in 0.1N HCl. But a two-fold increase in the human urinary excretion of erythromycin was found following oral administration of erythromycin lactobionate coated with cholesteryl acetate compared to the uncoated material (136). Thus, the protective nature of lipid coatings on acid-labile drugs has been demonstrated for these antibiotics.

2.7.3 Lipid-Drug Solid Dispersions

The presystemic inactivaton of progesterone has been shown to be significantly reduced when this drug was solvent-deposited as a solid solution with cholesterol or its acetate ester onto lactose particles(137). This has led to increased blood levels of the drug. Morphine administered orally undergoes extensive first-pass metabolism. Solid dispersions of morphine:tristearin (1:1) provided higher fractions of unmetabolized morphine after oral administration compared to free morphine sulfate solution or

morphine base administered as a suspension. It has been proposed that this effect was due to the inhibition of intestinal wall enzymes by the emulsified lipid, allowing a fraction of morphine to escape inactivation during its transport from the GI tract (138). The oral absorption of testosterone, which is known to undergo first-pass metabolism, was also increased from solid dispersions with lipids and lipid-surfactant combinations (139). In this instance, improved dissolution of testosterone increased its absorption. Kim and Jarowski (140) investigated the *in vitro* dissolution rate of hydrocortisone from systems containing cholesterol or cholesteryl esters. As the ratio of lipid to drug increased hydrocortisone dissolution rate decreased (except in the case of cholesteryl laurate). However, after being solvent deposited on powdered lactose faster dissolution of hydrocortisone occurred. This effect was attributed to the increased surface area attained by drug molecules on the lactose particles.

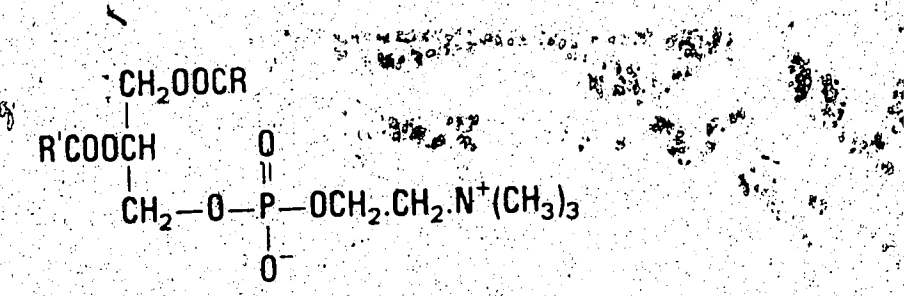
2.8 PHOSPHOLIPIDS AS EXCIPIENTS IN SOLID DOSAGE FORMS

Although a number of lipids have been tested as possible modifiers of drug dissolution or bioavailability the application of phospholipids for this purpose has been very limited. Duncan *et al.*, (141) found higher blood levels of griseofulvin in rats after the oral administration of a griseofulvin suspension containing 0.5% lecithin. A mixture of indoprofen with excipient blends composed of various

ratios of lecithin and spray dried whey produced increased dissolution of this drug and the optimum concentration of lecithin in the blend was found to be 20% (53). Thus far, no attempt has been made to formulate solid dosage forms of drugs which include phospholipids as adjuvants.

Phospholipids are one of the major structural components of the cell membrane. The phospholipid content of mammalian organs varies from organ to organ and species to species. A comprehensive list of the distribution of phospholipids in cell membranes has been published (142).

Lecithin was first isolated from egg yolk and brain by Gobley (143). Chemically it is 1,2-diacyl-sn glycero-3 phosphocholine having the structure



where, R and R' are fatty acyl substituents.

Other major phospholipids present in tissues include phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylserine (PS). Lysolecithin also occurs in tissues but at very low levels as compared to lecithin. Although the bulk of membrane phospholipids are zwitterionic, most biomembranes contain a minor proportion of phospholipids which are anionic at physiological pH, for

e.g. phosphatidylinositol biphosphate.

Phospholipids are known to exhibit a thermotropic phase transition as shown by differential thermal analysis and differential scanning calorimetry. They show an endothermic phase transition from crystalline gel to liquid crystal stage at a characteristic temperature known as the phase transition temperature, T_c (144). Below this temperature, the fatty acyl side chains are in a closely packed, relatively ordered, extended all-trans conformation known as the gel state (Figure 1-A). As the temperature is raised they progress to a more disordered, loosely packed, gauche conformation and are capable of high intra- and inter-molecular motions known as the fluid state (Figure 1-B). These molecular motions can be measured by ESR or NMR probes. Cholesterol gradually reduces the T_c as its mole fraction increases and at about 50 mole %, it results in the abolition of the T_c (144). The T_c also depends on other factors such as the nature of the fatty acid chains, and the polar head group.

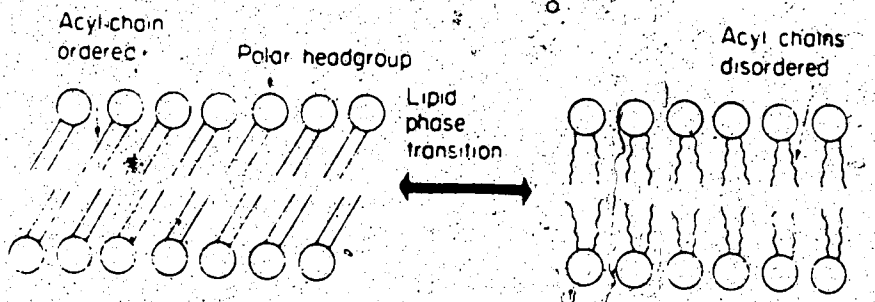


Figure 1. States of phospholipid molecules before (A) and after (B) phase transition.

3. EXPERIMENTAL

3.1 MATERIALS

Griseofulvin was obtained in a micronized state.[1] Pure synthetic phospholipids were obtained from Sigma Chemical Co.,[2] as follows: L-α-dimyristoyl-, L-α-dipalmitoyl-, L-α-distearoyl-phosphatidylcholine(DMPC, 98%; DPPC, 99%; and DSPC 99% respectively). cholesterol[2] (CHOL); egg phosphatidylcholine[2](EPC), 98%; stearylamine[2] (SA); dicetylphosphate[2] (DCP), 99+%; polyoxyethylene 40 monostearate[3] (POS); polyethylene glycol 400[4] (PEG400); polyethylene glycol 4000[4] (PEG 4000); polyvinylpyrrolidone[4](m.w.=25000)(PVP); polyoxyethylene sorbitan monooleate[4] (Polysorbate 80); m-phenylphenol[5] were used as received.

HPLC grade acetonitrile was obtained from Fisher Scientific Co.[6], or, B.D.H Chemicals.[7] De-ionized, distilled water was used to prepare the dissolution media. Diethylether[4] and Metofane[8] were used as supplied. Ethanol (98%), methylene chloride[4], chloroform[4], acetone[4] and all other chemicals were reagent grade. Heparin lock flush solution (Hapalean)[9] 1000 units/ml was diluted with normal

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[1] Glaxo Canada Ltd., Toronto, Canada.
[2] Sigma chemical Company, St. Louis, Missouri, USA.
[3] Myrj 52, Atlas Chemicals, Brantford, Canada.
[4] Allen and Hanburys, Toronto, Canada.
[5] Eastman-Kodak Co., Rochester, N.Y., USA.
[6] Fisher Scientific Co., N.J., USA.
[7] BDH Chemicals, Toronto, Canada.
[8] Pitman-Moore Ltd., Mississauga, Ontario, Canada.
[9] Organon Canada Ltd., Toronto, Canada.

saline to obtain 100 or 10 units/mL. Hydrocortisone acetate (HCA) was used as received [10].

3.2 METHODS

3.2.1 Preparation and Analysis of Griseofulvin Formulations

3.2.1.1 General Procedure for the Preparation of Coprecipitates

All coprecipitates were prepared by the 'solvent method'. The required amount of griseofulvin was dissolved in 10 mL of chloroform or in any other selected solvent in a jacketed beaker by constant stirring. Weighed amounts of the lipid or lipids were dissolved with the aid of gentle stirring. The solvent was then removed at 40-45°C under a nitrogen atmosphere with constant stirring. Further drying was accomplished in a vacuum desiccator over anhydrous calcium sulfate for 12-15 hours.

Coprecipitates containing different phospholipids (DMPC, DPPC, DSPC, DPPE, EPC) and various combinations of DMPC with other lipids such as CHOL, SA, DCP were examined. In some studies the coprecipitates contained in addition to griseofulvin and DMPC various agents including PEG 400, PEG 4000, PVP, L-cysteine, chloralose, succinic acid, mannitol, lactose, urea, deoxycholic acid, silica, or chloral hydrate. Two weight ratios of griseofulvin:(DMPC + agent) were selected namely 9:1 and 4:1. The weight ratios of DMPC:agent

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[10] Novapharm, Toronto, Ontario, Canada

selected were 1:1, and 1:3.

3.2.1.2 Preparation of Physical Mixtures

Samples were prepared by gently triturating appropriate quantities of chloroform-treated griseofulvin and phospholipid in a mortar. These samples were stored in a desiccator until ready for use.

3.2.1.3 UV Analysis of Griseofulvin

Fifty milligrams of micronized griseofulvin were dissolved in 50 mL of ethanol and 10 mL of this solution were diluted to 100 mL with KCl-HCl buffer at pH 2.0. Further dilutions were made with buffer to obtain concentrations ranging from 1 mcg to 30 mcg/mL. The absorbance of the solutions was measured at λ_{max} = 293 nm and a calibration curve was constructed. A regression equation for the straight line was computed. The calibration curve was verified using solutions of griseofulvin of known concentration and was found to agree well with the value calculated from the regression equation.

3.2.1.4 Content Uniformity Test on Coprecipitates

Coprecipitate systems were prepared from chloroform in the ratio of griseofulvin:D[illegible] 19:1 and 4:1 by weight. A quantity of the sample theoretically equivalent to 10 mg of griseofulvin was weighed and dissolved in acetonitrile. Accurate volumetric dilutions were made to give a final theoretical concentration of 2.0 mcg/mL of griseofulvin. The samples were analyzed using HPLC according to the procedure

described in a later section.

3.2.2 Physical Characterization of Griseofulvin Formulations

3.2.2.1 Differential Thermal Analysis

A Quantitative Differential Thermal Analyzer[11] equipped with an automatically programmable furnace was used in this study. A dual pen strip chart recorder[12] was used for recording the thermal changes of the sample. The instrument was calibrated with calorimetric standards of known heats of fusion (ΔH_f) according to the method of Guillory (145) using 15.0 mg of each standard substance. Calorimetric standards of indium, lead, tin and naphthalene were obtained from Fisher Scientific Company.[13] The empty pan was used as reference. The calibration coefficient, C, which is characteristic of the instrument, was determined using the formula,

$$C = \frac{\Delta H_f \; M \; P}{A \; \Delta T_s \; T_s} \qquad (22)$$

where, ΔH_f is the heat of fusion (J/g), M is the mass of sample, P is the program rate (deg/min), A is the peak area (sq.in), ΔT_s is the differential temperature sensitivity (deg/in), and T_s is the reference temperature sensitivity (deg/in). The peak areas were determined by planimeter and a

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[11] Model 300, Fisher Scientific Co., N.J., USA.
[12] Model 7128A, Hewlett Packard, USA.
[13] Fisher Scientific Co., N.J., USA.

plot of C versus peak temperature was constructed as the calibration curve.

In order to quantitatively determine ΔH_f samples (± 0.1 mg) were sealed in the sample pan, heated at the predetermined heating rate, and thermograms recorded. The areas under the endothermic peaks which appeared in the thermograms were normalized to a chart speed of 0.25 inch per min. Using the calibration curve, the C value corresponding to the sample peak temperature was obtained. Once this was determined ΔH_f was calculated.

3.2.2.2 Thermalmicroscopy

A hot-stage microscope[1] was employed for the determination of thaw and melting points of the binary mixtures prepared. The heating rate was maintained at 2°C/min just below the predetermined thaw temperature. About 1 to 2 mg of the sample was used for each test. The samples were mounted on a glass slide with a cover glass. As the samples were heated, the temperature at which the boundary of the crystal showed changes was taken as the thaw point and the complete conversion of the crystal into a melt was taken as the melting point.

3.2.2.3 Powder X-Ray Diffraction Studies

Powdered samples for low angle powder x-ray diffraction studies were uniformly dispersed on a glass slide. A piece of transparent cellulose tape was used to secure the powder

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[1] Model FP 52, Mettler Analytical and Precision Balances, Zurich, Switzerland.

in position. X-ray diffraction spectra were obtained employing CuK_α radiation run at 2°/min and a 2θ angle, in a X-ray Diffractometer[15] located in the Chemical and Mineral Engineering Department.

3.2.2.4 Photomicrographic Analysis

Samples of powder were examined microscopically at x80 or x320 magnification using a binocular microscope[16] equipped with an automatic camera. Microscope slides were prepared using light mineral oil and samples were photographed (1/8 or 1/15 sec. exposure time) using Kodak Plus-X (125 ASA) films. When the dissolution process was being observed crystals were exposed to water and photographed at various time intervals (1/8 or 1/15 sec. exposure time).

3.2.2.5 Determination of the solubility of griseofulvin

Two hundred milligrams of micronized or chloroform-treated griseofulvin were sprinkled on 900 mL of HCl-KCl buffer pH 2.0 at 37°C while the spin filter was rotating at 600 RPM. The dissolution was monitored until no further change in the absorbance was detected for a period of 2-3 hours indicating attainment of equilibrium i.e. solubility. The solubility of chloroform-treated griseofulvin was also determined in buffer containing 35 mg of DMPC under exactly the same conditions.

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[15] Model PW 1380, Philips Electronics, Holland.
[16] Carl Zeiss Co., Berlin, W.Germany

3.2.2.6 Partition Coefficients of Griseofulvin in the Liposome-Water System

Stock solutions of griseofulvin (71.4 mcg/mL) and DMPC (10mg/mL) were each prepared in chloroform. Aliquots of each stock solution were transfered to round-bottom flasks to contain 5.0, 3.0, 2.5, 0.5, 0.25, 0.075, 0.05 mg of DMPC and 71.4 mcg of griseofulvin in the dried film after the removal of the solvent by rotary evaporation. The films were dried in a vaccum oven[7] at 40°C for 12-14 hours. Five millilitres of HCl-KCl buffer pH 2.0 at 37°C were added to each flask, vortex-mixed with 1 or 2 glass beads until homogeneous. The liposomes were equilibrated at 37°C in a shaking water-bath[8] for 22 hours. The suspensions were then centrifuged [9] at 40,000 RPM for 30 min. The absorbance of the supernatant was measured and the concentration of griseofulvin in the aqueous phase was determined from the calibration curve. The concentration in the phospholipid phase was determined by difference. Partition coefficients, K_w^L, therefore, were determined from

$$K_w^L = \frac{(C_T - C_w)\ W_1}{C_w\ W_2} \qquad (23)$$

where, C_T is the concentration of griseofulvin before

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[7] Model 524, Precision Scientific Co., Chicago, IL 60647, USA.
[8] Dubnoff Metabolic Shaking Incubator, Precision Scientific Co., Chicago, IL 60647, USA
[9] Model L8-55, Ultracentrifuge, Beckman Instruments, Palo-Alto, CA 94304, USA.

equilibration, C_w is the concentration of griseofulvin in the supernatant after equilibriation, W_1 is the weight of the aqueous phase, and W_2 is the weight of the lipid in the sample.

3.2.3 Dissolution Studies

3.2.3.1 Preparation of Dissolution Media

A. Buffer solution pH 2.0: Unless otherwise specified, the dissolution medium was prepared by dissolving potassium chloride(KCl)(14.91 g) in one litre of deionized distilled water, to which were added 236 mL of 0.2 N HCl. The volume was made up to 4 litres with water and the pH was measured in a pH meter[30] calibrated with a standard buffer solution of pH 4.0.

B. Buffer Solution, pH 5.0: Monopotassium phosphate (KH_2PO_4)(36 g) and disodium phosphate ($Na_2HPO_4.2H_2O$)(0.38 g) were dissolved in sufficient water to make 4 litres. The pH was measured as before.

3.2.3.2 Dissolution Under Non-Sink Conditions

The dissolution rate measurements were carried out using a modified version of the spin-filter dissolution apparatus[31] of Shah and Nelson (73). The dissolution flask was immersed in a water bath situated on top of the magnetic stirring control unit through which water (37± 0.5°C) was

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[30] Fisher "Accumet", Fisher Scientific Co., USA.
[31] Magne-Drive, Model 74, Coffman Industries Inc., Kansas City, USA.

circulated with the aid of an external temperature control unit[22]. The dissolution medium (900 mL) was continuously circulated (40mL/min.) through a 1 cm silica glass microcell in a double beam spectrophotometer[23] with the aid of a peristaltic pump.[24] The absorbance was monitored at λ_{max} = 293 nm and recorded on a printer[25] and a recorder.[26] The reference cell contained the dissolution medium. The absorbance values were converted to concentration using the calibration curve previously constructed. The speed of rotation of the stainless steel filter was maintained at 600 RPM. When powdered drug samples were used the wire-mesh tablet basket was removed. The tubing (latex rubber tubing around the peristaltic pump, teflon tubing from sampling port to the flow cell) were made as short as possible. (30 and 125 cm, respectively) to reduce the lag time for observing the absorbance of griseofulvin. Under these conditions adsorption of griseofulvin was found to be absent.

Dissolution tests were conducted with griseofulvin in excess of its solubility i.e. under non-sink conditions unless otherwise specified. A weight of dried, powdered sample equivalent to 50 mg. of griseofulvin (coprecipitate, solvent-treated griseofulvin, physical mixture, or commercial micronized griseofulvin) was used. Samples were

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[22] Model D8, Haake Instruments, Berlin, W.Germany
[23] Model 25, Beckman Instruments, Irvine, CA 92713, USA.
[24] 'minipuls 2', Gilson Medical Electronics, France.
[25] Model 39, Beckman Instruments, Irvine, CA 92713, USA.
[26] Model 24-25 ACC, Beckman Instruments, Irvine, CA 92713, USA.

sieved through US standard sieves (80-120 mesh) prior to weighing. Sieved samples were sprinkled on the surface of the stirred dissolution medium at the beginning of the reaction.

3.2.3.3 Dissolution Under Sink Conditions

A weight of dried, powdered sample equivalent to 1.5 mg of griseofulvin, sieved as described above was sprinkled on the surface of the dissolution medium containing 0.001% polysorbate 80. Micronized griseofulvin, chloroform-treated griseofulvin, and griseofulvin:DMPC (19:1, 4:1, and 1.5:1 weight ratio) coprecipitates were examined under these conditions.

3.2.3.4 Dissolution of a Tablet Formulation

A. Preparation of Excipient Blend

Formula :

Lactose USP	25.000 g
Corn Starch USP	20.875 g
PVP 25000	2.625 g
Talc	1.500 g

Lactose, 10.44 g of corn starch, and PVP were sieved through a # 40 mesh US standard sieve and then thoroughly mixed in a mortar for 5 min. Absolute ethanol (2.5 mL) was added and the mass further triturated until the required consistency was obtained. The wet mass was subsequently sieved through a # 40 mesh sieve and then dried at 70°C for

1 hour in a hot air oven. The dried granules and talc were then sieved, again through a # 40 mesh sieve, and then blended for 15 min. in a bottle attached to a twin shell dry blender[27]. The lubricated granules ('Excipient blend') were then stored in a tightly closed container until ready for use.

B. Tablet Manufacture

Tablets were punched on a single punch tabletting machine[28] by manual rotation of the fly wheel. Micronized griseofulvin or griseofulvin:DMPC (4:1) coprecipitate were sieved through a # 40 mesh sieve and mixed with an appropriate amount of the excipient blend to prepare tablets weighing 150 mg each and containing 50 mg of griseofulvin. Tablets containing coprecipitates were compressed at 3.5 or 5.5 kg hardness as determined by Stokes Hardness Tester. Tablets of micronized griseofulvin were compressed at 5.5 kg hardness only. Placebo tablets using only the excipient blend were also prepared. Weight variation and hardness test were carried out on ten tablets individually and the results were statistically analysed using standard procedures. The dissolution test was conducted using the spin-filter test apparatus in dissolution medium at pH 2.0 as previously described. Tablet disintegration times were also recorded.

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[27] The Patterson-Kelley Co. Inc., Stroudsburg, PA, USA.
[28] Model F3, Manesty Machines, Liverpool, England.

3.2.3.5 Dissolution of Hydrocortisone acetate (HCA) Formulations

A. Calibration Curve for HCA determination

Ten milligrams of HCA was dissolved in 4.0 mL of ethanol and diluted to 100 mL with pH 5.0 HCl-acidified water. Further dilutions were made with water to obtain concentrations ranging from 1 mcg to 20 mcg/mL. The absorbance of the solutions was measured at λ_{max} = 242 nm and plotted against concentration to obtain the calibration curve. A regression equation for the straight line was computed.

B. Preparation and Dissolution of HCA Formulations

Coprecipitates of HCA:DMPC (4:1 weight ratio) were prepared from chloroform or 6 mL of a mixture of DMF:chloroform (2:1 v/v) following the general procedure. Similarly solvated HCA was obtained by treating HCA with DMF. The samples were dried *in vacuo* for 12-14 hours then sieved through US standard sieves (80-120 mesh) prior to weighing. Sieved samples equivalent to 50 mg of HCA were sprinkled on the surface of the stirred dissolution medium (acidified water at pH 5.0 and 37°C). The absorbance of HCA was monitored at 242 nm.

3.2.4 Bioavailability Studies

Adult, albino, male, Sprague Dawley rats weighing between 268 and 355 g (avg.=312 g) were used. The *in vivo* bioavailability was obtained after oral administration of

micronized griseofulvin, [illegible]roform-treated griseofulvin, griseofulvin:DMPC coprecipitates (19:1, 4:1 weight ratios), and griseof[illegible]in:DMPC (4:1 weight ratio) physical mixture and the extent and the rate of absorption of griseofulvin was determined from p[illegible]sma samples.

3.2.4.1 Analytical Procedures

A. High Pressure Liquid Chromatographic Analysis

A number of methods for the determination of griseofulvin in urine, plasma, and solid dosage forms have been reported. These include gas-liquid chromatography (146, 147), spectrofluorometry (148), high pressure liquid chromatography (149 to 152) and thin layer chromatography (153, 154). In the present study a modified method of Zia *et al.*, (152) was used.

The chromatographic system consisted of a solvent delivery system[29], a fixed wavelength uv detector[30] (280 nm), a Rheodyne injector[31] with a 20 mcL loop, a 15 cm C_{18} column[32], and an integrator-plotter[33]. The mobile phase consisting of 45% v/v acetonitrile in 0.1 M acetic acid at pH 3.5 was freshly prepared and filtered through a 0.22 μ Millipore filter under vacuum just prior to use. The flow rate was set at 1.0 mL/min.

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[29] Model M45, Waters Assoc. Inc., Milford, MA 01757, USA.
[30] Model 440, Waters Assoc. Inc., Milford, MA 01757, USA.
[31] Model 7125, Spectra Physics, Cotati, California, USA.
[32] "Novapak", Waters Assoc. Inc., Milford, MA 01757, USA.
[33] Chromatopac Model C-R3A, Shimadzu Corporation, Kyoto, Japan.

(i) Construction of a Calibration Curve

Stock solutions of micronized griseofulvin (200 mcg/mL) and internal standard, m-phenylphenol (200 mcg/mL)(147) were prepared in acetonitrile. Subsequently, the following dilutions were made: 2.0 mL of each stock solution were diluted to 100 mL with acetonitrile (Solution A). Blank plasma (100 mcL) was deproteinized with 100 mcL of solution A, vortexed for 10 secs, centrifuged[34] then 20 mcL of the supernatant were injected using a 100 mcL syringe[35]. Chromatograms complete with peak areas were recorded in triplicate and the averages computed; 1.0 mL of griseofulvin stock solution and 2.0 mL of the internal standard stock solution were diluted to 100 mL with acetonitrile (Solution B). Blank plasma (100 mcL) was deproteinized with 100 mcL of Solution B, centrifuged as before and the supernatant was injected. Triplicate analysis were made and the results averaged. The integrator automatically constructed a two point calibration curve using the average values obtained from the above injections then reported a response factor for the calibration curve which was stored for future use.

Samples of blank plasma were spiked with various known concentrations of griseofulvin and internal standard in acetonitrile. The samples were vortexed for 10 secs, centrifuged and 20 mcL of supernatant were injected as before. At the end of the run the integrator reported the concentration of the drug which was found to agree with the

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[34] Microcentrifuge, Model 235A, Fisher Scientific Co., USA.
[35] Hamilton Co., Reno, Nevada 89510, USA.

spiked concentration.

(ii) Determination of Griseofulvin in Unknown Samples

Initial screening experiments showed no significant difference in the griseofulvin concentration when one or two volumes of acetonitrile were used for the deproteinization of plasma samples. Plasma samples containing unknown amounts of griseofulvin were deproteinized using one or two volumes of the internal standard solution, (4 mcg/mL)in acetonitrile, vortexed for 10 secs, centrifuged and 20 mcL of the supernatant was injected as before. The appropriate dilution factor was applied to the recorded concentration to obtain the actual concentration of griseofulvin in the plasma samples.

B. Mass Spectrometric Analysis

Plasma samples obtained from a rat dosed with an aqueous suspension of griseofulvin were deproteinized using acetonitrile, centrifuged and the supernatant injected into HPLC as described before. The peak eluting at 3.5 min was collected from several such injections. The combined eluate was dried in a vacuum concentrator[3*]. The residue was dissolved in 25 mcL of methylene chloride and 2 mcL was injected into the gas chromatograph[3'] attached to the mass spectrometer[3*], a data system[3'], and a hard copy unit[4*]. A

[3*] Model SVC 100H, Savant Instruments, Inc., Farmingdale, N.Y., USA.
[3'] Model 5710 A, Hewlett-Packard, Avondale, PA 19311, USA.
[3*] Model 5980 A, Hewlett-Packard, Palo-Alto, CA 94304, USA.
[3'] 5934 A, Hewlett-Packard, Palo-Alto, CA 94304, USA.
[4*] Model 4631, Tektronix, Beaverton, Oregon, USA.

10 metre long DB1 (100% dimethylpolysiloxane) column[1] was used. The temperature was programmed to increase linearly from 100° to 280°C at a rate of 16°C/min. The carrier gas was helium at a flow rate of 2.0 mL/min. A gas chromatogram and a mass spectrum were subsequently obtained. A gas chromatogram and a mass spectrum were also obtained for a pure sample of griseofulvin dissolved in methylene chloride by injecting 2 mcL under identical conditions.

3.2.4.2 Bioavailability Determination

A. Cannulation of the Jugular Vein

The rats were anesthetized using diethylether. The animals were then shaved on the dorsal and ventral sides of the neck, and the shaved area was swabbed with 70% isopropanol. Metofane (methoxyflurane) was used during surgery to maintain anaesthesia.

A 1 mL syringe was fitted on one end with a 23G needle and PE50[2] tubing. To the other end of this tubing, soft silastic[3] tubing 2.5 cm in length was attached. Heparinized saline (10 units heparin/mL) was drawn into the syringe and kept ready for the cannulation procedure.

A small incision was made in the shaved area just above the jugular vein. The vein was exposed, then two pieces of surgical suture thread were placed 1 cm apart below the

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[1] J&W Scientific, Rancho Cordova, CA 95670, USA.
[2] 'Intramedic' polyethylene tubing, Becton Dickinson and Co., Parsippany, N.J 07054.
[3] 'Silastic', Dow Corning Corporation, Midland, M.I 48640, USA.

vein. During surgery the tissues were bathed with a small amount of normal saline to prevent shrinkage. The upper thread was then tied firmly around the vein to prevent any blood flow. A small incision was made in the jugular vein and immediately the silastic tube of the cannula was inserted 15-20 mm into the vein, then the lower thread was tied firmly to hold the cannula in place. After minor adjustments, the other end of the cannula was brought to the dorsal side, through the body wall, and out thorugh the skin. Finally, all of the incisions were sutured and the cannula was flushed with heparinized saline (100 units/mL).At this point the open end of the cannula was blocked with a needle blocker. After recovery the animals were housed in metabolic cages until experiments were begun on the following day.

B. Oral dosing and Blood Sampling

The rats were fasted 12 to 16 hours prior to dosing; water was allowed *ad libitum*. The animals were administered a single dose of 100 mg of the drug or its equivalent per Kg body weight by oral intubation prepared as an aqueous suspension, just prior to dosing. The suspensions were prepared in microcentrifuge tubes (1.5 mL), the amount of powder adhering to the tube and the dosing needle was estimated according to the following procedure. The dosing needle and syringe were rinsed three times with 0.2 mL quantities of water which were then transfered into the centrifuge tube. The water was evaporated at 105°C in a hot

air oven[**] and the weight of the tube was determined. By substracting the weight of the empty tube, the amount of sample adhering to the syringe, dosing needle and the centrifuge tube were obtained. This was found to be negligible in all cases (0.2 to 1.4 mg).

A sample of blood (300 mcL) was taken from the jugular vein just before dosing and at 1,2,3,5,7,11 and 24 hours after dosing. The cannula was flushed with normal saline solutions (containing 10 units of heparin/mL) following each sampling. The sample was centrifuged, the plasma was separated and immediately frozen in a freezer until required for analysis.

[**] Model 17, Precision Scientific Co., Chicago, IL 60647, USA.

4. RESULTS

4.1 IN-VITRO STUDIES

4.1.1 Analysis of Griseofulvin

4.1.1.1 Ultraviolet Spectrophotometric Analysis

A linear calibration curve which obeyed Beers law over the concentration range of 1 to 30 mcg/mL was obtained for griseofulvin and is shown in Figure 2. Regression analysis of the experimental points yielded slope = 0.0668, intercept = 0.0059, and correlation coefficient, r = 0.9999. Reproducibility at the lower end of the range was 99.2%. It was linear up to 40 mcg/mL with a deviation at the upper end of the range of [illegible]%. The molar extinction coefficient, ϵ, was calculated from

$$\epsilon = A/bC$$

where, A is the recorded absorbance at λ_{max} = 293 nm, b is the optical path length and is equal to 1 cm, C is the molar concentration of griseofulvin in the aqueous solution. The average value of ϵ was calculated to be 23,847 L/mol/cm (n=7). This compares with 24,900 L/mol/cm for griseofulvin in ethanol (155) and 24,100 L/mol/cm in methanol (156). The variation of ϵ values obtained from calibration curves determined at different times was ±1.3%.

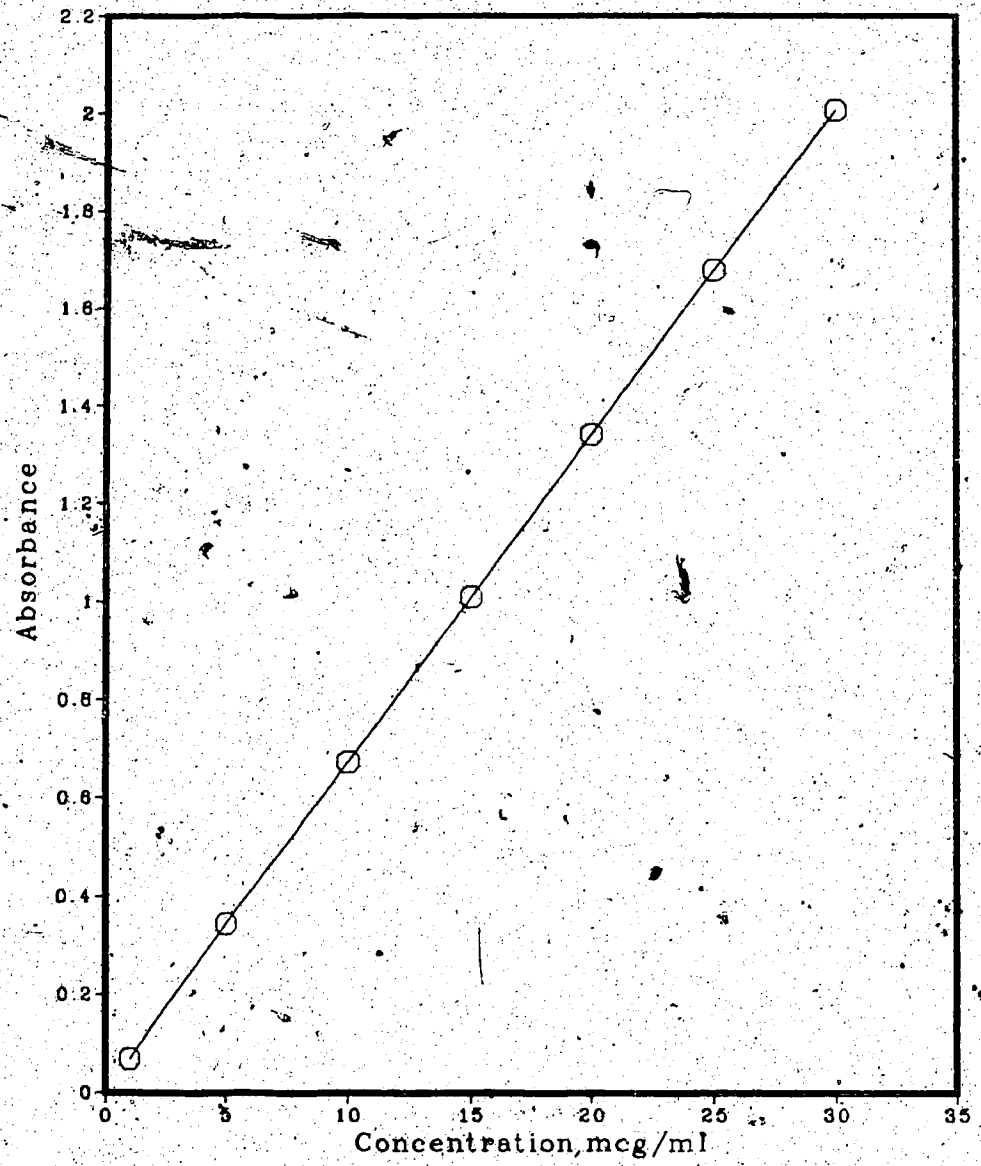

Figure 2. Beers Plot for the quantitation of griseofulvin in HCl-KCl buffer at pH 2.0 (λ_{max}= 293 nm).

4.1.1.2 High Pressure Liquid Chromatographic (HPLC) Analysis

Results of the analyses of griseofulvin from 19:1 and 4:1 weight ratio griseofulvin:DMPC coprecipitates are summarized in Table 1. It can be observed that the agreement between theoretical and observed concentrations of griseofulvin is 99+% and; furthermore, sampling different portions of a batch (samples 1 and 2) demonstrate a high degree of uniformity in griseofulvin content (98+%). A typical chromatogram resulting from these analyses is shown in Figure 3. The retention time of griseofulvin was found to be 3.5 min. and that of the internal standard 5.0 min.

4.1.2 Physical Characterization of Griseofulvin Formulations

4.1.2.1 Differential Thermal Analysis (DTA)

The data for the calibration of the Fisher Quantitative Differential Thermal Analyzer is presented in Table 2. The calibration curve obtained from a plot of calibration coefficient versus the peak temperature is depicted in Figure 4. To confirm the validity of the calibration curve, the heat of fusion of a reference substance, benzophenone, was determined. The observed value of 101.8 Joules/g is in good agreement with a previously reported value of 98.4 Joules/g (157).

Thermograms of untreated griseofulvin, griseofulvin treated with different solvents, and griseofulvin:DMPC coprecipitates are shown in Figures 5 and 6 respectively. The thaw and melt temperatures are shown in Table 3. The

Table 1

HPLC Analysis of Griseofulvin from Coprecipitates of Griseofulvin:DMPC[1]

Coprecipitate Composition (Wt. Ratio)	Batch No.	Sample No.	Griseofulvin Conc. (mcg/mL)	
			Theoretical	Observed
19:1	1	1	2.00	1.9980
	1	2	2.00	1.9764
	2	1	2.00	2.0247
	2	2	2.00	1.9838
4:1	1	1	2.00	2.0421
	1	2	2.00	2.0412
	2	1	2.00	2.0153
	2	2	2.00	1.9871

[1]The internal standard was m-phenylphenol and the injection volume was 20 mcL.

Table 2

Calibration Data for the Fisher Quantitative Differential Thermal Analyzer using Different Calorimetric Standards.

Calorimetric Standard	Peak Temperature (°C)	Heat of Fusion (J/g)	Calibration Coefficient (J/Deg/min)
Naphthalene	84.00	146.69	10.21
Indium	155.75	28.41	10.67
Tin	232.25	59.58	12.13
Lead	325.62	23.01	14.23

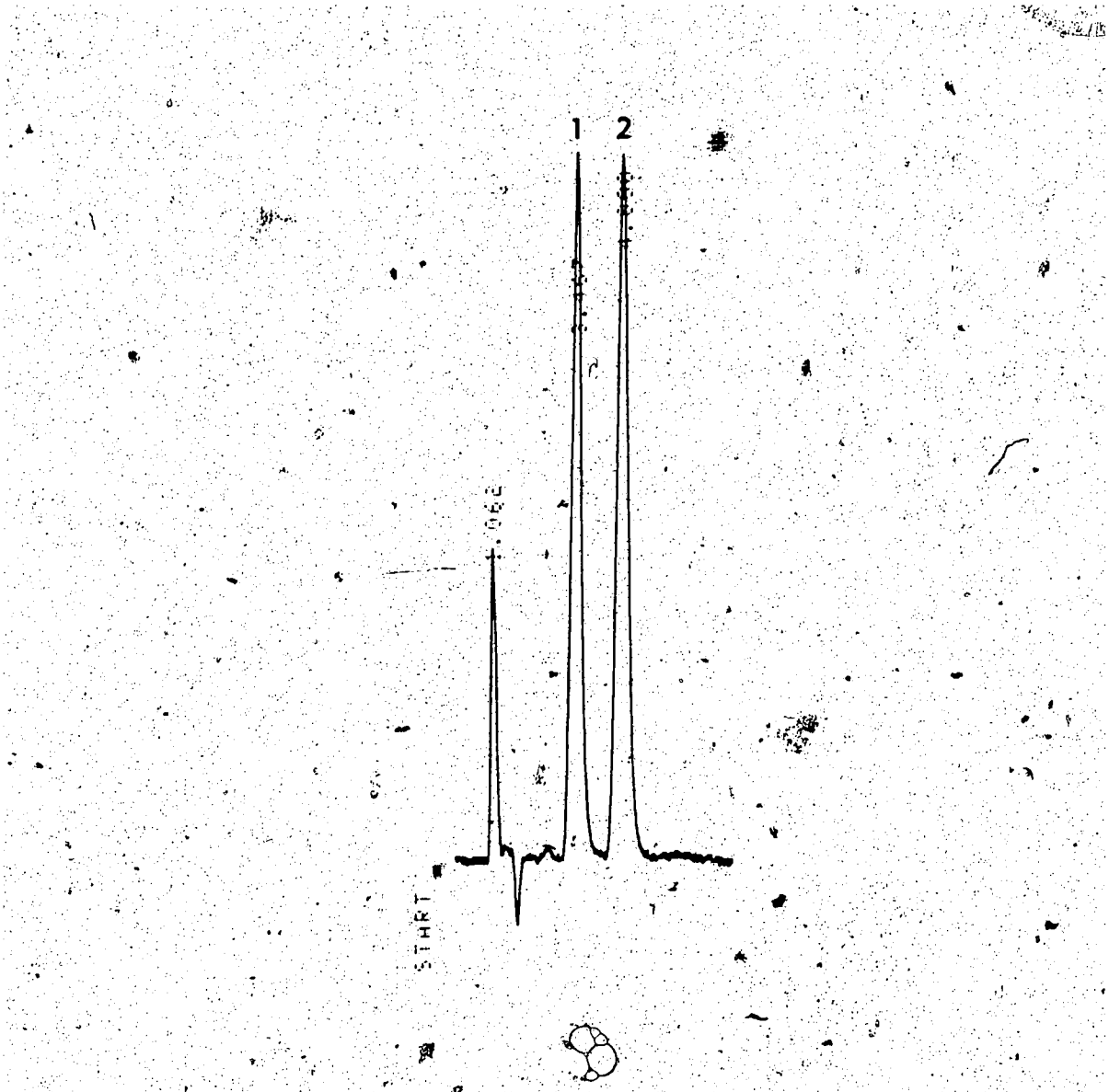

Figure 3. HPLC chromatogram obtained following the injection of a solution of griseofulvin:DMPC (4:1 weight ratio) coprecipitate in acetonitrile. Peak 1- Griseofulvin, Peak 2- internal standard. 20 mcL were injected on a 5μ, C_{18} Novapak Column. The flow rate of the mobile phase (45% acetonitrile in 0.1 molar acetic acid, pH=3.5) was 1 mL/min.

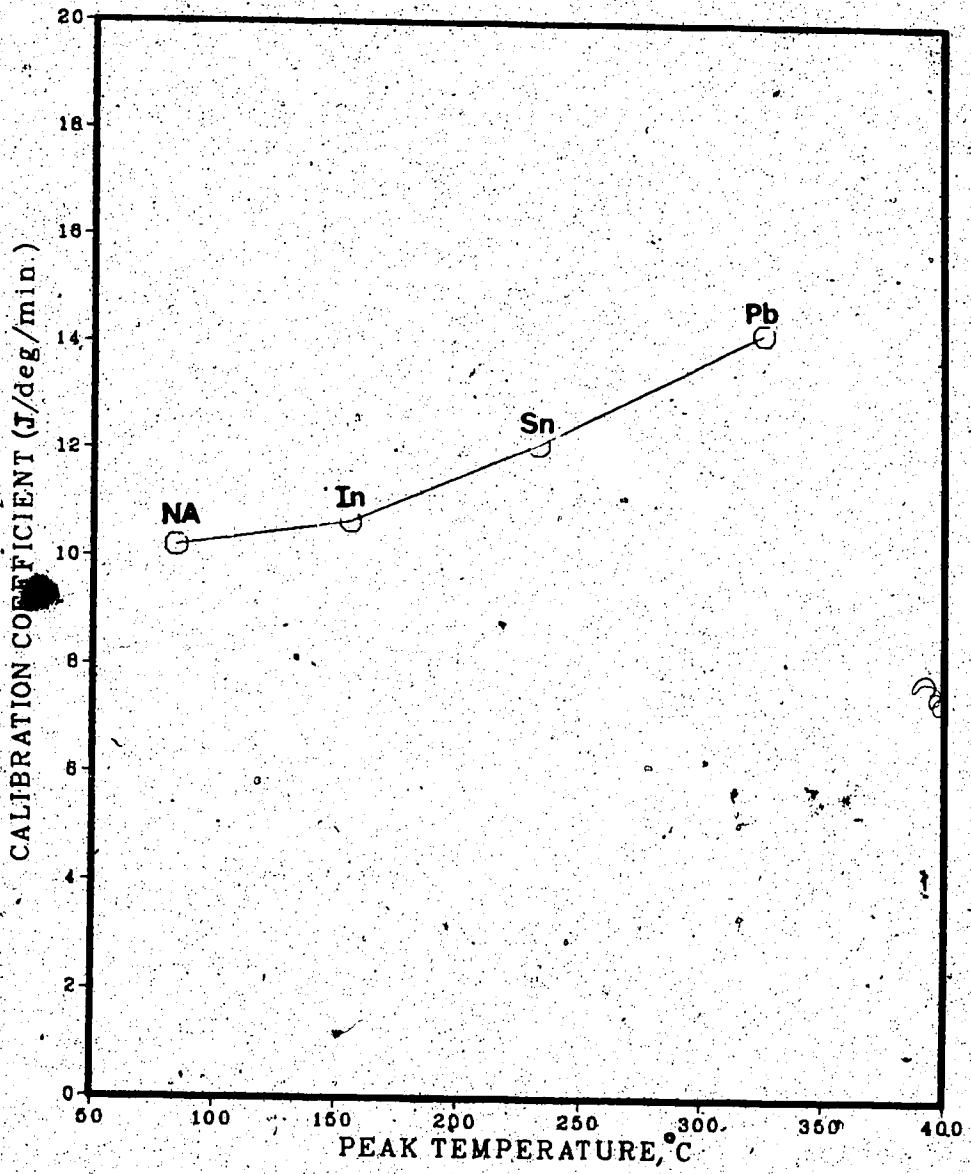

Figure 4. Calibration curve for the Fisher Quantitative Differential Thermal Analyzer under the following conditions: Sample size: 15 mg, Reference: Empty pan, Heating rate: 10°C/min. Chart speed: 0.25 in/min., sensitivity: ΔT = 0.3°C/in., and T[illegible]°C/in. using the calorimetric standards naphthalene (N[illegible] In, Sn, Pb.

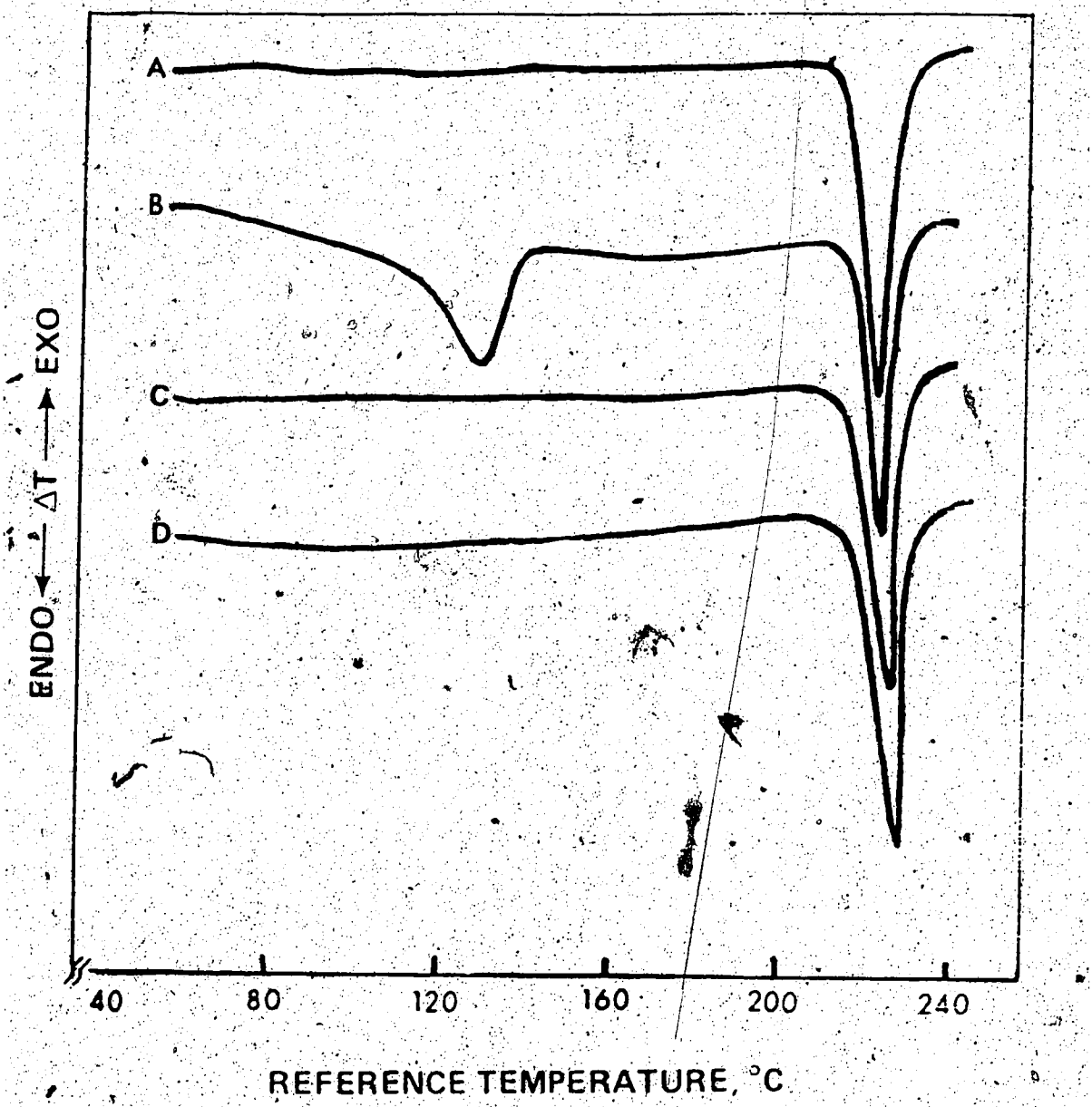

Figure 5: DTA thermograms of A: Untreated B: Chloroform-treated C: Ethanol-treated and D: Methylene chloride-treated griseofulvin.

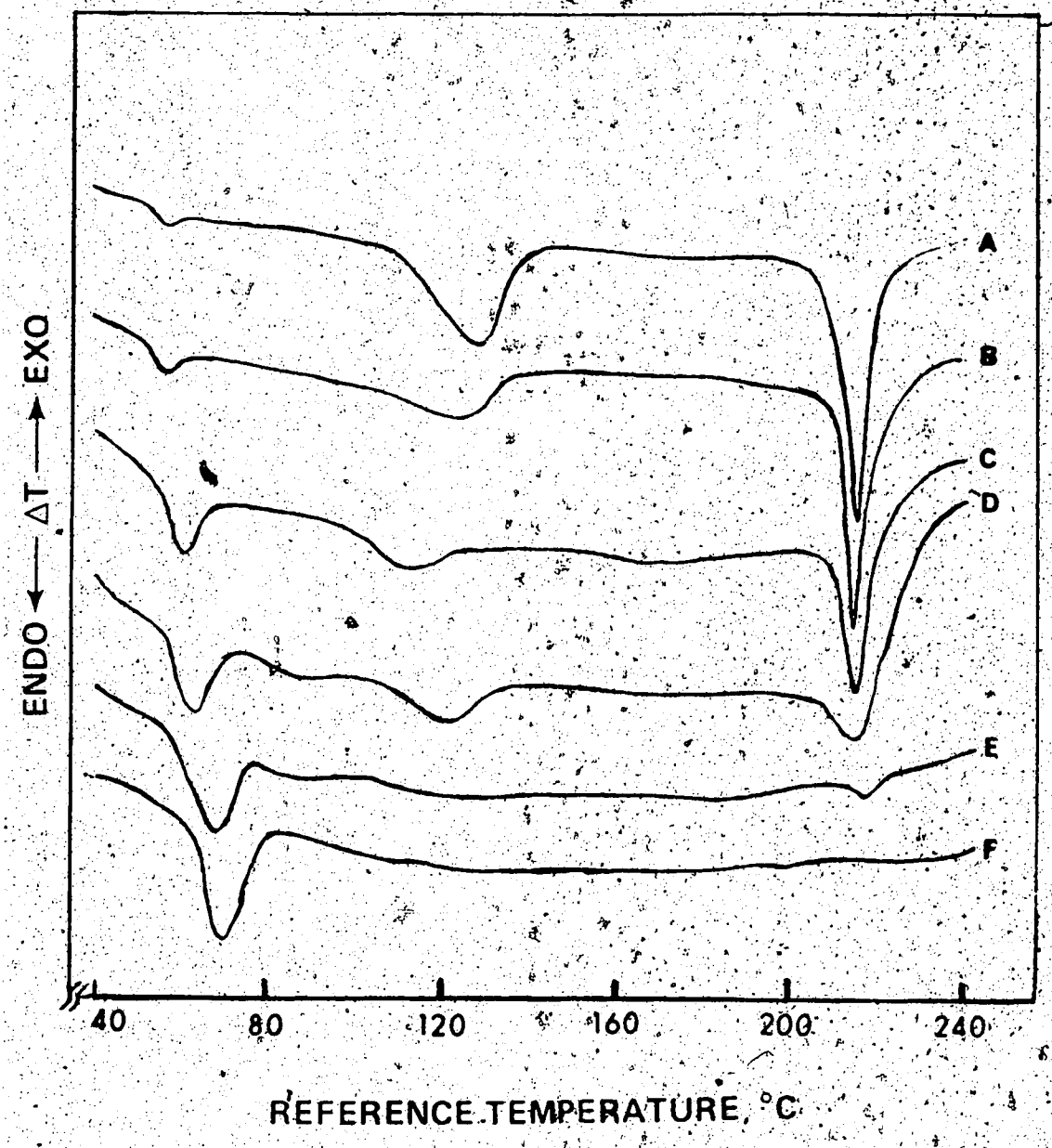

Figure 6. DTA thermograms of chloroform-treated griseofulvin:DMPC coprecipitates of various weight ratios. A-19:1; B-4:1; C-1.5:1; D-0.67:1; E-0.25:1; and F-0:1.

thermogram of untreated griseofulvin contains a major endotherm at 221°C (158). The thermogram of chloroform-treated griseofulvin (henceforth referred to as solvated griseofulvin) displayed two peaks- a major endothermic peak occurring at 221°C corresponding to the melting point of griseofulvin and a broad endothermic peak at 115 to 128°C. A similar second peak has been reported at 95° to 130°C for solvated griseofulvin (95). This peak has been attributed to the desolvation of solvated griseofulvin. The presence of the same desolvation peak in the thermograms of the griseofulvin:DMPC coprecipitates (Figure 6) also suggests that griseofulvin exists in the solvated state in the coprecipitates. This peak could be repeatedly produced even after rigorous drying conditions *in vacuo* for 12-14 hours indicating a high stability of the solvated state of griseofulvin in the solid state. Such a phenomenon has also been reported by others (159, 160). Thermograms of solvent-treated griseofulvin using ethanol, or methylene chloride displayed a single peak at 220°C. indicating that these solvents do not form solvates with griseofulvin. The amount of chloroform retained in the dried coprecipitates was also estimated from sample weights and was found to be in a 1:1 molar ratio (Table 4) with griseofulvin.

A comparison of fusion temperatures and ΔH_f for griseofulvin:DMPC weight ratios used in this study as coprecipitates or physical mixtures is given in Table 5. These DTA results clearly demonstrate similarities in the

Table 3

Thaw and Melt Temperatures of Griseofulvin:DMPC Coprecipitates obtained from DTA.

Coprecipitate Composition (Wt. ratio)	DMPC		Griseofulvin	
	Thaw Point °C	M.P. °C	Thaw Point °C	M.P °C
1:0	...	...	214.25	221.10
19:1	52.66	56.75	211.95	219.00
9:1	51.66	56.33	211.63	217.00
4:1	50.88	56.83	211.38	217.40
2.33:1	50.63	57.75	207.50	216.90
1.5:1	50.63	57.75	209.50	215.25
1:1	56.33	62.00	201.75	214.25
0.67:1	55.13	60.30	201.63	212.40
0.43:1	56.34	62.00	...	...
0.25:1	57.88	63.50	...	...
0.11:1	58.00	66.50	...	...
0:1	53.67	68.33	...	...

Table 4

Quantitative Determination of the Amount of Chloroform Retained in the Griseofulvin:DMPC Coprecipitate Crystal lattice.

Coprecipitate Composition (Weight Ratio)	Analytical Weight of Griseofulvin in the Coppt. (g)	Amount of $CHCl_3$ retained in the Coppt. (g)	Griseofulvin: $CHCl_3$ (Mole Ratio)
1:0	0.400	0.134	1.13:1.12
19:1	0.380	0.128	1.08:1.07
4:1	0.320	0.108	0.91:0.90
1.5:1	0.240	0.080	0.68:0.67

Table 5

Fusion Temperatures and Heats of Fusion of Griseofulvin:DMPC Physical Mixtures and Coprecipitates.

Composition (Wt. Ratio)	Fusion Temp (°C)		ΔH_f (Joules/g)	
	Coppts.	Phys. Mixts.	Coppts.	Phys. Mixts.
1:0	221.8	221.8	118.8	118.8
19:1	219.0	219.3	107.5	82.4
9:1	220.0	218.8	100.4	82.0
4:1	217.4	216.0	87.0	80.8
1.5:1	215.3	214.5	76.1	85.8
0:1	67.9	67.9	71.5	71.5

fusion temperatures of both formulations but marked differences in ΔH_f, an indication of differences in their energy states. There is a gradual and uniform decrease in ΔH_f with coprecipitate compositions whereas there is a drop in ΔH_f to a constant value with any physical mixture combination. This reflects obvious differences in the manner in which DMPC is associated with griseofulvin in these two formulations.

4.1.2.2 Thermalmicroscopy (TM)

Results obtained from TM measurements of griseofulvin:DMPC coprepecipitates varying in composition from 1:0 to 0:1 by weight are presented in Table 6. As the samples were heated it could be observed that DMPC and griseofulvin exhibited independent thaw and melt temperatures. During heating of these systems it was observed that between 100-130° opaquing of the crystals occurred followed by structural collapse. Previous reports of similar observations suggested that (161) the occurrence of opaqueness is the result of desolvation. The melting point of griseofulvin and DMPC also decreases slightly as its fraction in the coprecipitate diminishes (Table 6) and is indicative of the effect of an impurity on the melting point of pure compound exhibiting partial miscibility with the impurity.

Thermalmicroscopic measurements contributed to and also confirmed the results of thaw and melt temperatures of the various samples obtained from DTA. The combined data were

Table 6

Thaw and Melt Temperatures of Griseofulvin:DMPC Obtained from Thermalmicroscopy.

Coprecipitate Composition (Wt. Ratio)	DMPC		Griseofulvin	
	Thaw Point °C	M.P. °C	Thaw Point °C	M.P. °C
1:0	...	...	214.17	220.90
19:1	54.00	56.50	212.90	221.00
9:1	57.00	58.50	213.05	218.25
4:1	55.00	56.50	214.15	217.80
2.33:1	55.75	59.00	214.75	215.50
1.5:1	57.75	60.00	212.15	215.60
1:1	54.50	61.00	212.50	215.00
0.67:1	54:90	60.75	209.00	215.00
0.43:1	56.50	61.00	209.50	212.50
0.25:1	53.00	63.00	210.50	214.50
0.11:1	54.50	64.00	209.00	213.00
0:1	56.00	65.00	...	...

used to construct phase diagram for griseofulvin:DMPC coprecipitate systems. In addition, thermalmicroscopy allowed direct observation of the phase changes taking place during sample heating and for some samples (e.g. 0.43:1, 0.25:1, 0.11:1) it enabled m.p. determination that was unobtainable from DTA.

4.1.2.3 The Phase Diagram

The phase diagram as shown in Figure 7 is characterized by a linear solidus curve (lower line) and a linear liquidus curve (upper line) demonstrating a binary system in which there is very little miscibility of the components. Consequently, it is concluded that this solid dispersion system does not form any significant eutectic or solid solution. The thaw and melt temperatures of each component exhibit small differences and the slight slopes of the solidus and liquidus curves further indicate that the melting point of each is lowered only slightly by the addition of the other. The phase diagram is an example of a monotectic system, the type of which also has been shown to describe the solid dispersion behaviour of other drug-carrier systems (162).

4.1.2.4 Powder X-Ray Diffraction Studies.

Powder X-ray diffraction spectra of the pure components, griseofulvin, and DMPC are shown in Figure 8. The spectrum of untreated crystalline griseofulvin is characterized by major high intensity peaks at 16.4, 23.7,

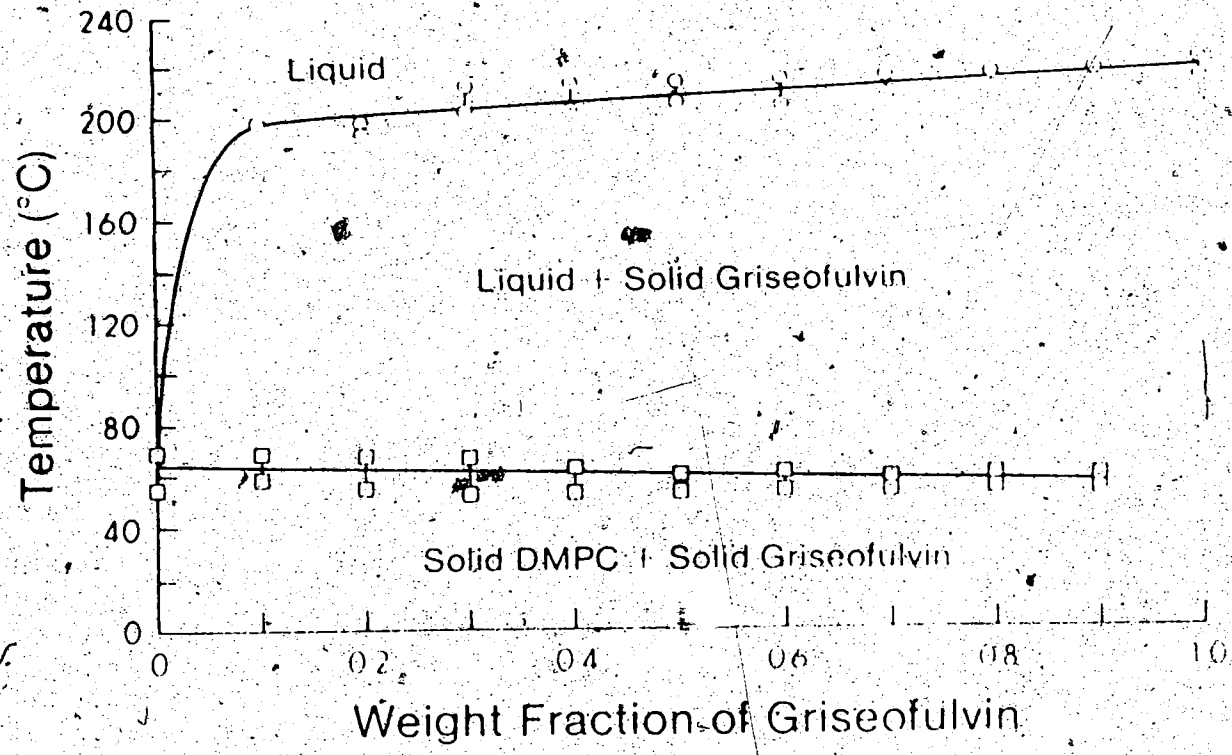

Figure 7. Phase Diagram of Griseofulvin:DMPC coprecipitates constructed using data from DTA and TM.

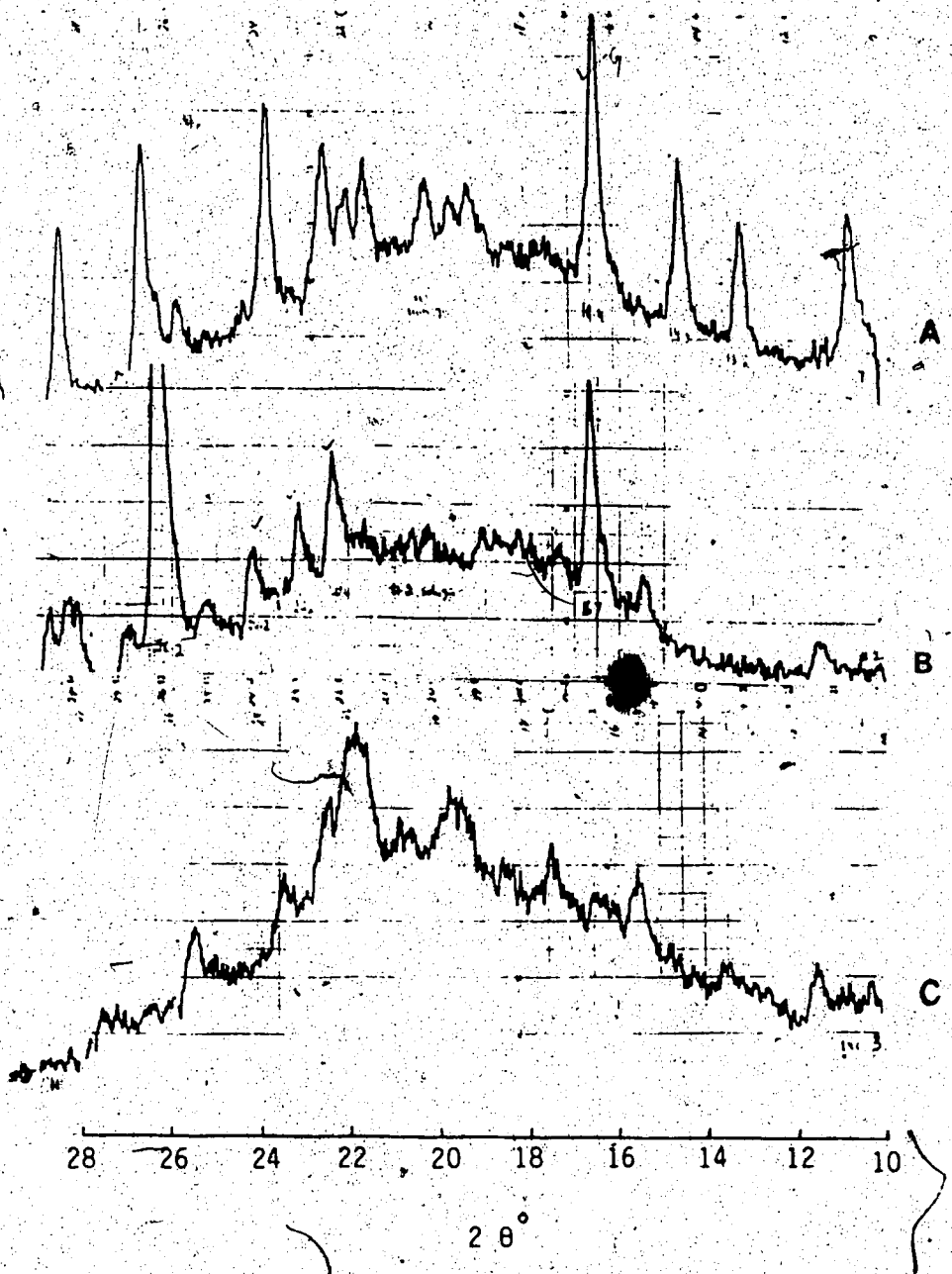

Figure 8. Powder X-Ray Difraction Spectra of, A:untreated griseofulvin, B:chloroform treated griseofulvin, and C:DMPC.

26.6, and 28.5(2θ°) and low intensity minor peaks at 10.7, 13.2, 14.7, 19, 19.5, 20.5, 22, 22.5, 23, and 25.8(2θ°). After crystallization of griseofulvin in chloroform with which it forms a solvate (163) the spectrum remained unchanged with respect to the major peaks at 16.4 and 26.6(2θ°) but pronounced changes in the occurrence of the other major peaks and reduction in intensity, elimination and shifting of the minor peaks resulted. Raman spectra of griseofulvin and solvated griseofulvin obtained by others (160) indicate the same incidence and type of crystalline structures and it has been suggested that such modification of the spectrum of griseofulvin may be due to expansion of the griseofulvin crystalline lattice by chloroform and to changes in the packing arrangement of the crystals (161). Thus, it would appear that the crystalline properties of griseofulvin are evident whether in the non solvated or solvated state. In contrast, the diffraction pattern of DMPC resembles that of an amorphous material (117).

Powder X-ray diffraction spectra of griseofulvin:DMPC coprecipitates are shown in Figure 9. With respect to each of these compositions, the crystalline state of griseofulvin still prevailed, as exemplified by the major peaks at 16.4 and 26.6 (2θ°). This evidence strongly suggests that the griseofulvin is not converted to another polymorphic form or to an amorphous state in the coprecipitate containing relatively high amounts of DMPC (griseofulvin:DMPC 1.5:1, weight ratio).

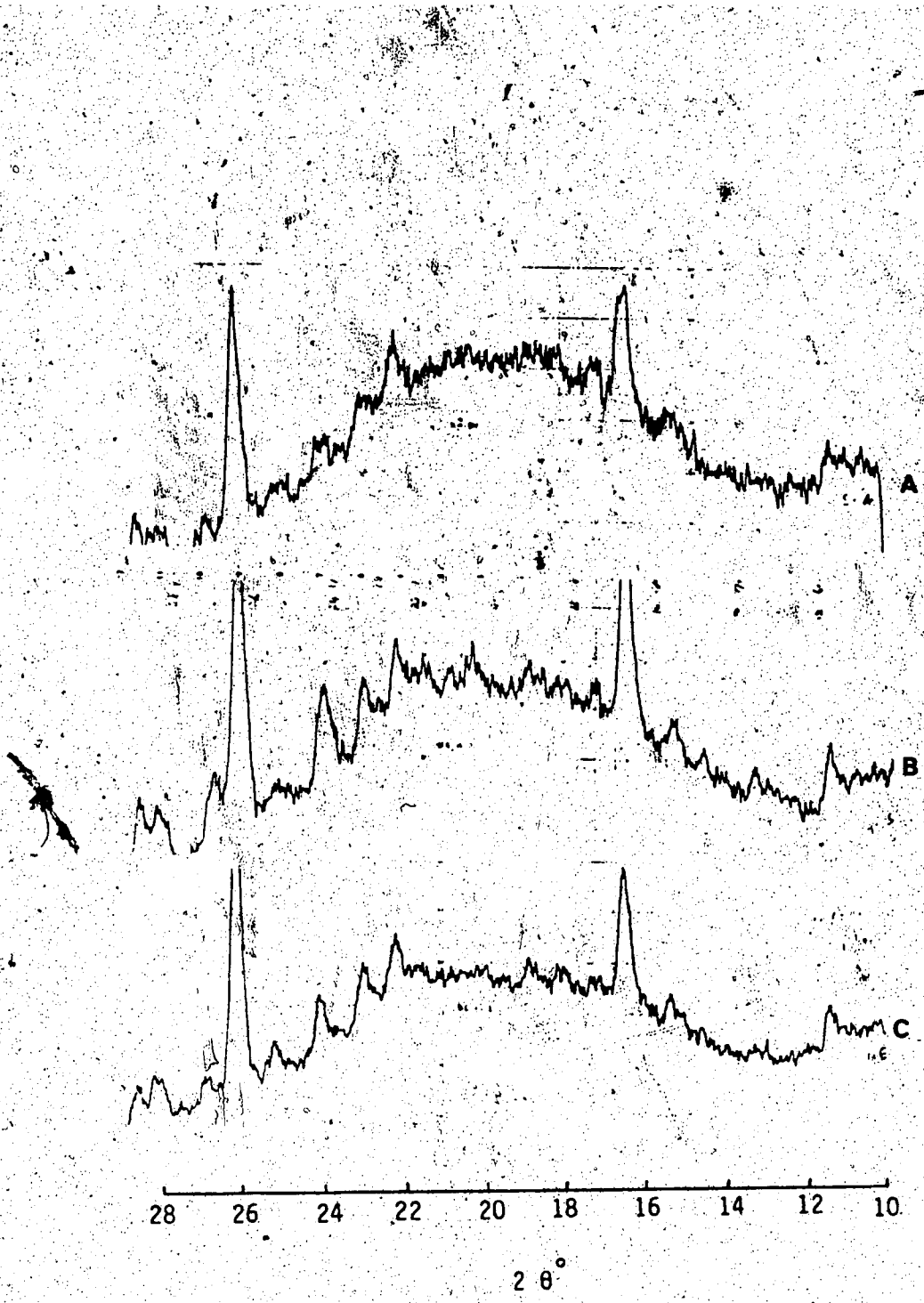

Figure 9. Powder X-Ray Diffraction Spectra of Griseofulvin:DMPC coprecipitates of various weight ratios. A-19:1, B-4:1, and C-1.5:

4.1.2.5 Photomicrographic Analysis

Examined under the microscope, the untreated micronized griseofulvin suspended in light mineral oil was observed as being highly aggregated fine crystals (Figure 10-A) whereas chloroform-treated griseofulvin appeared as larger rectangular crystals (Figure 10-B). Similarly, griseofulvin:DMPC coprecipitates also appeared as rectangularly-shaped (Figure 10-D-F) crystals which suggests that chloroform has been included in the crystal lattice of the coprecipitate as a solvate of griseofulvin.

In contrast to the static state of coprecipitate crystals in light mineral oil, suspending crystals of similar composition in water on a slide and inspection under the microscope revealed a sequence of events as the crystals underwent dissolution. Approximately 2 min. after the addition of water to coprecipitate crystals of griseofulvin:DMPC (1.5:1), the dynamic formation of multitudes of finger-like structures could be seen on the crystal surfaces (Figure 11). These grew rapidly, became extended and protruded outwards into the aqueous medium with time. Application of gentle pressure brought about extensive disintegration of the crystal (Figure 12) and the myelinic structures gradually transformed into spherical structures (possibly liposomes) freely dispersed in the aqueous medium. These same phenomena were observed if any of the griseofulvin:DMPC coprecipitate compositions was similarly treated. Although the growth of myelinic structures was also

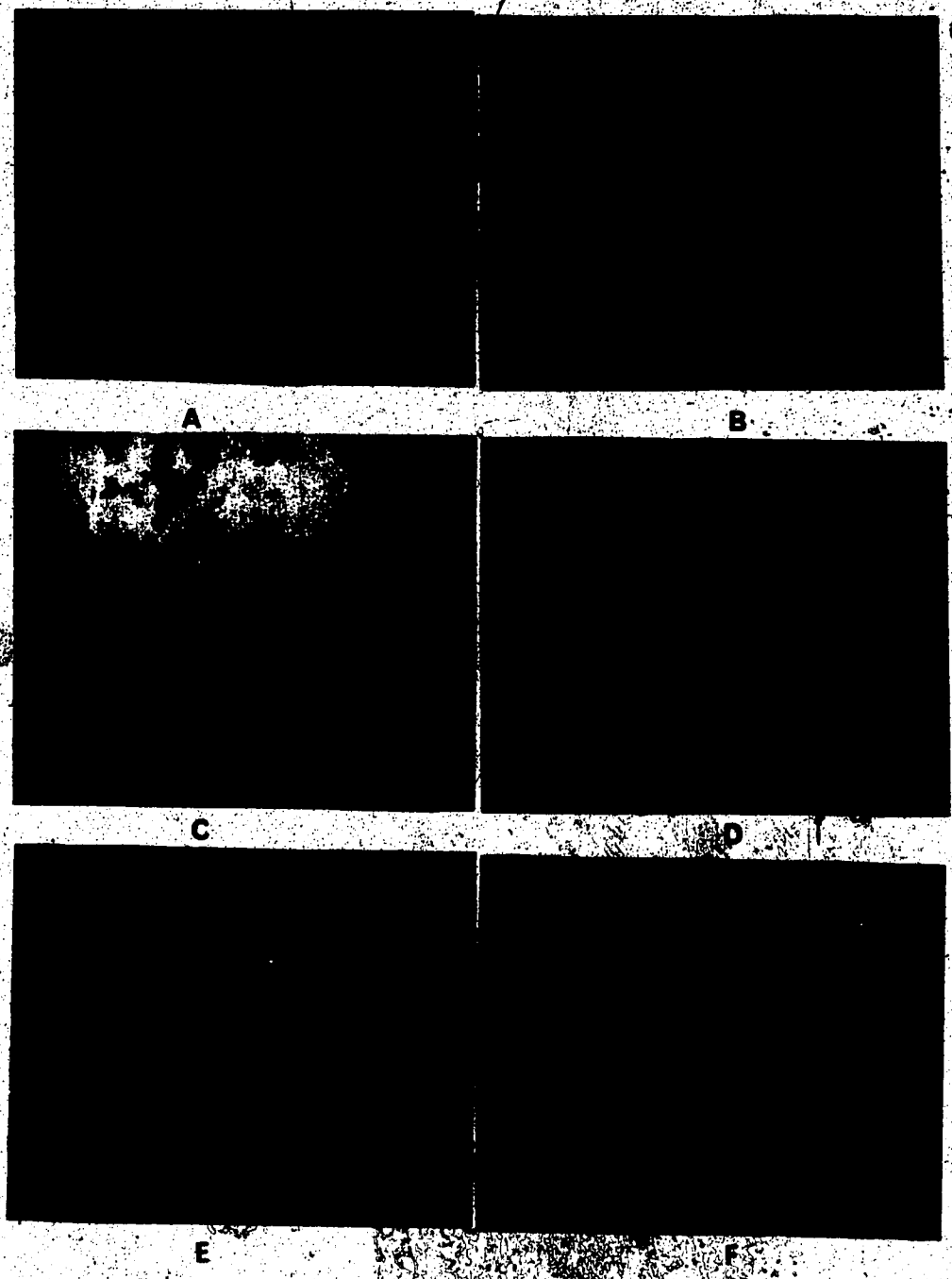

Figure 10. Photomicrographs of A-untreated griseofulvin, B-solvated griseofulvin, C-DMPC, D,E and F griseofulvin:DMPC coprecipitates (19:1, 4:1, and 1.5:1 weight ratios respectively) in light mineral oil (x80 magnification).

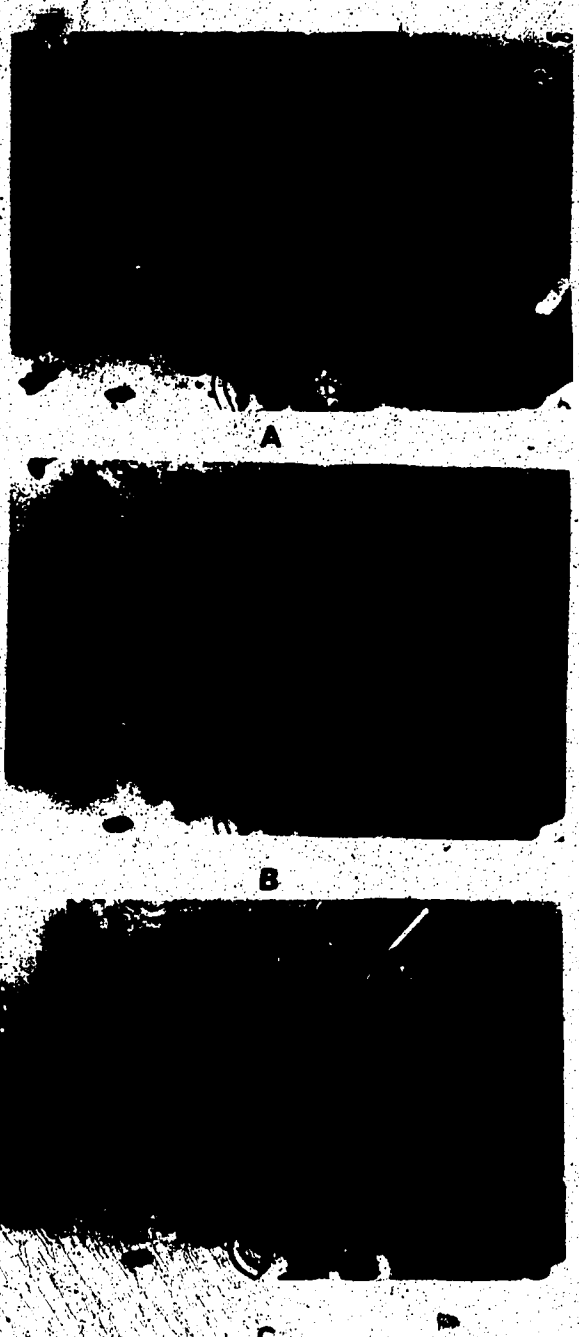

Figure 11. Photomicrographs showing the growth of myelinic structures from the surfaces of crystals of griseofulvin:DMPC (1.5:1 weight ratio) coprecipitate after 2 min.(A), 4 min.(B), and 6 min.(C) exposure to water (x320 magnification).

Figure 12. Photomicrograpah of a griseofulvin:DMPC (1.5:1 weight ratio) coprecipitate showing crystal break-up on exposure to water (x320 magnification).

observed using griseofulvin:DMPC physical mixtures it occurred more slowly but disintegration of the crystals into fine particles did not occur.

Recently, a similar observation has been made and a detailed study of the myelinic structure growth has been reported in egg yolk lecithin/hydrophilic liquid systems (164). The morphological features were classified into three steps 1) growth of simple rod-like myelinic figures in the first 10 minutes, 2) growth of more complicated forms such as helical, coil forms and, 3) association and fusion of the myelinic figures into a mosaic structure, after a growth period ranging from several hours to days.

4.1.3 Solubility of Griseofulvin in Various Media

The determination of the solubility of griseofulvin in aqueous buffer (HCl-KCl) pH 2.0 and 37°C did not reveal any major differences between the untreated and solvated forms of griseofulvin. The solubilities were found to be 15.9 and 18.7 mg/L, respectively. The solubility of solvated griseofulvin at 37°C in aqueous buffer (HCl-KCl) pH 2.0 containing 35 mg DMPC per 900 m[illegible]aximum amount obtainable in the dissolution medium fr[illegible].5:1 coprecipitate) was unchanged at 18.8 mg/L. Although the dissolution rate of solvated griseofulvin has been discussed (4,165) its solubility has not been reported before. The solubility of griseofulvin in water, buffer, and various media are presented in Table 7. The solubilities are in agreement with

Table 7

Solubility of Griseofulvin in Various Dissolution Media at 37°C

Dissolution Medium	Solubility mg/L	Reference
Water	10.0	6
	12.5	189
	15.0	45
	31.8	184
Sod. Phos. Buffer (pH 7.2)	32.2	184
Water + 2% w/v PEG 2000	10.0	6
Water + 5% w/v PEG 2000	20.0	6
Water + 2% POS	150.0	6
Water + 5% POS	200.0	6
Water + 40% Dimethylformamide	481.1	184

the solubility range of 10 to 15 mg/L reported in the literature. However, in two cases the solubilities reported are higher (31.8, and 32.2 mg/L) (166). Not enough information was available in order to be able to comment on this discrepancy. However, the addition of 5% PEG 2000 or 5% POS to water produced a 2- and 20-fold increase in the solubility of griseofulvin respectively. The particularly large increase in solubility in the latter case is most likely due to the high wetting and micelle forming ability of the surface active POS. DMPC is a poor wetting agent because there are insufficient molecules adsorbed at the solid-liquid interface owing to its extreme tendency to form colloidal aggregates. Association with these colloidal aggregates leading to increased solubility was not observed since, the process was not preceded by increased dissolution of griseofulvin. Therefore, it is concluded that the mere presence of DMPC in the dissolution medium does not have any solubilization effect on griseofulvin.

4.1.4 Partition Coefficient of Griseofulvin in DMPC Liposomes

Partition coefficients (K) of griseofulvin in the DMPC liposome-water system are graphically depicted in Figure 13. Log K values of griseofulvin exhibit a DMPC concentration-dependence. At low concentrations of DMPC the log K value was as high as 4.27, but decreased rapidly with increase in DMPC concentration to a minimum and then

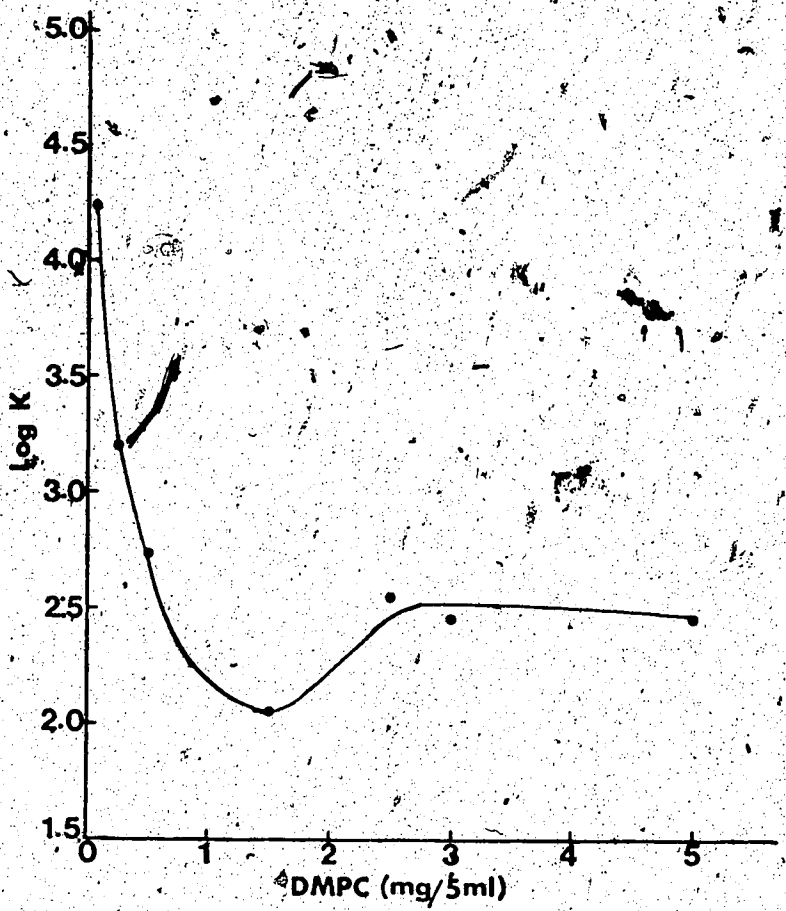

Figure 13. Partition Coefficients (log K) of griseofulvin in DMPC liposome-water system at 37°C.

increased slightly eventually attaining a constant value. Similar results were obtained whether griseofulvin was incorporated initially in the lipid film or in the aqueous phase during liposome preparation.

4.1.5 Dissolution of Griseofulvin:DMPC Physical Mixtures

The dissolution of griseofulvin from its 19:1 and 4:1 physical mixtures with DMPC is illustrated in Figure 14. The dissolution profile of solvated griseofulvin is also included for comparison. It is apparent that no significant differences exist in these dissolution profiles with respect to the initial release and the amount in solution after 60 min. Hence, at the concentrations of DMPC employed there is no significant interfacial tension reduction at the solid-water interface and no solubilization of griseofulvin from physical mixtures. Various reports in the literature have shown that carrier agents in physical mixtures of drugs exert only slight effects on the dissolution rate (114, 167, 122) except when the carrier possesses pronounced surface activity (6).

4.1.6 Dissolution of Griseofulvin:DMPC Coprecipitate Systems

Solid dispersion systems have often exhibited increased dissolution as a result of increased solubility of the drug in the dispersion medium (93). This occurs in studies in which excess drug has been added creating a condition of supersaturation and is regarded as being dissolution under a

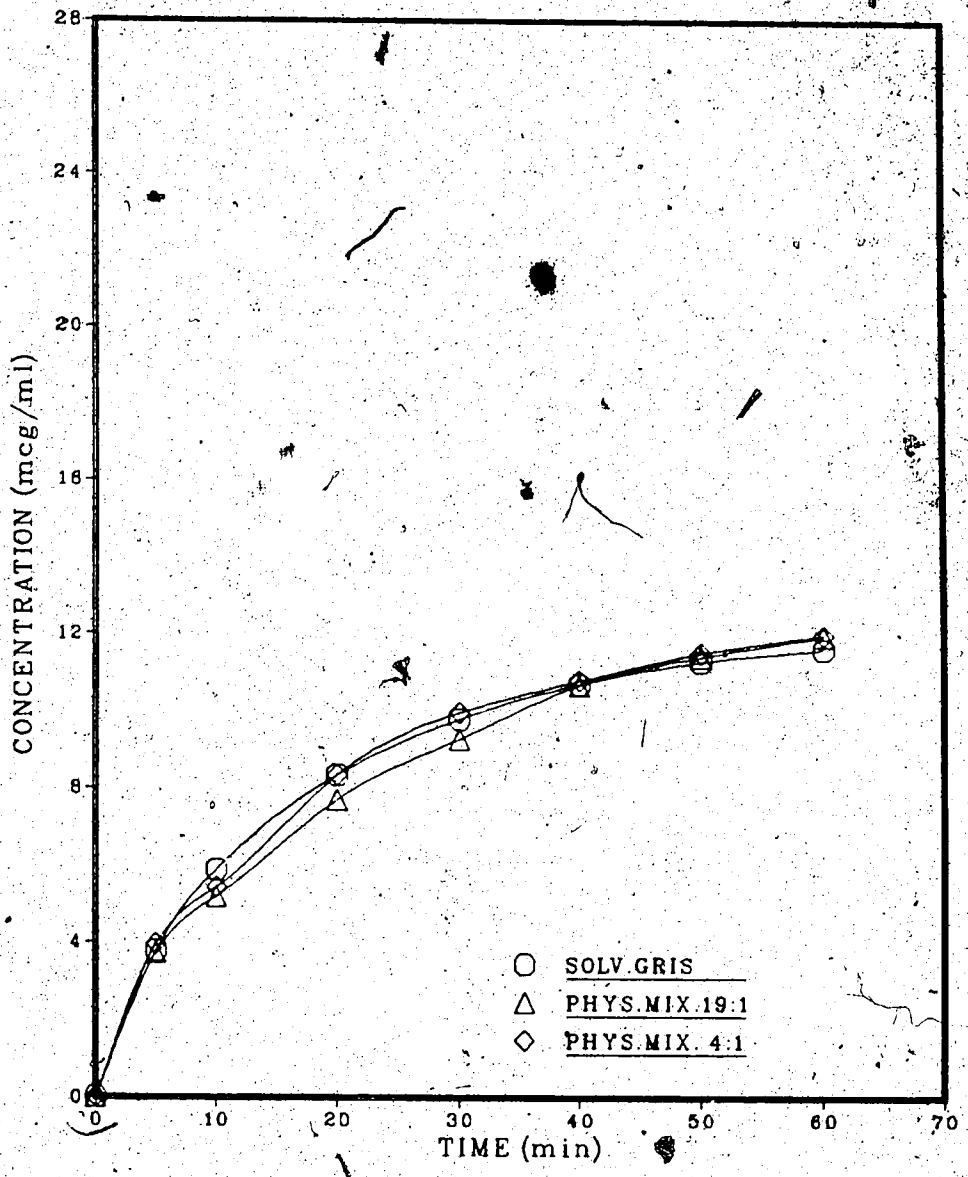

Figure 14. Dissolution profiles of griseofulvin formulations prepared as physical mixtures with DMPC in various ratios in pH 2.0 HCl-KCl buffer at 37°C. (n=2, Mean±Range are plotted). Error bars are shown unless they are smaller than the symbols.

non-sink condition Throughout this study all experiments were conducted under non-sink conditions using an amount of sample equivalent to 50 mg of griseofulvin in 900 mL of dissolution medium, except as reported in section 4.1.11.

4.1.6.1 Effect of pH

Griseofulvin is a drug which is reported to have a low pH dependency with respect to solubility (166). Thus it is not surprising that the dissolution curves shown in Figure 15 indicate that the dissolution of solvated griseofulvin or griseofulvin:DMPC coprecipitates in dissolution media at pH 2.0 and pH 5.0 is also pH independent. The amounts of griseofulvin dissolved at various times were consistently approximately 3-5% lower at pH 5.0 than at pH 2.0. This is likely due to the difference in the ionic strength of the buffers used (the ionic strength of HCl-KCl buffer=0.12 and that of phosphate buffer=0.35). This behavior is consistent with the slight effects of pH previously shown for the dissolution of griseofulvin (6).

4.1.6.2 Effect of Particle Size

It is well understood from dissolution theory that a reduction in particle size yields increased dissolution of drug. In a solid dispersion system, of 10% griseofulvin:succinic acid dissolution was found to be particle size-dependent (104). However, this was not the case for a sulfathiazole:urea solid dispersion perhaps because the drug was in a solid solution state (168). Figure

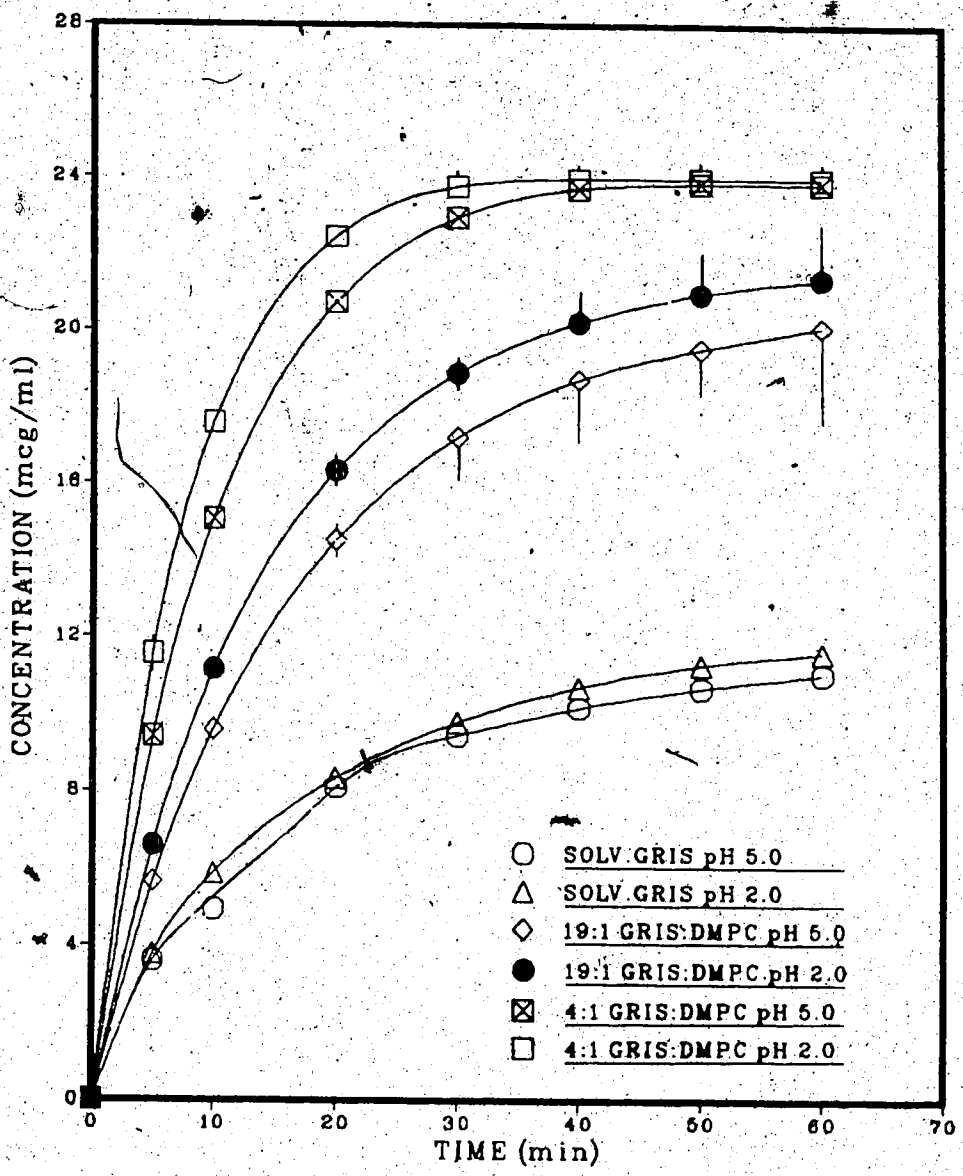

Figure 15. Dissolution of solvated griseofulvin, and griseofulvin:DMPC coprecipitates in pH 2.0 KCl-HCl buffer and pH 5.0 phosphate buffer at 37°C. (n=3, Mean±SEM at pH 2.0 and n=2, Mean±Range at pH 5.0 are plotted).

16 shows the dissolution of various particle size fractions of griseofulvin:DMPC coprecipitates (4:1) to be particle-size dependent. Thus a fine particle size of griseofulvin in coprecipitates plays an important role in the dissolution behavior.

4.1.6.3 Griseofulvin:DMPC Ratios

Dissolution profiles of griseofulvin : DMPC coprecipitates of various ratios are shown in Figure 17. The initial dissolution rates (IDR, as determined within the first 5 min. of dissolution) and the amounts dissolved after 60 min. for the different formulations are presented in Table 8. The IDR of a griseofulvin:DMPC (19:1) coprecipitate was found to be approximately 1.7 times higher than that of untreated griseofulvin. Increasing the concentration of DMPC in the coprecipitate yielded non linear increase in the IDR. For instance, the 4:1 griseofulvin:DMPC ratio yielded a 3-fold increase in the IDR as compared to the untreated griseofulvin sample but the (1.5:1) griseofulvin:DMPC coprecipitate had a slightly lower IDR. The increased lipid nature of the coprecipitate at this composition probably contributed to this behavior. In a similar manner testosterone solid dispersions containing cholesterol, β sitosterol, or cholesteryl stearate studied by Babar and Jarowski (139) also exhibited a slight increase then a decrease in IDR with an increase in the lipid content of the solid dispersions. The amount of griseofulvin dissolved after 60 min. from coprecipitates as shown in Table 8 was

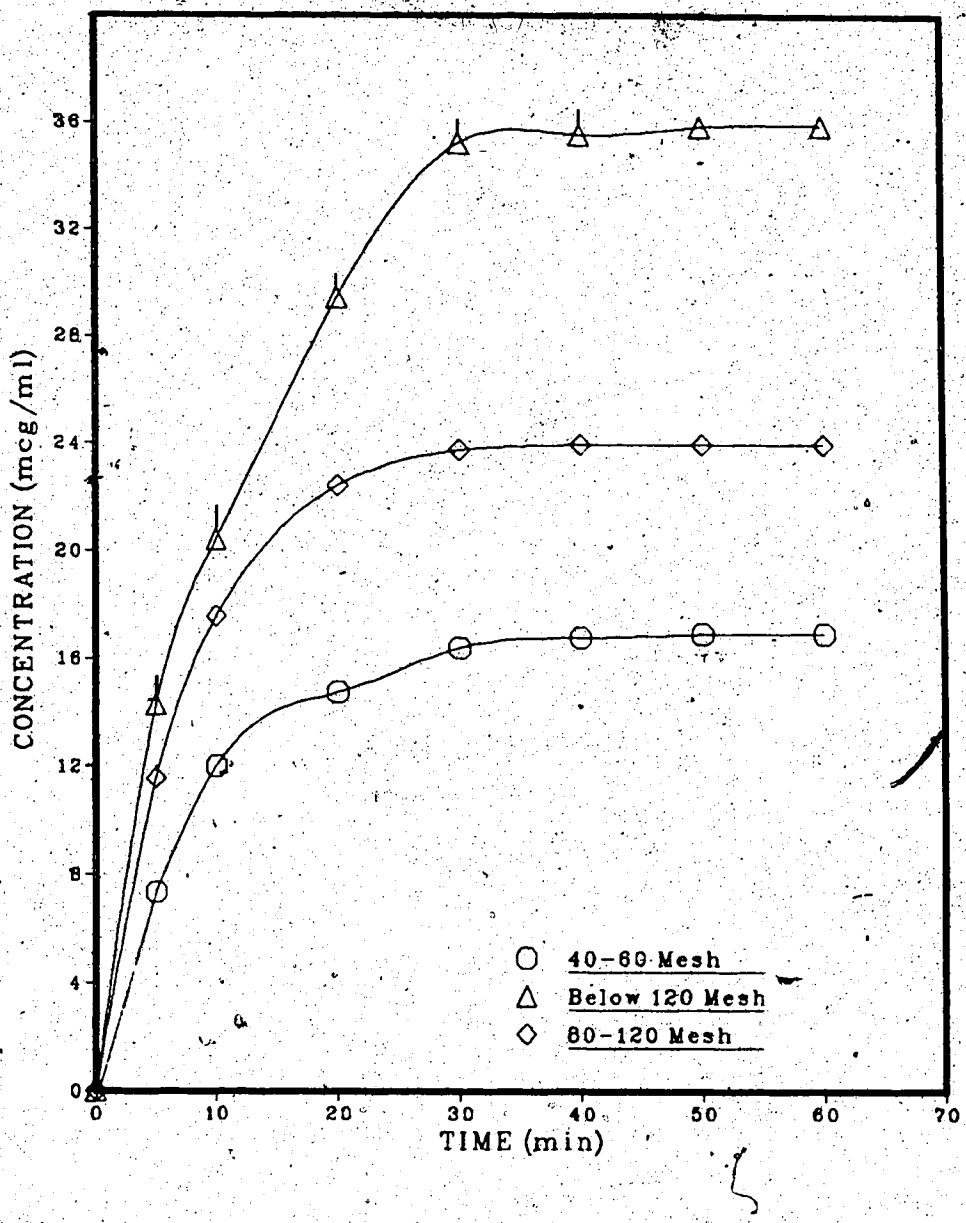

Figure 16. Dissolution of griseofulvin from griseofulvin:DMPC coprecipitates (4:1 weight ratio) as a function of various particle size distributions in pH 2.0 HCl-KCl buffer at 37°C. (n=2, Mean±Range except 80-120 mesh where n=3, Mean±SEM are plotted).

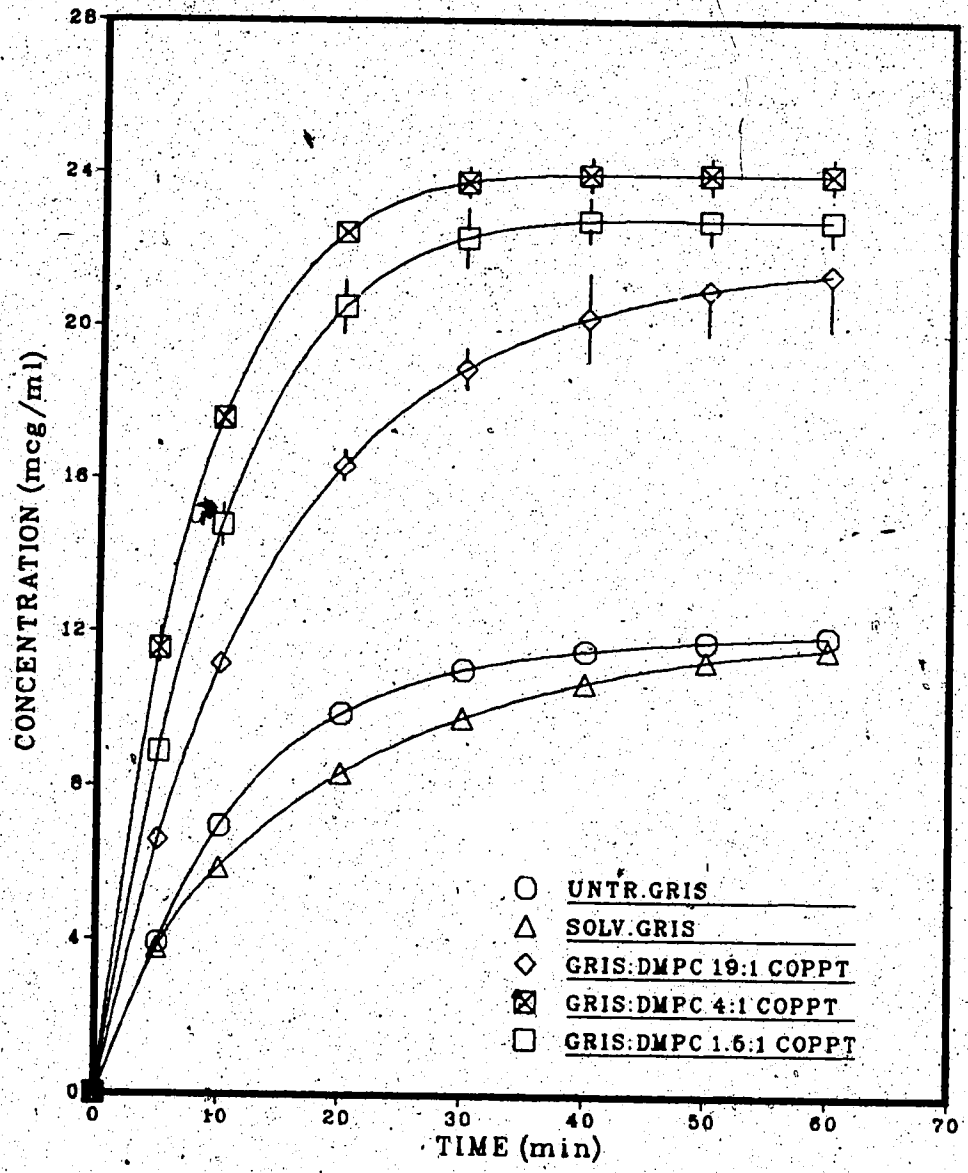

Figure 17. Dissolution profiles of griseofulvin and griseofulvin:DMPC coprecipitates in pH 2.0 HCl-KCl buffer at 37°C. (n=3, Mean±SEM are plotted).

Table 8

Dissolution Parameters for Griseofulvin and Various Griseofulvin:DMPC Coprecipitates in Aqueous KCl-HCl Buffer at pH 2.0 and 37°C.

Composition	IDR' (mcg/mL/min)	Amount Dissolved in 60 min. (mg)
Untreated Griseofulvin	0.78±0.05	10.74±0.13
Solvated Griseofulvin	0.75±0.05	10.46±0.12
Griseofulvin:DMPC (19:1)	1.32±0.02	19.22±1.33
Griseofulvin:DMPC (4:1)	2.31±0.09	21.59±0.43
Griseofulvin:DMPC (1.5:1)	1.78±0.02	20.50±1.31

'Based on the calculated amount released during the first 5 min.

The values represent mean±SEM, n=3.

also greater compared to untreated or solvated griseofulvin by approximately two-fold, indicating solubilization of griseofulvin in the dissolution media.

The dissolution data were subjected to analysis of variance at three time points namely; 5, 30 and 60 min. to represent the initial, middle, and terminal portions of the dissolution rate profiles. A significant F value was found and, therefore, in order to determine significant difference between the means, Duncan's multiple range test was applied (169). The coprecipitate systems behaved significantly different ($p=0.05$) from either untreated or solvated griseofulvin at all time points. Mayersohn and Gibaldi (4) reported a lower dissolution rate of the griseofulvin solvate versus untreated griseofulvin although no statistical tests were included. Our studies show that solvated griseofulvin had a significantly lower dissolution rate only at 30 min. On the other hand, Fung and Nealon (165) did not find any significant difference between the dissolution rates of the solvate and the anhydrous, non-microcrystalline form of griseofulvin. Hence, it is not possible to draw a definitive conclusion with respect to solvated or non-solvated forms of griseofulvin, but in the case of certain other drug solvates higher dissolution rates have been observed in comparison to non-solvated forms (75).

4.1.6.4 Effect of Solvent Selection on Griseofulvin : DMPC Coprecipitates

The consequences of using a solvent other than chloroform on coprecipitate formation and subsequent dissolution of griseofulvin were determined. Results using methylene chloride and ethanol, which do not form solvates with griseofulvin, are shown in Figure 18 and 19. Methylene chloride-treated griseofulvin gave slightly increased dissolution whereas ethanol-treated griseofulvin gave slightly decreased dissolution compared to untreated or chloroform-treated griseofulvin. However, the various solvent-treated coprecipitate systems did not significantly alter the dissolution of griseofulvin except when chloroform was employed as the coprecipitating solvent even though the various crystals possessed similar shapes and sizes when observed under the microscope.

4.1.6.5 Effect of Polysorbate 80 in the Dissolution Medium

The addition of a surface-active agent to the dissolution medium has the effect of reducing particle aggregates and increasing the effective surface area of the powder sample to the dissolution medium, thus increasing dissolution (Eq. 4). This is demonstrated particularly for untreated micronized griseofulvin in Figure 20. (cf Figure 17). On the other hand, the dissolution of solvated griseofulvin is affected much less by polysorbate 80 because its crystals are larger and not highly aggregated.

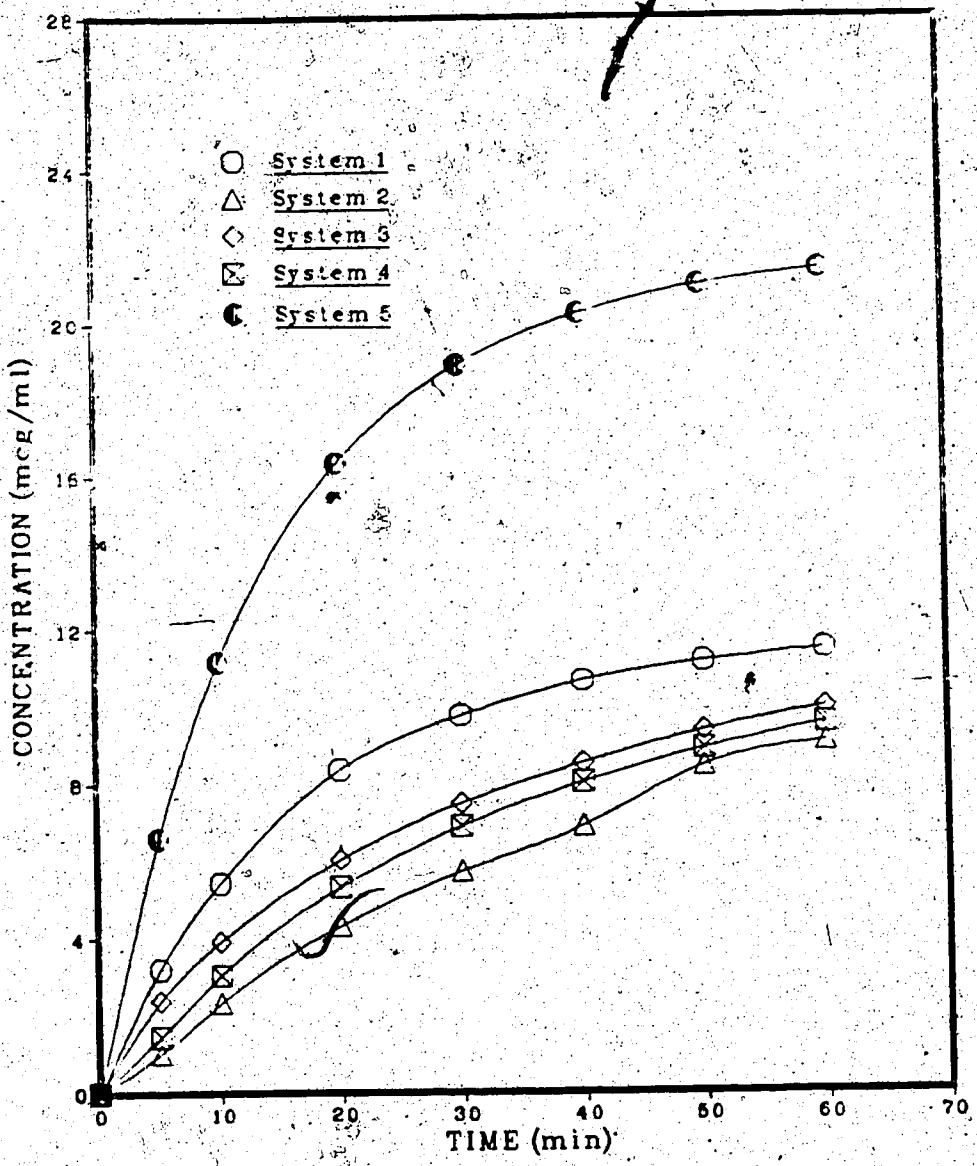

Figure 18. Effect of solvent selection on the dissolution of solvent-treated griseofulvin and griseofulvin:DMPC (19:1 weight ratio) coprecipitates in pH 2.0 HCl-KCl buffer at 37°C. (n=2, Mean±Range are plotted).

Key: System 1 and 2-griseofulvin treated with methylene chloride and ethanol respectively; system 3,4, and 5-griseofulvin: DMPC (19:1) coprecipitated with methylene chloride, ethanol, and chloroform respectively.

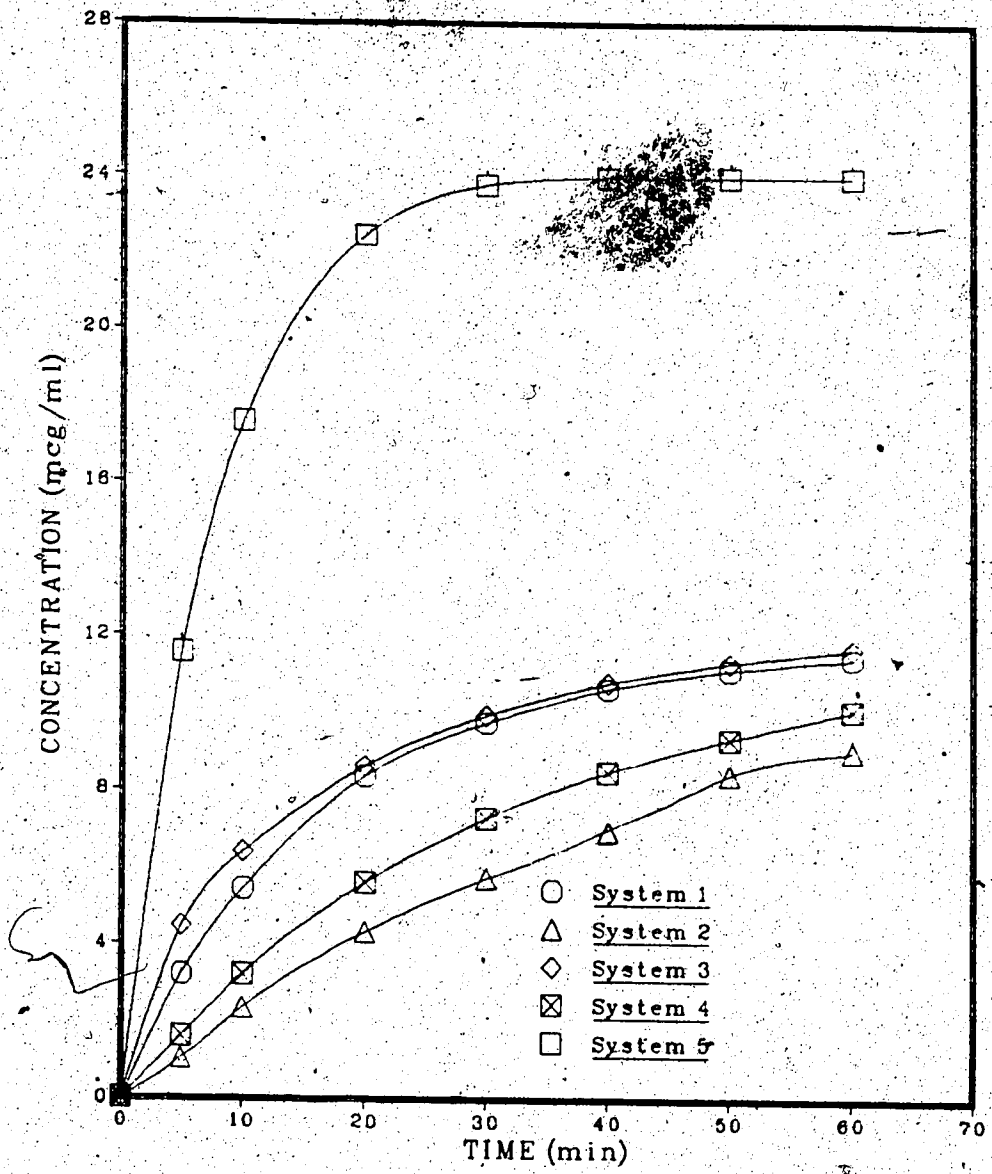

Figure 19. Effect of solvent selection on the dissolution of solvent-treated griseofulvin and griseofulvin:DMPC coprecipitates (4:1 weight ratio) in pH 2.0 HCL-KCL buffer at 37°C. (n=2, Mean±Range are plotted).

Systems 1 and 2 - griseofulvin treated with methylene chloride and ethanol respectively; systems 3,4 and 5-griseofulvin:DMPC (4:1) coprecipitated with methylene chloride, ethanol, and chloroform, respectively.

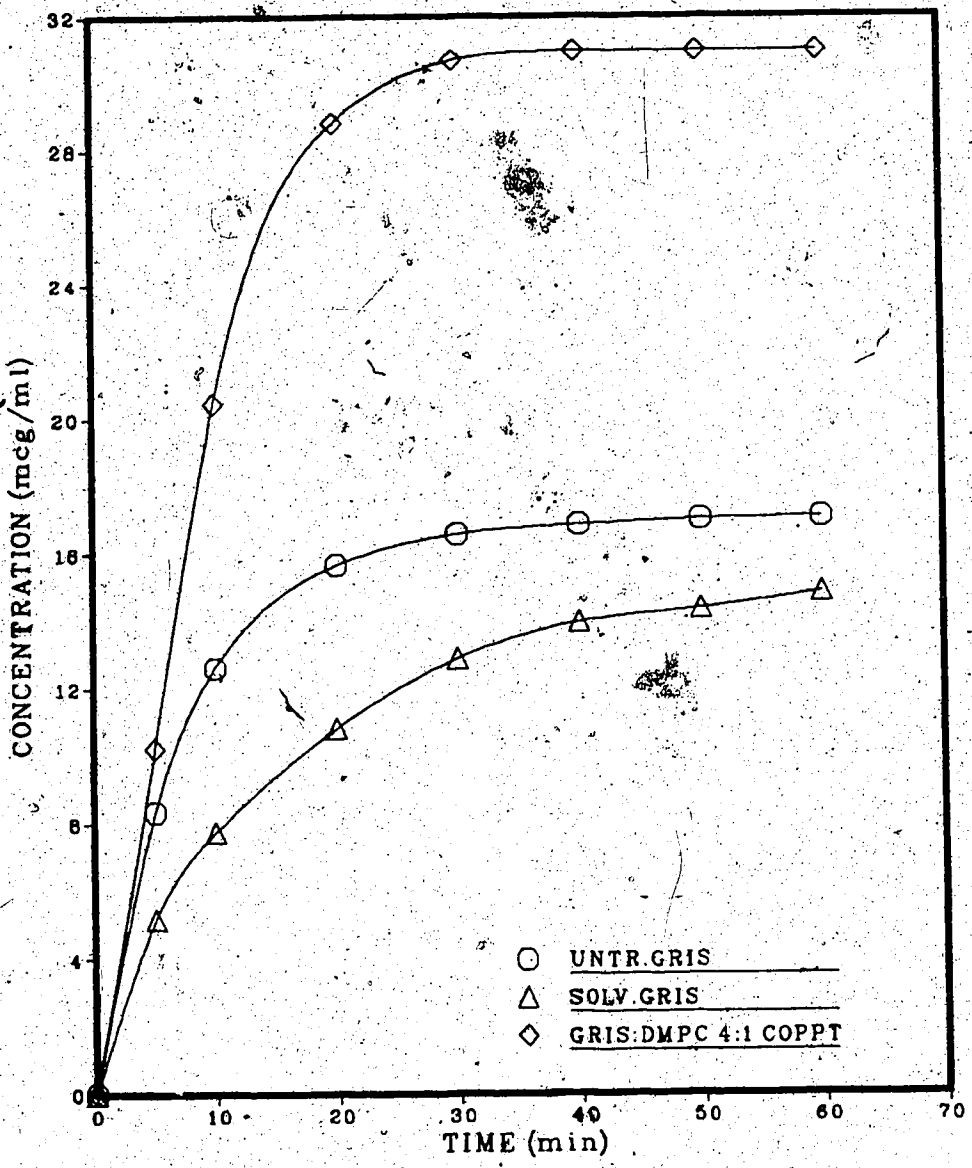

Figure 20. Dissolution profiles of griseofulvin systems in HCl-KCl buffer at pH 2.0 containing 0.1% w/v polysorbate 80 and 37°C. (n=3, Mean±SEM are plotted).

In contrast, the dissolution of griseofulvin:DMPC coprecipitate (4:1) is significantly increased in polysorbate 80 solution compared to buffer solution (cf. Figure 17). Hence, this dissolution improvement of griseofulvin from griseofulvin:DMPC coprecipitates is due to deaggregation and effective surface area increase of very fine particles of griseofulvin during dissolution.

4.1.7 Dissolution of Griseofulvin from Coprecipitates Prepared with Various Phospholipids

The dissolution of griseofulvin from coprecipitates containing either DMPC, DPPC, DSPC, DPPE or EPC is compared in Figures 21 and 22. Generally, increasing the fatty acid chain length of the phospholipid slightly decreased the IDR at 19:1 and 4:1 weight ratios, but also the amount dissolved over the first hour of dissolution was reduced, in some cases to approximately one-half of that obtained employing DMPC. The dissolution profile of DPPC coprecipitates displayed continuously increasing dissolution up to 60 min. unlike the plateauing behavior exhibited by the other coprecipitates.

The dissolution of griseofulvin:EPC coprecipitates was similar to that obtained with griseofulvin:DMPC coprecipitates (Figure 22) but, the combination of griseofulvin and DPPE did not produce any improvement in the dissolution of griseofulvin. This suggests that there is a molecular structure requirement of the phospholipid for

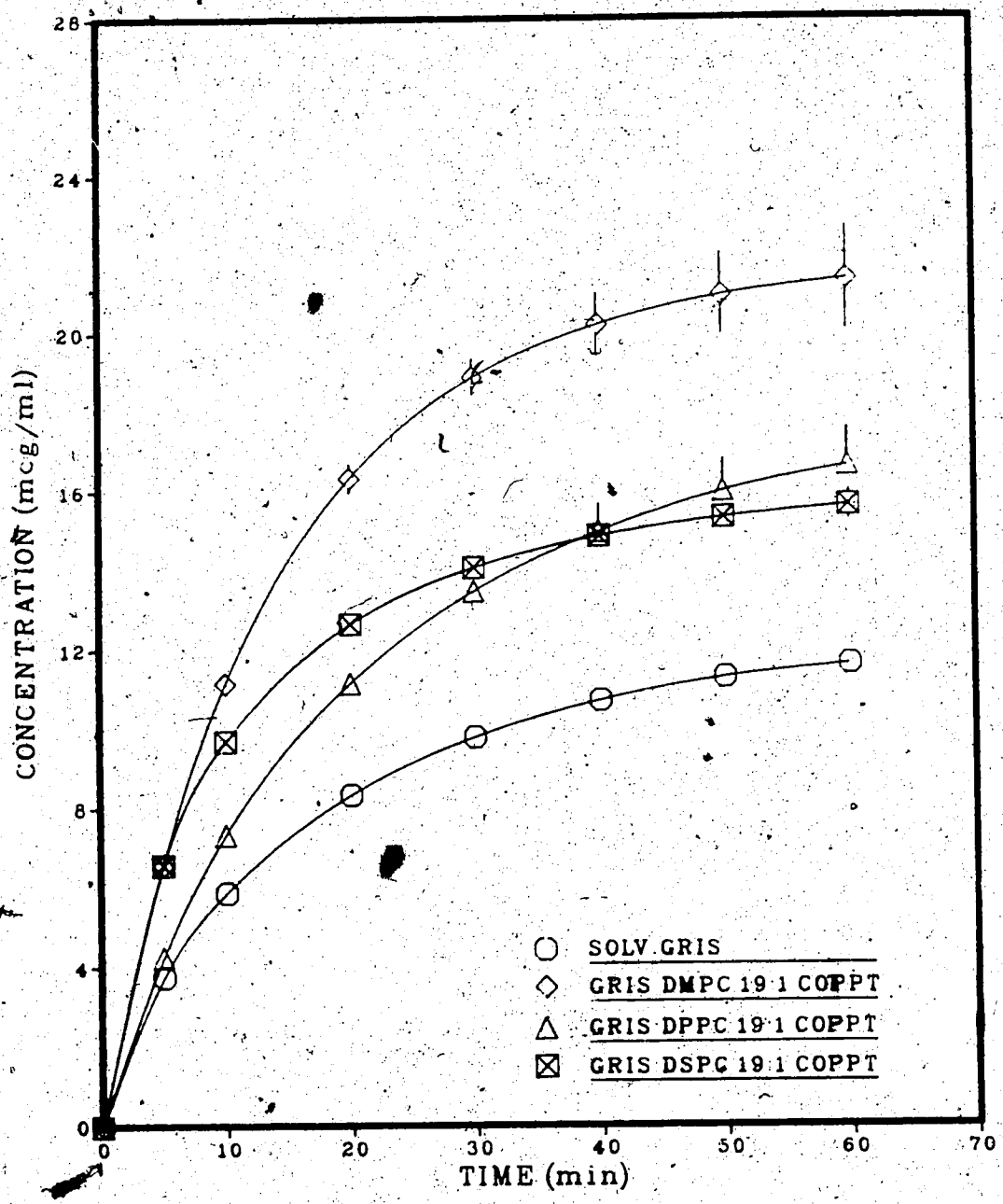

Figure 21. Dissolution of griseofulvin:phospholipid coprecipitates (19:1 weight ratio) at pH 2.0 (HCl-KCl buffer) and 37°C. (n=2, Mean±Range except in solv.gris and DMPC coprecipitate where n=3, Mean±SEM are plotted).

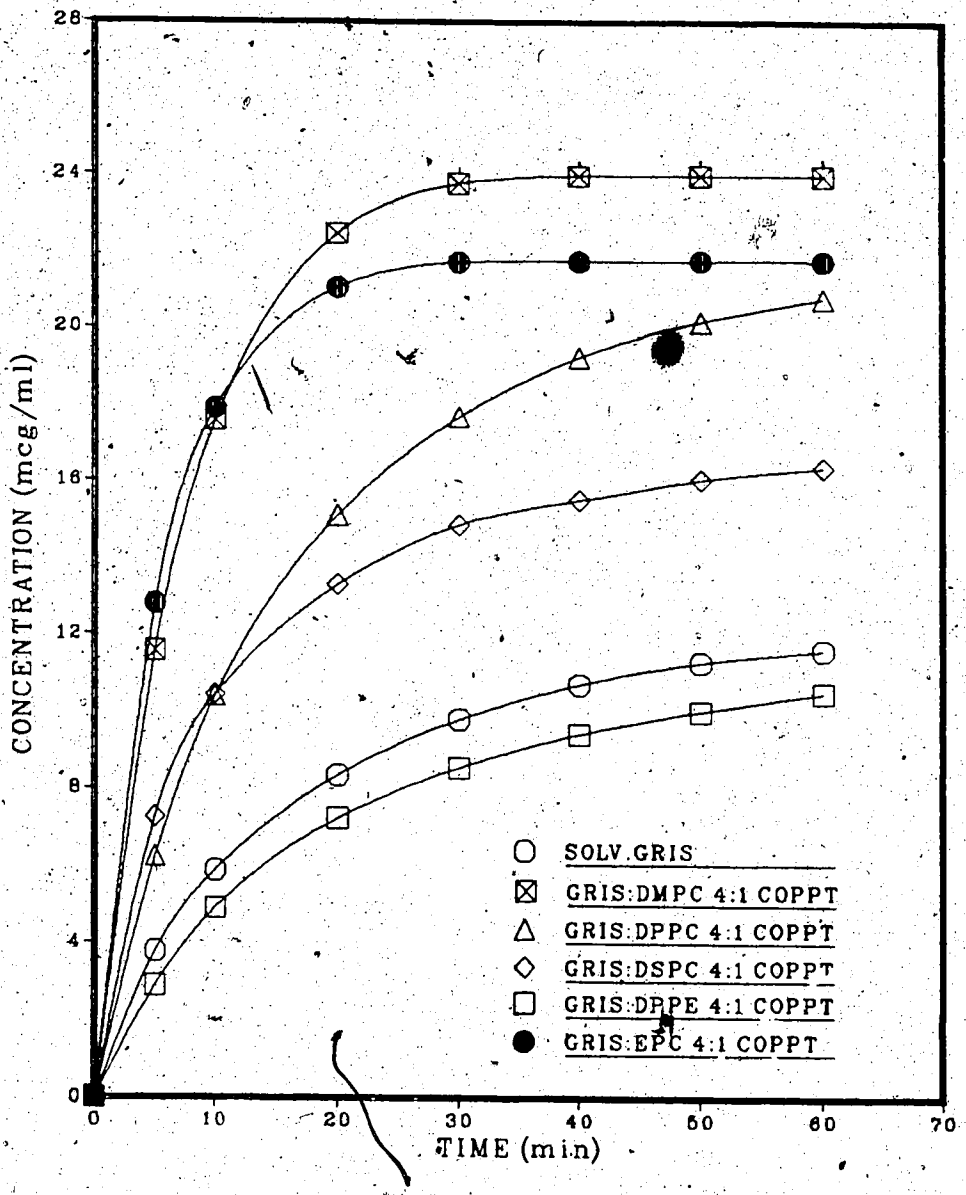

Figure 22. Dissolution of griseofulvin:phospholipid coprecipitates (4:1 weight ratio) at pH 2.0 (HCl-KCl buffer) and 37°C. (n=2, Mean±Range except in solv.gris, DMPC, and DPPE coprecipitates where n=3, Mean±SEM are plotted).

inclusion in the crystal lattice of solvated griseofulvin.

4.1.8 Dissolution of Griseofulvin from Coprecipitates Prepared with Various Combinations of DMPC and other Agents

4.1.8.1 Enhancers of Dissolution as Substitutes for Chloroform

It was observed in section 4.1.6.4 that only chloroform enhanced the dissolution of griseofulvin in coprecipitates with DMPC. Table 9 summarizes the results of twelve attempts at replacing chloroform in the griseofulvin:DMPC crystal lattice with another agent with the aim of still obtaining similar levels of dissolution improvement. Hence, these results further illustrate that the requirement of chloroform is quite specific. None of these attempts was successful. Therefore it is concluded that the coprecipitate crystals formed from the compositions shown do not exist in a high energetic state compared to those prepared from chloroform and are unable to undergo rapid dissolution upon contact with water. Hence, chloroform because of its ability to form a solvate with griseofulvin remains to be the only 'enhancer' of griseofulvin dissolution in the presence of a phospholipid.

4.1.8.2 Griseofulvin:DMPC:Cholesterol

The effect of the addition of CHOL to griseofulvin:DMPC coprecipitates on the dissolution of griseofulvin was

Table 9

Effect of Various Agents as Enhancers of Griseofulvin Dissolution from Griseofulvin:DMPC Coprecipitates.

Griseofulvin: DMPC:Agent (Wt. Ratio)	Agent	Copreci-pitating Solvent[1]	IDR[2] (mcg/mL/min)	Amount Dissolved in 60 min. (mg)
9:0.5:0.5	PEG 400	1	0.35	8.9
"	PEG 4000	1	0.32	8.7
"	PVP	1	0 43	8.9
"	L-cysteine	2	0.33	9.4
"	Chloralose	2	0.41	9.7
"	Succinic acid	2	0.26	8.2
"	Lactose	3	0.35	8.2
4:0.25:0.75	Mannitol	2	0.26	6.7
"	Urea	2	0.19	7.8
"	Deoxycholic Acid	2	0.16	6.5
"	Fumed silica	3	0.50	7.8
4:0.5:0.5	Chloral Hydrate	4	0.036	8.3

[1] 1 = absolute ethanol; 2 = absolute ethanol:acetone (1:4 v/v); 3 = methylene chloride; 4 = absolute ethanol:methylene chloride (1:2 v/v)

[2] Based on the calculated amount released during the first 5 min.

examined in order to determine the role played by CHOL on IDR and dissolution after 60 min. In Figures 23 and 24 it can be seen that CHOL itself slightly increases the IDR but otherwise does not improve the dissolution of solvated griseofulvin. However, it exerts profound changes in the dissolution profiles of coprecipitates which contain DMPC. CHOL in low concentrations has little effect on the IDR but causes dissolution to continue to increase almost at a constant rate and to promote a substantial increase in the amount of griseofulvin dissolved at 60 min. above the amounts obtained when DMPC alone was used in the coprecipitate. Thus, DMPC plays a direct role in augmenting the IDR of griseofulvin through the creation of a fine particle size of drug but also through a solubilizing mechanism which increases the total amount of drug in solution. It appears that CHOL addition contributes to the solubilizing ability of DMPC in the overall dissolution process. Values of IDR, and amount dissolved in 60 min. are compared in Table 10 which shows a consistent pattern at 19:1 and 4:1 weight ratios of the influence of CHOL on dissolution. The action of CHOL to control the release of griseofulvin is more clearly illustrated in Figure 25. The fraction of griseofulvin dissolved passes through a maximum when CHOL is approximately one third of the total lipid content. However, the maximum is broadened and shifted slightly to higher CHOL concentrations when coprecipitates have a greater portion of total lipid.

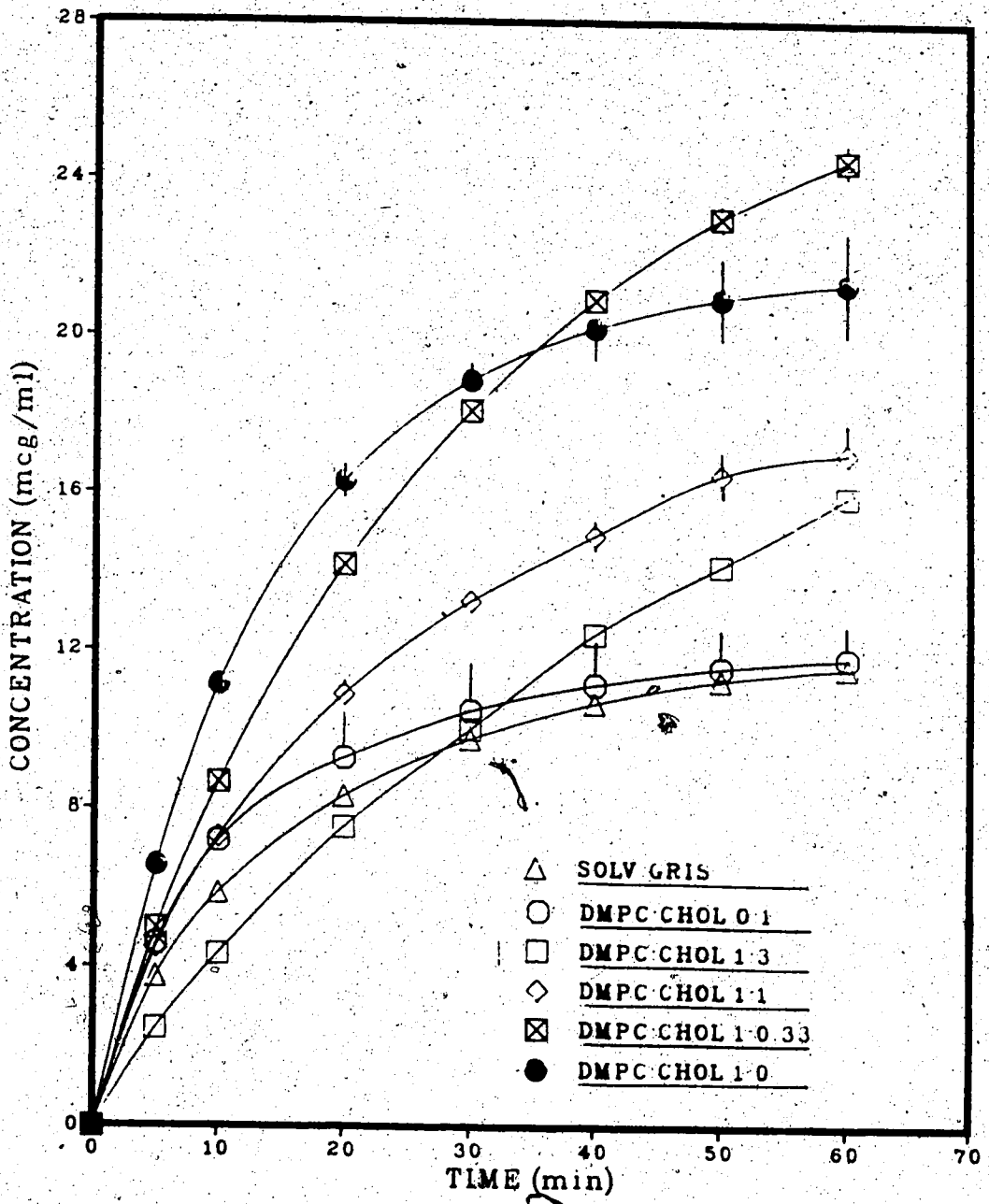

Figure 23. Dissolution of griseofulvin from griseofulvin:DMPC:CHOL coprecipitates at a 19:1 griseofulvin:lipid weight ratio at pH 2.0 (HCl-KCl buffer) and 37°C. DMPC:CHOL combinations are expressed on a mole ratio basis. (n=2, Mean±Range except in solv.gris and DMPC:CHOL(1:0) coprecipitate where n=3, Mean±SEM are plotted).

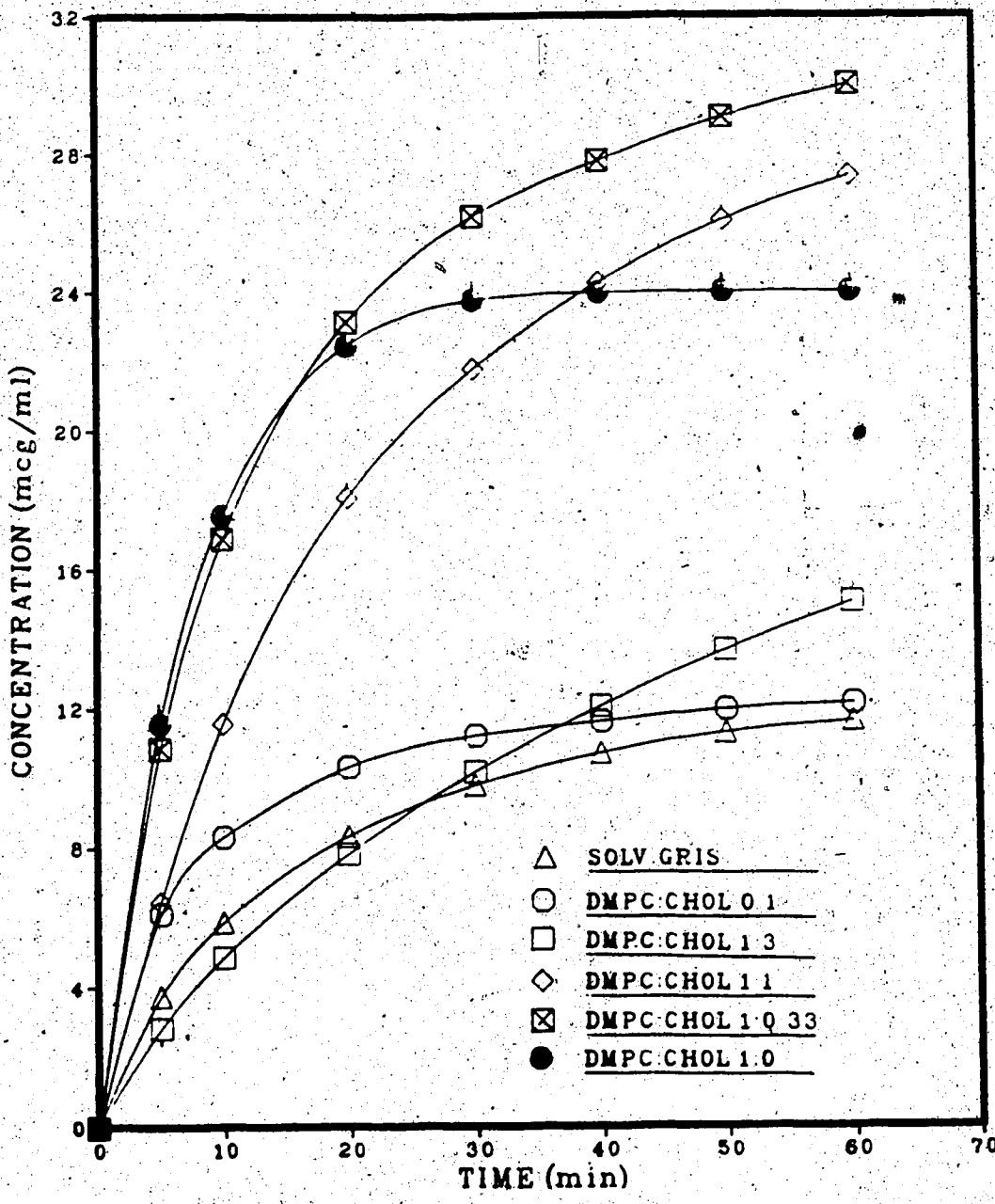

Figure 24. Dissolution of griseofulvin from griseofulvin:DMPC:CHOL coprecipitates at a 4:1 griseofulvin:lipid weight ratio at pH 2.0 (HCl-KCl buffer) and 37°C. DMPC:CHOL combinations are expressed on a mole ratio basis. (n=2, Mean±Range except in solv.gris and DMPC:CHOL(1:0) coprecipitate where n=3, Mean±SEM are plotted).

Table 10

Initial Dissolution Rate and Amount of Griseofulvin Dissolved After 60 min. from Griseofulvin:DMPC:CHOL Coprecipitates[1] in HCl-KCl Buffer at pH 2.0 and 37°C.

Griseofulvin: Lipid (Wt. Ratio)	DMPC:CHOL (Mole Ratio)	IDR' (mcg/mL/min)	Amount Dissolved in 60 min. (mg)
19:1	1.0	1.32±0.02	19.22±0.43
	1:0.33	0.98±0.01	22.04±0.92
	1:1	0.90±0.05	15.34±0.79
	1:3	0.50±0.00	14.36±0.06
	0:1	0.76±0.09	10.67±0.85
4:1	1:0	2.31±0.09	21.59±0.43
	1:0.33	2.17±0.02	27.00±0.96
	1:1	1.28±0.03	24.61±0.81
	1:3	0.62±0.03	13.54±0.52
	0:1	1.22±0.05	10.92±0.22

'Based on the calculated amount released during the first 5 min.

The values represent mean±range, n=2.

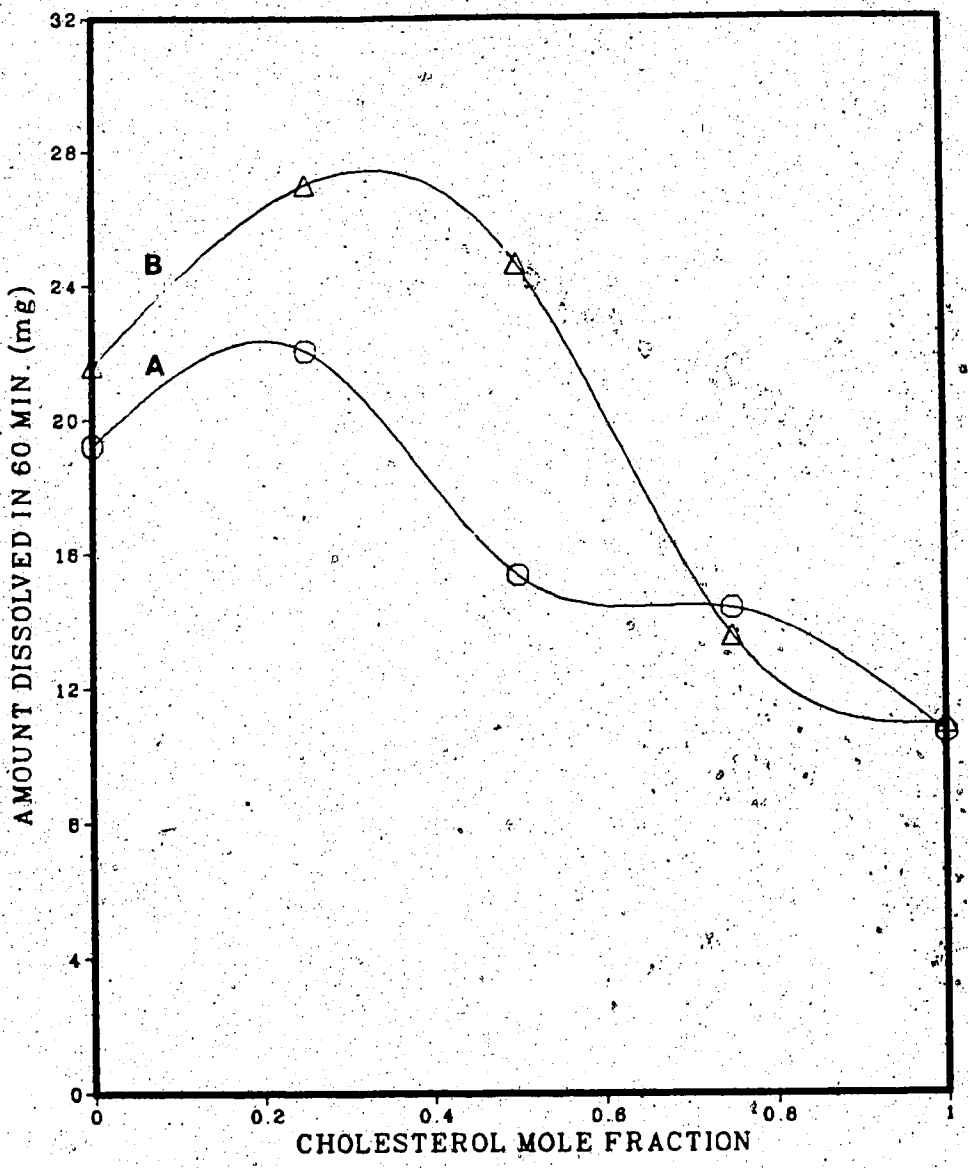

Figure 25. The effect of cholesterol addition on the amount of griseofulvin dissolved from coprecipitates of griseofulvin:DMPC at pH 2.0 (HCl-KCl) buffer and 37°C. A-19:1 and B-4:1 weight ratio of griseofulvin:total lipid.

4.1.8.3 Griseofulvin: DMPC:Stearylamine

The effect of inclusion of stearylamine (SA) as a positively charged lipid in coprecipitates of griseofulvin:DMPC is shown in Figure 26. Generally, DMPC:SA combinations alter the dissolution of griseofulvin in a manner similar to DMPC:CHOL combinations but to a lesser degree. At higher ratios SA retards dissolution similar to CHOL but unlike CHOL dissolution from coprecipitates at lower ratios exhibited plateauing of the dissolution profile similar to that of DMPC.

4.1.8.4 Griseofulvin : DMPC : CHOL : SA and Griseofulvin : DMPC : CHOL : DCP

The effects of a 3-component lipid combination on griseofulvin dissolution is shown in Figure 27 for a 4:1 griseofulvin:lipid weight ratio and a 7:2:1 mole ratio of lipid components. It is apparent that CHOL exerts a predominant behavior in the dissolution profile. Dicetylphosphate (DCP) increases the lipid content of the coprecipitate more than SA because of its two fatty acyl chains and, consequently, reduces the overall dissolution in a manner expected by increasing the CHOL content (see Figure 24).

4.1.9 Dissolution of Griseofulvin Coprecipitates with PEG 4000 or POS

The dissolution of griseofulvin from coprecitates with POS has previously been shown to be better than with PEG

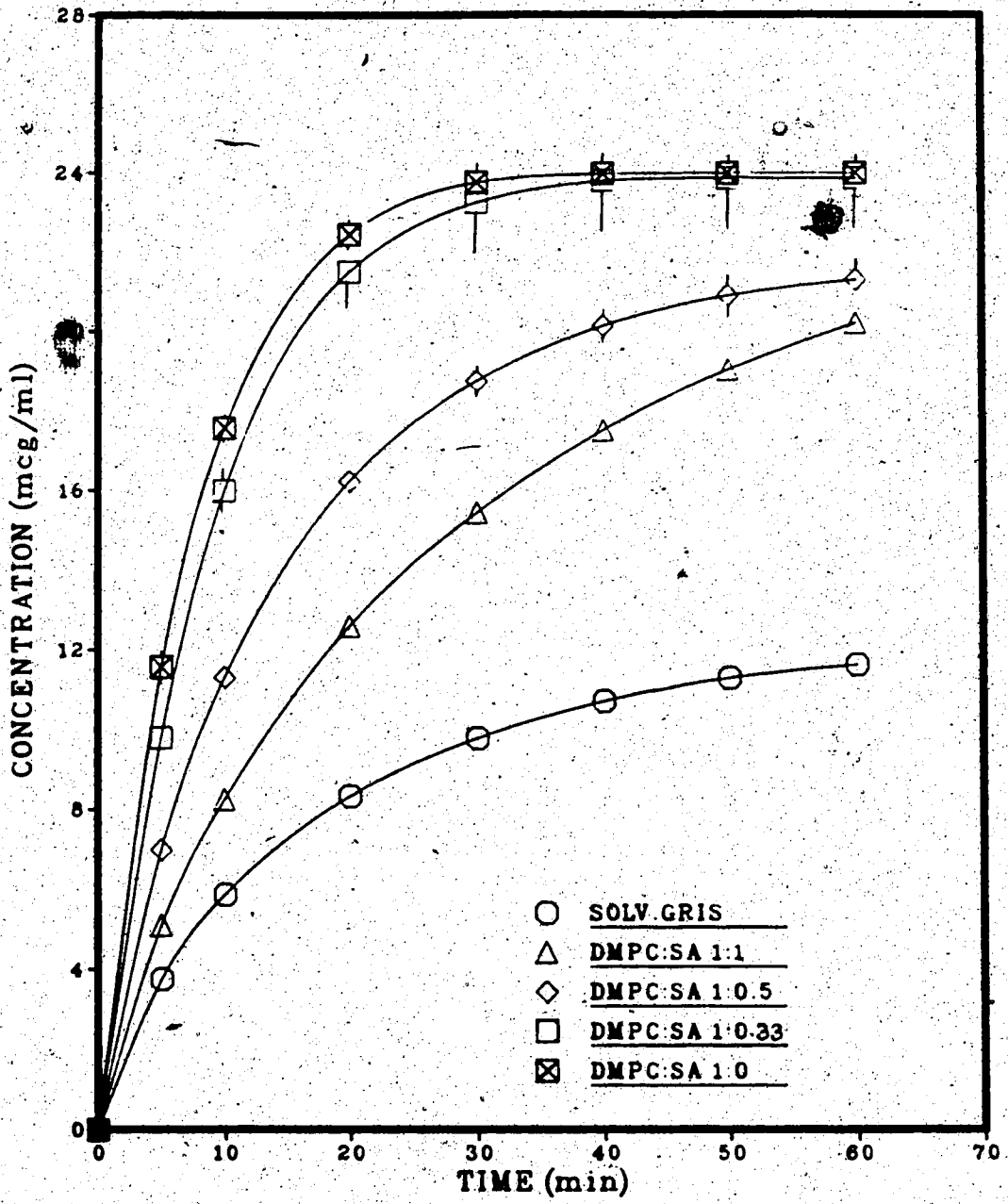

Figure 26. Dissolution of griseofulvin from griseofulvin:DMPC:SA coprecipitates at a 4:1 griseofulvin:lipid weight ratio at pH 2.0 (HCl-KCl buffer) and 37°C. DMPC:SA combinations are expressed on a mole ratio basis. (n=3, Mean±SEM are plotted).

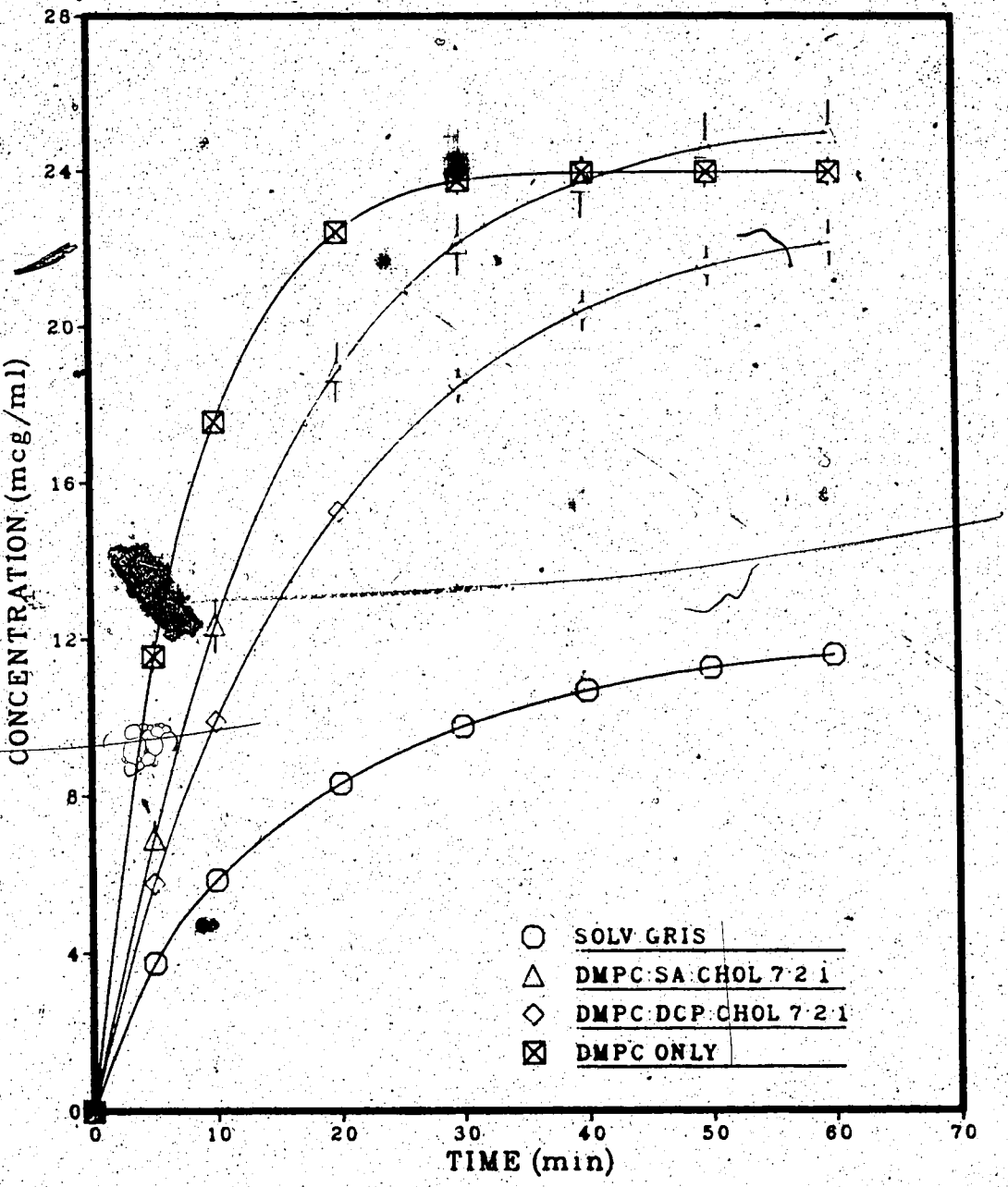

Figure 27. Dissolution of griseofulvin from 4:1 (weight ratio) of griseofulvin:lipid coprecipitates at pH 2.0 (HCL-KCL buffer) and 37°C. Lipid compositions are expressed on a mole ratio. (n=3, Mean±SEM are plotted).

2000 (6). The drug carrier ratios were 0.14:1 and 1.5:1 for each of these carriers and ethanol was used as the coprecipitating solvent. Rapid dissolution of griseofulvin:PEG 4000 solid dispersions has been shown at high concentrations of the carrier at which solid solution of the drug occurs (5). A comparison of the dissolution of griseofulvin from coprecipitates containing either PEG 4000 or POS is shown in Figure 28. It can be seen that inclusion of PEG 4000 at the ratios shown has a negligible effect on the dissolution of griseofulvin. On the other hand, the highly surface active POS (6) substantially increases the dissolution of griseofulvin. It is also noted that the activity of POS in the coprecipitates is higher when chloroform is used as a solvent compared to methylene chloride. Thus, POS may participate in the coprecipitated crystal with griseofulvin and chloroform in a manner similar to DMPC and other phospholipids. In contrast, PEG 4000 does not possess any appreciable surface activity and, hence, is not as effective in increasing dissolution of griseofulvin. The action of POS in the present work is to increase the solubility of griseofulvin (6) in water as well as to increase the effective surface area of the crystallites of griseofulvin in the coprecipitates and, consequently, increase its dissolution.

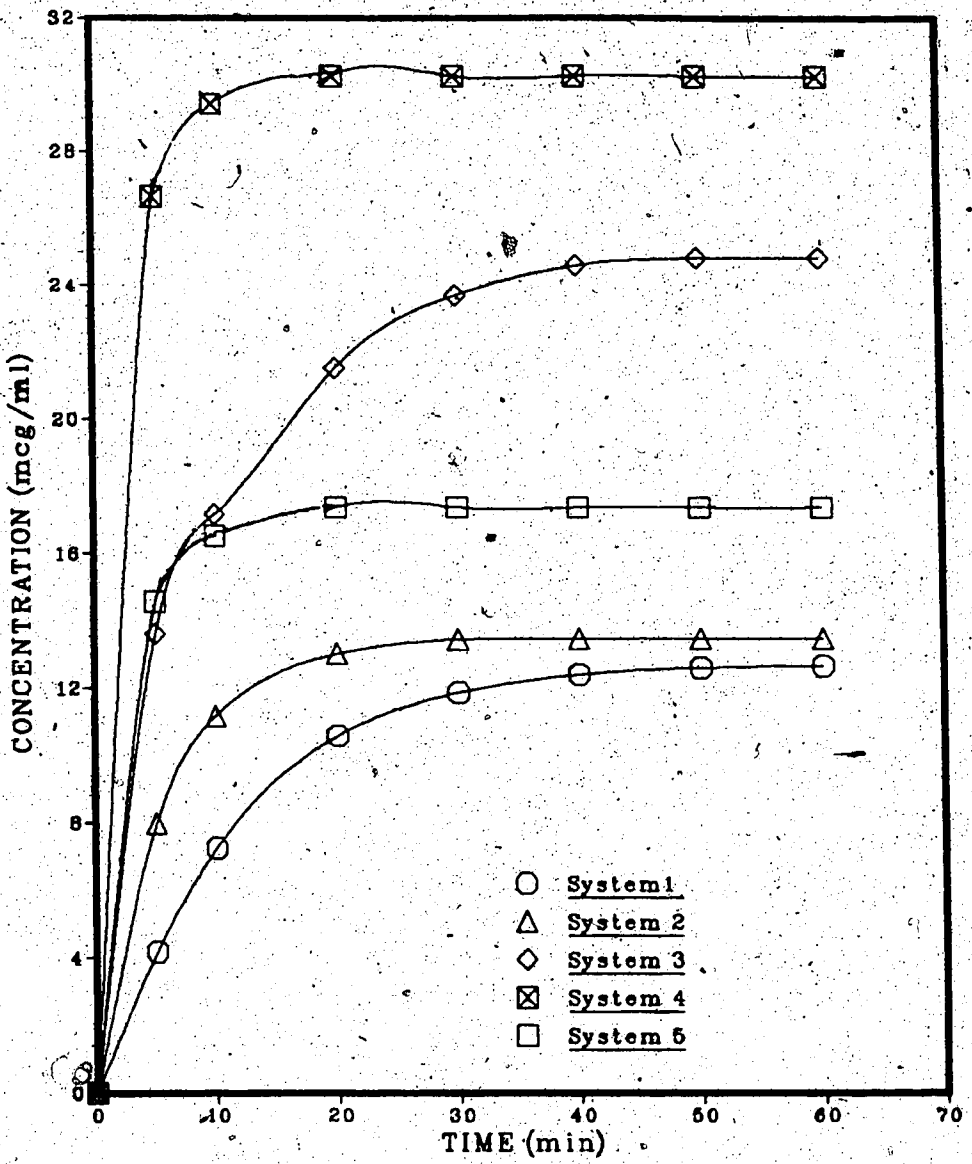

Figure 28. Dissolution of griseofulvin from griseofulvin:PEG 4000 or POS coprecipitates in pH 2.0 (HCl-KCl) buffer at 37°C. (n=2, Mean±Range are plotted). Key: Systems 1 and 2 - Gris:PEG 4000 (19:1 and 4:1 resp.); 3 and 4 Gris:POS (19:1 and 4:1 resp.); and 5 - Gris:POS (4:1) coprecipitated with methylene chloride. Systems 1 to 4 were coprecipitated with chloroform.

4.1.10 Effect of Aging on the Behavior of Griseofulvin:DMPC Coprecipitates

Solid dispersion systems exist in a highly energetic state due to the large surface area of the particles involved and are often shown to be unstable (113). Reduction of energy is brought about within the system by particle aggregation and growth. The consequence of this happening in the griseofulvin:DMPC coprecipitate system and its effect on griseofulvin dissolution has been examined.

A batch of griseofulvin:DMPC coprecipitate (4:1) was tested for dissolution at the end of 10, 30 and 112 days by collecting the required sieve fraction of the sample just prior to the dissolution test. The results are shown in Figure 29. The dissolution rate and extent decreased progressively and dropped to almost the same as that of untreated griseofulvin after aging for 112 days. To verify if the decrease in dissolution was due to repeated handling while sieving, it was necessary to determine the effect of aging on undisturbed samples. Hence, three batches of griseofulvin:DMPC (4:1) coprecipitates were aged for 10, 30, and 112 days. Following which they were tested for dissolution. The results obtained from this study are shown in Figure 30. Even in this case, there is a progressive reduction in the dissolution of griseofulvin. The determination of the weight of a batch of coprecipitate at regular intervals stored under vacuum showed a progressive loss indicating the loss of chloroform. The x-Ray

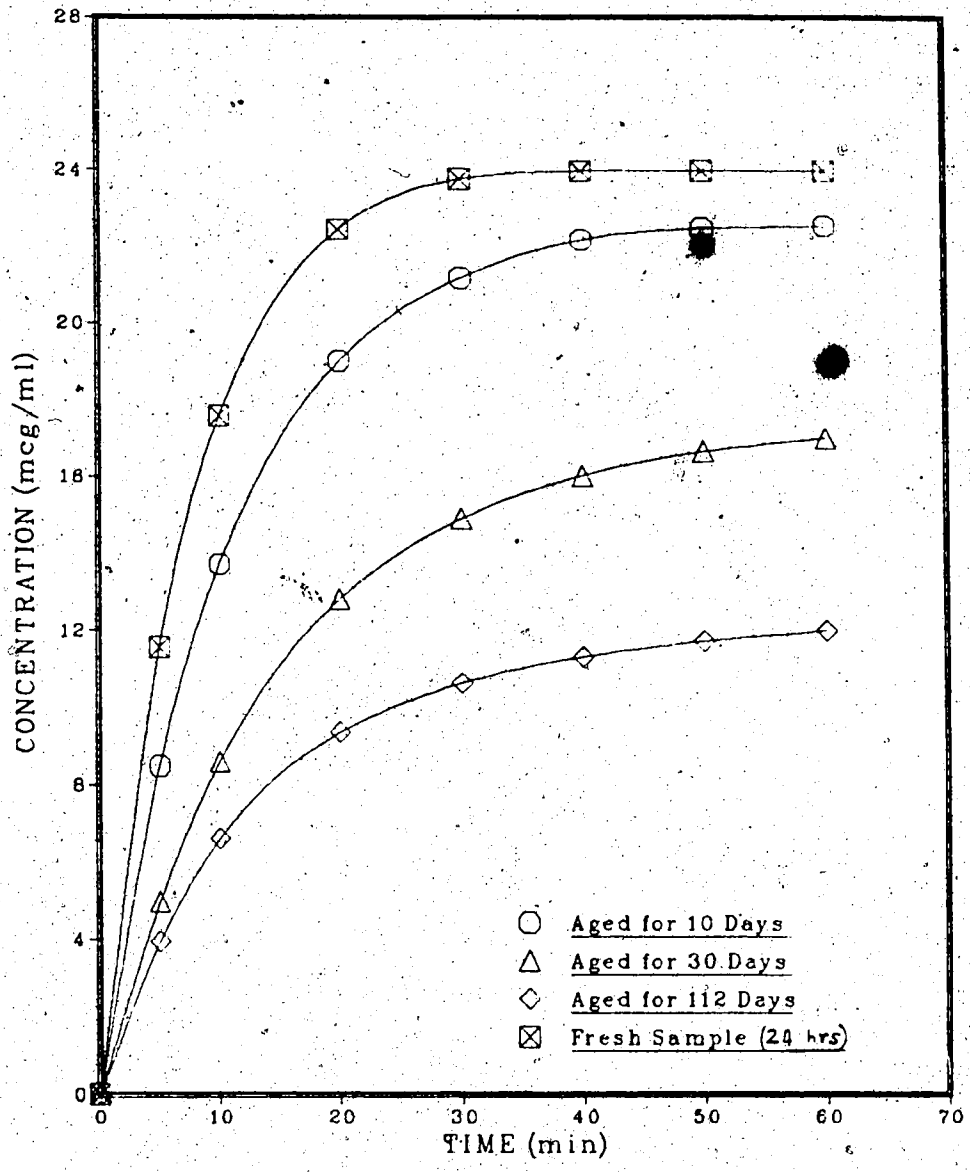

Figure 29. Dissolution of griseofulvin from aged griseofulvin:DMPC (4:1 weight ratio) coprecipitate in pH 2.0 (HCL-KCL) buffer at 37°C.

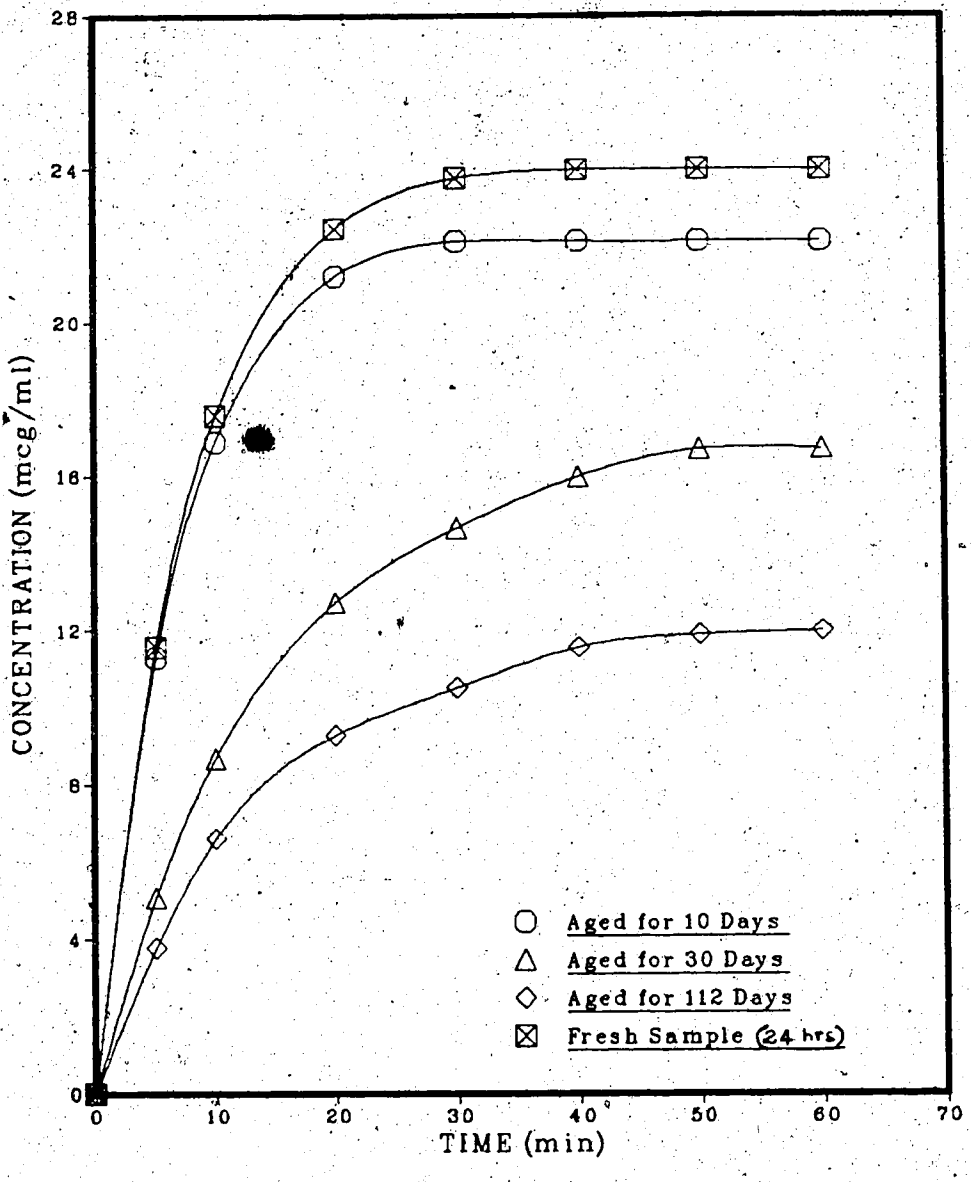

Figure 30. Dissolution of griseofulvin from griseofulvin: DMPC (4:1 weight ratio) allowed to age undisturbed, in pH 2.0 (HCL-KCL) buffer at 37°C.

diffraction spectrum of a coprecipitate left undisturbed for 112 days indicated changes in its diffraction pattern as compared to the spectrum of a fresh sample (Figure 31). The characteristic peaks of untreated griseofulvin appeared in the aged sample (peaks at 10.7, 13.2, 14.8, 16.4, 20.5, 22, 22.5, 23, 23.7, 25.8, and 26.6°) and the general appearance of the spectrum resembled that of untreated griseofulvin rather than solvated griseofulvin. Thus it appears that upon aging solvated-griseofulvin in the coprecipitates is converted to the nonsolvated form leading to a decrease in dissolution.

4.1.11 Kinetic Analysis of Dissolution of the Griseofulvin : DMPC Coprecipitate System Under Sink Conditions

Dissolution rate-limited absorption implies that there is no build-up of drug concentration in the gastrointestinal fluids i.e., the fluids act as a perfect sink. Frequently it is necessary to use exceedingly large volumes of dissolution medium in order to maintain sink conditions (166). Another approach to maintaining sink conditions is to carry out dissolution tests using an amount of sample less than 10 to 20% of its solubility. Accordingly, dissolution tests were conducted using an amount of sample equivalent to 1.5 mg of griseofulvin (170). The dissolution results obtained from the griseofulvin:DMPC coprecipitates are shown in Figure 32. A pronounced increase in the initial dissolution rate of griseofulvin from the coprecipitates was observed as

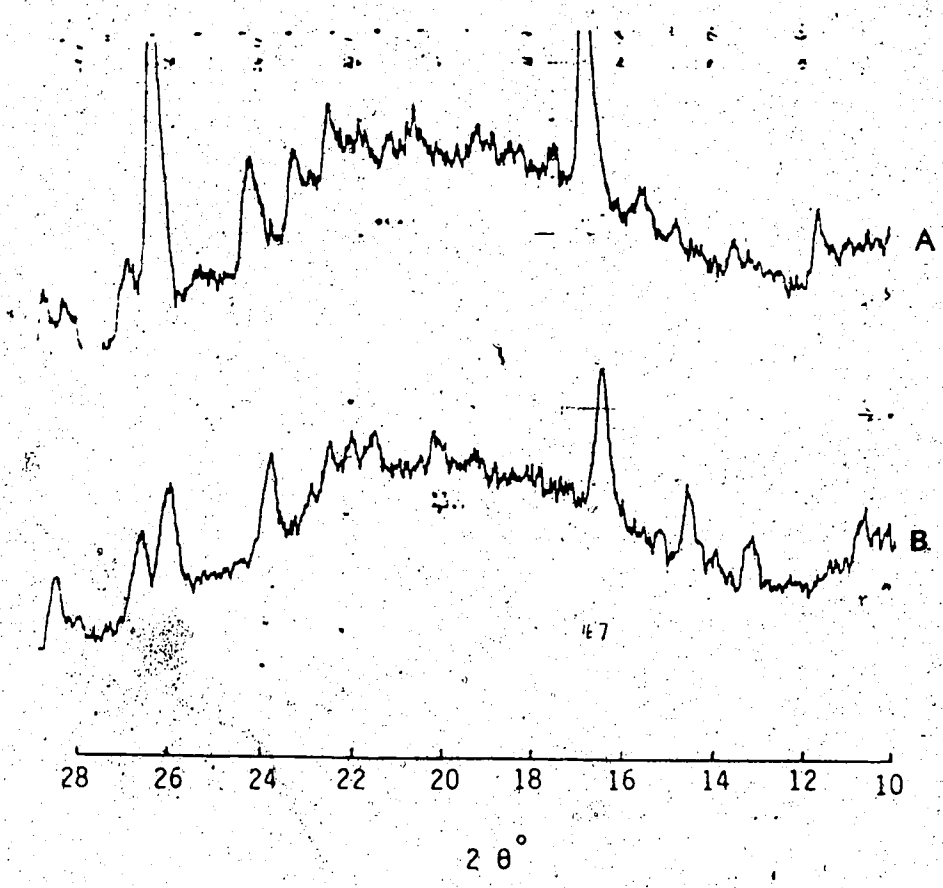

Figure 31. X-Ray diffraction spectrum of a griseofulvin DMPC coprecipitate (4:1), A-Fresh sample, and B-sample aged for 112 days.

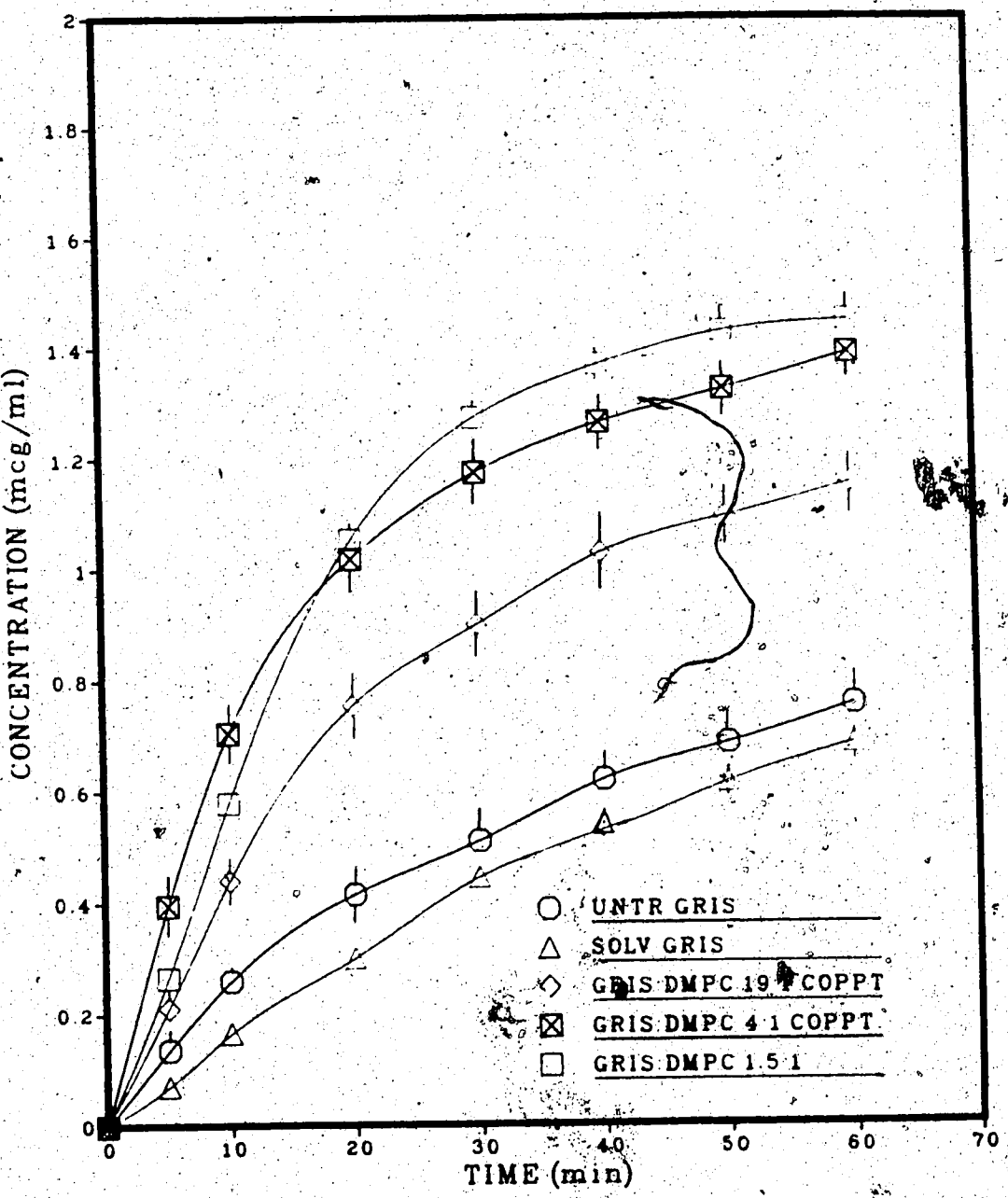

Figure 32. Dissolution Rate profiles of untreated, solvated griseofulvin and its coprecipitates with DMPC under sink conditions in HCl-KCl buffer at pH 2.0 and 37°C. (n=3, Mean±SEM are plotted).

compared to untreated and solvated griseofulvin. Initial screening experiments showed that the presence of polysorbate 80 exerted only a slight wetting effect on the powders. The initial dissolution rates and the percent of griseofulvin in solution at the end of 60 min. are summarized in Table 11. Increases of 1.6-, 2.8-, and 2-fold in the IDR was observed for 19:1, 4:1, and 1.5:1 (griseofulvin:DMPC) coprecipitates, respectively, as compared to untreated griseofulvin. Also, more drug underwent dissolution from all of the coprecipitates at the end of 60 min. as compared to untreated and solvated griseofulvin. Analysis of variance on these systems showed a highly significant F value at the 0.05 level. Further comparison of the formulations using Duncans test revealed that the coprecipitate systems were all significantly different from untreated and solvated griseofulvin at all time points tested for significance, namely 5, 30, and 60 min. The large increase in the dissolution rate from the coprecipitates cannot be explained on the basis of improved wetting by DMPC since in the concentrations employed its surface activity is quite low.

4.1.11.1 First-Order Kinetic Model

According to the first order rate equation (Eq. 18) a plot of log(100% - %Dissolved) versus time is linear. However, as shown in Figure 33, a change in slope of the plots occurs

Table 11

Dissolution of Griseofulvin Formulations Under Sink Conditions at pH 2,0 and 37°C.

Formulation*	IDR' (mcg/mL/min)	Amount Released After 60 min. (%)
1	0.027±0.007	45.1±3.6
2	0.015±0.003	41.5±1.7
3	0.043±0.006	69.3±3.1
4	0.078±0.010	83.7±2.8
5	0.054±0.004	87.3±2.8

* 1, untreated griseofulvin; 2, solvated griseofulvin; 3, 4, and 5, griseofulvin:DMPC coprecipitates 19:1, 4:1, and 1.5:1 respectively.

'Based on the calculated amount released during the first 5 min.

The values represent mean±SEM, n=3.

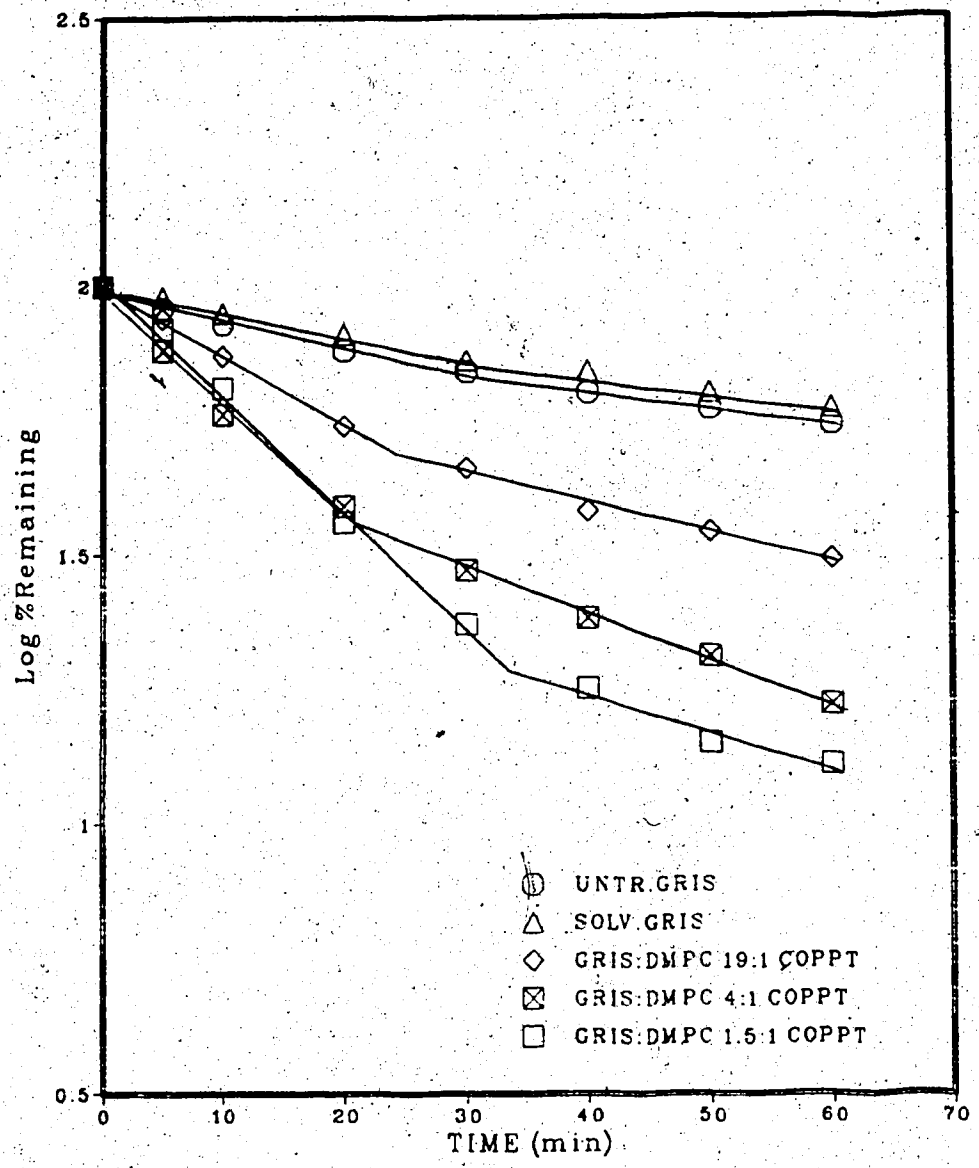

Figure 33. Dissolution of griseofulvin formulations in aqueous buffer under sink conditions plotted according to first-order kinetic model.

between 23.0 and 35.0 min. during the dissolution test. The values of K determined from the slopes of these two curves (K' and K''), and T'_{40}, T''_{40} (time taken for 40% dissolution of drug) calculated from

$$T_{40} = 0.511/K$$

for rate constants (K' and K'' respectively) are summarized in Table 12. A 2-, 4-, and 5-fold increase in K' is observed with the 19:1, 4:1, and 1.5:1 griseofulvin:DMPC coprecipitates as compared to solvated griseofulvin. The increases in K' are also reflected in corresponding decreases in T'_{40} values. However, the difference between solvated griseofulvin and coprecipitates decreases toward the end of the dissolution test period, as noted from the K" and T''_{40} values.

4.1.11.2 Second Order Dissolution Kinetics

A plot of $W/W_e(W_e-W)$ against t according to Eq. 19 is shown in Figure 34. The apparent rate constants and other parameters derived from these data are shown in Table 13. Regression analysis of the yielded correlation coefficients greater than or equal to 0.99 in all cases.

The time taken for 40% of the drug to dissolve, T_{40}, is calculated from the equation

$$T_{40} = 0.444/K_2$$

Table 12

Evaluation of the Dissolution of Griseofulvin Formulations Under Sink Conditions According to First-Order Kinetics.

Formulation*	K' (min.$^{-1}$)	T'_{40} (min.)	K'' (min.$^{-1}$)	T''_{40} (min.)	T_{40} (min.)
1	0.0111	46.0	0.0068	75.1	47.3
2	0.0105	48.7	0.0075	68.1	56.3
3	0.0258	19.8	0.0109	46.9	16.8
4	0.0376	13.6	0.0186	27.5	9.5
5	0.0516	9.9	0.0157	32.5	11.5

K' is the apparent first order rate constant determined between 5 and 30 min. and K'' determined between 40 and 60 min. from the slopes of the lines in Figure 33 in which the means of three dissolution determinations were plotted.

T'_{40} and T''_{40} are the times for 40% dissolution calculated using K' and K'' respectively.

T_{40} is the time for 40% dissolution determined experimentally.

* = Key same as in Table 11.

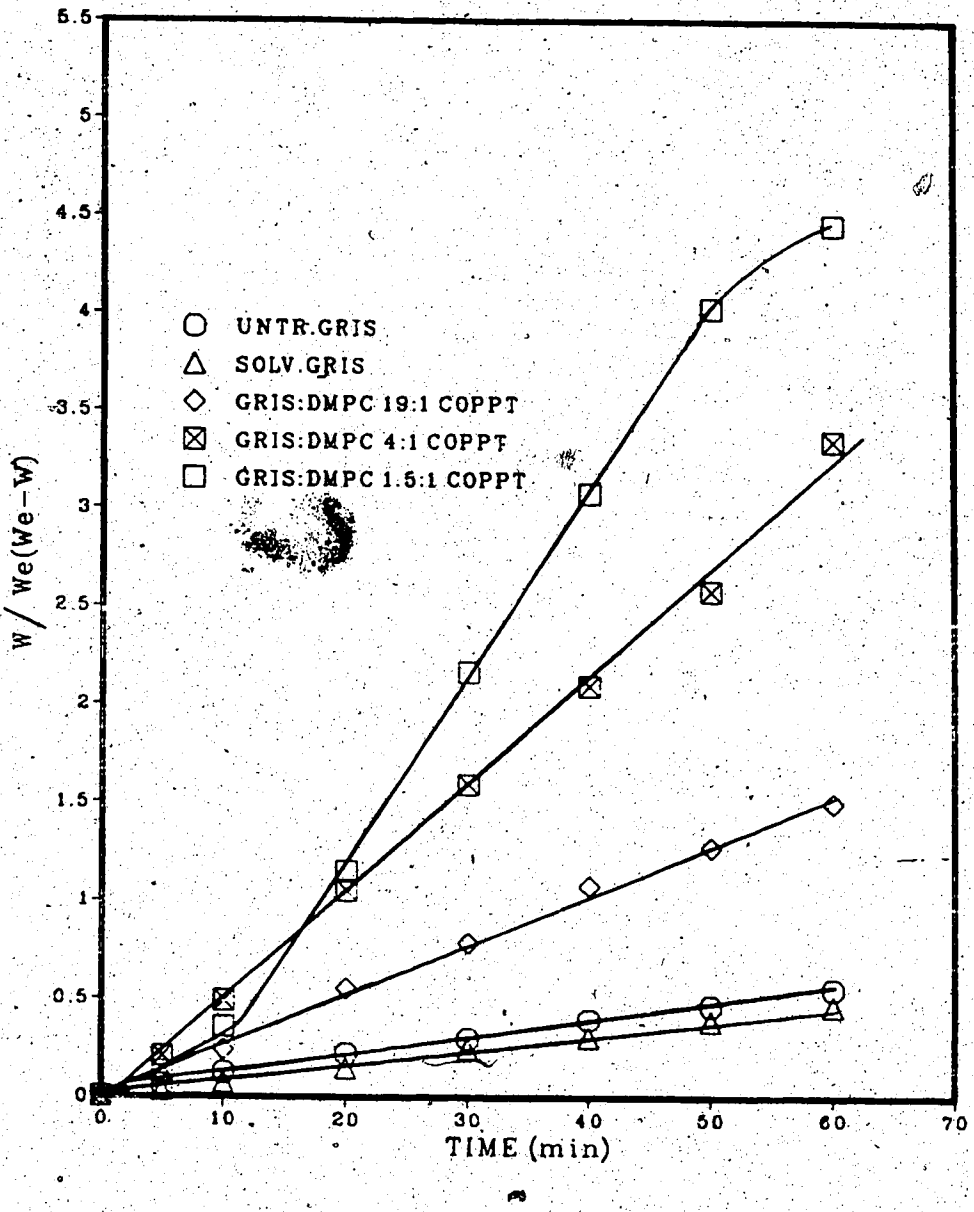

Figure 34. Dissolution of griseofulvin formulations under sink conditions plotted according to second-order kinetics.

Table 13

Evaluation of the Dissolution of Griseofulvin Formulations Under Sink Conditions According to Second-Order Kinetics.

Formulation*	K_2 (mg.$^{-1}$min.)	Calculated T_{40}(min)	Observed T_{40}(min)
1	0.0088	50.5	47.3
2	0.0079	56.2	56.3
3	0.0256	17.3	16.8
4	0.0553	8.0	9.5
5	0.0842	5.3	11.5

* Key same as Table 11.

K_2 is the apparent second order dissolution rate constant obtained from the slopes of the lines in figure 34 in which the means of three dissolution determinations were plotted.

T_{40} is the time taken for 40% of the drug to dissolve .

As shown in Table 13, there is approximately a 3-, 7-, and 10-fold increase in the second-order dissolution rate constant and corresponding decreases in the calculated values of $T_{.0}$ with the 19:1, 4:1, and 1.5:1 griseofulvin:DMPC coprecipitates as compared to solvated griseofulvin.

4.1.11.3 Weibull Distribution Function

The dissolution of griseofulvin formulations examined kinetically by the Weibull distribution equation (Eq. 14) is described in Figure 35 and Table 14. An auxillary ordinate scale on the right hand side of Figure 35 has been added in order to be able to calculate the time required for any given percent of drug to dissolve. Table 14 contains the data derived from the various dissolution parameters as defined by Eq. 14. The values of the shape parameter b are all <1 indicating that the curves have a steeper slope than is consistent with exponential behavior. The $T_{.0}$ and T_d values are much smaller than those of untreated and solvated griseofulvin.

4.1.12 Dissolution of a Tablet Formulation of Griseofulvin : DMPC Coprecipitate

The dissolution of griseofulvin from tablets containing untreated griseofulvin, and griseofulvin:DMPC (4:1) coprecipitates is shown in Figure 36. It is apparent that the dissolution behavior from tablets differs from the powder and that tablet hardness is critical. In contrast to

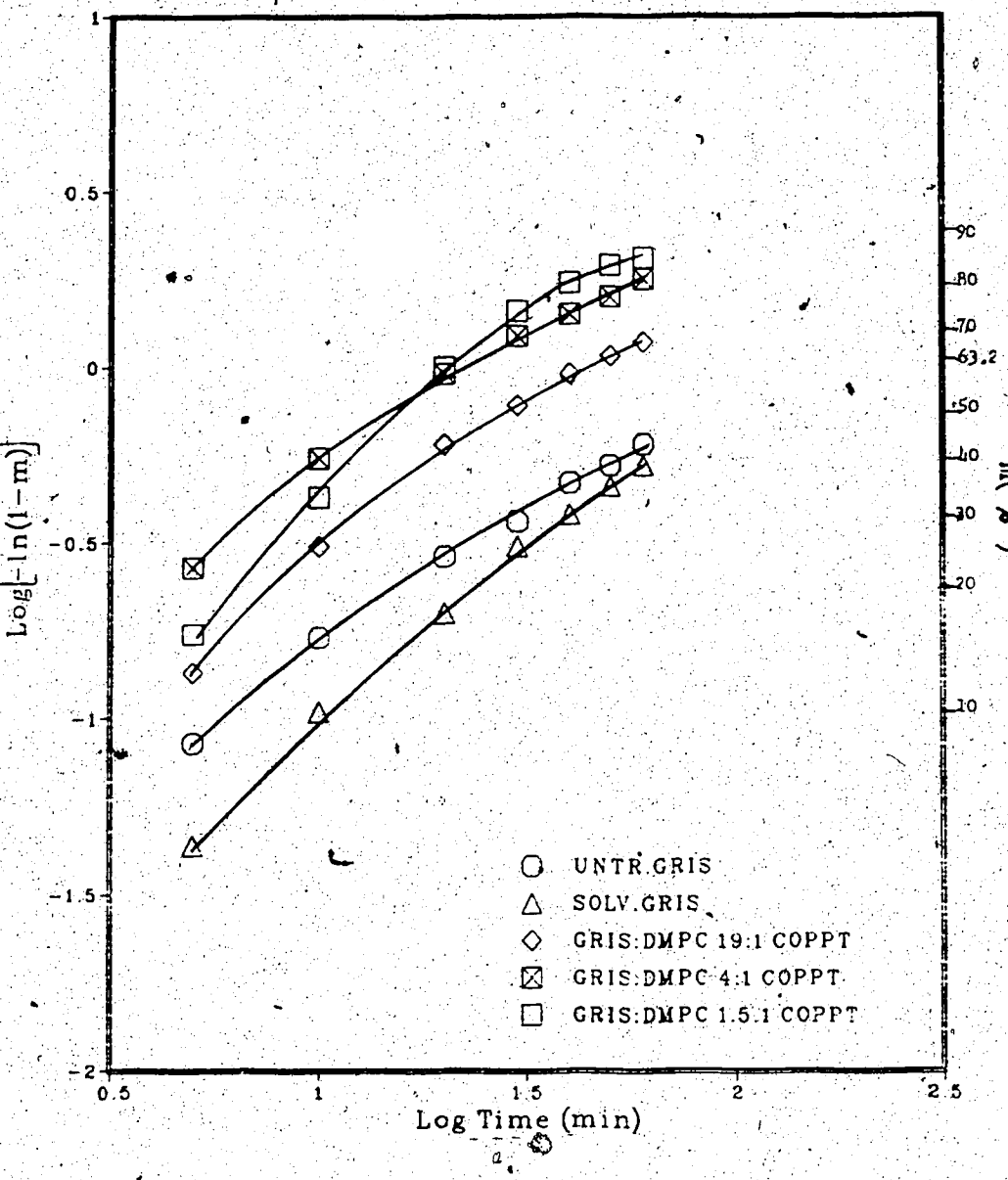

Figure 35. Dissolution of griseofulvin formulations under sink conditions plotted according to the Weibull Function.

Table 14

Evaluation of the Dissolution of Griseofulvin Formulations Under Sink Conditions According to the Weibull Distribution Function.

Formulation*	log a	b	T_{40} (min.)		Td (min.)	
			Pred.	Obs.	Pred.	Obs.
1	1.57	0.77	49.0	47.3	109.9	
2	2.00	0.99	58.9	56.3	104.1	
3	1.41	0.86	16.6	16.8	43.1	33.2
4	1.04	0.74	9.7	9.5	24.9	16.8
5	1.40	0.99	11.9	11.5	25.9	17.3

* Key same as Table 11.

T_{40} is the time taken for 40% dissolution.

Td is the time taken for 63.2% dissolution.

The values were obtained from Figure 35 in which the means of three dissolution determinations were plotted.

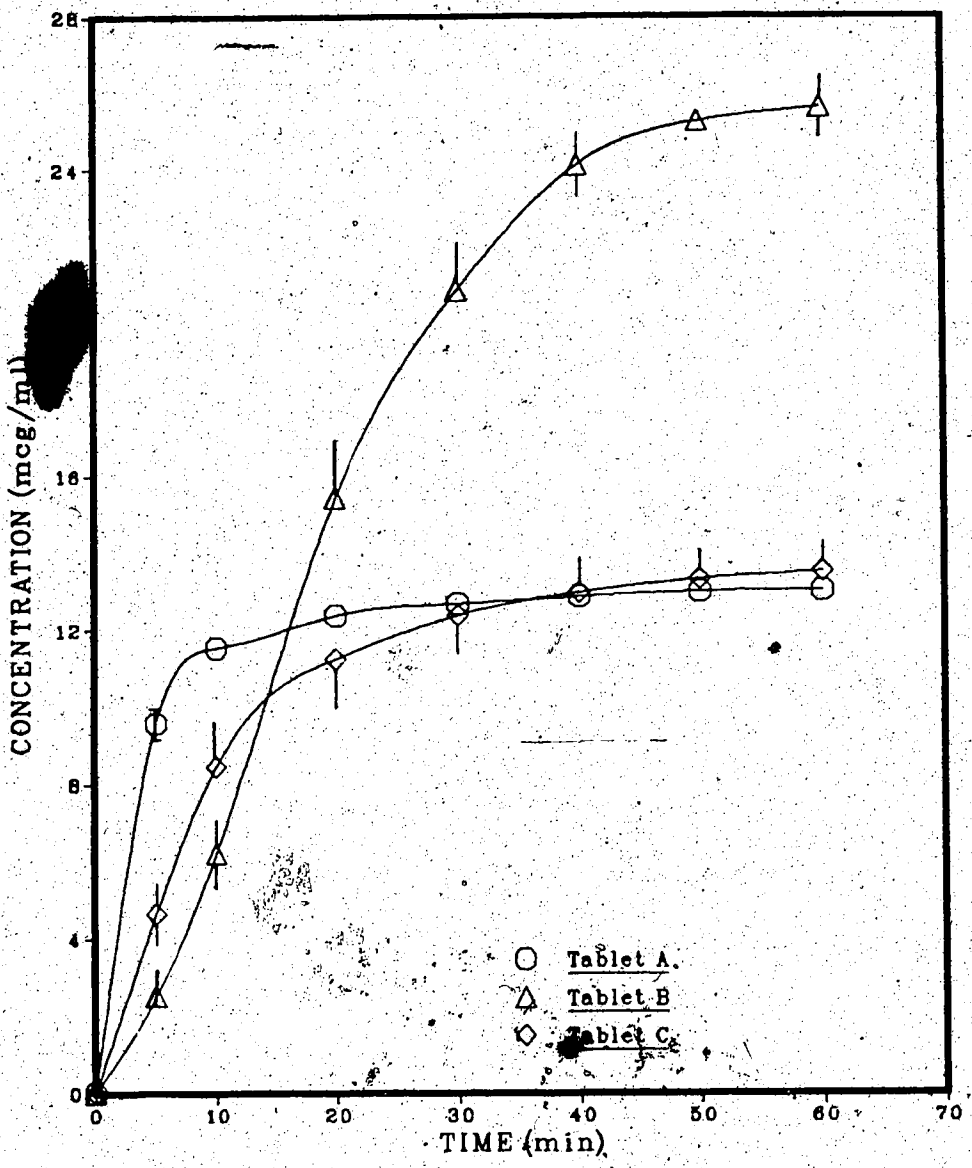

Figure 36. Dissolution of griseofulvin from tablets containing 50 mg equivalent of griseofulvin in HCl-KCl buffer, pH 2.0 and 37°C.(n=2, Mean±Range are plotted). Key-Tablet A, untreated griseofulvin (5.5 kg hardness); B, and C, griseofulvin:DMPC (4:1 weight ratio) coprecipitates (3.5 and 5.5 kg hardness respectively).

the powder, IDR is greatest from tablets of micronized griseofulvin of equal compressions (5.5 Kg hardness) but at a lower compression (3.5 Kg hardness) IDR is relatively slow but it continues at a nearly constant rate for about 30 min. before leveling off. In this respect, the tablet and powder formulations of griseofulvin:DMPC coprecipitates behaved in a similar fashion. The disintegration times of all tablets was found to be 30 seconds to 1 min. The placebo tablets showed a very small variation in the weights (2.1%) and also hardness (1.75%).

4.1.13 Hydrocortisone acetate (HCA):DMPC Coprecipitates

4.1.13.1 UV Analysis

A linear calibration curve which obeyed Beers law over the concentration range of 1 to 20 mcg/mL was obtained for HCA and is shown in Figure 37. Regression analysis of the experimental points yielded slope = 0.0357 intercept = 0.0046 and correlation coefficient, r = 0.9998.

4.1.14 Dissolution Behavior

HCA is reported to form a solvate with dimethylformamide (DMF) (171). The dissolution of untreated HCA was found to be lower than that of HCA-DMF solvate as shown in Figure 38. HCA:DMPC (4:1) coprecipitate from chloroform did not differ in its dissolution properties as compared to untreated powder. However, HCA:DMPC (4:1) coprecipitated from DMF:chloroform showed an increase in

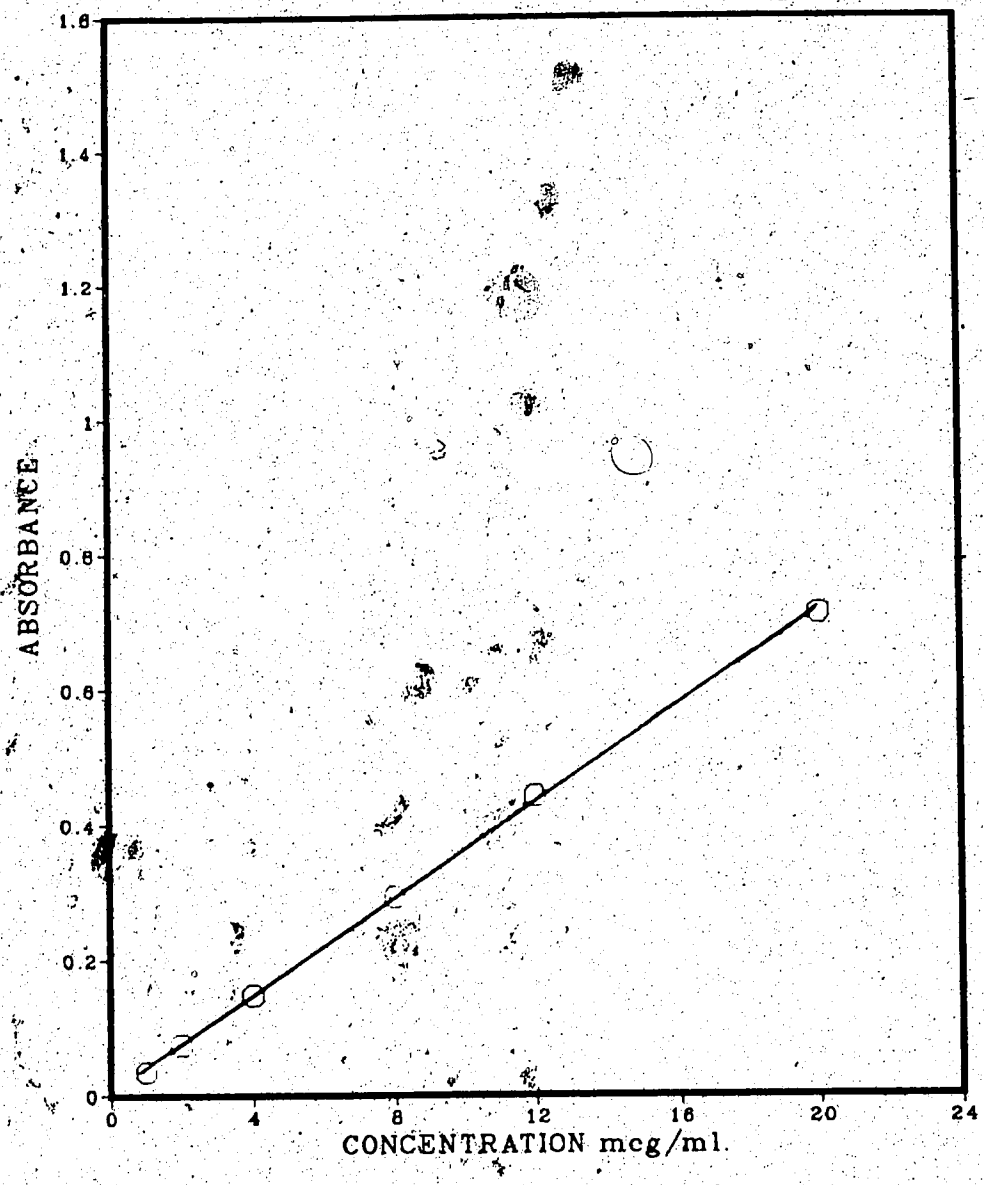

Figure 37. Beers Plot for the quantitation of hydrocortisone acetate in deionizecd distilled water at pH 5.0 (λ_{max}= 242 nm).

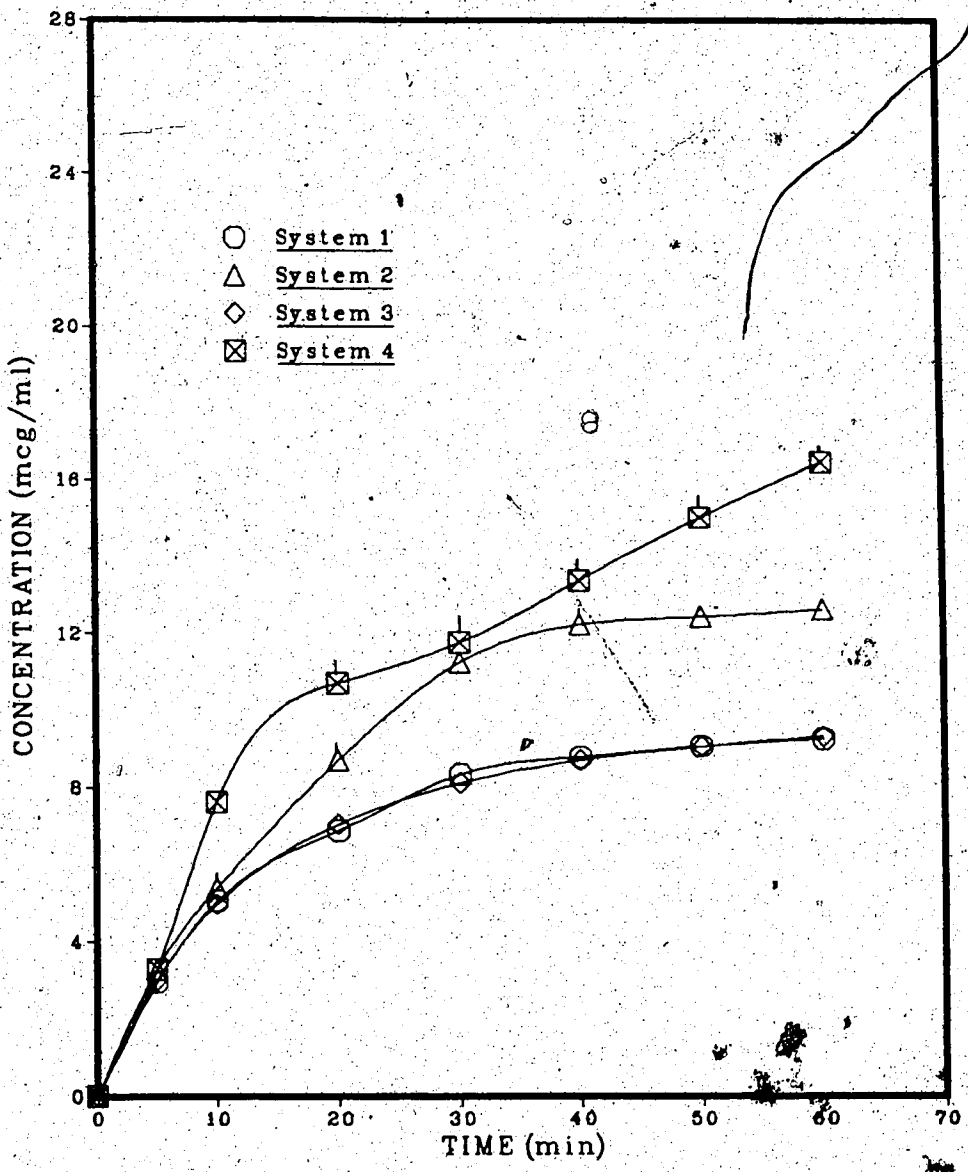

Figure 38. Dissolution of hydrocortisone acetate in deionized distilled water pH 5.0, at 37°C. System 1-untreated; 2-DMF-treated; 3, and 4 HCA:DMPC(4:1 weight ratios) coprecipitated with chloroform and DMF:chloroform (2:1 v/v), respectively. (n=2, Mean±Range are plotted).

dissolution as compared to both the solvated and untreated forms of the drug. Furthermore, the curve did not plateau at 60 min. The concentration reached at the end of 60 min. was greater than the reported solubility of HCA in water (10 mg/L) (172).

4.2 IN-VIVO STUDIES

4.2.1 HPLC Analysis of Griseofulvin in Rat Plasma

4.2.1.1 Determination of a Calibration Curve

A two - point calibration curve using the internal standard method was constructed by injecting blank plasma samples spiked with solutions of griseofulvin and m-phenylphenol (internal standard) in acetonitrile. The two samples used for injection contained 1.0 and 2.0 mcg/mL of griseofulvin. Both samples contained 2.0 mcg/mL of the internal standard. The ratios of the peak areas of griseofulvin to internal standard for the two samples are shown in Table 15. Using these ratios, the integrator calculated and printed the response factor which was retained in memory. The coefficient of variation of the peak area ratios was 2.78% and 1.66%, respectively.

The linearity of the relationship between peak area ratios and griseofulvin concentration was determined as follows: The ratios of the peak areas of plasma samples spiked with varying concentrations of griseofulvin (0.3 mcg/mL to 6.0 mcg/mL) and a fixed concentration of internal

Table 15

Precision of a Two-Point Calibration Curve for HPLC Determination of Griseofulvin in Plasma Samples.

Injection #	Peak Area Ratios	
	1st Point[a]	2nd Point[b]
1	0.8447	1.7793
2	0.8730	1.7589
3	0.8925	1.8174
Mean±SEM	0.8701±0.014	1.7852±0.017

[a] 1 mcg/mL griseofulvin, 2 mcg/mL m-phenylphenol (internal standard).

[b] 2 mcg/mL griseofulvin, 2 mcg/mL m-phenylphenol (internal standard).

standard (2 mcg/mL) were obtained by injecting in triplicate, and averaging the results. Regression analysis of peak area ratios versus griseofulvin concentration yielded a correlation coefficient of 0.9999.

4.2.1.2 Verification of the Calibration Curve

Further confirmation of the calibration curve using plasma samples spiked with known concentrations of griseofulvin and the internal standard in acetonitrile are given in Table 16. Agreement between the theoretical and observed concentrations, was greatest at the higher concentration (-0.9%) and somewhat less at the lower concentrations (3.28% and -3.04%). The detection limit was 0.05 mcg/mL. Hence, the method was considered to be sufficiently accurate for the quantitation of griseofulvin in plasma samples.

Acetonitrile was found to be better than ethanol for deproteinization since the latter yielded a supernatant containing very fine particulate matter, an observation also reported by others (149). Acetonitrile is the solvent of choice in the HPLC analysis of griseofulvin in plasma samples as well as for a number of other drugs.

4.2.1.3 Analysis of Rat Plasma Samples

A typical chromatogram obtained following HPLC injection of the supernatant of a deproteinized blank plasma sample is shown in Figure 39. The blank plasma does not show any peak in the region where griseofulvin and internal

Table 16

Verification of the Two-Point Calibration Curve for HPLC Determination of Griseofulvin in Plasma Samples.

Theoretical Griseofulvin Concentration (mcg/mL)	Observed Griseofulvin Concentration (mcg/mL)	Mean±SEM Observed Conc. (mcg/mL)	Percent Deviation
6.00	5.7830		
6.00	5.8122	5.8454±0.050	-2.58
6.00	6.0048		
2.00	2.0205		
2.00	1.9447	1.9827±0.016	-0.9
2.00	1.9920		
2.00	1.9735		
1.00	1.0102		
1.00	1.0145	1.0269±0.018	+2.69
1.00	1.0018		
1.00	1.0810		
0.50	0.5192		
0.50	0.5232	0.5164±0.005	+3.28
0.50	0.5068		
0.25	0.2352		
0.25	0.2740	0.2424±0.007	-3.04
0.25	0.2451		
0.25	0.2362		
0.25	0.2454		
0.25	0.2183		

standard appear (3.5 and 5.0 min. respectively), suggesting no possible interference in the quantitation of griseofulvin. A chromatogram obtained following the injection of the supernatant of a blank plasma sample spiked with griseofulvin, 4-demethyl griseofulvin (4-DMG), and the internal standard in acetonitrile is shown in Figure 40. The peaks at 2.4, 3.5, and 5.0 min. were identified as those of 4-DMG, griseofulvin, and internal standard, respectively. Good base line separation of the griseofulvin and internal standard peaks was obtained (151).

A typical chromatogram obtained from the analysis of the supernatant of deproteinized rat plasma sample obtained from a rat which had been dosed orally with an aqueous suspension of griseofulvin is shown in Figure 41. Griseofulvin is metabolized to 4- and 6-demethylgriseofulvin in the rat. However, 4-DMG is excreted as a glucuronide conjugate and 6-DMG is in the free form (29). In reverse phase (RP) chromatography 4 DMG-glucuronide was not retained by the 15 cm column. Separation of 6 DMG and 4 DMG was achieved by RP chromatography using a 30 cm column. The retention time of 6-DMG was lower than that of 4-DMG. However, using a shorter column (15 cm), it is unlikely that 4-DMG and 6-DMG would have very different retention times. The peak at 2.3 min. (Fig. 41) is therefore attributed to 6-DMG. Complete separation of this peak was not attempted since it was not intended to quantitate the metabolite in the current study.

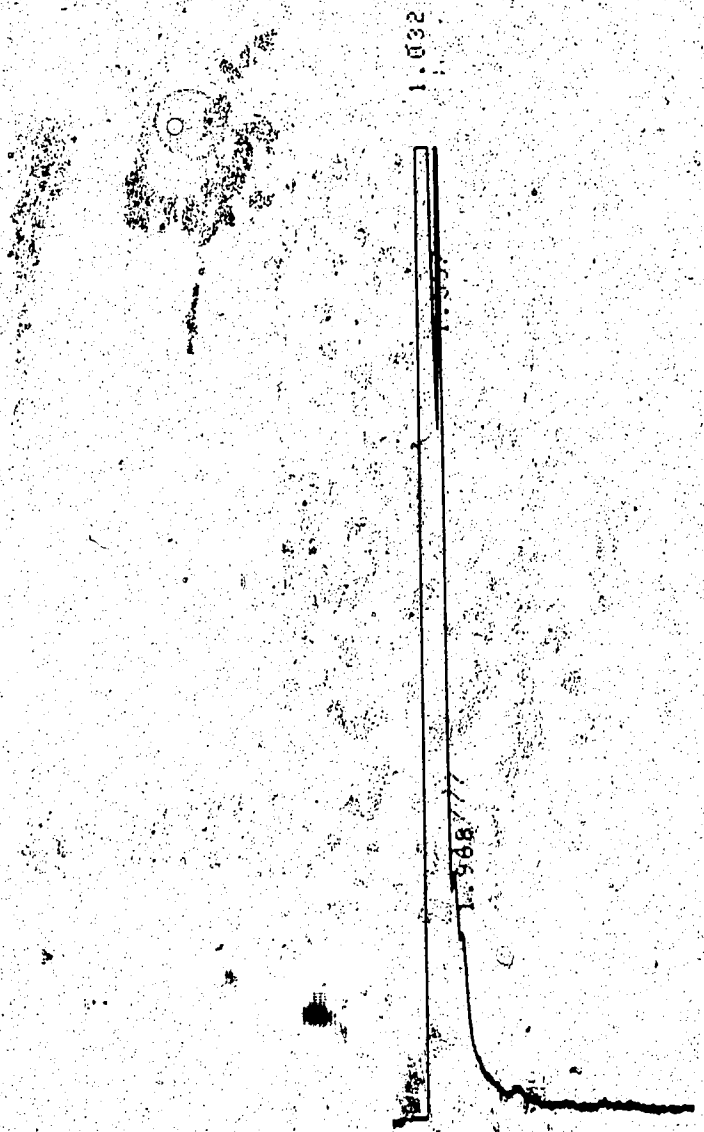

Figure 39. HPLC chromatogram obtained from the deproteinized (with acetonitrile) blank plasma sample of the rat. 20 mcL was injected on a 5μ, C_{18} Novapak Column. The flow rate of the mobile phase (45% acetonitrile in 0.1 molar acetic acid, pH=3.5) was 1.0 mL/min.

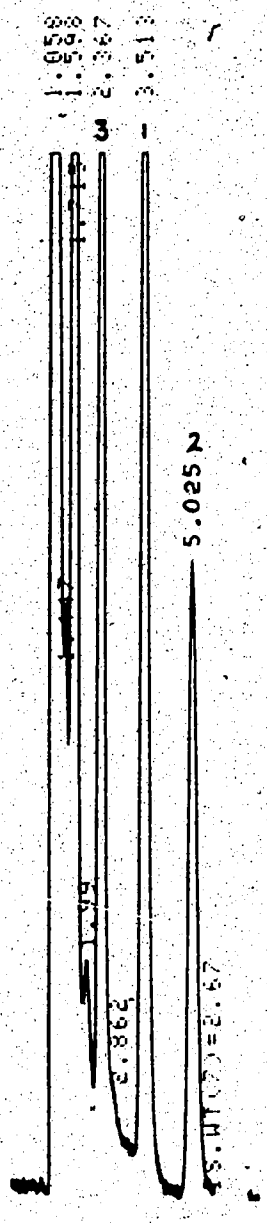

Figure 40. HPLC chromatogram obtained from a blank plasma sample spiked with acetonitrile solution of griseofulvin (Peak 1), m-phenylphenol (internal standard) (Peak 2), and 4-demethylgriseofulvin (Peak 3). 20 mcL was injected on a 5 μ, C_{18} Novapak Column. The flow rate of the mobile phase (45% acetonitrile in 0.1 molar acetic acid, pH 3.5) was 1.0 mL/min.

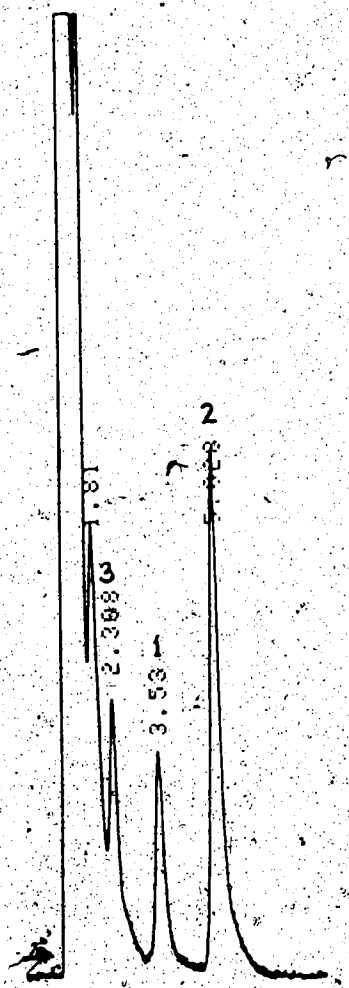

Figure 41. HPLC chromatogram obtained by the injection of 20 mcL of the supernatant, from deproteinized rat plasma dosed with an aqueous suspension of griseofulvin. Peak 1- griseofulvin, Peak 2- internal standard, and Peak 3- 6-demethylgriseofulvin.

The retention time of griseofulvin compares well with that identified in Figure 40. To confirm whether or not 6-DMG interfered with griseofulvin a chromatogram was obtained using a mobile phase of 45% acetonitrile in deionized, distilled water (pH 7.0). Since 6 DMG is a phenolic metabolite, (pK_a = 4.5) it remains ionized at pH 3.0 and unionized at pH 7.0 and, hence, it is not retained by the column (152). The retention times of griseofulvin (a neutral compound) and the internal standard (pK_a = 9.64) were not expected to change with a change in the mobile phase since the degree of ionization remains the same for both at pH 3.5 and 7.0. This hypothesis was found to be correct as shown in Figure 42. It is apparent the peak at 2.4 min. virtually disappeared and the peaks at 3.5 min. (gris) and 5.0 min. (int.Std) were unaltered. This indicates the presence of only griseofulvin in the peak eluting at 3.5 min.

Further evidence to this can be drawn from the results obtained by subjecting the 3.5 min. peak to GC-MS as shown in Figures 43-46. The chromatogram of the sample shows a peak with a retention time of 14.0 min. (Figure 43) which agrees well with the retention time of standard griseofulvin (14.1 min., Figure 45). The mass spectrum of the sample shows that the four major fragments having a m/z value of 69, 138, 214, and 215 conform to the four major fragments shown by the standard sample of griseofulvin. The fragment of mass 352.0 corresponds to the molecular weight of

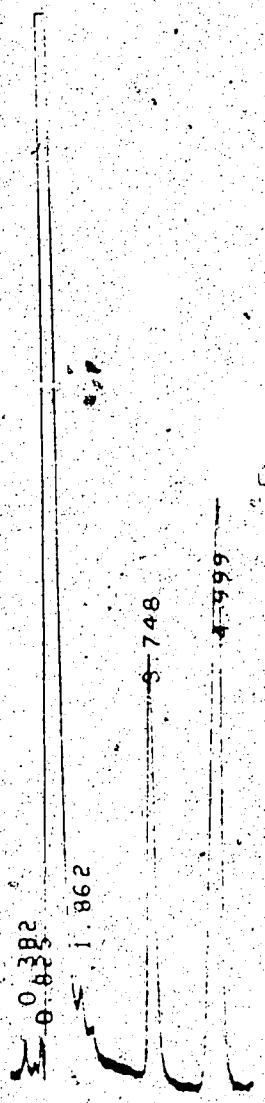

Figure 42. HPLC chromatogram of obtained from a deproteinized plasma sample of rat dosed with an aqueous suspension of griseofulvin. Peak 1- griseofulvin, and Peak 2- internal standard. 20 mcL was injected on a 5μ, C_{18} Novapak column. The mobile phase was 45% acetonitrile in deionized distilled water (pH=7.0) at a flow rate of 1.0 mL/min.

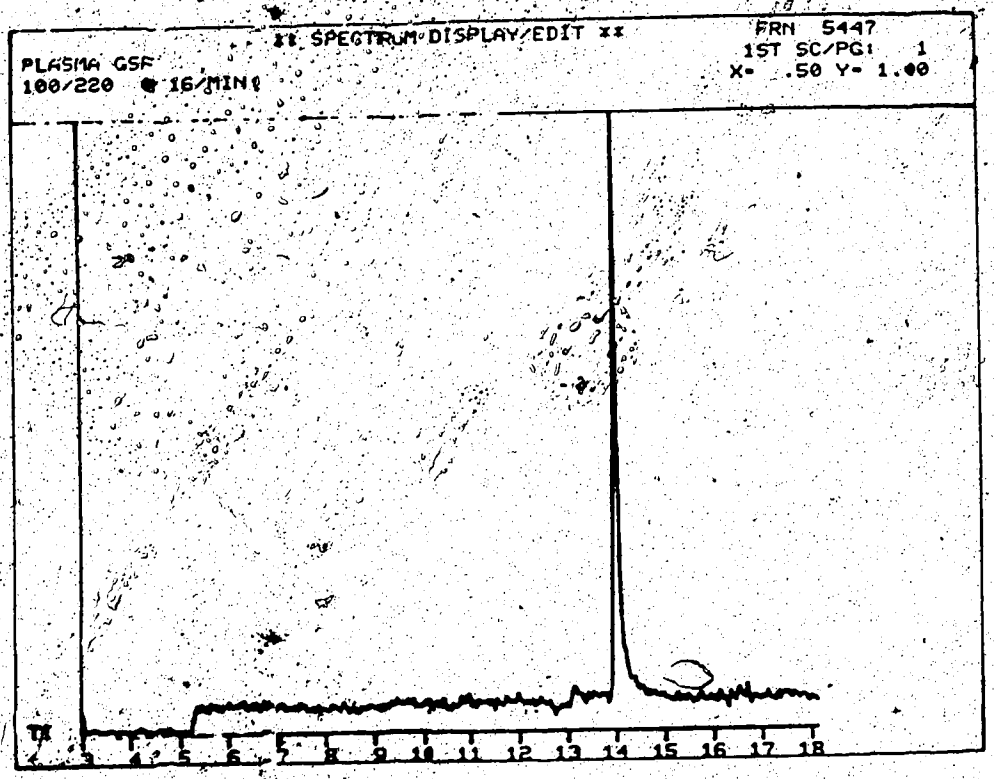

Figure 43. Gas chromatographic analysis of the peak eluting at 3.5 min. in HPLC (evaporated to dryness and reconstituted using dichloromethane) following injection of a 2 mcL sample.

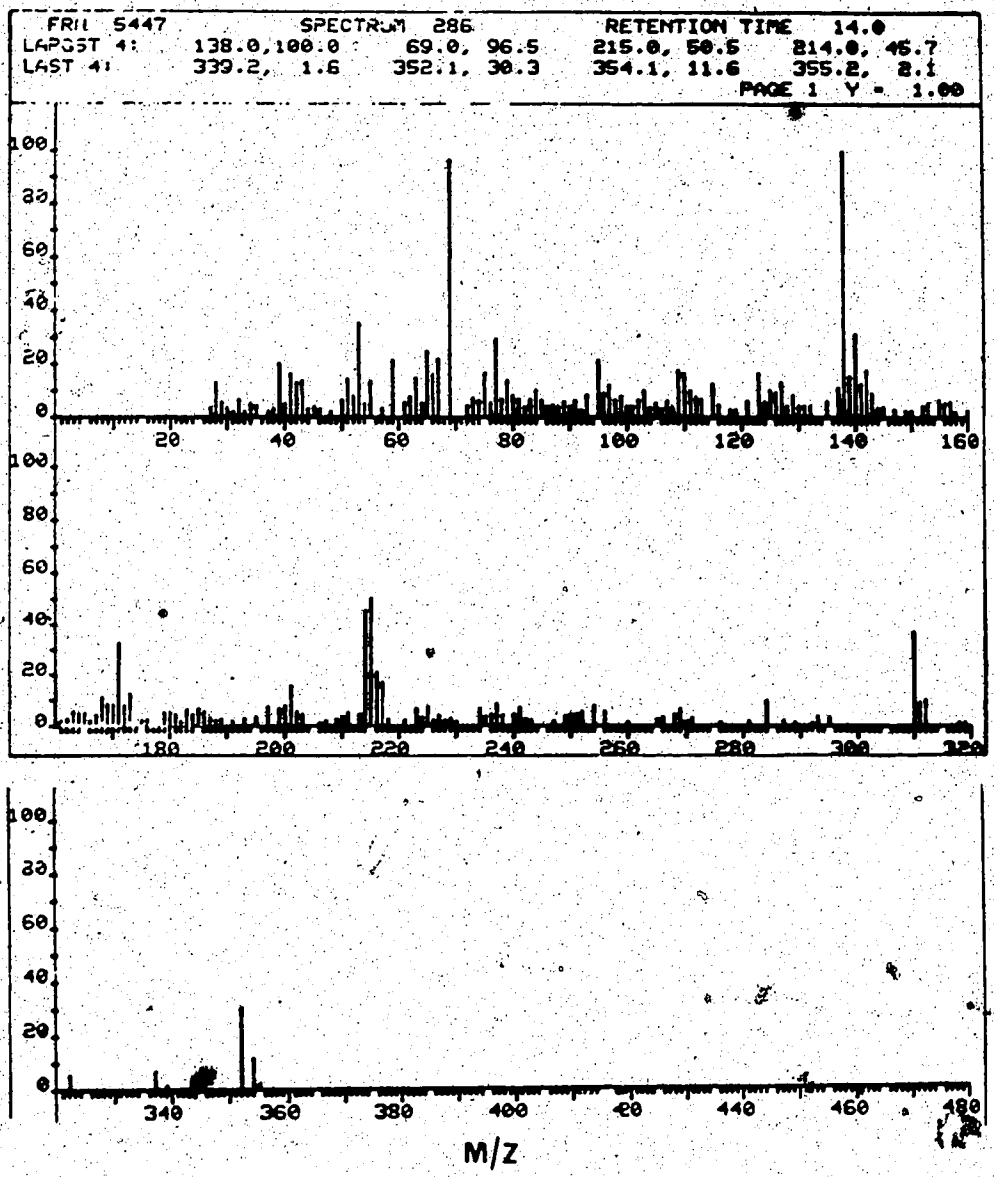

Figure 44. Mass spectra of the peak eluting at 3.5 min. in HPLC (evaporated to dryness and reconstituted using methylene chloride).

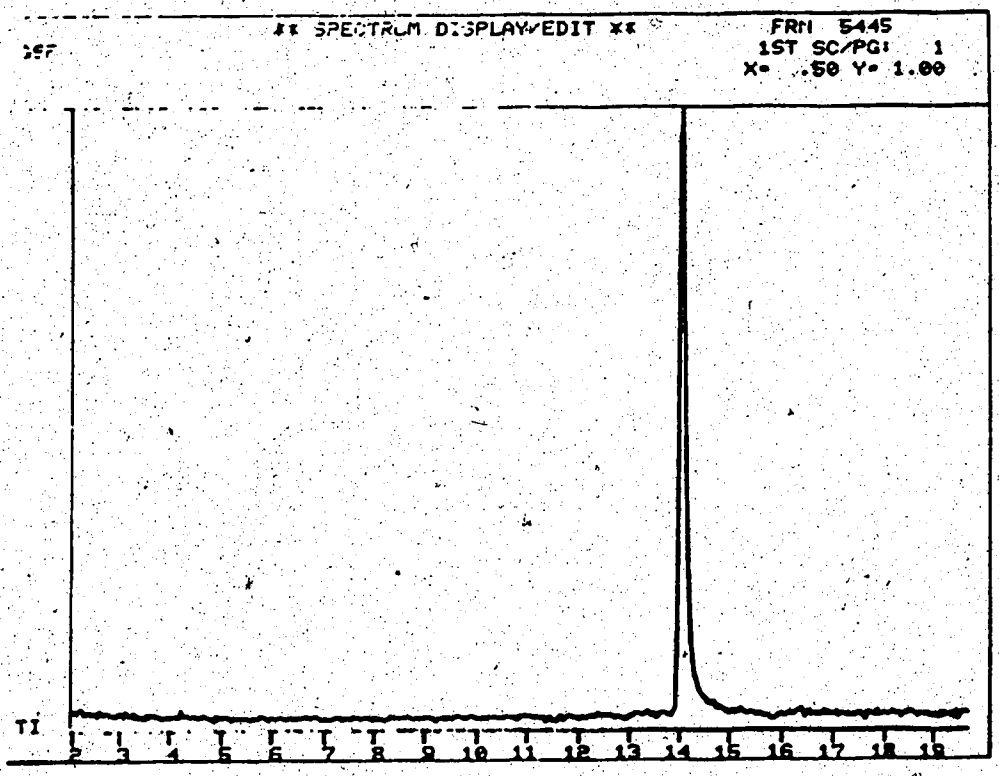

Figure 45. Gas chromatogram of 2 mcL of a solution of a pure sample of griseofulvin dissolved in methylene chloride.

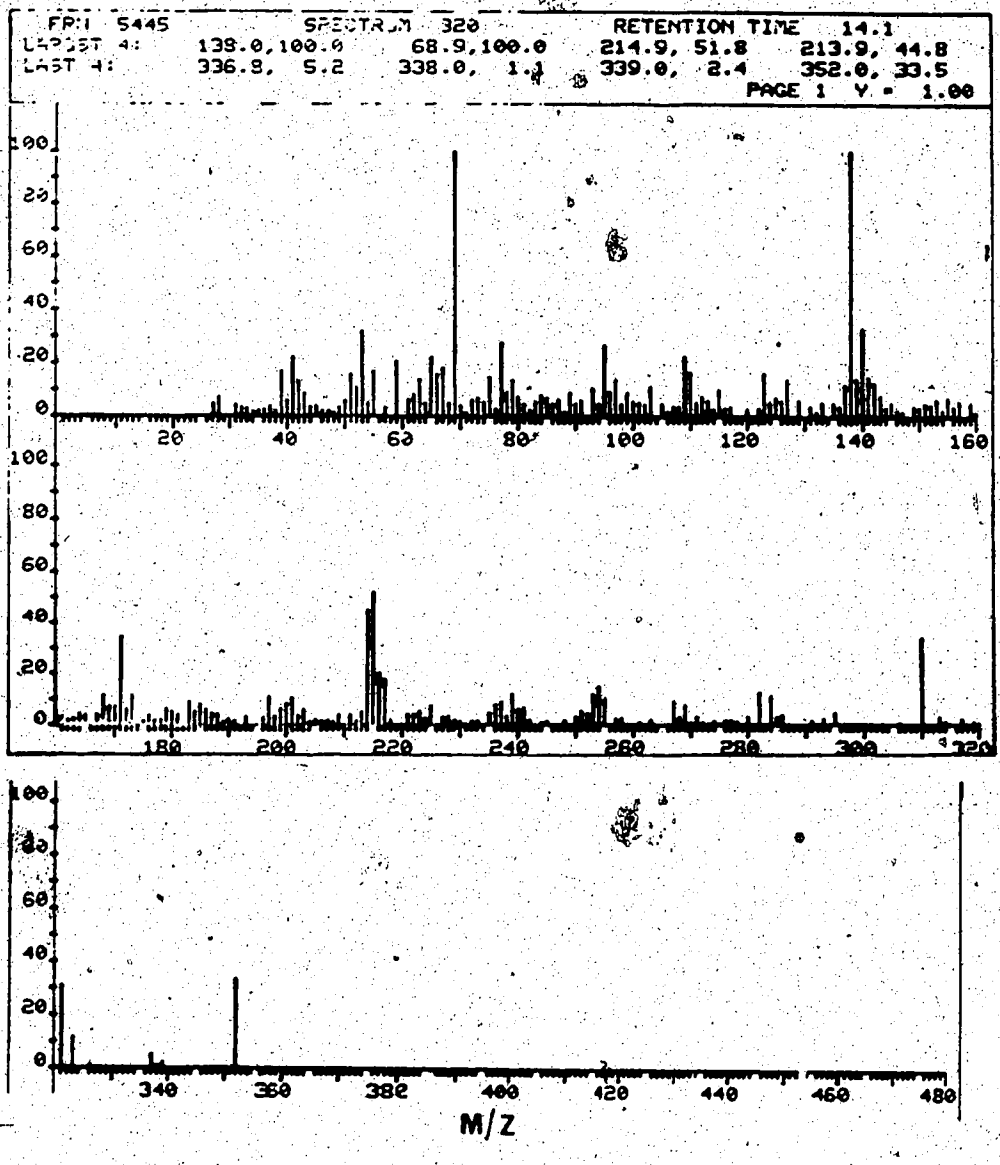

Figure 46. Mass spectrum of a pure sample of griseofulvin in methylene chloride.

griseofulvin.

4.2.2 Bioavailability Studies in the Rat Model

Bioavailability studies on the various formulations were compared using the rat model and sampling plasma at various time intervals (see Methods). Typical plasma concentration-time profiles obtained after the oral administration of various formulations as aqueous suspensions are shown in Figure 47. Plasma concentration-time profiles for griseofulvin were analyzed using the average values of individual peak plasma concentrations (C_{max}), the time to peak (t_{max}), area under the plasma level-time curve (AUC), 0 to 24 hours, and the plasma concentration after 1 hour (C_{1hr}). The results are summarized in Table 17. The results were tested for statistical significance using one-way ANOVA (173). A significant F value was obtained in all cases except t_{max} To compare the means, Duncan's Multiple Range Test (169) was used. These results are presented in Table 18.

The C_{max} obtained from solvated griseofulvin, although slightly higher, was not significantly difference statistically from the value obtained from untreated griseofulvin. A significant increase in the C_{max}, C_{1hr} was observed with coprecipitate systems compared to untreated and solvated griseofulvin. For instance, a 2-fold increase in C_{max} and a 2.3- and 2.8- fold increase in AUC was observed from the 19:1 and 4:1 griseofulvin:DMPC coprecipitates,

Table 17

Peak Plasma Levels (C_{max}), Time to Peak (t_{max}), Plasma Level at 1 hour (C_{1hr}), and Area Under the Plasma Level-time Curve (AUC) from 0 to 24 hrs Following Oral Administration of 100mg/Kg dose of Griseofulvin Formulations as Aqueous Suspensions to Rats.

Formulation*	Mean±SEM' Values of C_{1hr} (mcg/mL)	C_{max} (mcg/mL)	t_{max} (hrs)	AUC (mcg/mL/hr)
1	0.58±0.13	1.49±0.36	3.00±0.63	13.05±3.01
2	0.57±0.08	1.68±0.22	6.40±1.94	17.96±2.21
3	1.23±0.26	3.19±0.55	5.00±0.89	30.64±5.11
4	1.62±0.19	3.38±0.43	5.80±1.62	36.34±4.71
5	0.19±0.11	1.51±0.30	4.75±1.03	15.33±4.36

* 1, Untreated griseofulvin; 2, Solvated Griseofulvin; 3, 4, Griseofulvin:DMPC Coprecipitates, 19:1 and 4:1 weight ratios, respectively; and 5, griseofulvin:DMPC 4:1 physical mixture.

'n = 5 except in formulation 5 where n = 4.

Table 18

Duncan's Multiple-Comparison-of-Means Test on the Bioavailability Parameters, Obtained Following the Administration of Various Formulations of Griseofulvin as Aqueous Suspensions to Rats.

Bioavailability Parameter	Duncans Test Results
C_{1hr}	4>3> 1>2
C_{max}	4>3> 2>1
t_{max}	2>4> 3>1
AUC*	4>3> 2>1

* Formulation 1, Untreated griseofulvin; 2, solvated griseofulvin; 3 and 4, 19:1 and 4:1 weight ratio griseofulvin:DMPC coprecipitates, respectively.

The numbers of the formulations underlined are not significantly different from one another at the 0.05 significance level.

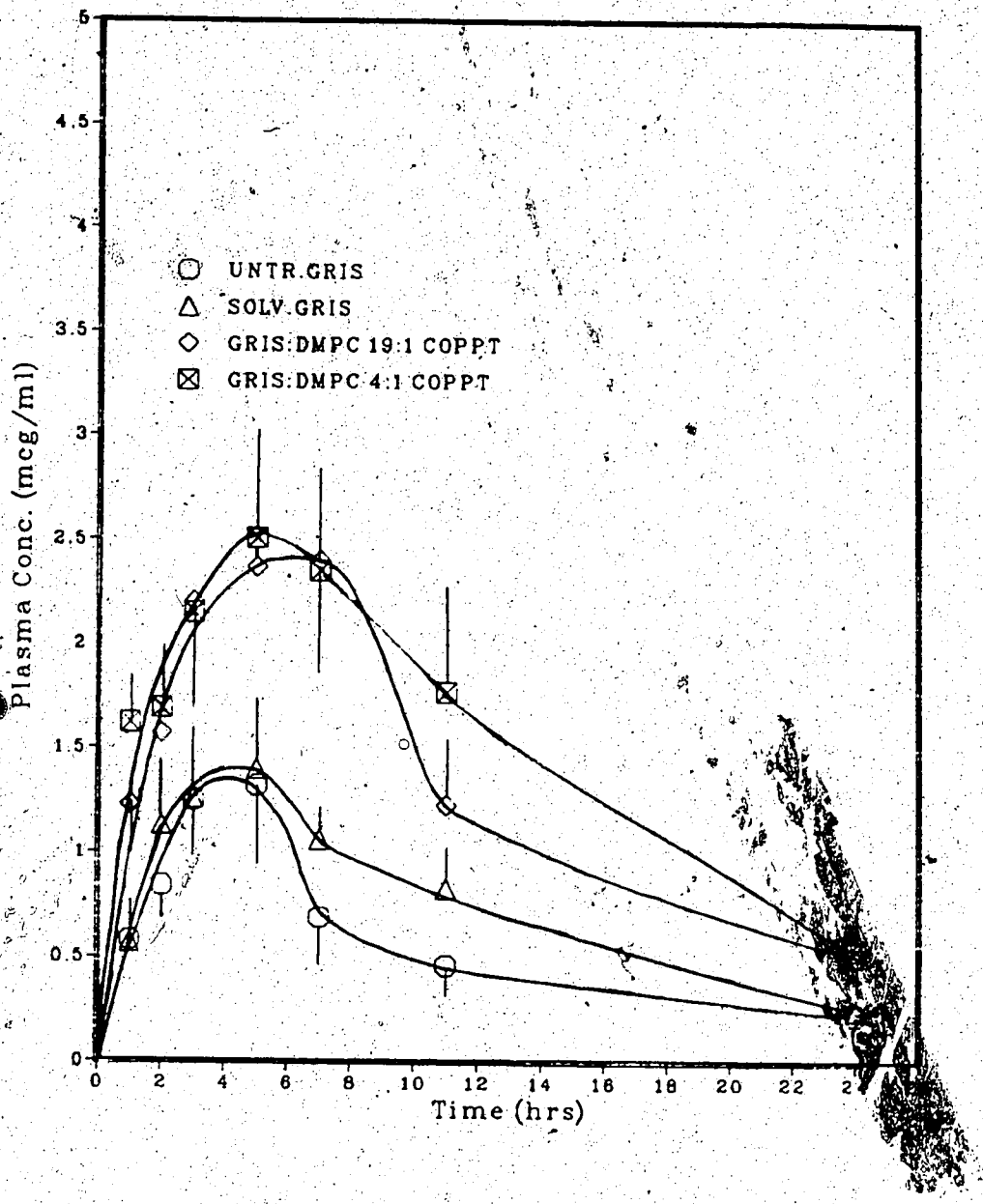

Figure 47. Plasma concentration-time profiles obtained following oral administration of griseofulvin formulation to rats as aqueous suspensions. (n=5, Mean±SEM are plotted).

respectively. However, the AUC obtained from the coprecipitates did not differ significantly from each other but both were significantly higher than those obtained from untreated or solvated griseofulvin. The administration of solvated griseofulvin as a physical mixture with DMPC did not exhibit increased bioavailability, i.e. C_{max}, t_{max}, and AUC of the physical mixtures were all similar to the values obtained after administering only solvated griseofulvin.

5. DISCUSSION

The dissolution rate studies showed that the release of griseofulvin from a coprecipitate containing a small fraction of DMPC (griseofulvin:DMPC 19:1) was as dramatic as from other coprecipitates containing larger fractions of the phospholipid (Figure 17). Normally, solid dispersions contain a large fraction of carrier e.g. drug:carrier ratios of 1:10 (114, 174) or 1:100 (11). However, unlike the griseofulvin:PEG 6000 or succinic acid solid dispersion (5,104) decreasing the carrier content when it is phospholipid does not necessarily result in decreased dissolution.

An important consideration in the characterization of solid dispersions is to identify the crystalline state of the drug. Powder X-ray diffraction spectra of griseofulvin: DMPC coprecipitates (Figure 8,9) show that for each of these compositions the crystalline state of griseofulvin still prevails. This is strong evidence that the griseofulvin is neither converted to an amorphous state nor remains as a solid solution in the coprecipitate containing relatively high amounts of DMPC (e.g. griseofulvin:DMPC, 1.5:1). This is further supported by the evidence that the phase diagram (Figure 7) was monotectic and also indicated no significant solid solution formation. A lack of mutual solubility of griseofulvin has been shown with other carriers as well (103).

Recently York and Grant (89) have introduced a dimensionless term called "disruption index" (d.i) for quantifying solid state disorder induced by an additive or impurity (the guest) in the crystal lattice of a host substance. The d.i value is defined as the "rate of change of entropy of the solid with respect to the ideal entropy of mixing of the components of the solid ΔS^m_{ideal}". The slope of a plot of entropy of fusion of the solid ΔS^f versus ΔS^m_{ideal} for mole fraction of impurity <0.05 is a close approximation of d.i. The authors applied the d.i. calculations to griseofulvin:DMPC systems and found that the relationship between ΔS^f and ΔS^m_{ideal} was linear up to mole fraction of 0.12 of DMPC in the coprecipitate (griseofulvin:DMPC 4:1 weight ratio). The relationship diverged from linearity at higher mole fraction of DMPC namely 0.25 (griseofulvin:DMPC 1.5:1 weight ratio). The slope of the linear part of the relationship was found to be 5.09 indicating a five-fold increase in crystal lattice disorder induced by the additive as compared with ideal molecular substitution, mixing, or dilution. It was suggested that this leads to increased thermodynamic activity of the crystals resulting in increased dissolution of griseofulvin from the coprecipitates.

However, as pointed out by the authors, the disadvantage of such a quantification using DTA data is that heating the sample may change the order of the crystal lattice by increasing thermal motion and/or by annealing the

solid during the heating process. This could then lead to reduced reliability of d.i. values. Different techniques used to identify degree of crystallinity of solids include density measurements, X-ray diffraction, IR spectroscopy, NMR spectroscopy, electron microscopy, DTA, solution calorimetry and kinetic studies. But frequently divergent values of the crystallinity of a sample are obtained from different techniques (175).

The importance of the role of chloroform in preparing the coprecipitate is underlined by the results of several dissolution studies. As shown in Figures 18 and 19 when chloroform is replaced by methylene chloride or ethanol coprecipitates offered no advantage over physical mixtures or drug alone.

The DTA thermograms indicated the presence of griseofulvin in a solvated state with chloroform and in a non-solvated state when treated with methylene chloride or ethanol. Similarly, the thermograms of griseofulvin:DMPC coprecipitates revealed the existence of griseofulvin in a solvated state. The presence of griseofulvin in the solvated state was also indicated by the X-ray diffraction pattern as shown in Figure 9. The spectra of coprecipitates resemble that of solvated griseofulvin rather than untreated griseofulvin. This suggests that griseofulvin, chloroform and DMPC are combined in a specific manner enabling rapid release of griseofulvin when contact is made with water. Similar observations have been reported previously

solid dispersions of riboflavin and benzoic acid combined with isopropyl alcohol which performed better than in combination with benzene (176).

The specific behavior of the griseofulvin-chloroform solvate is further illustrated when POS is used in place of DMPC as evidenced in Figure 28. The action of POS on the dissolution of griseofulvin from the coprecipitate when chloroform is used as a solvent, is in a way the same as the action of DMPC i.e. POS participates in the crystal lattice of coprecipitates with griseofulvin in a similar manner to phospholipids.

HCA was found to form a solvate with DMF in the presence or absence of DMPC but not with chloroform. HCA-DMF solvate was slightly more soluble in deionized water as compared to untreated HCA. The dissolution rate and solubility of HCA:DMPC (4:1) coprecipitates from chloroform did not differ from that of untreated HCA. But a coprecipitate of the same ratio prepared from DMF:chloroform (2:1 v/v) produced an increase in the dissolution rate in the first 10 min. as well as the amount in solution after 60 min. These results further substantiate the necessity of the drug to be in a solvated state in order for DMPC to be included in the crystal lattice and then to cause an increase in the dissolution rate and solubility of HCA.

5.1 Effect of Lipid Composition on Dissolution of Griseofulvin

As shown in Figures 21 and 22, the release of griseofulvin varies depending on the type of phospholipid used. If it is assumed that EPC, DPPC, and DSPC behave like DMPC in the formation of the crystal lattice of the coprecipitate, the decreased release of griseofulvin when combined with a phospholipid having longer chain is likely determined by the relative ease of dispersion of the phospholipid upon contact with the aqueous phase, which, in turn, is related to its T_c. The T_cs of EPC and DMPC are reported to be -10°C and 23°C respectively (177). However, the T_c of DPPC and DSPC are 41°C and 58°C, respectively (177). Thus EPC and DMPC would exist in a liquid crystalline state at 37°C, but DPPC and DSPC would exist in a gel state at 37°C. Therefore, increasing the amount of DSPC in the coprecipitate from 19:1 to 4:1 makes little difference in the release of griseofulvin because it does not readily disperse below its T_c. Hence, the effect of phospholipids on dissolution of griseofulvin appears to be related to their individual abilities to rapidly and spontaneously undergo dispersion in aqueous media. In this regard it is known that DPPE alone does not readily disperse in water (178) and, consequently, it does not influence the dissolution of griseofulvin from coprecipitates (Figure 22).

Another approach taken to alter the lipid composition was the addition of cholesterol in the lipid phase in

combination with DMPC. The incorporation of cholesterol in liposomes is known to alter their permeability properties. Below the T_c cholesterol increases the fluidity of the model membranes but above the T_c it increases the structural order (18). This behavior is possibly transferred to the dissolution of griseofulvin:DMPC coprecipitates. As shown in Figures 23 and 24 the addition of cholesterol to DMPC produces markedly different release patterns of griseofulvin depending on the mole ratio of DMPC and cholesterol, and the total lipid content of the coprecipitate. The coprecipitates exhibit decreased dissolution rates at early times but give increased amounts of drug released after 60 min. particularly when cholesterol is present in relatively small fractions. These changes could be due to the changes in the rate of formation of liposomes and subsequent uptake of molecular griseofulvin.

5.2 Dissolution of griseofulvin from non-solvated systems

The incorporation of inert carriers having high water solubility and of varying molecular sizes did not increase the dissolution of griseofulvin from coprecipitates with DMPC in the absence of chloroform in the crystal lattice (Table 9). This suggests the inability of these molecules to penetrate the strong crystalline structure of griseofulvin. These substances were unable to modify the energetic state of griseofulvin as observed with the incorporation of DMPC in the presence of chloroform in the crystal lattice.

Previously most of these agents have been shown to increase the dissolution of drugs although only at high ratios (111, 114). At the concentrations used in the current study these water soluble additives were unable to augment dissolution of griseofulvin even in the presence of DMPC when chloroform was not used as the coprecipitating solvent.

Fumed silica offers a very large surface area to which drug molecules can be deposited as ultrafine particles (53) allowing a large number of contact points between the dissolution medium and the drug. The results in Table 9, show that the IDR was the highest from this system compared to all the other combinations of agents studied. However the dissolution is much less than that obtained from DMPC coprecipitates. Griseofulvin:silicon dioxide (1:10) is reported to have increased dissolution as compared to micronized griseofulvin (53). But this is not the case with solvated griseofulvin whose crystals are larger and more regular-shaped but also adsorption at the surfaces of silicon dioxide particles did not increase the dispersion of DMPC and, in turn, the dissolution of griseofulvin.

5.3 Dissolution of Griseofulvin Tablets

The dissolution behavior of dosage forms prepared from powders could be markedly different due to the various steps involved in their manufacture. This is an important consideration in the scale-up procedures before leading to an eventual dosage form. This hypothesis was tested by

comparing the performance of coprecipitate powders with tablets.

The dissolution of griseofulvin from tablets as shown in Figure 36, revealed a decrease in the dissolution rate with increase in compression force. However, the disintegration time was not affected (all tablets disintegrated within 1 min.). This suggests a structural change in the crystal lattice upon compression in which presumably the fine crystallites of griseofulvin present in the coprecipitate powder are compressed to form larger particles . Alternatively compression has simply fractured the crystal lattice causing the loss of chloroform and subsequent conversion to the non-solvated form of griseofulvin. The end result would be a decrease in effective surface area and, hence, dissolution. Depending on whether particle bonding or particle crushing is affected various types of response curves between dissolution rate and compression force have been discussed by Carstensen *et al.*, (179). Since the optimum hardness of a tablet to have good strength and friability is in the range of 6 to 8 Kg, the coprecipitate systems under these conditions do not yield increased dissolution rates and so the dosage form of choice which provides the optimum performance of these coprecipitates is a powder administered, for example in a hard gelatin capsule.

5.4 Aging of Coprecipitates

As shown in Figures 29 and 30, after aging the coprecipitates the dissolution rate and extent gradually decreased with time and at the end of 112 days reduced to that of untreated griseofulvin. The X-ray diffraction spectrum of the aged sample (112 days) was changed from that of a fresh sample and resembled that of untreated griseofulvin (Figure 31) indicating that solvated griseofulvin had changed to a non-solvated form in aged samples. This would explain the observed decreased dissolution after aging for 112 days.

Solid dispersion systems are highly energetic and are unstable in most cases (93,113). Such instability leads to coarsening of particles under a driving force of interfacial energy reduction. There are a number of reports in literature that show decreased dissolution on aging for e.g. tablets made from chlorpropamide:urea solid dispersions showed decreased dissolution after aging (180) although solid dispersion in the powder form also gave variable dissolution results depending on the composition (2). Similarly, the dissolution rate of a phenobarbital-urea system decreased on aging for [illegible]s (124). Aged samples of hydrocortisone:PVP solid disp[illegible]s indicated decreased supersaturation on aging, possibly due to self-association of the drug in the solid dispersion (181).

5.5 Mechanism of Dissolution

Some of the most common reasons for increased dissolution rates from solid dispersions have been the formation of solid solution, eutectics, conversion to an amorphous form, solubilizatiion effect by the carrier or increased hydrophilicity of the drug due to coating by polymers. As discussed before the griseofulvin:DMPC system showed no evidence of any of the above mechanisms.

According to the film theory of dissolution, factors affecting the rate of dissolution include the diffusion coefficient, the diffusion layer thickness, the surface area and the difference between the saturation concentration in the diffusion layer and the bulk solution concentration. Under identical conditions of dissolution testing for the same compositions of griseofulvin:DMPC, differences in dissolution behaviour between coprecipitates and physical mixtures or solvated griseofulvin are probably closely related to inherent differences in particle size.

It has been known that heating solvated griseofulvin can lead to desolvation and conversion to very small particles of anhydrous griseofulvin (163) and in the process a 100% increase in surface area is realized. Cheng *et al.*, (161) in a detailed study reported that the desolvation process requires substantial reorganization of the crystals. This results in many regions which nucleate separately and form small particles. It appears that in coprecipitate crystals a phospholipid alters the crystal structure in such

a way to cause fracture and disintegration upon its spontaneous hydration after making contact with water. The evidence of this comes from microscopical observations of the coprecipitates in which very fine crystallites of griseofulvin were found to be released as the original crystals fractured in the dissolution medium. When physical mixtures are used or when coprecipitates were prepared from other solvents, the phospholipid undergoes dispersion in a similar manner but it does not bring about crystal disruption because it is not intimately involved in the crystal lattice. Hence, no increase in dissolution rate or extent are realized from those systems.

The solubilization effect in the diffusion layer surrounding the particles may be exerting a substantial effect in the dissolution process. More specifically, it is the rapid, spontaneous dispersion of DMPC into colloidal aggregates of phospholipid bilayers or liposomes and the simultaneous incorporation of griseofulvin molecules into this partitioning system that is analogous to an increase in the saturation concentration in the diffusion layer. In the case of physical mixtures, DMPC is not in a suitable physical state for spontaneous dispersion and, in addition, griseofulvin molecules must first be dissolved from the solid state before becoming sequestered by the preformed liposomes, a process which itself is relatively slow.

Microscopic observation of coprecipitate crystals undergoing dissolution in water as shown in Figure 11

revealed multitudes of long finger-like bodies forming at the crystal surfaces, growing and extending outward into the dissolution medium as part of a rapid dynamic process of dispersion which may be considered as part of the dissolution process. A similar observation was made upon examination of the physical mixture although in this case dissolution was not improved. Although these microscopic observations were made in isolation in what may be considered a static system it demonstrates a behavior of phospholipid solid dispersion formulations which under appropriate conditions can lead to improved dissolution.

The supersaturation of griseofulvin in the pH 2.0 dissolution medium following dissolution from coprecipitates containing phospholipid may be the result of two mechanisms of solution. Firstly, as shown in Figure 13, a very high partition coefficient of griseofulvin in low concentrations of DMPC liposomes suggests that substantial sequestering of griseofulvin might occur as a result of the rapid dispersal of the phospholipid into liposomes at the very early stages of dissolution. e.g., at a DMPC concentration of 0.01 mg/mL the entrapment was found to be 1.04 moles of griseofulvin/mole of DMPC. In an idealized situation 100% release of DMPC from 19:1, 4:1, or 1.5:1 (griseofulvin:DMPC weight ratios) coprecipitates into the dissolution medium would provide 3.8×10^{-6}, 1.8×10^{-5}, and 4.8×10^{-5} moles of DMPC, respectively. The amount of griseofulvin which would be associated with these quantities of DMPC would be

3.9×10^{-4}, 1.9×10^{-3}, and 5.0×10^{-3} moles, respectively. These values in turn correspond to 0.0014, 0.0066, and 0.017g of griseofulvin, respectively. Therefore a 11%, 42%, and 107% increase in the apparent solubility of griseofulvin is expected from respective 19:1, 4:1, and 1.5:1 coprecipitates. However, the increases observed were 21%, 26%, and 30%, respectively. This lack of agreement may be attributed to the following: (i) it is unlikely that a 100% release of the phospholipid is obtained in the dissolution medium since at the end of the dissolution test undissolved particles still remained, and (ii) the partition coefficients are equilibrium values in contrast to the dynamic conditions during the dissolution process. These factors could therefore cause over- or under-estimation of the apparent solubilities. Further evidence to support the fact that griseofulvin is associated with the dispersed phospholipid is obtained from the observation that when the dissolution medium after 60 min. was centrifuged at 40,000 RPM the concentration of griseofulvin in the supernatant decreased. This indicates that some of the griseofulvin was removed in the phospholipid pellet and, therefore, it had been associated with the phospholipid as small transparent vesicles in the dissolution medium.

Secondly, the increase in solubility may be partially attributed to dissolution of very fine particles as described by the Kelvin equation (179) given by

$$S = S_{\infty} \exp \frac{2\gamma M}{r\rho RT} \qquad (24)$$

where, S is the solubility of a microscopic particle, S_{∞} is the solubility of a macro particle, γ is the crystal-solvent interfacial tension, M is the molecular weight, r is the particle radius, R is the universal gas constant, ρ is the density of the particle, and T is the absolute temperature. Hence the small particle released into the dissolution medium, in addition to providing increased dissolution could also lead to an increase in the solubility of the drug. For example, if is estimated that a 25% decrease in particle radius leads to an 8% increase in the solubility of the drug. But the exact contribution of this process to the increased solubility could be calculated.

5.6 Selection of a Dissolution Kinetic Model

The dissolution data obtained under sink conditions were treated to explain the profiles in terms of a suitable model. The approaches tried included a first-order kinetic model (70), a second-order kinetic model (71) and a distribution function model proposed by Weibull (68).

As shown in Figure 33, the dissolution curves plotted according to first order kinetics show a change in slope between 23.0 and 35.0 min. of the dissolution test. Theoretically if the systems followed first order kinetics, a single linear curve would be expected. When two lines result as shown, two apparent rate constants can be derived.

Depending on which apparent rate constant is used, different values of T_{40} will be obtained as shown in Table 12. It is clear from the table that there is a large deviation between the observed and the calculated values of T_{40}. Therefore the systems do not seem to follow a simple first-order kinetics and the assumption that the surface area available for dissolution decreases exponentially with time is not true for these coprecipitate systems. Similar conclusions have been drawn for hydrocortisone:lipid solid dispersion systems (140).

The second-order plot as shown in Figure 34, were linear ($r>0.99$ in all cases). The calculated values of T_{40} agree very well with the observed values except for 1.5:1 coprecipitate. The deviation with this could be explained by analyzing the S-shaped curve. At initial time points the curve is non-linear and the observed T_{40} falls in this range. The calculated T_{40} however, is obtained from the slope of the entire S-shaped curve. The second order knetic model holds good for all the formulations except 1.5:1 coprecipitate. This suggests that the dissolution from these coprecipitates is not only dependent upon the surface area but also on the weight of drug available for dissolution. Similar results have been obtained by others for hydrocortisone:lipid systems (140).

As shown in Figure 35, it can be seen that the treatment of dissolution data according to Weibull distribution function yielded lines exhibiting an upward

curvature. These curves can be easily straightened by shifting all data points horizontally by the same interval (69). However, this distorts the original data. Hence, this was not carried out.

The advantage of this distribution function is that it is possible to calculate the time required for any percent of drug dissolved by setting up an auxilliary ordinate scale on the right hand side (Figure 35). As shown in Table 14, the T_{40} values predicted from this equation agree very well with the value obtained experimentally with negligible deviation. However, the observed and calculated values of T_d do not agree very well. These results indicate the applicability of the Weibull distribution function for all formulations studied during the initial period of the dissolution test. Deviation from Weibull distribution occurs at later times.

5.7 In Vivo Studies

The comparisons of micronized griseofulvin, solvated griseofulvin, griseofulvin:DMPC (19:1, 4:1) coprecipitates administered orally as aqueous suspensions to rats clearly indicated improved bioavailability from the coprecipitates.

The sharp increase in C_{1hr} values obtained from the coprecipitates corresponds to the rapid dissolution observed from the phospholipid containing formulations. A small fraction of the level observed was probably a result of drug dissolving in the aqueous suspension medium just prior to

administration (<30 seconds). However, the rapid blood levels observed over the first hour occur because of rapid dissolution of griseofulvin in the fluids of the GI tract of the rat.

The time to reach the maximum blood concentrations t_{max} is a function of the rate of absorption and the concentration levels obtained, C_{max}. In our studies, the t_{max} was the highest for solvated griseofulvin and lowest for untreated micronized drug possibly because of the larger effective surface area of the latter being exposed to the gastric fluids (Table 17). The C_{max} from untreated griseofulvin compares well with the value reported by others (133). However, slightly higher levels were obtained by Kraml *et al.*, (99). This lack of agreement is most likely due to differences in particle size distribution. They also found no significant difference in C_{max} when untreated griseofulvin was administered with or without a surfactant in the aqueous suspension. The longer t_{max} obtained from the coprecipitates arises because of more drug going into solution and thus, it takes more time to reach peak blood levels.

The AUC of untreated and solvated griseofulvin were not significantly different although the value of the latter was higher (Table 18). Similar values have been reported by others (132). However the results do not agree with those obtained by Bates *et al.*, (43), who observed a statistically different increase in the bioavailability from solvated

griseofulvin as compared to untreated griseofulvin in humans. This lack of agreement could be due to species variation and to a very high inter animal variation in our results obtained in studies with the untreated griseofulvin samples, and which may have over-shadowed differences in bioavailability between untreated and solvated griseofulvin. The AUC from the coprecipitates were higher than those obtained from untreated or solvated griseofulvin. Until now increased bioavailability has only been demonstrated from solid dispersions containing much higher amounts of carrier. For instance, griseofulvin:carrier ratio of 1:10 as observed with PEG 6000 (5), ground mixture with microcrystalline cellulose (42), surface deposited on spray dried whey or colloidal silicon dioxide (53). An exception to this is the ratio of 100:1 using hydroxypropyl cellulose reported by Fell *et al.*, (182) which also yielded increased bioavailability of griseofulvin.

The administration of solvated griseofulvin as a physical mixture with DMPC did not exhibit increased bioavailability but this was to be expected from the dissolution studies. The C_{max} and AUC were both similar to the values obtained after the administration of solvated griseofulvin.

It is interesting to speculate that the improved bioavailability observed from coprecipitates is not only due to rapid dissolution and a fine particle size but also due in part to transport of liposome-entrapped drug across the

gut wall. The oral administration of solute-loaded liposomes would suggest that this is possible (183). A thorough review of the use of liposomes for oral drug delivery has been published (184).

In summary, the utility of phospholipids as carriers in improving the bioavailability of griseofulvin has promise. Under appropriate conditions bioavailability improvement is achievable as a result of rapid dissolution and increased amount of drug in solution. Using phospholipids, both of these parameters can be altered and controlled to some extent in order to obtain the desired results.

5.8 In Vitro - In Vivo Correlations

Generally, two types of correlations are used to compare *in vitro* and *in vivo* results namely Quantitative and Rank order. In quantitative correlation an *in vitro* variable (X) is related to a *in vivo* variable (Y) using equations such as

$$Y = b.X$$

or

$$Y = a + bX$$

where b is the slope.

In Rank-Order correlation the *in vivo* variable (y) may show 3 different types of relationships with *in vitro* variable (X), namely 1) increase with increase in X, 2) decrease with increase in X, or 3) increase with decrease in

X. The variables are assigned a rank and the ranks are tested statistically.

There seems to be no general rule as to which *in vitro* or *in vivo* parameters should be correlated. A number of parameters have been used by others. For example:

a) *in vitro* parameters: percent dissolved in 30 min., 60 min. (185, 186), amount dissolved in 30 min. (187, 188), statistical moment functions (189), time taken for 16% of the drug to dissolve (190), dissolution rate constant (192).

b) *in vivo* parameters: C_{max} (186, 188), AUC (85), percent dose absorbed in one hour, six hours (185), 10% AUC (191) $C_{max/2}$ (190).

The time for 50% of the drug to dissolve *in vitro* is probably the best variable to be correlated since its value indicates the central tendency of the dissolution data as suggested by Wagner (193). This has been used by others (194, 191). A more meaningful parameter which considers the entire dissolution profile is the Dissolution Efficiency (DE) suggested by Khan (195). The concept is logical in the sense that since drug availability *in vivo* is estimated by integrating the AUC, it is preferable to express the *in vitro* results in the same manner. It is defined as "the area under the dissolution curve up to a certain time t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time". Mathematically, this is given by

$$\% DE = \int_0^t Y \, dt \, \frac{100}{Y_{100}t} \qquad (25)$$

where, DE is the dissolution efficiency, Y is the % dissolved at a given time t, and Y_{100} is 100% of the drug dissolved in time t.

As shown in Table 18, the AUC, C_{max}, and C_{1hr} obtained after the dose of untreated griseofulvin are not significantly different from those of solvated griseofulvin. This is in good agreement with the results obtained from *in vitro* dissolution test under both sink and non-sink conditions, which also showed no significant differences between the two formulations (except at 30 min. under non sink condition). However, a rank order correlation could not be seen i.e., the dissolution rate of solvated griseofulvin was always lower than that of untreated griseofulvin at all the three time points tested. Therefore, lower values of the *in vivo* parameters - AUC, C_{max}, and C_{1hr} would be expected from solvated griseofulvin. However, the reverse was found. This deviation could be due to the following reasons. It has been reported that the dissolution of the solvated griseofulvin in 0.1 N HCl at pH 1.3 is less than that of untreated griseofulvin. However, the solvated form is more soluble; it also dissolves at a faster rate than the untreated form in a dissolution medium containing 20 mM sodium deoxycholate at pH 7.5 (85). This suggests that solvated griseofulvin dissolves at a much faster rate in the small intestine where bile and other physiological

surfactants are also available. This could explain the observed results of increased AUC, C_{max}, and C_{1hr} from solvated griseofulvin as compared to untreated griseofulvin, thereby, causing a change in the expected rank order correlation.

The *in vitro* results showed a significant increase in the dissolution rate of griseofulvin from DMPC coprecipitates as compared to solvated or untreated griseofulvin under both sink and non-sink conditions. Therefore, it is anticipated that the oral administration of coprecipitates would increase the values of the *in vivo* parameters, AUC, C_{max}, and C_{1hr}. The observed results are in good agreement with this hypothesis. However, the *in vitro* results showed a significant difference between two of the coprecipitates tested, namely 19:1 and 4:1, and the 4:1 coprecipitate yielding greater dissolution at all the three time points tested. The *in vivo* results failed to show significant differences between these two coprecipitates, but the rank order was the same. Such a deviation may be due to a high inter-animal variability which overshadows differences between the two formulations. Also, a higher difference *in vitro* may be required in order to observe a significant difference *in vivo*.

Three *in vitro* parameters each from dissolution rate determinations under sink and non-sink conditions were tested for correlation with three *in vivo* parameters- AUC, C_{max}, and C_{1hr}. The *in vitro* parameters selected under sink

conditions were (1) amount dissolved in 30 min. (D_{30}), (2) amount dissolved in 60 min. (D_{60}), and (3) % dissolution efficiency (%DE) as listed in Table 19. The three parameters selected from dissolution rate studies under non-sink conditions were D_{30}, D_{60}, and IDR from 0 to 5 min. as shown in Table 20. The values of (D_{30}), (D_{60}), %DE and IDR increase in the order solvated griseofulvin, untreated griseofulvin, 19:1, and 4:1 (griseofulvin:DMPC, weight ratio) coprecipitates. Therefore the 4:1 coprecipitate is expected to produce the highest blood levels following oral administration.

The results in Table 21 indicate that all of the three *in vitro* parameters under sink conditions correlate with C_{1hr}, with a correlation coefficient, r, of >0.99 in all cases. The correlation of AUC and C_{max} with each of *in vitro* parameters was slightly lower, r being in the range of 0.95 to 0.96. The results indicate that DE% gives a slightly lower correlation in all cases, presumbly due to more stringent conditions of its calculation, based on the entire dissolution profile. However, D_{30}, and D_{60} which are just one point determinations on the dissolution profile also predict the *in vivo* bioavailability quite closely.

Similarly, under non-sink conditions of dissolution, D_{30} and D_{60} values correlate with all the three *in vivo* parameters. But, the IDR does not show a good correlation with C_{max} and AUC, but shows good correlation with C_{1hr}. The results are shown in Table 22. Thus, rapid dissolution of

Table 19

In Vitro Parameters of Griseofulvin Dissolution Under Sink Conditions.

Formulation*	(D_{30}) (mg)	(D_{60}) (mg)	DE (%)
1	0.46	0.68	28.7
2	0.40	0.62	24.0
3	0.81	1.04	48.0
4	1.06	1.25	62.0

D_{30} is the amount dissolved in 30 min.

D_{60} is the amount dissolved in 60 min.

DE is the dissolution efficiency.

* 1, Untreated griseofulvin; 2, Solvated griseofulvin; 3, and 4, Griseofulvin:DMPC coprecipitates 1:9:1 and 4:1 weight ratios.

Table 20

In Vitro Parameters of Griseofulvin Dissolution Under Non-Sink Conditions.

Formulation*	IDR (mcg/mL/min)	(D_{30}) (mg)	(D_{60}) (mg)
1	0.78	9.94	10.50
2	0.75	8.81	10.46
3	1.32	16.99	19.22
4	2.32	19.24	21.59

D_{30} is the amount dissolved in 30 min.

D_{60} is the amount dissolved in 60 min.

* Key same as in Table 19.

Table 21

Correlation Analysis of *in vivo* Parameters with *in vitro* Parameters of Griseofulvin Dissolution Under Sink Conditions for Four Formulations*.

in vitro parameter	*in vivo* parameters		
	C_{1hr}	C_{max}	AUC
D_{30}	0.9972	0.9540	0.9584
D_{60}	0.9972	0.9642	0.9618
DE %	0.9947	0.9499	0.9505

In Vivo parameters were taken from Table 17.

All values are significant at $p=0.05$

Formulations 1-4 as listed in Table 19.

Table 22

Correlation Analysis of *in vivo* Parameters with *in vitro* Parameters of Griseofulvin Dissolution under Non-Sink Conditions for Four Formulations*.

In vitro parameter	*In vivo* parameters		
	C_{1hr}	C_{max}	AUC
D_{30}	0.9884	0.9803	0.9614
D_{60}	0.9899	0.9925	0.9808
IDR	0.9608	0.8669'	0.9130'

In Vivo values are taken from Table 17.

'Except these all values are significant at p=0.05

Formulations 1-4 as listed in Tabl

griseofulvin within the first 5 min. enables rapid absorption of the drug. In other words a good correlation exists between the high IDR and high plasma level of griseofulvin observed from coprecipitates soon after administration.

These results demonstrate that the oral absorption of griseofulvin is dissolution-rate limited. Similar results have been obtained by others (187, 51). Although a good correlation was found between the dissolution rate of tablets and C_{1hr} in humans, no correlation was found between the dissolution rate with other *in vivo* parameters by others (53).

6. SUMMARY AND CONCLUSIONS

The absorption of griseofulvin, an antifungal antibiotic, is known to be dissolution-rate limited. Its absorption is reported to be incomplete and erratic. Hence, it was selected as the model drug to study the effect of phospholipids on its dissolution and absorption.

Coprecipitate formulations were prepared by the solvent method in various weight ratios of griseofulvin:phospholipid. Physical characterization of these formulations was carried out employing DTA, TM, phase diagram analysis, powder x-ray diffraction and photomicrographic observation. The dissolution behavior of the various formulations was determined using the spin-filter dissolution apparatus in a dissolution medium of HCl-KCl pH 2.0 at 37°C. The effect of formulation on the oral absorption of griseofulvin in rats was measured and the *in vivo* absorption parameters were correlated with *in vitro* dissolution parameters. Based on these studies it is possible to draw the following conclusions.

1. A significant increase in the dissolution rate and extent was observed from the coprecipitates as compared to micronized or solvated griseofulvin or physical mixtures.
2. The dissolution of griseofulvin was dependent upon the average particle size of the coprecipitates.
3. The dissolution of griseofulvin coprecipitate containing a small fraction of DMPC (griseofulvin:DMPC 19:1) was as

dramatic as from other coprecipitates containing higher fractions of DMPC.

4. The phase diagram obtained from DTA and TM did not reveal the formation of eutectic or solid solution. DTA did not show polymorphism of griseofulvin.
5. X-ray diffraction spectra showed the presence of griseofulvin crystallites in the coprecipitates indicating no conversion to amorphous form.
6. Photomicrographs showed that the crystals under appropriate conditions fractured to release fine particles on exposure to water.
7. Photomicrographs also revealed the formation of myelinic structures (possibly liposomes).
8. Partition coefficient studies showed a DMPC concentration dependent entrapment of griseofulvin. The logK values were high at low DMPC concentration.
9. Griseofulvin:POS coprecipitates (4:1) yielded increased dissolution of griseofulvin, whereas griseofulvin:PEG 4000 coprecipitates at a 4:1 ratio did not.
10. The inclusion of various water-soluble agents in combination with DMPC and non-solvated griseofulvin did not improve the dissolution of griseofulvin.
11. The existence of griseofulvin in the solvated state was essential in order to obtain increased dissolution from coprecipitates with DMPC.
12. Phospholipids in a liquid-crystalline state at 37°C exerted a more pronounced increase in dissolution of

griseofulvin from the coprecipitates than those which existed in the gel state.

13. Incorporation of CHOL in the lipid phase with DMPC showed that despite slower initial dissolution higher amounts were in solution after 60 min. as compared to systems containing only DMPC in the lipid phase. Depending on the CHOL content these systems exhibited controlled drug delivery.

14. The dissolution behaviors of coprecipitates containing positively or negatively charged lipids were similar to that of coprecipitates containing only DMPC.

15. The kinetics of dissolution under sink conditions was adequately described by second-order kinetics or the Weibull distribution function with the exception of the griseofulvin:DMPC 1.5:1 (weight ratio) coprecipitate.

16. *In vivo* oral absorption studies gave significantly greater values of AUC, $C_{1\ hr}$, and C_{max} for griseofulvin coprecipitates than for solvated or non-solvated griseofulvin or physical mixture.

17. Significant correlation was obtained between *in vitro* and *in vivo* parameters confirming the dissolution rate-limited absorption of griseofulvin.

18. Coprecipitates performed better in the powder form than in the tablet form suggesting casule as being a suitable dosage form in case of eventual formulation.

19. The dissolution of hydrocortisone acetate:DMPC coprecipitates in the solvated state with DMF was also

increased in comparison to solvated hydrocortisone acetate.

20. The use of lipidic molecules such as lecithin or fatty esters in a coprecipitate of a solvated drug has the potential of improving its dissolution and bioavailability. However, like many solid dispersions of drugs, processing and aging reduces the effectiveness of these systems and therefore they should be freshly prepared.

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