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UNIVERSITY OF ALBERTA

PREPARATION, CHARACTERIZATION AND BIOAVAILABILITY OF DRUG:PHOSPHOLIPID SOLID DISPERSIONS

BY

GOPI KRISHNA VUDATHALA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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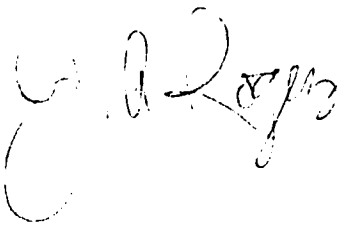

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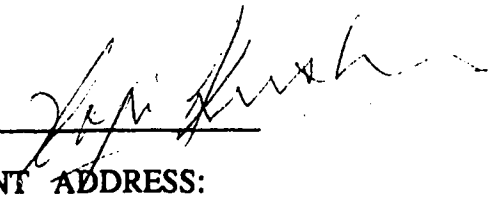
(SIGNED) 

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DEDICATED TO

MY PARENTS AND WIFE, AND

TO MY DEAR FRIEND WHO WAS KILLED IN AN INDIAN AIRLINES

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ABSTRACT

Coprecipitates of chloroform-solvated griseofulvin (Gris), dimyristoyl-phosphatidylcholine (DMPC) or egg phosphatidylcholine (EPC), and cholesterol (CHOL) have been characterized by dissolution studies, differential thermal analyses (DTA), and weight loss determinations and evaluated as a function of the storage time. Coprecipitates of Gris:lipid, 4:1 weight ratio (1:0.33 DMPC:CHOL mole ratio) exhibited maximum dissolution and the lowest extent of aging. Storage of EPC:CHOL coprecipitates up to 90 days resulted in increased dissolution of Gris. Chromatographic evidence and oxidation indices of EPC suggested that the unexpected behavior of these systems was possibly a result of the degradation of EPC. The incorporation of CHOL in phenylbutazone-PEG 6000 coprecipitates also reduced the aging of these systems, indicating the possibility of this application of CHOL to other types of solid dispersions. Coprecipitates of fludrocortisone acetate (FA) and phospholipids also exhibited increased dissolution properties with no aging after storage for four months. The inclusion of various polymers in FA coprecipitates (0.01 to 1 mole fraction % of DMPC) including polyvinylpyrrolidone (PVP), dextran or poly(L-lactic acid), altered the dissolution behavior in a controllable manner. The fusion temperatures and enthalpies of the coprecipitates varied depending on the type and concentration of polymer. The kinetics of dissolution of FA coprecipitates containing DMPC and low concentrations of PVP could best be described by the Weibull distribution function.

Microspheres of Gris coprecipitates (Gris:DMPC:CHOL) were prepared with poly(L-lactic acid) using the solvent evaporation method. The kinetics of release of Gris from microencapsulated coprecipitates was identical to that from coprecipitates alone. Coprecipitate microspheres were stable when suspended in PEG 600 for one week. Microsphere preparations flowed more freely than coprecipitate powder and

possessed better stability during processing than coprecipitates of Gris. Bioavailability studies indicated higher C_{lh} and C_{max} values for all the Gris:phospholipid compositions tested compared to the pure drug. The dissolution of 90-day aged Gris:DMPC coprecipitate was similar to that of the pure drug but the Gris:DMPC:CHOL coprecipitates showed no significant differences between the 1-day and 90-day aged samples. Good correlations between the in vitro and *in vivo* parameters were obtained.

LIST OF ABBREVIATIONS

AUC	Area Under the plasma concentration - time Curve.
$[CHCl_3]$	Chloroform concentration.
CHOL	Cholesterol.
C_{1h}	Plasma concentration 1 hour after dosing.
C_{max}	Peak plasma concentration.
DMPC	DiMyristoylPhosphatidylCholine.
DPPC	DiPalmitoylPhosphatidylCholine.
DTA	Differential Thermal Analysis.
EPC	Egg PhosphatidylCholine.
FA	Fludrocortisone Acetate.
Gris	Griseofulvin.
HPLC	High Pressure Liquid Chromatography.
IDR	Initial Dissolution Rate.
MC	Methylene Chloride.
mg	Milligram.
mL	Milliliter.
M.P.	Melting Point.
PEG 600	PolyEthyleneGlycol 600.
PVP 10	PolyVinylPyrrolidone 10,000.
PVP 24	PolyVinylPyrrolidone 24,000.
PVP 40	PolyVinylPyrrolidone 40,000.
T_c	Phase transition temperature.
t_{max}	Time to reach peak plasma concentration.
UV	Ultra Violet

CHAPTER 1

INTRODUCTION

Systemic absorption of most drug products consists of a succession of rate processes which include (1) disintegration of the drug product and subsequent release of the drug; (2) dissolution of the drug in an aqueous environment; and (3) absorption across cell membranes into the systemic circulation. The rate at which the drug reaches the blood circulation is determined by the slowest step in the sequence, which can also be termed the "rate-limiting" step. Except for sustained-release or prolonged-action products, disintegration of a solid dosage form occurs prior to drug dissolution and drug absorption. The rate at which poorly water-soluble drugs dissolve is often the slowest step and therefore exerts a rate-limiting effect on drug bioavailability (Shargel and Yu, 1985).

In the case of drugs with dissolution rate-limited absorption, reduction of particle size often increases the rate of dissolution and amount of drug absorbed. For example, the therapeutic dose of griseofulvin was reduced to 50% by micronization, and a more constant and reliable blood level was produced (Atkinson et al., 1962). Also, the dose of spironolactone could be decreased by half with just a slight reduction of particle size (Levy, 1962). More sophisticated means of physically reducing particle size has been by solid dispersion formulation (Chiou and Riegelman, 1971a). Solvent deposition onto inert solid particles has also increased the surface area available for dissolution (Yang et al., 1979). Solid dispersions have been investigated for many years. The main categories of solid dispersions include: i) eutectics, ii) amorphous precipitates, iii) solid solutions and iv) glass solutions.

Dramatic increases in dissolution of drugs have been shown from these types of formulations. For example, a 900-fold increase in the dissolution rate of chlorpropamide from dispersions of 30% w/w in urea has been reported (Ford and

Rubinstein, 1977). There have been several reports of improved dissolution of griseofulvin (Gris) from solid dispersions with inert adjuvants, such as polyvinylpyrrolidone (PVP) (Mayersohn and Gibaldi, 1966), polyethylene glycol (PEG) (Chiou and Riegelman, 1970), polyoxyethylene 40 monostearate (POS) (Kaur et al., 1980), pentaerythritol (Chiou and Riegelman, 1969), and succinic acid (Goldberg et al., 1966). The mechanisms of increased dissolution rates of solid dispersions of drugs in PVP carriers have been reviewed by Simonelli et al., (1969). The main advantages of using water-soluble polymers as carriers are their nontoxicity and general applicability to most drugs (Chiou and Riegelman, 1971a). In spite of the many apparent advantages of solid dispersions, only two products are commercially available, namely, Nabilone-PVP (Cesamet; Lilly) and Griseofulvin-PEG (Gris-PEG; Sandoz-Wander). The main problems in commercialization of these formulations are their instabilities during processing and in long-term storage. Some studies have identified and quantified these problems but attempts to reduce or eliminate them have not been addressed. This thesis describes means of slowing down or eliminating the aging process for a specific type of solid dispersion system. Thus, coprecipitates of griseofulvin and phospholipid prepared from a solvating solvent increased the dissolution of griseofulvin compared to micronized or solvated griseofulvin, or physical mixtures of griseofulvin and phospholipid (Venkataram and Rogers, 1984). The dissolution of griseofulvin from coprecipitates containing only a small fraction of a phospholipid, such as dimyristoylphosphatidylcholine (e.g. 5 percent) was increased 3-fold with only modest increases beyond this for higher phospholipid contents. It was established that solvated drug was necessary to produce these results with phospholipids (Venkataram, 1986). Also, incorporation of cholesterol at an

optimal ratio with the phospholipid was shown to decrease the initial rate of release of griseofulvin and the dissolution curve did not plateau even after 60 min (Venkataram and Rogers, 1985).

Thus far the application of phospholipids to drug solvates has involved only griseofulvin and the aging characteristics or processing characteristics of these systems have not been thoroughly examined. Hence, the overall aims of this project were to 1) investigate the applicability of this approach to other drug solvates, 2) examine ways of overcoming the aging problem, and 3) explore additional formulation means using microencapsulation to improve the handling and processing stability of these solid dispersions. The following objectives were identified to achieve these aims:

1. To study the role of cholesterol on the aging of griseofulvin in coprecipitates with phospholipids.
2. To formulate other drugs which form solvates with phospholipids, quantitate their dissolution properties, and their aging characteristics.
3. To study the effect of adding polymers to the coprecipitates in order to regulate the rate of drug dissolution i.e. release.
4. To develop and study a microencapsulated form of the solid dispersion of the drug with phospholipid.
5. To develop *in vitro-in vivo* correlations for the behavior of these formulations in an animal model e.g. the rat.

1.1 SOLID DISPERSION TECHNOLOGY

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 melting-solvent method (Chiou and Riegelman, 1971). The term 'coprecipitate' is often used to refer to a solid dispersion prepared by the solvent method. The purpose of the carrier in a solid dispersion is to present the drug in a very fine state for dissolution and to improve the dissolution by wetting, solubilization and dispersion mechanisms. Hence, the selection of carrier is crucial in obtaining the optimum results for a particular drug. Therefore, a poorly water-soluble drug combined with a water-soluble carrier generally increases dissolution whereas a water-soluble drug combined with a slightly water-soluble carrier results in slower availability of drug in solution.

1.1.1 CLASSIFICATION OF SOLID DISPERSIONS:

Solid dispersions systems have been classified (Chiou and Riegelman, 1971) as follows: 1) simple eutectic mixtures, 2) solid solutions, 3) glass solutions, 4) amorphous drug precipitates in crystalline carriers, 5) compound or complex formations between the drug and the carrier, and 6) any combinations of the above.

1.1.1.1 Simple Eutectic Mixtures:

Generally, eutectic mixtures are produced when two melted components show complete miscibility in the liquid state but negligible solid-solid solubility after rapid solidification. Thermodynamically, such a system is regarded as an intimately blended physical mixture of its two crystalline components (Goldberg et al., 1965). When a eutectic mixture of a poorly-soluble drug is exposed to aqueous solution, it

usually undergoes rapid dissolution thereby releasing the ultrafine particles of drug. The large surface area of drug exposed to the aqueous medium results in a more rapid dissolution rate and can also lead to increased solubility of the drug according to the Kelvin equation (Martin et al., 1983a). Earlier, Goldberg et al., (1966) ascertained from microthermal studies that griseofulvin formed a solid solution with succinic acid at a concentration of 25% w/w. Later, however this system was claimed by Chiou and Niazi (1973) to be a simple eutectic mixture with negligible solid solubility when examined by powder X-ray diffraction and differential thermal analysis (DTA). The dissolution rates of griseofulvin from fused compositions of griseofulvin and succinic acid were found to be inversely proportional to the concentration of griseofulvin in the dispersion, and was deduced to be primarily due to the finer crystals formed in a low concentration dispersion (Chiou and Niazi, 1976).

1.1.1.2 **Solid Solutions:**

A solid dispersion in which a solute or drug is dissolved in the carrier in the solid state is referred to as a solid solution. Sometimes, it is called a 'mixed crystal' because the two components crystallize together in a homogeneous one-phase system (Findlay, 1951). A solid solution of a poorly-soluble drug in a rapidly dissolving carrier achieves faster dissolution rates than an eutectic because the particle size of the drug in the solid solution is reduced to the minimum physical state, i.e. the molecular state (Goldberg et al., 1965). It has been shown that in a solid solution of a binary system the phase diagram is characterized by thaw and melt temperatures which coincide. For example, marked increases in the dissolution rates of sparingly water-soluble digitoxin, 17-methyl testosterone, hydrocortisone acetate and

prednisolone acetate after dispersion in PEG 6000 have been reported (Chiou and Riegelman, 1971). This is believed to be due to formation of colloidal or molecular dispersion of the drug in the carrier.

1.1.1.3 **Amorphous precipitations in a crystalline carrier:**

The drug may sometimes precipitate as an amorphous form in the crystalline carrier, unlike that in eutectics. Since the amorphous form is the highest energy form of a pure solid drug, it undergoes faster dissolution than any crystalline form. For example, amorphous Novobiocin has a ten-fold higher solubility than its crytalline form which is responsible for yielding faster dissolution rates of the drug and improved bioavailability (Mullins and Macek, 1960). It has been postulated that drugs which have high tendencies to be supercooled have greater tendencies to solidify as amorphous forms in the presence of a carrier (Chiou and Riegelman, 1971).

1.1.1.4 **Glass Solutions:**

A solid glass solution is a homogenous glassy system in which a solute dissolves in a glassy solvent. Often it is characterized by transparency and brittleness below the glass-tranforming temperature T_g. Glass systems produce weak and diffuse powder X-ray diffraction patterns whereas crystalline systems usually give strong and sharp powder X-ray diffraction peaks. If a poorly water-soluble drug is incorporated as a glass solution with a water-soluble, glass-forming carrier, the drug rapidly dissolves in the aqueous medium because of the rapid dissolution of the carrier (Chiou and Riegelman, 1969).

The bonding energy in the glass solution is more characteristic of the cohesive molecular energy of the solvent carrier, unlike that within a solid solution, in which

bonding is larger due to larger adhesive molecular energies. Therefore, theoretically, the dissolution rates of drugs in glass solutions are faster than in solid solutions. Compounds like sucrose, glucose, ethanol and 3-methyl-hexane have been shown to form glasses upon cooling from the liquid state. Furthermore, polymers possessing linear, flexible chains can also freeze into a glassy state of transparency and brittleness (Ubbelohde,1965). A marked increase in the dissolution rate of griseofulvin from its citric acid glass solution has been reported (Chiou and Riegelman, 1969).

1.1.1.5 **Compound or Complex Formations:**

Strictly, complex formation between a drug (D) and an inert soluble carrier (C), D_nC_m, does not fall within the classification of solid dispersions. Ultimately, the availability of a drug from a compound or complex depends on its solubility, dissociation constant, and the intrinsic absorption characteristics of the complex. Complex formation between griseofulvin and fatty acids have been reported (Grant and Mehdizadeh, 1984).

Complex formations are usually characterized by an enclosure of the drug molecule in the carrier molecules e.g. clathrate formation. One or more molecules of drug can be enclosed within the carrier molecules in this manner (Fromming, 1973). For example, cyclodextrins, prepared by the microbiological degradation of starch, have been used to improve the dissolution and absorption of poorly water-soluble drugs. The α-, β- and γ- forms have shown the capability to enclose several types of drug molecules, such as papaverine, ephedrine, phenylethyl barbituric acid and salicylic acid (Fromming, 1973).

1.1.1.6 **Combinations and Miscellaneous Mechanisms:**

Often a solid dispersion does not entirely belong to any of the groups discussed

above but is made up of combinations of different groups. The observed increase in dissolution and absorption rates may, therefore, be the contribution of different mechanisms (Chiou and Riegelman, 1971). For example, griseofulvin dispersed at high concentrations in PEG may exist either as individual molecules or as microcrystalline particles. Similarly, sulfathiazole dispersed at high concentrations in PVP may exist as molecular sulfathiazole and sulfathiazole-PVP complex, or amorphous and polymorphic sulfathiazole, or possibly as an amorphous sulfathiazole-PVP complex.

1.1.2 PHYSICAL CHARACTERIZATION OF SOLID DISPERSIONS

There are several methods used to physically characterize solid dispersion systems. 1) Thermoanalytical methods, including differential scanning calorimetry (DSC), differential thermal analysis (DTA), and thermal microscopy; 2) microscopic analysis, including particle light scattering and scanning electron microscopy; 3) powder X-ray diffraction; 4) spectroscopic methods, such as I.R., solid state NMR; 5) dissolution rate determination; 6) thermodynamic methods; and 7) dynamic dialysis to characterize the formation of highly supersaturated solutions after dissolution of solid dispersions (Chiou and Riegelman, 1971). Usually, no one method is entirely reliable, and a combination of two or more methods is often required to completely characterize a solid dispersion. Most frequently used methods among the above are thermoanalytical, powder X-ray diffraction and dissolution rate.

1.1.2.1 **Thermoanalytical Methods:**

1.1.2.1.1 Thermomicroscopic or Hot Stage Microscopic Analysis: A hot-stage polarized microscope was used by Goldberg et al., (1966) to generate phase diagrams

of several binary systems. The thaw and melt points are determined by visual observation. This method is simple and requires only a small amount of sample. However, it is somewhat inaccurate, is limited to thermally-stable compounds, and does not provide the thermodynamics of the melting process. The technique has been often used to support results from DTA or DSC measurements (Ford and Rubinstein, 1978).

1.1.2.1.2. Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC): These are sensitive, effective thermal methods for studying phase equilibria of pure compounds or mixtures. Differential effects associated with physical or chemical changes, are automatically recorded as a function of temperature or time as the substance is heated at a uniform rate. DTA is defined as "a technique for recording temperature differences between a substance and a reference material against either time or temperature as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate" (Pope and Judd, 1977). DSC differs from DTA in that it is "a technique for recording the energy necessary to establish a zero temperature difference between a substance and a reference material against either time or temperature, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate" (Pope and Judd, 1977). Both methods are equally suitable for qualitative applications. In addition to obtaining thawing and melting information, polymorphic transitions, energies of evaporation, sublimation, desolvation and various types of decomposition can be quantitatively detected (Bloch and Speiser, 1987). DTA has been used routinely to characterize different types of solid dispersions (Chiou and Riegelman, 1971b). In DTA, the total energy change which occurs upon

heating a sample at constant rate, ΔH, is proportional to the area under the DTA peak generated as follows:

$$\Delta H = K \int_{t_1}^{t_2} \Delta T \, dT = KA \qquad [1]$$

where **K** is the heat transfer coefficient or instrument calibration constant, **dT** is the temperature change of the sample and reference, ΔT is the temperature differential, **A** is the area of the DTA peak and **t** is the time (Borchardt and Daniels, 1957). A detailed description of the determination of ΔH is given in Appendix 5.

1.1.2.2 Powder X-Ray Diffraction Analysis:

The degree of X-ray diffraction from a sample in a powder X-ray diffractometer is measured as a function of 2θ, where θ is the angle of diffraction. The X-rays are reflected from crystal surfaces, the intensity of which is a function of the degree of crystallinity of the sample. Thus, it can provide information on the existence of polymorphs, solvates, complexes, amorphism or the extent of solid solution (Bloch and Spieser, 1987). Single crystal X-ray crystallography determines bond angles and interatomic distances whereas powder X-ray diffraction deals with crystal-lattice parameters. Changes in the diffraction pattern of the latter indicate changes in the 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 (Willard et al., 1958),

$$M\lambda = 2 d \sin \theta \qquad [2]$$

where **M** represents the order of diffraction.

X-ray diffraction techniques have been frequently used to characterize solid dispersions in conjunction with other methods, such as, thermal microscopy and DTA (Chiou, 1977; McGinnity et al., 1984).

1.1.3 DISSOLUTION

Knowledge of the dissolution behavior of solid drugs and their formulations is important in determining the relative bioavailabilities of drugs in various dosage forms. Thus, this subject has been covered in detail.

1.1.3.1 **Dissolution Theory:**

Frequently, dissolution is the rate-limiting or rate-controlling step in the bioabsorption of drugs of low solubility. The rate at which a solid dissolves in a solvent was proposed quantitatively by Noyes and Whitney in 1897. It was stated that "the rate at which a solid substance dissolves in its own solution is proportional to the difference between the concentration of the saturated solution and the concentration of that solution", and is expressed mathematically as,

$$\frac{dC}{dt} = k\,(C_s - C) \qquad [3]$$

where **C** is the concentration at time **t**, and C_s is the equilibrium solubility of the solute at the experimental temperature, **dC/dt** is the dissolution rate, and **k** is a proportionality constant. The model used assumed that a thin layer of saturated solution is formed initially at the crystal-liquid interface and the rate of dissolution is governed by the rate of diffusion from this layer to the bulk of the solution, and that there is negligible change in the surface area with time during dissolution. The

surface area (S) was then incorporated into Eq.(3) to give

$$\frac{dC}{dt} = k_1 S\,(C_s - C) \qquad [4]$$

where $\mathbf{k_1}$ is the intrinsic dissolution rate constant. Brunner (1904) and Nernst (1904) applied Fick's Law of Diffusion to establish a relationship between the constant (k_1) in Eq.(4) and the diffusion coefficient of the solute, thus transforming Eq.(3) to:

$$\frac{dC}{dt} = \frac{DS}{Vh}\,(C_s - C) \qquad [5]$$

where **D** is the diffusion coefficient of the solute, **V** is the volume of the dissolution medium, and **h** is the thickness of the diffusion layer. This has been referred to as the "Nernst-Brunner Film Theory" of dissolution (Wurster and Taylor, 1965). Other dissolution theories have been reviewed extensively by Higuchi (1967), Wagner (1971), Leeson and Carstensen (1974).

Hixson and Crowell (1931) derived an equation for the dissolution of drug powders consisting of uniformly sized particles that expresses the rate of dissolution based on the cube root weight of particles ("Hixson-Crowell Cube Root Law") (Martin et al., 1983b). Other dissolution models of interest which have been developed include Danckwerts model (1951), the convective-diffusion model (Neilsen, 1961), the Higuchi-Heistand model (1963), and the multiparticulate dissolution model (Pedersen, 1977).

1.1.3.2 **Dissolution Methods:**

There has been an abundance of literature dealing with dissolution methods since 1960. An extensive survey of dissolution apparatus' was made by Pernarowski in

1974, but since then many attempts have been made to develop a reliable, reproducible and efficient method to measure in vitro dissolution parameters that could effectively describe the bioavailabilities of drugs. A dissolution requirement was first introduced into the USP XVIII and NF XIII in 1970 and since then dissolution tests have superceded disintegration tests in most drug monographs. Recognition of variations in bioequivalence or bioavailability of some drug products have provided added impetus to the adoption of dissolution testing in product development, quality assurance, and compendial standards.

A. Rotating Basket Method (Method 1):

This method was adopted by the USP and NF in 1970 as the first official compendial dissolution test method. The details and specifications of this method are described in the USP XXI.

B. Paddle Method (Method 2):

This method was introduced in the USP XX in 1980 where a full description can be found. The USP now specifies those drugs for which dissolution tests are required in its monographs.

Although numerous dissolution test apparatus' are reported in the literature, many, including those adopted by the official compendia, do not meet all of the criteria as stated by Shah et al., (1973). For instance, the nature and extent of solvent agitation, the effect of the apparatus on the test sample during the dissolution study, and liquid sampling techniques used in these systems can influence the dissolution and the reliability of the method. Recently, a standardized Flow-cell Method, official in the European Pharmacopoeia, has been suggested as an alternative to existing Pharmacopoeial dissolution testing methods (Langenbucher et

al., 1989).

One of the methods recommended for approval by the FDA (Cox et al., 1979) and widely applied in formulation development is the "Spin-Filter Stationary Basket" apparatus developed by Shah et al., (1973). Essential features of the apparatus are a stationary sample basket, a large volume fluid container, and a rotating filter assembly. The rotating filter system functions as a liquid agitation device as well as an efficient fluid sampling system. Some of the major advantages of the apparatus are (a) precision-controlled variable intensity of mild laminar liquid agitation; (b) continuous or intermittent filtration of representative samples through an *in situ* microporous, non-clogging rotating filter, (c) minimal mechanical impacts, abrasion and wear of the solid sample with unaltered microenvironment, and (d) simultaneous determinations of disintegration - dissolution rates of capsules and tablets. The spin-filter apparatus has been found to provide a reliable and convenient means for determining in vitro dissolution parameters of tablets, capsules, powders, suspensions and most other solid dosage forms (Nelson and Shah, 1974, Carstensen et al., 1978).

1.1.3.3 **Factors Affecting Dissolution Rate:**

Those factors affecting dissolution rate are described by the Nernst-Brunner equation (Eq. 5, p.13). However, a variety of environmental factors must be controlled or known. This subject has been reviewed by Hoener and Benet, (1990).

1.1.4 PHOSPHOLIPIDS AS EXCIPIENTS IN SOLID DOSAGE FORMS

The effect of lipids on drug dissolution in the GI tract has been addressed to some extent previously (Bates and Sequeira, 1975). However, there are reports on the specific application of phospholipid (lecithin) to alter the dissolution behavior of

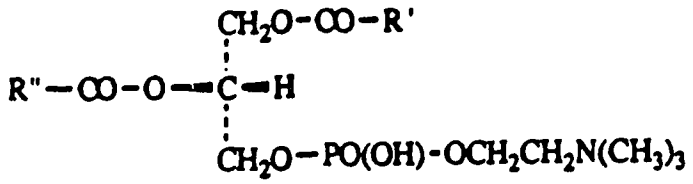
poorly water-soluble drugs. Improved bioavailability of griseofulvin in rats was observed after oral administration of a griseofulvin suspension containing 0.5% lecithin (Duncan et al., 1962). The improved dissolution (Venkataram and Rogers, 1984), release characteristics (Venkataram and Rogers, 1985) and bioavailability of griseofulvin from griseofulvin-phospholipid coprecipitate systems have been demonstrated (Venkataram, 1986). Recently, the improved solubilities of indomethacin, ketoprofen and flurbiprofen have been reported from (1:2 mole ratio) coprecipitates with phosphatidylcholine (Fujii et al., 1988a). These authors also reported significant increases in the dissolution behavior and rat plasma concentrations of (1:3 mole ratio) phenytoin-phosphatidylcholine solid dispersions (Fujii et al., 1988b). These effects were considered to have occurred as a result of the existence of amorphous drug in phosphatidylcholine. The dissolution of griseofulvin from griseofulvin-hydrogenated soya phospholipid coprecipitates was reported to be accelerated as a result of a decrease in crystallinity of griseofulvin and possible aggregation of phospholipid with griseofulvin (Nishihata et al., 1988).

Phospholipids are one of the major structural components of cell membranes. A comprehensive list of the distribution of phospholipids in cell membranes is available (Hawthorne and Ansell, 1982). Phosphatidylcholine (PC) was first isolated from egg yolk and brain (Gobley, 1850).

The chemical name of phosphatidylcholine is:

1, 2-diacyl-sn-glycero-3-phosphocholine.

Its molecular structure is represented as follows (next page):



where R' and R" are the fatty acyl substituents. In phosphatidylethanolamine and phosphatidylserine, the choline moiety is replaced by ethanolamine and serine, respectively. Other phospholipids that occur in tissues include phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylglycerol (PG). Lysolecithin (where R' or R" = OH) is a significant component of blood plasma but occurs in tissues at very much lower levels than lecithin. In most naturally occurring lecithins the R' position is a saturated fatty acid and R" is an unsaturated fatty acid, but there are notable exceptions (Hawthorne and Ansell, 1982).

Bilayers composed of a single phospholipid species undergo a well-defined thermotropic phase transition (in the fully hydrated state) in which the lipid chains change from an ordered gel state to a fluid or liquid crystalline state. The temperature at which this occurs is called the "phase transition temperature" (T_c). Determination of the T_c is possible using DTA or DSC. The transition temperatures and phase behavior differ with composition but each depends strongly on the water content. With increasing water content, the transition temperature decreases progressively, reaching a limiting value at 25-30 wt% water (Cevc and Marsh, 1987).

Also, the phase transition entropy and enthalpy are found to depend approximately linearly on the fatty acid chain length. Below the phase transition temperature, the fatty acyl side chains are in a closely packed, relatively ordered,

extended all-trans conformation giving rise to the 'gel state'. As the temperature rises, the packing of the fatty acyl chains decreases and over a short temperature range (1-2°) the molecules become highly disordered (T_c). As the temperature is increased above the T_c, the phospholipid molecules again assume a regularly ordered arrangement and have the properties of a liquid-crystalline state. The extent of the molecular motions can be detected by electron spin resonance (ESR) or nuclear magnetic resonance (NMR) probes and the fluidity can be monitored by fluorescence depolarization analysis using a suitable fluorescent probe. The T_c is a function of the nature of the fatty acyl chains, and the polar head group.

Phospholipids have seldom been used in solid dosage forms, although it has been known for some time that under appropriate conditions, they are able to spontaneously form liposomes from a solid film and entrap polar or nonpolar molecules. 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 (Venkataram, 1986).

1.1.4.1 **Griseofulvin-Phospholipid Coprecipitates**

Recent studies have demonstrated the potential of improving the solubility, dissolution and bioavailability of griseofulvin from griseofulvin-phospholipid coprecipitate systems (Venkataram, 1986). The dissolution rate of griseofulvin from coprecipitates containing as little as 5% DMPC was 3-fold greater than that of micronized or solvated griseofulvin or its physical mixture in pH 2.0 HCl-KCl buffer at 37°C. No eutectic or solid solution formation in these coprecipitates was indicated from the phase diagram. X-ray diffraction spectra indicated the presence of griseofulvin crystallites in the coprecipitates. The existence of griseofulvin in the

chloroform-solvated state was deemed essential for enhancement of dissolution. The improved dissolution rate from coprecipitates has been thought to occur from metastable drug-phospholipid-chloroform crystals as a result of the rapid hydration of lecithin at the crystal surfaces resulting in rapid, extensive disintegration into extremely fine particles of drug and formation of myelinic structures at the crystal surfaces which are thought to contain entrapped griseofulvin. The result is an effective increase in the saturation concentration of drug in the theoretical diffusion layer during the dissolution process (Eq.5) (Venkataram and Rogers, 1984). However, the performance of the coprecipitates was found to be affected by processing and aging of the coprecipitates. Thus, aged coprecipitates were found to undergo a slower rate of dissolution compared to fresh samples. The oral administration of freshly-prepared coprecipitates to rats yielded a significant increase in the relative bioavailabiiity of griseofulvin which correlated with the dissolution behavior (Venkataram and Rogers, 1988). When part of the phospholipid (in the coprecipitates) was replaced with cholesterol, the initial dissolution rates decreased whereas the fraction dissolved after 60 minutes increased. In this manner, it was suggested that adjustment of the cholesterol content could be a means of controlling the rate of release of griseofulvin from these systems (Venkataram and Rogers, 1985).

1.1.5 PHYSICAL STABILITY OF SOLID DISPERSIONS

Although the solid dispersion appears to be a potentially useful dosage form to increase dissolution and absorption rates of poorly water-soluble drugs, unfortunately it is often physically unstable during storage under various conditions. Surprisingly,

the effects of aging on their fast-release characteristics and physical stabilities have not been examined in detail.

1.1.5.1 **Aging Effects of Eutectic Mixtures:**

Dispersed-phase particles tend to coarsen on aging because the interfacial energy of the system is reduced by the concomitant reduction in interface area (Graham and Kraft, 1966). This phenomenon has been known to occur in eutectic systems, with or without solid solution formation. The extent of coarsening increases with time and aging temperature. Hence, the dissolution rates from the fused eutectic of phenobarbitone-urea were reported to decrease with age due to the coarsening of eutectic particles (El Banna et al., 1974). In a different manner phenylbutazone oxidized to oxyphenbutazone in solidified phenylbutazone-urea melts after storage due to an in situ increase in the pH. In another example, studies on the sulfathiazole-urea solid dispersion system have shown that aging of the eutectic mixture at 105° for 1 hour reduced the dissolution rate of sulphathiazole, due in part to the conversion of the metastable form II polymorph to the stable form I, and also to conversion of amorphous sulfathiazole to crystalline form II polymorph (Chiou and Niazi, 1971).

1.1.5.2 **Aging Effects of Solid Solutions:**

A major aging problem associated with solid solutions is the precipitation of drug from supersaturated solid solutions concomitant with physicochemical changes which take place (Chiou and Riegelman, 1971). Precipitation occurs when the concentration of the solute exceeds its equilibrium solubility. Chiou (1977) proposed that an amorphous form of griseofulvin precipitated from griseofulvin-PEG 6000 melts after cooling and that aging of these formulations depended on the

concentration of griseofulvin in the system. Similarly, indomethacin was found to age in indomethacin-PEG 6000 solid dispersions (Ford and Rubinstein, 1979). Aging of these systems markedly reduced their dissolution rates but the changes taking place were complex and dependent on drug concentrations and storage conditions. In contrast, no significant changes in dissolution rate, X-ray diffraction spectra or scanning electron micrographs of hydrocortisone-PEG 4000 systems were observed on aging (Hajratwala and Ho, 1984). Aging had not been considered a problem in studies on drug-polyethylene glycol solid dispersions (Ravis and Chen, 1981) and recent studies on freshly-prepared and aged tolbutamide-polyethylene glycol solid dispersions appear to support this (Alonso et al., 1988). However, it should be noted that aging may be important for some drug-PEG systems but not others and further studies are required to establish the same.

1.1.5.3 **Aging Effects of Glass Solutions:**

Storage of glass solutions of iopanoic acid or chloramphenicol palmitate-PVP 10,000 (5% w/w) dispersions at ambient temperatures over several months resulted in some degree of crystallization (Chiou and Riegelman, 1971). Likewise, recrystallization of griseofulvin in griseofulvin-PVP 30 dispersions (60% w/w) up to 92% was reported after storage for 23 d at 65% RH (Fromming and Hosemann, 1985). Aging of glass solutions has been shown to cause variable effects, for example, an initial decrease in dissolution rate of 60% chlorpropamide-urea melts after 2 h aging of the metastable glass solid reported by Ford and Rubinstein (1977) was attributed to partial crystallization of the drug. However, after aging for 4 days, the dissolution rate increased. Likewise, Hajratwala and Ho (1984) observed an initial decrease in the dissolution rate of hydrocortisone from hydrocortisone-PVP

solid dispersions followed by an increase after longer storage times. In both cases, as more of the drug was converted to the amorphous state (X-ray data) from the glassy state, aging revealed an increased dissolution rate. On the other hand, Merkle (1982) explained the differences in dissolution from freshly prepared and aged hydrocortisone-PVP glassy solids by lower thermodynamic activities of the resulting supersaturated solutions due to self-association of the drug molecules.

1.1.5.4. **Aging Effects of Metastable Forms:**

As is well known, drugs existing as metastable polymorphs can undergo transformations to more stable forms which usually have lower solubilities and dissolution rates. Thus, metastable forms of drugs prepared as solid dispersions will behave in a similar manner (Haleblian and McCrone, 1969; Haleblian, 1975). Fairly rapid (i.e. 24 h) transformations between the metastable and stable crystalline forms have been observed in solidified melts of griseofulvin and Pluronic F68 (Fromming et al., 1981). In spite of this, however, increased dissolution rates were observed from these microcrystalline systems.

1.1.5.5 **Aging Effects of Inclusion Compounds:**

Pure urea inclusion complexes are relatively stable in the solid state. They can be stored at a relative humidity of 70-75% without noticeable deterioration. Tablets of urea inclusion compounds prepared with water-free additives have been reported to be stable for some years (Fromming and Hosemann, 1985). However, the presence of starch or lactose and high water contents have resulted in fast rates of deterioration of the included compound. In comparison, solid cyclodextrin inclusion compounds are very stable and are unaffected by high humidity, water content, or additives. As an example, the inhibition of photo-oxidation of clofibrate included in

β- and γ- cyclodextrin has been reported (Uekama et al., 1983).

1.1.5.6 **Aging of Griseofulvin-Phospholipid Coprecipitates:**

The griseofulvin:DMPC (4:1 w/w) coprecipitate has been shown to age as observed from the gradual reduction in the dissolution rates and extent with time (Venkataram, 1986). Thus, the dissolution profile of griseofulvin from the coprecipitate was similar to that of griseofulvin alone after aging for 112 days. X-ray diffraction evidence of the aged sample demonstrated conversion to a crystalline structure not unlike that of pure griseofulvin. Desolvation of the chloroform-solvated griseofulvin in the coprecipitate was established as being the cause for the decreased dissolution in the aged sample (Venkataram and Rogers, 1984).

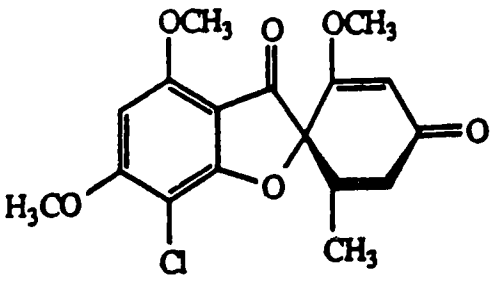
1.2 SELECTION OF MODEL DRUGS

Many drugs exhibit poor bioavailability characteristics, the reasons for which include: poor dissolution due to low water solubility, high first-pass effects, and degradation at physiological sites of absorption. Examples of drugs known to show erratic absorption behavior include: griseofulvin (Lin and Symchowicz, 1975), digoxin (Reddy et al., 1976), ergotamine (Anderson et al., 1981), phenytoin (Pentikainen et al., 1975), reserpine (Stoll et al., 1969), and sulfamethoxazole (Sekikawa et al., 1982). These drugs are, therefore, good candidates for studying the effects of formulation on bioavailability.

1.2.1 **Griseofulvin:**

Chemical Name: 7-chloro-2',4,6-trimethoxy-6'β-methylspiro {benzofuran-2(3H), 1'-[2]-cyclohexene}-3,4'-dione.

Chemical Structure:



Griseofulvin is a systemic, antifungal antibiotic of low water solubility which has been reported to undergo dissolution rate-limited oral absorption (MacKaman, 1980, Yamamoto et al., 1974., Atkinson et al., 1962 and Rowland et al., 1968). Its absorption is also influenced by gastric emptying rate (Jamali and Axelson, 1977). It is erratically and incompletely absorbed after oral administration and variations in absorption range from 27% to 72% (Rowland et al., 1968). 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 (Bates et al., 1977).

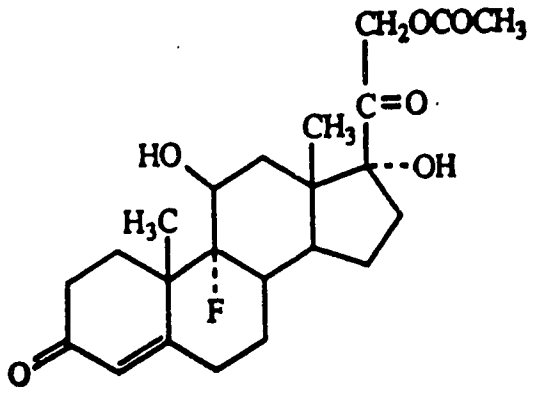
Factors mainly influencing the absorption of griseofulvin include host species variations (Lin and Symchowicz, 1975; Chiou and Riegelman, 1970, 1971b), the dosage regimen (Atkinson et al., 1962), fat intake (Kabasakalian et al., 1970; Crounse, 1963; Ogunbona et al., 1985), and formulation effects (which are discussed in detail in subsequent sections). Bates et al., (1975) reported an improved dissolution rate and oral absorption from the griseofulvin-chloroform solvate compared to the non-solvated micronized griseofulvin following oral administration

to humans. This is contrary to that found in rats by Venkataram and Rogers (1988). Solvates are crystalline forms of drugs combined with one or more molecules of solvent in the crystal lattice (Haleblian, 1975). It has been determined that the existence of griseofulvin in the solvated state was essential in order to obtain increased dissolution from coprecipitates containing phospholipids (Venkataram and Rogers, 1985). Non-solvated griseofulvin coprecipitated with phospholipid had no significant advantage compared to physical mixtures or the drug alone. The mechanisms involved in the dissolution of solvated griseofulvin from coprecipitates containing phospholipid have been described by Venkataram and Rogers (1984).

1.2.2 **Fludrocortisone Acetate:**

Chemical Name: 21-Acetoxy-9α-fluoro-11β, 17α-dihydroxy pregn-4-ene-3, 20-dione.

Chemical Structure:



Proprietary Names: Alfa-Fluorone, Alflorone acetate, Florinef acetate, F-Cortef acetate, Scherofluron.

Kuhnert-Brandstätter et al., (1968, 1971) studied extensively the solvates of steroids. Among these, estradiol was found to form solvates with 30 solvents which

were tested. Other steroids forming solvates include hydrocortisone acetate-dimethyl formamide (Shell, 1955), deoxycorticosterone acetate-dimethyl formamide (Kuhnert-Brandstätter et al., 1971) fluprednisolone-tert-butylamine (Haleblian et al., 1971), the tert-butylacetates of prednisolone and hydrocortisone (Biles, 1963), fluocortolone-ethanol or propanol (Kuhnert-Brandstätter et al., 1971) and fludrocortisone acetate-ethyl acetate or pentanol (Shefter and Higuchi, 1963).

Fludrocortisone acetate is a powerful synthetic steroid with both mineralocorticoid and glucocorticoid activity (Whitworth et al.,1983). It acts predominantly as a glucocorticoid in the rat and as a mineralocorticoid in man and sheep (Distler and Phillip, 1976). The physiologic action of fludrocortisone is similar to that of hydrocortisone, however, the effects of fludrocortisone, particularly with respect to electrolyte balance and carbohydrate metabolism, are considerably more pronounced and prolonged. In small oral doses, it produces marked sodium retention and increased potassium excretion. It also causes a rise in blood pressure, apparently because of these effects on electrolyte levels. In larger doses, fludrocortisone inhibits endogenous adrenal cortical secretion, thymic activity and pituitary corticotropin excretion, promotes the deposition of liver glycogen; and, unless protein intake is adequate, induces negative nitrogen balance (Compendium of Pharmaceuticals and Specialities, 1988). It is indicated in partial replacement therapy for primary and secondary adrenocortical insufficiency in Addision's disease and for the treatment of salt losing adrenogenital syndrome. It has been suggested that the lowest possible dose of this corticosteroid be used in order to control the condition being treated, because of its variety of adverse effects.

Fludrocortisone acetate was chosen as a model drug to be coprecipitated with

phospholipids due to its poor aqueous solubility (40 mg/L) and its tendency to form solvates with pentanol and ethyl acetate (Shefter & Higuchi, 1963). A three-fold increase in dissolution of its ethyl acetate solvate has been observed within 7 minutes compared to the pure drug. Ethylacetate is a relatively innocuous solvent, lying on the borderline between toxicity classes 2 and 3 (Gosselin et al., 1984) while ethyl alchohol has a toxicity rating of 2.

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CHAPTER 2

EFFECT OF CHOLESTEROL ON THE AGING OF GRISEOFULVIN-PHOSPHOLIPID COPRECIPITATES

2.1. INTRODUCTION

The bioavailability and pharmacodynamics of many drugs can be advantageously modified and controlled by formulation into appropriate dosage forms. In this regard, the bioavailability of poorly water-soluble drugs has been shown to be improved by the co-administration of a lipid which increases the rate of dissolution (Bloedow and Hayton, 1976). Recently, coprecipitates of solvated griseofulvin and lecithin have been shown to increase both the initial dissolution rate and the amount of Gris dissolved (Venkataram and Rogers, 1984) and to improve the bioavailability of Gris after administration to rats (Venkataram and Rogers, 1988). Furthermore, it was discovered that substitution of up to about one-third of the lecithin with cholesterol decreased the initial dissolution rate of griseofulvin but increased the amount dissolved after 60 min (Venkataram and Rogers, 1985).

Serious limitations to the commercial use of coprecipitates, as well as several other solid dispersion systems reported in the literature, are their sensitivities to processing and storage resulting in loss of the solid dispersion advantages (Ford and Rubinstein, 1977, 1979, 1981; Khalil and Mortada, 1978; Merkle, 1982; Hajratwala and Ho, 1984; Vila-Jato and Alonso, 1986; Alonso et al., 1988; Fromming and Hosemann, 1985). These coprecipitates exist in a highly energetic state and solvates, in particular, can lose weight due to a slow release of solvent to the atmosphere. Based on earlier results with cholesterol, it seemed possible to reduce the aging

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characteristics of solid dispersions with the addition of this lipid. Our objectives, therefore, were to reduce the loss of solvent from griseofulvin:lecithin coprecipitates and at the same time maintain approximately the same level of dissolution behavior after extended storage periods.

2.2. EXPERIMENTAL

2.2.1. Materials

Micronized griseofulvin (Gris) (Glaxo, Canada, 99.3%), phenylbutazone (PBZ) (Novopharm Ltd.), L-α-egg phosphatidylcholine (EPC, extracted from eggs according to Singleton et al., 1965) and recrystallized from diethyl ether until chromatographically pure), L-α-dimyristoylphosphatidylcholine (DMPC, approx. 99%), polyethylene glycol (PEG, MW 6000) and cholesterol (CHOL, 99+%) (Sigma Chemical Co.) were used as received. All other reagents and solvents were of analytical grade. Demineralized, distilled water from an all-glass still was used to prepare the dissolution media.

2.2.2. Preparation of Coprecipitates

Solid dispersions of Gris:lipid (4:1 weight ratio) and various lecithin:CHOL mole ratios were prepared by the solvent evaporation method from chloroform (40°C) under a gentle stream of nitrogen. Traces of chloroform were removed under vacuum for 12-16 h then the coprecipitates were weighed and stored in a desiccator over anhydrous calcium sulfate at room temperature (24°C). Samples were removed after 1, 10, 30, and 90 days and examined for weight loss, Gris content by UV spectrophotometric analysis, and the chloroform content was calculated by difference.

PBZ- PEG 6000 solid dispersions containing lipids were prepared by the melting method. Subsequently, an 80/120 mesh (U.S. standard sieves) sample was taken for dissolution and differential thermal analysis (DTA).

2.2.3. **Dissolution Studies**

The spin-filter dissolution test apparatus (Magne-drive, Clow Coffman Industries, Kansas) equipped with a 1 micron nominal porosity stainless steel filter screen was used under the following conditions: 500 rpm filter rotation speed; 900 ml dissolution medium consisting of pH 2.0 HCl-KCl buffer solution (Sorensen's phosphate buffer, pH 7.4 for PBZ), 37°C. This apparatus can be used advantageously to provide a reliable and convenient means for determining in vitro dissolution characteristics of powders (Shah et al., 1973).

Samples of formulations equivalent to 50 mg Gris or PBZ were dispersed in the dissolution medium. The dissolution medium was circulated through a microcell in a Beckman Model 25 spectrophotometer (λ = 293 nm Gris/ 264 nm PBZ) and concentrations of drug as a function of time were determined from absorbance readings and a calibration curve. The presence of small amounts of chloroform, DMPC, CHOL or other lipids (triolein, palmitic acid) in the dissolution medium did not interfere with the analysis of Gris or PBZ, respectively. Light scattering from submicron size particles, if any, did not affect the absorbance readings. Experiments were carried out in quadruplicate. Variation of results was less than 7 % of the mean value in all cases.

2.2.4. Differential Thermal Analysis

A sample equivalent to 10 mg Gris was hermetically sealed in an aluminum pan and subjected to a heating rate of 10°C/min (Fisher Thermalyzer, Series 300 QDTA, Fisher Scientific Co.) with an empty pan as reference. Thermograms were run at differential temperature sensitivity of 0.3°C/in. The reference temperature sensitivity was 13.7°C/in. Heats of fusion were determined from peak areas (planimeter method) and calibration coefficients derived from fusion temperatures and the calibration curve. The averages of triplicate determinations were obtained even though variations from the mean were less than 5 percent.

2.3. RESULTS AND DISCUSSION

2.3.1. Gris:DMPC Compositions

The aging characteristics of various coprecipitates of Gris:DMPC:CHOL at a drug:lipid weight ratio of 4:1, but containing various mole ratios of DMPC:CHOL, are illustrated in Fig. 2.1A and 2.1B. The dissolution profiles typically describe an initial rapid dissolution rate followed by a gradual leveling off after about 20 to 40 min. It is apparent from these data that Gris underwent increased dissolution from coprecipitates that contained DMPC compared to the pure drug, but compositions which included CHOL in the lipid component at a 1:0.33 mole ratio caused the dissolution of Gris to approximately double. However, as the CHOL content increased the fraction of Gris dissolved after 120 min decreased and Gris:CHOL coprecipitates at a 4:1 weight ratio exhibited dissolution behavior similar to that of pure or solvated Gris, although the initial dissolution rate (5 min) was slightly

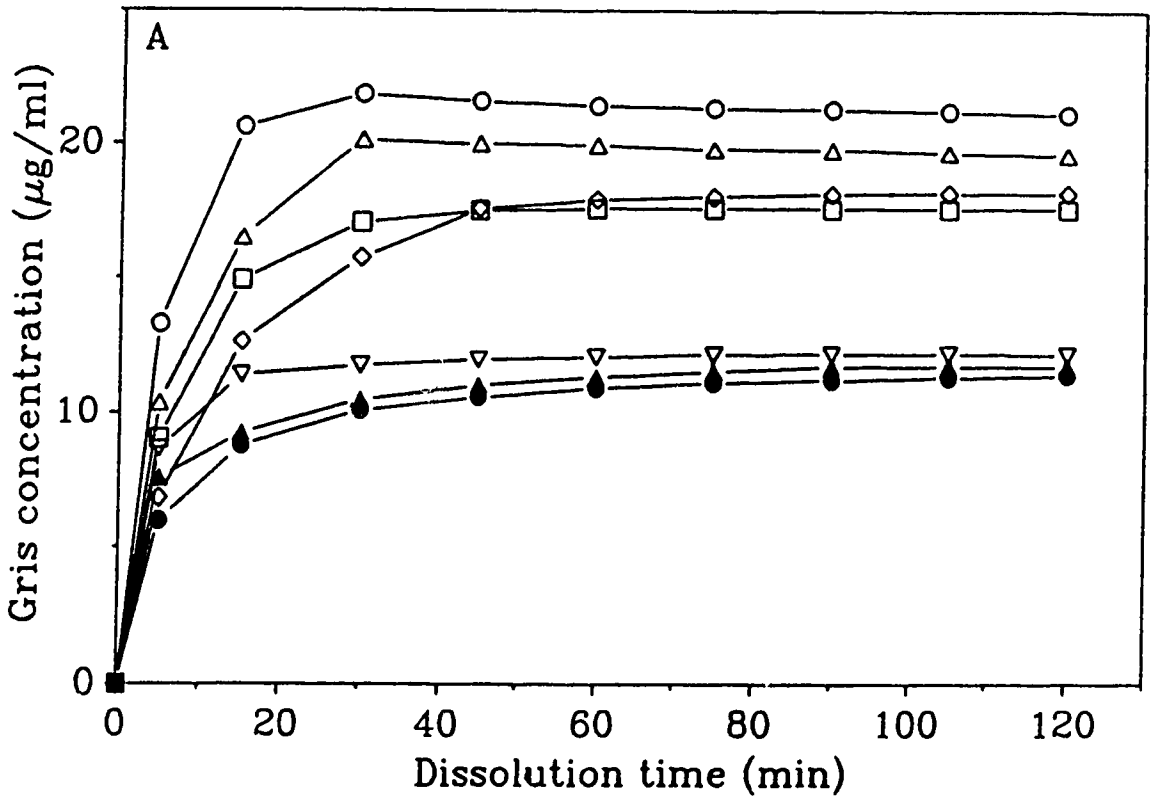

Figure 2.1A Dissolution of Gris:DMPC:CHOL coprecipitates after aging for one day. ▵ , 4:1(1:0); ○ , 4:1(1:0.33); □ , 4:1(1:1); ◇ , 4:1(1:3); ▽ , 4:1(0:1); ▲ , chloroform-solvated Gris; ● , micronized Gris. The weight ratio Gris:lipid was constant but the mole ratio DMPC:CHOL (in brackets) was varied in the coprecipitates.

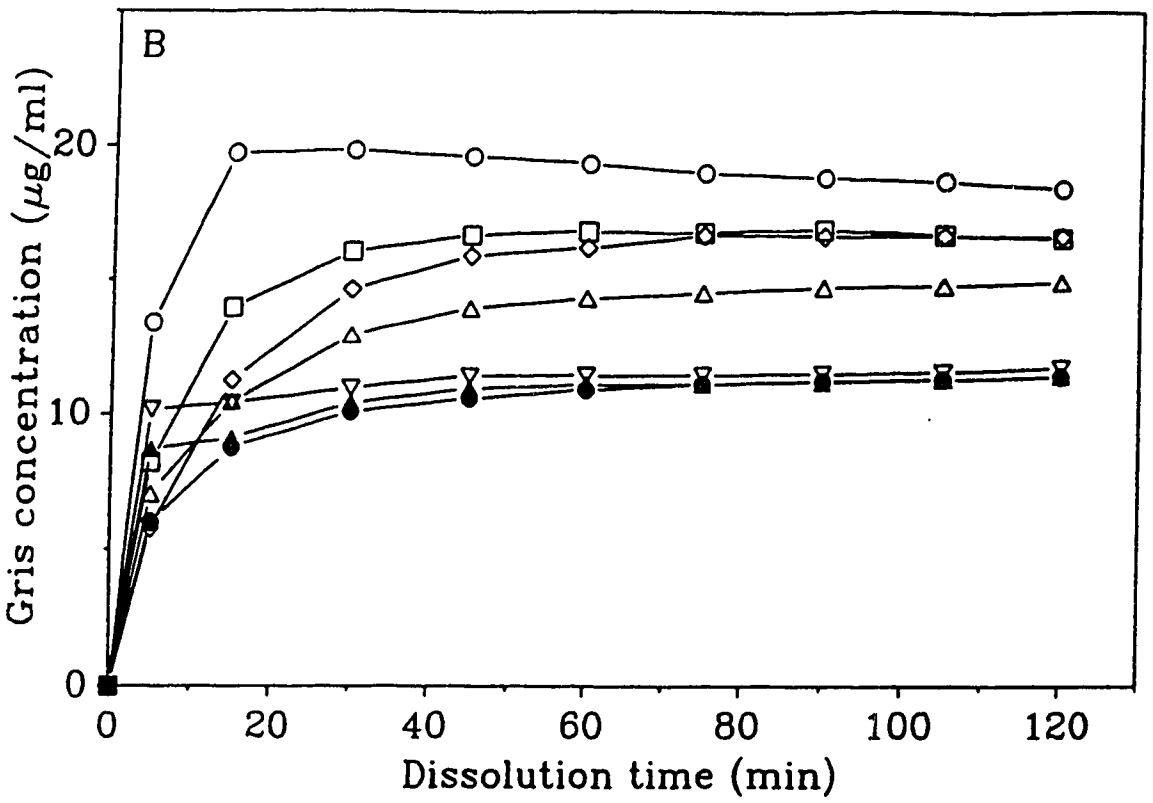

Figure 2.1B Dissolution of Gris:DMPC:CHOL coprecipitates after aging for 90 days. ▵, 4:1(1:0); ○, 4:1(1:0.33); □, 4:1(1:1); ◇, 4:1(1:3); ▽, 4:1(0:1); ▲, chloroform-solvated Gris; ●, micronized Gris. The weight ratio Gris:lipid was constant but the mole ratio DMPC:CHOL (in brackets) was varied in the coprecipitates.

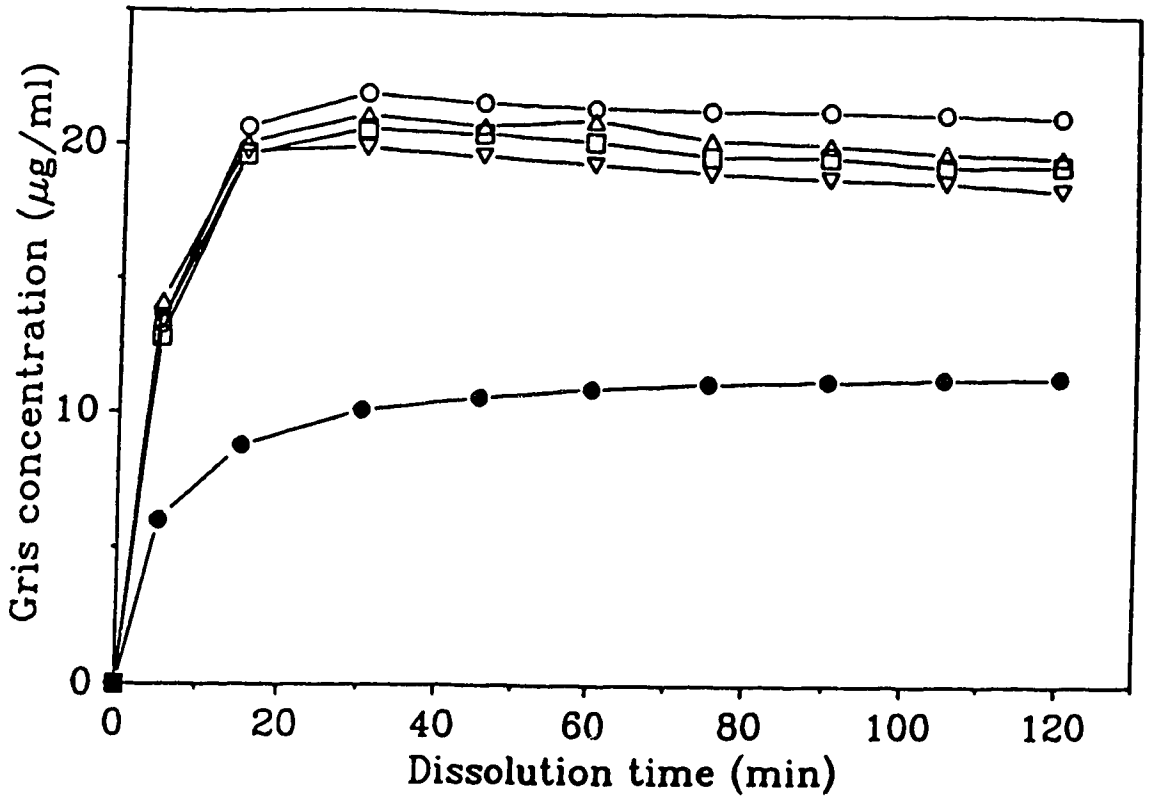

Figure 2.2 Effect of aging at room temperature on the dissolution of Gris:DMPC:CHOL 4:1(1:0.33) coprecipitates. ○ , 1 day; △ , 10 days; □ , 30 days; ▽ , 90 days; ● , micronized Gris (shown for reference).

TABLE 2.1 Comparison of the dissolution of Gris from Gris:DMPC:CHOL (Gris:lipid, 4:1 w/w) coprecipitates aged for 1 or 90 days.

DMPC:CHOL (mole ratio)	Maximum % Dissolved (2 h) (Mean±SD; n=4) 1 day	90 days	% Change	A[a]	B[b]
1:0	38.9±1.3	22.7±0.9	-42	S	--
1:0.05	36.6±0.4	23.9±0.8	-35	S	I
1:0.20	46.8±2.3	38.6±0.9	-18	S	S
1:0.33	43.9±1.5	36.9±1.5	-16	I	S
1:1	33.4±1.3	29.2±2.0	-13	I	S
1:3	33.9±2.2	28.5±1.9	-16	I	S
0:1	21.8±0.1	18.9±0.9	-13	S	S
EPC:CHOL					
1:0.33	53.7±3.2	72.0±1.2	+34	S	--

[a]Statistical analysis between 1 and 90 days among coprecipitates.

[b]Statistical analysis at 90 days between coprecipitates containing CHOL versus control (1:0). S and I indicate significant and insignificant differences, respectively, using the paired Student's t test at $P<0.05$.

greater. A comparison of Fig. 2.1A and 2.1B also indicates that aging of samples for 90 days did not significantly alter the dissolution profiles obtained from pure Gris, solvated Gris or Gris:CHOL (4:1 weight ratio) coprecipitates. In contrast, aging of Gris:DMPC (4:1 weight ratio) coprecipitates caused the dissolution of Gris, in terms of the maximum percent dissolved, to decrease by 42 percent (Table 2.1). However, when CHOL was included, at a DMPC:CHOL 1:0.33 mole ratio, aging was considerably reduced (Fig. 2.2) and caused only a 16 percent decrease in dissolution of Gris which was determined to be statistically insignificant ($P > 0.05$). Likewise, coprecipitates in which the DMPC:CHOL mole ratio was 1:1 or 1:3 did not show significant aging effects. Thus, inclusion of CHOL had the apparent effect of stabilizing the coprecipitates during the aging process.

Since the coprecipitates contain chloroform bound within the crystals, which participates in the crystalline structure together with phospholipid (Venkataram and Rogers, 1984), the loss of chloroform with time would be expected to be a factor in aging of the coprecipitates. Figure 2.3 shows residual chloroform content and the rates of loss of chloroform from the coprecipitates of various compositions with time. It is apparent that the Gris:DMPC:CHOL 4:1(1:0.33) coprecipitate retained the largest amount of chloroform (approximately 23 percent). All formulations, except Gris:CHOL 4:1 weight ratio, underwent an initial rapid loss of chloroform, but thereafter, underwent very little loss of chloroform, except for Gris:DMPC 4:1 and Gris:CHOL 4:1 weight ratio compositions, which continued to lose chloroform at a more rapid rate. Table 2.2 gives the fusion temperatures, heats of fusion, chloroform

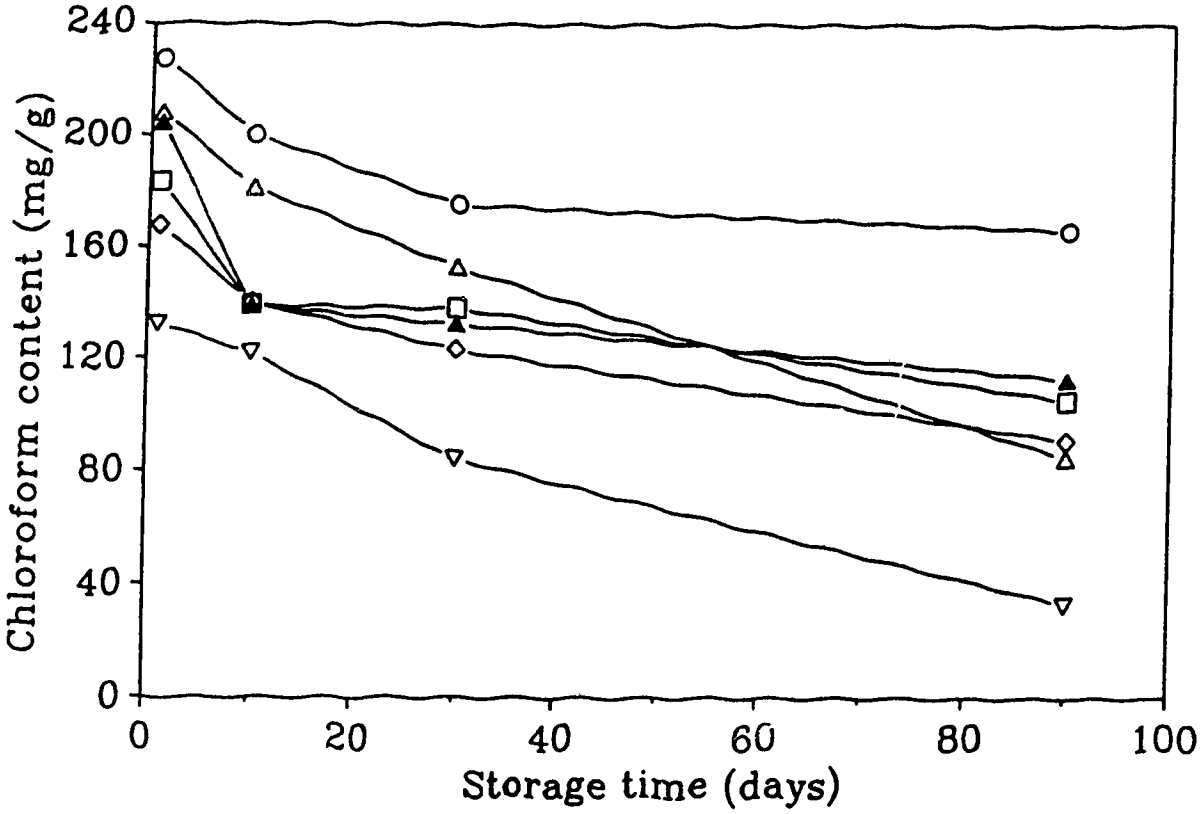

Figure 2.3 Plot of residual chloroform content in Gris:DMPC:CHOL coprecipitates as a function of storage time. Symbols are the same as in Fig. 2.1A.

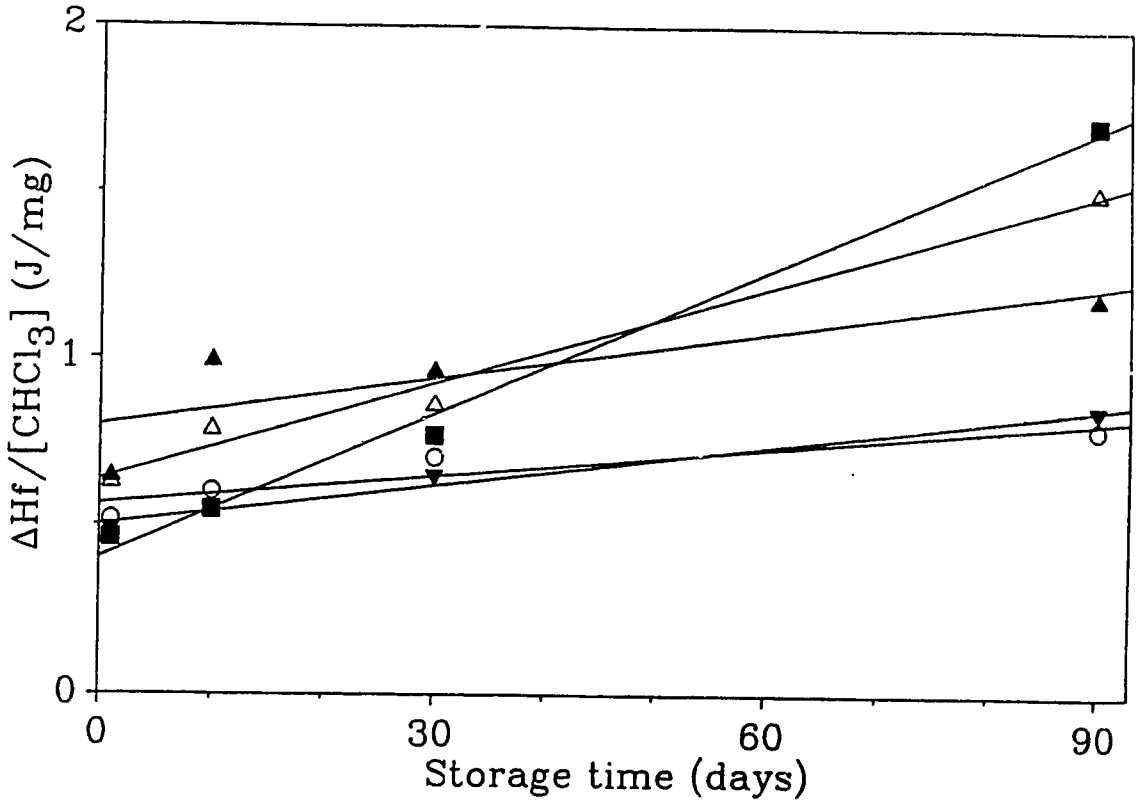

Figure 2.4A Plot of the aging factor, defined as $\Delta H_f/[CHCl_3]$ (J/mg), as a function of the storage time of: ▲ , Gris solvate (0.83); Gris:DMPC: CHOL: ▵ , 4:1(1:0), (0.99); ■ , 4:1(1:0.05), (0.99); ▼ , 4:1(1:0.2), (0.99); ○ , 4:1(1:0.33), (0.93). Correlation coefficients in parenthesis follow the composition.

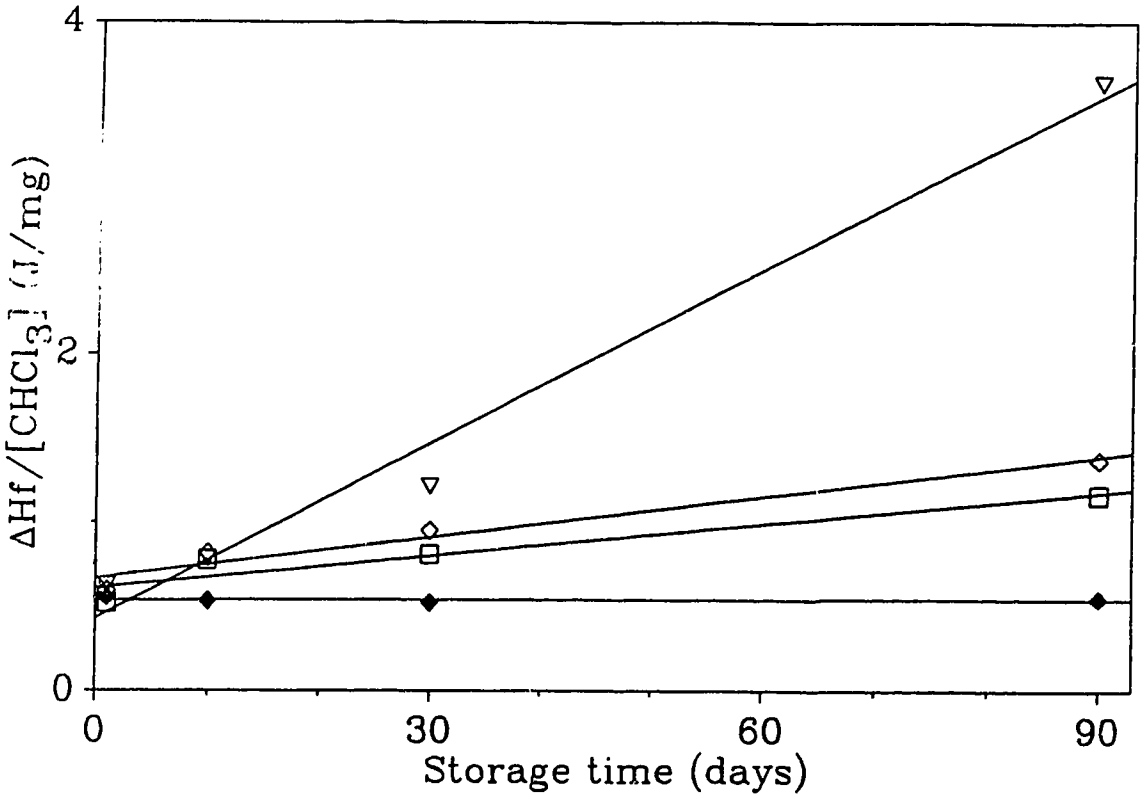

Figure 2.4B Plot of the aging factor, defined as $\Delta H_f/[CHCl_3]$ (J/mg), as a function of the storage time of Gris:DMPC:CHOL: □ , 4:1(1:1), (0.95); ◇ , 4:1(1:3), (0.98); ▽ , 4:1(0:1), (0.99); ♦ , Gris:EPC:CHOL, 4:1(1:0.33), (0.22). Correlation coefficients in parenthesis follow the composition.

TABLE 2.2 Fusion temperatures, heats of fusion, chloroform content and dissolution of Gris:DMPC:CHOL 4:1(1:0.33) coprecipitates as a function of aging

Time (days)	Fusion temp. (°C)	Heat of fusion (J/g)	Chloroform content (mg/g)	Maximum % dissolved (2 h) (mean±SD, n=3)
1	216	118	228	43.9 ± 1.5
10	214	120	200	39.8 ± 0.7
30	215	123	175	39.6 ± 2.0
90	217	132	166	36.9 ± 1.5

The fusion temperature and heat of fusion of pure Gris was 221° and 130 J/g, respectively.

TABLE 2.3 Comparison of the changes in the physical characteristics of coprecipitates of Gris:Phospholipid:CHOL as a function of CHOL content after aging for 90 days

DMPC:CHOL	Fusion temp. (°C)			Heat of fusion (J/g)			Chloroform content (mg/g)		
(mole ratio)	1 day	90 days	%change	1 day	90 days	%change	1 day	90 days	%change
1:0	216	220	1.9	132	149	13	208	85	59
1:0.05	219	217	1.0	93	127	37	202	74	63
1:0.20	219	217	1.0	99	124	25	204	146	28
1:0.33	216	217	0.5	118	132	12	227	166	27
1:1	214	219	2.5	96	123	28	183	105	43
1:3	211	217	3.0	99	125	26	168	91	46
0:1	204	214	5.0	83	121	46	133	33	75
EPC:CHOL									
1:0.33	219	216	1.0	123	117	5	223	212	5

contents, and maximum amount of Gris dissolved for the 4:1(1:0.33) coprecipitate. From the data it would appear that there is a trend between each of these parameters, except for the fusion temperatures. Furthermore, a comparison of the fusion temperatures, heats of fusion, and chloroform contents among the formulations (Table 2.3) indicates that the 4:1(1:0.33) composition underwent the least change of these parameters with age. Consequently, linear regression determinations of an empirical aging factor for each composition, defined as the ratio of ΔH_f to the chloroform content, were plotted as a function of the storage time in Fig. 2.4A and 2.4B. It is clear from these plots that the tendencies of DMPC coprecipitates to age were least for compositions of Gris:DMPC:CHOL 4:1(1:0.2), and 4:1(1:0.33).

The influence of CHOL on aging is further illustrated in Fig. 2.5 from a plot of the aging coefficient (slopes of regression lines in Fig. 2.4A and 2.4B) from which it may be concluded that the introduction of small amounts of CHOL to the lipid component beyond a threshold level, but over a narrow range, increased the storage stability of the coprecipitates. However, at higher levels of CHOL aging coefficients were gradually increased. A comparison of the dissolution behavior of the drug and coprecipitates with the aging factor is shown in Table 2.4. The linear correlations for all coprecipitates argues strongly in favor of the influence of the relationship of the chloroform content and the ΔH_f on the dissolution behavior.

2.3.2. Gris:EPC Compositions

The coprecipitate composition of Gris:EPC:CHOL, 4:1(1:0.33) exhibited an

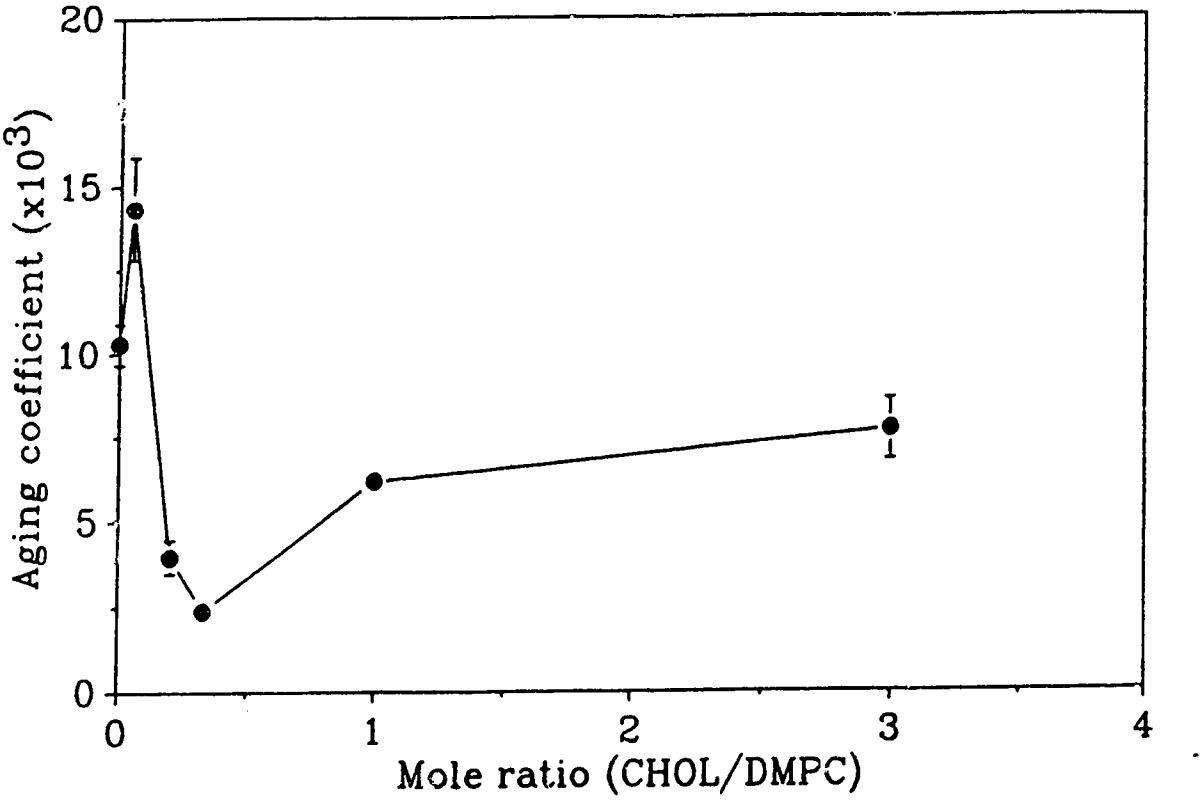

Figure 2.5 Plot of the aging coefficient (slopes of regression lines in Fig. 2.4A and 2.4B) versus the CHOL/DMPC mole ratio in coprecipitates at constant 4:1 Gris:lipid weight ratio.

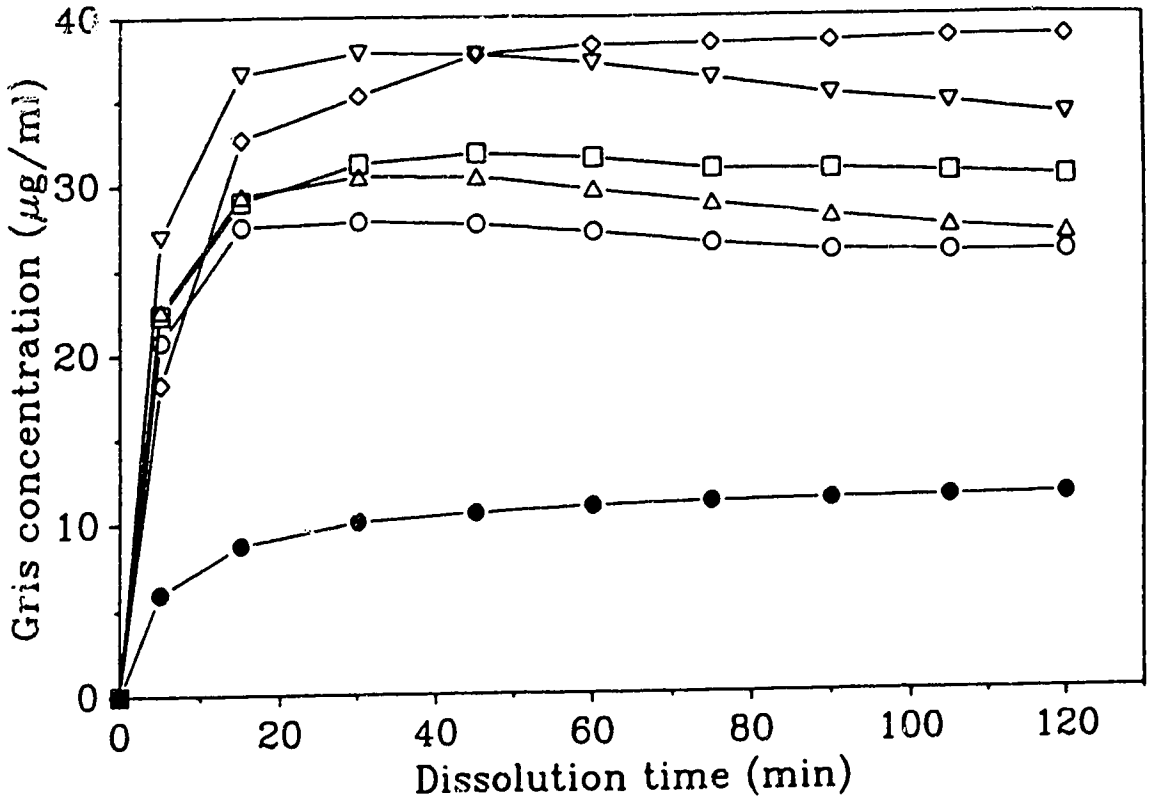

Figure 2.6 - Dissolution behavior of Gris:EPC:CHOL 4:1(1:0.33) coprecipitates as a function of aging at room temperature. ○ , 1 day; ▲ , 10 days; □ , 30 days; ▽ , 90 days; ◇ , 135 days; ● , micronized Gris (shown for reference).

TABLE 2.4 Linear regression analysis of dissolution (% dissolved after 2 h) of Gris-DMPC:CHOL coprecipitates[a] as a function of the aging factor[b]

DMPC:CHOL (mole ratio)	Intercept	Slope	r	P[c]
Gris.Solvate	-7.03	0.057	0.34	0.66
1:0	2.19	-0.043	0.74	0.26
1:0.05	1.32	-0.023	0.80	0.19
1:0.20	2.07	-0.034	0.84	0.16
1:0.33	1.91	-0.032	0.88	0.12
1:1	4.18	-0.108	0.85	0.14
1:3	5.06	-0.130	0.98	0.01
0:1	8.30	-0.347	0.94	0.05

[a]Gris:Lipid (4:1 w/w); r = corr. coeff. (n = 4).

[b]defined as $\Delta H_f/[CHCl_3]$. [c]Probability values based on the F test.

apparent unusually different dissolution behavior compared to Gris:DMPC CHOL systems and this is described in Fig. 2.6. The dissolution properties of Gris:EPC: CHOL coprecipitates actually improved with age, such that after 90 days the amount dissolved increased approximately 35 percent although the initial dissolution rates at each time interval were about the same. However, aging up to 135 days did not yield any further changes in the dissolution of Gris. Also, it was established that the chloroform content and the ΔH_f changed only slightly (ca. 5%) (Table 2.3) resulting in an aging factor which did not change after 90 days of storage (hence, a poor correlation) as shown in Fig. 2.4B.

An investigation of the possible reasons for the observed increase in dissolution behavior revealed that degradation products of EPC may be responsible. Samples of freshly purified EPC (Singleton et al., 1965), oxidized EPC (modified from Sotnichenko et al., 1984), EPC in a freshly-prepared Gris:EPC coprecipitate, and EPC in an aged sample of Gris:EPC coprecipitate, when analyzed by TLC (silica gel 60 F_{254} plastic sheets, Merck; ethanol:ethyl acetate:water, 4:14:3 v/v) indicated spots arising from the aged sample coinciding with oxidized EPC but having considerably reduced amounts of material compared to the freshly-prepared coprecipitate. Methods of determination of the extent of oxidation of EPC include measurement of the peroxide value, increase in the UV absorption at 233 nm due to increasing diene conjugation, and the thiobarbiturate estimation of free and bound malonaldehye. A convenient method described by Klein (1970) defines the "oxidation index" (OI) which is the ratio of absorbance at 233 nm to that at 215 nm.

Consequently, determination of the oxidation indices for EPC at various stages of degradation was made and the results are presented in Table 2.5. It can be seen that the lowest OI occurred with purified EPC, the highest occurred with fully oxidized EPC with intermediate values being obtained for different aged samples. It was observed that a substantial amount of oxidation had occurred in the freshly-prepared coprecipitate, probably because this sample was not continuously under a N_2 atmosphere during preparation and, consequently, the OI increased gradually with the age of the sample. Also, degradation proceeds in the presence or absence of CHOL. The exact mechanisms responsible for the increased dissolution from these systems with aging are not understood, however, indirect evidence of the presence of oxidized EPC suggests that this occurrence may play a role in the altered behavior of these systems.

In a study involving solid dispersions of phenylbutazone-PEG 6000 (1:5 w/w), it was observed that the incorporation of 0.05 M CHOL did not alter the dissolution profile of phenylbutazone even after storage for 80 days (Fig. 2.7). Phenylbutazone:-PEG 6000 (1:5 w/w) systems have been known to age on storage, probably due to complex interactions of the drug with the polymer (Khalil and Mortada, 1978). The addition of 0.05 M CHOL to these systems possibly reduces the interactions of the drug and the polymer. However, 0.1 M CHOL did not seem to inhibit the aging process (Fig 2.8). Other lipids, such as triolein and palmitic acid at 0.1 M concentrations appeared to increase the dissolution of phenylbutazone from aged solid dispersions. The effect of CHOL on these non-solvated solid dispersion systems may

TABLE 2.5 Comparison of oxidation indices (OI) of EPC under various conditions and in Gris:EPC coprecipitates

EPC System	OI[a]	Mean ± Range
Purified EPC	0.097 0.101	0.099 ± 0.002
Gris:EPC (4:1) (freshly-prepared)	0.859 0.757 0.631	0.749 ± 0.114
Gris:EPC (4:1) (aged for 5 days)	1.082 1.100	1.091 ± 0.012
Gris:EPC (4:1) (aged for 10 days)	1.184 1.183	1.183 ± 0.000
Gris:EPC:CHOL [4:1(1:0.33)] (aged for 10 months)	1.308 1.303	1.305 ± 0.002
Oxidized EPC	1.877 1.948	1.913 ± 0.035

[a]OI = ratio of absorbances of solutions in ethanol at 233 and 215 nm, respectively (Klein, 1970).

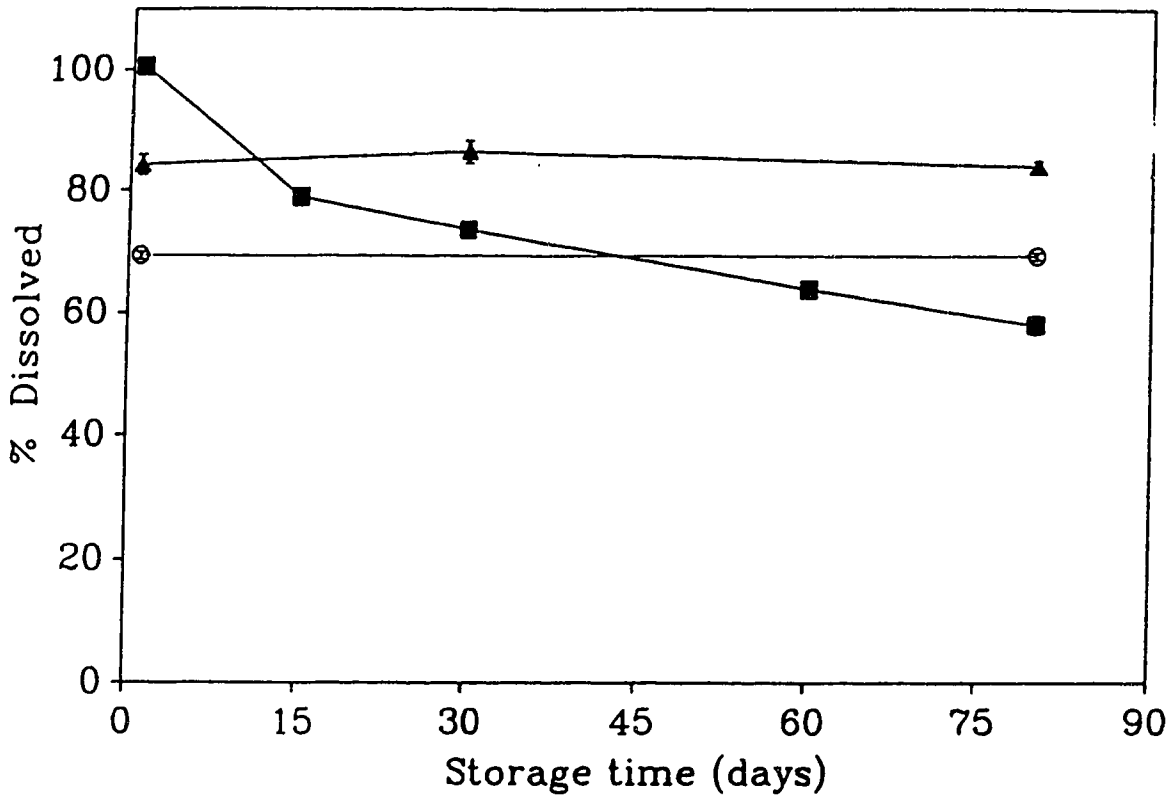

Figure 2.7 Aging profiles of phenylbutazone-PEG 6000 (1:5 w/w) solid dispersions containing cholesterol in various mole ratios (PBZ:CHOL); o , 1:0; ▲ , 1:0.05; ■ , 1:0.1.

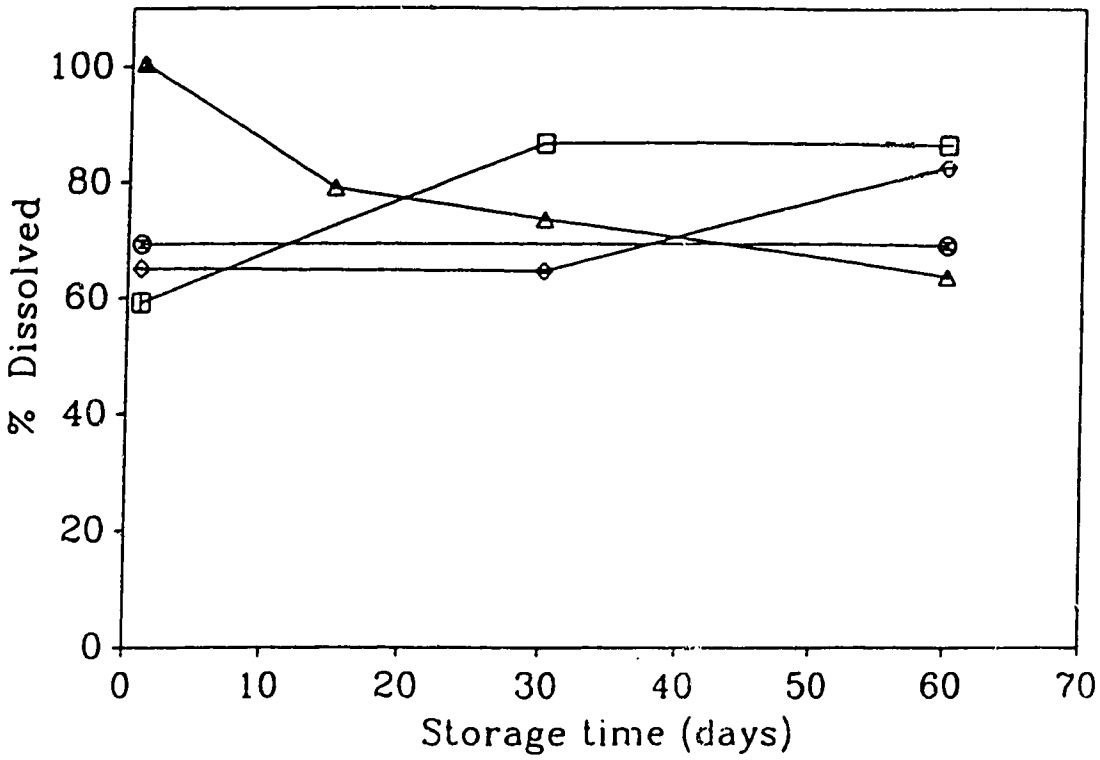

Figure 2.8 Aging profiles of phenylbutazone-PEG 6000 (1:5 w/w) solid dispersions containing 1:0.1 (PBZ:lipid) mole ratio of: ▲, CHOL; □, Triolein; and ◇, Palmitic acid. ○, PBZ shown for reference.

be concentration dependent as observed with the solvated Gris:DMPC:CHOL coprecipitates.

This study has shown that coprecipitates of a poorly water-soluble drug, such as griseofulvin prepared with a phospholipid from a solvating solvent, improves the dissolution of this drug and that the aging characteristics of the coprecipitates can be considerably slowed by the incorporation of CHOL. The quantities of lipid additives used are very small in comparison to other solid dispersion systems that have been developed with water-soluble components, e.g. polyethylene glycol. Furthermore, the choice of phospholipid can make a remarkable difference in the outcome. This has further implications for other drugs which are able to form solvates, and work is continuing in the direction of testing other compounds and determining a suitable means for commercialization.

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CHAPTER 3

DISSOLUTION OF FLUDROCORTISONE ACETATE FROM PHOSPHOLIPID COPRECIPITATES

3.1 INTRODUCTION

Several formulation approaches have been used to improve the bioavailability of poorly water-soluble drugs, including the preparation of solid dispersions and the use of lipid vehicles. Recent studies have demonstrated the potential of improving the dissolution and release characteristics of griseofulvin from griseofulvin-phospholipid coprecipitates, and the specific application of this type of formulation to drug solvates[1,2].

This approach has been shown to involve incorporation of the added lipids in the crystalline structure of the drug solvate which, upon contact of the crystals with water, almost spontaneously disperse into extremely fine particles followed by hydration of the phospholipid which rapidly forms myelinic structures at the crystal surfaces. This process, along with partitioning of drug in the myelinic structures, account for the rapid initial dissolution rate of the drug and for the several-fold increase in the amount of drug dissolved over a period of 1-2 h[1,2].

The dissolution of other drug solvates, including the steroids, may also benefit by formulation with phospholipids. Preliminary tests demonstrated that the dissolution of hydrocortisone from its dimethylformamide solvate increased significantly after incorporation of only 5 percent dimyristoylphosphatidylcholine[3]. Fludrocortisone acetate forms a pentanol or an ethyl acetate solvate[4] and its

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dissolution from these solvates has been reported[5]. Although fludrocortisone acetate exhibits improved dissolution from its solvate, it is poorly soluble in water (40 mg/L)[6]. Hence, the ethyl acetate solvate of fludrocortisone acetate was selected for formulation development as a phospholipid coprecipitate. Furthermore, since it would be beneficial to not only have increased dissolution properties but, also, controlled release behavior, the effect of incorporation of selected polymers in the coprecipitates on the release of the drug was a second objective of this investigation.

3.2 EXPERIMENTAL SECTION

3.2.1 Materials

Fludrocortisone acetate (FA), polyvinylpyrrolidone, (PVP 10, MW 10,000) (PVP 40, MW 40,000), dextran (D-40, MW 39,400; D-2m, MW 2 million), L-α-dimyristoyl phosphatidylcholine (DMPC, 99%), L-α-dipalmitoyl phosphatidylcholine (DPPC, 99%) were obtained from Sigma Chem. Co., St. Louis, MO. L-α-egg phosphatidylcholine (EPC) was extracted from eggs according to Singleton et al[7]., and purified by recrystallization in acetone. Polyvinylpyrrolidone (PVP 24, MW 24,000, Aldrich Chem. Co), and poly (L-lactic acid) (PLA, MW 146,000, Hexcel Medical) were used as received. All solvents were of reagent grade and were used without further purification. Demineralized-distilled water was used throughout to prepare solutions.

3.2.1 Preparation of Coprecipitates

Drug and phospholipid (and polymer when included) were dissolved in ethyl

acetate-ethanol (5:1 v/v) mixture. The solution was warmed to 40°C and the solvent was evaporated under a gentle stream of nitrogen. Residual excess solvent was removed by placing it in a vacuum desiccator containing anhydrous calcium sulphate at room temperature for 12-16 hours.

3.2.3 Analysis of Coprecipitates

The drug content in coprecipitates was determined by dissolving 10 mg in ethanol, determining the absorbance of a suitably-diluted solution at 238 nm (Beckman Model 25 spectrophotometer), and interpolating concentrations (mg/ml) from a standard calibration curve.

3.2.4 Differential Thermal Analysis

An 80/170 mesh (U.S. standard sieves) sample, equivalent to 10 mg FA, was sealed in an aluminum pan and subjected to a heating rate of 10°C/min (Fisher Thermalyzer, series 300 QDTA, Fisher Scientific Co.) using an empty pan as reference. Thermograms were run at a reference temperature sensitivity of 13.7°C/in. and a differential temperature sensitivity of 0.3°C/in. Heats of fusion were calculated from peak areas (planimeter, Gelman Instrument Co.) and calibration coefficients were derived from fusion temperatures and the calibration curve. The analyses were conducted in triplicate.

3.2.5 Automated Dissolution Studies

Application of the spin-filter dissolution test apparatus[8] (Magne-drive, Clow Coffman Industries, Kansas) equipped with a 1 micron nominal porosity stainless steel filter screen for the dissolution studies has been previously described[1]. The

filter was operated at a rotation speed of 600 rpm in 900 ml of dissolution medium which consisted of an HCl-KCl (0.02 M) buffer solution at pH 2.0 and maintained at 37°C. Some tests were run in 15 percent ethanol at 20°C due to the compound's limited solubility in water. Sieved samples (80/170 mesh) of formulations equivalent to 50 mg FA were dispersed in the dissolution medium, which was continuously circulated by a peristaltic pump (Gilson minipuls 2) through a microcell in the spectrophotometer, and concentrations of FA were determined as a function of time as described earlier. The presence of small amounts of phospholipid or polymers in the formulations did not interfere with the analysis of FA. All determinations were carried out in triplicate and average values $\pm$ SD reported.

3.2.6 **Kinetic Studies**

The kinetics of dissolution were examined by several means including the following.

3.2.6.1 First-Order Dissolution Kinetics[9]:

The linear first-order rate equation as applied to the dissolution of a powder formulation is given by:

$$\text{Log}(100 - \%\ \text{Dissolved}) = \log M - kt/2.303 \qquad [1]$$

where **M** is a constant and has dimension of mass, **k**, is the apparent first-order dissolution rate constant, and **t** is the dissolution time.

3.2.6.2 Second-Order Dissolution Kinetics[10]:

The second-order rate equation for dissolution from a powder sample is given by:

$$W/W_e\ (W_e - W) = k_2 t \qquad [2]$$

where **W** is the weight of 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.

3.2.6.3 Weibull Distribution Dissolution Kinetics[11]:

A more general function which may be applied successfully to all common types of dissolution curves, in particular the exponential and the sigmoid form has been described by Weibull[12]. A form of the Weibull equation can be written as follows:

$$\log [-\ln (1-m)] = b \log (t - T_i) - \log a \qquad [3]$$

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 characterizes the curve as being curved upwards ($b > 1$), or exponential ($b = 1$) or with a steeper initial slope than in the exponential case ($b < 1$). It is often convenient to replace the scale parameter, **a**, by means of the more informative dissolution time, T_d, defined as:

$$a = (T_d)^b \qquad [4]$$

which is read from the graph as the time value corresponding to the ordinate, $-\ln(1-m) = 1$. Since $-\ln(1-m) = 1$ is equivalent to $m = 0.63212$, T_d represents the time necessary to dissolve 63.2% of the material and is, thus, comparable to the frequently quoted t_{50} value. Further, the Weibull distribution combines the advantages of the first-order and the log-normal presentation.

3.2.6.4 Dissolution Efficiency (DE)[13]:

A parameter suitable for the evaluation of in vitro dissolution, referred to as "dissolution efficiency", has been suggested by Khan & Rhodes[14] and 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, within the same time frame, i.e. y_{100}. Thus,

$$\% \text{ DE} = \int_0^t \frac{y \, . \, dt}{y_{100} \, . \, t} \, . \, 100 \qquad [5]$$

where y is the amount released. A constant time interval, i.e. 90 min, was chosen for comparing various formulations by this means. The cut-and-weigh method was used to determine the area under the dissolution curve as this method has been found to be most accurate, reproducible and convenient[13].

3.3 RESULTS AND DISCUSSION

A comparison of the relative dissolution behavior of FA alone, as a solvate, or as a coprecipitate with DMPC in a dissolution medium of pH 2.0 at 37°C is shown in Figure 3.1. The FA or FA-solvate dissolved rapidly then levelled off at about 28 μg/ml although the FA-solvate had considerably greater dissolution properties over the first 10-15 min. The maximum concentration of FA achieved in solution is lower than the reported solubility of FA (40 mg/L) which is probably due to the low pH of the medium. In contrast, a marked improvement in dissolution of FA from DMPC coprecipitates was observed and is consistent with previous observations involving griseofulvin[1]. Table 3.1 compares the dissolution of FA quantitatively

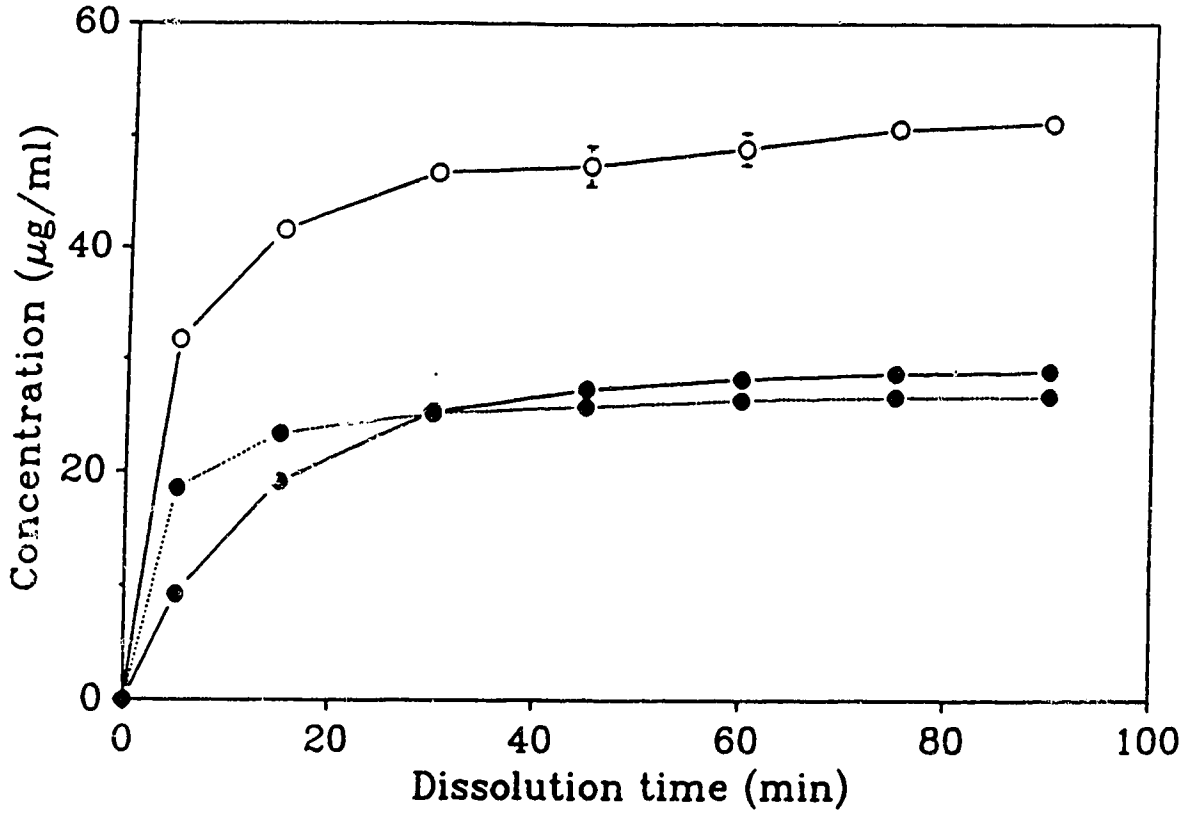

Figure 3.1 Dissolution profiles of: -●-, FA; ..●.., FA solvate; and o, FA:DMPC (4:1 w/w) coprecipitate.

Table 3.1 Dissolution of FA, FA-ethyl acetate solvate and FA:DMPC (4:1 w/w) coprecipitates.

No.	Composition	Dissolution medium	% Dissolved (90 min)	IDR (μg/ml/min)
1.	FA	pH 2.0, 37°C	52.1 ± 0.6	1.8 ± 0.0
2.	FA	15% EtOH, 20°C	73.8 ± 0.2[c]	3.8 ± 0.0[c]
3.	FA/EtAc solvate	pH 2.0, 37°C	48.1 ± 0.5[c]	3.7 ± 0.0[c]
4.	FA/EtAc solvate	15% EtOH, 20°C	73.2 ± 0.2[c]	5.9 ± 0.1[c]
5.	FA:DMPC(4:1w/w)	pH 2.0, 37°C	92.0 ± 0.1[c]	6.4 ± 0.2[c]

[a]Means ± SD, n=3. [b]Initial dissolution rate within first 5 minutes.

[c]Value significantly different from that obtained for composition no.1 ($p < 0.05$, paired Student's t test).

under different conditions of temperature and dissolution medium composition. This shows that the initial dissolution rate (IDR) of FA at pH 2.0,37°C doubled when the dissolution medium was 15 percent ethanol at 20°C or when FA-solvate was used. Further, the IDR of the solvate was increased by approximately 35 percent in ethanol solution. This compares with a 3.5-fold increase in IDR of the FA:DMPC (4:1w/w) coprecipitate and a 40 percent increase in the amount of FA dissolved after 90 min (cf. 20 percent increase of FA or FA-solvate dissolved in ethanol solution). Others had attempted to incorporate phosphatidylcholine in solid dispersions of non-solvated drugs and achieved only modest increases in dissolution[15,16]. On the other hand, the dissolution of FA compares favorably with the results obtained for griseofulvin[1]. Figure 3.2 compares the effect of the type of phospholipid on the dissolution of FA from coprecipitates. It can be seen that under the conditions of dissolution, DPPC exerts a substantial increase in the IDR but only a modest increase in the amount dissolved at 90 min. This can be contrasted with the effects produced by either DMPC or EPC. Again, a similar pattern has been observed previously with griseofulvin coprecipitates[2]. These differences, in each case, are attributed to the state of fluidity of the phospholipid at 37°C, and is related to the respective phase transition temperatures of the phospholipid[17].

The dissolution of FA was also studied as a function of the concentration of DMPC in the coprecipitates and this is shown in Fig. 3.3. It is noted that the incorporation of low amounts of DMPC markedly increased dissolution but that successive increases above 20 percent had little additional effect. This is interpreted

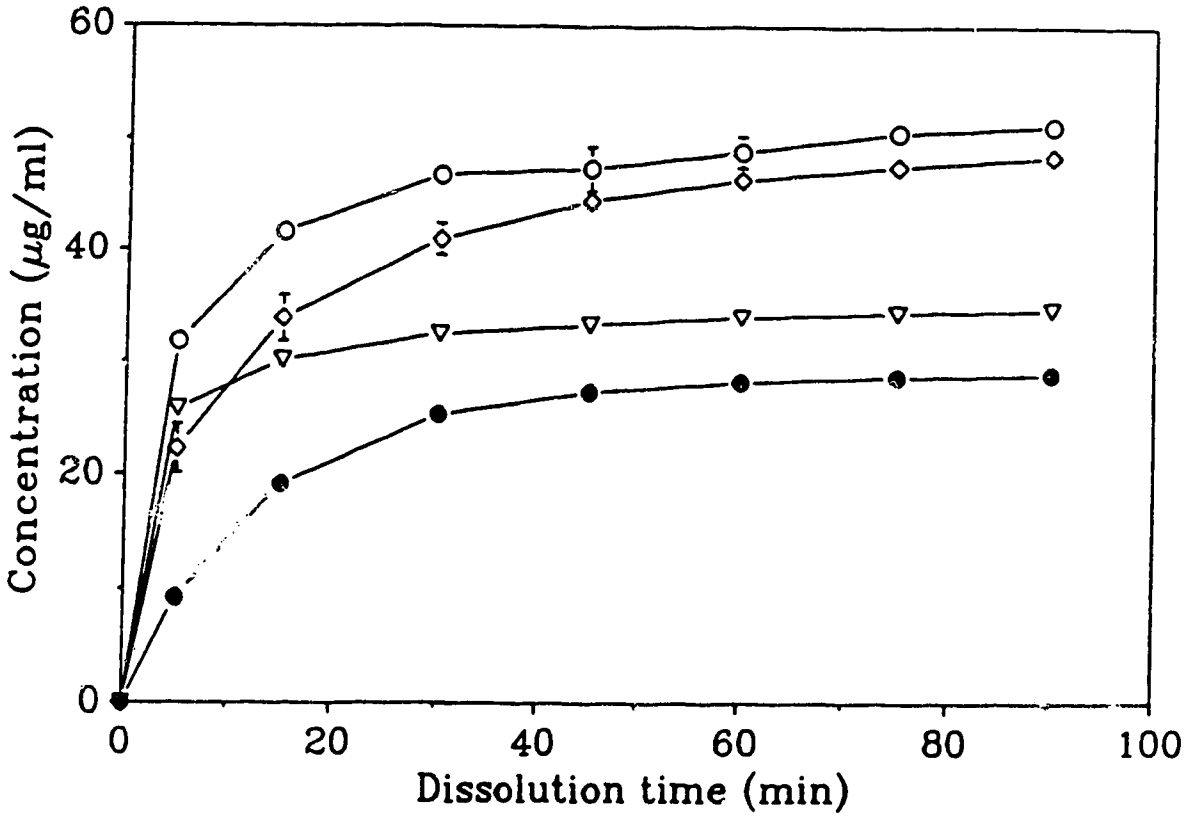

Figure 3.2 Dissolution profiles of FA:phospholipid (4:1 w/w) coprecipitates: ▽, DPPC; ○, DMPC; ◇, EPC; and ●, FA alone.

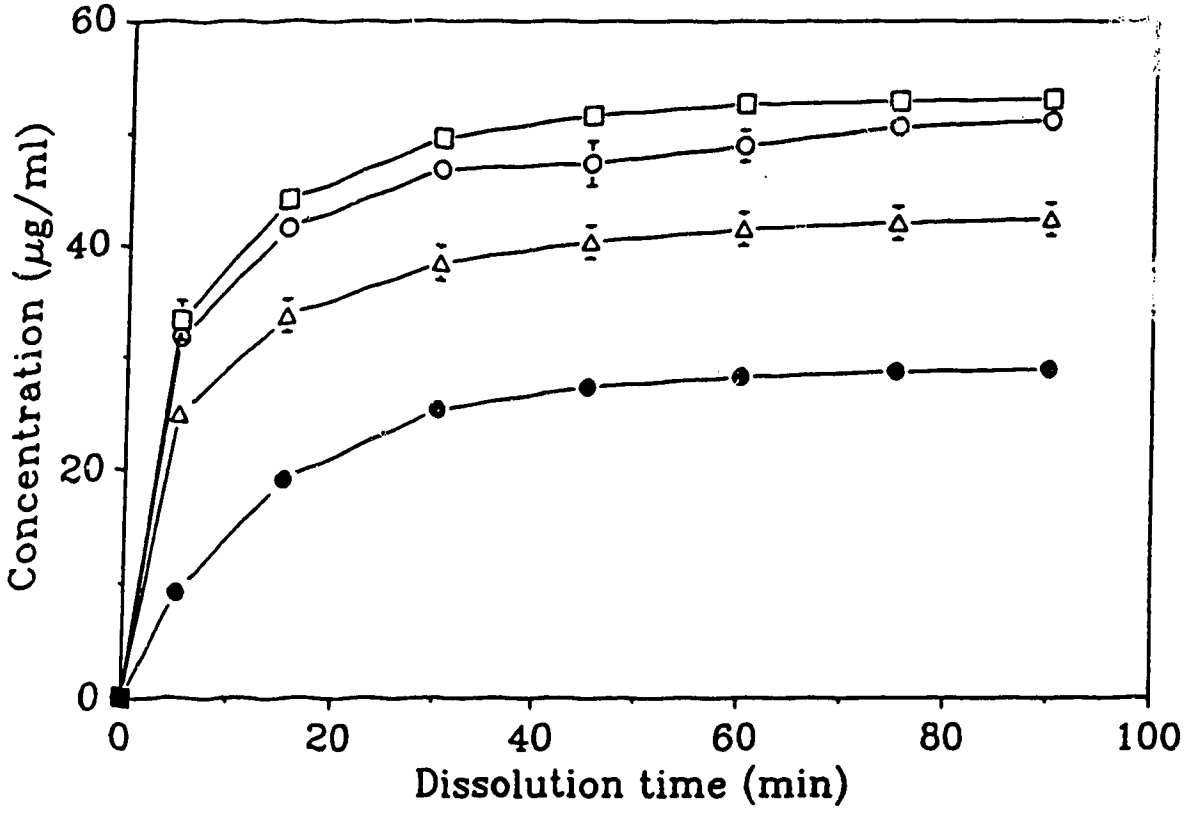

Figure 3.3 Dissolution profiles of FA:DMPC (w/w) coprecipitates: ▵, 9:1; ○, 4:1; □, 1:1; and ●, FA alone.

to mean that there is a limit to the quantity of DMPC that can be included in the FA solvate crystal lattice and that the excess beyond this limit, which becomes dispersed in the aqueous medium probably as liposomes, plays almost no role in the dissolution process. Using non-solvated griseofulvin-soya phospholipid coprecipitates, Nishihata et al.[18] obtained an 18 percent or a 26 percent increase in dissolution from 2:1 or 1:2 mixtures, respectively, and an actual decrease in dissolution was observed when 1:7.5 ratios were used.

Some physicochemical characteristics of the FA coprecipitate systems obtained by differential thermal analysis are described in Table 3.2. It is apparent that the largest single factor affecting the DTA parameters is the solvation of FA (except for the heats of fusion in formulations 6 and 7) with little influence exerted by the type or amount of phospholipid. Desolvation from the FA-solvate or FA-phospholipid coprecipitates results in a change of the polymorphic form of the drug and thus alters the melting point. The heats of fusion of coprecipitates containing DPPC or EPC deviate from that of the other formulations but in opposite directions, possibly reflecting the different physical states of the bilayers of these phospholipids at the experimental temperature[2].

Coprecipitates Containing Polymers: The effects of the addition of polymers on the dissolution behavior and the physical states of FA:DMPC (4:1w/w) coprecipitates are described in Table 3.3 and Table 3.4. At the ratios employed, D-2m and PLA, but not D-40, caused a decrease in dissolution which was also paralleled by a decrease in the heat of fusion, but not melting point which remained unchanged. This suggests

Table 3.2 Differential thermal analysis data of FA, its ethyl acetate solvate and coprecipitates with phospholipids.

No.	Composition	Desolvation[a] Temp.°C	Drug Thaw[a] Temp.°C	Drug Melt[a] Temp.°C	Heat of Fusion KJ/mol
1.	FA	---	204.0±0.0	209.0±0.0	31.48
2.	FA solvate	105.3±0.9	215.6±0.9	225.3±2.6	29.23
3.	FA:DMPC(9:1w/w)	106.0±0.0	219.7±1.2	229.7±0.3	30.65
4.	FA:DMPC(4:1w/w)	110.0±0.0	219.5±1.5	226.5±1.5	31.99
5.	FA:DPPC(4:1w/w)	107.5±0.5	220.0±3.0	227.2±1.8	26.57
6.	FA:EPC(4:1w/w)	104.0±2.0	220.5±0.5	229.0±1.0	42.66

[a]Means ± SD, n=3.

Table 3.3 Effect of incorporation of polymers in FA:DMPC (4:1 w/w) coprecipitates on dissolution of FA.

No.	Composition	DMPC:Polymer (mole fraction%)	%Dissolved[a] (90 min)	IDR[ab] (μg/ml/min)
1.	FA:DMPC	(1:0)	92.0 $\pm$ 0.1	6.4 $\pm$ 0.2
2.	FA:DMPC:D-2m	(1:0.01)	75.9 $\pm$ 3.9[c]	3.8 $\pm$ 0.0[c]
3.	FA:DMPC:D-40	(1:0.1)	92.3 $\pm$ 1.0	6.8 $\pm$ 0.7
4.	FA:DMPC:PLA	(1:0.1)	78.1 $\pm$ 0.0[c]	4.5 $\pm$ 0.0[c]

FA:DMPC-polymer weight ratio is 4:1. [a]Means $\pm$ SD, n=3. [b]Initial dissolution rate within first 5 minutes. [c]Value significantly different from that obtained for composition no.1 ($p<0.05$, paired Student's t test).

Table 3.4 Differential thermal analysis data of FA:DMPC coprecipitates with polymers.

No.	Composition	Desolvation[a] Temp. °C	Drug Thaw[a] Temp. °C	Drug Melt[a] Temp. °C	Heat of Fusion KJ/mol
1.	FA:DMPC	110.0±0.0	219.5±1.5	226.5±1.5	31.99
2.	FA:DMPC:D-2m	105.0±0.0	218.0±2.0	227.0±0.0	26.70
3.	FA:DMPC:D-40	105.0±1.4	217.5±0.7	227.0±0.0	34.60
4.	FA:DMPC:PLA	102.0±0.0	220.8±0.8	229.0±1.0	26.70
5.	FA:DMPC:PVP 10	103.0±0.0	217.0±0.0	228.0±0.0	30.59
6.	FA:DMPC:PVP 24	93.0±0.0	206.0±2.0	217.7±0.7	34.06
7.	FA:DMPC:PVP 40	108.0±0.0	216.0±1.0	226.2±0.7	33.17

FA:DMPC-polymer weight ratio is 4:1, DMPC:polymer mole fraction % is (1:0.1) in all cases except no.2 (1:0.01). [a]Means ± SD, n=3.

that D-2m and PLA interfered with the role of DMPC in the crystal lattice. On the other hand, D-40 likely only coated the crystals and contributed to an increase in the heat of fusion without exerting any significant effect on the mechanism of dissolution imparted by DMPC (as described in ref. 1).

The effects of a series of PVPs on the dissolution and physical parameters of FA:DMPC (4:1w/w) coprecipitates can be observed in Figure 3.4 and Table 3.5, and Table 3.6, respectively. The dissolution behavior typically follows a pattern of decrease with an increase of PVP content up to 1 mole fraction % but adding 10 mole fraction % PVP leads to the result of negligible change when PVP 10 or 40 was used. However, when PVP 24 was present at either 1 or 10 mole fraction %, the %DE results were comparable. The dissolution efficiency calculations using Eq. 5 also reflect these differences in the coprecipitates containing PVP, although some subtle differences at 1 mole fraction % PVP are now discernible. In particular, the minimum in dissolution of FA from FA:DMPC coprecipitates was found to occur with 1 mole fraction % of PVP 10 or PVP 24 but with 0.1 mole fraction % of PVP 40. The reversibilty of this trend at 10 mole fraction % by PVP 10 or PVP 40 but not PVP 24 is unclear, except that it is presumed that PVP 24 possesses a more favorable size and molecular configuration to be incorporated in the crystal lattice structures of the coprecipitates than the other two. This conclusion is supported partly by the DTA studies (Table 3.4) which also shows significant reductions in the thawing, melting, and desolvation temperatures of coprecipitates containing PVP 24, but not those containing PVP 10 or PVP 40. Further, variable viscosity effects with

Table 3.5 Dissolution data expressed as dissolution efficiency of FA (%DE) from FA:DMPC-PVP (4:1 w/w) coprecipitates as a function of the molecular weight and molar concentration of PVP

DMPC:PVP	% Dissolution Efficiency		
(mole fraction %)	PVP 10	PVP 24	PVP 40
0.00	82.5	82.5	82.5
0.01	78.3	78.3	77.6
0.10	73.6	72.8	68.6
1.00	62.8	67.7	69.3
10.00	78.9	68.8	81.0

[a]Means of n=3.

Table 3.6 Evaluation of the dissolution kinetics of FA, its ethyl acetate solvate and its coprecipitates according to the Second order and Weibull Distribution functions.

Composition	Second Order		Weibull Distribution			
	k_2[a] x 10^{-4}	Pred T_{40}[b]	log a	b	Pred T_{40}	Obs T_{40}
FA	1.9	23.4	1.24	0.48	19.5	31.1
FA Solvate	0.8	55.5	1.15	0.16	15.8	11.4
FA:DMPC[c]	2.3	1.9	0.54	0.37	3.5	3.3
FA:DMPC:PVP 10[c]	1.0	4.4	0.65	0.40	4.6	4.6
FA:DMPC:PVP 24[c]	2.7	1.7	0.72	0.53	5.3	5.3
FA:DMPC:PVP 40[c]	7.7	5.8	0.83	0.59	6.7	6.7

[a]Second order rate constant. [b]Time for 40% dissolution. [c]FA:DMPC-polymer weight ratio is 4:1, DMPC:polymer mole fraction % is 1:0.1. b is the slope.

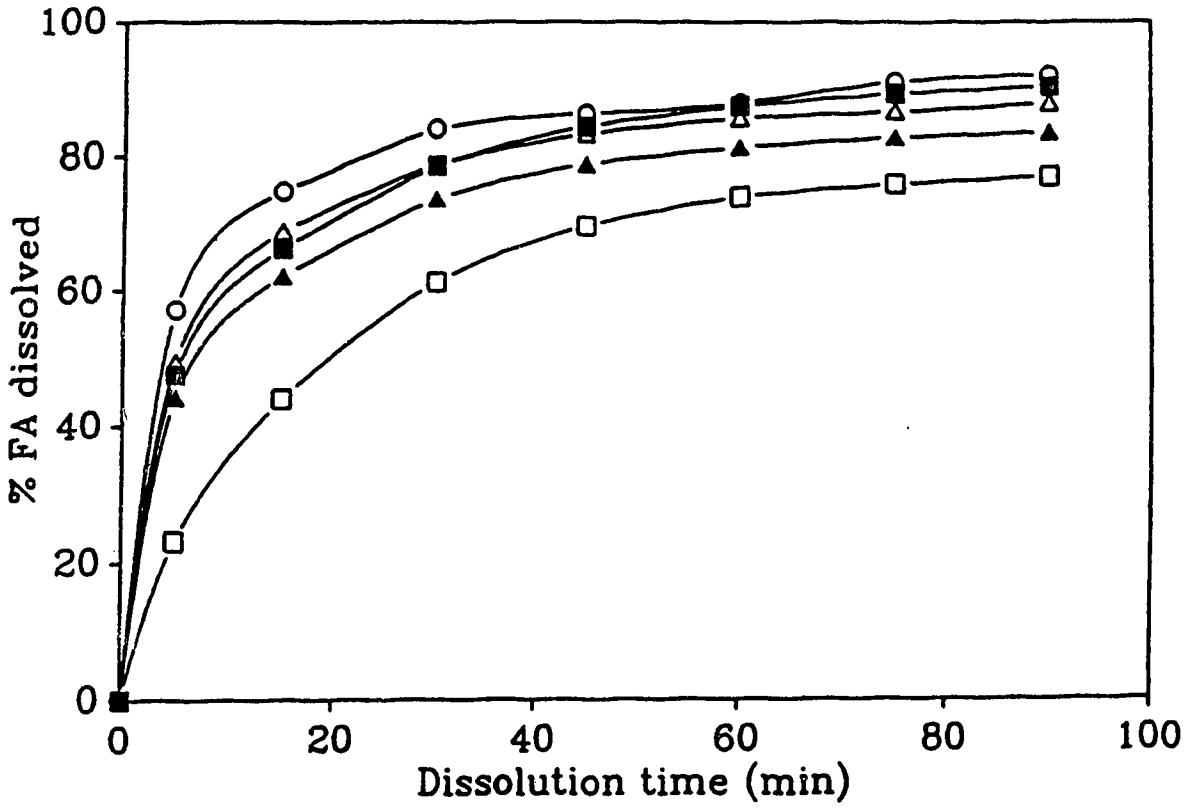

Figure 3.4 Dissolution profiles of FA:DMPC (w/w) coprecipitates at various compositions of PVP 10, expressed as mole fraction percent of DMPC: ▲, 0.01%; ▲, 0.1%; □, 1.0%; ■, 10.0%; and ○, FA:DMPC (4:1 w/w).

increase in PVP molecular weight have been reported[19], which may account for this finding. The elevated heats of fusion in coprecipitates containing PVP 24 or PVP 40 suggest that these polymers contribute energy to the material via intrinsic cohesive molecular bonding characteristics of the excess polymer coating the particles.

Kinetics of Dissolution: The dissolution of the various coprecipitates was examined kinetically using several approaches. The data have been plotted according to second order and Weibull distribution kinetic treatment (Eq. 2 and 3), respectively, in Figures 3.5 and 3.6, and a comparison of the calculated values for the derived-constants of these expressions is found in Table 3.6. Generally, the kinetics would appear to be well-described using either approach when the correlation coefficients of the plots are compared, although the Weibull distribution approach also yielded better correlations for the dissolution of FA and the FA-solvate. A comparison of the second order and Weibull distribution data for the coprecipitates in terms of the predicted time for 40 percent dissolution revealed that the Weibull distribution kinetic approach yielded better agreement with the observed values. In addition, the kinetic data for the FA solvate were fitted better by Weibull distribution kinetics but both treatments applied to FA gave poor agreement with the observed T_{40}. It is, however, recognized that the Weibull distribution has three adjustable parameters in the equation which probably accounts for the better fit and predictability. The data had yielded poor correlations following first-order kinetic treatment and, hence, comparisons were not presented. In a similar fashion, the kinetics of griseofulvin:phospholipid coprecipitates were best described by the Weibull

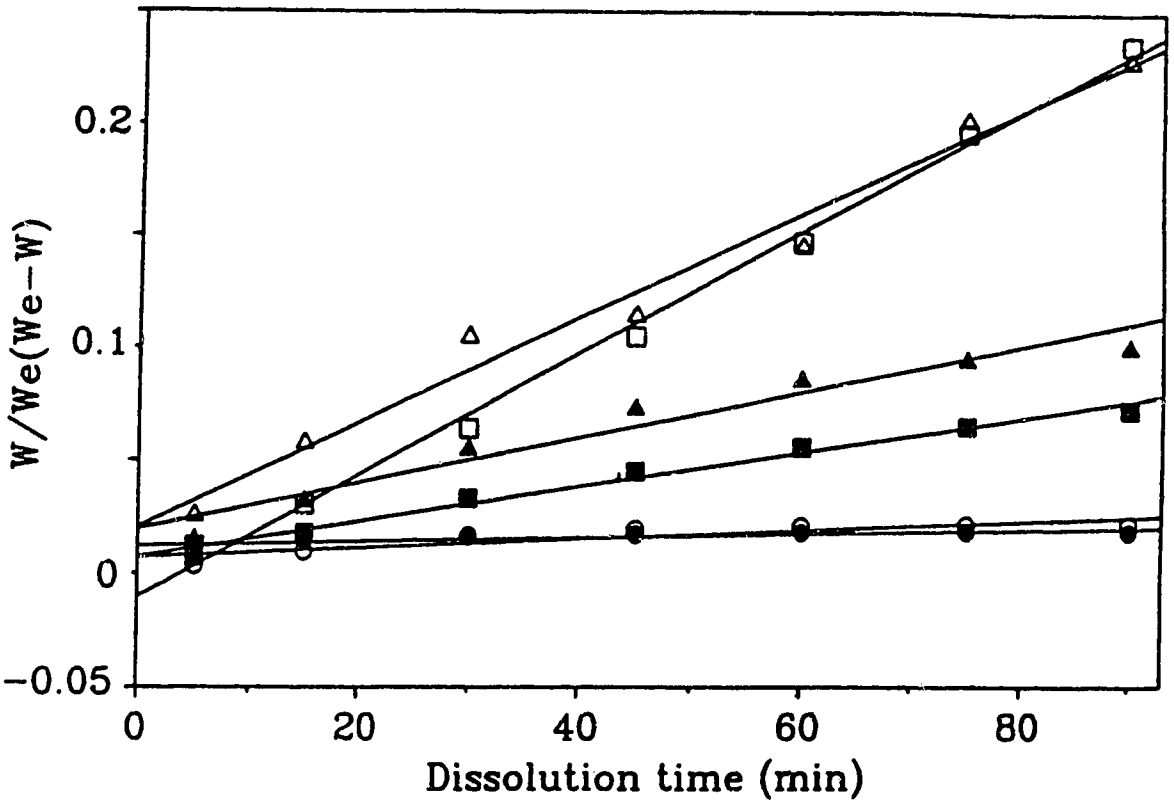

Figure 3.5 Second order kinetic plot for dissolution of FA formulations.

o , FA (.89); ● , FA solvate (.85); ▵ , FA:DMPC (.99); ▲ , FA:DMPC:PVP 10 (.97); □ , FA:DMPC:PVP 24 (.99); ■ , FA:DMPC:PVP 40 (.99). 'r' values from linear regression analysis are shown in parenthesis. Concentration of PVP in coprecipitates is 0.1 % mole fraction of DMPC.

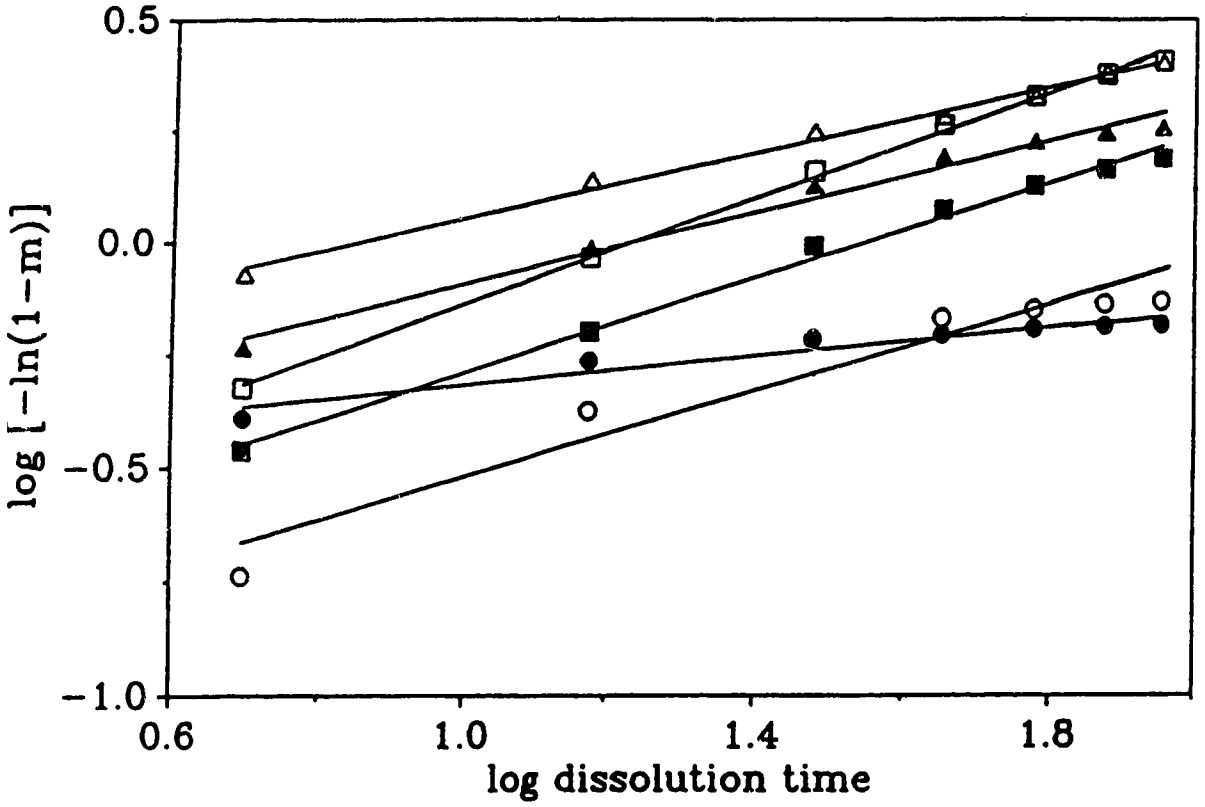

Figure 3.6 Weibull distribution plot for dissolution of FA formulations.

○ , FA (.96); • , FA solvate (.96); ▲ , FA:DMPC (.99); ▲ , FA:DMPC:PVP 10 (.99); □ , FA:DMPC:PVP 24 (.99); ■ , FA:DMPC:PVP 40 (.99). 'r' values from linear regression analysis are shown in parenthesis. Concentration of PVP in coprecipitates is 0.1 % mole fraction of DMPC.

distribution approach[20] suggesting similar mechanisms in the dissolution behavior of these formulations.

This approach to formulation is consistent with preparations of high drug content (80 - 95 percent) compared to other solid dispersions of water-soluble carriers (5 - 10 percent drug content). The FA:phospholipid coprecipitate dissolution behavior remained remarkably constant over a period of four months, whereas the griseofulvin:phospholipid coprecipitate system aged[1]. However, the aging could be reduced by varying the lipid composition, namely, the addition of cholesterol[21]. These encouraging results offer the possibility of delivering poorly water-soluble or erratically-absorbed drugs more efficiently and more economically than the other solid dispersion systems using water-soluble carriers.

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CHAPTER 4

MICROENCAPSULATION OF SOLID DISPERSIONS. GRISEOFULVIN:PHOSPHOLIPID COPRECIPITATES

4.1 INTRODUCTION

The preparation of solid dispersions of drugs with water-soluble or water-dispersible carriers is intended to either increase the dissolution of the drug or, at least, to modify the kinetics of release of the drug in some predictable manner (1). One of the drawbacks of this type of formulation, however, has been the instability of the physical state of the powder during processing and during storage, i.e. the system ages (2,3). Attempts to resolve these problems have been very few. However, recently, the reduction of aging of a specific type of solid dispersion system has been demonstrated by modifying the carrier composition (4).

The application of microcapsule and microsphere technology has successfully produced better physical properties of drugs for manufacturing tablets or capsules because of having superior flow characteristics and compressibilities than the standard granulation. In addition, the microparticles processed in this manner can be formulated to yield drug release kinetics which are more uniform and prolonged (5-7). Another potential use of microencapsulation in the formation of small particles is believed to be the stabilization of solid dispersions, thereby increasing the utility of these dispersions both after processing and during storage. Therefore, a study was undertaken to prepare microencapsulated coprecipitates of the griseofulvin: phospholipid system which previously was shown to have superior dissolution

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A version of this chapter was presented to the Fifth Annual Meeting of the AAPS at Las Vegas, Nov 1990, and has been submitted to Pharm. Res. for publication.

behavior compared to micronized griseofulvin (8,9).

4.2 EXPERIMENTAL

4.2.1 Materials:

Poly (L-Lactic Acids) (PLA), MW = 50,000 and 146,000 were obtained from ICN Biochemicals, OH, USA, and Hexcel Medical, CA, respectively. Micronized griseofulvin (Gris, 99.3%, Glaxo, Canada), cholesterol (CHOL, 99%), L-α-dimyristoylphosphatidylcholine (DMPC, 99%) and polyvinyl alcohol (PVA, avg. MW = 10,000) were obtained from Sigma Chemical Co. Chloroform and methylene chloride were of analytical grade and used without further purification.

4.2.2 Microsphere Preparation:

Microspheres were prepared under atmospheric conditions by a modified process of Beck et al.(10). The procedure involved dissolving the polymer, drug and lipids (4:1 Gris:lipid weight ratio where the lipid component consisted of 1:0.33 DMPC:CHOL mole ratio) in chloroform, adding a 0.5% PVA aqueous solution, then emulsifying with magnetic bar stirring at a constant rate of 300 rpm. The agitation was continued for 3 hours under atmospheric conditions to allow the solvent to evaporate. The microspheres formed were then allowed to settle and the supernatant was replaced with 1M sodium chloride solution. The microspheres were agitated for a further 30 min then filtered (Whatman No. 5 filter paper), washed with distilled water, rinsed with 10 percent ethanol (to remove excess Gris crystals) and dried under reduced pressure in a desiccator at 24°C for 16 hours. The 146,000 MW PLA was employed in all microsphere experiments except when the 50,000 MW PLA

was used for comparison.

4.2.3 **Microsphere Characterization:**

The loading of Gris in the microspheres was determined by dissolving 10 mg of microspheres in 1 ml chloroform and then diluting to 10 ml with 95% ethanol. Solutions for analysis were obtained by a 1:10 dilution with pH 2.0 buffer solution then absorbances were measured spectrophotometrically at 293 nm (Beckman Model 25 spectrophotometer) and the amount of Gris determined from a standard calibration curve. The DMPC content of the microspheres was determined using a modified method of Raheja et al. (11).

Particle size distributions of the respective formulations were obtained by microscopic examination. Also, information about microsphere size and morphological characteristics was obtained using scanning electron microscopy (Philips SEM 505, Holland; courtesy of SMRI, University of Alberta).

4.2.4 **Drug Release Studies:**

Drug release from the microspheres was determined as a function of time using the spin-filter dissolution test apparatus (Magne Drive, Clow-Koffman Industries, Kansas) equipped with a 1-micron nominal porosity filter screen and 900 ml of 37°C HCl-KCl pH 2.0 buffer stirred at 600 rpm. Normally, a weight of microspheres equivalent to 1.5 mg of Gris was suspended in the dissolution medium and from the filtrate circulated through the spectrophotometer, the time-dependent release of Gris was determined. Drug release and dissolution studies were also conducted using hard gelatin capsules (size 0) containing microspheres or Gris

powder suspended in PEG 600, which had been stored for one week. The averages of triplicate experiments have been reported.

4.3 RESULTS AND DISCUSSION

4.3.1 Preparation of Microspheres:

The optimum concentration of PVA as the emulsifier was determined to be 0.5 percent; higher concentrations of PVA led to excessive aggregation of microspheres which is consistent with the findings of others (12). The encapsulation efficiency of Gris in the microspheres varied between 58% and 100% depending on the amount added and the solvent used as shown in Table 4.1. The yield of microspheres was between 10% and 62% which at the higher end was greater than expected. Microscopic observation revealed some crystals of Gris adhering to the surfaces of the microspheres even though they had been washed with 10 percent ethanol (Fig. 4.1C). At higher Gris concentrations excess crystals actually interfered with microsphere formation. The adherence of crystals to microspheres is reported to be a common problem (13).

4.3.2 Characteristics of Microspheres:

The scanning electron micrographs (Fig. 4.1A) show that empty PLA microspheres had perfectly spherical shapes with smooth surfaces. In comparison, the Gris (10.2 percent loading) (Fig. 4.1B) or solid dispersion-loaded (18 percent loading) microspheres (Fig. 4.1C) were also spherically-shaped but with rippled surfaces. Generally, the occurrence of free Gris crystals on the surfaces of the

TABLE 4.1. Characteristics of griseofulvin-loaded poly(L-lactic acid) microspheres[a].

Microsphere Contents	Gris Conc. % Theoretical	Gris Conc. % Observed[b]	Specific surface[c] (cm^2/cm^3)	Particle Diameter[b] (μm)	Gris Crystals[d]
Empty	---	---	2919	17.1±1.4	---
Gris:DMPC:CHOL	12.65	7.4±0.5	2787	19.2±1.9	+
[4:1(1:0.33)]	15.81	10.2±1.1	1874	20.1±2.1	++
"	21.83	18.0±0.4	1238	26.2±2.0	++
"	25.00	21.8±1.4	1205	30.3±2.8	+++
Gris-solvate	25.00	16.7±0.2	961	28.1±2.6	+++
Methylene chloride-treated Gris	25.00	25.5±1.6	872	46.8±3.4	---

[a]146,000 mean MW; [b]Mean±SD, n=3; [c]Surface area per unit volume. [d]Extent of formation of free Gris crystals; ---, none; +, low; ++, medium; +++, high.

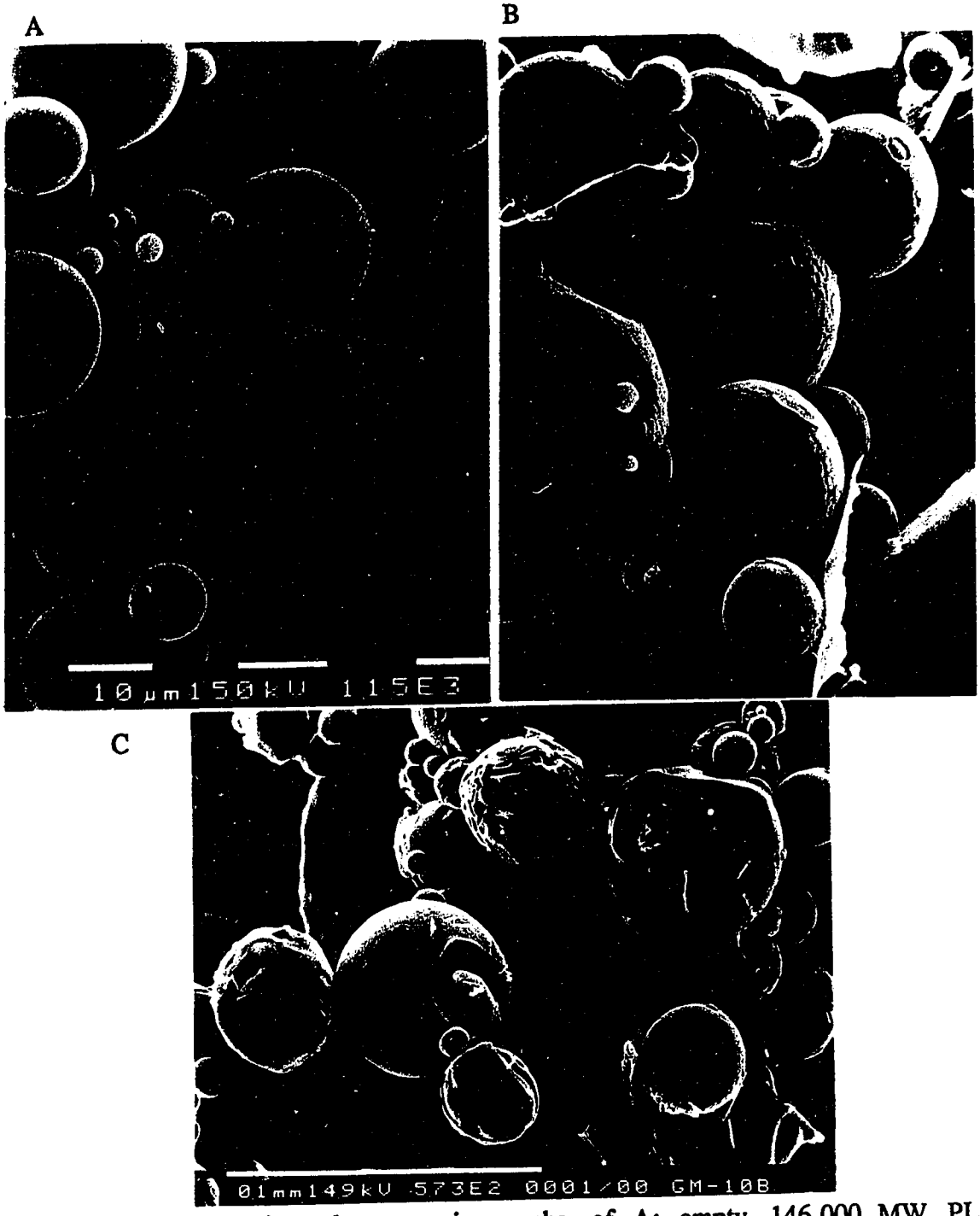

Figure 4.1 Scanning electron micrographs of A: empty 146,000 MW PLA microspheres; B. microspheres containing 16.7 % micronized Gris; C: microspheres of Gris:DMPC:CHOL 4:1(1:0.33) coprecipitate containing 10.2 % Gris. [courtesy of SMRI, University of Alberta].

microspheres was low.

Table 4.1 and Fig. 4.2 indicate that the particle size of the microspheres increased linearly with Gris content ($r = 0.99$), increasing from 17 μm for empty microspheres to 30 μm for microspheres containing 21.8 percent Gris as a coprecipitate. The largest microsphere size of 47 μm was obtained using methylene chloride as a solvent and 25 percent Gris loading. The corresponding specific surface (defined as surface area per unit volume) also decreased.

4.3.3 **Gris Release Studies:**

The time-dependent release of Gris from Gris coprecipitate-loaded microspheres relative to pure Gris is described in Fig. 4.3 as a function of the total Gris loading. These release profiles are typical of those obtained previously (8) in the dissolution of Gris from powdered coprecipitates suggesting that during microsphere formation the coprecipitates form in a manner similar to that in the absence of PLA. The amount of Gris released after 60 min from microspheres containing Gris coprecipitates was 35, 65, and 235 percent higher for 7, 10, and 18 percent Gris loading, respectively, compared to pure Gris microspheres at 16.7 percent loading. On the other hand, the release of pure Gris from microspheres was found to be slightly less than the amount of Gris in solution after dissolution of its powder (Fig. 4.4) whereas the release from microencapsulated Gris coprecipitates was the same as the dissolution of Gris from powdered coprecipitates of the same composition. This is further evidence that Gris coprecipitates can be loaded into microspheres without significant alteration of their dissolution properties. Analyses

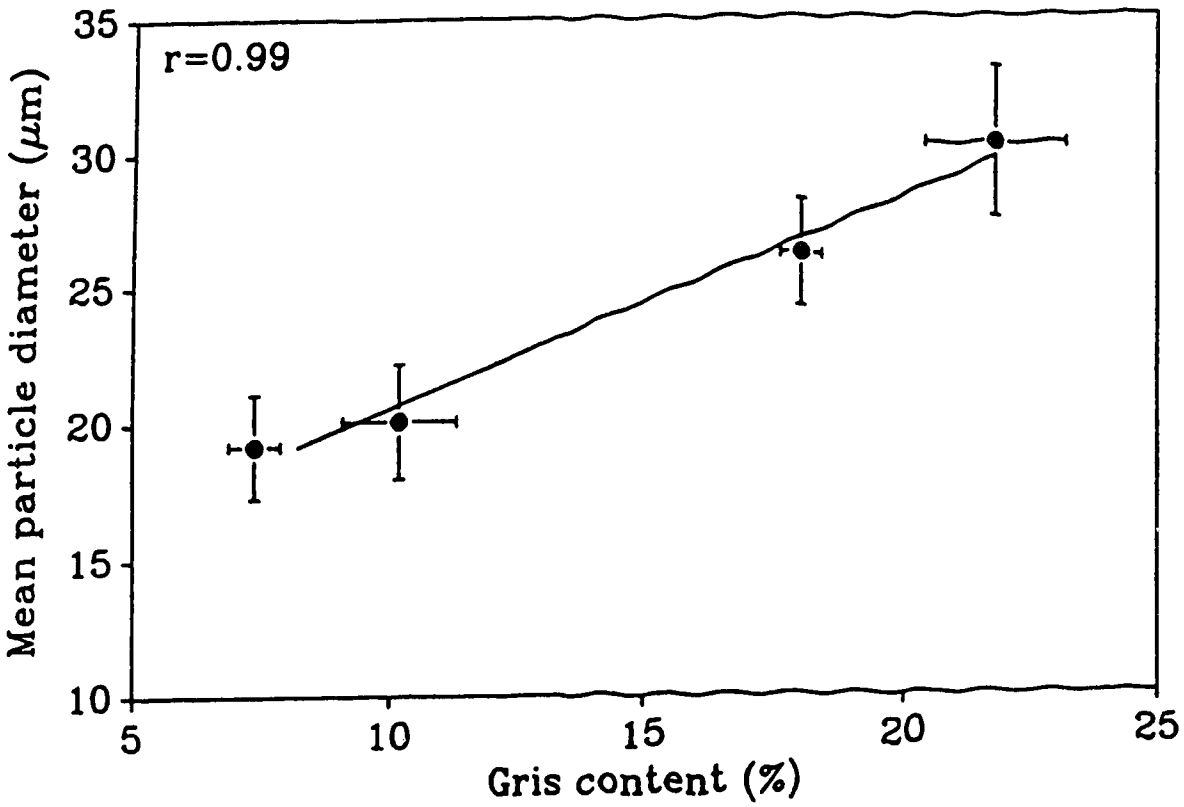

Figure 4.2 Dependence of PLA microsphere mean size on Gris:DMPC:CHOL 4:1(1:0.33) coprecipitate concentration expressed as a function the Gris content. Correlation coefficient, r = 0.99.

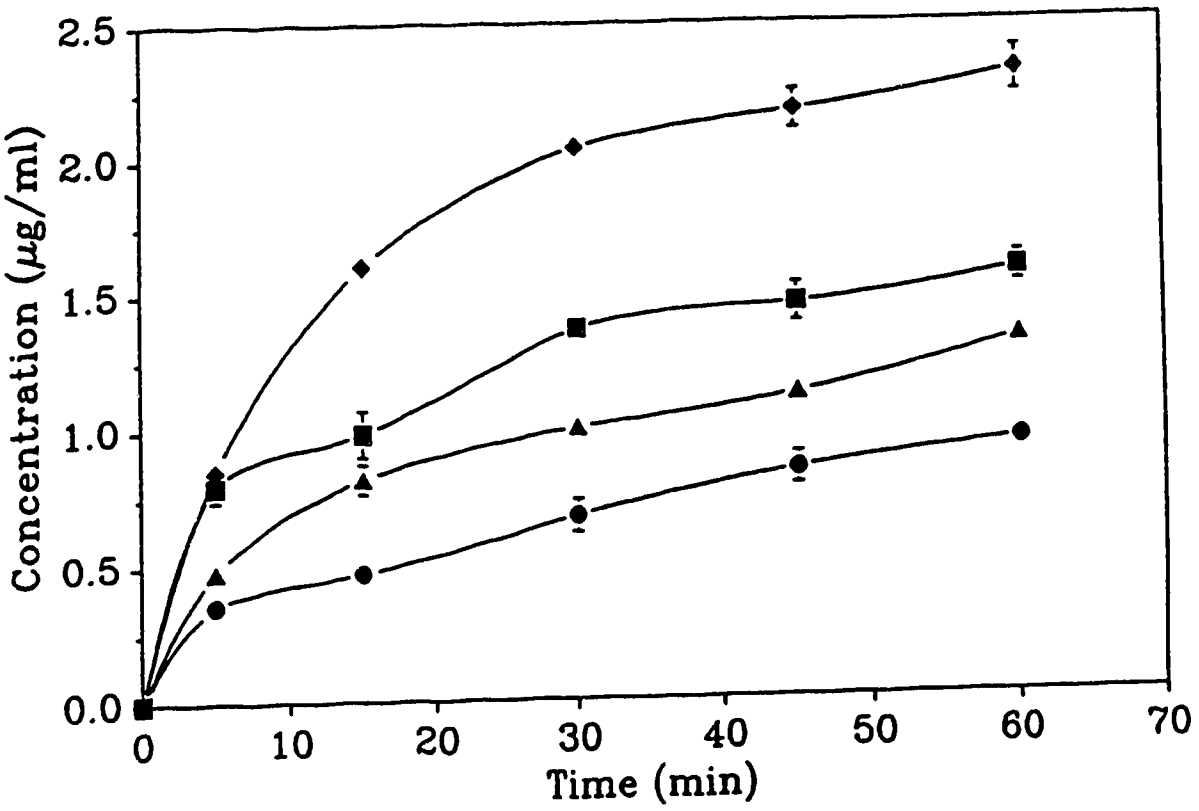

Figure 4.3 Release of Gris from PLA microspheres containing Gris or Gris:DMPC:CHOL 4:1(1:0.33) coprecipitate (SD) concentration expressed as a function of Gris content. ▲, 7.0% Gris SD; ■, 10.2% Gris SD; ♦, 18.0% Gris SD; ●, 16.7% Gris only.

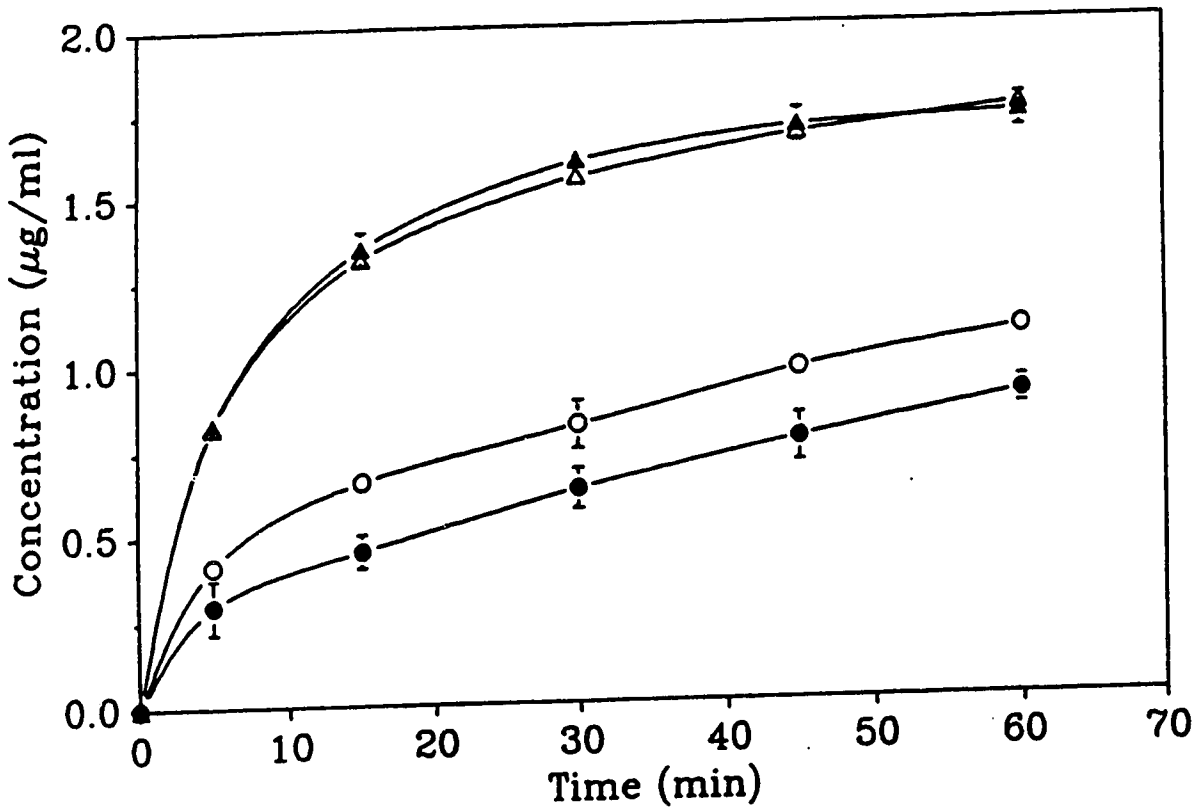

Figure 4.4 Comparison of release of Gris from PLA microspheres with the dissolution of Gris formulations of the same composition. ○, ●, 16.7% Gris; △, ▲, 21.8% Gris equivalent SD. Closed symbols represent microspheres.

of DMPC in the microspheres exhibited large variations between samples (20-50% of the total drug content), but in spite of this the enhanced release of coprecipitated Gris from the microspheres was not compromised. The storage of hard gelatin capsules containing either Gris, microspheres of Gris, or microspheres of coprecipitated Gris suspended in PEG 600 for one week was intended to determine whether the coprecipitates would retain their dissolution characteristics. The results are shown in Fig. 4.5. It can be seen that this treatment of Gris dramatically improved its dissolution and yielded the same level of Gris concentration as obtained from the microspheres after about 30 min, which was approximately two-fold that obtained from the dissolution of micronized Gris powder. The microspheres of Gris coprecipitate yielded a 10 percent higher level of Gris in solution under the same conditions which compares with a three-fold increase in dissolution of Gris from microspheres of coprecipitates over that of microspheres of pure Gris (Fig. 4.3) which had not been stored in PEG 600. The main difference between the dissolution curves of Fig. 4.3 and Fig. 4.5 is the rapid apparent dissolution observed from the PEG 600-treated microspheres during the first 15 min compared to the untreated microspheres. This is indicative of a solution concentration of Gris in the PEG 600 which occurred during the storage period (Gris solubility in PEG 600 is the same as in water, 15 mg/L), providing rapid high levels of Gris in the dissolution medium, after which Gris underwent either slow release from microspheres or dissolution in the dissolution medium at a relatively constant low rate. The release of Gris from microspheres prepared with different molecular weight PLAs are shown in Fig. 4.6.

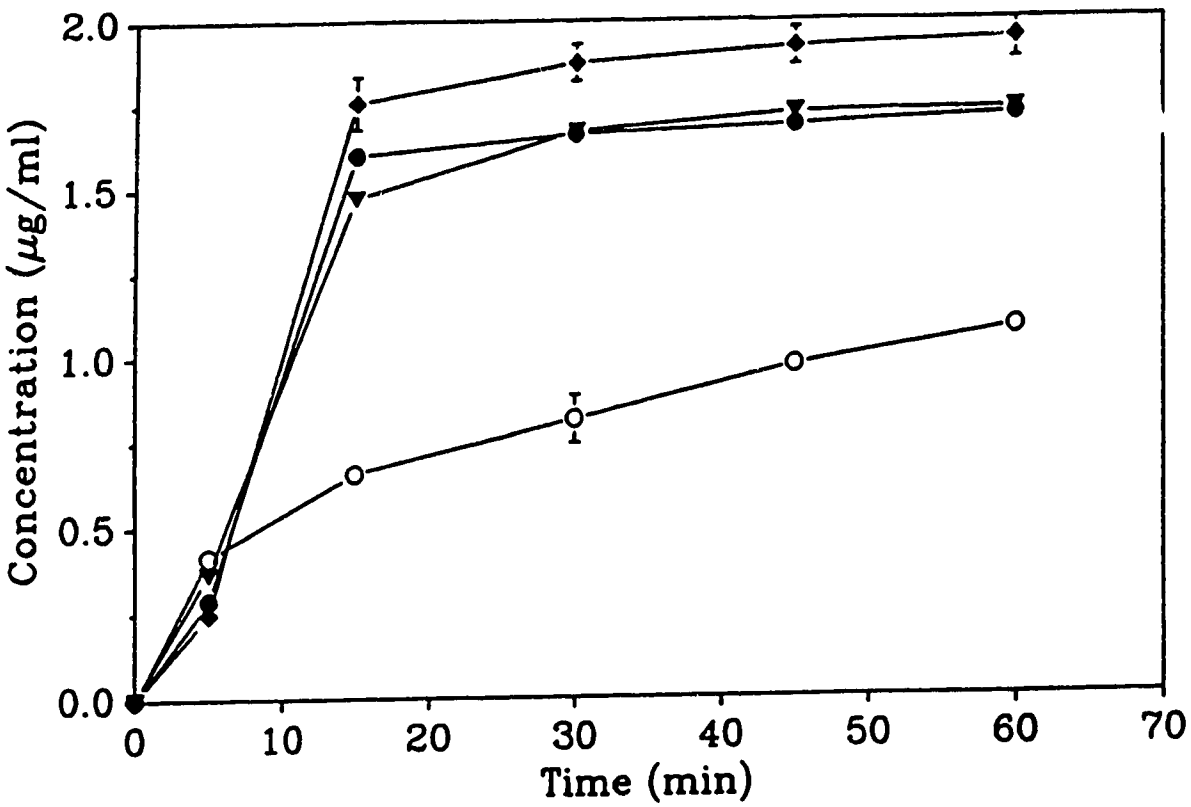

Figure 4.5 Release rates of Gris formulations from hard gelatin capsules of PEG 600 suspensions stored for one week. • , Gris microspheres; ♦ , microspheres of Gris:DMPC:CHOL 4:1(1:0.33) coprecipitate; ▼ , micronized Gris. The dissolution of micronized Gris powder (-o-) at pH 2.0, 37° is shown for comparison.

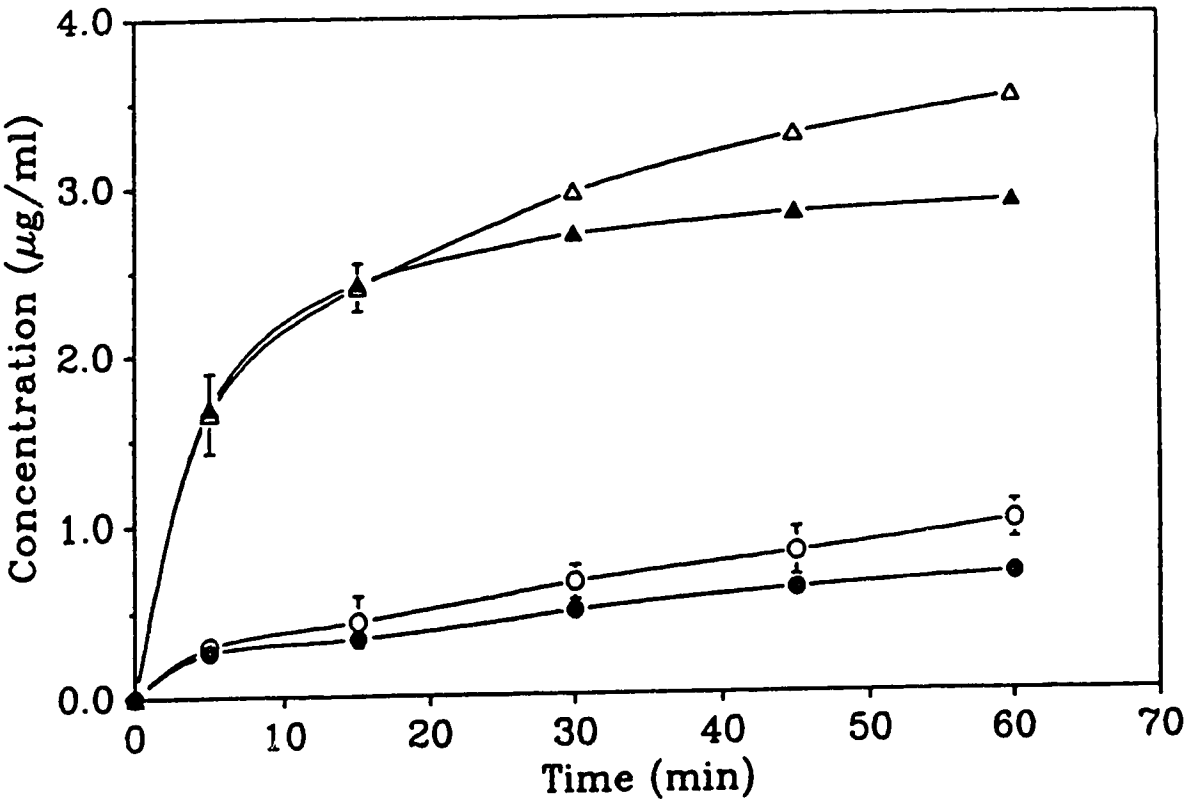

Figure 4.6 Molecular weight-dependance of the release of Gris from PLA microspheres containing 16.7% Gris or Gris:DMPC:CHOL 4:1(1:0.33) coprecipitate (SD) (18% Gris equivalent). ○, ●, Gris; △, ▲, SD. Open symbols- MW 50,000; closed symbols- MW 146,000. In this study 6 mg equivalent of Gris in microspheres was added to the dissolution medium.

The differences in the release profiles of Gris from 50,000 or 146,000 MW PLA microspheres was negligible but the constant release from Gris coprecipitate microspheres, after the initial rapid release phase (~20 min), diverged with the ratio of the rate constants from the 50,000 and 146,000 MW microspheres being 3.3.

It can be concluded that the release of Gris from coprecipitates incorporated in PLA microspheres was found to be similar to the dissolution of the coprecipitate whereas the release of Gris was slowed down when incorporated in microspheres. However, Gris or Gris microspheres exhibited equal dissolution and release behavior after being suspended in PEG 600 in a hard gelatin capsule for one week and was only 10 percent less than that found for microspheres of Gris coprecipitate under the same conditions. The release of Gris was only slightly reduced by its incorporation in 146,000 MW compared to 50,000 MW PLA microspheres but the coprecipitate microspheres yielded considerably different release kinetics after the initial rapid release phase indicating the possibility of preparing controlled drug delivery systems of coprecipitates by varying the molecular weight of the polymer. Thus, the microencapsulation of solid dispersion systems offers new opportunities for their application in improving the bioavailability of poorly water-soluble drugs and for the development of this type of formulation as pharmaceutical products.

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CHAPTER 5

ORAL BIOAVAILABILITYOF GRISEOFULVIN FROM AGED GRISEOFULVIN:LIPID COPRECIPITATES. IN VIVO STUDIES IN RATS

5.1 INTRODUCTION

Poorly water-soluble drugs often have poor bioavailabilities due to low and erratic absorption. Those drugs which undergo dissolution rate-limited gastrointestinal absorption generally obtain improved dissolution and bioavailability by reduction of particle size. For example, the therapeutic dose of micronized griseofulvin was able to be reduced to 50 percent[1], and additional benefits included steady and predictable blood levels. However, micronization of drugs often leads to aggregation and agglomeration of particles resulting in poor wettabilities[2]. Solid dispersions of poorly water-soluble drugs with water-soluble carriers have reduced the incidence of these problems and improved dissolution[3].

One of the earliest solid dispersion systems prepared to enhance dissolution rates and absorption behaviour of a drug was the solidified urea melt of griseofulvin[4]. Subsequently, it has been established that variations in dissolution of drugs from solid dispersions are not only dependent on the water-soluble carrier but also on the preparation technique. Unfortunately, many solid dispersions are physically unstable due to the high surface energy of the particles produced and, consequently, have a short shelf-life. It is mainly for this reason that very few commercial solid dispersion products are currently available. Solid dispersion systems which improved the dissolution of solvated griseofulvin, but which also were subject to aging, were those formulated containing phospholipids[5]. Recently, it has been reported that the aging

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A version of this chapter has been submitted for publication to J. Pharm. Sci.

of these coprecipitates could be drastically reduced or even halted by adding a critical amount of cholesterol[6]. The bioavailabilities of griseofulvin: dimyristoylphosphatidylcholine coprecipitates in rats and *in vitro-in vivo* correlations have been previously reported by Venkataram and Rogers[7]. The present report describes the extension of these studies to griseofulvin coprecipitate formulations which contain other phospholipids, cholesterol, and which compares the bioavailabiilities of 1-day versus 90-day aged coprecipitates.

5.2 EXPERIMENTAL SECTION

5.2.1 Materials:

Micronized griseofulvin (Gris) (Glaxo, Canada, 99.3%), L-α-dimyristoyl-phosphatidylcholine (DMPC, approx. 99%) and cholesterol (CHOL, 99+ %) (Sigma Chem. Co., St. Louis, MO) were used as received. L-α-egg phosphatidylcholine (EPC) was extracted from eggs according to Singleton et al.[8], and recrystallized from diethyl ether until chromatographically pure. Acetonitrile (BDH Canada Ltd.) was chromatographic grade; chloroform (BDH, Canada) and acetic acid (Fisher Scientific Co., Canada) were reagent grade.

p-Phenylphenol (Eastman Kodak Co., USA) was used as the internal standard in the chromatographic analyses. Heparin lock-flush solution (Hepalean, Organon Teknika Inc., Canada, 1000 USP units/mL) was diluted with normal saline (Travenol Canada Inc.) to obtain a concentration of 100 units/mL. The anesthetics diethylether (Fisher Scientific Co., Canada), methoxyflurane N.F. (Metofane, Pitman-Moore, Ltd.,

Canada) and halothane (Halothane Labs Inc., USA) were used as supplied.

5.2.2 **Preparation of Coprecipitates:**

Solid dispersions of Gris:lipid (4:1w/w) containing various lecithin:CHOL mole ratios were prepared from chloroform by the solvent evaporation method (40°C) under a gentle stream of nitrogen. Traces of chloroform were removed under vacuum for 12-16 h, then the coprecipitates were weighed and stored in a desiccator over anhydrous calcium sulfate at room temperature (24°C). Samples were removed after 1 and 90 days and examined for weight loss, Gris content, chloroform content, then an 80/120 mesh (U.S. standard sieves) sample was taken for *in vitro* dissolution or *in vivo* studies.

5.2.3 **Dissolution Studies**

The spin-filter dissolution test apparatus (Magne-drive, Clow Coffman Industries, Kansas) equipped with a 1 μm nominal porosity stainless steel filter screen was used under the following conditions: 500 rpm filter rotation speed; 900 ml dissolution medium of pH 2.0 HCl-KCl buffer solution at 37°C.

Samples of formulations equivalent to 50 mg Gris were dispersed in the dissolution medium during spin-filter rotation. The dissolution medium was circulated through a microcell in a Beckman Model 25 spectrophotometer at 293 nm and concentrations of Gris were determined from absorbances as a function of time by interpolation of absorbances from a calibration curve. The co-dissolution of small amounts of chloroform, DMPC, or CHOL did not interfere with the analyses of Gris. Experiments were carried out in quadruplicate and results were averaged. Variation

of results was less than 7 percent of the mean in all cases.

5.2.4 **In Vivo Studies in Rats:**

Adult, albino, male Sprague-Dawley rats weighing between 257 to 354g were cannulated via the external jugular vein as previously described[7] (courtesy of SMRI, University of Alberta), then fasted for 14 to 16 h prior to dosing but water was allowed *ad libitum*. Freshly-prepared (hereinafter referred to as 1-day aged) coprecipitate formulations and 90-day aged formulations were analyzed for Gris content then 80/120 sieve fractions were used to prepare aqueous suspensions immediately prior to dosing. Each rat was administered a single oral dose (equivalent to 100 mg of Gris/kg body weight in 0.5 mL of water) of the suspension by intubation. Blank blood samples (200 μL) were withdrawn prior to dosing, then samples withdrawn at 1, 2, 3, 5, 7, 11, and 24 hours post dosing. Each time the cannula was flushed with 200 μL heparinized saline. The blood samples were centrifuged, 100 μL plasma were separated then immediately frozen until required for analysis.

The plasma samples were deproteinized using 100 μL of acetonitrile containing p-phenylphenol, vortexed, centrifuged and 20 μL of the supernatant were analyzed by HPLC using a method modified from Zia et al.[9] The chromatographic system consisted of an HPLC pump (Waters Model 501), a 15 cm (C_{18}) reversed-phase chromatography column (Novapak, 5 μm, Waters Associates Inc.), a 20-μL injector loop attached to a Rheodyne injector (Model 7125, Cotati, CA), a variable wavelength UV detector (Waters, Lambda Max-Model 481 LC

spectrophotmeter) set at 290 nm, and an integrator-printer (Waters 740 Data Module). The mobile phase was 45% acetonitrile in 0.1M acetic acid (pH 3.5) at a flow rate of 1 mL/min.

5.3 RESULTS AND DISCUSSION

5.3.1 Dissolution Studies:

The dissolution behavior of the various formulations under non-sink conditions at pH 2.0 and 37° are compared in Table 5.1 using the in vitro parameters of dissolution corresponding to the amount dissolved in 5 min (D_5), the amount dissolved in 30 min (D_{30}), and the percent dissolution efficiency (%DE)[10]. It is immediately observed that these parameters were greater for all 1-day aged coprecipitates (formulations B, C, and D) than for micronized Gris (formulation A), in agreement with previous findings[5]. However, a 90-day aged Gris:DMPC coprecipitate (formulation G) yielded *in vitro* parameters statistically similar to micronized Gris (paired student's t-test, $p<0.05, n=4$). This is attributed to the loss of bound chloroform from within the crystal lattice which leads to a crystalline form of Gris similar to micronized Gris[6]. In contrast, 90-day aged Gris:DMPC:CHOL coprecipitates (formulation H) yielded in vitro parameters which were statistically unchanged from 1-day aged coprecipitates (formulation C) and also exhibited minimal loss of bound chloroform. Coprecipitates of Gris:EPC:CHOL not only possessed increased dissolution (formulation D, 1-day aged) at the optimum phospholipid:CHOL, 1:0.33 mole ratio, but the dissolution improved with age (formulation I) as previously discussed[6].

Table 5.1 Dissolution of griseofulvin formulations under non-sink conditions at pH 2.0 and 37°C.

Formulation[a]	D_5(μg/mL)	D_{30}(μg/mL)	% DE
A	5.96	10.09	18.13
B	10.36	20.15	33.49
C	13.29	21.86	36.87
D	20.80	27.82	46.36
G	7.01	12.96	23.63
H	13.40	19.84	33.27
I	26.95	37.82	62.63

[a]A, micronized Gris; B & G, Gris:DMPC (4:1w/w) coprecipitate; C & H, Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitate; D & I, Gris: EPC:CHOL [4:1(1:0.33)] coprecipitate. B, C, D are 1-day aged; G, H, I are 90-day aged.

5.3.2 **Bioavailability Studies:**

The sensitivity of the HPLC analysis of Gris using p-phenylphenol as internal standard was determined to be 0.05 μg/mL. This is identical to that obtained using m-phenylphenol as internal standard previously[7,11] and is more sensitive than 0.1 μg/mL using warfarin as internal standard in the analysis of Gris in human plasma[12].

Typical chromatograms obtained following the injection of the supernatants of centrifuged blank rat plasma spiked with Gris and internal standard, then mixed with acetonitrile, are shown in Figure 5.1. The retention times of Gris and the internal standard were 3.0 min and 4.5 min, respectively. Figure 5.1C resulted from analysis of the supernatant of a deproteinized rat plasma sample following oral dosing with an aqueous suspension of a Gris formulation, confirming that neither plasma components nor the internal standard interfered with the Gris peak. Previously, it had been established that the possible presence of the metabolite, 6-demethylgriseofulvin, did not overlap with the Gris peak[7].

Peak area ratios of Gris and internal standard were determined in triplicate from Gris-spiked plasma samples over the concentration range of 0.5 to 10.0 μg/mL at internal standard concentrations of 5 μg/mL. The calibration curve of average peak area ratios versus Gris concentration was linear with a correlation coefficient of 0.9999. Validation of the calibration curve was carried out by daily analyzing freshly-prepared standard solutions in random fashion. The coefficient of variation was found to be $< 5\%$ on all occasions.

Plasma concentration-time profiles of various Gris formulations (1-day aged

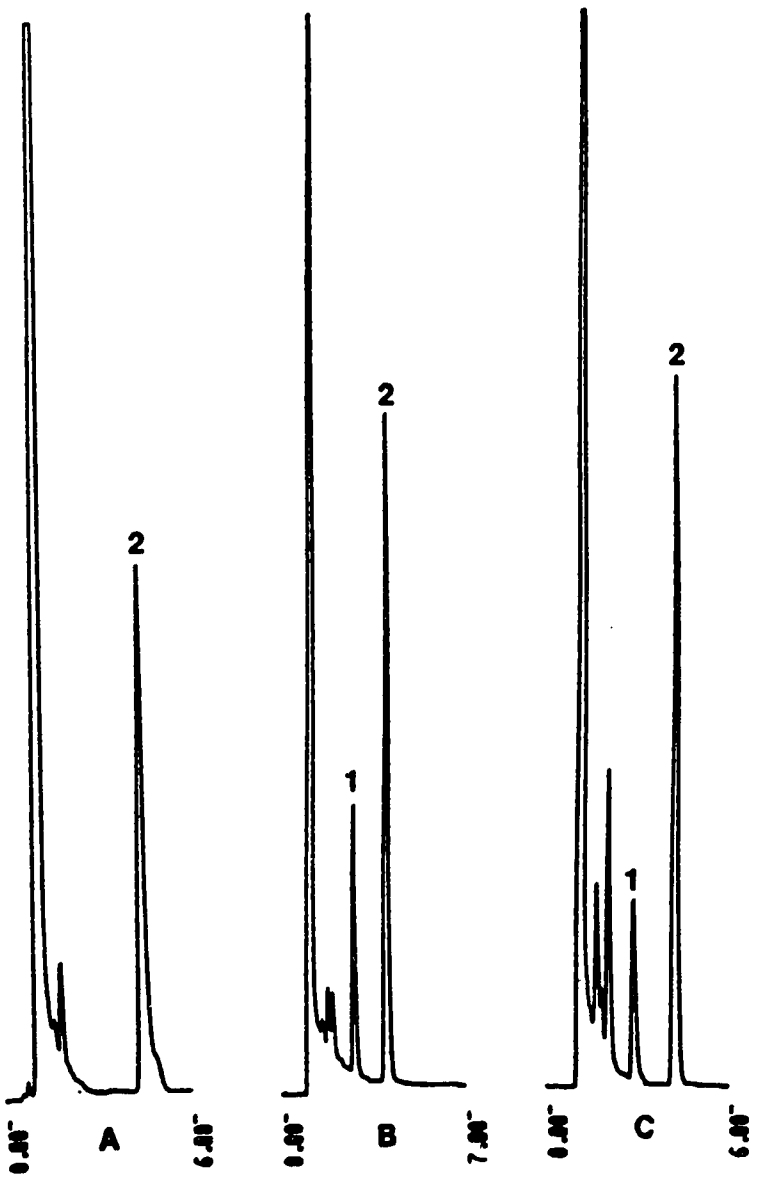

Figure 5.1 Typical HPLC chromatograms of the supernatant of spiked rat plasma in acetonitrile solution. A. p-phenylphenol (internal standard, peak 2); B. Gris (peak 1) and p-phenylphenol (peak 2); C: Deproteinized plasma sample from a rat previously dosed with an aqueous suspension of Gris.

and 90-day aged) following oral administration of aqueous suspensions to rats are shown in Figures 5.2, 5.3 and 5.4. The Gris:DMPC coprecipitates exhibited a much faster rate of absorption and achieved C_{max} (peak plasma concentration) in approximately 2 h whereas micronized Gris exhibited slow absorption and reached C_{max} in approximately 5 h (Fig. 5.2). The 90-day aged coprecipitates produced a lower C_{max} than the 1-day aged coprecipitates, similar to that obtained for Gris, but t_{max} (time to reach peak level) occurred earlier, similar to 1-day aged coprecipitates, indicating that only a small fraction of the dose administered existed in the fast dissolving state. In contrast, the concentration-time profile of the 90-day aged Gris:DMPC:CHOL coprecipitate was not significantly different from that of the 1-day aged formulation (Fig. 5.3). However, Figure 5.4 shows significantly **higher** plasma levels of the 90-day aged Gris:EPC:CHOL coprecipitate versus the 1-day aged, corresponding to the greater dissolution behavior as shown in Table 5.1.

Plasma concentration after 1 hour (C_{1h}), C_{max}, t_{max}, and area under the plasma concentration-time curve (AUC) are shown for micronized Gris, 1-day aged and 90-day aged coprecipitates in Table 5.2. Comparisons were made between the 1-day aged coprecipitate formulations and micronized Gris, and between 1-day aged and 90-day aged formulations of the same compositions. Statistical analysis by paired Student's t-test[13] and multiple comparison of means test of these data are summarized in Tables 5.3 and 5.4, respectively. Significant increases in the C_{1h} and decreases in t_{max} were observed for formulations B, C and D compared to micronized Gris (formulation A). This can be attributed to the high initial dissolution rate of

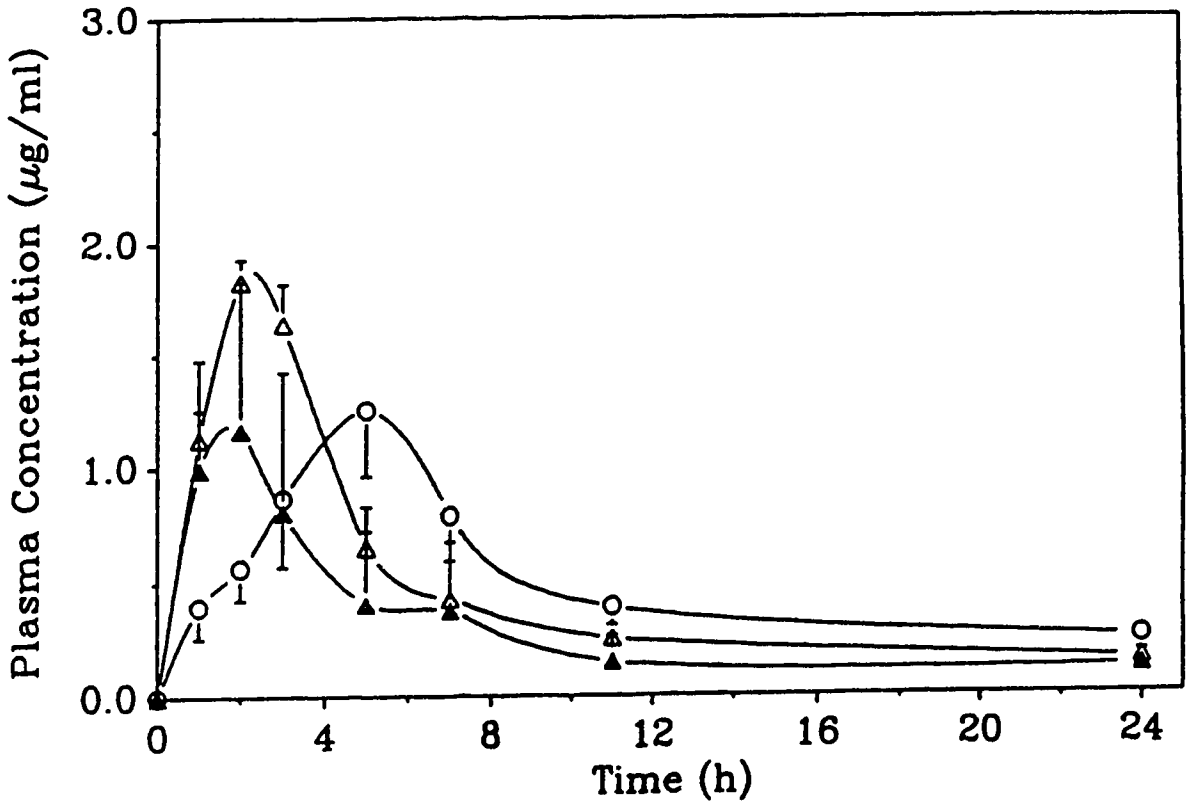

Figure 5.2 Rat plasma concentration-time profiles after oral administration of suspensions of 100 mg/kg equivalent of Gris formulations: o , micronized Gris; ▵ , 1-day aged Gris:DMPC (4:1 w/w) coprecipitate; and ▲ , 90-day aged Gris:DMPC (4:1 w/w) coprecipitate. n = 5.

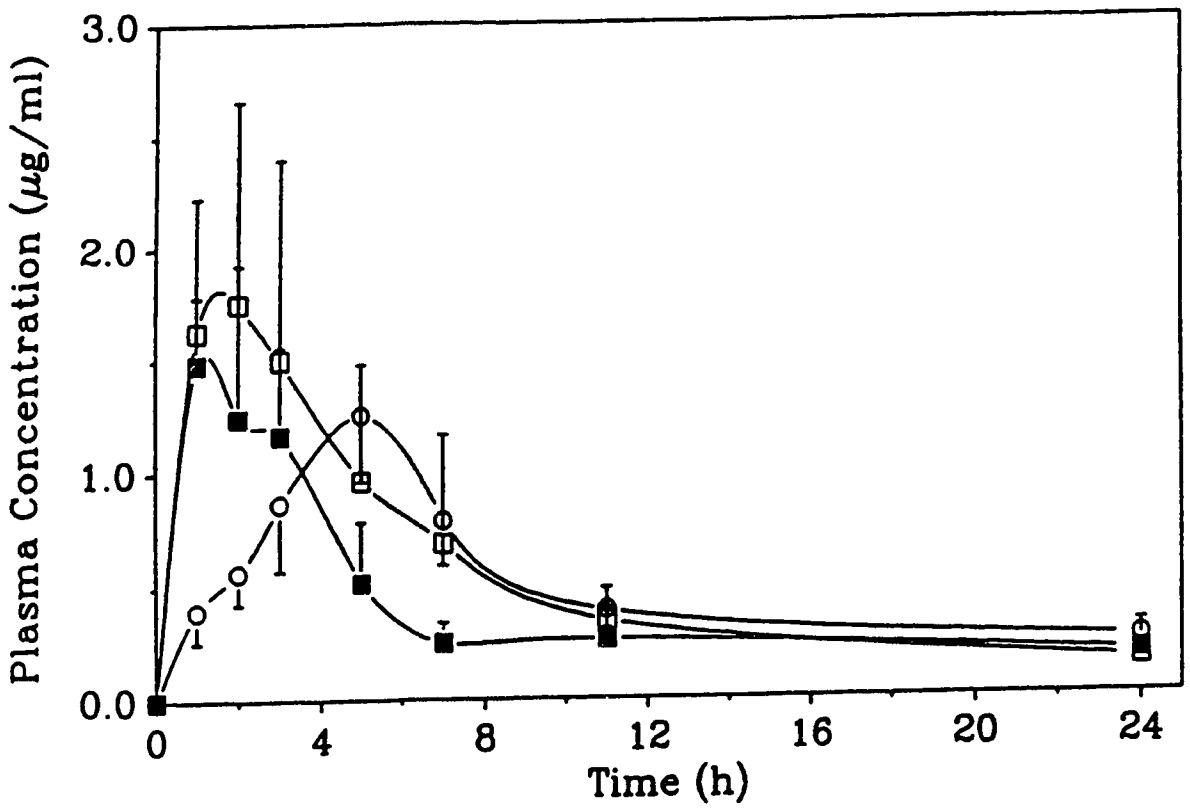

Figure 5.3 Rat plasma concentration-time profiles after oral administration of suspensions of 100 mg/kg equivalent of Gris formulations: o , micronized Gris; □ , 1-day aged Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitate; and ■ , 90-day aged Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitate. n = 5.

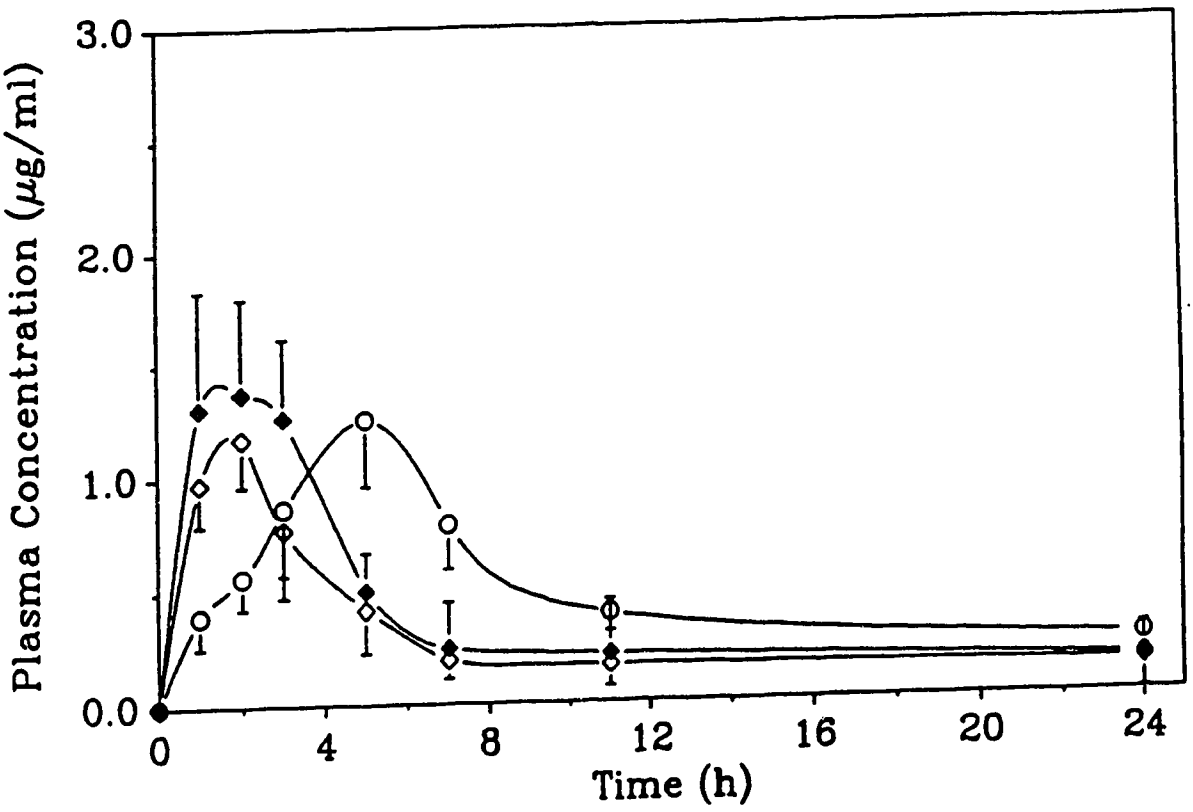

Figure 5.4 Rat plasma concentration-time profiles after oral administration of suspensions of 100 mg/kg equivalent of Gris formulations: o , micronized Gris; ◊ , 1-day aged Gris:EPC:CHOL [4:1(1:0.33)] coprecipitate; and ♦ , 90-day aged Gris:EPC:CHOL [4:1(1:0.33)] coprecipitate. n = 5.

Table 5.2. Peak plasma level (C_{max}), time-to-reach peak level (t_{max}), plasma level at 1 hour (C_{1h}), and area under the rat plasma concentration-time curve (AUC) following oral administration of Gris formulations[a].

Formulation[b]	C_{1h} (μg/mL)	C_{max} (μg/mL)	t_{max} (h)	AUC[c] (μg/mL/h)
1-day aged				
A	0.39±0.13	1.32±0.19	5.40±0.80	12.11±0.91
B	1.13±0.12	1.83±0.10	2.20±0.40	11.16±0.94
C	1.62±0.60	1.97±0.76	1.80±0.75	13.55±4.84
D	0.98±0.19	1.23±0.16	1.60±0.49	7.04±1.45
90-day aged				
G	0.99±0.49	1.19±0.64	1.60±0.49	7.37±3.54
H	1.49±0.29	1.60±0.39	1.40±0.49	9.90±2.56
I	1.31±0.52	1.72±0.10	1.60±0.80	9.17±2.75

[a]means±SD, n=5. [b]A, micronized Gris; B & G, Gris:DMPC (4:1w/w) coprecipitate; C & H, Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitate; D & I, Gris:EPC:CHOL [4:1(1:0.33)] coprecipitate. B, C, D are 1-day aged; G, H, I are 90-day aged. [c]for 24 h following oral administration.

Table 5.3. Statistical analysis (paired Student's t- test) of in vivo parameters obtained for various Gris formulations

Formulations[a]	C_{1h}	C_{max}	t_{max}	AUC
A vs B	*	*	*	-
A vs C	*	-	*	-
A vs D	*	-	*	*
B vs G	-	* $p<0.1$	* $p<0.1$	* $p<0.1$
C vs H	-	-	-	-
D vs I	-	*	-	-

[a]A, micronized Gris; B & G, Gris:DMPC (4:1w/w) coprecipitate; C & H, Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitate; D & I, Gris:EPC:CHOL [4:1(1:0.33)] coprecipitate. B, C, D are 1-day aged; G, H, I are 90-day aged.

*significantly different at $p<0.05$ unless otherwise indicated.

Table 5.4. Least Significant Difference (LSD) determined by multiple comparison-of-means test ($p<0.05$) on 1-day aged and 90-day aged Gris formulations.

Parameter	Formulations[a,b]
C_{1h}	D < G < <u>B < I < H < C</u>
C_{max}	G < D < <u>H < I < B < C</u>
t_{max}	<u>H < D < G < I < C < B</u>
AUC	D < G < I < <u>H < B < C</u>

[a]A, micronized Gris; B & G, Gris:DMPC (4:1w/w) coprecipitate; C & H, Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitate; D & I, Gris:EPC:CHOL [4:1(1:0.33)] coprecipitate. B, C, D are 1-day aged; G, H, I are 90-day aged.

[b]formulations underlined are not significantly different from one another at $p<0.05$.

the coprecipitate formulations, and, consequently, a lower t_{max} versus micronized Gris[7]. A statistical difference in C_{max} was found for formulation B but not formulations C or D, and in the AUC for formulation D but not B or C (Table 5.3). When the t_{max} values of all the Gris:lipid formulations are compared among themselves, no significant differences were found (Table 5.4).

In a comparison of 1-day and 90-day aged Gris:DMPC (4:1w/w) coprecipitates (formulations B vs G, Table 5.3) there were significant differences in C_{max}, AUC and t_{max} as expected from the *in vitro* studies. In contrast, formulations C and H (Gris:DMPC:CHOL [4:1(1:0.33)] coprecipitates) correlated with the 1-day aged in vitro data (Table 5.1) but no differences were found statistically when formulation C was compared with formulation H using the *in vivo* parameters in Table 5.3. This is interpreted to mean that the reduction in solvent loss from the coprecipitates containing 33 percent CHOL in the lipid phase minimized the aging process and, consequently, contributed to maintaining the improved dissolution behavior and absorption efficiency of these formulations. Comparison of formulations D and I revealed a significant increase in C_{max} with concomitant increases in C_{1h} and AUC. This apparently unusual increase in bioavailability with aging of the Gris:EPC:CHOL coprecipitates is attributed to degradation of EPC[6]. However, the exact mechanisms responsible for the increased dissolution and bioavailability of formulation I are not completely understood.

5.3.3 In Vitro-In Vivo Correlations:

Correlations were also obtained from plots of *in vitro* parameters versus *in vivo*

parameters, using pairings of D_5,C_{1h}, D_{30},C_{max}, and %DE,AUC for each formulation. The correlation coefficients ranged from 0.93 to 0.97 indicating that generally dissolution of Gris formulations can predict their behavior *in vivo*. Secondly, correlations of *in vitro* parameters with *in vivo* parameters for 1-day aged formulations (formulations A, B and C) and 90-day aged formulations (formulations A, G and H) were also obtained. The three *in vitro* parameters correlated highly with C_{1h} or C_{max} ($r = 0.96 - 0.99$, 1-day aged coprecipitates; $r = 0.90 - 0.98$, 90-day aged coprecipitates) but the correlation with AUC was poorer ($r = 0.88$, $r = 0.77$, respectively), possibly due to the different natures and pH of the dissolution media of the *in vitro* and *in vivo* studies, as well as the lack of a sampling point between the 11 h and 24 h samples.

It is concluded that the incorporation of CHOL in Gris:DMPC coprecipitates decreased aging thus maintaining the improved dissolution behavior as observed with freshly-prepared coprecipitates (Table 5.1, ref. 6). Furthermore, this benefit was extended to the *in vivo* situation in the rat since C_{1h}, C_{max}, and t_{max} of the 90-day aged coprecipitate Gris formulations also improved compared to micronized Gris. The unusual increase in dissolution behavior of aged Gris:EPC:CHOL coprecipitates also corresponded to a greater bioavailability (AUC) of Gris in rats. At least in this case, the aging process was not detrimental to achieving improved bioavailability. The addition of CHOL to other solid dispersion systems that age may exert a similar beneficial effect and, indeed, preliminary results with the phenylbutazone:polyethylene glycol system suggest that this is the case.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

6.1 GENERAL DISCUSSION

The formulation of drugs as solid dispersions offers considerable potential to increase the dissolution rates and bioavailabilities of drugs, the absorption of which are rate-limited by solubility or dissolution. However, the solvent method or fusion method used to prepare these systems are not free of problems, such as total removal of the coprecipitating solvent and thermal instability of drug. In addition, deterioration of the solid dispersion during storage, i.e. aging, is a difficulty encountered with some formulations. In this regard, small drug:carrier ratios are believed to favor stability of solid dispersions (Ford, 1986), but this usually leads to relatively large unit dosage forms. Thus, there would appear to be an advantage in having a rapidly-dissolving solid dispersion system of high drug content. This has been found possible using only 5 percent phospholipid incorporated in certain drug solvates (Venkataram and Rogers, 1984).

The antifungal antibiotic, griseofulvin, is known to exhibit poor bioavailability due to its low water solubility and poor dissolution characteristics. The stable crystal lattice of this drug can, however, be disrupted by proton-donating solvents, such as chloroform (Sekiguchi et al., 1964), and fatty acids which form hydrogen-bonded complexes with griseofulvin (Grant and Abougela, 1982; Grant and Mehdizadeh, 1984), or by certain other solvents, such as benzene and dioxane (Sekiguchi et al., 1976). The griseofulvin:chloroform solvate is well-known (Sekiguchi et al., 1964) and has been reported to have increased the solubility, dissolution rate and bioavailability of griseofulvin (Bates et al., 1977). In contrast, Venkataram and Rogers (1984)

reported a slightly decreased dissolution rate and bioavailability for the solvate compared to pure griseofulvin.

A detailed crystal structure evaluation of the griseofulvin:chloroform solvate (Cheng et al., 1979) may provide clues to the expected physical behavior of griseofulvin in this context. The chloroform molecules in the solvate lie in layers perpendicular to the *c*-axis of the crystal and it is reported that desolvation proceeds in a planar manner from the crystal surfaces towards the center of the crystal. The desolvation process converts a single crystal of solvate to a composite of small anhydrous crystals which accounts for the increased dissolution rate and bioavailability.

Although some of the most common reasons for the observed increased dissolution rates from solid dispersions have been due to solid solution, eutectic formation, conversion of drug crystals to an amorphous form, or due to the solubilization effect of the carrier, earlier investigations of Gris:DMPC coprecipitates (Venkataram and Rogers, 1984) did not reveal any evidence that these processes played a significant role except, perhaps, a solubilization effect of drug in the theoretical stationary layer. Partitioning of drug in liposomes of DMPC, which rapidly form upon contact of the crystals with water, could increase the solubility of drug and, hence, dissolution according to the Nernst-Brunner equation (Eq. 5) (Venkataram and Rogers, 1985). Furthermore, thermodynamic properties of the coprecipitate crystals indicated a degree of metastability. York and Grant (1985) determined the "disruption index" of Gris:DMPC coprecipitates and found a five-fold

increase compared to pure griseofulvin. Further, Venkataram (1986) reported lower heats of fusion for these coprecipitates. Similarly, metastable crystals of riboflavin coprecipitated with benzoic acid in isopropyl alcohol have been reported (El-Sayed et al., 1982). On the other hand, the dissolution increase of indomethacin, ketoprofen and flurbiprofen from phosphatidylcholine solid dispersions was attributed to the existence of amorphous forms of the drug in the coprecipitates (Fujii et al., 1988).

The properties of solid dispersions which give rise to improvements in dissolution are presumably also responsible for the aging of these systems which, generally, is pervasive. In solid dispersions where the drug has been shown to be in an amorphous or molecularly-dispersed state (i.e. solid solution) when freshly-prepared, the material has undergone hardening (Chiou and Riegleman, 1969), crystallization (Ford and Rubinstein, 1979), or self-association (Merkle, 1982) with age. Likewise, coprecipitates of drug:phospholipid as reported by Venkataram and Rogers (1984) aged with a concomitant loss of bound chloroform and reduced the dissolution properties. To this author's knowledge no attempts to reduce or eliminate the aging of solid dispersions have been reported in the literature, except that as described for the griseofulvin:DMPC:chloroform system using CHOL (Vudathala and Rogers, 1991).

It is informative to discuss at this point the role of additives, and CHOL in particular, on the aging stability of the coprecipitates used by Venkataram and Rogers (1984) and to show how this approach might be generally applicable to other

solid dispersion systems. Thus, it was first observed that the addition of CHOL in small amounts increased the dissolution behavior of Gris:DMPC coprecipitates and a maximum in this trend was reached after one-third of the DMPC had been replaced by CHOL, beyond which lower dissolution rates were observed (Venkataram and Rogers, 1985). These systems were not tested for aging but this effect of CHOL on the coprecipitate crystal behavior suggested that CHOL might have an influence on the aging behavior of these systems. Indeed, this has now been demonstrated and the aging has been quantitated. The slowing of the aging process has been described by the 'aging factor' (Chapter 2) which was found to be linearly correlated with the storage time and dissolution. The maximum effect was observed when the DMPC:CHOL mole ratio was 1:0.33.

In order to further examine the possibilities for other solid dispersions, CHOL was incorporated in fused phenylbutazone-PEG 6000 systems and, again, decreased aging was observed. The addition of triolein or palmitic acid to this solid dispersion system had the effect of increasing the rate and extent of dissolution on aging. This is parallel to the increased dissolution of Gris observed from the Gris:EPC:CHOL systems with aging, although the mechanisms occurring with palmitic acid systems are unclear. In this system aging is believed to result from complex formation via the acidic hydrogen of phenylbutazone (pK_a = 4.4) and the ethereal oxygen of PEG (Khalil and Mortada, 1978). CHOL and the other lipids apparently interfered with this interaction, thereby delaying the aging effect. Also, in the case of Gris:DMPC coprecipitates it can be argued that the incorporation of CHOL stabilized the

crystalline structural lattice against loss of chloroform through channels in the crystal, which had the effect of maintaining the dissolution properties of the coprecipitate.

Not all coprecipitates of drug solvates with phospholipid undergo aging. This is clearly the case with fludrocortisone acetate-ethyl acetate solvate in which phospholipid (DMPC) was incorporated. However, this system is another clear-cut example of the increased dissolution of a drug from its solvate with the addition of phospholipid (42 percent increase versus 18 percent for Gris:DMPC coprecipitates). The same level of dissolution from these coprecipitates was repeatedly demonstrated even after aging for 135 days.

Generally, it appears that the dissolution kinetics of drug:phospholipid coprecipitates can be described by second order or Weibull distribution functions and predictions can be made with a fair amount of accuracy. The kinetics of drug release obtained for fludrocortisone acetate:DMPC coprecipitates and Gris:DMPC coprecipitates (Venkataram, 1986) appears to substantiate this.

The application of the coprecipitate formulations for the improvement of efficiency of absorption was demonstrated in rats from *in vitro-in vivo* correlations. No significant differences were observed between 1-day and 90-day aged coprecipitates of Gris:DMPC:CHOL.

A particularly interesting development occurred from aged Gris:EPC:CHOL coprecipitates. It appeared that degraded EPC in the coprecipitates after aging altered the crystalline energies yielding increased dissolution of drug. This occurred without loss of chloroform, again demonstrating the stabilizing role of CHOL.

Additional means of altering the release rate of drug from the coprecipitates was to incorporate various polymers. In this regard, it was shown that the rate and extent of dissolution of Gris could be decreased as a function of the polymer concentration and polymer type (using either dextran, poly(L-lactic acid) or polyvinylpyrrolidone), presumably by reducing the diffusivity of the drug through a more viscous polymer solution (Kellaway and Najib, 1983). Thus, further means of controlling the availability of drug was made possible.

The mechanisms involved with these systems are dissimilar to other mechanisms reported for the improved absorption of drug from lipid vehicles (Bloedow and Hayton, 1976; Bates et al., 1977) or cholesteryl ester dispersions of hydrocortisone (Kim and Jarowski, 1977) or antibiotics (Patel and Jarowski, 1975). The dissolution of phospholipid coprecipitates described here is based on two concurrent mechanisms, namely, rapid break-up of crytals to ultrafine crystallites due to rapid hydration of phospholipid on crystal surfaces and rapid transfer of dissolving drug into phospholipid bilayers formed as myelinic structures during hydration. Previously described mechanisms only involved lipid dissolution of drug followed by absorption of microdroplets of lipid solution after bile acid emulsification or a surface tension lowering effect which increased wetting of the drug crystals and, consequently, increased dissolution.

The purpose of these studies was to develop an improved formulation of poorly water-soluble drugs which provides better dissolution and bioavailability characteristics. It is recognized that many of the solvents employed in this context

are or may be unsuitable for human consumption. The residual amount of chloroform in Gris coprecipitates was about 23 percent whereas only 6-8 percent ethyl acetate remained in fludrocortisone acetate coprecipitates. Nevertheless, a fundamental principle has been demonstrated and tested. However, a further attempt at developing these coprecipitate systems for practical administration purposes was made. Thus, microencapsulated coprecipitates were formulated in order to improve the stability, release characteristics, flow properties and handling capabilities of the powders. The process is relatively simple and leads to a dried powder which possesses superior flow properties and stability of the coprecipitate when suspended in a solvent such as PEG 600. In either case, the microspheres can be prepared in a granulation for hard gelatin capsule manufacture or as a suspension in PEG 400 or 600 in soft gelatin capsule manufacture. The properties of these dosage forms would not be unlike that of the coprecipitate powder itself in terms of dissolution and bioavailability except for a lag time period during which the gelatin capsules dissolve before releasing their contents. On the other hand, others have employed spray-drying to microencapsulate solid dispersions using enteric-coating polymers or colloidal silica (Takeuchi et al., 1987), or polyvinylpyrrolidone (Corrigan et al., 1984) in order to maintain the amorphous form of the drug, and have also obtained spherical and free-flowing particles with retention of good dissolution properties.

6.2 CONCLUSIONS

1. The addition of CHOL to Gris:DMPC coprecipitates (4:1 w/w) markedly reduced the aging (i.e. reversion of increased dissolution properties of Gris to its original poor dissolution behavior).
2. At a critical ratio of DMPC:CHOL (1:0.33 mole ratio) aging was insignificant even after 90 days as the dissolution behavior had not changed. This coincided with minimum loss of chloroform and minimum change in the heat of fusion (ΔH_f) of the coprecipitates.
3. An aging factor, defined as $\Delta H_f/[CHCl_3]$, as a function of the storage time clearly differentiated the relative aging tendencies of all formulations studied. Furthermore, among compositions of Gris:DMPC:CHOL, the 4:1[1:0.33] composition exhibited the lowest aging coefficient (= slope of aging factor vs storage time plot).
4. Gris:EPC:CHOL, 4:1[1:0.33]coprecipitates yielded an aging coefficient of zero after 135 days but produced an unexpected increase in the dissolution of Gris over the aging period. Indirect evidence suggested that oxidation of EPC was responsible for the observed increase in dissolution of Gris as a function of aging of the coprecipitates.
5. The extent of dissolution of FA from FA:DMPC coprecipitates improved by approximately 42% (cf. 18% for Gris:DMPC coprecipitates), but the dissolution of these did not alter with age.
6. The incorporation of polymers (Dextran, PLA or polyvinylpyrrolidones) at low

concentrations in the FA:DMPC (4:1 w/w) coprecipitates led to a decrease in the initial dissolution rates (13.5% to 60%) and percent dissolved (1% to 16%) as a function of concentration (0.01% to 7.5%) and polymer type.

7. The kinetics of dissolution from drug:phospholipid coprecipitates can be generally described by the second order or Weibull distribution functions.
8. The maximum encapsulation of Gris as coprecipitate in microspheres was 22 % by weight.
9. The relationship between microsphere size (A, μm) and Gris content (B, %) could be described by: $A = 0.778 \times B + 12.787, (r = 0.99)$.
10. The rate of release of Gris from microencapsulated Gris coprecipitate was higher than that of microencapsulated Gris alone, and increased as a function of the Gris loading.
11. The rate of release of microencapsulated Gris (PLA, MW 146000) was less than the dissolution rate of Gris whereas the rate of release of Gris from micoencapsulated Gris coprecipitate was approximately equal to the dissolution of Gris from coprecipitates.
12. Microspheres prepared from PLA (MW 146,000) yielded a free flowing powder, which was more easily manageable during processing, and were stable when suspended in PEG 600 in hard gelatin capsules.
13. *In vivo* studies were consistent with the observations made *in vitro* on the aging of Gris:phospholipid coprecipitates.
14. Good correlations between the *in vitro* and *in vivo* parameters were obtained.

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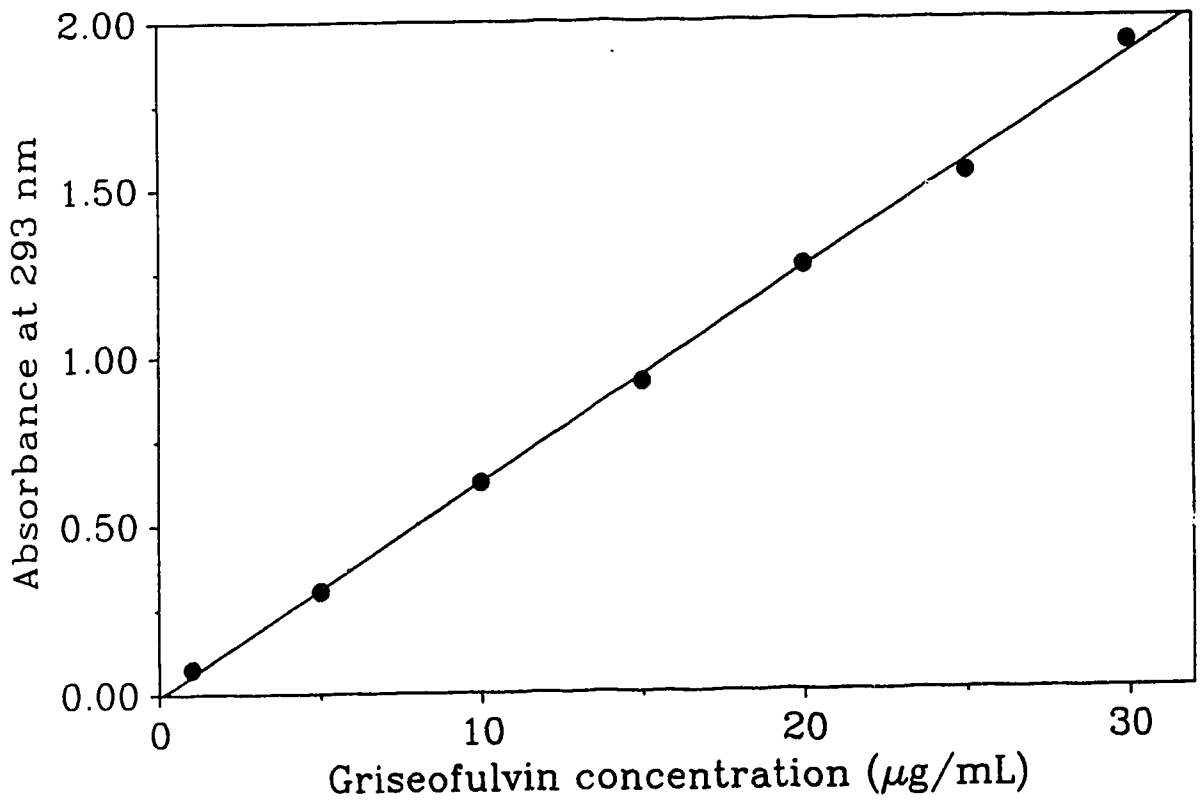
APPENDIX 1

UV Spectrophotometric Analysis of Griseofulvin

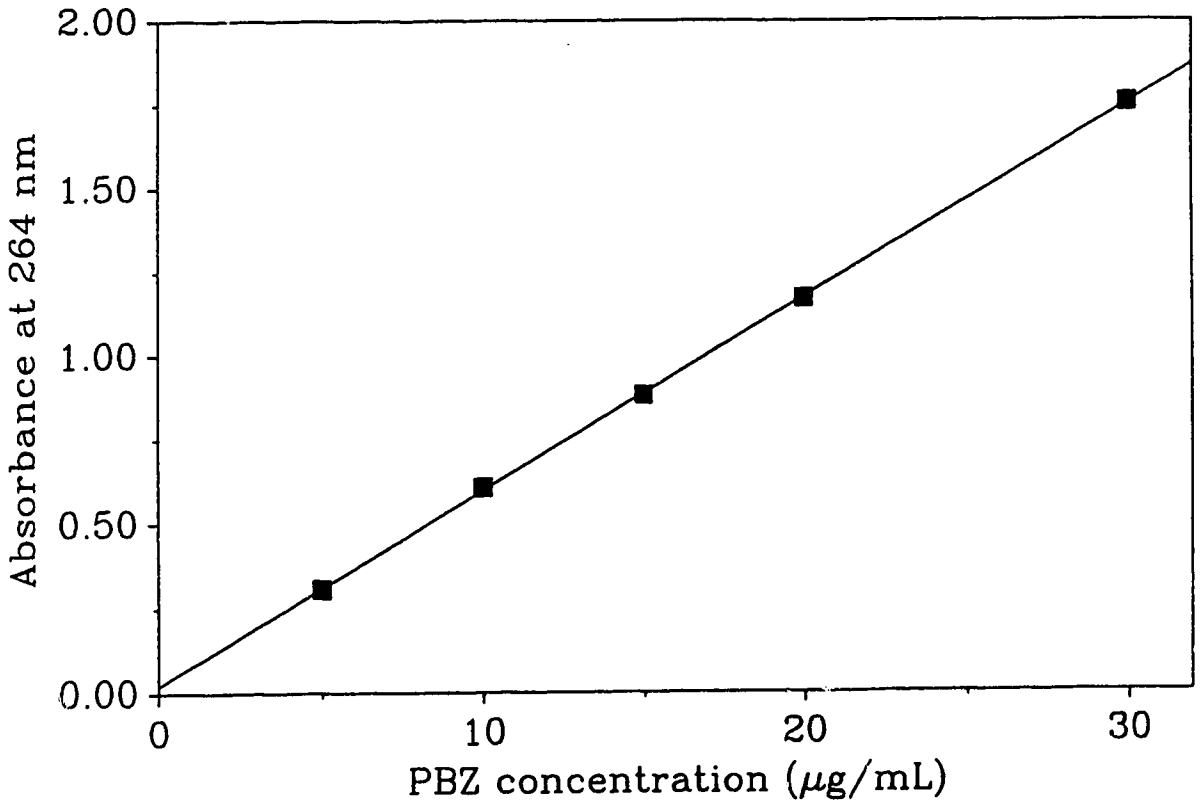
A linear calibration curve which obeyed Beer's law over the concentration range of 1 to 30 μg/ml was obtained for griseofulvin as shown below. Regression analysis of the experimental values yielded slope = 0.0632, intercept = -0.0070, and correlation coefficient r = 0.9994. Reproducibilty of the experimental values was always > 99 %.



APPENDIX 2

UV Spectrophotometric Analysis of Phenylbutazone

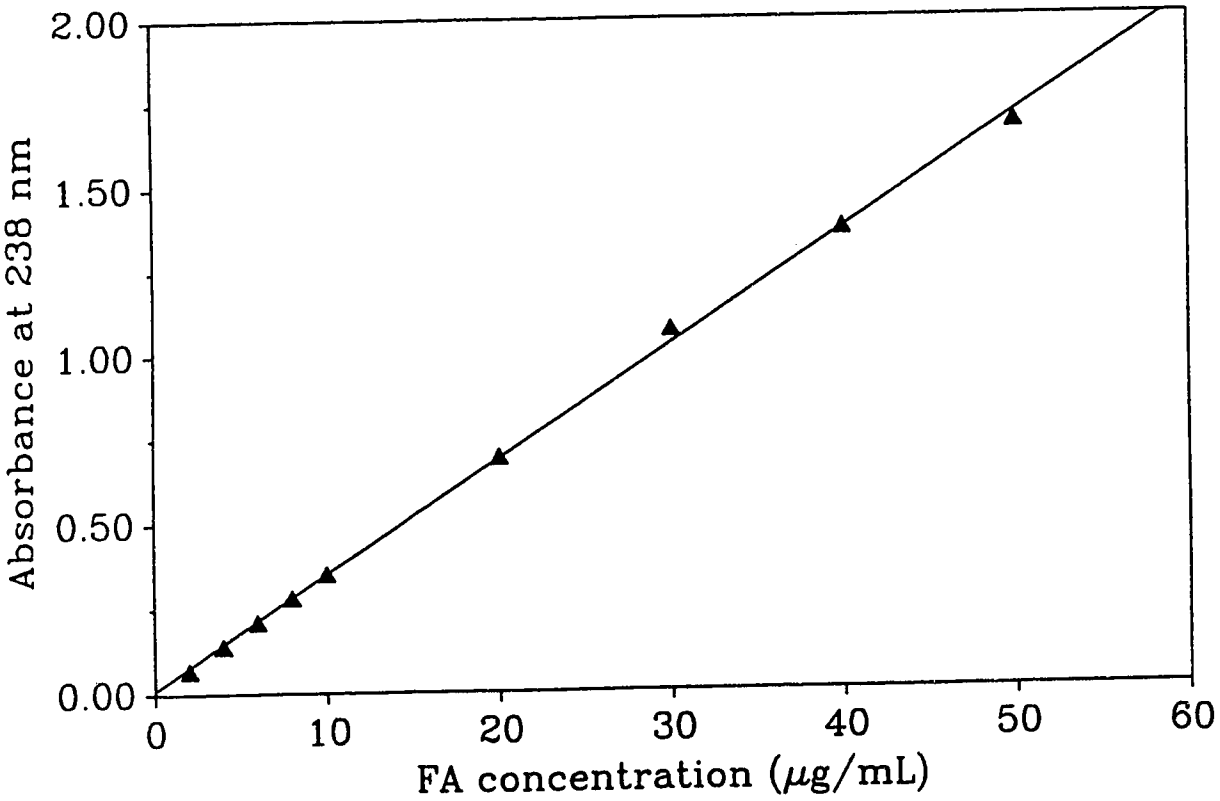
A linear calibration curve which obeyed Beer's law over the concentration range of 5 to 30 μg/ml was obtained for phenylbutazone as shown below. Regression analysis of the experimental values yielded slope = 0.0577, intercept = 0.0210, and correlation coefficient r = 0.9999. Reproducibilty of the experimental values was always > 99.5 %.



APPENDIX 3

UV Spectrophotometric Analysis of Fludrocortisone Acetate

A linear calibration curve which obeyed Beer's law over the concentration range of 1 to 50 μg/ml was obtained for fludrocortisone acetate as shown below. Regression analysis of the experimental values yielded slope = 0.0339, intercept = 0.0115, and correlation coefficient r = 0.9995. Reproducibilty of the experimental values was always >99.2%.



APPENDIX 4

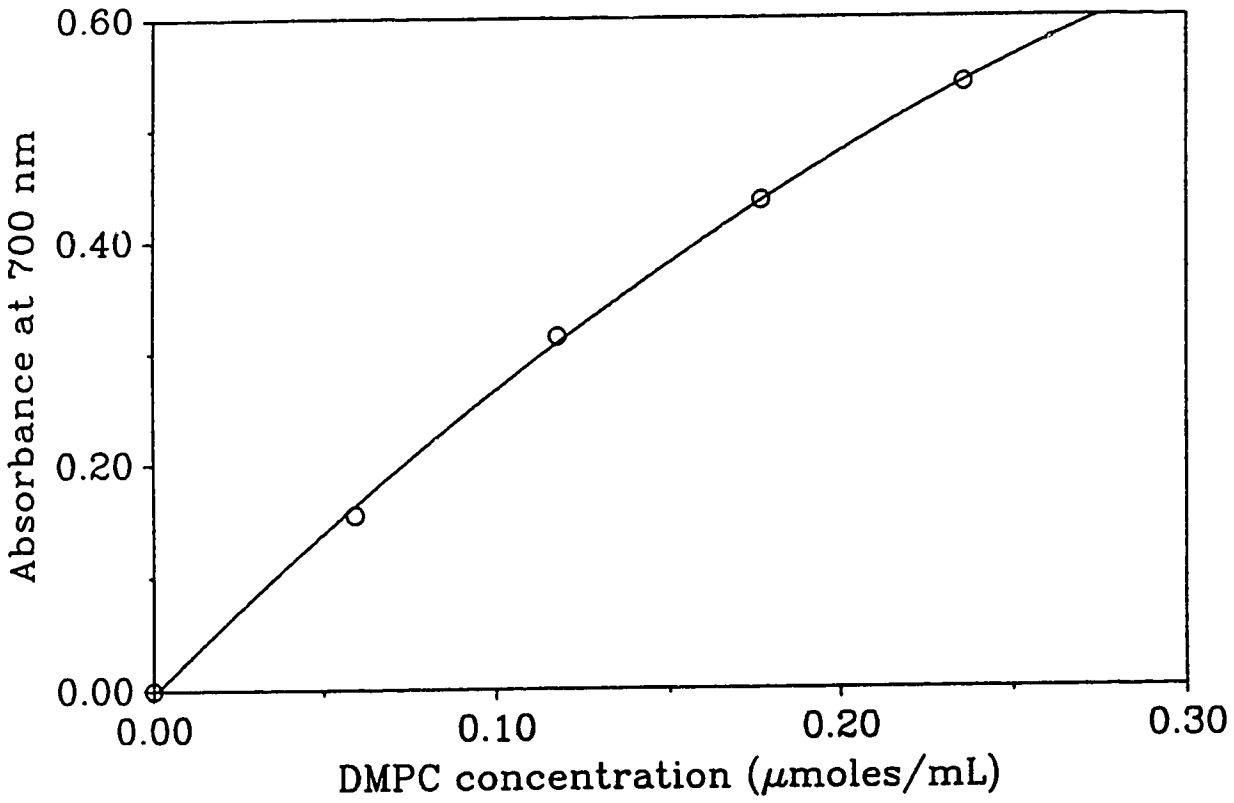
Analysis of Dimyristoylphosphatidylcholine

Procedure: To 2 ml of sample, 0.2 ml of concentrated perchloric acid was added and and the mixture hydrolysed in an oven at 180°C for at least 2 hours. The sample was then cooled to room temperature and 2 ml reducing agent (consisting of 10 g/L sodium bisulfite, 2 g/L sodium sulfite and 168 mg/L 1-amino-2-napthol-4-sulfonic acid in water) was added and mixed. Further, 2 ml of molybdate reagent (consisting of 4.4 g/L ammonium molybdate and 14 ml/L concentrated sulfuric acid in water) was added and mixed again. The sample solution was then boiled in a water for 10 min. The absorbance of the solution was then measured at 700 nm versus an untreated reagent blank in a Beckman 25 spectrophotometer. The concentration of DMPC was calculated from a calibration curve obtained over the range of 0.05 to 0.25 μmoles/mL by the equation:

$$C = 0.3061 * A + 0.2314 * A^2 + 0.0017$$

where C is the DMPC concentration and A is the absorbance with r = 0.9995.

DMPC Calibration Curve:



APPENDIX 5

DTA Calibration and Analysis

The Quantative Differential Thermal Analyzer (Model 300, Fisher Scientific Co. NJ., U.S.A.) was calibrated with calorimetric standards of known heats of fusion, using 10 mg each of naphthalene (NA), indium (In), tin (Sn) and lead (Pb). The empty pan was used as reference.

Calorimetric Standards were obtained from Fisher Scientific Co.,NJ, U.S.A. The Calibration constant (K) for each of the standards was calculated by the equation

$$K = \frac{\Delta H_f \, M \, P}{A \;\; \Delta T_s . \, T_s}$$

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 a planimeter (Gelman Instrument Co.,) and a plot of K versus peak temperature was constructed as the calibration curve.

In order to determine the ΔH_f of the samples. 10 mg equivalent of the drug was hermetically sealed in an aluminium pan, heated at a predetermined heating rate and thermograms were recorded. Using the calibration curve, the K value corresponding to the sample peak temperature was determined and ΔH_f calculated from the equation.

$$\Delta H_f = \frac{K \, A}{M}$$

where **A** is the peak area (sq.in) and **M** is the mass of sample (g).

DTA Calibration Curve: