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Effect of cholesterol on the aging of griseofulvin-phospholipid coprecipitates

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Summary

Coprecipitates of griseofulvin (Gris), dimyristoylphosphatidylcholine (DMPC), egg phosphatidylcholine (EPC) and cholesterol (CHOL) prepared from chloroform solutions were subjected to dissolution studies, differential thermal analysis (DTA), and weight loss determinations after various storage times. The addition of CHOL to certain compositions of Gris : DMPC coprecipitates increased the dissolution of Gris, reduced the residual chloroform content and decreased the aging towards lower dissolution behavior. Coprecipitates of Gris : lipid, 4 : 1 weight ratio (1 : 0.33 DMPC : CHOL mole ratio) exhibited maximum dissolution and the lowest degree of aging. Fusion temperatures, heats of fusion (ΔH_f), and chloroform contents of the coprecipitates varied with CHOL content and with aging but neither individually correlated with the dissolution data. Instead, the aging factor $= \Delta H_f/[CHCl_3]$ linearly correlated with the storage time and with dissolution. When DMPC was replaced with EPC at the optimum mole ratio of 1 : 0.33 EPC : CHOL, aging of the coprecipitate up to 90 days resulted in increased dissolution of Gris, the level of which remained constant for samples aged up to 135 days. Chromatographic evidence and oxidation indices of EPC suggest that the unexpected behavior of these systems is possibly a result of the degradation of EPC. Generally, the results indicate that over a narrow range of concentration, CHOL addition augments the dissolution of freshly-prepared Gris : phospholipid coprecipitates and reduces their desolvation on storage thereby minimizing the aging of these systems and maintaining the improved dissolution behavior of Gris.

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

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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; Fromming and Hosemann, 1985; Vila-Jato and Alonso, 1986; Alonso et al., 1988). 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 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.

Materials and Methods

Materials

Micronized griseofulvin (Gris) (Glaxo, Canada, 99.3%), egg L-α-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%), 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.

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. Subsequently, an 80/120 mesh (U.S. standard sieves) sample was taken for dissolution and differential thermal analysis (DTA).

Dissolution studies

The spin-filter dissolution test apparatus (Magne-drive, Clow Coffman Industries, KS) 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 consisting of pH 2.0 HCl-KCl buffer solution, 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 were dispersed in the dissolution medium. The dissolution medium was circulated through a microcell in a Beckman Model 25 spectrophotometer ($\lambda = 293$ nm) and concentrations of Gris as a function of time were determined from absorbance readings and a calibration curve. The presence of small amounts of chloroform, DMPC, or CHOL in the dissolution medium did not interfere with the analysis of Gris. Experiments were carried out in quadruplicate. Variation of results was less than 7% of the mean value in all cases.

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%.

Results and Discussion

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. 1A and B. The dissolution profiles typically describe an initial rapid dissolution rate followed by a gradual leveling off after about 20–40 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 greater. A comparison of Fig. 1A and B 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 percentage dissolved, to decrease by 42% (Table 1). However, when CHOL was included, at a DMPC : CHOL 1 : 0.33 mole ratio, aging was considerably reduced (Fig. 2) and caused only a 16% decrease in dissolution of Gris which was determined to be statistically insignificant ($P < 0.05$). Likewise, coprecipitates in which the DMPC :

TABLE 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 vs control (1 : 0). S and I indicate significant and insignificant differences, respectively, using the paired Student's *t*-test at $P < 0.05$.

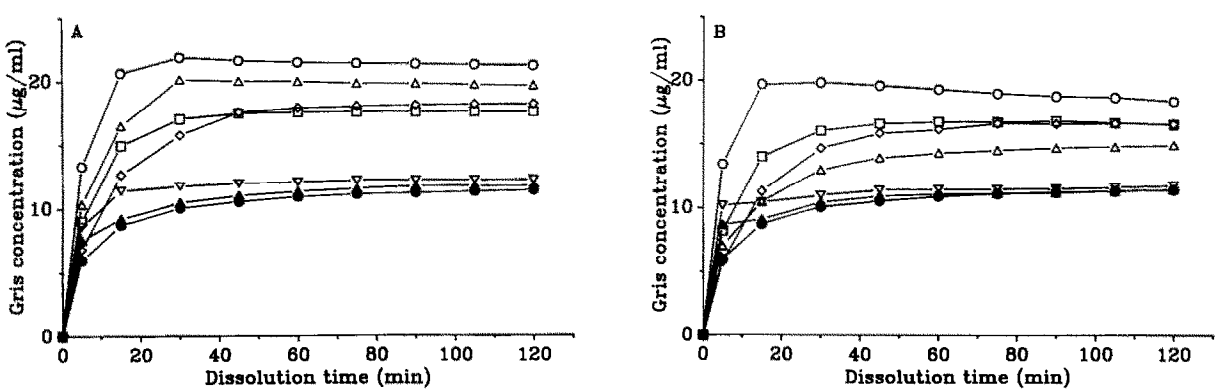


Fig. 1. (A) Dissolution of Gris : DMPC : CHOL coprecipitates after aging for 1 day. (△) 4 : 1(1 : 0); (○) 4 : 1(1 : 0.33); (□) 4 : 1(1 : 1); (◇) 4 : 1(1 : 3); (▽) 4 : 1(0 : 1); (▲) chloroform-solvated Gris; (●) micronized Gris. The weight ratio Gris : lipid was constant but the mole ratio DMPC : CHOL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

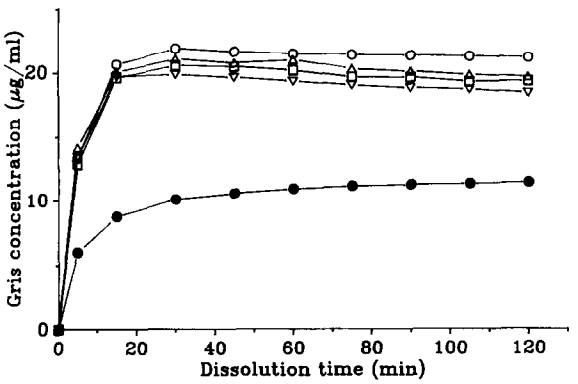


Fig. 2. Effect of aging at room temperature on the dissolution of Gris : DMPC : CHOL 4 : 1(1 : 0.33) coprecipitates. (○) 1 day; (△) 10 days; (□) 30 days; (▽) 90 days; (●) micronized Gris (shown for reference).

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. Fig. 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 (approx. 23%). 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 Gris : DMPC 4 : 1 and Gris : CHOL 4 : 1 weight ratio compositions which continued to lose chloroform at a more rapid rate. Table 2 gives data of the fusion temperatures, heats of fusion, chloroform contents, and maximum amount of Gris dissolved for the 4 : 1(1 : 0.33) coprecipitate and 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 3) indicates that the 4 : 1(1 : 0.33) composition underwent the least change of these parameters with age. Conse-

TABLE 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 temperature (°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 temperatures and heat of fusion of pure Gris was 221° C and 130 J/g, respectively.

TABLE 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 (mole ratio)	Fusion temperature (° C)			Heat of fusion (J/g)			Chloroform content (mg/g)		
	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

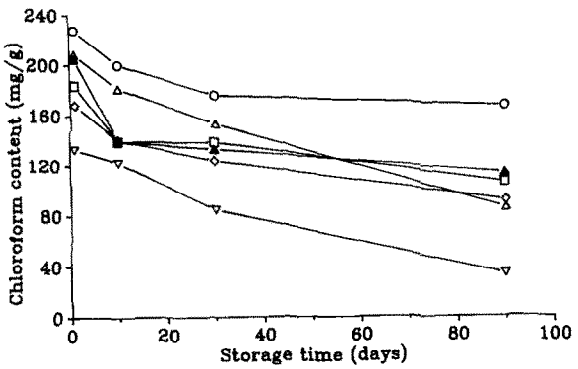


Fig. 3. Plot of residual chloroform content in Gris : DMPC : CHOL coprecipitates as a function of storage time. Symbols as in Fig. 1.

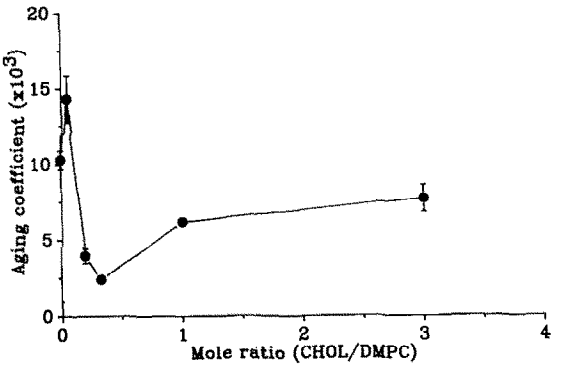
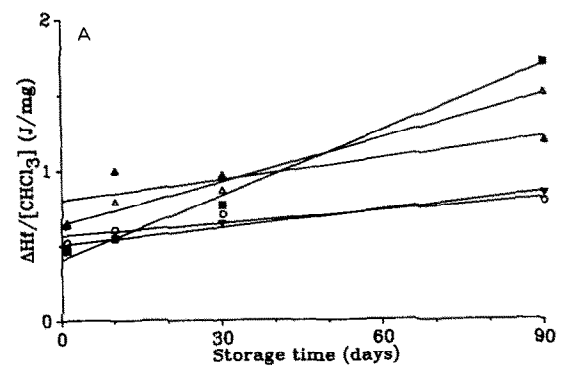


Fig. 5. Plot of the aging coefficient (slopes of regression lines in Fig. 4) vs the CHOL/DMPC mole ratio in coprecipitates at constant 4 : 1 Gris : lipid weight ratio.

quently, 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. 4A and B. 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. 5 from a plot of the aging coefficient (slopes of regression lines in Fig. 4A and B) 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. Comparison of the dissolution behavior of the drug and coprecipitates with the aging factor is shown in Table 4. The linear correlations for all coprecipitates argue strongly in favor of the influence of the relationship of the chloroform content and the ΔH_f on the dissolution behavior.

Gris : EPC compositions

The coprecipitate composition of Gris : EPC : CHOL, 4 : 1(1 : 0.33) exhibited an apparent unusually different dissolution behavior compared to Gris : DMPC : CHOL systems and this is described in Fig. 6. The dissolution properties of



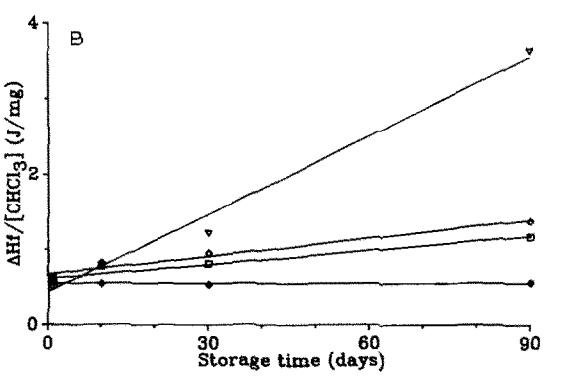


Fig. 4. (A) Plot of the aging factor, defined as $\Delta H_f/[CHCl_3]$ (J/mg), as a function of the storage time of: (▲) Gris solvate (0.83); Gris:DMPC : CHOL: (△) 4 : 1(1 : 0) (0.99); (■) 4 : 1(1 : 0.05) (0.99); (▼) 4 : 1(1 : 0.2), (0.99); (○) 4 : 1(1 : 0.33) (0.93). Correlation coefficients in parentheses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

TABLE 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 = correlation coefficient ($n = 4$).
[b] defined as $\Delta H_f/[CHCl_3]$.
[c] Probability values based on the F test.

Gris : EPC : CHOL coprecipitates actually improved with age, such that after 90 days the amount dissolved increased approx. 35% 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 (~ 5%) (Table 3) resulting in an aging factor which did not change after 90 days of storage (hence, a poor correlation) as shown in Fig. 4B.

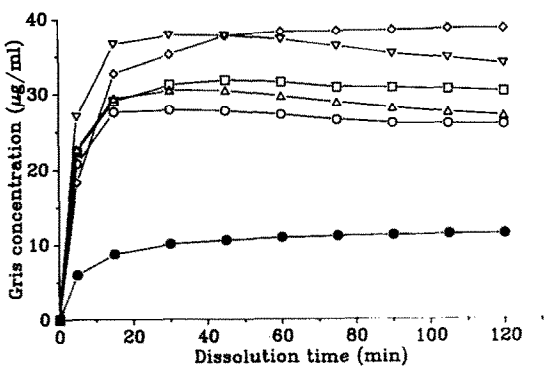


Fig. 6. Dissolution behavior of Gris : EPC : CHOL 4 : 1(1 : 0.33) coprecipitates as a function of aging at room temperature. (○) 1 day; (△) 10 days; (□) 30 days; (▽) 90 days; (◇) 135 days; (●) micronized Gris (shown for reference).

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 malonaldehyde. 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 5. It can be seen that the lowest OI occurred with purified EPC, the highest occurred with fully oxidized EPC with intermediate values being ob-

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

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

tained 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 an 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 involved which are 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.

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.

Acknowledgements

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