Dissolution of Fludrocortisone from Phospholipid Coprecipitates

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Abstract □ The physical properties and dissolution behavior of phospholipid coprecipitates of fludrocortisone acetate (FA) prepared from ethyl acetate, as well as the effect of added polymer, have been determined. The fraction dissolved after 90 min and the initial dissolution rate (IDR) of coprecipitates containing dimyristoyl phosphatidylcholine (DMPC) (4:1, w/w; FA:DMPC) were 77% and 3.5-fold greater than for FA at pH 2.0 and 37 °C. The mechanisms of dissolution were similar to those previously established for griseofulvin, but no aging occurred over 4 months at room temperature in a desiccator. The addition of 0.01 mol% of dextran (MW = 2 million) or 0.1 mol% of poly(lactic acid) reduced the fraction of FA dissolved in 90 min by 15% and reduced the IDR by 35%. The addition of poly(vinylpyrrolidone) (PVP) resulted in a minimum of dissolution efficiency at 1 mol% of PVP 10 (MW = 10 000) or PVP 24 (MW = 24 000) and at 0.1 mol% PVP 40 (MW = 40 000). Only PVP 24 influenced the melting point and heat of fusion of the coprecipitates (determined by differential thermal analysis). Coprecipitate dissolution was reasonably described by either second-order or Weibull distribution kinetic models. These results support the application of high drug-containing solid dispersions using phospholipids to increase the dissolution behavior of poorly water-soluble drug solvates and the possibility of modifying drug release by the incorporation of small amounts of polymers.

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 have shown 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. Upon contact with water, the solvate crystals almost spontaneously disperse into extremely fine particles; this is 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, accounts for the rapid initial dissolution rate of the drug and for the severalfold increase in the amount of drug dissolved over a period of 1–2 h.[1,2]

The dissolution of other drug solvates, including 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% dimyristoyl phosphatidylcholine.[3] Fludrocortisone acetate forms a pentanol or an ethyl acetate solvate,[4] and its 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, because it would be beneficial to have not only 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.

Experimental Section

Materials—Fludrocortisone acetate (FA), poly(vinylpyrrolidone), (PVP 10, MW = 10 000 or PVP 40, MW = 40 000), dextran (D-40, MW = 39 400; D-2m, MW = 2 million), L-α-dimyristoyl phosphatidylcholine (DMPC, 99%), and L-α-dipalmitoyl phosphatidylcholine (DPPC, 99%) were obtained from Sigma Chemical. L-α-Phosphatidylcholine (EPC) was extracted from eggs according to Singleton et al.[7] and purified by recrystallization in acetone. Poly(vinylpyrrolidone) (PVP 24, MW = 24 000; Aldrich Chemical Company) 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 to prepare solutions.

Preparation of Coprecipitates—Drug and phospholipid (and polymer when included) were dissolved in an 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 solvent was removed by placing it in a vacuum desiccator containing anhydrous calcium sulfate at room temperature for 12–16 h.

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 (μg/mL) from a standard calibration curve.

Differential Thermal Analysis (DTA)—An 80/170 mesh (U.S. standard sieves) sample, equivalent to 10 mg of FA, was sealed in an aluminum pan and subjected to a heating rate of 10 °C/min (Fisher Thermalyzer, series 300 QDTA, Fisher Scientific Company), using an empty pan as a 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 Company), and calibration coefficients were derived from fusion temperatures and a calibration curve. The analyses were conducted in triplicate.

Automated Dissolution Studies—Application of the spin-filter dissolution test apparatus[8] [(Magne-drive, Clow Coffman Industries, Kansas), equipped with a stainless steel filter screen (1-μm nominal porosity)] has been previously described.[1] The filter was operated at a rotation speed of 600 rpm in 900 mL of dissolution medium that, except for some test runs in 15% ethanol at 20 °C, consisted of an HCl:KCl (0.02 M) buffer solution at pH 2.0 and was maintained at 37 °C. Sieved samples (80/170 mesh) of formulations equivalent to 50 mg of FA were dispersed in the dissolution medium, which was continuously circulated by a peristaltic pump (Gilson Minipuls 2, 40 mL/min) through a microcell in the spectrophotometer, and concentrations of FA were determined as a function of time as before. The presence of small amounts of phospholipid or polymers in the formulations did not interfere with the analysis of FA. Concentrations of polymers included in the coprecipitates were expressed as mol% of DMPC. All determinations were carried out in triplicate and averaged.

Kinetic Studies—The kinetics of dissolution were examined by several methods.

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First-Order Dissolution Kinetics[9]—The linear first-order rate equation as applied to the dissolution of a powder formulation is given by:

$$\log(100 - \%\ \text{Dissolved}) = \log M - kt/2.303 \quad (1)$$

where M is an integration constant and has dimensions of mass, k is the apparent first-order dissolution rate constant, and t is the time.

Second-Order Dissolution Kinetics[10]—The second-order rate equation for dissolution from a powder sample is given by:

$$W/W_e(W_e - W) = k_2 t \quad (2)$$

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.

Weibull Distribution Dissolution Kinetics[11,12]—A form of the Weibull equation can be written as follows:

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

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 ($b > 1$), 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 the more informative dissolution time, T_d, which is defined as:

$$a = (T_d)^b \quad (4)$$

where T_d 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.

Dissolution Efficiency[13]—A parameter suitable for the evaluation of in vitro dissolution, referred to as "dissolution efficiency" (DE), has been suggested by Khan and Rhodes.[14] It is defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution, within the same time frame (i.e., y_{100}). Thus:

$$\%\text{DE} = 100 \int_0^t \frac{y \cdot dt}{y_{100} \cdot t} \quad (5)$$

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.[13]

Results and Discussion

A comparison of the relative dissolution behavior of FA alone, as a solvate (FA-solvate), or as a coprecipitate with DMPC in a dissolution medium of pH 2.0 at 37 °C is shown in Figure 1. The FA or FA-solvate dissolved rapidly and then leveled off at ~28 μg/mL, although the FA-solvate dissolved faster than FA over the first 10–15 min. The maximum concentration of FA achieved in solution is lower than the reported solubility of FA (40 μg/mL), which is probably due to the low pH of the medium and poor wettability of the powder. In contrast, a marked improvement in dissolution of FA from DMPC coprecipitates was observed and is consistent with previous observations involving griseofulvin.[1] Table I compares the dissolution of FA quantitatively under different conditions of temperature and dissolution medium composition. This shows that the initial dissolution rate (IDR) of FA-solvate at pH 2.0 and 37 °C or FA in 15% ethanol at 20 °C was double that of FA at pH 2.0 and 37 °C, and the IDR of FA-solvate in 15% alcohol solution was ~35% greater than

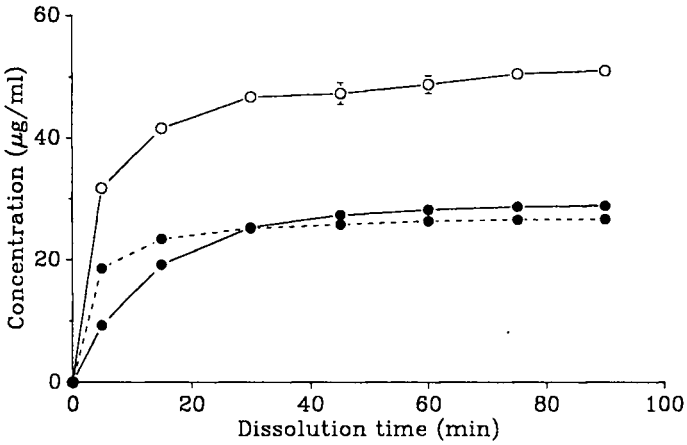

Figure 1—Dissolution profiles of (—●—) FA, (– – ● – –) FA-solvate, and (○) FA:DMPC (4:1, w/w) coprecipitate at pH 2.0 and 37 °C.

Table I—Comparative Dissolution of Fludrocortisone Acetate (FA), FA-Ethyl Acetate Solvate, and FA:DMPC (4:1, w/w) Coprecipitates

No.	Composition	Dissolution Medium	% Dissolved (90 min)[a]	IDR, μg/mL/min[a,b]
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-EtOAc solvate	PH 2.0, 37 °C	48.1 ± 0.5[c]	3.7 ± 0.0[c]
4	FA-EtOAc solvate	15% EtOH, 20 °C	73.2 ± 0.2[c]	5.9 ± 0.1[c]
5	FA:DMPC (4:1, w/w)	pH 2.0, 37 °C	92.0 ± 0.1[c]	6.4 ± 0.2[c]

[a] Mean ± SD; n = 3. [b] Initial dissolution rate within first 5 min. [c] Value significantly different from that obtained for composition no.1 ($p < 0.05$, paired t test).

that of the solvate at pH 2.0 and 37 °C. This compares with a 3.5-fold increase in the IDR of the FA:DMPC (4:1, w/w) coprecipitate and a 40% increase in the amount of FA dissolved after 90 min at pH 2.0 and 37 °C (cf. 20% increase of FA or FA-solvate dissolved in ethanol solution). Others have attempted to incorporate phosphatidylcholine in solid dispersions of nonsolvated 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]

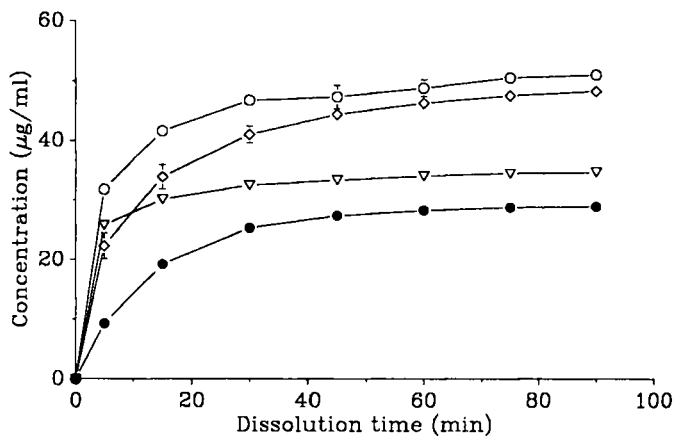

Figure 2—Dissolution profiles of FA:phospholipid (4:1, w/w) coprecipitates containing various phospholipids at pH 2.0 and 37 °C. Key: (▽) DPPC; (○) DMPC; (◇) EPC; (●) no phospholipid.

Table II—Differential Thermal Analysis Data of Fludrocortisone Acetate (FA), Its Ethyl Acetate Solvate, and Coprecipitates with Phospholipids

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

[a] Mean ± SD; n = 3.

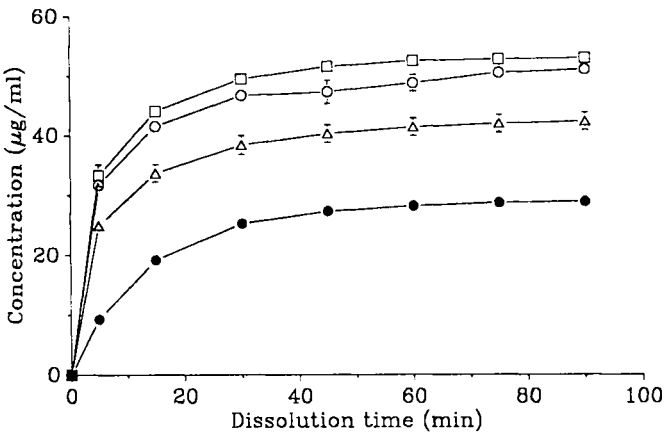

Figure 3—Dissolution profiles of FA:DMPC coprecipitates at pH 2.0 and 37 °C at weight ratios of (△) 9:1, (○) 4:1, (□) 1:1, and (●) 1:0.

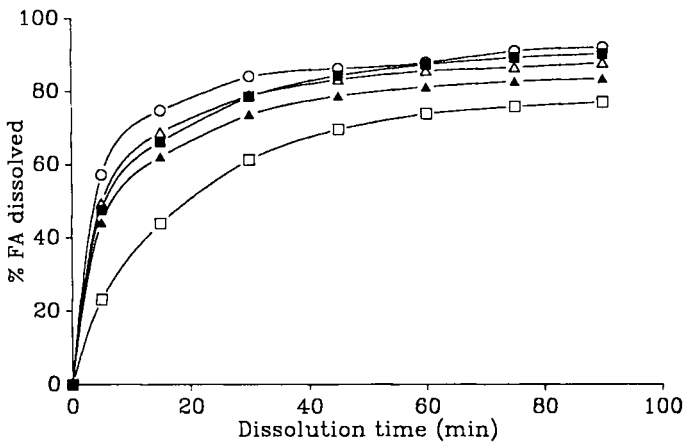

Figure 5—Typical dissolution profiles of FA:DMPC (4:1, w/w) coprecipitates at various compositions of PVP 10, expressed as mol% of DMPC. Key: (○) 0%; (△) 0.01%; (▲) 0.1%; (□) 1.0%; (■) 10.0%.

Figure 2 describes the dissolution profiles of FA observed for coprecipitates using various phospholipids. The IDR was rapid and substantially increased over that of FA, but was approximately the same regardless of the phospholipid used. However, the total dissolution after 90 min when DPPC was incorporated was significantly less (one-fifth) than that produced by either DMPC or EPC. A similar behavior was observed previously with griseofulvin coprecipitates[2] and has been attributed to the respective states of fluidity of the phospholipids at 37 °C.[17]

The DMPC concentration also exerted a notable effect on the dissolution of FA from coprecipitates. Figure 3 shows that the incorporation of low amounts of DMPC markedly increased dissolution, but that concentrations >20% produced little additional improvement. This is interpreted to mean that there is a limit to the quantity of DMPC that can be included in the FA-solvate crystal lattice,[1] and that the excess beyond this limit, which is deposited on the crystal surfaces, plays almost no role in the dissolution process. Using nonsolvated griseofulvin:soya phospholipid coprecipitates, Nishihata et al.[18] obtained an 18 or a 26% increase in dissolution from 2:1 or 1:2 mixtures, respectively, and an actual decrease in dissolution was observed when a 1:7.5 ratio was used.

The energetics of the various formulations as obtained from DTA are described in Table II, and typical thermograms are illustrated in Figure 4. A sharp endothermic peak was observed for FA from which the thaw and melt temperatures shown in Table II were obtained. It is apparent, however, that solvated and coprecipitated FA produced a melting endotherm ~20 °C higher than that of FA alone, indicating the existence of a more coherent crystalline structure, and an endotherm at ~110 °C arising from desolvation. The desol-

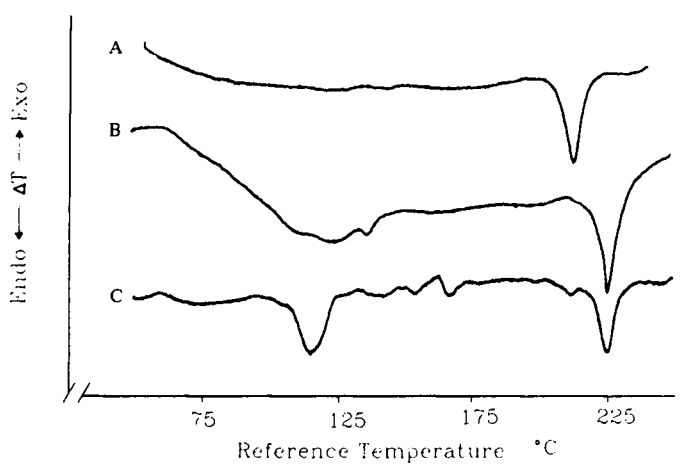

Figure 4—DTA thermograms of (**A**) FA, (**B**) FA-ethyl acetate solvate, and (**C**) FA:DMPC (4:1, w/w) coprecipitate.

Table III—Effect of Incorporation of Polymers in FA:DMPC (4:1, w/w) Coprecipitates on the Dissolution of Fludrocortisone Acetate

No.	Composition[a]	DMPC:Polymer mole ratio	% Dissolved (90 min)[b]	IDR, μg/mL/min[b, c]
1	FA:DMPC	1:0	92.0 ± 0.1	6.4 ± 0.2
2	FA:DMPC:D-2m	1:0.0001	75.9 ± 3.9[d]	3.8 ± 0.0[d]
3	FA:DMPC:D-40	1:0.001	92.3 ± 1.0	6.8 ± 0.7
4	FA:DMPC:PLA	1:0.001	78.1 ± 0.0[d]	4.5 ± 0.0[d]

[a] FA:DMPC-polymer weight ratio was 4:1. [b] Mean ± SD; n = 3. [c] Initial dissolution rate within first 5 min. [d] Value significantly different from that obtained for composition no. 1 ($p < 0.05$, paired t test).

Table IV—Differential Thermal Analysis Data of FA:DMPC Coprecipitates with Polymers

No.	Composition[a]	Desolvation Peak Temp., °C[b]	Drug Thaw Peak Temp., °C[b]	Drug Melt Temp., °C[b]	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

[a] The FA:DMPC-polymer weight ratio was 4:1; DMPC:polymer mole ratio was 1:0.001 in all cases except no. 2 (1:0.0001). [b] Mean ± SD; n = 3.

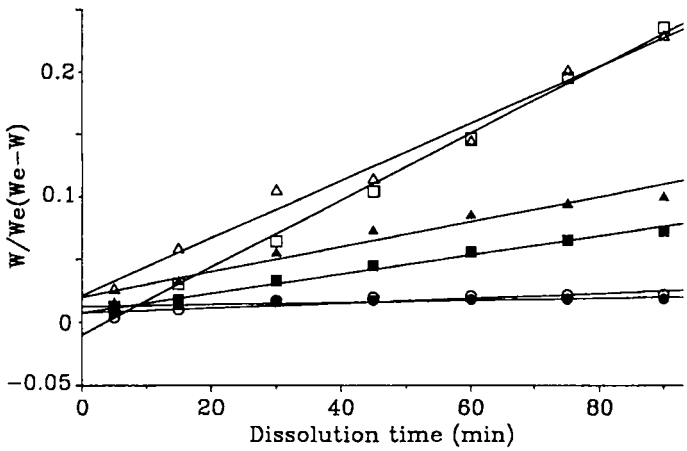

Figure 6—Second-order kinetic plot for dissolution of FA formulations. Key: (○) FA (0.89); (●) FA-solvate (0.85); (△) FA:DMPC (0.99); (▲) FA:DMPC:PVP 10 (0.97); (□) FA:DMPC:PVP 24 (0.99); (■) FA:DMPC:PVP 40 (0.99). The r values from linear regression analysis are shown in parentheses, and the concentration of PVP in coprecipitates was 0.1 mol% of DMPC.

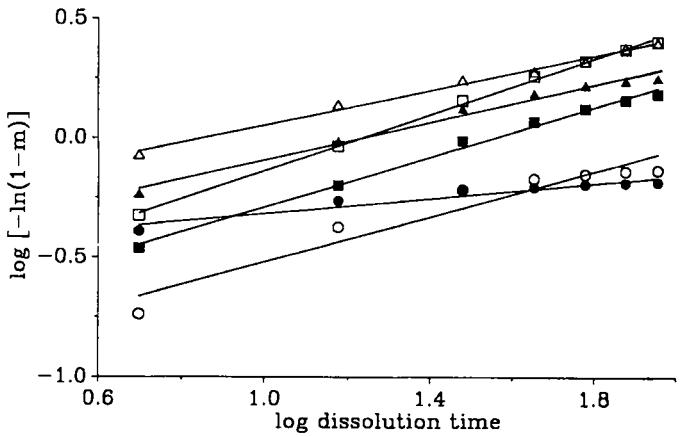

Figure 7—Weibull distribution plot for dissolution of FA formulations. Key: (○) FA (0.96); (●) FA solvate (0.96); (△) FA:DMPC (0.99); (△) FA:DMPC:PVP 10 (0.99); (□) FA:DMPC:PVP 24 (0.99); (■) FA:DMPC:PVP 40 (0.99). The r values from linear regression analysis are shown in parentheses, and the concentration of PVP in coprecipitates was 0.1 mol% of DMPC.

vation, thaw, and melt temperatures of all coprecipitates and solvated FA were relatively constant, as were the heats of fusion, except for coprecipitates containing DPPC or EPC. These differed from the other formulations, but in opposite directions, suggesting decreased or increased binding of these phospholipids within the crystalline network, respectively, compared with DMPC.

Coprecipitates Containing Polymers—The dissolution and DTA results in Tables III and IV, respectively, provide some evidence of how the addition of certain polymers at low concentrations can alter the physical state and dissolution behavior of FA:DMPC (4:1, w/w) coprecipitates. At the ratios employed, D-2m and PLA, but not D-40, caused a decrease in dissolution. This was paralleled by a decrease in the heat of fusion, but not the melting point, which remained essentially unchanged. These results suggest that D-2m and PLA reduced the interaction of DMPC in the coprecipitates, thereby resulting in a slower dissolution rate. On the other hand, it is likely that D-40 only coated the coprecipitate 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 previously[1]).

The effects of a series of PVPs on the dissolution of FA:DMPC (4:1, w/w) coprecipitates can be observed in Figure 5 and Table V, and their physical parameters are given in Table IV. The incorporation of PVP (up to 1 mol% of DMPC) decreased the dissolution of FA, whereas incorporating 10 mol% reversed the trend, with the result that a negligible change in the dissolution of FA occurred using either PVP 10 or 40. The %DE calculations using eq 5 also show a minimum in the dissolution of FA from FA:DMPC coprecipitates at 1 mol% of PVP 10 or PVP 24 and at 0.1 mol% of PVP 40. The reversibility of this trend at 10 mol% by PVP 10 or PVP 40, but not PVP 24, is unclear. This occurrence is partly supported by the DTA studies (Table IV), which also show significant reductions in the thawing, melting, and desolvation temperatures of coprecipitates containing PVP 24, but not those containing PVP 10 or PVP 40. 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 (eqs 2 and 3, respectively) in Figures 6 and 7, respectively. A comparison of the calculated values for the derived constants of these expressions is found in Table VI. Generally, the kinetics would appear to be well described using either approach when the predicted T_{40} values are compared, although the Weibull

Table V—Dissolution Efficiency (%DE) of FA:DMPC (4:1, w/w) Coprecipitates as a Function of the Molecular Weight and Molar Concentration of Poly(vinylpyrrolidone) (PVP)

PVP Concentration, mol% of DMPC	% Dissolution Efficiency[a]		
	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; n = 3.

Table VI—Evaluation of the Dissolution Kinetics of FA, FA-Ethyl Acetate Solvate, and FA:DMPC Coprecipitates According to Second-Order and Weibull Distribution

Composition	Second Order		Weibull			
	$k_2 \times 10^{4a}$	Pred $T_{40}{}^b$	log a	b^d	Pred T_{40}	Obs T_{40}
FA	1.9	23.4	1.24	0.48	19.5	31.1
FA-EtOAc solvate	0.8	55.1	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 (mg/min). [b] Time (min) for 40% dissolution. [c] FA:DMPC-polymer weight ratio was 4:1; DMPC:polymer mole ratio % was 1:0.001. [d] The slope.

distribution approach also yielded better correlations for the dissolution of FA and the FA-solvate. Also, the Weibull distribution kinetic approach yielded better agreement than did the second-order treatment of the predicted time for 40% dissolution from coprecipitates with the observed values. In addition, the kinetic data for the FA-solvate were better fit by Weibull distribution kinetics, but both treatments applied to FA gave poor agreement with the observed T_{40}. Attempts at fitting the data to first-order kinetics yielded poor correlations; hence, comparisons were not presented. In a similar fashion, the kinetics of griseofulvin:phospholipid coprecipitates were best described by the Weibull distribution approach,[19] suggesting similar mechanisms in the dissolution behavior of these formulations. Consistent with this approach to formulation are preparations having a high drug content (80–95%) compared with other solid dispersions of water-soluble carriers (5–10% drug content). The FA:phospholipid coprecipitate dissolution behavior remained remarkably constant over a period of 4 months at room temperature in a desiccator, whereas the griseofulvin:phospholipid coprecipitate system aged.[1] However, the aging could be reduced by varying the lipid composition by the addition of cholesterol.[20] These encouraging results offer the possibility of delivering poorly water-soluble or erratically absorbed drugs more efficiently and more economically than other solid dispersion systems using water-soluble carriers.

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