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Preformulation study of etoposide: II. Increased solubility and dissolution rate by solid-solid dispersions

Jaymin C. Shah [a,*], Jivn R. Chen [b], Diana Chow [c]

[a] *Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA*

[b] *Sage Pharmaceuticals Inc., Shreveport, LA, USA*

[c] *Department of Pharmaceutics, College of Pharmacy, University of Houston, Houston, TX, USA*

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Abstract

Etoposide, an anticancer drug, has low and erratic oral bioavailability which is due to low aqueous solubility, slow dissolution rate and instability in acidic pH. The study objective was to enhance the aqueous solubility and dissolution rate of etoposide by solid-state modifications, which was attempted by preparation of solid-solid dispersions by coprecipitating the drug with polyethylene glycols (PEG) of different molecular weights in various ratios. The solubility and dissolution rate of etoposide from the coprecipitates were evaluated. The coprecipitate of etoposide with PEG 8000 (1:10, PEG weight fraction of 0.91) increased its solubility 2-fold and dissolution rate 42-fold (190.7 μg/ml and 0.42 mg/min per cm^2 vs 93.8 μg/ml and 0.01 mg/min per cm^2 of etoposide pure powder, respectively). The coprecipitates with other PEGs (PEG 1500, PEG 3400, PEG 6000) and PVP 40000 also increased etoposide dissolution rate to a great extent.

Keywords: Etoposide; Solid-solid dispersion; PEG coprecipitate; Preformulation; Solubility; Dissolution rate

1. Introduction

Etoposide is an anticancer drug for the treatment of small cell lung cancer and testicular carcinoma (Issell and Crooke, 1979; Clark and Slevin, 1987). Several investigational oral formulations of etoposide had been developed but all suffered the disadvantage of low and erratic oral bioavailability (Clark and Slevin, 1987). The currently available commercial oral formulation, a soft gelatin capsule filled with etoposide solution in cosolvent mixture (comprising benzyl alcohol, ethanol, PEG 400 and Tween 80), was reported to have an absolute oral bioavailability of 50%, ranging from 25 to 75% (Clark and Slevin, 1987; Product Insert, 1987).

In our previous study (Shah et al., 1989), various physicochemical properties of etoposide were examined, and the low aqueous solubility, degradation at pH 1.3 and slow intrinsic dissolution rate of etoposide were suggested as the probable causes of low and erratic oral bioavailability. The solubility of etoposide at 37°C was extremely low,

* Corresponding author.

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148–153 μg/ml, very inadequate to result in complete dissolution of the usual oral dose of 100 mg in gastric fluids (Issell et al., 1984). The intrinsic dissolution rate was 0.01 mg/min per cm^2, far less than 1.0 mg/min per cm^2, suggesting that oral absorption was dissolution rate limiting (Kaplan, 1972; Shah et al., 1989).

Attempts have been made to increase the aqueous solubility of etoposide by chemical modifications (Kreis and Soricelli, 1979; Rose et al., 1990), cosolvent approach (Clark and Slevin, 1987) and use of hydrotropic agents (Darwish et al., 1989). The current parenteral and oral formulations of etoposide use a mixture of cosolvents to solubilize etoposide but they are reported to result in precipitation of drug on dilution (Clark and Slevin, 1987; Product Insert, 1987). Various approaches have been suggested to increase the solubility of drugs but the property responsible for poor solubility must be first identified, and then modified to enhance solubility (Yalkowsy, 1981). Etoposide has a high melting point (240–250°C), which is indicative of strong crystal lattice energy. This high melting point is one of the factors responsible for lower ideal solubility and hence poor aqueous solubility (Yalkowsy, 1981). Therefore, any approach which disrupts the crystalline nature and/or results in a lower crystal lattice energy would be more successful in improving aqueous solubility of etoposide from the solid state. The metastable polymorphs and the amorphous form melt at lower temperatures and thus tend to have higher aqueous solubilities. Polymorphs have been reported in various classes of drugs and have been demonstrated to increase aqueous solubility and dissolution rates of drugs (Byrn, 1982; Carstensen, 1990).

Another physical approach is to disrupt the crystalline nature of drug by solid-state dispersion of the drug into water-soluble carrier molecules which replace the drug molecule in the crystal lattice. This may result in a total or partial loss of crystallinity, resulting in a significant increase in solubility and dissolution rate. Dispersions and solid solutions of griseofulvin (Chiou and Riegleman, 1969, 1971), glybornuride (Vila-Jato and Alonso, 1986), diazepam (Ford and Elliott, 1985) and indomethacin (Anastasiadou et al., 1983) have increased the solubility and dissolution rate of the respective drugs.

We selected the physical approach of solid dispersions to enhance the aqueous solubility and dissolution rate as our objective was to develop a solid oral formulation. In this study, solid dispersions of etoposide were prepared by the solvent (methanol) coprecipitation method with PVP 40 000 and PEGs of different molecular weights in various ratios, and the best solid dispersion was identified based on the maximum improvement of etoposide solubility and dissolution rate and the physical nature and property of the coprecipitate.

2. Materials and methods

Etoposide powder was used as supplied by Bristol Myers (Syracuse, NY). Methanol, and acetonitrile were HPLC grade. PEGs 1500, 3400, 6000, and 8000 and PVP 40 000 were used as obtained from Aldrich Chemical Co.

2.1. Assay

A stability indicating HPLC assay of etoposide previously developed by us and reported earlier (Chow et al., 1987) was used for this study. The HPLC assay is a reversed-phase separation on an octyl column (5 μm, 15 cm × 4.6 mm i.d.) with an isocratic mobile phase consisting of acetonitrile-acetic acid-water (27:1:72). Etoposide was detected and quantified at 230 nm. In addition, a spectrophotometric assay at 286 nm was developed for etoposide dissolution study. The assay was validated by simultaneous analysis of samples once by both HPLC and spectrophotometric assays.

2.2. Preparation of coprecipitates

The coprecipitates were prepared by dissolving weighed amounts of etoposide and PEG in 5 ml of methanol using sonication. Methanol was evaporated with a steady stream of nitrogen gas for 8–12 h, until a dry cake was obtained. The coprecipitates of etoposide with each PEG were prepared with various PEG weight fractions,

ranging from 0.25 to 0.91 (etoposide/PEG, 3:1 to 1:10). The coprecipitate with PVP 40 000 was prepared in the ratio of 1:10 only. The coprecipitates so obtained were stored in tightly capped glass vials at room temperature. The absence of any degradation product's peak in the HPLC chromatograms indicated that etoposide was stable during the preparation, and the measurements of solubility and dissolution. Physical mixtures of etoposide and PEGs were prepared by mixing weighed amounts of each in the glass vial by trituration. Since etoposide is a very fine milled powder (less than 5 μm), no attempt was made to further reduce its particle size. Physical mixtures were compared with coprecipitates of the same ratio for enhancement of etoposide aqueous solubility.

2.3. Solubility and dissolution rate of etoposide from coprecipitate

The solubility of etoposide from the coprecipitate was determined by agitating excess of coprecipitate in 5 ml of distilled water in a glass vial at room temperature for 24 h (previously determined to be adequate time for equilibration). The solution was then filtered through 0.45 μm membrane filter and analyzed by HPLC. Solubility of etoposide from the physical mixture was similarly determined.

Intrinsic dissolution rate of etoposide from coprecipitate was determined using Wood's apparatus by a method similar to that used for etoposide pure powder reported in an earlier publication (Shah et al., 1989).

2.4. Particulate dissolution of coprecipitate with 91% PEG 8000

Particulate dissolution was studied by adding excess (> 2 mg) of etoposide to 10 ml of distilled water. In the case of coprecipitate and physical mixture, excess (> 22 mg of each, equivalent to 2 mg of etoposide) was used. The suspension was rotated on a rotatory mixer. Samples of 0.5 ml were taken at various time intervals, filtered through 0.45 μm membrane filter and analyzed by the UV assay.

2.5. Differential scanning calorimetry (DSC)

DSC was conducted to ascertain the nature of the solid state of etoposide in the coprecipitates. The thermograms of etoposide, PEG 8000, and the coprecipitates were obtained on Perkin Elmer DSC-4 model with system 4 microprocessor. The weighed powder (about 5–10 mg) was sealed in one aluminum pan, and another pan with air served as the control. The samples were heated from 50 to 300°C at a heating rate of 20°C/min. Inert atmosphere was maintained above the sample by constantly flushing the headspace with nitrogen gas. The thermograms were normalized with respect to the sample size and temperatures at which any thermal events occurred were noted. The thermograms were integrated and the peak areas calculated.

2.6. Particle size distribution of etoposide powder

Particle size distribution of etoposide powder was estimated using a Coulter Counter Model ZF (Coulter Electronic, Hialeah, FL) after suspending about 3.3 mg of powder in 20 ml of Isotone solution. The data are reported as the mean of four measurments.

2.7. Statistical analysis

The differences in the solubility of etoposide from coprecipitate and physical mixture with PEGs were evaluated by two-sample t-test at $p = 0.05$. The effects of PEG molecular weight and weight fraction on the solubility of etoposide were analyzed by two-way ANOVA at $p = 0.05$. The effect of the various weight fractions of PEG 8000 on the dissolution rate was evaluated by one-way ANOVA at $p = 0.05$, and two-tailed Student Newman Keul's test was used for ranking the dissolution rates.

3. Results

3.1. Solubility of etoposide from coprecipitate

The aqueous solubilities of etoposide at room temperature from coprecipitates and physical

mixtures with PEG 1500, 3400, 6000, and 8000 in various PEG weight fractions are shown in Fig. 1a and 1b. The solubilities of etoposide from coprecipitates and physical mixtures with PEGs in all ratios were higher than that of pure etoposide powder. However, there was no statistically significant difference in the solubility of etoposide from coprecipitate and the respective physical mixture for all the PEGs studied at all the PEG weight fractions.

Solubility of etoposide from coprecipitates and physical mixtures was affected by the molecular weight and the weight fraction of the PEG (ratio of etoposide to PEGs). The solubility of etoposide increased with increasing PEG weight fractions, but not in a linear fashion (Fig. 1a,b). Similarly, the higher the molecular weight of PEG, the greater was the enhancement of solubility from both the physical mixture and coprecipitate. The notable exceptions were the physical mixtures and coprecipitates of PEG 1500, which exhibited higher solubilities of etoposide than PEG 3400 and 6000 physical mixtures and coprecipitates. In addition, the solubility from the PEG 1500 physical mixtures and coprecipitates increased almost linearly with PEG weight fractions. Of all the PEGs studied, PEG 8000 consistently resulted in higher solubilities of etoposide, from both the coprecipitates and physical mixtures, in all the ratios studied. The greatest enhancement in etoposide solubility (191 μg/ml) was achieved from coprecipitate with PEG 8000 in the ratio of 1 : 10.

3.2. Dissolution rate of etoposide from coprecipitates

The dissolution profiles of etoposide from coprecipitates with PEGs 3400, 6000, 8000 and PVP 40 000 were studied with Wood's apparatus and are depicted in Fig. 2. The coprecipitates of PEG 6000 and 8000 were dry, free-flowing powders, while the coprecipitate of PVP 40 000 was glassy and extremely hygroscopic. The coprecipitates with PEG 1500 and 3400 were soft and waxy. The measurement of dissolution rate of PEG 1500 coprecipitates was precluded because rigid disks could not be prepared. All the polymers increased etoposide dissolution rate, however, the maximum increase in dissolution rate was obtained using PEG 3400. PEG 8000 coprecipitate was selected for further investigation in various weight fractions of PEG because the rigid disks attainable with this polymer yielded more reproducible dissolution data.

The dissolution profiles of etoposide from PEG 8000 coprecipitates are shown in Fig. 3. The intrinsic dissolution rates of etoposide from PEG 8000 coprecipitates determined from the slopes are listed in Table 1. The weight fraction of PEG

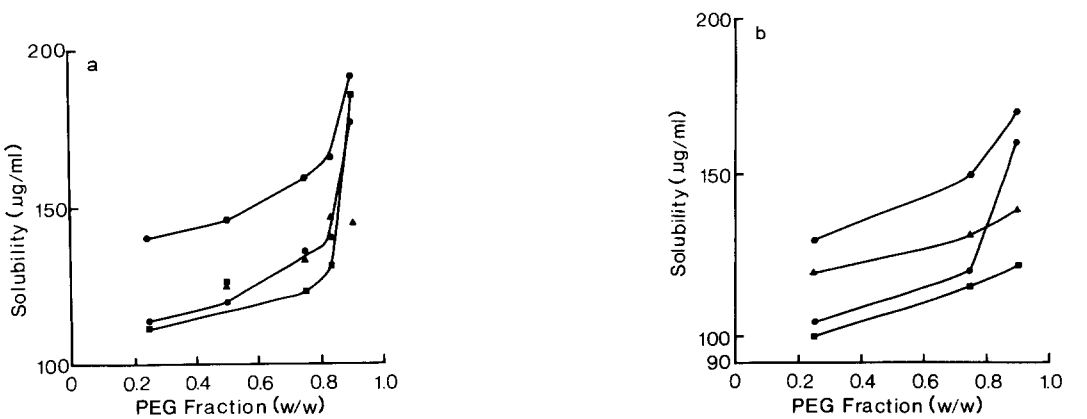

Fig. 1. (A) Solubility of etoposide from coprecipitates with (a) PEG 1500 (▲), (b) PEG 3400 (■), (c) PEG 6000 (●), and (d) PEG 8000 (●), with various PEG weight fractions (n = 3). (B) Solubility of etoposide from physical mixtures with (a) PEG 1500 (▲), (b) PEG 3400 (■), (c) PEG 6000 (●), and (d) PEG 8000 (●) with various PEG weight fractions (n = 3).

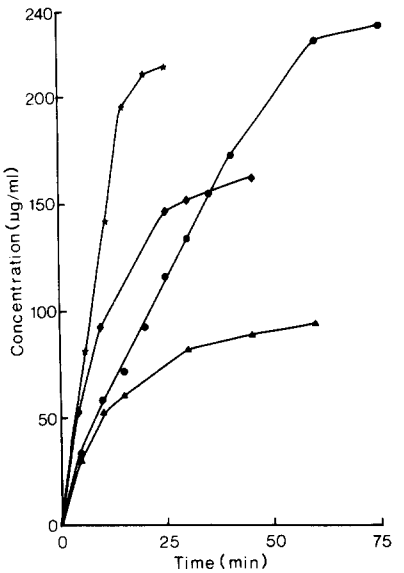

Fig. 2. Intrinsic dissolution profiles of etoposide from coprecipitates with (a) PEG 3400 (★), (b) PEG 6000 (▲), (c) PEG 8000 (◆), and (d) PVP 40000 (●), in the ratio of 1 to 10 (PEG weight fraction of 0.91) ($n = 1$).

8000 affected the dissolution rate and the effects were ranked into three groups. The greatest enhancement in dissolution rate, 42-fold, was achieved with PEG weight fraction of 0.91, followed by 4–5-fold enhancement with weight fractions of 0.75 and 0.83. The PEG weight fractions of 0.25 and 0.50 exerted no significant effect.

Table 1
Dissolution rates of etoposide (E) from coprecipitates (COPP) with various PEG 8000 weight fractions

PEG 8000 weight fraction	Dissolution rate, dc/dt (μg/ml per h)	Intrinsic dissolution rate, $[(D/h)\cdot C_s]$ (mg/min per cm^2)	Ratio of dc/dt (COPP) /dc/dt(E)
0	5.4 ± 0.6 [a]	0.01 ±0.001	1.00
0.25	7.4 ± 0.6	0.013 ± 0.001	1.36
0.50	11.6 ± 0.9	0.021 ± 0.001	2.13
0.75 [b]	21.1 ± 5.8	0.038 ± 0.01	3.88
0.83 [b]	26.9 ± 6.6	0.048 ± 0.01	4.90
0.91 [c]	238.3 ± 25.7	0.423 ± 0.05	42.30

[a] Mean ± S.D, for $n = 3$ different batches of coprecipitates.
[b] Dissolution rate significantly different from that of etoposide and its coprecipitates with PEG 8000 weight fractions of 0.25 and 0.50 at $p = 0.05$ by Student Newman Keul test.
[c] Dissolution rate at PEG weight fraction of 0.91 is significantly different from the rest by Student Newman Keul test at $p = 0.05$.

The relative dissolution rate as a function of PEG 8000 weight fractions is plotted in Fig. 4. The dependence of the relative dissolution rates from coprecipitates on the PEG weight fraction is apparent, yet, more data for PEG weight fractions between 0.83 and 0.91 and exceeding 0.91

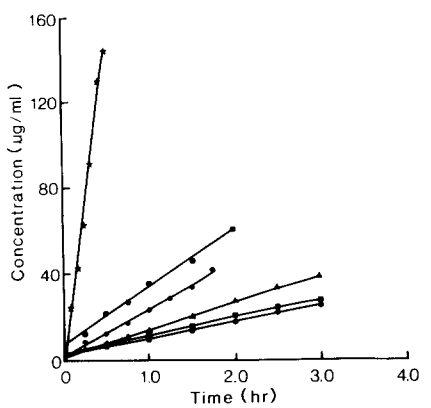

Fig. 3. Intrinsic dissolution profiles of etoposide from coprecipitates with PEG 8000, with PEG weight fractions of (a) 0.25 (■), (b) 0.50 (▲), (c) 0.75 (✚), (d) 0.83 (⬢), and (e) 0.91 (★) compared with that of etoposide (●) ($n = 3$).

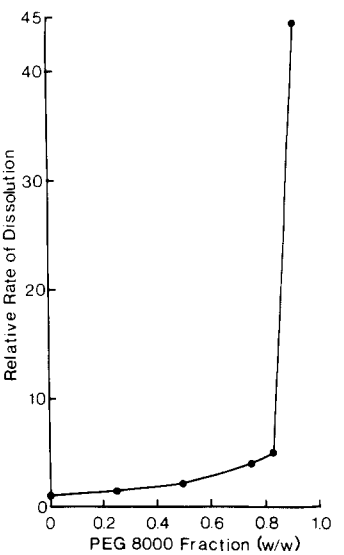

Fig. 4. Relative dissolution rates of etoposide as a function of PEG 8000 weight fraction in coprecipitates ($n = 3$).

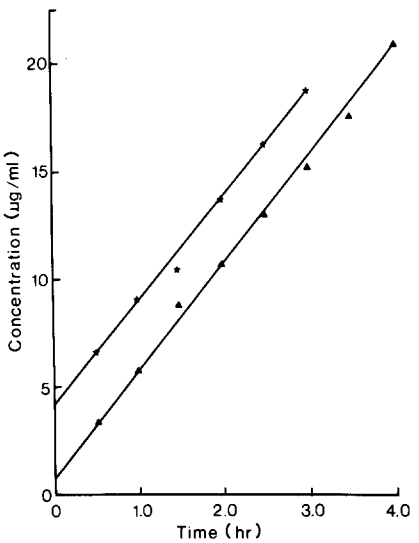

Fig. 5. Intrinsic dissolution profile of etoposide in (a) 1% PEG 8000 solution (★), and (b) distilled water (▲) as the dissolution medium ($n = 1$).

are required to establish the relationship completely.

The dissolution rate of etoposide into 1% PEG 8000-containing aqueous solution was 5.20 μg/ml per h, similar to that into PEG 8000-free aqueous dissolution medium, 5.42 μg/ml per h (Fig. 5). This suggested that the enhanced etoposide dissolution rate from coprecipitate was not due to the dissolution of PEG 8000 from the coprecipitate into the aqueous dissolution medium, and subsequently facilitating the dissolution of etoposide by solubilization.

3.3. Particulate dissolution of etoposide from physical mixture and coprecipitate with 91% PEG 8000

The dissolution of etoposide from physical mixture and coprecipitate with PEG 8000 is depicted in Fig. 6. The C_{max} of etoposide occurred in 5 min from physical mixture and coprecipitate, but at 15 min from powder. All the three preparations resulted in supersaturation of the solution with etoposide but the concentrations from the coprecipitate were 2–3-times higher than those from the physical mixture and the powder. This supersaturated solution with coprecipitate exhibited significantly higher solubility of etoposide (280 μg/ml) compared to the equilibrium solubility of etoposide (191 μg/ml) from the coprecipitate with PEG 8000, 1:10 determined in the earlier experiment (Fig. 1a). The dissolution profile of the physical mixture was similar to that of etoposide powder.

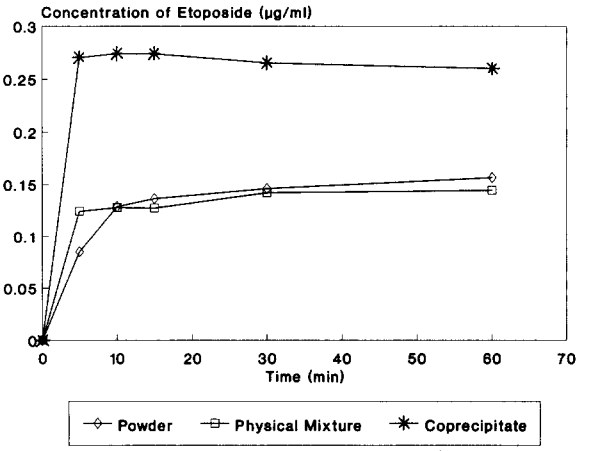

Fig. 6. Particulate dissolution of (a) etoposide powder, (b) physical mixture, and (c) coprecipitate with 91% PEG 8000 ($n = 3$).

3.4. Particle size distribution

The partial particle size distribution curve of etoposide in Fig. 7 shows that majority of particles are below 4 μm in diameter, and there are

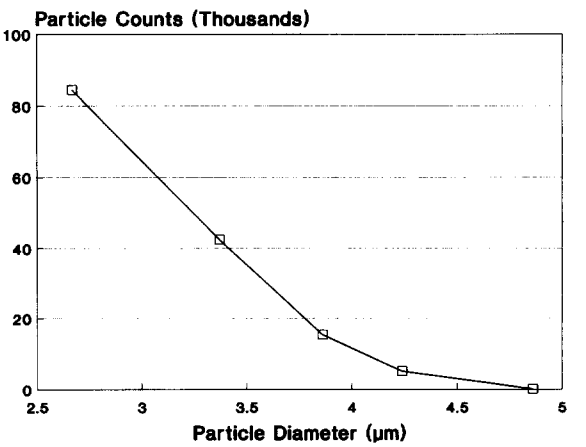

Fig. 7. Particle size distribution of etoposide by Coulter counter ($n = 4$).

no particles above 5 μm. Therefore, etoposide powder is an extremely fine milled powder, and thus it is not possible to reduce its particle size further, even with a fluid-energy mill (Parrott et al., 1986; Barber, 1993). Since physical mixtures were prepared by trituration of etoposide with PEGs, the particle size would be similar and hence no difference in dissolution rate may be expected except due to better dispersal and wetting with the PEGs. Although the true particle size of etoposide in the coprecipitate cannot be measured, it can be safely assumed that etoposide may be in the molecularly dispersed form, or might be in the sub-micron range, since etoposide powder itself is in the micron range (< 5 μm). Furthermore, this may be responsible for the significant enhancement of dissolution rate from the coprecipitate as compared to the powder and the physical mixture.

3.5. DSC

The thermograms of PEG 8000 had a melting endotherm at 69°C while etoposide showed a melting endotherm at 283°C. The thermograms of coprecipitates at the 3:1 and 1:1 ratios of etoposide and PEG 8000 consisted of the melting endotherms of both PEG 8000 and etoposide without significant alteration of melting points, indicating the absence of solid-state interaction. The thermogram of coprecipitate at the 1:3 ratio of etoposide to PEG 8000 had numerous very small endotherms in the temperature range of 140–220°C, with the absence of the etoposide melting endotherm at 270–290°C. The thermograms of coprecipitates at the 1:5 and 1:10 ratios of etoposide to PEG 8000 had neither the melting endotherm of etoposide nor the small endotherms seen in the thermogram of 1:3 coprecipitate. There was insignificant alteration of PEG 8000 melting point in all the coprecipitates. This suggests that there may not be a eutectic or a solid-solution formation between etoposide and PEG 8000. However, the absence of etoposide melting endotherm in coprecipitates (1:3, 1:5 and 1:10) and the appearance of small endotherms at earlier temperatures in the thermogram of coprecipitate 1:3 are indicative of loss of crystalline nature of etoposide in coprecipitate at and above the 1:3 ratio of etoposide to PEG 8000.

4. Discussion

Etoposide coprecipitates were prepared by the solvent-coprecipitation method. The co-melting method was not used because of the instabilities of PEGs and PVP 40 000 at the melting point of etoposide, greater than 240°C. The physical nature of the coprecipitate depended on the nature of the polymer used. PEG 1500 and 3400 produced waxy coprecipitates due to their inherent waxy nature, while PEG 6000 and 8000 produced dry free-flowing coprecipitates. PVP 40 000 yielded glassy and hygroscopic coprecipitate, and became translucent after absorbing moisture.

The enhancement of solubility and dissolution of dispersed drug in coprecipitate depended on the type and weight fraction of the carrier molecule. All the polymers significantly increased the solubility and the dissolution rate of etoposide at the polymer weight fraction of 0.91. The equilibrium aqueous solubilities of etoposide from physical mixture and coprecipitate of PEG 8000 were similar, however, in the particulate dissolution experiment, a concentration of 280 μg/ml was attained in less than 10 min from coprecipitate. In the intrinsic dissolution experiments also, a concentration of 220 μg/ml was attained in 20 min from a limited surface area disk. These results suggest that coprecipitate leads to rapid supersaturation, an observation which has been reported in the literature for solid-solid dispersions of various drugs (Simonelli et al., 1969; Chiou and Riegleman, 1971). However, the increase in dissolution rate from coprecipitate was not solely due to the increase in solubility for the following reasons. Firstly, the increase in equilibrium solubility was 2-fold, while the increase in dissolution rate was 42-fold. Secondly, the physical mixture with the same amount of PEG increases solubility to the same extent, yet the particulate dissolution studies indicated that the dissolution rates from physical mixture and pure drug substance were similar and significantly

lower than that from coprecipitate. Furthermore, pure etoposide's dissolution rate in the medium containing 1% PEG 8000 was similar to that in distilled water, confirming that the mere presence of PEG 8000 in solution does not increase dissolution rate. The results also indicate that formation of a coprecipitate was a prerequisite for enhancing the dissolution rate of the dispersed drug. Thus, it appears that coprecipitation alters the solid state of drug such that the dissolution enhancement is significantly higher than can be explained by the magnitude of the increase in solubility alone. The alteration of solid state of etoposide in the coprecipitates at 1:3, 1:5 and 1:10 ratios was confirmed by the absence of its melting endotherms by DSC.

Since the solubilities of etoposide from the coprecipitate and physical mixture of PEG 8000 were similar, the differences in the dissolution rate may be due to the differences in the particle size of etoposide. However, as indicated in section 3, etoposide powder as such is finely milled with no particles greater than 5 μm in diameter. Therefore, the particle size of etoposide in the coprecipitate must be in the sub-micron range. It has been suggested that the higher dissolution rates of the dispersed drugs from the solid dispersions are a result of molecular dispersion, i.e., the lowest possible particle size (Chiou and Riegleman, 1971). The formation of sub-micron range dispersion and/or molecular dispersion of etoposide in PEG 8000 at 1:10 ratios in the coprecipitate is very likely and is supported by the DSC thermograms which have no melting endotherms of etoposide. Molecularly dispersed drug will not demonstrate a melting endotherm as the crystal lattice no longer exists.

Another mechanism for this preferential enhancement of dissolution rate over solubility from coprecipitate may be due to the formation of a eutectic, or a solid solution (Chiou and Riegleman, 1971). Such a solid solution or a eutectic cannot be prepared by just physically mixing the two components, and hence, the physical mixture failed to increase the dissolution rate. The DSC thermograms of etoposide coprecipitates also failed to support the formation of a eutectic or a solid solution of etoposide with PEG 8000. Polymer layer controlled, high energy dispersion of drug was claimed to be responsible for high relative dissolution rates from a dispersion of sulfathiazole in PVP (Simonelli et al., 1969). The relative dissolution rate (Fig. 4; Table 1) of etoposide behaved very similarly to that of a sulfathiazole-PVP system, indicating a similar mechanism of dissolution rate enhancement.

In summary, coprecipitate of etoposide with PEG 8000 in the ratio of 1:10 increased the solubility 2-fold, and dissolution rate 42-fold, respectively, and may have a great potential to significantly improve oral bioavailability. Etoposide coprecipitates with PEG 3400 and PVP 40 000 may also improve oral bioavailability due to the significant enhancement in the dissolution rate of drug.

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