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SOLUBILITY AND DISSOLUTION OF ETOPOSIDE FROM SOLID DISPERSIONS OF PEG 8000

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ABSTRACT

The Solubility and dissolution of etoposide from solid dispersion of PEG 8000, prepared by the fusion method, were investigated. Stability studies revealed that the etoposide was stable in water for three days at 37 ± 0.5°C alone and as a physical mixture with PEG 8000. However, nearly 5% decomposition was oberved in aqueous solutions made from solid dispersions. TLC, IR and HPLC studies showed both the drug and carrier were stable during the fusion process. Aqueous solubility of etoposide from solid dispersions with etoposide:PEG 8000 ratios of 1:5, 1:10, 1:20, 1:30 and 1:40, was studied at 37± 0.5°C, and found to be significantly higher than that of etoposide alone or from its physical mixtures with PEG 8000. These dispersions increased the solubility of etoposide by 32.3%, 96.8%, 133.5%, 280.7% and 326.6% respectively compared to that of etoposide alone, whereas only 1:40 etoposide:PEG 8000 physical mixture demonstrated a significant increase in etoposide solubility (16.1%). Dissolution studies, on the solid dispersions in water at 37± 0.5°C, revealed a marked increase in the dissolution rate of etoposide from 1:20, 1:30 and 1:40 solid dispersions with 100% drug dissolving within 1 minute; dissolution time for 1:5 and 1:10 dispersions, and all

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physical mixtures was 3 minutes while etoposide alone required 30 minutes for complete drug dissolution. The melting behavior of the etoposide-PEG 8000 mixtures and subsequent thermal analysis of the melts suggested that the increase of solubility of etoposide was mostly due to the formation of a solid solution of etoposide in PEG 8000.

INTRODUCTION

Etoposide, (VePesid®; VP-16, MW = 588.58) a new antineoplastic agent, is a semisynthetic derivative of epipodophyllotoxin with the molecular formula, $C_{29}H_{32}O_{13}$. It was shown to have cytotoxic activity against testicular and small-cell lung cancers, lymphoma, leukemia and Kaposi's sarcoma associated with AIDS (1). Etoposide has a basic pharmaceutical formulation problem of poor solubility, which could be a source of bioavailability problems including the low oral bioavailability (50 - 60%). Its solubility in water at 37° C has been reported to be 148.5-167.25 μg/ml and does not vary over the pH range 2 to 6 (2). The low solubility has been attributed to very poor wettability, and ease of aggregation/ agglomeration/ lumping caused by an electrostatic surface charge generated during handling or processing operation and subsequent stronger Van der Waals' attraction between nonpolar molecules (3). In view of these characteristics, formation of solid dispersion of etoposide with a hydrophilic carrier was attempted (4, 5) in order to enhance its aqueous solubility. Besides achieving an extremely fine state of subdivision of ingredients, such formations provide for an intimate contact between the drug and the carrier molecules or molecular aggregates, thereby contributing to increased wettability and dispersibility of the hydrophobic drug in a hydrophilic environment. PEG 8000, the general formula $HOCH_2(CH_2OCH_2)_xCH_2OH$, was used as the carrier because of its high water solubility, low melting point (< 65° C) and its viscous property which can reduce crystallization of the drug (6).

MATERIALS AND METHODS

Materials: Etoposide powder, used as recieved, was supplied by Britol-Myers Company (Syracuse, NY). PEG 8000 was purchased from Sigma Chemical Company (St.Louis, MO), Chloroform and methanol

were from Mallinckrodt Chemical Works (St.Louis, MO) and J.T. Baker Chemical Co. (Phillipsburg, NJ), respectively. All other chemicals/solvents were of reagent grade and were obtained commercially.

Instruments: A UV/Vis spectrophotometer (Spectronic 710, Bausch & Lomb, San Leandro, CA), a sieve vibrator (Sepor Laboratory Supply, Wilmington, CA), HPLC (Spectra-Physics Inc., San Jose, CA), an IR spectrophotometer (Model 283, Perkin-Elmer Corporation, Norwalk, CT), TLC plates (Analtech Inc., Newark, DE), a Beckman-43 pH meter (Beckman Instruments, Inc. Irvine, CA), a shaker (New Brunswick Scientific, Edison, NJ), USP/NF dissolution assembly (Hanson Research Corporation, Northridge, CA), a differential scanning calorimeter (DSC-50, Shimadzu Corporation, Japan) and a capillary tube melting-point apparatus (Arthur H. Thomas Co., Philadelphia, PA), were used in this study.

Assay of Etoposide: Etoposide was assayed by using UV/Vis spectrophotometer at a wavelength of 230 nm (7). UV spectrum of PEG 8000 showed very weak absorption at 230 nm. The absorbance reading of etoposide in etoposide-PEG 8000 water solutions was corrected by subtracting the contribution by PEG 8000 according to its calibration curve. HPLC assay (7) was utilized for stability studies on etoposide.

Preparation of Solid Dispersions: A series of solid dispersions of etoposide in PEG 8000 (1:5, 1:10, 1:20, 1:30 and 1:40) were prepared. Accurately weighed quantities of etoposide and PEG 8000 in the required ratios were blended thoroughly and heated with stirring (one minute) in glass petri dishes (5 cm diameter) on an electrical hot plate at the temperature just in excess of the melting point of the carrier (62° C). The solid dispersion obtained by cooling and keeping the molten mixture in a desiccator for two days at ambient teperature was pulverized and an 80-100 mesh (150-180 micron) fraction was then collected with the aid of a sieve vibrator, and used for further studies.

Stability Studies

a. Stability of etoposide during the process of making dispersion:

UV, IR, TLC and HPLC characteristics of pure & processed samples of etoposide were studied. In the UV study, the spectra of the

pure and dispersed drugs in water were scanned from 280 to 200 nm (scanning speed: 20 nm/min and the chart speed: 2.54 cm/min). In the IR study, a potassium bromide disc of etoposide was scanned from 4000 to 200 cm^{-1}. In the TLC study, methanol solution of pure and dispersed drugs were spotted on a plate (alumina GF, 250μ, activated at 110° C for 20 minutes), developed with chloroform/methanol (9/1, v/v), and subsequently colored with iodine. Samples used in TLC study were also analyzed by HPLC(7)

b. Stability of etoposide in aqueous solution

The aqueous stability of etoposide itself and in the physical mixtures and solid dispersions of PEG 8000 was examined to ascertain whether aqueous solutions of etoposide in PEG 8000 remained stable during the subsequent equilibrium solubility determinations. The 50 ml-sample solutions containing approximately 20 μg/ml etoposide were prepared and placed in a room maintained at of 37 ± 0.5°C . Over a 3-day period, 3-ml and 20 μl samples were withdrawn at designated time intervals and assayed by UV and HPLC for etoposide. The pH of the freshly-prepared aqueous solutions of etposide, PEG 8000, and their corresponding solid dispersions was also measured.

Solubility Studies: The solubility of etoposide alone was determined at 37 ± 0.5°C by adding a known excess of etoposide to 3 ml water in a 20-ml screw-capped glass vial placed on a shaker. The equalibration, and subsequent filtration through 0.22-micron filter paper (Filter type GS, white, plain, 25-mm diameter, Millipore Filter Corporation, Bedford, MA) and sampling were carried out in a constant temperature room at 37 ± 0.5°C. After 72 hours, drug concentration in each sample was determined spectrophotometrically as stated earlier.

Dissolution studies: Dissolution studies were carried out on etoposide alone, etoposide from dispersions of PEG 8000 and from physical mixtures with PEG 8000 using the modified USP/NF dissolution assembly at 37 ± 0.5°C in water. An accurately weighed quantity of the sample equivalent to 2.25 mg etoposide was added to the 300 ml water in the flask under constant stirring (250 rpm). 5 ml samples were withdrawn at designated intervals, filtered through 0.22-micron filter

paper and then assayed for etoposide spectrometrically. Each sample was replaced by an equal volume of water (37°C) to keep the total volume constant. A cumulative correction was made for the removed samples to determine the total amount dissolved (8).

Thermal Analysis: A differential scanning calorimeter was used to determine the melting temperatures or the physicochemical interactions of the components in solid dispersions. A 2-mg sample was accurately weighted in an aluminum sample pan, scanned from 40 to 360° C at a rate of 10°C/min in a static nitrogen atmosphere. The melting temperature of the physical mixtures and dispersions was also visually determined by the conventional capillary tube melting-point apparatus.

RESULTS AND DISCUSSION

Solid Dispersion Formation: The solid dispersions of etoposide in PEG 8000, prepared at 62 ± 2°C, were waxy, irrespective of the proportion of the drug , and needed 1 to 1.5 days for complete solidification. The appearance of the molten mixtures suggested that some of the etoposide had dissolved in the molten PEG 8000. Usually, a supersaturation of drug can be obtained by rapid quenching of the molten mixture since solute molecules are arrested in the matrix by instantaneous solidification (9). In view of the low melting point of the carrier, melts could be cooled to room temperature rapidly by placing them on a counter top for two minutes at room temperature, prior to storage in a desiccator. This procedure might not have caused the same level of supersaturation attainable from very rapid cooling. No discoloration was observed in any of the dispersions.

Stability Studies

a. Stability of etoposide during the process of making a dispersion

A comparison of the UV and IR spectra of the pure and processed drugs in PEG 8000 dispersions suggested lack of decomposition and absence of any chemical interaction between the drug and the carrier. The TLC plates revealed upon iodination only a single brown spot with an identical R_f value (0.69) for both the pure and processed etoposide samples, while a known sample of thermally decomposed etoposide produced multiple spots. The PEG 8000 produced a trailing spot with an R_f value of 0.59. HPLC retention time of both pure and processed eto-

Table I
pH Values of Aqueous Solutions of Etoposide, PEG 8000 and Their Dispersions

Product	pH Value[a]
Distilled water	5.59 ± 0.03
Etoposide (30ug/ml)	5.58 ± 0.02
Etoposide (60ug/ml)	5.60 ± 0.05
Etoposide (150ug/ml)	5.54 ± 0.03
PEG 8000 (10mg/ml)	6.26 ± 0.04
Etps/PEG,Disp 1:5 (50ug/ml)	5.70 ± 0.04
Etps/PEG,Disp 1:40 (50ug/ml)	5.87 ± 0.05

[a] Mean ± Standard deviation

posdie was 5.39 ± 0.10 minutes, and no other peaks appeared in these chromotographs. This TLC and HPLC analysis (capable of differentiating drug from its decomposition products) further demonstrated the thermal stability of etoposide and the carrier during the preparation of the dispersion systems.

b. Stability of etoposide in aqueous solution

Stability studies by both UV and HPLC indicated that etoposide was stable in an aqueous solution alone and in a physical mixture with PEG 8000 at 37 ± 0.5°C at the end of 3 days. However, etoposide in 1:20 dispersion exhibited nearly 5.0% decomposition by HPLC at the end of 3 days.

It was reported by Chow (2) that pH affected the etoposide stability to a great extent at pH 1.3 and pH 10, the degradation half-life was less than 4 hours at 25°C and maximal stability at 25°C was reached at pH 5 to 6.15 with half lives of 63 and 49.5 days respectively. Chow also reported that etoposide degradation was negligible at pH 3.05 to 7.30 within 48 hours (7). Although PEG 8000 is a nonelectrolyte, sometimes, presence of nonelectrolytes in high concentration could alter pH of the system. The decomposition of any substance in solution could also alter pH. The pH

Table II
Aqueous Solubility of Etoposide from Etoposide-PEG 8000 Systems at 37° ± 0.5°C

Product	Solubility[a] (ug/ml)	% Increase[b]
Etoposide	150.1 ± 2.7	--
1:5 PhM	150.9 ± 5.7	N.S.[c]
1:10 PhM	155.3 ± 7.0	N.S.[c]
1:20 PhM	159.7 ± 5.7	N.S.[c]
1:30 PhM	164.8 ± 10.2	N.S.[c]
1:40 PhM	174.2 ± 6.7	16.1± 3.8
1:5 Disp	298.5 ± 13.2	32.3± 4.4
1:10 Disp	295.4 ± 6.2	6.8 ± 2.1
1:20 Disp	351.3 ± 26.6	133.5 ± 7.6
1:30 Disp	571.4 ± 23.3	280.7 ± 4.1
1:40 Disp	640.3 ± 21.5	326.6 ± 3.4

[a]Average of two samples ± Standard Deviation
[b]Compared with the solubility of pure etoposide
[c]No significant difference

of all aqueous solutions was, therefore, monitored. As shown in Table I, the pH of freshly prepared aqueous solutions of pure etoposide, PEG 8000,and solid dispersions of etoposde in PEG 8000 was around 5.5, well within the range desired for maximum stability (7). The pH measurement is also important because etoposide is a weak acid and its solubility would change with a change in pH.

Solubility Studies: Table II and Fig.1 show the solubility of etoposide from physical mixtures and dispersions of PEG 8000 at 37 ± 0.5°C. The one-way ANOVA revealed no significant solubility increase among the physical mixtures of 1:5, 1:10, 1:20 and 1:30 compositions, only 1:40 physical mixture demonstrated significant increase (16.1%) in the etoposide solubility.

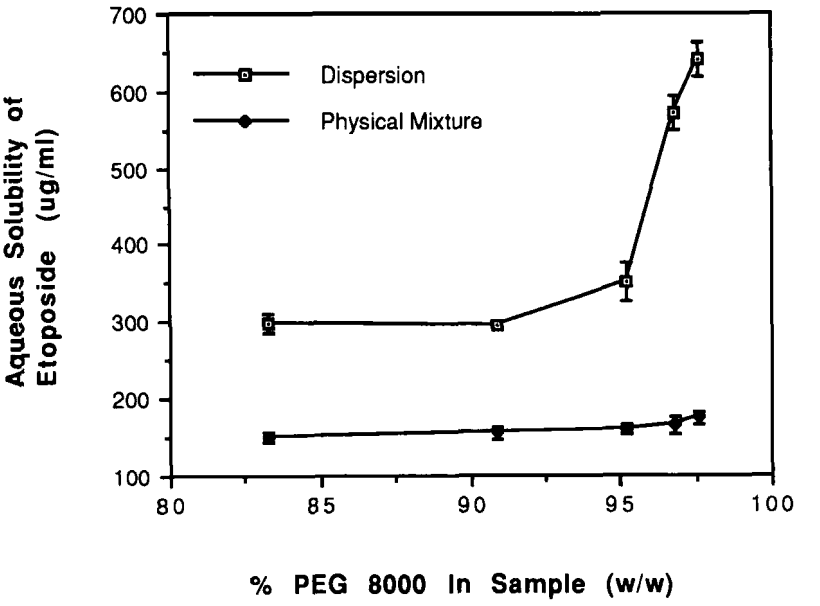

FIGURE 1
Effect of PEG 8000 on Solubility of Etoposide (Comparison of Dispersion and Physical Mixture)

The aqueous solubility data of etoposide from solid dispersions revealed that etoposide-PEG 8000 dispersions were extremely effective in enhancing the solubility of etoposide, particularly the PEG 8000 rich compositions (1:20, 1:30 & 1:40). This increase of etoposide solubility as a function of PEG 8000 concentration in the dispersion may be attributed partly to the breaking of water structure by the excipient, and thereby creating a more energetically favorable environment for solubilization of a relatively hydrophobic molecule such as etoposide (10). The etoposide appeared to dissolve partially in the molten PEG 8000 which upon cooling gave solid solution, a mixed crystal in which the two components crystallized together in a homogeneous one-phase system. At high drug concentration, such as 1:5 dispersion, only a small portion of etoposide would form solid solution (the remainder would be only solid suspension), however, with the increase in the proportion of PEG 8000, for instance, 1:40 dispersion, considerably more drug would be in solid

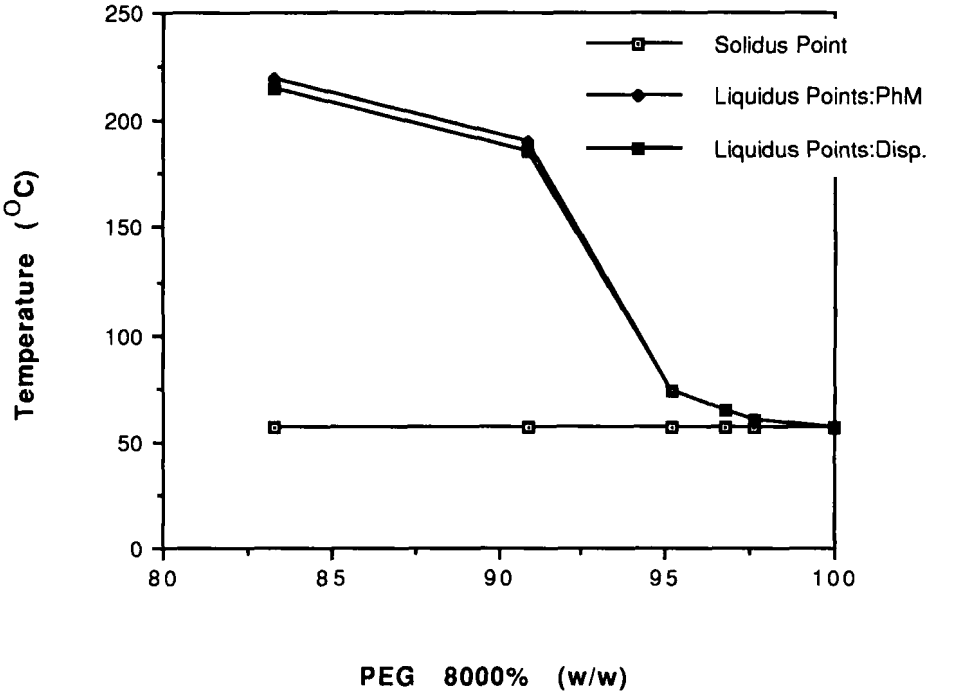

FIGURE 2
Phase Diagram of Etoposide-PEG 8000 Systems (Comparison of Dispersion and Physical Mixture)

solution, causing further enhancement of drug solubility. In the solid solution, particle size of the drug is reduced to its minimum state, its molecular size, which is in turn responsible for the increased dissolution rate and solubility of the drug in water. The phase diagram in Fig.2 shows that when concentration of PEG 8000 is greater than 95%, considerably more drug dissolves in PEG 8000 and liquidus phase is obtained below 75°C.

Dissolution Studies : Table III shows the dissolution profile of etoposide in water at 37 ± 0.5°C in PEG 8000 systems. Under experimental conditions, etoposide alone required 30 minutes to dissolve completely, while all the physical mixtures, and 1:5 and 1:10 dispersions were approximately equally effective with 100% of the drug dissolving within three minutes. For 1:20, 1:30 and 1:40 dispersions, the

Table III
Aqueous Dissolution Profile of Etoposide from Physical Mixtures and Dispersions with PEG 8000 at 37 ± 0.5°C

Product	% Amount Dissolved[a] at following time (Minute)				
	1	3	5	10	30
Etoposide	53.1±1.7	60.0±2.4	85.3±1.7	90.2±1.7	**100.9**±0.7
1:5 PhM[b]	88.6±0.8	**98.6**±2.1	98.1±5.2		
1:10 PhM[b]	95.1±0.9	**100.0**±4.3	99.4±4.4		
1:20 PhM[b]	92.2±3.3	**100.0**±2.5	100.0±3.4		
1:30 PhM[b]	96.1±3.9	**100.0**±5.4	99.4±2.6		
1:40 PhM[b]	89.3±4.8	**100.0**±6.4	99.1±7.4		
1:5 Disp[c]	91.9±5.0	**100.0**±5.6	99.4±4.8		
1:10 Disp[c]	87.5±2.8	**100.0**±0.8	99.5± 0		
1:20 Disp[c]	**100.0**±1.2	100.0±3.5			
1:30 Disp[c]	**100.0**±2.2	99.1±1.5			
1:40 Disp[c]	**99.0**±2.7	100.0±1.6			

[a]Mean ± Standard Deviation
[b]Physical mixture of etoposide and PEG 8000
[c]Solid dispersion of etoposide in PEG 8000

time for complete dissolution was about 1 minute. The dissolution enhancement especially in the case of 1:5 - 1:40 dispersions may be due to some of the same factors responsible for solubility enhancement discussed earlier. These data suggest that PEG 8000 at concentration level of 83% or greater is very effective in promoting dissolution of etoposide regardless of whether drug is present as a physical mixture or solid dispersion, although solid dispersion is superior.

The well-known Noyes-Whitney equation predicts that the dissolution rate of a drug is a function of the concentration gradient and the surface area of the dissolving particles. In the case of solid dispersions, with increasing proportion of PEG 8000, effect of solubility and the surface area would approach their maximum since the drug would be

molecularly dispersed in the highly hydrophilic carrier. This can not be accomplished with physical mixtures.

Thermal Analysis: The DSC thermograms of various etoposide solid dispersions with PEG 8000 were run. With etoposide content of less than 9.1% (1:20, 1:30 and 1:40 dispersions) only a single endotherm could be seen corresponding to the PEG 8000 endotherm at 62°C. With the capillary tube method, the melting point was visually estimated and phase diagram constructed (Fig. 2). A complete melting of the sample in the capillary tube was observed between 61-72°C for the 1:20, 1:30 and 1:40 systems suggesting dissolution of etoposide in the fused PEG 8000. The melting range was especially narrow for the 1:30 and 1:40 systems that is why phase diagram did not show any solidus points for these compositions. At 1:5 and 1:10 ratios, the drug amount was too high to dissolve completely in the molten PEG 8000, and the liquidus points were reached above 200°C, corresponding to the melting point of etoposide in the presence of PEG 8000.

The melting behavior of the etoposide-PEG 8000 mixtures and subsequent thermal analysis of the melts suggested that the increase of solubility of etoposide was mostly due to the formation of a solid solution of etoposide in PEG 8000. Studies with physical mixtures suggested that the carrier-influenced changes in the affinity between a relative hydrophobic drug and water molecules also played a role.

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