Drug Development and Industrial Pharmacy, 25(1), 63–67 (1999)

RESEARCH PAPER

# Metastable Polymorph of Etoposide with Higher Dissolution Rate

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## ABSTRACT

*Etoposide, an anticancer drug, has low oral bioavailability because of low aqueous solubility, slow dissolution rate, and instability in acidic pH. Our objective was to enhance the aqueous solubility and dissolution rate of etoposide by polymorph formation. Preparation of various polymorphs of etoposide was attempted by crystallizing etoposide from organic solvents. Physicochemical properties of the crystals, namely, crystal habit, thermal behavior with hot-stage microscopy, thermal analysis by differential scanning calorimetry, IR spectrum, and solubility and dissolution rates, were examined. Based on the physicochemical characteristics, a metastable polymorph of etoposide was identified when it was crystallized from isopropanol. The metastable polymorph had an equilibrium solubility and intrinsic dissolution rate of 221 μg/ml and 16.3 μg/min/cm², respectively; 1.9 and 1.7 times that of etoposide powder at 25°C, respectively.*

## INTRODUCTION

Etoposide is a drug of choice for the treatments of small cell lung cancer and testicular carcinoma (1). Several investigational oral formulations of etoposide exhibited low and erratic oral bioavailability in humans (2). The currently available oral formulation is a soft gelatin capsule filled with etoposide solution in a cosolvent mixture (comprising benzyl alcohol, ethanol, PEG 400, and Tween 80) and was reported to have an oral bioavailability of 50% (2,3). In a previous investigation (4), 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 its low oral bioavailability. The solubility of etoposide at 37°C was extremely

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low, 0.15 mg/ml, which is very inadequate to result in complete dissolution of the usual oral dose of 100 mg in gastric fluids (5). The intrinsic dissolution rate was 0.01 mg/min/cm$^2$, far less than 1.0 mg/min/cm$^2$, suggesting that oral absorption was dissolution rate limiting (4,6). Attempts have been made to increase the aqueous solubility of etoposide by chemical modifications, cosolvent approach, solid-state modification, and the use of hydrotropic agents (2,7–10). 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 (2,3). The coprecipitate of etoposide with PEG 8000 increased its solubility 2-fold and dissolution rate 42-fold (10).

Polymorphs have been reported in various classes of drugs, and the metastable polymorphs and the amorphous forms that melt at lower temperatures have been shown to increase aqueous solubility and dissolution rate of drugs (11–13). Recently, hydrates of etoposide with slightly higher aqueous solubilities were reported (14). To identify and characterize potential polymorphs and/or amorphous forms of etoposide with high aqueous solubility, etoposide was crystallized from organic solvents. The crystals so obtained were characterized for crystal habit, thermal behavior with hot-stage microscopy, and thermal analysis by differential scanning calorimetry (DSC) and IR spectrum. However, based on the crystal habit, DSC thermogram, solubility, and dissolution behavior, crystals from isopropanol when compared with etoposide powder appear to be a metastable polymorph of etoposide, as described below.

## MATERIALS AND METHODS

Etoposide powder was used as supplied by Bristol Myers (Syracuse, NY). Ethanol, methanol, chloroform, acetonitrile, and isopropanol were all HPLC grade.

### Assay

A stability indicating HPLC assay previously developed for etoposide was used for the solubility and dissolution study (15).

### Preparation of Crystals

Etoposide powder was dissolved in isopropanol at subsaturation concentrations, and then the solution was poured into petri dishes. The solvent was allowed to evaporate at room temperature and atmospheric pressure to obtain crystals. The crystals were allowed to dry at room temperature for 2 hr and then characterized as described below.

### Crystal Habit and Hot-Stage Microscopy

Crystals were examined and photographed under a microscope using both regular and plane-polarized light at 10× magnification. The microscope was also fitted with a Koeffler hot stage. The crystals were heated on the hot stage at a rate of 2–5°C/min, and observations were made of any significant thermal event such as melting, recrystallization, and/or loss of solvent, which were correlated with DSC thermograms. Photographs were taken under polarized light at various temperatures to record the observation with a camera mounted on the microscope. Etoposide powder used to obtain the crystals was similarly studied using hot-stage microscopy.

### Differential Scanning Calorimetry

The thermograms of crystals and etoposide powder were obtained on Perkin Elmer DSC-4 model with a system 4 microprocessor. The weighed crystals or powder (about 5–10 mg) were sealed in one aluminum pan, and another pan with air served as the control. The crystals were heated from 50 to 300°C at a heating rate of 5°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 the temperatures at which any thermal event occurred were noted. The thermograms were integrated and the peak areas calculated to obtain the heat for the respective thermal events.

### IR Spectra

IR spectrum was obtained with a Hewlett Packard IR spectrometer from a disk manually compressed from a dispersion of crystals or powder in KBr.

### Intrinsic Dissolution Rates

The intrinsic dissolution rates of crystals were determined from a drug disk of constant surface area using a Wood's apparatus by a method similar to that used for etoposide powder in an earlier publication (4,10).

### Particulate Dissolution and Equilibrium Solubility

An excess amount (5 mg) of etoposide powder or crystals from isopropanol were agitated with 10 ml of dis-

tilled water on a rotatory mixer at room temperature. Samples of 0.5 ml were taken at various time intervals, filtered through a 0.45-μm membrane filter (Gelman), and analyzed by the HPLC assay.

## RESULTS

### Crystal Habit and Behavior on Hot-stage Microscope

The etoposide powder from which the crystals were prepared appeared to be micronized and did not exhibit any birefringence under polarized light and thus its crystal habit could not be determined. The crystals from isopropanol were large, triangular to prismatic shaped, and exhibited birefringence in polarized light. The crystals from isopropanol were very pure as seen by the high birefringence of the sample and the sharp melting of all the crystals in the temperature range of 215–220°C. In contrast, etoposide powder melted between 260 and 280°C. The melt was yellowish brown in color, indicating charring.

### Differential Scanning Calorimetry

Thermogram of crystals from isopropanol is compared with that of etoposide powder in Fig. 1. The thermogram of etoposide powder had a sharp exothermic peak at 194.7°C and a sharp melting endotherm at 275.9°C. Thermogram of crystals from isopropanol showed no thermal events with a flat baseline and ended with a sharp melting endotherm at 217.9°C, significantly lower than the melting point of etoposide powder. There was no significant difference between the heat of fusion for the two melting endotherms for powder and crystals from isopropanol.

### IR Spectra

IR spectra were obtained from etoposide crystal-KBr disks. Minimal pressure was applied during grinding and disk compression to avoid possible polymorphic transformation (16). The fingerprint region, 1000–1500 $cm^{-1}$ of the IR spectrum, was compared with that of etoposide powder. The spectrum of crystals from isopropanol was distinct from the powder in having an additional peak at 1315 $cm^{-1}$. No sharp peak at about 2900 $cm^{-1}$, corresponding to the—OH stretching frequency of isopropanol, was present, indicating probably absence of solvent in the crystal lattice. Otherwise, the spectrum of crystals was identical and coincided with that of etoposide pure powder.

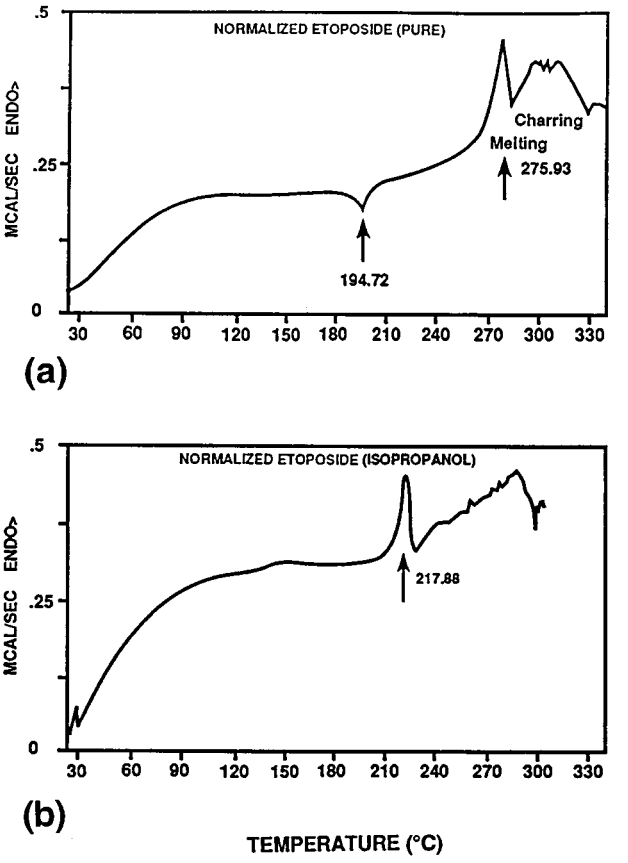


**Figure 1.** DSC thermograms of (a) etoposide powder and (b) crystals from isopropanol.

### Intrinsic Dissolution Rate

The dissolution profiles of etoposide crystals and powder are compared in Fig. 2, and the dissolution rates based on slopes of the initial linear region are tabulated in Table 1. The intrinsic dissolution rate of crystals from isopropanol was 1.7-fold higher than that of pure etoposide powder. The dissolution experiment had been conducted for 75 hr to achieve saturation solubility limit according to the Noyes–Whitney equation. At the end of 75 hr, the concentration of etoposide in solution from the crystals from isopropanol was twice that achieved with etoposide pure powder.

### Particulate Dissolution and Solubility Experiment

A solubility experiment was conducted for the powder and crystals, but samples were taken at various time inter-

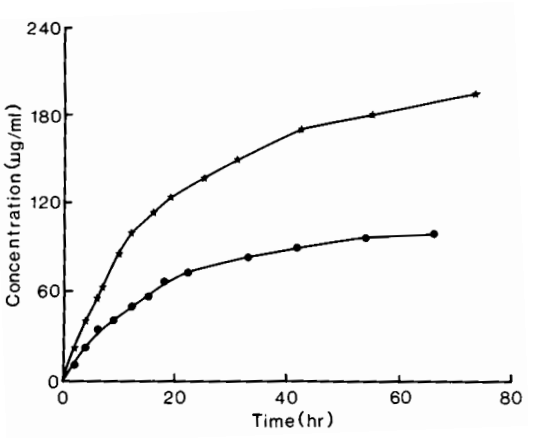


**Figure 2.** Intrinsic dissolution profiles of etoposide powder (●) and crystals from isopropanol (✯) ($n = 3$).

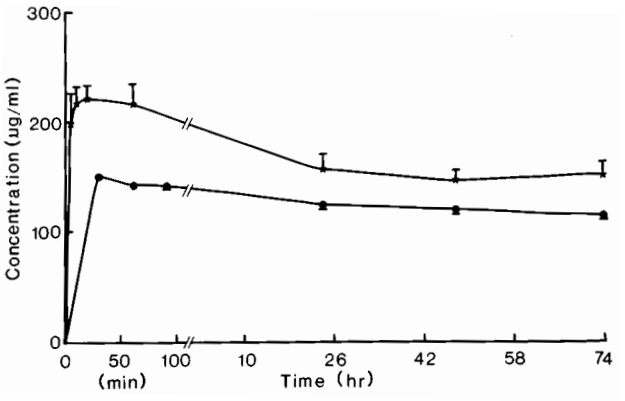


**Figure 3.** Particulate dissolution of etoposide powder (●) and crystals from isopropanol (✯) ($n = 3$).

vals to determine if crystals from isopropanol was metastable and exhibited a change in solubility with time. The resulting dissolution profiles are shown in Fig. 3. Saturation occurred with crystals from isopropanol, resulting in a concentration of 221 μg/ml in 20 min, as compared with 150 μg/ml for etoposide powder. However, the concentration declined to about 150 μg/ml at 24 hr and remained at that concentration thereafter for 72 hr (Fig. 3), indicating transformation and hence metastable nature of the crystals from isopropanol. This indicated that the apparent solubility of etoposide from the metastable polymorph was 221 μg/ml and that it may decrease and eventually reach the saturation solubility of etoposide. The equilibrium solubility of etoposide powder was 114.5 μg/ml at 25°C.

In summary, crystals from isopropanol were a low-melting metastable polymorph of etoposide with higher solubility (1.9-fold) and dissolution rate (1.7-fold) than etoposide powder.

## DISCUSSION

The thermogram of etoposide bulk powder had a significant negative endotherm at 195°C followed by melting at 275.93°C, much higher than that reported in the Merck Index. The analytical profile of etoposide (17) described the DSC thermogram with an exothermic peak between approximately 190 and 210°C and a melting endotherm between 255 and 264°C with a maximum at 258°C. Jasti et al. (14) attributed a similar endo-

***Table 1***

*Physicochemical Properties of Etoposide Powder and Crystals from Isopropanol*

| Physicochemical Property | Etoposide Powder | Crystals from Isopropanol |
|---|---|---|
| Dissolution rate (μg/ml/hr) | 5.42 ± 0.58 | 9.10 ± 0.56[a] |
| Intrinsic dissolution rate (μg/min/cm$^2$) | 9.60 ± 1.00 | 16.30 ± 1.00[a] |
| Intrinsic dissolution rate constant (cm/min) | 0.084 ± 0.008 | 0.074 ± 0.007 |
| Particulate dissolution and equilibrium solubility experiment | | |
| Aqueous solubility (μ/ml) | 114.5 ± 4.0 | 221.0 ± 11.7[a] |

Values are means ± SD; $n = 3$.

[a] Values are significantly different at $p = 0.05$ by Student's two sample $t$-test when compared with that of etoposide powder.

therm–exotherm event between 198 and 206°C to melting of polymorph Ia and crystallization of the melt into polymorph IIa, which melted on further heating at 269°C. Thus, although the thermograms in this study match with those reported earlier, the reported melting of polymorph Ia and crystallization of IIa could not be confirmed with observations on the hot-stage microscope. Although the Merck Index reports that etoposide powder is recrystallized from methanol, the powder obtained from the manufacturer was found to be micronized and did not exhibit crystallinity under polarized light. Earlier, particle sizing of the etoposide powder revealed that more than 90% of the particles were smaller than 5 μm in size (10). It appears that etoposide may have been milled to reduce the particle size and, in the milling process, experienced a stress or a transition in the solid-state form.

The stress introduced in the crystal lattice by milling is a well-known phenomenon (13,16). This stress or transformation may be responsible for the exothermic peak in the thermogram, poor crystallinity observed under polarized light, and a higher melting point (275.9°C) observed for etoposide powder. This stress may be also responsible for the supersaturation observed in the solubility experiment (Fig. 3). Thermogram of crystals from isopropanol did not exhibit any previously described thermal events for the powder and had a flat baseline ending with a sharp melting endotherm at 217°C. Therefore, crystals from isopropanol may be a low-melting polymorph of etoposide that melts before it undergoes transition. The melting endotherm of crystals from isopropanol was confirmed with the hot-stage microscopy observations.

Crystals from isopropanol had higher aqueous solubility (1.9-fold) and higher intrinsic dissolution rate (1.7-fold). It is apparent from the particulate dissolution data that the polymorph (crystals from isopropanol) is metastable and results in supersaturation rapidly (221 μg/ml in 20 min), followed by slow conversion to the stable form and eventually will attain the aqueous solubility of the stable form of etoposide. Also in the intrinsic dissolution experiments, the concentration achieved by crystals at the end of 75 hr was twice that from powder (193 versus 99 μg/ml). The increase in dissolution rate (1.7-fold) is due to this significantly higher solubility (1.9-fold) of polymorph, as expected based on the Noyes–Whitney equation. As mentioned above, the thermogram of crystals from isopropanol does not exhibit any transition, although it is metastable and has a lower melting point than the powder. However, the transformation of crystals from isopropanol may be accelerated by contact with the aqueous medium. This solid-state transformation could be verified by isolating the solid and investigating the solid state with DSC and x-ray diffraction. The sharpness of the melting endotherm of the polymorph indicated purity and high degree of crystallinity (18).

Although the polymorph exhibited higher aqueous solubility, the improvement in oral bioavailability of etoposide may be marginal because the dissolution rate of etoposide is increased only 1.7-fold. In contrast, a 42-fold increase in dissolution rate was observed by solid-state dispersion of etoposide in PEG 8000, which resulted in improved oral bioavailability in rat (10,19).

## REFERENCES


1. B. F. Issell and S. Crooke, Cancer Treat. Rev., 6, 107–124 (1979).
2. P. I. Clark and M. L. Slevin, Clin. Pharmacokinet., 12, 223–252 (1987).
3. Product Insert of Vepesid (1987).
4. J. Shah, J. R. Chen, and D. Chow, Pharm. Res., 6, 408–412 (1989).
5. B. F. Issell, A. R. Rudolph, and A. C. Louie, in *Etoposide (VP-16)* (B. F. Issell, F. M. Muggia, and S. K. Carter, eds.), Academic Press, Orlando, 1984, pp. 4–6.
6. S. K. Kaplan, Drug Metab. Rev., 1, 15–34, (1972).
7. W. Kreis and A. Soricelli, Clin. Oncol., 95, 233–237 (1979).
8. W. R. Rose, G. A. Basler, P. A. Trail, M. Saulnier, A. R. Cosswell, and A. Casazza, Invest. New Drugs, 8, S25–S32 (1990).
9. I. A. Darwish, A. T. Florence, and A. M. J. Saleh, Pharm. Sci., 78, 577–581 (1989).
10. J. C. Shah, J. R. Chen, and D. Chow, Int. J. Pharm., 113, 103–111 (1995).
11. S. R. Byrn, *Solid State Chemistry of Drugs*, Academic Press, New York, 1982, pp. 79–148.
12. J. T. Carstensen, in *Modern Pharmaceutics* (G. S. Banker and C. T. Rhodes, eds.), Marcel Dekker, New York, 1990, pp. 239–262.
13. J. Haleblian and W. J. McCrone, Pharm. Sci., 58, 911–930 (1969).
14. B. R. Jasti, J. Du, and R. C. Vasavada, Int. J. Pharm., 118, 161–167 (1995).
15. D. Chow, J. Shah, and J. R. Chen, J. Chromatogr., 396, 217–223 (1987).
16. H. K. Chan and E. Doelkar, Drug Dev. Ind. Pharm., 11, 315–332 (1984).
17. J. J. M. Holthius, J. J. Bosch, and A. Bult, in *Analytical Profiles of Drug Substances* (K. Florey, ed.), Academic Press, New York, 1989, 18, 121–151.
18. Perkin-Elmer Application Notes, DSC-4 Instrument Manual.
19. J. C. Shah, J. R. Chen, and D. Chow, Int. J. Pharm., 84, 223–232 (1992).