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Characterization of thermal behavior of etoposide

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Abstract

Etoposide is a potent cytotoxic agent useful in the treatment of various malignancies. Earlier work in our laboratory with this drug as obtained from the source (etoposide I) revealed that it undergoes thermal transitions during the course of heating to 300°C. In this study these thermal events were characterized by subjecting etoposide I to differential thermal analyses, powder X-ray diffractometry, mass spectrometry, infrared analysis and HPLC. Etoposide I, a monohydrate, was found to undergo a dehydration reaction in the range of 85–115°C to yield etoposide Ia, which upon further heating melted at 198°C and crystallized to a new polymorph, etoposide IIa at 206°C. Etoposide IIa was found to melt at 269°C and converted to its hydrated form, etoposide II, when exposed to the atmosphere at room temperature. This hydrate was also found to undergo a dehydration reaction at 90–120°C to yield etoposide IIa. The X-ray diffraction studies confirmed the existence of etoposide I, Ia and II, IIa as two different crystal arrangements. Nearly identical infrared spectra and retention times with the HPLC indicated that the two forms were not chemically different. The polymorphic transition was found to be irreversible and a monotropic type of transition. TGA and mass spectral analyses of etoposide I and II suggested that the water was present in their crystal lattices. Etoposide II was found to have greater aqueous solubility than that of etoposide I at 0, 22, and 37°C.

Keywords: Etoposide; Thermal transition; DSC; Thermal gravimetric analysis; X-ray diffractometry; IR spectroscopy; Mass spectrometry; Polymorphism; Solubility

1. Introduction

Etoposide [4′-demethylepipodophyllotoxin-9-(4,6-*o*-ethyllidene-β-D-glucopyranoside) (Vepesid®; VP-16-213)] an antineoplastic agent, is a semisynthetic derivative of podophyllotoxin. The molecular formula of etoposide is $C_{29}H_{32}O_{13}$, and its molecular weight, 588.58. It was shown to have cytotoxic activity against testicular and small-cell lung cancers, lymphoma, leukemia and Kaposi's sarcoma associated with AIDS (O'Duyer et al., 1985). Etoposide has low aqueous solubility and surface electrical charges that might contribute to low and erratic bioavailability (Shah et al, 1989). Recent attempts at enhancing its dissolution and aqueous solubility include the use of mixed-solvent systems (Gate et al., 1984; Sato et al., 1984;) and of glass dispersions in PEG 8000 (Du and Vasavada, 1993) or xylitol (Du and

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Vasavada, 1990). Differential scanning calorimetry (DSC) of etoposide in our laboratory has revealed evidence of thermal events in the range of 85–115 and 190–210°C, and at 269°C prior to decomposition at about 290°C. In particular, an exotherm at 206°C suggested conversion to a different crystalline form. An investigation of these thermal events was warranted, since this could lead to a form of etoposide with more desirable physical chemical properties and thus might aid in the development of an improved delivery system.

The importance of polymorphism in relation to drug delivery and therapeutic effectiveness is well recognized and documented in the literature (Haleblian and McCrone, 1969; Haleblian, 1975). The thermodynamic relationships involving polymorphism and solubility of two forms of methylprednisolone were examined by Higuchi et al. (1963). Polymorphism has been extensively studied in a variety of drugs. The high and low melting forms of phenylephrine oxazolidine were shown to have the same IR spectra and X-ray diffraction patterns but different crystal habits, thermal properties, solubilities and intrinsic dissolution rates (Qiu et al., 1993). A new polymorph of indomethacin has recently been reported (Lin, 1992). Thermally induced polymorphic transitions of tobramycin were recently characterized by Dash and Suryanarayanan (1991). A solvent-dependent polymorphic transition of a diuretic, frusemide, was reported by Doherty and York (1988). Different polymorphs of a given compound may, in general, be as different in structure and properties as the crystals of two different compounds. Solubility, melting point, hardness, crystal shape and electrical properties, etc., may all vary with the polymorphic form. Differences in the crystal structure could lead to differences in the bioavailability of the different forms of the same drug, e.g., flupredinisolone (Haleblian et al., 1971).

2. Materials and methods

Etoposide, supplied by Bristol and Myers Co., Syracuse, NY (etoposide I), analytical grade acetonitrile and glacial acetic acid obtained from J.T. Baker Co., Phillipsburg, NJ, and deionized-distilled water were used in the studies.

2.1. Preparation of etoposide I and II

Etoposide I refers to material obtained from Bristol Myers and Co. and it was used as such. Etoposide II was prepared by heating etoposide I to 220°C at 10°C/min, holding at this temperature for 5 min and cooling back to room temperature under ordinary atmospheric conditions. Both were found to be hydrated forms. They were stored in a dessicator over anhydrous calcium sulfate at room temperature.

2.2. DSC

A differential scanning calorimeter (DSC-50, Shimadzu Corp., Japan) was connected to a thermal analysis operating system and was calibrated with indium (Shimadzu). Typically, about 2 mg of sample was weighed into an aluminum sample pan, and the pan crimped nonhermatically and scanned from 30 to 300°C in a static nitrogen atmosphere at a heating rate of 10°C/min, unless otherwise stated. An empty aluminum pan was used as the reference. The collection and integration of the thermograms were computerized and the recording was done on the plotter.

2.3. Thermal gravimetric analysis

A Perkin-Elmer thermal gravimetric analyzer (TGA) with a 7 series thermal analysis system was used to calculate percentage weight changes. About 5 mg of the sample was weighed into a platinum pan and heated from 30 to 300°C, at a rate of 10°C/min, under a static nitrogen atmosphere.

2.4. Infrared spectral analysis

The Perkin-Elmer Model 283 double-beam spectrophotometer was used for infrared spectral analysis. A thin KBr pellet was prepared using a hydraulic press. After calibration with polystyrene

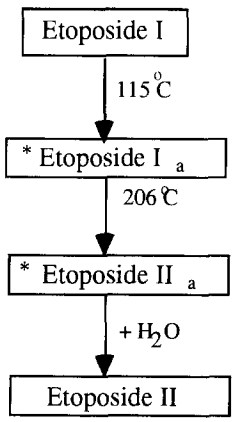
films infrared absorption spectra were recorded over 4000–200 cm^{-1} wavenumber.

2.5. HPLC analysis

A liquid chromatograph (Waters Model 590) equipped with autosampler system (Waters WISO 710B), a variable UV detector (Waters M 490), a C_8 reverse-phase column (Millipore, o.d. 6.35 mm and length 25 cm) and chart recorder was used. A modified isocratic mobile phase (Chow and Shah, 1987) consisted of acetonitrile-acetic acid-water (34:1:65), pH 4.0. The flow rate was 1.5 ml/min, and the eluents were monitored at 230 nm. 8-Methoxypsoralen was used as the internal standard.

2.6. Mass spectral analysis

A UTHE Technology International Model 100B quadrupole mass spectrometer with a sensitivity of 10 mV/division and a chart speed of 5 cm/min was used to scan the samples. The ability of the instrument to detect water was validated by using potassium dihydrogen tartrate tetrahydrate. The samples were placed into a specially made, dried sample holder. High vacuum was applied to obtain low pressures. The spectrum of each sample was taken immediately and again following the application of vacuum for 24 h. The samples were then heated to 140°C. The spectra taken with the sample valve shut off to the detector served as the blank. The data were compared with standard samples for the purpose of identification.



* Etoposide Ia and IIa are anhydrous forms of Etoposide I and II,resp.

Fig. 1. Schematic diagram showing the thermal behavior of etoposide.

2.7. X-ray diffraction studies

Samples were exposed to CuKα radiation (40 kV, 30 mA) in a wide-angle X-ray diffractometer (Model D500, Siemens). The instrument was operated in the step-scan mode, in increments of 0.05° 2θ. The angular range was 5–40° 2θ and counts were accumulated for 1 s at each step. A regular X-ray sample holder was used for the analysis.

2.8. Solubility studies

The solubility of etoposide was determined at 0 ± 0.5, 22 ± 0.5 and 37 ± 0.5°C in water by adding a known excess of etoposide to 5 ml water in a 20-ml screw-capped glass vial placed on a shaker. After 72 h equilibration, and subsequent filtration through 0.22 μm filter paper (filter type GS, white, plain, 25 mm diameter, Millipore Filter Corp., Bedford, MA) samples were withdrawn and assayed for etoposide content. Drug concentration in each sample was determined spectrophotometrically at 230 nm using a Beckman Model 25 double-beam spectrophotometer.

3. Results and discussion

The flow chart in Fig. 1 describes the thermal behavior of etoposide. Heating of etoposide I to 300°C in the DSC resulted in endotherms at 85–115, 198 and 269°C and an exotherm at 206°C (Fig. 2, trace I). On the basis of the DSC and TGA data (Fig. 3, trace I), the endotherm over the range of 85–115°C was attributed to a dehydration reaction which could be reversed upon cooling and exposing the sample to atmosphere.

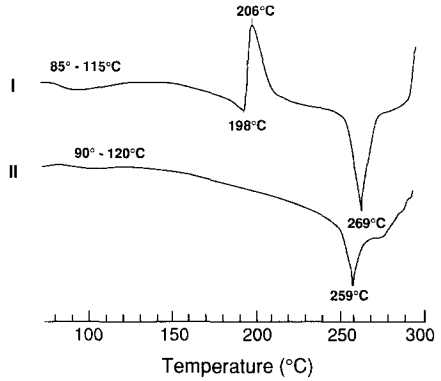


Fig. 2. DSC curves of etoposide I and etoposide I heated to 220°C, held for 5 min and cooled to room temperature at a heating rate of 10°C/min.

Etoposide I, when subjected to TGA, suffered an average weight loss of 2.9% between 50 and 120°C. Fig. 3 shows that there was no significant weight loss thereafter until decomposition began at approx. 290°C. Since this value exceeded the theoretical weight loss value of 2.8% corresponding to 1 mol of water, the difference was attributed to the presence of surface water. The nature of solvate in etoposide I was also determined by mass spectral analysis. The mass spectra of etoposide I heated to 120°C after evacuation for 15 min at 5 mmHg, showed a peak corresponding to mass 18, suggesting incorporation of water into the crystal lattice. Since this temperature was above the boiling point of water, the dehydration would have been accompanied by vaporization of water. Furthermore, TGA showed that the weight loss was monophasic, corresponding to a dehydration reaction (Wendlandt, 1986). The weight loss over a broad temperature range, 50–120°C in this case, was expected because TGA was carried out in an open container (Thermal Analysis Newsletter, 1967).

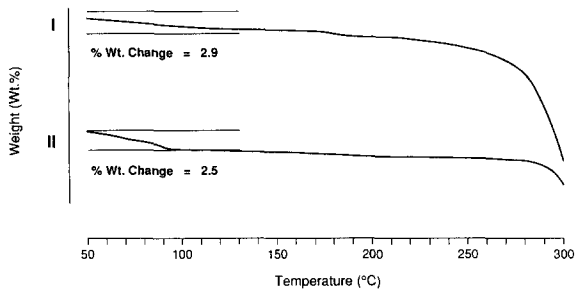


Fig. 3. Thermogravimetric curves of etoposide I and II.

The endotherm at 198°C (Fig. 2, trace I) corresponds to the melting of etoposide Ia, the anhydrous form of etoposide I, which soon begins to crystallize to a different polymorphic form, etoposide IIa (crystallization exotherm at 206°C). The thermal events in the range of 190–210°C were continuous with no clear separation. The third endotherm at 269°C was attributed to the melting point of the newly formed etoposide IIa. It was of interest to determine whether the transitions (190–210°C) observed during heating of etoposide I in the DSC could be reveresed upon cooling the sample. Therefore, etoposide I was heated to 220°C at 10°C per min, held for 5 min in order to allow complete conversion, and gradually cooled to room temperature. The absence of the corresponding thermal events during the cooling process suggested that the transitions in question were not readily reversible. However, etoposide IIa was found to add water when exposed to the atmosphere and to convert to a hydrate, etoposide II. Etoposide II did not convert to etoposide I even during storage over a period of 6 months. This was confirmed by reheating the compound II to 300°C, whereupon the endotherm at 198°C or the exotherm at 206°C did not occur (Fig. 2, trace II), however, a dehydration event could be seen over the temperature range of 90–120°C and the compound melted at 259°C. The slightly lower melting temperature could be due to the presence of a degradation product which could be detected by HPLC as mentioned later but could not be removed by recrystallization from water.

When etoposide II was subjected to TGA, an average weight loss of 2.5% was observed between 50 and 120°C (Fig. 3, trace II). Fig. 3 shows that, as in the case of etoposide I, there was no significant weight loss thereafter until decomposition began at approx. 290°C. The slightly lower than theoretical weight loss value of 2.8% was attributed to the presence of an impurity (8.6%) due to thermally induced degradation. The nature of solvate in etoposide II was also deter-

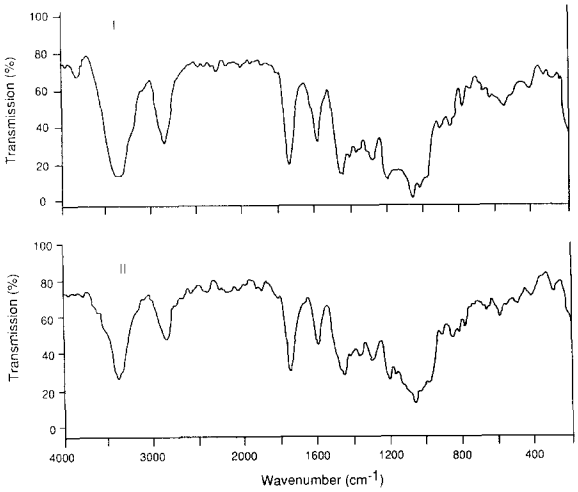


Fig. 4. Infrared spectra of etoposide I and II.

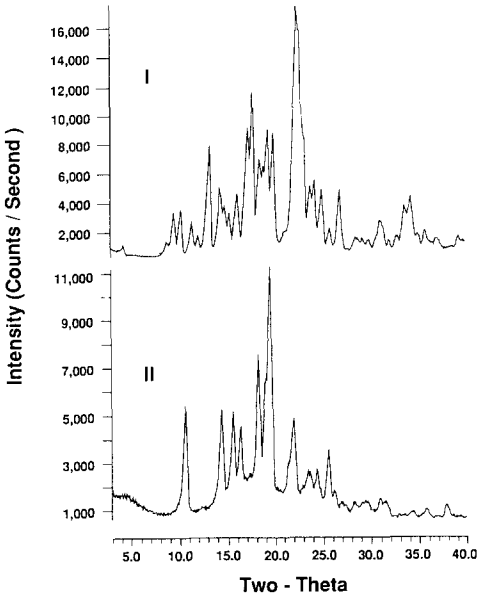


Fig. 5. X-ray diffractograms of etoposide I and II.

mined by mass spectral analysis. The mass spectrum as in the case of etoposide I showed a peak corresponding to mass 18 when heated to 120°C, suggesting that the water was embedded in the crystal lattice in the form of hydrate.

The infrared spectra of both etoposide I and II were nearly identical (Fig. 4). Strong distinguishing absorption peaks at 3350, 2880, 1730, 1590, 1450, 1380, 1070 and 850–920 cm^{-1} that corresponded to -OH, $-CH_2$ stretching, lactone group, aromatic groups, -OH, -C-O from $-OCH_3$, AR-OH and AR-CH functional groups, respectively (Silverstein et al. 1981; Sternhell and Kalman, 1986) were present in the spectra of both compounds as shown. The HPLC analysis showed that the two forms of etoposide have similar retention times (5.35 and 5.36 min) and absorption peaks. During HPLC analysis of etoposide II, however, a small degradation peak corresponding to an 8.6% impurity was observed at 5.86 min. The results from IR and HPLC studies strongly suggested that the two forms of etoposide were not chemically different.

The powder X-ray diffraction pattern of form I was much different from that of form II (Fig. 5, traces I and II). However, the sharpness of the peaks in the diffraction pattern and an examination of samples in the polarized light confirmed the crystalline nature of both forms. The prominent peaks with d spacing of 9.21, 6.66, 2.66, 2.61 Å present in etoposide I (Table 1) were conspicuously absent in the etoposide II diffraction pattern. On the other hand, the diffraction pattern of etoposide II showed a sharp peak with a d space of 2.38 Å. In addition, a marked increase or decrease in the relative intensities of several peaks was also noted. Together, this evidence

Table 1
X-ray diffraction data of etoposide I, Ia, IIa and II

d spacing (I) (Å) [a]			
Etoposide I	Etoposide Ia	Etoposide IIa	Etoposide II
9.21 (16)	9.34 (11)	20.18 (<1)	16.26 (<1)
6.66 (44)	6.71 (55)	8.41 (35)	8.33 (44)
5.14 (51)	5.15 (94)	6.19 (46)	6.17 (41)
4.99 (65)	4.98 (100)	5.69 (59)	5.69 (41)
4.58 (50)	4.57 (76)	5.42 (63)	5.42 (34)
3.99 (147)	4.02 (68)	4.86 (79)	4.86 (63)
3.98 (50)	3.97 (79)	4.66 (65)	4.67 (54)
3.96 (87)	3.86 (43)	4.53 (100)	4.55 (100)
3.86 (51)	3.75 (321)	4.01 (38)	4.05 (38)
3.67 (30)	3.69 (30)	3.45 (21)	3.49 (26)
3.56 (26)	3.57 (23)	2.33 (5)	2.39 (21)
2.66 (20)	2.63 (8)		
2.61 (23)			

[a] (I) Relative intensity (%).

suggested that the two diffraction patterns represented two different crystal arrangements. Adequate quantities of etoposide Ia and IIa were prepared for X-ray diffraction studies by holding for 5 min etoposide I at 120 and 220°C, respectively. Powder X-ray diffraction patterns of etoposide Ia and etoposide IIa were found to be similar to those of etoposide I and etoposide II respectively (Table 1). An examination under polarized light confirmed their crystalline character. Based upon the crystal lattice studies, Garner (1955) has identified three possible cases following the dehydration of hydrates: (1) crystal lattice of the residue is nearly identical to that of the original hydrate; (2) the residue is poorly crystalline; and (3) the residue recrystallizes with a different crystal lattice. The X-ray diffraction data presented here indicated that the dehydration of etoposide I to Ia belongs to the first case. Our data also indicated that the crystal lattice of Etposide IIa is nearly identical to that of etoposide II.

Polymorphism is the ability of any element or compound to crystallize as more than one distinct crystal species (e.g., carbon as cubic diamond or hexagonal graphite). The differences in the polymorphs exist because of the different arrangements of the molecules of that compound in the solid state which in turn result from resonance structures, rotation of parts of the molecule about certain bonds, and minor distortions of bond distances and angles. These distortions of molecular shape result from polarizability effects of one molecule on another due to the change in relative positions of adjacent molecules in the two different crystal arrangements (Haleblian and McCrone, 1969). The identification of the type of polymorphic transition (e.g., enantiotropic or monotropic) is not always easy. The type of transition in this case (transition from etoposide Ia to etoposide IIa) was monotropic for the following two reasons: (1) no solid-solid transitions were observed in the experimental temperature range; and (2) the transitions observed were irreversible (Dash and Suryanarayanan, 1991). The heat of transition rule proposed by Burger and Ramberger was also used to identify the type of polymorphic transition (Burger and Ramberger, 1979). According to the heat of transition rule 'if an exothermal transition is observed at some temperature, it may be assumed that there is no transition point below it, i.e., the two forms are related monotropically or the transition temperature is higher.' According to this rule, the anhydrous form of etoposide (form Ia) has no transition point below 206°C. Above this temperature, the only event observed was the melting of the anhydrous etoposide II (form IIa) apart from decomposition at about 290°C, thus supporting our conclusion that the observed polymorphic transition was monotropic.

The solubility of etoposide I, the hydrated form of the polymorph, etoposide Ia was found to be 35.7, 75.7, and 151.1 μg per ml at 0 ± 0.5, 22 ± 0.5 and 37 ± 0.5°C, respectively. The solubility of etoposide II was found to be 9, 21 and 80% higher than that of etoposide I at the same temperatures. The solubility ratios of the two forms at these temperature were found to be 1.10, 1.21 and 1.80, respectively. Etoposide I and II did not interconvert in the solution phase during the course of our solubility study. The crystals isolated from the solution phase at the end of a solubility study of each form were analyzed and identified by DSC. Furthermore, the solubility of etoposide I and II did not change over a 144 h period at 37°C. If etoposide II underwent transition to etoposide I in the aqueous phase, the solubility should have decreased. In general, the higher solubility form is thermodynamically unstable relative to the form with lower solubility, i.e., etoposide II is the metastable form (Lindenbaum et al., 1985). The free energy differences between the hydrated forms of the polymorphs (forms I and II) were calculated from their aqueous solubilities according to the formula:

$$\Delta F_T = RT \ln(\text{solubility of the metastable form} / \text{solubility of the stable form})$$

where T is the temperature at which solubilities were determined and R denotes the gas constant. This formula is an approximation, where the concentration has been substituted for activity. Since the aqueous solubility of the drug is low, the error associated may not be significant (Dash and

Suryanarayanan, 1991). The free energy differences based on the solubilities at 0 ± 0.5, 22 ± 0.5 and 37 ± 0.5°C were found to be 51.7, 111.7 and 362.1 cal/mol, respectively.

In summary, etoposide I and II have been shown to be hydrated forms of polymorphs etoposide Ia and IIa, respectively. Each of these forms has been isolated and characterized. The polymorphic transition was concluded to be monotropic. Etoposide II was found to have higher aqueous solubility than etoposide I.

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