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WIN 63843 polymorphs: prediction of enantiotropy

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Abstract

An experimental anti-viral compound (WIN 63843) which exists in at least three polymorphic forms was characterized by thermal analysis, X-ray powder diffraction, and Fourier transform infrared spectroscopy. The melting points and heats of fusion of forms I and III were utilized in predicting a solubility ratio of these polymorphs. This theoretical ratio showed excellent agreement with the experimental ratio in each solvent system. The highest melting form (form I) is more soluble at room temperature. This observation indicates that an enantiotropic relationship exists between forms I and III. The enantiotropic relationship can be predicted from thermal analysis data.

Keywords: Polymorphism; Physical characterization; Solubility; Stability

1. Introduction

Polymorphism is a factor which influences the bioavailability, stability, and processibility of drug substances (Haleblian and McCrone, 1969; Haleblian, 1975; Shefter, 1981; York, 1983). The physical data on each form can be used to select the most advantageous form for drug development. The polymorph with the lowest solubility will be the most thermodynamically stable form at a particular temperature. Heat of fusion and melting point data can be used to predict the relative solubility and hence relative stability of different polymorphs. The utility of these calculations has been presented (Lindenbaum, 1992) for auranofin and chloramphenicol palmitate. WIN 63843 (Fig. 1) is an anti-viral compound which exists in at least three different polymorphic forms. The DSC data for WIN 63843 are utilized in predicting relative solubility in the present work.

2. Materials and methods

2.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a Perkin Elmer DSC-7 instrument. Samples were encapsulated (2–5 mg) in volatile sample pans and scanned initially at a rate of 5°C/min under nitrogen purge gas. The system was calibrated with indium (m.p. 156.6°C) prior to use. The accuracy of the system was verified by measuring the melting point of water. Samples of crystal forms I and III were rescanned in tripli-

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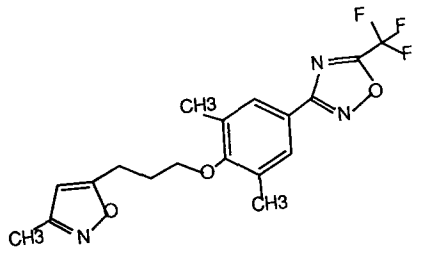

Fig. 1. WIN 63843 chemical structure.

cate at 10°C/min to allow calculation of standard deviations for heat of fusion and melting point.

2.2. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra were obtained on a Nicolet 730 system. Samples were run as solid KBr dispersions. The samples were analyzed prior to solubility testing then re-analyzed after exposure to each solvent. Comparison of the spectra before and after solubility testing allowed the verification of polymorphic stability.

2.3. X-ray powder diffraction

X-ray powder diffraction (XRPD) data was obtained on a Scintag XDS system with CuKα radiation (wavelength = 1.54 Å) and a solid state germanium detector. The powder was ground, spread onto a zero background quartz plate and scanned at a rate of 5°/min.

2.4. Preparation of crystal forms

Forms I and III were prepared by recrystallization of WIN 63843 in ethanol. The form I sample was formed by dissolution in ethanol at 55–65°C and cooling to 18°C. The form III sample was crystallized by dissolution in ethanol at 55–65°C and crystallization at 0°C. Form II was prepared by dissolving WIN 63843 in methanol and evaporating the solvent at 23°C.

2.5. Solubility

Excess solid (forms I and III) was added to 4 ml screw-cap glass vials containing 2 ml solvent. The contents were then mixed on a laboratory rotator for approx. 24 h. The samples were filtered through a 0.45 μm filter and diluted for HPLC analysis. Three solvent systems were investigated: 50% (v/v) 2-propanol/water, 5% (w/v) sodium lauryl sulfate (aqueous), and 5% (w/v) Ammonyx LO (30% lauryl dimethylamine oxide-Stepan Co.) in water.

HPLC assays were performed as follows: column, Zorbax Phenyl; mobile phase, acetonitrile/water/phosphoric acid (85%) 510:490:0.4; flow rate, 2 ml/min; detection, UV at 247 nm. A Waters 510 pump, 700 WISP, and 490E detector were used. Solubility determinations were obtained for polymorphs I and III in triplicate in each solvent. A preliminary study indicated that filtration did not influence drug concentration. HPLC grade solvents were obtained from Fisher Scientific.

3. Results and discussion

XRPD patterns for crystal forms I–III are shown in Fig. 2. Notable differences include the peaks at approx. 9.2 and 10.5° 2θ which were present in the form I and II patterns, respectively, but absent in the form III pattern. The pattern for form III shows a peak at approx. 8.2° which is absent in the pattern for form I and not significant in the form II pattern.

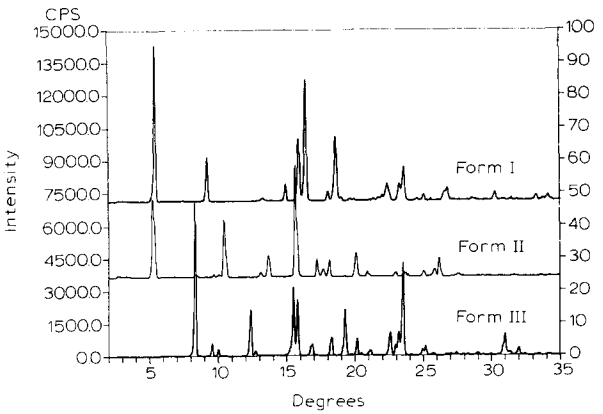

Fig. 2. X-ray powder diffraction patterns obtained for forms I–III of WIN 63843.

The most intense peak in the form I pattern (at approx. 5.3°) is absent in the form III pattern. The most intense peak in the form III pattern (at approx. 8.2°) is absent in the form I pattern. If form I was contaminated with form III or form III was contaminated with form I, then the most intense peak of the contaminant would have been observed. In addition, if grinding had caused transformations, the newly formed contaminant polymorphs would have been detected by the presence of their respective highest intensity peaks.

The most intense peak in the form I pattern (at approx. 5.2°) is similar in location to the second most intense peak in the form II pattern. However, the second most intense peak in the form I pattern (at approx. 16.5°) is absent in the form II pattern. If form II contained form I at a significant level, a peak would have appeared at approx. 16.5° in the form II pattern. Likewise, the most intense peak in the form II pattern (at approx. 15.5°) is absent in the form I pattern.

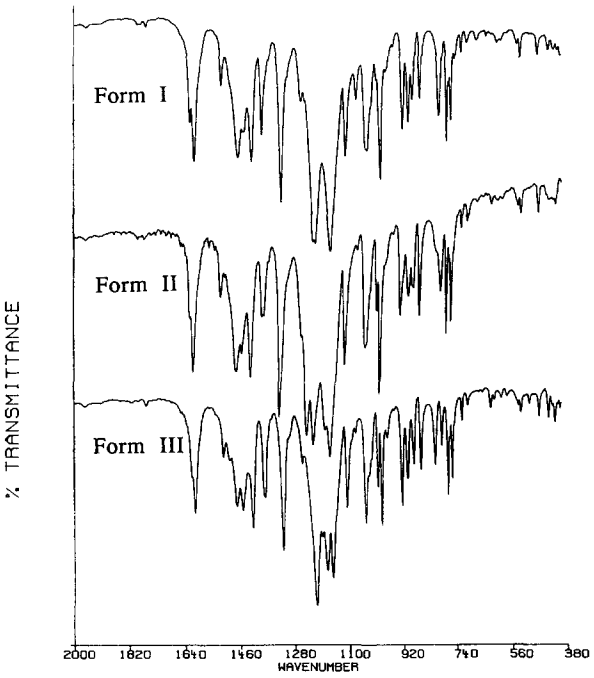

Fig. 3. Fourier Transform infrared spectra obtained for forms I–III of WIN 63843.

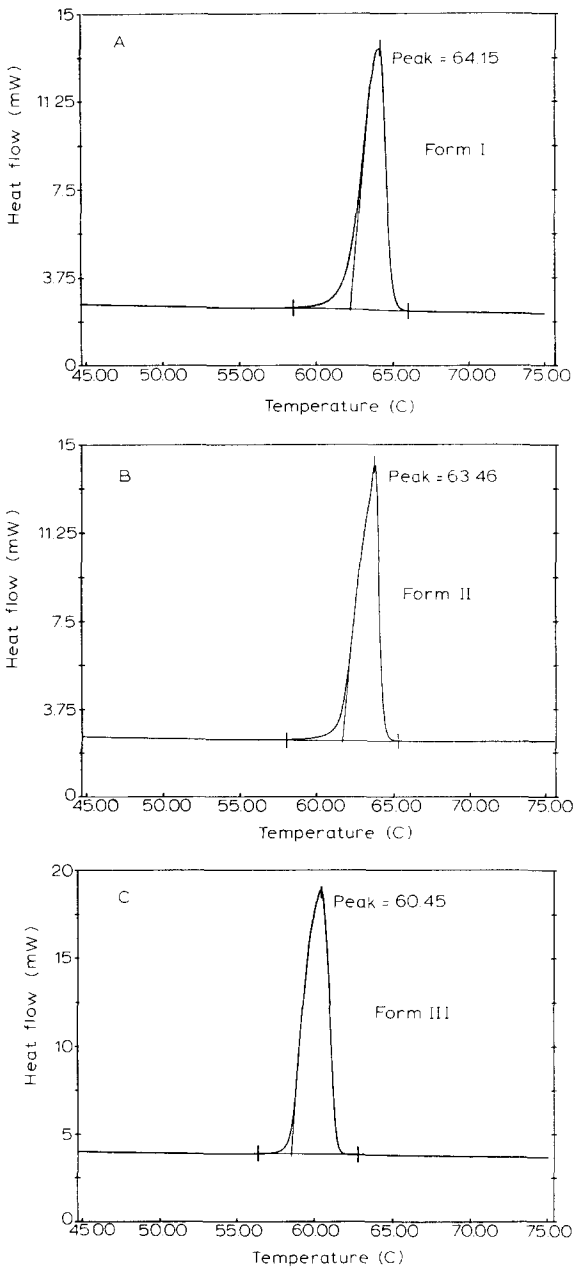

Fig. 4. Representative DSC thermograms for forms I–III of WIN 63843 (5°C/min).

The patterns are clearly unique and indicated, in conjunction with FTIR and DSC results, that the three solid forms are predominantly different polymorphs.

Fourier transform IR spectra for crystal forms I–III are compared in Fig. 3. The region below 2000 cm^{-1} was expanded to show differences between the spectra. Significant differences were observed at numerous wavenumbers; the region between 1100 and 1280 cm^{-1} was particularly useful in discriminating forms I–III. Since absorbance of the carbon-oxygen linkage is expected in this region, differences in the spectra of the three forms appear to be related to different conformations of the C-O bond. The ether linkage (Fig. 1) is capable of rotation and is a likely participant in polymorphic structural differences. It should be noted that compression may sometimes influence polymorphic form and therefore FTIR is recommended as a complementary rather than a primary technique. However, since the spectra for the three forms are significantly different, compression clearly does not cause a complete change in polymorphic composition. It is not known whether small changes can be observed for WIN 63843.

DSC scans (5°C/min) for polymorphs I–III showed a single melting peak, indicating a single crystalline phase (Fig. 4A–C). The observed melting points of forms I–III were 64.2 ± 0.5, 63.5 ± 0.2, and 60.9 ± 0.5°C, respectively.

Samples of form II were observed by DSC to transform to form III at room temperature and were therefore not included in the solubility study. Two melting peaks were observed after several weeks at room temperature, the first peak initiating in the region of form III and the second peak in the region of form II. After 2 years the form II sample had completely transformed to form III (FTIR analysis). The transformation was verified by hot-stage microscopy.

DSC data for forms I and III, scanned in triplicate at 10°C/min, are tabulated in Table 1. Precision of the data was improved with respect to the data obtained at 5°C/min.

The mean melting point (peak temperature at 10°C/min) observed for form I was 64.5°C, 3.3°C higher than that of form III. However, the melting point alone should not be used to predict stability, since in an enantiotropic system the relative stability and solubility will change with temperature. In enantiotropic systems a transition temperature exists, below the melting point, where the free energy versus temperature curves cross for the different polymorphs. In monotropic systems the curves will not cross below the melting point and relative stability will not change with temperature (Shefter, 1981). The heat of fusion observed for form I, 75.7 J/g, was somewhat lower than that of form III (83.6 J/g). When a higher melting polymorphic form has a lower heat of fusion the polymorphic forms usually have an enantiotropic relationship.

Table 1
Differential scanning calorimetry data (10°C/min)

Sample ID	Melting peak (°C)	Heat of fusion (J/g)
Form I	64.67	75.61
	64.33	76.45
	64.46	75.10
	64.5 ± 0.2	75.7 ± 0.7
Form III	61.16	83.65
	61.43	83.45
	60.87	83.55
	61.2 ± 0.3	83.6 ± 0.1

The solubility equation:

$$RT \ln S = -\Delta H(T(\mathrm{m}) - T)/T(\mathrm{m}) + \text{heat capacity term} + \text{non-ideal terms}$$

where S is the equilibrium solubility at temperature T, ΔH denotes the heat of fusion, $T(\mathrm{m})$ is the melting temperature of solid phase and T represents the temperature of interest, can be written for each polymorphic form and the two equations subtracted (Lindenbaum, 1992). One then assumes that heat capacity changes between the solid phase and molten phase are small. Since this assumption is generally valid, the second term on the right is removed. After subtraction, an equation which predicts the relative solubilities of the polymorphs is obtained:

$$RT \ln [S(\mathrm{I})/S(\mathrm{III})] = [\Delta H(\mathrm{III})(T(\mathrm{m}) - T)/(T(\mathrm{m}))] - [\Delta H(\mathrm{I})(T(\mathrm{m}) - T)/(T(\mathrm{m}))]$$

Note that the non-ideal terms have been subtracted out (Lindenbaum, 1992). If we enter the values obtained by DSC for melting point and

Table 2
Solubility by HPLC at 23°C (mg/ml)

Sample	50% prop./H_2O	5% SLS	5% Ammonyx LO
Form I	2.86 ± 0.01	1.68 ± 0.02	0.340 ± 0.002
Form III	2.74 ± 0.03	1.63 ± 0.01	0.331 ± 0.003

heat of fusion for forms I and III and choose a temperature, a theoretical solubility ratio is obtained. At 23°C, the predicted ratio $S(I)/S(III) = 1.04 + 0.04/-0.03$. The error limits are based on the standard deviations of melting point and heat of fusion for each polymorphic form.

The mean solubilities of crystal forms I and III in 50% 2-propanol/water, 5% sodium lauryl sulfate, and 5% Ammonyx LO are tabulated in Table 2. It should be noted that the solid remaining after several solubility experiments was screened for polymorphic changes by FTIR; no transformations were observed.

In each solvent system the higher melting form (form I) was more soluble. The experimental solubility ratio of form I/form III at 23°C, calculated for each solvent system with the values in Table 2, is listed in Table 3.

One can calculate a range of experimental solubility for each solvent system based on mean and standard deviations for each form. The following ranges are obtained: 50% propanol/H_2O, 1.03–1.06, 5% SLS 1.01–1.05, and 5% Ammonyx 1.01–1.04. The predicted ratio of $1.04 + 0.04/-0.03$ falls within the range for all three solvent systems. In addition, the average experimental solubility ratio for the three solvent systems (1.03 ± 0.01) shows excellent agreement with the predicted ratio of $1.04 + 0.04/-0.03$. This agreement illustrates the utility of DSC in the prediction of relative solubility and hence relative stability of different polymorphic forms.

Table 3
Experimental solubility ratio for different solvent systems at 23°C

Solvent	Experimentally obtained solubility ratio ($F(I)/F(III)$)
50% 2-propanol/water	1.04
5% SLS	1.03
5% Ammonyx LO	1.03

In order to be enantiotropic, the form I and III samples must show a change in relative stability as temperature is changed. Form III is clearly the most stable form at room temperature by virtue of the lower solubility values observed for form III. To prove form III is less stable at elevated temperature, a suspension of form III (which was seeded with form I) was prepared in mineral oil, stressed at 40°C, and analyzed by XRPD. After two weeks, the suspension had completely transformed to form I. This experiment illustrated that form I was the most stable form at 40°C and hence an enantiotropic relationship existed. Using the equation derived previously, the solubility ratio (I/III) becomes less than 1.0 as temperature is increased to 40°C. Therefore, this transformation would be expected.

4. Conclusions

It is essential to know the relative stability of different polymorphic forms of a compound; the information is a factor to be used in deciding which polymorphic form to recommend for development. Although the highest melting form is often regarded as the most stable, this is only true for monotropic systems. In enantiotropic systems, the higher melting form may be more soluble at a particular temperature and therefore less stable. If the higher melting form has a lower heat of fusion, the system can be enantiotropic.

The ability to predict the relative solubility of polymorphic forms I and III of WIN 63843 using melting point and heat of fusion data obtained from DSC experiments was illustrated. In this system enantiotropic behavior was both predicted and observed. The predicted ratio of polymorphic solubility (form I/form III = $1.04 + 0.04/-0.03$) was observed experimentally in three solvent systems.

References

Haleblian, J., Characterization of habits and crystalline modifications of solids and their pharmaceutical applications. *J. Pharm. Sci.*, 64 (1975) 1269–1288.

Haleblian, J. and McCrone, W., Pharmaceutical applications of polymorphism. *J. Pharm. Sci.*, 58 (1969) 911–929.

Lindenbaum, S., Calorimetric technique applied to pharmaceutical chemistry (Seminar – Thermal methods in pharmaceutical research), Springfield, NJ, 1992.

Shefter, E., Solubility by solid state manipulation. *Techniques of Solubilization of Drugs*, Ch. 5, Dekker, New York, 1981.

York, P., Solid state properties of powders in the formulation and processing of solid dosage forms. *Int. J. Pharm.*, 14 (1983) 1–28.