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Evaluation of reversible polymorphic phase transitions by thermal analysis

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Crystalline states of 1,2-dihydro-6-neopentyl-2-oxonicotinic acid, an investigational antidiabetic drug, were evaluated by thermal analyses. Two polymorphs were detected for the drug, Form I (m.p. 193 °C) and Form II (m.p. 196 °C). Interconversion of the polymorphs upon cyclic solid-melt transitions provided confirmation of the crystal forms. Solidification of the melt was observed to occur either at 162 or 182 °C with the formation of Form I or Form II crystals, respectively. Form I underwent partial conversion to Form II upon heating at 10 °C min^{-1} when nucleating crystals of Form II were present in the sample. Differential scanning calorimetry (DSC) thermograms were recorded for different lots of the drug, solvent-recrystallized samples, and a series of known mixtures of Form I and II polymorphs. The study illustrates the usefulness of cyclic heat-cool studies to characterize polymorphic crystal forms of drugs.

Many drug substances are known to exist in more than one crystalline state (Verma & Krishna 1966). These forms can often exhibit distinctly different physical properties such as density, hardness, crystal shape, solubility, stability, vapour pressure, optical and electrical properties, etc. to the extent that the stability, processability, and bioavailability of a solid dosage form may depend on which crystalline state of the drug is used (Haleblian & McCrone 1969; Haleblian 1975). It is necessary, therefore, to characterize the crystalline nature of every new drug during the early phase of formulation development and to establish appropriate test specifications to assure lot-to-lot conformity of the drug crystal form.

The present study was undertaken as part of the preformulation evaluation of an investigational hypoglycaemic agent, 1-2-dihydro-6-neopentyl-2-oxonicotinic acid. It is weakly acidic with a pK_a of 5·62 and an intrinsic solubility of about 375 μg mL^{-1}. Polymorphic forms of the drug and polycrystalline mixtures were characterized by thermo-analytical methods.

Materials and methods

Test samples. Samples from several lots of the antidiabetic drug were analysed. Pure Form I and II crystals were prepared and tested along with mixtures of Form I and Form II. Polycrystalline mixtures were prepared by blending the two polymorphs obtained by solvent recrystallization. Efforts were made to obtain Form I by solvent crystallization with a variety of solvents. The procedure involved rapid cooling of a saturated solution, prepared at an elevated temperature, in an acetone-dry ice bath. Precipitates were filtered and air-dried. Pure Form I crystals were produced by recrystallization from a saturated solution of the drug in methanol or ethanol, and were identified by X-ray. A batch-size lot, identified as pure Form I by X-ray, was crystallized from ethanol–water (3:1). Recrystallization from acetone–water (2:1) yielded pure Form II crystals, while mixtures of Form I and II were obtained upon crystallization from chloroform or ethyl acetate.

Apparatus. Samples were subjected to thermomicroscopic, thermogravimetric (TGA), and DSC analyses. A Mettler FP_2 Hot Stage Polarizing microscope, a DuPont thermal system consisting of a 990 Thermal Analyzer, 950 Thermogravimetric Analyzer, and a 910 Pressure DSC cell were used.

Procedure. Normally, polymorphic conversions can be studied by thermomicroscopic observations of the solid-melt, melt-solid transitions. However, due to the close proximity of the melting points of the two crystal forms of the drug, detection of such transitions was not possible. Alternatively, DSC scans, obtained by subjecting the sample to repeated programmed heat-cool cycles were evaluated. Thermogravimetric and thermomicroscopic analyses were carried out at a heating rate of 5 °C min^{-1}. Both heating and cooling rates were held at 10 °C min^{-1} for the DSC analyses.

The thermogravimetric profile showed no noticeable weight loss up to 220 °C, indicating the absence of residual solvent. Decomposition was not evident until a temperature of approximately 240 °C was reached. DSC heat-cool cyclic studies were performed by heating the sample, below ~210 °C, until melting was completed, then cooling to record the exothermic recrystallization transition.

Results and discussion

Two crystalline forms of the drug, a stable Form II melting at 196 °C and a metastable Form I melting at 193 °C were identified. Fig. 1 shows the DSC thermograms of these two crystal forms. From the peak area of the endotherms, the heat of fusion was estimated at 4·62 kcal (19·33 kJ) mol^{-1}. Verification of the crystalline nature of the forms was confirmed by cyclic heat-cool studies.

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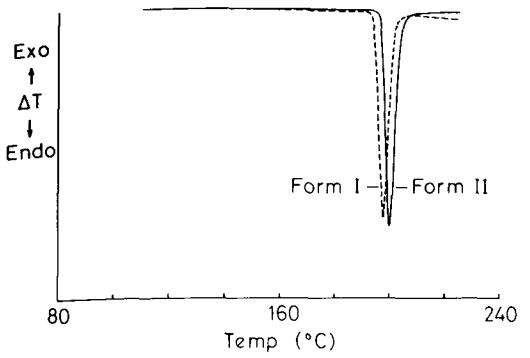

FIG. 1. DSC thermograms of Form I and Form II.

During cyclic DSC heat-cool treatment, most of the lots examined displayed a single endothermic melt transition at 196 °C, corresponding to the melting point of Form II. Fig. 2 shows a typical set of thermograms obtained upon heat-cool treatment of a Form II sample. The melt, upon cooling, exhibited an exothermic transition at 162 °C which corresponds to the solidification of the drug into the lower melting polymorph, Form I. Upon reheating the sample melted at 193 °C. Thus it was randomly possible to convert Form II to Form I by thermal melt-solid transitions.

A sample of pure Form I was prepared in the laboratory by recrystallization of the drug from either methanol or ethanol. The crystalline form of this sample was confirmed by X-ray studies (Chao & Vail, personal communication). The thermograms recorded for this sample exhibited a single melt endotherm at 193 °C,

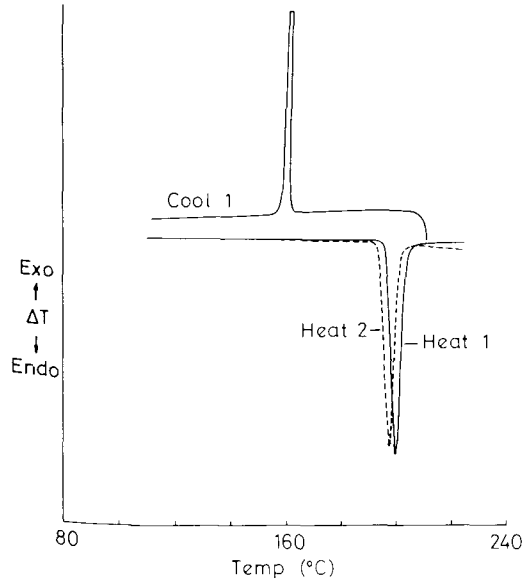

FIG. 2. Cyclic DSC heat-melt transitions of Form II.

which upon cooling solidified at 162 °C with the reformation of Form I crystals. Reheating resulted in an endothermic transition at 193 °C. During the second cooling cycle, however, solidification occurred at 182 °C yielding Form II. Heating for a third time produced an endotherm at 196 °C. The data clearly indicate that the two polymorphs can be interconverted by thermal phase transitions and that crystallization of the drug into either Form I or Form II depends on the melt-solid transition temperature. Crystallization of the melt at 162 °C results in Form I, while crystallization at 182 °C produces Form II crystals. A schematic representation of these reversible transformations is:

$$\text{Form II} \underset{182\,^\circ\text{C}}{\overset{196\,^\circ\text{C}}{\rightleftarrows}} \text{Melt} \underset{162\,^\circ\text{C}}{\overset{193\,^\circ\text{C}}{\rightleftarrows}} \text{Form I}$$

A batch size lot, crystallized from ethanol–water (3:1) and identified as Form I by X-ray (Chao & Vail, personal communication), was subjected to rapid cyclic heat-cool DSC studies. On heating, this sample exhibited two endotherms at 193 and 196 °C, which correspond to the melting transitions of Form I and Form II, respectively. The melt, upon cooling, showed an exothermic transition at 162 °C. When the sample was reheated a single endothermic peak resulted at 193 °C. Upon subsequent cooling, the melt this time underwent an exothermic transition at 182 °C. Reheating produced an endotherm at 196 °C, indicating that Form I had been converted to Form II. When the melt was again cooled, it recrystallized at 162 °C, as represented by an exothermic transition. An endotherm at 193 °C was evident during the fourth heating cycle, showing that a transformation to Form I had occurred. Thermomicroscopic observations of these transitions were consistent with the occurrence of melt-solid transformations during endothermic and exothermic transitions.

Samples prepared by recrystallization of the drug from chloroform and from ethyl acetate gave two peaks on heating, at 193 and 196 °C. A series of mixtures of Form I and Form II crystals, of known composition,

Table 1. Peak area ratios for various mixtures of Forms I and II.

% Drug in mixtures by weight		Peak area by DSC		Peak area Form I/ Peak area Form I + Form II
Form I	Form II	Form I	Form II	
9·6	90·4	2	63	0·0308
18·5	81·5	4	50·5	0·0734
33·4	66·6	5	30·5	0·141
45·8	54·2	6	30·5	0·164
69·6	30·4	10	35	0·222
72·2	27·8	12	35	0·255
78·7	21·3	13·5	34·5	0·281
86·1	13·9	16	33	0·327
90·3	9·7	20	30	0·400
94·9	5·1	20·5	22	0·482

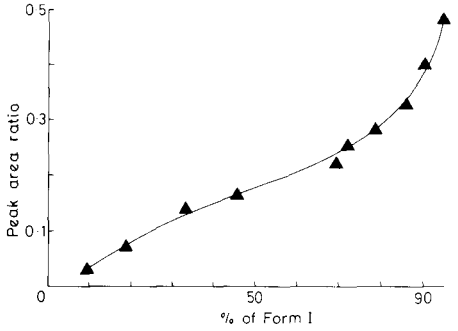

FIG. 3. Graph of peak area ratio (see Table 1) against the percentage weight of Form I in the mixture.

were prepared and subjected to DSC analyses. From the scans of these mixtures, peak areas corresponding to endothermic transitions were measured and a tabulation of peak area ratios obtained for the mixtures are given in Table 1. The relative peak area of Form I was plotted as a function of the percent Form I in the mixture (Fig. 3). The shape of the curve suggests that in the presence of Form II crystals there is some premelt solid-solid conversion of Form I to Form II. This premelt conversion and the closeness of the two endotherms inhibits the estimation of composition of mixtures of polymorphs of this drug. It may however, be feasible to apply this DSC method of analyses to polymorphic mixtures of other drugs.

Conclusions. The drug studied was found to exist in two polymorphic states, Form I (m.p. 193 °C) and Form II (m.p. 196 °C). These polymorphs are interconvertible by solid-melt, melt-solid transitions. Cyclic DSC heat-cool studies were employed to characterize these polymorphic transitions. The study demonstrates application of the DSC method to examine reversible crystalline phase transitions of polymorphs.

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Determination of the aryltetralin lignan content of podophyllum resins and roots/rhizomes

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A simple, quantitatively reproducible method for determining the aryltetralin lignan content of the resins and roots/rhizomes of *Podophyllum hexandrum* and *P. peltatum* is described. The method confirms that *P. hexandrum* resins and roots/rhizomes contain approximately four times the quantity of lignans as do those of *P. peltatum* and also that there is a significant variation in the lignan content of *P. hexandrum* resins.

Although the resin from *Podophyllum hexandrum* Royle and *P. peltatum* L. is the subject of an official monograph in many pharmacopoeias, none standardize the resin on lignan content. Methods for isolating and determining *individual* lignans have been published (Treppendahl & Jakobsen 1980; Cairnes et al 1981) but, as yet, none has tackled the problem of pharmacopoeial resins. This is particularly important because, whereas most pharmacopoeias only allow the resin to be manufactured from *P. peltatum*, the British Pharmacopoeia 1980 allows the resin from both *P. hexandrum* and *P. peltatum*. Previous reports have indicated that both the root/rhizome and the resin of *P. hexandrum* contain approximately four times the quantity of lignans found in those from *P. peltatum* (Kelly & Hartwell 1954 (crystallization or derivitization methods); Jackson & Dewick 1984 (preparative TLC)). A procedure is now described which allows the determination of the total lignan content in both podophyllum resins and podophyllum roots/rhizomes.

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Method

Total lignan content of resin. Approximately 0·5 g resin, accurately weighed, was dissolved in a small volume of ethanol (96% v/v) and sufficient ethanol (96% v/v) was added to produce 100 mL. To 10·0 mL of this solution, in a separator, 190 mL water was added and this mixture was extracted with 6 × 30 mL dichloromethane: the combined dichloromethane layers were extracted with 10 mL 0·2 M NaOH followed by 5 × 10 mL water. Each of the six aqueous layers was washed with the same 20 mL dichloromethane. The dichloromethane fractions were combined, filtered through a plug of cotton wool and evaporated to dryness. The residue was dissolved in ethanol (96% v/v) and sufficient ethanol