Pharmaceutical Research, Vol. 4, No. 5, 1987

Report

Polymorphism of 1,2-Dihydro-6-neopentyl-2-oxonicotinic Acid: Characterization, Interconversion, and Quantitation

Robert S. Chao[1,2] and Kenneth C. Vail[1]

Received April 20, 1987; accepted June 18, 1987

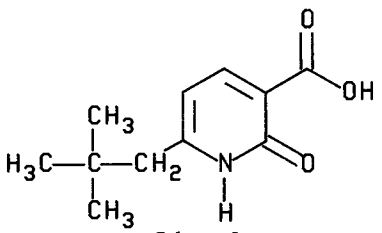
Two polymorphs of 1,2-dihydro-6-neopentyl-2-oxonicotinic acid have been characterized by X-ray diffraction (XRD), infrared spectroscopy (IR), differential scanning calorimetry (DSC), and thermal (hot-stage) microscopy (HSM). In batch-scale preparation, form I was crystallized in ethanol–water (3:1), while form II was obtained by recrystallization from acetone–water (2:1). The melting points for forms I and II are 193 and 196°C, respectively. Thermal studies (DSC and HSM) showed that form II melts at 196°C, while form I melts at 193°C, immediately followed by a resolidification and remelt at 196°C. The conversion of form II to form I was accomplished by recrystallization from ethanol or methanol, and the form I-to-form II transition was obtained by controlled heating of form I around 194°C. Quantitative XRD was used to determine the polymorphic composition, with a detection limit of less than 1% of the minor form and a linearity of 0–10% form I in form II (correlation coefficient of 0.999).

KEY WORDS: oxonicotinic acids; polymorphism; phase transition; polymorphic quantitation.

INTRODUCTION

1,2-Dihydro-6-neopentyl-2-oxonicotinic acid (Scheme I) is being investigated for its hypoglycemic activity. Two crystalline forms have been isolated and identified during the preformulation studies. Polymorphs were previously reported in other hypoglycemic agents such as acetohexamide (1), chlorpropamide (2), and tolbutamide (3). The primary objective of this study is to characterize the two known polymorphs using spectroscopic, optical, and thermal methodologies [i.e., X-ray diffraction (XRD), infrared spectroscopy (IR), differential scanning calorimetry (DSC), and thermal (hot-stage) microscopy (HSM)].

Since mixtures of the known forms have been observed, the development of a sensitive quantitative method capable of determining the minor polymorph at less than 1% level would be desirable. Finally, since an understanding of the interconversion of polymorphic forms can aid in the development of production processes, the study is extended to provide an interconversion scheme for production of either polymorph at a high purity.



Scheme I

[1] Product Control Division, The Upjohn Company, Kalamazoo, Michigan 49001.

[2] To whom correspondence should be addressed.

EXPERIMENTAL

Materials and Reagents

Form I, form II, and a polymorphic mixture (or Sample A, I:II = 2.2:97.8) were supplied by the Fine Chemical Division of The Upjohn Company. Unless otherwise specified, all polymorph samples referred to in the text are from batch products.

All solvents employed in laboratory-scale recrystallization experiments were of analytical grade. Solvents include acetone, ethanol, ethylacetate, carbon tetrachloride, chloroform, hexane, acetonitrile, tetrahydrofuran, and chlorobenzene.

Instrumentation

X-Ray Diffraction. X-ray diffraction patterns were obtained on a Siemens D-500 diffractometer using copper radiation with a nickel filter (CuKα = 1.5418 Å). Beam apertures of 1°, a detector aperture of 0.05°, and a continuous scan rate of 2° two-theta/min were employed. For peak area measurements a continuous scan rate of 0.5° two-theta/min was used. Samples were ground to fine powders and then packed into aluminum trays.

Infrared Spectroscopy. Infrared spectra were recorded using a Digilab FTS-10 spectrometer equipped with a Data General Nova-2 computer. All samples were prepared as mineral oil mulls. Spectra were obtained at 4-cm^{-1} resolution over the 600- to 4000-cm^{-1} spectral region.

Thermal Microscopy. All microscopic observations were made using a Nikon polarized light microscope and a Mettler FP5 hot stage. Samples were finely ground and suspended in high-purity silicone oil (Dow 360) and sandwiched

between a glass slide and a coverslip. Two heating rates were employed. A rapid heating was performed from 50 to 200°C at 10°C/min. A slower heating rate was performed from 188 to 198°C at 1°C/min.

Differential Scanning Calorimetry. The DSC profiles were recorded on a Dupont 1090 thermal analyzer equipped with a Dupont 910 DSC cell. The nitrogen gas flow in the cell was 50 ml/min. Three scan rates (1, 5, and 10°C/min) were employed to investigate the thermal behavior of the polymorphs.

RESULTS AND DISCUSSION

Crystal Habit

Both polymorph I and polymorph II appear macroscopically as white crystalline powders. Microscopic examination was performed on fine powders with a Nikon polarized microscope at a magnification of 110× to 400×. Both polymorphs appeared as large regular rods and laths.

X-Ray Diffraction

X-ray diffraction is one of the most sensitive methods for characterizing solid materials because the results are derived directly from the atomic arrangement of the crystalline specimens. The use of the powder X-ray diffraction pattern as a means of "fingerprint" identification is a general practice for solid material evaluation in pharmaceutical laboratories because the peak locations and relative intensities in an XRD pattern are characteristics of its crystal structure (4). For this oxonicotinic acid analogue, two polymorphs (forms I and II) were readily distinguished by their XRD patterns as shown in Fig. 1. Table I lists the two-theta angles, d-spacings, and relative intensities of the 10 most intense lines in the respective XRD patterns obtained by manual measurements. Also denoted in Table I are the unique diffraction lines in both crystal forms.

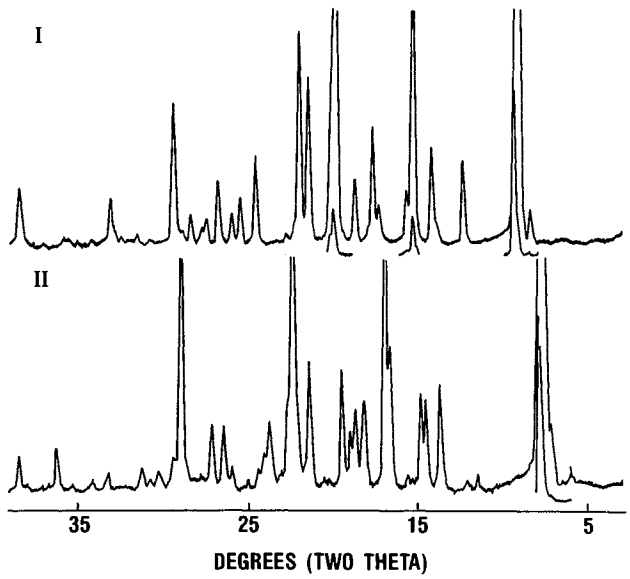


Fig. 1. X-ray diffraction patterns of form I (top) and form II (bottom).

Table I. XRD Data for the Ten Most Intense Lines of Forms I and II

Form I			Form II		
2θ (°)	d (Å)	I/I_1 (%)[a]	2θ (°)	d (Å)	I/I_1 (%)[a]
9.40[b]	9.408	100	7.90[b]	11.191	100
12.40[b]	7.138	10	13.70[b]	6.463	12
14.20[b]	6.238	12	14.50	6.109	10
15.30[b]	5.791	21	14.85[b]	5.965	11
17.70[b]	5.011	14	16.65[b]	5.324	16
19.95[b]	4.450	26	16.95[b]	5.231	29
21.50	4.133	20	19.50[b]	4.552	13
22.05[b]	4.031	25	21.40	4.152	15
24.60	3.619	10	22.40[b]	3.969	37
29.40	3.038	16	28.95[b]	3.084	31

[a] I_1 is the intensity of the strongest diffraction line.
[b] Unique reflections.

Infrared Spectroscopy

Infrared spectroscopy has long been employed for the discrimination of crystalline forms, as spectral features vary at frequencies corresponding to the changes in intermolecular bonding. IR spectra of forms I and II are shown in Fig. 2. Characteristic differences appear in the 3100- to 3300-cm^{-1} (NH,OH stretching), 1700- to 1750-cm^{-1} (C=O stretching), 1150- to 1200-cm^{-1} (C−O stretching), and 800- to 900-cm^{-1} (fingerprint) regions. The majority of these differences may be related to variations in hydrogen bonding between the two polymorphs. Lesser spectral variations are also observed in the 1630- to 1660-cm^{-1} (amide I), 1380- to

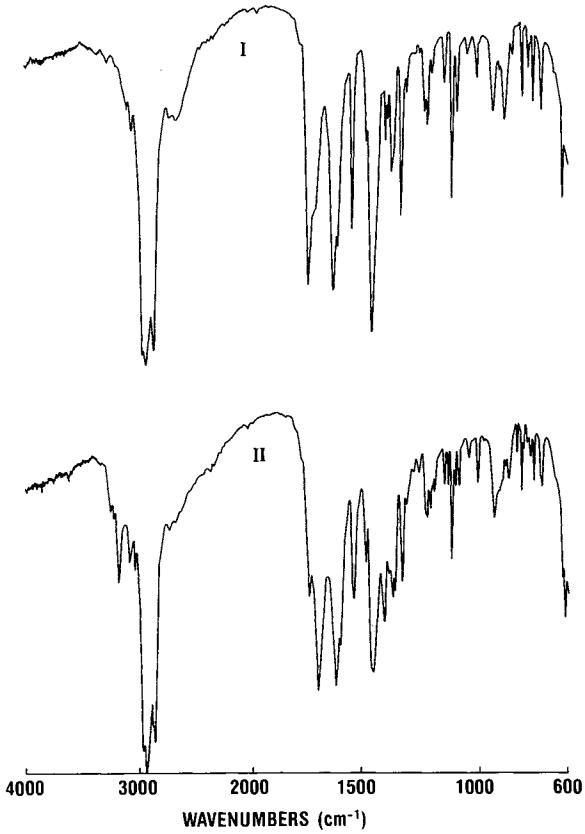


Fig. 2. Infrared spectra of form I (top) and form II (bottom).

1420-cm^{-1} (carboxylate), and 1250- to 1300-cm^{-1} (amide III) regions.

To ensure the spectroscopic differences observed from two specimens are indeed due to polymorphism, infrared absorption was also used for quick confirmation of chemical identity. The possibility of different compounds or tautomerism is eliminated, as identical IR features are obtained after both forms have been recrystallized from ethanol.

Differential Scanning Calorimetry

Thermal analysis is the measurement of the physicochemical behavior of material as a function of temperature. It provides valuable information on melting point, purity, phase transitions, and polymorphism (5–7).

Figure 3 shows thermograms for specimens of form I and form II at three heating rates: 1, 5, and 10°C/min. The fact that DSC thermograms of form II remain unchanged at various heating rates demonstrates the thermal stability of this polymorph and the reproducibility of the DSC technique. It should be noted that the endotherm is sharper (but weaker in intensity) at slower heating rates relative to its faster counterparts. Such a phenomenon is generally recognized as thermal lag (5) and is attributed to the temperature difference between the test specimen and the heater.

As for the batch scale sample of form I obtained from ethanol/water recrystallization, the slow scan at 1°C/min produces one endotherm at 196°C which is identical to the melting temperature of form II. However, faster scans (5 and 10°C/min) illustrate that the specimen underwent an endothermic peak at 193°C, immediately followed by an exotherm corresponding to the formation of a thermally stable form. The stable form melts at 196°C, as does the form II.

The appearance of dual endotherms (at the fast heating rate) demonstrates the characteristic thermal behavior of polymorph I. The lower endotherm at 193°C can be considered as the melting of form I, while the endotherm at 196°C is actually the melting of form II. At a scan rate of 1°C/min, the heat of fusion for both form I and form II is identical and estimated at 19.2 kJ mol^{-1}.

Thermal Microscopy

The thermal behavior of both polymorphs was examined by thermal microscopy with conditions as outlined under Experimental. Samples were finely ground to obtain homogenous particle size. Preliminary runs were scanned from 50 to 200°C/min rate. Close examination in the vicinity of the melting range of both forms was made at a slower heating rate of 1°C/min from 188 to 198°C.

The melting of polymorph II starts at 193.6°C and completes at 195.8°C. Melting of polymorph I begins at 192.8°C and completes at 193.4°C; however, crystallization of a new solid is evident at 193.2°C at the expense of the melt of the original specimen and the crystallization quickens after 193.4°C. The melt of the new crystals is apparent at 195°C and complete around 196.2°C, essentially the same as for polymorph II.

The thermal microscopic examination of both forms strongly support the DSC observations.

XRD Quantitation

As mentioned, different recrystallization solvent systems were used to obtain form I (ethanol–water) and form II (acetone–water). The polymorphic composition of most batches produced have been readily distinguished by IR and XRD. However, an early batch, hereafter designated sample A, was characterized as a mixture of predominately form II with a small amount of form I by XRD. The XRD of sample A is shown in Fig. 4. The low level of form I was not, however, detectable by IR or DSC. Since polymorphs of a given compound possess identical X-ray mass absorption coefficients, the diffraction intensities due to a specific polymorph are directly proportional to the composition of that polymorph in a mixture. XRD is ideally suited in this study for it can be employed not only to detect mixtures but to quantitate the relative proportions of the polymorphs in mixtures.

To ensure the reproducibility of diffraction intensities, all samples in this study were ground to a fine powder to eliminate preferred orientation effects. No indication of preferred orientation was observed.

A series of mixtures of polymorphs I and II (0–10% I in II) was prepared by milling for 5 min in plastic vials with glass beads. The characteristic diffraction peak chosen for quantitative purposes was 9.40° two-theta for form I. This is the most intense peak in the XRD patterns of form I and is

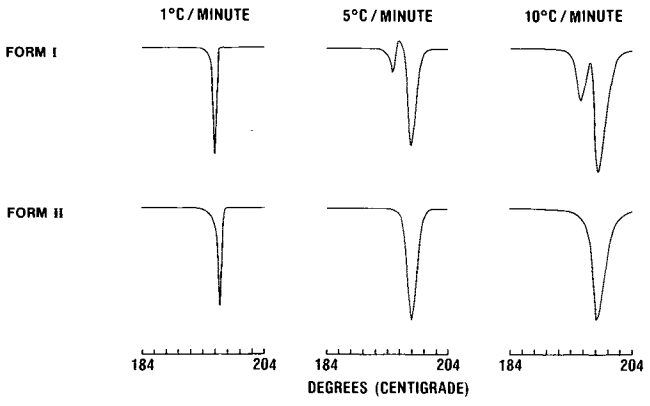


Fig. 3. DSC thermograms obtained at various heating rates (1, 5, and 10°C/min).

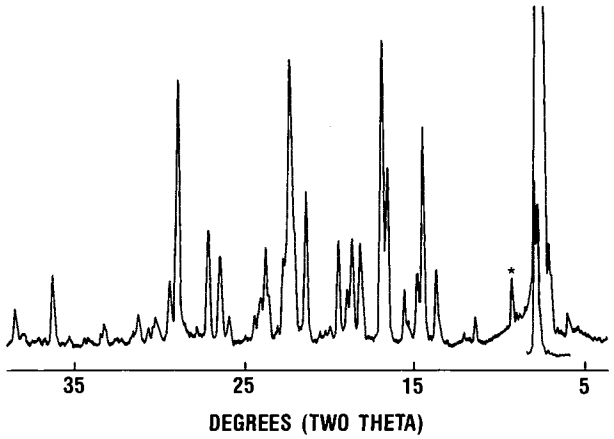


Fig. 4. X-ray diffraction pattern of "sample A," identified as predominately form II with a low level of Form I. The asterisk denotes characteristic form I peak at 9.4° two-theta.

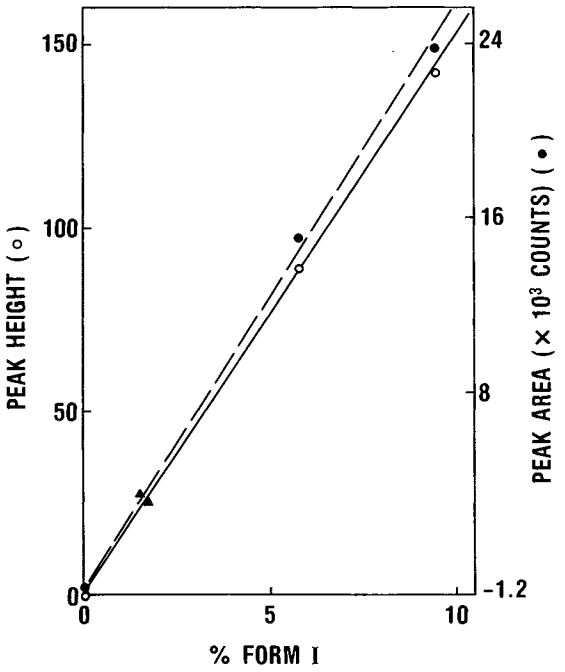


Fig. 5. Plot of XRD intensity at 9.4° two-theta. Peak height (○) and peak area (●) versus percentage of form I. (▲) Results for "Sample A."

well resolved from the characteristic peaks of form II. Both peak height and peak area measurements of the characteristic diffraction peak were made. The peak heights were measured manually and the peak areas determined by integration of counts over the angular range of the peak using the Siemens diffractometer software. Figure 5 shows the calibration curve plotted for form I content in mixtures from both peak height and peak area measurements. Excellent linearity was observed with both measurements, with a correlation coefficient of 0.999 and intercepts not significantly different from zero. The precision is about 2% based on four replicate peak area determinations for each sample. By applying this calibration curve, sample A, the mixture batch, was determined to contain 2.2% of form I by peak area and 2.1% of form I by peak height measurements. It has been estimated that form I levels as low as 0.5% can be determined by XRD in this case. However, it should be noted that the sensitivity of XRD quantitation may vary for polymorphic mixtures of other compounds, as their corresponding diffraction patterns may differ.

II → I Polymorphic Transition

A solubility study screening a number of organic solvents indicated that form II was significantly more soluble in ethanol (>25 mg/ml) than form I. Thus recrystallization of form I was readily produced in the following manner: 200 mg of form II was added to 10 ml of absolute ethanol. Samples were shaken for complete dissolution and filtered. The ethanol was then evaporated at room temperature. The dried solids were confirmed as form I by both infrared and XRD.

I → II Polymorphic Transition

Recrystallization experiments failed to produce a pure form II. In view of the thermal behavior from DSC and thermal microscopic analyses of both forms, a polymorphic I–II interconversion mechanism based on the thermodynamic equilibrium was considered.

Both thermal studies (DSC and HSM) indicated a rather significant melt—recrystallization was observed from 193 to 196°C when form I was heated. A quantity of form I (approximately 200 mg) was therefore heated and held at 194°C. The sample was completely converted to form II as confirmed by XRD and IR.

ACKNOWLEDGMENTS

The authors wish to thank D. S. Aldrich and R. D. White for helpful discussions.

REFERENCES

1. S. S. Al-Saieq and G. S. Riley. *Pharm. Acta. Helv.* 57(2):43–46 (1982).
2. D. L. Simmons, R. J. Ranz, and N. D. Gyanchandani. *Can. J. Pharm. Sci.* 8(4):125–127 (1973).
3. J. R. Leary, S. D. Ross, and M. J. K. Thomas. *Pharm. Weekblad Sci. Eds.* 3:62–66 (1961).
4. H. P. Klug and L. E. Alexander, *X-Ray Diffraction Procedures for Polycrystalline and Amorphous Materials*, 2nd ed., John Wiley and Sons, New York, 1974, Chap. 7.
5. J. L. McNaughton and C. T. Mortimer. *Differential Scanning Calorimetry*, Physical Chemistry Series 2, Vol. 10, Butterworths, London, 1975.
6. W. P. Brennan. Applications of thermal analysis in the pharmaceutical industry, a review, *Perkin-Elmer Therm. Anal. Newsletter.* No. 17, Aug. (1976).
7. C. Plato and A. R. Glasgow, Jr. *Anal. Chem.* 41:330–336 (1969).