Neotame Anhydrate Polymorphs I: Preparation and Characterization

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Purpose: To prepare, characterize, and compare polymorphs of neotame anhydrate.
Methods: Neotame anhydrate polymorphs were prepared from amorphous or crystalline anhydrate by crystallization or suspension in various organic solvents, or by dehydration of neotame monohydrate. The following techniques were used for characterization: differential scanning calorimetry, thermogravimetry, hot-stage microscopy, powder X-ray diffractometry (PXRD), ^{13}C solid-state nuclear magnetic resonance (SSNMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy, dynamic water vapor sorption/desorption, and density measurements.
Results: Seven polymorphs (Forms A–G) of neotame anhydrate were prepared and show different thermal properties and PXRD patterns. Two enantiotropically related pairs were identified: B and C; E and A. ^{13}C SSNMR and FTIR spectroscopy clearly distinguish between Forms A, D, F, and G, which show similar needle-shaped morphology but distinct differences in dynamic water vapor sorption/desorption and density. The ^{13}C SSNMR chemical shifts suggest conformational polymorphism. The stability in the presence of water vapor follows the rank order, G > A > D ~ F, which resembles the rank orders of the molar volume and of the polarity of the solvents from which they crystallized.
Conclusions: The neotame anhydrate polymorphs appear to show different molecular conformations. The less dense polymorphic structures crystallize from solvents of greater polarity and sorb water vapor less rapidly and less completely. Two enantiotropic pairs were discerned.

KEY WORDS: neotame; polymorph; density; dynamic water vapor sorption/desorption; infrared spectroscopy; powder X-ray diffractometry; ^{13}C solid-state nuclear magnetic resonance spectroscopy.

INTRODUCTION

Polymorphism is defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the same molecules in the crystal lattice (1). As a consequence of these differences, polymorphism affects the following physical properties (1) of the substance: crystal lattice properties, and spectroscopic, thermodynamic, kinetic, surface, and mechanical properties. Prominent among the thermodynamic and kinetic properties are solubility, dissolution rate, bioavailability, and stability.

Polymorphs of a compound may be prepared by various methods (1). The classical methods include sublimation (2), crystallization from a single solvent (3), evaporation of a binary mixture of solvents (4), vapor diffusion (5), thermal treatment (6), crystallization from the melt (7), rapidly changing solution pH to precipitate acidic or basic substances (8), desolvation of crystalline solvates (9), and grinding (10). To prepare the metastable forms, some special methods may be employed. The nucleation process for a metastable form can be selectively directed on a molecular substrate of defined composition and structure (11). In addition, conformational mimicry, using an additive with a similar conformation to that of the stable form, may inhibit its nucleation and crystal growth, and will consequently stabilize the metastable polymorph (12). Furthermore, tailor-made additives also may be used to obtain metastable phases (13) and to study the kinetics of nucleation (14) and crystal growth (15).

The common techniques for characterizing polymorphs include (a) thermal analysis, such as differential scanning calorimetry (DSC) (16) and thermal microscopy (17); (b) single crystal and powder X-ray diffractometry (18); (c) vibrational spectroscopy, including infrared spectroscopy (19) and Raman spectroscopy (20); (d) ^{13}C solid-state nuclear magnetic resonance (SSNMR) spectroscopy (21,22); and (e) solution properties, such as solution calorimetry (3), solubility (23), and intrinsic dissolution rate (24). The present study applies a number of these techniques to probe the polymorphic behavior of the anhydrous dipeptide, neotame. This substance may be considered to be a model synthetic dipeptide.

Neotame (*N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine 1-methyl ester, Fig. 1) is an alkylated derivative of a dipeptide sweetener, aspartame, and has intense sweetening potency. Discovered by Nofri and Tinti, neotame is currently being developed by the NutraSweet Company (25). Neotame was recently submitted to the U.S. Food and Drug Administration for approval as a general-use sweetener in food and beverages. The sweetness of neotame is 8000 times more potent than sucrose, and approximately 40 times the sweetness potency of aspartame (26). The most stable known crystal form of neotame under ambient conditions is the monohydrate (27). On drying the monohydrate under vacuum (~1 torr) for 3 days, various crystalline forms of anhydrous neotame have been detected by SSNMR spectroscopy (22). In a high humidity environment, these anhydrates take up water and convert to the monohydrate (22). Neotame also exists as solvates, such as a methanol solvate (28).

MATERIALS AND METHODS

Materials

Neotame monohydrate with a water content of 4.64% w/w was supplied by the NutraSweet Company (Mount Prospect, Illinois). Acetonitrile, ethyl acetate, and tetrahydrofuran (THF) were purchased from Fisher Scientific (Fair Lawn,

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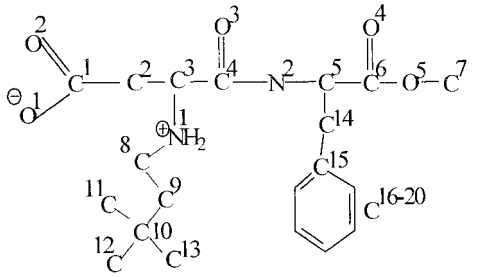

Fig. 1. Structure and atomic labeling of the neotame molecule.

New Jersey). Heptane was purchased from EM Science (Gibbstown, New Jersey). Carbon tetrachloride was purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). All the organic solvents were of analytical grade or better and were dried by molecular sieves (3 or 4 Å).

Experimental Techniques

Hot stage microscopy (HSM, Mettler FP 80, Mettler Instrument Corp., Hightstown, New Jersey) was used to observe the visual change in the crystals at different temperatures.

The water content of the crystalline samples was determined by Karl-Fischer titrimetry (KFT) employing a Moisture Meter (model CA-05, Mitsubishi Chemical Industries Ltd., Tokyo, Japan). The sample was dissolved in anhydrous methanol.

Powder X-ray diffraction (PXRD) patterns were determined at room temperature using an X-ray diffractometer (Siemens, model D-5005, Germany) with Cu Kα radiation at 40 mA, 45 kV. The samples were packed into a plastic holder and were scanned from 2° to 40° 2θ at a step size 0.05° with a dwell time 1 s. Variable temperature X-ray diffraction patterns were obtained using a different X-ray diffractometer (Scintag, model XDS 2000, Cupertino, California) also with Cu Kα radiation at 30 mA and 45 kV. The samples were packed into a copper holder and were scanned from 2° to 40° at a step size of 0.03 with a dwell time of 1.8 s.

Differential scanning calorimetry (DSC) employed a differential scanning calorimeter (Du Pont, model 910, TA Instruments, New Castle, Deleware) equipped with a data station (Thermal Analyst 2000, TA Instruments, New Castle, Deleware). All DSC runs were performed under a nitrogen purge at 50 ml/min and at a heating rate of 10°C/min except where otherwise noted. The calorimeter was calibrated with indium.

Thermogravimetric analysis (TGA) employed a thermogravimetric analyzer (Du Pont, model 951, TA Instruments, New Castle, Deleware) linked to a data station (Thermal Analyst 2000, TA Instruments, New Castle, Deleware). All TGA runs were performed under a nitrogen purge at 50 ml/min and at a heating rate of 10°C/min.

SSNMR spectra with cross-polarization and magic-angle spinning were acquired at 75.4 MHz using a Chemagnetics CMX-300 spectrometer (Fort Collins, Colorado) and a Chemagnetics probe equipped with a Pencil™ spinning module. Samples were packed in 7.5 mm zirconia rotors using Kel-F endcaps and spun at ~5 kHz at the magic angle. Spectra were acquired with total sideband suppresion, a 3.0 s recycle delay, and a decoupling field of approximately 60 kHz. The ^{1}H pulse was 4.5 μs and the contact time was 5 ms. The spectra were externally referred to tetramethylsilane using the methyl peak of hexamethylbenzene (17.35 ppm).

For solid-state Fourier transform infrared (FTIR) spectroscopy, approximately 3 mg of solid sample were gently ground with a pestle in an agate mortar and then was mixed with 300 mg of ground dry potassium bromide powder (Spectra-Tech, Stamford, Connecticut). The mixture was loosely packed into the sample container of the FTIR spectrometer and the sample surface was leveled. The diffuse reflectance spectra of the samples under dry air were recorded by a FTIR spectrophotometer (Nicolet Magna 750 FTIR, Nicolet Instrument Corp., Madison, Wisconsin) equipped with a HgCdTe compound semiconductor detector and Windows-based OMNIC® software (Nicolet Instrument Corp., Madison, Wisconsin) for data collection and analysis. The spectra was recorded from 700 to 4000 cm^{-1} at a resolution of 2 cm^{-1} for 32 scans. A background spectrum of the potassium bromide powder without the sample was recorded under the same instrumental conditions and was subtracted from each sample spectrum.

The morphology of the polymorphs was analyzed by scanning electron microscopy (SEM) (Hitachi S-800, Tokyo, Japan) at an accelerating voltage of 5 kV. The samples were sputter-coated with platinum to a thickness of 80 Å.

A helium-air pycnometer (Autopycnometer, model 1320, Micromeritics, Norcross, Georgia) was used to measure the true densities of the polymorphs of neotame anhydrate.

A vapor sorption microbalance (DVS-1000, Surface Measurement Systems, United Kingdom) was used to study the dynamic adsorption and desorption of water vapor by neotame anhydrate polymorphs. The experimental temperature was maintained at 23°C, while the weight of the sample was recorded and converted to the percentage of weight gain in an isothermal plot, as the relative humidity (RH) was increased from 10% to 90% and then decreased to 10% in steps of 10% RH. At every step of RH from 10% to 80%, the differences of eight pairs (sorption and desorption) of the weight percentages of adsorbed water vapor were used to draw a hysteresis plot.

Preparation of Neotame Anhydrate Polymorphs, Forms A–G

Form A was prepared by crystallization of amorphous neotame anhydrate (28) during suspension in ethyl acetate for about 1 week at 23°C. Form A also can be obtained by slowly heating neotame monohydrate from 50 to 70°C in a water bath under nitrogen purge for 24 h.

Form B crystallized from a saturated solution of neotame monohydrate (7.2 g) in THF (4.0 ml) at 23°C. After 2 weeks the gel that had initially formed was resuspended in THF from which Form B crystallized and was filtered off. At 105°C, Form B is converted to Form C. Because of the difficulty of preparation and the chemical impurity, only very limited quantities of Form B and C were obtained. The chemical impurity present in Form B was detected by high performance liquid chromatography and was identified as the hydrolysis product of neotame, *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine. The crystal structure of this compound corresponds to this chemical name and will be described in a subsequent report.

Form D was obtained by crystallization, filtration, and then drying of a suspension of amorphous neotame anhydrate

in cyclohexane, hexane, or heptane at room temperature after 2 weeks.

Form E was prepared by dehydration of neotame monohydrate in DSC at a slow heating rate. Form E has not been obtained in bulk due to its instability under ambient conditions.

Form F was produced by a method analogous to that for Form D except that the organic solvent was carbon tetrachloride.

Form G crystallized from a suspension or solution of Form A or amorphous neotame anhydrate in acetonitrile after 1 week.

During the preparation of the above polymorphs, suspension indicates that excess of solid neotame was added to the individual solvents to ensure the presence of suspended solid phase. Stirring may be applied, but is not necessary. Among the seven anhydrate polymorphic forms, only Forms A, D, F, and G could be prepared in bulk, and hence were characterized in detail.

RESULTS AND DISCUSSION

DSC of Forms A, D, F, G (Fig. 2) show only melting endotherms at 92, 94, 92, and 112°C, respectively, indicating no phase transitions before melting. However, Form B undergoes an endothermic transition at 105°C to Form C, which subsequently melts at 166°C followed immediately by decomposition at 171°C. Form E is produced by dehydration of neotame monohydrate at 60°C in DSC at a heating rate of 2°C/min, followed by an endothermic transition to Form A at 71°C and subsequently by the melting of Form A. The endothermic transitions (29,30) show that Forms B and C, Forms E and A, are enantiotropically related. The TGA curves of the polymorphs show no weight loss below their melting points, suggesting no decomposition before melting.

Figure 3 shows the PXRD patterns of all the anhydrate polymorphs. The distinctive differences among these patterns show that the polymorphs possess different crystal structures. Among the seven forms, A, E, and G are highly crystalline, as shown by the sharp diffraction peaks in their individual PXRD patterns. However, the PXRD patterns of Forms B, C, D, and F show the presence of some amorphous content, as indicated by the broad peaks from 10° to 30° 2θ. The amorphous phase may be introduced with the starting material for the polymorph preparation, by incomplete crystallization, or by heating. In addition, the amorphous phase may also arise as defects in the crystals, perhaps introduced during the process of crystallization. For example, Form D was prepared by suspension of amorphous anhydrate in cyclohexane, hexane, or heptane, but because of the poor solubility of neotame in these alkane solvents, the crystallization process was slow. Perhaps, by the time of harvest, crystallization was not complete. In another example, neotame may undergo degradation in THF, to yield a small amount of a product of a similar molecular structure that could influence the nucleation and crystal growth of neotame, and even be included as impurity

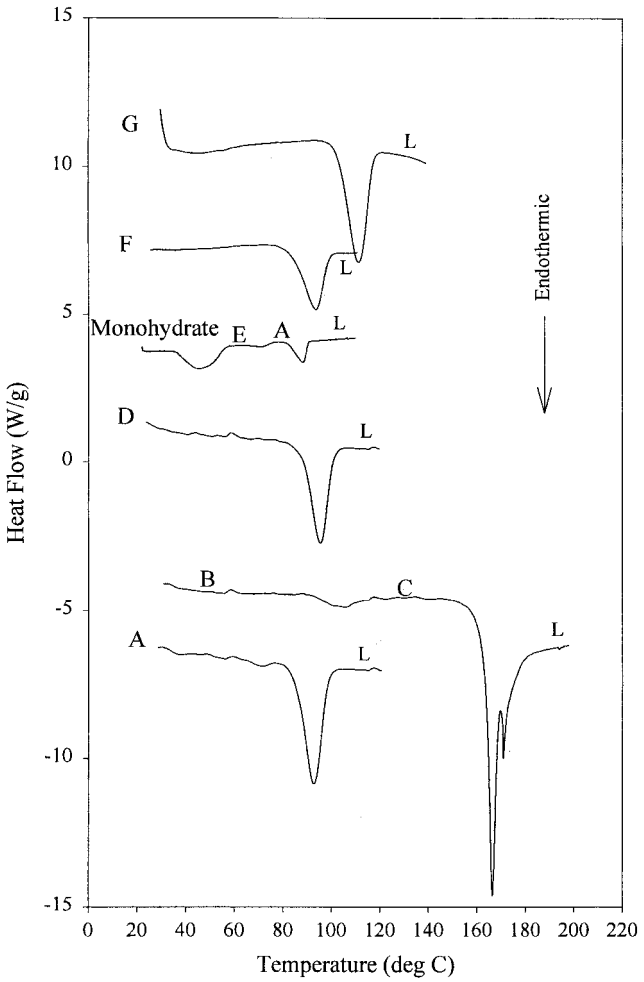

Fig. 2. DSC curves of neotame anhydrate polymorphs A–G. Each curve is labeled with the corresponding polymorph and the liquid state, L.

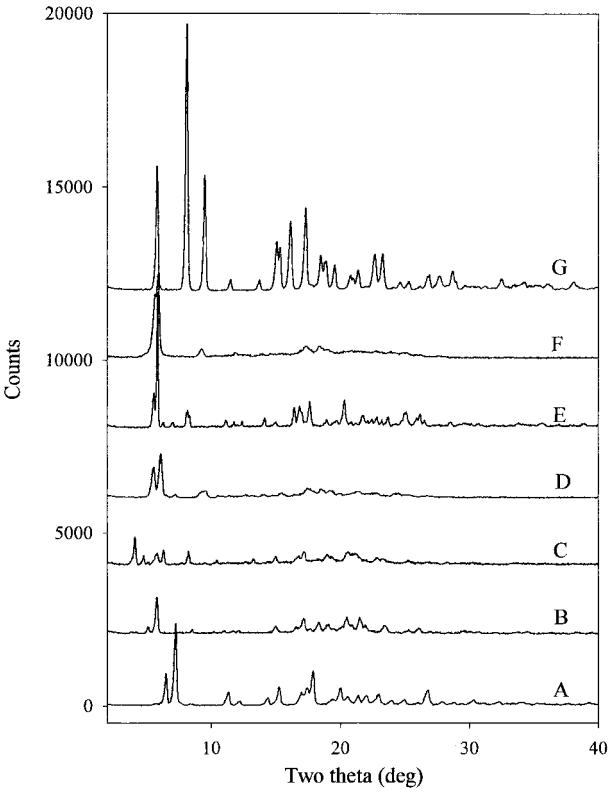

Fig. 3. PXRD patterns of neotame anhydrate polymorphs A–G.

defects in Form B crystals. Furthermore, during the preparation of Form F, neotame with carbon tetrachloride forms fine crystals, observable under an optical microscope, which may be a solvate and which, during filtration and drying, may undergo desolvation to produce a powder, Form F. The escape of carbon tetrachloride molecules from the solvate crystals itself may also cause defects thereby decreasing the crystallinity of Form F.

Because of the difficulties in preparing Forms B, C, and E in bulk, only Forms A, D, F, and G were characterized further by additional experimental techniques.

SEM shows that the crystals of the four neotame anhydrate polymorphs, A, D, F, and G, crystallize in a needle-shaped habit (Fig. 4). However, Forms A and G appear to consist of much larger crystals than Forms D and F. These differences in size are believed to result from differences in crystallization conditions and are not inherent properties of the polymorphs.

^{13}C SSNMR spectroscopy with cross polarization and magic angle spinning is emerging as a powerful technique in solid-state pharmaceutics, especially in the study of polymorphism. It is a nondestructive technique that has the ability to probe the chemical environment of each carbon nucleus, and therefore to identify the number of crystallographically inequivalent sites in a unit cell and to understand a molecular structure on the basis of the chemical shifts of the individual resonances (18,22). The SSNMR spectra of Forms A, D, F, and G are shown in Fig. 5. The peak assignments are based on the previous studies with neotame and aspartame (22). In the case of Form G, the carboxylate carbon C1 is assigned to the resonance farthest downfield at 174.7 ppm, and another carboxylate carbon, C6, is assigned to the peak at 173.3 ppm. The carbonyl carbon C4 produces two peaks at 172.1 and 172.3 ppm with an approximate equal intensity due to coupling to ^{14}N. The phenyl ring carbons (C15–C20) are assigned to the peaks between 120–140 ppm. Only C15 can be unambiguously assigned to the peak at 138.4 ppm; the others are difficult to assign. All the other carbons, including C2, C3, C5, C7–C13, are assigned to the peaks below 60.0 ppm. The peaks for other forms can be assigned similarly.

Because the resonance of C15 is relatively well separated from those of the other carbon atoms, its chemical shift is chosen to distinguish the different polymorphs. The chemical shifts of C15 for Forms A, F, and G are 135.5, 137.6, and 138.4 ppm, respectively, corresponding to a single resonance for each individual form and to one crystallographically inequivalent molecule in their crystals. However, Form D has two resonances, at 139.4 and 137.8 ppm, corresponding to two crystallographically inequivalent sites in Form D crystals. Because the chemical shift of ^{13}C in SSNMR spectroscopy also detects the electronic environment of the carbon nuclei in the molecule and because C15 in Forms A, D, F, and G has

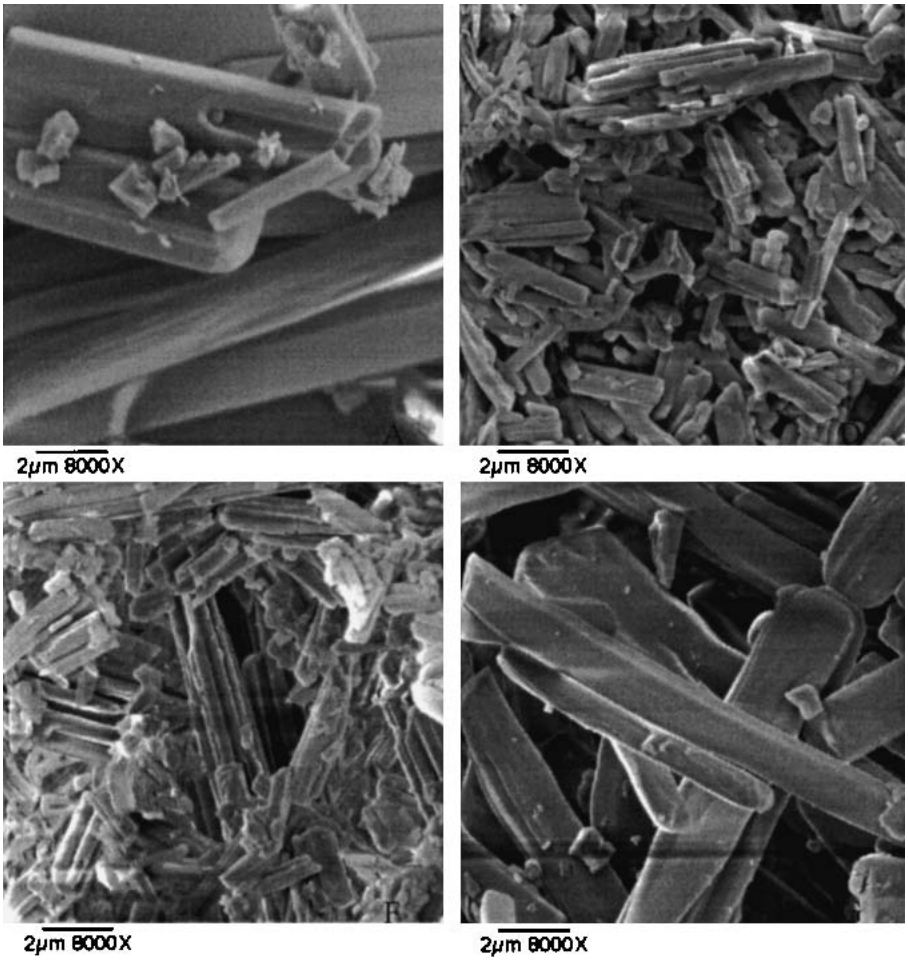

Fig. 4. SEM of neotame anhydrate polymorphs A (top left), D (top right), F (bottom left), and G (bottom right).

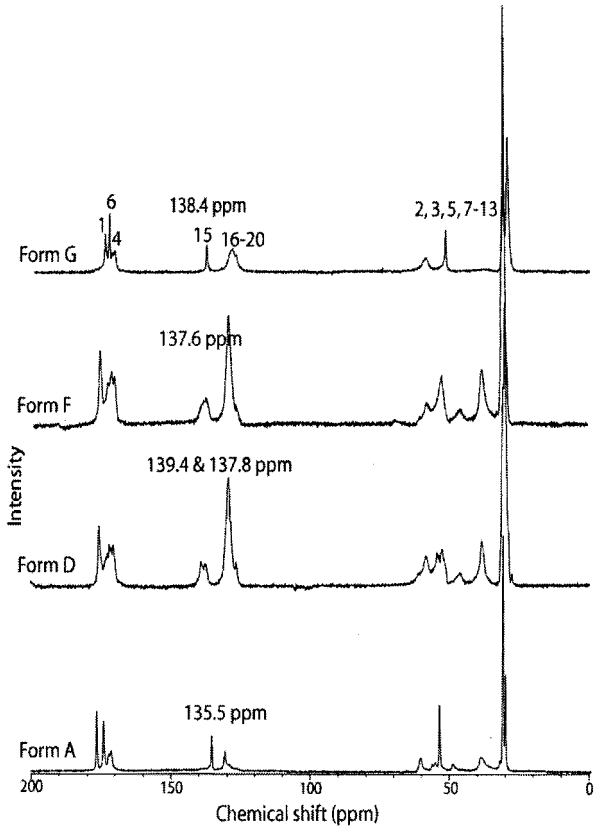

Fig. 5. SSNMR spectra of neotame anhydrate polymorphs A, D, F, and G.

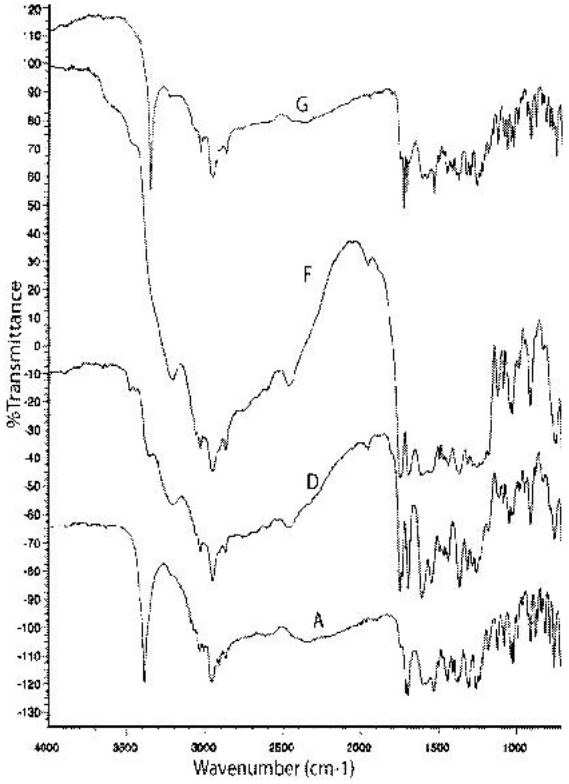

Fig. 6. FTIR spectra of neotame anhydrate polymorphs A, D, F, and G.

different chemical shifts, the neotame molecules may take different conformations in these polymorphs. In other words, conformational polymorphism is possible among the crystal forms of neotame anhydrate. The existence of conformational polymorphism will be tested by structural determination using single crystal X-ray diffractometry. The C15 signals of Forms D and F are broader than those of Forms A and G, confirming the presence of amorphous content in D and F, suggested by their PXRD patterns.

Infrared, especially FTIR, spectroscopy has long been employed to distinguish polymorphs (18). The shift of an absorption band in the FTIR spectrum usually indicates a change in force constant, corresponding to a change in the environment of the corresponding bond, such as the bond length, bond angle, or molecular conformation. The FTIR spectra of Forms A, D, F, and G are shown in Fig. 6. Form A has a strong IR absorption band at 3390 cm^{-1} and Form G has one at 3347 cm^{-1}, whereas Forms D and F have none in the same region. This sharp peak is possibly due to strong hydrogen bonding between the carboxylic or carbonyl oxygen atoms (O^{1-4}) and the hydrogen atoms attached to nitrogen (N^{1-2}) in the two forms. In the carbonyl region, 1650–1800 cm^{-1}, all these four forms have two peaks: Form A at 1712 and 1694 cm^{-1}; Form D at 1748 and 1696 cm^{-1}; Form F at 1747 and 1695 cm^{-1}; and Form G at 1726 and 1703 cm^{-1}. The differences in peak positions indicate different environments of the carbonyl groups in these four polymorphs of neotame anhydrate and probably arise from differences in conformation and crystal packing.

The densities, in grams per cubic centimeter, of the four forms are: 1.202 ± 0.003 (n = 3) for Form A; 1.211 ± 0.003 (n = 3) for Form D; 1.225 ± 0.005 (n = 3) for Form F; 1.181 ± 0.008 (n = 5) for Form G. This rank order, Forms F > D > A > G, is that of the packing efficiency of the neotame molecule in its anhydrate polymorphs. A high packing efficiency appears to result from low polarity of the crystallization media of the individual polymorphs. Forms A and G crystallize from the more polar solvents, ethyl acetate and acetonitrile; Form F crystallizes from the nonpolar, but polarizable solvent carbon tetrachloride; whereas Form D crystallizes from the least polar and least polarizable solvents, cyclohexane, hexane, or heptane. In explanation, weak intermolecular interactions between neotame and solvent molecules may lead to relatively strong intramolecular interactions between the neotame molecules themselves, which, consequently, enhances their packing efficiency in the crystal.

The dynamic vapor sorption microbalance was employed to quantify the affinity of each polymorph for water vapor. The sorption and desorption curves of Forms A, D, F, and G (Fig. 7) show hysteresis. The maximum water uptake of these forms at high relative humidity (RH) approaches 6% w/w, which corresponds to 4.6% w/w for neotame monohydrate plus some adsorbed water. This result suggests that neotame anhydrate Forms A, D, F, and G are converted to the monohydrate completely at certain RH values, namely, at 80% RH for Form A, at 70% RH for Forms D and F, and at 90% RH for Form G. These results show that, in the presence of water vapor, the ease of conversion of neotame anhydrate to the monohydrate follows the rank order, F ~ D > A > G, corresponding to decreasing affinity for water. For all these four anhydrate polymorphs, the isothermal hysteresis value, (weight at desorption–weight at adsorption) × 100%/dry

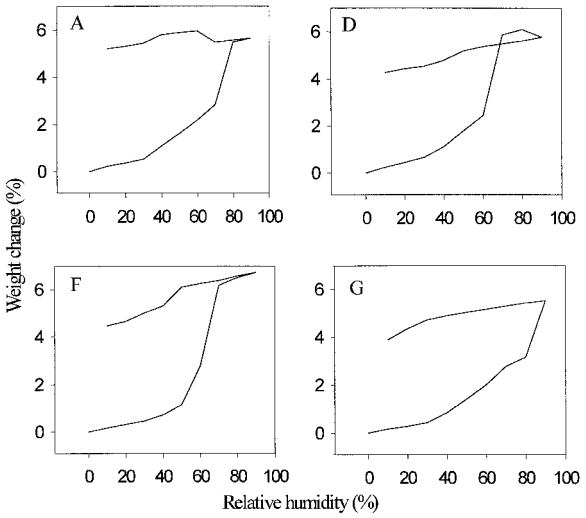

Fig. 7. Dynamic water vapor sorption and desorption curves of neotame anhydrate polymorphs A, D, F, and G at 25°C.

sample weight, decreases from about 4–5% with the increasing RH (Fig. 8). The isothermal hysteresis value for each anhydrate polymorph decreases to zero at the RH value just above the phase transition to the monohydrate (Form A at 80% RH, Forms D and F at 70% RH, Form G at 90% RH). The rank order of these RH values, Forms F ~ D < A < G, corresponds to decreasing affinity of these polymorphs for water. Form G shows isothermal hysteresis significantly above zero (2.26%) at 80% RH, indicating incomplete conversion of Form G to the monohydrate at this point. The molar volume (molecular weight/density) shows a reverse rank order, G > A > D > F. Thus, a more open structure appears to correspond to slower and less complete sorption of water vapor. This apparently contradictory result may arise from the greater separation of the hydrophobic groups in the more open, less dense polymorphic structures.

CONCLUSIONS

Seven polymorphs, A–G, of neotame anhydrate were prepared and investigated. However, only Forms A, D, F, and G were obtained in bulk and could be physically characterized in detail. Forms B and C were found to be enantiotropically related but to contain a decomposition product. Form E was observed in DSC but was unstable under ambient conditions. Forms E and A also were found to be enantiotropically related. The crystal densities of the polymorphs, F > D > A > G, appear to be inversely related to the polarity of the solvents from which they crystallized.

The polymorphs of neotame anhydrate appear to be conformational polymorphs, based on the sensitivity of the ^{13}C SSNMR chemical shift of C15. Dynamic water vapor sorption/desorption of Forms A, D, F, and G show distinctive behavior, from which the stability of the four forms under various RH values are inferred. The polymorphs are converted to neotame monohydrate at the following RH values: 80% for Form A, 70% for Forms D and F, 90% for Form G. However, more openness of molecular packing does not correspond to faster and more complete sorption of water vapor.

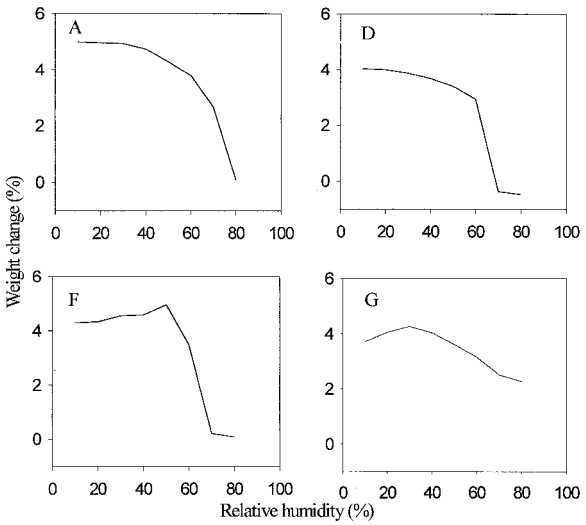

Fig. 8. Isothermal hysteresis of neotame anhydrate polymorphs A, D, F, and G during dynamic water vapor sorption/desorption at 25°C.

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REFERENCES

1. H. G. Brittain. *Polymorphism in Pharmaceutical Solids,* Marcel Dekker, NY, New York, 1999.
2. S.-Y. Tsai and S.-C. Kuo, and S.-Y. Lin. Physicochemical characterization of 9,10-anthraquinone-2-carboxylic acid. *J. Pharm. Sci.* **82**:1250–1254 (1993).
3. L. S. Wu, G. Torosian, K. Sigvardson, C. Gerard, and M. A. Hussain. Investigation of moricizine hydrochloride polymorphs. *J. Pharm. Sci.* **83**:1404–1406 (1994).
4. A. Burger and A. W. Ratz. Polymorphism and dissolution behavior of midodrine hydrochloride. *Pharm. Ind.* **50**:1186–1189 (1988).
5. R. F. Shanker. *Micro-techniques for physicochemical measurements*. AAPS Symposium on Pharmaceutical Development Contributions During the Drug Discovery Process, Miami Beach, Florida, 1995.
6. M. Otsuka and Y. Matsuda. Effects of environmental temperature and compression energy on polymorphic transformation during tableting. *Drug. Dev. Ind. Pharm.* **19**:2241–2269 (1993).
7. M. Yoshioka, B. C. Hancock, and G. Zografi. Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. *J. Pharm. Sci.* **83**:1700–1705 (1994).
8. W. C. Stagner and J. K. Guillory. Physical characterization of solid iopanoic acid forms. *J. Pharm. Sci.* **68**:1005–1009 (1979).
9. W. L. Rocco. Solid state characterization of stanozolol. *Drug. Dev. Ind. Pharm.* **20**:1831–1849 (1994).
10. M. Otsuka, K. Otsuka, and N. Kaneniwa. Relation between polymorphic transformation pathway during grinding and the physicochemical properties of bulk powders for pharmaceutical preparations. *Drug. Dev. Ind. Pharm.* **20**:1649–1660 (1994).
11. P. W. Carter and M. D. Ward. Directing polymorph selectivity during nucleation of anthranilic acid on molecular substrates. *J. Am. Chem. Soc.* **116**:769–770 (1994).
12. R. J. Davey, N. Blagden, G. D. Potts, and R. Docherty. Polymorphism in molecular crystals: stabilization of a metastable form by conformational mimicry. *J. Am. Chem. Soc.* **119**:1767–1772 (1997).
13. X. He, U. J. Griesser, J. G. Stowell, T. B. Borchardt, and S. R. Byrn. Conformational color polymorphism and control of crystallization of 5-methyl-2-[(4-methyl-2-nitrophenyl)amino]-3-thiophenecarbonitrile. *J. Pharm. Sci.* **90**:371–388 (2001).
14. B. A. Hendriksen and D. J. W. Grant. The effect of structurally

related substances on the nucleation kinetics of paracetamol (acetaminophen). *J. Cryst. Growth* **156**:252–260 (1995).
15. D. Lechuga-Ballesteros and N. Rodríguez-Hornedo. The influence of additives on the growth kinetics and mechanism of L-alanine crystals. *Int. J. Pharm.* **115**:139–149 (1995).
16. D. Giron. Thermal analysis and calorimetric methods in the characterization of polymorphs and solvates. *Thermochim. Acta* **248**: 1–59 (1995).
17. M. Kuhnert-Brandstätter, A. Kofler, A. Vlachopoulos, and A. Lobenwein. Microscopic characterization and identification of drugs by use of UV spectrophotometry. *Sci. Pharm.* **38**:154–163 (1970).
18. H. G. Brittain. *Physical Characterization of Pharmaceutical Solids,* Marcel Dekker, New York, NY, 1995.
19. S. Byrn, R. Pfeiffer, M. Ganey, C. Hoiberg, and G. Poochikian. Pharmaceutical solids: a strategic approach to regulatory considerations. *Pharm. Res.* **12**:945–954 (1995).
20. B. A. Bolton and P. N. Prasad. Laser Raman investigation of drug-polymer conjugates: sulfathiazole-povidone coprecipitates. *J. Pharm. Sci.* **73**:1849–1851 (1984).
21. L. R. Chen, B. E. Padden, S. R. Vippagunta, E. J. Munson, and D. J. W. Grant. Nuclear magnetic resonance and infrared spectroscopy analysis of nedocromil hydrates. *Pharm. Res.* **17**:619–624 (2000).
22. B. E. Padden, M. T. Zell, Z. Dong, S. A. Schroeder, D. J. W. Grant, and E. J. Munson. Comparison of solid-state ^{13}C NMR spectroscopy and powder X-ray diffraction for analyzing mixtures of polymorphs of neotame. *Anal. Chem.* **71**:3325–3331 (1999).
23. H. Jahansouz, K. C. Thompson, G. S. Brenner, and M. J. Kaufman. Investigation of the polymorphism of the angiotensin II antagonist agent MK-996. *Pharm. Dev. Technol.* **4**:181–187 (1999).
24. J. C. Shah, J. R. Chen, and D. Chow. Metastable polymorph of etoposide with higher dissolution rate. *Drug Dev. Ind. Pharm.* **25**:63–67 (1999).
25. C. Nofre and J.-M. Tinti. Method of preparing a compound derived from aspartame, useful as a sweetening agent. *U.S. Patent 5510508.*
26. I. Prakash, I. Bishay, and S. Schroeder. Neotame: synthesis, stereochemistry, and sweetness. *Synthetic Commun.* **29**:4461–4467 (1999).
27. I. Prakash. Method for preparing and purifying an N-alkylated aspartame derivative. *U.S. Patent 5728862.*
28. Z. Dong, V. G. Young, Jr., B. E. Padden, S. A. Schroeder, I. Prakash, E. J. Munson, and D. J. W. Grant. Crystal structure and physical characterization of neotame methanol solvate. *J. Chem. Crystallogr.* **29**:967–975 (1999).
29. A. Burger and R. Ramberger. On the polymorphism of pharmaceuticals and other molecular crystals. I. Theory of thermodynamic rules. *Mikrochim. Acta (Wien)* 2:259–271 (1979).
30. A. Burger and R. Ramberger. On the polymorphism of pharmaceuticals and other molecular crystals. II. Applicability of thermodynamic rules. *Mikrochim. Acta (Wien)* 2:273–316 (1979).