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Investigation of Polymorphism in Aspartame and Neotame Using Solid-State NMR Spectroscopy

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Received 25 February 2000; accepted 2 May 2000

Abstract—We have been studying the artificial sweeteners aspartame (L-aspartyl-L-phenylalanine methyl ester) and neotame (*N*-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine methyl ester) as compounds which exhibit polymorphism. ^{13}C CP/MAS NMR shows that aspartame exists in three distinct forms at room temperature, depending on preparation conditions. For two of the forms, there exists three resonances for each carbon, indicating three crystallographically inequivalent sites and therefore three distinct conformations and/or arrangements of aspartame molecules within the unit cell. Two-dimensional exchange spectroscopy using high-speed MAS and very high-power ^{1}H decoupling on uniformly ^{13}C labeled aspartame is a very powerful tool for unambiguously assigning each resonance in the NMR spectrum of aspartame. Even for forms of aspartame that possesses multiple crystallographically inequivalent sites, it is possible to identify connectivities between the nuclei of each conformation and/or arrangement of molecules using two-dimensional NMR techniques. ^{13}C CP/MAS NMR also shows that neotame exists in multiple solid forms. The most stable form of neotame under ambient conditions is a monohydrate. However, other forms can be prepared by heating or using reduced pressures. We have found that high-speed magic-angle spinning can cause a change in polymorphic forms. Three different forms were produced upon spinning at 29 kHz for several days. The monohydrate was identified as the second form produced. Also, altering the crystallization and drying conditions can generate mixtures of the solid forms of neotame. When the monohydrate form of neotame was heated under vacuum, a mixture of anhydrate forms was produced. In the reconversion to the monohydrate upon exposure to moisture under ambient conditions no significant changes were observed in the powder X-ray diffraction patterns during part of the reconversion process. This suggests that no change in form had occurred. The ^{13}C CP/MAS NMR spectra, however, indicated the presence of many forms of neotame during the reconversion. One possible reason that solid-state NMR spectroscopy detected the changes in forms and powder X-ray diffraction did not is that the conformation of the neotame molecules changes between forms but the unit cell parameters do not change significantly.

Introduction

Polymorphism is typically defined as the ability of a compound to crystallize in more than one distinct crystal form. Pseudopolymorphism is similar to polymorphism, except that the material crystallizes in a different solvation state.[1] Approximately 30% of organic compounds are believed to exhibit polymorphism.[2] The differences between polymorphs lie in variations in molecular conformation or packing, which may cause physical properties such as density, melting point, and solubility to differ between polymorphs. Pseudopolymorphs usually have a different molecular conformation or packing, although occasionally there is no change in conformation and/or packing upon solvation. Polymorphism and pseudopolymorphism are important in the formulation of pharmaceuticals, pigments and dyes, explosives, and agrochemicals, where careful control over production must be enforced to ensure that polymorphic or pseudopolymorphic transformations do not occur.[3] Throughout the rest of this paper, both polymorphism and pseudopolymorphism will be referred to as polymorphism.

Characterization of polymorphic forms of a compound is typically accomplished using a combination of diffractometry (single crystal and powder X-ray diffraction), calorimetric methods (DSC and TGA), and spectroscopic techniques (infrared and NMR spectroscopy).[4] Single crystal X-ray diffraction is usually used to determine the structure of crystalline organic materials.[5,6] In order to determine the crystal structure using single-crystal X-ray diffraction, however, it is necessary for a large single crystal to be grown.[7] Some polymorphic forms do not form large enough crystals for single crystal X-ray diffraction to be performed. Powder X-ray diffraction is currently the most widely used method for distinguishing different forms of crystalline organic solids. If two samples with the same

Keywords: polymorphism; magic-angle spinning; artificial sweeteners; solid-state NMR.
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0040–4020/00/$ - see front matter
PII: S0040-4020(00)00461-0

chemical structure give different powder X-ray diffraction patterns, then they are generally considered polymorphs. The determination of polymorphism using powder X-ray diffraction is difficult when phenomena such as particle size or crystallite orientation effects interfere.[8] These effects can cause different powder patterns to be observed, allowing two samples to be incorrectly identified as polymorphs when they are actually the same form.[9]

Solid-state ^{13}C NMR spectroscopy using cross-polarization[10] (CP) and magic-angle spinning[11] (MAS) is a powerful method for investigating polymorphism in crystalline organic materials.[3,4,7,12–24] The chemical shift observed in the CP/MAS experiment is very informative about the crystalline structure, since the electronic environment of the observed nucleus is affected by the local conformation and/or arrangement.[23,24] In addition, solid-state NMR can be used to identify the number of crystallographically inequivalent sites, since each crystallographically unique molecule in the unit cell gives rise to an NMR signal.[21]

We have chosen aspartame and neotame as model systems to study. Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a commonly used synthetic dipeptide sweetener in low calorie food products such as diet soft drinks, because it is about 15–200 times sweeter than sucrose.[25] Neotame (*N*-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine methyl ester) is an alkylated derivative of aspartame. Discovered by Nofri and Tinti,[26] neotame is currently being developed by the Nutrition and Consumer Product Sector of the Monsanto Company. Neotame has recently been submitted to the United States Food and Drug Administration for approval as a general-use sweetener in food and beverages. Neotame has approximately 40 times the sweetness potency of aspartame. The method for preparing and purifying neotame has been published.[27]

In this paper we address three problems associated with analyzing crystalline organic solids using solid-state NMR spectroscopy and powder X-ray diffraction. The first is the assignment of peaks in the solid-state NMR spectrum. The second is the possibility of polymorphic changes occurring during the acquisition of the solid-state NMR spectrum. The third is the ability of powder X-ray diffraction to detect all of the forms present in the sample.[28]

The first problem is the assignment of chemical shifts in solid forms of a compound. Isotropic ^{13}C chemical shifts in the solid state may vary by up to 10 ppm from their corresponding solution values, and there may be multiple resonances for each crystallographically inequivalent carbon. Assignments are typically made in solid-state NMR experiments using information from solution-state NMR experiments, solid-state experiments such as interrupted decoupling,[29] and effects such as peak splitting due to $^{13}C-^{14}N$ coupling.[30,31] However, the information provided by these experiments is limited. For example, it is impossible to definitively assign carbons that have the same number of protons and similar chemical shifts.

One method for unambiguously assigning the resonances is to uniformly ^{13}C label the compound and to rely on dipolar couplings for the transfer of magnetization between neighboring nuclei in a two-dimensional (2D) exchange experiment.[32a,b] Cross peaks typically indicate carbons that are either directly bonded or are two to three carbons away. Often experiments such as RFDR,[33] MELODRAMA,[34] DRAWS,[35] C7,[36] and POST-C7,[37] are used to refocus dipolar couplings and to enhance magnetization transfer. These techniques have been used quite successfully to assign small peptides.[38] Unfortunately, the peaks in the spectra are usually significantly broadened by $^{13}C-^{13}C$ and $^{13}C-^{1}H$ dipolar couplings. It is also possible to use NMR experiments such as INADEQUATE to trace through bond connectivity of ^{13}C labeled materials using scalar couplings.[39] In crystalline organic compounds, especially those that have multiple peaks for each carbon due to crystallographically inequivalent sites, there may be several peaks separated by <3 ppm that must be resolved to observe their cross peaks. Zilm and coworkers have found that line widths comparable to unlabeled compounds can be obtained in uniformly ^{13}C-labeled crystalline organic compounds using spinning speeds on the order of 35 kHz with ^{1}H decoupling powers on the order of 250 kHz.[40]

Three distinct forms of aspartame are known to exist, two hemihydrate polymorphs (Forms I and II) and a 2.5 hydrate (Form III). Only the crystal structure of Form I has been reported.[41] Information about the structure of aspartame was derived from differences in the one-dimensional NMR spectra. Two-dimensional NMR spectra were used to unambiguously assign the resonances for each form of aspartame.

The second problem is the possibility of polymorphic form changes occurring as a result of high-speed magic-angle spinning. Changes in polymorphic forms of drugs are known to occur as a function of temperature and/or pressure. For example, aspartame changes from one hemihydrate form (Form I) to the other hemihydrate form (Form II) after being placed in a ball mill for half an hour. At spinning speeds in excess of 25 kHz, the centripetal forces and frictional heating may be sufficient to induce a polymorphic change in a material.

We have observed that when neotame is exposed to MAS rates of >25 kHz, three different forms are observed as a function of time. The initial form observed in the NMR spectrum is produced by recrystallization from water at $>70°C$. After spinning at 29 kHz for several hours, the spectrum changes and corresponds to neotame monohydrate, for which the crystal structure and solid-state NMR spectrum are known. Upon spinning for several days, yet another transformation is observed, resulting in a previously unknown form of neotame.

The third problem is the ability of powder X-ray diffraction to detect all of the forms present in a sample. Neotame monohydrate was converted under vacuum to a mixture of anhydrate forms and then reconverted to the monohydrate upon exposure to moisture at ambient conditions. Several new forms of neotame were discovered but to date only the monohydrate and the amorphous anhydrate have been obtained as pure forms. No significant changes were observed in the powder X-ray diffraction patterns during part of the reconversion process, suggesting that no change

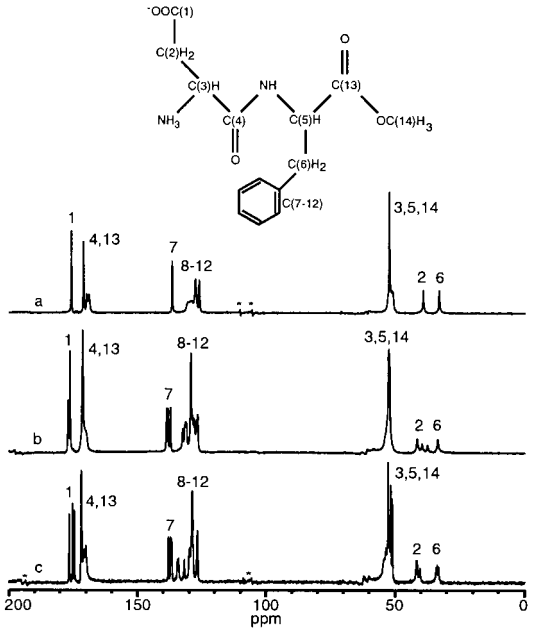

Figure 1. ^{13}C CP/MAS NMR spectra of the three forms of aspartame. (a) Form I, hemihydrate, recrystallized from a quaternary solvent mixture. (b) Form II, hemihydrate, as received from NutraSweet Kelco Co. (c) Form III, dihemihydrate, prepared by placing Form II in an environment of high relative humidity (<98%) for 5 days.

in form had occurred. The ^{13}C CP/MAS NMR spectra, however, indicated the existence of multiple forms of neotame during the reconversion. One possible explanation for the observation of different forms in the ^{13}C CP/MAS NMR spectrum but no detectable changes in the powder X-ray diffraction pattern is that the conformation of the neotame molecules is changing between forms but the unit cell parameters are not significantly different between forms.

Experimental

Solid-state NMR spectroscopy

All ^{13}C spectra were acquired at 75.4 MHz with a Chemagnetics CMX-300 solid-state NMR spectrometer and were externally referenced to TMS using the methyl peak of hexamethylbenzene (17.35 ppm). One-dimensional spectra were acquired using a Chemagnetics Pencil Probe with 7.5 mm zirconia rotors using Kel-F end caps and spun at the magic angle (between 4 and6 kHz). One-dimensional spectra were acquired with total sideband suppression[42] (TOSS), a 3.0 s recycle delay, and a decoupling field of approximately 60 kHz. The ^{1}H 90° pulse was 4.5 μs and the contact time was 5 ms. Typically, 2048 transients were acquired for each spectrum. The dephasing time for interrupted decoupling spectra was 50 μs. All one-dimensional spectra were scaled to the largest peak in each spectrum. The chemical shifts reported are reproducible to ±0.1 ppm.

One and two-dimensional spectra of ^{13}C-labeled aspartame and neotame were acquired using a 2.5 mm Varian spinning module with variable-amplitude cross polarization (VACP)[43] (5 ms contact time) and two-pulse phase modulation (TPPM)[44] decoupling. Two different two-dimensional experiments were carried out. Radio frequency driven dipolar recoupling (RFDR) was used to observe short range (1–2 bond) connectivities. Exchange via spin diffusion was used to observe longer-range couplings (up to 6 bond). 96 transients were block averaged as 6 groups of 16 transients for each t_1 slice to minimize t_1 noise. The initial t_1 time was 1 μs for all two-dimensional experiments, and mixing times ranged from 20 ms (128 rotor cycles) using RFDR to 0.5–2.5 s without dipolar recoupling. Sine bell apodization (center=0.30) was applied to all 2D spectra in both dimensions during processing; 512 points were acquired in both dimensions using a sweep width of 15 kHz. Symmetrization was performed on the spectra of Form II of aspartame. All cross peaks observed in these spectra were also present in the unsymmetrized spectra.

Powder X-ray diffraction

All powder patterns were acquired at 45 kV and 40 mA with Cu Kα radiation with a Siemens D5005 diffractometer. Counts were measured using a scintillation detector. Samples were packed into a plastic holder and scanned from 2θ=5–50°, increasing at a step size of 0.03° with a counting time of 2 s. All powder X-ray diffraction patterns have been scaled to 1000 counts.

Karl–Fischer titrimetry

Bulk water content was determined with a Mitsubishi Moisture Meter (model CA-05). Samples in the range of 2–20 mg were weighed by difference and quickly transferred to the titration vessel containing anhydrous methanol prior to coulometric titration. All water contents are reported as weight/weight ratios, each as an average of three trials with sample standard deviation.

Preparation of different polymorphic forms

Aspartame Form I can be prepared in two different ways: by placing aspartame as received in a ball mill for 30 min, or by recrystallizing aspartame as received from a quaternary solvent mixture of 50% H_2O:10% DMSO:20% EtOH:20% Acetone.[41] Aspartame Form II is the material as received from Nutrasweet. This form can be generated by recrystallizing from water and allowing to dry at ambient conditions (<60% RH) for 5 days. Uniformly ^{13}C-labeled aspartame was prepared by Nutrasweet according to the literature procedure for unlabeled aspartame. For two dimensional exchange experiments, uniformly ^{13}C-labeled aspartame was diluted to 20% with unlabeled aspartame and prepared using the same procedures as for the unlabeled material.[45]

Neotame monohydrate and neotame anhydrate were both obtained from the Nutrasweet Company. The monohydrate is stable under ambient conditions; no special storage or handling procedures were used. The anhydrate was stored in a 0% relative humidity environment over phosphorus pentoxide. The dehydration resulting in different anhydrate forms is discussed in the following section. A sample of uniformly ^{13}C-labeled neotame in unlabeled neotame was prepared at approximately 20% w/w dilution. This material

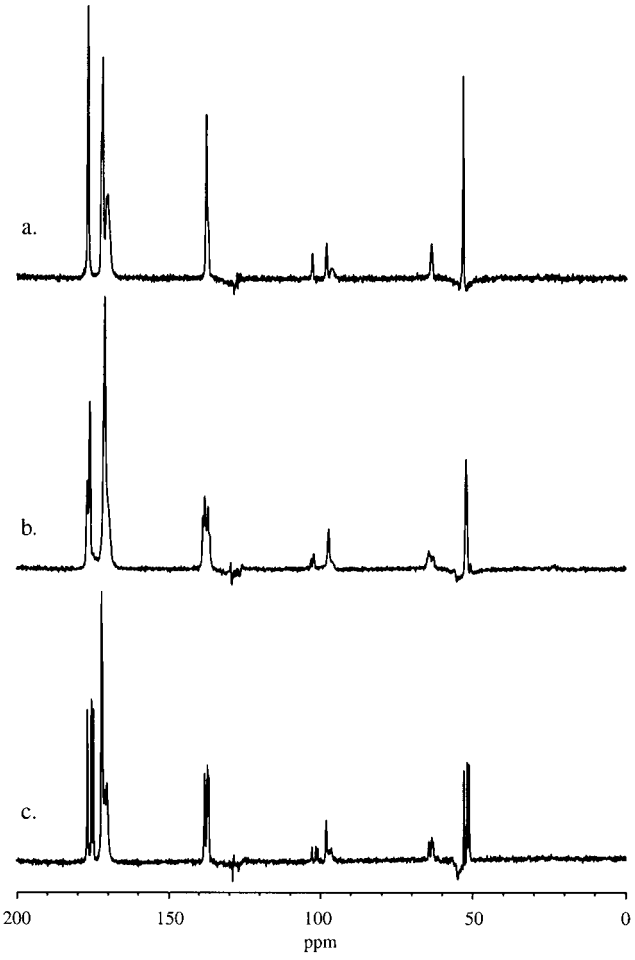

Figure 2. ^{13}C CP/MAS NMR spectra acquired with interrupted decoupling of the three forms of aspartame: (a) Form I, hemihydrate; (b) Form II, hemihydrate; (c) Form III, dihemihydrate. The interrupted decoupling pulse sequence suppresses resonances due to methine and methylene carbons.

was prepared by dissolving 0.1607 g of unlabeled neotame and 0.0398 g of uniformly ^{13}C-labeled neotame in hot water (approximately 2 mL) and pouring the solution into a Petri dish for recrystallization. Within 24 h the water had evaporated to dryness and the crystals were available for harvest. This material was packed into a 2.5 mm rotor for analysis.

Results and Discussion

Assignment of peaks in solid-state NMR spectrum

Shown in Fig. 1 are the structure of aspartame and the ^{13}C CP/MAS NMR spectra of the three forms of aspartame. The three spectra are clearly different, indicating that the conformation and/or arrangement of molecules in the unit cell varies significantly between forms. For Form I there is one peak per carbon, indicating only one crystallographically inequivalent molecule in the unit cell. This fact is consistent with the published crystal structure obtained by single-crystal X-ray diffraction of this form. For Forms II and III there are three resonances for several of the carbons (e.g. carbon 7), indicating at least three crystallographically inequivalent molecules per unit cell. The large number of closely spaced resonances makes assignment of the spectra in Fig. 1 difficult.

Fig. 2 shows the interrupted decoupling spectra of the three forms of aspartame. The interrupted decoupling experiment eliminates resonances due to methine and methylene carbons, making quaternary and methyl carbons easier to assign. Despite the removal of the methine and methylene resonances from the interrupted decoupling spectra in Fig. 2, it is still extremely difficult to unambiguously assign each resonance.

Tentative peak assignments were made based upon the interrupted decoupling experiments and peak splitting due to $^{13}C-^{14}N$ dipolar coupling and were confirmed using 2D exchange experiments (vide infra).[29–32] The spectra in Fig. 1 can be divided into three distinct regions. The region from 170–180 ppm contains resonances due to carbons 1, 4, and 13. The peak for carbon 4 in Fig. 1a is identified by a characteristic 1:2 splitting due to coupling with ^{14}N.[29–32] Of the three carbons, only the peaks assigned to carbon 1 are significantly affected by changing forms. In Form I, a single resonance is observed for carbon 1, for Form II, two resonances are observed with a 1:2 intensity ratio, and for Form III, three resonances of equal intensity are observed. This indicates that for Form I, there is only one unique

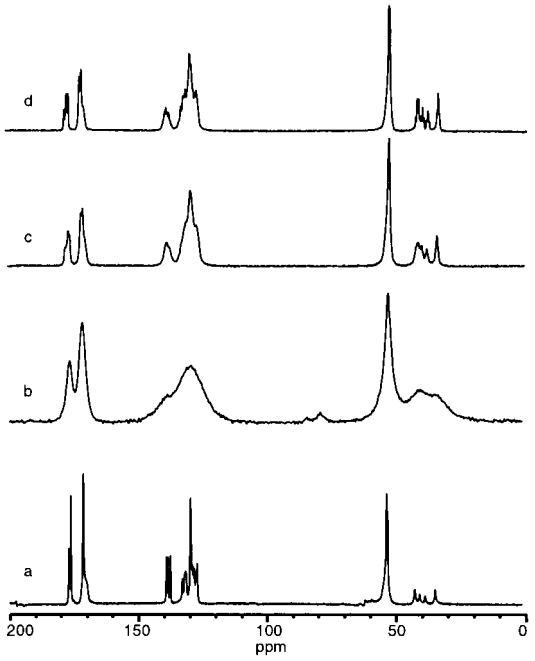

Figure 3. ^{13}C CP/MAS NMR spectra of Form II of aspartame acquired using different spinning speeds and decoupling powers. (a) Unlabeled aspartame, 7 kHz MAS and 63 kHz ^{1}H decoupling. (b) 100% Uniformly ^{13}C labeled aspartame, 7 kHz MAS and 63 kHz ^{1}H decoupling. (c) Uniformly ^{13}C labeled aspartame diluted to 20% in unlabeled aspartame, 24 kHz MAS and 150 kHz ^{1}H decoupling with TPPM. (d) Uniformly ^{13}C labeled aspartame diluted to 20% in unlabeled aspartame, 27 kHz MAS and 263 kHz ^{1}H decoupling with TPPM.

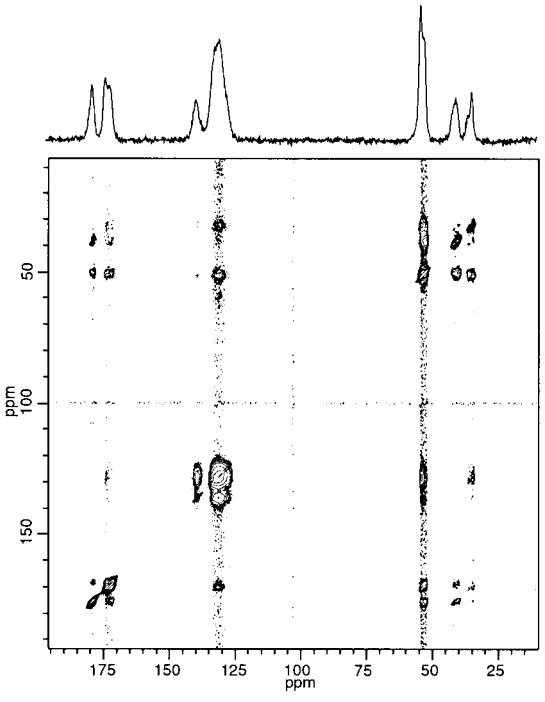

Figure 4. Two-dimensional exchange spectrum of 20% uniformly ^{13}C labeled aspartame (Form I) diluted in unlabeled aspartame acquired at a spinning speed of 20 with 200 kHz ^{1}H decoupling and a mixing time of 500 ms.

molecular conformation in the unit cell, which is confirmed by the crystal structure of this form. Forms II and III both contain three crystallographically inequivalent molecules in the unit cell. In Form II, two of the inequivalent molecules must have very similar molecular conformations since they have the same chemical shift, while the third inequivalent molecule likely has a slightly different conformation. In Form III, all three inequivalent molecules in the unit cell have slightly different conformations, resulting in three resonances of equal intensity for carbon 1 in the NMR spectrum. The aromatic region of the spectrum from 125–140 ppm changes dramatically with each form. The peaks corresponding to carbon 7 in all three forms were assigned using interrupted decoupling. In Fig. 1a some of the aromatic resonances are broadened, presumably due to molecular motion interfering with averaging of either $^{13}C–^{1}H$ dipolar interactions or chemical shift anisotropy. It is not possible to assign each resonance for carbons 8–12 in the aromatic region of the spectrum because of the significant peak overlap in this region. In the third region of the spectrum, carbons 3, 5, and 14 are all within 5 ppm of each other. The sharp peaks at ca. 51 ppm are assigned to carbon 14 based on interrupted decoupling experiments. The resonances for carbons 3 and 5 are broadened due to coupling to ^{14}N. Assigning the peaks due to carbons 2 and 6 represents one of the difficulties in assigning NMR spectra in the solid state. In solution, the difference in chemical shift between the two resonances is 0.7 ppm, but in the solid state it is close to 6 ppm. In Form I, the peaks have shifted about 3 ppm from their values in the solution state, but the direction of shift for each of the resonances (upfield or downfield) cannot be determined. In Form II (Fig. 1b) there are at least three peaks for each carbon, two of which apparently overlap for carbons 2 and 6. Form III also contains more than one resonance for both carbons 2 and 6, although because of the overlap of these resonances it is difficult to resolve exactly how many resonances are present in this region. Complete assignment of the one-dimensional NMR spectra of the three forms of aspartame is not possible without the use of two-dimensional NMR techniques.

Fig. 3 shows the ^{13}C CP/MAS NMR spectra of ^{13}C-labeled and unlabeled samples of Form II of aspartame. The spectrum of the unlabeled sample acquired using typical conditions (7 kHz spinning speed, 63 kHz decoupling field) is shown in 3a. This is the same spectrum as in Fig. 1b. The spectrum of 100% ^{13}C-labeled sample acquired under identical conditions (7 kHz spinning speed, 63 kHz decoupling field) is shown in 3b. The resolution is significantly degraded in 3b, primarily because of increased $^{13}C–^{13}C$ and $^{13}C–^{1}H$ dipolar interactions. Zilm and coworkers have shown that is possible to obtain high-resolution ^{13}C NMR spectra of uniformly ^{13}C-labeled compounds by using high-speed MAS combined with extremely high-power ^{1}H decoupling.[40] The spectrum of 20% uniformly ^{13}C-labeled aspartame diluted in a matrix of unlabeled aspartame acquired with 24 kHz spinning speed and 150 kHz ^{1}H decoupling with TPPM is shown in Fig. 3c. The resolution has been significantly improved. Resolution close to that of the unlabeled compound can be obtained by using even more extreme decoupling and spinning speeds. The spectrum of 20% uniformly ^{13}C-labeled aspartame diluted in a matrix of unlabeled aspartame

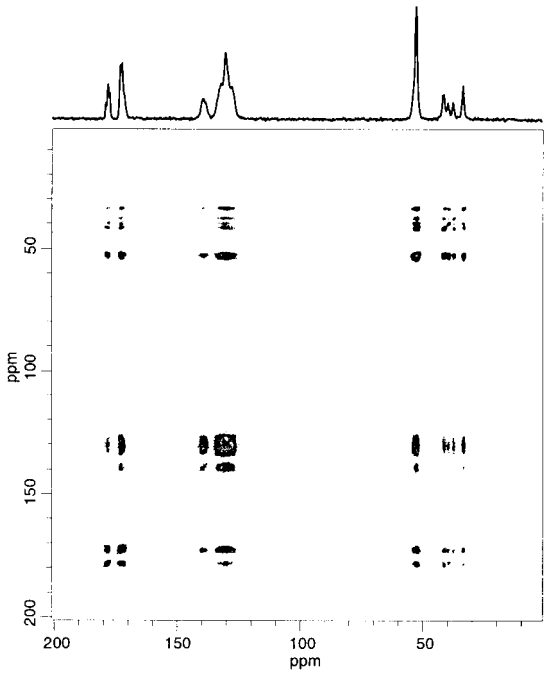

Figure 5. Two-dimensional exchange spectrum of 20% uniformly ^{13}C labeled aspartame (Form II) diluted in unlabeled aspartame acquired at a spinning speed of 26 with 263 kHz ^{1}H decoupling and a 2.5 s mixing time.

acquired with 27 kHz spinning speed and 263 kHz ^{1}H decoupling with TPPM is shown in 3d. ^{13}C–^{13}C *J*-coupling is clearly evident in this spectrum. We have found that ^{1}H decoupling power is more critical than high spinning speeds in improving resolution, and that TPPM is essential to obtain high-resolution spectra.

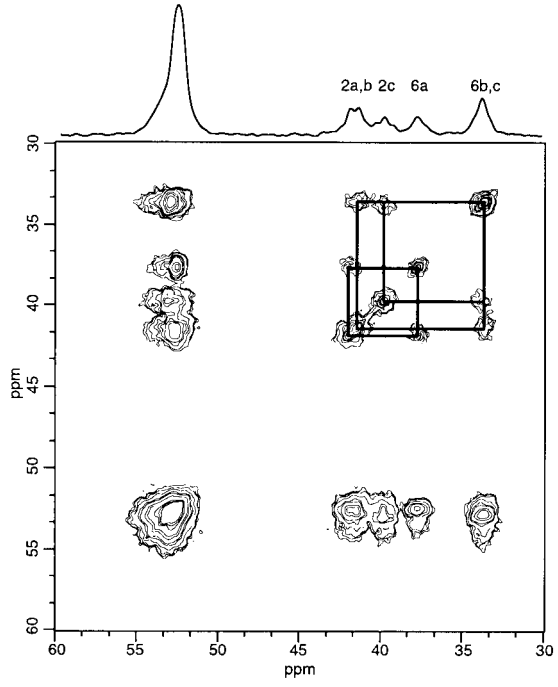

Figure 6. Expansion of Fig. 5, showing the region from 30–60 ppm in both dimensions. Connectivity between crystallographically inequivalent sites is indicated by boxes connecting cross-peaks.

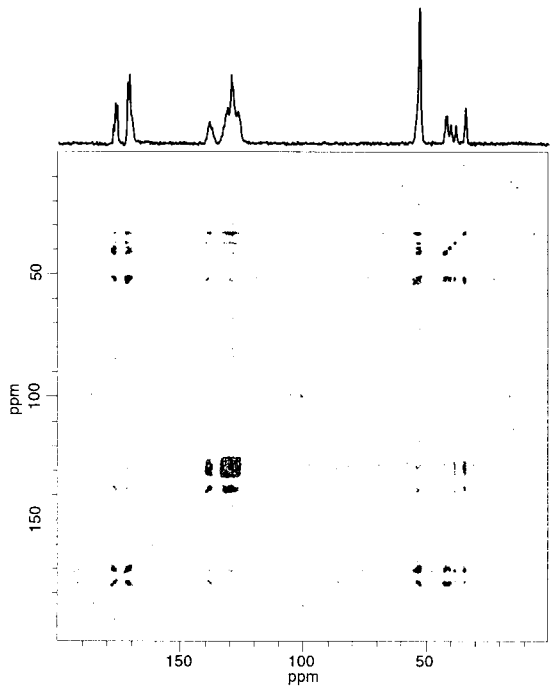

Figure 7. Two-dimensional RFDR spectrum of 20% uniformly ^{13}C labeled aspartame (Form II) diluted in unlabeled aspartame acquired at a spinning speed of 15 with 263 kHz ^{1}H decoupling and a mixing time of 20 ms (128 rotor cycles).

Incorporation of uniformly ^{13}C-labeled aspartame in a matrix of unlabeled aspartame allows two-dimensional ^{13}C–^{13}C correlation experiments to be carried out to trace the connectivity in the different forms of aspartame. Fig. 3 demonstrates that it is possible to obtain sufficient resolution to be able to unambiguously assign each resonance due to crystallographically inequivalent sites in the different forms of aspartame. The presence of resolved *J*-couplings means that COSY or INADEQUATE experiments could be used to assign the spectra. However, we have chosen to use two-dimensional chemical exchange or NOESY type experiments in which magnetization is transferred via spin diffusion rather than a chemical exchange process. Another concern was the possibility of intermolecular dipolar coupling, which would complicate the interpretation of the two-dimensional NMR spectrum by introducing cross-peaks between resonances in crystallographically inequivalent molecules. In order to overcome this difficulty, the ^{13}C-labeled aspartame was diluted to 20% in unlabeled aspartame. A dilution of 5% is considered sufficient to remove intermolecular couplings. However, in order to obtain adequate sensitivity, it was necessary to use a 20% dilution. Any intermolecular cross-peaks should still be much smaller than intramolecular cross-peaks.

Fig. 4 shows the two-dimensional exchange NMR spectrum of aspartame crystallized as Form I. A 20% dilution of ^{13}C-labeled aspartame in unlabeled aspartame was used. This experiment was performed at 20 kHz spinning speed, 200 kHz ^{1}H decoupling with TPPM, and a 0.5 s mixing time, which allows almost complete intramolecular spin diffusion to occur. For this experiment, cross peaks are strongest between carbons whose resonance frequencies are closest together, for example, carbons 2 and 6. From

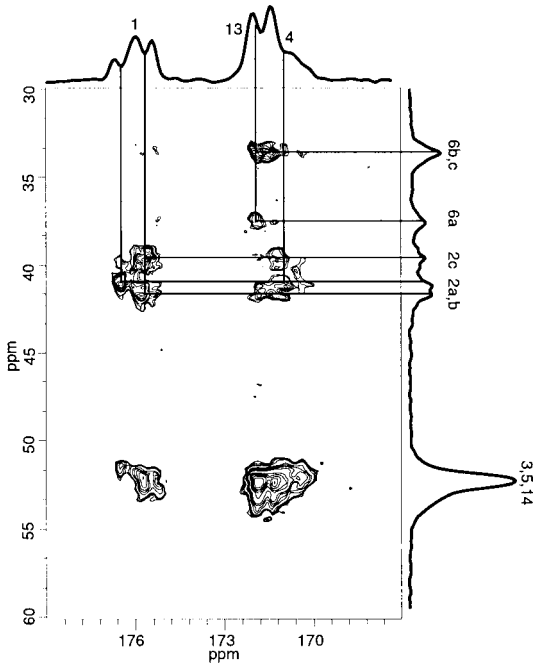

Figure 8. Expansion of Fig. 7, showing the region from 30–60 ppm in the first dimension and 167–179 ppm in the second dimension. Connectivity is indicated by lines drawn to cross-peaks.

this spectrum, the resonances can be assigned by tracing connectivity patterns throughout the molecule. Cross peaks were observed up to four carbons away. For this particular form, there is only one peak per carbon. Strong cross-peaks are observed between C1 and C2, C3, C4; C2 and C3, C4; C5, and C6, C(8–12), C13; C7 and C(8–12); and C(8–12) and C13. This information was used to make the chemical shift assignments in Fig. 1. The resolution of the spectrum in Fig. 4 is sufficient to assign all of the carbons except carbons 8–12 in the aromatic ring.

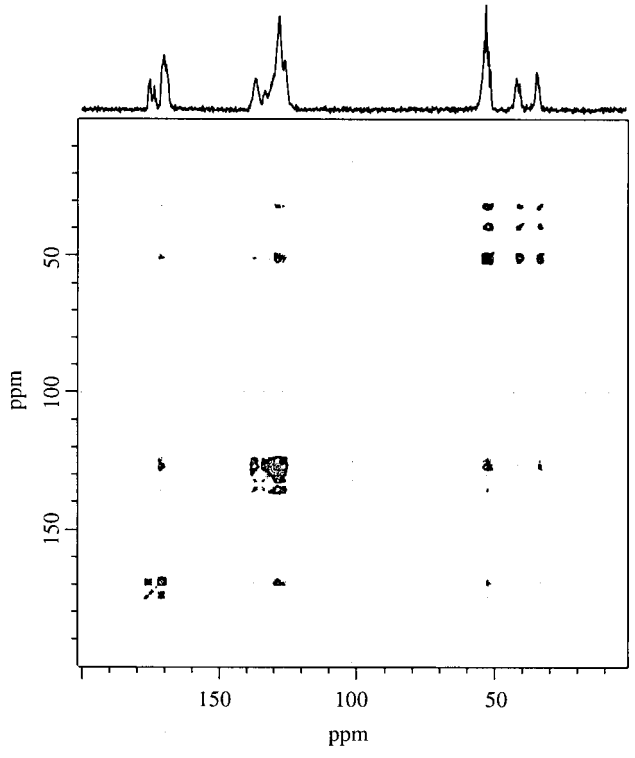

Figure 9. Two-dimensional exchange spectrum of 20% uniformly ^{13}C labeled aspartame (Form III) diluted in unlabeled aspartame acquired at a spinning speed of 26 with 263 kHz ^{1}H decoupling and a 2.5 s mixing time.

Fig. 5 shows the two-dimensional exchange spectrum of aspartame crystallized as Form II. A 20% dilution of ^{13}C-labeled aspartame in unlabeled aspartame was used. This experiment was performed at 26 kHz spinning speed, 263 kHz ^{1}H decoupling with TPPM, and a 2.5 s mixing time. Cross-peaks are observed in this experiment between: C1 and C2, C3, C4; C3 and C4; C5 and C6, C13; C6 and C7, C8–12, C13; and C7 and C8–C12. In addition, the resolution in the spectrum allows the individual resonances due to multiple crystallographically inequivalent sites to be identified. The region between 30 and 60 ppm in Fig. 6 shows the cross peaks between C2, C3, C5, and C6. Cross-peaks are evident between C2–C6, C2–C3, and C5–C6. The spectrum of unlabeled aspartame Form II suggests that both carbon C2 and C6 should have three peaks, because there are three crystallographically inequivalent sites in the unit cell. Two of the peaks apparently overlap to give an integrated intensity of 2:1. In Fig. 6, the correlations are shown by boxes which connect the cross-peaks. The larger peak of C2 is correlated with both the large and small peaks of C6, while the smaller peak of C2 is correlated with the large peak of C6.

Fig. 7 shows the two-dimensional exchange spectrum of 20% uniformly ^{13}C-labeled aspartame in Form II diluted in a matrix of unlabeled aspartame acquired with 15 kHz spinning speed, 263 kHz ^{1}H decoupling with TPPM, and a 20 ms mixing time utilizing RFDR (radio frequency driven dipolar recoupling, 128 rotor cycles) to refocus the ^{13}C–^{13}C dipolar interactions. The resulting spectrum contains primarily one-bond couplings, although some longer-range couplings are also present. Between 30 and 40 ppm, four peaks are observed in the unlabeled material (Fig. 1b). Fig. 8 shows the cross-peak region of carbons C1, C4, and C13 correlated with carbons C2, C3, C5, and C6. Cross-peaks are evident between C1–C2, C1–C3, C3–C4, C5–C13, and C6–C13. The spectrum of unlabeled aspartame shows that for both carbons C1 and C2 there are two peaks with an apparent integrated intensity ratio of 2:1. The larger peak contains resonances from two of the three inequivalent sites. A reasonable hypothesis would be that the larger peaks of C1 (176.3 ppm) and C2 (41.6 ppm) represent two molecules with similar conformations, and that the small peaks of C1 (177.1 ppm) and C2 (39.7 ppm) arise from the third inequivalent molecule. From Fig. 8 it is clear that the larger peak of C1 is correlated with the large and small peaks of C2, and the smaller peak of C1 is correlated with the large peak of C2.

From the results of these two separate two-dimensional experiments, it is now possible to state that for the three crystallographically inequivalent molecules present in Form II, one has chemical shifts for carbons C1–C2–C6 of 177.1, 41.6, and 37.6 ppm, the second has chemical shifts of 176.3, 41.6, and 33.7 ppm, and the third has chemical shifts of 176.3, 39.7, and 33.7 ppm, respectively.

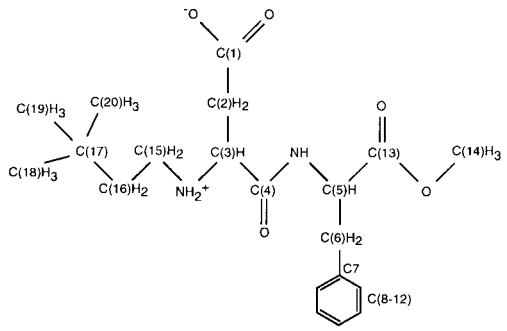

Figure 10. The molecular structure of neotame, with numerical labeling of the individual carbon nuclei.

Fig. 9 shows the two-dimensional exchange spectrum of aspartame crystallized as Form III. A 20% dilution of ^{13}C-labeled aspartame in unlabeled aspartame was also used in this experiment. This experiment was also performed at 26 kHz spinning speed, 263 kHz ^{1}H decoupling with TPPM, with a 2.5 s mixing time, which allows almost complete intramolecular spin diffusion to occur. The most interesting part of this spectrum is the phenyl region, located between 120–140 ppm. In Forms I and II, the resonances for carbons 8–12 are tightly clustered together and separated from the resonance for carbon 7. While this makes it easy to definitively identify carbon 7, it is not possible to obtain any information about carbons 8–12. In Form III, the resonances for carbons 8–12 are much more dispersed. In principle this should allow complete assignment of the phenyl region for Form III.

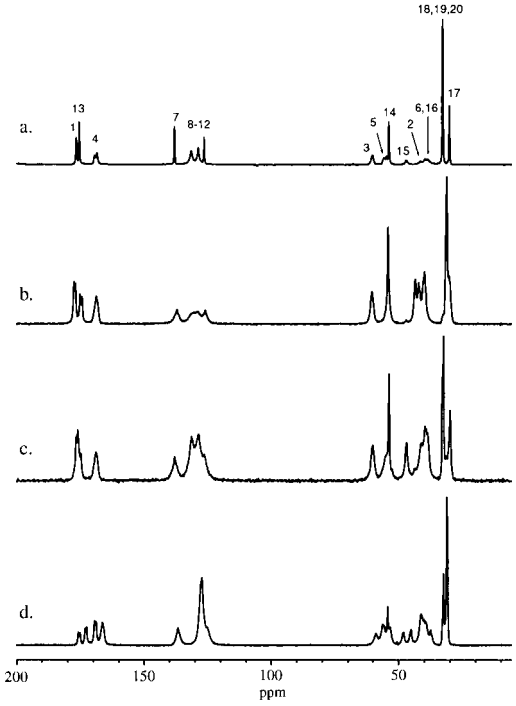

Figure 11. ^{13}C CP/MAS NMR spectra of neotame: (a) Original monohydrate material, 2048 scans acquired using conventional 7.5 mm spinning module at approximately 6 kHz. (b) Recrystallized 20% uniformly ^{13}C-labeled material, 256 scans acquired using 2.5 mm spinning module at 19.9 kHz. (c) After several hours at 29 kHz, 256 scans at 29 kHz. (d) 14336 scans after 6 days at 29 kHz.

However, as can be seen in Fig. 9, the cross peaks are not sufficiently resolved to assign this region of the spectrum.

Despite the quality of the correlations obtained, there are still difficulties associated with making complete assignments of each resonance in the spectrum. First, there is still not sufficient resolution for some of the peaks to be discriminated in the NMR spectrum (C8–C12). Since these peaks are not clearly resolved in the NMR spectrum of the unlabeled material, it is unlikely that assignments for these peaks are possible. Second, chemical shift overlap, such as occurs for C3, C5, and C14, makes it difficult to correlate individual resonances. Third, certain carbons have peaks that do not change significantly between forms, such as carbons C4 and C13. Finally, certain cross-peaks are difficult to observe because of low sensitivity, such as C6–C7, and hence are obscured by the noise.

Polymorphic changes induced by high-speed magic-angle spinning

The structure of neotame is shown in Fig. 10. The ^{13}C CP/MAS NMR spectrum of the monohydrate is shown in Fig. 11a along with the tentative peak assignments. The assignments are based on interrupted decoupling experiments and previous studies with aspartame. The carboxylate carbon, C1, is assigned as the resonance farthest downfield at 176.7 ppm, and the ester carbon, C13, is assigned as slightly upfield of C1 at 175.6 ppm. Coupling of the other carbonyl carbon, C4, to the attached nitrogen produces two peaks at 169.6 and 168.7 ppm with an approximate intensity ratio of 2:1. This splitting is due to dipolar coupling to the quadrupolar ^{14}N which is not averaged with MAS. The phenyl region of the spectrum, C7–12, is difficult to assign except for C7 at 138.3 ppm, because C7 is not surpressed in the interrupted decoupling experiments. The two methine carbons can be distinguished based on comparison with the aspartame spectra in which the peaks for C3 and C5 overlap; for neotame, C3 is attached to a nitrogen that broadens and shifts the resonance downfield to 60.5 ppm. C5, at 56.0 and 55.1 ppm, can be distinguished from C14 at 54.1 ppm by the coupling to the attached nitrogen. C15 is likely to be downfield at 47.2 ppm compared to C16, due again to deshielding by the attached nitrogen. The other methylene carbons are difficult to assign; C6 and C16 are indistinguishable at 39.9 and 38.9 ppm, while C2 is probably slightly downfield at 41.7 ppm. The three methyl carbons, C18–20 at 33.1 ppm, and the quaternary carbon to which they are attached, C17 at 30.4 ppm, are easily assigned based on interrupted decoupling experiments and their expected chemical shifts. All of these assignments are tentative.

Fully ^{13}C-labeled neotame was prepared in order to assign the resonances of the monohydrate form, as have been done for aspartame. Samples were prepared by recrystallization, using 80% original unlabeled monhydrate and 20% fully ^{13}C-labeled monohydrate. Shown in Fig. 11b–d are the ^{13}C CP/MAS NMR spectra acquired at 19 kHz (b) and 29 kHz (c, d) of fully ^{13}C-labeled neotame which had been diluted in a matrix of unlabeled neotame. Although the spectra contain ^{13}C–^{13}C spin–spin couplings, it is clear that the spectrum in Fig. 11b is not the same as the

Figure 12. ^{13}C CP/MAS NMR spectra acquired with TOSS of: (a) neotame monohydrate; (b) neotame anhydrate (monohydrate was placed under reduced pressure (~100 Torr) at 60°C for approximately 12 h); (c) neotame anhydrate generated by placing the monohydrate under vacuum (~1 Torr) for three days; and (d) neotame anhydrate generated by melting the monohydrate at 90°C under vacuum (~1 Torr) for one day.

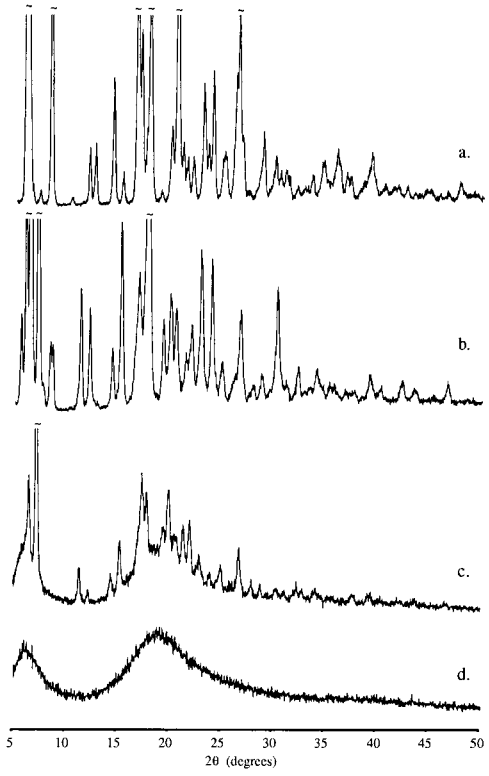

Figure 13. Powder X-ray diffraction patterns of: (a) neotame monohydrate; (b) neotame anhydrate (monohydrate was placed under reduced pressure (~100 Torr) at 60°C for approximately 12 h); (c) neotame anhydrate generated by placing the monohydrate under vacuum (~1 Torr) for three days; and (d) neotame anhydrate generated by melting the monohydrate at 90°C under vacuum (~1 Torr) for one day.

monohydrate form (Fig. 11a). The most distinguishing regions of the spectrum are the methyl carbons (30–35 ppm) and what we have assigned as C15, at 47 ppm. It is clear that both regions are significantly different in the two forms. After spinning the sample for several hours at 29 kHz, the spectrum has changed significantly (Fig. 11c). This spectrum now corresponds to the monohydrate form (Fig. 11a). After six days, another spectrum was acquired, as shown in Fig. 11d. This spectrum is also significantly different than the spectrum of the monohydrate form. The presence of two peaks between 45–50 ppm suggest that there may be two crystallographically inequivalent sites in this particular form, or that it is a mixture of two forms. During this entire conversion process, the sample was continuously spinning in the sealed MAS rotor.

The observation of three different forms of neotame upon spinning is highly surprising. Previously, we have never observed form interconversion upon magic-angle spinning. However, we have observed that most forms tend to convert to the monohydrate, and therefore the transformation of the form observed in Fig. 11b to the monohydrate form (Fig. 11c) is unexpected, but not unusual. The conversion of the monohydrate form to the form observed in Fig. 11d is highly surprising. There are several possible explanations for form interconversion during high-speed magic-angle spinning. The first, and most likely, is that frictional heating is increasing the sample temperature by 20–30°C, which is inducing a polymorphic change in the material. We have previously found that spinning speeds ~19 kHz in a 3.2 mm spinning module increase the sample temperature by 20°C.[46] Another possible explanation is the centripetal force applied by the walls of the rotor. We are currently performing experiments to characterize the forms observed in Fig. 11b and 11d, and to determine the mechanism for their formation. However, form interconversion with high-speed magic-angle spinning is clearly a concern in obtaining high-resolution solid-state NMR spectra of uniformly ^{13}C-labeled compounds.

Identification of multiple polymorphic forms using ^{13}C CP/MAS NMR and powder X-ray diffraction

The ^{13}C CP/MAS NMR spectrum of the monohydrate has only one resonance for each carbon, which suggests that there is only one molecular conformation of neotame present in the unit cell of the monohydrate. This situation is not unusual, as one form of aspartame hemihydrate also has only one crystallographically inequivalent molecule in the unit cell.[18,19] The powder X-ray diffraction pattern of the monohydrate is shown in Fig. 13a. Both the ^{13}C CP/MAS NMR spectrum and the powder X-ray diffraction pattern have sharp peaks, which indicate a highly crystalline compound. The water content of the monohydrate is 4.8±0.1%.

The monohydrate converts to other forms when conditions such as atmospheric pressure and temperature are altered because of its ability to lose water of hydration. One obvious method of converting the monohydrate to other forms is dehydration. When the monohydrate is placed in a vacuum oven at 60°C and ~100 Torr for 12 h, the resulting material has a ^{13}C CP/MAS NMR spectrum (Fig. 12b) and powder

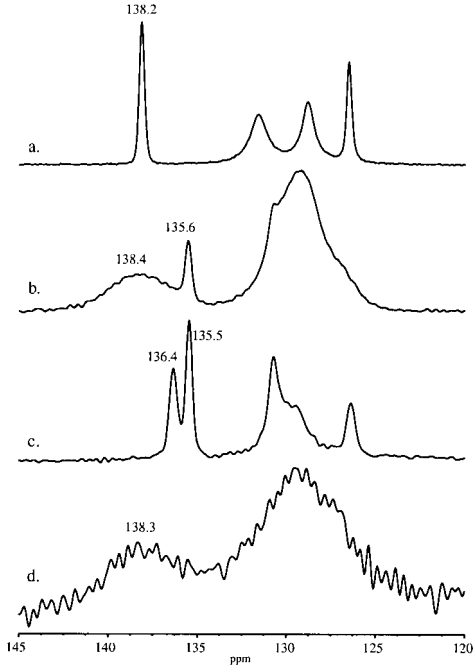

Figure 14. Phenyl region of the ^{13}C CP/MAS NMR spectra acquired with TOSS of: (a) neotame monohydrate; (b) neotame anhydrate (monohydrate was placed under reduced pressure (~100 Torr) at 60°C for approximately 12 h); (c) neotame anhydrate generated by placing the monohydrate under vacuum (~1 Torr) for three days; and (d) neotame anhydrate generated by melting the monohydrate at 90°C under vacuum (~1 Torr) for one day.

X-ray diffraction pattern (Fig. 13b) distinctly different from those of the monohydrate. In the ^{13}C CP/MAS NMR spectrum, many of the sharp peaks are superimposed on much broader resonances. The broader resonances arise from the presence of a significant amorphous phase in the sample. A similar conclusion is reached based on the powder X-ray diffraction pattern in which there is a broad halo beneath the sharp diffraction peaks. The water content of this new material is 0.34±0.04%, which indicates that the neotame forms associated with the two resonances are anhydrous. The residual water content is likely to result from water sorbed by the material.

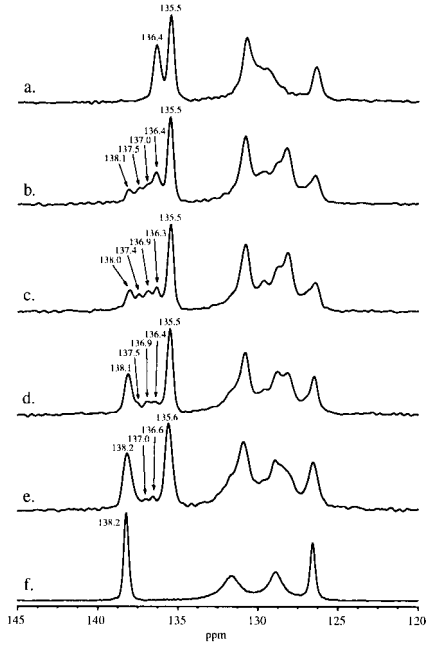

Figure 15. Phenyl region of the ^{13}C CP/MAS NMR spectra of: (a) neotame anhydrate generated by placing the original monohydrate under vacuum (~1 Torr) for three days; (b) after being sealed in a jar for two days; (c) after four days; (d) after six days; (e) after eight days; and (f) after being placed in a relative humidity environment of 84% for 12 days.

Neotame monohydrate can also be dehydrated using a lower pressure vacuum (with or without heat). Fig. 12c shows the ^{13}C CP/MAS NMR spectrum of a sample that was kept under a 1 Torr vacuum for three days. The corresponding powder X-ray diffraction pattern is shown in Fig. 13c. Again, both the ^{13}C CP/MAS NMR spectrum and the powder X-ray diffraction pattern are distinctly different from those of the monohydrate. The water content of this sample is 0.7±0.1%. C7 exhibits two resonances, one of which is attributed to the crystalline anhydrate at 135.5 ppm that we observe in the sample containing the mixture of amorphous and crystalline forms. The other peak for C7 at 136.4 ppm is probably due to a polymorph of the crystalline anhydrate. The peaks have an approximate intensity ratio of 2:1, suggesting that there is approximately half as much of the form producing the peak at 136.4 ppm than there is of the form producing the peak at 135.5 ppm. Therefore, if the peak at 136.4 ppm is due to a hydrated form (e.g. hemihydrate or monohydrate), a larger percentage of water would be expected in the sample. We anticipate that the cross polarization efficiencies of two crystalline forms of the same compound are similar enough to result in quantitative ratios for the same carbons in the two forms, although we have not performed the necessary studies to verify that conclusion. In the powder X-ray diffraction pattern (Fig. 13c), there is overlap of some of the peaks with those in the sample shown in Fig. 13c. This overlap is consistent with the ^{13}C CP/MAS NMR spectrum in Fig. 12c, which shows two crystalline forms while Fig. 12b shows only one. Some of the peaks in the powder X-ray diffraction pattern of Fig. 13c do not match those in Fig. 13b and are possibly due to the presence of the second crystalline form.

Another form of neotame that has been obtained as a pure phase is the amorphous anhydrate. This phase was made by melting the monohydrate at 90°C under vacuum at 1 Torr for approximately 24 h followed by quenching in liquid nitrogen. Given the extreme conditions used to prepare this sample, it is assumed that the low water content of 1.0±0.1% is due to sorbed water. The ^{13}C CP/MAS NMR spectrum and powder X-ray diffraction pattern can be seen in Figs. 12d and 13d. There are no sharp peaks in either the ^{13}C CP/MAS NMR spectrum or the powder X-ray diffraction pattern, which suggests that the sample is entirely amorphous. The two broad peaks in the powder pattern of the amorphous anhydrate match the broad peaks in the powder pattern in Fig. 13b, which was thought to indicate the presence of amorphous anhydrate.

We have observed that the location of the resonance for carbon C7 in the ^{13}C CP/MAS NMR spectra of neotame is indicative of the form of neotame present in the sample. For this reason the phenyl regions of the ^{13}C CP/MAS NMR

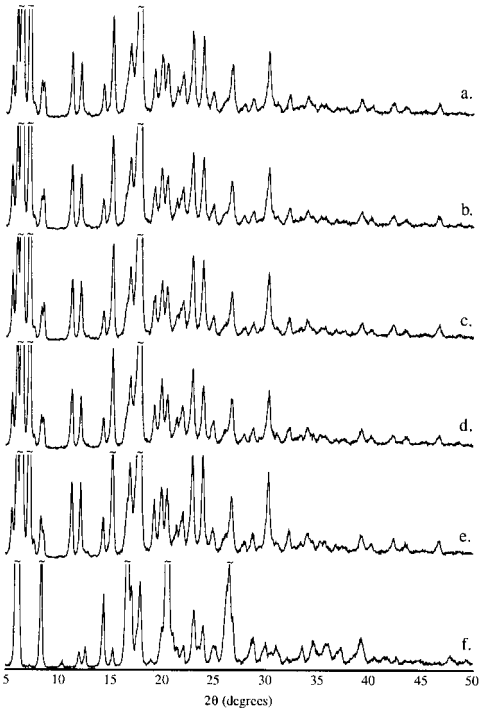

Figure 16. Powder X-ray diffraction patterns of: (a) neotame anhydrate generated by placing the original monohydrate under vacuum (~1 Torr) for three days; (b) after being sealed in a jar for two days; (c) after four days; (d) after six days; (e) after eight days; and (f) after being placed in a relative humidity environment of 84% for 12 days.

spectra are expanded in Fig. 14. This region, approximately 135–139 ppm, can be used to identify the forms present in a particular sample. For instance, the sharp peak at 138.2 ppm for C7 in the monohydrate (see Fig. 14a) is not present in Fig. 14b, which indicates that the converted sample does not contain any crystalline monohydrate. However, the spectrum in Fig. 14b does exhibit two resonances for C7. The broad peak centered on 138.4 ppm cannot be ascribed to a monohydrate form, given the low water content of this sample. Rather, this broad peak is likely due to an amorphous anhydrate phase. The much sharper peak at 135.6 ppm probably corresponds to a crystalline anhydrate, based on the low water content of the sample. The broad resonance for C7 centered around 138.3 ppm in the spectrum of the amorphous anhydrate matches the broad resonance for C7 in Fig. 14b as well, confirming the presence of the amorphous anhydrate in that sample.

Additional experiments indicate that the multiple forms of neotame reconvert over time to the stable monohydrate under ambient conditions. Our objective was to observe this conversion with both ^{13}C CP/MAS NMR spectroscopy and powder X-ray diffraction. The starting material chosen was the sample shown in Figs. 12c, 13c and 14c because it contained two different crystalline anhydrate forms, indicated by two sharp resonances for C7. The phenyl region of the ^{13}C CP/MAS NMR spectrum and the powder X-ray diffraction pattern have been reproduced in Figs. 15a and 16a. As mentioned previously, the water content of this material was 0.7%. The material was then sealed in a glass jar with a foil lid. Each day for the next eight days, samples were removed for ^{13}C CP/MAS NMR spectroscopy, powder X-ray diffraction, and Karl Fischer analysis, and the rest of the material was resealed in the jar. The results of these experiments are shown in Figs. 15b–e and Figs. 16b–e.

After two days, the ^{13}C CP/MAS NMR spectrum (Fig. 15b) showed significant differences from that of the initial material (Fig. 15a) while the powder X-ray diffraction pattern (Fig. 16b) remained unchanged. The water content increased slightly to 1.0±0.7%. The intensity of the resonance at 135.5 ppm (Fig. 15b) did not change significantly while the peak at 136.4 ppm dramatically decreased. New resonances appeared at 137.0, 137.5, and 138.1 ppm. The peak at 138.1 ppm is possibly due to the monohydrate, but the origin of the peaks at 137.0 ppm and 137.5 ppm is likely due to additional forms of neotame not previously observed. Whether these resonances are due to anhydrous or hydrated forms is difficult to determine because the small quantities present will not significantly change the water content.

Four days after the material had been removed from the vacuum, the water content remained relatively unchanged at 0.96±0.03%. Changes are evident in the ^{13}C CP/MAS NMR spectrum (Fig. 15c), but again there is no significant difference in the powder X-ray diffraction pattern (Fig. 16c). The resonance at 136.3 ppm continues to decrease in intensity, while the peaks at 136.9, 137.4, and 138.0 ppm increase in intensity. These results suggest that the crystalline anhydrate at 136.3 ppm is unstable under ambient conditions and is converting to the forms indicated by the three downfield resonances.

After six days the ^{13}C CP/MAS NMR spectrum (Fig. 15d) was still changing while the powder X-ray diffraction pattern (Fig. 16d) was not. The water content was 1.2±0.1%. The resonance at 135.5 ppm has the same relative intensity as before. The peak at 138.1 ppm increased in intensity and the peak at 136.4 ppm continued to decrease. However, the resonances at 136.6 and 137.0 ppm lost some intensity which indicated that these forms were probably converting rapidly to the form indicated by the peak at 138.1 ppm.

Eight days after the material had been removed from the vacuum the same trends were observed. The water content remained relatively unchanged at 1.21±0.01% with no significant differences observed in the powder X-ray diffraction pattern (Fig. 16e). In the ^{13}C CP/MAS NMR spectrum (Fig. 15e), the peak at 138.2 ppm increased in intensity while the resonances at 136.6, 137.0, and 137.5 ppm decreased to levels that could not be detected easily.

To confirm that the material fully reconverts to the monohydrate, the lid was removed from the jar which was then placed in a sealed vessel over a saturated solution of potassium chloride that maintained the relative humidity at 84%. After 12 days, the water content of the material had increased to 5.2±0.2% and both the ^{13}C CP/MAS NMR spectrum (Fig. 15f) and powder X-ray diffraction pattern (Fig. 16f) matched those of the monohydrate. These observations indicated that all of the new forms revert to the monohydrate after 12 days at 84% relative humidity.

The fact that the powder X-ray diffraction pattern did not change during the eight days while the conversion was occurring is surprising. The ^{13}C CP/MAS NMR spectra clearly indicate that neotame has multiple forms. We wanted to ensure that form interconversion was not occurring during the acquisition of the powder X-ray diffraction pattern and that the samples removed from the jar for the three different analyses were identical. While the jar was stirred thoroughly each day, it is possible that only part of the material in the jar (perhaps that near the surface) converted and that a large proportion of this material was inadvertently packed into the solid-state NMR rotor. The possibility arises that the material being packed into the powder X-ray diffraction holder may not have converted, which would explain the lack of change in the powder patterns. To test this possibility, the analytical procedure was altered on the eighth day. Instead of taking separate samples from the jar for both ^{13}C CP/MAS NMR and powder X-ray diffraction, only one sample was taken and was subjected to both techniques. The results obtained using this protocol are shown in Figs. 15e and 16e. No changes in the reported trends were observed. This result confirmed that the method used to obtain the samples was not the source of the discrepancy.

One possible explanation for the absence of change in the powder X-ray diffraction patterns is that one or more forms is not observed using powder X-ray diffraction. Each component of a mixture of forms should have a characteristic diffraction pattern independent of the other components. Based on this fact, powder X-ray diffraction has long been used for quantitative analysis. However, it is known that minor components (usually $<0.5\%$ w/w) in mixtures of crystalline solids are often undetectable by powder X-ray diffraction.[8] The amounts of the minor components in the mixtures of forms of neotame under analysis are generally larger than 0.5% based on the approximate integrated intensities of the NMR resonances. For example, the ^{13}C CP/MAS NMR spectrum of the eight-day sample (Fig. 15e) shows that approximately 30% of the sample has a different form from the starting material (Fig. 15a). The powder X-ray diffraction pattern of this sample (Fig. 16e) however, is not significantly different from that of the starting material (Fig. 16a). Another explanation is that only the molecular conformation is changing between the minor forms in the mixture and the unit cell parameters are not significantly different. ^{13}C CP/MAS NMR spectroscopy is sensitive to changes in local order, such as differences in molecular conformation, while powder X-ray diffraction is sensitive to changes in long-range order, such as differences in d-spacings. The results indicate that the changes in molecular conformation between the minor components in the mixtures of neotame are not sufficient to alter the lattice planes in the crystal structure.

We have shown that the existence of multiple forms of neotame is required to explain the ^{13}C CP/MAS NMR results. A monohydrate has been generated as a pure phase and has a characteristic resonance for carbon C7 at 138.2 ppm. This monohydrate is the most stable form of neotame in the presence of moisture. An amorphous anhydrate has also been prepared as a pure phase and gives a very broad peak for C7 centered around 138.4 ppm. The stability of the amorphous anhydrate is not exactly known but it is thought to crystallize very slowly and eventually convert to the monohydrate. A crystalline anhydrate has been observed in a mixture of the crystalline and amorphous anhydrate and also in a mixture of two crystalline anhydrates; this form yields a characteristic resonance for C7 at 135.5 ppm. This crystalline anhydrate is probably the most stable form of neotame under reduced pressure ($\sim$100 Torr) but is also relatively stable under ambient conditions. The second crystalline anhydrate has only been observed in a mixture of the crystalline anhydrates and is only stable under vacuum ($\sim$1 Torr); it gives a characteristic resonance for C7 at 136.5 ppm. The relative stabilities of the two crystalline anhydrates at vacuum ($\sim$1 Torr) is unknown but work is currently underway to determine whether one form converts to the other over time.

Two additional resonances have been observed in the quaternary phenyl region of the spectrum, one at 136.9 ppm and the other at 137.5 ppm. These peaks have thus far been observed only in mixtures of multiple forms during the reconversion of neotame. The low water contents of the samples in the conversion experiment suggest that these two additional forms are anhydrous. However, the relatively low concentrations ($<10\%$) of these forms precludes determining the water stoichiometry for these samples. Regardless of the stoichiometric water content of these forms, they do not seem stable under vacuum or under ambient conditions. These two forms are only present while the two anhydrates mentioned in the previous paragraph convert to the monohydrate.

There is reason to believe that the resonance appearing at 138.2 ppm during the reconversion of neotame is not the monohydrate previously observed at that chemical shift. While the assignment of this peak is still unclear, the low water content of samples that contain this form suggests this form is not likely to be the monohydrate. It is possible that this resonance is due to another anhydrate and that, during the conversion, the neotame molecules assume the same molecular conformation as the monohydrate without the stoichiometric water. This hypothesis explains why the powder X-ray diffraction patterns do not change during the conversion. The molecular conformation of neotame changes even without the water of hydration but the unit cell parameters do not change until water is present during the rehumidification. Work is currently under way to generate pure phases of each of the new forms, to determine their water contents, and to acquire their ^{13}C CP/MAS NMR spectra and powder X-ray diffraction patterns.

Unlike two of the three forms of aspartame,[17,18] none of the multiple forms of neotame appear to have multiple peaks in their ^{13}C CP/MAS NMR spectra due to crystallographically inequivalent sites. The monohydrate (C7 peak at 138.2 ppm), the crystalline anhydrate (C7 peak at 135.5), and the other crystalline anhydrate (C7 peak at 136.4 ppm) clearly have one resonance for each carbon. The only possibility for multiple peaks due to crystallographically inequivalent sites are the two unknown forms indicated by the peaks for C7 at 136.9 and 137.4 ppm. It is possible that

these resonances result from one form of neotame that contains two distinct molecular conformations. The two peaks maintain the same behavior during conversion, because they simultaneously increase and decrease together during the experiment (Fig. 15). However, it is difficult to make a definitive prediction primarily because of the low intensities of these peaks. It is equally probable that the two peaks result from two different polymorphs of neotame. We are currently working to generate pure phases of the new forms of neotame that will provide evidence to answer these questions.

Conclusions

The results presented here show that solid-state NMR spectroscopy is a powerful tool for studying molecular conformation among polymorphs of crystalline organic materials, both in bulk and in mixtures of solid forms. Differences in the one-dimensional NMR spectra between polymorphic forms allow conclusions about differences in conformation and packing to be drawn. Two-dimensional NMR experiments on uniformly ^{13}C-labeled materials using very high spinning speed and decoupling power to average $^{13}C-^{13}C$ and $^{13}C-^{1}H$ dipolar interactions allow resonances to be assigned and connectivity to be traced in molecules which contain crystallographically inequivalent sites. The ultimate goal of our research is to use the information from solid-state NMR spectra to obtain structural information about polymorphs for which crystal structures are not available. In order to achieve this goal, it is first necessary to be able to elucidate as much information as possible from the solid-state NMR spectrum. Using very high spinning speed and decoupling power, we have shown that it is now possible to fully assign most resonances due to crystallographically inequivalent sites in complex crystalline organic materials. However, care must be taken that form interconversion does not occur due to high-speed magic-angle spinning. Because of the advantages of using solid-state NMR spectroscopy, it is important that powder X-ray diffraction no longer be regarded as the exclusive technique in the determination of the existence of polymorphism. ^{13}C CP/MAS NMR and powder X-ray diffraction must be used as complementary techniques in the structural characterization of polymorphs.

Acknowledgements

The authors would like to thank Dr Cindy Ridenaur at Varian NMR for the use of a 2.5 mm spinning module and probe. We would like to thank The Nutrasweet Company for providing resources for the synthesis of ^{13}C-labeled aspartame and for the neotame materials. We would like to thank the 3M Company for financial support and the University of Minnesota for a Grant-in-Aid. We would also like to thank Professors Klaus Schmidt-Rohr and Raj Suryanarayanan for insightful discussions.

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