Two-Dimensional High-Speed CP/MAS NMR Spectroscopy of Polymorphs. 1. Uniformly ^{13}C-Labeled Aspartame

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Abstract: Three forms of crystalline aspartame have been observed: two hemihydrate polymorphs and a dihemihydrate. The ^{13}C CP/MAS NMR spectra of two of the forms of aspartame showed that certain carbons have up to three resonances due to different conformations/arrangements of molecules in the asymmetric unit cell. Techniques for assigning resonances based upon the number of attached protons or *J* couplings were not effective because the multiple resonances arise from the same carbon in the molecule. We used two-dimensional exchange experiments on uniformly ^{13}C-labeled aspartame to assign the spectra of aspartame. Experiments performed with typical MAS rates (7 kHz) and ^{1}H decoupling powers (63 kHz) of uniformly ^{13}C-labeled aspartame were uninformative because ^{1}H−^{13}C and ^{13}C−^{13}C dipolar couplings significantly broadened these resonances. Increasing the spinning rate to 28 kHz and the ^{1}H decoupling power to 263 kHz increased the resolution sufficiently to observe crystallographically inequivalent sites. Two-dimensional radio frequency driven dipolar recoupling (RFDR) and exchange experiments using very high spinning speed and decoupling power gave complimentary assignment information for short (1−2 bond) and long (>3 bonds) range interactions in the two polymorphic forms. For one form of aspartame, peaks were assigned to aspartame molecules in three inequivalent crystalline environments.

Introduction

Approximately 30% of organic compounds are believed to crystallize in two or more forms that differ in the conformation and/or arrangement of the molecules in the crystal lattice.[1,2] This effect, known as polymorphism, is important in the formulation of pharmaceuticals, pigments and dyes, explosives, and agrochemicals. Polymorphic transformations during processing can be problematic, because they may cause physical properties such as density, melting point, and solubility to change.[3]

Polymorphism has been investigated extensively, including fundamental studies of the formation of polymorphs,[4,5] computational predictions of polymorphic structure,[6,7] and physical characterization.[8−12] Characterization of polymorphic forms is typically accomplished using a combination of diffractometry (single crystal and powder X-ray diffraction) and spectroscopic techniques (such as infrared spectroscopy). Single-crystal X-ray diffraction is perhaps the best method for characterizing the structures of polymorphs, since it provides detailed structural information for the compound being investigated.[13,14] However, it requires a single crystal of the appropriate dimensions and of high crystallinity for X-ray analysis, which is often difficult for certain polymorphic forms.[15] Powder X-ray diffraction (PXRD) is commonly used for the characterization of polymorphs. Because PXRD is sensitive to long-range order, particle size or preferred orientation effects may cause an incorrect identification of polymorphic forms.[16] Spectroscopic techniques such as infrared (IR) spectroscopy can provide useful information about the hydrogen-bonding environment of polymorphs.[17,18]

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy using cross polarization[19] and magic-angle spinning[20] has

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emerged as a very powerful technique for analysis of polycrystalline organic compounds that exhibit polymorphism.[3,7,15,17,21−30] Solid-state NMR can be used to identify the number of crystallographically inequivalent sites in the unit cell[29] and to understand the molecular structure based on the chemical shift of the individual resonances.[30] Before structural information can be obtained from the solid-state NMR spectrum, each of the peaks in the spectrum must be assigned. A fundamental problem with assigning chemical shifts in solid forms of a compound is that the 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 carbon due to crystallographically inequivalent sites in the unit cell. Assignments are typically made in solid-state NMR experiments using information from solution-state NMR experiments, solid-state experiments such as interrupted decoupling,[31] and effects such as peak splitting due to $^{13}C-^{14}N$ coupling.[32,33] 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.[34] Cross-peaks typically indicate carbons that are either directly bonded or are 2−3 carbons away. Often experiments such as RFDR,[35] MELODRAMA,[36] DRAWS,[37] C7,[38] and POST-C7[39] are used to refocus dipolar couplings and to enhance magnetization transfer. These techniques have been used quite successfully to assign small peptides.[40] 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.[41] In biological samples where crystallinity is often low, the lines are broad, especially compared to highly crystalline organic compounds. There is usually sufficient resolution in the cross-peaks of a 2D NMR spectrum to identify resonances that overlap on the diagonal. 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 co-workers have found that line widths comparable to those of unlabeled compounds can be obtained in uniformly ^{13}C-labeled crystalline organic compounds by using spinning speeds on the order of 35 kHz with ^{1}H decoupling powers on the order of 250 kHz.[42]

Recently much effort has been put forth using computational methods to predict crystal structures of small organic molecules for which single crystals suitable for single-crystal X-ray diffraction studies were not available.[8−12,43−46] The use of two-dimensional solid-state NMR studies on uniformly labeled materials can provide unique information about the structure of these materials by providing information on connectivity. In addition, NMR spectroscopy is sensitive to the local electronic environment of a molecule, so changes in conformation or packing of a compound in the solid state can significantly affect chemical shifts. This information can be used in conjunction with computational methods to eliminate unlikely high-energy conformations and shorten the structure prediction process.

In this paper we used two-dimensional ^{13}C CP/MAS NMR with high spinning speed and high ^{1}H decoupling power to study polymorphic forms of aspartame. Aspartame (L-aspartyl L-phenylalanine methyl ester) is a commonly used sweetener in low calorie food products such as diet soft drinks, because it is about 150−200 times sweeter than sucrose.[47] Three distinct forms of aspartame are known to exist, two hemihydrate polymorphs (Forms I and II) and a dihemihydrate (Form III).[26] Form I is prepared by recrystallization from a quaternary solvent mixture[48] or by grinding Form II in a ball mill for 30 min.[26,27] Form III is obtained by recrystallization from water under ambient conditions. Form III may also be prepared by exposing either Form I or Form II to a high humidity environment (>98% relative humidity)[49] for 5 days.[26] Only the crystal structure of Form I has been reported.[48] We used two-dimensional exchange with and without dipolar recoupling to study Forms I and II of aspartame. Connectivity between resonances due to crystallographically inequivalent molecules in the unit cell was observed for one form of aspartame. This is the first time that connectivity between resonances was followed through each of the crystallographically inequivalent molecules in a polymorphic form of a compound.

Experimental Section

Solid-State NMR Spectroscopy. All ^{13}C spectra were acquired at 75.4 MHz (7 T static magnetic field) with a Chemagnetics CMX-300 solid-state NMR spectrometer and were externally referenced to TMS

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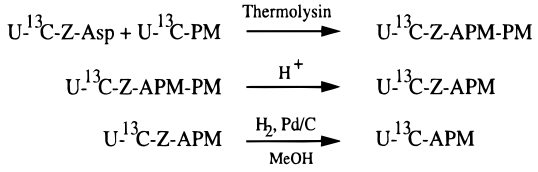
by using the methyl peak of hexamethylbenzene (17.35 ppm). Three different spinning modules were used in this investigation: a 7.5 mm Varian spinning module was used for experiments with spinning rates up to 7 kHz, a 3.2 mm Varian spinning module was used for spinning rates up to 24 kHz, and a 2.5 mm Varian spinning module was used for spinning rates up to 28 kHz. A 63 kHz decoupling field was used with the 7.5 mm spinning module, a 200 kHz decoupling field was used with the 3.2 mm spinning module, and a 263 kHz decoupling field was used with the 2.5 mm spinning module. Spectra acquired with the 3.2 mm and 2.5 mm spinning modules utilized variable-amplitude cross polarization (VACP)[50] (5 ms contact time) and two-pulse phase modulation (TPPM)[51] decoupling. 2048 data points were acquired for one-dimensional experiments, with a sweep width of 30 kHz.

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) with 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 with use of a sweep width of 15 kHz. Symmetrization was performed on the spectra of Form II. All cross-peaks observed in these spectra were also present in the unsymmetrized spectra.

Synthesis of Uniformly ^{13}C-Labeled Aspartame. ^{13}C-labeled aspartame was prepared according to the literature procedure for nonlabeled aspartame as shown in Scheme 1.[52] Uniformly ^{13}C-labeled *N*-α-carbobenzyloxyl-L-aspartic acid (U-^{13}C-Z-Asp) and uniformly ^{13}C-labeled L-phenylalanine methyl ester·HCl (U-^{13}C-PM·HCl) were purchased from Cambridge Isotope Laboratories. Thermolysin (43 U/mg of solid) was obtained from Sigma, and 5% palladium on carbon was obtained from Aldrich. TLC was performed on precoated silica gel plates (Merck) in a *n*-BuOH:H_2O:AcOH (3:1:1) solvent system. HPLC was performed on a Beckman ultrasphere octyl semipreparative column with 15% acetonitrile in sodium phosphate buffer pH 2.2 as the mobile phase. High-resolution mass spectrometric analysis (EI+) was performed on a Finnigan MAT-95 HRMS instrument.

Scheme 1



U-^{13}C −*N*-α-Carbobenzyloxy-L-aspartyl-L-phenylalanine, Methyl Ester-L-phenylalanine, Methyl Ester Complex (U-^{13}C-Z-APM·PM Complex). Water (2.5 mL) was added to a mixture of U-^{13}C-Z-Asp (248 mg; 0.92 mmol) and U-^{13}C-Z-PM·HCl (500 mg; 2.22 mmol). The pH of this solution was adjusted to 6.5 with 10 M HCl and the final volume adjusted to 3 mL. Calcium acetate (9 mg; 20 mM) was added to this solution. The reaction was initiated by addition of 30 mg of Thermolysin. The reaction mixture was placed in a shaker incubator at 37−38 °C overnight. After this time, the consistency of the off-white mixture was between an off-white very thick suspension and solid. The mass was broken up with a glass rod and washed five times by centrifugation with 5 mL of ice-cold water each time. Samples taken showed identical TLC results as the unenriched analogue. Rfs: Z-APM, 0.75; PM, 0.56.

U-^{13}C−N-α-Carbobenzyloxy-L-aspartyl-L-phenylalanine, Methyl Ester- (U-^{13}C-Z-APM). The free U-^{13}C-Z-APM was obtained by triturating the U-^{13}C-Z-APM·PM complex with ice-cold 0.25 M HCl (4 mL) for 10 to 15 min. The resulting solid was washed and centrifuged five times with ice-cold water (5 mL each). Rf: Z-APM, 0.75.

U-^{13}C-L-aspartyl-L-phenylalanine, Methyl Ester- (U-^{13}C-APM). U-^{13}C-Z-APM was placed in a Parr bottle containing water (2 mL) and methanol (10 mL). After the mixture was flushed with argon, 5% Pd/C was added (50 mg). The mixture was then hydrogenated for 2 h. After this time, no residual starting material was observed by TLC. The slurry was diluted by additional methanol (35 mL) and filtered over Celite. The solvent was removed under vacuum. The white solid was resuspended in water and lyophilized. U-^{13}C-APM was obtained as a white fluffy powder in 67% overall yield (190 mg; 0.62 mmol): mp 240−241 °C. Anal. Calcd for $C_{14}H_{18}N_2O_5$: N, 9.09. Found: N, 8.93. HRMS (EI+) *m/z* calcd for $C_{14}H_{18}N_2O_5$ 308.1685, found 308.1659. HPLC indicated a purity of 99.69%. U-^{13}C-APM co-eluted with standard APM on HPLC at 27.7 min under the conditions described above.

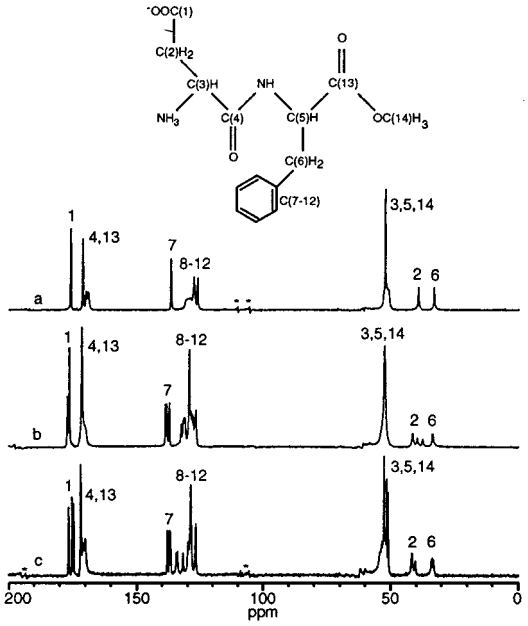


Figure 1. ^{13}C CP/MAS NMR spectra of the three forms of aspartame: (a) Form I, hemihydrate, recrystallized from a quaternary solvent mixture; (b) Form II, hemihydrate, as received from NutraSweet Kelco Co.; (c) Form III, dihemihydrate, prepared by placing Form II in an environment of high relative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

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.[27,48] Aspartame Form II is the material as received from Nutrasweet. This form can be generated by recrystallization from water and drying at ambient conditions (<60% RH) for 5 days. For two-dimensional exchange experiments, uniformly ^{13}C-labeled aspartame was diluted to 20% with unlabeled aspartame and prepared by using the same procedures as for the unlabeled material.

Results and Discussion

Figure 1 shows the structure of aspartame and the ^{13}C CP/MAS NMR spectra of the three forms of aspartame acquired using total sideband suppression (TOSS) at a MAS rate of 6 kHz with 63 kHz ^{1}H decoupling.[53] 1024 transients were averaged with a 5 ms contact time and a 3 s pulse delay. 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

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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. Tentative assignments were made based upon interrupted decoupling experiments and peak splitting due to $^{13}C-^{14}N$ dipolar coupling and were confirmed by 2D exchange experiments (vide infra).[31−33] The spectrum can be divided into three distinct regions. The region from 170 to 180 ppm contains carbons 1, 4, and 13. The peak for carbon 4 in Figure 1a is identified by a characteristic 1:2 splitting due to coupling with ^{14}N.[32,33] Of the three carbons, only the peaks assigned to carbon 1 are significantly affected by changing forms. The aromatic region of the spectrum from 125 to 140 ppm changes dramatically with each form. The peaks corresponding to carbon 7 in all three forms were assigned using interrupted decoupling. In Figure 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. 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 solution, but the direction of shift for each of the resonances (upfield or downfield) cannot be determined. In Form II (Figure 1b) there are at least three peaks for each carbon, two of which apparently overlap for carbons 2 and 6. Complete assignment of these spectra is not possible without the use of two-dimensional NMR techniques.

Figure 2 shows the ^{13}C CP/MAS NMR spectra of 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 part a. The spectrum of 100% labeled sample acquired under identical conditions (7 kHz spinning speed, 63 kHz decoupling field) is shown in part b. The resolution is significantly degraded in Figure 2b, primarily because of increased $^{13}C-^{13}C$ and $^{13}C-^{1}H$ dipolar interactions. Zilm and co-workers 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.[42] 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 Figure 2c. 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 acquired with 27 kHz spinning speed and 263 kHz ^{1}H decoupling with TPPM is shown in Figure 2d. $^{13}C-^{13}C$ *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.

2D exchange experiments were used to unambiguously assign the NMR spectrum because most of the peaks in the NMR spectra of uniformly ^{13}C-labeled compounds were resolved.

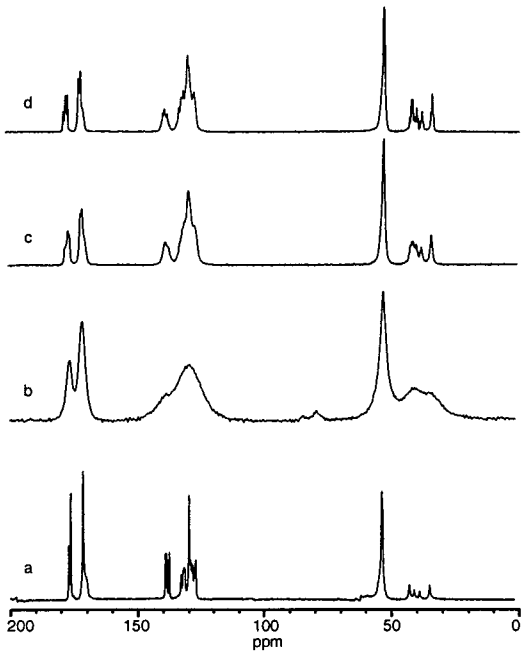

Figure 2. ^{13}C CP/MAS NMR spectra of Form II of aspartame acquired with use of different spinning speeds and decoupling powers: (a) unlabeled aspartame, 7 kHz MAS and 63 kHz ^{1}H decoupling; (b) 100% uniformly ^{13}C-labeled aspartame, 7 kHz MAS and 63 kHz ^{1}H decoupling; (c) uniformly ^{13}C-labeled aspartame diluted to 20% in unlabeled aspartame, 24 kHz MAS and 150 kHz ^{1}H decoupling with TPPM; and (d) uniformly ^{13}C-labeled aspartame diluted to 20% in unlabeled aspartame, 27 kHz MAS and 263 kHz ^{1}H decoupling with TPPM.

Having resolved *J* couplings present means that COSY and INADEQUATE are possible methods for assigning the spectrum. Another method, which we have employed in this paper, is to rely on dipolar couplings between ^{13}C nuclei to trace the path of magnetization through the molecule. The sequence used is equivalent to a 2D chemical exchange or NOESY sequence except that magnetization is transferred via spin diffusion rather than chemical exchange. Magnetization transfer can be enhanced by using the dipolar recoupling sequences previously mentioned (RFDR, DRAWS, MELODRAMA). RFDR was chosen for these experiments, although any of the above sequences would also work. Another concern was the possibility of intermolecular dipolar coupling, which would complicate the interpretation of the NMR spectrum by introducing cross-peaks in the spectrum between molecules in crystallographically inequivalent sites. A dilution of $<5\%$ uniformly ^{13}C-labeled material is usually considered sufficient to avoid intermolecular interactions. We chose a 20% dilution as a compromise between increasing sensitivity and avoiding potential intermolecular interactions. At 20% dilution any cross-peaks due to intermolecular interactions should be small.

Figure 3 shows the two-dimensional exchange spectrum of aspartame crystallized in Form I. A spinning rate of 20 kHz with 200 kHz ^{1}H decoupling and a mixing time of 500 ms was used. From this spectrum, the resonances can be assigned by tracing connectivity patterns through 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; C3 and C4; C5 and C6, C(8−12), C13; C6 and 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 Figure 1. The resolution of the spectrum in Figure 3 is sufficient to assign all of the

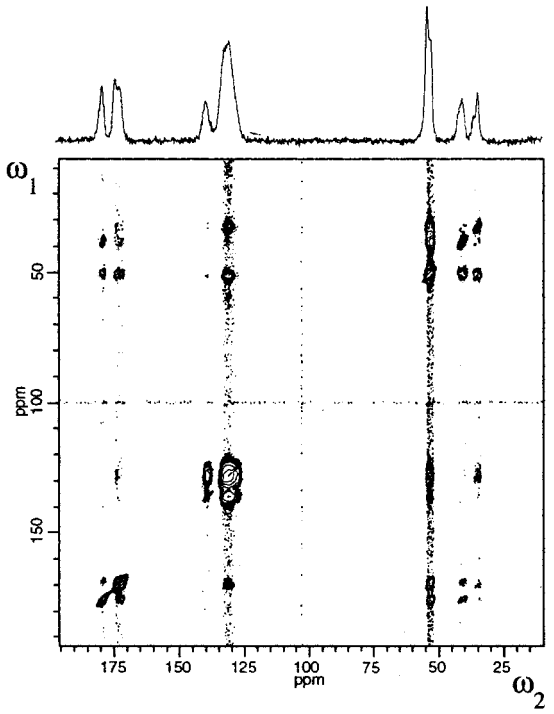

Figure 3. Two-dimensional exchange spectrum of 20% uniformly ^{13}C-labeled aspartame (Form I) diluted in unlabeled aspartame acquired at a spinning speed of 20 kHz with 200 kHz ^{1}H decoupling and a mixing time of 500 ms.

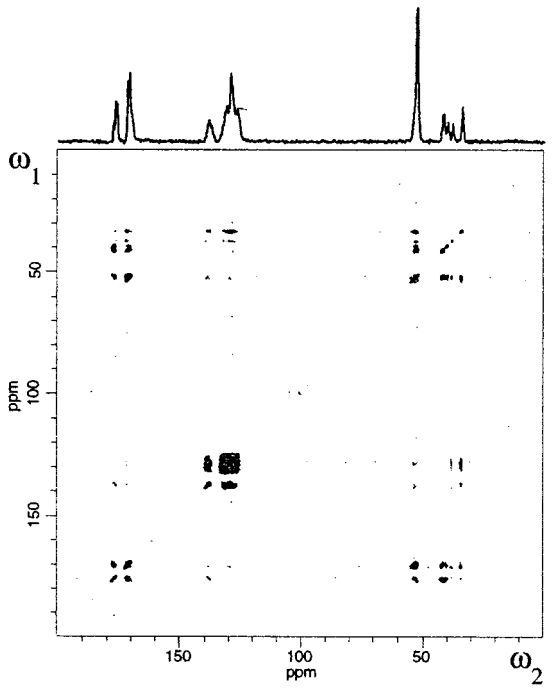

Figure 4. Two-dimensional RFDR spectrum of 20% uniformly ^{13}C-labeled aspartame (Form II) diluted in unlabeled aspartame acquired at a spinning speed of 15 kHz with 263 kHz ^{1}H decoupling and a mixing time of 20 ms (128 rotor cycles).

carbons except carbons 8−12 in the aromatic ring. The resolution in this spectrum is not adequate to distinguish individual resonances for the same carbon caused by multiple crystallographically inequivalent sites such as in Forms II and III.

Figure 4 shows the 2D exchange spectrum of 20% uniformly ^{13}C-labeled aspartame in Form II diluted in a matrix of unlabeled

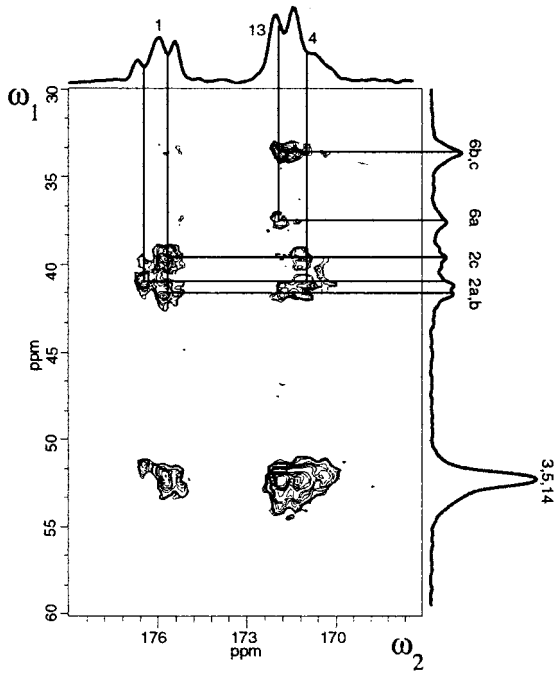

Figure 5. Expansion of Figure 4, showing the region from 30−60 ppm in the first dimension and 167−179 ppm in the second dimension. Connectivity is indicated by lines drawn to cross-peaks.

aspartame. This spectrum was acquired with 15 kHz spinning speed, 263 kHz ^{1}H decoupling with TPPM, and a mixing time of 20 ms utilizing RFDR (128 rotor cycles) to refocus the ^{13}C−^{13}C dipolar interactions. The resolution is significantly better than that obtained in Figure 3. The resulting spectrum contains primarily one-bond couplings, although some multiple bond couplings are also evident. Cross-peaks are evident between C1 and C2, C3, C4; C2 and C3, C4; C3 and C4; C5 and C6, C13; C6 and C7, C8−C12, C13; and C7 and C8−12. The resolution in the spectrum allows the individual resonances due to the multiple crystallographically inequivalent sites to be identified. In the region 30−40 ppm, four peaks are present along the diagonal, corresponding to the four peaks observed in the unlabeled material (Figure 1b). In other regions of the spectrum the resolution is not sufficient to identify individual resonances. For example, the three peaks due to carbon 7 are not clearly resolved, nor is it easy to distinguish the two peaks of carbon C1. Figure 5 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. In Figure 5 the correlations between carbons C1 and C2 can be seen. The larger peak of C1 is correlated with both the large and small peaks of C2. The smaller peak of C1 is correlated with the large peak of C2. The correct molecular assignments are the following: conformation 1, C1 (176.3 ppm) and C2 (41.6 ppm); conformation 2, C1 (176.3 ppm) and C2 (39.7 ppm); and conformation 3, C1 (177.1 ppm) and C2 (41.6 ppm). This somewhat surprising result could only be obtained by having sufficient resolution to separate the peaks due to the crystallographically inequivalent sites. The 1D projections are broadened

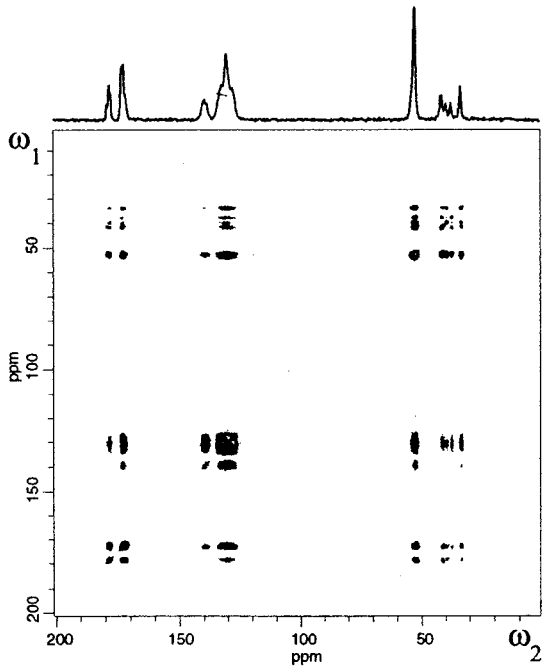

Figure 6. Two-dimensional exchange spectrum of 20% uniformly ^{13}C-labeled aspartame (Form II) diluted in unlabeled aspartame acquired at a spinning speed of 26 kHz with 263 kHz ^{1}H decoupling and a 2.5 s mixing time.

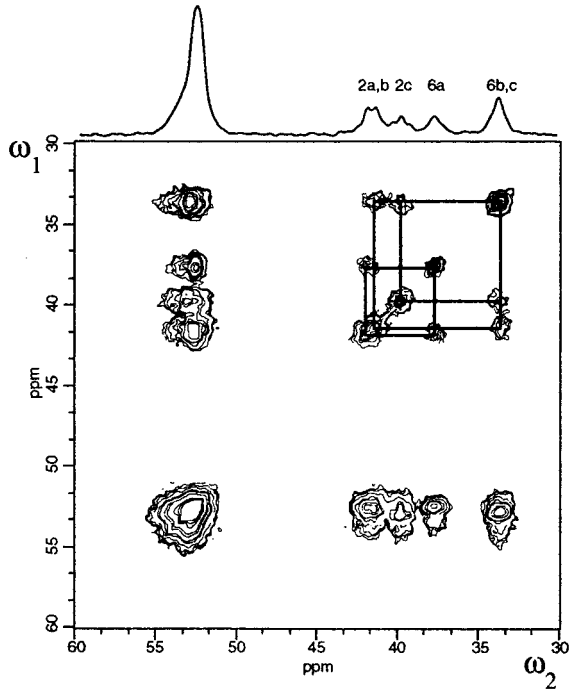

Figure 7. Expansion of Figure 6, showing the region from 30−60 ppm in both dimensions. Connectivity between crystallographically inequivalent sites is indicated by boxes connecting cross-peaks.

due to the convolution of ^{13}C−^{13}C J couplings and multiple resonances due to crystallographically inequivalent sites. The center of the cross-peaks for carbons such as carbon 1 line up with the expected center of these resonances.

The spectrum in Figure 4 should be sufficient to permit the assignment of all of the peaks in the NMR spectrum by following the connectivity pattern indicated by the cross-peaks. However, there are several problems with making full assignments of the spectrum: (1) there is still not sufficient resolution for some of the peaks to be identified in the NMR spectrum (e.g. C8−C12); (2) overlap of chemical shifts such as in carbons C3, C5, and C14 makes it extremely difficult to correlate individual resonances; and (3) many carbons have peaks which do not significantly change in the different forms, such as carbons C4 and C13. For these reasons the assignment information provided by the spectrum in Figure 4 is limited. However, further information can be obtained by relying on spin diffusion to transfer magnetization between all of the carbons in the molecule. Having correlations between all of the carbons in a molecule is usually not helpful in assigning the NMR spectrum of a single compound. However, in this form of aspartame, we can consider each of the three crystallographically inequivalent molecules as representing a unique molecular conformation, with each conformation having its own set of chemical shifts. Since we have an equal mixture of three different conformations, we will rely on the cross-peaks to correlate which peak goes with which conformation. For example, we would like not only to determine if a particular peak is due to carbon 1, but also which of the three peaks assigned to carbon 2 correlates with that particular peak.

Figure 6 shows the 2D exchange spectrum of 20% uniformly ^{13}C-labeled aspartame Form II diluted in a matrix of unlabeled aspartame acquired with 26 kHz spinning speed, 263 kHz ^{1}H decoupling with TPPM, and a mixing time of 2.5 s. The mixing time was chosen to permit almost complete intramolecular spin diffusion to occur. Unlike the previous 2D exchange spectrum acquired with dipolar recoupling in which cross-peaks were strongest between carbons one to two bonds away, the cross-peaks are strongest between carbons whose resonance frequencies are closest together. For example, strong cross-peaks are observed between carbons C2 and C6, although they are separated in the molecule by several bonds. At very fast spinning speeds, both ^{13}C−^{13}C and ^{13}C−^{1}H dipolar couplings are significantly averaged. At these spinning speeds spin diffusion rates are determined by both $1/r^3$ and the degree of overlap of the two resonances. At fast spinning speeds, the overlap is very small for carbons with very different resonance frequencies, and is larger as the difference in frequencies decreases. For example, cross-peaks between carbons 1 and 2 were not present at mixing times <1 s (spectrum not shown), but cross-peaks between carbons 2 and 6 were present. The difference in resonance frequencies is >10 kHz for carbons 1 and 2 and <600 Hz for carbons 2 and 6. We can use the cross-peak information between carbons 2 and 6 to assign the three different conformations of carbon 6. Figure 7 shows the cross-peak region of carbons C2, C3, C5, and C6. Cross-peaks are evident between C2−C6, C2−C3, and C5−C6. As noted previously, the spectrum of unlabeled aspartame suggests that both carbons 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 ratio of 2:1. In Figure 7 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. The smaller peak of C2 is correlated with the large peak of C6. On the basis of these correlations, it is now possible to state that for the three crystallographically inequivalent molecules, 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. An additional correlation that may be possible to obtain in the future is C6−C7, which is now limited primarily by sensitivity, because both peaks are very small and because the corresponding cross-peaks are currently obscured by the noise.

Conclusions

The results presented in this paper show that solid-state NMR is a powerful tool for studying molecular conformation among polymorphs. It is possible using very high spinning speed and decoupling power to effectively average $^{13}C-^{13}C$ and $^{13}C-^{1}H$ dipolar interactions to obtain high-resolution CP/MAS NMR spectra of uniformly ^{13}C-labeled materials, such as aspartame. Two-dimensional solid-state NMR exchange experiments of uniformly ^{13}C-labeled materials are shown to be a valuable tool for tracing connectivity and assigning resonances in molecules which contain crystallographically inequivalent sites. Our ultimate goal is to use information gained from solid-state NMR spectra to obtain structural information about polymorphs for which crystal structures are not available. The first step toward achieving this objective is to correctly assign each resonance in the NMR spectrum. Using very high spinning speed and very high decoupling power, we have shown that it is now possible to make these assignments using standard two-dimensional exchange methods on uniformly labeled compounds.

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