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^{13}C CP/MAS: Application to Glycine

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ABSTRACT:

In a previous article (Concepts Magn Reson May, 2004), the initial setup of the ^{13}C CP/MAS experiment was described. The advantages of the experiment over direct ^{13}C excitation were also given. In this article, the experiment is applied to three polymorphic structures of glycine. Temperature calibration of the experiment to investigate molecular dynamics as a function of temperature is also described. While the ^{13}C CP/MAS experiment is widely applicable, it can not be universally used. The ^{13}C CP/MAS experiment works at ambient temperature for both the α and γ polymorphic structures of glycine. However, the cross-polarization technique fails to provide a signal at low temperature from either polymorph. The failure of the technique for both polymorphs arises from the shortened ^1H $T_{1\rho}$ at low temperatures. This shortened $T_{1\rho}$ results from the hindered rotation of the $-\text{NH}_3$ group. The technique even fails at ambient temperature for β glycine. Again, the reason is a shortened ^1H $T_{1\rho}$.

KEY WORDS:

solid-state NMR; ^{13}C CP/MAS; cross-polarization; magic angle spinning; glycine; variable temperature; polymorphic structures

Introduction

In a previous article (1), the initial setup of the ^{13}C cross-polarization/magic angle spinning (CP/MAS) experiment was described. The one dimensional experiment offers high-sensitivity spectra of natural abundance ^{13}C in solids by providing a polarization transfer from abundant proton spins, by repetition rates determined by the short(er) relaxation times of protons, and by eliminating broadening from both chemical shift anisotropy and dipolar coupling. The experiment is useful for studying both polymorphic structures and molecular dynamics. While the ^{13}C CP/MAS experiment is widely applicable, unfortunately it can not be universally used. Occasionally the experiment will fail to give a ^{13}C signal, with the failure usually a result of molecular dynamics. However, direct excitation of the ^{13}C still provides a signal, though with the loss of the advantages of the cross-polarization. As a particular illustration of the failure of the technique to provide a ^{13}C signal, three polymorphic structures of glycine are studied.

Two of the polymorphic structures are investigated as a function of temperature to elucidate the molecular dynamics. In addition, the temperature calibration of the experiment is described.

Glycine ($\text{C}_2\text{H}_5\text{NO}_2$) is structurally the simplest amino acid. It has no center of chirality. While glycine can exist as a neutral molecule in the gas phase, it exists as a zwitterion in solution and in the solid state.

In the solid state, glycine crystallizes in three forms (α , β , and γ) which have been studied by x-ray (2,3,4) and neutron diffraction (5,6). The α form (Figure 1) consists of hydrogen bonded double layers of molecules which are packed by van der Waals forces. The unstable β form, whose single molecular layers possesses an internal arrangement the same as the α form, readily transforms into the α form in air (3). The γ form (Figure 2) crystallizes with a trigonal hemihedral symmetry. While the γ form is stable at room temperature, it is reported to irreversibly convert to the α form upon heating above 165 C. Polycrystalline samples can be distinguished on the basis of x-ray powder diffraction (7) and by infrared (IR) spectroscopy (3,8).

Glycine has many commercial uses (9) such as flavor masking and enhancement (i.e., carbonated soft drinks based on saccharin), pH buffering and stabilization applications (in antiperspirants, cosmetics, and toiletries), pharmaceutical applications (as an excipient) and as a chemical intermediate. Glycine is also the object of interstellar spectroscopic searches (10) to help answer the question of whether the seeds of life were created in space or on earth.

For nuclear magnetic resonance (NMR) spectroscopists, glycine can serve as a standard for checking and calibrating spectrometer performance or, utilizing the various isotopic enrichments commercially available, for quickly implementing and demonstrating a variety of techniques, such as rotational resonance (11), triple resonance experiments (REDOR) (12), etc. In particular, the characterization of the α and γ polymorphs of glycine by ^{13}C CP/MAS has been previously reported (13). A ^{13}C CP/MAS spectrum of a mixture of the α and γ polymorphs of glycine is shown in Figure 3. The glycine molecules in both forms exist as zwitterions, the only difference in the two forms being the nature of the hydrogen bonding.

Sample Preparation

Relatively pure samples of the three forms of glycine are easily prepared. However, occasionally two or more forms will crystallize simultaneously. The α form can simply be recrystallized by slow evaporation from aqueous solution. This literally means just dissolving the glycine in distilled water in a dish and letting it sit undisturbed until the water has evaporated. The β form requires only slightly more effort. After dissolution of glycine in distilled water, the β glycine precipitates from solution upon rapid addition of ethanol. If the β glycine is not filtered, and the water and ethanol are left to evaporate, there may be some contamination from α glycine. However the β glycine is unstable. It can convert to the α form by mechanical shock or even if left over time in the open air. Finally the γ glycine can be recrystallized with slow cooling from 5% aqueous acetic acid. In addition to characterization by powder diffraction previously mentioned, the literature values for the N-H stretching frequency in the infrared spectra can be used to identify the purified forms. The N-H stretch appears at 3164 cm^{-1} for α glycine, 3191

cm⁻¹ for β glycine, and at 3105 cm⁻¹ for γ glycine. The thermodynamic properties of the polymorphs, along with the sample preparation, have also been reported in the literature (14).

Spectrometer Calibration

The NMR data were obtained with a Bruker Avance 300 NMR spectrometer. The ¹³C CP/MAS spectra were acquired using a ¹H ninety degree pulse width of 3.75 microseconds (μ s), a Hartmann-Hahn contact time of 3 milliseconds (ms), a data acquisition time of 65 ms, and a recycle delay of five times the spin-lattice relaxation time of the protons. These measured relaxation times are given in Table 1. The Bruker probe has a 4 millimeter (outside diameter) rotor which was spun at 5 kHz. A detailed procedure for setting up the ¹³C CP/MAS experiment is given in Reference 1.

Molecular dynamics can be investigated by studies as a function of temperature. Variable temperature work in ¹³C CP/MAS presents several challenges for the spectroscopist. In addition to the concern for calibration of the thermocouple over the temperature range investigated, one must also consider frictional heating of the rotor resulting from aerodynamic drag, radiofrequency induction heating resulting primarily from the ¹H decoupling, and Joule-Thompson cooling and heating (15). The solid-state ²⁰⁷Pb chemical shift of lead nitrate serves as a convenient chemical shift thermometer, both for static and MAS measurements (16-22). A review of solid-state ²⁰⁷Pb NMR spectroscopy, including a section on the lead shift as a thermometer, is given by Dybowski and Neue (23). This approach is convenient when MAS probes can be tuned to both nuclei.

Calibration with the ²⁰⁷Pb chemical shift of lead nitrate begins with the assignment of the measured shift at a known temperature. Then, by measuring the shift at other temperatures, as indicated by the thermocouple in the probe, the actual sample temperature can be calculated. From this information, a calibration chart of the actual sample temperature versus the measured temperature of the probe thermocouple can be created. If the experiment is repeated with the same experimental conditions, then the actual temperature of the sample will be known.

This calibration described above begins with the method for static samples as outlined by Beckmann and Dybowski (22). That is, place a static sample of lead nitrate in the MAS rotor in the probe and allow the sample to equilibrate at the ambient temperature of the probe. A single pulse experiment can be used to acquire the axially symmetric chemical shift powder pattern of the lead nitrate. The prominent spectral feature of the maximum peak near the position of the nonunique chemical shift tensor element can be assigned a value based on the known temperature to provide an absolute scale of temperature. The temperature variation of this spectral feature of the static sample is given (22) by

$$\sigma_{\text{peak}} = -\{3670.6 \pm 1.0 \text{ ppm}\} + \{0.666 \pm 0.003 \text{ ppm/K}\}T \quad (1)$$

with the chemical shifts referenced to tetramethyllead at 295 K and the temperature in K. For the measurements reported in this article, before the NMR experiments were run with

a sample, a second nonmagnetic thermocouple was threaded down the bore of the magnet and through the top of the MAS probe to make a temperature measurement inside the sample coil while the probe was equilibrated in the magnet. This second temperature measurement, performed without a sample and without pulsing, allows an estimation of the accuracy of the thermocouple in the probe at ambient temperatures. After confirming the reading of the thermocouple in the probe in this manner, a measurement on a static lead nitrate sample with chemical shift assignment bypasses problems associated with temperature gradients or other factors arising from MAS or radiofrequency sample heating (as the spectrum is acquired on a static sample with a single pulse of typically only a few microseconds).

With this chemical shift assignment, the calibration chart of the sample temperature versus the thermocouple temperature can be made for the MAS experiment under the conditions to be used, i.e., the spin rate (which will determine the bearing and drive gas flows), the variable temperature gas flow, and even including the effects of radiofrequency decoupling (even though ^1H decoupling is not needed for ^{207}Pb of lead nitrate). For these MAS experiments (over a range from 123 K to 423 K), the sample temperature can be calculated from the measured isotropic shift of the lead nitrate with the linear relation given by Neue and Dybowski (20)

$$\delta_{\text{iso}}(T) = a_0 + b_0 T \quad (2)$$

with $a_0 = -3714.66$ (+/- 0.2) ppm and $b_0 = 0.76$ (+/- 0.01) ppm/K.

Application to Glycine

With the temperature calibration in hand, the ^{13}C CP/MAS spectra of α -glycine can be acquired as a function of temperature, as illustrated in Figure 4. This sample consists of a physical mixture of α -glycine with samarium acetate, whose ^{13}C shifts exhibit Curie law behavior and can also act as a NMR thermometer if one's probe does not tune to ^{207}Pb (24). By 213 K, all spectral traces of the α -glycine have disappeared. However, the spectrum of the samarium acetate is still discernable and indicates that indeed the ^{13}C CP/MAS experiment and hardware are still properly functioning. In other words, the ^{13}C CP/MAS experiment is correctly set up, yet no ^{13}C CP/MAS signal is obtained from the sample of interest.

The question arises as to why the ^{13}C CP/MAS signal from glycine disappears. The answer to this requires a deeper examination at the cross-polarization experiment. The experiment, shown in Figure 5, begins with a ^1H ninety degree pulse on the decoupler channel. The phases of the radiofrequency pulses are defined relative to the axes of the rotating frames of the ^1H and ^{13}C nuclei. While the static laboratory magnetic field provides a common Z axis for both the ^1H and ^{13}C nuclei, the different magnetogyric ratios of the two nuclei mean that the X and Y axes of each rotating frame are not necessarily coincident. In other words, while the rotating frames of both nuclei have a common Z axis, the X and Y axes of each of the two rotating frames may be thought of as independent. If the ^1H ninety degree pulse has a radiofrequency phase of X, then the proton magnetization will be along the -Y axis in the rotating frame. The phase of the ^1H radiofrequency pulse is then switched to the -Y phase in order to spin lock the

magnetization while the ^{13}C radiofrequency pulse is turned on simultaneously. During this time (typically ranging from 0.5 ms to 10 ms), the polarization is transferred from the protons to the carbons. Finally, the ^{13}C radiofrequency pulse is turned off to allow acquisition of the ^{13}C data while the ^1H decoupler remains on during the acquisition. Experimentally the important NMR relaxation time constants are the ^1H and ^{13}C spin-lattice relaxation times (T_1) and the ^1H and ^{13}C spin-lattice relaxation times in the rotating frame ($T_{1\rho}$) (which indicate how long the respective magnetizations may be spin locked). Obviously, if either the ^1H or ^{13}C T_1 s are too short, the spins will return to their equilibrium state along the Z axis before polarization transfer can occur. Also, if the magnetization can not be spin locked long enough for polarization transfer to occur, the cross-polarization experiment will fail.

The answer as to why the α -glycine signal disappears at 213 K and the γ -glycine signal disappears at 268 K lies in simply measuring the relaxation times. These results are given in Table 1. The ^{13}C CP/MAS signals disappear when the ^1H $T_{1\rho}$ s become too short (0.6 ms) for the polarization transfer to occur. It should be explicitly stated that since the ^{13}C CP/MAS experiment failed to give a signal at the low temperatures, these relaxation times were measured by direct excitation techniques. While the reason for the disappearance of the ^{13}C CP/MAS signals at low temperatures for both the α -glycine and γ -glycine is now known, what is going in the crystals to give rise to such results? This question was answered by Andrew (25) in a study of ^1H spin-lattice relaxation and molecular motion in polycrystalline amino acids.

In Andrew's study (25), the ^1H T_1 s were acquired on static, polycrystalline samples as a function of temperature, with results reproduced in Figure 6. These amino acids (which contain no methyl groups) all have a single relaxation minimum, which Andrew fit using the theory from Kubo and Tomita (26). Their theory was an early formulation of the nuclear magnetic resonance phenomenon, including spin lattice relaxation, in terms of rigorous quantum mechanics. In particular, they considered the case of spin-lattice relaxation resulting from dipolar broadening under the influence of random reorientations of molecules or molecular groups in solids, including molecular rotation. Andrew recast their result as the following

$$\frac{1}{T_1} = C\tau_c \left[\frac{1}{1 + (\omega\tau_c)^2} + \frac{4}{1 + (2\omega\tau_c)^2} \right] \quad (3)$$

with the constant C having units of sec^{-2} . Andrew showed that measurements fit well with the assumption of a single correlation time of the form

$$\tau_c = \tau_0 e^{(E/kT)} \quad (4)$$

where E is the activation energy, k is Boltzmann's constant, and T is the temperature. In the limit of fast motion at high temperature ($\omega^2\tau_c^2 \ll 1$), then $T_1^{-1} \sim \tau_c$. In this limit, the relaxation rate is independent of the measurement frequency. In the limit of slow motion at low temperature ($\omega^2\tau_c^2 \gg 1$), the $T_1^{-1} \sim (\omega^2\tau_c)^{-1}$. In this limit, the rate explicitly depends upon the resonance frequency of the measurement. The theory predicts a

minimum rate for $\omega\tau \sim 1$. The thermally driven reorientation of the $-\text{NH}_3$ group causes time-dependent fluctuations in the local field due to the protons. With the strong ^1H homonuclear dipolar coupling to the rest of the protons, this relaxation mechanism provides a single relaxation rate for all the protons. Andrew measured an activation energy for glycine of 28 ± 1 kJ/mole.

McDermott (27) measured the ^1H T_1 s for both α -glycine and γ -glycine under MAS with a "standard cross-polarization inverse-recovery pulse sequence." The reported values for the activation energy are 23 ± 2 kJ/mole for α -glycine and 30 ± 3 kJ/mole for γ -glycine. While Andrew did not specify the form of glycine used in his study, his activation energy agrees within experimental error with that for γ -glycine. McDermott's results were also derived by application of the Kubo and Tomita theory. McDermott pointed out that the constant C of equation 3 has been approximated using the "rather unrealistic assumption" that the molecule is "non-associated, i.e., ignoring the effect of additional couplings to nearby molecules." Both α -glycine and γ -glycine exhibit hydrogen bonding to neighboring molecules. Nevertheless, the theory has worked well in this application.

It should be noted that it is the hydrogen bonding that slows the rotation of the $-\text{NH}_3$ group and raises the minimum in the relaxation time T_1 much closer to ambient temperature, as opposed to the situation commonly encountered for rotating methyl groups where the minimum in the relaxation time is usually found at much lower temperatures. It is the fluctuating local fields from the protons due to this hindered rotation that so effectively reduces the spin lattice relaxation times. Specifically, Andrew's ^1H spin-lattice measurement, made at 60 MHz, shows a minimum T_1 of approximately 50 milliseconds around 280 K (Figure 6).

For the sake of completeness, both the ^{15}N T_1 s and ^{15}N $T_{1\rho}$ s for α -glycine and γ -glycine were measured at ambient temperature via ^{15}N CP/MAS experiments. The results show unexpectedly short values for a small natural abundance, low gamma nucleus in a highly crystalline solid. The values for ambient temperature are given in Table 1. These fast relaxation rates result from the same fluctuating proton local fields due to the hindered rotation of the $-\text{NH}_3$ group. The relaxation rates for ^{15}N at the low temperatures where the ^{13}C CP/MAS signals disappear were not measured since cross-polarization from the protons could not be used. The low frequency and low natural abundance with the anticipated increases in the relaxation times simply proved too great a challenge for successful measurement at low temperatures.

The ^{13}C chemical shift anisotropy for the carbonyls can be measured. Mehring (28) gives the observed shielding σ_{zz} of a spectral line expressed in terms of the Euler angles (α, β) (29) which relate the principal axes of the tensor (σ_{11} , σ_{22} , σ_{33}) to the laboratory by

$$\sigma_{zz} = \sigma_{11} \cos^2\alpha \sin^2\beta + \sigma_{22} \sin^2\alpha \sin^2\beta + \sigma_{33} \cos^2\beta \quad (5)$$

In a polycrystalline powder, all angles (α, β) are equally probable and must be summed over. Eden (30) has written a series of articles on computer simulations in solid-state NMR, including the chemical shift anisotropy and powder averaging. The computer program used for simulations in this article is available online from the research group of Dominique Massiot (31). The spectral fit for α -glycine is shown in Figure 7. The principal components of the chemical shift tensors are given in Table 2. The overlay of

the carbonyl spectra for α -glycine and γ -glycine is shown in Figure 8. The overlay of the two spectra shows that the difference in the isotropic shifts of the two compounds comes primarily from the change in the σ_{22} principal components.

Quantum mechanics provides a formulation for the calculation of physical properties, including chemical shielding (32). In the case of the hydrogen atom (a two-body problem, the proton and the electron), this may be solved analytically. However, a many-electron problem presents a calculational challenge, as this would require knowledge of the distance r_{ij} between electron i and electron j for calculating the potential energies. Hartree (33) proposed writing the wavefunction as a product of one-electron wavefunctions and looking at the interaction of one electron with the effective field of all the other electrons, known as the self-consistent field method. Fock (34) pointed out that one really does not know which electron is in which orbital, specifically that the wavefunction must be antisymmetric with respect to interchange of electrons. While a number of methods for better handling of electron correlation have been developed, Salhub and co-workers used a "sum-over-states density functional perturbation theory" (SOS-DFT) specifically for NMR shielding tensor calculations (35).

Calculations for solid α -glycine using SOS-DFPT (35) show the effect of hydrogen bonds on the components of the carboxyl ^{13}C chemical shift tensor. The molecular models and results are reproduced in Figure 9. The symbols δ_{ij} are used to designate the principal components of the chemical shift tensor. The principal axes are chosen such that δ_{11} is close to the bisector of the OCO angle, δ_{33} is perpendicular to the CO_2 plane, and δ_{22} is orthogonal to δ_{11} and δ_{33} with numerical values relative to liquid benzene.

Upon inclusion of hydrogen bonding to the carboxyl (as opposed to a single zwitterionic molecule), the order of δ_{22} and δ_{33} is reversed. However, it is not until the inclusion of hydrogen bonding to the positive $-\text{NH}_3$ end of the molecule that a significant improvement relative to the previous models is shown and a reasonably good agreement with the experimental values of the carboxyl chemical shift tensor is reached. Salhub and co-workers point out that "this electronic perturbation is transmitted through three bonds to the carboxyl carbon: C-C-N-H!" They also note that the other atoms in this chain are also affected but that their shielding tensors are "much less sensitive than those for the carboxyl carbon." This is attributed to negative ions being typically more polarizable than neutral or positively charged fragments. The experimentally measured change in both the isotropic shift and the chemical shift anisotropy for the ^{15}N is given in Table 2. (Most biochemical applications of ^{15}N NMR have used liquid NH_3 as a secondary reference for ^{15}N in aqueous solutions (36). This referencing has been used here rather than that based on nitromethane. It should also be noted that the relative ^{15}N shifts agree reasonably well with those reported in Reference 8; however, a difference in absolute values results from their use of a saturated aqueous solution of $^{15}\text{NH}_4\text{NO}_3$ as a zero ppm reference.)

With regard to the carboxyl chemical shift tensor, it is rather striking that hydrogen bonding, particularly that of three bonds away, would have such a large and rather selective effect on the δ_{22} component. The effect on the δ_{11} component is small. The δ_{33} component shows virtually no change. This large change in the δ_{22} component is indeed seen in the comparison of the carboxyl chemical shift tensors for α -glycine and γ -glycine shown in Figure 8. Facelli (32) has used calculations to study the dependence of the principal values of the ^{13}C chemical shieldings in carboxylic acids as a function of the

difference between the two C-O bond distances. The difference between the C-O and the C=O bond distances is a "direct measurement of the change in the hybridization of the carbon atom from pure sp^2 hybridization to a situation in which the π electron is equally delocalized between the two C-O bonds." In particular, the shielding component perpendicular to the OCO plane (δ_{33}) is almost independent of the change in bond distances while the other two components are monotonic with the change. Thus the difference between α -glycine and γ -glycine in the hydrogen bonding arrangement can explain the changes in the components of the chemical shift tensor.

Thus far, the discussion has been limited to the α and γ polymorphs of glycine. Both of these forms cross-polarize at ambient temperature. The loss of a ^{13}C CP/MAS signal occurs at low temperatures. However, β glycine does not even cross-polarize at ambient temperature. The reason for this is that the ^1H $T_{1\rho}$ of 0.4 ms (as shown in Table 1) at ambient temperature is too short for the polarization transfer. As pointed out in the Introduction, β glycine is unstable. For this reason, the relaxation times were measured by direction excitation methods on a static sample. Under MAS, β glycine rapidly converts into α glycine, as shown in Figure 10.

Summary

The ^{13}C CP/MAS experiment can be used to investigate both polymorphism and molecular dynamics (with variable temperature) in the solid state. The experiment has a significant sensitivity advantage over direct excitation of the ^{13}C nuclei due to both the polarization transfer from the protons to the carbons and the generally shorter spin-lattice relaxation rates of the protons in comparison to those of the carbons. However, even when the experiment is correctly set up and the hardware is properly functioning, a ^{13}C CP/MAS signal from the sample of interest may not always be obtained. This can result from the molecular physics. When this situation arises, it is necessary to use direct excitation (Bloch decays) to acquire the data. In short, while ^{13}C CP/MAS is widely applicable, it cannot be universally applied. However, the failure to obtain a ^{13}C CP/MAS signal may indicate some interesting molecular dynamics.

For the specific example of glycine, the ^{13}C CP/MAS signals from the α -glycine and γ -glycine polymorphs both disappear at low temperatures. The reason for the disappearing signals is due to the ^1H $T_{1\rho}$ s shortening to the point where ^1H magnetization can not be spin locked long enough for the polarization transfer from the protons to the carbons to occur. This shortening of the ^1H $T_{1\rho}$ is a result of the hindered rotation of the $-\text{NH}_3$ group. For β glycine, the ^1H $T_{1\rho}$ at ambient temperature is too short to allow the polarization transfer to occur.

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Table 1: Relaxation Times

Glycine	Temperature	^1H T_1	^1H $T_{1\rho}$	^{13}C T_1	^{13}C $T_{1\rho}$	^{15}N T_1	^{15}N $T_{1\rho}$
α	296 K	0.25 s	44 ms	12 s (C=O)	180 ms (C=O)	240 ms	93 ms
				11 s (NCH ₂)	43 ms (NCH ₂)		
α	213 K	23 s	0.6 ms	220 s (C=O)	44 ms (C=O)		
				210 s (NCH ₂)	20 ms (NCH ₂)		
β	296 K	29 s	0.4 ms	8 s (C=O)	12 ms (C=O)		
				2.3 s (NCH ₂)	300 ms (NCH ₂)		
γ	296 K	4.0 s	4.1 ms	55 s (C=O)	270 ms (C=O)	150 ms	18 ms
				22 s (NCH ₂)	53 ms (NCH ₂)		
γ	268 K	23 s	0.6 ms	260 s (C=O)	70 ms (C=O)		
				144 s (NCH ₂)	22 ms (NCH ₂)		

Relaxation times for α and γ were measured with MAS. Relaxation times for β were measured with a static sample.

Table 2: Chemical Shifts

Glycine	¹³ C Carbonyl (isotropic) ppm	σ_{11} ppm	σ_{22} ppm	σ_{33} ppm	
α	176.5				MAS
	177.0	243.5	178.9	108.7	Static
	176.5	244	180	105	Ref. 12
β	175.5				MAS
	175.4	243.5	176.5	106.0	Static
γ	174.5				MAS
	174.9	243.2	174.9	106.6	Static
	174.6	243	173	108	Ref. 12
	¹⁵ N (isotropic) ppm				
α	32.3				MAS
	32.5	40.2	33.1	24.3	static
γ	32.9				MAS
	32.8	40.5	33.6	26.7	static

Figure 1: The crystal structure of α -glycine. The zwitterionic glycine molecules align end to end, with the second layer antiparallel to the first. There are four molecules per unit cell. The space group is $P2_1/n$.

Figure 2: The crystal structure of γ -glycine. The zwitterionic glycine molecules align with trigonal hemihedral symmetry. There are three molecules per unit cell. The space group is $P3_2$.

Figure 3: ^{13}C CP/MAS spectrum of a mixture of the α and γ polymorphs of glycine.

Figure 4: ^{13}C CP/MAS spectra from a physical mixture of α -glycine and samarium acetate. Spectrum A is at 294 K. The spectral intensity of α -glycine in spectrum B at 223 K is significantly diminished. At 220 K in spectrum C, the carbonyl of α -glycine is barely visible. By 213 K, all traces of the α -glycine resonances have disappeared from spectrum D. Acquisition conditions are given in the text.

Figure 5: ^1H - ^{13}C cross-polarization pulse sequence. The ^1H ninety degree pulse brings the proton magnetization into the X-Y plane. The ^1H radiofrequency pulse is then phase shifted by 90 degrees to spin lock the proton magnetization. Simultaneously the ^{13}C radiofrequency pulse is applied matching the Hartmann-Hahn condition. This causes the polarization transfer from the protons to the carbons. When the Hartmann-Hahn (contact) pulse is turned off, the ^1H radiofrequency pulse is left on during the ^{13}C data acquisition in order to provide ^1H decoupling.

Figure 6: The variation of proton spin-lattice relaxation time T_1 with inverse temperature T^{-1} for polycrystalline amino acids. Curve 1: glycine (60.2 MHz); curve 2: DL-serine (60.2 MHz); curve 3: DL-serine (38.8 MHz). The full lines are theoretical curves calculated in the manner described in the text (Reference 25). Reprinted with permission from E. R. Andrew, et al, *Molecular Physics*, 1976, Vol. 31, No. 5, 1479-1488.

Figure 7: ^{13}C CP spectrum (top) of the carboxyl of a static sample of α -glycine. The theoretical fit of the ^{13}C chemical shift anisotropy from the software program DMFIT (Reference 31) is shown below. The numerical results for the principal components of the shielding tensor are given in Table 2.

Figure 8: ^{13}C CP spectrum of the carboxyl of static samples of α -glycine and γ -glycine. The change in the σ_{22} component of the shielding tensors is listed in Table 2.

Figure 9: Comparison between the principal components of the glycine carboxyl ^{13}C chemical shift tensor (ppm with respect to liquid benzene) calculated with the SOS-DFPT approach and experimental data. Reprinted with permission from V. Malkin, et al, Journal of the American Chemical Society, 1995, Volume 117, 3294-3295. Copyright 1995 American Chemical Society.

Figure 10: ^{13}C CP/MAS spectra of the three polymorphs of glycine. The β glycine shows an isotropic shift for the carbonyl in between those of α and γ glycine. However, β glycine is unstable and converts to α glycine under continued MAS.

Robert E. (Bob) Taylor earned his Ph.D. in 1980 working on the development of the Combined Rotation and Multiple Pulse Spectroscopy (CRAMPS) technique in the laboratory of Professor Bernie Gerstein in the Department of Chemistry at Iowa State University in Ames. For a number of years he worked as an analytical NMR spectroscopist applying solution-state, solid-state, and microimaging techniques to problems in the petrochemical and agricultural chemical industries. Seeking to escape the vagaries of corporate America, he joined what was then Bruker Instruments Incorporated as an applications scientist to see the world....and did. Growing weary and giving up his frequent flier miles, he left Bruker to work in the NMR Facility of the Chemistry Department at Texas A&M University. Upon realization that one must actually graduate from A&M to be a true Aggie, he left in 2001 for his current position in the NMR Facility in the Department of Chemistry and Biochemistry at the University of California, Los Angeles. His areas of responsibility include solid-state NMR and EPR.