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Application of Two-Dimensional ^{13}C Solid-State NMR to the Study of Conformational Polymorphism

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Abstract: Two-dimensional ^{13}C solid-state nuclear magnetic resonance (2D SSNMR) spectroscopy was applied to the study of three conformational polymorphs of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile. The separation of sidebands by order experiment (de Lacroix, S. F.; Titman, J. J.; Hagemeyer; A.; Spiess, H. W. *J. Magn. Reson.* **1992,** *97*, 435−443) allowed the separation of isotropic and anisotropic chemical shift information over two dimensions, making it possible to distinguish individual carbon atoms and analyze the full chemical shift anisotropy patterns using magic angle spinning (MAS) at moderate spinning speeds. The C-3 carbon of the thiophene ring, α to the nitrile carbon, was chosen to probe the differences in chemical environment between forms. The three forms exhibited a large variation in both measured and theoretically predicted isotropic and anisotropic chemical shifts for this carbon site. The results summarized in this paper demonstrate the utility in using 2D SSNMR methods for studying polymorphism in crystalline compounds with moderate molecular weights.

Introduction

A number of organic solids exhibit conformational polymorphism.[1] Conformational polymorphs have the same chemical structure, but differ in molecular conformation. Polymorphism is especially important in pharmaceutical solids since polymorphs typically exhibit differences in stability and bioavailability. Since conformational differences can result in variations in intramolecular distances and local electronic structure, NMR is an ideal and sensitive probe for this type of behavior and can provide complementary information to that obtained from other structural techniques, such as X-ray methods. One way in which NMR can be used to detect conformational differences is by observing changes in the chemical shift. In addition to the isotropic chemical shift information that can be measured in a liquid-state NMR experiment, solid-state NMR allows the measurement of chemical shift anisotropy (CSA), which provides additional structural information. CSA is a second rank tensor interaction that arises in solid samples from a dependence of the chemical shift on molecular orientation with respect to the external static magnetic field. Using SSNMR to study molecular structure and conformation through CSA currently is of growing interest and can be aided by the concurrent use of ab initio methods.[2] Toward the study of polymorphism, a number of studies have used 1D CPMAS to observe structural changes through the CSA tensor.[3] However, even in moderately complex organic solids the overlapping spectral features make analysis nearly impossible, even with the application of very high spinning speeds.

To remedy this situation, a number of schemes have been developed to separate isotropic and anisotropic chemical shift information under magic angle spinning (MAS) conditions.[4−16] The easiest of these to implement is the 2D NMR experiment in which the isotropic and anisotropic information can be obtained simultaneously for each distinct site in the sample, without special hardware requirements. An example of such an experiment is the 2DTOSS[17] pulse sequence developed by Spiess and co-workers which separates CSA information by sideband order in the indirectly detected frequency dimension and provides the full CSA patterns in the second dimension.[14]

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(2) See, for example: (a) Havlin, R. H.; Le, H. B.; Laws, D. D.; deDios, A. C.; Oldfield, E. *J. Am. Chem. Soc.* **1997**, *119*, 11951. (b) Heller, J.; Laws, D. D.; Tomaseli, M.; King, D. S.; Wemmer, D. E.; Pines, A.; Havlin, R. H.; Oldfield, E. *J. Am. Chem. Soc.* **1997**, *119*, 7827. (c) Liu, F.; Orent, A. M.; Alderman, D. W.; Grant, D. M. *J. Am. Chem. Soc.* **1997**, *119*, 8981. (d) Orendt, A. M.; Hu, J. Z.; Jiang, Y. J.; Facelli, J. C.; Wang, W.; Pugmire, R. J.; Ye, C. H.; Grant, D. M. *J. Phys. Chem. A* **1997**, *101*, 9169.

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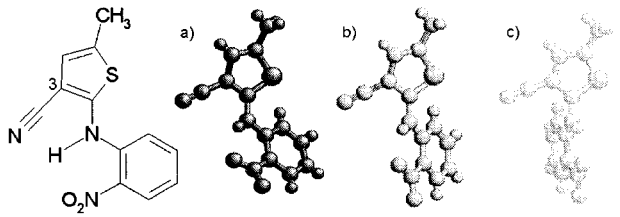

Figure 1. Chemical structure and three forms of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile, collectively referred to as ROY. (a) Yellow form with a phenyl-thiophene coplanar angle of approximately 106°, (b) orange form with a coplanar angle of 56°, and (c) the red form with a coplanar angle of 46°. The number "3" is placed near the C-3 carbon to indicate that this is the probe nucleus of interest.

The previous application of this technique was dedicated to the study of amorphous polymers, but the sequence is also quite useful for studying crystalline materials.

In this paper we report our experiments using the 2DTOSS sequence to analyze three crystalline polymorphic forms of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile, which we believe constitutes the first application of 2D SSNMR to the study of conformational polymorphism. This compound has five known polymorphic forms, three of which are shown in Figure 1 and are known collectively as ROY due to their red, orange, and yellow colors. The major structural difference in these compounds is the coplanar angle between the phenyl and thiophene rings. The form with the largest coplanar angle, 106°, is yellow in color, the next largest, 56°, is orange, and the smallest, 46°, is red.[18] The ROY compounds have been previously characterized using X-ray diffraction, FTIR, and CPMAS NMR.[18] The ^{13}C CPMAS spectra indicate that the thiophene carbon α to the nitrile carbon, referred to as C-3, has an isotropic peak that is isolated from the other resonances and differs noticeably between polymorphic forms. For these reasons, we chose to focus on this carbon as a probe for the conformational differences between the polymorphs. Future work in our laboratory will investigate the other carbon sites. The ROY compound has a total of 12 chemically distinct carbon sites, making the 1D CPMAS spectra complicated by dozens of spinning sidebands in a spinning regime slow enough for sideband analysis. The ROY compounds are therefore ideal for demonstrating the utility of the 2DTOSS sequence for studying polymorphism. In addition to our NMR work, we have also applied density functional methods to compare observed and predicted conformational dependencies of the chemical shifts and to investigate a possible origin for these dependencies.

Experimental Section

All polymorphic forms of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile were gifts from Eli Lilly and Company and had ^{13}C in natural abundance (1%). The yellow form is readily grown from a slurry of any other form at room temperature. The red form is obtained by crystallization from ethanol at a concentration of about 70 mg/mL. The orange form can be grown from ethanol or by slow evaporation from methanol.

The 2DTOSS pulse sequence is illustrated in Figure 2 and is described in detail by de Lacroix et al.[14] Briefly, the preparation period begins with ^1H to ^{13}C cross-polarization to generate transverse ^{13}C magnetization. A TOSS (TOtal Suppression of Sidebands) sequence,[5] consisting of a series of four specially timed π pulses, is then applied to rotate the magnetization trajectory of each crystallite so that each

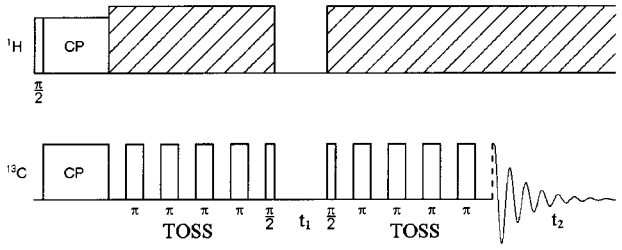

Figure 2. 2DTOSS sequence developed by de Lacroix et al. A detailed description of this sequence can be found in refs 14 and 20.

trajectory has the same time-averaged position.[19] As a result, TOSS generates a net magnetization vector that would effectively precess at only the isotropic chemical shift frequency by imparting to each crystallite a phase that is dependent on its position in the rotor and its orientation in the external static magnetic field. Immediately after the first TOSS period, one component (x or y) of the magnetization is stored along the z-axis with a $\pi/2$ pulse and the ^1H decoupling is turned off in order to eliminate any remaining magnetization in the $x-y$ plane. The other component of the magnetization is measured in a second experiment, and the two components are combined in the hypercomplex data processing. Since the magnetization is aligned along z during t_1, no isotropic chemical shift evolution takes place during this time period. However, each crystallite does accumulate a rotor dependent phase of $\omega_r t_1$, where ω_r is the spinning speed, which affects the orientational-dependent anisotropic chemical shift frequency during t_2. Following the t_1 delay, a $\pi/2$ pulse returns the magnetization to the transverse plane, and a second TOSS sequence is executed. The phase of the magnetization for a given crystallite just prior to acquisition is correlated to the amount of rotor phase accumulated during the t_1 period. By accumulating data spaced equally over one rotor cycle, the full spinning sideband manifold is reconstructed in the ω_1 dimension without contributions from the isotropic chemical shift. For organic solids, T_1 is usually much longer than $1/\omega_r$; thus, in effect, the modulation in t_1 is essentially fully periodic. It is therefore sufficient to use only as many t_1 increments as sidebands desired in the ω_1 dimension, spacing the t_1 timings in regular increments over one rotor period. The resulting 2D FID has purely anisotropic modulation in t_1 and the full chemical shift modulation in t_2. Fourier transformation of the 2D data set over the t_2 dimension produces a series of MAS spectra in which each sideband of order N has a pitch or phase of $N\omega_r t_1$. A subsequent transform over the t_1 dimension yields a 2D spectrum that separates the spectra by sideband order in the ω_1 dimension, and produces the full MAS spectrum of each spinning sideband order in the ω_2 dimension.

All of the ^{13}C spectra were recorded on a commercial Varian Unity Plus spectrometer at room-temperature operating at 75.44 MHz with a 7-mm Varian CPMAS probe. Samples were spun at 1500 Hz in each 2DTOSS experiment. Slow spinning speeds were employed to ensure that each CSA pattern had a sufficient number of sidebands for analysis. Proton and carbon 90° pulse widths were 5 μs, and the ^{13}C contact time was 2.5 ms. The TOSS pulse timings used in our experiments had a total duration of 2 rotor cycles per TOSS period.[20] Composite π pulses and a phase cycling scheme were implemented to reduce imperfections in the TOSS sequence.[21,22] A table of pulse phases used in this sequence can be found in ref 14. A total of 16 t_1 increments were recorded, including both the real and the imaginary components of the signal, and at least 135 transients were taken for each t_1 increment. The ROY compounds had long ^1H T_1 times ranging from 30 to 60 s such that recycle delays of 40−70 s were necessary for optimal signal averaging. As a result, each experiment ran for approximately 40 h.

(17) In the original manuscript by de Lacroix et al., the authors made no mention of a name for this technique. For brevity, we refer to this sequence as the 2DTOSS sequence.

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The isotropic peak assignments in the ^{13}C SSNMR spectra were made by comparison with the corresponding solution phase spectra. All chemical shift values are reported relative to TMS. The chemical shifts were referenced using an internal adamantane standard for which the chemical shift values have been recorded previously.[23]

Experimental CSA tensor values were determined using an iterative fitting program based on the formalism developed by Herzfeld and Berger,[24] and the analysis was performed on a desktop PC. A Simplex routine was used to adjust the fitted tensor values until the numerically calculated sideband intensities agreed well with experimental values.[25] Calculated sideband intensities all fit to within 3% of the volume of the isotropic peak. An error of 3.5 ppm in all measured tensor values was estimated by adjusting tensor values in small increments until the minimum square difference statistic, χ^2_{min}, had doubled.

Theoretical calculations were performed on an IBM RS-6000 computer cluster using the Gaussian 94 program.[26] All of the calculations used a density functional approach with the hybrid B3LYP functional[27,28] and the Gaussian basis set, 6-311G.[29,30] Structures determined by X-ray methods were used as input coordinates for all of the calculations, and the proton positions were relaxed by partial geometry optimizations. NMR shielding calculations were performed with gauge-invariant atomic orbitals.[31] Initially, a constant offset of 190.2 ppm was applied to reference the shielding values to TMS. However, this correction had a noticeable bias toward overestimating large shift values, and thus a linear correlation of $\sigma_{shift} = -0.969\sigma_{shielding} + 182.8$ ppm, obtained from the difference between observed shifts and predicted shieldings for the C-3 tensor values and the methyl isotropic peaks, was applied. Similar linear correlations have recently been used by Grant and co-workers.[2d]

Results

A spectrum generated by the 2DTOSS sequence is shown in Figure 3 for ROY-orange. At the top of the 2D spectrum is a projection of the data along the ω_2 dimension. The projection is equivalent to the standard 1D MAS spectrum and illustrates the need for using a 2D separation experiment for CSA analysis. Each trace along the ω_2 dimension, for a given ω_1, contains the sidebands of order $N = \omega_1/\omega_r$ for each chemically distinct ^{13}C site. The MAS pattern for each resonance resides along a direction parallel to the diagonal in the 2D spectrum. Small contours located outside of these diagonal regions are noise peaks that are larger than the chosen display threshold. As can be seen from the spectrum, sites such as C-3 which have a moderate to large size CSA produce many spinning sidebands at low spinning speeds, and hence have many peaks along the diagonal. Sites with smaller CSA, such as the methyl peak found at $\omega_1 = 0$, $\omega_2 = 18$ ppm, produce almost no sideband intensity.

A 1D MAS spectrum can be constructed for each site by selecting the corresponding sideband peak in each trace along ω_2 and placing the bands together. The result of this process is shown in Figure 4a for the C-3 site of the ROY-orange form. The dashed lines indicate that no information exists between the sidebands due to the manner in which the spectrum was constructed. The CSA tensor values are extracted from the spinning sideband intensities using the fitting process outlined above. An example of a result from this process is shown in Figure 4b for C-3 of the ROY-orange form. A line broadening of 75 Hz was used in the simulated spectrum to match experimental line widths. The fitted CSA tensor values for all of the ROY forms are tabulated in Table 1.

In Figure 5, the isotropic trace from the 2DTOSS spectrum is shown for each of the three polymorphic forms of ROY. The

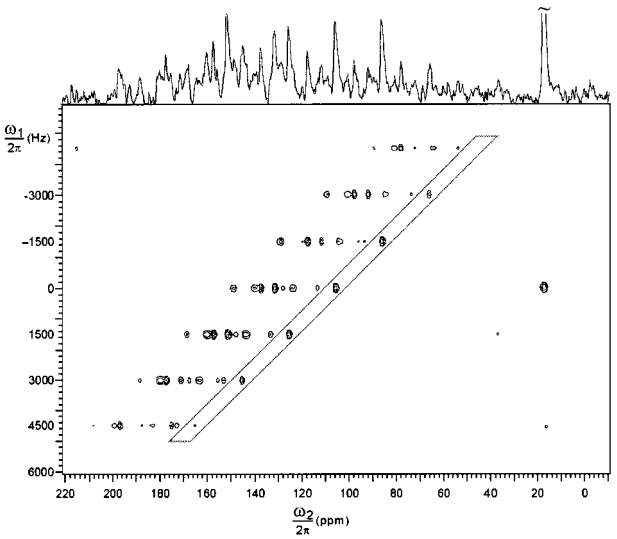

Figure 3. 2DTOSS spectrum of the ROY-orange form. Chemical shift information is separated by sideband order in the ω_1 dimension and by full CSA pattern in the ω_2 dimension. A box is drawn around the peaks that constitute the 1D MAS spectrum for the C-3 site.

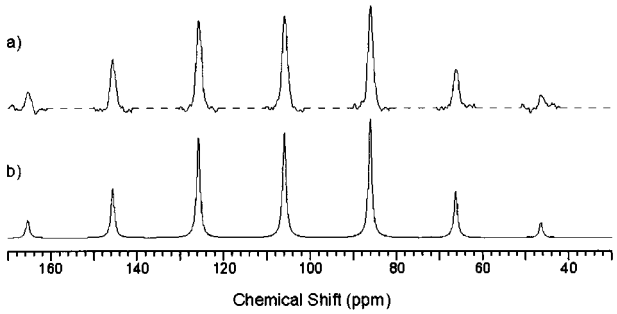

Figure 4. Fit to the 1D MAS spectrum for C-3 in the ROY-orange form. (a) 1D MAS spectrum is constructed by piecing together successive slices of the 2DTOSS spectrum along the ω_1 dimension. (b) Simulated spectrum generated from a best fit to the CSA tensor values.

Table 1. C-3 Site CSA Tensor Values for the Three Forms of ROY Reported in ppm from the ^{13}C Resonance of TMS[a]

form	$\delta_{11}(\sigma_{11})$	$\delta_{22}(\sigma_{22})$	$\delta_{33}(\sigma_{33})$	$\delta_{iso}(\sigma_{iso})$
red	49.2(48.8)	90.7(93.2)	155.0(150.8)	98.3(97.6)
orange	50.2(49.8)	102.7(99.8)	165.7(167.3)	106.2(105.6)
yellow	43.3(44.1)	105.8(107.5)	179.2(180.6)	109.4(110.7)

[a] For the experimentally determined tensor values (the values on the left), the isotropic shifts were obtained from $\omega_1 = 0$ slices of the 2DTOSS spectra and the traceless tensor values were calculated by fitting the MAS sideband intensities. An error of 3.5 ppm in all measured tensor values was estimated by doubling the fitting statistic, χ^2_{min}. In parentheses are the corresponding adjusted tensor values calculated using Gaussian 94.

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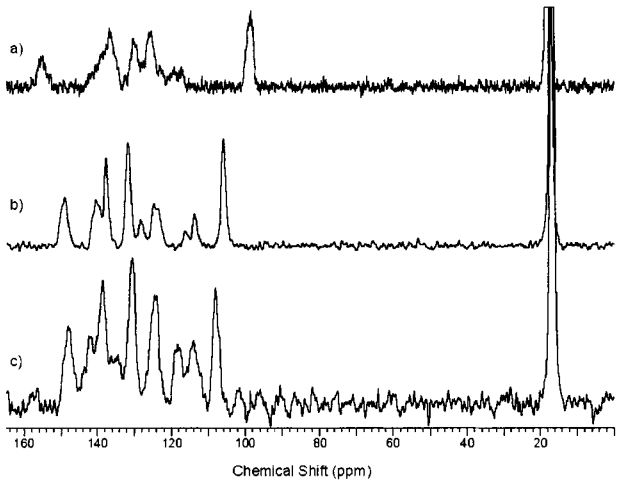

Figure 5. Isotropic spectra obtained by taking $\omega_1 = 0$ slices of the 2DTOSS spectra for ROY (a) red, (b) orange, and (c) yellow. The C-3 site has an isotropic shift of 98 ppm for ROY-red, 106 ppm for ROY-orange, and 109 ppm for ROY-yellow.

$\omega_1 = 0$ trace of a 2DTOSS spectrum is equivalent to the spectrum generated by a 1DTOSS[5] experiment and can be useful for peak assignments and comparisons to liquid-state spectra. Since the 2DTOSS experiment does not rely on rotor speed to produce isotropic spectra, there are no mechanical limitations on which ^{13}C sites can be analyzed due to the magnitude of the CSA.

The results from the density functional analysis of the C-3 chemical shift tensor values are also summarized in Table 1. After application of the linear regression, calculated tensor values matched closely with the observed values, having an rms deviation of only 2.1 ppm.

Discussion

It is readily apparent that both isotropic and anisotropic chemical shifts vary noticeably between polymorphs. Figure 5 illustrates isotropic peak rearrangement in the aromatic region of the spectrum, from 100 to 160 ppm. Isotropic resonances that overlap cannot be separated by the 2DTOSS sequence and therefore many sites in this region are difficult to analyze. The C-3 site, however, is easily distinguishable and appears to be sensitive to the differences in conformation between forms. A gradual progression in its isotropic resonance is observed as the phenyl-thiophene coplanar angle becomes larger in magnitude. The isotropic peak for C-3 moves downfield by almost 10 ppm in going from ROY-red to ROY-yellow. The anisotropic chemical shift also changes in magnitude between forms and appears to be more sensitive to the structural differences than the isotropic chemical shift. The tensor values listed in Table 1 show that the breadth of the CSA for C-3 increases in magnitude by 30 ppm and the line shape appears to become more asymmetric as the coplanar angle increases.

In contrast, some carbon sites in the different forms are not as sensitive to molecular conformation. The methyl peak at 18 ppm, for example, shows little difference in isotropic chemical shift. Unfortunately, the methyl peak yields only small, first-order spinning sidebands at 1500 Hz, making determination of the anisotropic chemical shift by spinning sideband analysis impractical.

One explanation that could account for the difference in chemical shift sensitivity between the C-3 and methyl carbons to the changes in conformation is the transfer of electron density between the phenyl and thiophene rings in the molecules. In an LCAO-MO sense, the p-orbitals of the atoms in the two rings become aligned in the more coplanar polymorphs, which could allow greater transfer of π electrons between the rings, resulting in a net increase in electron density at the C-3 carbon site. Hence C-3 for the ROY-red polymorph would experience the greatest diamagnetic shielding and would be found the furthest upfield. The methyl group, however, would not participate in the transfer of electron density; hence, its resonance would be relatively unaffected by the changes in conformation. A Mulliken charge density analysis[32] at the C-3 nucleus supports this idea. Calculations show that there is an increase in electron density at the C-3 site by as much as 10% of an electron charge, going from the yellow form to the red form. However, the charge density at the methyl carbon site is relatively constant between forms, with differences of less than 1% of a charge unit. The theoretical modeling of the electron density at the C-3 site is partially validated by the close agreement between the experimentally determined and theoretically calculated chemical shift principal tensor values found in Table 1.

Of the various 2D isotropic/anisotropic techniques, we found the 2DTOSS sequence to be the easiest to implement since there is no need for special hardware and the pulse programming is relatively straightforward. Also, since the number of t_1 increments needs only to be as large as the number of sidebands expected in the 1D MAS spectrum, the experiment is relatively short in length compared to other 2D methods. For a molecule with proton $T_1 < 1$s, a 2DTOSS spectrum containing up to third-order sidebands and acquiring 128 scans per t_1 increment would take about 30 min to acquire with natural abundance ^{13}C samples. Due to the low spinning speed requirements for CSA analysis, large sample volume rotors could be used to further decrease the acquisition time.

Conclusions

We have shown that the 2DTOSS experiment, which represents one of a class of isotropic/anisotropic chemical shift separation experiments, comprises a viable method to study conformational polymorphism. By using the 2DTOSS sequence, we were able to readily determine anisotropic chemical shifts, which, for the example of the C-3 carbon site on the ROY compounds, can be more sensitive to molecular conformation than isotropic chemical shifts. This type of information cannot feasibly be extracted from any 1D experiment without isotopically labeling the individual ^{13}C sites.

2D SSNMR is a powerful method for analyzing differences in chemical environment and molecular structure and will provide a new method for determining molecular conformations in polymorphism. By coupling information derived from the application of 2DTOSS experiments with ab initio or density functional methods, quantitative structural information can be obtained. Work in our lab along these lines is progressing to provide a fuller picture of the ROY compounds. Additionally, experiments designed to address the utility of other 2D techniques that provide complementary structural information, such as homonuclear and heteronuclear distances, will be examined in future work and may further expand the applicability of SSNMR for analyzing conformational polymorphism.

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