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Quantitative Structural Constraints for Organic Powders at Natural Isotopic Abundance Using Dynamic Nuclear Polarization Solid-State NMR Spectroscopy**

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Abstract: *A straightforward method is reported to quantitatively relate structural constraints based on ^{13}C–^{13}C double-quantum build-up curves obtained by dynamic nuclear polarization (DNP) solid-state NMR to the crystal structure of organic powders at natural isotopic abundance. This method relies on the significant gain in NMR sensitivity provided by DNP (approximately 50-fold, lowering the experimental time from a few years to a few days), and is sensitive to the molecular conformation and crystal packing of the studied powder sample (in this case theophylline). This method allows trial crystal structures to be rapidly and effectively discriminated, and paves the way to three-dimensional structure elucidation of powders through combination with powder X-ray diffraction, crystal-structure prediction, and density functional theory computation of NMR chemical shifts.*

Although single-crystal X-ray diffraction (SCXRD) is currently unrivalled for the structural elucidation of organic compounds, it is typically inadequate when single crystals of sufficient size (or quality) are not available. Thus, structure determination of microcrystalline powders remains highly challenging. In this context, NMR crystallography has emerged as a powerful technique by paving the way to ab initio structure determination of organic powders.[1] Precisely, powder X-ray diffraction (PXRD) and crystal-structure prediction methods[2] can be combined to generate trial crystal structures that are refined using NMR experiments. One possible approach compares experimental chemical shifts with those computed by density functional theory (DFT) for a pool of trial crystal structures, the best match yielding the correct (or most likely) structure. However, because chemical shifts are not directly related to the crystal structure, their use as structural refinement constraints is not entirely satisfactory. In fact, ^{13}C NMR chemical shifts were shown to be insufficiently sensitive for structure discrimination, whereas ^{1}H NMR chemical shifts (which are sensitive to the crystal packing) showed limitations in the case of a decreased number of assigned resonances.[1a] Accessing alternative structural constraints is therefore desirable. In contrast to liquid-state NMR, however, where such constraints can be derived by the nuclear Overhauser effect[3] or through residual dipolar coupling[4] experiments, similar experiments to provide meaningful structural parameters using solid-state NMR (SSNMR) techniques are still sought.

Studies on inorganic solids[5] indicate that dipolar couplings could be valuable candidates, but the high density of spins for abundant nuclei in organic compounds (^{1}H, or ^{13}C for uniformly enriched samples) drastically complicates the spin dynamics and prevents the measurement of long-range homonuclear spin coupling constants.[6] Analyzing rare spins such as ^{13}C or ^{15}N in samples at natural isotopic abundances (NA) could alleviate this issue but the low NMR sensitivity precludes it. Recent work has shown, however, that the SSNMR sensitivity of microcrystalline samples (with crystal widths ranging from 0.05 to 10 μm) could be enhanced by high-field low-temperature magic-angle spinning (MAS) dynamic nuclear polarization (DNP).[7] DNP relies on the microwave-driven transfer of the electron spin polarization to nuclei, giving a maximum NMR signal enhancement equal to the ratio of the electron to nuclear magnetogyric ratios (approximately 660 for ^{1}H nuclei). As a result, sensitivity enhancements of one to two orders of magnitude can now be envisaged,[8] allowing 2D dipolar and/or scalar correlation SSNMR experiments based on the detection of rare spins to be recorded on NA samples within reasonable times.[7,9] In particular, DNP-enhanced 2D double-quantum (DQ) dipolar correlation experiments have been used to probe qualitatively one-bond or two-bond ^{13}C–^{13}C correlations in NA powders (by comparing cross-peak amplitudes obtained for specific mixing times),[7a,b] and to estimate the distance between ^{29}Si–^{29}Si sites on the surface of silica nanoparticles (using numerical simulations).[9] Overall, 2D DQ ^{13}C–^{13}C dipolar correlation experiments, while extremely challenging for NA samples because of the low ^{13}C natural abundance (1.1%), could provide significant constraints for structure determination of organic powders. However, the required framework quantitatively relating ^{13}C–^{13}C cross-peak amplitudes to structural parameters has not been reported yet.

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Herein, we show how DNP-enhanced 2D ^{13}C–^{13}C DQ dipolar correlation experiments can be used to straightforwardly obtain quantitative data that are sensitive to the crystal structure of NA organic powders. We focused on NA theophylline powder (Figure 1 a). Theophylline is a pharmaceutical compound representative of the current challenges found in structure determination: it has several polymorphic forms,[10] and, although it may form crystals suitable for SCXRD, its crystallization is far from straightforward. Moreover, the above-mentioned ab initio NMR crystallography approach based on ^{1}H chemical shifts refinement failed to determine its correct crystal structure.[1a] Herein, the anhydrous polymorph of NA theophylline was first impregnated with a tetrabromoethane solution of the TEKPol biradical[11] (used as a source of electron spin polarization) and subsequently analyzed by DNP at 105 K. We obtained a DNP signal enhancement of 12 and an overall sensitivity enhancement of approximately 50 (see the Supporting Information),[7a,b,12] allowing DNP-enhanced 2D ^{13}C–^{13}C DQ dipolar correlation spectra of NA theophylline to be recorded in about 7 h (instead of about 2 years at room temperature without DNP). Two such spectra with a short and a longer DQ excitation times (τ_{DQ}), respectively, are shown in Figure 1 a and b. The strongest correlations in Figure 1 a arise mainly from ^{13}C–^{13}C spin pairs separated by short distances (C1–C5, C3-C5), whereas weaker correlations because of ^{13}C–^{13}C spin pairs separated by longer distances (C5–C6 and C7, C5–C5) are detected in Figure 1 b.

More quantitatively, the amplitudes of each pair of ^{13}C–^{13}C correlation peaks in a series of 2D DQ dipolar correlation spectra were measured and plotted as a function of τ_{DQ}, yielding DQ build-up curves that were scaled to the ^{13}C CPMAS spectrum (CPMAS = cross-polarization magic angle spinning) recorded under identical conditions (Figure 2). As a result of the low ^{13}C natural abundance, the spin system only consists of isolated ^{13}C–^{13}C spin pairs. These data could thus be fitted with a straightforward analytical function based on the sum of the distinct ^{13}C–^{13}C dipolar couplings that contribute to the correlation peak under study. This differs considerably from the complex many-body spin dynamics observed in spin networks (^{1}H, or ^{13}C for uniformly enriched samples). Figure 2 shows the analytical function plotted for the internuclear distances taken from the reference crystal structure of anhydrous theophylline previously determined by SCXRD and deposited in the Cambridge Structural Database (CSD entry code: BAPLOT06).[10a–c] This analytical function was also multiplied by a constant to scale the data and by an exponential decay to account for magnetic relaxation (see the Supporting Information).[13] The agreement between the experimental and the analytical ^{13}C–^{13}C DQ build-up curves in Figure 2 is excellent, showing trends that are clearly different depending on the measured ^{13}C–^{13}C DQ correlation peak. Importantly, these curves could only be perfectly reproduced by considering both the molecular conformation and the crystal packing of the theophylline crystal structure, which influenced the nature (intra- versus intermolecular) of the involved dipolar couplings. This confirmed that ^{13}C–^{13}C DQ dipolar build-up curves obtained by DNP-enhanced SSNMR provided meaningful quantitative data that were sensitive to the crystal structure of the powder sample under study. In fact, attempts to reproduce these curves with alternative (but highly similar) theophylline crystal structures proved unsuccessful.

First, the sensitivity of our method to molecular conformation was studied by considering a virtual crystal structure in place of the reference structure (BAPLOT06).

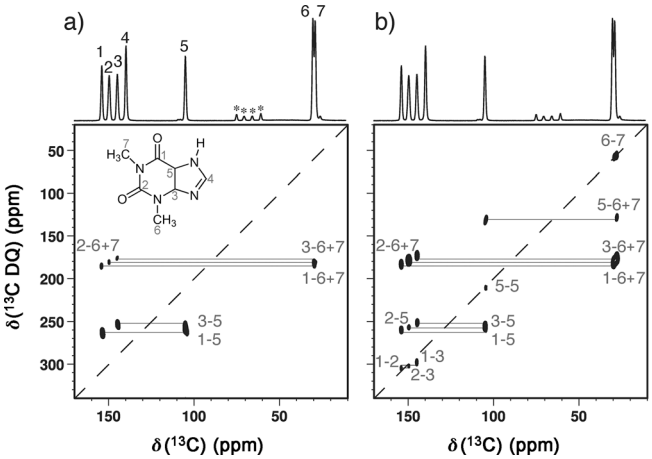

Figure 1. DNP-enhanced 2D ^{13}C–^{13}C DQ dipolar correlation spectra of the anhydrous polymorph of NA theophylline impregnated with a TEKPol tetrabromoethane solution. Correlations between pairs of ^{13}C resonances appear in the DQ dimension at the sum of their chemical shifts. The τ_{DQ} value is a) 0.5 and b) 2 ms, leading to the observation of correlation peaks between ^{13}C spins separated by short and longer distances, respectively. The ^{13}C CPMAS spectrum is shown in the top projections and has been assigned according to the theophylline molecular structure (left inset; * denotes spinning side bands).

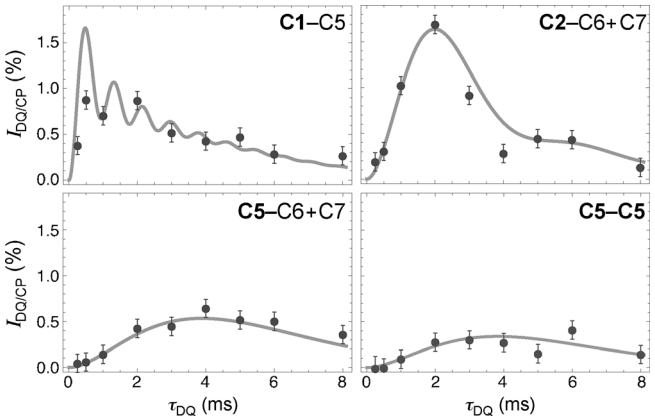

Figure 2. DQ build-up curves for selected pairs of ^{13}C dipolar correlation peaks. Selected pairs of peaks are indicated by numbers on the top right of each graph, with the number in bold referring to the detected resonance. The experimental data points (●) were obtained by integrating the correlation peaks obtained in a series of DNP-enhanced 2D ^{13}C–^{13}C DQ correlation spectra (Figure 1) recorded for different τ_{DQ} values. The solid lines represent an analytical function that considers the ^{13}C–^{13}C pairs involved in a specific correlation peak (see the Supporting Information for details and other curves).

The virtual structure differed purposely from the reference only by an all-carbon-atoms root-mean-square-deviation (RMSD) of 15 pm, while exhibiting otherwise identical space group and unit cell parameters. An RMSD value of 15 pm is the precision that can be achieved with state-of-the-art NMR crystallography approaches based on chemical shift refinement.[1] Figure 3a compares the DQ build-up curve

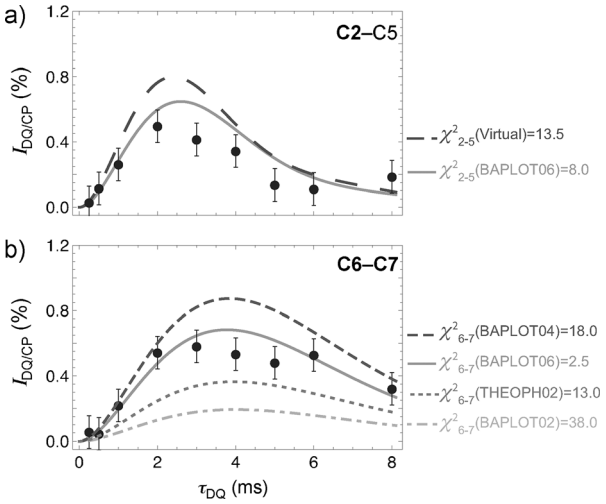

Figure 3. Sensitivity of the DQ build-up curves to the theophylline crystal structure. In (a) and (b), the solid line is the analytical DQ build-up curve calculated for the reference structure (BAPLOT06). The agreement between the calculated and experimental curves for a given *i*–*j* spin pair is evaluated using the goodness-of-fit coefficient $\chi^2_{i\text{-}j}$. a) C2—C5 experimental (●) and analytical (solid and dashed lines) DQ build-up curves. The virtual structure differs from the reference structure by an all-carbon atoms RMSD value of 15 pm. b) C6—C7 DQ build-up curves. The analytical curves were calculated for different theophylline polymorphs.

obtained experimentally for the C2–C5 correlation peak with those calculated from the virtual and reference crystal structures. This comparison could be more suitably evaluated using the goodness-of-fit coefficient of the involved C2–C5 spin pair ($\chi^2_{2\text{–}5}$), which was higher for the virtual (13.5) than for the reference (8.0) crystal structure. This confirmed that the DQ build-up curves, especially those involving medium- to long-range internuclear distances, could be used to detect small changes in molecular conformation (typical of conformational polymorphs). Second, the sensitivity to the crystal packing was studied by considering three different packing polymorphs of theophylline: the monoclinic,[10d] the monohydrate,[10b] and the high-temperature[10b] polymorphs (CSD entry codes: BAPLOT02, THEOPH02, and BAPLOT04, respectively). These polymorphs have almost identical molecular conformations but different unit-cell parameters. Moreover, BAPLOT02 and THEOPH02 have different space groups (*P*21/*c* and *P*21/*n*, respectively), whereas BAPLOT04 and BAPLOT06 have the same space group ($Pna2_1$). For these polymorphs, deviations between calculated and experimental DQ build-up curves were measured for correlation peaks that primarily depend on intermolecular distances. This is shown in Figure 3b, where the analytical C6–C7 DQ build-up curves calculated for each theophylline polymorph are compared to the experimental data. The range of $\chi^2_{6\text{–}7}$ values clearly shows that this correlation is sensitive to the crystal packing of the polymorphs. A similar trend was found for C5–C5 (data not shown). Overall, a global χ^2 parameter including all ^{13}C–^{13}C spin pairs could be defined, which allowed these crystal structures to be sorted out and the reference crystal structure to be identified (Table 1).

Table 1: Goodness-of-fit coefficient (χ^2) for ^{13}C–^{13}C DQ build-up curves based on theophylline crystal structures.[a]

Theophylline crystal structure	χ^2
BAPLOT06[b]	63.0
Virtual structure (^{13}C RMSD = 15 pm)[c]	84.0
BAPLOT02[d]	98.0
BAPLOT04[d]	94.0
THEOPH02[d]	79.0

[a] χ^2 values calculated using 8 distinct correlation peaks. [b] Reference crystal structure determined by SCXRD. [c, d] Trial crystal structures selected to study the sensitivity of our method to molecular conformation[c] and crystal packing.[d]

In summary, we have shown that ^{13}C–^{13}C DQ dipolar build-up curves recorded by DNP-enhanced SSNMR on NA powders can be straightforwardly analyzed, providing quantitative data that are sensitive to the sample molecular conformation and crystal packing. Using this method, it is possible to rapidly and effectively discriminate between trial crystal structures of organic powders generated by NMR crystallography approaches. This technique will thus be highly valuable to existing PXRD, crystal-structure prediction, and DFT-based chemical-shift prediction methods for the 3D structure elucidation of organic powders at natural isotopic abundance.

Keywords: natural isotopic abundance · solid-state NMR spectroscopy · spin–spin coupling · structure elucidation

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[14] CCDC-878133 (BAPLOT06), 862238 (BAPLOT04), 858440 (BAPLOT02), and 878134 (THEOPH02) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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