Solid-State NMR Spectroscopy

DOI: 10.1002/anie.200702582

Distinguishing Anhydrous and Hydrous Forms of an Active Pharmaceutical Ingredient in a Tablet Formulation Using Solid-State NMR Spectroscopy**

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The phenomena of polymorphism and pseudopolymorphism (the formation of hydrates/solvates) and their influence on the chemical and physical properties of molecular crystals are well known.[1] This is especially true for pharmaceutical compounds, where polymorphic or pseudopolymorphic changes in active pharmaceutical ingredients (APIs) can have significant effects on bioavailability.[2] Identification and characterization of (pseudo-)polymorphs is therefore essential during all stages of the development and manufacture of pharmaceuticals; of key importance is the form adopted in a tablet formulation in which the API is combined with a mixture of filler compounds (excipients). ^{13}C cross-polarization (CP) magic-angle spinning (MAS) solid-state NMR spectroscopy has proven itself as a powerful workhorse experiment for this purpose;[3–5] however, very little use has been made of ^{1}H solid-state NMR spectroscopy due to the large anisotropic broadening that results from extensive dipolar-coupled proton networks. However, advances such as fast MAS and improved homonuclear decoupling techniques have led to increased resolution for organic compounds.[6]

In particular, ^{1}H double-quantum (DQ) MAS NMR spectroscopy[6–8] has become a powerful approach, providing valuable information about, for example, a molecular tweezer host–guest complex,[9] intramolecular hydrogen-bonding in a biological molecule,[10] water and hydroxy-group environments in polyoxoniobate materials,[11] and surface organometallic species.[12] More recently, the ^{1}H DQ CRAMPS (combined rotation and multiple-pulse spectroscopy) technique[13–15] has been shown to dramatically increase resolution, thus allowing well-resolved spectra to be obtained for model compounds.[13–16]

Herein, we show how the presence of a specific pseudopolymorph of an API containing about 20 carbon atoms is identified in a tablet formulation using ^{1}H DQ CRAMPS NMR spectroscopy. This is demonstrated for an API currently under development, using only 30 mg of sample, and with no need for special preparation (that is, isotopic labeling). We present high-resolution 2D NMR spectra recorded in under two hours, a time equivalent to that needed for a high-quality 1D ^{13}C CP MAS spectrum.

^{1}H DQ CRAMPS NMR spectra, recorded using the pulse sequence in reference [15], of pure anhydrous and monohydrate forms of the API are shown in Figure 1a and 1b, respectively. For the monohydrate form, broader signals indicate a slightly less crystalline sample. X-ray single-crystal structure analyses reveal different intermolecular hydrogen-

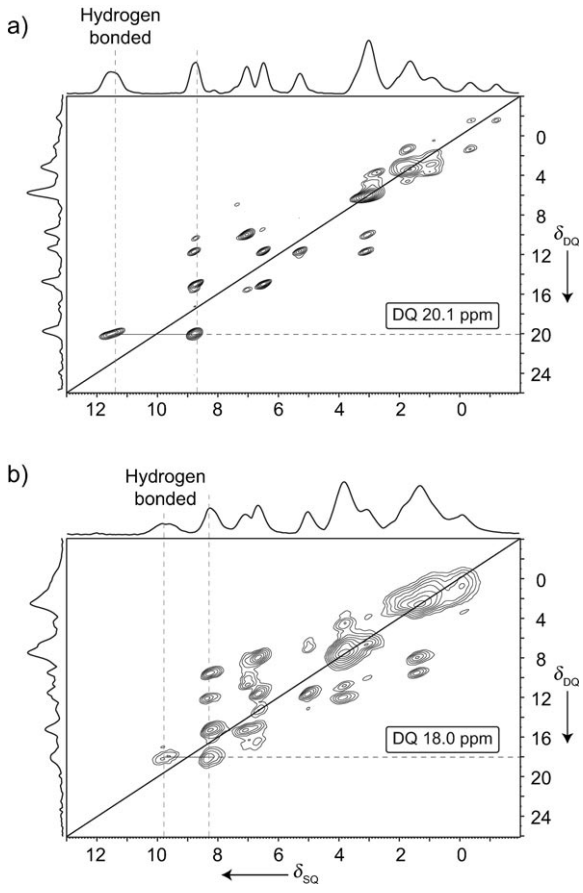

Figure 1. 600 MHz ^{1}H DQ CRAMPS NMR spectra together with skyline projections of a) the anhydrous and b) the monohydrate form of the API under consideration. The pair of DQ signals in both spectra corresponding to the intramolecular proximity of hydrogen-bonded protons with high-ppm resonance signals to the same nearby proton are highlighted. Each spectrum was recorded in 105 min. Base contours are shown at 11% of maximum intensity. All axes in ppm.

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[**] Funding from EPSRC, BBSRC, and AstraZeneca (CASE studentship) is acknowledged.

InterScience
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bonding arrangements for the anhydrous and hydrous forms, which is reflected in the different chemical shifts of the high-ppm resonance signals at $\delta = 11.4$ and 9.8 ppm, respectively. While good resolution is observed for the other signals, as is the case for published spectra of a dipeptide[15] and a pencillin salt,[16] we focus our discussion herein on the DQ signals involving hydrogen-bonded protons. For a typical organic solid, the presence of DQ signals in a ^{1}H DQ solid-state NMR spectrum implies a ^{1}H–^{1}H separation of less than 3 Å.[7,8] For the hydrogen-bonded protons, the observed DQ signals at $\delta_{DQ} = 11.4 + 8.7 = 20.1$ ppm and $\delta_{DQ} = 9.8 + 8.2 = 18.0$ ppm in Figure 1a and 1b, respectively, both correspond to such an intramolecular proximity to the same nearby proton.

The ^{1}H DQ CRAMPS NMR spectra of the pure anhydrous and hydrous form of the API in Figure 1 constitute detailed fingerprints that can be used to demonstrate the presence or absence of a particular pseudopolymorph in a tablet formulation comprising a mixture of the API and excipients. Figure 2 shows a ^{1}H DQ CRAMPS NMR spectrum of a crushed tablet which was stressed (exposed to 75% humidity at 40°C) for one week. (We note that a spectrum (not shown) recorded for a non-stressed crushed tablet of the same composition was identical.) In the spectrum, signals arising from the excipients are visible in addition to those from the API; for example, signals at $\delta_{DQ} = 4.3 + 4.3 = 8.6$, $6.4 + 6.4 = 12.8$ and $10.1 + 4.3 = 14.4$, $10.1 + 6.4 = 16.5$ and $10.1 + 10.1 = 20.2$ ppm are attributed to the main excipient calcium phosphate, $CaHPO_4 \cdot 2H_2O$. Resonances of other excipients are visible as broad diagonal signals in the aliphatic region (shown by asterisks in Figure 2). Despite the presence of excipients in the tablet, the spectrum allows the clear identification of only resonances that are characteristic of the anhydrous form of the API, notably the indicated pair of cross-peaks at $\delta_{DQ} = 11.4 + 8.7 = 20.1$ ppm (compare to Figure 1a). Importantly, note the *absence* of DQ cross-peaks at $\delta_{DQ} = 9.8 + 8.2 = 18.0$ ppm that would be indicative of the hydrous form of the API.

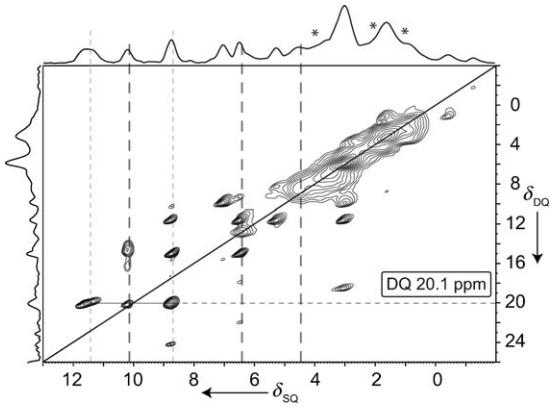

Figure 2. A ^{1}H DQ CRAMPS NMR spectrum of a crushed tablet containing the API and some excipients. Long dashes indicate the positions of signals from the calcium phosphate excipient and asterisks indicate signals from other excipients. Short dashes indicate DQ signals involving the hydrogen-bonded protons of the anhydrous API (see Figure 1a). The base contour is shown at 11% of maximum intensity.

These observations are clearly evident from Figure 3, which compares the traces at $\delta_{DQ} = 20.1$ ppm for the pure anhydrous API and the stressed tablet (a, b), and the traces at $\delta_{DQ} = 18.0$ ppm for the pure hydrous API and the stressed tablet (c, d). We emphasize that a high-resolution 2D ^{1}H DQ NMR spectrum is essential to distinguish the API signals from those of the excipients: note the similarity in the SQ chemical shift of the $\delta = 10.1$ ppm signal of the calcium phosphate excipient and the $\delta = 9.8$ ppm signal for hydrogen-bonded protons of the hydrous API. In a 1D spectrum these two signals could be confused, suggesting the presence of hydrated API in the stressed tablet. However, the proton–proton proximity information gained by the 2D DQ approach used herein removes any uncertainty in the assignment of these signals.

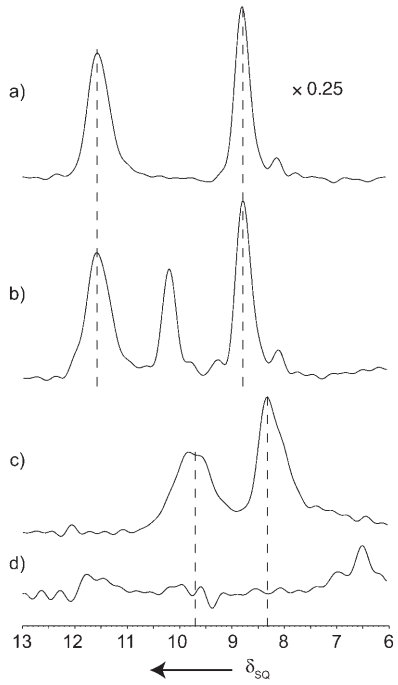

Figure 3. Traces extracted from ^{1}H DQ CRAMPS NMR spectra shown in Figures 1a (a), 2 (b, d), and 1b (c) demonstrate the presence of the anhydrous API ($\delta_{DQ} = 20.1$ ppm, a, b) and indicate the absence of the hydrated API ($\delta_{DQ} = 18.0$ ppm, c, d) in the tablet.

We have shown that ^{1}H DQ CRAMPS solid-state NMR spectra (recorded using 30 mg of sample in 105 minutes at natural abundance) conclusively demonstrate the presence of only a single specific pseudopolymorph in a tablet formulation; this new approach should be adopted as a routine tool in the characterization of pharmaceuticals.

Experimental Section

All experiments were performed on a Bruker Avance II+ spectrometer operating at a ^{1}H Larmor frequency of 600 MHz and a MAS frequency of 12.5 kHz using a Bruker 4-mm probe. In each experi-

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ment approximately 30 mg of sample was used; the sample was restricted to the center of the rotor using spacers. ^{1}H DQ CRAMPS NMR spectra were recorded using the pulse sequence described in reference [15]. The windowless e-DUMBO-1_{22}[17] and windowed DUMBO-1[18,19] schemes were used for homonuclear dipolar decoupling during t_1 and t_2, respectively. The θ_1 and θ_2 pre-pulses were both of duration 0.7 μs. In t_2, one complex data point was acquired in each acquisition window (2.2 μs). The DUMBO blocks were 24 μs long (corresponding to one basic DUMBO cycle). Including the pre-pulses, the effective dwell time in t_2 was 29.1 μs (maximum $t_2 = 15$ ms). DQ excitation and reconversion was achieved using three elements of POST-C7,[20] corresponding to a recoupling time of 69 μs. A 16-step nested phase cycle was used to select $\Delta p = \pm 2$ on the DQ excitation pulses (four steps), and $\Delta p = -1$ on the z-filter 90° pulse (four steps). ^{1}H radio-frequency field strengths of 87.5 and 100 kHz were used for POST-C7 and DUMBO decoupling, respectively. The States-TPPI method was used to achieve sign discrimination in the F_1 dimension. In all experiments, 16 transients were coadded for each of the 196 t_1 slices. The t_1 increment corresponded to the length of two DUMBO decoupling cycles, 48 μs. The recycle delay was 2 s. Scaling factors for the t_1 and t_2 dimensions were determined experimentally to be 0.57 and 0.63, respectively.

Received: June 13, 2007
Published online: September 11, 2007

Keywords: analytical methods · drug design · NMR spectroscopy · polymorphism

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