

## Sampling Methods for Quantification of Solid-state Phases in Powder Samples with Solid-state NMR Spectroscopy

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Received November 18, 2008, Accepted March 16, 2009

To minimize the variance in the quantification of solid-state phases in powder samples, gently placing polycrystalline samples one next to another directly in a sample holder is better than trying to mix them homogeneously prior to transferring to a sample holder. However, the solid-state cross polarization-magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy results demonstrated that it is essential in this sampling method to place all the samples in the location of consistent signal sensitivity. The same sampling method may be employed in other spectroscopic quantification techniques of solid-state phases if the method to limit the sample to the location with uniform signal sensitivity in the sample holder is adapted to each technique.

**Key Words:** Solid-state NMR, Quantification, Solid-state phase, Powder sample, Polymorph

### Introduction

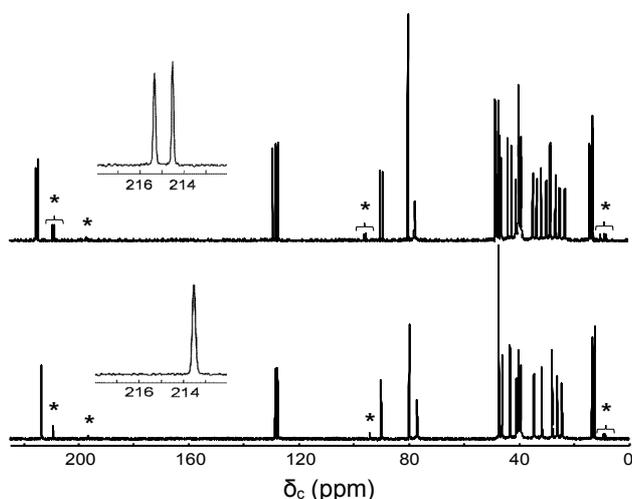
Many important chemical and physical properties of solid-state materials, such as pharmaceutical activity, catalytic activity, biocompatibility, solubility, semi-conductivity, mechanical strength, elasticity and various thermal properties including thermal expansion, are closely related with solid-state structures and phases, including amorphous or glassy phase.<sup>1-11</sup> For example, the polymorphs for each drug substance, *i.e.*, the active pharmaceutical ingredient, need to be identified and quantified before the commercial use of drug substances. The characterization and quantification of each phase in a sample have enabled even a precise diagnosis of diseases such as urolithiasis and a prophylaxis,<sup>1</sup> an estimation of the lixiviation performance of the host for radioactive elements,<sup>2</sup> and the deciphering of the magma mixing phenomena.<sup>3</sup> Consequently, many studies have quantified solid-state structures and phases by various spectroscopic methods.<sup>1-12</sup> However, powder sample mixing to obtain calibration curves and to apply a

standard addition method<sup>9</sup> causes significant variance in the quantification of solid-state phases, in contrast to liquid samples, due to the difficulty in ensuring a homogeneous mixing of solid-state polycrystalline powders. Especially with limited sample amounts, a larger error may be produced due to the presence of remnants on the surface of mixing tools. In some cases, the physical force required for mixing might even induce unwanted phase changes.<sup>5</sup> Here, we demonstrate an alternative sampling method, in which one polycrystalline powder is gently placed next to another directly in a sample holder, with solid-state cross polarization-magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy.

In this work, the case study was carried out with an active pharmaceutical ingredient, (7 $\alpha$ ,17 $\alpha$ )-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one (Org OD 14), which was reported to have two different crystal structures, forms I and II, and their <sup>13</sup>C CP-MAS spectra were reported.<sup>12</sup> Carbonyl peaks at 215.3 and 214.5 ppm for form I and at 213.5 ppm for form II, resolved down to the baseline and not overlapped with other peaks as shown in Figure 1, were used for quantification. When the samples were mixed in a mixing tool, it was difficult to ensure that the entire sample was transferred to a sample rotor, which was the sample holder for the CP-MAS NMR experiments, without any trace of the sample remaining on the surface of the mixing tools. In addition, the line widths were broadened by approximately 23% for the samples ground in a mortar and pestle. The samples were therefore gently placed one next to another in the sample rotor directly. The rotor filled with the sample was weighed in comparison with an empty rotor to measure the sample amount in the rotor.<sup>14</sup> The amount of sample portion additionally added was determined likewise.

### Experimental

All solid-state NMR spectra were acquired on a DSX-400 spectrometer (Bruker Biospin GmbH, Germany) with a magnetic field strength of 9.4 T and zirconia rotors of 4 mm outer diameter at room temperature. <sup>13</sup>C CP-MAS spectra

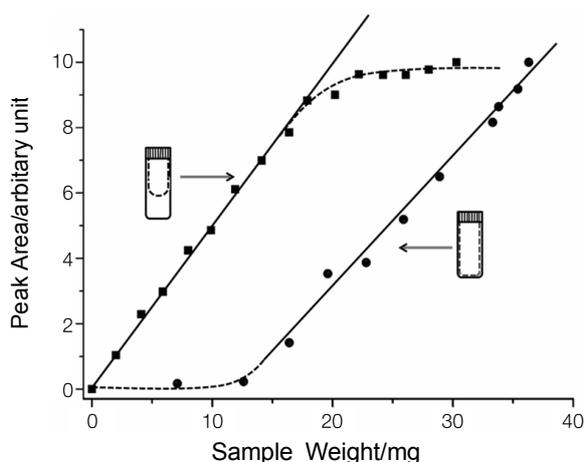


**Figure 1.** <sup>13</sup>C CP-MAS spectra of forms I (top) and II (bottom) of Org OD 14 sample. Expanded carbonyl peak regions are shown in the insets and spinning side bands are marked with asterisks.

were acquired with a 6.5 s pulse sequence repetition delay, a 2.8  $\mu$ s proton pulse length (90° flip), an optimized TPPM decoupling pulse of 5.65  $\mu$ s, a 2.5 ms contact time, a dwell time of 12.6  $\mu$ s, a spinning rate of 12 kHz, a time domain data size of 8 kilobytes, and 4 dummy scans for each datum file. The NMR signal was acquired with a scan number of 5,120 for each datum file and the files of individual sample were generated and added until the desired signal-to-noise ratio (S/N) (~50 or higher) was reached. For the S/N calculation, noise level was taken from the spectrum region of 216 ~ 220 ppm and signal levels from the chosen carbonyl peaks. Data were zero filled up to 32 kilobyte before Fourier transformation. All chemical shifts in ppm were referenced to external tetramethylsilane. The crystalline purities of reference samples for forms I and II were ensured to be higher than 99% by the absence of the carbonyl peak of the other crystalline phase in the  $^{13}\text{C}$  CP-MAS NMR spectra, even at an S/N ratio above 100.

### Results and Discussion

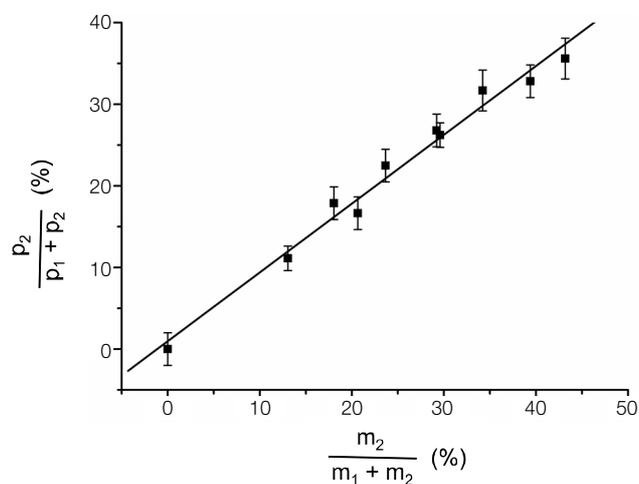
When a regular rotor was used, the signal was unfortunately not proportional to the amount of sample added, especially for small amounts, as shown in Figure 2. This was due to the non-uniform CP efficiency and signal collection from each sample location in an NMR coil.<sup>15,16</sup> If only a spherical space at the center of the rotor is used, sample inhomogeneity might not be a significant problem for quantification. However, the experimental time becomes too long to take a spectrum with a reasonable S/N. In addition, if a rotor with spacers both at the bottom and upper part is used, it was noticed that applying a standard addition method results in a significant error due to the powder samples adhering on the surface of the upper spacer even after disassembling the rotor in order to add another portion of standard powders. The samples adhered to the upper space can fall off from the spacer during the sampling



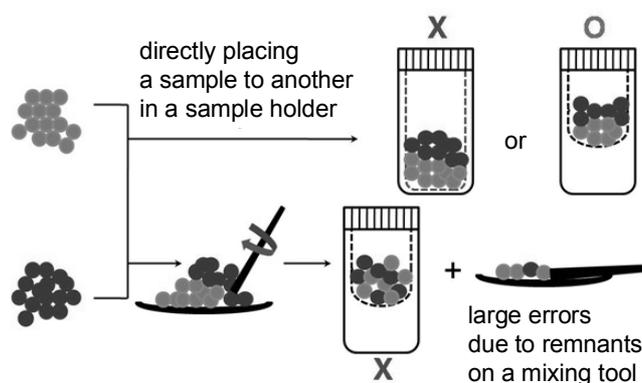
**Figure 2.** Carbonyl carbon peak areas in  $^{13}\text{C}$  CP-MAS spectra are plotted versus sample weights in a rotor. Only samples with form II were used. Solid circle data were obtained with a regular rotor and solid square data with a rotor with a bottom spacer, as indicated by the schematics in the plot. The solid lines are the least square fits of the data in linearity while the dotted lines are provided to guide the eyes.

procedure. Thus, rotors filled with spacers at the bottom only, as shown in Figure 2, were tested. The peak area versus sample weight showed linearity but the slope started to decline when the sample weight exceeded approximately 15 mg and continued declining more drastically as the weight exceeded 20 mg. This confirmed that the sensitivity drops more as the added sample is placed farther away from the center region of the rotor. Therefore, Figure 2 also indicates that a total sample amount of less than 10 mg fills the rotor bottom in a regular rotor but only the center region in a rotor with a bottom spacer.

As an example, an unknown Org OD 14 sample was identified to have form I : form II = 68  $\pm$  3 : 32  $\pm$  3 using the calibration curve in Figure 3 and the standard addition method. The CP rates for different carbonyl carbons are not



**Figure 3.** The calibration curve, generated by least-squares fitting of the  $^{13}\text{C}$  CP-MAS spectral data of the standard samples of forms I and II.  $p_1$  and  $p_2$  are the carbonyl peak areas while  $m_1$  and  $m_2$  are sample weights of forms I and II, respectively. The slope of the calibration curve is 0.843 and the correlation coefficient is 0.991.



**Figure 4.** Schematic indicating that gently placing polycrystalline samples one next to another directly in a sample holder is better than trying to mix them homogeneously in a mixing tool, due to the unknown amount remaining on the mixing tool. However, for this sampling method, it is essential to place all the samples in the location of consistent signal sensitivity. For the NMR rotors shown in the figure, only the sample in the central region was detected with uniform sensitivity.

necessarily the same. Therefore, direct measurement of the peak areas does not support the quantification of the polymorphs in the sample. In fact, the slope of the calibration curve of  $p_2/(p_1 + p_2)$  versus  $m_2/(m_1 + m_2)$  was 0.843 as shown in Figure 3, implying that the carbonyl carbons in form II had a smaller signal than those in form I in the  $^{13}\text{C}$  CP-MAS spectra even for the same number of carbonyl carbons.  $p_1$  and  $p_2$  are the carbonyl peak areas while  $m_1$  and  $m_2$  are the sample weights of forms I and II, respectively. The peak areas of the carbonyl signals of the unknown Org OD 14 sample were measured and the  $p_2/(p_1 + p_2)$  value was calculated to be  $0.28 \pm 0.02$ . The corresponding  $m_2/(m_1 + m_2)$  value of  $0.33 \pm 0.03$  was obtained using the calibration curve. Thus, forms I and II were present in  $67 \pm 3\%$  and  $33 \pm 3\%$ , respectively, of the Org OD 14 sample. The form I reference sample was added to the Org OD 14 sample and an NMR spectrum was taken each time for the standard addition method. A plot of  $p_1/(p_1+p_2)$  versus (weight of added form I reference sample)/(total sample weight) was obtained. The intercept on the ordinate at  $x = 0$  was  $0.73 \pm 0.03$ , corresponding to  $27 \pm 3\%$  of the carbonyl peak area from form II. Therefore, the calibration curve indicated that form II was present in  $31 \pm 3\%$  of the sample, which was equivalent, within the error range, to the result of  $33 \pm 3\%$  obtained with the calibration curve only.

### Conclusion

The present solid-state  $^{13}\text{C}$  CP-MAS NMR case study on the compound of two different crystal structures confirmed that gently placing polycrystalline samples one next to another directly in a sample holder is better than trying to mix them homogeneously in a mixing tool by physical force. However, for this sampling method, it is essential to place all the samples in the location of consistent signal sensitivity as schematically summarized in Figure 4.

**Acknowledgments.** N. V. Organon is gratefully acknowledged for providing the reference samples. This work was supported financially through the grants B2831B and PG7049.

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