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# A Technique for In Situ Monitoring of Crystallization from Solution by Solid-State $^{13}C$ CPMAS NMR Spectroscopy

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We report a technique for carrying out in situ solid-state NMR studies of crystallization from solution, allowing the evolution of different solid state structures (polymorphs) produced during the crystallization process to be identified. The technique exploits selectivity in NMR properties (specifically, the efficiency of cross-polarization from $^{1}H$ to $^{13}C$) between molecules in the solid and solution states, such that the first solid particles produced during the crystallization process are observed *selectively*, without detecting any signal from dissolved solute (or solvent) molecules. The application of the technique is demonstrated to reveal new insights concerning an isotope effect on the polymorphic outcome of crystallization of glycine from water. As revealed by this example, the in situ solid-state NMR approach reported here creates significant new opportunities for probing and understanding details of the evolution of solid state structures produced during crystallization from solution.

## Introduction

In general, direct in situ studies of chemical processes provide considerably greater opportunities to understand fundamental details of the underlying principles than ex situ studies of the same processes. However, it is essential that suitable techniques are available to allow a process of interest to be studied under in situ conditions, given the essential requirement that the technique must be able to probe the process within a suitable environment and on an appropriate time scale. Although solid-state NMR is a powerful and versatile technique for studying structural and dynamic properties of solids, its adaptation for in situ studies[1–13] is associated with specific technical challenges, arising from the requirement to locate the sample in a confined and relatively inaccessible space inside the NMR magnet and the fact that high-resolution solid-state NMR usually requires rapid sample rotation. In this paper, we report a technique for in situ solid-state NMR studies of crystallization processes. As an illustrative example of the application of this technique, we apply it to yield new insights concerning an isotope effect on the preferred polymorphic form obtained in the crystallization of glycine from water.

By exploiting selectivity in NMR properties [specifically, the efficiency of cross-polarization (CP) from $^{1}H$ to $^{13}C$] that arises from differences in the dynamics of molecules in the solid and solution states, our experiments allow the first solid particles produced in the crystallization process to be observed *selectively*, without detecting any signal from dissolved solute (or solvent) molecules. Thus, even if only a small fraction of the solute species has crystallized out of solution, it is only those molecules present in solid particles that contribute to the measured spectrum. The dissolved solute molecules, present in much higher amount, are effectively "invisible". In contrast, for many other in situ techniques (e.g., X-ray or neutron scattering), the underlying process does not discriminate between dissolved solute molecules, solid particles and solvent molecules (although selectivity in neutron scattering from solute and solvent can be achieved in some cases by use of appropriate isotopic combinations). Until now, the prospect of using solid-state NMR for in situ studies of crystallization has been limited by the difficulty of sealing a solution inside a rotor to allow magic angle spinning (MAS) to be carried out at several kHz without leakage of the solution from the rotor. In this context, MAS is required to give sufficiently narrow lines to allow the assignment of solid phases present at different stages of the crystallization process. Recently, suitable rotor technology has been developed for sealing solutions inside rotors for MAS experiments, enabling the work described here.

Crystallization of glycine ($H_2NCH_2CO_2H$) currently attracts much interest, in part because of the role of glycine as a prototypical system in polymorphism research. Under ambient conditions, three polymorphs of glycine ($\alpha$, $\beta$ and $\gamma$) are known, with the order of stability[14–17] $\gamma > \alpha > \beta$. Crystallization from aqueous solution at neutral pH preferentially forms the metastable $\alpha$ polymorph.[15,18] However, crystallization from deuterated water has been reported[19] to induce formation of the $\gamma$ polymorph, although systematic studies of this phenomenon have only recently been reported.[20] These studies have shown that even at deuteration levels as low as 1%, the probability of obtaining the $\gamma$ polymorph increases significantly. In general, the $\beta$ polymorph is not observed in crystallization of glycine from neutral, bulk water.[21] Two further polymorphs ($\delta$ and $\epsilon$) have been reported[22] at high pressure (in excess of 1.9 GPa).

## Experimental Section

For our in situ solid-state NMR studies of crystallization, a rotor assembly suitable for the study of heterogeneous liquid/solid systems was purchased from Varian Inc. To increase sensitivity, we used a sample of glycine $^{13}C$-labeled (99%; Isotec) in both carbon positions. High-resolution solid-state $^{13}C$ NMR showed that this sample was a mixture of the $\alpha$ and $\gamma$

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polymorphs. As the sample of glycine had natural $^1H/^2H$ abundances, dissolution in $D_2O$ leads to $^1H/^2H$ exchange between the acidic hydrogen sites of glycine and water, leading to a resultant percentage deuteration for a 6 mol $dm^{-3}$ solution (see below) of 86% for all exchangeable hydrogen sites in the system.

In the crystallization experiments reported here, the concentration of glycine was ca. 6 mol $dm^{-3}$, prepared by packing glycine powder into the rotor, with water then added by pipet. Before sealing, the rotor was placed in a centrifuge and spun (ca. 500 rpm) for a few seconds to drive the water toward the bottom of the rotor. The glycine/water sample was kept initially at 65 °C for 2 h (the temperature required[23] for complete dissolution of a 6 mol $dm^{-3}$ solution is 60 °C). $^{13}C$ CPMAS NMR spectra recorded after two hours at 65 °C showed no evidence for undissolved solid glycine (thus ensuring that no crystals were present that could act as seeds for the crystallization process). The temperature was then decreased to 20 °C over several minutes. When the temperature reached 30 °C, recording of the $^{13}C$ NMR spectra was started [measurements were not started at higher temperatures as the tuning of the probe was optimized for 20 °C (the temperature for most of the crystallization experiment)]. The temperature reached 20 °C within the time taken to record the first spectrum, and thus, apart from the first spectrum, our experiments probe the crystallization process as a function of time at constant temperature. All solid-state $^{13}C$ NMR spectra were recorded using ramped CP[24] from $^1H$ to $^{13}C$ (contact time 0.9 ms),$^1H$ decoupling (80 kHz) and MAS (8 kHz). We estimate that the pressure at the wall of the rotor does not exceed ca. 5 MPa and is well below the pressure required to generate the $\delta$ and $\epsilon$ polymorphs of glycine.[22]

## Results and Discussion

It is known from previous studies[25] that the $^{13}C$ resonances for the carboxylate group in the α and $\gamma$ polymorphs of glycine occur at ca. 177 ppm and ca. 175 ppm, respectively, and are sufficiently well resolved to provide a basis for distinguishing the two polymorphs (in contrast, the $^{13}C$ resonances for the $CH_2$ group are not resolved). The carboxylate region of the $^{13}C$ CPMAS NMR spectra recorded as a function of time in our in situ studies of crystallization of glycine from $H_2O$ and $D_2O$ are shown in Figure 1a,b respectively. Crystallization in $H_2O$ gives rise to a peak at ca. 177 ppm, assigned as the α polymorph, with no detectable amount of the $\gamma$ polymorph produced during the experiment.[26] Crystallization from $D_2O$ is also found to lead to the initial rapid formation of the α polymorph. However, after some time, a signal at ca. 175 ppm characteristic of the $\gamma$ polymorph emerges, which then increases in intensity while the intensity of the peak due to the α polymorph decreases. The final polymorph obtained in each case (α polymorph in $H_2O$; $\gamma$ polymorph in $D_2O$) is in agreement with the preferred polymorphic outcome observed in conventional laboratory crystallization experiments.

The relative amounts of the α and $\gamma$ polymorphs present as a function of time in $D_2O$ can be established from integrated peak intensities corrected to allow for the different CP efficiencies of the α and $\gamma$ polymorphs. To establish the relative CP efficiencies, a powder mixture of the α and $\gamma$ polymorphs (using a sample of glycine with natural isotopic abundances) was prepared, with the relative amounts of the two polymorphs established by Rietveld refinement[27] from powder X-ray diffraction data. The $^{13}C$ CPMAS NMR spectrum of this solid mixture was also recorded (using the same data collection

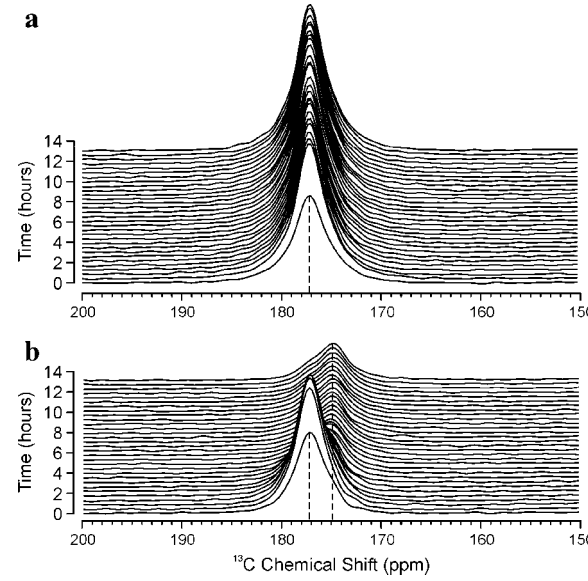


**Figure 1.** Solid state $^{13}C$ CPMAS NMR spectra (carboxylate region) of the solid component produced from solutions of glycine in (a) $H_2O$ and (b) $D_2O$, recorded as a function of time at 20 °C.

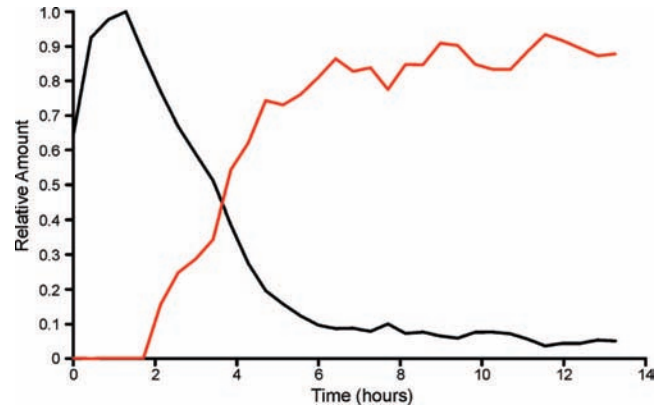


**Figure 2.** Relative amounts of the α (black) and $\gamma$ (red) polymorphs of glycine as a function of time during crystallization from $D_2O$, established from the solid-state $^{13}C$ NMR spectra shown in Figure 1b.

parameters used in the in situ crystallization experiments) and the relative intensities of the peaks characteristic of the α and $\gamma$ polymorphs were measured. Under the conditions of our $^{13}C$ CPMAS NMR measurements, the CP efficiency is higher for the α polymorph by a factor of 3.3.

The spectra in Figure 1b were fitted to a model of two Lorentzian lineshapes [the line widths of the fitted peaks are largely independent of time and solvent ($H_2O$ or $D_2O$)]. After correcting the measured integrated intensities for the relative CP efficiencies as discussed above, the relative amounts of the α and $\gamma$ polymorphs present as a function of time for crystallization in $D_2O$ were determined, and are shown in Figure 2. The data are normalized so that the maximum total amount of glycine present during the experiment corresponds to a value of 1. Initially, the α polymorph is produced rapidly, reaching a maximum amount at ca. 1.5 h. After this point, the amount of the α polymorph decreases, while the $\gamma$ polymorph emerges and increases in relative amount. The total amount of solid glycine present is approximately constant, and there is no evidence from the $^{13}C$ NMR spectra for the formation of any intermediate phase, consistent with the rate of formation of the $\gamma$ polymorph mirroring the rate of decay of the α polymorph in Figure 2. This observation implies that the α polymorph

transforms directly to the $\gamma$ polymorph, presumably by a solution mediated process rather than a direct solid state transformation.[28]

We emphasize that sufficiently good time resolution in the in situ experiment is required to observe the formation of transient polymorphs in such crystallization processes. In the present case, the time to record each spectrum (ca. 26 min) is adequate to observe the initial formation of the $\alpha$ polymorph in crystallization from $D_2O$, with this polymorph existing for ca. 1.5 h (at 20 °C) before the appearance of the $\gamma$ polymorph.

## Concluding Remarks

This work provides the first demonstration that solid-state NMR is a viable in situ technique for probing the evolution of solid phases produced during crystallization from solution, and clearly this technique has considerable potential to yield insights on transient phases produced in a wide range of crystallization processes. The only significant restriction is that the material under investigation must contain suitable NMR-active nuclei in sufficiently high abundance, for which specific isotopic enrichment may be advantageous.

Furthermore, the specific example considered here to illustrate this technique has yielded the first direct insights into the reasons underlying the isotope effect observed in the crystallization of glycine from water. Our observation that initial rapid formation of the $\alpha$ polymorph occurs in both $H_2O$ and $D_2O$ implies that nucleation of the $\alpha$ polymorph is favored in both cases, and indicates that the different final polymorphs obtained in crystallization from $H_2O$ and $D_2O$ is *not* due to these solvents favoring different nucleation pathways. Instead, the key observation is that crystallization from $D_2O$ initially produces the $\alpha$ polymorph, with the preferential formation of the $\gamma$ polymorph in $D_2O$ arising from a subsequent transformation of the initially formed $\alpha$ polymorph to the $\gamma$ polymorph. The fact that the $\gamma$ polymorph is formed subsequently in $D_2O$ but not in $H_2O$ suggests that this polymorphic transformation is influenced strongly by the isotopic nature of the system. Further investigations are in progress to understand the nature of this isotope effect in more detail.

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## References and Notes

(1) Hunger, M.; Horvath, T. *J. Chem. Soc., Chem. Commun.* **1995**, 1423.
(2) Haw, J. F.; Nicholas, J. B.; Xu, T.; Beck, L. W.; Ferguson, D. B. *Acc. Chem. Res.* **1996**, *29*, 259.
(3) Xu, T.; Haw, J. F. *Top. Catal.* **1997**, *4*, 109.
(4) Haw, J. F.; Goguen, P. W.; Xu, T.; Skloss, T. W.; Song, W. G.; Wang, Z. K. *Angew. Chem., Int. Ed.* **1998**, 948.
(5) Gladden, L. F. *Top. Catal.* **1999**, *8*, 87.
(6) Ivanova, I. I. *Colloids Surf. A: Physicochem. Eng. Aspects* **1999**, *158*, 189.
(7) Han, X. W.; Yan, Z. M.; Zhang, W. P.; Bao, X. H. *Curr. Org. Chem.* **2001**, *5*, 1017.
(8) Hunger, M. *Catal. Today* **2004**, *97*, 3.
(9) Hunger, M. *Microporous Mesoporous Mater.* **2005**, *82*, 241.
(10) Hunger, M.; Wang, W. *Adv. Catal.* **2006**, *50*, 149.
(11) Kuhn, L. T.; Bargon, J. *Top. Curr. Chem.* **2007**, *276*, 125.
(12) Xu, M.; Harris, K. D. M.; Thomas, J. M.; Vaughan, D. E. W. *ChemPhysChem* **2007**, *8*, 1311.
(13) Xu, M.; Harris, K. D. M.; Thomas, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 5880.
(14) Perlovich, G. L.; Hansen, L. K.; Bauer-Brandl, A. *J. Therm. Anal. Cal.* **2001**, *66*, 699.
(15) Boldyreva, E. V.; Drebushchak, V. A.; Drebushchak, T. N.; Paukov, I. E.; Kovalevskaya, Y. A.; Shutova, E. S. *J. Therm. Anal. Cal.* **2003**, *73*, 409.
(16) Boldyreva, E. V.; Drebushchak, V. A.; Drebushchak, T. N.; Paukov, I. E.; Kovalevskaya, Y. A.; Shutova, E. S. *J. Therm. Anal. Cal.* **2003**, *73*, 419.
(17) Price, S. L.; Hamad, S.; Torrisi, A.; Karamertzanis, P. G.; Leslie, M.; Catlow, C. R. A. *Mol. Simul.* **2006**, *32*, 985.
(18) Towler, C. S.; Davey, R. J.; Lancaster, R. W.; Price, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 13347.
(19) Iitaka, Y. *Acta Crystallogr.* **1961**, *14*, 1.
(20) Hughes, C. E.; Hamad, S.; Catlow, C. R. A.; Harris, K. D. M.; Griffiths, P. C. *Faraday Discuss.* **2007**, *136*, 71.
(21) Torbeev, V. Y.; Shavit, E.; Weissbuch, I.; Leiserowitz, L.; Lahav, M. *Cryst. Growth Des.* **2005**, *5*, 2190.
(22) Dawson, A.; Allan, D. R.; Belmonte, S. A.; Clark, S. J.; David, W. I. F.; McGregor, P. A.; Parsons, S.; Pulham, C. R.; Sawyer, L. *Cryst. Growth Des.* **2005**, *5*, 1415.
(23) Dalton, J. B.; Schmidt, C. L. A. *J. Biol. Chem.* **1933**, *103*, 549.
(24) Metz, G.; Wu, X. L.; Smith, S. O. *J. Magn. Reson. A* **1994**, *110*, 219.
(25) Taylor, R. E. *Concepts Magn. Reson.* **2004**, *22A*, 79.
(26) The peak for the $\alpha$ polymorph can be fitted well by a single Lorentzian line. The addition of a second peak at the position characteristic of the $\gamma$ polymorph does not lead to any statistically significant improvement in the fit to the experimental data.
(27) Rietveld, H. M. *J. Appl. Crystallogr.* **1969**, *2*, 65.
(28) We have observed that dry powder samples of the $\alpha$ polymorph can transform to the $\gamma$ polymorph over several days or weeks. However, the transformation observed in the present work is substantially faster (a few hours), suggesting that a different mechanism of transformation is operative in the crystallization system studied here.

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