Polymorphism in the Indomethacin Molecule

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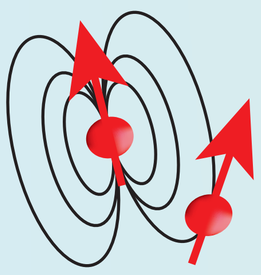
Solid State NMR

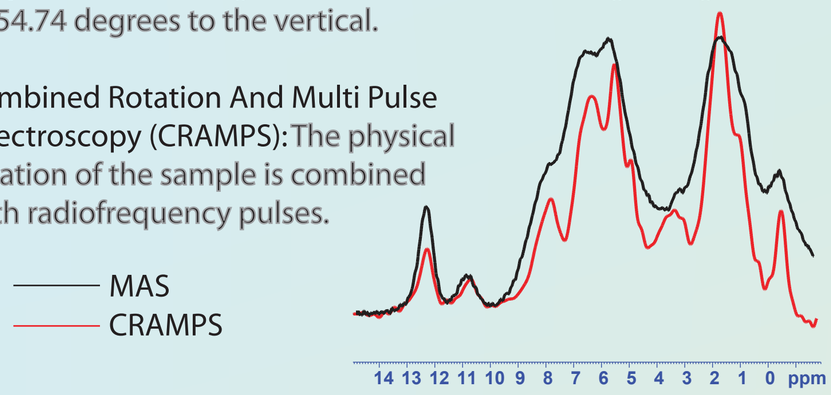
Solution State NMR is now a well established technique used by Chemists worldwide for a variety of purposes. NMR of the solid state, however, is theoretically and practically more difficult and is still in its early years of development. One of the key problems to overcome in solid state NMR is peak broadening caused by both the Chemical Shift Anisotropy (CSA) of the molecule and the dipolar interactions between local spins.



Novel experimental methods have been implemented to imitate the fast molecular tumbling present in solutions (resulting in the averaging out of the dipolar and CSA Hamiltonians). These Include;

Magic Angle Spinning (MAS): The sample is spun at high frequencies (kHz range)whilst inclined at the "magic" angle of 54.74 degrees to the vertical.

Combined Rotation And Multi Pulse Spectroscopy (CRAMPS): The physical rotation of the sample is combined with radiofrequency pulses.



Hydrogen spectra for the Alpha polymorph demonstrating the resolution that can be achieved using 30kHz MAS (black line) and 12.5 kHz CRAMPS (red line).

Numerous NMR techniques have been developed to discern structural information in the solid state. Both Hydrogen and Carbon NMR are key for organic molecules. The low natural abundance of Carbon-13 means that techniques like Cross Polarisation MAS (1) and INEPT (2) must be used to improve the signal to noise ratio. A Hydrogen technique is explained in detail in another section.

Aims and Objectives

The aim of this project is to develop NMR techniques to discern structural information about Pharmaceutical molecules of known crystal structures demonstrating polymorphism.

The techniques will then be applied to amorphous solids of unknown structure.

What is polymorphism?

A molecule displays polymorphism if there exits crystalline forms of the solid with different unit cell configurations.

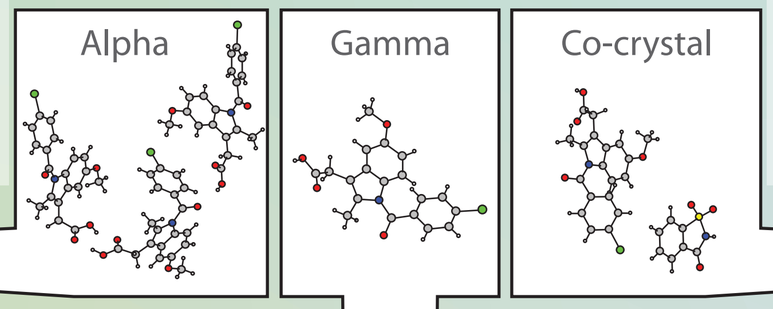
Why are polymorphs important?

The chemical and biological behaviour can vary between different polymorphs of the same substance. Knowing which polymorph is present is therefore very important.

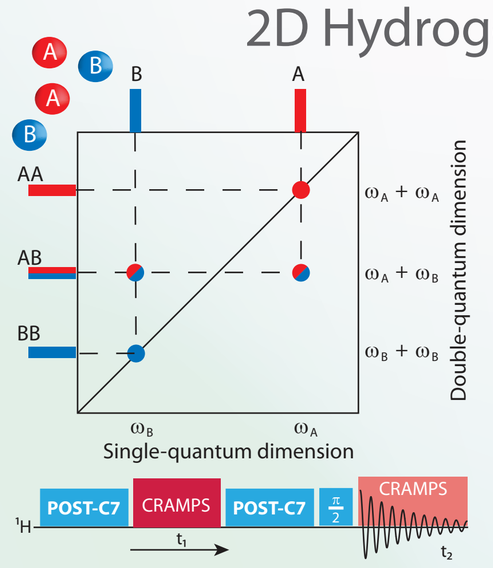
Indomethacin

Indomethacin is a non-steroidal anti-inflammatory pharmaceutical drug used for pain relief from conditions like Arthritis.

Indomethacin exists in many solid forms including polymorphic forms. Of these, the Alpha and Gamma structures were used in this investigation (shown below) along with an Indomethacin-Saccharin co-crystal produced by manually grinding Indomethacin with the sweetener Saccharin (3). Gamma has one Indomethacin molecule per unit cell, whilst Alpha has three.



2D Hydrogen Experiments



The high abundance of Hydrogen-1 in organic molecules leads to large dipolar coupled networks of protons.

Dipolar coupling,

$$\left|\mathbf{D}_{ij}\right| \propto \frac{\gamma_i \gamma_j}{\left|\mathbf{r}_{ij}\right|^3},$$

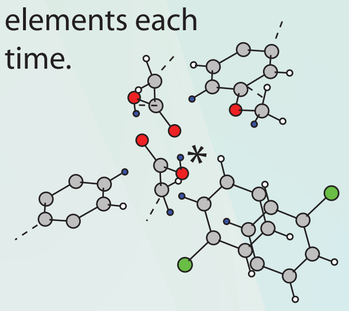
contains information on separation of nuclei.

2D Double Quantum Coherence (DQC) CRAMPS experiments display information on coupling whilst retaining good resolution. Like coupled nuclei peak on diagonal whilst unlike coupled nuclei peak either side of the diagonal.

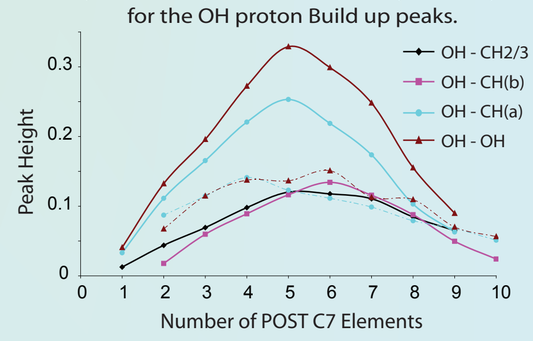
Can investigate Build up of DQC by incrementing the number POST C7 re-coupling elements.

Simulations

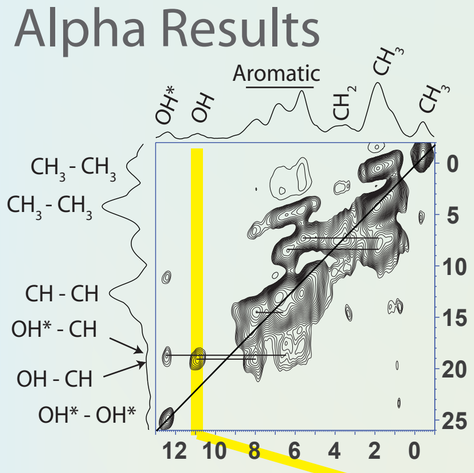
We can investigate Build-up using Spinevolution (a density matrix simulation package (4)) and compare these results to the experimental results. Only small sections of the 2D spectrum are interesting so we can take a slice at the chemical shift of a particular nucleus and investigate its DQC build up peaks. The nucleus of interest is selected, along with the 7 nearest Hydrogen nuclei (see diagram) and the simulations are run, incrementing the number of POST C7 elements each time.



Indomethacin Gamma: Experimental (dashed line) versus Simulation (solid line) for the OH proton Build up peaks.



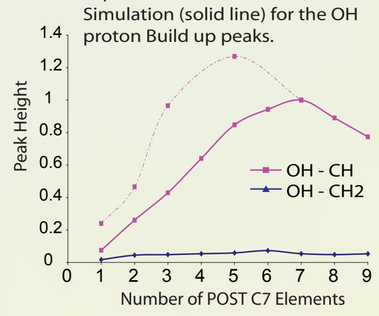
Alpha Results

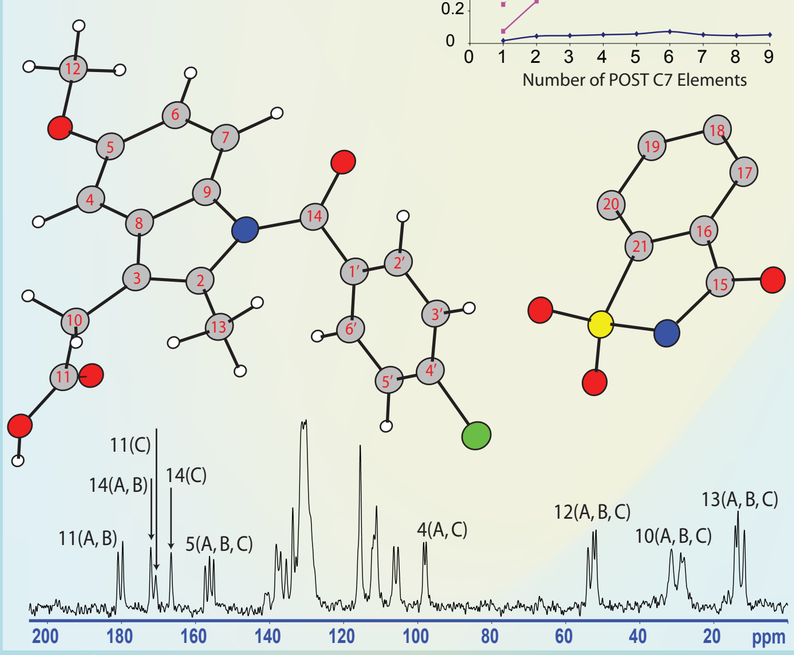


The Alpha ^{1}H 2D spectrum (left) is much more clustered than the Gamma because the number of Hydrogen nuclei in the asymmetric unit cell has increased. Build-up is shown below for the OH proton.

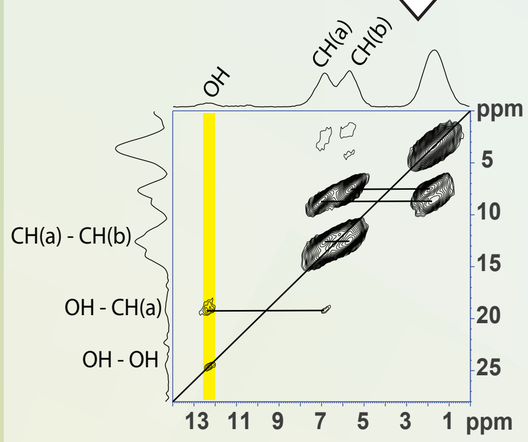
Because there are now three Indomethacin molecules per unit cell, the peaks in the Carbon spectrum (below) appear as triplets.

Experimental (dashed line) versus Simulation (solid line) for the OH proton Build up peaks.



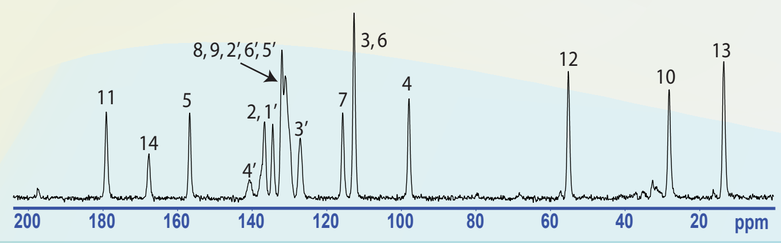


Gamma Results

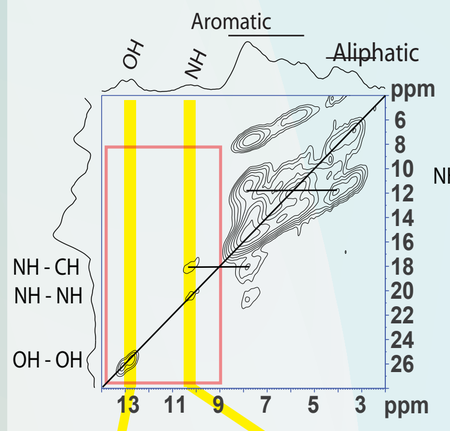


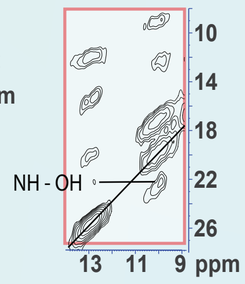
The ^{1}H 2D DQC Gamma spectrum is shown (left). Experimental versus simulated build-up can be seen for the OH proton (the highlighted slice) and is shown in the simulation section of the poster.

For the Carbon-13 spectrum, peak identification was made possible using a combination of CASTEP and INEPT results.



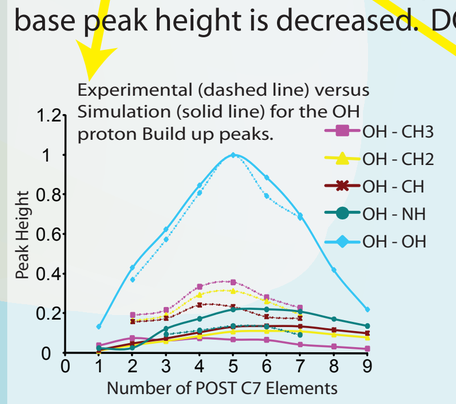
Co-crystal Results



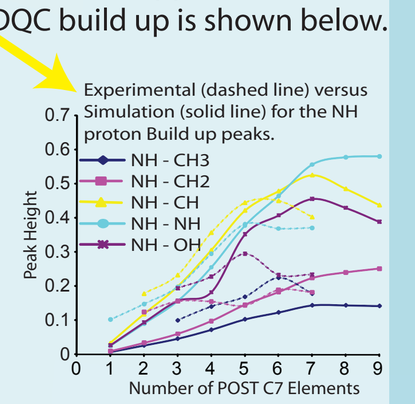


The presence of Saccharin introduces a NH proton into the ^{1}H 2D DQC spectrum. The close proximity of the indomethacin and saccharin molecule is evident from the NH-OH cross peak which appears when the base peak height is decreased. DQC build up is shown below.

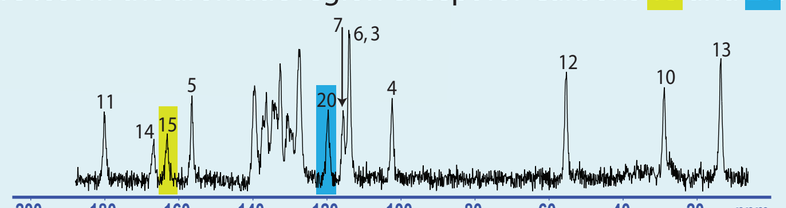
Experimental (dashed line) versus Simulation (solid line) for the OH proton Build up peaks.



Experimental (dashed line) versus Simulation (solid line) for the NH proton Build up peaks.



New peaks appear in the Carbon-13 spectrum due to the presence of Carbon nuclei from the Saccharin molecule. Most are lost in the aromatic region except for Carbons 15 and 20.



Summary

Both ^{1}H 2D DQC CRAMPS and Carbon-13 spectra can be used to differentiate between polymorphs and other solid forms of the Indomethacin molecule.

Furthermore, semi-quantitative structural information can be gained from ^{1}H DQC build-up.

Finally, a combination of CASTEP results (ref) and INEPT results allow for accurate Carbon-13 peak assignment.

Acknowledgements

Many thanks to the University of Warwick NMR department, especially Steven Brown and Jonathan Bradley.

References

(1) and (2) see M.Levitt, *Spin Dynamics - Basics of Nuclear Magnetic Resonance*, 2nd ed. p440.
(3) S.Basavoju, D.Boström, S.P.Velaga, *Pharmaceutical research*. 25 (2008) 530-41.
(4) M.Veshtort, R.G.Griffin, *J. Magn. Reson.*, 178 (2006) 248-282.