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Pharmaceutical hydrates at ambient conditions from high-pressure seeds: a case study on GABA monohydrate§

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The monohydrate form of the neurotransmitter γ-amino butyric acid (GABA) has been crystallised in the 0.4-0.8 GPa pressure range, recovered to ambient pressure and then used as a seed. Theoretical calculations indicate that this hydrate is only thermodynamically favoured over the two anhydrous forms at high pressures.

§Celebrating 300 years of Chemistry at Edinburgh

γ-Amino butyric acid (GABA) is a non-standard gamma-amino acid and the main inhibitory neurotransmitter in the central nervous system.1 GABAergic drugs have sedative and anti-convulsive effects; they are employed for the treatment of neurological disorders such as epilepsy, anxiety and Parkinson's disease.2-4

GABA exists as a neutral molecule exhibiting extensive conformational flexibility in the gas phase5 and can exist as a zwitterion (Fig. 1) or a cation in solution and the solid state. Two anhydrous polymorphs containing the zwitterionic form have been reported for GABA: a stable monoclinic6 and a metastable tetragonal polymorph,7,8 both of which have been crystallised from aqueous solutions. Whilst a hydrate structure of GABA had never been observed, GABA zwitterions have been suggested to form stable clusters with water (GABA.2H2O and GABA.5H2O) in solution.9

Intrigued by the fact that GABA forms stable clusters with water in aqueous solutions and yet by the absence of GABA hydrates in the solid state, we set out to investigate which solid forms would result from in situ high-pressure crystallisation experiments of aqueous GABA solutions. Previous studies of small organic compounds indicate that under these conditions water tends to be included into their crystal structures. This has been shown for several small organic molecules10-12 including the pharmaceuticals paracetamol,13 piracetam,14 ciprofloxacin,15 and the GABA analogue gabapentin.16

A GABA monohydrate was reproducibly obtained by in situ high-pressure crystallisation and crystal growth in a diamond-anvil cell (DAC) of the Ahsbahs type17 (Fig. 2) from a variety of aqueous solutions in the 0.4 - 0.8 GPa pressure range.1 The high-pressure structure was elucidated by single-crystal X-ray diffraction (SXRD) at the ANKA synchrotron.5

Recovery of GABA monohydrate to ambient pressure proceeded in a straightforward manner and at ambient-temperature conditions (Fig. 2; the DAC was rapidly opened to prevent extensive dissolution and the crystal immersed in mounting oil. Subsequent to recovery, SXRD data were collected at 150 K on our home diffractometer, confirming that no phase transition had taken place.1 High-pressure crystallisation of GABA monohydrate was repeated several times, always yielding the monohydrate and all crystals were easily recovered. Recovered crystals were then used to seed saturated aqueous solutions of GABA at ambient conditions, as confirmed by SXRD. Although no extensive crystallisation screening was conducted, we were only able to obtain the monohydrate form with the help of hydrate seeds obtained from the high-pressure crystallisations. In the absence of these seeds, all our crystallisations at ambient conditions yielded anhydrous monoclinic GABA.

Fig. 1 Chemical diagram of the GABA zwitterion with carbon backbone naming.

Fig. 2 Optical images of GABA monohydrate a) at 0.48 GPa in the DAC and b&c) recovered to ambient temperature and rotated on its side by 90°.
Waals corrections linked by antiparallel H-bonded and double-stranded.

Average increased, however, the hydration enthalpy becomes increasingly more negative, up to -9 kJ/mol at 0.8 GPa. Average

Δenthalpy of hydration of GABA,

Periodic DFT calculations (PBE)

PIXEL calculations indicate that the strongest dimer interaction in the crystal structure is associated with the GABA centrosymmetric H-bonded dimer (-389.3 kJ/mol). Whilst all interaction energies between molecular pairs linked by H-bonding are strong and stabilising, four other non H-bonded molecular pairs rank amongst the six energetically most significant interactions because of their favourable dipolar arrangements (-114.7 - -72.6 kJ/mol). These interactions are characterised by a significant Coulombic energy contribution to the total interaction energy and a very small repulsive term (Table S4 and Fig. S3, ESI†).

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Periodic DFT calculations (PBE) with the Grimme van der Waals corrections were performed in order to calculate the enthalpy of hydration of GABA, ΔH_{hyd}, as a function of pressure (Fig. 4). At 0 K, ΔH_{hyd} is defined as the difference between the enthalpy of the GABA hydrate minus the sum of the enthalpies of the most stable GABA and ice polymorphs at a given pressure. A negative ΔH_{hyd} indicates that the hydrate structure is more stable than the anhydrous form plus ice at a given set of conditions. Whist recent ambient-pressure calculations have shown that cocrystal, solvate and hydrate formation are indeed driven by thermodynamics, to the best of our knowledge this is the first time that such calculations have been performed under a pressure range.

The change of the enthalpy of hydration with pressure is depicted in Fig. 4. At ambient pressures, close to 0 GPa, there is no driving force for hydrate formation (ΔH_{hyd} = ~0 kJ/mol). The enthalpy of the hydrate is equal to that of ice XI and the stable monoclinic GABA polymorph at 0 K. As the pressure is increased, however, the hydration enthalpy becomes increasingly more negative, up to -9 kJ/mol at 0.8 GPa. Average cocrystallisation energies lie around -11 kJ/mol according to a recent study with a similar computational model. Our theoretical calculations nicely corroborate the experimental observations: GABA monohydrate is obtained at pressures between 0.4-0.8 GPa, for which the ΔH_{hyd} lies between -5 up to -9 kJ/mol, and the monohydrate can be recovered at ambient pressures because it is energetically close to monoclinic GABA plus ice XI at those conditions (ΔH_{hyd} = ~0 kJ/mol). Although there is no driving force for hydrate formation at ambient conditions, if seeds of the hydrate are present in solution, growth of the hydrate occurs because the hydrate is energetically close to anhydrous GABA plus ice.

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Whether an opportunity or a nuisance, the study of

Whether those hydrates can be recoverable to ambient pressures and be used as seeds may be indeed anticipated with the calculations presented herein.

The phenomenon of hydrate formation has long been the subject of intensive research from both the academic and industrial communities. Approximately one third of organic molecules in the Cambridge Structural Database and a similar percentage of pharmaceuticals, crystallise as hydrates. Hydrates actually "form an integral part of many pharmaceutical dosage forms". Whether an opportunity or a nuisance, the study of
hydrate formation in the pharmaceutical industry is a necessity. In our study we have illustrated the benefits of performing crystallisation experiments under high-pressure conditions followed by the recovery of forms to ambient conditions for their use as seeds. Following that procedure, we were able to consistently produce a hydrate, otherwise elusive, at ambient conditions. Whether original seeds come from other isomorphic materials or from crystallisations at high-pressure conditions as shown herein, the seeding techniques can promote the realisation of otherwise unobservable forms under ambient conditions.

While a particular form may not find industrial applications, our study demonstrates how knowledge of the structural landscape of a compound can be extended, potentially providing useful information for devising improved manufacturing strategies and for patent protection.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Full crystallographic details and CIF files for the high-pressure and low-temperature structures, crystal packing description and figures, comparison of torsion angles in GABA structures deposited in the CSD, details of PIXEL calculations, and details of the computational study. See DOI: 10.1039/b000000x/

‡ High-pressure crystallisation experiments: aqueous solutions (6-12 M) and 2: 1 MeOH : H2O solutions (4 M) were loaded in Beryllium-free DACs of the Absbhs type (45° half-cell opening angle) equipped with 600 µm culet diamonds and an Inconel gasket with a starting diameter hole of ca. 300 µm. On increasing pressure, precipitation of polycrystalline material was observed and a single crystal was grown by cycling the temperature inside the DAC.

Crystal data for GABA monohydrate at 0.44 GPa, CCDC deposition number 969073: C3H4N4O3H2O, M = 121.14, a = 14.3465(10) Å, b = 9.05, β = 94.8993(13)°, γ = 90°, V = 1151.6(5) Å3, T = 296(2) K, space group P41212, Z = 8, calculated density = 1.371 g cm−3, 3751 reflections measured, 463 independent reflections (Rint = 0.051). The final R1 values was 0.04 (I > 2σ(I)). The final wR2 value was 0.11 (all data).

Crystal data for GABA monohydrate at 150K, CCDC deposition number 969074: CH3NO4•H2O, M = 121.14, a = 14.3799(9) Å, b = 9.6552(4) Å, c = 14.4202(9) Å, a = 90°, β = 94.8993(13)°, γ = 90°, V = 1169.05(13) Å3, T = 150(2) K, space group P21212, Z = 8, calculated density = 1.371 g cm−3, 3128 reflections measured, 1669 independent reflections (Rint = 0.051). The final R1 values was 0.038 (I > 2σ(I)). The final wR2 value was 0.10 (all data).


