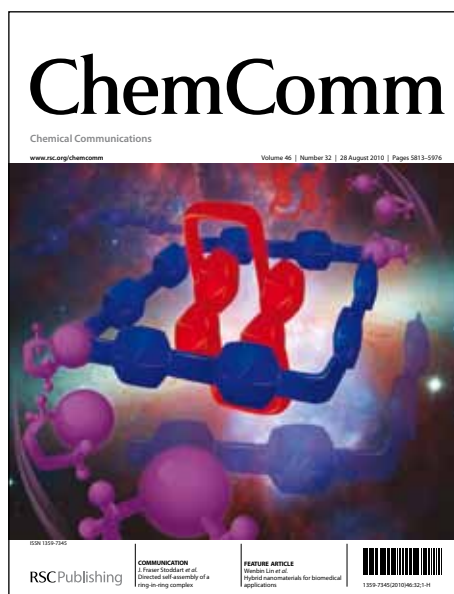


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ARTICLE TYPE

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. 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 molecules^{10–12} 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.[‡] The high-pressure structure was elucidated by single-crystal X-ray diffraction (SXRD) at the ANKA synchrotron.[‡]

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.[‡] 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.

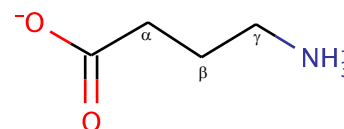


Fig. 1 Chemical diagram of the GABA zwitterion with carbon backbone naming.

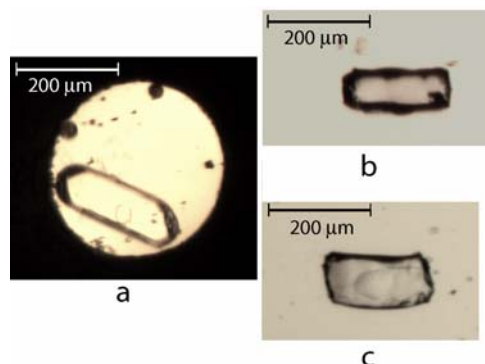


Fig. 2 Optical images of GABA monohydrate a) at 0.48 GPa in the DAC and b)&c) recovered to ambient temperature and rotated on its side by 90°.

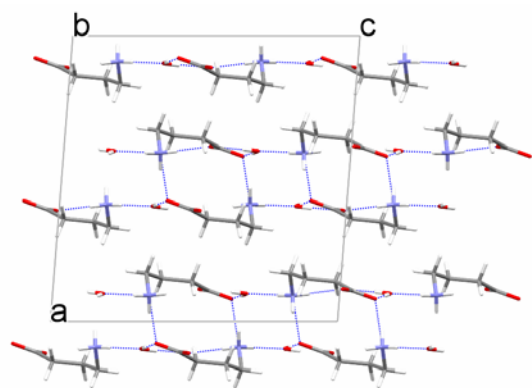


Fig. 3 2-D layered structure of GABA monohydrate viewed along the b -axis. H-bonds are depicted as dotted lines.

A representation of the crystal structure of the GABA monohydrate is given in Fig. 3. GABA displays a folded conformation most similar to the one observed in the tetragonal polymorph (Table S3, ESI[†]). Using graph-set notation,¹⁸ the main building block of the crystal structure can be described as being composed of centrosymmetric $R^2_2(14)$ -GABA dimers which are linked by antiparallel H-bonded and double-stranded $C(7)$ chains running along the b -axis (Fig. 3 and S1, ESI[†]). Each strand is strengthened by accepting two H-bonds from a water molecule, which in turn links double strands related by glide symmetry. The resulting 2-D H-bonded layered structure propagates along the c -axis (Fig. 3).

PIXEL calculations¹⁹ 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 Coloumbic energy contribution to the total interaction energy and a very small repulsive term (Table S4 and Fig. S3, ESI[†]).

Periodic DFT calculations (PBE)²⁰ with the Grimme van der Waals corrections²¹ 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. Whilst recent ambient-pressure calculations have shown that cocrystal, solvate and hydrate formation are indeed driven by thermodynamics,^{22,23} 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 ($\Delta H_{\text{hyd}} = \sim 0$ 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.²⁴ 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 to ambient pressures because it is energetically close to monoclinic GABA plus ice XI at those conditions ($\Delta H_{\text{hyd}} = \sim 0$ 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.

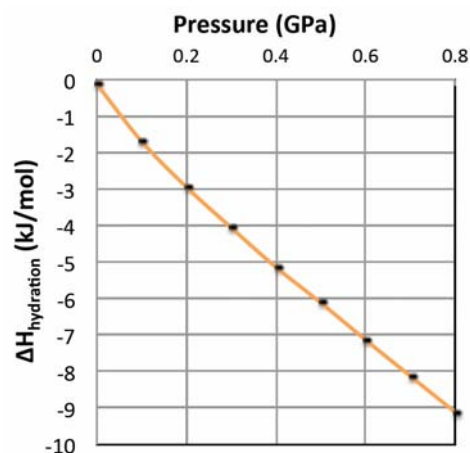


Fig. 4 Calculated hydration enthalpy of GABA as a function of pressure.

But, what is the reason for the GABA hydrate becoming more stable than the anhydrous form plus ice at higher pressures? As the pressure is increased, the enthalpies of all the involved forms become less stabilising because molecules are forced closer together and repulsions become more important (Fig. S6, ESI[†]). We noticed that compressing water within ice costs relatively more enthalpy than compressing water within the GABA monohydrate structure. Compression of a water molecule in ice from 0 to 0.8 GPa occurs at a structural cost of shortening 4 H-bonds by 0.035 Å each and an enthalpic cost of 14 kJ/mol. In contrast, compression of water within the GABA hydrate from 0 to 0.8 GPa occurs at a structural cost of shortening 3 H-bonds by an average of 0.019 Å each and an estimated enthalpic cost of 5 kJ/mol. Hence, this indicates that because of the poorer compressibility of water within ice,²⁵ hydration of GABA becomes thermodynamically favoured at higher pressures. This is likely to be the case for many other pharmaceuticals; in fact, high-pressure crystallisations are known to produce hydrates otherwise unseen under ambient conditions.¹⁰⁻¹⁶ 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,²⁶ and a similar percentage of pharmaceuticals,²⁷ crystallise as hydrates. Hydrates actually "form an integral part of many pharmaceutical dosage forms".^{27,28} 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 isomorphous materials²⁹ or from crystallisations at high-pressure conditions as shown herein, the seeding techniques can promote the realisation of otherwise unobservable forms under ambient conditions. Whilst 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 Ahsbahs type¹⁷ (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: C₄H₉NO₂·H₂O, *M* = 121.14, *a* = 14.276(6) Å, *b* = 5.6339(3) Å, *c* = 14.3645(10) Å, *α* = 90°, *β* = 94.598(13)°, *γ* = 90°, *V* = 1151.6(5) Å³, *T* = 296(2) K, space group *C2/c*, *Z* = 8, calculated density = 1.397 g cm⁻³, 3751 reflections measured, 463 independent reflections (*R*_{int} = 0.051). The final *R*_i values was 0.04 (*I* > 2σ(*I*)). The final *wR*(*F*²) value was 0.11 (all data). Crystal data for GABA monohydrate at 150K, CCDC deposition number 969074: C₄H₉NO₂·H₂O, *M* = 121.14, *a* = 14.3799(9) Å, *b* = 5.6552(4) Å, *c* = 14.4202(9) Å, *α* = 90°, *β* = 94.499(3)°, *γ* = 90°, *V* = 1169.05(13) Å³, *T* = 150(2) K, space group *C2/c*, *Z* = 8, calculated density = 1.377 g cm⁻³, 8128 reflections measured, 1669 independent reflections (*R*_{int} = 0.0256). The final *R*_i values was 0.038 (*I* > 2σ(*I*)). The final *wR*(*F*²) values was 0.10 (all data).

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