Pre-nucleation aggregation based on solvent microheterogeneity

Jones, C., Walker, M., & Edkins, K. (2019). Pre-nucleation aggregation based on solvent microheterogeneity. *Chemical Communications*. <https://doi.org/10.1039/C9CC01455A>

Published in:
Chemical Communications

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights
Copyright 2019, Royal Society of Chemistry.
This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

COMMUNICATION

Pre-nucleation aggregation based on solvent microheterogeneity

Christopher D. Jones,^a Martin Walker,^b Yitian Xiao,^a and Katharina Edkins^{*a}Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Investigation into pre-nucleation aggregates indicating hydrate formation of caffeine and theophylline in aqueous acetonitrile showed hydrate crystallisation at much lower water fraction than significant solute self-association. Molecular dynamics simulations indicate that the solvent separates on the molecular scale and that solute molecules preferentially localise on the phase interface.

Hydrates, *i.e.* crystal forms incorporating water from the crystallisation process, are amongst the most difficult pharmaceutical materials for formulation and processing due to their inherently low water solubility.¹ Efforts to identify these crystal forms early-on in the drug development, or even to predict them, range from extensive crystallisation screens² to elaborate *in silico* crystal structure prediction.^{3, 4} But a truly effective method has yet to be discovered. Of the estimated one third of APIs with hydrated solid forms,⁵ the methylated xanthines caffeine (1) and theophylline (2) are arguably the most extensively characterized (Fig. 1). Caffeine, a commonly consumed stimulant, forms a channel hydrate with a water/xanthine molar ratio of 0.8,^{6, 7} but keep in line with earlier publications, we will refer to this crystal form as monohydrate. The structure is stabilized by π -stacking and $\text{CH}\cdots\text{O}$ hydrogen-bonded dimer formation of the xanthine molecules, and H-bonding of water to the unsubstituted nitrogen atom.^{8, 9} The monohydrate of the bronchospasmolytic theophylline has a very similar channel structure, but shows stronger $\text{NH}\cdots\text{O}$ H-bonded dimer formation of host molecules.¹⁰ While theophylline monohydrate is physically more stable against dehydration than the caffeine monohydrate, both systems undergo complete dehydration at low humidity (<20% and <35% relative humidity, respectively).^{11–13} In addition to their hydrates, caffeine can form three anhydrous modifications,^{14, 15}

Figure 1. Molecular structures of caffeine (1, R=CH₃) and theophylline (2, R=H).

while theophylline exists in up to seven different anhydrous crystal forms,^{16–18} some of which can only be obtained as epitaxial adducts.^{19–22} Neutron total scattering experiments and molecular dynamics (MD) simulations show that caffeine forms extended stacks surrounded by poorly defined hydration shells in pure water solutions.^{23–25} This supramolecular stacking is driven by a reduction in area of the water-solute interface and a related enthalpic stabilization of the solvent. This has been suggested to be the initial stage of hydrate nucleation.^{26–28} However, the presence of water in solutions does not always favour hydrate formation. Slurry experiments in aqueous solvent mixtures reveal that the hydrates of xanthines and several other APIs are stable only above a critical water activity, corresponding to different water concentrations in different solvents.^{29–31}

To gauge the thermodynamic crystallisation products from aqueous acetonitrile, supersaturated solutions of caffeine (0.36 M) and theophylline (0.11 M) were stored at 20 °C for one week, and the crystalline products were analysed by powder X-ray diffraction (see ESI Fig. S1). Pure monohydrate for both solutes was obtained at mole fractions of water, $X_{\text{H}_2\text{O}}$, above 0.29, whilst mixtures of hydrate with anhydrous form β (caffeine) or form II (theophylline) were obtained at $X_{\text{H}_2\text{O}}$ between 0.15 and 0.29. Below $X_{\text{H}_2\text{O}} = 0.15$, the pure anhydrous crystal forms crystallised. Due to the highly non-ideal behaviour of acetonitrile/water mixtures, the water activity, a_w , rises from 0 to 0.82 in the range $X_{\text{H}_2\text{O}} = 0 - 0.4$.³² The thermodynamic concept a_w quantifies the availability of water to hydrate

^a School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom. Email: k.edkins@qub.ac.uk

^b Department of Chemistry, Durham University, South Road, Durham DH1 3LE, United Kingdom.

Electronic Supplementary Information (ESI) available: supplementary figures to the manuscript, experimental section. See DOI: 10.1039/x0xx00000x

Figure 2. Self-association K constants of caffeine (black squares) and theophylline (red triangles) vs. mole fraction water (X_{H_2O}) of the acetonitrile solvent as measured by NMR titrations.

material, *i.e.* form hydrogen bonds to solute or at interfaces, or participate in reactions in aqueous solvent mixtures and solutions. The activity a_w is independent of the solvent system used and gives a measure of how water-like the aqueous fraction behaves compared to pure water.³³ The reason for the rapid increase in a_w in aqueous acetonitrile at low X_{H_2O} is due to the nanoscale demixing of the components. This leads to frustrated hydrogen bonding for the water molecules and thus a higher propensity of interaction with solute molecules. For theophylline, the threshold a_w for hydrate crystallisation is reported to be 0.64 at 30 °C for acetone-water and methanol-water mixtures,²⁹ which fits well with our measurement of $a_w = 0.67$ at $X_{H_2O} = 0.29$ in acetonitrile at 20 °C. No comparable study has been performed for caffeine, but this system seems to follow the same trend as theophylline.

Since both caffeine and theophylline monohydrates show dimer formation in their crystal structure, we are interested to probe for these dimers in solution as indicators of hydrate nucleation. ¹H-NMR spectra of the compounds in concentrations ranging from 1 mM to the solubility limit in acetonitrile- d_3 were recorded at various fixed X_{D_2O} . Chemical shifts were referenced to the signal of residual protiated acetonitrile ($\delta_H = 2.0$), and a calibration curve was used to account for changes in solvent density for different X_{D_2O} (Fig. S2). Self-association constants K were estimated by simultaneous fitting of the methyl proton signals (Fig. 2) using an isodesmic polymerization model.³⁴ At low X_{D_2O} , the K values for caffeine (1.0 – 2.0 M^{-1}) are slightly higher than those for theophylline, which appears to undergo negligible self-association. Surprisingly, K rises rapidly for both solutes above $X_{D_2O} = 0.77$ with the endpoint K values for pure D_2O close to those reported.^{35–37} This behaviour does not reveal a significant difference in self-aggregation between the two compounds. In addition, the low value of the binding constants relates to a surprisingly low Gibbs free energy of the interactions (just under 2 kJ mol⁻¹), which suggests that the interaction motif probed is different to the hydrogen-bonded dimer observed in the crystal

structures. As we reported in an earlier study, the dimer motif in theophylline monohydrate is with an energy of approximately -30 kJ mol⁻¹ the second strongest individual interaction in the crystal structure³⁸ and can be classified as a strong neutral hydrogen bond.³⁹ The low Gibbs free energy of the solution interaction points towards a different interaction motif.

Even though the increase of self-association and the onset of hydrate crystallisation do not coincide and are on the first glance mutually exclusive, the behaviour can be explained by earlier observations of the molecular characteristics of acetonitrile-water mixtures.^{33, 40} In the range from $0.2 < X_{H_2O} < 0.8$, they show macroscopic homogeneity but microscopic phase separation with transient water clusters in a coherent acetonitrile matrix, a phenomenon called microheterogeneity.⁴¹ At lower X_{H_2O} the microscopic demixing leads to clustering and isolation of the water molecules and thus frustration of their hydrogen bonding network, which explains the steep increase in water activity as described above.⁴² Once the transient water clusters have formed, the behaviour of the mixture does not change significantly upon addition of further water. Finally, above $X_{H_2O} = 0.80$, the microheterogeneity is lost as neighbouring clusters merge into a percolated network and the overall properties of the solvent mixtures are dominated by water characteristics.⁴³ This change in the local environment affects the solute behaviour of both hydrophilic and hydrophobic species, impacting a wide range of reaction and aggregation processes. For example, both the K_a of 2-naphthol and the hydrolysis rate constant of *t*-butyl chloride in acetonitrile increase dramatically at $X_{H_2O} > 0.80$.⁴⁴

To gain further insight into the behaviour of caffeine and theophylline in aqueous acetonitrile, 0.1 M solutions of varying X_{H_2O} were analysed in atomistic molecular dynamics (MD) simulations. It is noted that H_2O and D_2O display similar trends in activity with varying concentration, although D_2O demixes more readily.⁴⁵ The simulations were performed in Gromacs 4.9.2⁴⁶ using the General Amber Force Field⁴⁷ a time step of 2 fs and fixed bond constraints. The solutions were modelled by solvating 60 solute molecules in a 10 nm cubic box with a random configuration of TIP5P water⁴⁸ and acetonitrile, matching the experimental density of the solvent mixture. After energy minimization and equilibration for 5 ns at 293 K and 1 bar, the systems were simulated for a further 5 ns under the same conditions. Solute-solute and solute-solvent interactions were analysed at 100 ps intervals and averaged over three replicate runs for each solvent composition.

As expected, pronounced clustering of water molecules was observed at all X_{H_2O} . Even at low X_{H_2O} , water and acetonitrile do not show molecular mixing but local demixing. Water molecules interact more strongly with each other than with acetonitrile molecules leading to clustering. However, these clusters are too small to show the H-bonded network of bulk water leading to H-bond frustrated water molecules, exhibiting the sharp rise in a_w (*vide supra*). At X_{H_2O} above 0.3, the clusters become large enough to exhibit bulk water characteristics while H-bond frustrated water molecules can only be found on the surface of the clusters. This is the region of microheterogeneity. Both

COMMUNICATION

Figure 3. MD simulations of a) caffeine and b) theophylline in acetonitrile/water mixtures at $X_{\text{H}_2\text{O}} = 0.5$ mole fraction and a concentration of 0.1 M. Acetonitrile molecules are represented in red, water in cyan and solute in light grey (space-filling). Quantitative data extracted from MD simulations of c) number of hydrogen bonds per molecule and d) proportion of molecules in clusters.

caffeine and theophylline preferentially localize at the water-acetonitrile interface without being individually solvated in either phase even when $X_{\text{H}_2\text{O}} = 0.9$. In the range $X_{\text{H}_2\text{O}} = 0.1 - 0.8$, water occupies 10 – 26% of the solvent-accessible area for theophylline but just 4 – 19% of the equivalent area for caffeine. Thus, the area of the solute-water interface represents an optimal balance between water-water and solute-water hydrogen bonding and increases only slightly as water is added (Fig. 3, S3).

In the range of $X_{\text{H}_2\text{O}}$ up to 0.3, the MD simulations show for both caffeine and theophylline a sharp rise in solute-water H-bonding before plateauing between $0.3 < X_{\text{H}_2\text{O}} < 0.8$, the microheterogeneous region (Figure 3c). At this critical value of $X_{\text{H}_2\text{O}}$, water forms one hydrogen bond with caffeine and 2.0 – 2.5 with theophylline. This could explain the crystallisation behaviour of both hydrates at lower $X_{\text{H}_2\text{O}}$, as the activity at which caffeine and theophylline hydrates become the only crystallisation products coincides exactly with the plateau in hydrogen bonding. Surprisingly, the number of homomeric theophylline hydrogen bonds decreases from 0.5 per molecule to zero, so dimer formation is unlikely to occur in solution, as evidenced by the low binding constants in the NMR titrations. The main interaction between the solute molecules is π -stacking to minimize solvent disruption (Figure 3d and S6). For theophylline, the proportion of molecules involved in stacking rises from 85% in pure acetonitrile to 90% at $X_{\text{H}_2\text{O}} = 0.2$ and remains approximately constant above that. By contrast, caffeine solutions exhibit a gradual increase in stacking from 75% to almost 90% up to $X_{\text{H}_2\text{O}} = 0.8$. In both systems, stacking becomes much more prevalent if $X_{\text{H}_2\text{O}}$ rises beyond 0.8, affecting 95% of the solute at $X_{\text{H}_2\text{O}} = 1$. This transition can be attributed to the loss of discrete water clusters, which

subsequently forces solute molecules to occupy a relatively water-rich solvent environment. This is directly comparable to the self-association constant K measured by NMR, which shows a sharp increase at $X_{\text{H}_2\text{O}} > 0.77$. It is thus clear that the microheterogeneities in the solvent mixture act as a template for solute aggregation, which will have a large influence on crystallisation behaviour.

The behaviour of either solute is only marginally influenced by concentration with the main impact being on the cluster size and thus solvent accessible surface (Fig. S4, S6, S7).

The influence of microheterogeneity on molecular assembly raises questions to be considered in the crystallisation and crystal growth community. The case of microheterogeneity in acetonitrile-water mixtures is well documented, although its effect on crystallisation has been overlooked, but there are also reports of DMSO-water,⁴⁹ acetone-water,⁴⁹ methanol-water^{50, 51} and DMF-water⁵² mixtures showing either microscopic phase separation or considerable clustering. These mixtures are frequently used for crystallisation. How many other solvent mixtures show microscopic structuring that can influence the molecular arrangement in solution and thus crystal nucleation? We should pay more attention to the solvent systems we use for crystallisation on the laboratory scale for research and development as well as the large scale of manufacturing. Furthermore, such effects are likely to be of relevance to all supramolecular chemistry in binary solvent mixtures.

In conclusion, we have shown that both caffeine and theophylline crystallise readily into their hydrated crystal forms from aqueous acetonitrile with $X_{\text{H}_2\text{O}} > 0.3$. This solvent composition corresponds to a water activity of 0.6 matching the threshold for hydrate crystallisation observed in other solvent systems. On the microscopic scale, however, the solute

molecules interact only weakly with each other at these low $X_{\text{H}_2\text{O}}$, while a significant increase in self-association can be observed by ^1H -NMR spectroscopy at $X_{\text{H}_2\text{O}} > 0.77$. With the help of MD simulations, we show that in the range between these values, both caffeine and theophylline localise at the interface of water clusters present in the microheterogeneous mixed solvent system. This result raises the question of the influence of microheterogeneity on supramolecular assembly in mixed solvent systems, which can have subsequent effect on sensing, material science and synthesis.

We thank the Engineering and Physical Science Research Council for funding (EP/P002870/2), raw data is available free of charge upon request.

Conflicts of interest

There are no conflicts to declare.

References

- R. K. Khankari and D. J. W. Grant, *Thermochim. Acta*, 1995, **248**, 61–79.
- A. Y. Lee, D. Erdemir and A. S. Myerson, *Annu. Rev. Chem. Biomol. Eng.*, 2011, **2**, 259–280.
- D. E. Braun and U. J. Griesser, *Cryst. Growth Des.*, 2016, **16**, 6111–6121.
- D. E. Braun, P. G. Karamertzanis and S. L. Price, *Chem. Commun.*, 2011, **47**, 5443–5445.
- F. Tian, H. Y. Qu, A. Zimmermann, T. Munk, A. C. Jørgensen and J. Rantanen, *J. Pharm. Pharmacol.*, 2010, **62**, 1534–1546.
- U. J. Griesser and A. Burger, *Int. J. Pharm.*, 1995, **120**, 83–93.
- J. Pirttimäki and E. Laine, *Eur. J. Pharm. Sci.*, 1994, **1**, 203–208.
- L. Carlucci and A. Gavezzotti, *Chem. Eur. J.*, 2005, **11**, 271–279.
- H. G. M. Edwards, E. Lawson, M. de Matas, L. Shields and P. York, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1985–1990.
- D. J. Sutor, *Acta Crystallogr.*, 1958, **11**, 83–87.
- K. P. Nartowski, Y. Z. Khimyak and D. J. Berry, *CrystEngComm*, 2016, **18**, 2617–2620.
- J. F. Krzyzaniak, G. R. Williams and N. Ni, *J. Pharm. Sci.*, 2007, **96**, 1270–1281.
- K. L. Vora, G. Buckton and D. Clapham, *Eur. J. Pharm. Sci.*, 2004, **22**, 97–105.
- G. D. Enright, V. V. Terskikh, D. H. Brouwer and J. A. Ripmeester, *Cryst. Growth Des.*, 2007, **7**, 1406–1410.
- E. Dichi, B. Legendre and M. Sghaier, *J. Therm. Anal. Calorim.*, 2014, **115**, 1551–1561.
- K. Fücke, G. J. McIntyre, C. Wilkinson, M. Henry, J. A. K. Howard and J. W. Steed, *Cryst. Growth Des.*, 2012, **12**, 1395–1401.
- Y. Ebisuzaki, P. D. Boyle and J. A. Smith, *Acta Cryst. Sect. C*, 1997, **53**, 777–779.
- R. Bobrows, L. Seton and N. Dempster, *CrystEngComm*, 2015, **17**, 5237–5251.
- M. D. Eddleston, K. E. Hejczyk, A. M. C. Cassidy, H. P. G. Thompson, G. M. Day and W. Jones, *Cryst. Growth Des.*, 2015, **15**, 2514–2523.
- M. D. Eddleston, K. E. Hejczyk, E. G. Bithell, G. M. Day and W. Jones, *Chem. Eur. J.*, 2013, **19**, 7883–7888.
- M. D. Eddleston, E. G. Bithell and W. Jones, *J. Pharm. Sci.*, 2010, **99**, 4072–4083.
- C. Roy, A. Vega-González and P. Subra-Paternault, *Int. J. Pharm.*, 2007, **343**, 79–89.
- L. Tavagnacco, P. E. Mason, G. W. Neilson, M.-L. Sabounji, A. Cesàro and J. W. Brady, *J. Phys. Chem. B*, 2017, DOI: 10.1021/acs.jpcc.7b07798.
- L. Tavagnacco, Y. Gerelli, A. Cesàro and J. W. Brady, *J. Phys. Chem. B*, 2016, **120**, 9987–9996.
- L. Tavagnacco, J. W. Brady, F. Bruni, S. Callear, M. A. Ricci, M. L. Sabounji and A. Cesàro, *J. Phys. Chem. B*, 2015, **119**, 13294–13301.
- A. J. Cruz-Cabeza, R. J. Davey, S. S. Sachithanathan, R. Smith, S. K. Tang, T. Vetter and Y. Xiao, *Chem. Commun.*, 2017, **53**, 7905–7908.
- R. Sanjeewa and S. Weerasinghe, *Comput. Theor. Chem.*, 2011, **966**, 140–148.
- A. Cesaro, E. Russo and V. Crescenzi, *J. Phys. Chem.*, 1976, **80**, 335–339.
- M. D. Ticehurst, R. A. Storey and C. Watt, *Int. J. Pharm.*, 2002, **247**, 1–10.
- H. Zhu, C. Yuen and D. J. W. Grant, *Int. J. Pharm.*, 1996, **135**, 151–160.
- J. B. Bogardus, *J. Pharm. Sci.*, 1983, **72**, 837–838.
- G. Bell, A. E. M. Janssen and P. J. Halling, *Enzyme Microb. Technol.*, 1997, **20**, 471–477.
- M. J. Blandamer, J. B. F. N. Engberts, P. T. Gleeson and J. C. R. Reis, *Chem. Soc. Rev.*, 2005, **34**, 440–458.
- P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305–1323.
- N. D'Amelio, L. Fontanive, F. Uggeri, F. Suggi-Liverani and L. Navarini, *Food Biophys.*, 2009, **4**, 321–330.
- D. D. Andrejuk, A. A. H. Santiago, V. V. Khomich, V. K. Voronov, D. B. Davies and M. P. Evstigneev, *J. Mol. Struct.*, 2008, **889**, 229–236.
- I. Horman and B. Dreux, *Helv. Chim. Acta*, 1984, **67**, 754–764.
- K. Fücke, G. J. McIntyre, C. Wilkinson, M. Henry, J. A. K. Howard and J. W. Steed, *Cryst. Growth Des.*, 2012, **12**, 1395–1401.
- G. A. Jeffrey, *An Introduction to Hydrogen Bonding*, OUP, Oxford, 1st edn., 1997.
- Y. Marcus, *J. Phys. Org. Chem.*, 2012, **25**, 1072–1085.
- Y. Marcus and Y. Migron, *J. Phys. Chem.*, 1991, **95**, 400–406.
- H. T. French, *J. Chem. Thermodyn.*, 1987, **19**, 1155–1161.
- J. Chen and P. H. L. Sit, *Chem. Phys.*, 2015, **457**, 87–97.
- A. Wakisaka, Y. Shimizu, N. Nishi, K. Tokumaru and H. Sakuragi, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 1129–1135.
- J. Szydlowski and M. Szykuła, *Fluid Phase Equilibria*, 1999, **154**, 79–87.
- D. Van der Spoel, E. Lindahl, B. Hess, G. Groenhof, A. E. Mark and H. J. C. Berendsen, *J. Comput. Chem.*, 2005, **26**, 1701–1718.
- D. A. Case, T. E. Cheatham, T. Darden, H. Gohlke, R. Luo, K. M. Merz, A. Onufriev, C. Simmerling, B. Wang and R. J. Woods, *J. Comput. Chem.*, 2005, **26**, 1668–1688.
- M. W. Mahoney and W. L. Jorgensen, *J. Chem. Phys.*, 2000, **112**, 8910–8922.
- S. E. McLain, A. K. Soper and A. Luzar, *J. Chem. Phys.*, 2007, **127**, 12.
- S. Dixit, J. Crain, W. C. K. Poon, J. L. Finney and A. K. Soper, *Nature*, 2002, **416**, 829.
- L. Dougan, S. P. Bates, R. Hargreaves, J. P. Fox, J. Crain, J. L. Finney, V. Réat and A. K. Soper, *J. Chem. Phys.*, 2004, **121**, 6456–6462.
- L. Zoranić, R. Mazighi, F. Sokolić and A. Perera, *J. Chem. Phys.*, 2009, **130**, 124315.