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Ostwald's Rule and Enantiotropy: polymorph appearance in the crystallisation of *p*-aminobenzoic acid.

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The development of rational crystallisation strategies in polymorphic systems requires the experimental manipulation of both kinetics and thermodynamics. We show for the first time the results of the interplay between these competing driving forces in an enantiotropic system, *p*-aminobenzoic acid. The outcomes are unexpected with temperature having no impact.

The enormous scientific and commercial significance of crystalline polymorphism underlines the importance of rational crystallisation strategies for preparing structurally pure polymorphic materials from solution. In 2000 Threlfall noted (1) that 'Most of the accounts which purport to address this issue prove on close examination to be plausible deductions from a limited set of specific experimental observations, but unrelatable to the general problem of the interaction between thermodynamic and kinetic factors …..'. In fact our best guide in this context remains Ostwald's Rule of Stages first put forward in 1897(2). For the many systems in which polymorphic forms are monotropically related much recent work (*eg*. glycine (3), L-glutamic acid (4), 2,6 dihydroxybenzoic acid (5), *o* and *m*-aminobenzoic acids (6,7), mannitol (8), benzamide (9)) offers continued support of the Rule with initial crystallisation of metastable phases over a wide range of conditions. Limited data on the water/inosine system (10) showed this to be the case for the crystallisation of the two monotropically related polymorphs but below 10°C, where the enantiotropically related dihydrate exists, direct crystallisation of this stable phase was possible. Apart from this and the much earlier report of Sato and Boistelle (11) on polymorphic mixtures of stearic acid, we are unaware of any systematic studies concerning the relationship between polymorph appearance, solvent, supersaturation and temperature in an *enantiotropic* system. It is our belief, surprisingly that this current work is the first to explore this topic for a molecular material crystallising from solution.

As a model system we have chosen *p*-aminobenzoic acid (PABA) crystallising from aqueous and ethanolic solutions. The two enantiotropically related polymorphs, α and β have a transition temperature 13.8°C (12)) with known crystal structures (13, 14). α is the stable form above 13.8°C and β below. The two forms are based on differing hydrogen bonded motifs and have distinct needle and rhombic morphologies (15). Gracin and Rasmuson (16) reported that in aqueous solutions both forms, α and β, can be crystallised, while only α PABA appears from organic solvents, over a wide temperature range. Svard *et al* (17) confirmed this in 330 cooling crystallisation experiments in the temperature range 15-30°C from methanol, acetonitrile and ethyl acetate. Most recently Sullivan *et al* (18) used the induction time probability technique to determine the nucleation rates of α PABA from 2-propanol, acetonitrile and ethyl acetate and demonstrated the importance of desolvation and self-assembly in the nucleation pathway. Again, in these experiments performed at 20°C, over the supersaturation range 1.1 to 1.5, only α crystallised. In this current work we set out to make a full record of solvent, supersaturation and temperature effects on the crystallisation of PABA. By performing crystallisation experiments above, at and below the transition temperature (5 to 25°C) at well-defined supersaturations from both aqueous and ethanolic solutions it was anticipated that this enantiotropic system would allow us to report on the balance of kinetics and thermodynamic that control polymorph appearance.

The solubility data used to define experimental supersaturations were taken from previous publications. In the case of ethanol the data of Hao *et al* (12) were used together with their value of 13.8°C for the transition temperature. In the case of aqueous solutions however, because the solubility is low and the solubilities of the forms close together the available data are less equivocal in defining the transition temperature (15). To circumvent this we have force-fitted the aqueous solubility data of Gracin and Rasmusson (15) through 13.8°C using the relationship $X = Ae^{-BT}$ with X in g/kgH_2O and T in °C. The best fit values of A (g/kgH_2O) and B ($^oC^{-1}$) for α and β solubilities were 2.01, -0.037 and 1.89, -0.042 respectively as seen in Figure 1. Crystallisation experiments were performed using a temperature jump method in which 30g of water or ethanol and a known weight (typically between 0.1 and 0.2g for water and 3-6g for ethanol) of PABA, determined by the desired supersaturation, were held in a 100ml jacketed vessel at 15°C above the saturation temperature in order to create a solution of known composition.

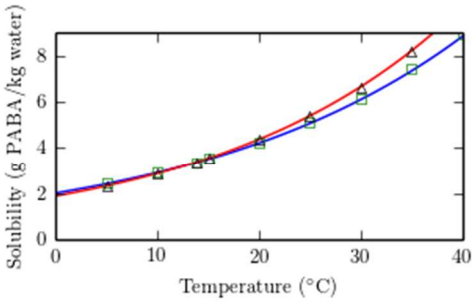

Figure 1. Aqueous solubility of α (blue line) and β(red line) forms fitted through the transition temperature of 13.8°C. Green squares α form and black squares β form. Data are taken from ref 16.

By switching thermostats and employing magnetic stirring this solution was then cooled to the desired crystallisation temperature in a maximum time of 4 minutes. Once crystallisation had commenced (typically 30mins for β and 10mins for α) a sample of slurry was removed immediately for characterisation of the polymorph produced. In some aqueous solution experiments light transmission through the solution was monitored as a means of assessing the induction time taken for the first crystals to appear. Experimental compositions and temperatures in relation to the solubility curves are shown in Figure 2 for both aqueous and ethanolic experiments. Crystallisation temperatures both above (15, 25, 26°C) at (13.8°C) and below (5,10°C) the transition temperature were chosen.

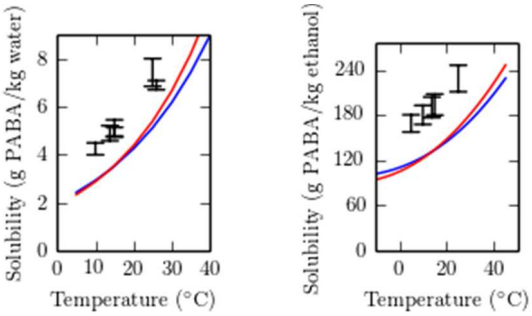

Figure 2. The experimental landscape for crystallisation of PABA from aqueous solutions (LHS) and from ethanolic solutions (RHS). Vertical bars show experimental temperatures and horizontal cross lines indicate range of starting compositions used. The solubility curves for the two forms are shown by the red (β) and blue (α) lines.

In all experiments solutions were supersaturated with respect to both forms. Supersaturation values quoted (S) are with respect to the α form at all temperatures, as the ratio of the supersaturated to saturated compositions in g/kgsolvent. The products of these experiments were characterised by examination under the optical microscope (Zeiss Axioplan 2 Microscope with Linksy software to capture and edit images) and by pXRD (Rigaku Miniflex benchtop XRPD machine in the Bragg-Brentano geometry with all powder samples scanned between 5° and 40° 2θ at a rate of 1.5° per minute with a step size of 0.03°).

For experiments in which the stable form appeared it was deemed important to establish whether this was the result of primary nucleation or of form conversion from the metastable form. For this reason experiments were also performed to assess the time taken for transition between the forms at both 25°C (β to α) and at 10°C (α to β). The same vessel and similar slurry densities were used as in the crystallisation experiments. Experiments were left to run for a maximum time of 24 hours after which the remaining solids were characterised by pXRD. No transformations between forms at either temperature were observed over the 24 hour period. This result is important since, given that typical crystallisation times were of the order of minutes, it allows us to conclude that the occurrence of a stable form reflects a primary nucleation process rather than being the result of a rapid transformation from a metastable state.

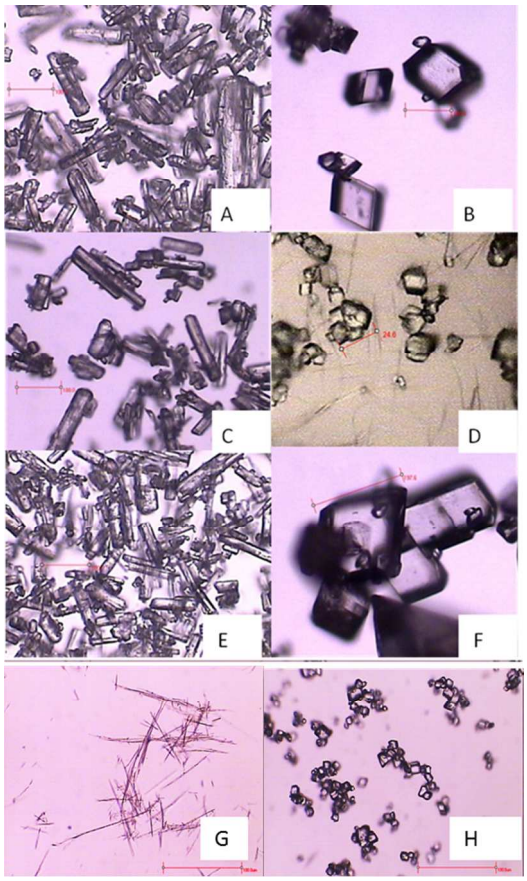

Figure 3. Optical micrographs of products crystallised at various temperatures and supersaturations. A. α-form obtained at 26°C, S=1.35; B. β-form obtained at 26°C, S =1.27; C. α-form obtained at 24°C, S=1.48; D. Concomitant nucleation of α and β at 24°C, S=1.40; E. α-form obtained at 15°C, S=1.48; F.β-form obtained at 15°C, S=1.37; G α-form obtained at 10°C, S = 1.6; H. β-form obtained at 10°C, S = 1.3. Scale bars – A, B, C, E, G, H all 100μm; D, 25 μm; F, 200 μm

Figure 3 shows typical optical micrographs of product crystals precipitated from aqueous solutions. It is clear that in agreement with previous reports (15, 16), crystallisation from aqueous solution can yield both α and β forms. However in these new results a clear pattern is evident as shown in full in Table 1 for data measured at 15°C. Here, at low supersaturations (1.3 - ~1.45) β appears while at high supersaturations (1.5 -1.6) α is the consistent outcome.

Intermediate levels (~1.48) can yield mixtures of forms. Similar behaviour was observed over the entire temperature range studied. Thus in water at any temperature a supersaturation of < ~1.35 will yield pure β crystals and a supersaturation > ~1.4 a pure α product; intermediate supersaturations will give mixtures. Hence it is clear that whether above, at, or below the transition temperature, the temperature itself plays no part in determining the outcome – this is controlled only by the supersaturation, with α always appearing at high supersaturation and β at low. Table 1 also provides values of the measured induction times, τ. From a plot of $\ln(1/\tau)$ vs $1/\ln^{-2}S$ (19) the effective interfacial tensions, γ_{eff}, can be estimated for each polymorph. These data points fall on two intersecting straight lines, the slopes of which yield γ_{eff} values of 3.2 and 3.5mJm^{-2} for α and 1.7 and 2.4mJm^{-2} for β at 26 and 15°C respectively. These values for α are larger than those reported recently by Sullivan *et al* (18) in organic solvents (1.33-2.24mJm^{-2}). Given the significantly lower solubility of PABA in water compared to organic solvents this seems a reasonable outcome.

Temperature (°C)	Supersaturation	Form	Induction Time (s)
15	1.60	α	540
15	1.51	α	634
15	1.48	α or β	800
15	1.41	β	1080
15	1.37	β	1124
15	1.29	β	1250

Table 1. The polymorphic outcomes of experiments performed at 15°C from aqueous solutions. Some values of induction time are also given.

In ethanol at identical low (1.3) and high (1.6) supersaturations only the α form was ever observed, independent of the experimental temperature. This result seems to be consistent with previous studies in organic solvents discussed above in which α was the only form observed.

Overall these results demonstrate for the first time the conflicting influences of temperature, solvent and supersaturation (ie of thermodynamics and kinetics) on crystallisation in an enantiotropic system. Specifically, they confirm, in a precise way the combined influences of these experimental variables on the crystallisation of PABA polymorphs. It is now clear that at comparable high supersaturations ethanol and water behave identically, yielding the α polymorph. At low supersaturation on the other hand while ethanol continues to yield α, aqueous solutions yield β. Given other reports of PABA crystallisation from organic solvents (16, 17, 18) it is thus apparent that water is unique in being the only solvent from which both polymorphs may be consistently prepared. This is thus a significant and well documented example of a supersaturation dependant solvent effect in polymorph appearance. Additionally and surprisingly the crystallisation outcome in both ethanol and water is independent of temperature. A strategic approach to the development of a crystallisation process might reasonably have used Ostwald's Rule as a guiding heuristic. However it is clear from these data that this would have been unsuccessful since it is essentially governed by kinetics alone (20) and would predict that below the transition temperature the metastable α would be the form to crystallise first and that above the transition temperature the metastable β would be favoured. At the transition temperature (13.8°C) where the solubilities and supersaturations are uniquely identical for both forms we may have expected to obtain a mixture of α and β. This is not the case: the experiments only match the Ostwald Rule expectation in crystallisation from ethanol below 13.8°C where the metastable α appears. Above the transition temperature, α becomes the stable form and its appearance from ethanol is in direct violation of the Rule. In aqueous solutions the Rule is simply not obeyed either below or above the transition temperature. Here from solutions supersaturated with respect to both forms the outcome switches depending on composition: this is not accounted for by Ostwald. Such a major deviation from expectations would make the *a priori* definition of temperature and composition for a polymorph specific crystallisation process impossible. Given that the Rule seems to be generally well adhered to in monotropic polymorphic systems this raises the questions as to whether this apparent delicate balance between kinetics and thermodynamic only reveals itself in enantiotropic systems when crystallisation occurs relatively close to a transition temperature. Clearly more data on related systems is needed to resolve this issue.

Since both α and β crystals can grow at reasonable rates from aqueous solutions (ie they reach macroscopic sizes in reasonable experimental times) it is assumed that the unique property of water in promoting β is related to the nucleation process. We know from previous work (18) that the growth of α nuclei is controlled by desolvation of the carboxylic acid group and acid dimer formation and hence we make the logical deduction that while in organic solvents this desolvation pathway leads only to α dimers, in aqueous solutions it must be more finely balanced with the additional possibility of forming the β tetramer motif. Put another way, in ethanol the transition state can lead only to one product while in water there are two self-assembly pathways leading to two different products depending on concentration. Overall this example serves to highlight further our current lack of molecular scale understanding of the nucleation process and the need for further such investigations of crystallisation in polymorphic systems.

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1. T. Threlfall, *Org. Process Res. & Dev*. 2000, **4**, 384-390.
2. W. Ostwald, *Z. Phys. Chem*., 1897, **22**, 289-330.
3. C.S. Towler, R. J. Davey, R. W. Lancaster, C.J. Price, *J. Amer. Chem. Soc.* 2004, **126** 13347-13353.
4. M. Kitamura, *J Cryst. Growth*, 1989, **96**, 541-546.
5. R. J. Davey, N. Blagden, S. Righini, H. Alison, M. J. Quayle, S. Fuller, *Cryst. Growth & Design*, 2001, **1**, 59-65.
6. M. Svard, F. L. Nordstr€om, T. Jasnobulka, A. C. Rasmuson, *Cryst. Growth & Design,* 2010, **10**, 195-204.

7. S. Jiang, P. J. Jansens, J. H. ter Horst, *Cryst. Growth & Design*, 2010, **10**, 2541-2547.
8 .J. Cornel, P. Kidambi, M. Mazzotti, Ind. Eng. Chem. Res. 2010, **49**, 5854–5862.
9. N. Blagden, R. Davey, G. Dent, M. Song, W. I. F. David, C. R. Pulham, K. Shankland, *Cryst. Growth & Design*, 2005, **5**, 2218-2224.
10. R.A. Chiarella, A.L. Gillon, R.C. Burton, R. J. Davey, G. Sadiq, A. Auffret, M. Cioffi, C. A. Hunter, *Faraday Discussions* 2007, **136**, 179-193.
11. K. Sato, R. Boistelle, *J. Cryst. Growth*, 1984, **66**, 441-450.
12. H. Hao, M. Barrett, Y. Hu, W. Su, S. Ferguson, B. Wood, B. Glennon, *Org. Process Res. Dev*. 2012, **16**, 35–41.
13. T. F. Lai and R. E. Marsh, *Acta Cryst*., 1967, **22**, 885- 893.
14. S. Gracin, A. Fischer *Acta Cryst*., 2005 **E61**, 1242–1244.
15. R. A.Sullivan, R. J..Davey, *Cryst. Eng. Comm*. 2015, **17**,1015-1023.
16. S. Gracin, Å. C. Rasmuson, *Cryst. Growth& Design*, 2004, **4**, 1013-1023.
17. M. Svard, F. L. Nordstrom, E. Hoffmann, B. Aziza, Å. C. Rasmuson, *CrystEngComm*, 2013, **15**, 5020–5031.
18. R. A. Sullivan,R . J. Davey, G. Sadiq, G. Dent, K. R. Back, J. H. ter Horst, D. Toroz, R. B. Hammond, *Cryst. Growth & Des*., 2014, **14**, 2689−2696.
19. R. J. Davey and J. Garside, *From Molecules to Crystallizers*, Oxford University Press, Oxford, 2000.
20. .J.Bernstein, R.J.Davey, J. O. Henck, *Angew Chem Int Edit* 1999, **38**, 3440-3461.

