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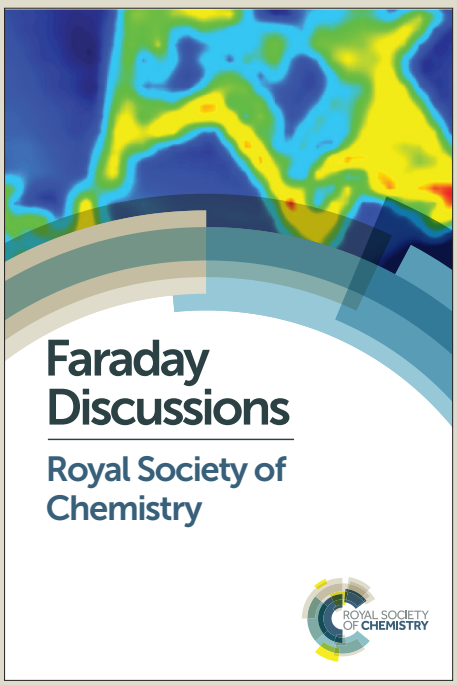


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# Crystal Nucleation from Solutions – transition states, rate determining steps and complexity.

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**Abstract.** This introductory paper offers a contemporary view of crystal nucleation. We begin with a molecular interpretation of the transition state and then revisit the use of classical nucleation theory as a means of obtaining molecular scale information from kinetic data. Traditional physical organic chemistry has always utilised the combination of kinetics and thermodynamics in order to gain insight over reaction pathways. Here we demonstrate for the cases of sucrose and *p*-aminobenzoic acid how solution chemistry, crystallography and kinetics come together to provide self-consistent pictures of the molecular scale processes occurring during nucleation. In this and a number of other systems desolvation of specific functional groups is highlighted as the rate determining step. Finally we move on to discuss the question of complexity, both from a phase and molecular perspective.

## 1. Introduction.

In an obscure publication by Dunning and Shipman (1, also available as supplementary information) published as part of the proceedings of the Tenth International Conference of Agricultural Industries held in Madrid in 1954, the authors considered many key features of a nucleation process. For example, they dealt with the issue of the transition state, offered a simple yet wonderfully insightful derivation of Classical Nucleation Theory (CNT) and described an experimental technique whereby they measured the nucleation rates of sucrose from aqueous solution over a range of temperatures. Using this combination of data and theory they concluded that desolvation was the rate determining step and that due to the complexity of its size and shape the sucrose molecule encountered an entropic barrier to its nucleation. Our current introductory paper pays homage to Dunning and Shipman's work and uses their themes – transition state; nucleation theory; experimental methods; rate determining steps and complexity- as a basis for the structure of this paper.

Of course Dunning and Shipman did not produce their work in a vacuum. They relied on the results of a century of study of crystallisation processes. In this world Friedrich Wilhelm Ostwald had been 'king', establishing many of the key aspects of crystallisation that we take for granted: the importance of the phase diagram in defining crystallisation conditions, the role of supersaturation in the control of nucleation, the concept of the metastable zone and of course his Rule of Stages relating to the appearance of crystal forms in systems with multiple solid phases. This was largely summarised in his 1897 paper (2) and it brought to an end a period during which crystallisation had been viewed as a largely uncontrollable and irreproducible process. However, it was not until 1926 (3) that Max Volmer developed a kinetic theory describing the relationship between supersaturation, interfacial tension and the rate of nucleation, initially for drops but later adapted in 1934 (4) by Kaischew and Stranski

to cover the case of crystal nucleation. Overall this kinetic theory describes rather well the experimental observations of Ostwald and remains a major achievement in the field.

Whether or not these two scientists, Volmer and Ostwald ever met to discuss the questions in hand is unknown. Ostwald was born in 1853 and was Volmer's senior by some 32 years. By the time Volmer began his career in Berlin in 1916, Ostwald had been retired from Leipzig University for ten years. On chronological grounds a meeting seems unlikely. A search of the internet offered no further comment on this possibility but rather surprisingly did reveal a PDF (English translation) of the 1939 book (5) written by Volmer and entitled 'Kinetics of Phase Formation'. Although this book is frequently referenced we had never seen it before – it is illuminating. Reading Volmer's historical introduction seems to confirm that the two had never met. Of Ostwald he notes *'Ostwald's importance to the field rests upon the order and clarity he introduced into the chaotic multiplicity and confusion of existing data'* On the question of the Rule of Stages he is critical, claiming that Ostwald ignored exceptions to the Rule revealing his prejudice in its favour. However it is his comment on Ostwald's response to the work of Gibbs that seems to confirm that the two never met. On metastability Volmer comments that *'the only valuable theoretical work remains completely unknown. It is to be found in the second part of Gibbs' great treatise 'On the Equilibrium of Heterogeneous Substances' and is conveniently accessible in W. Ostwald's well-known German translation 'Thermodynamische Studien'. It is strange that Ostwald who was especially interested in the subject and who must have thoroughly penetrated the meaning of the work in the course of translating it, should have failed to recognise its importance. In his textbook of general chemistry which contains a brief summary of Gibbs' works, he merely says 'Passing over a few sections of more abstract subject matter concerned with the possibility of formation of a new phase within a homogeneous liquid ... ect'* . Thus Volmer offers Ostwald a metaphorical

'slap on the wrist' but ironically of course he (presumably) relied on Ostwald's German translation of Gibbs work to enable him to develop what has now become CNT.

In recent years interest has been aroused by the possibility that CNT needs to be modified following the experimental discovery of pre-nucleation clusters, mesostructured solutions and liquid phase precursors to crystal nucleation. We do not discuss these issues but recognise that they are well covered in this discussion by the papers of Vorontsova *et al* (5114), Sauter *et al* (5038) Voelke *et al* (4679) and Jawor-Baczynska *et al*, (5156).

In what follows we will deal firstly with the contrasting physics and chemistry approaches to the transition state. Secondly we will say something about the relationship of this approach to CNT. As part of this we look briefly at experimental methods used and reprise Dunning and Shipman's conclusions in their interpretation of sucrose nucleation. One significant issue discussed is the extent to which solution and solid state chemistry can help us to formulate a molecular picture of the nucleation transition state and for the case of *p*-aminobenzoic acid we see how kinetic and transition state ideas come together to provide a self- consistent interpretation of data. We then move into the arena of nucleation in complex systems – complex in the sense of both molecular structure and phase behaviour.

## 2. The Transition State.

Dunning and Shipman's free energy co-ordinate diagram is reproduced here as Figure 1. As physical chemists we are familiar with this simple form of the energy-reaction landscape, separating, as it does, reactants and products via a transition state situated at the highest point of the curve. In the case of nucleation this represents the passage from a metastable (supersaturated) solution over an activation barrier to the final state in which crystals are

present. Dunning and Shipman equate the transition state to a cluster of molecules which CNT identifies as the critical sized cluster (or embryo). They write *'However, there will be a certain sized embryo, the solubility of which is just equal to the concentration of the supersaturated solution. Embryos smaller than this critical size tend to redisperse - and those larger tend to grow. This critical size is the bottleneck of the process and corresponds to the activated state in ordinary kinetics.'* Versions of Figure 1 have been used by other authors in attempting to describe the nucleation process – in their 1999 paper on concomitant polymorphism Bernstein *et al* (6) used a similar figure to describe crystallisation in polymorphic systems and in 2002 Desiraju (7) used a version in his 'Cryptic Crystallography' commentary in Nature Materials to distinguish kinetic and thermodynamic products in crystallisation. In both these papers the transition state was again defined as the critical cluster of molecules. This notion of the transition state as a supramolecular assembly having the packing of the final crystal structure derives from Volmer's physics approach and while

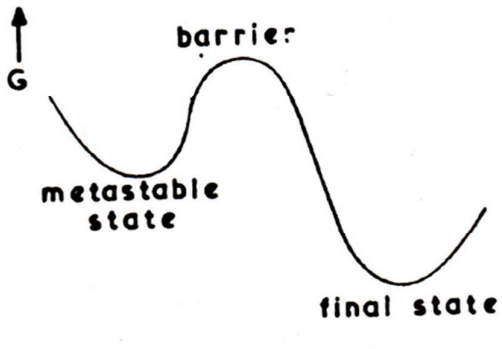


**Figure 1. The reaction co-cordinate – free energy landscape as visualised by Dunning and Shipman (1).**

successful in terms of deriving a kinetic theory it tells us nothing of the essential chemistry of the transition. In reaction chemistry, where molecules are built from covalent bonds, the idea of the transition state has led to an understanding of reaction rates based on molecular properties. The approach of the chemist is nicely summarised in Kirby's 1994 review (8):

*'Transition states are by far the most difficult of the relevant species which are experimentally accessible. Formally they can only be studied kinetically, because their only experimental manifestation is their effect on the rate of the reaction. A transition state, which lies by definition between the starting materials and products for a particular step of a reaction, is supposed to be closer in structure, because closer in free energy, to the higher energy species of the two. ….Crystal structures give us information specifically about ground states, averaged over a period of hours or even days; whereas transition states for bond making or breaking, as defined by transition state theory, do not exist for any significant length of time.'*

Hence the critical nucleus as a transition state poses a conundrum – while it does indeed represent a maximum in the free energy-size co-ordinate, it is also a reflection of the ground state packing of the product crystals. If on the other hand we consider the formation of non-covalent interaction to be nucleation's equivalent of covalent bond formation in reaction chemistry then for nucleation in solution it would surely seem reasonable to define the solvent as the leaving group in this reaction that sees two solute molecules come together either to form a dimer or to enlarge an existing cluster. A transition state viewed in this way will be closer in structure to the supersaturated state and might be approximated by the following intellectual process. Starting from knowledge of the first solvation shell of the solute in solution, one solvent molecule is removed and replaced with a partially solvated solute molecule so as to create an intermolecular contact that is present in the ground state crystal structure. This may be thought of conceptually as the first of many such steps leading to a critically sized cluster. However, it leads our thinking away from concerns over the size, packing and surface free energy of a cluster and forces us to focus on the essential chemistry that must be performed before a cluster can emerge. It answers questions such as – which might be the most difficult solvent interactions to break? Can I choose a solvent that changes

the chemistry and hence the transition state? Can I direct the formation of the solute- solute interaction that I want and hence control crystal structure? We have considered such questions before but never in such a formal way (9, 10). Here we consider three examples. In each case the ground state crystal structure is known from crystallography while the solution chemistry essential for this process comes from a number of different sources and is, where possible, supported by known structures of relevant solvated crystal forms.

### 2.1 Trimesic acid crystallising from DMSO.

The case of trimesic acid (TMA) crystallising from DMSO has been discussed previously (11). In this system there are two known solvates, the TMA.3DMSO trisolvate (XASFAA,11) which appears as a metastable phase with a lifetime of about 1 hour and the ultimate product of such a crystallisation, the monosolvate TMA.DMSO (TMADMS01,12). In the context of a transition state both of these structures are essentially ground states but we will consider the more stable monosolvate to be the true ground state in this system. In the trisolvate the DMSO is disordered and there are no acid-acid hydrogen bonds between TMA molecules. Indeed, in terms of FTIR the carbonyl environment in this trisolvate is identical to that in the solution from which it crystallises (11). For this reason we take the crystal structure of the trisolvate as an approximate model for the supersaturated solution and this gives the TMA with its first solvation sphere as seen on the left hand side of Figure 2. To transform this arrangement into the monosolvate by the addition of a second TMA molecule requires desolvation of one acid group and the creation of a new –C=O…H-O- hydrogen bonded contact with an incoming TMA molecule. This creates the image of the transition state as seen in the centre of Figure 2. Repetition of this process can then lead to a cluster having the packing of the ground state, monosolvate structure.

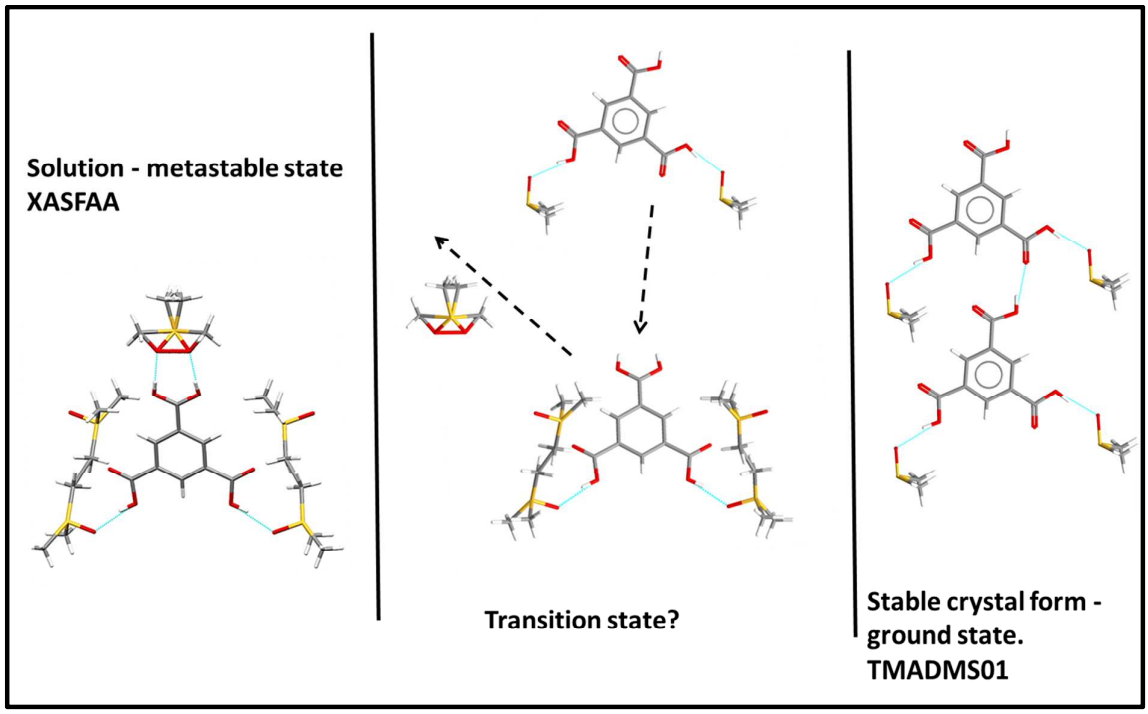


**Figure 2. The creation of a potential transition state (centre) from a combination of the solution co-ordination (left) and the ground state crystal structure (right). The case of trimesic acid and dimethylsulphoxide.**

### 2.2. Hexamethylenetetramine crystallising from aqueous solutions.

Hexamethylenetetramime (HMT) crystallises in a body centred cubic lattice from aqueous solutions (HXMTAM07, 13, 14). This is shown as the ground state on the right hand side of Figure 3. There are no known polymorphs but a hexahydrate exists at temperatures below 13.5$^{o}$C (HXMTHH,15). The co-ordination of HMT in aqueous solutions is known rather precisely (16) from neutron scattering studies in the form of radial distribution functions. Thus in Figure 3 we show on the left hand side the solution co-ordination via the spatial distribution of both HMT around HMT and the water oxygen, (Ow) around HMT. It is clear that closest to a central HMT are water molecules, four of which are H-bonded to nitrogens and 4 interacting with the faces of the HMT cage. Beyond these eight that comprise the solvation shell are 4 HMT molecules poised ready to form –C-H…N- contacts as in the

crystal structure. Figure 3 then utilises the similarity in structure between the hexahydrate and the solution phase solvation shell to create a central image in which a leaving water molecule allows the formation of the strongest HMT-HMT contact yielding the transition state. It appears that in this case the solution co-ordination of HMT favours the creation of the ground state packing, a view supported by previously measured nucleation kinetics which show significant rates even at extremely low supersaturations with almost no thermodynamic dead zone (14).

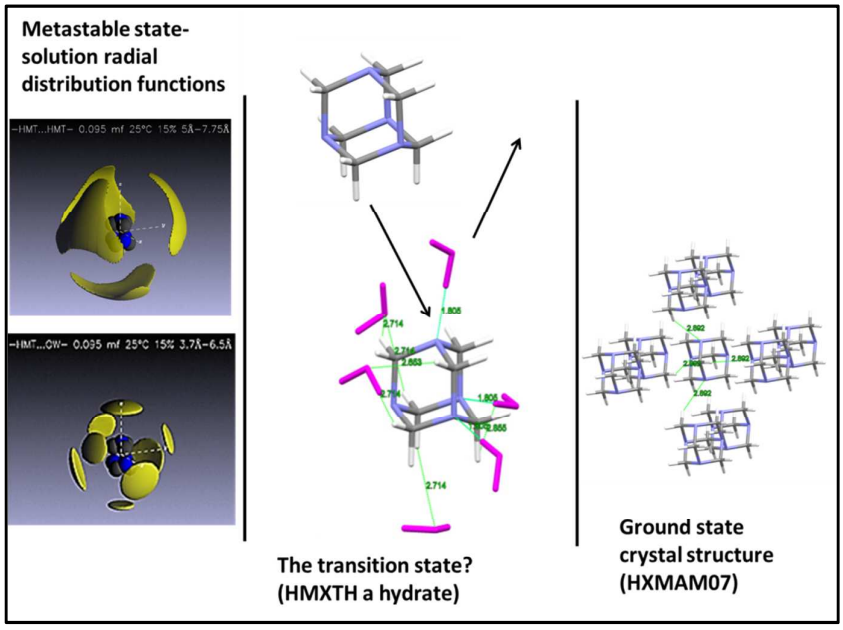


**Figure 3 The creation of a potential transition state (centre) from a combination of the solution co-ordination (left) and the ground state crystal structure (right). The case of HMT and water.**

### 2.3. 5-Fluorouracil crystallising from water and nitromethane.

In the case of 5-fluorouracil two polymorphs are known. Form I crystallises from water while Form II is prepared from dry nitromethane. Evidently even small amounts of water are sufficient to switch the outcome from Form II to I (17). In order to understand this subtle effect Hamad *et al* (18) performed molecular dynamics studies which linked the solution co-ordination of 5-fluorouracil to the polymorphic outcome. We have used these data as the

basis for Figure 4. This takes the radial distribution functions computed in water together with the water positions observed in ZAYLOA (a hydrated co-crystal of 5-fluorouracil and theophylline, (19)) and the ground state Form I to create a transition state. In this case, following the reasoning of Hamad *et al* (18) it appears that the transition is driven by the combination of the hydrophobic -F…F- interaction between neighbouring molecules and the difficulty of removal of water from the carbonyl and amine groups. This latter prevents the formation of the doubly H-bonded ribbon seen in Form II – this can only be formed by changing the nature of the transition state via the solvent. Thus, the use of dry nitromethane, which cannot form strong H-bonded interactions with the carbonyl groups of 5-fluorouracil, enables amide dimers to form the extended H-bonded ribbons of Form II.

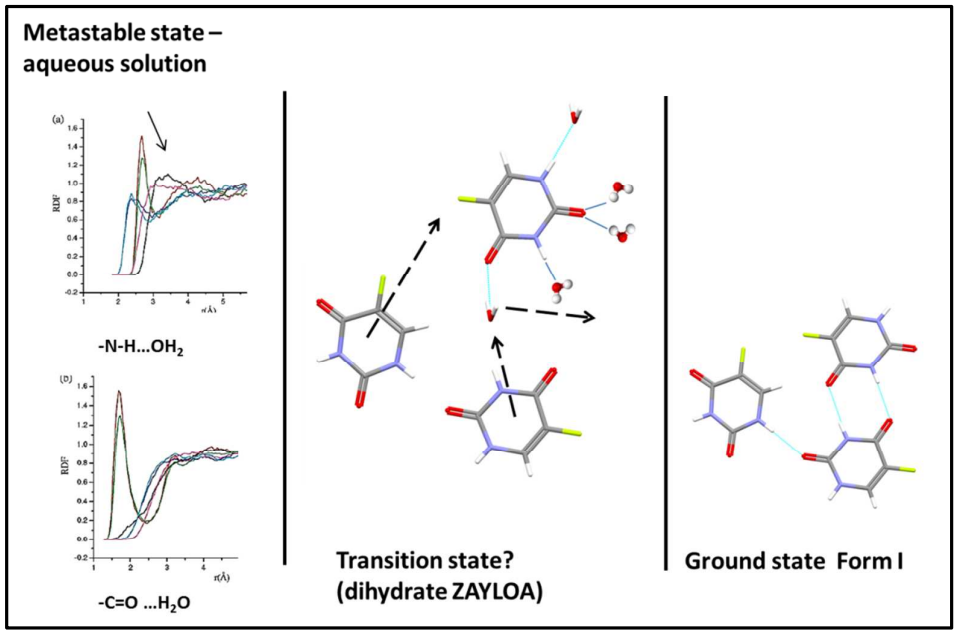


**Figure 4 The creation of a potential transition state (centre) from a combination of the solution co-ordination (left ( taken from ref. 18 and reproduced with kind permission of ACS)) and the ground state crystal structure (right). The case of 5-fluorouracil and water.**

In these three examples we have utilised the combination of solution and solid state chemistry in order to create an impression of a potential transition state. This appears to be a promising

approach in that it directs our thinking away from questions of clusters and packing (which we can never resolve) towards the essential chemistry of the processes. It is evident that the NMR and NEXAFS techniques as reported in papers by Hughes *et al* (5046) and Thomason *et al* (4678) may have much to contribute to this topic. In the next section we take these ideas a step further by combining such structural insights with kinetic data.

### 3. Kinetic data and rate determining steps.

Dunning and Shipman employ a direct approach to the measurement of nucleation rate. Using a specially constructed counting cell, solutions were subjected to different supersaturations and temperatures and the number of crystals appearing with time counted manually. These data yielded directly the nucleation rate. In more recent times workers have sought to perform measurements that reflect more directly the stochastic nature of nucleation. Turnbull (20) was probably the first to use liquid droplets to observe nucleation of metals and the 1978 paper of Keller *et al* (21) describes very well how such a technique may be modified using emulsions to provide nucleation rates. Essentially many, many drops are kept under identical conditions and the probability that a drop contains a crystal is measured. From such data the nucleation rate may be obtained. More recently the use of emulsions has been superseded by high and medium throughput techniques using well plates (22), microfluidic devices (23, this discussion Hammadi *et al* paper 4683) and induction time measurements (24, this discussion Brandel and terHorst paper 4680). Whatever the methodology, the aim is to obtain *isothermal* nucleation rate versus supersaturation data to confront with an appropriate kinetic model. For nucleation the only model available is CNT (25) which gives a relationship between nucleation rate, J and supersaturation ratio, S of the form:

$$J = A_o S \ln S^2 \exp\left(-\frac{B}{\ln S^2}\right) \qquad (1)$$

The pre-exponental factor, $A_o$ is related to z, the Zeldovich factor, *f**, the molecular attachment frequency of building units to a growing nucleus and $C_o$ the concentration of nucleation sites. The former of these are clearly supersaturation dependant and using Kashchiev's evaluation of z (26) and realising that *f**should be a linear function of supersaturation leads to:

$$\ln\frac{J}{S\ln^2 S} = \ln A_0 \; - \; \frac{B}{\ln^2 S} \qquad (2)$$

A plot of $\ln(J/S\ln^2 S)$ against $\ln^{-2} S$ should be linear. The slope yields the thermodynamic factor, B from which the effective interfacial tension can be derived while the intercept gives $\ln A_o$. The product $f^* C_0$ can then be determined from the relationship:

$$f^* C_0 = \frac{A_0}{z} S\ln^2 S = \sqrt{12\pi B} A_0 S \qquad (3)$$

Dunning and Shipman use their measured data to obtain the temperature dependence of $A_0$ from which they estimated the activation energy of the kinetic process to be 67kJmol$^{-1}$. This was attributed to the transport of a sucrose molecule from solution onto the nucleus and concomitant removal of four water molecules (ie 67kJmol$^{-1}$ ~ 4 hydrogen bonds). Such a desolvation process might be expected to be reflected in the aqueous solubility and it turns out that there is in fact a good linear correlation (not shown) between $\log A_0$ and the free energies of solvation $RT\ln X_s$ calculated using measured values of $X_s$, the aqueous mole fraction solubility of sucrose. Combining these data and interpretation with the known crystal structure of sucrose (SUCROS08, (27)) suggests that the process shown in Figure 5 is the rate determining step in sucrose nucleation. Here the necessity to remove a total of 4 water molecules in order for a molecule to join a cluster along its *b*-axis is evident, matching rather well Dunning and Shipman's suggestion.

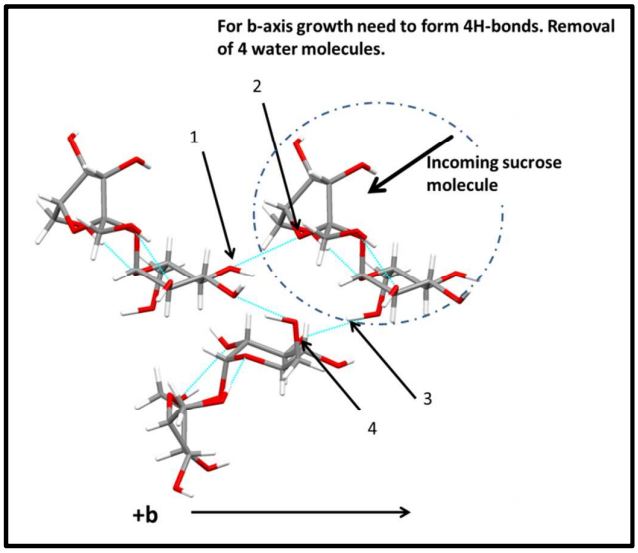


**Figure 5. The removal of 4 water molecules - the rate determining step in sucrose nucleation. The arrows and numbers highlight the 4 groups likely to be solvated by water molecules.**

### 3.1 The nucleation of *p*-aminobenzoic acid (pABA).

Overall given access to reliable and comparable kinetic data it seems that if we follow the kinetic rigour of Dunning and Shipman, together with additional insight into solution chemistry it should be possible to define the rate determining step and to visualise a potential transition state which is consistent with both structural and kinetic factors. In this section we reprise and extend the recent report of Sullivan *et al* (28) concerning the solvent dependent nucleation of the α polymorph of pABA. This is a structure based on the carboxylic acid dimer (AMBNAC07, (28)). The induction time method was used (24) to measure nucleation rates at 20$^{o}$C in 3 solvents, 2-propanol, ethylacetate and acetonitrile and following equations 1 to 3 the supersaturation dependence of f*$C_o$/M was extracted. The authors made one major assumption that for data measured by the same researcher, in the same laboratory and using the same equipment $C_o$, the number of (heterogeneous) nucleation sites is essentially fixed so that relative values of f*Co/M reflect the solvent

effect on f*, the attachment frequency of a molecule to a cluster. The results showed that this frequency was lowest in 2-propanol and highest in acetonitrile and these data are shown in Figure 6 along with associated macroscopic crystal growth rate measurements. The latter are important since they provide an effective means of independently checking the relative magnitudes of the derived attachment kinetics since growth of a crystal is a macroscopic reflection of the growth of clusters. α-pABA crystals grow as b-axis needles with their width corresponding to the a-axis (29). The growth rate data measured on single seed crystals at 20$^{o}$C in a temperature controlled growth cell (see this discussion Toroz *et al* paper 5128 ) are shown for both growth directions (*a* and *b*). It is clear that the relative order of growth rates with solvent follows the same pattern as the nucleation data with growth slowest from 2-propanol and fastest from acetonitrile. The growth curves show significant dead zones at low supersaturations suggesting surface nucleation controlled growth and their size show a similar solvent dependence. Taken together these data provide clear evidence that the kinetic factors in nucleation are controlled by the solvent choice. The available solution chemistry sheds further light on this. FTIR studies of the carbonyl group in ethanol solution show two bands (1685 and 1715cm$^{-1}$) having relative intensities which do not change with concentration in the range 0.056-0.9M. In agreement with available neutron scattering data for benzoic acid in methanol (30) this confirms that there are no carboxylic acid dimers in such solutions. Equivalent FTIR studies in acetonitrile show (1680 and 1710cm$^{-1}$; 0.0012-0.38M) that with increasing concentration the relative intensity of the 1710:1680 bands decreases from 15 to 1.4. This is a clear indication that the lower of the bands relates to a solute-solute interaction while the higher band is due to solvation. In ethylacetate interpretation of the spectra are hampered by overlap with the solvent. Related calculations of the solvation free energies (using a thermodynamic integration technique (28)) of both monomer and dimer in the

three solvents confirmed the experimental result showing that in 2-propanol the solvated acid group was more stable than the dimer, in ethylacetate the two species seem finely balanced and in acetonitrile solvated dimers were slightly more stable than monomers. These results taken together suggest desolvation as the rate determining step in the growth of clusters. Plots of the relative values of f* in the three solvents show a poor correlation (Figure 7, left) with overall solvation as reflected in the relative solubilities ($\ln X_s$) but a good correlation (Figure 7, right) with the calculated free energies for specific desolvation of the acid group. Hence we conclude that desolvation of the acid group and formation of acid dimers is the slowest, rate determining step in the nucleation pathway. This is certainly consistent with the relative growth rates of the slowest growing *a* direction since it is in the direction that dimers are created at the growing surfaces (29).

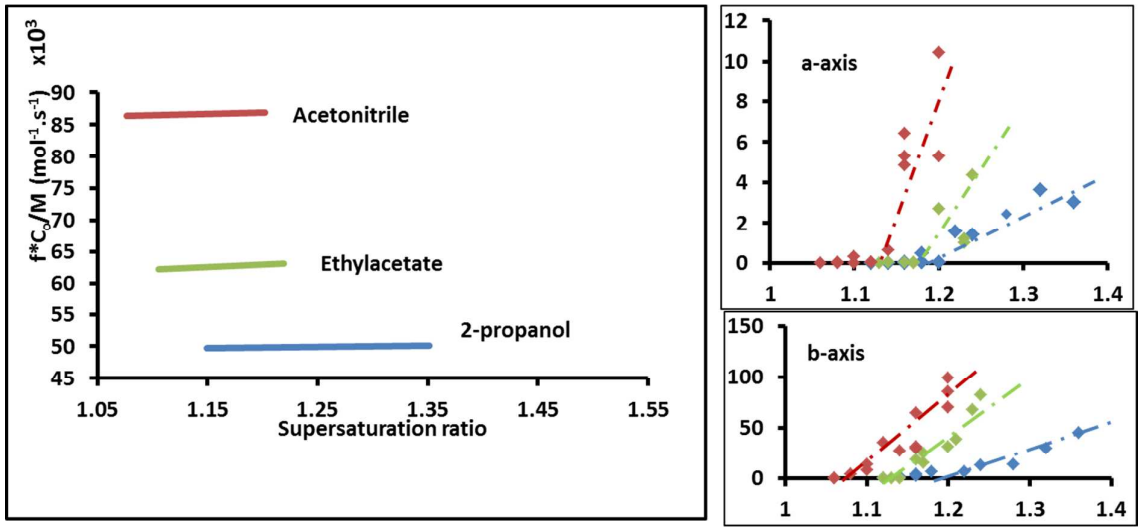


**Figure 6. Nucleation attachment frequencies (left) crystal growth rates (right, vertical axes) both as functions of supersaturation in the three solvents, 2-propanol (blue), ethylacetate (green) and acetonitrile (red). Growth rates are µm/minute.**

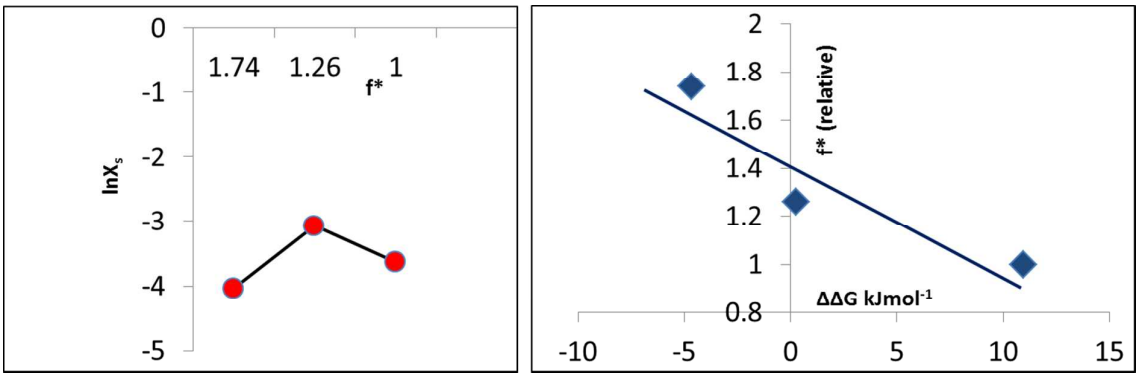


**Figure 7. Correlations of relative values of f* for pABA in different solvents with solvation parameters. Left with solubility and right with the solvation free energy of the carboxylic acid dimer.**

Taking this information together and using the previously measured radial distribution functions for benzoic acid in methanol as a model of the solution we arrive at Figure 8, a potential transition state for nucleation of pABA from 2-propanol.

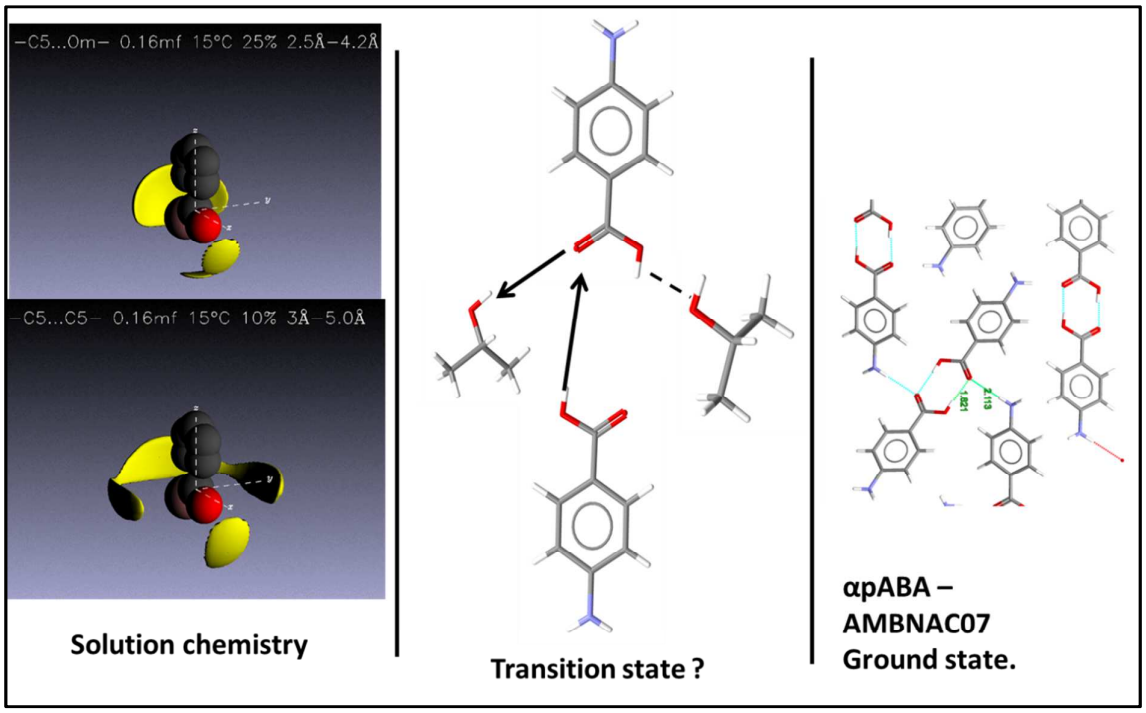


**Figure 8. A potential transition state for pABA nucleating from 2-propanol (centre). Solution co-ordination, left, and ground state α polymorph, right.**

As a corollary to this insight, in their original work Sullivan *et al* (28) argued that if indeed this was the transitions state then the activation barrier for attachment could be lowered by use of a solvent in which the solute was pre-assembled (dimerised). Taking benzoic acid in toluene (pABA is insoluble in non-polar solvents) they showed (FTIR) that these solutions contained preformed acid dimers and that the value of $f^*C_o/M$ was almost two orders of magnitude higher than in pABA in 2-propanol. This result showed, perhaps for the first time, that directed self-assembly is indeed possible through solvent selection.

A similar importance of desolvation as the rate determining step in nucleation has further been suggested by recent studies of salicylic acid (31) and riseridone (this discussion Mealy et al paper 5165). In both cases although the rate of nucleation has been related to the chosen solvent it does not correlate with overall solubility but rather with strong solvent binding at a specific site on the solute molecule. In the case of salicylic acid this implicates desolvation of the carboxylic acid group as the rate determining step while for riseridone it is the carbonyl group.

## 4. Complexity.

There are a number of possible sources of complexity in crystal nucleation. Dunning and Shipman were most concerned with entropic effects. We discuss these here in the context of molecular conformation but mention first complexity originating from phase behaviour.

### 4.1 Phase Complexity.

Clearly polymorphism would be an obvious example of complex nucleation behaviour in which the pathway can follow more than one route (32). We shall not discuss this issue further here rather we concern ourselves with nucleation in ternary systems since these are of significant interest from the perspective of chiral separation, co-crystal discovery and

molecular salt selection. We note that the famous Etter rules (33) for order of hydrogen bond formation are derived partly from experiments performed in ternary systems and yet we are not aware of any related, detailed nucleation studies. Three reports are worthy of mention, all involving cooling crystallisation. Of particular interest is the outcome of crystallisation experiments performed within the 3 phase region (s) of the ternary diagram where two solids phases are of equal stability and in equilibrium with solution having a eutectic (invariant) composition. Firstly, the case of mandelic acid is recalled which, in the ternary system R/S/$H_2O$, can crystallise either as pure enantiomers or as a stable (or metastable polymorph) racemic compound (34). Secondly we mention carbamazepine/saccharin/methanol which crystallises as pure components together with a stable and metastable polymorph of the 1:1 cocrystal (35) and thirdly the case of *p*-toluenesulfonamide(TSA)/triphenylphosphineoxide($Ph_3PO$) /acetonitrile which crystallises as pure components together with 1:1 and 3:2 co-crystals (36) is discussed.

For mandelic acid (34) ternary compositions were selected to give solutions at 40$^{o}$C which on cooling to 25$^{o}$C allowed crystallisation within the three phase region (S/RS/solution). In this case the enantiomeric excess of the crystallised solids was measured as a function of time and the initially nucleated product was found to be rich in pure S suggesting that nucleation of S and (R,S) crystals is not concomitant but rather the first phase to be nucleated is the pure enanatiomer, not the racemic compound. Only at longer equilibration times does the enantiomeric excess fall to the expected value.

In the carbamazepine-saccharin case the outcome of crystallisations (35) performed at constant solvent loadings but varying solute ratios was reported. In the two phase regions as expected only the pure forms appear. In 3 phase regions however, as the ternary diagram is traversed from carbamazepine rich to saccharin rich then close to the boundaries with the two phase regions only the pure co-former nucleates. In moving towards the co-crystal only

region the appearance of the stable and metastable co-crystal polymorphs appears to depend on composition. Thus in moving across the ternary diagram at fixed solvent level we find that, although the phases all have the same stability in the three phase regions, kinetics favours the closest pure form. This behaviour is essentially mirrored in the (TSA)/($Ph_3PO$)/acetonitrile system (36), which is complicated further by the existence of both 3:2 and 1:1 co-crystals. In traversing the pure TSA/3:2 cocrystal region from left to right, the proportion of co-crystal in the product increases. In the region where $Ph_3PO$ and the 1:1 co-crystal are stable it seems to be possible to nucleate mixtures which include the metastable 3:2 co-crystal along with pure $Ph_3PO$ and the 1:1 co-crystal. Overall it appears that in these complex systems nucleation continues to occur stepwise with kinetic processes driving the outcomes so that even in regions where two phases have equal stability adjustment of composition can yield non-equilibrium mixtures.

**4.2 Molecular Complexity.**

Dunning and Shipman foresaw the possibility that because sucrose is a *'large and complicated molecule' that it might have difficulty 'dovetailing' into the growing cluster. This might give rise to unexpected entropy effects'*. Of course entropic effects will arise for a number of reasons (37) associated with enhanced molecular mobility in the liquid phase. Most recently this issue has been considered specifically in the context of conformational flexibility which is seen as a potential constraint on the ease of crystallisation of large molecules (38, 39). Here we discuss one specific case of the molecule shown in Figure 9. This is a pharmaceutical intermediate ((3S)-oxolan-3-ylN-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)(4-itrobenzene)sulfonamido]- 1-phenylbutan-2-yl]carbamate; ethanol, which will be abbreviated to ONSC-E. In terms of complexity this molecule has at least 11 rotatable bonds and three chiral centres. If the general assumption is made that each rotatable bond has 3 low energy configurations and that they alter independently, then the theoretical maximum

number of conformers for this molecule is $3^{11}$ = 177, 147. Obviously its potential energy surface is not accessible in the normal way by considering each bond in turn. As an alternative means of exploring conformational space a Monte Carlo search method was used (HyperCube, 'HyperChem 8.0', 2010.) together with the MM+ forcefield to generate a list of possible low energy conformations. These were further optimised using *ab initio* calculation in Gaussian with each geometry being optimised at the HF/STO-3G level, followed by a single point energy calculation carried out at the B3LYP/6-31+G(d) level. This provided a list of 2611 conformations which were further reduced by removal of duplicates to give a final list of 1850. From the lowest energy conformation only one other conformation was found to be within 2.5 kJ/mol and hence, at least in vacuum the population will be highly biased towards these two conformers, neither of which match that in the crystal structures discussed below.

In terms of crystallisation ONSC is readily crystallised from solvents. In this study, acetone, ethylacetate, ethanol, methanol, 1-butanol, diethylether, 1-hexanol, acetonitrile, chloroform/ethanol, ethylacetate/ethanol were all tested. Crystals obtained were needle like with typical widths of 10μm. Overall it proved extremely difficult to grow crystals wider than this. In cases where suitable crystals for XRD were recovered the crystal structure was always found to be solvated. Thus for example from ethanol/chloroform an ethanol solvate was obtained; from methanol a methanolate and from acetonitrile a hemihydrate. The ethanolate and methanolate are isostructural, being channel solvates. The conformation of ONSC-E in the three crystal forms is compared in Figure 9 with that of the lowest energy gas phase conformation discussed above. It is noted that in the crystal the –OH group of ONSC-E hydrogen bonds to the solvent whilst in the low energy vacuum conformer it forms an intra-molecular H-bond with the $–SO_2$ group.

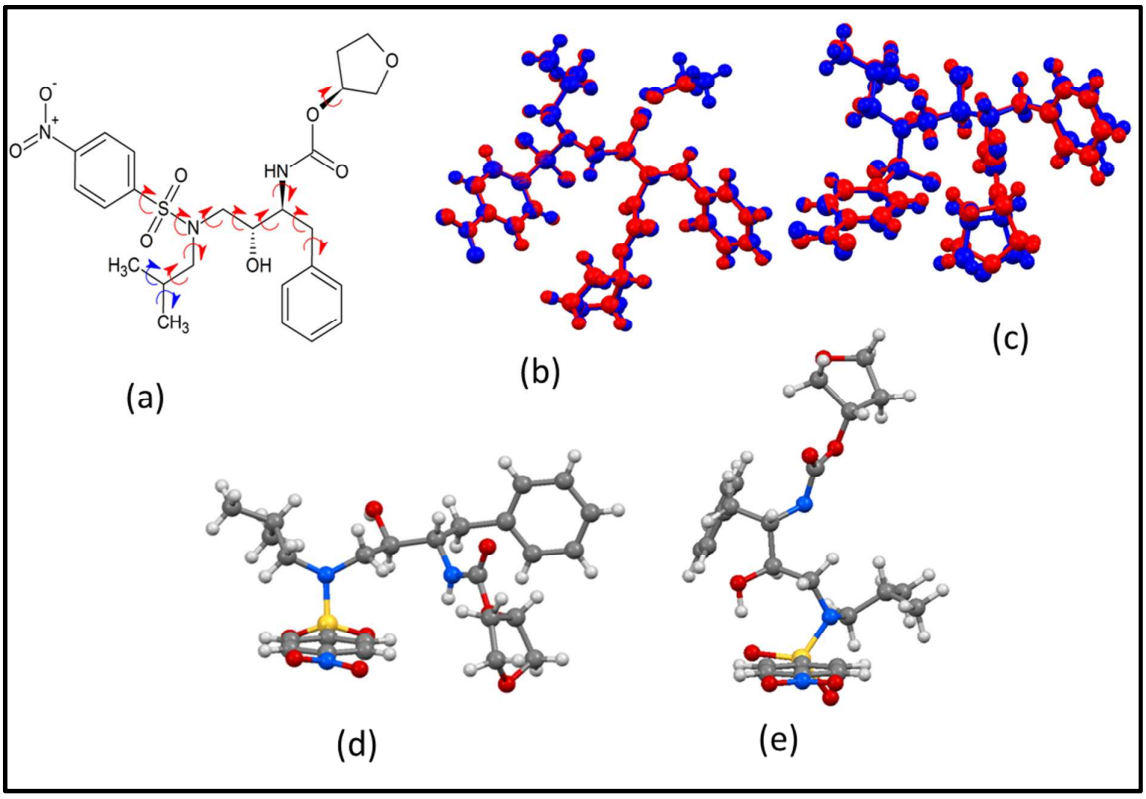


**Figure 9. (a) The molecular structure of ONSC with rotatable bonds highlighted in red. (b) an overlay of ONSC ethanolate (blue) and methanolate (red) conformations (c) an overlay of ONSC ethanolate (blue) and hemihydrate (red) conformations. (d) ONSC-E crystal structure conformation (e) lowest energy structure from Monte Carlo search.**

Detailed crystallisation and solubility studies in the ternary system ONSC-E/ethylacetate/ethanol allowed the phase diagram in Figure 10 to be determined at 25°C. This confirmed the lack of a non-solvated form and defined the composition of stability of both the hemihydrate and the ethanolate. The temperature dependence and metastable zone width of the ethanolate in 3.7:1 ratio (v/v) of ethylacetate to ethanol solvent is also shown in Fig 10. It is evident from these data that the stability domain of the ethanolate is very large and that its crystallisation behaviour as evidenced by the MSZ width data is highly regular and in no way abnormal. An attempt was made to measure the growth rate of a crystal in its needle direction (a-axis). A growth rate of 0.3μm/min was obtained at a supersaturation ratio of 3.46.

It is noted that this is significantly slower than, for example, the b-axis growth rate of α-glycine, which grows at 61μm/min (40) at the lower supersaturation ratio of 1.58.

The nucleation kinetics of the ethanolate were determined at 15$^{o}$C using the induction time method (23) and the Crystal16 at supersaturation ratios ($x/x_{sat}$) of 2.47, 2.55, 2.68, 2.78, 2.94 and 3.01. The fitted growth times form these data were between 3000 and 6000s. These are relatively long compared with benzoic acid (12s) and this is consistent with the observation of unusually low growth rates of single crystals. Again using equations 1 to 3 it was possible to deduce the value of $f^*C_o/M$ (though correlations were poorer than in the pABA case above due to the volatility of the solvent giving rise to crowning effects during measurement). As seen in Figure 10 these appear to be of the same order of magnitude as benzoic acid. The effective interfacial tension was found to be 2mJ/m$^2$, typical for such a low solubility system. Thus, despite the apparent number of degrees of freedom of this molecule its crystallisation behaviour, as a solvate show little out of the ordinary – its metastable zone is well characterised and its nucleation behaviour appears in line with benzoic acid and *p*aminobenzoic acid. There is one major exception to this in that while its nucleation characteristics are normal, its growth rate appears to be exceptionally low. Whether or not this is in some way linked to conformation is yet to be established. What is clear, however, is that much of this regularity of behaviour arises from the role played by the solvent in moderating the conformation of the molecule. The lowest energy conformer found by computation does not appear in a crystal structure and no crystals of any non-solvated form were ever seen. In the context of this paper it might be concluded that the transition state for nucleation involves such favourable solvent binding that the result is always a solvate. It would appear that such is the stability of this solute-solvent dimer that conformation is, surprisingly, not an issue during nucleation it only becomes a problem during growth.

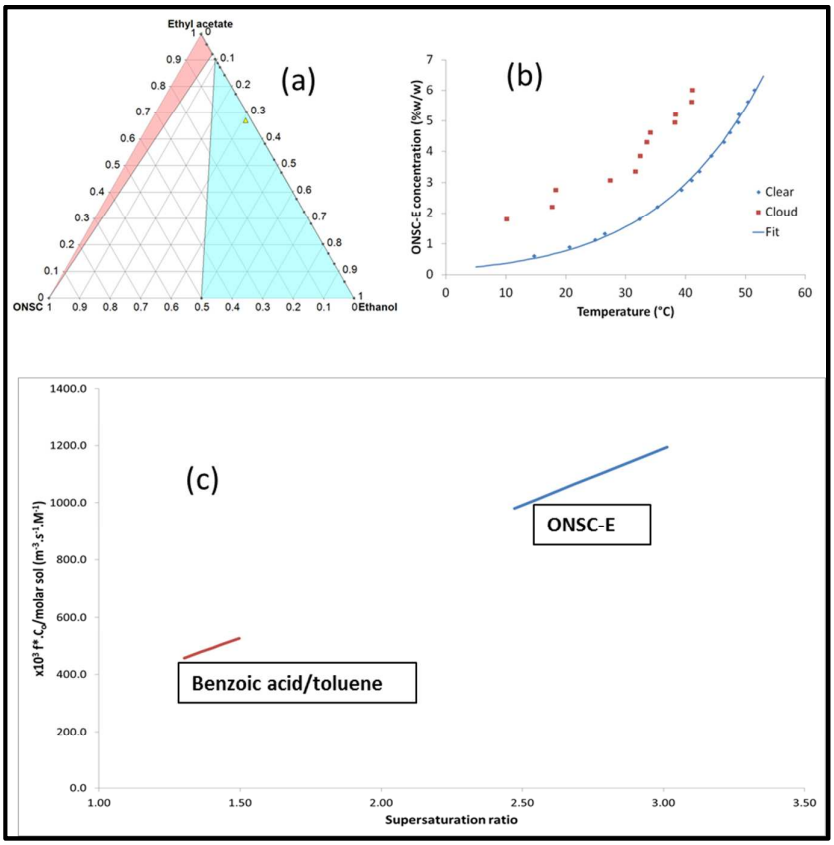


**Figure 10. (a) Phase diagram for the ternary system ONSC-ethylacetate-ethanol at 25°C (mole fraction). The blue section highlights the region where the ethanol solvate is the stable form crystallised, and the red section where the hemihydrate is obtained. The yellow triangle indicates typical crystallisation conditions for the ethanolate in the system. (b) Solubility and MSZW of ONSC-E in the 3.7:1 (v/v) ethylacetate to ethanol system measured on the Crystal16. (c) the attachment frequency of ONSC-E nucleating from ethanol/ethylacetate solutions. Benzoic acid data is included for comparison.**

## 5. Conclusions.

Here we have suggested a way of combining data from different sources to give insight into the chemistry of nucleation – the processes which must happen in order for a molecule to move from its solvent environment into a cluster. Re-examination of the sucrose data of Dunning and Shipman reminds us of the role that carefully measured *isothermal* kinetic data can play in developing an understanding of nucleation processes. We have shown how all the structural and kinetic factors can come into play in the case of pABA. This leads ultimately to

the demonstration of the key determining role played by desolvation of specific functional groups and of the use of solvent selection as a way of achieving preassembly and enhanced nucleation attachment frequencies. Complexity comes in many forms and we have dealt here with two types that feel most relevant in today's climate – phase complexity and molecular conformational. Clearly little is really known about competitive nucleation in ternary systems where both pure phases and compound solids can form. Given the commercial importance of molecular salts and co-crystals this may be an area worthy of pursuit. As far a molecular conformation is concerned this is high on the agenda as an issue relating to the development of drugs with ever increasing molecular weights. However, there have been few studies in which problems of nucleation or crystal growth of relevant molecules have been reported and in the example of ONSC-E described here it seems that despite its 11 torsions it finds a way to crystallise using the solvent as a 'conformational' auxiliary.

Reflecting on Dunning and Shipman's 1954 study of nucleation in aqueous sucrose solutions it seems to us that their work showed remarkable foresight in dealing with many issues, - the transition state, rate determining steps, molecular complexity- which remain highly relevant sixty years later. At the same time we are reminded of the strong tradition of physical chemistry in which kinetic measurements play a critical role in understanding the nature of transformative pathways be they related to the creation of covalent chemical bonds or of packing interactions in crystalline products. Elucidation of the rate determining step can only be achieved through the combined use of kinetic and structural data. Today with the access to new, high throughput experimental possibilities for measuring nucleation rates, routine probes for exploring solution chemistry and the ever expanding crystal structure database (CSD), progress in unravelling the secrets of nucleation seems inevitable.

Finally, in praise of nucleation we offer a concluding Haiku (41). A snapshot in 17 syllables:

*From chaotic mess*

*the magical transition*

*yields forms evolving.*

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