The crystallisation and stability of a polymorphic salt, ethylene diammonium dinitrobenzoate

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The crystallisation of the polymorphic salt, ethylene diammonium dinitrobenzoate, is reported. In particular it is noted that in this monotropic system it is possible at certain temperature, pH and supersaturation conditions, for the stable form to crystallise directly rather than *via* the metastable triclinic polymorph. The underlying reason for this is sought *via* studies of transformation kinetics and speciation of the salt components. The ideas developed are extended to demonstrate, for the first time, the selection of additives for the control of selective polymorph crystallisation of a molecular salt.

Introduction

The importance of solvents and the use of additives for controlling the polymorphic outcome of crystallisation experiments are well known. For example, the works of Weissbuch *et al.*[1] and Davey *et al.*[2,3] include many examples demonstrating the possibility that solvents and additives may be utilised to modify the relative crystallisation kinetics of two polymorphic phases so as to inhibit the appearance of a stable form and allow the appearance of a metastable phase. These studies have been performed and effects demonstrated using neutral molecules and single component crystals. In the world of pharmaceutical materials, however, crystalline forms having at least two components in the form of molecular salts are most common.[4] Much has been written concerning the processes of selection and screening for this class of material, but it is only recently that solubility, supersaturation and crystallisation issues have been discussed in detail.[5] Here we extend these studies to include the role of pH and the presence of stereochemically related additives on the polymorphic outcome and subsequent phase transformation for the two polymorphic forms of the 1 : 2 salt of ethylenediamine and dinitrobenzoic acid.

Fig. 1 shows the molecular structure of this salt for which two polymorphs are recorded in the Cambridge Crystallographic Database: a monoclinic form (VUJXIH01; $P2_1/c$) and a triclinic form (VUJXIH; $P\bar{1}$).

The structure of the monoclinic form, grown by evaporation of an ethanolic solution of 3,5-dinitrobenzoic acid and ethylenediamine, was solved by Lynch and Smith[6] in 1994 and a further structure solution published by Burchell[7] in 2001. The structure of the triclinic form, grown by evaporation of a mixture of calcium dinitrobenzoate and ethylenediamine in ethanol, was solved by Nethaji *et al.*[8] in 1992. Since this latter structure lacked the determination of hydrogen atom positions it was re-solved as part of this work (VUJIH03[9]) to allow eventual comparison of the hydrogen bonding motifs in the two forms. The structures are built utilizing the $R_4^4(12)$ motif comprising two anions and two cations held by the coulombic interactions of the charged benzoate and protonated amine groups. The geometry of the motifs differs between the structures with the cation and anion being coplanar in the triclinic form and in orthogonal planes in the monoclinic structure. For both structures this motif is then linked into chains through coulombic interactions by sharing of diammonium ions as seen in Fig. 2(a) and (d) for the monoclinic and triclinic forms, respectively. In the triclinic form the chains lie in the $(40\bar{2})$ plane and are stacked along the *a* axis through combined $NH_3^+\cdots NO_2$ and π–π interactions (3.36 Å) (Fig. 2(e)). In the monoclinic form the chains run along the *a* axis and are again interlinked by $NH_3^+\cdots NO_2$ and π–π interactions (3.43 Å) (Fig. 2(b)). Comparison of the interactions between the chains in each form shows that, in both, $NH_3^+\cdots NO_2$ and π–π stacking interactions are involved. The latter interaction is likely to be more significant in the monoclinic structure, however, since it involves contact between the aromatic π electrons in one layer and the electron deficient nitro group in the layer above rather than the aromatic C–H in the triclinic structure. In the triclinic structure only four of the dinitrobenzoate oxygen atoms are involved in hydrogen bonding to five cations: each carboxylate group is H-bonded to two different ethylenediammonium ions while each of the nitro groups uses only one oxygen to interact with a further three cations (Fig. 2(f)). This is in contrast to the monoclinic structure, in which *all* the oxygen atoms on both the carboxylate group and the nitro groups are hydrogen bonded to a total

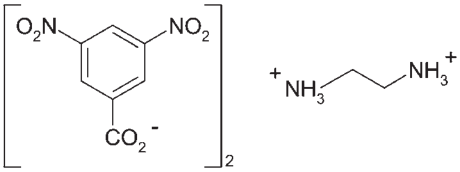

Fig. 1 The molecular structure of ethylenediammonium 3,5-dinitrobenzoate.

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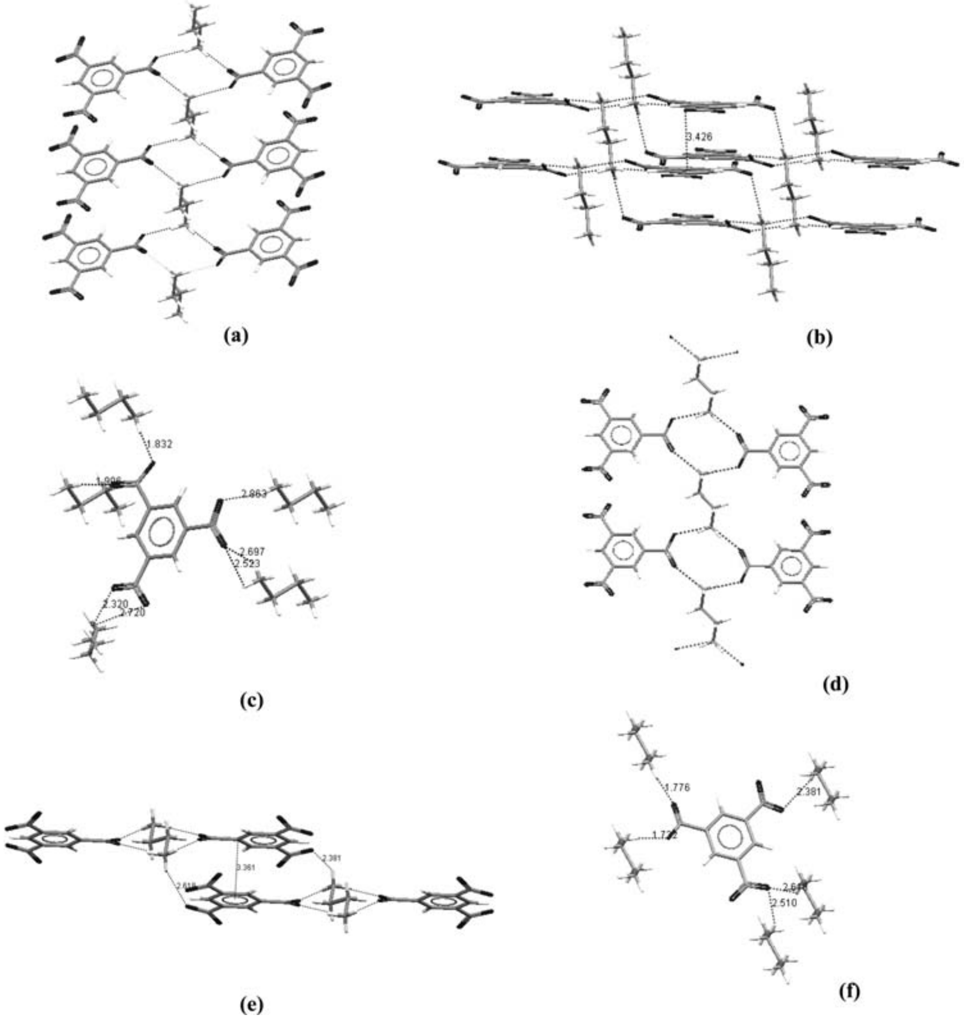

Fig. 2 The crystal structures of the polymorphic forms of ethylenediammonium 3,5-dinitrobenzoate: (a) $R_4^4(12)$ chain, (b) assembly of chains, and (c) close contacts of the benzoate anion for the monoclinic form. (d) $R_4^4(12)$ chain, (e) assembly of chains, and (f) close contacts of the benzoate anion for the triclinic form.

of five ethylenediammonium ions (Fig. 2(c)). Previously measured solubility data[5] showed that the two forms are monotropically related with the monoclinic being the stable form.

Experimental

Crystallisation experiments were carried out as described fully elsewhere.[5] Briefly, the pH was adjusted to a required value by stirred addition of 37% HCl to basic (aqueous NaOH) solutions of acid and base. When additives were used, 2% of a molar equivalent of the additive (to the host) was dissolved with the acid or base at the beginning of the experiment. The supersaturation, calculated from the concentration of ionic species and the solubility product,[5] was 2.0 with respect to the monoclinic form and 3.1 with respect to the triclinic form in these experiments. A temperature of 50 °C and pH 7 were used. Samples of the solid forms present were analysed by FTIR spectroscopy both on crystallisation and 3 h after crystallization in order to determine the polymorphic form. This was possible as a result of differences in the nitro and aromatic group stretching vibrations (nitro: 1549 and 1518 cm^{-1} for the monoclinic and 1533 cm^{-1} for the triclinic; unique aromatic peaks present in the triclinic form at 1126 and 1051 cm^{-1}). For one additive, 3-nitrobenzoic acid, both the concentration of additive and supersaturation were varied with all solutions supersaturated with respect to both forms.

The effect of pH on the rate of transformation of the metastable triclinic form to the stable monoclinic form was measured at 25 and 50 °C. Slurries of the triclinic form in water were prepared at a range of pH values and solid samples were taken approximately every 12 h at 25 °C and every 15 min at 50 °C. These were analysed by FTIR, optical microscopy and later by XRPD, to determine how the polymorphic form changed with time. Conversion times of the triclinic to the monoclinic form were estimated as the midpoint between consecutive samples which showed a switch from the triclinic form to 100% monoclinic form. For example, for the transformation of the triclinic form to the monoclinic form at pH 5.12 and 25 °C, a sample removed after 168 h was the triclinic form. The next sample removed after 240 h was the monoclinic form. The transformation time was estimated as $1/2(240 + 168) = 204$ h with an uncertainty of $\pm 1/2(240 - 168) = 36$ h.

'Effective' supersaturations in these experiments were calculated with respect to the monoclinic form as if all solids were dissolved and were in the range 3.80–4.1.

Results and discussion

Crystal morphology of the triclinic form

In pH controlled crystallization from aqueous solution the triclinic form appeared as rectangular plates (Fig. 3(a)). Strong preferred orientation in the X-ray powder diffractograms of unground plates (not shown) allowed the Miller index of the largest crystal faces to be determined as {001}, while single-crystal X-ray diffraction indicated the longest axis (fastest growth direction) to be the *a* axis. As discussed above and shown in Fig. 3(a), this results from a combination of π–π stacking of the benzoate ions and $NH_3^+\cdots NO_2$ contacts.

Crystal morphology of the monoclinic form

The morphology and pink colour of the monoclinic form, shown in Fig. 3(b), make it easy to distinguish from the pale yellow crystals of the triclinic form. The *a* axis is known to be the fastest growth direction from single-crystal X-ray diffraction experiments. Fig. 3(b) and the previous discussion show this to be the result of combined $NH_3^+\cdots COO^-$, $NH_3^+\cdots NO_2$ and π–π interactions.

Crystallisation of polymorphic forms

The crystallization of this salt under defined conditions of supersaturation, pH and temperature has been reported previously.[5] At 25 °C and pH between 4 and 8 the metastable triclinic form appeared at all values of supersaturation. However, at 50 °C with pH values in excess of 6 and for solutions supersaturated with both triclinic and monoclinic forms, direct crystallization (within minutes) of the monoclinic form was observed at supersaturations up to about 2.5 with respect to the monoclinic form.[5]

While the initial crystallization of metastable phases is common, and indeed reflected in Ostwald's rule,[10] the direct appearance of a stable phase in a polymorphic system is less commonly reported. In order to gain further understanding of the possible factors leading to this phenomenon two issues are considered here: first the rate of conversion from the triclinic to the monoclinic form and second the impact of pH-dependent solution speciation in this system. The rationale for the former is that if the transformation between forms occurs on a timescale of the same order as the crystallization time then the apparent initial appearance of the monoclinic form may simply result from a rapid form interconversion from triclinic to monoclinic unobserved on the time scale of these experiments. The latter scenario follows from the case of glycine polymorphs,[11] in which solution speciation was found to yield a self-poisoning impurity which selectively inhibited the crystallisation of the metastable α form. In the current case it may thus be that the appearance of the monoclinic from is the result of a pH-dependent charged species selectively inhibiting the formation of the metastable triclinic structure.

Polymorphic conversions

Fig. 4(a) and (b) show the results for conversion at 25 °C, pH 5–8 and for 50 °C, pH 5–7, respectively. Clearly these transformation times decrease both with increasing pH and temperature. The former effect can account for up to a four-fold reduction in transformation time at each temperature, perhaps as a result of higher solubility, while increased temperature typically reduced the time from hundreds of hours to hundreds of minutes. It is clear from these data that crystallisation of the monoclinic form at 50 °C and pH 7 cannot be the result of conversion from the triclinic form as this process took 45 min

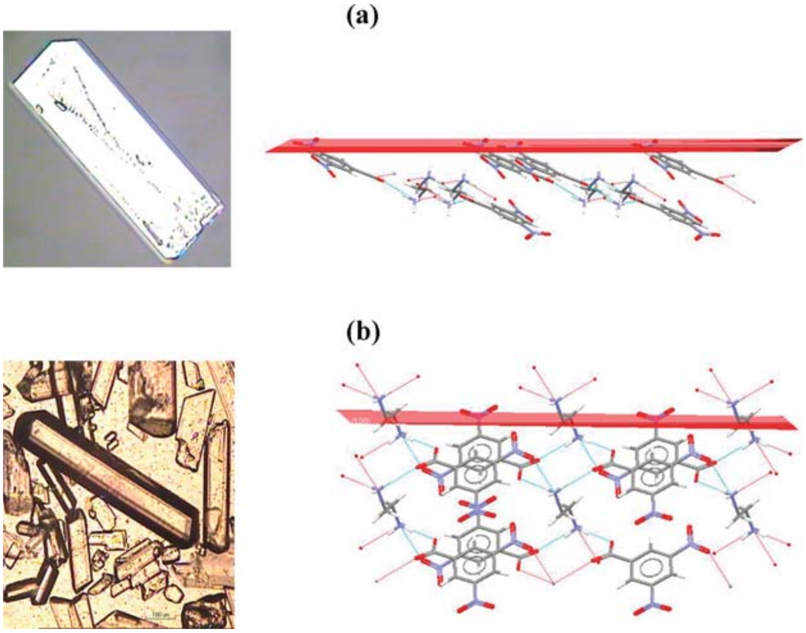

Fig. 3 The morphology and fast growing faces of the polymorphic forms. (a) Morphology and (100) surface of the triclinic form. (b) Morphology and (100) surface of the monoclinic form.

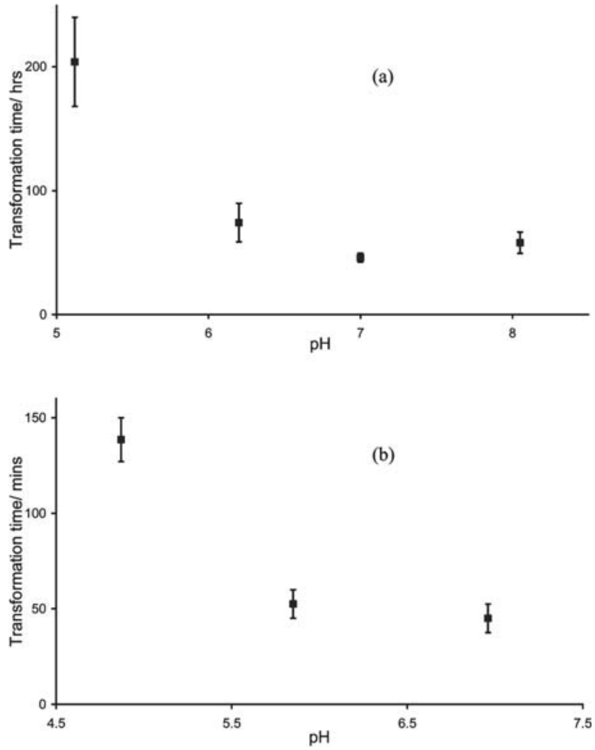

Fig. 4 The rates of polymorph conversion from the triclinic to monoclinic form at (a) 25 and (b) 50 °C as a function of pH.

at a supersaturation of 4 while crystallisation under these conditions even at a lower supersaturation of 2 took only 10 min. The monoclinic form must therefore nucleate and crystallise directly without the initial appearance of the triclinic form.

At 50 °C and pH 7 the conversion was further studied by optical microscopy. Fig. 5 shows images of crystals of the triclinic form converting to the monoclinic form over a period of 45 min. Large triclinic crystals were observed to begin to dissolve after 15 min with a noticeable size reduction after 30 min. At this time a few small prisms of the monoclinic form are also visible and these grow with time so that after 45 min all crystals were observed to be prisms of the monoclinic form. These crystals have increased in size after 60 min. This indicates that transformation from the triclinic form to the monoclinic form is a solution-mediated process,[12] a feature confirmed by the observation that dry samples of the triclinic form take several months to convert to the monoclinic form at room temperature. This is an important outcome since it implies that, during both the initial crystallization and the subsequent transformation, crystal growth occurs from solution and hence that both processes will be susceptible to interference by dissolved additives and impurities.

Speciation effects

Following previous studies[11] on the effects of speciation on glycine crystallization, Fig. 6 shows the speciation diagram for ethylenediamine and 3,5-dinitrobenzoic acid calculated at 50 °C from the available pK_a values[13–15] (3.07 for 3,5-dinitrobenzoic acid and 6.33 and 9.00 for ethylenediamine) and Table 1 gives numerical values for the percentages of the various species present at pH 5 and 7. Not surprisingly, the most significant differences are in the relative amounts of mono- and diprotonated ethylenediamine; the unionised acid and base account for less than 2% of the total amounts at both pH values. Thus at the same supersaturation but different pH, while the driving force for crystallization is fixed, the proportions of deprotonated acid to diprotonated base change such that at pH 7 crystallization could potentially be limited by the availability of diprotonated base, an effect that would of course not be polymorph or temperature specific.

On the other hand, the monoprotonated amine, which increases in proportion with increase in pH may be an effective crystallisation inhibitor. In the above examination of the crystal structures of the two forms it was noted that in the triclinic form the ions had fewer intermolecular H-bonds than in the monoclinic suggesting that the triclinic form might more readily accommodate species showing slight modification of the host molecular structure. This potential selectivity is further reinforced when examining the nature of the fast growth directions of the two forms in Fig. 3. This shows how in the triclinic

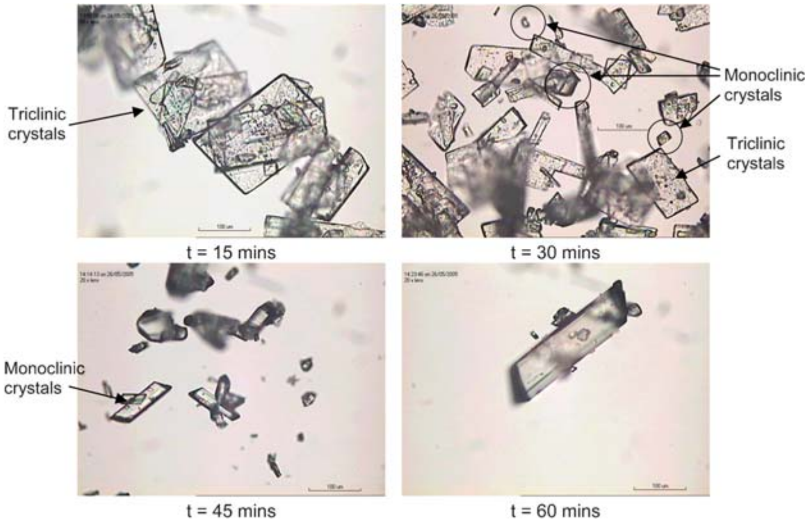

Fig. 5 Optical micrographs illustrating the solution-mediated nature of the triclinic to monoclinic phase transition.

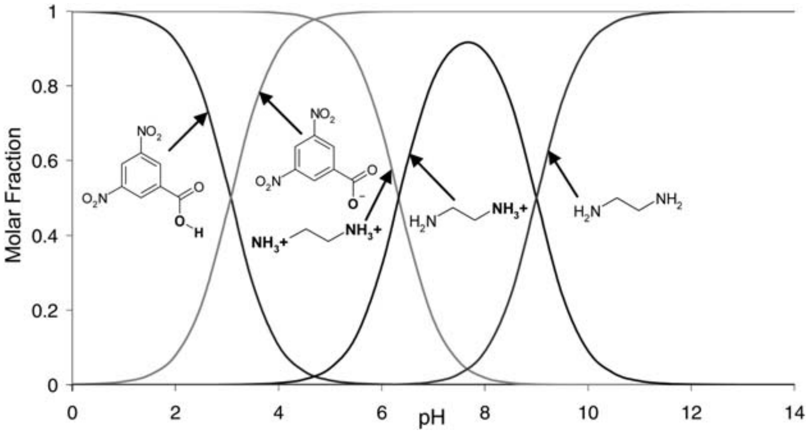

Fig. 6 The speciation diagram for 3,5-dinitrobenzoic acid and ethylenediamine in water at 50 °C.

structure the diammonium cations lie close to the plane of the (100) face with the nitro groups oblique to it, while in the stable monoclinic form both the nitro and protonated amine groups protrude almost normally along the fast growth direction. These differences suggest that it should be possible, in principle, for a solution species or a chosen additive (see below) to selectively inhibit the crystallization of the two forms. The monoprotonated ethylenediamine ion would be one such species since the protonated amine group could bind to the crystal surface in the same way as the diprotonated ion while the unprotonated group could then potentially prevent further growth as it would lack the necessary coulombic charge to hydrogen bond to the carboxylate and nitro groups in the required way. This reasoning however suggests selective inhibition of the monoclinic form over the triclinic, the reverse of the experimental observation at this pH and temperature. It is also evident that such a mechanism could not explain the appearance of the monoclinic form at 50 °C but not 25 °C.

The inability of either mechanism to account for the observed impact of temperature and pH on the appearance of polymorphs in this system is disappointing but further indicative of our overall lack of understanding of the process of nucleation.[16] On the other hand, it is clear from the forgoing analysis that it should be possible to select additives that would discriminate between the two forms and in particular inhibit the growth of the stable monoclinic structure over the triclinic. This is discussed in the next section.

Selection of additives

In this section the selection of additives to inhibit crystallisation of the monoclinic form and allow crystallisation of the triclinic form is discussed. Given the forgoing discussion concerning the tolerance of the triclinic structure and the differences in the intermolecular interactions along the fast growth directions, two classes of additives were selected as mimics for the host anion and cation. First, a number of benzoic acids: 3-nitrobenzoic acid (3-NBA), 4-amino-3-nitrobenzoic acid (4-A-3-NBA), 4-chloro-3-nitrobenzoic acid (4-C-3-NBA), 3-aminobenzoic acid (3-ABA) and 3,5-dichlorobenzoic acid (3,5-CBA), and second, the substituted diamine 1,2-diaminopropane (1,2-DAP). It was intended that all additives would adsorb along the fast growth direction during crystallization, offering a steric barrier and modifying intermolecular interactions in such a way as to preferentially inhibit growth of the monoclinic form allowing the triclinic form to appear. Thus for example, changing the aromatic substituents or removing a nitro group from the benzoate ion offers a steric barrier to growth along the *a* axis of the monoclinic form, and also preferentially reduces the strength of its $NO_2\cdots$ stacking interactions by changing the availability of π electrons. Removing one of the nitro substituents would also affect the $NH_3^+\cdots NO_2$ interactions but this would be equivalent for both forms. Modifying the cation will have the most effect on the monoclinic structure by clashing with nitro groups while in the triclinic structure its additional methyl group can fit between layers. Overall, then it is anticipated that these additives will selectively inhibit the crystallization of the monoclinic over the triclinic form.

Table 1 The speciation of ethylenediammonium 3,5-dinitrobenzoate in aqueous solution at pH 5 and 7: percentages of the acid and base existing as each species

	Speciation (%)				
pH	AH	A^-	BH_2^{2+}	BH^+	B
5	1.2	98.8	95.5	4.5	0.0004
7	0.01	>99.9	17.5	81.7	0.8

The simplest example of the anionic additives is 3-nitrobenzoic acid which lacks one of the nitro groups of the host and was chosen on the basis that it can bind to the fast growth faces of each form through its carboxylate group and its missing nitro group will have most effect on the growth of the monoclinic form. Fig. 7 shows how this might occur with the 3-nitrobenzoic acid docked into each structure.

The pK_a values for the additives selected are given in Table 2. Clearly, at the experimental pH of 7 all these species are present in the desired deprotonated, carboxylate form. The range of p*K*s suggests different availability of π electrons, however. In the case of 3-NBA, 4-C-3-NBA and 3,5-CBA with slightly higher p*K*s than the host it is expected that electronic charge on the phenyl ring will be slightly increased relative to 3,5-dinitrobenzoic acid. 4-A-3-NBA and 3-ABA have similar p*K*s to the host with the amine groups deprotonated and capable of hydrogen bonding. 1,2-diaminopropane will exist as a mixture of mono- and diprotonated forms.

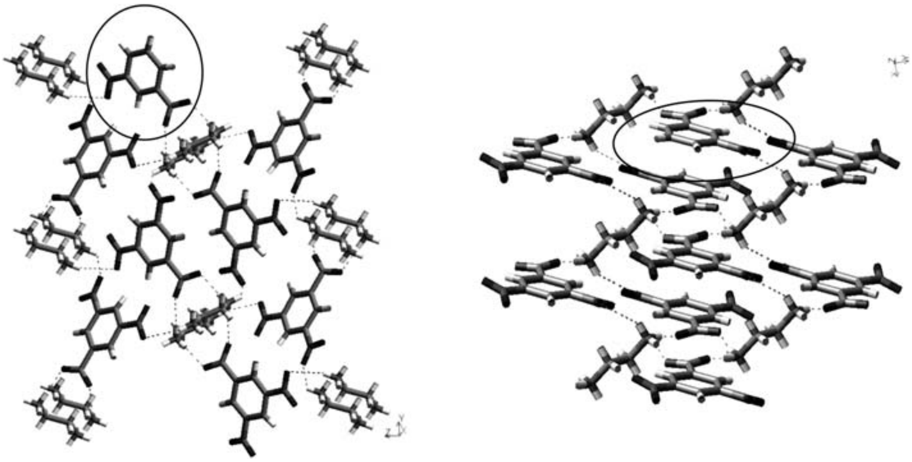

Fig. 7 3-Nitrobenzoic acid (circled) docked into the fast growth directions of (a) the monoclinic form and (b) the triclinic form.

Table 2 Additives chosen as growth inhibitors

Additive	pK_a
3,5-Dinitrobenzoic acid	2.82
3-Nitrobenzoic acid	3.46
4-Amino-3-nitrobenzoic acid	(2.52) 4.19
4-Chloro-3-nitrobenzoic acid	3.34
3-Aminobenzoic acid	(2.17) 4.85
3,5-Dichlorobenzoic acid	3.56
1,2-Diaminopropane	9.82 6.61

Additive potency

For experiments carried out at a supersaturation of 2 in the absence of additives the monoclinic form appeared within 10 min. The transformation between forms under equivalent conditions is estimated, from Fig. 4(b), to take about 1 h (Fig. 5–7). All of the additives tested under these conditions were found to inhibit crystallisation of the monoclinic form allowing the triclinic form to appear first, as predicted. At this 2% loading, however, it was not possible to prevent transformation to the monoclinic form although it was delayed for up to about 3 h in all cases. In the case of 3-nitrobenzoic acid further experiments were carried out to determine the impact of additive concentration and supersaturation on the polymorphic outcome. Table 3 and Fig. 8 show these results for additive concentrations in the range 5–20% and supersaturations from 0.95 to 3.14. The identity of the initial form and a photograph of its morphology is given for each set of conditions. At high percentages of additive, concerns that different salts could be forming in addition to ethylenediammonium 3,5-dinitrobenzoate was ruled out by XRPD characterization of the solids formed. Below a supersaturation of 2.0 the monoclinic form crystallized at all additive levels and at supersaturations above 3 the triclinic form appeared. In these ranges the behaviour is as it would in the absence of additives.[5] It is in the supersaturation range 2–3 and with additive loadings up to 10% that 3-nitrobenzoic acid is most active. Here the crystallizing form switches from the stable monoclinic to the metastable triclinic form and crystallisation times are reduced to a few minutes.

Conclusions

This work has extended previous controlled crystallization experiments with ethylenediammonium 3,5-dinitrobenzoate.[5] In particular the reasons for direct crystallization of the stable monoclinic form in this polymorphic system have been investigated. Despite exploring both the rates of transformation between the forms and the existence of potential charged inhibitor species the underlying cause of this phenomenon remains unresolved. In an extension of the study, however, consideration of crystal morphologies allowed the selection of

Table 3 The impact of 3-nitrobenzoic acid and supersaturation (S) on the polymorphic outcome

% 3-Nitrobenzoic acid	S (mono)	Form crystallised	Crystallisation time/min
0	2.09	Monoclinic	10
5.0	0.95	Monoclinic	15
5.0	2.01	Triclinic	4
5.3	2.08	Triclinic	5
5.4	2.20	Triclinic	5
5.0	2.90	Triclinic	3
10.0	1.71	Monoclinic	30
10.0	1.91	Monoclinic	45
10.0	2.99	Triclinic	0
10.0	3.14	Triclinic	8
20.0	1.91	Monoclinic	19

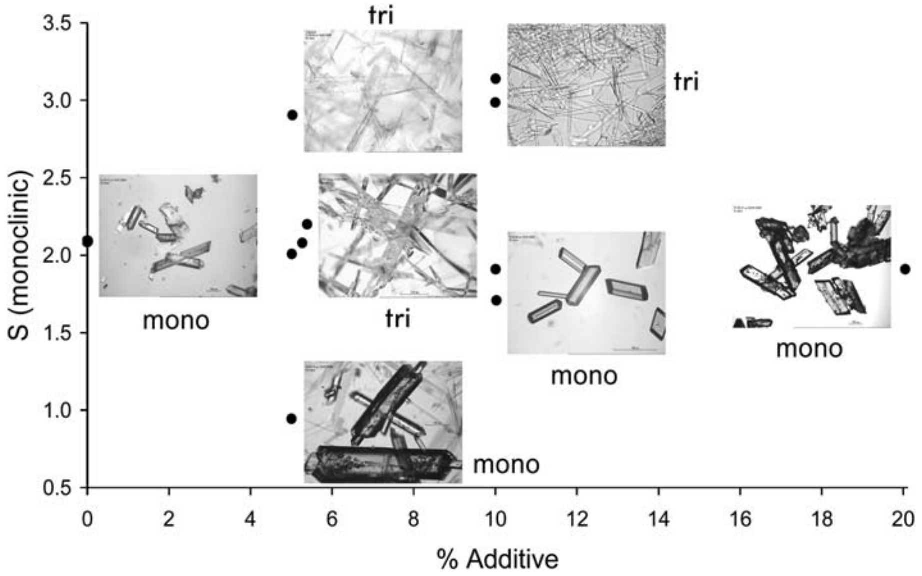

Fig. 8 The effect of concentration of 3-nitrobenzoic acid and supersaturation on the polymorphism and morphology of crystals of ethylenediammonium 3,5-dinitrobenzoate at pH 7. Images were recorded on crystallisation. The label mono and tri indicate which form crystallised first.

additives which selectively inhibit the crystallization of the monoclinic form. This is the first example of selective inhibition of a two-component crystal.

Acknowledgements

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