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A single substance can have multiple crystal forms: some useful, some awkward and even some dangerous. We outline recent approaches to the control of polymorphs.

Polymorph control: past, present and future

Antonio Llinàs and Jonathan M. Goodman

Pfizer Institute for Pharmaceutical Materials Science and Unilever Centre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom

The appearance and disappearance of polymorphs is no longer a mysterious and inexplicable process. Although methods for polymorph control are still imperfect, there is a large armoury of methods that can be used to tackle this important and challenging problem. We survey the methods and their successes over the last few years.

Introduction

The need to control polymorph formation has been an important issue in the chemical industry for over a century. Although identical in chemical composition, polymorphs differ in bioavailability, solubility, dissolution rate, chemical stability, physical stability, melting point, colour, filterability, density, flow behaviour, and many other properties. A hundred years ago, this was particularly important in the development of azo pigments and copper phthalocyanide for the dye industry [1]. More recently, the polymorphism of drugs has been the subject of intense interest in the pharmaceutical industry. In particular, the variation in solubility between different polymorphs is important for pharmaceuticals, as it can affect drug efficacy, bioavailability and safety.

Despite significant investment in processes to find all the stable polymorphs of active pharmaceutical ingredients (API), new polymorphs can still appear without warning. Polymorphs can convert spontaneously from less stable to more stable forms, and the most stable polymorph will be the least soluble. Because solubility may be a limiting factor in the efficacy of the API, it is best to try to discover and to characterize the most stable form as early as possible, ideally while a drug candidate is still in the discovery phase, so that this form is the one used for subsequent testing. There is no method that can provide absolute confidence the most stable polymorph of a compound has been obtained, and mistakes can be costly [2]. New forms may appear as a result of a change in the manufacturing process, even if the change is just in the equipment used to dry the final drug substance. A change may even occur whilst materials are being stored, with no recorded change in the storage conditions [3]. The appearance of a new polymorphic form of an API is a major concern for pharmaceutical companies.

The subject of polymorph control is developing rapidly, drawing ideas from scientists in all areas of molecular sciences. In 1995, Dunitz and Bernstein's account of polymorphs focused on seeding effects [4]. Over the last decade, many new methods of controlling polymorph formation have been developed, and a huge number of new polymorphic forms have been characterized, partly as a result of improvements in the methods for the characterization of polymorphs [5].

ANTONIO LLINÀS

ANTONIO LLINÀS is a Senior Research Associate at the University of Cambridge. He obtained his PhD from the University of Huddersfield working with Mike Page, designing new β-lactam antibiotics and inhibitors for β-lactamases and peptidases. He has held lecturing positions in organic and physical chemistry at the University of Balearic Islands and the University of Huddersfield. He is currently responsible for running the solubility-measurement facility in the Pfizer Institute for Pharmaceutical Molecular Science. His recent research involves studying physicochemical properties of pharmaceuticals, including structure–activity relationships, polymorphism and the design and validation of new analytical techniques. Since 2006, he has been chairman of the PhysChem Forum committee.

JONATHAN GOODMAN

JONATHAN GOODMAN is a Senior Lecturer at the University of Cambridge's Department of Chemistry, working in the Unilever Centre for Molecular Science Informatics. His research career began at Cambridge with a PhD linking synthetic and computational studies of aldol reactions, working with Ian Paterson. He then studied molecular recognition and molecular modelling with Clark Still at Columbia University, before returning to Cambridge. He is a fellow of Clare College, where he directs studies in physical natural sciences. His research in molecular structure, reactivity and informatics has lead to over a hundred research papers.

Corresponding author: Goodman, Llinàs, A. (Antonio.Llinas@ch.cam.ac.uk), J.M. (J.M.Goodman@ch.cam.ac.uk)

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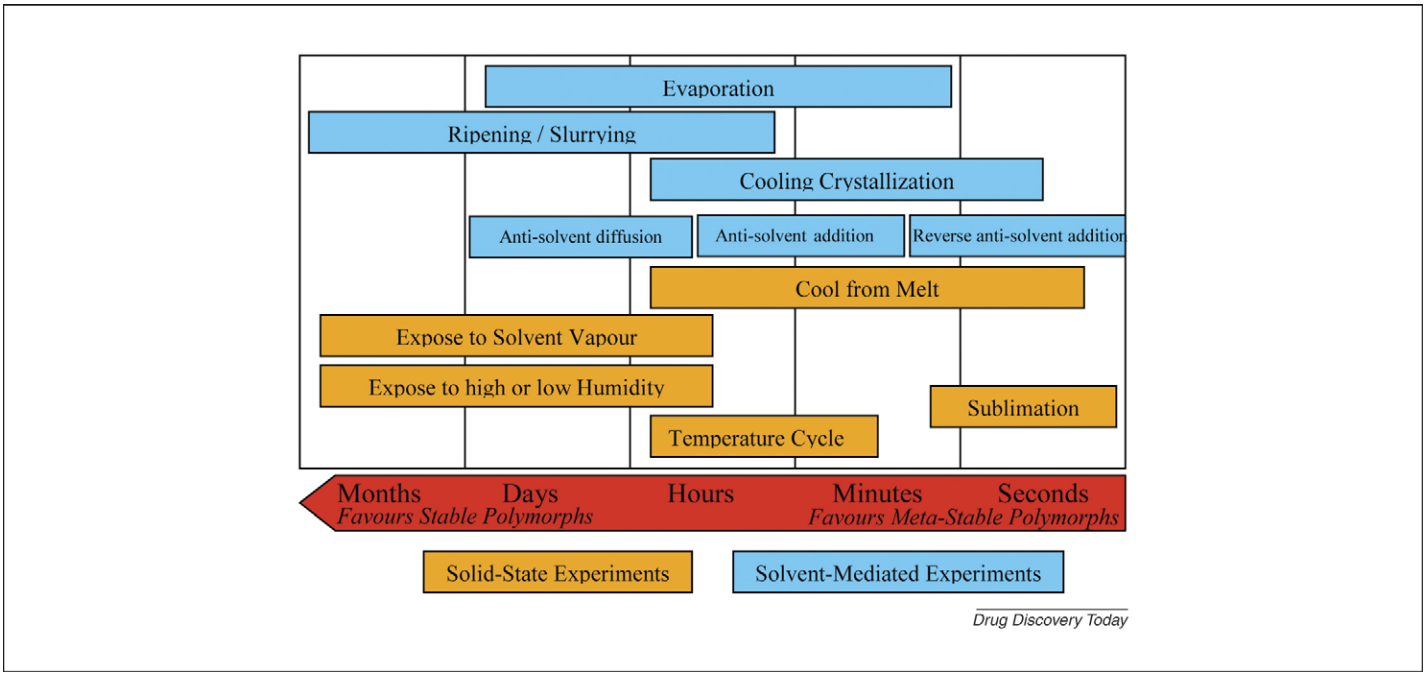


FIGURE 1

Crystallization experiments showing the timescales that can be employed to favour stable or metastable polymorphs. Figure based on ref. [8].

The growth of crystals from solution is often the method of choice for purification, particularly for pharmaceutical compounds. Whilst some materials form crystals readily, others can be extremely troublesome to crystallize [6]. Despite advances in solving crystal structures from powder diffraction in recent years, growth of single crystals remains important if the solid-state structure of a compound is required. Crystallization occurs in two steps: first, crystal nuclei are formed; second, some of these nuclei grow into larger single crystals. If a system obeys Ostwald's rule of stages, metastable forms will be obtained first [7]. Single crystals will usually be produced readily from systems that can form a metastable solution over a wide range of conditions. Systems in which the metastable region is very restricted require careful control of the crystallization conditions to achieve single crystals rather than fine precipitates. Figure 1 shows the time scale for the most traditional crystallization experiments [8]. In general, fast crystallization processes have a greater tendency to form metastable polymorphs than slow processes (Boxes 1–4).

In this review, we begin with an overview of recent advances in the use of seeding and other additives for polymorph control. We then consider the effects of modifying the solvent, physical confinement of the growing crystals, and methods of obtaining supersaturated solutions. Finally, we consider the use of radiation to stimulate nucleation, and methods of running many crystallization experiments together in a high-throughput process.

Additives

The structure of the compounds in this section are shown in Box 5.

Seeding

Seeding a solution with a crystal of the product is a well-established method to induce crystallization. It has also been used to encourage the formation of particular polymorphs, provided that a seed-crystal of the product is available. This process is not always deliberate [9]. In order to use the seeding technique successfully it is useful to know the width of the metastable zone, the range of conditions over which the solution can be supersaturated [10]. This can be done by carefully monitoring the moment of nucleation whilst a compound dissolves and re-precipitates during the heating and cooling cycle, or else by comparing values for kinetic and thermodynamic solubility [11]. Seeding is likely to be effective when the solution is within the metastable zone. If seeding is attempted when the solution is too dilute, then the seed crystal will dissolve and be lost. If the seeding is tried after the first traces of nucleation from the supersaturated solution, the effect of the seed may be swamped by other crystals forming in the solution.

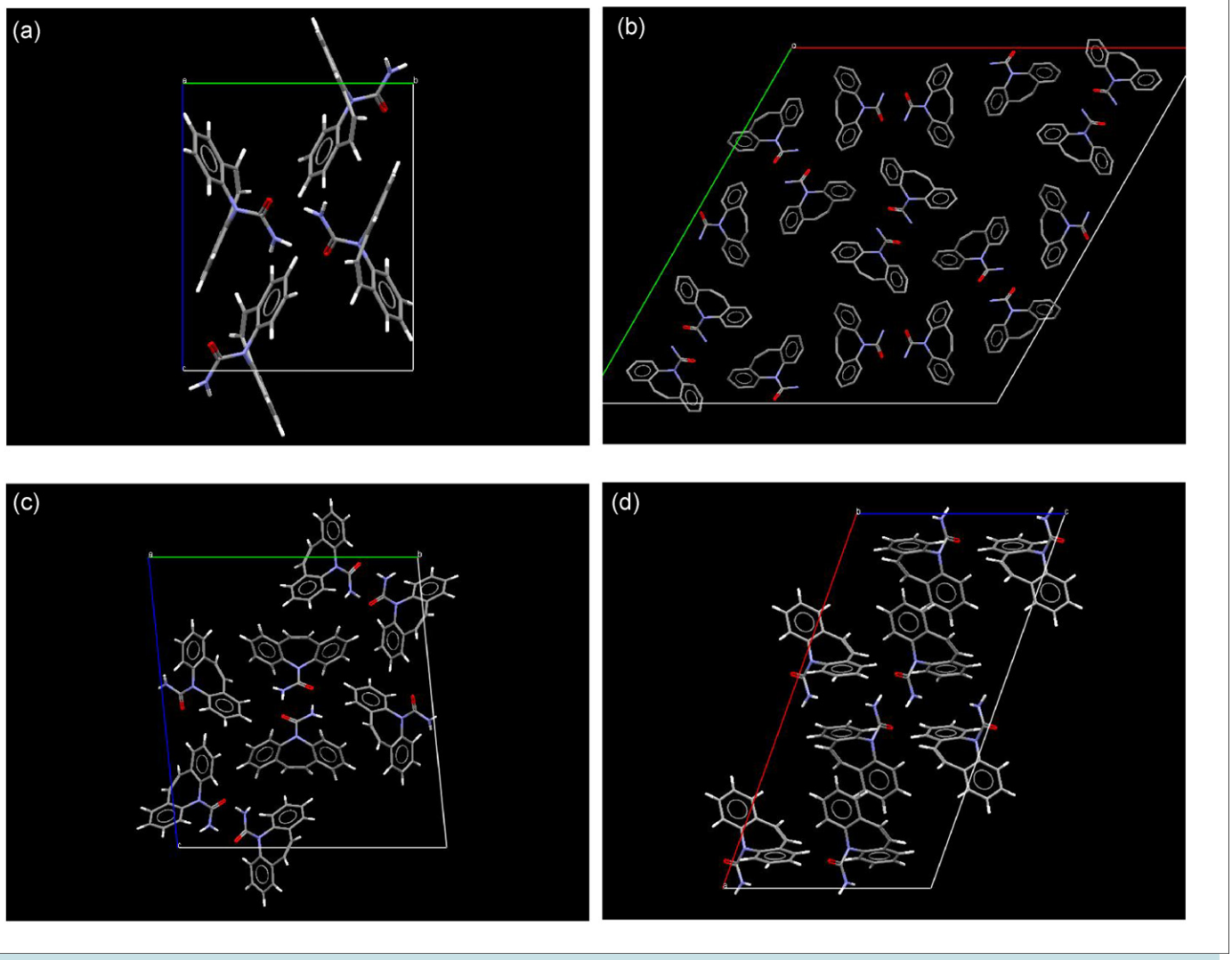
The first nucleation is likely to lead to a metastable polymorph (Ostwald's rule), which forms through a kinetically driven process. These crystals will eventually transform to more stable ones. A common method to obtain seeds of an unstable polymorph is by quench-cooling of the pure substance in its liquid form. A metastable form of paracetamol (Form II) has been reproducibly obtained by quench-cooling molten paracetamol [12].

Seeding can also be used to start the crystallization of single enantiomers from racemic mixtures. This has been recently used by Tamura and colleagues [13] who successfully induced preferential enrichment for certain racemic samples. They were also able to form the desired metastable δ-polymorph of a racemic sulfonate by adding previously formed single crystals of the δ-polymorph to a supersaturated solution [14]. The authors conclude that the seeds not only promoted the heterogeneous nucleation of the δ-form but also inhibited the nucleation or crystal growth process of the more stable α-form.

Kline *et al.* [15] have reported a seeding technique to prepare Form A and Form B of two peroxisome proliferator-activated receptor (PPAR) inhibitors (PF00287586 and AG035029) for the

BOX 1

Carbamazepine



Marketed as Epitol® and Tegretol®, carbamazepine is prescribed as an anti-convulsant, anti-manic and in the treatment of trigeminal neuralgia. The polymorphs of carbamazepine have been studied extensively. Four polymorphs have been discovered to date (among many hydrates and co-crystals). The most stable at room temperature is a monoclinic form **(a)** ($P2_1/c$). The trigonal form **(b)** crystallises in the $R\bar{3}$ space group and undergoes solvent-mediated conversion to the monoclinic form at room temperature, although recently Jones' group has showed that this form could contain solvent in the pores, which would be of great importance in its formation and stability [135]. Form I, a triclinic form **(c)**, was obtained by sublimation and it has the highest density (1.31 g/cm^3) of all the forms. The C-centered monoclinic Form IV **(d)** ($C2/c$) was obtained recently by Matzger and co-workers using the polymer templating technique described in the text. Seeds of this form were used to obtain gram quantities of the new polymorph. The monoclinic, the trigonal and the triclinic forms have been selectively produced using SEDS and the four polymorphs have been obtained by RESS.

treatment of diabetes. A high-throughput solvent-mediated screening was performed to find a solvent which inhibited the transformation of Form A to Form B. Knowledge of the width of the metastable zones was used to give a reliable procedure for preparing both forms of the inhibitors.

Soluble additives

The design of tailor-made additives to obtain a particular crystal form or to inhibit the growth of an unwanted form has proved to be a very successful strategy [16–20]. These additives are designed to interfere with the nucleation or growth rates of a particular form. This technique relies on the idea that in a supersaturated solution the molecules assemble into clusters. Some of these clusters have similar structures to the macroscopic crystals into which they will develop. An additive can inhibit the growth of some of these early nuclei, without interfering with the growth of the other phases. Figure 2 shows a schematic overview of this process. A supersaturated solution of a compound A will form several different nuclei, which we label $(\alpha)_n$ and $(\beta)_n$. If the system is left undisturbed, the stable polymorph will eventually grow forming the α-polymorph. If an additive designed to inhibit the growth of the α-form is added to the solution at the right time, it

BOX 2

McCrone's rule

Walter McCrone, a spectroscopist from Chicago, said in 1965 that the number of polymorphs a compound has is proportional to the amount of money and time that has been spent investigating the molecule [136]. Carbamazepine is a counter example to McCrone's rule, as only four polymorphs are known, despite the extraordinary effort that has gone into investigating its properties. Aspirin is also a counter example: it has just one known polymorph [137].

will selectively bind to the nuclei $(\alpha)_n$ inhibiting their growth and allowing the less stable polymorph, $(\beta)_n$, to develop into a crystal.

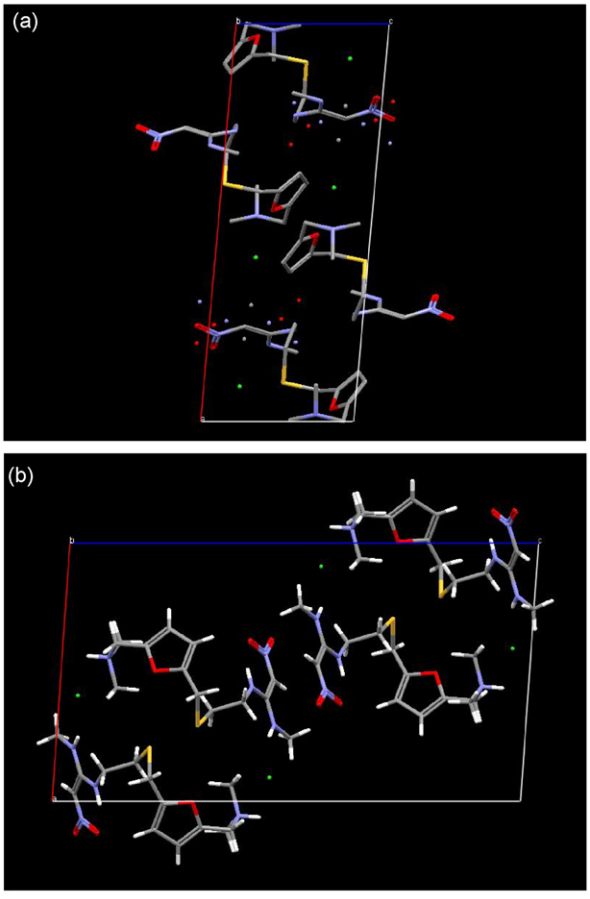
In order to design a substrate to encourage the growth of a particular polymorph by inhibiting its competitors, the intermolecular relationships between the substrate and polymorph should be understood as fully as possible. An additive is chosen by its ability to mimic specific motifs of the molecular assemblies of the desired host polymorph while disrupting the growth of the other polymorphs. For this technique to be effective, the additive has to inhibit, at least, the fastest growing face of the crystals of the unwanted polymorph without affecting the fastest growing faces of the crystal of the desired polymorphic form. Molecular modelling has been useful for designing the right additive. Davey *et al.* [21] selectively inhibited the stable β-polymorph of L-glutamic acid and, hence, favoured the α-form. This work was based on a molecular mechanics conformation analysis of L-glutamic acid and of possible additives. The resulting conformational minima were tested using semi-empirical molecular orbital calculations (MOPAC) [22]. The additives worked well, indicating that predictions can be useful in this field.

More recently, Deij *et al.* [23] have published a method, based on Monte Carlo crystal growth simulations, to determine the site configurations that play a role in the crystal growth process. This method uses the growth site statistics of these configurations to quantify the contribution of each site. Tailor-made additives can then be designed to interact with the most important site configurations.

Reaction byproducts or impurities present in the system [24–28] may also have an effect on polymorph control. For example, the

BOX 3

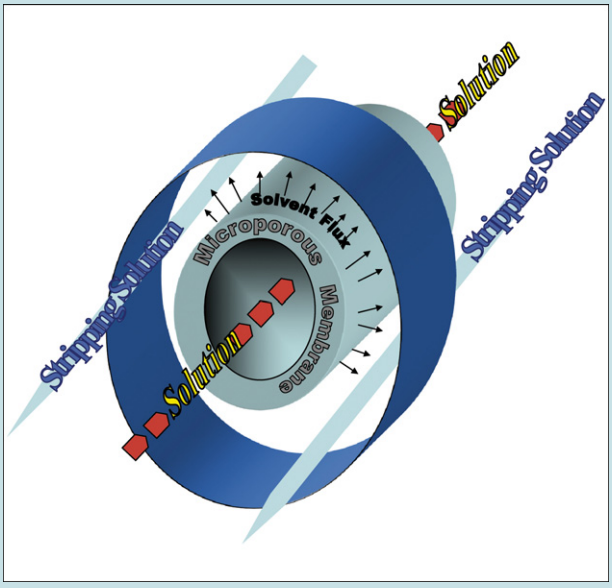
Ranitidine hydrochloride



Ranitidine hydrochloride, an anti-ulcer drug, was marketed by Glaxo under the brand name of Zantac®. The patented form of ranitidine hydrochloride was polymorph I **(a)** [138]. Later, Glaxo patented a new polymorph of ranitidine hydrochloride, Form II **(b)** [139]. This patent might have protected Form I too, because anyone trying to make Form I would probably make a mixture also containing Form II. However, Novopharm was able to market pure Form I.

BOX 4

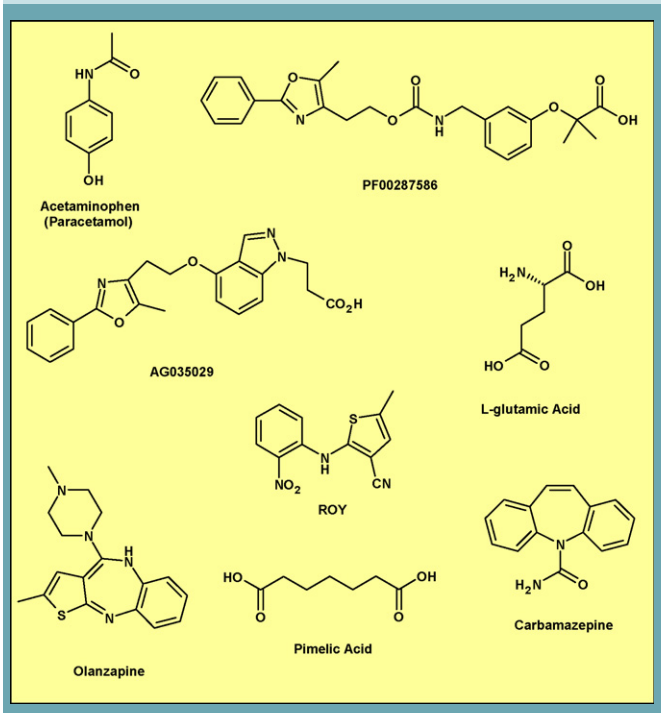
Crystallisation using microporous membranes



The microporous membrane separates two isothermal solutions. Appropriate choice of the outer solution, the stripping solution, leads to the progressive concentration of the inner solution. This method is able to control accurately the rate of solvent transfer which affects the morphology, the crystallinity and the polymorph of the product. By controlling the rate of concentration change, thermodynamic or kinetic control can be achieved. At low solvent transfer rates, the amount of supersaturation is low and the more stable polymorphs have time to grow at the expense of the less stable forms. At high solvent transfer rates, the amount of supersaturation is high and this induces nucleation, favouring the appearance and growth of a metastable polymorph. In Di Profio et al.'s paper [97] the fine control of the solvent exchange rate through the membrane allowed the formation of either the γ-glycine polymorph or the α-glycine polymorph, as required.

BOX 5

Chemical structures (Section 'Additives')



metastable form of sulphathiazole is stabilized by the presence of 1 mol% of ethamidosulphathiazole, a hydrolysis sulphathiazole by-product [27].

The tailor-made additives technique has been applied to stabilize metastable crystal forms [29,30], in the kinetic resolution of enantiomers [20,31–33] leading to new potential crystallization processes for chiral enrichment, and to encourage twinning of molecular crystals [30,34]. Additives have also been used as motif-cappers, able to stop the extension of the structural motifs at the surface of a growing crystal [35]. The field has been reviewed quite recently by Davey *et al.* [36] and Weissbuch *et al.* [37].

Templating

Heterogeneous nucleation relies on specific interactions between the surface, which induces nucleation, and the early aggregate, which will grow into a crystal. Several different materials have been used to study the effects and direct the crystallization of different polymorphs. Ward [38] used pimelic acid as a substrate to grow selectively the metastable (YN, oriented yellow needles) polymorph form of ROY (5-methyl-2-[(2-nitrophenyl)amino]3-thiophenecarbonitrile), a precursor of the antipsychotic agent olanzapine, which forms six conformational polymorphs. Only the YN polymorph, in the observed orientation, achieved good epitaxial match to $(1\,0\,1)_{PA}$ faces of the single crystal of pimelic acid. Other authors have used metastable forms as templates to grow a more stable polymorphic form [39,40] or conversely, to grow the metastable polymorphs using 2D epitaxial nucleation growth on the stable forms [41]. Computational strategies can be especially useful combined with this technique. This should allow efficient screening for substrates prior to performing the experiments with libraries [38].

SAM templating

Self-assembled monolayers (SAM) [42] and Langmuir–Blodgett films [43] have been used to control nucleation, orientation, polymorphism and morphology. Many materials have been grown in a controlled way on SAMs, including proteins, enantiomerically pure amino acids, semiconductors, and biomaterials. Recently Dressler and Mastai [44] have demonstrated that SAM surfaces can act in a manner similar to other additives, stabilizing the metastable form of a crystal (the α-form of L-glutamic acid) using a self assembled multilayer of a phenylalanine derivative, without needing to use well-oriented and molecularly designed SAMs.

Polymer templating

Polymers can also be used as templates to aid polymorph selection through heterogeneous nucleation. The use of polymer-induced heteronucleation has been shown to facilitate the production of single crystals [45–47]. Lang *et al.* [46] introduced the method of using polymers for the selection and discovery of polymorphs, and this method has attracted a lot of attention. The metastable orthorhombic form of acetaminophen was grown as single crystals, by evaporation of aqueous solution in the presence of certain polymers (nylons, isotactic polypropylene, chlorinated polyethylene, poly(tetrafluoroethylene), poly(2,3,5-tribromostyrene) and poly(vinylchloride) [46].

This method seems particularly promising because no previous knowledge of the lattice or surface chemistry of the polymorph is required, it is compatible with several high-throughput polymorph-screening techniques, and it often produces single crystals. Price *et al.* [45] have applied this approach to several compounds and has obtained all stable polymorphs from a single set of solvent and temperature conditions. A new polymorph of carbamazepine was identified, and the two stable polymorphs of acetaminophen and the six stable forms of ROY were also produced. This approach has also been applied to other fields, including the preparation of extended solids, such as metal-organic frameworks (MOFs) [47], and in the selection and discovery of polymorphs of platinum complexes [48].

Solvent control

The structure of the compounds in this section are shown in Box 6 and Box 7.

Targeted solvents

The use of solvents or solvent mixtures to promote or inhibit certain crystal motifs has been successfully applied to polymorph formation. The mechanism for the selection of different forms by targeted solvent crystallization is similar to other additives, except that the solvent is present in huge excess. The growth of a macroscopic crystal from these nuclei will depend on the geometry of the solvation and desolvation process of incoming molecules and outgoing solvent molecules [49]. A good level of control and selection of the desired polymorph can be achieved if the system has well documented crystal structures and phase transformations. Blagden and Davey's review [50] describes how different solvents can be used as templating agents for a specific motif. A good

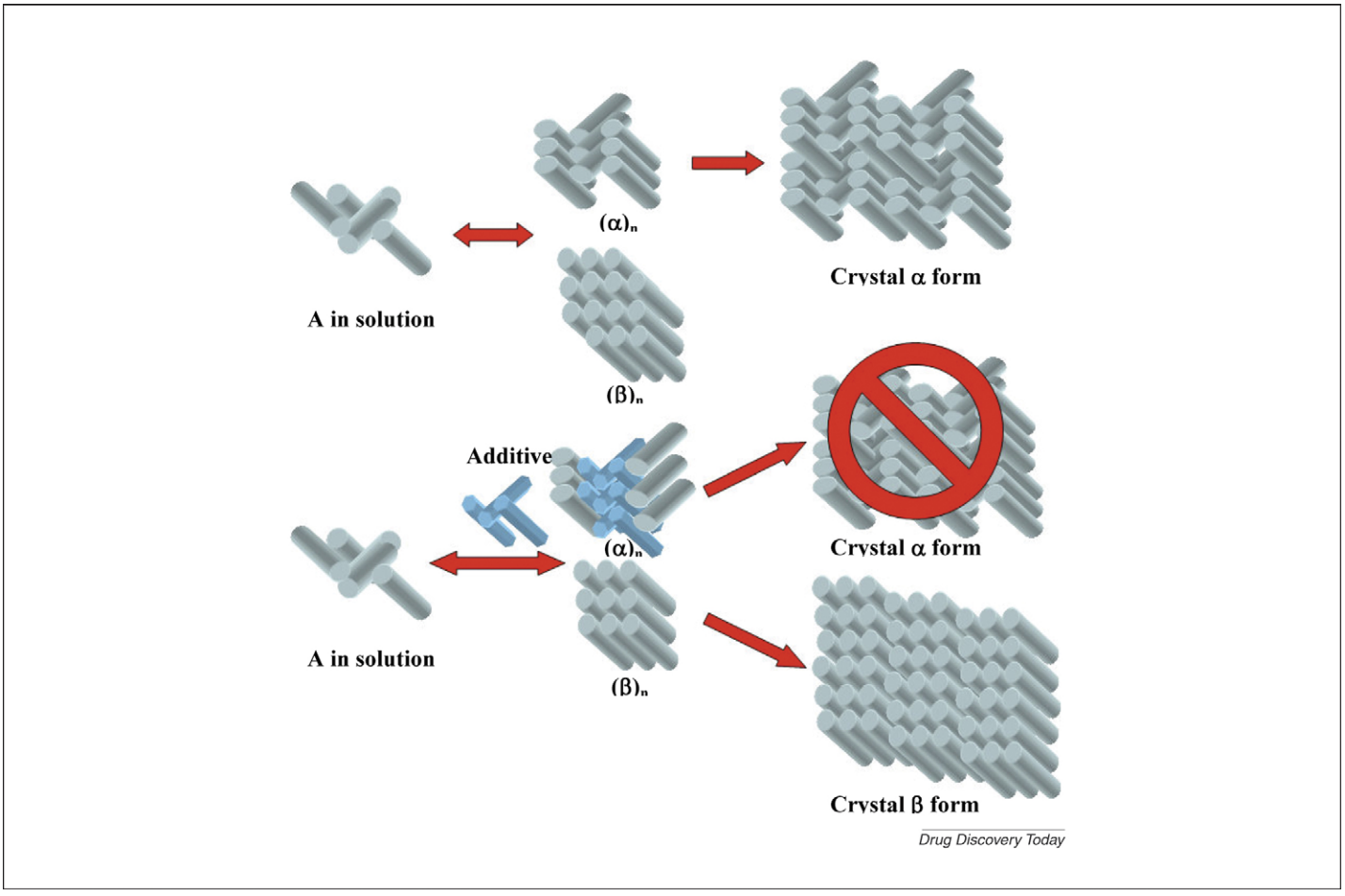


FIGURE 2

Schematic mechanistic pathway of the polymorph control using an additive.

example of this approach is their study of 2-amino-4-nitrophenol (ANP). They used solvents with nitro groups to stabilize chain motifs leaving the amino groups exposed; solvents with amino groups to stabilize chains to leave nitro groups exposed; solvents with hydroxy groups to form chains that leave hydroxyl groups exposed; aromatic solvents to promote open ring motifs. Structure prediction has proved useful in this field [51]. Molecular dynamics simulations have been used to show that relevant solvent-surface interactions are not limited to hydrogen bonding, but also include electrostatic and van der Waals interactions [52].

Using solvent control, Weissbuch *et al.* [53] have investigated the growth kinetics of the three polymorphs of glycine coupled with an analysis of the action of the solvent at the various crystal faces. The study explains why the more thermodynamically stable α- and γ-glycine polymorphs do not generally precipitate in aqueous solutions, even though the addition of alcohol reduces the solubility of glycine by ten fold.

Kitamura *et al.* [54] have studied the effect of solvent in the polymorphic crystallization of BPT propyl ester (propyl 2-(3-cyano-4-(2-methylpropoxy)phenyl)-4-methylthiazole-5-carboxylate). When acetonitrile was used as the solvent, they obtained only the more stable polymorph. However, the metastable form appeared when ethanol and cyclohexane were used instead. Trifkovic *et al.* [55] have shown that crystallization of ranitidine hydrochloride using a non-polar solvent, which leads to strong hydrogen bonding interactions, led to a polymorph containing the enamine tautomer (Form I). When a more polar solvent, such as water or methanol, was used, the ranitidine–ranitidine hydrogen-bonding interactions were disrupted and the nitronic acid tautomer (Form II) was obtained instead.

Mechanical grinding

Mechanical grinding is a mechano-chemical alternative to solution-based crystallization, and can be used to obtain crystalline complexes such as salts, charge-transfer complexes and co-crystals. The kinetics of this process seems to be enhanced by the addition of a few drops of solvent, a technique referred to as solvent drop grinding. Using the solvent drop grinding technique, Rafilovich and Bernstein [56] reported the crystal structures of four polymorphs of benzidine. In the course of a series of crystallization experiments to obtain new co-crystals of caffeine and maleic acid, Day *et al.* [57] recently reported a second polymorphic form of maleic acid, more than a century after the first polymorph was reported.

X-ray crystal structures do not always lead to incontrovertible conclusions. Vishweshwar *et al.* [58] reported a new polymorph of aspirin during the attempted co-crystallization of aspirin and levetiracetam, in the presence of a molar equivalent of acetamide from hot acetonitrile. A later re-analysis by Bond *et al.* [59] showed that the X-ray data could have an alternative interpretation.

Nevertheless the effect of mechanical grinding on the generation and control of different polymorphs has promise for the future.

Co-crystals are not as widely studied as single component crystals, but the formation of co-crystals has attracted some attention from the pharmaceutical industry. A co-crystal can be defined as 'a system containing two or more components together' [9] although this definition may be too broad to be useful as it includes molecular adducts, salts, solvates, and hydrates. Bis *et al.* [60] define a co-crystal as 'a multiple component crystal in which all components when pure are solid under ambient conditions' and Remenar *et al.* [61] use the definition: 'crystalline phases engineered by combining molecules selected to match hydrogen-bond donors with acceptors and by considering structural complementarities'. Whatever the details of the definition, co-crystals offer an attractive alternative to classical APIs because they represent an effective means of altering a drug's physical properties [61,62]. These materials are sometimes labeled as polymorphs, but pseudo-polymorphs are now a more acceptable term [63–66]. The search for co-crystals can lead to the discovery of new polymorphic forms of the components of the co-crystal [45,46,58]. The technique of co-crystal formation by solid-state grinding has been reviewed recently [67,68].

Supercritical fluids

The morphology of solid dosage forms is very important to the pharmaceutical industry. Uniform size distribution and sphericity improve the flow properties of powders and the ease of making tablets. Micronisation, the reduction of particle size to a few micrometers or even to the nanometer range, increases the surface area and dissolution rate of the drug, thus increasing its bioavailability. Electrostatic charges lead to aggregation and agglomeration, however, and these can decrease the drug's performance. Classical micronisation techniques can lead to thermal and mechanical stress on the particles, to changes in crystallinity, to an increased tendency to aggregate, and to contamination from solvents. Supercritical fluid technology was developed to avoid these problems and obtain particles with useful characteristics. The use of supercritical fluids is usually a single-step process that is carried out at mild temperatures, and is a green technology because the fluid can be recovered and recycled. There are two main groups of methods to crystallize a solid using supercritical fluids: RESS (rapid expansion of a supercritical solution) and SAS (supercritical antisolvent solution) [69]. Since Krukonis [70] applied the supercritical fluid technique to produce fine particles with a narrow size distribution, both RESS and SAS techniques have been applied to manufacture pure polymorphs, especially a recent variation of the SAS technique called SEDS (solution enhanced dispersion by supercritical solution). By controlling temperature, pressure, flow rate and solvent, different polymorphs can be generated and isolated. Forms I, III and IV of sulfathiazole were isolated using this technique, using methanol as solvent [71,72]. At high temperature, Form I was prepared, whilst at low temperature, Form II and IV were isolated. If acetone was used as solvent, no significant temperature dependence was found and only Form I could be produced [71,72].

Changing crystallization kinetics, solvent and temperature leads to the formation of different polymorphs of carbamazepine [73]. Salmeterol xinafoate (SX), an anti-asthma drug, has been extensively studied by SEDS, because it has to be administered by inhalation and so the particle size and morphology must be carefully controlled [74]. More recently, specific polymorphs of SX have been produced by varying the process conditions of SEDS [75], and demonstrated that Form I had a superior crystalline purity than the commercial micronised one [76]. Hydrocortisone [77], flunisolide [78], budesonide [78], terbutaline sulphate [79], natural carotene [80], and many other pharmaceutical drugs [81] have been successfully studied using this approach, showing that this technique can not only control the crystal habit and morphology of the chosen drug but also generate and precisely control the crystallization of the desired polymorph.

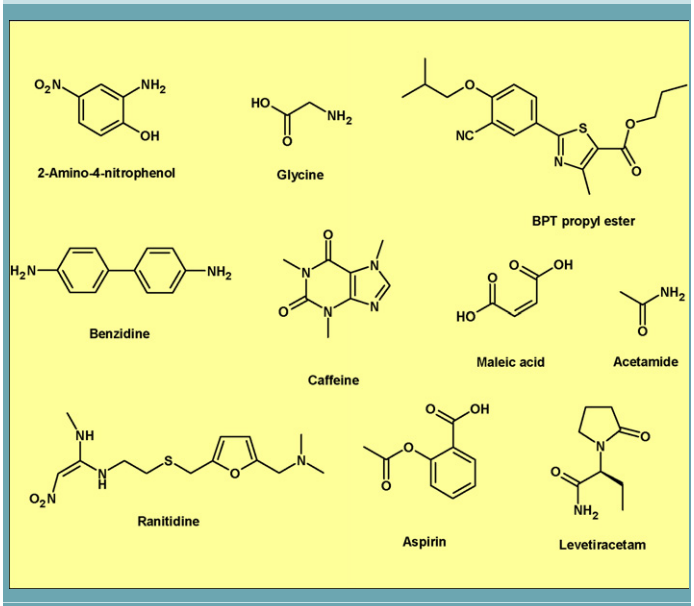
RESS and a variant (SESS: slow expansion of supercritical solution [82]) have also been applied to polymorphic conversion. Three of the four polymorphs of tolbutamide (Forms I, II and IV), and one of the three polymorphs of barbital (Form II), could be consistently produced by choosing appropriate conditions for RESS [83]. Gosselin obtained four different polymorphs of carbamazepine [84] by varying pressure and temperature. Bouchard has recently obtained pure α-, β- and γ-glycine by controlling the flow rates and ethanol concentration in the system [85]. RESS has been applied to many compounds including poly(L-lactide) [86], felodipine [87], and phenylbutazone [88]. The metastable polymorph of chlorpropamide (CPD) was generated by this method using crystal doping [89]. RESS has also been used to obtain composite crystals. Physical mixtures such as acetaminophen, ascorbic acid, urea, and chloramphenicol have been obtained using crystallization in supercritical fluids [90]. The application of supercritical fluids to polymorphism control, therefore, shows considerable promise [91].

Supersaturation

The structure of the compounds in this section are shown in Box 6 and Box 7.

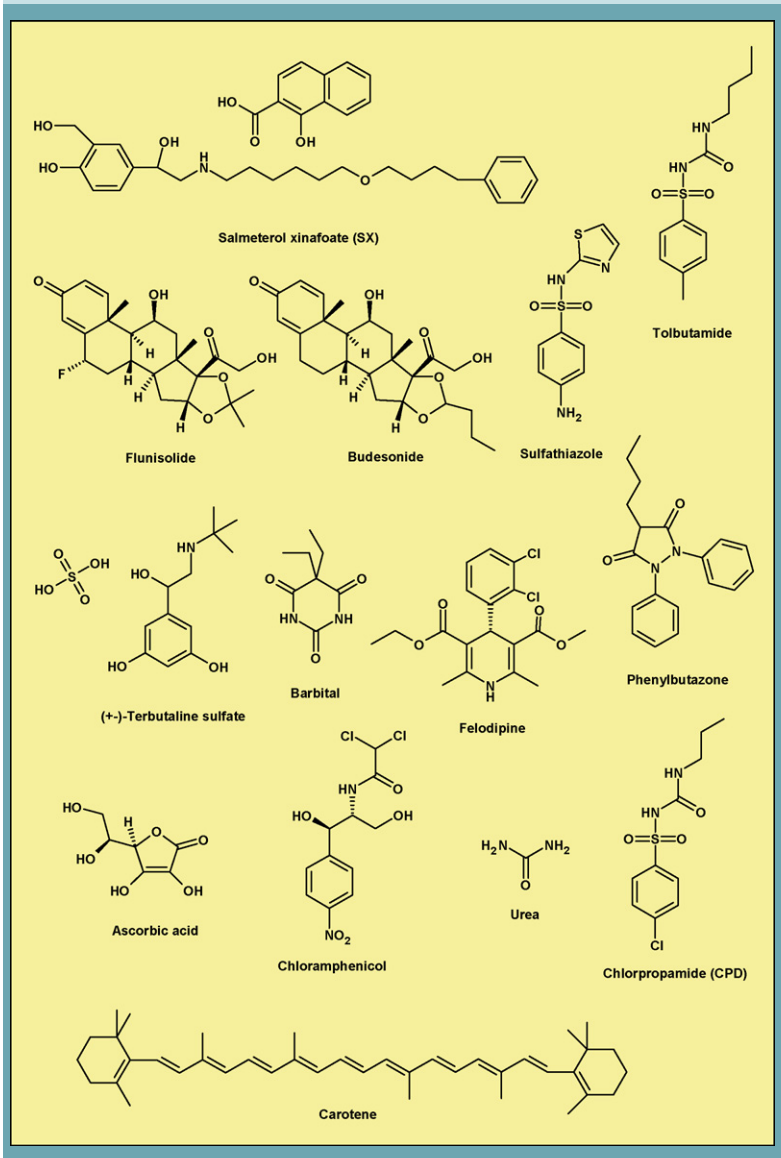
BOX 6

Chemical structures (Section 'Solvent control')



BOX 7

Chemical structures (Section 'Solvent control – supercritical fluids')



The degree of supersaturation of a solution can affect the preference for different polymorphs. In solutions which contain much more dissolved material than a saturated solution (a high supersaturation factor), any nucleation point is likely to lead to the formation of crystalline material, but not always to the most stable polymorph.

Crystallization using microporous membranes

Microporous membranes have recently been used in the development of a new method of crystallisation. The membrane acts as a physical support separating two isothermal solutions, and allows solvent to move between them [92]. This method has the advantage of being able to control the rate of solvent transfer accurately and this affects the morphology and the crystallinity of the product obtained [93], in both inorganic [94] and organic materials [95,96]. Di Profio *et al.* [97] have shown how this technique can be applied to the generation of specific polymorphs of glycine. The kinetics of nucleation is related to the width of the metastable zone, which can be increased by adjusting the solvent transfer rate. By controlling these parameters thermodynamic or kinetic control is achieved. At low solvent transfer rates the more stable polymorph has time to grow at the expense of the less stable form; high solvent transfer rates induce nucleation at higher values of supersaturation, favouring the appearance and growth of metastable polymorphs. Lee and co-workers [98,99] have investigated the effect of the solvent evaporation rate on the polymorph distribution of α- and β-glycine. In Di Profio *et al.*'s paper [97] the fine control of the solvent transport rate through the microporous membrane always yielded the γ-glycine polymorph. In static membrane crystallization experiments, careful control of the concentration of the stripping solution inside the membrane always produced the α-glycine polymorph. It is too early to fully assess the

value of this approach to the pharmaceutical industry, but the application of this technique could have a big impact in polymorph selection and production.

pH control: (PC)^2

Potentiometric cycling for polymorph creation (PC)^2 controls the level of supersaturation by altering the pH of the solution. This approach, which requires the solute to have a pK_a in an appropriate range for the solvent and available buffers, was originally designed to measure the intrinsic solubility of ionisable compounds, and is described in detail elsewhere [11,100,101]. The (PC)^2 method explores the metastable zone, starting from the kinetically driven highest-energy polymorph which precipitates at high supersaturation, through to more stable forms which precipitate at lower supersaturation. This is achieved by cycling the system between supersaturation and subsaturation by pH variation. Metastable polymorphs encountered during this process are not, in general, stable enough to isolate, but from time to time a metastable polymorph arises which is stable enough to characterize. Continued cycling between supersaturated solution and subsaturated solution will push this metastable form towards more stable ones. Threlfall analysed the necessary conditions to obtain different polymorphs under kinetic or thermodynamic control, stating that, in the latter case the nature of the solvent is immaterial in respect of the polymorph produced [102]. The (PC)^2 method is able to reproducibly generate the same series of polymorphs on successive runs. A related method which uses pH-metric titration to generate the most stable polymorph of a substance has recently been published [103].

Confinement

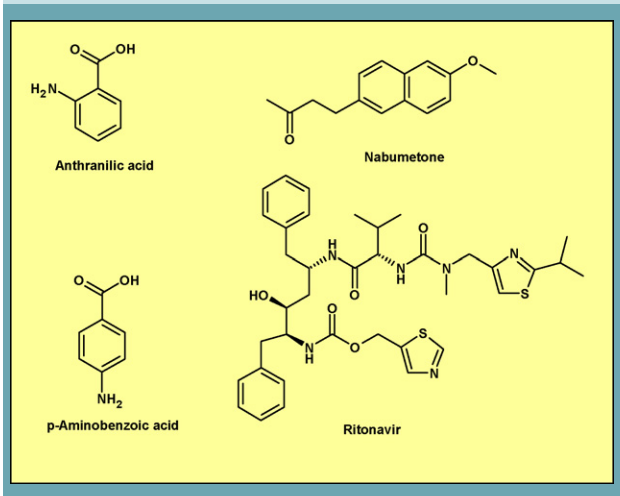
The structure of the compounds in this section are shown in Box 8.

Capillary crystallization

The capillary technique is a method to achieve large supersaturation factors whilst making heterogeneous nucleation difficult. Small volumes (1–50 μl) of solution are held in capillary tubes, usually the same used for powder X-ray diffraction, with an inner diameter of around 0.7–1 mm. The volume of the tubes is so small that each rarely contains more than one nucleation site. This means that less stable polymorphs can grow without having to compete with more stable polymorphs nucleated in the same vessel [104]. Using this method, Hilden *et al.* [104] has been able to characterise all six forms of ROY and even observe the seventh one [105]. The technique has also generated a new polymorph for nabumetone [106]. This method offers the advantage of being able to generate the high-energy forms of the system studied using a near-isothermal approach and requiring only a very small amount of material. Unfortunately, multiple experiments often have to be performed because the nucleation sites cannot be controlled.

BOX 8

Chemical structures (Section 'Confinement, external effects and HTC')



Crystal nucleation in nanoscopic confinement

Different polymorphs may have different nucleus sizes and this characteristic can be exploited to discriminate between polymorphs. Using nanoporous polymers and glass matrices, Ha *et al.* [107] studied the influence of pore confinement on polymorph selection. Crystallization experiments on anthranilic acid in controlled conditions showed that in the presence of non-porous glass beads or commercially available 55 nm controlled pore glass (CPG), only Form III of anthranilic acid was observed. When a 23 nm CPG was used, the presence of the metastable Form II was also observed. When the crystallization was carried out in the presence of 7.5 nm CPG, only the metastable polymorph Form II was detected. The effect of the pore size on the formation of one polymorph or another may be due to the smaller critical nucleus size of the metastable Form II than the other forms [107]. It is also possible that the CPG is acting as a templating agent, but this does not explain why similar effects are observed with nanoporous polymers. Once seed crystals of the desired polymorph have been generated, the matrix can be fractured or dissolved to release the seed crystals which would otherwise be difficult to generate.

Contact line crystallization

Crystallization around the edge of a drop [108], is a well known phenomenon, and very recently it has been applied to the selective crystallization of metastable polymorphs. Capes and Cameron [109] have grown a metastable form of paracetamol (Form II) at the edge of a meniscus. The novelty of this method is not the growth of Form II itself, which has been previously grown in several conditions and using different techniques, but the fact that Form II is not converted to the more stable Form I via the solvent mediated phase transformation which normally occurs if the metastable crystals are not harvested from the solution fast enough. The explanation is that as Form II nucleates at the contact line the level of the solution drops through evaporation. As Form II grows the water is effectively removed from around the crystals as they grow, preventing the transformation to the more stable Form I.

External effects

The structure of the compounds in this section are shown in Box 8.

Sonocrystallization

Ultrasonic irradiation has been used for enhancing the rate of crystallization of metals, inorganic salts [110], and co-crystals

[111]. Salting-out crystallization is rapidly completed with the aid of ultrasound [112]. This method has been commonly used during the nucleation phase of the crystallization process, allowing the nucleation to take place at lower supersaturation levels. Sonocrystallization has also been applied to increase the amount of material recovered from solution. In a study of lactose, 90% of the lactose was recovered from solution in just two minutes of sonication time. Without sonication, only 55–60% was recovered after three days [113].

The mechanism for these effects is still not fully understood. Three factors may be responsible for the contribution of ultrasound to crystallization: first, nucleation is enhanced by the shear forces that are produced when the bubbles produced by cavitation collapse [114]; second, the rate of crystallization is enhanced by the fragmentation of seed crystals [115,116]; third, a better uniformity in crystal size distribution is obtained [113]. The influence of ultrasound on polymorphism has also been studied recently. Gracin *et al*. obtained the metastable form of *p*-aminobenzoic acid under sonicated conditions [117]. Louhi-Kultanen *et al*. [118] studied the influence of the sonocrystallization on glycine at two different ranges in temperature (40–50 °C and 20–30 °C). The purity of the main glycine polymorph (α) was diminished at the lower temperature range. Ultrasound decreased the amount of the α-polymorph at the higher temperature range. At the lower temperature range the α form was obtained almost exclusively.

The advantages of this method are the generation of smaller crystals with a better shape, uniform crystal morphology, less agglomeration and the generation of polymorphic forms close to the ground state. For a single probe with one kilowatt of power [112], the ultrasound has a range of about ten centimeters [119]. As a result, the practical limit on the effective sonicated volume is about 500 ml. This limit can be bypassed by using flow cells, either in a batch mode or in a continuous mode, or by using an external in-line ultrasound source [116].

Non-photochemical laser induced nucleation (NPLIN)

Infrared radiation can affect crystallization. Garetz *et al*. [120] discovered that intense pulses of near infrared from a laser could induce the nucleation of supersaturated urea solutions. In the absence of the laser beam the crystallization of urea took days or even weeks, but in presence of light it took place within the duration of a laser pulse (9 ns), reducing the nucleation induction time by a factor of 10^{13}. This reduction in the nucleation induction time is rather impressive, but NPLIN can do a lot more. Sun *et al*. [121] also showed that linear and circular polarizations induce different types of alignment and induce the nucleation of different polymorphs of the amino acid glycine. This technique represents a new method to control nucleation in supersaturated solutions and opens a new field in polymorphism.

A particular variant of this technique has recently being patented [122]. The patent relates to a method of selecting and controlling polymorph formation by illuminating a material with non-absorbed polarized light as the material is thermally driven through a phase-transition. The inventors give just one example, nabumetone, and this is only characterised by Raman spectroscopy. If the method has wide application, it would be a major step forward in polymorph generation.

High-throughput crystallization

The structure of the compounds in this section are shown in Box 8.

Traditionally, automated processes have not been a major concern for the pharmaceutical industry which has been dominated by small batch procedures. However, the lonely chemist monitoring one variable at a time on the bench is out of date. Nowadays the pharmaceutical industry needs to test thousands of samples a day using different combinations of temperature, rate of cooling and heating, concentration, additives, rate of mixing, concentration of excipients and solvents. This must all be done in the shortest time possible, and using the smallest amount of compound possible. To address these challenges high-throughput crystallization (HTC) systems have been developed [123–131], permitting rapid and more comprehensive exploration of solid form diversity with only small amounts of API. HTC uses large arrays of conditions and compositions and runs all of the processes in parallel.

The variables that need to be explored are selected and an initial survey may be used to refine the values used for each variable. The number of the experiments necessary to perform increases rapidly with the number of variables [132]. As the number of variables is increased, fractional factorial design may be needed to remove subsets of experiments, which are unlikely to be productive, while ensuring a uniform coverage of the rest of the variable space. Evolutionary optimization procedures use information from a small number of experiments to choose values for the next experiments. These may be combined with simplex optimization methods to identify the experimental conditions that are closest to the optimal outcome [133].

Morissette *et al*. [134] performed more than two thousand experiments trying to find all polymorphs of the HIV protease inhibitor ritonavir using only 2 g of the API. They covered the solvent space in a very efficient manner, characterizing solvents by their physical properties, including Hilderbrand and Hansen solubility parameters, log *P*, hydrophile–lipophile balance, boiling point and melting point, and building a 24 solvent library. This process generated 51 crystalline samples, a 2.5% hit rate. From these, five different crystalline forms were identified, including two known polymorphs and three unknown forms.

HTC is a good way to attack the very complex problem of crystallization. The problem is so complex that it is not possible to exhaustively investigate all possible combinations of solvents, additives, temperature gradients, mechanical and confinement effects, radiation, pH control, and concentration control, and so the outcome depends on the variables chosen and the procedures used to optimize them. The choice of a different path through the space of variables could lead to a very different outcome. The successes of the method, however, show that finding useful results is much easier than finding all possible outcomes.

Conclusions

Polymorph control has moved on over the last decade from being a mysterious process in which seeding was a key technique, to one for which there is a large armory of methods that may be used to attack this central problem in pharmaceutical science. It is clear that in the last decade big steps have been made towards the understanding and control of polymorphism. This review shows

how the interplay between molecular recognition, thermodynamics and kinetics can be carefully controlled to obtain the desired crystal forms.

Our understanding of molecular recognition, molecular assemblies, nucleation and crystal growth coupled with the development of new analytical techniques and increasingly powerful prediction tools are leading to a new generation of tailored compounds with fine-tuned properties. Some of the techniques described in this review have only been developed and, therefore, only a few applications have been yet reported. This is a very exciting and fast moving field of research.

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References

1 Erk, P. *et al.* (2004) The innovative momentum of crystal engineering. *Cryst. Eng. Comm.* 6, 474–483
2 Chemburkar, S.R. *et al.* (2000) Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development. *Org. Proc. Res. Dev.* 4, 413–417
3 Brittain, H.G. and Fiese, E.F. (1999) Effect of pharmaceutical processing on drug polymorphs and solvates. In *Polymorphism in Pharmaceutical Solids* (Brittain, H.G., ed.), pp. 331–362, Marcel Dekker, Inc.
4 Dunitz, J.D. and Bernstein, J. (1995) Disappearing polymorphs. *Acc. Chem. Res.* 28, 193–200
5 Bugay, D.E. (2001) Characterization of the solid-state: spectroscopic techniques. *Adv. Drug Deliv. Rev.* 48, 43–65
6 Bernstein, J. (2005) Cultivating crystal forms. *Chem. Commun.* 5007–5012
7 Bernstein, J. (2002) *Polymorphism in Molecular Crystals*. Clarendon Press p. 23
8 Anderton, C. (2005) A valuable technique for polymorph screening. *Eur. Pharm. Rev.* 68–74
9 Dunitz, J.D. (2003) Crystal and co-crystal. A second opinion. *Cryst.Eng. Comm.* 5, 506
10 Beckmann, W. (2000) Seeding the desired polymorph: Background, possibilities, limitations, and case studies. *Org. Proc. Res. Dev.* 4, 372–383
11 Llinàs, A. *et al.* (2007) A new method for the reproducible generation of polymorphs: two forms of sulindac with very different solubilities. *J. Appl. Crystallogr.* 40, 379–381
12 Al-Zoubi, N. and Malamataris, S. (2003) Effects of initial concentration and seeding procedure on crystallisation of orthorhombic paracetamol from ethanolic solution. *Int. J. Pharm.* 260, 123–135
13 Tamura, R. *et al.* (2006) Induction and inhibition of preferential enrichment by controlling the mode of the polymorphic transition with seed crystals. *Chem. Eur. J.* 12, 3515–3527
14 Miura, H. *et al.* (2003) Crystallization of a desired metastable polymorph by pseudoseeding, crystal structure solution from its powder X-ray diffraction data, and confirmation of polymorphic transition. *Cryst. Growth Des.* 3, 959–965
15 Kline, B.J. *et al.* (2006) Polymorph and particle size control of PPAR compounds PF00287586 and AG035029. *Org. Proc. Res. Dev.* 10, 203–211
16 Li, T. *et al.* (2001) Influence of tailor-made additives on etching patterns of acetaminophen single crystals. *Pharm. Res.* 18, 398–402
17 Weissbuch, I. *et al.* (2001) Crystal morphology control with tailor-made additives; a stereochemical approach. In *Advances in Crystal Growth Research* (Sato, K., ed.), pp. 381–400, Elsevier
18 Rubin-Preminger, J.M. and Bernstein, J. (2005) 3-Aminobenzenesulfonic acid: a disappearing polymorph. *Cryst. Growth Des.* 5, 1343–1349
19 Kwon, O.P. *et al.* (2006) Morphology and polymorphism control of organic polyene crystals by tailor-made auxiliaries. *Cryst. Growth Des.* 6, 2327–2332
20 Kellogg, R.M. *et al.* (2007) Dutch resolution of racemates and the roles of solid solution formation and nucleation inhibition. *Top. Curr. Chem.* 269, 159–197 (Novel Optical Resolution Technologies)
21 Davey, R.J. *et al.* (1997) Polymorphism in molecular crystals: Stabilization of a metastable form by conformational mimicry. *J. Am. Chem. Soc.* 119, 1767–1772
22 Stewart, J.J.P. (1990) MOPAC: a semiempirical molecular orbital program. *J. Comput. Aided Mol. Des.* 4, 1–105
23 Deij, M.A. *et al.* (2007) Toward rational design of tailor-made additives using growth site statistics. *Cryst. Growth Des.* 7, 778–786
24 Mohan, R. *et al.* (2001) Effect of additives on the transformation behavior of L-phenylalanine in aqueous solution. *Ind. Eng. Chem. Res.* 40, 6111–6117
25 Masui, Y. *et al.* (2003) Control of crystal habit and size of cefmatilen hydrochloride hydrate with a habit modifier. *Org. Proc. Res. Dev.* 7, 334–338
26 Beckmann, W. *et al.* (2001) Crystallisation of the stable polymorph of hydroxytriendione: seeding process and effects of purity. *Org. Proc. Res. Dev.* 5, 387–392
27 Blagden, N. *et al.* (1998) Disappearing polymorphs and the role of reaction by-products: the case of sulphathiazole. *Int. J. Pharm.* 172, 169–177
28 Weissbuch, I. *et al.* (2006) Self-poisoning at {0 1 1} faces of a-resorcinol crystals may explain its unidirectional growth in the vapor phase: a molecular modeling study. *Cryst. Growth Des.* 6, 625–628
29 He, X. *et al.* (2001) Stabilization of a metastable polymorph of 4-methyl-2-nitroacetanilide by isomorphic additives. *Cryst. Growth Des.* 1, 305–312
30 Davey, R.J. *et al.* (1999) Stabilizing a solid-solid interface with a molecular-scale adhesive. *Nature* 402, 797–799
31 Mughal, R.K. *et al.* (2007) Application of crystallization inhibitors to chiral separations. 1. Design of additives to discriminate between the racemic compound and the pure enantiomer of mandelic acid. *Cryst. Growth Des.* 7 (2), 218–224
32 Mughal, R.K. *et al.* (2007) Application of crystallization inhibitors to chiral separations. 2. Enhancing the chiral purity of mandelic acid by crystallization. *Cryst. Growth Des.* 7, 225–228
33 Poornachary, S.K. *et al.* (2007) Molecular speciation controlling stereoselectivity of additives: Impact on the habit modification in alpha-glycine crystals. *Cryst. Growth Des.* 7, 254–261
34 Lieberman, H.F. *et al.* (1998) Molecular configuration at the solid-solid interface: twinning in saccharin crystals. *J. Am. Chem. Soc.* 120, 686–691
35 Blagden, N. *et al.* (2005) Ordered aggregation of benzamide crystals induced using a 'motif capper' additive. *Cryst. Growth Des.* 5, 467–471
36 Davey, R.J. *et al.* (2002) Crystal engineering – nucleation, the key step. *Cryst. Eng. Commun.* 257–264
37 Weissbuch, I. *et al.* (2003) Toward stereochentical control, monitoring, and understanding of crystal nucleation. *Cryst. Growth Des.* 3, 125–150
38 Mitchell, C.A. *et al.* (2001) Selective nucleation and discovery of organic polymorphs through epitaxy with single crystal substrates. *J. Am. Chem. Soc.* 123, 10830–10839
39 Stoica, C. *et al.* (2005) Heterogeneous 2D nucleation of the stable polymorphic form on the metastable form. *J. Cryst. Growth* 275, e1727–e1731
40 Rodriguez-Hornedo, N. *et al.* (1992) Phase transition and heterogeneous/epitaxial nucleation of hydrated and anhydrous theophylline crystals. *Int. J. Pharm.* 85, 149–162
41 Stoica, C. *et al.* (2005) Epitaxial 2D nucleation of metastable polymorphs: A 2D version of Ostwald's rule of stages. *Cryst. Growth Des.* 5, 975–981
42 Love, J.C. *et al.* (2005) Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chem. Rev.* 105, 1103–1169
43 Kuzmenko, I. *et al.* (2001) Design and characterization at crystalline thin film architectures at the air–liquid interface: simplicity to complexity. *Chem. Rev.* 101, 1659–1696
44 Dressler, D.H. and Mastai, Y. (2007) Controlling polymorphism by crystallization on self-assembled multilayers. *Cryst. Growth Des.* 7, 847–850
45 Price, C.P. *et al.* (2005) Crystalline polymorph selection and discovery with polymer heteronuclei. *J. Am. Chem. Soc.* 127, 5512–5517
46 Lang, M.D. *et al.* (2002) The use of polymer heteronuclei for crystalline polymorph selection. *J. Am. Chem. Soc.* 124, 14834–14835
47 Grzesiak, A.L. *et al.* (2006) Polymer-induced heteronucleation for the discovery of new extended solids. *Angew. Chem. Int. Ed.* 45, 2553–2556
48 Grzesiak, A.L. and Matzger, A.J. (2007) Selection and discovery of polymorphs of platinum complexes facilitated by polymer-induced heteronucleation. *Inorg. Chem.* 46, 453–457
49 Blagden, N. (2001) Crystal engineering of polymorph appearance: the case of sulphathiazole. *Powder Technol.* 121, 46–52
50 Blagden, N. and Davey, R.J. (2003) Polymorph selection: challenges for the future? *Cryst. Growth Des.* 3, 873–885

51 Blagden, N. *et al.* (2001) Can crystal structure prediction be used as part of an integrated strategy for ensuring maximum diversity of isolated crystal forms? The case of 2-amino-4-nitrophenol. *PCCP* 3, 3819–3825
52 Stoica, C. *et al.* (2004) Understanding the effect of a solvent on the crystal habit. *Cryst. Growth Des.* 4, 765–768
53 Weissbuch, I. *et al.* (2005) Solvent effect on crystal polymorphism: why addition of methanol or ethanol to aqueous solutions induces the precipitation of the least stable beta form of glycine. *Angew. Chem. Int. Ed. Engl.* 44, 3226–3229
54 Kitamura, M. *et al.* (2006) Solvent effect on polymorphism in crystallization of BPT propyl ester. *Cryst. Growth Des.* 6, 1945–1950
55 Trifkovic, M. *et al.* (2007) Polymorphic generation through solvent selection: ranitidine hydrochloride. *Org. Proc. Res. Dev.* 11, 138–143
56 Rafilovich, M. and Bernstein, J. (2006) Serendipity and four polymorphic structures of benzidine $C_{12}H_{12}N_2$. *J. Am. Chem. Soc.* 128, 12185–12191
57 Day, G.M. *et al.* (2006) Investigating the latent polymorphism of maleic acid. *Chem. Commun.* 54–56
58 Vishweshwar, P. *et al.* (2005) The predictably elusive form II of aspirin. *J. Am. Chem. Soc.* 127, 16802–16803
59 Bond, A.D. *et al.* (2007) On the polymorphism of aspirin: crystalline aspirin as intergrowths of two 'polymorphic' domains. *Angew. Chem. Int. Ed.* 46, 618–622
60 Bis, A.J. *et al.* (2006) Concomitant and conformational polymorphism, conformational isomorphism, and phase relationships in 4-cyanopyridine·4,4′-biphenol cocrystals. *Cryst. Growth Des.* 6, 1048–1053
61 Remenar, J.F. *et al.* (2003) Crystal engineering of novel cocrystals of a triazole drug with 1,4-dicarboxylic acids. *J. Am. Chem. Soc.* 125, 8456–8457
62 Walsh, R.D.B. *et al.* (2003) Crystal engineering of the composition of pharmaceutical phases. *Chem. Commun.* 2, 186–187
63 Seddon, K.R. (2004) Pseudopolymorph: a polemic. *Cryst. Growth Des.* 4, 1087
64 Desiraju, G.R. (2004) Counterpoint: what's in the name? *Cryst. Growth Des.* 4, 1089–1090
65 Bernstein, J. (2005) ... And another comment on pseudopolymorphism. *Cryst. Growth Des.* 5, 1661–1662
66 Nangia, A. (2005) Pseudopolymorph: retain this widely accepted term. *Cryst. Growth Des.* 6, 2–4
67 Stahly, G.P. (2007) Diversity in single- and multiple-component crystals. The search for and prevalence of polymorphs and cocrystals. *Cryst. Growth Des.* 7, 1007–1026
68 Vishweshwar, P. *et al.* (2006) Pharmaceutical co-crystals. *J. Pharm. Sci.* 95, 499–516
69 Fages, J. *et al.* (2004) Particle generation for pharmaceutical applications using supercritical fluid technology. *Powder Technol.* 141, 219–226
70 Krukonis, V. (1984) Supercritical fluid nucleation of difficult-to-comminute solids. In *Proceedings of the Annual Meeting AIChE,* paper 140 f, T-214
71 Kordikowski, A. *et al.* (2001) Polymorph control of sulfathiazole in supercritical CO_2. *Pharm. Res.* 18, 682–688
72 Hooton, J.C. *et al.* (2006) A comparison of morphology and surface energy characteristics of sulfathiazole polymorphs based upon single particle studies. *Eur. J. Pharm. Sci.* 28, 315–324
73 Edwards, A.D. *et al.* (2001) Crystallization of pure anhydrous polymorphs of carbamazepine by solution enhanced dispersion with supercritical fluids (SEDS). *J. Pharm. Sci.* 90, 1115–1124
74 Beach, S. *et al.* (1999) Control of the physical form of salmeterol xinafoate. *Org. Proc. Res. Dev.* 3, 370–376
75 Tong, H.H. *et al.* (2001) Characterization of two polymorphs of salmeterol xinafoate crystallized from supercritical fluids. *Pharm. Res.* 18, 852–858
76 Tong, H.H. *et al.* (2003) Thermal analysis of trace levels of polymorphic impurity in salmeterol xinafoate samples. *Pharm. Res.* 20, 1423–1429
77 Velaga, S.P. *et al.* (2002) Preparation and characterisation of hydrocortisone particles using a supercritical fluids extraction process. *Int. J. Pharm.* 231, 155–166
78 Velaga, S.P. *et al.* (2002) Supercritical fluids crystallization of budesonide and flunisolide. *Pharm. Res.* 19, 1564–1571
79 Rehman, M. *et al.* (2004) Optimisation of powders for pulmonary delivery using supercritical fluid technology. *Eur. J. Pharm. Sci.* 22, 1–17
80 He, W.Z. *et al.* (2006) Supercritical antisolvent micronization of natural carotene by the SEDS process through prefilming atomization. *Ind. Eng. Chem. Res.* 45, 2108–2115
81 Toropainen, T. *et al.* (2006) Preparation of budesonide/gamma-cyclodextrin complexes in supercritical fluids with a novel SEDS method. *J. Pharm. Sci.* 95, 2235–2245
82 Raveendran, P. *et al.* (2005) Crystallization and processing of carbohydrates using carbon dioxide. *Green Chem.* 7, 129–131
83 Shinozaki, H. *et al.* (2006) Micronization and polymorphic conversion of tolbutamide and barbital by rapid expansion of supercritical solutions. *Drug. Dev. Ind. Pharm.* 32, 877–891
84 Gosselin, P.M. *et al.* (2003) Polymorphic properties of micronized carbamazepine produced by RESS. *Int. J. Pharm.* 252, 225–233
85 Bouchard, A. *et al.* (2007) Selective production of polymorphs and pseudomorphs using supercritical fluid crystallization from aqueous solutions. *Cryst. Growth Des.* 7, 1432–1440
86 Sane, A. and Thies, M.C. (2007) Effect of material properties and processing conditions on RESS of poly(L-lactide). *J. Supercrit. Fluids* 40, 134–143
87 Chiou, A.H. *et al.* (2006) Micronization and microencapsulation of felodipine by supercritical carbon dioxide. *J. Microencapsul.* 23, 265–276
88 Moribe, K. *et al.* (2005) Micronization of phenylbutazone by rapid expansion of supercritical CO_2 solution. *Chem. Pharm. Bull.* 53, 1025–1028
89 Vemavarapu, C. *et al.* (2002) Crystal doping aided by rapid expansion of supercritical solutions. *AAPS Pharm. Sci. Technol.* 3, E29
90 Shekunov, B.Y. *et al.* (2003) Formation of composite crystals by precipitation in supercritical CO_2. *Cryst. Growth Des.* 3, 603–610
91 Pasquali, I. *et al.* (2006) Solid-state chemistry and particle engineering with supercritical fluids in pharmaceutics. *Eur. J. Pharm. Sci.* 27, 299–310
92 Curcio, E. *et al.* (2003) A new membrane-based crystallization technique: tests on lysozyme. *J. Cryst. Growth* 247, 166–176
93 Curcio, E. *et al.* (2006) Influence of the structural properties of poly(vinylidene fluoride) membranes on the heterogeneous nucleation rate of protein crystals. *J. Phys. Chem. B* 110, 12438–12445
94 Drioli, E. *et al.* (2004) Integrated system for recovery of $CaCO_3$, NaCl and $MgSO_4 \cdot 7H_2O$ from nanofiltration retentate. *J. Membr. Sci.* 239, 27–38
95 Di Profio, G. *et al.* (2005) Trypsin crystallization by membrane-based techniques. *J. Struct. Biol.* 150, 41–49
96 Simone, S. *et al.* (2006) Polymeric hydrophobic membranes as a tool to control polymorphism and protein-ligand interactions. *J. Membr. Sci.* 283, 123–132
97 Di Profio, G. *et al.* (2007) Selective Glycine Polymorph Crystallization by Using Microporous Membranes. *Cryst. Growth Des.* 7, 526–530
98 Lee, A.Y. *et al.* (2005) Crystallization on confined engineered surfaces: a method to control crystal size and generate different polymorphs. *J. Am. Chem. Soc.* 127, 14982–14983
99 Lee, A.Y. *et al.* (2006) Factors affecting the polymorphic outcome of glycine crystals constrained on patterned substrates. *Chem. Eng. Tech.* 29, 281–285
100 Llinàs, A. *et al.* (2007) Diclofenac solubility: Independent determination of the intrinsic solubility of three crystal forms. *J. Med. Chem.* 50, 979–983
101 Stuart, M. and Box, K. (2005) Chasing equilibrium: measuring the intrinsic solubility of weak acids and bases. *Anal. Chem.* 77, 983–990
102 Threlfall, T. (2000) Crystallisation of polymorphs: thermodynamic insight into the role of solvent. *Org. Proc. Res. Dev.* 4, 384–390
103 Fioritto, A.F. *et al.* (2007) Solubility measurement of polymorphic compounds via the pH-metric titration technique. *Int. J. Pharm.* 330, 105–113
104 Hilden, J.L. *et al.* (2003) Capillary precipitation of a highly polymorphic organic compound. *Cryst. Growth Des.* 3, 921–926
105 Yu, L. (2002) Color changes caused by conformational polymorphism: optical-crystallography, single-crystal spectroscopy, and computational chemistry. *J. Phys. Chem. A* 106, 544–550
106 Chyall, L.J. *et al.* (2002) Polymorph generation in capillary spaces: the preparation and structural analysis of a metastable polymorph of nabumetone. *Cryst. Growth Des.* 2, 505–510
107 Ha, J.-M. *et al.* (2004) Polymorph selectivity under nanoscopic confinement. *J. Am. Chem. Soc.* 126, 3382–3383
108 Deegan, R.D. *et al.* (1997) Capillary flow as the cause of ring stains from dried liquid drops. *Nat. Biotechnol.* 389, 827–829
109 Capes, J.S. and Cameron, R.E. (2007) Contact line crystallization to obtain metastable polymorphs. *Cryst. Growth Des.* 7, 108–112
110 Sirota, N.N. (1996) Crystallization processes. In *ACS Monograph*. Plenum Press
111 Bucar, D.-K. and MacGillivray, L.R. (2007) Preparation and reactivity of nanocrystalline co-crystals formed via sonocrystallization. *J. Am. Chem. Soc.* 129, 32–33
112 Li, H. *et al.* (2003) Rapid sonocrystallization in the salting-out process. *J. Cryst. Growth* 247, 192–198
113 Bund, R.K. and Pandit, A.B. (2007) Sonocrystallization: effect on lactose recovery and crystal habit. *Ultrason. Sonochem.* 14, 143–152
114 Luque de Castro, M.D. and Priego-Capote, F. (2007) Ultrasound-assisted crystallization (sonocrystallization). *Ultrason. Sonochem.* 14, 717–724
115 Chow, R. *et al.* (2004) The microscopic visualisation of the sonocrystallisation of ice using a novel ultrasonic cold stage. *Ultrason. Sonochem.* 11, 245–250
116 Kim, S. *et al.* (2003) Crystallization process development of an active pharmaceutical ingredient and particle engineering via the use of ultrasonics and temperature cycling. *Org. Proc. Res. Dev.* 7, 997–1001

117 Gracin, S. *et al.* (2005) Influence of ultrasound on the nucleation of polymorphs of *p*-aminobenzoic acid. *Cryst. Growth Des.* 5, 1787–1794

118 Louhi-Kultanen, M. *et al.* (2006) Crystallization of glycine with ultrasound. *Int. J. Pharm.* 320, 23–29

119 McCausland, L.J. *et al.* (2001) Use the power of sonocrystallization for improved properties. *Chem. Eng. Prog.* 97, 56–61

120 Garetz, B.A. *et al.* (1996) Nonphotochemical, polarization-dependent, laser-induced nucleation in supersaturated aqueous urea solutions. *Phys. Rev. Lett.* 77, 3475–3476

121 Sun, X. *et al.* (2006) Supersaturation and polarization dependence of polymorph control in the nonphotochemical laser-induced nucleation (NPLIN) of aqueous glycine solutions. *Cryst. Growth Des.* 6, 684–689

122 Tuschel, D. and Bangalore, A.S. (2006) Chemimage Corporation, USA. Control and monitoring of non-resonant radiation-induced nucleation, crystallization, and polymorph formation in, for example, nabumetone, WO2006052464, Available on: http://v3.espacenet.com/textdoc?DB=EPODOC&IDX=WO2006052464&F=1

123 Blundell, T.L. *et al.* (2002) High-throughput crystallography for lead discovery in drug design. *Nat. Rev. Drug Discov.* 1, 45–54

124 Peterson, M.L. *et al.* (2002) Iterative high-throughput polymorphism studies on acetaminophen and an experimentally derived structure for form III. *J. Am. Chem. Soc.* 124, 10958–10959

125 Stewart, L. *et al.* (2002) High-throughput crystallization and structure determination in drug discovery. *Drug. Discov. Today* 7, 187–196

126 Storey, R.A. *et al.* (2003) Integration of high throughput screening methodologies and manual processes for solid form selection. *Am. Pharm. Rev.* 6, 104–105 pp. 100, 102

127 Morissette, S.L. *et al.* (2004) High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. *Adv. Drug. Deliv. Rev.* 56, 275–300

128 Rupp, B. (2005) Automated high throughput drug target crystallography. *Am. Pharm. Rev.* 8, 127–128 130-131

129 Segelke, B.W. *et al.* (2006) Publ., United States Patent Application Relational database management system for automated random crystallization screening, US 2006228756, Available on: http://appft1.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PG01&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&s1='20060228756'.PGNR.&OS=DN/20060228756&RS=DN/20060228756

130 Hilfiker, R. *et al.* (2006) Approaches to polymorphism screening. *Polymorphism* 287–308

131 Chayen, N.E. (2007) Optimization techniques for automation and high throughput. In *Methods in Molecular Biology* (Vol. 363), pp. 175–190, Clifton, NJ

132 Yu, L.X. *et al.* (2003) Scientific considerations of pharmaceutical solid polymorphism in abbreviated new drug applications. *Pharm. Res.* 20, 531–536

133 Gabrielson, J. *et al.* (2002) Multivariate methods in pharmaceutical applications. *J. Chemometrics* 16, 141–160

134 Morissette, S.L. *et al.* (2003) Elucidation of crystal form diversity of the HIV protease inhibitor ritonavir by high-throughput crystallization. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2180–2184

135 Cabeza, A.J.C. *et al.* (2007) Solvent inclusion in form II carbamazepine. *Chem. Commun. (Cambridge, UK)* 1600–1602

136 McCrone, W.C. (1965) In *Physics and Chemistry of the Organic Solid State*, (Vol. II) (Fox, D. *et al.* eds), pp. 725–767, Interscience Publishers

137 A new polymorph of aspirin has been reported [58] but subsequent work suggests it is not a true polymorph [59]. Is Form II a new polymorph of aspirin? According to Bond, Boese and Desiraju '... aspirin crystals display both arrangements within a single crystal, should aspirin really be described as polymorphic? What is certain is that the PZ description of their crystal as a polymorph distinct from Form I is not justifiable'

138 Ishida, T. *et al.* (1990) Structure of ranitidine hydrochloride. *Acta Cryst.* C46, 1893–1896

139 Hempel, A. *et al.* (2000) Ranitidine hydrochloride, a polymorphic crystal form. *Acta Cryst.* C56, 1048–1049