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High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids

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Abstract

The concepts of high-throughput (HT) screening and combinatorial synthesis have been integrated into the pharmaceutical discovery process, but are not yet commonplace in the pharmaceutical development arena. Emerging strategies to speed pharmaceutical development and capture solid form diversity of pharmaceutical substances have resulted in the emergence of HT crystallization technologies. The primary type of diversity often refers to polymorphs, which are different crystal forms of the same chemical composition. However, diverse salt forms, co-crystals, hydrates and solvates are also amenable to study in HT crystallization systems. The impact of form diversity encompasses issues of stability and bioavailability, as well as development considerations such as process definition, formulation design, patent protection and regulatory control. This review highlights the opportunities and challenges of HT crystallization technologies as they apply to pharmaceutical research and development.
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Keywords: High-throughput; Crystallization; Polymorph; Solvate; Salt; Co-crystal

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1. Introduction

Active pharmaceutical ingredients (APIs) are frequently delivered to the patient in the solid-state as part of an approved dosage form (e.g., tablets, capsules, etc.). Solids provide a convenient, compact and generally stable format to store an API or a drug product. Understanding and controlling the solid-state chemistry of APIs, both as pure drug substances and in formulated products, is therefore an important aspect of the drug development process. APIs can exist in a variety of distinct solid forms, including polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids. Each form displays unique physicochemical properties that can profoundly influence the bioavailability, manufacturability purification, stability and other performance characteristics of the drug [1]. Hence, it is critical to understand the relationship between the particular solid form of a compound and its functional properties. Discovery and characterization of the diversity of solid forms of a drug substance provide options from which to select a form that exhibits the appropriate balance of critical properties for development into the drug product. Importantly, the desired properties may vary with each mode of delivery (i.e., oral, pulmonary, parenteral, transdermal, etc.), such that the solid form may differ for each optimized dosage form. Given these options, the choice and design of pharmaceutical solid forms can be critically important to successful drug development.

Solid form discovery and design depends on the nature of the molecule of interest and type of physical property challenges faced in its development. The preferred solid form is generally the thermodynamically most stable crystalline form of the compound [1,2]. However, the stable crystal form of the parent compound may exhibit inadequate solubility or dissolution rate resulting in poor oral absorption, particularly for water-insoluble compounds. In this case, alternative solid forms may be investigated. For ionizable compounds, preparation of salt forms using pharmaceutically acceptable acids and bases is a common strategy to improve bioavailability [1,3,4]. Like the parent compound, pharmaceutical salts may exist in several polymorphic, solvated and/or hydrated forms.

Most APIs and their salts are purified and isolated by crystallization from an appropriate solvent during the final step in the synthetic process. A large number of factors can influence crystal nucleation and growth during this process, including the composition of the crystallization medium and the process(es) used to generate supersaturation and promote crystallization [1,5–13]. The most notable variables of composition and processing are summarized in Table 1. Solid form screening is used to understand the effects that these variables have on the polymorphic outcome of a crystallization experiment, so that a robust process can be identified to produce the desired crystal form. Traditionally, the study of solid form diversity of active compounds has relied on the use of a variety of common process methods for generation of new forms, coupled with modern characterization methods for analysis of the solids produced [2,14]. Most often, however, a combination of solvent recrystallization (cooling or evaporative, as well as slurry conversion) and thermal analysis (e.g., hot stage microscopy, differential scanning calorimetry) are employed for initial form screening. Such methods are inherently slow and only allow exploration of a small fraction of the composition and process space that can contribute to form diversity. Before suggesting a form for development, scientists may have carried out only a few dozen crystallization experiments and possibly prepared a handful of different salts of a compound. The main reasons for the limited number of experiments are the constraints on availability of compound and scientists' analytical capacity in a given time frame, and they are therefore often forced to make form selection decisions on incomplete data. Accordingly, it is not surprising that unexpected and undesired outcomes can, and do, occur later on in development.

Despite more than a century of research [15], the fundamental mechanisms and molecular properties that drive crystal form diversity, specifically the nucleation of polymorphic forms, are not well under-

Table 1
Crystallization composition and processing variables [1,2,8]

Composition type		Process variables[a]				
Polymorph/ solvates	Salts/ co-crystals	Thermal	Anti-solvent	Evaporation	Slurry conversion	Other variables
▪ Solvent/ solvent combinations	▪ Counter-ion type	▪ Heating rate	▪ Anti-solvent type	▪ Rate of evaporation	▪ Solvent type	▪ Mixing rate
▪ Degree of supersaturation	▪ Acid/base ratio	▪ Cooling rate	▪ Rate of anti-solvent addition	▪ Evaporation time	▪ Incubation temperature	▪ Impeller design
▪ Additive type	▪ Solvent/ solvent combinations	▪ Maximum temperature	▪ Temperature of anti-solvent addition	▪ Carrier gas	▪ Incubation time	▪ Crystallization vessel design (including capillaries, etc.)
▪ Additive concentration	▪ Degree of super-saturation ▪ Additive type and concentration ▪ pH ▪ Ionic strength	▪ Incubation temperature(s) ▪ Incubation time	▪ Time of anti-solvent addition	▪ Surface-volume ratio	▪ Thermal cycling and gradients	

[a] Applicable to all types of screens.

stood [13,16]. As a result, predictive methods of assessing polymorphic behavior of pharmaceutical compounds by ab initio calculations remain a formidable challenge. Even in cases where the existence of a crystalline form is predicted, the stability relative to other crystalline packing arrangements has been difficult to estimate with accuracy [17]. Moreover, the prediction of packing structures for multicomponent (e.g., solvates, hydrates, co-crystals) or ionic systems is not yet possible [17]. Due to these limitations, solid form discovery remains an experimental exercise, where manual screening methods are employed to explore form diversity of a compound.

Control over solid form throughout the drug development process is of paramount importance. Reliable preparation and preservation of the desired form of the drug substance must be demonstrated, and has become increasingly scrutinized by regulatory agencies as more sensitive and quantitative solid-state analytical methods have become available [18]. Many strategies to influence and control the crystallization process to produce the solid form of interest have been reported. Some examples include stereochemical control using tailor-made auxiliaries [19–21], targeted solvent recrystallization [22–24], and templating using a variety of surfaces (e.g., organic single crystal substrates [25], surfaces of metastable crystal faces [25,26], inorganic crystal surfaces [27] and polymeric materials [28]). Recent studies have also begun to uncover the role of reaction byproducts and other impurities in determining polymorphic outcome and crystal properties [29–32], and in fact, it has been shown that in some cases such species can stabilize metastable crystal forms [33,34]. In addition, new processing methods continue to be developed to improve discovery and characterization of new forms, including precipitation by supercritical fluid [35,36], laser induced nucleation [37–39] and capillary crystallization [40–42]. However, there remains a lack of fundamental understanding of the nucleation process and the specific factors that contribute to crystallization of diverse forms of a compound [13,21,23]. In order to fully control the crystallization process, the link between the physical or chemical processes that influence nucleation and crystal growth needs to be better established. It is in this area that new experimental methodologies have the potential to enable development of this knowledge base.

There is reason to believe that the already complicated landscape of pharmaceutical solid forms will become even more complex in the future. It is now increasingly appreciated that hydrogen bonded co-crystal structures between active agents and molecules other than water or solvent can be prepared. For example, co-crystals of aspirin, *rac*-ibuprofen and

rac-flurbiprofen have been prepared by disrupting the carboxylic acid dimers using 4,4′ -bipyridine [43]. These structures are formally molecular compounds (or co-crystals) but do not involve formation of covalent bonds or charge transfer from or to the active substance. Recent demonstrations of these principles with drug compounds have been published [43–45].

Exploration of a given compound's polymorphs, hydrates, solvates, salts, co-crystals and combinations of all of these appears intractable by conventional experimental methods, and as the number of potential methods for exploring and controlling crystal form diversity continue to expand, existing strategies will become increasingly inadequate. In an effort to understand form diversity in a more comprehensive manner, high-throughput (HT) crystallization systems have recently been developed. This methodology uses a combinatorial approach to solid form generation, where large arrays of conditions and compositions are processed in parallel. Experiments are performed at small scale to reduce the material demand and to afford the largest number of conditions possible. The large number of crystallization trials performed in these experiments reflects the reality that nucleation rate has an extremely non-linear dependence on the experimental conditions, and as such, the probability of a chance occurrence of a particular form is increased by a HT approach. Supersaturation (solubility) and induction time of the various possible solid forms are independently controlled by these conditions, resulting in highly non-linear time dependence of crystallization. In addition, the combinatorial approach permits exploration of a chemical continuum, where use of many solvent mixtures may allow one to assess what underlying physical or chemical processes are required to produce a particular solid form. Once a variety of conditions that can be used to produce a given crystal form on the microscale are identified in the HT screen, scale-up studies are typically conducted to optimize the process for laboratory scale production.

In this review, the development and application of novel HT crystallization technologies for exploration of solid form diversity are discussed. The operational features of a fully integrated, automated HT crystallization system are presented, highlighting the design requirements for hardware and software components, as well as general specifications for consumables. Case studies are used to illustrate the benefits and capabilities of the approach, including salt selection in early lead optimization (ELO) and pre-clinical development, polymorph and solvate screening in highly polymorphic systems, comprehensive discovery of crystal forms to reduce the risk of late displays of polymorphism, comparison of experimental and predictive methods of solid form discovery, and engineering of co-crystals. The need for post-screening characterization of crystal forms to enable ranking and selection of the most suitable form for development is briefly reviewed. Finally, the implications of HT crystallization technologies on the future of solid form screening processes, intellectual property protection and regulatory compliance are discussed.

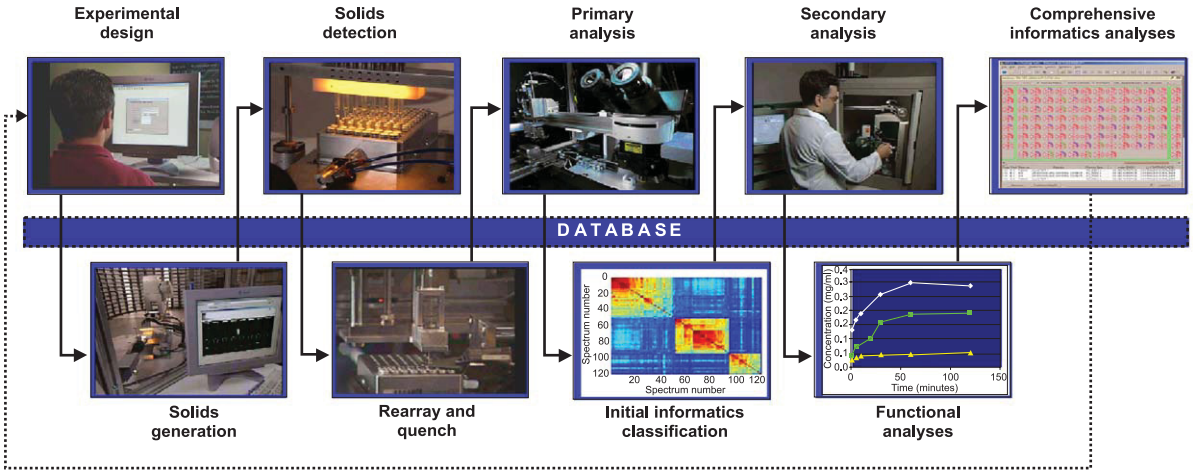
2. Development of high-throughput crystallization technologies

HT crystallization systems have been developed to more rapidly and comprehensively explore the multiparameter space that contributes to solid form diversity [40,46–51]. In its simplest description, HT crystallization can be broken down into three key experimental steps: *design* of experiment (DOE), *execution* of experimental protocols and *analysis* of data. Systems designed to carry out these experiments generally consist of both hardware and software components that drive and track experimentation, and permit data storage, retrieval and analysis. Such systems should be designed to be flexible and scalable to ensure that a variety of experimental procedures can be carried out either serially or concurrently. Thus, the system can be employed at various stages of drug development, where differences exist in the quality and quantity of compound available. While it is highly desirable to have the ability to mine and model experimental data, and to use the subsequent knowledge to guide further experiments, not all HT crystallization systems are equipped with these features. In Section 3, the hardware and software considerations for design and development of a fully integrated, informatics-driven HT crystallization system are described.

While the concepts of HT screening are widely applied in the pharmaceutical industry, most notably in the drug discovery arena [52], the application of

HT approaches to drug development, in particular solid form screening, are just beginning to be realized. These latter approaches, however, are more akin to HT experimentation than HT screening. Hence, several important distinctions, which reflect on the design of HT experimental systems, need to be made. First, the goal of HT screening is to get a small number of successful outcomes, which are then passed on to the next stage of development. Little effort is typically made to learn why certain outcomes were positive and why others were negative. In contrast, HT experimentation, such as HT crystallization, is carried out with the goal of having each point in the experiment produce multiple types of data that can be interpreted, and the interpretation used to guide the experimental process to a successful conclusion. Second, unlike traditional HT screening assays where experiments are generally conducted under constant experimental conditions, HT crystallization experiments for solid form discovery are best conducted using a variety of process methods, each having varying experimental conditions (e.g., temperature variations as a function of time) over the course of the experiment. These additional process variables permit maximal diversity in the experimental space, increasing the likelihood that comprehensive coverage will be achieved. Finally, there is a distinction to be made in terms of relative "hit rates". In both HT screening and HT crystallization, a "hit" can be thought of as a set of conditions that gives rise to a desired result. In HT screening, the desired result is typically an activity, or potency, that exceeds a predefined threshold. In HT crystallization, a hit is defined as the formation of a solid. The typical observed hit rate of HT screening is on the order of 0.1% of the total number of samples analyzed. In contrast, HT crystallization experiments can yield hit rates ranging from tens of percents to nearly 100%, depending on the type of experiment and the process mode(s) used. For example, while only a handful of compounds from a selection of thousands may exhibit the required potency, 10–50% of crystallization trials may yield solids. In fact, the range of wells that yield solids is very wide, depending on process mode and experimental time scale, as will be discussed in subsequent sections. The impact of these differences is manifested in the design and operational requirements of HT experimentation systems.

A fully integrated HT crystallization system consists of a number of components, including experimental design and execution software, robotic dispensing and handling hardware, automated high-speed micro-analytical tools, end-to-end sample tracking and integrated cheminformatics analysis software for data visualization, modeling and mining. A schematic overview detailing the workflow of such a system is depicted in Scheme 1 [53]. These features are supported by a comprehensive informatics foun-



Scheme 1. A schematic illustration of the workflow of a fully integrated HT crystallization system [53].

dation that is used to handle the large quantities of data generated. Specifically, informatics tools are used to design statistically relevant and diverse experiments, drive the automation hardware to perform the specified operations, and provide an analytical function to analyze, compare and sort the results of experiments. An important feature of these systems is the ability to mine and model experimental data and use the knowledge generated to guide further experiments. These functions are supported by use of a relational database that provides a mechanism of communication between system components.

When designing a HT crystallization experiment, or set of experiments, a large variety of parameters of composition and process are involved. Experimental designs must be aimed at covering a large multifactorial parameter space, with the goal of determining which experimental factors affect the desired outcome. In practice, it is desirable to place constraints on the experimental space, making common statistical design methods such as full or partial factorial designs inappropriate or impractical. For example, hardware limitations, including minimum and maximum dispense volumes or masses and accessible temperature ranges, as well as constraints related to chemical compatibility (i.e., reactivity of components, miscibility, etc.) or toxicity limits of components (if appropriate), need to be considered. Thus, alternative DOE methods that can accommodate such constraints are required. D-optimal design [54,55] is an example of a DOE algorithm that can take a set of constraints, such as the ones described above, in combination with a target analytical model and determine the optimal set of experimental points to test. Another commonly used DOE algorithm is diversity generation, with which the experimentalist selects a set of pertinent chemical properties and uses the algorithm to evenly spread experimental points over the chosen property space. In addition, some systems utilize a solubility calculator tool to estimate the solubility of the API in the given solvent/additive mixture. The calculated information is then used to select the appropriate concentration of API in each mixture so that it is supersaturated with respect to the reference phase at the harvest temperature. Here, the driving force for crystallization can also be varied by tailoring the composition of each sample based on the API solubility in that mixture. With such DOE tools, experiments may be designed to effectively and simultaneously explore the diverse composition and process space described in Table 1.

Ideally, DOE algorithms should also incorporate prior knowledge or experimental results, which have been stored in a database as a set of rules or models, to limit an experimental space to have certain predicted characteristics. For example, over the course of time, a regression model may be developed between a set of known or calculated chemical properties and a parameter of experimental interest. The model could be used during the design of a new experiment in order to test only those chemicals that are predicted to give a desirable result. Since a large number of factors need to be considered during experimental design, the DOE interface available to the scientist must not only be flexible and easy to use, but must also offer tools that aid design efficiency and effectiveness and permit input of scientific knowledge generated over time.

At the end of the experimental design process, the resulting set of experimental conditions is translated into a series of commands for the HT systems, and stored in a relational database for later retrieval by the software that controls the automation. When an experiment is activated, the overall operation of the automation systems is managed by the HT informatics system, which is responsible for physical operation of the HT platforms as well as data tracking and storage.

Execution of experimental commands is carried out by automated laboratory equipment that comprises the HT crystallization system. Specialized automated systems perform several of the functions in a sequence of events that make up the experiment. Each station is controlled through an interface to the informatics system that ensures the samples are processed at the correct stations, in the correct order, with the selected experimental parameters being followed. Parameters of operation are recorded, including the time at which an action is taken. After execution of the experimental steps, the software interface retrieves any pertinent information generated by the automated platform, such as assay results or operational parameters, stores these data in the relational database, and updates the status of the experiment to reflect the completion of operations.

In general, the hardware required for a HT crystallization system is comprised of four major functional elements: sample preparation, solids generation, solids detection and sample analysis. Sample preparation

involves adding the compound of interest (API) to the diverse set of conditions used to conduct crystallization studies. Typically, the API is dispensed as a solution in a suitable solvent, followed by solvent removal to yield the solid API. Solvent removal can be achieved by passive evaporation or by controlled active evaporation (e.g., use of a vortex dryer). Alternatively, the API can be delivered in the solid state with suitable powder handling systems. Depending on the amount of saturation desired, the crystallization vessel used, and the API's solubility in solvents or solvent mixtures of interest, API masses ranging from a few hundreds of micrograms to several milligrams will be present in each vessel. Once the API has been delivered to the crystallization vessels (tubes, vials or microwell plates), combinations of solvents and/or additives are added to each vessel. By taking advantage of the power of combinatorial approaches, large numbers of unique combinations can be dispensed from manageable sets of starting materials.

Compatibility of equipment components (syringes, dispense tips, tubing, etc.) and consumables (plates, tubes, etc.) with solvents and other compounds is a key hurdle faced in the development of combinatorial crystallization for small molecules. Unlike protein crystallization systems [56,57], which are commonly based on the sitting-drop method in aqueous media, small molecule crystallization employs a range of crystallization additives and processes. The additives include organic solvents with varying properties (e.g., alcohols, acetone, hexane, ethyl acetate, etc.), water, acids, bases and co-crystal formers, as well as other compounds (e.g., small molecule templating agents, surfactants, pharmaceutical excipients, etc.). This wide range of materials needs to be handled by appropriate liquid handling techniques to enable the combinatorial assembly previously mentioned. Ideally, liquid transfers are achieved using multichannel pipettors with individually controllable channels. Depending on the crystallization vessel design, the volumes of reagents dispensed will be as low as a few microliters to as high as several hundred microliters.

Potential for cross-contamination and tendency toward unwanted solvent evaporation from crystallization wells are challenges that need to be addressed in a HT crystallization system. A large number of the solvents used to crystallize small molecules have high vapor pressure under ordinary laboratory conditions. Sealing of the crystallization vessels is key to being able to control composition during crystallization from these solvents. Due to solvent fugacity, vessels need to be protected from ingress of the components of neighboring wells. These problems have been solved by different means, such as sealing of individual tubes with a Teflon-backed crimp seal [40] or O-rings/gasket seals and clamped covers [47,51].

HT crystallization must enable several process modes that are compatible with the compound (e.g., chemical stability, thermal stability, etc.). In some cases, multiple modes of operation may be combined. The most common modes of solids generation will be discussed below, including thermal cooling crystallization, anti-solvent and evaporative crystallization. Less common process modes include melt crystallization, flash or quench cooling and template-directed crystallization. It is important to note that generation of maximal diversity in solid form requires multiple modes of operation [6,18,58].

In thermally induced cooling crystallization, samples created in the sample preparation process described above are subjected to temperature ramps. Prior to beginning the temperature ramp, samples are exposed to an elevated temperature for a short period of time in order to dissolve the API in the crystallization medium. Although dissolution can be achieved most simply by diffusion and convection from the heating process, addition of external energy can speed up the process (e.g., sonication). Samples may be optically inspected (see Fig. 1) and vessels that contain undissolved solids can be flagged in the database for further analysis. For instance, undissolved samples may be treated as slurry conversion experiments and monitored over time for crystal form changes. The thermal cycle is then initiated, using controlled cooling to induce supersaturation. In this mode of crystallization, samples continually experience an under cooling and, based on the level of supersaturation in the vessel, may recrystallize at a given temperature after a period of time. Thermal crystallization tends to generate a cumulative number of samples that are produced over time in a fashion approximating a square root function, as illustrated in Fig. 2. This means that initially there is a small bolus of ''hits'', after which the rate of crystallization tails off over a period of time, typically in days to weeks. This results in a manageable hit rate

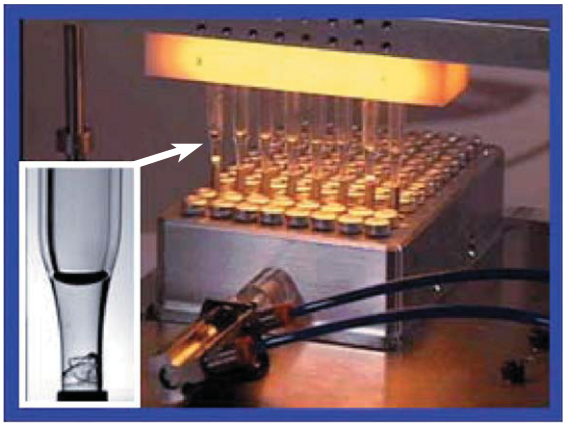


Fig. 1. Photo of optical inspection station. (Inset shows close up of crystallization vessel that contains crystals.) (Courtesy of Trans-Form Pharmaceuticals, 2002.)

for analysis, on the order of approximately 10% in aggregate. This mode of solids generation has the lowest throughput rate, typically, because experiments span days to weeks, with system residence times of months being possible.

In contrast, anti-solvent addition, also known as "crash-out" (or "drown out") crystallization, relies on the fact that an API is soluble to varying degrees in the crystallization medium, but is largely insoluble in a particular solvent or solvents (e.g., the anti-solvent). As a result, this mode of crystallization can operate at high-throughput rates, with samples being turned around hourly. When crystallization vessels containing API in reagent mixtures are exposed to aliquots of anti-solvent, nearly all vessels will contain API that has precipitated out of solution. This creates a challenge to the analytical process, as the near 100% hit rate leads to a large bolus of samples. There are, however, advantages to this mode of solids generation, such as the ability to produce microfine crystallites and amorphous solids, should they be desired.

Lastly, evaporative crystallization can be carried out on the combinatorial array of samples. This mode of operation relies on gradually increasing the concentration of API in the vessel to achieve supersaturation and to increase the degree of supersaturation (by preferential evaporation) in order to induce crystallization. Concentration of samples can be achieved either passively or actively by controlled flow of inert gas while maintaining temperature. With evaporative methods, differential rates of solvent loss from mixtures result in unknown composition of the crystallization medium at the time of crystal nucleation. In addition, the degree of supersaturation changes over the course of the experiment, often resulting in the appearance of multiple crystal forms. The evaporative mode of solids generation typically produces throughput and hit rates intermediate between the thermal and anti-solvent processes.

As suggested above, in appropriately configured HT crystallization systems, several process modes may be used in series or in parallel [40]. Frequently, the preparation of replicate plates (in some systems "daughter" plates [47,51]) is necessary for parallel processing by different process modes. Systems may be additionally equipped with the ability to serially process sample arrays using different process modes [59]. This feature is particularly attractive for cases where only small quantities of sample are available, increasing the drive to generate useful information from every sample. Here, samples may be processed by optimal modes first (e.g., thermal crystallization), then a secondary process step can be applied to maximize the hit rate. Another example where this feature is useful is in the case of salt selection, especially in early drug discovery. Upon the addition of salt forming acids or bases, the solubility of the compound is modulated by in-situ salt formation, often resulting in reduced or non-existent driving forces for crystallization (e.g., subsaturation) of the salt species, particularly in polar

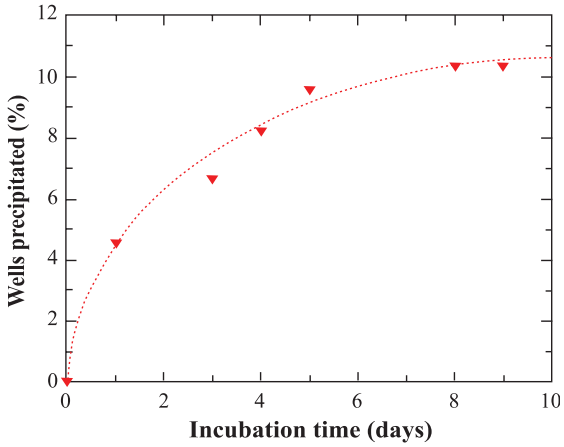


Fig. 2. Typical rate of appearance of solids during a thermally driven HT crystallization experiment [65].

solvents. It should be noted that rapid onset of supersaturation can be experienced in any of the process modes discussed and can result in oiling out or precipitation of amorphous solids, rather than generation of crystalline solids. Thus, it is important to monitor and control the crystallization conditions throughout the experiment.

In general, the percentage of wells that yield solids varies, depending on process mode and experimental time scale. For example, evaporative modes usually result in a solid in virtually every vessel, while slow undercooling results in far fewer (on the order of low percents). The differences in hit rates between these process methods arise in part from the differences in the supersaturation attained. For evaporative crystallization, supersaturation is achieved in all cases as the concentration of the active compound is continuously increased as solvent is evaporated. In contrast, the composition of wells processed by thermal crystallization is fixed. In some cases, because there is limited data on the precise state of supersaturation for each of the large variety of experimental compositions and potential crystal forms, some wells may remain subsaturated during the process. For these wells, additional process steps, such as partial evaporation or anti-solvent addition, may be employed to generate supersaturation to yield a solid. In contrast, as mentioned previously, a fraction of the wells may not go fully into solution at elevated temperatures. In this case, the temperature of the system may be raised to achieve full dissolution, additional solvent may be added to solubilize residual solids or the samples may simply be monitored for slurry conversion over time. To overcome these challenges, we have developed a solubility calculator tool using group contribution theory to estimate the solubility of the reference solid phase at specified temperatures in each solvent composition. These data are then used at the DOE step to define the viable concentrations of the active compound for crystallization (i.e., minimum concentration required to achieve saturation and maximum solubility limit or concentration) in each solvent mixture. Additionally, the timescale of the experiment has a significant impact on the observed hit rate. Hit rates will approach 100% for viable crystallization conditions in the limit of infinite time, but in practice most experiments are conducted over days to weeks, so observed hit rates reflect this temporal influence. In fact, similar behavior is observed in manual experimentation. Note that only some HT crystallization systems are configured to permit selective sampling of "hits", providing the ability to further incubate un-crystallized samples to monitor for slow growing crystal forms.

Solids detection can be achieved by examining each sample using machine vision systems. Samples may be monitored over time to detect precipitation in vessels that were previously devoid of solids. This simple, yet robust process can rapidly and non-destructively determine state changes in the crystallization vessels and signal when a particular vessel or set of vessels is ready for solid-state analysis. Depending on the sample array configuration, the signaling of "hits" results in harvesting of samples by one of two approaches. In the "cherry-picking" approach, only those samples that have been flagged as containing solids are selected for further processing [40]. In contrast, using a sacrificial approach the entire plate must be moved forward after a predetermined fraction of the samples in that array have produced precipitates [47,51]. The latter, of course, can be carried out without an online detection system. Here, samples can be processed in batches, without regard to whether there are actually solids present in a vessel. This simple process approach is effective, but has significant limitations, the primary of which being that samples are destroyed after a fixed amount of time regardless of their state. Hence, it is advantageous to employ an online detection and harvest system so that samples can be differentially and asynchronously processed, with only those vessels containing solids undergoing analysis [40,60].

Sample analysis is the final action in execution of the HT crystallization process. Depending on the mode of operation and the choice of analytical measurements employed, this process may involve several steps. Most HT crystallization systems use Raman spectroscopy and/or powder X-ray diffraction (PXRD) for primary analysis of harvested solid-state samples. Both techniques have advantages and disadvantages in terms of their ability to discriminate between forms of a solid (i.e., polymorphs, salt forms, solvates, hydrates) [1,14,61]. The rate of generation of samples for analysis likely dictates which technique is used for the primary approach. Generally speaking, Raman spectroscopy can be employed in a more rapid fashion than PXRD, since acquisition times for Raman are considerably less dependent on sample size, as is depicted in

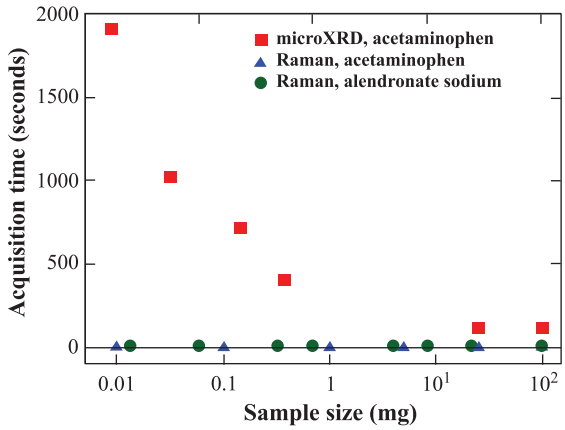


Fig. 3. Comparison of acquisition times of Raman and X-ray powder diffraction data as a function of mass of API [65]. (Data collected on D/Max Rapid, Contact Rigaku/MSC, 9009 New Trails Drive, The Woodlands, TX, USA 77381-5209).

Fig. 3. In addition, plate-based PXRD methods are susceptible to problems with preferred orientation effects, which may prevent accurate classification of samples. As a result, Raman spectroscopy methods are often used as a primary means of characterization in HT crystallization systems. Although one disadvantage of the Raman technique is interference due to fluorescent samples, the wavelength of the excitation laser can be changed to the near-IR to reduce fluorescence of problematic samples. Recent advances in PXRD instrumentation, brought on by the increasing demands of HT crystallization, make it possible to achieve similar analysis timescales with PXRD and Raman, on the order of less than one minute per sample depending on the capabilities of particular instruments used. Clearly, the best option is to employ both methods for initial sample evaluation, which can be realized with the appropriate informatics structure, as described in Section 3.

Once the primary solid-state characterization data are collected and stored, samples are generally classified into groups (or bins) that display similar characteristics (e.g., Raman spectra or powder X-ray diffraction patterns) using informatics tools. A variety of methods can be used to accomplish the binning. For instance, Raman spectra may be compared (based on relevant features or over the entire spectral range) and clustered using calculated similarity measures, such as Tanimoto coefficients. In one method [40,60,61], each Raman spectrum, which represents the contents of an individual well at a given time, is filtered to remove background and to accentuate Raman peaks and shoulders. Peaks are then located and assigned a wavenumber using standard derivative methods and the amplitude of each peak is calculated. These data are used to calculate a similarity (or distance) measure related to the Tanimoto coefficient, from which the Raman spectra are binned into groups of similar samples using a classification algorithm such as hierarchical clustering. This method often uses peak positions, rather than amplitudes to discriminate between different patterns in order to reduce the significance of potential preferred orientation effects, which can result in modulation of relative peak intensity for certain crystallographic planes. The window over which two peaks are considered to be at the same position (e.g., 1 cm^{-1} wavenumber), as well as a minimum height for a filtered peak to be considered for clustering, can be selected by the user, allowing regions of interest (e.g., spectral ranges) to be explored in greater detail. With appropriate settings, a Raman spectrum that has only one peak or feature in a slightly different location than observed in other patterns can be differentiated and binned as unique, indicating a different or new crystal form. During clustering, each spectrum is assigned an arbitrary number, i.e., a sorted spectrum number, for ease of tracking, and the resultant clusters are graphed as shown in Fig. 4, where the red-colored regions repre-

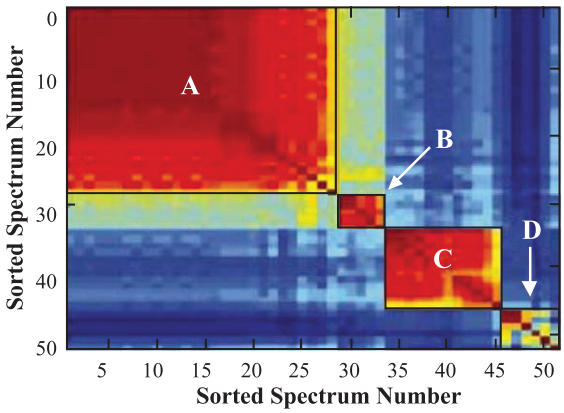


Fig. 4. Raman cluster diagram showing *n*-by-*n* matrix of sorted spectrum numbers for all samples resulting from the HT polymorph screen of Ritonavir. Clusters are indicated by warm-colored (red) regions, which have been outlined to guide the eye, and indicate different solid forms [65].

sent bins of similar samples. Alternatively, the results from several analytical methods such as Raman and PXRD can be used to simultaneously classify samples.

Regardless of the choice of primary analytical method, and in keeping with traditional methodologies for solid form screening, it is necessary to further characterize the solids generated in HT crystallization systems to accurately determine their solid form and properties. Most HT systems integrate multiple analytical methods as part of the screening process. These so-called secondary analytical methods often include thermal property measurement (e.g., melting point) and optical microscopy (for crystallinity, habit, etc.). Depending on how the samples are processed and the degree of computerized support, these techniques may be applied to all samples, or a subset of selected samples. For systems that analyze all samples by secondary techniques, several HT plate-based methods for optical microscopy and melting point determination have been developed [47,51]. It is important to note that, in this case, all samples are destroyed during characterization of the melting point. When replicates are retained, the functional properties such as dissolution rate and hygroscopicity can be analyzed using either manual or HT methods. (For more information on functional analysis, see Section 4 on post-screening analyses and form selection.)

With the aid of informatics tools, the data sets obtained can be used to generate information about the experimental space. Software interfaces that allow access to the data permit classification and regression analysis to be performed. The results are displayed in high-dimensional visualization tools that can be used to guide further experiments toward optimizing processes to make each form. For instance, sample composition and processing information can be linked to the resulting crystal form and morphology. Correlation of trends between experimental factors and the products can lead to hypotheses that can be used to direct the design of follow-up experiments. An example of this was reported by Peterson et al. [40], where the knowledge gained from iterative experiments was used to drive new experimental designs, which ultimately yielded the desired outcome, i.e., the isolation and characterization of the highly unstable form III of acetaminophen (paracetamol).

While these new methodologies provide unprecedented capabilities for solids form discovery, it is clear that there remains a need for some level of manual processing, particularly in the case of detailed form characterization such as single crystal structure determination, scale-up of the desired form and understanding the effects of downstream processing on potential form conversion. HT methods provide the landscape of possible forms and their properties and should be used in conjunction with traditional methods to enable rapid, efficient selection of the optimal form for development.

3. Applications of high-throughput crystallization screening in pharmaceutical research and development: case studies

HT technologies offer unprecedented capabilities for form discovery and characterization. Potential applications range across the entire pharmaceutical value chain, including screening of active molecules in discovery during ELO, form selection for preclinical candidates, final form optimization for early clinical candidates, process chemistry development of crystallization processes for bulk drug and intermediates, as well as identification of new or enabling solid forms for product life cycle management. While numerous impact points have been identified, only limited information on the use and performance of HT form screening systems is available in the literature, indicating that the benefits of these new methodologies have just begun to be realized. In the following sections, case studies on the application of HT crystallization systems are reviewed. Special attention is given to the implications of new form discoveries.

3.1. High-throughput salt selection

Preparation of salt forms of an active compound is commonly used to modulate physicochemical properties. In most cases, the goal is to increase solubility (or dissolution rate) to improve bioavailability or to enhance the manufacturability of poorly soluble ionizable compounds [1,3,4]. Salts may also be employed to increase chemical stability [3] or to reduce the solubility of a given compound for certain applications (e.g., sustained release dosage forms) [62]. Thus, it is important to consider the route of administration and

dosage form requirements when selecting a salt form for development. Since the choice of counter-ion affects the properties of salt forms [3,4], salt selection studies involve the preparation of a number of different salts using a variety of pharmaceutically acceptable acids or bases with differing properties (e.g., acidity/basicity, molecular size, shape, flexibility, etc.). The relevant physicochemical properties of each salt are characterized, including degree of crystallinity, hygroscopicity, aqueous solubility, crystal habit, and physical and chemical stability. Based on these properties of the salt forms, their suitability for development can be evaluated. Several strategies for streamlining and optimizing salt selection procedures have been reported, including in-situ techniques for ranking the solubility of salts [63], tiered approaches in which the least time-consuming studies are carried out first and used to remove from consideration salts that are not viable [64]. One issue not readily considered by existing strategies is the polymorphism and solvate forming behavior of the different salt forms of a compound, which could be used as an additional criterion when more than one salt may be viable, but the degree of polymorphism and solvate formation of each may become a criterion for form selection.

HT crystallization technologies have been used to more rapidly and comprehensively identify the range of salt forms that may be prepared for a given compound or series of compounds, and characterize their crystal form diversity (polymorphs, solvates, hydrates). However, only a few studies have been published or presented. Several HT salt selection studies on well-characterized pharmaceutical compounds have been carried out to demonstrate the power of these technologies in solid form discovery. For example, in a small HT study (i.e., 96 wells) on the antibacterial sulfathiazole, salt formation was explored using varying stoichiometric ratios of pharmaceutically acceptable organic and mineral bases in an array of solvent conditions [65]. The screen resulted in the rapid identification and characterization of 10 salt forms and showed that the salts exhibited a range of melting points depending on the counter-ion type and stoichiometric ratio. Similar HT salt selection experiments on caffeine and naproxen resulted in the identification of numerous salts of each compound [47,50,51].

In the discovery phase, HT crystallization has been used to identify soluble salt forms of compounds during ELO to facilitate early animal dosing, thereby providing the ability to uncover underlying chemical and/or biological responses elicited by candidate molecules, including toxicity or efflux [46,59]. Such information permits rapid identification of problematic compounds or scaffolds, allowing resources to be directed to projects with greater opportunity for success. HT crystallization can facilitate selection of leads that are more likely to survive preclinical development. HT crystallization has been used successfully to identify multiple new salt forms and the polymorphs and solvates of each compound belonging to two discovery programs using less than 200 mg of compound per screen [59]. Approximately 150–200 experiments were performed on each compound using a library of pharmaceutically acceptable acids or bases with an array of solvent compositions and process conditions. Each screen resulted in discovery of multiple new salt forms, and in some cases polymorphs and solvates. Interestingly, similar salt types were identified for each compound in a given series, as illustrated in Fig. 5, where the frequency of occurrence is plotted as a function of counter-ion for each discovery series. Clear trends in the degree of solid form diversity of salt forms, including polymorphism and solvation behavior, were also evident within each compound series. These data indicate the potential for identifying salts suitable for most compounds tested in a particular scaffold or series, based on analysis of only a portion of the series, i.e., a platform-based approach to salt selection, provided the chemistry surrounding the ionizable functionality is not significantly altered during further structure–activity relationship (SAR) development. Furthermore, solubility measurements of each salt form in physiologically relevant fluids allowed ranking of salt forms in a given series, and comparison of salts between series was also possible. The average turnaround time per screen was approximately 2 weeks, such that feedback on the physicochemical properties of each compound was provided to the medicinal chemists on a similar time scale as potency, selectivity and metabolism screens.

Salt selection is normally part of the standard preformulation studies carried out during preclinical development, where rapid identification of the possible salts of a compound and their properties can facilitate product development. To further facilitate

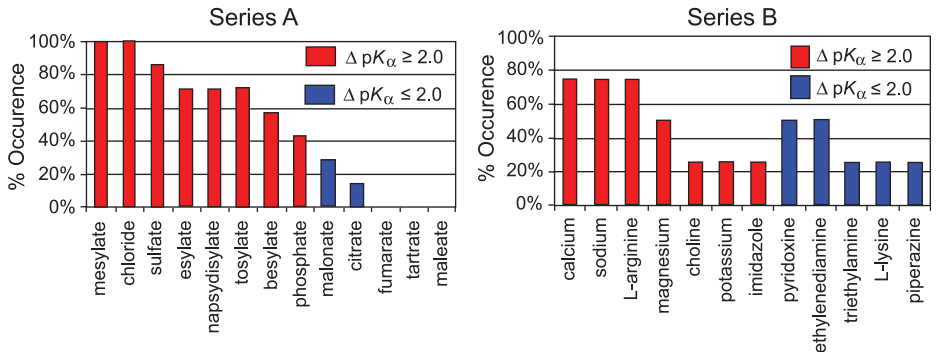


Fig. 5. Frequency of occurrence (%) plotted as a function of the counter-ion of the salt for compounds from discovery series A and B [59].

such studies, a microplate technique capable of investigating an array of conditions has been developed to determine which counter-ion and solvent conditions can be used to prepare crystalline salts of the compound [66]. Each plate is prepared by first depositing approximately 0.5 mg of compound into each well using an appropriate amount of stock solution. The counter-ion type is systematically varied along the rows of the plate and different crystallization solvents are deposited down the columns of the plate. Crystallization is monitored by optical microscopy over the course of the evaporative crystallization, which can be accelerated by flowing a stream of dry nitrogen over the plate. Once salt forms are identified, they are scaled up for more detailed characterization.

The microplate approach was demonstrated by Bastin et al. [66] through several examples, however little detail of the specific screening protocol and results was provided. All three of the reported examples are on compounds that are weak bases with pK_a between 4.1 and 5.3. Only a small number of stable, crystalline salts could be prepared for the two very weak bases (i.e., $pK_a < 4.25$), as opposed to the larger variety found for the stronger base. In each case, the salt forms were scaled-up for more detailed analysis and comparison to the respective free base compound to determine the optimal form for development. This approach provides a useful mechanism for preliminary, small-scale salt formation studies. Both the crystallization media and process modes accessible by the technique are somewhat limited, resulting in a narrow exploration of experimental conditions for salt formation. For example, only solvents compatible with plate materials can be used, thereby reducing the probability that a crystalline phase can be identified. In addition, current protocols only provide for evaporative crystallization, likely due to difficulties with sealing of the plates. In this case, the composition of the crystallization medium is not well controlled. The utility of HT crystallization in ELO, although demonstrated by initial reports of feasibility, is less well documented than the use of HT on later stage compounds.

3.2. Solid form discovery in highly polymorphic systems

The statement by the late Walter McCrone in 1965 that "the number of forms of a given molecule is proportional to the time, money and experiments spent on that compound" [67] has gained credence in recent years, as illustrated by the significant increase in reported crystal form diversity of pharmaceutical solids. Depending on when alternative solid forms of a compound are identified, the appearance of a novel form may or may not be a welcomed discovery. Occurrence of a new form in research or early development is potentially enabling. At later stages, the appearance of new forms, particularly stable ones that are not bioequivalent or deemed unprocessable, can have catastrophic consequences for product performance as well as regulatory compliance (e.g., control of crystal form). Additionally, recent rulings on the use of alternative, commercially viable solid forms not protected by patents from

innovator companies have opened the market to generic competition [68–79]. In order to mitigate these risks, and to save time and reduce costs, many pharmaceutical companies have begun to re-evaluate their strategies for solid form screening and are looking to HT crystallization technologies to address the needs for more rapid and comprehensive exploration. In this section, the application of HT crystallization to highly polymorphic systems is reviewed, including specific cases of compounds exhibiting latent polymorphism.

Polymorphic systems are quite common among many types of organic crystals [7]. For the purposes of this review, compounds exhibiting more than three polymorphic forms will be classified as being "highly polymorphic". While only a handful of well-known organic compounds are considered for practical purposes to be non-polymorphic, e.g., aspirin [80,81], sucrose and naphthalene [7], it should be stressed that one will never be able to exclude the possibility of polymorphs appearing, even a century after the initial discovery of the compound. So far, no polymorphs of aspirin have been found, despite the proposal by Payne et al. [80] that polymorphic forms may exist. In contrast, acetaminophen form III was observed by Burger in 1982 using thermal microscopy [82], but it took another 20 years for a crystal structure to be proposed [40]. Many reports exist on the polymorphic nature of specific drug compounds with one or two alternative packing modes for the same chemical composition. However, literature examples of compounds with more than three packing modes are considerably rarer, as will be summarized shortly. It should be noted that the increased number of reports on highly polymorphic compounds in recent years is likely the result of enhanced screening practices and more sensitive characterization techniques.

Highly polymorphic compounds present several challenges in drug development. First, the generation of different forms is often not a simultaneous event, but rather a gradual evolution of form diversity leading to the branding of a compound as being highly polymorphic. Consequently, once more than one form is identified, concern is raised that additional forms may eventually be discovered. For instance, the 13 polymorphs of phenobarbitone evolved over ca. 13 years [7], and a fourth polymorph of carbamazepine was reported in 2002, a full two decades after the publication of the structures of the initial three forms [83]. Second, selection of the preferred form of a highly polymorphic compound for development demands a complex set of thermodynamic and kinetic investigations, due to the geometric increase in the number of stability relationships that need to be established. More complexity arises when some polymorphic pairs are enantiotropic, exhibiting a switch in the identity of the stable form as a function of temperature. Third, concerns over bio-performance and the impact of a large number of polymorphs on processing lead to regulatory issues that need to be addressed. Decision trees [58] have been established to aid scientists in assessing the impact of polymorphic change and have been incorporated into the ICH guidelines [84]. Lastly, the analytical challenge of monitoring polymorph content in the dosage form increases as the number of possible forms grows, particularly with low dose compounds where the concentration of drug in the formulation is small.

The literature on highly polymorphic pharmaceuticals is relatively sparse, but several examples of compounds known to have four or more polymorphic forms are available in the literature and are summarized in Table 2. In addition to these drug examples, the pharmaceutical ingredients mannitol and aspartame have been shown to exhibit 4 and 5 polymorphs, respectively [7]. The phenomenon in inactive exci-

Table 2
Examples of highly polymorphic drug compounds in the literature

Compound	Number of reported polymorphs	Other forms	Reference(s)
Phenobarbitone	13		[7,p.255]
Cimetidine	7	Hydrates	[7,p.73]
'ROY'	7	7th form found after the initial publication	[111,112]
Sulfathiazole	5	Numerous solvates	[113]
Carbamazepine	4	Dihydrate and numerous solvates	[28,45,83,85]
MK-996	9	Hydrate	[87]
MK-A	4	2 hydrates and numerous solvates	[86]

pients may well be under-appreciated due to lack of study.

In general, pharmaceutical polymorphism is likely to be underreported in the literature, since much of the polymorphism research is carried out in companies. As a result of growing interest in the subject and advances in techniques to study polymorphism, it is expected that reports of extreme form diversity will grow. Conferences on the subject, such as the ACS ProSpectives symposium, reflect the appreciation for the complexities introduced by the appearance of polymorphism in important materials such as pharmaceuticals. Work has recently commenced to understand the opportunities and challenges of using HT technologies in pursuit of rapid identification and characterization of the large number of forms presented by highly polymorphic compounds. Three published case studies and two examples that are in press at the time of this review will be highlighted.

Form IV of carbamazepine was reportedly discovered as the result of crystallization trials in the presence of hydroxypropyl cellulose HPC [83]. Subsequent to this publication, Lang et al. [28] published the use of polymers to influence polymorphic form using a 96-well plate system for the screening of polymorphs of carbamazepine and acetaminophen. In all, 84 different polymers were employed to direct nucleation. Form IV of carbamazepine was found to crystallize from methanol in the presence of hydroxypropyl cellulose, poly(4-methylpentene), poly(*R*-methylstyrene) or poly(*p*-phenylene ether-sulfone). Using the same approach, the monoclinic and orthorhombic forms I and II, respectively, of acetaminophen were also isolated. While observation of metastable form III was not reported in this study, the strategy of employing polymeric additives is of interest, as it can direct the course of crystallization and because polymeric impurities may be in contact with a drug substance and/or formulation at various points in development.

Another approach, reported by Anquetil et al. [85], identified selective conditions for the crystallization of carbamazepine polymorphs forms I and III, as well as the dihydrate, from methanol and/or methanol/water solutions by thermal processing in a microliter cell format (i.e., 35–100 μl). Optical laser trapping was used in situ to target the microcrystals for real-time form analysis using Raman spectroscopy. The crystallization process was monitored optically and with Raman spectroscopy as a function of temperature and time. The study revealed the conversion of form I to form III, as evidenced by a change in characteristic crystal habit from needles to prisms. Raman spectroscopy on the solution phase measured the saturation solubility of each crystal form produced. Although only several experiments were carried out in this study, the authors advance the microfluidic cell format as a potentially viable system for HT polymorph screening.

A third report details the use of in situ Raman spectroscopy to optimize process conditions. The compound MK-A has four anhydrous polymorphs and several other forms, including two hydrates and numerous solvates [86]. The study gives an example of the complex thermodynamic relationships (monotropic and enantiotropic pairs) that can exist in highly polymorphic systems and demonstrates the power of in-situ methods for monitoring the crystallization process.

The angiotensin-II antagonist MK-996 is an example of a highly polymorphic compound (Table 2) [87]. The structure of MK-996, depicted in Fig. 6, contains seven rotatable bonds, the conformations of which could lead to many configurations for crystal packing. HT crystallization experiments with MK-996 in 96-well arrays comprising over 1500 discrete recrystallization trials from a set of 21 solvents or solvent mixtures yielded 186 solids, which were harvested over a period of 7 days [87]. PXRD analysis of these solids suggested the presence of at least 18 distinct

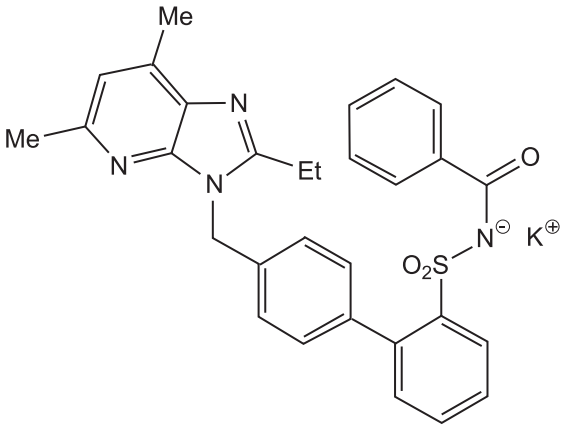


Fig. 6. The molecular structure of the angiotensin-II antagonist MK-996 [87].

forms, some resulting from solvent-mediated recrystallization. A hydrate (originally named form I), obtained by slurry conversion in the presence of aqueous solvent mixtures in the HT experiments, was the form previously selected for pharmaceutical development. Importantly, a form (form D) reported by the innovator [87] to be a "disappearing polymorph" [88] once form I appeared, was also found in the HT screen. Clearly, sufficient experimentation with rationally selected diverse conditions affords the possibility to regenerate elusive forms.

Sertraline HCl, the active ingredient in the antidepressant Zoloft®, is found in various crystal forms. The molecular structure for Sertraline HCl is illustrated in Fig. 7. Information on various solid phases can be found in patent disclosures filed by several companies [89–92]. Survey of these documents, which published between 1992 and 2001, reveals data for 27 purported crystal forms of Sertraline HCl, including 17 polymorphs, 4 solvates, 6 hydrates and the amorphous solid. Further analysis and comparison of characterization data for the various forms presented in the patents revealed that mixtures have been mistaken for real polymorphs on at least two occasions, and at least two polymorphs were disclosed more than once (by different workers each time). In addition, the hydrate forms reported were not readily identified as polymorphic and many of the forms are likely transient, e.g., only identified by variable-temperature and humidity-controlled XRD. With the help of HT crystallization, the extent of true polymorphism of the HCl salt was estimated at eight forms so far [92]. Two new solvates were also found in the HT studies. Care should be taken in isolation of such forms, particularly at small to intermediate scale, as desolvation of solvates due to aggressive drying during processing may cause one to overlook solvated forms [93]. Comparing the results of the HT study to the congruence of historical data, one can conclude that HT screening gives rise to relevant forms of the drug in a time frame of weeks rather than years. One metastable form, polymorph IV, remained elusive in the hands of the authors [92]. The lack of observation of form IV may be due to a subtle purity difference between early batches at Pfizer and the materials available for testing in the HT screen. Clearly, impurity effects should be explored further [32].

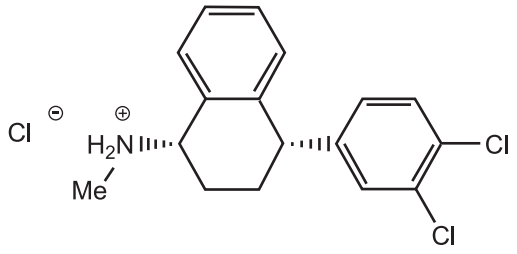


Fig. 7. The molecular structure of the selective serotonin reuptake inhibitor (SSRI) sertraline HCl.

To date, HT studies on highly polymorphic materials highlight the importance of varying processing conditions (including solvent conditions, degree of supersaturation, method of crystallization, desolvation of solvates, inclusion of additives, thermal microscopy, etc.) to find as many forms as possible. It has been shown that multiple process modes, including HT processing, coupled with detailed follow-up characterization studies of form stability, facilitate insight into crystal form diversity [40]. Such a multimode strategy becomes valuable in the quest for the most comprehensive dataset possible for a given pharmaceutical material.

Undoubtedly, the definition of highly polymorphic materials and their frequency will evolve in the age of HT crystallization [40,60] and with the aid of ever improved solid-state analytical capabilities [18,94,95]. The value of employing multiple processing techniques to elucidate as many crystal forms as possible will be demonstrated, as it is expected that no single technique will generate all forms of a given compound. Without doubt, HT crystallization strategies will be used, as a complement to other techniques, to identify issues of polymorphism early, thus allowing drug development scientists to react appropriately to information on form diversity of their compounds.

3.3. Avoiding latent polymorphism

Very few cases of latent polymorphism have been reported in the literature. It is likely that many more instances of the phenomenon have occurred, but unless product development was slowed, product performance was impacted, or generic competition was threatened, a spotlight is not usually cast on the issue. As an example of a public polymorph issue, form 2 of ranitidine hydrochloride was discovered 2–

3 years into development but it was (and is) the form still marketed by GlaxoSmithKline [75,76,96]. Paroxetine hydrochloride hemihydrate, the active ingredient in Paxil®, was discovered during development after only an anhydrate had been known for a number of years [97]. The hemihydrate is the form marketed by the innovator, but recent litigations have occurred between the innovator company and generic competition around the anhydrate form.

One of the most recognized cases of latent polymorphism occurred with Abbott Laboratories' Norvir®. Two years after entry into the market, a previously unknown, but thermodynamically more stable, polymorph of the active ingredient (Ritonavir) appeared. This new form (form II) was approximately 50% less soluble in the hydroalcoholic formulation vehicle, resulting in poor dissolution behavior and eventual withdrawal of the original Norvir® capsule from the market [98]. At some considerable cost, a new formulation of Norvir® using form II was eventually developed and launched [99]. In a recent HT crystallization study on Ritonavir, a total of five forms were found: both known polymorphs and three previously unknown forms [99]. The HT polymorph screen, which consisted of 2000 experiments was carried out with less than 2 g of the API and used multiple, and sometimes combined, process methods. The three new forms were described as a metastable polymorph, a crystalline solvate and a non-stoichiometric hydrate. Interestingly, the solvate was easily converted to form I via the hydrate phase using a simple washing procedure, and provided an unusual route to prepare the form I "disappearing polymorph" [88]. Since the crystals of form I prepared using this method retained the small needle morphology of the solvate, the authors suggest that the process may offer a potential strategy for particle size and morphology control. The results of this study emphasize the need for more comprehensive studies of form diversity in the early stages of drug development to avoid risks of form conversion downstream, and highlight the advantage of combining parallel HT crystallization experimentation with detailed physicochemical analyses to identify the diversity of solid forms in which a given molecule can exist. Clearly, late stage discovery of new forms or form conversion can have serious competitive and regulatory implications (e.g., process control), especially in cases where the new forms are not bioequivalent.

3.4. Prediction of crystallization and polymorphism: applications to pharmaceutical form studies

Crystal structure prediction is a challenging area of research. Due to the overwhelming influence of packing forces in determining crystal structure, it remains extremely difficult to predict the structural impact of subtle conformational effects and weak interactions between adjacent molecules in a crystalline arrangement. Although significant progress has been made in the last decade, crystal structures are by and large not reliably predictable from first principles [88]. While this important area of theoretical research is too large a topic to be considered in detail here, a brief overview of the successes and challenges will be presented, and the potential for using HT crystallization as a validation to aid model development will be highlighted. For a more detailed discussion on polymorph and crystal structure prediction, refer to the article by Price [100] in this issue.

Polymorph prediction of pharmaceuticals is thwarted by the complexity of active pharmaceutical molecules. The number of degrees of freedom in torsion angles and the molecule count in the unit cell (which can be deduced by such techniques as solid-state NMR [94]) are frequently too great to allow computations on a reasonable time scale. Additionally, predictions are typically carried out one space group at a time. This limitation is mitigated by the fact that over 90% of the organic compounds in the Cambridge Structural Database (CSD) [101] crystallize in only a few space groups [100]. We know of only one example where predictions have been extended to multicomponent systems [102]. The prevalence of multicomponents systems, some of which have charge transfer (salts) and many of which exist as hydrates, solvates or mixed hydrate/solvates, essentially limits the usefulness of the prediction methods to neutral compounds. Various other technical issues remain as the science of crystal structure prediction matures [100]. Some of these issues were highlighted in two blind tests that were conducted in recent years to determine the accuracy and robustness of crystal structure prediction [103]. In the latest round, 17 methods were used to predict structure, yielding only three correct predictions [104]. For one of the compounds used in the study, experimental characterization of a second, more stable, polymorph provided the key to the correct prediction by three participating

research groups. The structure could have easily been overlooked, leading to the misinterpretation of the results as an apparent failure of the computational methods. Thus, compounds that are amenable to structure prediction are not always studied experimentally to the extent necessary to ensure that the relevant forms have in fact been discovered and characterized ahead of computational studies.

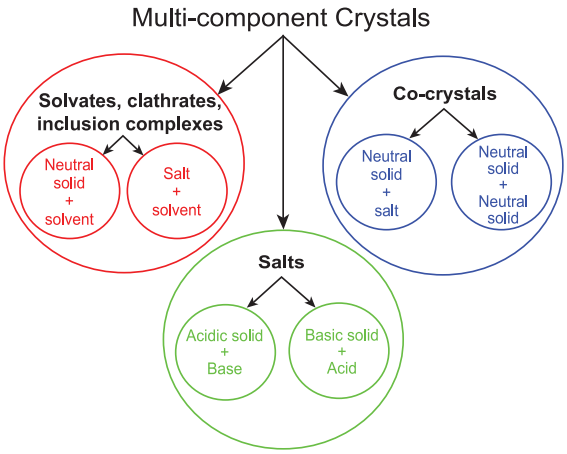
Despite the challenges, a few methods have been developed that allow structure prediction of small, relatively rigid organic compounds with only a few functional groups in several important space groups [17,105,106]. Polymorph Predictor™ has been implemented within the commercial software Cerius2 (C2 Polymorph by Accelrys). In general, current prediction methods generate large ensembles of different packing arrangements along with calculations of relative energetics. In reality, many of the calculated structures are not observed, giving the appearance of over-prediction of polymorphism. This was apparently the case with acetaminophen (paracetamol) [107]. In their study of the drug, Beyer et al. [107] calculated 14 structures, 2 of which were the known monoclinic (stable) and orthorhombic forms. The remaining 12 structures were considered as candidates for the metastable form III, which had been observed by thermal microscopy methods [82] but for which diffraction data were unavailable. Using calculations of mechanical properties and morphology, Beyer et al. separated the 12 energetically feasible structures into two groups, based on the likelihood of each structure to exist as a stable form. Shortly after the publication of the prediction study, the experimental powder pattern of form III became available [40]. Rietveld refinement and comparison of the experimental diffraction results with the theoretical powder patterns published by Beyer et al. yielded a monoclinic structure solution for form III. This structure is in fact part of the prediction set, but was considered an unlikely contender based on its extreme plate-like morphology. The potential for complementarity of HT crystallization and polymorph prediction is evident from these studies. In one sense, polymorph prediction can serve as a yardstick for "risk assessment" when it comes to form diversity, but inevitably one will require experimental data to assess the scope of polymorphism that can be elicited and the precise relative stabilities of different crystalline arrangements.

Opportunities do exist for current use of predictions in solid form discovery. For instance, certain hydrogen-bonding motifs or molecular layer types may be observed in predicted structures. Such information can be used to aid the design of crystallization experiments. It might be desirable to employ a particular type of interaction with salt selection or co-crystal formation by the strategic selection of crystallization conditions, solvents, additives and processing methods [22,23]. In addition, since transient or metastable crystalline species may be difficult to characterize accurately, one may use predicted structures to estimate various physical data. For example, powder diffraction patterns may be used to assist the accurate description of these metastable forms [40]. Continued development of theoretical methods coupled with validation of the predictions by extensive crystallization screening will lead to better models and computational methods. At present, experimental methods must still be relied upon to assess the potential form diversity of a given compound. It will be important to concurrently push the limits on theoretical prediction and HT crystallization, in order to advance our understanding of the nature and extent of polymorphism in pharmaceutical compounds.

3.5. Engineering of co-crystals

Co-crystals of drugs and drug candidates represent a new type of material for pharmaceutical development. They are part of a broader family of multicomponent crystals that also includes salts, solvates, clathrates, inclusion crystals and hydrates as shown in Scheme 2. The primary difference between solvates and co-crystals is the physical state of the isolated pure components: if one component is a liquid at room temperature, the crystals are designated as solvates; if both components are solids at room temperature, the crystals are designated as co-crystals. While at first glance these differences may seem trivial, they have profound impact on preparation, stability and ultimately on the ability to develop products.

In general, it is usually easier to initially prepare solvates than co-crystals, and indeed, solvates are often found as by-products of polymorph and salts screens. Co-crystals have been prepared by melt-crystallization, grinding and recrystallization from solvents [1]. Sol-



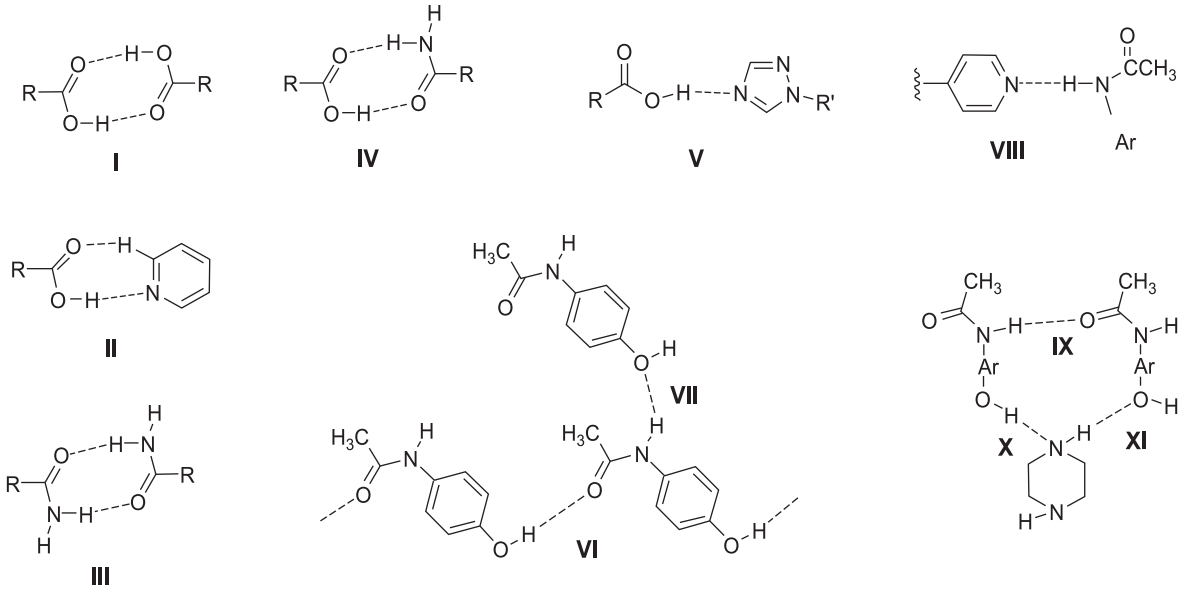
Scheme 2. Types of multicomponent crystals.

vent systems for co-crystals must dissolve all components, but must not interfere with the interactions necessary for co-crystal formation. The need to try many solvent combinations and the availability of multiple co-crystal formers creates a diversity that is ideally suited for exploration by HT systems.

Co-crystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. The number of pharmaceutically acceptable solvents is very small, and because solvents tend to be more mobile and have higher vapor pressure, it is not unusual to observe dehydration/desolvation in solid dosage forms. Solvent loss frequently leads to amorphous compounds, which are less chemically stable and can crystallize into less soluble forms. In contrast, most co-crystal formers are unlikely to evaporate from solid dosage forms, making phase separation and other physical changes less likely.

Examples of co-crystals have existed in conductive organic crystals, non-linear optical crystals, dyes, photographic materials pigments and agrochemicals for some time [7]. Two recent papers by Fleischman et al. [43, 45] emphasize the importance of understanding "supramolecular synthons" in synthesizing co-crystals containing pharmaceutical agents. For example, the ability to insert 4,4′ -bipyridine between the carboxylic acid dimers of aspirin, *rac*-ibuprofen and *rac*-flurbiprofen was recently reported [43]. The three examples clearly demonstrate the generality of the use of a pyridine-carboxylic acid heterosynthon II (Scheme 3) to replace a dicarboxylic acid dimer homosynthon I. A second study focused on finding multiple solvates and co-crystals of carbamazepine [45]. Carbamazepine polymorphs crystallize as amide dimers, each of which ties up the polar amide functional groups through homosynthon III. Crystal structures shows that each dimer contains a peripheral H-bond donor and acceptor pair that remain unused due to geometric constraints imposed by the drug molecule. Simple H-bond acceptor solvents like acetone and DMSO insert themselves to fill voids between the adjacent pairs of dimers [45]. Multiple co-crystals formers having hydrogen bond acceptors likewise insert themselves into the void. The homosynthon can also be broken to form heterosynthon IV, an amide-carboxylic acid dimer [45]. This was achieved to form solvates with acetic, formic and butyric acids, and co-crystals with trimesic and nitro-isophthalic acid.

A recent study of adducts of acetaminophen (paracetamol) with ethers and amines provides additional examples of supramolecular synthons for co-crystal formation [108]. While amide-amide homosynthon could have formed, both known forms of the pure material consist of linear head-to-tail chains held together through motif VI; the chains are cross-linked through synthon VII. The linear chain structure is preserved in co-crystals with 4,4′ bipyridine, but the cross-linking interaction VII is replaced by VIII, in which the 4,4′ bipyridine is hydrogen bonded to the amide hydrogen. The chains remain cross-



Scheme 3. Supramolecular synthons observed in co-crystals.

linked but only through pi-stacking interactions between 4,4′ bipyridine pairs on neighboring chains. In co-crystals with piperazine, the acetaminophen forms head-to-head chains through IX. Each chain is joined to the next through a layer of piperazine molecules that interact through heterosynthons X and XI. The paper also includes many solvates that will not be reviewed here, but their synthons should be applicable to co-crystal formation.

The above studies focused on demonstrating the use of supramolecular synthons to create novel crystalline phases. The variety of structures observed provides hope that some forms will have superior performance in pharmaceutical dosage forms. However, the studies stop short of providing data on the physical properties, such as solubility, necessary to evaluate their utility. Furthermore, only the saccharin and nicotinamide co-crystals of carbamazepine represent pharmaceutically acceptable co-crystals. Crystals containing two drugs may appear to be a good technique for making combination products of two drugs, but unless the two drugs are dosed only in stoichiometric ratios consistent with the co-crystal composition, such crystals would still need to be co-formulated with at least one of the bulk drugs in order to satisfy the clinical requirements.

We recently reported on the discovery and dissolution properties of pharmaceutically acceptable co-crystals consisting of hydrogen-bonded trimers of two molecules of *cis*-itraconazole and one molecule of a 1,4-dicarboxylic acid resulting from a HT crystallization screen [44]. The crystal structure of the succinic acid co-crystal (Fig. 8) revealed an unanticipated interaction between the triazole of itraconazole and the carboxylic acid (heterosynthon V in Scheme 3). The extended succinic acid molecule fills a pocket, bridging the triazole groups. The interaction between the 1,4-diacid and the strongest base on itraconazole (piperazine) is absent in the co-crystal structure. Other 1,4-diacids including fumaric acid, L-malic acid and L-, D- and DL-tartaric acids also yielded co-crystals with itraconazole, but co-crystals could not be made from maleic acid with *Z*-regiochemistry, or from 1,3- or 1,5-dicarboxylic acids. Hence, geometric fit appears to be more important than acid-base chemistry in directing crystallization of the compounds of itraconazole with 1,4-dicarboxylic acids.

Identification of multiple crystal forms of the same drug with acceptable solubility, dissolution rate and stability enables selection of the optimal form for dosage form development. To demonstrate this feature, the dissolution of itraconazole co-crystals in

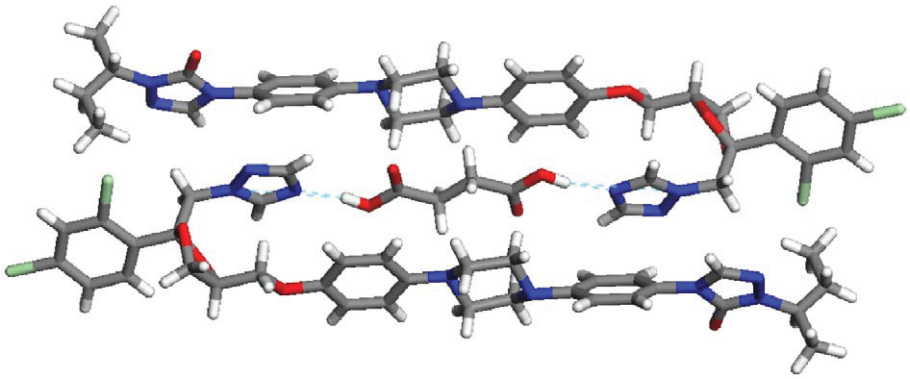


Fig. 8. Trimer unit of the itraconazole succinic acid co-crystal from single crystal X-ray structure (from [44], with permission).

aqueous medium was studied to assess their potential impact on bioavailability of the drug from a solid dosage form. Fig. 9 compares the dissolution profiles of the co-crystals into 0.1 N HCl to those of crystalline itraconazole-free base (95 % of all crystalline particles < 10 μm) and commercial Sporanox® beads (amorphous itraconazole). The malic acid co-crystal rivals the dissolution of the commercial product. In general, the co-crystals behave more similarly to Sporanox® than the crystalline-free base. The co-crystal forms achieve and sustain 4- to 20-fold higher concentrations than that achieved from the crystalline-free base. The practical implication is significant, since the ability to form a supersaturated solution, even transiently, can have dramatic impact on absorption and bioavailability.

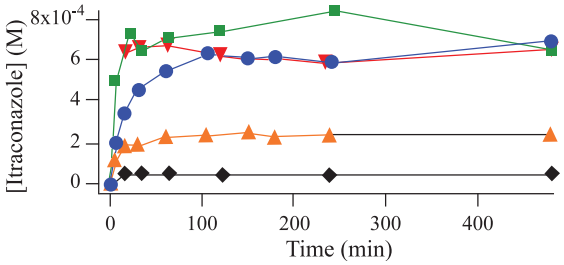


Fig. 9. Dissolution profiles into 0.1 N HCl at 25 °C plotted as itraconazole concentration ([itraconazole]) as a function of time for Sporanox® beads (■), crystalline itraconazole-free base (◆) and co-crystals of itraconazole with L-malic acid (▼), L-tartaric acid (●) and succinic acid (▲) (from [44], with permission).

Co-crystals represent a class of pharmaceutical materials of interest, both in terms of projected diversity and applicability. The study of co-crystals, along with polymorphs, solvates, salts and hydrates, is perfectly suited to HT crystallization experimentation and should be considered part of the form selection processes.

4. Post-screening analyses and form selection

Several functional characteristics must be considered in the selection of a suitable crystal form for a pharmaceutical dosage form. HT crystallization has the potential to create a larger pool of crystal forms for which functional parameters, such as dissolution rate, chemical stability, flow and compressibility, must be determined and compared. Strategies to accomplish ranking of the numerous forms must be devised. An example is the adaptation of HT for solubility measurement. The plot in Fig. 9 illustrates results of a plate-based kinetic dissolution assay in which various forms of a compound were placed in simulated gastric fluid and monitored for dissolution as a function of time. The schematic in Fig. 10 shows how such an analysis can be accomplished in a 96-well filter plate. The concentration at a given time point is determined after filtration of the suspension by quantification using either UV or HPLC with UV detection.

While the entire plate is filtered at one time, different time points can be achieved by timing the addition of dissolution medium such that the aliquot

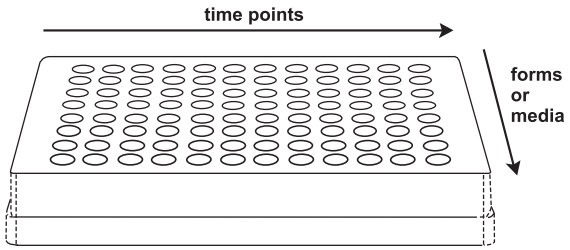


Fig. 10. Schematic of a 96-well dissolution filter plate.

for the longest time point desired is dispensed first and the shortest one comes last. Instead of varying the form along one axis of the plate, one can choose to study the dissolution of a single form into several different media (see Fig. 10). Equilibrium solubility can be determined in a variety of solvents and at different temperatures using a similar principle to the dissolution plate. A demonstration has been provided using automated React-IR analysis [109]. Other functional parameters, such as solid-state stability and thermal properties, can be adapted to HT. Such systems for ranking the stability of forms generated from HT crystallization await publication and review at a future date.

5. Summary and outlook

HT crystallization methodologies are capable of screening hundreds or thousands of crystallization conditions in parallel using small amounts of compound for the identification and characterization of diverse forms of active pharmaceutical ingredients. As demonstrated by numerous case studies from several stages of pharmaceutical development, such technologies have begun to show promise in enabling more comprehensive exploration of solid form diversity. The technologies are likely to provide a landscape of potential operating conditions from which scientists and engineers can design robust and scalable processes for transfer to manufacturing.

The ability to conduct extensive crystallizations with small amounts of material using a variety of solvents, additives and conditions necessarily generates large sets of data. However, the information by itself is of limited value, unless it can be properly analyzed. In order to extract maximum knowledge from the studies, it is essential to have the ability to design experiments, track samples in the process, collect the data in a relational database, and mine the information using statistical techniques and models in property space that assist the scientist to maximize the value of the data. Such models attempt to fit an output variable to physical properties or descriptors using techniques similar to those used in traditional quantitative structure activity relationships (QSAR). These models can be *carefully* extended to mixtures containing compounds that were not included in the original experiments if validation suggests that the models are sufficiently stable. Significant models that are found in the analysis of the data can be stored in the database for later retrieval and use to direct iterative experiments. The power of this approach becomes increasingly more visible when several properties are being co-optimized, as can be very important in the pharmaceutical development process where such properties as oral bioavailability, stability and processability need to be reconciled. The availability of a map of conditions that lead to the formation of different forms (salts, hydrates, solvates, polymorphs, co-crystals) of the drug can be valuable to the process chemists or engineers as they develop scalable processes to produce materials suitable for development and registration.

For many years, the value of composition of matter (CoM) patents on new chemical entities, including where appropriate, pharmaceutically acceptable salts, has been well appreciated. However, it is only within the last decade or so that the application of CoM patents has been significantly extended to cover all forms of the compound, including hydrates, solvates, co-crystals and polymorphs. Unlike salts, which for the most part can be prophetically claimed based on an understanding of the chemical structure of the compound and its ionization constants, the existence and identity of hydrates, solvates, co-crystals and polymorphs have defied prediction. Therefore, in order to obtain patent protection on these forms, some of which may have significantly different properties and relevance as development candidates, it is essential to prepare them, identify conditions for making them and evaluate their properties as valuable new pharmaceutical materials.

In general, discrete crystal forms are considered non-obvious and patentable. Given the diversity and greater complexity of chemical structures of today's

drug candidates [110], coupled with the advanced technology to identify novel forms, it is common to find multiple forms of drugs [61], some similar, some dramatically different in terms of their in vivo performance. These forms are all candidates for separate intellectual property protection. Therefore, it is incumbent on the innovator of a new drug candidate to identify and patent these forms in order to optimally protect their investment in the compound. Recent case studies suggest that identifying and patenting all forms of new chemical entities should be a primary strategy of all innovators of novel drugs. In this regard, the use of HT crystallization technologies for rapid, comprehensive discovery and characterization of solids form diversity offers significant advantages for the development of a strong intellectual property position.

With the advent of HT crystallization methods, appreciation for the landscape of physical form for drug development has begun to change. Use of these systems has the potential to facilitate drug development by saving valuable time in selecting the optimal physical or chemical form of a given compound. HT systems that generate rich datasets offer the ability to develop a more fundamental understanding of the crystallization process, based on knowledge generated from large numbers of experiments on diverse compounds. Having such information at an early stage minimizes the risk of process modifications resulting in form changes and provides the opportunity to gain more comprehensive intellectual property coverage. In addition, comprehensive form data help address important regulatory questions related to the number of solid forms of an API and the relationships between them.

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