DOI: 10.1021/cg900421v

CRYSTAL GROWTH & DESIGN Article
XXXX, Vol. XXX
000–000

# Polymorph Screening: Comparing a Semi-Automated Approach with a High Throughput Method

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*Received April 14, 2009; Revised Manuscript Received June 15, 2009*

**ABSTRACT:** Polymorph screening studies of sulfathiazole, mefenamic acid, flufenamic acid, and ROY were carried out using a semi-automated apparatus. Cooling crystallization and slurry aging experiments were conducted with varying process conditions and a selection of 16 diverse solvents to find as many polymorphic forms as possible. Results yielded four out of five polymorphs of sulfathiazole, both polymorphs and a solvate of mefenamic acid, four out of the seven stable forms of ROY, as well as the two most commonly encountered polymorphs and a solvate of flufenamic acid. The results obtained in this study were compared with a novel high throughput method based on patterned substrates of self-assembled monolayers.[17,32,38] It was shown that in the case of sulfathiazole and mefenamic acid the same number of polymorphs were obtained using the two approaches. In the case of ROY, the semi-automated approach was not able to produce three of the forms found using the patterned self-assembled monolayers (SAMs) method. These three forms were found in fewer than 1% of approximately 10 000 experiments performed using the high throughput approach and thus will be very difficult to find in the 58 experiments performed using the semi-automated approach. Results of this study demonstrate that the simple semi-automated approach of ~60 experiments described in this work is suitable for early stage polymorph screening as it was able to reproduce effectively the diversity of polymorphs in model compounds.



## Introduction

The ability of a compound to exist in more than one crystalline form is known as polymorphism. The phenomenon of a molecule existing in more than one solid-state structure is a result of differences in packing arrangement and/or molecular conformation.[1] Different polymorphs of the same compound exhibit different physical and chemical properties. One example of a compound showing such behavior is ritonavir, a protease inhibitor, developed by Abbott Laboratories. The appearance of a less-soluble second polymorph of ritonavir resulted in the need to reformulate the drug two years after it was launched.[2] In the case of acetaminophen, a well-known analgesic drug, form I of the compound lacks slip planes in its crystal structure, which make it unsuitable for direct compression into tablets. On the other hand, form II of the compound has well-developed slip planes which give it processing advantages over form I.[3]

The importance of discovering all polymorphs of an active pharmaceutical ingredient cannot be overstated. The late discovery of polymorphs can lead to a delay in the time to market for a drug. Once a drug is launched, discovery of new polymorphs can lead to patent protection issues. The U.S. Food and Drug Administration (FDA) also requires characterization of all possible polymorphs and identification of the stable form of a drug. Thus, polymorph screening is needed in the early stages of drug development.

The discovery of polymorphs requires extensive experimentation. Typically, a variety of factors such as supersaturation, agitation rate, cooling rate, solvent composition, temperature, seed crystals, additives, impurities, etc. are varied as they are known to affect crystallization.[4−7] Increasing the number of experiments leads to a higher possibility of identifying the majority of different polymorphs.[8] In a high throughput polymorphism study on acetaminophen, Peterson et al. obtained form II in only 29 out of 7776 trials.[9]

The use of technology to assist in parallel experimentation and polymorph screening is becoming increasingly common. Recently, Rubin et al. have presented a review of the emerging technologies supporting chemical process research and development and their impact on the pharmaceutical industry.[10] The use of automation to carry out experiments helps in reducing the time and labor required. As target drug materials are often available in limited quantities, methods that utilize minimal amount of material are particularly useful. Storey et al. presented an automated system for polymorph screening in combination with automated isolation of samples. High throughput powder X-ray diffraction (PXRD) was used to characterize the samples.[11] Raman spectroscopy has also been used to characterize crystals obtained from high throughput experiments and is particularly useful when the characterization needs to be rapid.[12] Recently, our group developed a small-scale automated solubility measurement apparatus, which offers substantial savings in material, time, and labor.[13] This apparatus can also be used for solvent screening before polymorph screening experiments are carried out.

The crystal form produced from solution is the result of competing thermodynamic and kinetic factors that govern crystallization of polymorphs. The polymorph with lower free energy is the thermodynamic stable form, whereas the other polymorphs are known as metastable forms. According to Ostwald's rule of stages, the metastable form is the first to crystallize, followed by transformation to the more stable form.[14] This transformation proceeds in many cases through a dissolution−recrystallization mechanism. Under certain conditions, the transformation process can be hindered or suppressed, leading to the generation of a metastable polymorph as the final crystal form.

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The main controlling factors in the crystallization of polymorphs include temperature, supersaturation, and type of solvent, as well as the addition of seed crystals, stirring rate, and interfaces.[15] It is well-known that in enantiotropic systems, the thermodynamic stability order among polymorphs can be inverted by shifting temperature above and below the transition temperature.[16] Moreover, the temperature can change the dissolution rate, and the kinetics of nucleation and growth of each polymorph retarding the appearance of certain polymorphs and promoting others. Also, it has been shown that a rapid generation of supersaturation provides crystals of different polymorphic forms when compared with those obtained with a slow increase in supersaturation.[17] In the case of the effect of solvents, the interactions between solute and solvent molecules result in solute molecules assembling in particular conformation structure an/or packing mode.[18]

There is, as yet, no failsafe method to predict the extent of polymorphism of a given compound. Hence, subjecting the active pharmaceutical ingredient (API) to a variety of crystallization conditions is the only method that can expose the diversity of its forms. High throughput polymorph screening methods allow researchers to carry out a large number of crystallization experiments while providing savings in time, material, and labor. Systems such as the fully automated crystallization platform CrystalMax, developed by Transform Pharmaceuticals Inc., are capable of carrying out more than 10 000 parallel crystallization experiments using $< 1$ mg of the active pharmaceutical ingredient (API) per trial.[12] Symyx Technologies, Inc. has also developed high throughput systems which include solid dispensers and liquid handlers with complete automation, as well as informatic capabilities to support polymorph screening studies.[19] However, the high cost of these systems makes them unaffordable for a number of research laboratories.

In this work, we evaluated a simple and relatively inexpensive semi-automated method to carry out initial polymorph screens. We assessed the React Array RS12 from Barnstead International as a platform for polymorph screening studies. We used the RS12 platform to evaluate the effect of initial temperature, cooling rate, and type of solvent on the crystallization of polymorphic forms of model APIs. Experiments on sulfathiazole (64), mefenamic acid (66), acetaminophen (66), flufenamic acid (68), and ROY (58) were carried out and compared to a high throughput method developed in this laboratory[17] employing patterned self-assembled monoloayers.

## Experimental Section

**Materials.** Sulfathiazole, 4-amino-*N*-(2,3-dihydro-2-thiazolyidene)-benzenesulfonamide, is an antibacterial drug. It possesses multiple solid forms and has been used as a model pharmaceutical compound in the study of polymorphism.[20,21] Sulfathiazole has five known polymorphs.[22] The Cambridge Structural Database reference codes for the five forms are Suthaz, Suthaz01, Suthaz02, Suthaz04, and Suthaz05. It is also known to form over 100 solvates due to its multiple hydrogen bonding capabilities.[23]

Mefenamic acid, 2-[(2,3-(dimethylphenyl)amino] benzoic acid, is a nonsteroidal anti-inflammatory, antipyretic, and analgesic agent used to release pain and inflammation. Mefenamic acid has two crystalline forms, form I and form II.[24] Forms I and II are enantiotropically related with a transition temperature between 86 to 87 °C. Form I is the stable form below this temperature while form II is stable above it.[25]

Acetaminophen is an important analgesic and antipyretic drug. It is used worldwide in the manufacture of tablets and other dosage forms. It has three known polymorphs, forms I, II, and III. Form I is the thermodynamically stable form at room temperature while form III is very unstable. Form I is readily obtained from aqueous solution; however, obtaining form II from solution has proved difficult. Form II is readily obtained by melt crystallization after melting form I.[3]

5-Methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile, commonly known as ROY for its red, orange, and yellow crystals, is a precursor to the antipsychotic agent olanzapine.[26] ROY is currently the most polymorphic system of known structures. ROY has 10 known polymorphs, seven with solved structures (Y-yellow prism, YN-yellow needle, YT04-Y04 transformed, ON-orange needle, OP-orange plate, ORP-orange red plate, and R-red prism) and three whose structures have not been solved (Y04-yellow (2004), RPL-red plate, and R05-red (2005)). At room temperature, Y is the most stable form.[27]

Flufenamic acid, 2-([3-(trifluoromethyl) phenyl] amino) benzoic acid, is a potent nonsteroidal drug with analgesic, anti-inflammatory, and antipyretic properties. It has been reported that FFA has at least eight polymorphs,[28] although forms III and I are the most commonly encountered. Most of the other polymorphs can only be obtained by sublimation, fusion, or a boiling solvent method, and cannot be isolated easily. Form III is the stable form at room temperature, and forms III and I are enantiotropic, with a transition temperature of 42 °C.

The pharmaceutical products sulfathiazole, mefenamic acid, acetaminophen, and flufenamic acid were purchased from Sigma Aldrich Chemicals and were used without further purification. ROY as forms R and Y was a gift from Eli Lilly & Company. Deionized water was obtained from a Barnstead Nanopure Infinity water purification system. *N*,*N*-Dimethylformamide (99.95%), dimethylsulfoxide (99.99%), ethanol (200 proof), and acetonitrile were supplied from Pharmco Products. *N*,*N*-Dimethylacetamide (99%), formamide (98%), and acetone (99.5%) were acquired from Sigma Aldrich Chemicals. 1,4-Dioxane (99%) and chloroform (99.8%) were obtained from Fisher Scientific. *n*-Propanol (99.9%) was purchased from Mallinckrodt. Benzonitrile (99%), methyl *tert*-butyl ether (99%), *N*-methyl pyrrolidone (99%), and *o*-tolunitrile (98%) were supplied from Acros Organics.

**Experimental Apparatus.** A Barnstead ReactArray Workstation was used to perform crystallization and slurry aging experiments in the present work. The workstation integrates a Gilson 175SW liquid handler and syringe pump with reaction and reagent racks. The dual-syringe pump has two syringes with capacities of 500 $\mu$L and 10 mL. The system has two RS12 reaction racks and each rack holds 48 glass vials arranged in 12 rows of 4 vials each. The volume of the vials is ~2 mL. Each row in a reaction rack can be given an independent temperature profile and the temperature range is −30 to 150 °C. The maximum controlled heating/cooling rate is 5 °C / min while the minimum is 0.1 °C /min. Micro magnetic stirring bars can be used for stirring with a stirring speed range of 250−1200 rpm. There are two reagent racks in the system that can hold 6 (~130 mL each) and 18 (~37 mL each) reagent vials, respectively. The system is connected to a computer and can be controlled through the ReactArray control software.

A Barnstead Clarity system was used for solubility measurement. The solubility measurement was carried out for solvent screening purposes before designing polymorph screening experiments. The system consists of a RS10 reaction block and a multi-IR unit connected to a computer and controlled by the RSPCclient software. Solubility data can be obtained from solution volumes as low as 1 mL. The RS10 block has 10 independently controlled cells with independent temperature zones and stirring rates. The temperature range is −30 to 150 °C. The maximum controlled heating/cooling rate is 5 °C /min while the minimum is 0.1 °C /min. The multi-IR unit consists of 10 IR turbidity probes. The software generates a plot of the IR value vs temperature, and a sharp increase in the IR value at a particular temperature indicates a solubility point.

Crystals obtained were characterized using Raman spectroscopy. Raman spectra were obtained using a Raman Microprobe from Kaiser Optical Systems, Inc. The Raman microprobe was equipped with a 450-mW external cavity stabilized diode laser as the excitation source, operating at 785 nm. The unit consisted of a Leica optical light microscope, a motorized translational stage, and a CCD camera. Data were collected with HoloGRAMS version 4.0

**Table 1. Initial Temperature for Cooling Crystallization Experiments**

| compound | solvent(s) | initial temperature (°C) high | intermediate | low |
|---|---|---|---|---|
| sulfathiazole | W, P | 90 | 65 | 30 |
| | A, AC | 48 | 38 | 30 |
| mefenamic acid | BZN, DMF, T, DMA | 145 | 90 | 30 |
| | A, P, MTBE, AN | 50 | 40 | 30 |
| acetaminophen | DMF, W, DO | 90 | 60 | 30 |
| | E, P, A, AN | 50 | 40 | 30 |
| ROY | BZN, DMF, T, DMA | 90 | 60 | 30 |
| | NMP, DO, AN | 75 | 60 | 30 |
| flufenamic acid | BZN, DO, T, DMA, | 90 | 60 | 30 |
| | A, P, CYC, AN | 60 | 45 | 30 |

and processed and analyzed using GRAMS (Thermo Electron Corporation).

**Procedure.** The polymorph screening crystallizations were performed using the Barnstead ReactArray Workstation. Two approaches were explored to produce different solid forms of the pharmaceutical products: cooling crystallization and slurry aging. In cooling crystallization, a solution was cooled at a controlled rate to create supersaturation and promote the formation of crystal polymorphs. The crystals were immediately characterized using Raman spectroscopy to try to prevent their transformation to a more stable form. In the slurry aging experiments, particles were suspended in different solvents for a long equilibration period to allow polymorphic transformation.

**Cooling Crystallization Experiments.** A total of 40 solutions of sulfathiazole (SZ) were prepared by placing a measured amount of solid in 2 mL glass vials. In order to estimate the initial concentration of the solutions, preliminary solubility tests were carried out using the small-scale automated apparatus developed in our group[13] to obtain solubility data as a function of temperature. A volume of 1.5 mL of solvent was automatically dispensed into each glass vial using the robot arm of the Barnstead System. The following solvents were used: water (W), *n*-propanol (P), acetone (A), and a mixture (3:2) of acetone/chloroform (AC). Each vial was heated to reach the initial temperature, as per the experimental design. Three different levels of initial temperature: high (HT), intermediate (IT), and low (LT) were explored as shown in Table 1. The heating rate was 5 °C/min. Stirring rate was constant during the experiment. Vials were maintained at the initial temperature for at least 30 min for complete dissolution. Then, solutions were cooled down to 10 °C at either a slow (1 °C/min) or fast (5 °C/min) cooling rate, according to the experimental design. Once the solutions had reached the final temperature, the reflux head was removed, and each vial was manually removed from the well plate. The crystals were harvested with a spatula and immediately analyzed with Raman Spectroscopy. The Raman spectra obtained were compared with standard reference spectra of the known polymorphs of the compound to identify the type of polymorph obtained.

Mefenamic acid (MA) was crystallized with the same procedure as described above. A total of 48 solutions were prepared with 8 different solvents: benzonitrile (BZN), *N*,*N*-dimethylformamide (DMF), *o*-tolunitrile (T), *N*,*N*-dimethylacetamide (DMA), acetone (A), *n*-propanol (P), methyl *tert*-butyl ether (MTBE), and acetonitrile (AN). Forty-two experiments were conducted using acetaminophen with seven different solvents: DMF, water (W), 1,4-dioxane (DO), ethanol (E), *n*-propanol (P), acetone (A), and acetonitrile (AN). A total of 42 experiments with ROY were conducted. BZN, DMF, *o*-tolunitrile (T), DMA, NMP, 1,4-dioxane (DO), and acetonitrile (AN) were the solvents used. Finally, 48 experiments were conducted using flufenamic acid with eight different solvents: benzonitrile (BZN), 1,4-dioxane (DO), *o*-tolunitrile (T), *N*,*N*-dimethylacetamide (DMA), acetone (A), *n*-propanol (P), cyclohexane (CYC), and acetonitrile (AN).

**Slurry Aging Experiments.** A total of 24 suspensions of sulfathiazole (SZ) were prepared by placing an excess of solid in 2 mL glass vials and adding 1.5 mL of the following solvents: DMSO, BZN, DMF, DMA, formamide (F), NMP, acetone (A), MTBE, *n*-propanol (P), water (W), ethanol (E), and cyclohexane (CH). Each experiment was duplicated.

**Table 2. Experimental Temperature for Slurry Aging Experiments**

| compound | solvent(s) | temperature (°C) |
|---|---|---|
| sulfathiazole | DMSO, BZN, DMF, DMA, F, NMP | 110 |
| | A, MTBE | 45 |
| | P, W, E, CH | 70 |
| mefenamic acid | BZN, DMF, T, DMA | 110 |
| | A, MTBE | 40 |
| | P, AN, W | 70 |
| acetaminophen | DMF, DMSO, F, NMP | 110 |
| | W, DO | 85 |
| | E, P | 60 |
| | A, MTBE | 45 |
| | CH, AN | 70 |
| ROY | AN | 70 |
| | F, W, DMSO, P, BZN, T, DO | 90 |
| flufenamic acid | BZN, DO, T, DMA | 90 |
| | A, P, CYC, AN, W, M | 60 |

Each vial was heated to reach the experimental temperature, as per the experimental design shown in Table 2. The heating rate was 5 °C/min. Stirring rate was constant during the experiment. Vials were maintained overnight at the experimental temperature. At the end of the experiment, the reflux head was removed and each vial was manually removed from the well plate. The crystals were harvested with a spatula and immediately analyzed with Raman spectroscopy. The Raman spectra obtained were compared with standard reference spectra of the known polymorphs of the compound to identify the type of polymorph obtained. Eighteen experiments were conducted using mefenamic acid with nine different solvents: BZN, DMF, *o*-tolunitrile, DMA, acetone, *n*-propanol MTBE, acetonitrile, and water, and 24 experiments were conducted using the compound acetaminophen with 12 different solvents: DMF, DMSO, water, 1,4-dioxane, ethanol, *n*-propanol, acetone, MTBE, cyclohexane, acetonitrile, formamide, and NMP. Sixteen experiments were conducted using ROY with eight different solvents: acetonitrile, formamide, water, DMSO, *n*-propanol, BZN, *o*-tolunitrile, and 1,4-dioxane. Finally, 20 experiments were conducted using flufenamic acid with 10 solvents: BZN, 1,4-dioxane, *o*-tolunitrile, DMA, acetone, *n*-propanol cyclohexane, acetonitrile, water, and methanol.

## Results and Discussion

**Sulfathiazole. Cooling Crystallization Experiments.** Four out of the five polymorphs of sulfathiazole were obtained in our experiments, as shown in Table 3. There is some confusion in the literature regarding the nomenclature for different polymorphs of sulfathiazole as noted by Blagden et al.[29] and Apperley et al.[21] We have used the notation of the Cambridge Structural Database reference codes in this report. The stability order of the polymorphs is Suthaz04 > Suthaz02 > Suthaz > Suthaz01 > Suthaz05.[22,29] Figure 1 shows the Raman spectra of four of the five polymorphs of sulfathiazole.

Suthaz02 and Suthaz04 were obtained in cooling crystallization experiments with water as the solvent. When solutions were cooled from high temperature Suthaz04 crystals were obtained in both fast and slow cooling experiments. Fast cooling from intermediate temperature gave Suthaz02 crystals in one vial and Suthaz04 crystals in the second vial. No crystals were obtained in fast cooling from low temperature experiments while slow cooling from low temperature gave Suthaz02 crystals. Blagden et al. have previously reported that crystallization of sulfathiazole from water favors Suthaz04.[30]

Suthaz01, Suthaz02, and Suthaz04 were obtained from *n*-propanol solutions. It has been reported in the literature that crystallization in *n*-propanol favors Suthaz01.[30,31] However, in our experiments three different polymorphs of sulfathiazole were obtained from *n*-propanol solutions.

**Table 3. Results Obtained from Sulfathiazole Cooling Crystallization Experiments**

| | initial temperature | | | | |
|---|---|---|---|---|---|
| | high | | intermediate | low | |
| solvent | 5 °C/min | 1 °C/min | 5 °C/min | 5 °C/min | 1 °C/min |
| water | Suthaz04 | Suthaz04 | Suthaz02 (vial 1) and Suthaz04 (vial 2) | no crystals | Suthaz02 |
| *n*-propanol | Suthaz02 (vial 1) and Suthaz04 (vial 2) | Suthaz02 (vial 1) and Suthaz04 (vial 2) | Suthaz01 (vial 1) and Suthaz04 (vial 2) | no crystals | Suthaz02 |
| acetone | Suthaz+Suthaz02 mixture (vial 1) and Suthaz02 (vial2) | Suthaz+Suthaz02 mixture | Suthaz+Suthaz02 mixture | Suthaz+Suthaz02 mixture | Suthaz02 |
| acetone/chloroform | Suthaz+Suthaz02 mixture (vial 1) and Suthaz02 (vial 2) | Suthaz+Suthaz02 mixture | Suthaz+Suthaz02 mixture | Suthaz+Suthaz02 mixture | Suthaz+Suthaz02 mixture |

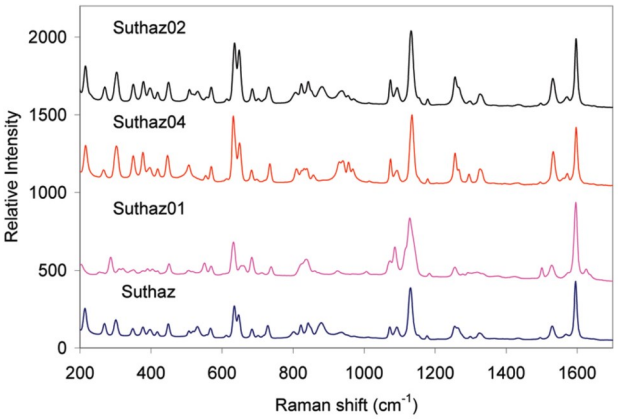


**Figure 1.** Raman spectra of sulfathiazole polymorphs.

Suthaz02 and mixtures of Suthaz and Suthaz02 were obtained. It has been previously reported that only Suthaz01 and Suthaz can be obtained from acetone, while Suthaz01, Suthaz04, and Suthaz can all be obtained from acetone/chloroform (3:2).[31]

**Effect of Solvent on the Polymorphic Outcome of Sulfathiazole.** Sulfathiazole has been previously used as a model compound to study the effect of solvent on crystallization of polymorphs.[30,31] It has been reported that crystallization of sulfathiazole from *n*-propanol solutions favors Suthaz01. However, in a paper on solvates of sulfathiazole, Bingham et al. have noted that sulfadrugs crystallize erratically from solution, despite the contrary impression that might be gained from the literature.[23] Lee et al. also reported that although the type of solvents employed can influence the crystallization outcome, sulfathiazole might not be an accurate example of this behavior.[32] Hughes et al. have also noted the erratic crystallization of sulfathiazole, usually as mixtures of polymorphs, from solution and how guaranteed recipes for producing single polymorphs are difficult to obtain.[33]

Our results using the semi-automated polymorph screening equipment also support the latter view as mixtures of polymorphs were frequently obtained and forms obtained from particular solvents were different than those previously reported. When carrying out experiments with *n*-propanol as the solvent, we were able to obtain forms Suthaz01, Suthaz02, and Suthaz04 contrary to previous reports.[30,31] In the case of water Suthaz04 and Suthaz05 have been reported to be the preferred forms; however, we obtained Suthaz02 and Suthaz04. Because of the stochastic nature of nucleation from solution extensive experimentation is needed to acquire a better understanding of solid form diversity particularly for compounds such as sulfathiazole. The use of automation in experimentation helps in carrying out a high number of experiments while providing savings in time and labor.

**Table 4. Results Obtained from Sulfathiazole Slurry Aging Experiments**

| solvent | polymorph |
|---|---|
| DMSO | no crystals |
| BZN | no crystals |
| DMF | no crystals |
| DMA | no crystals |
| formamide | no crystals |
| NMP | no crystals |
| acetone | Suthaz02 (100%) |
| MTBE | Suthaz02 (100%) |
| *n*-propanol | Suthaz02 (100%) |
| water | Suthaz02 (100%) |
| ethanol | Suthaz02 (100%) |
| cyclohexane | Suthaz02 (100%) |

**Slurry Aging Experiments.** Crystals were obtained in 50% of the experiments. In the remaining 50% of the experiments the solute was dissolved in the solvent. Because of the high solubility of sulfathiazole in some solvents it was not possible to form a slurry in the 2 mL reaction vials. No polymorphic transformation was observed in the slurry aging experiments, as shown in Table 4. The Raman spectra of all the crystals obtained matched that of form Suthaz02, which is the commercial form.

**Comparison of Results Obtained with a High-Throughput Approach.** Recently, our group has developed patterned substrates of self-assembled monolayers (SAMs) which can be used to carry out a large number of independent crystallization trials with a minimal amount of material.[17] We have previously used this method to perform polymorph screening experiments with sulfathiazole.[32] It is important to note here that the experiments carried out in each case were different; in the SAMs experiments evaporation of solvent was used to create supersaturation while cooling crystallization and slurry aging experiments were carried out in the present work. However, it is interesting to compare the results obtained from a polymorph screening perspective.

When comparing the results obtained in our current experiments with the SAMs experiments we find that in the case of sulfathiazole the same four polymorphs (out of the five known forms) were obtained using the two approaches. Although the amount of material required per crystallization trial is low when using our present approach, it is even lower in the SAMs experiments, for example, in the sulfathiazole cooling crystallization experiments, the amount of sulfathiazole required for each trial varied from 0.6 to 33.75 mg. In the case of SAMs, the material required per trial is often as low as 0.01−0.02 mg. When studying the effect of solvent on the

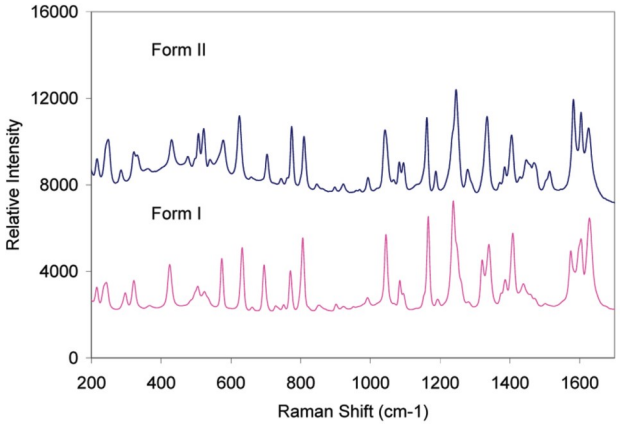


**Figure 2.** Raman spectra of mefenamic acid polymorphs.

polymorphic outcome and to compare the results with those obtained while carrying out conventional crystallization experiments, the present approach is more suited. This is because in the present approach each trial is similar to a conventional crystallization experiment while the scale of the experiments is smaller to provide savings in material and some degree of automation is added to provide savings in time and labor. In the case of SAMs template nucleation takes place and factors such as the monolayer may affect the polymorph obtained in an experiment. The total number of islands (crystallization trials) tested for sulfathiazole using patterned SAMs was 4200.

**Mefenamic Acid. Cooling Crystallization Experiments.** Crystals were obtained in 96% of the cooling crystallization experiments. Lee et al., reported three distinct characteristic peaks for the two polymorphic forms of mefenamic acid.[32] Raman characteristic peak positions are 623, 702, and 1581 $cm^{-1}$ for Form I, and 631, 694, and 1573 $cm^{-1}$ for Form II. Figure 2 shows the Raman spectra of mefenamic acid polymorphs. Comparing the experimental Raman spectra obtained in each experiment against the characteristic peak position, Form I was identified in 54% of the experiments, and Form II in 19% as shown in Table 5.

Both polymorphs were nucleated by cooling crystallization in methyl *tert*-butyl ether (MTBE). Form I was observed at a slow cooling rate from high and intermediate temperature. At a fast cooling rate, Form II was obtained from high and intermediate temperature. The metastable Form II was also observed when crystallized from low temperature at fast and slow cooling rates.

It is known that metastable solid forms are favored with the creation of high supersaturation. Lee et al. obtained the metastable $\beta$-glycine as a result of the high supersaturation generated on confined engineered surfaces.[17] Kitamura observed that only the metastable B-form of L-hystidine crystallized by rapid cooling a mixed solvent water−ethanol solution with high ethanol fraction.[34] The preferred appearance of the metastable form is observed when a less stable state can be reached faster because its kinetics is faster than the stable state. In our experiments, the appearance of the metastable form II of mefenamic acid with MTBE at a fast cooling rate at high, intermediate, or low temperature can be explained as the result of the high supersaturation that is generated from the rapid cooling.

The metastable form II of mefenamic acid was obtained from acetone by cooling crystallization in four out of six

**Table 5. Mefenamic Acid Polymorphs Obtained in Cooling Crystallization Experiments**

| | initial temperature | | | | | |
|---|---|---|---|---|---|---|
| | high | | intermediate | | low | |
| solvent | 5 °C/min | 1 °C/min | 5 °C/min | 1 °C/min | 5 °C/min | 1 °C/min |
| benzonitrile | I | I | I | II | I | I |
| DMF | II | II | II | II | II | II |
| *o*-tolunitrile | I | | I | I | I | I |
| DMA | | I | I | I | I | I |
| acetone | I | II | II | I | II | II |
| *n*-propanol | I | I | I | I | I | I |
| MTBE | II | I | II | I | II | II |
| acetonitrile | I | I | I | I | I | I |

**Table 6. Mefenamic Acid Polymorphs obtained in Slurry Aging Experiments**

| solvent(s) | polymorph |
|---|---|
| benzonitrile | I |
| DMF | II |
| *o*-tolunitrile | I and II |
| DMA | |
| acetone | I |
| propanol | I |
| MTBE | I |
| acetonitrile | I |
| water | I |

experiments. These results can be explained as an experimental example of Ostwald's rule, which suggests that metastable form is the first crystal form to crystallize, followed by solvent mediated transformation to the stable form. Moreover, the stable form I was observed when crystallized from high and intermediate temperature with a fast and slow cooling rate, respectively. The increase in the rate of transformation of form II into form I with increased temperature, reported by Osuka,[35] may explain the appearance of the stable form I at high and intermediate temperature.

Interaction between solvent and solute molecules can promote the nucleation of a metastable form and inhibit the formation of the stable form. Blagden[29] explained this phenomenon as a result of inhibiting nucleation and/or growth by adsorbing on the fastest growing faces of the crystal. The metastable Form II of mefenamic acid was obtained through cooling crystallization from DMF at all experimental conditions. These results agree with data obtained by Aguair,[36] Otsuka,[35] and Cesur,[37] who observed that crystallization of MA form II is induced by DMF. Together with the metastable form II, the presence of a solvate was observed, as indicated by additional vibrational bands in the Raman spectra. This solvate has been previously reported by Lee.[28]

Metastable form II of mefenamic acid was also obtained with benzonitrile when crystallized from intermediate temperature at a slow cooling rate. This may be due to the stochastic nature of nucleation of different polymorphic forms. Other solvents were screened with mefenamic acid, including *o*-tolunitrile, *N*,*N*-dimethylacetamide, *n*-propanol, and acetonitrile. Under the experimental conditions explored with these solvents, form I was solely observed.

**Slurry Aging Experiments.** Crystals were obtained in 89% of the slurry aging experiments, as shown in Table 6. Form I was obtained in most of the solvents, except for DMF and *o*-tolunitrile. In the case of DMF, it was previously observed in the cooling crystallization experiments that this solvent favored the formation of the metastable Form II.

A mixture of forms I and II was observed in the slurry aging experiments with *o*-tolunitrile at 110 °C as evidenced

**Table 7. Results Obtained from ROY Cooling Crystallization Experiments**

| | initial temperature | | | | | |
|---|---|---|---|---|---|---|
| | high | | intermediate | | low | |
| solvent cooling rate | 5 °C/min | 1 °C/min | 5 °C/min | 1 °C/min | 5 °C/min | 1 °C/min |
| benzonitrile | Y | Y | Y | Y | Y | Y |
| DMF | Y | Y | Y | Y | Y | Y |
| *o*-tolunitrile | Y | Y | Y | Y | OP + Y | Y |
| DMA | Y | Y | Y | Y | Y | Y |
| NMP | Y | Y | Y | Y | Y | Y |
| 1,4-dioxane | Y | Y | Y | Y | Y | Y |
| acetonitrile | Y | Y | R | Y | Y | Y |

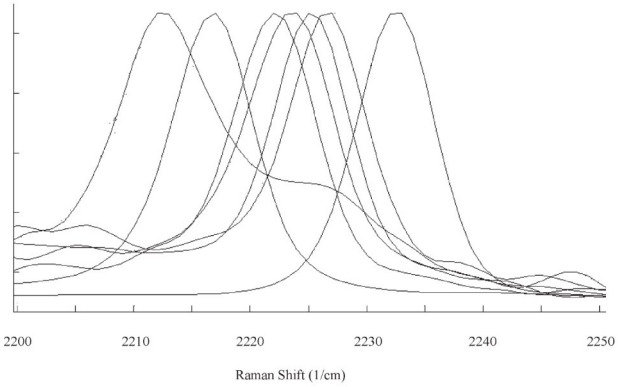


**Figure 3.** The nitrile stretch (2200−2250 $cm^{-1}$) of the Raman spectra of the seven stable ROY polymorphs (peaks from left to right: R, ORP, YN, ON, YT04, OP, and Y).

by the presence of the characteristic peaks of both polymorphs. As mefenamic acid is an enantiotropic system with transition temperature between 86 and 87 °C, partial transformation to form II at temperature higher than 87 °C would explain the presence of both polymorphic forms.

**Comparison of Results Obtained with a High-Throughput Approach.** When comparing the results obtained in the semi-automated system with the patterned SAMs[32] polymorph screening experiments, we find that in the case of mefenamic acid the same two polymorphs were obtained using the two approaches. The total number of islands (crystallization trials) tested for mefenamic acid using patterned SAMs was 1200. For a system such as mefenamic acid with a limited number of known forms a simple method such as the semi-automated approach described in this work seems to offer advantages when carrying out polymorph screening experiments as it is able to reproduce the diversity of polymorphs in a short time scale evaluating different process conditions and combination of solvents.

**Acetaminophen.** Only one polymorph, the stable form I, was identified for acetaminophen in both cooling crystallization and slurry aging experiments, even though a number of solvents (8 solvents for cooling crystallization and 12 solvents for slurry aging), as well as a range of process conditions were explored. The appearance of the metastable form II of acetaminophen seems to be very elusive as suggested by a previous high throughput polymorphism study on acetaminophen where Peterson et al. obtained form II in only 29 out of 7776 trials.[9]

**ROY. Cooling Crystallization Experiments.** Three out of the seven stable polymorphs of ROY, yellow prism, red prism, and orange plate, were obtained in our experiments, as shown in Table 7. The Raman spectra of the polymorphs of ROY are different from each other, and the peak positions

**Table 8. Results Obtained from ROY Slurry Aging Experiments**

| solvent(s) | polymorph |
|---|---|
| acetonitrile | red prism (vial 1) and yellow prism (vial 2) |
| formamide | yellow prism |
| water | yellow prism |
| *n*-propanol | orange needle |
| dimethylsulfoxide | orange needle (vial 1) and yellow prism (vial 2) |
| benzonitrile | orange needle |
| *o*-tolunitrile | orange needle |
| 1,4-dioxane | yellow prism |

in the nitrile stretch (2200−2250 $cm^{-1}$) have been used previously to distinguish between them.[26,27] Figure 3 shows the nitrile stretch of the Raman spectra of the seven stable polymorphs of ROY. ROY crystallized as the most stable yellow prism form in 40 out of the 42 vials. In one vial red prism crystals were obtained and a mixture of yellow prism and orange plate crystals was obtained in another vial.

We recently reported the results of almost 20 000 crystallization trials using patterned substrates of self-assembled monolayers, with ROY as a model compound.[38] Interestingly, during the 20 000 trials, orange plate was the only stable polymorph of ROY that was not obtained. It has previously been reported that solution-grown orange plate crystals are difficult to obtain without seeds.[39] Orange plate crystals have been obtained by room temperature seeding of an ethylene glycol solution saturated at 60 °C, with the seeds obtained by heating form R at 90 °C for 3 days.[40]

**Slurry Aging Experiments.** Three out of the seven stable polymorphs of ROY, yellow prism, red prism, and orange needle, were obtained in our experiments, as shown in Table 8. Red prism crystals were obtained in only one vial, while yellow prism and orange needle crystals were obtained in a number of experiments. The experimental temperature was 70 °C for the acetonitrile slurry and 90 °C for all other slurries, and the starting material for all our experiments was a mixture of forms red prism and yellow prism. ROY is an enantiotropic system and the free energy-temperature diagram of its polymorphs has been previously reported.[27] While yellow prism is the most stable form at room temperature, orange needle is the most stable at 90 °C. The appearance of orange needle crystals can be explained by dissolution and recrystallization to the most stable form at a particular temperature.[8,41] In other experiments in which yellow prism crystals were obtained, it is possible that the crystals could not transform to the orange needles overnight and may need more time to transform to the most stable form (at 90 °C).

**Comparison of Results Obtained with a High-Throughput Approach.** Recently, we used patterned substrates of SAMs to carry out polymorph screening experiments using ROY.[38] While we obtained four forms of ROY (Y, R, OP, ON) in our present experiments, we had obtained seven polymorphs

**Table 9. Flufenamic Acid Polymorphs Obtained in Cooling Crystallization Experiments**

| | initial temperature | | | | | |
|---|---|---|---|---|---|---|
| | high | | intermediate | | low | |
| solvent | 5 °C/min | 1 °C/min | 5 °C/min | 1 °C/min | 5 °C/min | 1 °C/min |
| benzonitrile | III | III | III | III | III | |
| 1,4-dioxane | I | I | III | III | III | III |
| *o*-tolunitrile | III | III | III | III | III | III |
| DMA | | solvate | solvate | solvate | solvate | solvate |
| acetone | III | III | III | III | | |
| *n*-propanol | I | I | III | III | | III |
| cyclohexane | III | III | III | III | | |
| acetonitrile | III | III | III | III | | |

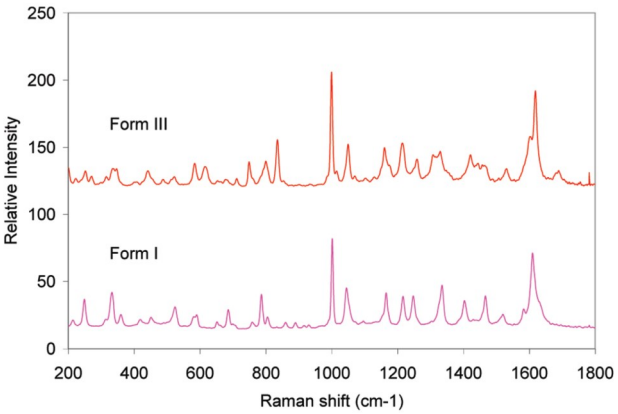


**Figure 4.** Raman spectra of flufenamic acid polymorphs.

(out of the 10 known forms) using patterned SAMs (Y, R, ON, YN, ORP, YT04, R05). For a system such as ROY with a very high number of known forms, a high throughput method seems to offer advantages when carrying out polymorph screening experiments. Also, while the amount of ROY required in the cooling crystallization experiments in the present work is relatively low and varied from 75 to 1000 mg per trial, the patterned SAMs were used to carry out almost 20 000 crystallization trials using approximately 200 mg of ROY. Although using patterned SAMs offered the above-mentioned advantages, for an enantiotropic system such as ROY high temperature slurry aging offers another route to obtain different polymorphs and these experiments can easily be carried out using the present approach.

**Flufenamic Acid. Cooling Crystallization Experiments.** Three different forms of flufenamic acid were obtained in cooling crystallization experiments: form I, form III, and a third form that was tentatively assigned to solvate and which is currently under further investigation to characterize. Table 9 summarizes the results of the experiments.

Crystals were obtained in 81% of the experiments, with 8, 10, and 63% corresponding to form I, solvate, and form III, respectively. Crystal form was identified with Raman spectroscopy. Figure 4 show the Raman spectra for flufenamic acid polymorphs.

FFA is an enantiotropic system where form III is stable at temperature lower than 42 °C and form I is stable at temperatures higher than 42 °C. Form I was obtained in cooling crystallization experiments at high temperature for both fast and slow cooling rates with 1,4-dioxane and *n*-propanol, whereas form III was obtained for intermediate and low temperature and the same solvents. This observation can be explained by the fact that the high temperature

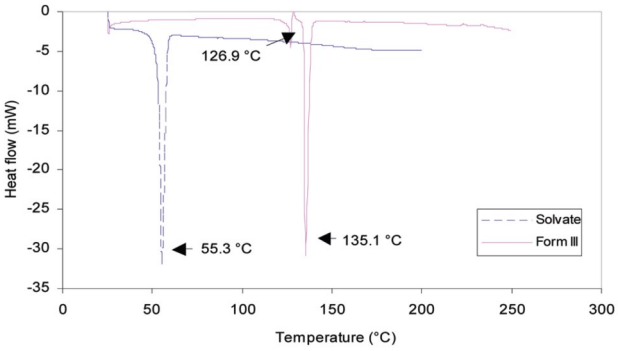


**Figure 5.** DSC curve of pure flufenamic acid form III and solvate.

**Table 10. Flufenamic Acid Polymorphs Obtained in Slurry Aging Experiments**

| solvent(s) | polymorph |
|---|---|
| benzonitrile | I |
| 1,4-dioxane | I |
| *o*-tolunitrile | I |
| DMA | solvate |
| acetone | III |
| propanol | I |
| cyclohexane | I |
| acetonitrile | I |
| water | I |
| methanol | III |

utilized in the experiments (90 °C for 1,4-dioxane and 60 °C for *n*-propanol) was higher than the transition temperature (42 °C); thus, it is possible that crystallization occurred at a temperature for which the thermodynamically stable form is form I.

The presence of a solvate was observed when DMA was the solvent at all initial temperatures and all cooling rates. The solvate was characterized by differential scanning calorimetry using a Mettler DSC 822e instrument with 10 °C/min heating rate. Figure 5 compares the differential scanning calorimetry (DSC) curves of the solvate and flufenamic acid form III. In the case of the solvate, DSC showed a melting point of 55.3 °C, whereas form III displayed an initial transition point at 126.9 °C, corresponding to the transition from form III to form I, followed by a melting point at 135.1 °C, which is similar to the thermal behavior reported by Romero, et al.[42] for flufenamic acid.

**Slurry Aging Experiments.** Form I was obtained in 70% of the slurry aging experiments, as shown in Table 10. The starting material for all the experiments was flufenamic acid form III, which is the stable from at room temperature. As described previously, forms I and III of flufenamic acid represent an enantiotropic system with a transition temperature of 42 °C. All slurry aging experiments were conducted at a temperature higher than the transition temperature; thus, results showed that in most of the cases the initial form III was transformed to the stable form I as expected at high temperature.

In the case of experiments conducted with dimethylacetamide, the new form (solvate) described in the cooling crystallization section was also obtained in slurry aging experiments.

## Conclusions

A semi-automated apparatus for polymorph screening has been evaluated and compared to a high throughput method. The system is capable of carrying out polymorph screening

experiments using ~2 mL solution volume per experiment. Different crystallization conditions can be evaluated by varying the heating/cooling rates and the initial and final temperatures. By immediately characterizing the crystals obtained in cooling crystallization experiments, their transformation to a more stable form may be prevented, thus offering an advantage over conventional experiments where the most stable form is often obtained. Only one Raman spectrum was collected per sample to minimize the time required to characterize all samples. Because of this, it is possible that in some cases mixtures of polymorphs may have been characterized as a single polymorph. This apparatus may be further improved by automation of sample recovery and characterization. This would provide savings in the time needed to characterize samples and enable the researcher to collect Raman spectra from different spots on each sample. Experiments were designed and carried out using this apparatus and four out of five polymorphs for sulfathiazole, both polymorphs and a solvate for mefenamic acid, four out of the seven stable forms of ROY, as well as the two most commonly encountered polymorphs and a solvate for flufenamic acid were obtained. The number of experiments conducted for sulfathiazole, mefenamic acid, ROY and flufenamic acid was 64, 66, 58, and 68, respectively. When comparing the results obtained in this study with a novel high throughput method based on patterned substrates of self-assembled monolayers recently developed in our group, it was shown that in the case of sulfathiazole and mefenamic acid the same number of polymorphs were obtained using the two approaches The number of experiments using the patterned SAMs was 4200 for sulfathiazole, 1200 for mefenamic acid, and 19556 for ROY. In the case of ROY, the semi-automated approach was not able to produce as many polymorphs as the patterned SAMs method because the increased number of trials carried out in the latter high throughput approach produced three additional polymorphs of ROY in very low number (less than 1% of 10 000 experiments). Both the semi-automated and high throughput approaches offer their own advantages. Using the patterned SAMs method may be beneficial if material is very limited or if a very high number of crystallization trials are needed. For systems with a high number of polymorphs, such as ROY, the patterned SAMs are more likely to produce a higher number of forms. The ability to use monolayers as templates may also lead to discovery of new forms, and there is a possibility, when using only a solvent based screen such as the one used in the present approach, that all of the forms will not be found. The semi-automated approach described here enables us to carry out cooling crystallization experiments and slurry aging experiments which cannot be carried out using patterned SAMs. The slurry aging approach is most suited to attempt to ensure that the most stable form at a given temperature has been found. This study demonstrates that the semi-automated approach described in this work is suitable for polymorph screening as it was able to reproduce effectively the diversity of polymorphs in model compounds, and it can be a cost-effective alternative to reduce the time scale required to identify all the possible phases of new pharmaceutical compounds.

**Acknowledgment.** The authors acknowledge the support of Barnstead International for partial support of this research.

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