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

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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 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 μL 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

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

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

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

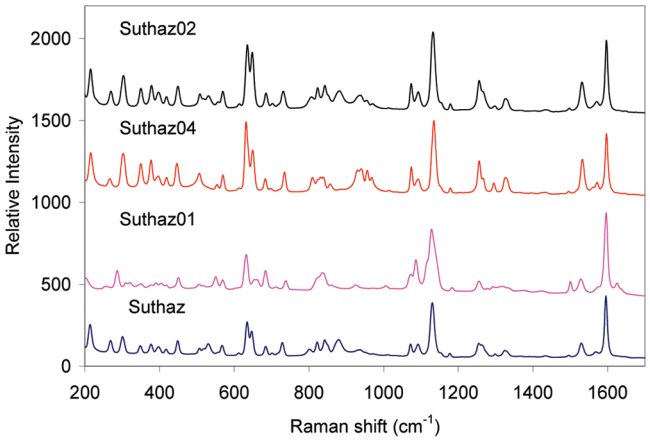

Figure 1. Raman spectra of sulfathiazole polymorphs.

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

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%)

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.

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

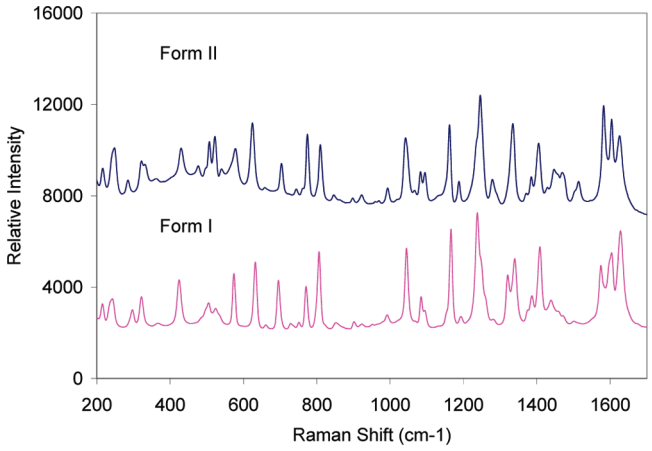

Figure 2. Raman spectra of mefenamic acid polymorphs.

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 β-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

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

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