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Research Paper

Concomitant Polymorphism in Confined Environment

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Purpose. The aim of this paper is to demonstrate that multiple crystal forms can be generated on patterned self-assembled monolayers (SAMs) substrates in single experiments in a given solvent system.
Methods. Functionalized metallic islands are fabricated and utilized as individual templates for crystal formation. Taking advantage of the different wetting properties that patterned surfaces offered, arrays of small solution droplets on the nano- and pico- liter scale were produced on the substrates. Different droplet dimensions were deposited on the substrate. As the solvent evaporates from the droplets, crystals were formed within the constrained volume. Crystal habits were examined with optical microscopy while the solid form was identified with Raman microscopy.
Results. With mefenamic acid (MA) and sulfathiazole as model pharmaceutical compounds, two and four different polymorphs, respectively, were observed under identical conditions. Moreover, it is established that the polymorphic distribution is highly dependent on the solvent evaporation rate and the solution concentration. These results imply that multiple crystal forms competitively nucleate in solution, and the probability of each form nucleating is strongly dependent on the supersaturation of the solution. Additionally, solvent was observed to play a role in controlling the solid state outcome.
Conclusions. Multiple crystal forms can concomitantly nucleate on patterned substrates. This technique can particularly be attractive to screen for polymorphs as elusive, metastable solid forms are favored with the creation of high supersaturation and can be stabilized due to the minimal volumes generated.

KEY WORDS: concomitant polymorphs; crystal form screening; nucleation; Raman microscopy.

INTRODUCTION

It is common that chemical compounds can adopt in more than one solid state structure, which is universally known as polymorphism (1,2). This has significant implications in the pharmaceutical industry as properties affected by different polymorphic forms include melting point, density, hardness, hygroscopicity, dissolution rate and solubility, which can in turn impact the process acceptability and bioavailability of a drug substance (3). In this regard, the selection of a desired solid state form for processing and the final product is one of the vital steps in drug development (4). The subject of polymorphism of molecular crystals with respect to pharmaceutical compounds has grown immensely the past few years as evident in the recent book by Rolf Hilfiker (5), the numerous special issue of journals dedicated to this particular topic (6) and annual reviews of the literature and patents that were published during 2004 (7) and 2005 (8).

Phase transformation of solid state forms mediated by liquid or vapor phase or stimulated by thermal and mechanical stress is a very common phenomenon (9). Unexpected conversion of solid state forms of a drug substance to undesired polymorphs during processing, storage and shipping is a huge challenge for the pharmaceutical industry, since a drug substance of an undesired form may give rise to different physiochemical properties (10). As such, the Food and Drug Administration (FDA) has strengthened regulation of the drug development process, requiring the characterization of all possible polymorphs and identification of the stable form of the target drug material (11).

Another important issue with respect to polymorphism is that different crystal forms can be considered a patentable invention. As such, generic pharmaceutical companies are increasingly devoting their time and effort in searching for novel crystal forms in order to allow them to gain early access into the market place (12). Consequently polymorph screening, comprehensively searching, isolating and characterizing all possible crystal structures that a drug substance can have, has been regarded as an indispensable step in the early and late stages of drug development (13).

Two or more crystal forms that nucleate and grow simultaneously under identical conditions are known as concomitant polymorphs. The appearance of mixed solid forms is due to competing kinetic and thermodynamic factors as each factor seeks to govern the crystallization of polymorphs. Occasionally, concomitant polymorphs may not be recognized or detected as kinetic forms that nucleated from and remain in contact with solution and may convert to the more stable form via a solution-mediated transformation

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Table I. Crystallographic Data of MA Polymorphs

	Form I[24]	Form II[23]
Crystal System	Triclinic	Triclinic
Space Group (Å)	P-1	P-1
a	14.556	7.6969
b	6.811	9.1234
c	7.657	9.4535
α	119.57	107.113
β	103.93	91.791
γ	91.30	101.481
Cell Volume (Å^3)	631.767	618.89
Z	2	2
R (F_o)	0.045	0.052

process by means of dissolution and recrystallization (14). Examples in which different polymorphs of a variety of chemical systems concomitantly crystallize can be found in an excellent review by Bernstein and coworkers (15).

Recently, we demonstrated that multiple forms of glycine simultaneously nucleated on patterned metallic gold islands (16,17). Moreover, it was observed that the polymorph distribution was dependent on the size of aqueous glycine droplets. With small square metallic islands (25 μm), the unstable β-form was obtained, whereas for larger islands, the solid state outcome favored the α-form. The bias toward the β-form was attributed to the rapid solvent evaporation which lead to high supersaturation that favored the formation of the higher energetic metastable phase (17). Surprisingly, the γ-form is also observed in several instances for the different island dimensions together with the other two polymorphs as this particular modification only nucleates under acidic or basic conditions (18).

In this paper, we report the crystallization of two pharmaceutical compounds, mefenamic acid (MA) and sulfathiazole, on functionalized metallic islands. It was observed that for MA, both anhydrous polymorphs can concomitantly nucleate, while for sulfathiazole four of the five anhydrous forms simultaneously crystallized on the patterned surfaces. Moreover, supersaturation and the type of solvent used influenced the solid state outcome. Lastly, we discuss how crystallization in confined volumes can be valuable for screening polymorphs particularly stabilizing elusive, metastable crystal phases.

MATERIALS AND METHODS

Materials

Mefenamic acid (MA) and sulfathiazole were purchased from Sigma Aldrich Chemicals and were used without further purification. Titanium (99.995%) and gold pellets (99.999%) were supplied from Plasmaterials, Inc., and Kutt J. Lesker Company, respectively. 4-Mercaptobenzoic acid (4-MBA, $C_6H_6O_2S$), and *n*-octadecyltrichlorosilane (OTS, $C_{18}H_{37}SiC_{13}$) were obtained from TCI America. Anhydrous ethanol (200 proof), acetonitrile (99.9%, *N*,*N*-dimethylformamide (DMF) (99.95%), dimethylsulfoxide (DMSO) (99.99%), and sulfuric acid were acquired from Pharmco Products. Anhydrous toluene (99.8%), hydrogen peroxide (30%), formamide (98%), and dimethylacetamide (DMA) (99%) were purchased from Sigma Aldrich Chemicals. Deionized (DI) water with a resistivity of 18 MΩ was obtained from a Barnstead Nanopure Infinity water purification system.

Gold Islands and Self-Assembled Monolayers (SAMs) Preparations

Microscope glass slides were immersed in "piranha solution" (3:1 concentrated H_2SO_4/30%H_2O_2) for 15 min. CAUTION: *Piranha solution reacts violently with organic materials and should be handled with extreme care*. The glass

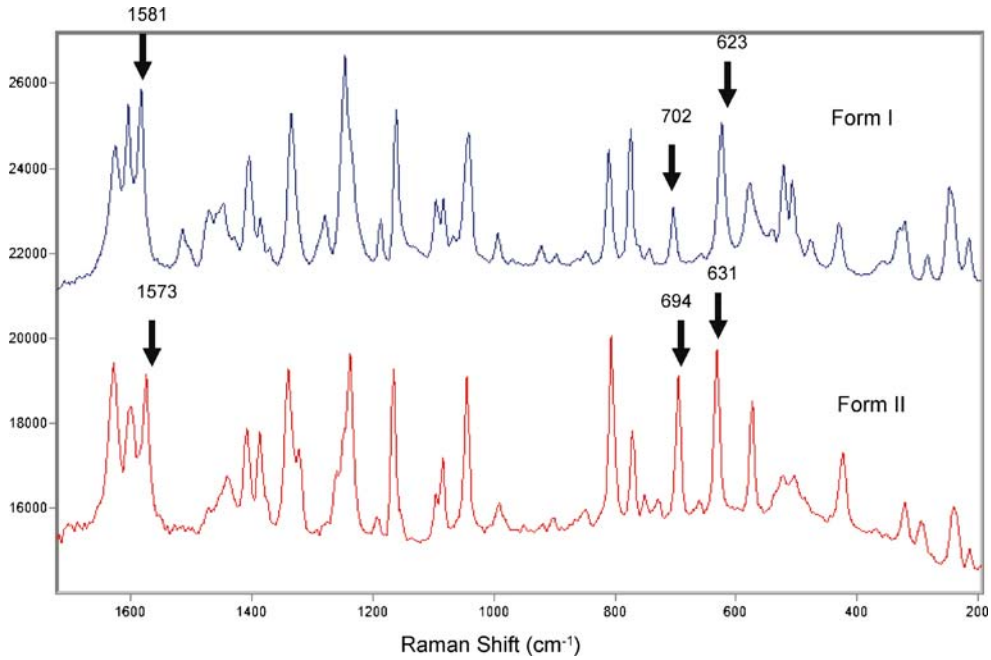

Fig. 1. Raman spectra of MA polymorphs.

Table II. MA Polymorph Distribution (in %) for Different Metallic Island Sizes from DMSO

Concentration (M)	25 μm		140 μm		250 μm		725 μm	
	Form I	Form II	Form I	Form II	Form I	Form II	Form I	Form II
0.207	NA		0	100	0	100	0	100
0.414	NA		0	100	0	100	0	100
0.621	NA		0	100	5	95	11	89
0.829	1	99	5	95	16	84	20	80

The number of crystals analyzed for each concentration and island dimensions is 100
Solubility of MA form I in DMSO (~325 mg/mL) at 23°C

substrates were thoroughly washed with water and rinsed with copious amount of ethanol, and blown dry with a jet of nitrogen. The deposition of metals on the glass substrates was conducted using an electron beam evaporator (Thermionics, base vacuum of 10^{-7} torr). To fabricate array of metallic islands, several different sizes (25, 140, 250, and 725 μm) of brass meshes with square holes (W. S. Tyler, U.S. Standard Nos. 500, 100, 60, and 25) were used and placed between the substrate and the metal source. The mesh essentially serves as a mask and prevents the metal from entirely evaporating to the surface. The dimensions of the metallic islands and the spacing distance between each island is dictated by the hole size and the width of the grid bars, respectively. The substrates were first coated with a thin layer of titanium (~50 Å) to promote adhesion and afterwards, gold (500 to 1,000 Å) was evaporated onto the surface. The film thickness was monitored with a quartz crystal microbalance.

Self-assembled monolayers were formed on the gold islands by immersing the substrates overnight (~18 h) in 1 to 10 mM ethanolic thiol (4-MBA) solutions. The substrates were removed from the solution, rinsed with copious amounts of absolute ethanol to remove unbound thiols, and blown dry with a jet of nitrogen. 4-MBA monolayers exclusively chemisorbed to the gold islands. Next, the second monolayer, OTS, was deposited onto the glass-exposed substrate by immersing the samples into an anhydrous toluene solution of OTS (2 mM) for 2 h under dry ambient conditions where the relative humidity was less than 20%. After removal from solution, the substrates were rinsed with toluene and ethanol, and dried in a stream of nitrogen. OTS covalently bonds to the glass surface and cross-linked among the silane molecules. With these two contrasting SAMs, patterned bifunctional surfaces with divergent wetting properties (lyophilic gold islands surrounded by lyophobic regions) were generated.

Crystallization Experiments

Patterned bifunctional gold island substrates were immersed in undersaturated and supersaturated solutions of MA and sulfathiazole at ambient temperature. As the substrate was slowly removed from solution, the solution preferentially wets the lyophilic square islands, in turn, producing arrays of hemispherical droplets. Crystallization ensued as the solvent evaporates from the droplets under ambient conditions (~23°C and 30% RH). Crystal nucleated in the droplets and are attached to each island after the solvent fully evaporated, forming an array of micron sized particles.

Characterization Methods

The habit and size of the crystals were acquired with a polarized light microscope (Nikon Eclipse ME600) and a stereomicroscope (Olympus SZX12), which were equipped with a video camera.

Raman spectra were obtained with a Raman Microprobe from Kaiser Optical System, Inc. The Raman microscope was equipped with a 450-mW external cavity stabilized diode laser as the excitation source, operating at 785 nm. The unit consisted of Leica optical light microscope, motorized translational stage and a CCD camera. Data were collected with HoloGRAMS Version 4.0, and processed and analyzed using GRAMS (Thermo Electron Corporation). The Raman spectra were scanned over a range of 200 to 3,200 cm^{-1}.

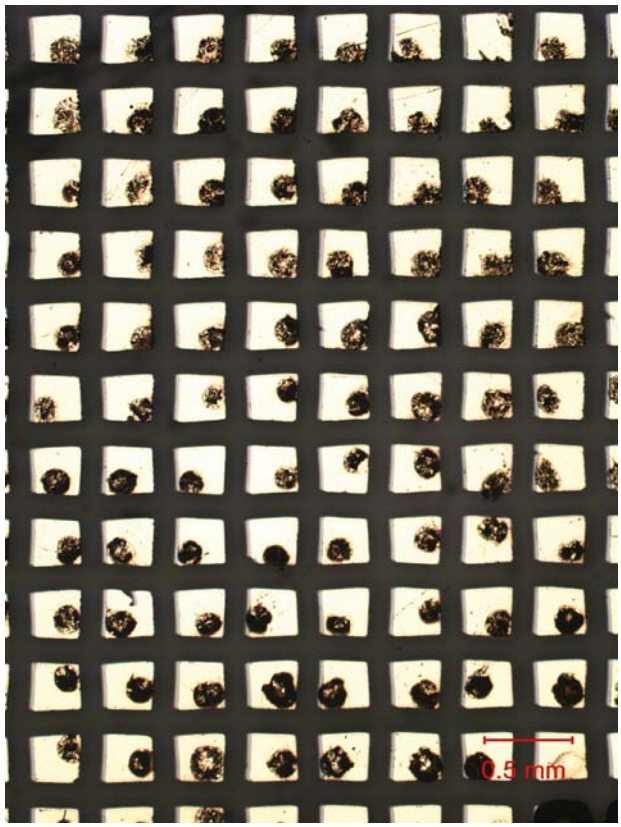

Fig. 2. Array of MA crystals nucleated on 250 μm metallic gold islands.

Table III. MA Polymorph Distribution (in %) for Different Metallic Islands Sizes from DMA

Concentration (M)	140 μm		250 μm		725 μm	
	Form I	Form II	Form I	Form II	Form I	Form II
0.207	0	100	0	100	0	100
0.414	0	100	0	100	0	100
0.621	0	100	0	100	0	100
0.829	0	100	0	100	5	95

The number of crystals analyzed for each concentration and island dimensions is 100

Powder X-ray diffraction patterns were acquired with a Rigaku Miniflex diffractometer using monochromatic Cu Kα-radiation with a nickel filter (λ=1.5405 Å) generated at 30 kV and 15 mA. The data were collected from 3 to 40° with a step size of 0.1° at a scan rate of 1.0°/min. The crystals were manually ground into fine powder and packed in an aluminum sample holder with a zero background silicon plate.

RESULTS AND DISCUSSION

Mefenamic Acid

Mefenamic acid (MA) is a nonsteroidal anti-inflammatory drug that is widely used to release pain and inflammation. It is poorly soluble in water and tends to stick to surfaces resulting in difficulties with tabletting and granulation processes (19). MA has two crystalline forms: form I and form II, where the physiochemical and solid state properties of both forms have been described previously (19,20). At room temperature, form I is the stable phase and can be prepared via recrystallization from acetone solution (21). Metastable form II can be generated by rapidly cooling supersaturated solutions of *N*,*N*-dimethylformamide (21) or by heating form I crystals above the transition temperature (22). Additionally, it has been recently demonstrated that structurally similar additives, flufenamic acid, can be used to induce the nucleation and growth of form II crystals (23). The single crystal data of both forms is summarized in Table I. Forms I and II are enantiotropically related as van't Hoff plot of the solubility data of each form reveal a transition temperature between 86 to 87°C (25,26). Above this cross-over temperature, Form II is the stable form, while below this temperature, Form I is most stable. Recently, it was observed that the rate of transformation between Form II to Form I is sensitive to solvent (27).

Undersaturated solutions of MA in dimethylsulfoxide (DMSO) were prepared and used to screen for polymorphs of MA on the bifunctional substrates. DMSO was selected due to MA's high solubility. Patterned substrates were immersed and slowly withdrawn from solution. Due to the contrasting wetting properties of the surface, the solution wetted the substrate but was constrained on the lyophilic metallic gold islands. This in turn created an array of uniform hemispherical droplets where the volume of each droplet can be controlled by varying the feature dimensions of the islands. With 25 to 725 μm square metallic islands, pico- to nano-liter volumes can be generated on each island. Crystallization was induced by solvent evaporation, as small islands tend to result in the creation of higher supersaturation compared with larger islands due to the rapid evaporation. After the solvent fully evaporated, crystals were deposited on the islands.

Optical and Raman microscopy were employed to characterize the particle shape and crystal form. Generally, different polymorphs have dissimilar Raman spectra due to differences in the vibrational energy of the molecules as a result of the different hydrogen bonding networks in the crystal lattice of each form. By comparing the Raman spectra to the spectrum of the known forms, this enabled the identification and classification of the crystals on the islands. Moreover, both approaches are nonintrusive as the solid did not have to be removed from the surface for solid state characterization. Fig. 1 shows the Raman spectra of the two

Table IV. Crystallographic Data of Sulfathiazole Polymorphs

CCDC Ref Code Form	Suthaz II	Suthaz01 I	Suthaz02 III	Suthaz04 IV	Suthaz05 V
Crystal System	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space Group (Å)	P21/c	P21/c	P21/c	P1121/n	P21/n
a	8.235	10.554	17.570	10.867	14.330
b	8.550	13.220	8.574	8.543	15.273
c	15.580	17.050	15.583	11.456	10.443
α	90.00	90.00	90.00	90.00	90.00
β	93.67	108.06	112.93	88.13	91.05
γ	90.00	90.00	90.00	90.00	90.00
Cell Volume ($Å^3$)	1,093	2,261	2,162	1,063	–
Z	4	8	8	4	8
ρ_{calc} (g/cm^3)	1.551	1.499	1.567	1.595	1.484

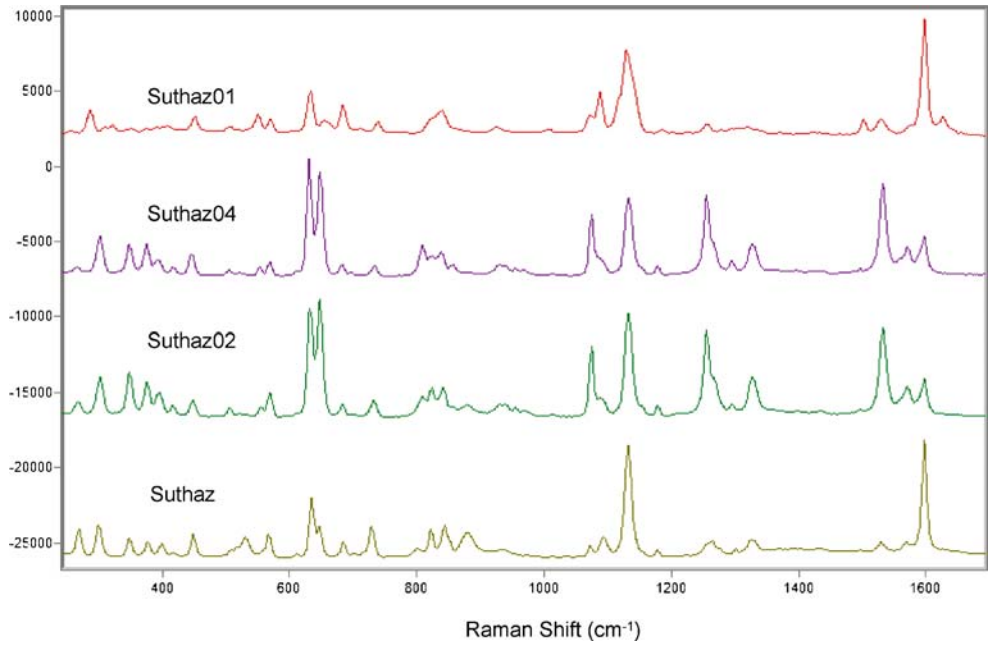

Fig. 3. Raman spectra of sulfathiazole polymorphs.

different forms of MA. Form I was prepared by recrystallization from acetone while the metastable form, form II, was obtained by heating form I to 160°C for one week. To confirm that the two polymorphs were indeed the correct form and not possibly a mixture of polymorphs, powder X-ray diffraction was also collected and compared to the simulated powder patterns. Both polymorphs were indeed phase pure and the correct solid forms. Examination of the Raman spectra for the two forms reveals three distinct vibrational bands as illustrated in Fig. 1. With these peak positions, the crystal polymorphic form produced on the patterned SAMs were identified.

Table II show the polymorph distribution of MA crystals nucleated on the functionalized metallic islands for different DMSO solutions. Fig. 2 shows an array of metallic islands with MA crystals. In contrast to the case of glycine (16, 17), multiple crystals nucleated on each island. On the small islands [25 μm], Form II predominantly nucleated with a small percentage of the stable phase, form I. As the dimensions of the gold islands increases, the appearance of form I crystals steadily increases as evident in the two most concentrated solutions. In addition to the feature size of the islands, the polymorph distribution of MA can also vary with solution concentration. For 250 and 725 μm metallic islands, as the concentration increases threefold or higher, the number of form I crystals rises from 0 to 16 and 20%, respectively. The large number of form II crystals observed contrasts with crystals produced from evaporation of DMSO at larger scales (e.g. microbeaker or evaporating dish), where at this scale form I is always observed. With dilute solutions and/or small islands, the metastable form of MA can be exclusively produced from DMSO.

In addition to DMSO, other solvents were screened with the patterned SAMs including *N*,*N*-dimethylformamide (DMF), dimethylacetamide (DMA) and acetonitrile. Table III summarizes the solid state outcome of MA from DMA. Under most of the conditions, form II was solely observed except at the concentrated solution with the largest island (725 μm) where the stable phase also concomitantly nucleated albeit a small percentage. Similarly to DMSO, evaporation driven crystallization at larger scales typically generated form I. In the case of DMF, a solvate form was observed on the metallic islands together with the metastable form. The solvate appears to quickly desolvate to form II when it is no longer in contact with the mother liquor. The crystal structure of this solvate has recently been solved and will be reported elsewhere. In acetonitrile, MA crystallized as form I for the different island dimensions and solution concentrations. Not surprisingly, solvent can play a role in controlling the polymorphic form as certain solvent can favor the formation of one particular polymorph over another (28).

Table V. Unique Raman Peak Positions of Sulfathiazole Polymorphs

	Wavenumbers (cm^{-1})									
Suthaz01	634	656	683	739	–	840	–	1,072	1,129	1,529
Suthaz	635	647	685	727	802	843	880	1,073	1,132	1,529
Suthaz02	633	648	684	732	809	842	880	1,074	1,133	1,531
Suthaz04	631	649	682	734	809	839	856	1,074	1,133	1,532

Table VI. Percentage of Sulfathiazole Oiling-out on Different Metallic Islands

Island Dimensions (μm)	Number of Islands	Oiling-Out (%)	Crystal (%)
140	3,000	95	5
250	1,000	70	30
725	200	15	85

Sulfathiazole

Sulfathiazole, 4-amino-*N*-(2,3-dihydro-2-thiazolyidene)-benzenesulfonamide, is a potent sulfonamide antibacterial drug. Like most sulfadrugs, it possesses multiple solid forms and has been considered a model pharmaceutical compound in the study of polymorphism in molecular crystals (29,30). Sulfathiazole has five distinct anhydrous crystalline forms (31). It is known as a promiscuous solvate former and has been reported to have over one hundred solvates due to its multiple hydrogen bonding capabilities (32). Table IV is a summary of the crystallographic data for each of the non-solvated polymorphs taken from the Cambridge Structural Database. There has been some confusion regarding the numbering of the different forms as noted by Blagden *et al.* (33) and Apperley *et al.* (30) due to inconsistencies with the solubility data of the different polymorphs. To minimize any confusion, we have adopted the notation of the Cambridge Structural Database reference codes used by Blagden *et al.* (33) to label each form. Based on the calculated unit cell densities, the thermodynamic stability order of all five polymorphic forms at 0 K should be Suthaz04 > Suthaz02 > Suthaz > Suthaz01 > Suthaz05, assuming that the crystal lattice energies are directly related to the densities (31,33). It is widely accepted that the polymorphic outcome of sulfathiazole can be controlled by the type of solvents used (34, 35). For instance, the most thermodynamically stable form, Suthaz04, is normally produced from acetone–chloroform mixtures, while Suthaz02, Suthaz, and Suthaz01 are crystallized from aqueous ammonia, nitromethane, and *n*-propanol, respectively (35). The fifth polymorph, Suthaz05, has only been crystallized from boiling water. Moreover, Suthaz05 is very unstable as it will rapidly convert to Suthaz01 at slightly lower temperatures in water. Consequently, it has been difficult to grow single crystals of Suthaz05 for single crystal X-ray analysis and the crystal structure has only been solved by high resolution synchrotron X-ray powder diffraction (31).

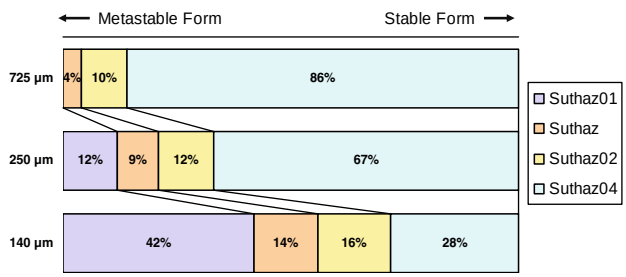

Fig. 4. Polymorph distribution of sulfathiazole crystals nucleated on different functionalized metallic islands.

Prior to screening for different polymorphs of sulfathiazole on patterned SAMs, four of the five polymorphs were successfully crystallized from the solvents described above. The lone exception was the elusive Suthaz05, where Suthaz02 or mixtures of Suthaz02 and Suthaz04 were consistently obtained from boiling water. In the same way as mefenamic acid, Raman microscopy was employed to characterize each crystal form, and powder X-ray diffraction patterns were also collected and compared with the simulated patterns to ensure that the crystals were not a mixture of polymorphs and the correct solid form was achieved. Fig. 3 shows the Raman spectra of four of the five neat polymorphs of sulfathiazole. Each form had a distinct spectra and Raman was able to discriminate them. Table V summarizes the specific peak positions that were used to identify each polymorph that nucleated on the bifunctional substrates.

Three different solvents (formamide, DMF and DMSO) were used to dissolve up sulfathiazole and were screened with the patterned substrates. In each of these solvent, a viscous gel on each island was observed rather than the formation of crystals. This might be due to the soluble nature of these solvents with sulfathiazole. To overcome this, water was introduced given that sulfathiazole is poorly soluble in aqueous media. In aqueous mixtures of both DMF and DMSO, oiling-out still occurred. However in supersaturated solutions of formamide–water solutions (4:1, *v*/*v*), crystals nucleated on the surface although not a very high percentage particularly for the 140 μm square islands (Table VI). Additionally it was observed that with less concentrated solutions, oiling transpired at a higher amount. While with solutions that are more supersaturated, spontaneous nucleation occurred frequently above ambient conditions. As the feature size of the islands decreases, the number of oiling cases significantly rises to greater than 90%. This is most likely due to the rapid evaporation in the smaller islands which does not allow sufficient time for sulfathiazole molecules to organize themselves into a crystal lattice. Generally from these solutions, spontaneous nucleation normally results in the formation of Suthaz02 with some trace of Suthaz04.

Fig. 4 shows the polymorphic distribution of sulfathiazole crystals from a supersaturated solution (0.2 M) of formamide/water (4:1, *v*/*v*). With the largest square islands, the three most stable polymorphs crystallized concomitantly. While with the two smaller metallic islands, four polymorphs nucleated simultaneously. The polymorph distribution appears to vary considerably with larger island dimensions favoring the stable crystalline phase, Suthaz04, whereas in the 140 and 250 μm square islands, the frequency of the two least stable forms steadily rises. The stability order of the polymorphic forms surprisingly corresponds with the crystal form distribution. Microscopic images of the four different polymorphs nucleated on the patterned SAMs are shown in Fig. 5. The elusive and least stable form, Suthaz05, was not observed in any of the different bifunctional substrates.

Self-assembled monolayers have been shown to direct the polymorph selectivity of molecular crystals (36). However in the case of sulfathiazole, the thin surfactant film (4-MBA) formed on the different gold island dimension were

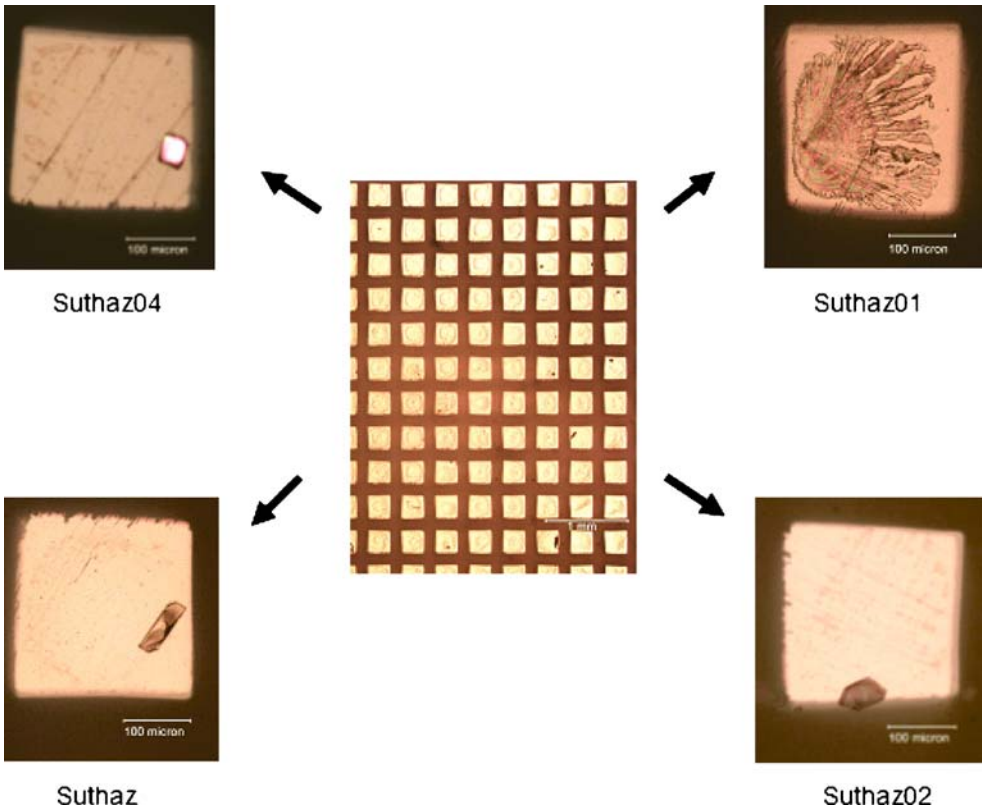

Fig. 5. Sulfathiazole crystals nucleated on 250 μm metallic gold islands.

the same but the solid state outcome differed considerably. The concomitant crystallization of the multiple polymorphs is most likely due to the generation of supersaturation rather than interfacial interactions between the monolayer film and the sulfathiazole molecules. To confirm this hypothesis, 140 μm metallic islands were withdrawn and immediately placed into two closed chambers of different volumes. With these chambers, the rate of evaporation was reduced as the solvent vapor saturated the environment inside the chamber. Accordingly, the evaporation rate inside the chamber with the lower volume should be expected to be slower compared to a chamber that has a higher volume. The crystallization outcomes are summarized in Table VII. The number of crystals formed on the patterned substrates increases as the rate of evaporation is reduced. More importantly, the polymorphic distribution becomes biased toward the stable form, Suthaz04, with slower rates, while the frequency of the two metastable phases, Suthaz02 and Suthaz, considerably decreased. With fast evaporation, the driving force for crystallization, supersaturation, is generated very quickly and high levels are attained which normally favors oiling-out particularly for molecules that requires extensive time for assembly into a lattice, or is kinetically conducive to the nucleation of high energy polymorphs.

Concomitant polymorphism of mefenamic acid and sulfathiazole on patterned gold islands of different lateral dimensions are a result of the competing nucleation rates of the various crystal forms. The domain for multiple solid forms to exist simultaneously is rarely known as many factors (e.g. solvent, temperature, cooling, rate of evaporation, and other conditions) can influence the crystallization outcome. This is clearly evident in both molecules as crystallization of sulfathiazole from single solvents lead to liquid–liquid phase separation while for mefenamic acid, Forms I and II nucleated on substrate immersed in acetonitrile and DMA, respectively. It is possible to speculate that in solution, multiple forms might have existed but due to the metastability of some of these polymorphs, they might have quickly converted to the more thermodynamically stable phase via a solution-mediated transformation process by means of disso-

Table VII. Polymorph Distribution (in %) of Sulfathiazole Crystals Nucleated on Metallic Islands as a Function of the Volume of the Closed Chamber

Volume (ml)	Oiling-Out (%)	Crystal (%)	← Metastable		Stable →	
			Suthaz01	Suthaz	Suthaz02	Suthaz04
–	95	5	42	14	16	28
150	94	6	35	5	15	45
40	85	15	23	1	4	72

lution and recrystallization. The disappearance of these kinetic forms can be minimized with the use of minimal solution volumes while still maintaining an environment that promotes the nucleation and growth of these polymorphs. Minimal volumes are not necessary but may be beneficial, particularly when crystallization occurs where the mother liquor is rapidly depleted, reducing the possibility of phase conversion mediated by solution. Functionalized metallic islands address this and offer the opportunity to perform hundreds and thousands of crystallization experiments at the nano- and pico-liter scale under identical conditions in a given substrate.

It is widely known that at a high supersaturation state, kinetic effects normally direct the solid state outcome of crystals produced from solution (37). This is true for mefenamic acid and sulfathiazole crystals constrained on patterned gold islands as well. With rapid solvent evaporation, the least stable phase of each compound nucleated more frequently than the more stable phases. The type of solvents employed can also influence the crystallization outcome as the appearances of certain polymorphs are solvent dependent. The classic example often cited is sulfathiazole, however, this may no longer be accurate as illustrated in this work whereby varying the degree of supersaturation can simultaneously nucleate four distinct polymorphic forms in different amounts, contrary to previous studies (34).

Due to the stochastic nature of nucleation of different polymorphic forms, an extensive number of crystallization trials should be conducted in order to acquire a better understanding of solid form diversity. Crystallization constrained on patterned substrates enables one to achieve this as it requires minimal amount of material, and can be easily coupled with optical and Raman microscopy, allowing for automated solid state characterization, akin to current high throughput polymorph screening methods.

Crystallization in confined spaces is not only a useful approach to produce nano- and micro-size particles (38), but it can also provide an alternative to traditional methods in searching for metastable polymorphs and novel crystal forms. It is well known that nucleation becomes more difficult as the container volume decreases. Our approach has been the manipulation of the rate of solvent evaporation on pico- and nano-liter droplets while providing the nucleation templating effect of the SAM's The rate of evaporation impacts the level of supersaturation generated with high supersaturation favoring the formation of high energy metastable polymorphic forms while with low supersaturations, the thermodynamically stable form typically nucleates Capillary crystallization employs a different strategy where slow solvent evaporation in constrained environments (capillaries) decreases the probability of nucleation since the volume is small and clean with no templating surface, thus allowing high supersaturations and metastable forms (39). Crystallization confined in nanopores is an example where polymorph selectivity can be directed by suppressing the nucleation of unwanted polymorphs whose critical nucleus size are larger than the pore dimensions. As a consequence, nucleation of polymorphic forms with critical sizes less than the pore size can be achieved (40). Confined crystallization in capillary vessels, nanopores and on patterned surfaces not only increases your chance of discovering new polymorphs but can aid in the nucleation and stabilization of known metastable modifications that might be elusive with conventional crystallization methods.

CONCLUSION

Multiple polymorphs of mefenamic acid and sulfathiazole nucleated concomitantly on patterned metallic gold islands under identical conditions. With these surfaces, an array of solution droplets ranging from the pico- to the nanoliter scale can be fabricated and served as independent nucleation sites where crystallization proceeds via solvent evaporation. Particle habit and solid state forms were easily characterized with optical and Raman microscopy, respectively. The type of solvents used and the level of supersaturation were two major factors in shaping the polymorph distributions of each compound. With increasing supersaturation, the frequency of the least stable polymorph steadily grows while the more stable forms are produced less regularly. Moreover, in the case of MA the solution concentration also affects the distribution with the formation of the stable polymorph favored at higher concentrations for larger square metallic islands. Depending on the lateral dimensions of the metallic islands, hundreds and thousands of crystallization (or oiling) events can take place with the use of one patterned substrate while all along requiring a minimal amount of material. Coupled with microscopic and spectroscopic tools, functionalized metallic islands has the potential to be automated in a high throughput fashion, akin to well-plates, to screen for conditions where crystallization may occur and to search for novel polymorphs.

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