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Polymer Templating of Supercooled Indomethacin for Polymorph Selection

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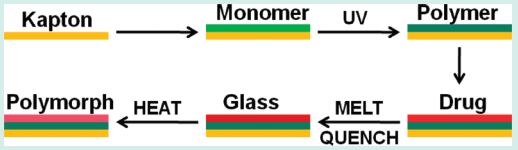
Supporting Information

ABSTRACT: Reported here is a relatively simple technique for polymorph screening of pharmaceutical compounds that are thermally stable. Polymer libraries have previously been used as surfaces to influence, or direct, the crystalline form adopted by an active pharmaceutical ingredient on crystallization from solution. In this current work, we demonstrate the polymorph-directing effect of homopolymer surfaces in the absence of solvent by recrystallization from the supercooled melt. When the nonsteroidal anti-inflammatory drug indomethacin is melted, cooled, and subsequently reheated above its glass transition temperature on an untreated surface, it has a proclivity to crystallize as its δ polymorph. On certain polymer surfaces, however, it preferentially crystallizes as the α polymorph, as a direct result of polymer templating. The method is well-suited to implementation in multiwell plate formats requiring only small amounts of material and enabling multiple experiments to be carried out in parallel with samples readily characterized using X-ray powder diffraction.



KEYWORDS: *indomethacin, polymorph screening, polymer templating, polymer-induced heteronucleation, glass transition*

It is well established[1] that drug polymorphism, the ability of a molecule to adopt more than one crystal structure, can have a marked effect on many important physical and chemical properties. As such, polymorph screening and discovery continues to attract significant attention from a number of research fields, using a diverse range of methods.[2−7] An innovative technique to emerge within the past decade is polymer templating (polymer-induced heteronucleation),[8,9] wherein the outcome of heterogeneous nucleation and subsequent crystal growth of a compound from solution can be templated by the surfaces of dispersed polymers. Notably, Price et al.[10] were able to isolate from solution new polymorphs of carbamazepine and sulfamethoxazole as single crystals on top of a combinatorially synthesized, cross-linked polymer library base. In this approach, the insoluble polymers in the microarray provide diverse surfaces with a variety of functionalities that can influence the nucleation, crystal morphology, and growth rate[11] of solute from solution placed in direct contact with the surface.

Here we report a polymorph screening methodology that utilizes a solvent-free melt-cooling crystallization technique in conjunction with polymer templating. Eliminating the use of solvent is in keeping with current drives toward "green" chemistry,[12] and removes the possibility of solvated crystal forms that often occur during polymorph screens.[13,14] The straightforward implementation of this method illustrates the relative ease with which polymer surfaces may be exploited as a rich source of diversity in the context of polymer screening, and thus its application as a complementary technique to traditional solution-based drug screening methods that assist with the identification and isolation of all possible solid drug forms (hydrates, solvates, salts, and cocrystals). The application of the method is demonstrated with the compound indomethacin (IMC; Scheme 1).

IMC is a nonsteroidal anti-inflammatory drug with at least three reported polymorphs; α, γ, and δ.[15−19] It has been demonstrated[20] that when supercooled liquid IMC is subsequently crystallized as a polycrystalline powder on a glass microscope slide by heating above the IMC glass transition temperature, T_g (41 °C), the IMC polymorph recrystallized from the glass varies according to the incubation temperature of the sample. Between 50 and 100 °C, over 90% of the crystallites in the recrystallized IMC samples were the δ polymorph (the remaining crystallites being α and γ).[20]

A selection of polymers was synthesized using a series of nine modified acrylate monomers (Scheme 1), intended to produce a range of chemical and physical properties across the resultant polymer surfaces presented for crystallization.[21] Several of the monomers used have structural features (e.g., ester, hydroxyl, amine, and aromatic moieties) that are complementary to groups on the IMC molecule (e.g., carboxylic acid, amide, methoxyl, chlorobenzene) and thus offer the potential for manipulation of the IMC crystal structure through complementary intermolecular interactions. Monomers were dosed onto a polyimide (Kapton) film mounted on a bespoke aluminum 28-position sample plate using the minimum volume of monomer required to achieve uniform polymerization and complete coverage of each well. The samples were then polymerized in situ in a UV oven.[22] The plate was removed from the UV oven, and a thin layer of γ IMC powder was

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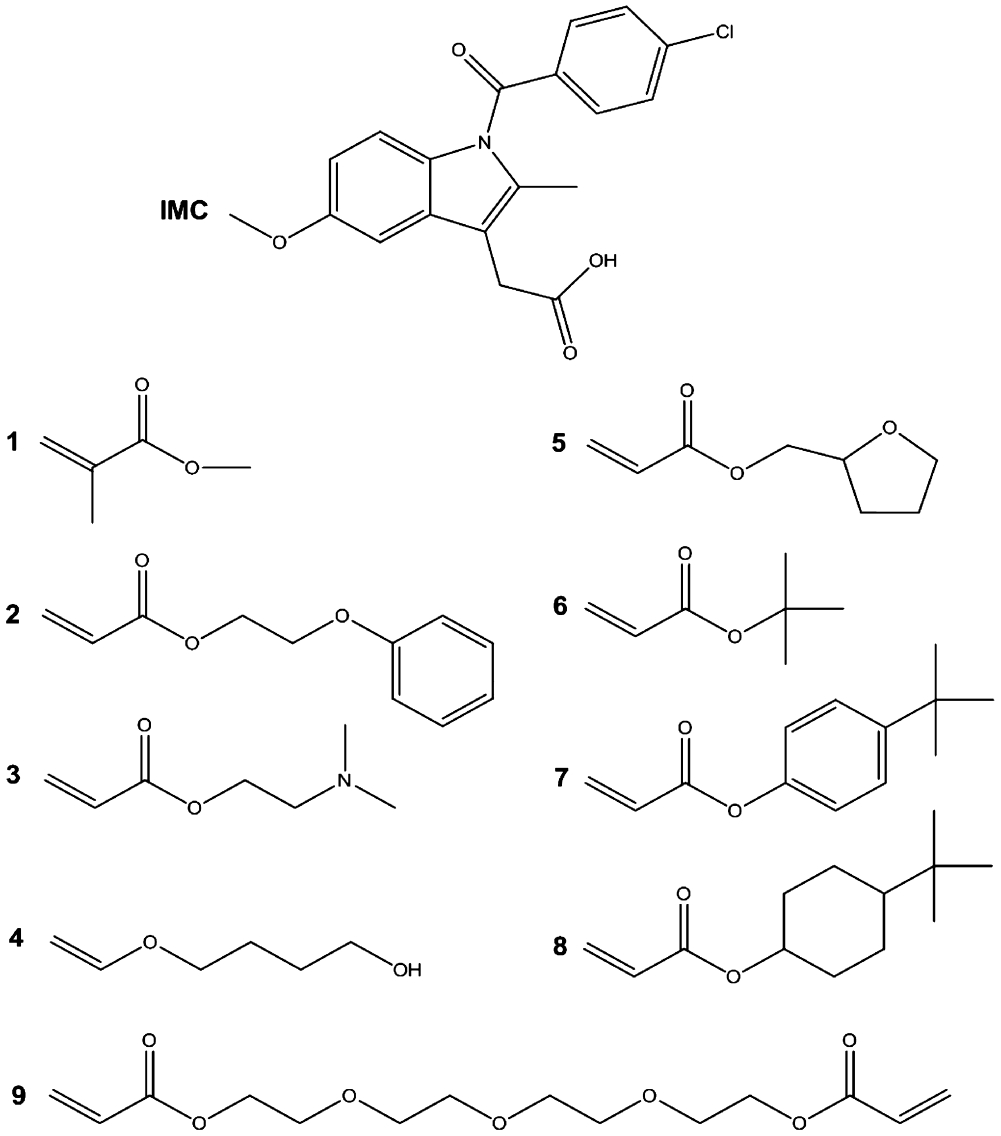
Scheme 1. Chemical Structure of Indomethacin (IMC) and Monomers (1−9) Used as Polymer Templates



placed on the homopolymer in each well and spread evenly over the surface. The whole plate was heated to 175 °C to melt the IMC, and then cooled to room temperature before being reheated above T_g to 70 °C to induce crystallization, which occurred within 24−48 h. Each plate was analyzed using foil transmission X-ray powder diffraction (XRPD).[23] Proportions of each polymorph within each sample were estimated using PolySNAP[24] by comparison of the XRPD pattern to reference patterns collected form pure samples of each IMC polymorph (Figure 1, see Supporting Information).

As described previously,[20] IMC recrystallizing at 70 °C from the quench-cooled melt tends to produce the δ polymorph, or to a lesser extent a mixed phase of δ, α, and γ. On top of most of the surfaces (both polymers and control) used in this work, IMC recrystallizes as δ IMC or a δ/α mixed phase. However, on two polymer supports detailed below, IMC recrystallizes reproducibly as the α polymorph, clearly showing a templating effect of α IMC by the polymers.

It is therefore of interest to investigate the effect of different polymer side chain functionalities on IMC polymorph selectivity at the same temperature.

IMC polymorphs were identified by their XRPD patterns. The reference patterns were produced using established methods: γ IMC was used as received; α was recrystallized from ethanol and water,[19] and δ was recrystallized from methanol and desolvated under vacuum.[25] Phase purity of the reference samples of γ and α IMC was confirmed by a Pawley-type fit[26] of the respective single crystal parameters[19,27] to the observed diffraction patterns using the DASH software package.[28] The reference pattern for the δ polymorph, for which there is no crystal structure, was compared with previous work.[20] The homopolymers remained amorphous throughout the experiments.

Recrystallization experiments were carried out in two stages. In the first stage, an initial polymorph screen comprising 42 melt-cooling crystallizations on two 28-well plates was implemented in which the Kapton base of wells either had no coating (control; 8 wells) or were coated with one of the polymers, **1**−**6** and **8** (3, 3, 3, 3, 4, 11, and 7 wells respectively). The plate layout and polymorph results are presented in Figure 2. The results from the blanks and polymers **1**, **3**, **4**, **6**, and **8** were largely consistent with previous work,[20] with the recrystallized samples identified either as pure δ IMC or as

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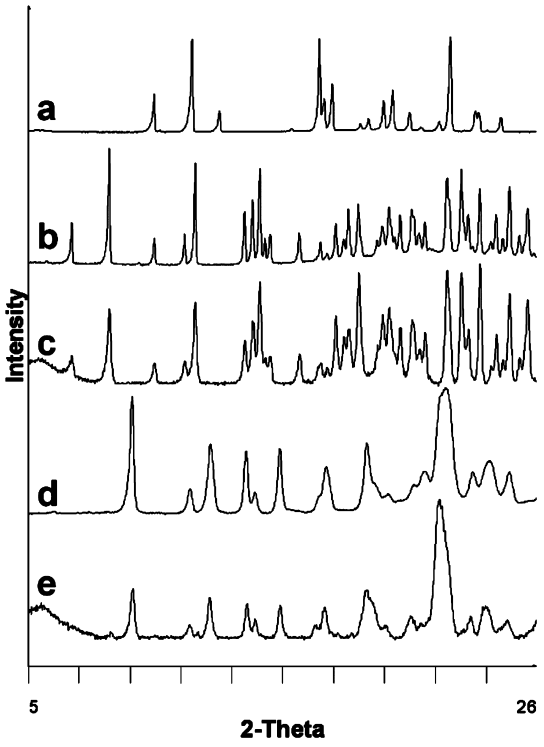

Figure 1. XRPD patterns of IMC for reference samples and recrystallized polymorphs. Stackplot showing (a) γ reference, (b) α reference, (c) α recrystallized from the melt on polymer **5**, (d) δ reference, and (e) δ recrystallized from the melt on blank Kapton.

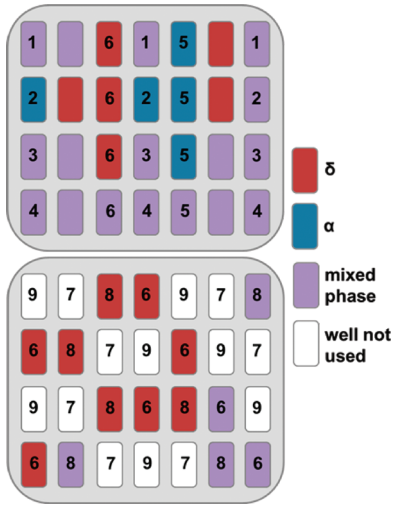

Figure 2. Observed IMC polymorphs on the first two polymer plates after one week. The IMC polymorph obtained from each well is denoted by color. The numbers identify the polymer substrates present in each well. Control wells (Kapton) are indicated as blank.

mixtures of δ and α ranging from 50:50 to 80:20 (ratio δ:α). On polymers **2** and **5** however, IMC was found to crystallize as pure α (or >90% α) in two out of three and three out of four trials, respectively. Monomers **7** and **9** degraded on heating and irradiation, and so these wells were unusable for further investigation. γ IMC was not observed from any of the homopolymers tested.

In the second stage of the experiment, crystallization on polymers **2** and **5** was repeated and scaled up to assess the reproducibility of the initial results. For comparison, crystallizations on polymer **6**, which returned the highest number of pure-phase δ samples in the initial screen, were also repeated as were the control experiments, conducted in the absence of polymers. From 33 crystallizations on polymer **2**, 27 were pure α IMC and 6 were a α/δ mixed phase (<80% α); from 37 crystallizations on polymer **5**, 33 returned a result of pure or predominantly (>90%) α IMC and 4 returned a mixed phase (<80% α); from 42 crystallizations on polymer **6**, 26 crystallized as pure or predominantly (>80%) δ IMC and 16 as a mixed phase (<80% δ); of 43 control experiments, 25 pure δ IMC and 18 mixed phase (<80% δ) samples were observed (Figure 3).

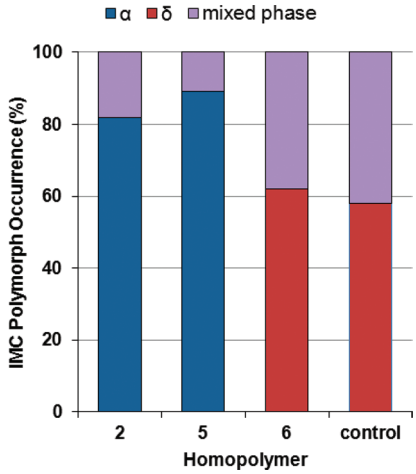

Figure 3. Total occurrences (%) of IMC polymorphs returned from polymers **2**, **5**, and **6** and control samples.

While each result was confirmed by XRPD, the silver-white α and beige δ polymorphs could also be distinguished by visual inspection. This is illustrated in Figure 4, which shows the

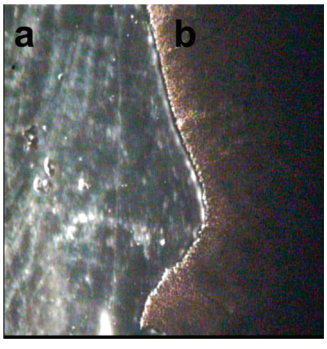

Figure 4. Photomicrograph of the boundary of (a) silver α IMC atop polymer **5** and (b) beige δ IMC atop blank Kapton film.

concomitant crystallization of α and δ IMC on top of polymer **5** and blank Kapton, respectively. The micrograph was taken at the Kapton-polymer boundary of a sample well in which there was incomplete polymer coverage of the Kapton film during preliminary work and provides a clear demonstration of the direct local effect of recrystallization of IMC from the melt on two different surfaces. On the basis of these results, crystallization of IMC on polymers **1**, **3**, **4**, **6**, and **8** does not significantly influence the outcome of IMC crystallization from the supercooled melt. However, polymers **2** and **5** have a significant influence on the crystallization outcome, favoring the

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formation of the α polymorph in preference to the expected δ form.

To explore this effect further, gradated sample plates were assembled, where a copolymer gradient was set up between polymers **6** and **5** across a row of five sample wells so as that **6**:**5** (%:%) monomer ratios were of the order 100:0, 80:20, 50:50, 20:80, and 0:100, and repeated in quintuplicate. IMC crystallized as the δ polymorph in sample wells bearing 100% polymer **6**. Copolymers containing polymer **5**—even at the lowest ratio (80% **6**: 20% **5**)—yielded the α form, irrespective of the presence of polymer **6** (see Supporting Information for further details on plate layouts and results). In five of the samples IMC crystallization was inhibited, arising from diffusion of molten IMC into the polymer base, and these results were discarded from the data set.

The nature of the polymorph directing effect was examined by performing atomic force microscopy (AFM) and contact angle goniometry (CAG) measurements on multiple samples of blank Kapton film and polymers **2**, **5**, and **6**. Surface roughness (R_a) tests for each sample were carried out before and after the same heating procedure used for the IMC recrystallizations. All of the samples had relatively flat topographies,[22] with R_a values in the range 0.8−1.8 nm (see Supporting Information). These results suggest polymer surface topography is not a factor in conferring polymorph selectivity. Contact angle (CA) measurements for each of the polymers and Kapton were also similar. Advancing contact angles for all samples showed that Kapton and polymers **2**, **5**, and **6** all have relatively hydrophobic surfaces before and after heating, returning values in the range 78−93° (see Supporting Information).[29] Homopolymer integrity was checked with Raman spectroscopy before and after heating to 175 °C, holding for 10 min and cooling naturally back to room temperature. No near-surface chemical changes or polymer degradation were observed in the spectra. The results from the AFM, CA, and Raman analyses point to the observed polymer templating effect as being a chemical, rather than physical, effect with the phenyl and tetrahydrofurfuryl side chains of polymers **2** and **5** yielding a surface more conducive to α IMC than δ IMC. Since the remaining polymer surfaces do not promote growth of α IMC the role, if any, of specific polymer backbone···IMC interactions with individual crystal faces of IMC polymorphs requires further investigation. Work is ongoing to determine the δ IMC crystal to assist with this.

This is the first report of the use of polymer surfaces for solvent-free templating of pharmaceutical polymorphs. The approach has clear potential for application as a complementary tool in polymorph screening studies for target molecules that do not decompose on melting, and have a melting point below the thermal decomposition point of the polymer substrate. The method is relatively straightforward to implement practically with results obtained within minutes-hours. It is also well-suited to multiwell plate formats enabling rapid, in situ sample characterization, and, compared with many other solution-based crystallization screening methodologies, requires relatively little sample preparation such as filtration. As diversity is critical to any successful screening strategy, the variation of monomer functional groups must be maximized to fully exploit polymer surface chemistry.

EXPERIMENTAL PROCEDURES

Polymer Synthesis. 28-position aluminum sample plates, compatible with a Bruker AXS D8-Advance diffractometer sample stage, were covered with polyimide (Kapton, 7.5 μm thickness) film and sealed with vacuum grease. A 150 μL portion of each monomer was added to individual sample wells in triplicate or quadruplicate, and the plate was UV irradiated in a Dymax UV oven for 1 min. This polymerization method is well-established, giving a degree of polymerization of approximately 80% for the monomers used.[30] The above was repeated as necessary to ensure statistically significant results for polymers which gave a positive result. Monomers used were methyl methacrylate (**1**), ethylene glycol phenyl ether acrylate (**2**), 2-(dimethylamino) ethyl acrylate (**3**), 1,4-butanediol vinyl ether (**4**), tetrahydrofurfuryl acrylate (**5**), *tert*-butyl acrylate (**6**), *tert*-butyl phenyl acrylate (**7**), 4-*tert*-butyl cyclohexyl acrylate (**8**), and tetra (ethylene glycol) diacrylate (**9**). Each monomer was prepared with 10% (w/v) 2,2-dimethoxy-2-phenylacetophenone as a photoinitiator. All reagents were purchased from Sigma-Aldrich (U.K.) and used as received.

IMC Crystallization. A thin layer (20−30 mg) of IMC (Roig Farma, Spain) was spread evenly over each polymer sample in plates treated as described above. Each plate was then added to an oven at 175 °C for approximately 2 min, or until the IMC had melted. The plates were removed from the oven and left to cool naturally at room temperature, facilitating the formation of IMC glass after a few seconds. XRPD analysis was carried out on all samples to confirm that all polymers and IMC were amorphous. Plates were then incubated in an oven at 70 °C and subsequent XRPD analysis was carried out daily for 1−2 weeks. Sample plates were cooled naturally to room temperature over a few minutes before analysis, which was carried out at room temperature. The temporary temperature drop from 70 °C to room temperature (below T_g) had no effect on IMC recrystallization (see Supporting Information).

ASSOCIATED CONTENT

Supporting Information

Plate layouts and results for gradated copolymer plates. Experimental procedures and parameters for XRPD, AFM, CAG, Raman spectroscopy, and microscopy. Results of AFM, CAG, Raman spectroscopy and PolySNAP analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Hilfiker, R., Blatter, F., von Raumer, M., Relevance of Solid-state Properties for Pharmaceutical Products. In *Polymorphism in the Pharmaceutical Industry*, 1st ed.; Hilfiker, R., Ed.; Wiley-VCH: Weinheim, Germany, 2006; pp 1−17.

(2) Weissbuch, I.; Popovitzbiro, R.; Lahav, M.; Leiserowitz, L. Understanding and control of nucleation, growth, habit, dissolution and structure of 2-dimensional and 3-dimensional crystals using tailor-made auxiliaries. *Acta Crystallogr., Sect. B: Struct. Sci.* **1995**, *51*, 115−148.

(3) Peterson, M. L.; Morissette, S. L.; McNulty, C.; Goldsweig, A.; Shaw, P.; LeQuesne, M.; Monagle, J.; Encina, N.; Marchionna, J.; Johnson, A.; Gonzalez-Zugasti, J.; Lemmo, A. V.; Ellis, S. J.; Cima, M. J.; Almarsson, O. Iterative high-throughput polymorphism studies on acetaminophen and an experimentally derived structure for form III. *J. Am. Chem. Soc.* **2002**, *124* (37), 10958−10959.
(4) Zaccaro, J.; Matic, J.; Myerson, A. S.; Garetz, B. A. Nonphotochemical, laser-induced nucleation of supersaturated aqueous glycine produces unexpected gamma-polymorph. *Cryst. Growth Des.* **2001**, *1* (1), 5−8.
(5) Arlin, J.-B.; Price, L. S.; Price, S. L.; Florence, A. J. A strategy for producing predicted polymorphs: catemeric carbamazepine form V. *Chem. Commun.* **2011**, *47* (25), 7074−7076.
(6) Capacci-Daniel, C.; Gaskell, K. J.; Swift, J. A. Nucleation and Growth of Metastable Polymorphs on Siloxane Monolayer Templates. *Cryst. Growth Des.* **2010**, *10* (2), 952−962.
(7) Liberski, A. R.; Tizzard, G. J.; Diaz-Mochon, J. J.; Hursthouse, M. B.; Milnes, P.; Bradley, M. Screening for polymorphs on polymer microarrays. *J. Comb. Chem.* **2008**, *10* (1), 24−27.
(8) Lang, M. D.; Grzesiak, A. L.; Matzger, A. J. The use of polymer heteronuclei for crystalline polymorph selection. *J. Am. Chem. Soc.* **2002**, *124* (50), 14834−14835.
(9) Grzesiak, A. L.; Matzger, A. J. Selection and discovery of polymorphs of platinum complexes facilitated by polymer-induced heteronucleation. *Inorg. Chem.* **2007**, *46* (2), 453−457.
(10) Price, C. P.; Grzesiak, A. L.; Matzger, A. J. Crystalline polymorph selection and discovery with polymer heteronuclei. *J. Am. Chem. Soc.* **2005**, *127* (15), 5512−5517.
(11) Diao, Y.; Myerson, A. S.; Hatton, T. A.; Trout, B. L. Surface Design for Controlled Crystallization: The Role of Surface Chemistry and Nanoscale Pores in Heterogeneous Nucleation. *Langmuir* **2011**, *27* (9), 5324−5334.
(12) Tanaka, K.; Toda, F. Solvent-free organic synthesis. *Chem. Rev.* **2000**, *100* (3), 1025−1074.
(13) Florence, A. J.; Johnston, A.; Price, S. L.; Nowell, H.; Kennedy, A. R.; Shankland, N. An automated parallel crystallisation search for predicted crystal structures and packing motifs of carbamazepine. *J. Pharm. Sci.* **2006**, *95* (9), 1918−1930.
(14) Johnston, A.; Florence, A. J.; Shankland, N.; Kennedy, A. R.; Price, S. L. Crystallization and crystal energy landscape of hydrochlorothiazide. *Cryst. Growth Des.* **2007**, *7* (4), 705−712.
(15) Yoshioka, M.; Hancock, B. C.; Zografi, G. Crystallization of Indomethacin from the Amorphous State below and above Its Glass-Transition Temperature. *J. Pharm. Sci.* **1994**, *83* (12), 1700−1705.
(16) Bogdanova, S.; Bontclaeva, E.; Avramova, N. Phase characterization of indomethacin in adsorbates onto hydroxyethylcellulose. *Drug Dev. Ind. Pharm.* **2007**, *33* (8), 900−906.
(17) Borka, L. Polymorphism of Indomethacin - New Modifications, Their Melting Behaviour and Solubility. *Acta Pharm.* **1974**, *11* (3), 295−303.
(18) Andronis, V.; Zografi, G. Crystal nucleation and growth of indomethacin polymorphs from the amorphous state. *J. Non-Cryst. Solids* **2000**, *271* (3), 236−248.
(19) Chen, X. M.; Morris, K. R.; Griesser, U. J.; Byrn, S. R.; Stowell, J. G. Reactivity differences of indomethacin solid forms with ammonia gas. *J. Am. Chem. Soc.* **2002**, *124* (50), 15012−15019.
(20) Wu, T.; Yu, L. Origin of enhanced crystal growth kinetics near T-g probed with indomethacin polymorphs. *J. Phys. Chem. B* **2006**, *110* (32), 15694−15699.
(21) Urquhart, A. J.; Taylor, M.; Anderson, D. G.; Langer, R.; Davies, M. C.; Alexander, M. R. TOF-SIMS analysis of a 576 micropatterned copolymer array to reveal surface moieties that control wettability. *Anal. Chem.* **2008**, *80* (1), 135−142.
(22) Urquhart, A. J.; Anderson, D. G.; Taylor, M.; Alexander, M. R.; Langer, R.; Davies, M. C. High throughput surface characterisation of a combinatorial material library. *Adv. Mater.* **2007**, *19* (18), 2486−2491.
(23) Florence, A. J.; Baumgartner, B.; Weston, C.; Shankland, N.; Kennedy, A. R.; Shankland, K.; David, W. I. F. Indexing powder patterns in physical form screening: Instrumentation and data quality. *J. Pharm. Sci.* **2003**, *92* (9), 1930−1938.
(24) Barr, G.; Dong, W.; Gilmore, C. J. PolySNAP: a computer program for analysing high-throughput powder diffraction data. *J. Appl. Crystallogr.* **2004**, *37*, 658−664.
(25) Crowley, K. J.; Zografi, G. Cryogenic grinding of indomethacin polymorphs and solvates: Assessment of amorphous phase formation and amorphous phase physical stability. *J. Pharm. Sci.* **2002**, *91* (2), 492−507.
(26) Pawley, G. S. Unit-Cell Refinement From Powder Diffraction Scans. *J. Appl. Crystallogr.* **1981**, *14*, 357−361.
(27) Kistenmacher, T. J.; Marsh, R. E. Crystal And Molecular Structure Of An Antiinflammatory Agent, Indomethacin, 1-(Para Chlorobenzoyl)-5-Methoxy-2-Methylindole-3-Acetic Acid. *J. Am. Chem. Soc.* **1972**, *94* (4), 1340−1345.
(28) David, W. I. F.; Shankland, K.; Cole, J.; Maginn, S.; Motherwell, W. D. S.; Taylor, R. *DASH user manual*; Cambridge Crystallographic Data Centre: Cambridge, England, 2001.
(29) Lamprou, D. A.; Smith, J. R.; Nevell, T. G.; Barbu, E.; Stone, C.; Willis, C. R.; Tsibouklis, J. A comparative study of surface energy data from atomic force microscopy and from contact angle goniometry. *Appl. Surf. Sci.* **2010**, *256* (16), 5082−5087.
(30) Yang, J.; Mei, Y.; Hook, A. L.; Taylor, M.; Urquhart, A. J.; Bogatyrev, S. R.; Langer, R.; Anderson, D. G.; Davies, M. C.; Alexander, M. R. Polymer surface functionalities that control human embryoid body cell adhesion revealed by high throughput surface characterization of combinatorial material microarrays. *Biomaterials* **2010**, *31* (34), 8827−8838.