This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard **Terms & Conditions** and the **Ethical guidelines** still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

CrystEngComm

ARTICLE TYPE

Template–induced polymorphic selectivity: The effects of surface chemistry and solute concentration on carbamazepine crystallisation

Jose V. Parambil,[a,b] **Sendhil K. Poornachary,**[c] **Reginald B.H. Tan**[b,c] **and Jerry Y.Y. Heng**[a]

Cooling crystallisation of carbamazepine solution in glass vials functionalised with different silane molecules resulted in preferential nucleation of metastable form II or stable form III polymorphs within a definite range of supersaturation. In
10 **contrast, the two crystal forms nucleated concomitantly on a control substrate under similar solution conditions.**

Polymorph control in pharmaceutical crystallisation is mainly ensured by seeding with the required crystal form and ensuring strict control of solution supersaturation. Seeding not only helps
15 in reducing the crystallisation batch time through secondary nucleation but also provides an additional control over particle size distribution.[1] Nevertheless, the properties of seed crystals, such as surface area, roughness and defects, have to be consistent throughout in order to minimise batch-to-batch variation in
20 product quality. Heterogeneous nucleation is thermodynamically favoured over homogeneous nucleation since the high surface energy component required to produce a stable nucleus is reduced by the contribution from a heterogeneous nucleating surface. As a result of the reduced surface energy component associated with
25 heterogeneous nucleation, most nucleation events can be envisaged to occur via heterogeneous nucleation following a two stage nucleation pathway.[2] One of the motivations for developing a template–induced crystallisation process using tailor–made surfaces is to improve upon reproducibility with respect to the
30 crystal form obtained. Epitaxial relationship between a template surface and nucleating crystal has been singled out to be the most critical factor in polymorph selective crystallisation by heterogeneous nucleation.[3] However, for certain systems, it has been reported that the influence of chemical interactions at the
35 heterogeneous nucleant interface may dominate in directing polymorphic nucleation.[4]

Template substrates used for inducing crystallisation of a specific polymorph range from crystalline inorganic surfaces,[5] single crystals or surfaces adsorbed with structurally–related
40 molecule or a different crystal form of the same molecule,[6] self-assembled monolayers (SAMs) of thiols[7] and chiral molecules,[8] polymer[9] and organosilane substrates.[4] Besides molecular functionality, porosity of the template substrate has been reported to influence crystallisation behaviour of both small molecules and
45 proteins.[10] However, the effect of solute concentration on template–mediated nucleation is often overlooked and no studies have been reported to date.

In this study, glass vials were chemically modified via silanisation procedure reported by Shah et al.[11] The silanised
50 surfaces were analysed using contact angle measurements and ellipsometry (see ESI†). Cooling crystallisation of carbamazepine

(CBZ), an anticonvulsant and mood-stabilising drug, from ethanol solutions of different initial concentrations was carried out in the silanised glass vials. CBZ was selected as the model
55 compound in these studies because of its well characterised polymorphic forms[12] and crystallisation behaviour from common organic solvents.[13] Moreover, recently a new polymorphic form of CBZ was reported to form on the crystal facet of an analogous molecule directed by epitaxial relationship.[6]

60 **Table 1** Surface energy components for functionalised templates calculated using the Owens-Wendt method.

| Head group	Cyano (-CN)	Mercapto (-SH)	Fluoro (-CF$_3$)
γ_d (mJ/m^2)	38.0	37.6	19.2
γ_p (mJ/m^2)	14.2	5.0	2.5
γ (mJ/m^2)	52.3	42.6	21.7

γ_d, γ_p and γ stand for dispersive, polar and total surface energy of the template surface respectively.

65 Surface energy of the modified glass substrates were calculated using Owens-Wendt method[14], and the polar and dispersive components of surface energy values are reported in Table 1.It can be seen that the cyano–functionalised surface is the most polar and also exhibits highest surface energy amongst the
70 three functionalised templates. The thickness of the silane layer on a silicon wafer substrate as determined by ellipsometry‡ was found to be 1.37±0.03 nm. Considering that the linear length of a stretched out silane molecule equals to *ca.* 7 Å, the silane layer thickness should correspond to 2–3 layers of silane molecules
75 depending on its molecular orientation on the surface.

The crystallisation experiments were conducted at 20°C with 3 mL solution with initial solution concentrations in the range of 45–65 mg/ml in glass vials placed inside a temperature controlled incubator. Induction time measurements correspond to the time
80 for observation of a first crystal (<10μm) nucleating from the supersaturated solution using a digital handheld optical microscope (VMS-004D, Veho U.K.). Induction time measurements can be affected by sample volume and experimental setup.[15] Hence, precautions were taken to ensure
85 identical conditions between control and silanised templates with surface chemistry being the only variable between vials. CBZ crystal polymorphs were distinguished from its growth morphology, with needle shaped form II and tetrahedral form III crystals. The polymorphic forms were further confirmed through
90 powder X-ray diffraction (see ESI†). Throughout this study, at least 10 trials of crystallisation experiments were carried out for each solute concentration, with each trial comprising 3 replicates

of the same template surface. Control experiments were performed in clean glass vials at similar solution conditions.

Results of CBZ crystallisation conducted in glass vials from an initial concentration of 60 mg/ml are shown in Figure 1. In the control glass vials, both form II and form III polymorphs crystallised concomitantly, eventually transforming into stable form III crystals in about 24 h. Crystallisation in mercapto and fluoro silanised glass vials nucleated more of form III crystals and very few form II crystals compared to control vials. In contrast, the cyano silanised vials predominantly resulted in nucleation of metastable form II crystals. By monitoring the images captured at different time intervals, it was revealed that the metastable crystals formed on cyano functionalised vials did not transform to the stable form even after 24 h. This could be reasoned due to desupersaturation of the solution primarily through nucleation of form II crystals. Consequently, form II crystals are prevented from undergoing polymorphic transformation in the absence of any stable form III crystal nuclei. On the other hand, on introducing form III seed crystals into the system, form II polymorph completely transformed to the stable form within 24 h.
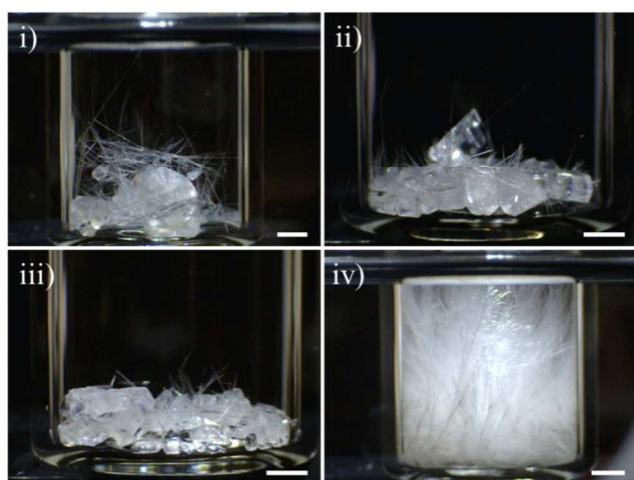
Figure 1. Carbamazepine crystallised from 60 mg/ml solution in glass vials silanised with different functional groups: i) control; ii) mercapto; iii) fluoro; iv) cyano. The crystals were photographed after 24 h from the start of crystallisation. Scale bar represent 200μm length.
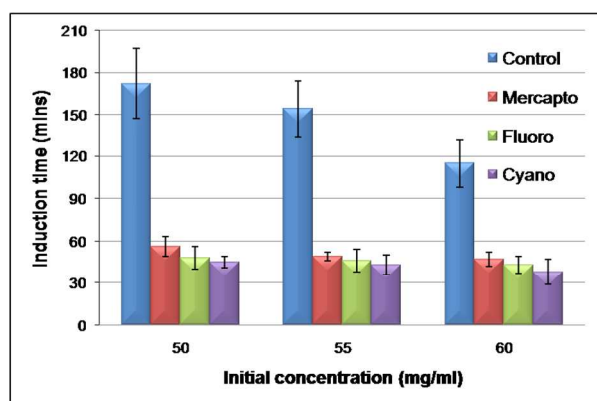

Figure 2. Induction period for nucleation of carbamazepine crystals in silanised glass vials at various solute concentrations.

The induction periods for nucleation of CBZ crystals at various solute concentrations and under the influence of different template surface chemistries are shown in Figure 2. With the increase in solute concentration from 50 mg/ml to 60 mg/ml, the induction period decreased from 172 ± 24 min to 115 ± 17 min. This data qualitatively agrees with the classical nucleation relationship between induction period and solution supersaturation. For an initial solute concentration of 60 mg/ml, the observed induction period was ~2 h in control glass vials as compared to ~45 min in each of the silanised glass vials. Likewise, at 55 mg/ml and 50 mg/ml solute concentrations, the induction period was shortened by 2−3 times, due to the influence of templates. The effect of template surfaces on the number of crystals formed was also pronounced. While a large number of either form II or form III crystals were observed in functionalised glass vials, only a few big crystals of form III together with form II needles were observed on the control surfaces (cf. Figure 1). While these kinetic data clearly shows that the silanised surfaces in general enhanced heterogeneous nucleation of CBZ crystals, no specific effect of the template surface chemistry on the induction times were observed.

Furthermore, template−mediated nucleation of CBZ crystal polymorphs was found to be influenced by the initial solute concentration (Figure 3). At 55 mg/ml, the polymorphic forms crystallised in the control and silanised glass vials respectively were akin to that observed at 60 mg/ml. At 50 mg/ml, nucleation in the control vessel was dominated by form III crystals, along with a few form II polymorph crystallised concomitantly. On mercapto and fluoro−functionalised surfaces, form III nucleation was clearly dominant over form II at this solute concentration. In contrast, cyano−functionalised surfaces produced form II crystals initially followed by nucleation and growth of form III. Consequently, the form II crystals underwent solution−mediated transformation to the stable form III within 24 h. Upon further reducing the initial solute concentration to 45 mg/ml, no apparent differences were observed in the polymorphic forms crystallised on the control and functionalised surfaces. Above 60 mg/ml, CBZ crystallisation was dominated by form II nucleation on both the control and functionalised surfaces. Thereafter the form II crystals remained stable for more than 24 h without undergoing polymorphic transformation.
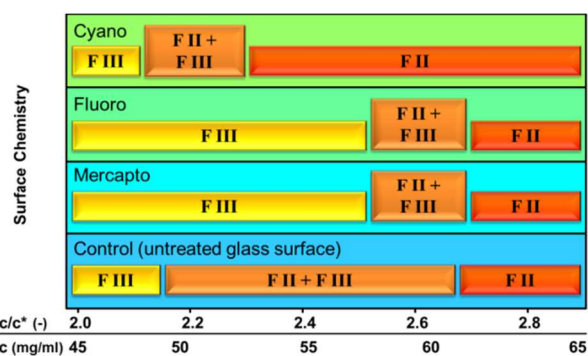
Figure 3. The effects of surface chemistry and initial supersaturation (c/c*, calculated based on the solubility of form III) on crystallisation of carbamazepine polymorphs from ethanol at 20°C. Initial solute concentration (mg/ml) is also shown on the horizontal axis for reference.

Preferential nucleation of polymorphs on templates with different chemistry could be attributed to specific molecular interaction at template interface. Due to the multilayer thickness of silane layer, an epitaxial relationship between crystal facet and template surface appear highly improbable. However, facet specific electrostatic interactions involving dipole moments and hydrogen bonding potentials emerge as the most probable

candidates that can result in polymorph selective nucleation. The polarity of the template surface can also play a role in resulting in a preferential orientation of crystals as reported for polymer heteronuclei.[16] Also, polarity of the solvents used in CBZ crystallisation has been established to affect the structure of prenucleation aggregates.[17] Nevertheless, a direct correlation between nucleant surface polarity and facet specific crystal properties is not identified.

From the experimental observations, it is conclusive that the template surface chemistry had effectively mediated preferential nucleation of CBZ crystal polymorphs from ethanol solutions only within a definite solute concentration range of 50–60 mg/ml. This corresponds to a supersaturation (c/c*) window of 2.1–2.6. Outside this solute concentration range, the kinetics of homogeneous and/or heterogeneous primary nucleation was dominant over template–mediated nucleation resulting in crystallisation of the stable and metastable polymorphs at the lower and higher supersaturations respectively. As the functional groups on silanised templates differ in polar and non-polar surface energies, it is likely that intermolecular interactions with nucleating cluster had potentially influenced the template–mediated nucleation of CBZ crystal forms. However, an epitaxial relationship between the template substrate and crystal polymorph seems elusive. As a logical extension of this work, chemical interactions between the template surface and various facets of CBZ crystal polymorph(s) are currently being analysed to gain further insights into the template–induced nucleation process. Building on this study, a design space for selective crystallisation of CBZ polymorphs is being developed by incorporating surface chemistry as a process parameter in the concentration–temperature phase diagram.

Notes and references

ªSurfaces and Particle Engineering Laboratory, Department of Chemical Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom. Tel: 44 (0)20 7594 0784; E-mail: jerry.heng@imperial.ac.uk
ᵇDepartment of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576ᵇ
ᶜInstitute of Chemical and Engineering Sciences, A*STAR (Agency for Science, Technology and Research), 1 Pesek Road, Jurong Island, Singapore 627833

† Electronic Supplementary Information (ESI) available: Details on silanisation method, silane structure, advancing contact angle measurements of four probe liquids on template surface, ellipsometry and PXRD data are provided in ESI. See DOI: 10.1039/b000000x/

‡ Ellipsometric data was fitted using combined Cauchy layer model with a refractive index of 1.45 for the silane and SiO_2 layers on Si wafer surface. The thickness of the silane layer was calculated by subtracting the SiO_2 layer thickness from the total Cauchy layer thickness.

1. S. L. Morissette, O. Almarsson, M. L. Peterson, J. F. Remenar, M. J. Read, A. V. Lemmo, S. Ellis, M. J. Cima and C. R. Gardner, *Advanced Drug Deliv Reviews*, 2004, **56**, 275-300.
2. D. Erdemir, A. Y. Lee and A. S. Myerson, *Accounts of Chemical Research*, 2009, **42**, 621-629.
3. K. Chadwick, A. Myerson and B. Trout, *CrystEngComm*, 2011, **13**, 6625; R. Hiremath, J. A. Basile, S. W. Varney and J. A. Swift, *Journal of the American Chemical Society*, 2005, **127**, 18321-18327; A. Y. Lee, A. Ulman and A. S. Myerson, *Langmuir*, 2002, **18**, 5886-5898.
4. J. Zhang, A. Liu, Y. Han, Y. Ren, J. Gong, W. Li and J. Wang, *Crystal Growth & Design*, 2011, **11**, 5498-5506; T. Delmas, U. V. Shah, M. M. Roberts, D. R. Williams and J. Y. Y. Heng, *Powder Technology*, 2013, **236**, 24-29; B. K. Olmsted and M. D. Ward, *CrystEngComm*, 2011, **13**, 1070.
5. L. Ruan, H. Ramezani-Dakhel, C. Y. Chiu, E. Zhu, Y. Li, H. Heinz and Y. Huang, *Nano letters*, 2013, **13**, 840-846; O. Werzer, B. Kunert, E. Roblegg, A. Zimmer, M. Oehzelt and R. Resel, *Crystal Growth & Design*, 2013, **13**, 1322-1328.
6. J. Urbanus, J. Laven, C. P. M. Roelands, J. H. Horst, D. Verdoes and P. J. Jansens, *Crystal Growth and* Design, 2009, **9**, 2762; J. Tao, K. J. Jones and L. Yu, *Crystal Growth and Design*, 2007, **7**, 2410; J.B. Arlin, L. S. Price, S. L. Price and A. J. Florence, *Chemical Communications*, 2011, **47**, 7074-7076.
7. A. Kwokal, T. T. H. Nguyen and K. J. Roberts, *Crystal Growth and Design*, 2009, **9**, 4324; R. Hiremath, S. W. Varney and J. A. Swift, *Chemical Communications*, 2004, 2676-2677.
8. D. H. Dressler, I. Hod and Y. Mastai, *Journal of Crystal Growth*, 2008, **310**, 1718-1724.
9. M. D. Lang, A. L. Grzesiak and A. J. Matzger, *Journal of the American Chemical Society*, 2002, **124**, 14834-14835.
10. U. V. Shah, D. R. Williams and J. Y. Y. Heng, *Crystal Growth & Design*, 2012, **12**, 1362-1369.
11. U. V. Shah, M. C. Allenby, D. R. Williams and J. Y. Y. Heng, *Crystal Growth & Design*, 2012, **12**, 1772-1777.
12. A. L. Grzesiak, M. Lang, K. Kim and A. J. Matzger, *Journal of Pharmaceutical Sciences*, 2003, **92**, 2260-2271.
13. C. A. Hunter, J. F. McCabe and A. Spitaleri, *CrystEngComm*, 2012, **14**, 7115.
14. D. K. Owens and R. C. Wendt, *Journal of Applied Polymer Science*, 1969, **13**, 1741-1747.
15. R. J. Davey, S. L. Schroeder and J. H. ter Horst, *Angew Chem Int Ed Engl*, 2013, **52**, 2166-2179.
16. E. Curcio, V. López-Mejías, G. Di Profio, E. Fontananova, E. Drioli, B. L. Trout and A. S. Myerson, *Crystal Growth & Design*, 2014, **14**, 678-686.
17. C. A. Hunter, J. F. McCabe and A. Spitaleri, *CrysEngComm*, 2012, 14, 7115-7117.
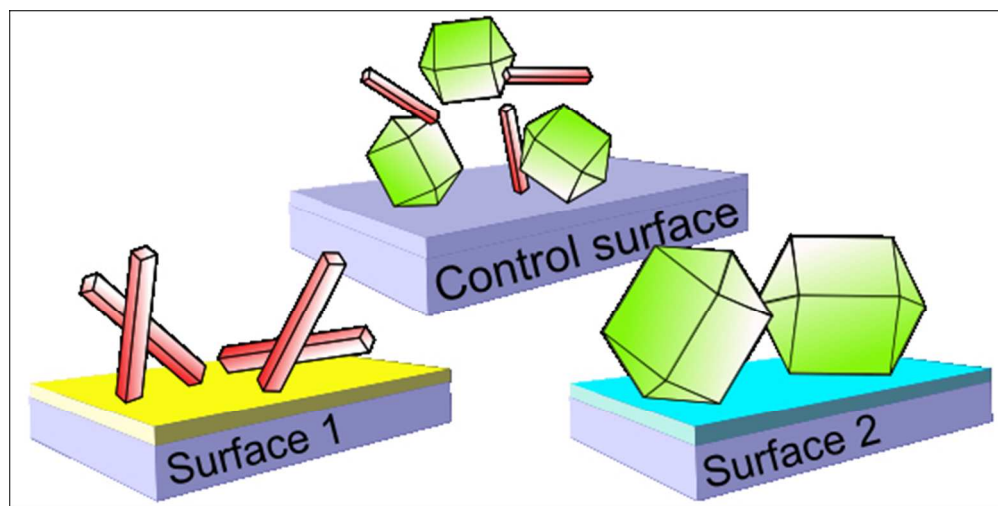
Crystallisation of form II, form III or forms II+III (concomitant) carbamazepine by varying nucleant surface chemistry and solute concentration.