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COMMUNICATION

Polymorphism Control of an Active Pharmaceutical Ingredient Beneath Calixarene-based Langmuir Monolayers

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This communication demonstrates the possibility to nucleate and grow different crystalline polymorphic forms of gabapentin (GBP) using, as nucleation templates, Langmuir monolayers of an amphiphilic calixarene at different packing densities.

Solid-state polymorphism has a pivotal role in pharmaceutical sciences as many active pharmaceutical ingredients (APIs) exist in several crystalline polymorphic forms, which may exhibit divergent bioavailability and pharmacokinetic properties.[1] Therefore, the US Federal Drug Administration (FDA) acknowledges each polymorph of an API as a patentable entity.[2] Besides strategies based on the modulation of chemical (*e.g.*, solvent, co-solvent, additive) and physical (*e.g.*, evaporation, sublimation, thermal treatment, pressure) crystallization conditions,[3] heterogeneous nucleation represents a valuable alternative to control crystal growth processes with a possible control over the polymorphism of the produced crystals. For example, polymorphism control can be achieved using crystallization templates such as supramolecular gels,[4] polymer surfaces,[5] single crystal faces,[6] self-assembled monolayers[7] and Langmuir films.[8] Considering the latter approach, Mann demonstrated that Langmuir monolayers can act as crystal nucleation templates of calcium carbonate in the vaterite polymorphic form.[8] Besides systems based on simple surfactants, the potential of more complex amphiphiles has also been evaluated.[9] Recently, we demonstrated that the crystallization kinetics of acetaminophen can be modulated using Langmuir monolayers of an amphiphilic calix[4]arene.[10] To our knowledge, no control over the polymorphism of APIs through the modulation of the packing of the monolayer has been demonstrated to date. This can be explained by the fact that even if the Langmuir monolayer approach theoretically provides the possibility to control the packing density of template amphiphiles at the air-water interface, strong intermolecular interactions among these molecules often dominate the interfacial self-assembly process. Consequently, this leads to interfacial molecular organizations where the packing state variations are limited. We and others demonstrated that Langmuir monolayers of tetra-dodecyl-*p*-carboxy-calix[4]arene, during the film compression, undergo an organizational phase transition.[10b,11] In this communication, we demonstrate that this phenomenon can be exploited to control the crystalline polymorphism of gabapentin.

5,11,17,23-tetra-carboxy-25,26,27,28-tetradodecyloxycalix-[4]arene, **1**, in the cone conformation, was synthesized as previously described;[10b] *cf.* Fig. 1. **1** was recrystallized from pyridine; the resulting weakly diffracting crystals were of suitable quality for single crystal X-ray diffraction studies. Structure was solved in a triclinic space group *P* -1 with one molecule of **1** and four molecules of pyridine in the asymmetric unit (ASU); *cf.* Fig. 1 and S1 in ESI.† **1** is seen to adopt a pinched cone conformation with the shorter arene-arene distance of 4.7 Å; this is in agreement with the tetra-butoxy-*p*-carboxylatocalix[4]arene structure reported by Dalgarno.[12] Symmetry expansion of the ASU affords a bilayer structure where the aliphatic chains are organized in an all-parallel fashion, with a slight tilt of 9.7° with regard to the bilayer axis.

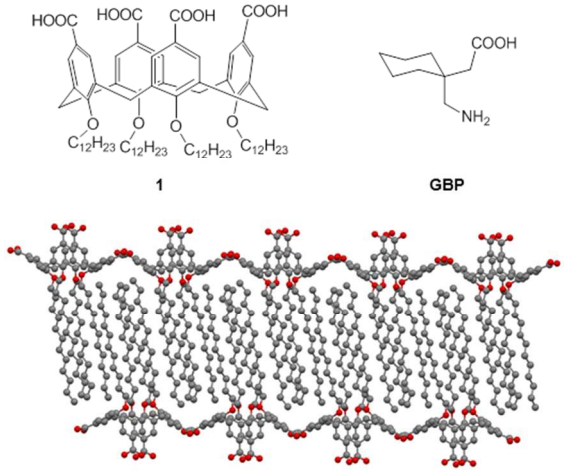

Fig. 1 Chemical structures of **1** and GBP (top) and packing arrangement of the crystal structure of **1** (bottom). Solvent molecules and hydrogen atoms are omitted for clarity.

The interactions of **1**, self-assembled as Langmuir monolayers, with GBP were studied by measuring surface pressure-area (Π/A) compression isotherms on a GBP subphase (5 g L^{-1}); the thickness of the film was measured in real-time during the compression using surface ellipsometry; *cf.* Fig 2. On pure water, the monolayer collapses at an apparent area of 99 ± 1 $Å^2$ $molecule^{-1}$ and a surface pressure of 51.5 ± 0.5 mN m^{-1}, which is consistent with a calixarene-based amphiphile in the cone conformation.[13]

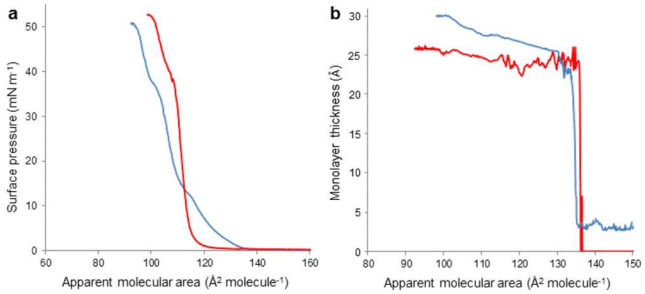

Fig. 2 Surface pressure-area compression isotherms (**a**) and film thickness measured by surface ellipsometry (**b**) at the air-water interface of **1** on pure water (–) and on a 5 g L^{-1} aqueous GBP solution (–).

The isotherm profile shows a phase transition at 37.5 ± 0.5 mN m^{-1} (107 ± 1 Å^2 $molecule^{-1}$) suggesting a molecular rearrangement of the amphiphiles, in agreement with previously reported data.[11] The ellipsometric measurements reveal that, before reaching an available area per molecule of 137 ± 0.5 Å^2 $molecule^{-1}$, no thickness can be measured. This suggests a macroscopic gas-phase behaviour of the amphiphiles. Nevertheless, Brewster angle microscopy experiments show, at 0 mN m^{-1}, the existence of liquid domains floating at the surface of the water subphase (Fig. S2). Their surface coverage is too low to generate a measurable surface thickness value and the signal remains in the background. Upon further compression, the thickness sharply increases to a value fluctuating around an average of 24 ± 1 Å and remains constant until the phase transition. After this phase transition, a slight but significant increase of 2 Å is measured and the monolayer thickness reaches 26 ± 1 Å. This value is reasonably consistent with the molecular structure of the calixarene macrocycle self-assembled as a densely packed monolayer with the C_4 pseudo-symmetry axis parallel to the interface. In the presence of GBP, the situation is significantly different. Indeed, even before the isotherm take-off, an average thickness of 2 Å could be measured, suggesting the presence of islands of liquid phase domains at the interface. At the isotherm take-off (136 ± 1 Å^2 $molecule^{-1}$), the thickness sharply increases to 24 ± 0.5 Å and, beyond this value, steadily increases up to 26 ± 0.5 Å until the phase transition at 36 ± 0.5 mN m^{-1}. Upon further compression, the thickness interestingly increases up to 30 Å, a thickness value that is definitely too high to be explained by the presence of only **1** at the interface. This higher thickness value can be attributed to a partial layer of GBP in contact with the Langmuir monolayer and thus suggests that the amphiphile organization, after the phase transition, significantly favours the interaction of the monolayer with GBP.

The 2D structure of monolayers of **1** was investigated by synchrotron-generated X-ray reflectivity (XR) and grazing incidence X-ray diffraction (GIXD). Inspection of the electron density (ED) profile shows negligible change in the ED of the head group region suggestive of only weak and sporadic adsorption of GBP to the monolayer on a dilute GPB subphase that has no effect on the structure of the calixarene monolayer (Fig. S3). GIXD of the monolayer of **1** (*cf.* Fig. 3), compressed at 25 mN m^{-1}, shows a broad peak (A1) at Q_{xy} ~ 1.40 Å^{-1} for Q_z < 0.2 Å^{-1} that corresponded to a *d*-spacing (= 2π Q_{xy}^{-1}) ~ 4.5 Å, a feature that was observed for a short chain surfactant on water surfaces[14] and was attributed to bulk hydrocarbon chains in the liquid state.[15]

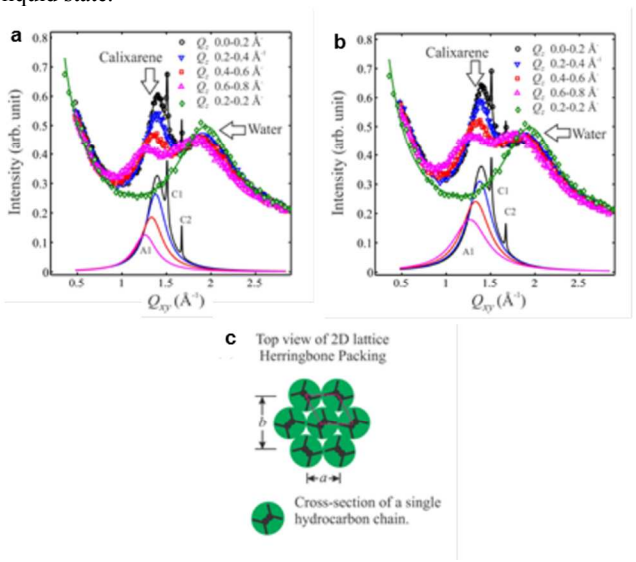

Fig. 3 GIXD patterns of monolayers of **1** on water (**a**) and on a 5 g L^{-1} aqueous GBP subphase (**b**) both compressed at 45 mN m^{-1}. Each data point is integrated over the Q_z range as indicated. The solid lines are the best fit of superposed Lorentzian functions. Solid lines are obtained after subtraction of water signal. Schematic representation of 2D herringbone packing of hydrocarbon chains, in a rectangular unit cell (**c**). The dashed-lines group 4 NN chains that represent a single molecule of **1**.

Overlay of the A1 peak integrated over different Q_z ranges shows that the A1 peak shifts to lower Q_{xy} with increasing Q_z, while its intensity (over Q_{xy}) falls off with Q_z. It is reminiscent of the diffractions from uniaxially oriented polymers, where the chain-chain correlations give rise to interference peaks along equatorial directions (i.e. Q_{xy}) and spread onto an arc toward meridian direction (i.e. Q_z) due to misorientation. The finite extension of intensity distribution may result from combined effect of finite length of hydrocarbon chains and finite degree of misorientation. The full width at half maximum (FWHM) of A1 (of Lorentzian line shape) is Q_{xy} ~ 0.2-0.3 Å^{-1}, corresponding the lateral short range order (SRO) correlation length l ~ 6-10 Å, so that A1 peak results from interference of hydrocarbon chains with nearest neighbour (NN) and at most with next nearest neighbour (NNN) chains. When the monolayer is compressed above the phase transition (π = 45 mN m^{-1}), two additional sharp Bragg-reflections (C1 and C2) emerge within Q_z < 0.2 Å^{-1} at Q_{xy} ~ 1.51 and 1.67 Å^{-1}, with a peak height ratio 2:1. Using a rectangular unit cell with *a* = 5.0 Å and *b* = 7.5 Å (Fig. 3c), the C1 and C2 peaks index as {11} and {02} crystalline planes, respectively, with d_{02} = 3.76 Å and d_{11} = 4.16 Å. This structure is consistent with the herringbone acyl-chain packing commonly observed in crystalline alkanes and in a few Langmuir monolayers of lipids.[16] Consequently, this highly ordered phase coexists with SRO previously discussed above the phase transition. Interestingly, although molecules of **1** possess a higher degree of molecular complexity compared to alkanes (i.e., four alkyl chains per single molecule), they show similar packing features when self-assembled as Langmuir monolayers. The GIXD pattern of the monolayer of **1** on a dilute aqueous GBP subphase (5 g L^{-1}) is similar to that on pure water, consistent with the reflectivity that showed a weak influence of GBP on the organization of the monolayer. This strongly suggests that the presence of GBP does

not drastically influence the hydrocarbon-chains packing of **1** at the air-water interface.

The ability of **1**-based monolayers to crystallize GBP at the air-water interface was investigated by spreading, at the surface of supersaturated GBP solutions (150 g L^{-1}†), adequate amounts of **1** corresponding on the Langmuir compression isotherm to values of 0, 1, 25 and 45 mN m^{-1}. Interestingly, while the control experiments did not contain any solids, even the vials with a limited amount of **1** spread at the interface (corresponding to a surface pressure of 0 mN m^{-1}) had crystalline material at the interface after 14 days, a representative photograph is given in Fig. S1. On the other hand, crystals in the control vials appeared as late as after 4 weeks, mostly in solution. Infrared (IR) spectroscopy (Fig. S4) revealed that while the crystals grown beneath monolayers below the surface pressure of the phase transition (0, 1, and 25 mN m^{-1}) were the polymorph γ,[17] those produced above this transition (45 mN m^{-1}) were the polymorph α;[18] both structures are anhydrous polymorphs of GBP, and no differences in crystallization kinetics were observed. The hydrate polymorphic form of GBP, named I, nucleated in the controls vials.[18] Single crystal X-ray diffraction experiments confirmed the polymorphs identities and face-indexing analyses revealed that, whereas the polymorph α nucleated with the (001) crystallographic plane parallel to the air-water interface, the polymorph γ grew with the (100) crystallographic plane parallel to the interface. Interestingly, both the polymorphs are organized in a bilayered fashion, with the planes of the bilayers parallel to the crystallographic planes. These layered packing supports our initial hypothesis on the possibility to use calixarene-based Langmuir monolayers as crystallization templates where the polar head groups of the amphiphile are expected to act as molecular recognition units, assembling the API in an organized fashion at the interface to initiate crystal growth (*cf.* Fig. 4).

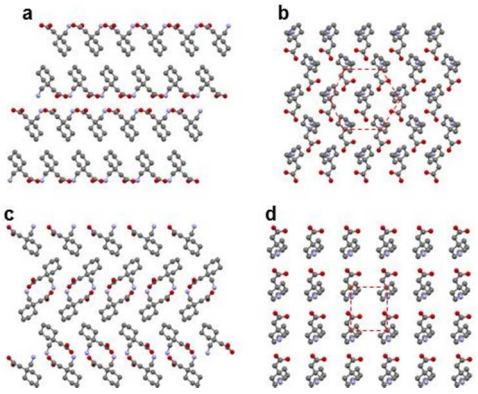

Fig. 4 Packing arrangements of GBP polymorphic form γ (**a**: side view, **b**: top view) and α (**c**: side view, **d**: top view).

In our attempts to assess the possibility of epitaxial match between **1**-based monolayers and GBP crystals, we applied the model developed by Hillier and Ward that allows studying epitaxial interactions between molecular overlayers and ordered substrates.[19] The difficulty to conclude from the GIXD data on the molecular organisation of the macrocycle and consequently of the polar recognition headgroups, prevented us from establishing a clear picture of the epitaxial match between the monolayer and the crystals of GBP.

In summary, we have demonstrated that Langmuir monolayers of a calixarene-based amphiphile at different interfacial compression states can trigger the crystallization of two polymorphic forms of GBP (α and γ). Interestingly, even the phase deprived of long-range crystalline order templates the crystallization of an anhydrous polymorph of the API.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details, crystal structures of **1**, photograph of GBP crystals**,** BAM micrographs, X-ray reflectivity, IR spectra of GBP. See DOI: 10.1039/b000000x/

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