A rare case of mesomorphic behaviour – molecular reorientation of itraconazole liquid crystal induced by a hygrothermal treatment

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Abstract

Liquid crystals constitute a unique class of soft state of matter exhibiting the counterintuitive combination of fluidity and order in addition to the unique feature of controllable molecular orientation. Herein, we introduce the use of moisture sorption approach to reversibly realign mesogen molecules within a liquid crystalline phase of itraconazole, assigned to as a nematic phase (ITR-N), obtained by spray drying. This unique, nematic-to-smectic switching behaviour of ITR was achieved via moisture triggered activation of molecular mobility of ITR molecules below the material's glass transition temperature (T_g). This phenomenon was characterised using small and wide angle X-ray diffraction, scanning electron microscopy and thermal analysis. The smectic phase was found to be formed by a 1:1 mole/mole ITR:water complex, an example of rare thermotropic mesomorphism of a solvent complex, and the water presence was found to be necessary for the material to retain the smectic order. The ordered sample, in the smectic phase, possessed a greater T_g than ITR-N due to an increase in order and subsequent increase in rigidity. This behaviour highlights the ability of ITR to accommodate stress conditions via molecular rearrangement and thus resist full crystallisation, which rationalises the importance of the liquid crystalline phase.

1. Introduction

Liquid crystals (LCs) are states of condensed matter that exhibit an intermediate molecular arrangement between the amorphous and crystalline states. They are often referred to as mesophases and they possess structural, mechanical and optical properties intermediate to those of the three dimensionally ordered solids and the isotropic liquids ¹. LCs can be categorised according to their structure, composition and method of production into thermotropic (TLCs) and lyotropic (LLCs) liquid crystals. TLCs can be further sub-classified according to the arrangement of mesogens into, mainly, nematic phases, which lack the positional order but the molecules tend to point in the same direction, and smectic phases exhibiting a layered arrangement of orientationally ordered molecules ^{2, 3}. Other molecular arrangements including cubic and hexagonal columnar phases can also be observed in TLCs ⁴. Similarly, LLCs can be mainly sub-classified into smectic, lamellar, cubic and columnar phases and are usually identified based on the texture and X-ray diffraction data ⁵. Some molecules, referred to as amphotropic, can exhibit both, TLC and LLC phases ⁴.

TLCs are usually formed via heating a crystalline solid material or cooling a melted mesogen, thus the formation of TLCs is a function of temperature and secondarily pressure ^{3, 6}. In contrast, lyotropic LCs are usually formed by the addition of a solvent to the mesogen, where the mesogen's concentration and temperature are the controlling parameters ³. Some pharmaceuticals have been reported to form LCs, including MK-571 (L-66071, a leukotriene D4 receptor antagonist), nafoxidine hydrochloride, palmitoyl propranolol hydrochloride, arspenamine, penbutolol sulfate and cyclosporine ^{3, 7}. Among those, the first three exhibit the amphotropic behaviour.

Water vapour sorption by pharmaceutical solids is known to cause deliquescence, hydrate formation and capillary condensation in porous materials as demonstrated for sodium salicylate and emetine dihydrochloride ^{8, 9}. However, a few reports on a crystal to liquid crystal (MK-571

⁷ and trifluoperazine dihydrochloride ¹⁰) and a liquid crystal to the isotropic phase transition (GNE068 ¹¹) following the water vapour uptake are available in the literature for pharmaceuticals.

In addition to the amphotropic behaviour, a polymorphic transformation, usually referred to as switching between different LC phases, is another observed phenomenon in mesophases and is achievable via molecular realignment. Molecular realignment can be achieved using a thermal treatment, including thermal expansion, and photo-induction ^{4, 12, 13}. Thermotropic mesomorphism through a guest-host relationship or solvent complexes was observed in 3-[2'-methyl-4'-(3-methylbenzyloxy)biphenyl-4-yloxy]propane-1,2-diol in the presence of protic solvents (glycerol and water), where only a limited quantity of these solvents was taken up by the compound instead of solvation/dissolution in the traditional sense ¹⁴. However, to the best of our knowledge, mesomorphic interconversion or switching via hygrothermal treatment has not been reported before.

Itraconazole (ITR) is an antifungal drug with molecules that are rod-like shaped. It is known to form thermotropic LC phases when cooled from a melt with two phase transitions observed at 90 and 74 °C ². These transitions were assigned to the formation of the nematic and smectic A LC phases, respectively ^{15, 16}. We have previously reported the formation of ITR LC nanoparticles using antisolvent precipitation ¹⁷. The effect of the solvent to antisolvent temperature difference (ΔT) on the structure of the LC phase of ITR was further investigated. A LC phase with the highest degree of order was observed at ΔT of 0 °C. This new liquid crystalline phase exhibited three equidistant quasi-Bragg peaks corresponding to the interplanar spacing of 28.0, 14.5 and 9.7 Å and it was assigned to an intercalated smectic A phase ¹⁸. Recently, we have reported on the existence of a new phenomenon of a liquid-liquid crystalline phase separation, where the liquid crystal phase was observed as an intermediate prior to the ITR crystal growth ¹⁹.

The aim of this work was to demonstrate and characterise a new effect, by which the alignment of mesogens in the nematic phase of ITR was altered using a hygrothermal treatment of the sample. The combination of moisture and temperature induced sub-T_g molecular motions and reorientation of molecules were observed and phases identified using wide angle (WAXS) and small angle (SAXS) X-ray diffraction. The thermal behaviour of the different ITR LC phases was investigated using high speed differential scanning calorimetry (HSDSC) and modulated differential scanning calorimetry (MDSC). This demonstration of the ability of mesogenic molecules in the nematic LC phase to realign to a more ordered smectic phase without crystallisation offers a new approach of formulating pharmaceuticals into LCs, where feasible.

2. Materials and Methods

2.1. Materials

ITR was a gift from Neuland Laboratories Ltd. (Welding, Hamburg, Germany). Poloxamer 407 (P407) was purchased from BASF Corp. (Ludwigshafen, Germany). Dichloromethane Chromasolv[®] for spray drying was purchased from Sigma-Aldrich (Dorset, UK). Water ($\geq 99.8\%$), employed in DVS experiments was acquired from Sigma-Aldrich (Dublin, Ireland). All other chemicals were of analytical grade and used as received.

2.2. Production of ITR samples

Mesomorphous microparticles of ITR (median particle size of 3.1 μm by laser diffraction) were prepared via spray drying using a laboratory scale Buchi B-290 mini Spray Dryer (Buchi Laboratories Technik AG, Flawil, Switzerland) operating in the open cycle mode configuration using nitrogen as a drying gas. A 4% w/v solution of pure ITR or ITR/P407 mixture (containing 95% w/w ITR and 5% w/w P407) in dichloromethane was spray dried using the following parameters: inlet temperature of 40 °C, outlet temperature of 34 °C, atomising gas flow of 40 mm (rotameter setting) corresponding to 473 normL/h, aspirator rate of 100% and a feed flow rate of 25% (10 ml/min).

2.3. Dynamic vapour sorption

ITR samples were analysed with a Dynamic Vapour Sorption (DVS) Advantage-1 automated gravimetric vapour sorption analyser (Surface Measurement Systems Ltd., London, UK). The temperature was maintained constant at 50.0 ± 0.1 °C. The analysis was performed in triplicate for each sample. In all measurements, around 10 mg of powder was loaded into a sample quartz pan and placed in the system. All samples were equilibrated at 0% of RH (0% p/p₀) until a constant mass change ($dm/dt \leq 0.002$ mg/min for at least 10 min) was achieved. The reference mass was recorded as a mass equilibrated at 0% RH. The samples were then subjected to one step water vapour sorption/desorption profiles at 50% or 95% RH for 5 hours. Photos were captured every two minutes during the course of sorption step using the built-in video camera to visualise the powder transformations in real-time.

A separate DVS analysis was carried out using the abovementioned conditions at 95% RH with the use of an in-house made sample holder (Figure SI.1) to enable the texture investigation using a glass cover slide as described later in Section 2.8.

DVS was utilised to determine the bulk moisture diffusion coefficient at different RH (50 °C). 20 ± 1 mg of ITR-N powder was placed in a quartz flat bottom sample DVS pan (9.3 mm in diameter). The filled pan was tapped gently followed by light compression using a flat edged spatula to form a 0.8 mm thin layer of powder. The total gas flow was parallel to the sample and it was set to 200 cm³/min for all experiments. The slope method was employed to calculate the diffusion coefficient (D_{ba})²⁰.

DVS was also used for the purpose of investigating the critical relative humidity for the moisture-induced molecular ordering (CRH_s) and the reversible deordering transition (CRH_d). In this type of measurements the samples were placed in the fine mesh basket pans at 50 °C. Following equilibration at 0% RH, the samples were exposed to a linearly increasing relative humidity environment up to 95% RH. Different ramping rates were applied (4, 15, 20, 40 and

60% RH/h). The RH_s and RH_d values were then plotted versus the relative humidity ramping rate and the intercept of the fitted line was designated as the critical RH_s (CRH_s) and critical RH_d (CRH_d), respectively ²¹.

2.4. Wide angle powder X-ray diffraction (WAXS) and small angle powder X-ray diffraction (SAXS)

WAXS measurements were performed over a 2θ range of $2-35^\circ$ or $5-35^\circ$, at 25°C , as described previously ^{18, 19}. Peak positioning and d-spacing measurements were performed using the PDXL comprehensive analysis software (Rigaku, Japan). SAXS studies were performed using a Bruker D8 Advance with a parallelised, monochromated Cu $K\alpha$ source (Goebel mirror, Ge(220) double bounce monochromator). Scanning with 666 s/point with effective 0.1 mm slits was applied ¹⁸.

2.5. Morphology of ITR microparticles

A Zeiss Supra variable Pressure Field Emission Scanning Electron Microscope (Germany) equipped with a secondary electron detector and accelerating voltage of 5 kV was used for the morphological examination of ITR microparticles. Samples were fixed on aluminium stubs using carbon tabs. Samples were sputter-coated with a layer of gold/palladium under vacuum before analysis.

2.6. High speed Differential Scanning Calorimetry (HSDSC)

A Perkin-Elmer Diamond DSC Pyris1 (Waltham, MA, USA) instrument with HyperScan was used. The purge gas was helium (60 ml/min). Samples, weighing 1-2 mg, were placed in non-hermetic standard aluminium pans. The heating rates employed were 100 and $300^\circ\text{C}/\text{min}$. The temperature calibration was performed using pure indium. Presented results are the average of at least duplicate analyses ²².

2.7. Modulated Differential Scanning Calorimetry (MDSC)

Approximately 3-5 mg of a powdered sample was analysed using a DSC Q200 (TA Instruments, UK) in a crimped Tzero aluminium pan. Samples were heated from 25 to 200 °C using a 5 °C/min heating rate, ± 0.796 modulation amplitude and 60 s modulation period. The data was analysed using Universal Analysis[®] (version 4.7A) software.

2.8. Polarised Light and Hot Stage Microscopy

Polarised light (PL) and hot stage (HS) microscopy were performed with an Olympus BX53 polarising optical microscope equipped with a U-POT cross polarizer, a Linkam hot stage and a Q IMAGING Fast 1394 camera (Olympus, Japan) at x500 magnification. The hot stage ramp, for thermomicroscopic investigations, was performed from 25 to 110 °C using a 5 °C/min heating rate.

3. Results and discussion

ITR has been reported to form two distinct, bulk thermotropic LC phases (nematic and smectic) upon a thermal treatment^{15, 23}. Recently, we have reported on the ability of ITR to form LC nanoparticles with nematic and smectic assemblies locked in the nanospheres¹⁸. The smectic arrangement was seen to disappear upon heating the material above the T_g of ITR (60 °C) confirming that molecular mobility is a pre-requisite for LC phase changes¹⁸. In this work, the ITR material was obtained by spray drying into microparticles, and its phase assigned to as a nematic phase supported by WAXS and SAXS data displayed in Figure 1. It should be highlighted that using WAXS and conventional DSC alone, this liquid crystalline phase can be misinterpreted as amorphous^{17, 24}. A similar issue with the correct phase identification has been reported for spray dried cyclosporine and quenched ursodeoxycholic acid²⁴. However, in our case, the presence of a broad diffraction peak in addition at 3.00° 2θ recorded by SAXS, characteristic of a nematic arrangement of ITR molecules, and birefringence by PLM (as shown in Figure SI.2) supports this phase assignment of spray dried ITR material, further referred to as ITR-N.

Initial experiments on studying the molecular mobility of ITR molecules in ITR-N were conducted at 50 °C and 50, 60, 75 and 95% RH (details of those experiments are available in the Supplementary Information file: “Investigation of ITR-N ordering using saturated salt solutions in an oven”). Following storing ITR-N at the abovementioned conditions for one hour, samples were taken for WAXS investigation. WAXS results, as displayed in Figure SI.2, illustrate the absence of the characteristic peaks of crystalline ITR (Figure SI.3) and the presence of an intense diffraction peak at around $6.05^\circ 2\theta$ in the diffractograms of samples stored above 50% RH. This quasi-diffraction peak at $6.05^\circ 2\theta$ is characteristic of the smectic A LC phase¹⁸. The positions of quasi-Bragg peaks were different to those of any Bragg peaks characteristic of the crystalline sample (Figure SI.3).

To trace the kinetics of formation of this liquid crystalline phase, the hygrothermal treatment was reproduced using DVS. For the DVS investigation, incubation at 50% and 95% RH (50 °C) was chosen as the lower humidity condition represents environment where no structural changes by WAXS were observed, while the higher humidity condition corresponds to environment causing the greatest structural change (monitored by the change in intensity of the peak at $6.05^\circ 2\theta$ compared to ITR-N).

Figure 1. (a) WAXS diffractograms of ITR-N, P, V and S with the peak at $6.05^\circ 2\theta$ displaying the highest peak intensity in case of S, (b) SAXS diffractograms showing the diffuse maxima at $3.00^\circ 2\theta$ for ITR-N and the increase in peak intensity in P and S. Arrows indicate (001), (002) and (003) quasi-Bragg diffraction peaks of LC phases; symbols S, V and P represent samples of ITR-N at the different stages of hygrothermal treatment by DVS as described in text. cps – counts per second, ncps – normalised counts per second.

The kinetic data obtained during water sorption of ITR-N at 95% RH (constant humidity condition by DVS) and 50°C , presented in Figure 2, revealed the presence of a marked weight change, which indicated a possible phase transition. The solid line traces the % mass change as a function of time, while the dashed line follows the relative humidity. The sorption profile of ITR-N at 95% RH exhibited three events following a lag time of 10, 18 and 38 min, which will henceforth be referred to as the peak (P), the valley (V) and the shoulder (S), respectively. There was no further phase change observed when the sample was subjected to a second cycle in DVS (the upper profile in Figure SI.4). No transitions leading to samples P, V and S were observed at 50% RH, where the maximum sorbed water was below 1% w/w (lower panel in Figure SI.4).

Figure 2. Water sorption kinetic profile for ITR-N at 95% RH (50 °C) for 300 min by DVS displaying the major transitions of ITR-N to P, V and S samples (left) and the corresponding images captured during the run (right).

WAXS and SAXS profiles characteristic of the three samples (P, V and S) are presented in Figure 1 and illustrate the presence of a phase transition occurring on the hygrothermal treatment applied. The intensity of the (002) LC peak in the low-angle region ($2\theta=6.05^\circ$), corresponding to an interlayer spacing of 14.6 Å, increased as ITR-N transitioned into P, then V and finally into S. In addition, the (003) peak at $2\theta=9.05^\circ$, corresponding to a 9.7 Å interplanar spacing, was observed in sample S. This layered mesophase has been previously assigned to an intercalated smectic A phase¹⁸. WAXS data is consistent with the visible changes to the ITR-N sample as seen in the captured video images presented in Figure 2. The captured photos illustrate apparent powder movements most likely associated with the water uptake (ITR-N $\rightarrow$ P). In contrast, no visible powder movements were observed as sample P transitioned into V, thus this transition is expected to be due to excess water expulsion following molecular ordering. This behaviour has not been reported before for mesophases and it is similar to the water expulsion observed during crystallisation of amorphous materials²¹. The ordered LC phase continued to sorb water until a total uptake of 2.5% w/w was achieved (sample S). This water sorption step is associated with powder mobility as can be inferred from the photos in Figure 2 and an increase in order as can be seen from WAXS data (Figure 1a). The SAXS data is displayed in Figure 1b and is consistent with the abovementioned results. For the ITR-N sample, a diffuse halo at around $2\theta=3^\circ$ ((001) quasi-Bragg peak) was observed. This halo is characteristic of the nematic phase at a calculated 2θ of 3.14° using the equation: $\theta = \frac{Q\lambda}{4\pi}$ ¹⁸. Here, Q is the diffraction vector and positioned at $2\pi/l_o$ where l_o is the molecule length and λ is the X-ray wavelength. The second diffuse maximum at Q of $2\pi/w_o$, where w_o

is the molecule width ⁴, was observed in the wide angle region ($2\theta=19^\circ$). As the sample transitions to higher ordered phases from P to V and then to S, the (001) peak becomes sharper and has a higher intensity (Figure 1b) compared to that of ITR-N indicating a phase switching ^{18, 25}.

Similar phase changes were observed for ITR-P407, however they were followed by a crystallisation event, which can be ascribed to the reported effect of P407 inducing crystallisation of ITR ¹⁸. The magnitude of the mass change during the transition from P to V was lower than that observed in the absence of P407 (Figure 3). This can be attributed to the hygroscopic nature of P407, which can lead to overestimation of the amount of water sorbed during each transition. The crystallisation event was confirmed by WAXS (Figure SI.5) and it was associated with powder shrinkage as can be seen in Figure 3. It should be emphasised that ITR-P407 also possesses a liquid crystalline assembly as can be inferred from WAXS and PLM results (Figures SI.5 and SI.6). These observations are in agreement with our results of phase determination reported for ITR NPs containing P407 ¹⁸. We therefore suggest that the characteristic display of transitions (from P to V and then to S) in DVS of nematic ITR phase may be used to detect, and possibly quantify, this phase in ITR samples.

Figure 3. Water sorption kinetic profile for ITR-P407 at 95% RH (50 °C) for 300 min displaying the transitions of ITR-P407 to P, V and S samples (left) and the corresponding images captured during the run (right).

The sorption diffusion coefficients calculated for ITR-N at 50 and 95% RH revealed that although 95% RH was associated with a higher diffusion coefficient (D_{ba}) in comparison to the 50% RH condition (Table 1), the values of D_{ba} determined “up to the peak” and “up to the shoulder” were not statistically significantly different, indicating that the nematic-smectic phase transition is not controlled by diffusion.

Table 1. Bulk moisture diffusion coefficient for ITR-N determined by DVS at 50 °C.

Sample	Up to peak (P) at 95% RH (m^2/s)	Up to shoulder (S) at 95% RH (m^2/s)	50% RH (m^2/s)
D_{ba}	$5.18E-09 \pm 3.52E-10$	$6.28E-10 \pm 1.93E-11$	$4.90E-09 \pm 6.79E-10$

A step-wise kinetic sorption-desorption-resorption DVS profile (using 10% RH increments) at 50 °C (Figure 4) indicated the existence of a phase transformation at 60% RH in the sorption step and, to our surprise, a phase transition event was observed in the resorption step at the same relative humidity of 60% RH, as indicated by the arrow in Figure 4, and indicating reversibility of this transition. This reversible transition can be seen explicitly when 5% RH increments were used (inset in Figure 4). This reversible event is characterised by a substantial weight gain followed by a weight loss and observed at a lower RH (50% RH) in the desorption step than the phase transition in the sorption step. This behaviour is comparable with the deliquescence/efflorescence transition and although ITR is not hygroscopic, the water loss and microstructural rearrangement are delayed ²⁶.

In contrast, no reversibility of the transition was seen when humidity was ramped directly to 95% RH in one step and then changed to 0% RH in one step (Figure SI.4) indicating that the phase reversal is kinetically limited by the equilibration with the vapour phase at a certain RH.

Figure 4. Water sorption kinetic profile (at 50 °C) for ITR-N obtained using a stepwise (10% RH) increase in humidity up to 95% RH and the reverse for desorption. The sample mass was allowed to reach equilibrium defined as $dm/dt \leq 0.002$ mg/min over 10 min before the RH environment was changed. The accompanying inset illustrates the transition when a stepwise increase/decrease of 5% RH was applied. The arrow indicates the existence of the transition in the second sorption step.

Data obtained using different ramping rates for the sorption and desorption steps were used to determine critical relative humidities (CRH) for both transitions (Figure SI.7). RH_s was taken as the point at the peak, just before the sample mass decrease, in the sorption step, while the

RH_d was taken as the point where the sample mass started to increase in the desorption step (both positions are indicated by arrows in Figure SI.8). The CRH_s and CRH_d values, indicative of the inherent relative humidities to cause the LC ordering transition in the sorption step and the reversing transition in the desorption step at 50 °C were $42.1 \pm 0.6\%$ and $31.1 \pm 0.1\%$, respectively. Both transitions were dependent on the humidity ramping rate with the reversing transition in the desorption step always occurring at a lower relative humidity compared to the ordering transition in the sorption step.

SE micrographs of ITR-N and samples P, V and S are displayed in Figure 5. The ITR-N particles appear as collapsed spheres, which upon water sorption swell until the P state is achieved (Figure 5b). No observable changes were recorded for sample V (Figure 5c), in comparison to P, which was consistent with the DVS video data, although a slight increase in the quasi-Bragg peak (002) intensity was observed by WAXS for sample V (Figure 1). On the other hand, the SE micrograph of the sample S revealed that particles sorbed more water than the sample V as shown in Figure 5d, where further swelling and particle fusion was observed. The sample recovered following a full DVS sorption-desorption cycle with a stepwise (10% RH, $dm/dt \leq 0.002$ mg/min for at least 10 min) change in humidity showed particle collapse and aggregation (Figure 5e). Although the collapsed particles comprising this sample displayed the nematic WAXS pattern (Figure SI.9), the original shape of the particles was not restored. The morphology of particles on resorption (90% RH, second DVS sorption cycle, as shown in Figure 4) is presented in Figure SI.10.

Figure 5. SE micrographs of (a) ITR-N, (b) sample P, (c) sample V (d) sample S and (e) sample post-DVS (DVS conditions as are described in text). The red line corresponds to 2 μm .

Figure 6 shows the PL micrographs of ITR-N and sample S as well as PLHS micrographs of sample S when heated in the hot stage at 5 $^{\circ}\text{C}/\text{min}$ up to 110 $^{\circ}\text{C}$. PL micrographs of the same area before (ITR-N) and after (sample S) exposure to 95% RH at 50 $^{\circ}\text{C}$ in DVS (Figure 6) revealed absence of any visible changes. However, the sample S exhibited a transformation upon heating at 70 $^{\circ}\text{C}$ and when the sample reached the first liquid crystalline transition, at 74 $^{\circ}\text{C}$, particles started to merge with the neighbouring small particles until larger, highly

birefringent droplets formed at 88 °C. Those droplets underwent a second liquid crystalline transition at 90 °C, except for a minute region marked with the red arrow in the 90 °C micrograph. This region appeared to be a crystalline domain in the hygrothermally treated sample, which was confirmed by a further enlargement of this region upon heating and indicates crystallisation of the surrounding region.

Figure 6 PL micrographs representing birefringence of nematic (ITR-N) and smectic (sample S) ITR TLC phases. The remaining micrographs illustrate the thermomicroscopic investigation of the sample S from 50 to 110 °C using HSM.

Results of thermal analysis using 100 and 300 °C/min scanning rates are displayed in Figure 7. Fast heating rates were used due to the increase in sensitivity of thermal events²⁷. At a heating rate of 100 °C/min, an endothermic event, displaying a peak at around 39 °C, was present in thermograms of the P, V and S samples (Figure 7a), while it was absent in the thermograms of ITR-N before DVS and also in the sample post -DVS (step-wise change in humidity of 10% RH, $dm/dt \leq 0.002$ mg/min for at least 10 min). This event was observed at a higher temperature (a peak around 47 °C) when the 300 °C/min heating rate was applied indicating the heating rate dependency of the event temperature (Figure 7b). Modulated DSC thermograms (Figure

SI.11) indicated that this is a non-reversing thermal event with a broad endotherm typical for desolvation process²⁸.

Glass transition temperatures (T_g s) varied depending on the scanning speed (Figure 7), which is in agreement with the observation reported by Lambert for polystyrene²⁹. The higher scanning rate (300 °C/min) was able to discriminate between the T_g s of the samples (Figure 7b). Surprisingly, the T_g of samples P, V and S were 65.1, 64.7 and 64.3 °C, respectively, and higher than that of ITR-N (57.1 °C) and the sample post-DVS (59.6 °C). This observation contradicts the well-known T_g lowering effect of moisture³⁰. We, however, attribute the increase in T_g in the samples P, V and S to the increase in order in the layered smectic arrangement compared to the nematic phase. This increase in the order is expected to be accompanied by an increase in rigidity and/or a decrease in flexibility of molecules in the samples, which in turn will decrease molecular mobility and an increase in the T_g . It is also well known that the presence of crystallites, in other words ordered domains, suppresses the change in specific heat at the T_g and results in an increase the T_g by acting as physical cross-links, which add rigidity to the amorphous regions³¹. Similarly, an increase in the T_g was observed upon immersing epoxy resins in water at elevated temperatures via promoting crosslinking and subsequently enhancing rigidity³². The T_g of the sample post-DVS was higher than that of the original ITR-N sample, 59.6 °C compared to 57.1 °C (at 300 °C/min), which indicates that although the sample lost almost all sorbed water, it did not fully return to its original state and that its hygrothermal history had an irreversible impact on its molecular arrangement.

Figure 7. HSDSC scans of ITR-N, P, V, S and the sample following a DVS cycle (DVS conditions are described in text) at 100 (a) and 300 °C/min (b). T_g – glass transition temperature, T_{LC1} – temperature of the first liquid crystalline transition. Broken lines are added for visual guide.

On the other hand, the lower heating rate of 100 °C/min was utilised to detect the effect of the hygrothermal history on physical destabilisation of the sample. Figure 7 illustrates the presence of a melting event recorded for the sample S, while such a transition was absent in the thermogram obtained at the 300 °C/min scanning rate. This clearly indicates that there was no sufficient time available to allow sample crystallisation at the faster heating rate (300 °C/min). A melting event of the sample post-DVS was observed using the 300 °C/min heating rate, which further confirms that the nematic arrangement in the sample was not fully restored following the desorption step.

4. Overall discussion

ITR was previously reported to exist in two different monotropic TLC phases, nematic and smectic when cooled from a melt with two phase transitions observed at 90 and 74 °C². This behaviour is uncommon since, although it possess some anisotropy, ITR molecule lacks

terminal linear aliphatic chain(s) which is usually a common feature of TLCs, however it is not a prerequisite^{14,16}. Recently, we were able to produce LC ITR nanoparticles with the LC phase, nematic or smectic A, controlled via changing precipitation conditions. Here, we observed a nematic-smectic switching due to interactions of the nematic mesomorphous phase with water upon exposure to moisture at 50 °C. At 95% RH, ordering occurred progressively, as monitored by DVS, throughout three distinct transitions to states assigned as P, V and S. The sample S contained 2.5% w/w water and was found to possess the highest mesomorphic order (smectic). In contrast, the 50% RH condition did not result in any phase changes. Switching back from the smectic to the nematic phase can be achieved via slow dehydration, here achieved by a step-wise (10% RH) change in humidity, equilibrating the mass of sample after each condition ($dm/dt \leq 0.002$ mg/min for at least 10 min) until 0% RH, indicating that the presence of moisture is crucial in the ordered phase.

The 2.5% w/w sorbed moisture (sample S) is equivalent to 1 mole of water per 1 mole of ITR. This low quantity of solvent in the sample excludes the existence of an LLC of ITR and suggests the presence of what was described by Kolbel et al. as thermotropic mesomorphism of solvent complexes¹⁴. This is the first account of this type of behaviour for itraconazole and for pharmaceutical materials in general.

As shown above, diffusion was not found to control the water induced order in the sample at 50% and 95% RH. RH ramping experiments indicated that the RH at which the progressive transitions occur depends on the RH ramping rate, where the faster this rate, the quicker those transitions are. The critical RH for the ordering (CRH_s) and deordering (CRH_d) transitions were calculated via extrapolating the ramping rate to 0% RH/h and were found to be $42.1 \pm 0.6\%$ and $31.1 \pm 0.1\%$, respectively. The CRH_d value is lower than the value for CRH_s , therefore this behaviour is similar to the deliquescence/fluorescence typically exhibited by hygroscopic materials.

ITR is a hydrophobic molecule with very low aqueous solubility¹⁷. However, recently we observed that ITR can interact with hydrogen-bond donors¹⁹. The two faces of water have been reported by Chandler in 2002, where the reason for separation of water and oil in ambient conditions was determined to be due to the favourable hydrogen bonding between water molecules and not due to repulsion between oil and water molecules³³. Therefore, interactions between ITR and water molecules in the gaseous phase are likely, as observed in the DVS studies described herein, however the formation of ITR nanoparticles with the smectic arrangement in an aqueous environment has also been observed previously¹⁸.

Thermal analysis provided a further insight into the molecular mobility of ITR. The use of different scanning rates elucidated the subtle differences between the different samples. The use of 300 °C/min indicated that samples P, V and S exhibited higher T_g s due to the increase in molecular order thus an increase in rigidity, which require more energy for the glass transition to occur. The post-DVS sample showed a melting event implying that the hygrothermal induced nematic-smectic transition was not fully reversible and the liquid crystalline arrangement was compromised by the switching process. This melting transition indicates that although the post-DVS sample had the same WAXS pattern as ITR-N, the presence of crystalline domains, below the detection limit of WAXS, within this sample can be responsible for the melting observed in DSC. This was further confirmed by the presence of a minute crystalline region above 90 °C when the sample was investigated using HSM.

5. Conclusions

Mesomorphous, nematic ITR showed an increase in long-range order upon moisture sorption in a range of humidity conditions at 50 °C. A reversal of ordering from the smectic to the nematic phase was described as reordering without complete restoration of the original structure of the material. This reversal of phases was depended on the hygrothermal conditions applied. DVS and SEM data suggested that the more ordered LC phase is formed not by ITR

molecules only, but rather 1:1 mole/mole ITR complex with water. Additionally, the presence of moisture led to a marked decrease in molecular mobility in the ordered phase and subsequently an increase in T_g , an observation counterintuitive to what typically is seen in disordered materials. The present study highlights the ability of liquid crystalline ITR to accommodate stress exerted by high relative humidity via molecular reorientation without full crystallisation.

ASSOCIATED CONTENT

The Supporting Information file is available free of charge on the ACS Publications website.

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A rare case of mesomorphic behaviour – molecular reorientation of itraconazole liquid crystal induced by a hygrothermal treatment

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Synopsis

This work describes the use of moisture sorption approach to reversibly realign mesogen molecules within a liquid crystalline phase of itraconazole (ITR). A nematic-to-smectic switching behaviour of ITR was achieved via moisture triggered activation of molecular mobility below the material's glass transition temperature. The smectic phase was a 1:1 mole/mole ITR:water complex, an example of thermotropic mesomorphism of solvent complex.