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Trinity College Dublin

Coláiste na Tríonóide, Baile Átha Cliath

The University of Dublin

Engineering of Itraconazole Nano- and Microparticles with Improved Pharmaceutical Performance

A dissertation submitted for
the degree of Doctor of Philosophy,
School of Pharmacy and Pharmaceutical Sciences
University of Dublin, Trinity College Dublin, Ireland

by

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under the direction and supervision of
Lidia Tajber, M. Pharm., M.A., Ph.D.

May 2016



PhD
PHARMACY +
PHARMACEUTICAL
SCIENCES



Thesis 11309

Dedication

This thesis is dedicated to my Parents, Abdulhamid Mugheirbi and Hawa Benghuzzi
for their endless love, support and encouragement.

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work, except where duly acknowledged.

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Naila A. Mugheirbi

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Naila A. Mugheirbi

Summary

Itraconazole (ITR) is an antifungal drug with limited bioavailability due to its poor aqueous solubility. In this work, ITR was used to investigate unexplored and/or previously reported as infeasible formulation approaches such as, the impact of nanonization at elevated temperatures, crystal habit tuning and solid state manipulation, including unexplored state of matter such as liquid crystals, on drug's pharmaceutical properties. A bottom up approach to the production of amorphous ITR nanoparticles (NPs), composed of 100% drug, with a particle diameter below 250 nm, using heat induced evaporative antisolvent nanoprecipitation (HIEAN) from acetone was developed. The NPs demonstrated improved apparent solubility and dissolution in simulated gastro-intestinal conditions when compared to spray dried ITR microparticles. NPs produced with polyethylene glycol (PEG) or its methoxylated derivative (MPEG) as a stabilizer possessed smaller particle size with narrower particle size distribution and enhanced apparent solubility. MPEG stabilized NPs gave the greatest ITR supersaturation levels (up to $11.6 \pm 0.5 \mu\text{g/ml}$) in simulated gastric fluids. Dynamic vapor sorption data indicated no solid state changes in NP samples with water vapor at 25 °C, while crystallization was apparent at 50 °C.

The synthesis and phase tuning of nanoparticulate ITR mesophases was also investigated to seek out an alternative pathway to the nucleation-based NP formation. ITR liquid crystalline (LC) phases were formed and nematic-smectic mesomorphism was achieved via controlling solvent:antisolvent temperature difference ($\Delta T_{S:AS}$). The use of $\Delta T_{S:AS}=49.5$ °C was associated with a nematic assembly, while intercalated smectic A layering was observed at $\Delta T_{S:AS}= \sim 0$ °C, with both phases confined in the nanospheres at room temperature. Poly(acrylic acid) in the solvent phase exhibited a concentration dependent interaction with ITR during formation of NPs. This nanodroplet-based approach enabled the preparation of a stable ITR nanodispersion using Poloxamer 407 at 80 °C, which was unachievable before using precipitation via nucleation.

Three distinct isomorphic crystal habits of ITR, a twinned blade-shaped (CH_{tw}), a plate-shaped (CH_{pl}) and a flat sheet-shaped with dendritic ends (CH_{sh}) were obtained by controlling crystallization conditions. A liquid-liquid crystalline phase separation was observed as an intermediate stage preceding crystal growth. The March-Dollase parameter was used as a quantitative description of the preferred orientation, where CH_{sh} exhibited the greatest preferred orientation. The three crystal habits were evaluated for their wettability and water vapor distribution, at 37

°C, using the Young-Nelson fitting model. CH_{tw} crystals sorbed a statistically significantly higher amount of water than CH_{pl} and CH_{sh}, which was attributed to the presence of crystal defects due to the twinning boundary. On the other hand, the amount of water adsorbed on the surface of CH_{pl} and CH_{sh} crystals was comparable and it was about twice that adsorbed on CH_{tw} crystals. This was related to the abundance of hydrophilic chemical functionalities on the (010) facet of CH_{pl} and CH_{sh} as supported by the full interaction map carried out using Mercury[®] software. This study expands investigations of the impact of crystal habit manipulation on API's functional properties beyond the well-known solubility improvement approaches.

The use of moisture sorption approach to reversibly realign mesogen molecules within a liquid crystalline phase of ITR, assigned to as a nematic phase (ITR-N), obtained by spray drying was introduced. This unique, nematic-to-smectic switching behavior 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 characterized 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 behavior highlights the ability of ITR to accommodate stress conditions via molecular rearrangement and thus its resistance to full crystallization, which rationalizes the importance of ITR liquid crystalline phases in pharmaceutical development.

In general, the production of liquid crystalline ITR NPs at high temperature which could reduce further solvent removal steps with the feasibility of manipulating the liquid crystalline phase in the produced NPs is of great importance to pharmaceutical field. The ability of ITR to accommodate different crystal habits, including twinning, based on the initial crystallization conditions along with the observed nematic to smectic mesomorphism could encourage researchers to explore different overlooked behaviours of ITR and other APIs.

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Presentations and publications

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Mugheirbi, N. A., Tajber, L., Mesophase and size manipulation of itraconazole liquid crystalline nanoparticles produced via quasi nanoemulsion precipitation. *European Journal of Pharmaceutics and Biopharmaceutics* **2015**, 96, 226-236.

Mugheirbi, N. A., Tajber, L., Crystal habits of itraconazole microcrystals: unusual isomorphic intergrowths induced via tuning recrystallization conditions. *Molecular Pharmaceutics* **2015**, 12, 3468-3478.

Mugheirbi, N. A., Fleischer, K, Tajber, L., A rare case of mesomorphic behavior – molecular reorientation of itraconazole liquid crystal induced by a hygrothermal treatment. *Crystal Growth & Design* **2016**, 16, 1329-1336.

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Poster presentation: "In situ monitoring of kinetics of azole anti-fungal nanoparticle formation. AAPS Annual Meeting, 25-29th October, Orlando, USA, 2015.

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Poster presentation: "In situ monitoring of kinetics nanoparticle formation. Summer school on crystal shape engineering, 6-10th July, Zurich, Switzerland, 2015.

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Poster presentation: Heat induced evaporative antisolvent nanoprecipitation of itraconazole. The PBP World Meeting, 31st March-3rd April, Lisbon, Portugal, 2014

List of abbreviations

API	Active pharmaceutical ingredient
ASD	Amorphous solid dispersion
ANOVA	Analysis of variance
BCS	Biopharmaceutics Classification System
CAP	Cellulose acetate phthalate
dm/dt	Change of mass per unit time
cps	Counts per second
DCS	Developability Classification System
DCM	Dichloromethane
DSC	Differential scanning calorimetry
DLS	Dynamic light scattering
DVS	Dynamic vapor sorption
EPAS	Evaporative precipitation into aqueous solution
Δ_{exo}	Exothermic direction
EOS	Extent of supersaturation
FaSSGF	Fasted state simulated gastric fluid
FaSSIF	Fasted state simulated intestinal fluid
FTIR	Fourier transform infrared spectroscopy
GIT	Gastrointestinal tract
T_g	Glass transition temperature
HeIM	Hellium ion microscopy
HPLC	High Pressure Liquid Chromatography
ITR	Itraconazole
LDV	Laser Doppler Velocimetry
LC	Liquid crystal
MPEG	Methoxy poly(ethylene glycol)
NPs	Nanoparticles
PLM	Polarized light microscopy
P407	Poloxamer 407
PDI	Polydispersity index
PEG	Poly(ethylene glycol)
PAA	Poly(acrylic acid)
PXRD	Powder X-ray diffraction
p/p_0	Relative partial pressure
SEM	Scanning electron microscopy

SIF	Simulated intestinal fluid
SCXRD	Single crystal X-ray diffraction
SAXS	Small angle X-ray scattering
TEM	Transmission electron microscopy
PTXRD	Transmission powder X-ray diffraction
USP	United States Pharmacopoeia
WAXS	Wide angle X-ray scattering
ZP	Zeta potential

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CHAPTER I: General Introduction

1.1. BCS and DCS classifications

A drug, also known as an active pharmaceutical ingredient (API), has to solubilize and present as molecules at the site of absorption in order to permeate several membrane barriers before interacting with the receptor (Kubinyi 1979). Therefore, drug dissolution is an essential step preceding absorption and clinical response for the vast majority of drugs administered orally. Gastro-intestinal "GI" drugs including antidiarricals, adsorbants and some laxatives are exceptions where dissolution is not a prerequisite for pharmacological activity since they exert their action locally (Amidon et al. 1995).

Solubility and dissolution are two distinct phenomena, however, they are closely related. Solubility is the maximum amount of a pure solute that a pure medium at specified conditions can hold in a solution. In other words, solubility in a specific medium is the saturation concentration in that medium. Therefore, solubility is a thermodynamic phenomenon. In contrast, dissolution is a kinetic process, thus the dissolution rate is a key consideration. In simple terms solubility is an endpoint, while dissolution is the process of reaching this end point (Allen 2012). It has been estimated that nearly 40% of the drug candidates entering the development pipeline have poor water solubility and subsequently low bioavailability (Lipinski 2002). Amidon et al. (1995) established a drug classification system correlating *in vitro* dissolution of a drug and its *in vivo* bioavailability. The so-called Biopharmaceutics Classification System (BCS) utilizes two key parameters, drug solubility and gastrointestinal permeability, to demonstrate the rate and extent of drug absorption. Thus, drugs were divided into high/low solubility-permeability classes as shown in Table 1.1. Based on the fundamental dissolution and permeability properties of physiologic importance, a drug is considered highly soluble when its highest dose strength is soluble in < 250 ml water over a pH range of 1 to 7.5. Similarly, High permeability drugs should possess an extent of absorption in humans higher than 90% of the administered dose.

Table 1.1. The theoretical basis for the Biopharmaceutics Drug Classification system.

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

According to the BCS classification, drugs belonging to BCS class II, with low solubility and high permeability, possess bioavailability that is limited by the dissolution rate in aqueous gastrointestinal fluids (Amidon et al. 1995).

The BCS classification was revised by Butler and Dressman (2010) to provide a greater focus on drug developability (Figure 1.1). The drugs falling into the BCS class II are subdivided into category IIa, dissolution rate limited, and category IIb, solubility limited. According to the revised model, a drug will be class IIa (dissolution rate limited) if the dissolution rate is too slow for all drug particles to dissolve during the transit time in the small intestine. On the other hand, the drug will be class IIb (solubility limited) if there is insufficient fluid within the GIT to dissolve the orally administered dose. This modified classification system utilizes more realistic volumes of fluid available in the GIT and the compensatory nature of permeability on low solubility (Butler and Dressman 2010).

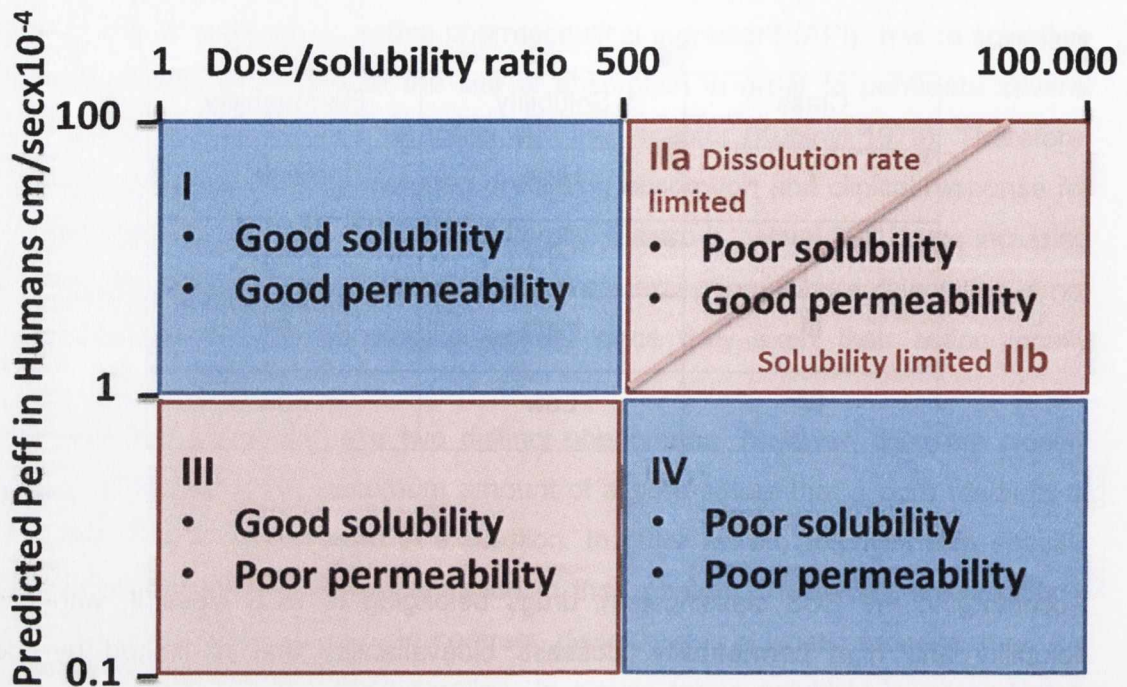


Figure 1.1. The modified BCS classification system, known as Developability Classification System (DCS). Adapted from Butler and Dressman (2010).

Considering the Noyes-Whitney equation, in conjunction with the Nernst-Brunner diffusion layer model, drug dissolution can be described as:

$$\frac{dM}{dt} = \frac{DA}{h} (C_s - C) \tag{Eqn 1.1}$$

Where $\frac{dM}{dt}$ is the dissolution rate of drug, D is the diffusion coefficient of drug, A is the surface area of the exposed solid, h is the thickness of the diffusion layer, C_s is the solubility of drug in dissolution medium and C is the concentration of the drug in the dissolution medium.

According to Taylor and Zhang (2016), under sink conditions where the drug concentration in the dissolution medium C is almost zero, this term can be excluded from the equation. Therefore, in such case, the drug dissolution rate is governed by D , h , A and C_s . Terms D and h are governed by the properties of the drug molecule and the conditions of the human GIT, respectively, and thus difficult to improve via formulation. Therefore, A and C_s , surface area and solubility, are the two parameters that could be manipulated when developing a formulation. Particle size reduction

has been used to increase the exposed surface area of APIs and consequently facilitate dissolution (Matteucci et al. 2007, Taylor and Zhang 2016). Solubility can be improved using several approaches as displayed in Figure 1.2.

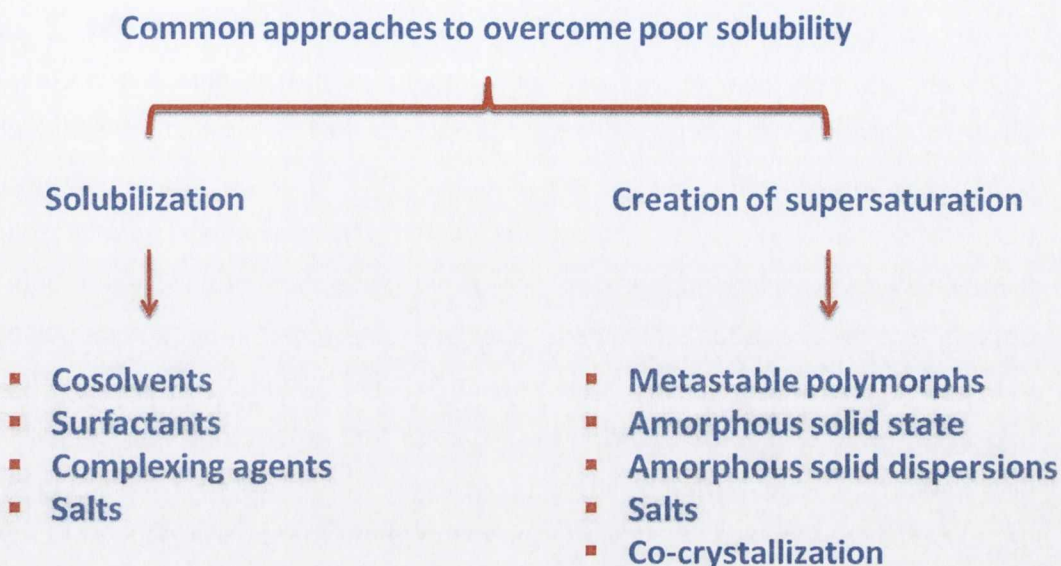


Figure 1.2. Commonly used approaches to improve pharmaceutical performance of poorly soluble APIs.

To date, two main approaches, solubilization and creation of supersaturation, have been employed to overcome solubility issues associated with BCS class II drugs (Poelma et al. 1991). Solubilization approaches, where the thermodynamic solubility is improved, involve using cosolvents, surfactants and complexing agents to increase the dissolved or dispersed drug in water (Poelma et al. 1991). Supersaturation generation, on the other hand, results in a higher concentration than the thermodynamic solubility of the crystalline drug or so called an increase in “apparent” solubility (Hancock and Parks 2000). This can be achieved via using metastable polymorphs, amorphous state, amorphous solid dispersions (ASDs), salts and co-crystals, as alternative to the thermodynamically favorable crystalline API (Pudipeddi and Serajuddin 2005, Murdande et al. 2010, Grohgan et al. 2013, Good and Rodríguez-Hornedo 2010, Serajuddin and Jarowski 1985).

A recent study by Sousa et. al. (2016) shows a comparison of the supersaturation potential of a salt, co-crystal, pure amorphous form and amorphous solid dispersion of 4-(4-chloro-5-(2,6-dimethyl-8-(pentan-3-yl)imidazo[1,2-b]-pyridazin-3-yl)thiazol-2-yl)morpholine, a weak basic corticotropin releasing hormone receptor 1 antagonist

(Sousa et al. 2016). This study illustrates that the upper limit of supersaturation that can be achieved upon dissolution of any of the solid-state form studied was determined by the amorphous solubility, above which, liquid-liquid phase separation (LLPS) and the formation of a drug-rich phase was observed. Although LLPS is a well-known phenomenon occurring in protein formulations, and described as the physical destabilization of protein solutions into a protein-rich and protein-poor phases, it has been extensively studied recently in the context of APIs' phase behavior (Bye et al. 2014, Taylor and Zhang 2016). It has been demonstrated that LLPS most commonly takes place if the system, typically drug-aqueous solution, exhibits a very high supersaturation or a rapid generation of supersaturation. The presence of crystallization inhibitors or excessive amount of impurities can also lead to a system separation into two liquid phases. Glass-liquid phase separation (GLPS) was reported to take place when the water saturated amorphous drug exhibits a T_g higher than the solution temperature (Taylor and Zhang 2016). Clearly LLPS is a depletion of supersaturation, however nanodroplets formed as a result of LLPS can serve as an API reservoir to maintain drug concentration in solution. The LLPS phenomenon was observed with the ASD and the salt form of 4-(4-chloro-5-(2,6-dimethyl-8-(pentan-3-yl)imidazo[1,2-b]pyridazin-3-yl)thiazol-2-yl)morpholine. In contrast, pure amorphous, free base and co-crystal forms of the drug did not reach the amorphous solubility and hence no LLPS was observed (Sousa et al. 2016). Although this study provide an insight into the importance of ASDs and salt forms in achieving supersaturation and thus improving drug dissolution, those two forms are extensively investigated in literature and more attention needs to be paid to the poorly known formulations based on liquid crystals (LCs) and non-crystalline nanoparticles (NPs).

1.2. Itraconazole

Itraconazole (ITR) is a broad spectrum triazole antifungal drug, which is effective against a variety of fungal genera such as *Aspergillus*, *Cryptococcus*, *Candida*, *Blastomyces*, and *Histoplasma capsulatum* var. *capsulatum* (Odds et al. 2000, Saag and Dismukes 1988). It disrupts the fungal membrane via inhibiting the fungal cytochrome P450 dependent 14- α -demethylase, an enzyme that is necessary for the synthesis of ergosterol from lanosterol (Grant and Clissold 1989).

ITR, Figure 1.3, is a weak base with two pK_a values of 2 and 3.7 (piperazine moiety) and $\log P = 6.2$ (Peeters 2002). It has a molecular weight of 705.6 g/mol (Peeters

2002). ITR has a very poor aqueous solubility ($\sim 5 \mu\text{g/mL}$ at $\text{pH}=1$ and $\sim 1 \text{ ng/mL}$ at $\text{pH}=7$), but good permeability ($\log D$ larger than 5) and hence belongs to the BCS class II (Peeters et al. 1996). According to DCS, ITR is classified as class IIb (solubility limited), where the dose and solubility are the two main product dependent variables (Figure 1.1, Butler and Dressman 2010). Having all the abovementioned properties, ITR has been the focus of extensive research investigations to demonstrate the advantages and disadvantages of various techniques used to improve pharmaceutical performance of BCS class II members (Dalvi and Dave 2010, Matteucci et al. 2006, Kumar et al. 2009, Beck et al. 2010, Badawi et al. 2011, Chen et al. 2004, Bosselmann et al. 2012, Janssens et al. 2008a, Janssens et al. 2008b). To confront the issue of poor biopharmaceutical properties, posaconazole (POS), a structural analog to ITR, was developed as a second-generation triazole antifungal. The fluorine and a furan ring, in POS, replaced the chlorine and the dioxolane ring, in ITR, respectively (Percival and Bergman 2014) as shown in Figure 1.3. POS is also a weak base, $\text{pK}_{\text{a}1} = 3.6$ (piperazine) and $\text{pK}_{\text{a}2} = 4.6$ (triazole), with a solubility of about $790 \mu\text{g/mL}$ at $\text{pH}=1$ (Fang et al. 2015). Despite the relatively higher solubility and extended spectrum of POS compared to ITR, the use of POS is limited to immune-compromised patients due to the extremely high cost of the marketed product Noxafil®.

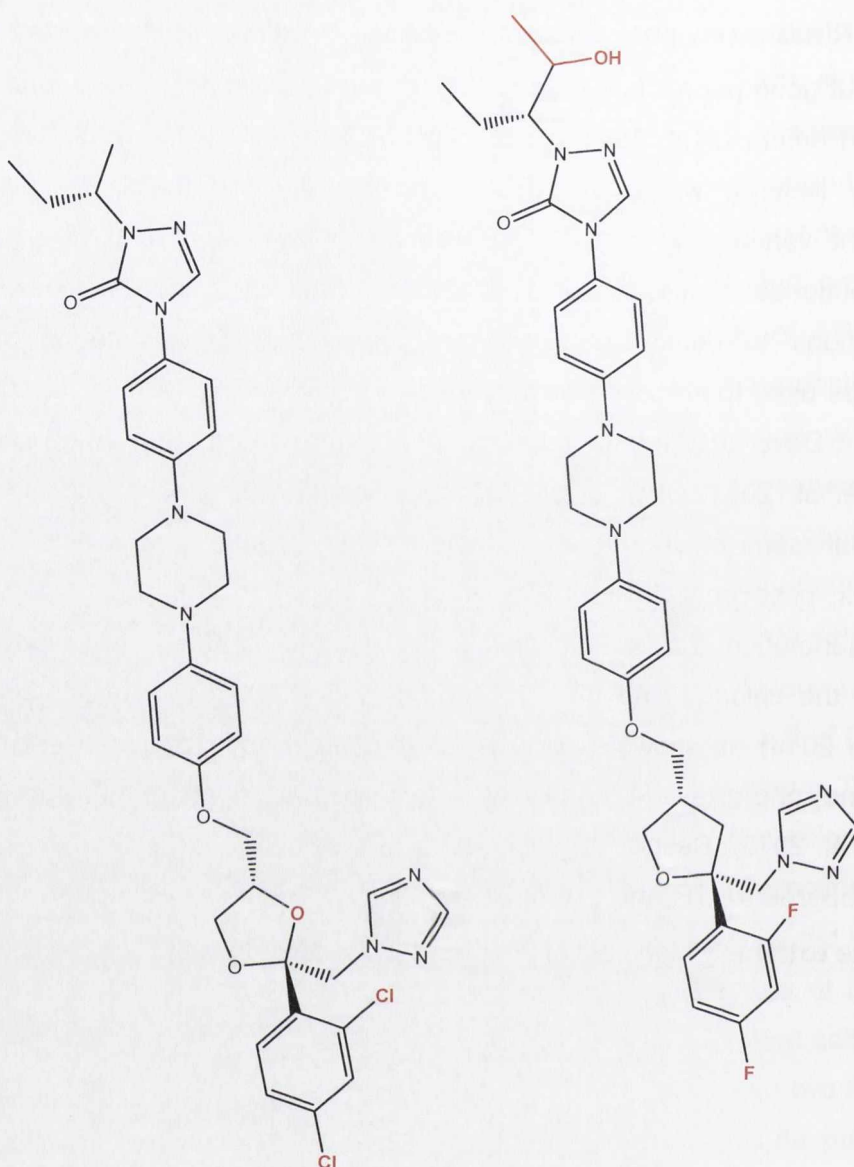


Figure 1.3. Chemical structure of ITR (left) and POS (right) with the structural differences between the two compounds highlighted in red (ChemBioDraw).

ITR is available commercially, under the trade name Sporanox[®], as a multiple unit (MU) solid oral dosage form, an intravenous solution and a liquid oral dosage form. The MU solid dosage form is an amorphous solid dispersion, based on hydroxypropylmethylcellulose HPMC 2910 E5, coated on sugar spheres, usually referred to as non-pareil seeds and administered as a capsule. The liquid dosage form is an oral solution manufactured via complexation of ITR with HP- β -cyclodextrin (Buchanan et al. 2007). Solid and liquid oral Sporanox[®] formulations have observed absolute bioavailabilities of 30 and 55%, respectively (Barrett et al. 2008). This necessitates the administration of a large dose to achieve the required

blood concentration, which could result in developing serious side effects along with drug wastage (Badawy et al. 1996). Therefore, according to the DCS, since ITR possesses solubility-limited bioavailability, improving the “apparent” solubility and the subsequent bioavailability could ultimately reduce the administered dose of ITR.

1.3. Literature review of approaches to improve pharmaceutical performance of ITR

1.3.1. Metastable solid state forms

1.3.1.1. Amorphous forms and amorphous solid dispersions (ASDs)

Solubility of amorphous drugs has been assumed to be around 100 to 1600-times higher than the crystalline forms based on free energy calculations as well as experimentally observed configurational heat capacities (Parks et al. 1934, Hancock and Parks 2000). There have been extensive investigations into miscibility of ITR with the commonly used carrier polymers in an attempt to develop binary and ternary solid dispersions. The vast majority of the published work, investigating binary and ternary ITR ASDs, also present the preparation and characterization of amorphous 100% (pure) ITR solid-state forms (Engers et al. 2010). However, this pure phase of disordered ITR was used mainly for comparative purposes. In general, the use of these forms is impractical since they are highly prone to crystallization and the inclusion of a crystallization inhibitor is of paramount importance. Binary and ternary ITR solid dispersions have been prepared using different techniques, such as, but not limited to, spray drying, hot melt extrusion, ultra-rapid freezing (Overhoff et al. 2007), high shear pelletization (Ye et al. 2007), supercritical CO₂ (Verreck et al. 2005), flash evaporation, freeze drying (Engers et al. 2010) and solvent evaporation (Matteucci et al. 2007, Maghraby et al. 2009). Reported binary ASDs include the use of stabilizing and solubilizing polymers such as polyvinylpyrrolidone (PVP) (Engers et al. 2010), hydroxypropylcellulose (HPC) (Chowdary and Rao 2000), hydroxypropylmethylcellulose 2910 E5 (HPMC) (Six et al. 2003b), ethylcellulose (EC 20 cps) (Verreck et al. 2007), hydroxypropylmethylcellulose phthalate (HPMC-P), Eudragit® L100-55 (an anionic copolymer based on methacrylic acid and ethyl acrylate) (Overhoff et al. 2007), Eudragit® L100 (Jung et al. 1999, Six et al. 2003a), polyvinylpyrrolidone vinylacetate (PVPVA64) (Verreck et al. 2005, Wang et al. 2005), polyvinylacetal diethylaminoacetate (AEA®) (Jung et al. 1999), polyethylene glycol (PEG) with

different molecular weights including PEG3500 (Kapsi and Ayres 2001), PEG6000 (Wang et al. 2004), PEG8000 and PEG20000 (Kapsi and Ayres 2001), inulin lauryl carbamate (Inutec SP1) (Van den Mooter et al. 2006), polyvinyl alcohol/polyethylene glycol commercially known as Kollicoat IR[®] (Janssens et al. 2007) and hydroxypropylmethylcellulose acetate succinate (HPMC-AS) (Engers et al. 2010). Ternary solid dispersions have also been investigated using polymers mixtures such as polyvinylpyrrolidone vinylacetate/polyoxyethylene (40) monostearate (PVP VA64/Myrj 52) (Wang et al. 2005), d-alpha-tocopheryl polyethylene glycol (TPGS 1000)/PVP VA64 (Janssens et al. 2008a), Eudragit[®] E100/PVP VA64, hydroxypropyl-β-cyclodextrin (HP-β-CD)/HPMC (Ramball et al. 2003), PEG 6000/HPMC 2910 E5 (Janssens et al. 2008b). In general, the aims of formulating ITR into solid dispersions was to obtain a drug-polymer system that can improve stability, which was achieved using stabilizing polymers such as HPMC 2910 E5 (Six et al. 2003b) and PVPVA 64 (Verreck et al. 2005, Wang et al. 2005) or to improve dissolution behavior such as Inutec SP1 (Van den Mooter et al. 2006). On the other hand, ternary solid dispersions focused on improving dissolution characteristics as well as maintaining the drug in the solid solution state as achieved by Janssens et al. (2008b) using PEG 6000/HPMC 2910 E5.

1.3.1.2. Crystalline solid forms

Crystalline solid forms are more stable than their disordered counterparts. However, thermodynamically stable crystalline forms of drugs suffer from compromised solubility due to their crystal lattice packing (Shah et al. 2006, Sousa et al. 2016). This endeavors scientists to establish metastable crystalline forms of APIs to seek out more soluble, yet relatively stable solid-state forms. A metastable polymorphic crystalline form of ITR is claimed to be successfully prepared, however no solubility advantage associated with this form has been reported (Werling et al. 2003). Nonetheless, solubility enhancement associated with metastable polymorphic forms of API is not adequate (Sousa et al. 2016).

Co-crystals are another example of solid forms that take the advantage of stability associated with the crystalline state and the enhanced solubility of high-energy co-crystals (Lindeman 2009). Studies on ITR co-crystals using a wide range of aliphatic dicarboxylic acid co-formers are available in the literature. Suitable co-formers include succinic, malic, adipic, oxalic, malonic, glutaric and pimelic acids (Ober et al. 2013, Shete et al. 2015, Shevchenko et al. 2012, 2013). Aliphatic dicarboxylic acid co-crystal solvates and hydrates include adipic acid co-crystal hydrate (ITR-ADI-

H₂O), oxalic acid co-crystal acetone solvate (ITR-OXA-ACE) and oxalic acid co-crystal tetrahydrofuran solvate (ITR-OXA-THF) were also reported (Shevchenko et al. 2013). Recently, co-crystals of ITR with amino acid co-formers were prepared using co-grinding. The investigated amino acids include aspartic acid, glycine, proline and serine (Shete et al. 2015).

The solubility of poorly soluble acidic and basic drugs can also be enhanced via salt formation using a counterion (Ledwidge and Corrigan 1998, Serajuddin 2007). Salts can be crystalline or amorphous (Paluch et al. 2010, 2011, 2013). In water, salts convert to the ionized species that exhibit higher solubility than the unionized drug. The hydrochloride salt of ITR (ITR-HCl) was found to enable delivery into and across the nail plate by iontophoresis (Kushwaha et al. 2015). Tao et al. (2009) reported on the production of ITR-dihydrochloride using a process of slow bubbling of anhydrous hydrogen chloride gas into a suspension of ITR in acetone. The produced dihydrochloride salt was associated with an improved dissolution profile compared to Sporanox[®] (Tao et al. 2009). Shevchenko et al. (2012) produced ITR-dihydrochloride via salting out from solution. In the same study, the process of slow bubbling of anhydrous hydrogen chloride was employed and this resulted in ITR-trihydrochloride. Both, ITR di- and tri-hydrochloric acid salts demonstrated higher intrinsic dissolution rates compared to the free base (Shevchenko et al. 2012).

1.3.1.3. Liquid crystalline forms

Liquid crystals (LCs) comprise an intriguing class of soft matter that exhibits the counterintuitive combination of fluidity and order characteristics of amorphous and crystalline materials, respectively. As a result, LC phases are known as mesophases and they possess structural, mechanical and optical properties intermediate to those of the three dimensionally ordered crystalline and amorphous states (Muller-Goymann 2001). Molecules with the ability to form LCs are known as mesogens and can be classified according to the anisotropy of their shape into rod-like and disc-like with the former exhibiting the highest molecular anisotropy (Tanaka et al. 2012). LC phases are often unintentionally formed during the manufacturing of other phases and are sometimes overlooked or confused with the amorphous phase (Patterson et al. 2002, Lechuga-Ballesteros et al. 2003).

LCs can be classified based on their structure, composition and method of production into thermotropic (TLCs) and lyotropic (LLCs) liquid crystals. TLCs can be further sub-categorized according to the mesogen arrangement into, mainly, nematic phases, which lack any positional order, however, mesogens tend to point

in the same direction, and smectic phases (one dimensional stack of layers) displaying a layered arrangement of orientationally ordered molecules (Six et al. 2001b, Bunjes and Rades 2005). The most commonly encountered smectic phases, smectic A (SmA) and the tilted smectic C (SmC) phases, are schematically represented in Figure 1.4 (Demus et al. 1998). Additionally, cubic (three dimensional) and hexagonal columnar (two dimensional lattice of rods) phases can also be observed in TLCs (Demus et al. 1998). 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 investigation (Holmberg 2002). Some molecules, referred to as amphotropic, can exhibit both, TLC and LLC phases (Demus et al. 1998).

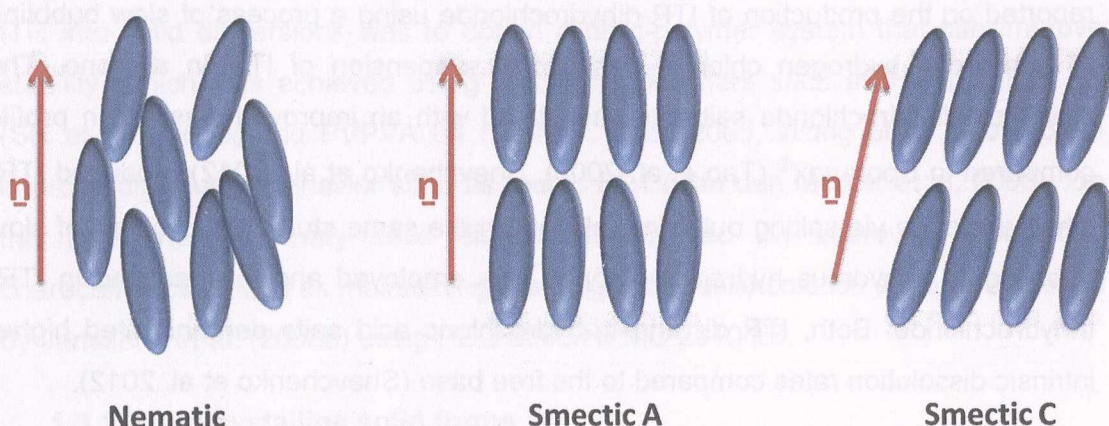


Figure 1.4. Schematic representation of the main thermotropic liquid crystalline phases of rod like mesogens. η is the director (adapted from Dierking 2014 and Demus 1998).

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 (Bunjes and Rades 2005, Shashidhar 1977). In contrast, LLCs are usually formed by the addition of a solvent to the mesogen, where the mesogen's concentration and temperature are the controlling parameters.

TLCs of ITR were first reported by Six et al. (2001a, 2001b). Two distinct, bulk thermotropic LC phases (nematic and smectic) were identified upon a thermal treatment of cooled ITR melt. The X-ray diffraction results of bulk ITR LC phase using a Cu Ka1 source ($k = 1.5406 \text{ \AA}$) revealed the presence of two diffuse maxima at around 7° and 18° 2theta at 82°C , which was assigned as a chiral nematic phase (Six et al. 2001a). Another published results using an Ag Ka source ($k = 0.5608 \text{ \AA}$) revealed the presence of a low angle diffuse maximum above and below the LC

transition at 89 °C, and the authors suggested the presence of an additional ordering within the sample (Tarnacka et al. 2013). The LC transitions at 74° and 89° 2 theta have been extensively utilized to examine the miscibility of ITR with additives (Six et al. 2003a, 2003b). However, the rationale of formulating ITR in a LC phase has not been explored.

1.3.2. Nanotechnology approaches

Nanotechnology-based approaches to enhance the pharmaceutical performance of poorly soluble APIs can be subdivided into two main categories: miniaturization and nanocarrier based. The former utilizes various techniques to produce discrete drug particulate (amorphous or crystalline) in the nano-size range, whereas the latter involves loading drug molecules into nano-sized carrier particles (Kumar et al. 2014, Neves et al. 2015, Crawford et al. 2015).

1.3.2.1. Nanonization (miniaturization)

The dissolution rate of poorly soluble APIs may be enhanced by size reduction due to the increase in surface area as described by Whitney-Noyes equation (Section 1.1) (Krishnaiah 2010, Taylor and Zhang 2016). Drug nanodispersions have shown better dissolution profiles compared to the nanocarrier based nanoparticles (Chen et al. 2011). Drug nanoparticles can be fabricated using top down, bottom up or combination (NanoEdge™) techniques (Figure 1.5) (Junghanns and Muller 2008). The top down approach is the most commonly used since it is a scalable technique that usually does not involve the use of organic solvents (Liu et al. 2011a). Methods include wet milling (Lee et al. 2010), microfluidization (Liu et al. 2011b) and high pressure homogenization (Chen et al. 2008, Owen et al. 2009). However, they are time and energy consuming methods (Shah 2006, Wiedmann et al. 1997, Yang et al. 2010, Tam et al. 2010). Moreover, the produced NPs are susceptible to contamination from the milling media or the homogenization chamber (Chow et al. 2007). On the other hand, the bottom up approach is considered as a simple and relatively low cost technique. The bottom up methodology includes mainly antisolvent precipitation and solvent evaporation (Matteucci et al. 2008, Badawi et al. 2011, Weers et al. 2007). However, the application of bottom up techniques to industry is relatively challenging due to the possible presence of organic solvent residues in the final formulation (Keck and Müller 2006, Chen et al. 2006).

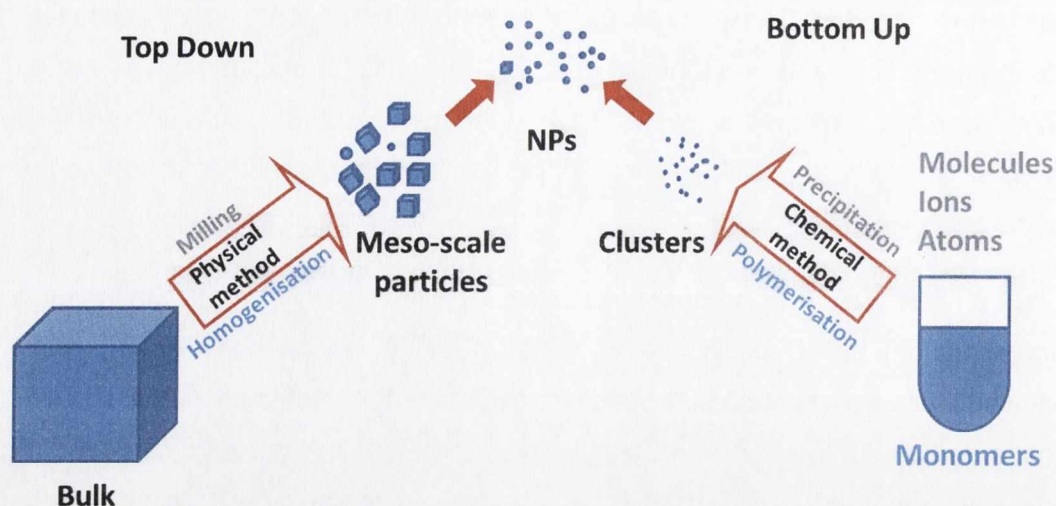


Figure 1.5. Schematic illustration of top-down and bottom up approaches for nanoparticle manufacturing. Adapted from Velikov and Pelan 2008 and Toshima and Yonezawa 1998.

A wide variety of techniques have been employed to fabricate discrete and aggregated/flocculated ITR NPs. Flocculated amorphous ITR NPs, produced via antisolvent precipitation, have shown an enhanced supersaturation and delayed crystallization when compared to the commercial product (Sporanox®), spray dried and rapidly frozen amorphous ITR (Matteucci et al. 2008). The higher *in vitro* supersaturation was observed as enhanced bioavailability *in vivo* in rats (Miller et al. 2012). Amorphous ITR NPs, produced using antisolvent precipitation, were also recovered as a dry powder using spray drying with alkyldiphenyloxide disulphonate and were found to possess superior dissolution and bioavailability compared to melt-quench and crystalline ITR (Kumar et al. 2014). In contrast, the improved *in vitro* dissolution of ITR nanocrystals was not translated into an increase in bioavailability when tested using dogs as animal model (Sarnes et al. 2014).

Media milling was utilized to fabricate nanoparticulate ITR-adipic acid co-crystal and their bioavailability was tested *in vivo* using dogs. This formulation demonstrated enhanced bioavailability since it combines the advantages of small particles size and the higher solubility of the co-crystal (De Smet et al. 2014).

1.3.2.2. Nanocarrier based formulations

Polymeric and lipid nanoparticles (NPs) have been extensively investigated as drug carriers owing to their subcellular size and the ability to improve intestinal absorption of poorly water-soluble APIs (Burapapadh et al. 2015). In addition, nanoparticles

may permeate epithelia more easily than microparticles and can be utilized to provide a controlled release of the encapsulated drugs.

Although a few reports of ITR loaded nano-sized delivery systems are available in the literature, a handful only were intended for oral administration (Yi et al. 2007). The rest were mainly investigated to enhance the parenteral delivery of ITR, for instance PEGylated-liposomal formulations (Curic et al. 2013) and polymeric micelles based formulations (Yi et al. 2007), and pulmonary delivery, such as lipid nanocarriers (Pardeike et al. 2011). An *in vivo* study by Yi et al. (2007) using orally administered polymeric micelles (PM), with 99.3% ITR loading efficiency, illustrated a comparable pharmacokinetic profile to the marketed ITR-cyclodextrin solution (Sporanox®). A study investigating the effect of ITR loaded pectin NPs on the drug dissolution has been recently published. This study illustrated 80% drug release from the NPs within two hours (Burapapadh et al. 2016).

1.3.3. Complexation

Pharmaceutical modification of drug molecules by inclusion and non-inclusion complexation has been extensively employed to improve solubility, dissolution rate, chemical and physical stability, absorption and bioavailability of APIs with poor aqueous solubility. α , β and γ -cyclodextrins (CDs) and their derivatives are among the most commonly used complexation agents (Loftsson et al. 2004, Strickley 2004). Complexes can be binary or ternary and mainly intended to be administered via oral and parenteral routes. Buchanan et al. (2007) reported an *in vivo* comparative study to investigate the effect of ITR complexation with hydroxybutenyl- β -cyclodextrin (HBenBCD) and hydroxypropyl- β -cyclodextrin (HPBCD), marketed as SporanoX®, on the solubility and oral bioavailability of the drug. The study illustrates that oral administration of the HBenBCD-based complex resulted in a two fold increase in oral bioavailability compared to SporanoX®. A few years later, the same group reported that β -cyclodextrin derivatives are able to form binary and ternary complexes (with the inclusion of Soluplus®) with ITR, which were found to exhibit favorable release profiles when compared to the marketed product. The ternary complex with Soluplus® not only improved the release profile but also the ability to reduce drug precipitation tendency upon transfer from acidic to basic pH (Taupitz et al. 2013).

1.4. Project objectives

The goal of this work is to investigate unexplored and/or previously reported as infeasible formulation approaches, including manipulating the state of matter of the drug and employing bottom up miniaturization techniques, to improve the (bio)pharmaceutical performance of ITR.

The use of high temperature antisolvent phase to produce drug nanoparticles, or so called evaporative precipitation into aqueous solution (EPAS), was first introduced by Chen et al. to produce cyclosporine NPs with a diameter of 253 nm (Chen et al. 2004). However, no successful production of ITR NPs using this method has been reported in the literature. In contrast, the evaporative precipitation into aqueous solution, using DCM/water (immiscible solvent:antisolvent) system, produced ITR microparticles with rapid dissolution properties as reported by Chen et al. (2005). Bosselmann et al. (2012) demonstrated the production of partially amorphous ITR NPs using a modified version of EPAS known as advanced evaporative precipitation in hot aqueous solution. The advanced EPAS employed in that study involved several steps and required pre-emulsification of dichloromethane solution of ITR with lecithin in aqueous solution of PVP and sodium deoxycholate. Therefore, it was of a great interest to investigate a miscible solvent:antisolvent system, that could enable the production of ITR NPs at an elevated temperature using a single step precipitation method. To this end, the use of acetone/water (solvent:antisolvent) system is investigated in Chapter 2.

Chapter 3 further investigates the effect of manipulating the antisolvent precipitation process parameters on the size and the state of matter of ITR. The motivation for this study lies in the fact that the spherical crystallization of drug substances from acetone/water system via the so called quasi emulsion solvent diffusion precipitation into microspheres was reported to be influenced by various process parameters (Kachrimanis et al. 1998, Cui et al. 2003, Nocent et al. 2001, Re et al. 1999, Yang et al. 2003). Therefore, the effect of those process parameters, including the antisolvent temperature, polymer concentration and viscosity manipulation on the process at the nanoscale is of a great importance. Additionally, ITR is capable of forming bulk nematic and smectic LC phases (Six et al. 2001a, 2001b). However, LC ITR NPs have not been reported before nor does the LC phase tuning in ITR NPs.

Chapter 4 focuses on investigating the ability of ITR to accommodate more than one

crystal habit, especially those including twinning and unusual intergrowths. In addition, the conditions and precursors, especially mesoscopic LC particles, which lead to the formation of those rare crystal habits is examined. The formation of unstable colloidal dispersions in the preliminary study of Chapter 3 was the incentive to carry out the study in Chapter 4. The investigated conditions were chosen based on the conditions that failed to produce stable ITR NPs. In this study, the March-Dollase (MD) parameter is employed to numerically describe the preferred orientation and thus the shape of the produced crystals. Finally, the effect of crystal shape on water molecule distribution upon moisture sorption is examined using the Young-Nelson model.

Chapter 5 concentrates on the arrangement of ITR molecules in spray-dried microparticles (MPs). Lechuga-Ballesteros et al. (2003) commented on the challenging assignment of nematic TLCs phases of APIs and the possibility of misinterpreting this phase as isotropic due to the lack of quasi-Bragg peaks in the nematic phase on X-ray diffractograms. In Chapter 5, the phase and phase change of spray dried ITR microparticles produced in Chapter 2 is thoroughly investigated. The rationale of the LC phase of ITR to the pharmaceutical industry, in relation to stability is assessed. The nematic-smectic mesophase switching in bulk samples, or so called mesomorphism, using heating/cooling was investigated by Six et al., (2001b). However, the mesomorphic phenomenon using a hygrothermal treatment has not been reported for ITR.

In Chapter 6, a comprehensive discussion of the observations encountered in Chapters 2 to 5 and summaries of the key advancements achieved in this thesis with recommendations for future research directions are provided.

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CHAPTER II: Heat Induced Evaporative Antisolvent Nanoprecipitation (HIEAN) of Itraconazole

2.1. Introduction

The increasing frequency of drug candidates with complex molecular structure and limited aqueous solubility is a major challenge to the pharmaceutical industry. The poor solubility of these drugs in the gastro-intestinal fluid is a substantial hurdle to their oral administration. Many therapeutically active compounds exhibiting dissolution rate-limited bioavailability fail commercialisation, which endeavors scientists to establish a number of strategies to improve the solubility and subsequent bioavailability of such drugs (Dressman et al. 2000, Gardner et al. 2004). Production of nanoparticles (NPs) is one of the commonly investigated approaches to improve the performance of drugs that possess high permeability but poor solubility (BCS class II drugs) and are likely to have dissolution-limited absorption rates. The breakdown of bulk material into particles in the nanometre range leads to a significant increase in the surface area and thus an improvement in dissolution rates (Liversidge et al. 1992).

Itraconazole (ITR) is a BCS class II drug with low aqueous solubility ($\sim 5 \mu\text{g/mL}$ at $\text{pH}=1$ and $\sim 1 \text{ ng/mL}$ at $\text{pH}=7$) (Peeters et al. 1996, Matteucci et al. 2009), which has been extensively used as a model drug to study the potential advantages of nanonisation. It is an azole antifungal agent, which is effective against a variety of fungal species such as *Aspergillus*, *Cryptococcus*, *candida*, *Blastomyces*, and *Histoplasma capsulatum var. capsulatum* (Odds et al. 2000, Saag and Dismukes 1988). However, the use of ITR is limited owing to its poor bioavailability and variable absorption (Kapsi and Ayres 2001) where the oral solid dosage form available on the market (Sporanox[®]) has an absolute bioavailability of 30% (Barrett et al. 2008).

A wide variety of techniques have been used to form ITR NPs. The top down approach is the most commonly used since it is a scalable approach that avoids the use of organic solvents (Liu et al. 2011) which includes wet milling (Lee et al. 2010) and high pressure homogenization (Chen et al. 2008, Owen et al. 2009). The majority of the commercially available NP-based formulations such as Rapamune[®], Emend[®], TriCor[®], Megace[®], and Triglide[®] are produced using these techniques (van Eerdenbrugh et al. 2008). However, they are time and energy consuming methods (Shah 2006, Wiedmann et al. 1997, Yang et al. 2010, Tam et al. 2010). Moreover, the produced NPs are susceptible to contamination from milling media or the homogenization chamber (Chow et al. 2007).

On the other hand, the bottom up approach is simple with relatively low cost and achieved mainly through antisolvent precipitation and solvent evaporation (Badawi

et al. 2011, Weers et al. 2007). However, the application of bottom up techniques to industry is relatively challenging owing to the possible presence of organic solvent residues in the final product (Keck and Müller 2006, Chen et al. 2006). Different solvent-antisolvent systems and stabilizers have been investigated for the production of ITR nanocrystals and amorphous NPs via antisolvent precipitation (Dalvi and Dave 2010, Matteucci et al. 2006, Kumar et al. 2009, Beck et al. 2010). Although nanocrystals exhibited an improved solubility and dissolution profile compared to the marketed product (Badawi et al. 2011), amorphous NPs are expected to have superior bioavailability due to the absence of a long-range order and periodicity in their structure (Shah et al. 2006). The formation of NPs involves several steps including supersaturation, nucleation and growth of the formed nuclei for nanocrystals, while supersaturation and precipitation is seen for amorphous NPs. Uncontrolled particle growth may result in the aggregation of NPs. The size, solid state and purity of the produced particles mainly depend on the rate and degree of the generated supersaturation (Mullin and Nývlt 1971, Jones and Mullin 1974). A quasi-emulsion solvent diffusion method was used by Re and Biscans (1999) to prepare ketoprofen microspheres using acetone as a solvent. Chen et al. (2002) introduced the use of antisolvent phase at high temperature (75 °C) to evaporate the solvent phase during antisolvent precipitation of NPs and this method resulted in the production of 253 nm cyclosporine NPs. The same method was utilized to produce ITR microparticles with rapid dissolution properties (Chen et al. 2004). To the best of found knowledge, no reports of ITZ NPs produced via this method are available in the literature. Bosselmann et al. (2012) demonstrated the production of partially amorphous ITR NPs using advanced evaporative precipitation in hot aqueous solution. The method employed in that study involved several steps and required pre-emulsification of dichloromethane solution of ITR with lecithin in aqueous solution of PVP and sodium deoxycholate.

In this work, the use of a simple heat induced evaporative antisolvent nanoprecipitation (HIEAN) for the preparation of disordered ITR NPs with and without a polymeric stabilizer is investigated. The effect of inclusion of poly(ethylene glycol) (PEG 2000) and its metoxylated derivative (MPEG) as stabilizers in the antisolvent phase is also studied. The solid-state nature of the produced NPs is determined using thermal analysis and powder X-ray diffraction. The effect of combining size reduction with the conversion into the disordered solid state on the solubility and dissolution profile of ITR in biorelevant media is examined. Finally, the

properties of NPs and the effect of incorporation of PEG and MPEG as stabilizers are assessed using dynamic vapor sorption analysis.

2.2. Materials and methods

2.2.1. Materials

Itraconazole (ITR) was a gift from Welding GmbH (Hamburg, Germany). Poly(ethylene glycol) (PEG) and poly(ethylene glycol) methyl ether (MPEG) with average molecular weights of 2,000 were purchased from Aldrich Chemical Co. Ltd. (Dorset, UK). Acetone Chromasolv[®] HPLC grade was obtained from Sigma-Aldrich (Dorset, UK). Acetonitrile HPLC grade was purchased from Fisher Scientific (Loughborough, UK). Simulated Intestinal Fluid (SIF) powder was acquired from Biorelevant.com Ltd (Croydon, Surrey, UK). Ethanol ($\geq 99.8\%$) employed in DVS experiments was purchased from Sigma-Aldrich Ltd. (Arklow, Ireland). All other chemicals and solvents were of analytical grade.

2.2.2. Heat induced evaporative antisolvent nanoprecipitation (HIEAN) of ITR

The antisolvent phase was either pure deionized water or 0.64 mg/ml PEG (2,000) or 1 mg/ml MPEG (2,000) solution in deionized water and maintained at 80 °C (Figure 2.1). The solvent solution was prepared by dissolving 6.4 mg/ml of ITR in acetone and this solution was maintained at 50 °C. An aliquot of the solvent phase was rapidly injected into the antisolvent phase that was continuously stirred at 13,000 rpm, using a 0.5 * 16 mm Sterican[®] needle using conditions (antisolvent phase and composition as well as solvent/antisolvent ratios) presented in Table 2.1. Once the whole volume of the ITR solution was injected, the mixture was immediately transferred to a jacketed cylinder and allowed to cool to 25 °C.

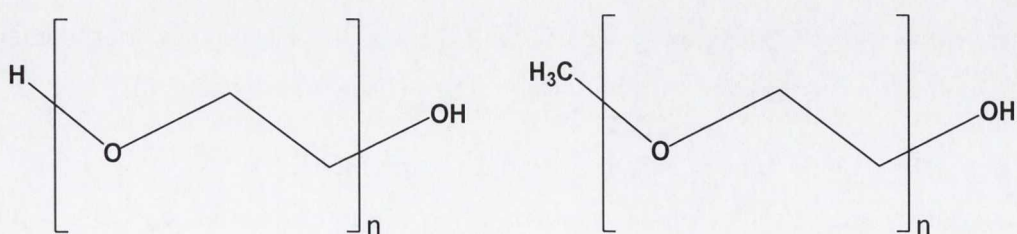


Figure 2.1. Chemical structure of PEG (left) and MPEG (right) (ChemBioDraw).

2.2.3. Spray drying microparticles of ITR

Microparticles (MPs) of ITR 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. 4% w/v ITR solution in dichloromethane was spray dried using the following parameters: inlet temperature of 40 °C, outlet temperature of 34 °C, atomizing 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).

2.2.4. Determination of solubility of crystalline ITR

Solubility of the crystalline ITR in acetone at different temperatures (0, 5, 10, 20, 30, 40, and 50 °C) was determined as follows. At each temperature, 100 mg of ITR was added to 10 ml acetone kept in a closed vial and left to equilibrate overnight with a continuous stirring at 1,000 rpm. Each sample was then filtered through a 0.45 µm syringe filter (VWR, Ireland) and the filtrate diluted 200 times using the mobile phase (40% phosphate buffer and 60% acetonitrile v/v, as described in Section 2.5). Solubility of crystalline ITR in water and antisolvent solutions of different stabilizers (PEG 2,000 and MPEG 2,000) at 80 °C and 25 °C was also measured by equilibrating 100 mg of the drug in the antisolvent solution for 2 hours at 80 °C followed by equilibration at 25 °C for two hours. After each equilibration step an aliquot was withdrawn and filtered using a 0.45 µm syringe filter (VWR, Ireland). For each sample, 1 ml of the filtrate was then evaporated in a microtube (VWR, Ireland), the residue was dissolved in 300 µl of the mobile phase and then analyzed using HPLC (Section 2.2.5).

Solubility of crystalline ITR in the fasted state simulated gastric fluid (FaSSGF, pH=1.6) and fasted state simulated intestinal fluid (FaSSIF, pH=6.5) was assessed by placing 10 mg of the drug in 10 ml of the simulated fluid (prepared according to manufacturer's instructions) at 37 °C and in each case the mixture was continuously stirred at 1,000 rpm. 2 ml aliquots were withdrawn from each vial after 12 and 24 hr and filtered through a 0.45 µm syringe filter. 0.6 ml of the filtrate was then diluted with 0.4 ml of the mobile phase and analyzed using HPLC (Section 2.2.5).

2.2.5. High performance liquid chromatography (HPLC)

The concentrations of ITR were measured using a Waters Symmetry® C₁₈ 5 µm (4.6*150 mm) column attached to a Waters HPLC system equipped with a Waters 1525 Binary HPLC pump, Waters 717 plus autosampler and operating with a Waters 2487 dual λ absorbance detector (λ=260 nm). For the solubility studies of

ITR in acetone, a 50 μ l injection volume was used with a flow rate of 2 ml/min for 7 min. For the solubility studies of ITR in the antisolvent phase a 100 μ l injection volume was used due to the low solubility of ITR in aqueous solutions. For the dissolution studies, 50 μ l injection volumes were used with a flow rate of 1 ml/min for 14 min to prevent ITR peak from overlapping with peaks of the excipients present in the dissolution media. HPLC was also used to detect the quantity of ITR in the PEG and MPEG stabilized NPs and in those experiments a 20 μ l injection volume was used.

2.2.6. Separation, freeze drying and washing of ITR NPs

The formed NP dispersions, as indicated later and except for the directly freeze dried sample (ITRf), were centrifuged at 12,000 rpm at 4 °C for 30 min using an Eppendorf 5810R centrifuge (Hamburg, Germany) (Badawi et al. 2011). The supernatant was removed and NPs in the pellet were frozen in liquid nitrogen and freeze dried at 35 mTorr vacuum pressure and -75 °C for 24 hr using a VirTis Bench Top 6K freeze dryer model EL (SP Scientific, USA). The effect of washing on the NP morphology was studied by washing the residue left after centrifugation with 5 ml deionized water. The suspension was then recentrifuged and freeze dried using the same conditions as mentioned above. Another sample was prepared and freeze dried without centrifugation to assess the necessity of this step.

2.2.7. Characterization of ITR particles

2.2.7.1. Particle size and morphology

The mean particle size and the polydispersity indices (PDI) of NPs were measured using Zetasizer Nano series (Malvern Instruments, UK). The samples were diluted 1:1 with the antisolvent solution and placed in a ZEN0112 low volume disposable sizing cuvette. All measurements were carried out at 25 °C with an equilibration time of 2 min. The analysis was performed in triplicate for each sample and the average was recorded along with the PDI values.

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 particles. Aliquot samples of the nanosuspensions were placed directly on aluminum stubs and dried using nitrogen purge. For the freeze and spray dried samples, powders were placed on carbon tabs fitted on aluminum stubs. The solid samples were sputter coated with gold/palladium under vacuum before analysis.

In an attempt to examine the surface texture of NPs in their native state before coating, helium ion microscopy (HeIM) was used where liquid samples were drop casted onto aluminum stubs, dried using nitrogen purge and examined using a Zeiss Orion plus (Peabody, MA, USA). All images were acquired using 10 μm aperture and 30 kV accelerating voltage. Beam currents were 0.5 to 0.9 pA. No coating and no charge compensation techniques were used.

2.2.7.2. Differential scanning calorimetry (DSC)

Accurately weighed samples (7-9 mg) were placed in pin-holed aluminum pans. The DSC measurements were performed using a Mettler Toledo DSC 821e (Greifensee, Switzerland), under nitrogen purge. A temperature range of 25-200 $^{\circ}\text{C}$ was applied with a heating rate of 10 $^{\circ}\text{C}/\text{min}$ (Liu et al. 2011). For the spray dried material a 5 $^{\circ}\text{C}/\text{min}$ heating rate was also used. The DSC system was controlled by Mettler Toledo STAR^e software (version 6.10). The starting ITR material, the stabilizers and physical mixtures were tested as reference samples. Integration under the crystallization peak shoulder at around 120 $^{\circ}\text{C}$ (Δh_{cryst}) and the melting endotherm at around 168 $^{\circ}\text{C}$ (Δh_{melt}) were utilized to estimate the percent crystallinity in the produced NPs using the following formula (Eqn. 2.1, Hancock and Parks 2000):

$$\% \text{ crystallinity} = \frac{\Delta h_{\text{melt}} - \Delta h_{\text{cryst}}}{\Delta h_{\text{meltITR}}} \quad \text{Eqn.2.1}$$

where $\Delta h_{\text{meltITR}}$ is the heat of melting for pure crystalline ITR.

2.2.7.3. Dynamic vapor sorption

Spray dried ITR and ITR NPs were analyzed with a Dynamic Vapour Sorption (DVS) Advantage-1 automated gravimetric vapor sorption analyzer (Surface Measurement Systems Ltd., London, UK). The temperature was maintained constant at 25.0 $\pm$ 0.1 $^{\circ}\text{C}$ (ethanol or water vapor) or 50.0 $\pm$ 0.1 $^{\circ}\text{C}$ (water vapor). The analysis was performed in triplicate for each sample. In all measurements, around 10 mg of the powder was loaded into a sample net basket and placed in the system. All samples were equilibrated at 0% of RH until constant mass ($\text{dm}/\text{dt} \leq 0.002 \text{ mg}/\text{min}$ for at least 10 min). The reference mass was recorded as a mass equilibrated at 0% RH. Samples were then subjected to either ethanol or water vapor sorption/desorption analysis in a range from 0% to 90% of relative pressure (p/p_0) in 10% p/p_0 steps. Samples recovered following the sorption-desorption cycle were analyzed using PXRD and DSC to examine their solid-state properties and to detect the possible crystallization of drug and/or polymer.

2.2.7.4. Quantification of ITR in NPs (drug loading)

HPLC (as described in Section 2.2.5) was used to quantify the amount of ITR in NPs. In these experiments, 1 mg of the produced NPs was dissolved in 10 ml of the phosphate buffer/acetonitrile mobile phase and analyzed using HPLC. The stabilizer-free NPs were assumed to have 100% ITR since no stabilizers were used in the preparation of these NPs.

2.2.7.5. Powder X-ray diffraction (PXRD)

PXRD measurements were performed using a Rigaku Miniflex II, desktop X-ray diffractometer (Japan) equipped with a Cu K α radiation X-ray source. The samples were mounted on a low-background silicon sample holder and scanned over a 2θ range of 5-40° (Paluch et al. 2010).

2.2.8. Determination of solubility of ITR nano- and micro-particles

ITR displays pH dependent solubility and dissolution with a very low solubility at high pH (Miller et al. 2012), thus in this study the solubility of the produced NPs and the spray dried microparticles were measured in pH 6.8 to assess any improvement in the solubility at high pH upon nanonisation. Simulated intestinal fluid (SIF, pH 6.8) was prepared according to the USP 26th edition (2003) and made of 6.805% w/v KH₂PO₄ and 0.896% w/v NaOH. The pH value of the medium was adjusted as necessary to pH 6.8 with 0.1 M NaOH. In each experiment, excess sample (25 mg) was added to 25 ml of the SIF placed in a temperature controlled jacketed cylinder, preheated to 37 °C and continuously stirred at 1,000 rpm. Sample aliquots (1 ml) were taken and filtered through a 0.1 μ m membrane filter (Sartorius Stedim, Germany) at a predetermined time intervals for 4 hours and analyzed with a Shimadzu Pharmspec UV-1700 UV-Visible spectrophotometer at 262 nm without dilution using low volume 10-mm quartz cuvettes. The analysis was performed in triplicate for each sample and the average concentrations were plotted against time. The undissolved residues were examined using PXRD to assess possible crystallization during solubility studies.

2.2.9. Dissolution studies

Dissolution profiles of the stabilizer-free, PEG and MPEG stabilized ITR NPs were compared to that of the spray dried ITR microparticles. From the solubility study, after 24 hr, the solubility of crystalline ITR in FaSSGF and FaSSIF was 2.36 and 0.26 μ g/ml, respectively. Therefore, 10 mg of ITR NPs or microparticles (equivalent to approximately 42 times of the solubility of crystalline ITR in FaSSGF, similar to the studies of Matteucci et al. (2009)) were added to 100 ml of FaSSGF at 37 °C

with a continuous stirring using an overhead paddle with a stirring rate of 100 rpm. Aliquots were withdrawn at 5, 10, 20, 30, and 60 min and filtered using 0.1 μm membrane filters (Sartorius Stedim, Germany). An equal volume of FaSSGF was replaced except for the 60 min sample where a concentrated solution of SIF, prepared based on an earlier experiment, was injected to shift the pH and the content of the dissolution media to that of FaSSIF and thus mimic the transition of the drug from the stomach to the small intestine. Following the pH shift, aliquots were withdrawn at 5, 10, 20, 30, 60, 120, and 180 min, filtered and an equal volume of FaSSIF was replaced. All filtered samples were diluted 3:2 v/v with the mobile phase and assayed using HPLC (Section 2.2.5). The analysis was performed in triplicate for each sample and the average was plotted against time. Dissolution data obtained was also used to evaluate the maximum concentration achieved, the time point at which this was achieved and the extent of supersaturation (EOS) as described previously by Matteucci et al. (2009).

2.2.10. Statistical analysis

Statistical analyses were performed via either a two sample t-test or one way ANOVA with the Tukey comparison test, as specified in relevant sections, using Minitab Release 16. For all tests, $p \leq 0.05$ was used as the criterion to assess statistical significance.

2.3. Results and discussion

2.3.1. ITR solubility studies and nanoparticle manufacturing conditions

In this study, ITR NPs were precipitated from acetone using heat induced evaporative antisolvent technique (HIEAN). The particles were produced by injecting a volume of acetonic drug solution to a volume of water or an aqueous solution (antisolvent) kept at 80°C. The concentration of ITR in acetone used (6.4 mg/ml) was selected based on the solubility studies conducted at different temperatures where the highest drug saturation was observed at 50 °C (7.34 ± 0.036 mg/ml). The concentration of the stabilizer in the antisolvent phase was chosen to be 10% relative to the drug concentration, similar to the conditions used by Liu et al. (2011). This concentration was observed here to provide the smallest particle size when PEG was used as a stabilizer and the particle size did not change significantly when this concentration was increased to 20% of the drug concentration. On the other hand, smaller particles were obtained as the concentration of MPEG in water was increased up to 1 mg/ml (Table 2.1). Following this increase in MPEG concentration,

there was no further size reduction observed. The solubility of crystalline ITR in the various antisolvent phases used in the HIEAN method was determined. In water at 25 and 80 °C it was very similar and was below 0.5 µg/ml (0.34 ± 0.002 and 0.37 ± 0.002 µg/ml, respectively). The addition of PEG (0.64 mg/ml) and MPEG (1 mg/ml) to water increased the solubility of ITR with the 80 °C incubation temperature yielding higher solubilities (0.46 ± 0.001 and 0.61 ± 0.003 µg/ml for the PEG and MPEG solutions, respectively) than those measured at 25 °C (0.38 ± 0.003 and 0.47 ± 0.006 µg/ml for the PEG and MPEG solutions, respectively).

Table 2.1. summarizes the experiments that were carried out to obtain NPs with smaller size and lower PDI values and shows that as the solvent/antisolvent ratio (v/v) increases (higher drug quantity is injected), larger and more polydispersed particles are produced. The 1:8 ratio v/v yielded particles in the nanometre range but they were larger than those produced when the 1:10 ratio v/v was used and since a small difference in particle size is expected to have a considerable impact on solubility and dissolution (Kondo et al. 1993), the 1:10 solvent/antisolvent (v/v) ratio was chosen. The successful production of NPs, especially the stabilizer-free batch, can be explained with the aid of the precipitation steps described earlier and the acetone induced droplet formation reported by Re and Biscans (1999). Minute droplets are generated upon injecting the solvent phase into the antisolvent phase (Re and Biscans 1999, Nocent et al. 2001, Yang et al. 2003). This process of droplet formation is promoted by the mechanical shear forces of injection and mixing (Yang et al. 2003).

Table 2.1. Outline of trial experiments, which were done to optimize the precipitation conditions and the size of NPs. PDI – polydispersity index, SD – standard deviation.

Formula	PEG conc. (mg/ml)	MPEG conc. (mg/ml)	Solvent/ antisolvent (v/v) ratio	Mean particle size $\pm$ SD (nm)	PDI $\pm$ SD
ITR only	0	0	1:8	797 $\pm$ 56	0.053 $\pm$ 0.040
ITR only	0	0	1:6.7	1489 $\pm$ 168	0.156 $\pm$ 0.078
ITR only	0	0	1:5	1708 $\pm$ 423	0.782 $\pm$ 0.377
ITR only NPs*	0	0	1:10	240 $\pm$ 3	0.087 $\pm$ 0.014
ITR PEG	0.64	0	1:8	780 $\pm$ 52	0.174 $\pm$ 0.042
ITR PEG	0.64	0	1:6.7	1169 $\pm$ 195	0.221 $\pm$ 0.078
ITR PEG	0.64	0	1:5	1539 $\pm$ 337	0.290 $\pm$ 0.170
ITR PEG NPs*	0.64	0	1:10	221 $\pm$ 2	0.041 $\pm$ 0.007
ITR PEG	1.00	0	1:10	222 $\pm$ 3	0.038 $\pm$ 0.011
ITR MPEG	0	0.64	1:10	220 $\pm$ 4	0.048 $\pm$ 0.025
ITR MPEG	0	0.64	1:8	378 $\pm$ 19	0.106 $\pm$ 0.054
ITR MPEG	0	0.64	1:6.7	609 $\pm$ 108	0.174 $\pm$ 0.038
ITR MPEG	0	0.64	1:5	794 $\pm$ 129	0.388 $\pm$ 0.237
ITR MPEG NPs*	0	1.00	1:10	216 $\pm$ 0.4	0.011 $\pm$ 0.007

* - final formulations selected for further testing

The very low solubility of ITR in water even at 80 °C results in solidification of the droplet forming a shell enclosing the supersaturated solvent phase. The high temperature of the antisolvent permits rapid evaporation of the solvent (acetone) through the shell, solvent/antisolvent boundary, and thus the NPs are formed very quickly (Nocent et al. 2001, Yang et al. 2003). The rapid formation of NPs is suggested to prevent the assembly of drug molecules into the well-ordered crystal structure.

The final manufacturing conditions used and the resulting mean particle sizes of the systems as measured by dynamic light scattering (DLS) are summarized in Table 2.1. The use of acetone at 50 °C as a solvent along with the use of 80 °C as the antisolvent temperature resulted in successful production of ITR NPs with mean particle sizes below 250 nm without the need of a stabilizer, thus ensures 100% drug loading, which, to the best of our knowledge, has not been reported before for

ITR NPs. The final formulation produced with PEG had a drug loading of $93.9 \pm 0.1\%$, while that with MPEG had $92.4 \pm 0.5\%$ ITR.

2.3.2. Particle size and morphology

As it can be inferred from the SE micrographs, all produced NPs were spherical in shape with some of them displaying holes (Figure 2.2a, b, c), which further supports the hypothesis of rapid solvent evaporation suggested earlier. The morphology of the NPs displayed in HeIM images was comparable to that from SEM, which excludes any artefacts due to the coating. The spray dried ITR particles were in the micron size range and possessed irregular mushroom-like shapes as shown by SEM in Fig. 1d. The particles were highly polydisperse and could reach up to 10 μm in size.

Based on the ANOVA test, the use of PEG as a stabilizer in the antisolvent phase had a statistically significant impact on the size of the produced NPs and enabled the production of less polydisperse NPs (Table 2.1). Liu et al. (2011) reported that PEG is not suitable for the production of ITR nanosuspensions using milling due to the absence of any hydrophobic groups which are required for the adsorption of the stabilizer on hydrophobic drugs such as ITR. Therefore, a more hydrophobic PEG derivative, methoxylated polyethylene glycol 2000 (MPEG 2000), was used to assess any possible effects of slight chemical modification of the stabilizer on quality of produced nanoparticles. As it can be inferred from the DLS data (Table 2.1), the fabricated NPs had a relatively small mean particle size (although not significantly different in comparison to the PEG sample) but lower PDI compared to those produced with PEG, which is further confirmed by the SEM data (Figure 2.2). It also indicates that although the solubility of ITR is higher in PEG and MPEG aqueous solutions, the 1:10 solvent to antisolvent v/v ratio still provides the required supersaturation for the NP production.

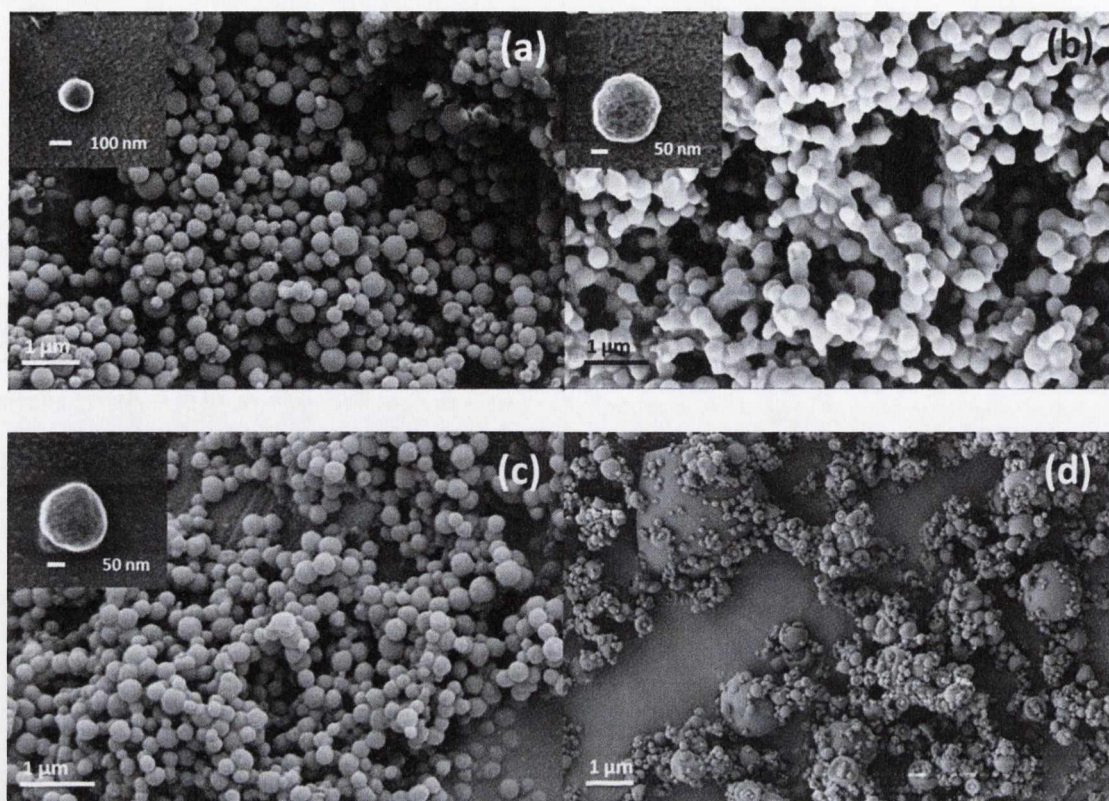


Figure 2.2. SE micrographs and Hel micrographs (in the upper left corner of SE micrographs for NPs) of: (a) stabilizer-free ITR NPs, (b) PEG stabilized NPs, (c) MPEG stabilized NPs and (d) spray dried ITR microparticles.

2.3.3. Impact of washing, centrifugation and freeze drying

Impact of secondary NP treatment, such as washing, centrifugation and freeze drying, on morphology of NPs was assessed as these processes are often used in collection of NP-based products (Umerska et al. 2012). Table 2.2 presents the investigated sample handling procedures. SE micrographs (Figure 2.3a and b) of the non-washed, non-centrifuged but freeze dried sample (ITRf) illustrate micro- and nano-structures of different shapes with some hollow spheres (white arrow) which are very close in shape to those obtained via spray drying of ITR/PEG6000/HPMC ternary dispersions (Janssens et al. 2008). Those non-uniform sample morphologies indicate that centrifugation is an essential step before freeze drying to collect the NPs. SE micrographs of the centrifuged samples illustrate that the shape and size of NPs did not exhibit any significant changes during freeze drying (Figure 2.3c and d).

Table 2.2 Manufacturing and separation conditions employed to optimize the quality of produced particles. In all cases the solvent and antisolvent temperatures were 50 and 80 °C, respectively, and a 1:10 solvent/antisolvent (v/v) ratio was used.

Sample	Stabilizer conc. (mg/ml)	Stabilizer phase	Centrifugation	Washing	Freeze drying
ITRf	N/A	N/A	No	No	Yes
ITRcf	N/A	N/A	Yes	No	Yes
ITRwcf	N/A	N/A	Yes	Yes	Yes
ITR/PEGcf	0.64	Antisolvent	Yes	No	Yes
ITR/PEGwcf	0.64	Antisolvent	Yes	Yes	Yes
ITR/MPEGcf	1.00	Antisolvent	Yes	No	Yes
ITR/MPEGwcf	1.00	Antisolvent	Yes	Yes	Yes

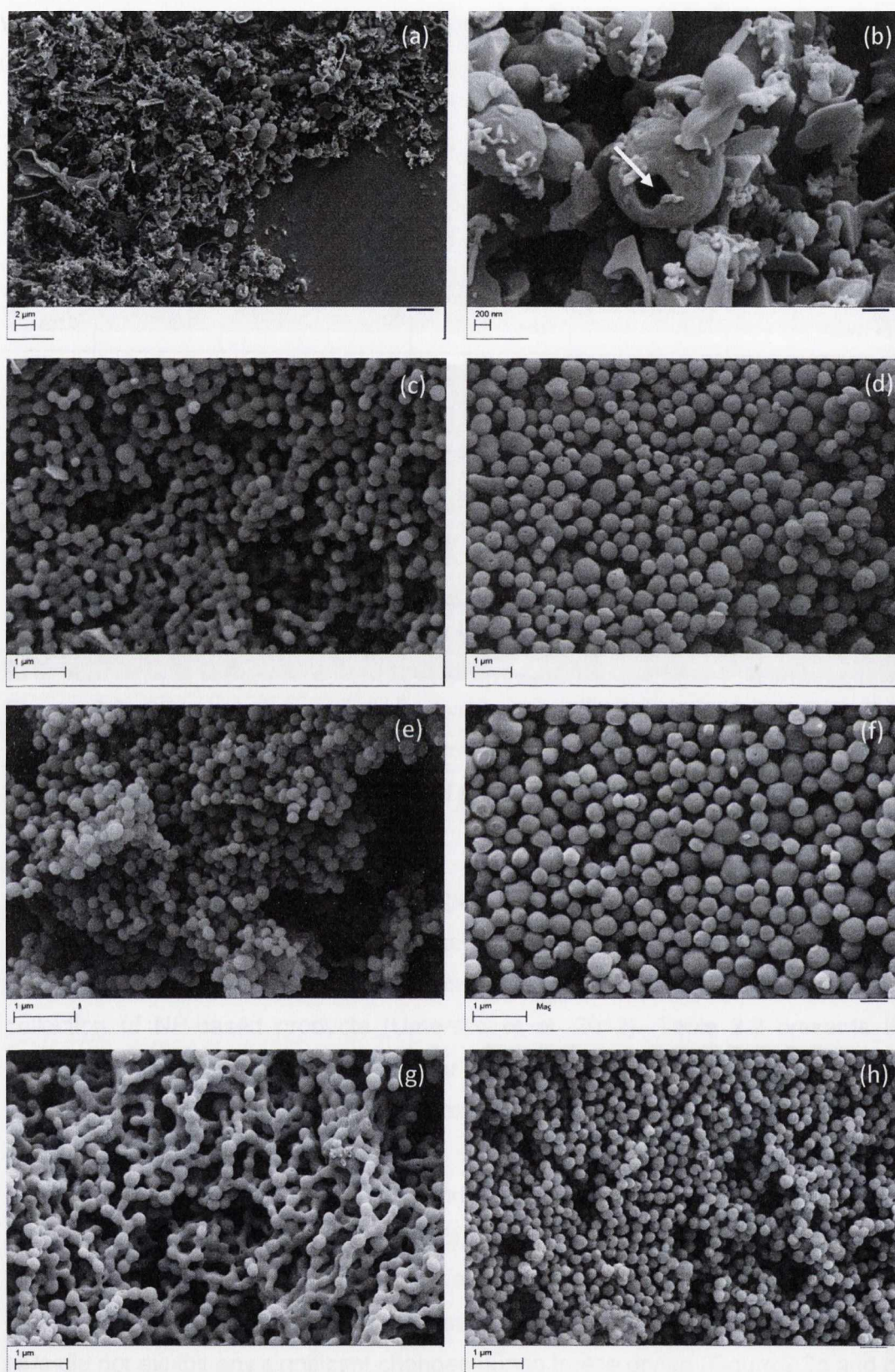


Figure 2.3. Scanning electron micrographs of (a, b) ITRf NPs (c) ITRcf NPs, (d) ITRwcf NPs, (e) ITR/PEGcf NPs (f) ITR/PEGwcf NPs, (g) ITR/MPEGcf NPs and (h)

ITR/MPEGwcf NPs. The white arrow shows an apparent hole in one of the particles, sample codes are explained in Table 2.2.

Some of the freeze dried NPs were hollow and their surface exhibited pores, which is a common phenomenon associated with lyophilisation (Mukai et al. 2004, Wu et al. 2010). In addition, unwashed NPs showed a network-like arrangement following freeze drying (Figure 2.3c, e and g), which was very close in morphology to the sample obtained by Yang et al. (2010) using ultra rapid freezing process with a ITR/mannitol solution. This network-like arrangement was not observed in the washed samples where intact and discrete NPs exhibiting better quality dominate the samples, which can be due to the removal of excess polymer and dissolved drug upon washing (Figure 2.3d, f and h).

2.3.4. Thermal analysis

The thermal behavior of the ITR NPs (without and with PEG or MPEG as stabilizers) as well as ITR:PEG (50:50 w/w) and ITR:MPEG (50:50 w/w) physical mixes is shown in Figure 2.4. The thermograms of the NPs were similar to that of the glassy ITR as described by Wang et al. (2004). The three NP samples exhibited a glass transition (T_g) event at approximately 60 °C, two endothermic transitions at 74 (T_{LC1}) and 90 °C (T_{LC2}) due to the formation of a chiral nematic mesophase (Six et al. 2001) and finally melting at ~168 °C. A crystallization event (T_c) before melting was observable in the three samples at different temperatures. For the ITR only sample the crystallization onset was at 105 °C and it shifted to higher temperatures in the stabilized samples to 108 °C and 113 °C for ITR/PEG and ITR/MPEG NPs, respectively. The degree of ITR crystallinity in each sample was also calculated using Eqn. 2.1 and it was found that the stabilizer-free sample had the highest degree of crystalline ITR of 24±1%, followed by the PEG stabilized sample with 22±1% crystalline ITR. MPEG stabilized sample had the lowest crystalline drug content with only 16±1% crystallinity. This difference in the degree of drug crystallinity in NPs may explain the shift of the crystallization exotherm towards lower temperatures for the 100% ITR NP sample with the highest amount of crystalline material, which acts as nuclei that accelerate crystallization of the amorphous fraction of the sample (Lefort et al. 2004). The spray dried microparticles were 100% disordered, based on calculations using Eqn 2.1, and exhibited a similar thermal behavior as NPs with a T_g at 60 °C followed by two endothermic transitions at 75 and 90 °C. The crystallization event started at 125 °C and the melting

endotherm at 168 °C. This melting endotherm was broader than that reported for the glassy itraconazole (Six et al. 2001) and those recorded for the NPs (Figure 2.4). Therefore, a second run with a heating rate of 5 °C/min was performed. With this heating rate it was possible to observe two peaks (with onsets at 161.0 and 167.6 °C). The peak at 161 °C may be due to melting of the less stable polymorph of ITR (Six et al. 2002, Werling et al. 2007), which formed during crystallization of spray dried ITR. Such a peak was not detected in any of the DSC thermograms of ITR NPs, which indicates that both microparticles and nanoparticles exhibited different thermal behavior. The second peak (at 167.6 °C) was the melting peak of the stable polymorph of ITR, as seen for NPs.

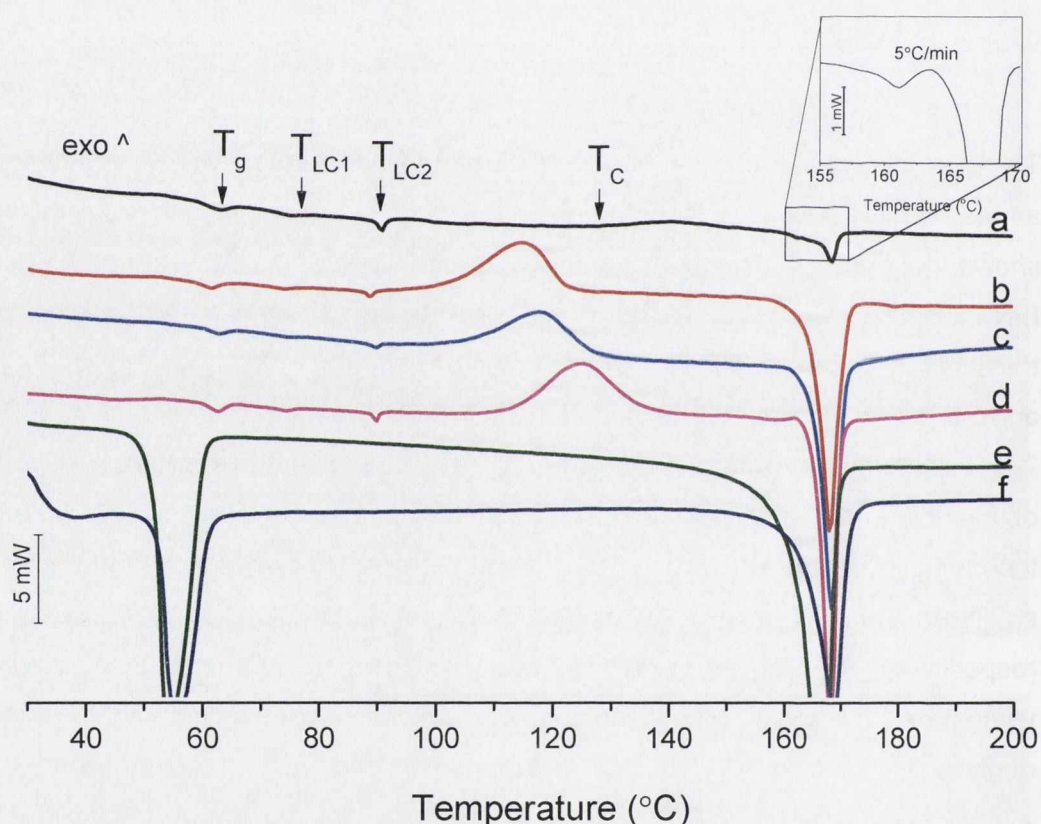


Figure 2.4. DSC thermograms of: (a) spray dried ITR MPs, (b) stabilizer-free ITR NPs, (c) PEG stabilized NPs, (d) MPEG stabilized NPs, (e) ITR and PEG 50:50 (w/w) physical mix and (f) ITR and MPEG 50:50 (w/w) physical mix. T_g – glass transition, T_{LC1} and T_{LC2} – events associated with liquid crystalline transitions and T_C – onset of crystallization.

2.3.5. PXRD analysis

The diffractogram of the starting ITR material was characterized by sharp Bragg peaks, which confirm its crystalline structure. PXRD patterns obtained for all ITR NPs, with and without a stabilizer, revealed the presence of a single liquid crystal peak at 6.05 2 θ degrees and absence of the characteristic peaks of crystalline ITR, thus confirming their disordered nature (Figure 2.5). No diffraction peaks for the polymers were detected. This was consistent with DSC data revealing either no detectable quantity of polymers bound to ITR or the presence of these polymers in an amorphous form. The first hypothesis can be excluded based on HPLC data, which proves that ITR/PEG and ITR/MPEG NP samples are not 100% ITR (drug loading of 93.91 ± 0.12 and $92.4 \pm 0.47\%$ for ITR/PEG and ITR/MPEG NPs, respectively). This suggests that both polymers were present in the amorphous form in the stabilized NPs, which was further assessed by the DSC analysis of the samples obtained following exposure to ethanol and water vapor in DVS (Section 2.3.8).

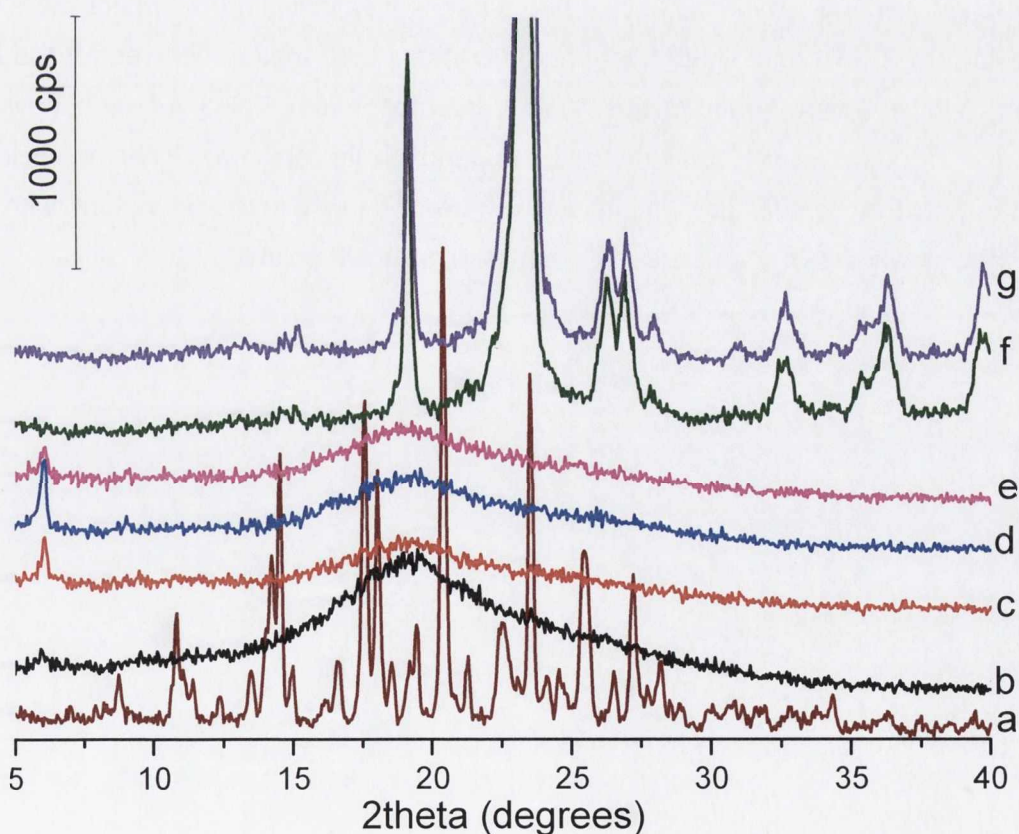


Figure 2.5. PXRD diffraction patterns of (a) crystalline ITR, (b) spray dried microparticles, (c) stabilizer-free ITR NPs, (d) PEG stabilized NPs, (e) MPEG stabilized NPs, (f) crystalline PEG and (g) crystalline MPEG.

2.3.6. Determination of NP solubility

Solubility studies performed for 4 hours demonstrated that the three NP samples: the stabilizer-free and the polymer stabilized, provided higher amounts of solubilized ITR than the spray dried microparticles. MPEG stabilized NPs showed higher apparent solubility with about 20% more ITR soluble compared to the PEG stabilized NPs in the first 10 minutes and reached a steady state of 1.45 ± 0.02 $\mu\text{g/ml}$ after 3 hours (Figure 2.6). PEG stabilized NPs showed a very close solubility profile to that of their MPEG stabilized counterparts in the first hour although they reached a steady state at lower concentrations of 1.08 ± 0.02 $\mu\text{g/ml}$ after 3 hours. This can be attributed to the fact that MPEG has an OH group methoxylated which render the polymer more hydrophobic compared to PEG, thus of a greater affinity to ITR. In addition, these NPs had a higher polymer content. This higher percentage of polymer may have also resulted in an improved wetting of NPs. The solubility profile of the 100% ITR NPs was not far from those of the PEG and MPEG stabilized NPs in the first hour, however a lower (in comparison to the polymer containing NPs) steady state concentration of 0.79 ± 0.01 $\mu\text{g/ml}$ after 3 hours was attained. In contrast, spray dried microparticles had a different solubility profile where there was a slower increase in solubility beyond 30 minutes than those observed for the NPs with a lower steady state concentration (0.54 ± 0.04 $\mu\text{g/ml}$). This superior behavior of NPs produced using HIEAN method compared to the disordered microparticles is owing to the combination of the advantages of the disordered nature with the nanosize which led to an increase in the drug apparent solubility.

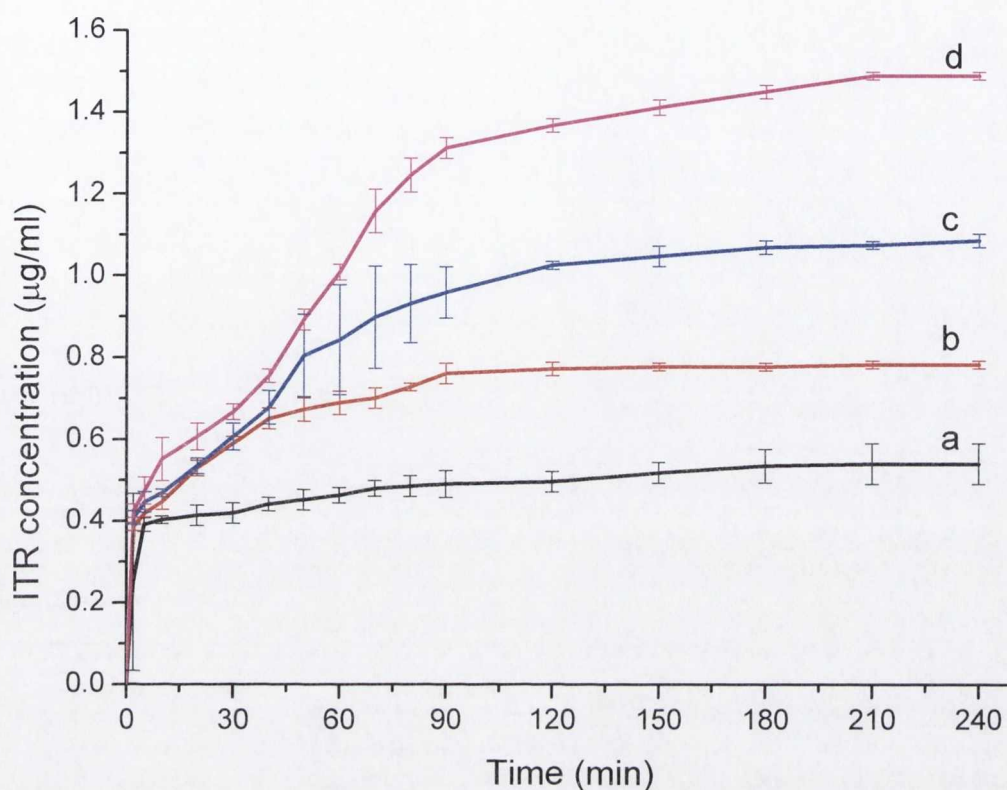


Figure 2.6. Solubility profiles of (a) spray dried microparticles, (b) stabilizer-free ITR NPs, (c) PEG stabilized NPs and (d) MPEG stabilized NPs.

PXRD patterns obtained for the remaining solids after the solubility studies (Figure 2.7) revealed that the ITR NPs without the polymeric stabilizer had a very weak tendency to crystallize during the experiments since they produced the characteristic halo of an amorphous material with very low intensity Bragg peaks. On the other hand, the polymer (PEG or MPEG) stabilized NPs ITR had a greater tendency to crystallize as shown in Figure 2.7. This can be due the higher solubility of the stabilized NPs, which is due to the presence of the polymer.

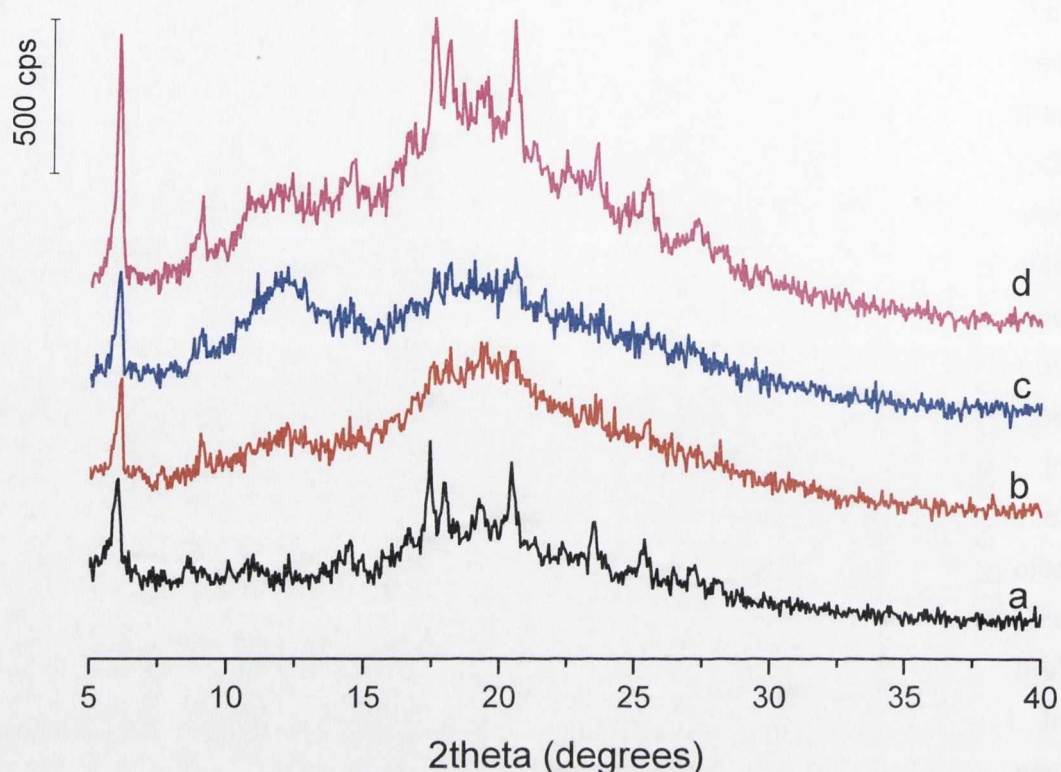


Figure 2.7. PXRD patterns of the remaining undissolved powders following solubility studies of (a) spray dried microparticles, (b) stabilizer-free ITR NPs, (c) PEG stabilized NPs and (d) MPEG stabilized NPs.

2.3.7. Dissolution studies

ITR NPs produced using the HIEAN method showed significant superiority over the spray dried microparticles during solubility investigation in high pH and dissolution studies conducted to assess the effect of this behavior on the dissolution properties of ITR mimicking GIT conditions. The saturation solubility of crystalline ITR in FaSSGF is approximately 2.36 ± 0.003 $\mu\text{g/ml}$. The dissolution profile of MPEG stabilized NPs illustrates a slow release of ITR from the formulation in the first 30 minutes in FaSSGF followed by a steep increase in the dissolved ITR until a peak of 11.6 ± 0.5 $\mu\text{g/ml}$ (~5 times solubility of crystalline ITR) is reached after 60 minutes (Figure 2.8). The slow release of ITR in the first 30 minutes was in accordance with the visual monitoring of the dissolution process (powder was floating on the surface for the first 30 minutes of the study) where the mixing speed of the paddle was not sufficient to wet the ITR NPs instantly. This delay in powder wetting is expected to affect dissolution timeline. Thus, to optimise the behaviour of those NP samples in the dissolution media, the incorporation of wetting agents might be necessary.

Following the pH shift, the large quantity of solubilized ITR was responsible for the sudden drop of ITR concentration in FaSSIF after 5 minutes due to the low saturation solubility of ITR in FaSSIF (approximately 0.26 ± 0.001 $\mu\text{g/ml}$). This low saturation solubility is the driving force for nucleation and growth via coagulation as described by Matteucci et al. (2009). The drop in concentration of ITR in the dissolution medium continued until the dissolved ITR reached 2.3 ± 0.2 $\mu\text{g/ml}$ after 3 hours in FaSSIF, which is most likely to be due to the crystallization of some ITR as demonstrated during the solubility study. PEG stabilized NPs yielded a peak concentration of 3.7 ± 0.7 $\mu\text{g/ml}$ after 5 minutes following the switch of the dissolution media to FaSSIF (although pH of the media increased). This peak was followed by a decrease in ITR level to 2.1 ± 0.03 $\mu\text{g/ml}$, about 8 times solubility in FaSSIF, within 60 minutes. ITR only NPs exhibited a dissolution profile closely resembling that of PEG stabilized ITR NPs with a peak concentration of 2.6 ± 0.3 $\mu\text{g/ml}$ achieved 5 minutes following the media pH shift. The results indicate that the absence of high supersaturation before shifting the media along with the presence of solubilizing excipients in FaSSIF, compared to the MPEG stabilized NPs, prevents sudden drug precipitation following pH shift and enables to sustain the high “apparent” solubility of ITR in pH=6.5. In addition, the relatively high levels of dissolved ITR from the stabilized NPs compared to the stabilizer-free NPs in the FaSSGF support the hypothesis of disordered drug present along with amorphous polymer on the surface of the NPs. This disordered ITR is wetted and dissolved in FaSSGF yielding 1.4 ± 0.01 and 1.2 ± 0.09 $\mu\text{g/ml}$ of ITR in the first 30 minutes from the MPEG and PEG stabilized NPs, respectively. Spray dried microparticles exhibited the lowest peak maximum (0.4 ± 0.01 $\mu\text{g/ml}$) 5 minutes following pH shift. This indicates that size reduction and thus an increase in powder surface area had a greater effect on dissolution properties of ITR in comparison to the solid state effects as the MPs were 100% amorphous, based on calculations from Eqn 2.1, compared to the NPs (Leuner et al. 2000). A decrease in ITR concentration to 0.14 ± 0.02 $\mu\text{g/ml}$ occurred within 60 minutes for ITR MPs following pH shift to 6.5. This can be explained by the partial crystallization observed from the PXRD analysis of the undissolved microparticles remained following solubility study.

To get an insight into the total amount of ITR released throughout the dissolution study, the extent of supersaturation (EOS) for each of the four samples was calculated and the results, along with maximum concentration achieved, expressed here as C_{max} , are presented in Table 2.3. A two sample t-test was also carried out for EOS and C_{max} . From the EOS data it can be inferred that the soluble ITR

available in the dissolution media from the MPEG stabilized NPs was twice that of the PEG stabilized batch. The polymer-free NPs and the spray dried microparticles provided considerably less soluble ITR. The effect of size reduction is demonstrated by the higher EOS of the stabilizer-free NPs compared to the MPs. However, the presence of a small quantity of polymer has a greater implication on the solubility than does the size only as indicated by the approximately twelve fold greater EOS of the MPEG stabilized NPs compared to their stabilizer-free counterparts. Statistical analysis indicates that all EOS and C_{max} values calculated for all the samples were statistically significantly different. The type of the polymer included had a significant impact on the behavior of the NPs in the dissolution studies as can be inferred from the improvement in the EOS and C_{max} associated with the exchange of PEG by MPEG in the NPs, although there was no significant difference in the size of the produced NPs as indicated earlier (Table 2.1).

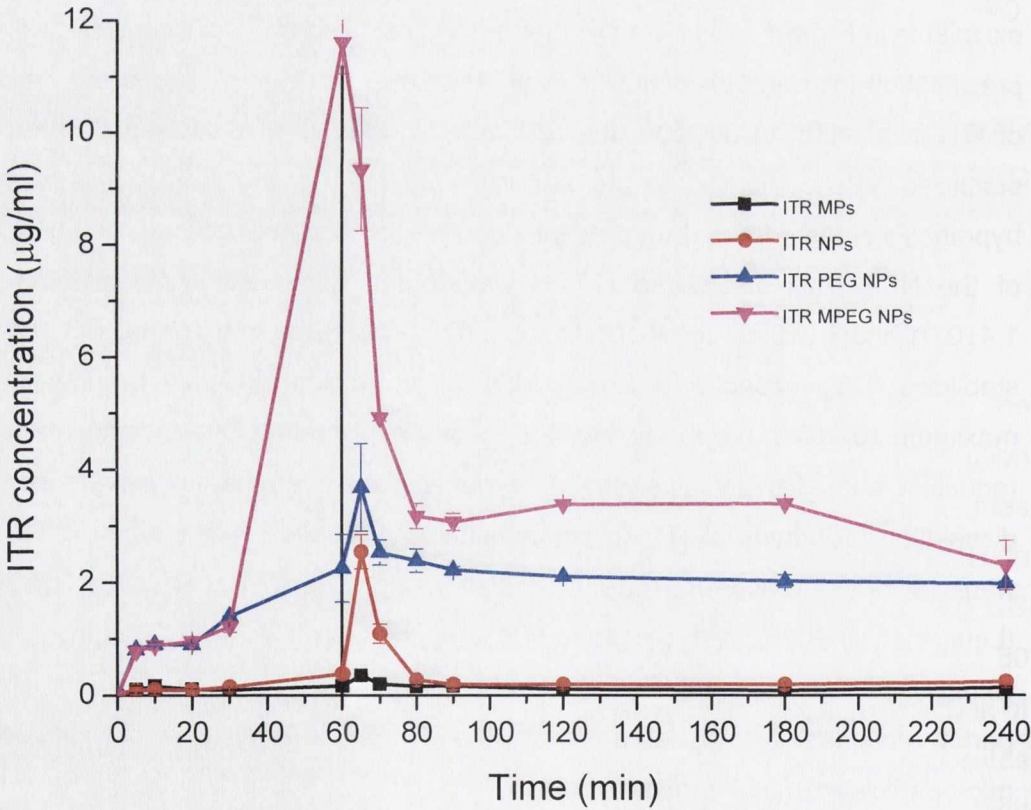


Figure 2.8. Dissolution profiles of ITR nano- and micro-particles. The vertical line indicates dissolution media change from FaSSGF to FaSSIF.

Table 2.3. Summary of the EOS (extent of supersaturation), $C_{\max}$ (maximum concentration), $T_{\max}$ (time to maximum concentration) parameters obtained from the dissolution studies. SD – standard deviation.

Sample	$EOS_{0-240\min} \pm SD$ ($\mu\text{g} \cdot \text{min}/\text{ml}$)	$C_{\max} \pm SD$ ($\mu\text{g}/\text{ml}$)
ITR NPs	68.0 ± 2.9	2.6 ± 0.3
ITR/PEG NPs	469.2 ± 7.6	3.7 ± 0.7
ITR/MPEG NPs	843.1 ± 24.6	11.6 ± 0.5
SD MPs	30.7 ± 3.6	0.4 ± 0.01

2.3.8. Dynamic vapor sorption

In Figure 2.9, DVS isotherms of a full cycle of sorption-desorption of the spray dried ITR and the ITR NPs in ethanol vapor are displayed. The isotherms revealed that the spray dried ITR (MPs), when exposed to a series of 10% step changes of ethanol vapor from 0 to 90% p/p_0 , gained a mass of $4.29 \pm 0.21\%$ of its dry weight up to 80% p/p_0 followed by a mass loss of $3.66 \pm 0.35\%$ in the 80-90% p/p_0 step. A lower mass gain, $3.41 \pm 0.13\%$ at 70% ethanol vapor in the sorption cycle, was showed by the ITR NPs produced using the HIEAN method, which indicates that they are less stable than the spray dried powder. Another mass gain of $0.16 \pm 0.03\%$ occurred by the end of the sorption cycle (between 80 and 90% p/p_0). The PEG and the MPEG stabilized NPs were characterized by a mass gain of 2.73 ± 0.12 and $2.53 \pm 0.07\%$ up to 60% p/p_0 , respectively, followed by a mass loss (2.16 ± 0.08 and $1.61 \pm 0.13\%$) in the 60-70% ethanol vapor step.

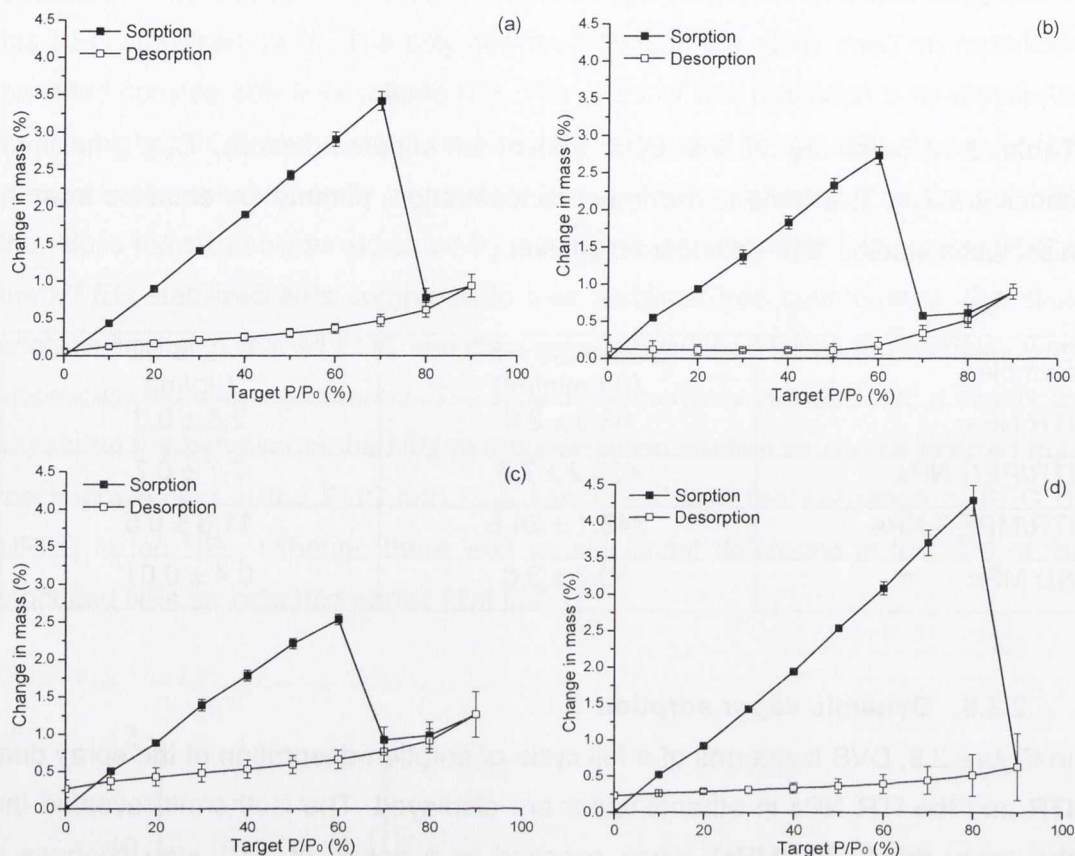


Figure 2.9. DVS sorption-desorption isotherm of (a) stabilizer-free ITR NPs, (b) PEG stabilized ITR NPs, (c) MPEG stabilized ITR NPs, and (d) spray dried ITR MPs. Experiments were carried out at 25 °C using ethanol.

According to DVS data, the spray dried ITR sorbed more ethanol vapor than the other samples because it was a fully amorphous material, according to Eqn 2.1, unlike the NPs produced by the HIEAN method where the crystalline content could act as nuclei for crystallization. Among the NPs, although they are less amorphous based on DSC data, the stabilizer-free NPs absorbed more ethanolic vapor than did the polymer stabilized NPs. This could be because the latter contain either PEG or MPEG, which also attract the ethanol vapor. All samples were fully crystalline after one sorption-desorption cycle upon examination by PXRD and DSC, where the characteristics crystalline ITR PXRD patterns (Figure 2.10) and the sharp endotherm at 168 °C in DSC thermograms were observed. In the polymer stabilized samples there was a small endotherm observed at 52 °C, which is most likely the characteristic melting peak of PEG or MPEG. This supports the hypothesis (stated earlier in Section 2.3.5) of the presence of amorphous polymers in the NPs, which crystallized upon exposure to ethanol vapor.

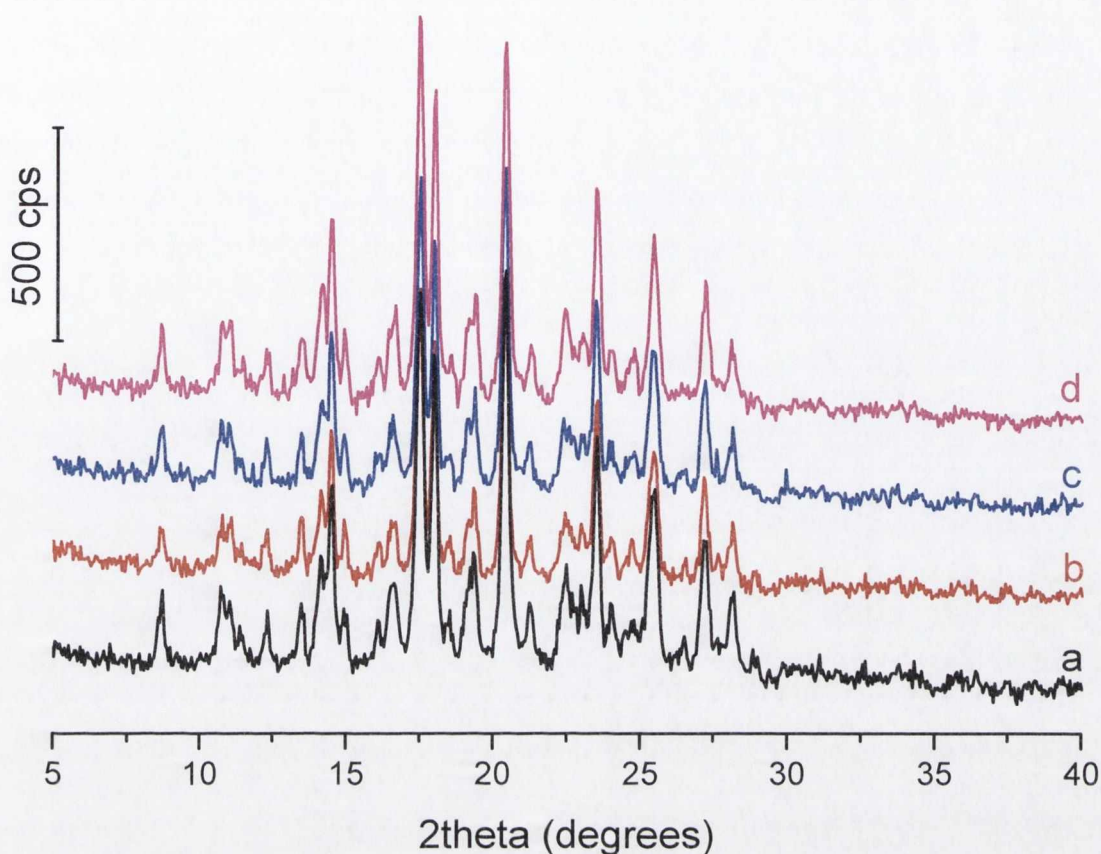


Figure 2.10. PXRD patterns of the remaining sample following DVS studies using ethanol at 25°C of (a) spray dried microparticles, (b) stabilizer-free ITR NPs, (c) PEG stabilized NPs and (d) MPEG stabilized NPs.

The moisture sorption-desorption isotherms using water vapor for the ITR NPs at 25 and 50 °C are illustrated in Figure 2.11 a, b, and c. All isotherms at 25 °C have a hysteresis, where the water content at each relative humidity step is higher during desorption than sorption. The condensed water on the surface can act as a driving force for water penetration which is not applicable for desorption (York 1981). In addition, in the PEG and MPEG stabilized NPs, the presence of a small quantity of the hygroscopic polymer can also attract more water during the sorption cycle and retain it during the desorption, which makes a greater hysteresis effect in isotherms of the stabilized NPs. This also explains the higher water uptake (mass gain) observed in the polymer stabilized NPs (2.63% and 2.59% for ITR/PEG and ITR/MPEG at 90% RH, respectively) compared to 2.00% for the polymer-free NPs. The effect of the hygroscopic polymer can be also inferred from the final weight of the sample after the sorption/desorption cycle, which is higher than that of the initial sample mass in the case of the polymer stabilized NPs, where the polymer retained

a higher quantity of water compared to the polymer-free NPs, where no observable weight gain was found at the end of the desorption cycle. There were no pores observed in the SE micrograph of the spray dried sample (Figure 2.2) and this, in combination with the lower surface area of MPs, might explain the lower hysteresis effect compared to that of the NPs (Columbano et al. 2002). The total mass gain (water uptake) was also lower in the spray dried sample, 1.8% at 90% RH.

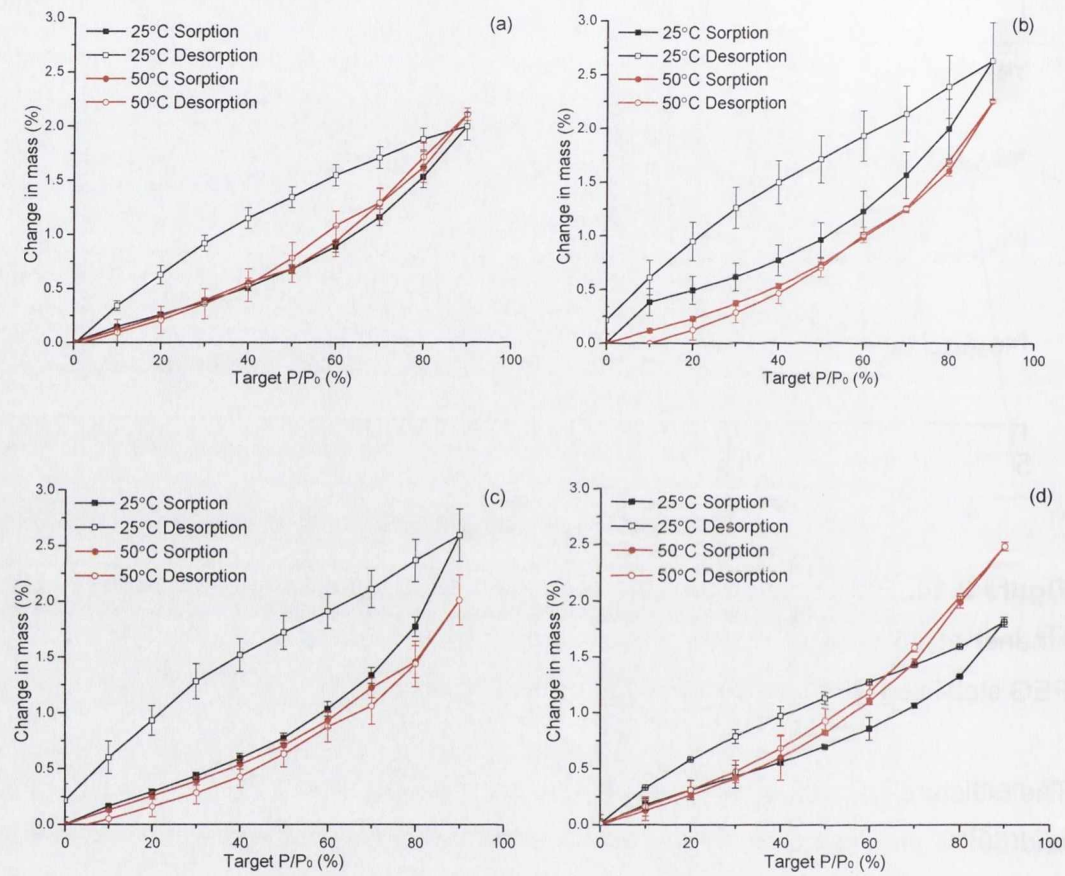


Figure 2.11. DVS sorption-desorption isotherm of (a) stabilizer-free ITR NPs, (b) PEG stabilized ITR NPs (c) MPEG stabilized ITR NPs and (d) SD ITR MPs. Experiments were carried out at 25 and 50 °C using water.

The moisture sorption and desorption isotherms generated with water vapor at 50 °C were type III isotherm according to the IUPAC system (Airaksinen et al. 2005) and characteristic of a weak adsorbate-adsorbent interaction which is consistent with the hydrophobic nature of ITR. The isotherms illustrate mass gains in all samples due to water uptake (Figure 2.11). The percentage of mass gain was different for each sample. In general, NPs exhibited lower mass gains compared to MPs. The maximum uptake among the NPs was by the PEG stabilized sample,

which was also characterized by the highest uptake at 25 °C (squares in Figure 2.11). In addition, there was a very small hysteresis effect observed for all of the isotherms obtained for the NP samples indicating that water sorption under these conditions was completely reversible. This can be attributed to analysis of the samples at 50 °C, which may facilitate water evaporation during desorption. Unlike the 25 °C data, the 50 °C isotherms of MPs indicate the highest weight gain in comparison to all samples investigated (about $2.49 \pm 0.04\%$ of its dry weight at 90% RH).

DSC and PXRD examination of all samples subjected to water vapor showed that all samples did not crystallize following exposure to water vapor at 25 °C in DVS. A slight shift in the crystallization exothermic peak temperature in DSC was observed for all samples. The MPEG stabilized sample, which appeared to have the highest crystallization temperature was found to have the lowest crystallization onset following DVS analysis (94 ± 0.3 °C), followed by the PEG stabilized NPs which crystallized at 98.9 ± 0.1 °C (onset). In both samples a melting peak at 52 °C was observed due to crystallized polymer. The stabilizer-free sample was the most stable among the NP samples and crystallized at 104.2 ± 0.3 °C (onset). The spray dried MPs were less affected by the exposure to water vapor at 25 °C in DVS and crystallization started at 125.2 ± 0.3 °C. The above observations illustrate the effect of polymer crystallization on thermal stability of the NPs, where the entrapment of water and rearrangement of polymer molecules facilitate ITR crystallization at lower temperatures compared to the polymer-free samples. Similarly, all samples exhibited the halo pattern with a single liquid crystal peak upon examination using PXRD (Figure 2.12), consistent with DSC data.

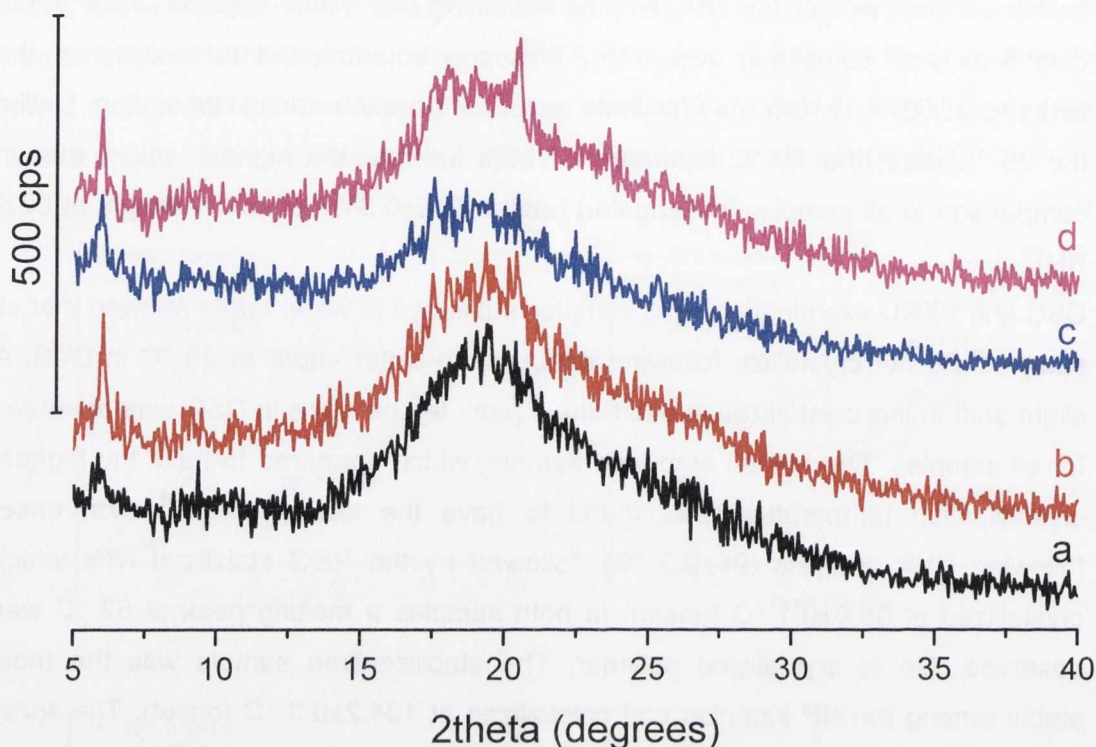


Figure 2.12. PXRD patterns of the remaining sample following DVS studies using water at 25°C of (a) spray dried microparticles, (b) stabilizer-free ITR NPs, (c) PEG stabilized NPs and (d) MPEG stabilized NPs.

Upon elevation of the DVS analysis temperature to 50 °C, a further decrease in the crystallization onset temperature was recorded for all nanoparticulate samples (ITR NPs = 97.9 ± 0.4 , ITR/PEG NPs = 97.1 ± 0.5 , ITR/MPEG = 93.2 ± 0.7 °C). On the other hand, when examined by PXRD, in the polymer stabilized NPs there were some residual peaks emerging from the halo pattern along with the single liquid crystal peak, which further explains the crystallization peak shift to lower temperatures in DSC. These residual crystalline peaks were hardly detected in the stabilizer-free NPs and the spray dried microparticles. On the other hand, the liquid crystal peak was nearly invisible in the diffractogram of microparticles, which could indicate a phase change. DSC thermogram showed a crystallization onset at a lower temperature, 120.1 ± 0.3 °C of the DVS sample compared to 125 ± 0.9 °C for the untreated ITR MPs and the enthalpy of melting of the metastable polymorph was slightly greater than that of the material before DVS experiment. Collectively, observations from DVS at 50 °C, DSC and PXRD suggest a possible structural collapse in part of the amorphous MP sample during the DVS cycle (Figure 2.13).

Structural collapse before crystallization was reported by Buckton and Darcy (1996), where exposure to 40% and 50% RH induced structural changes, but not crystallization of lactose.

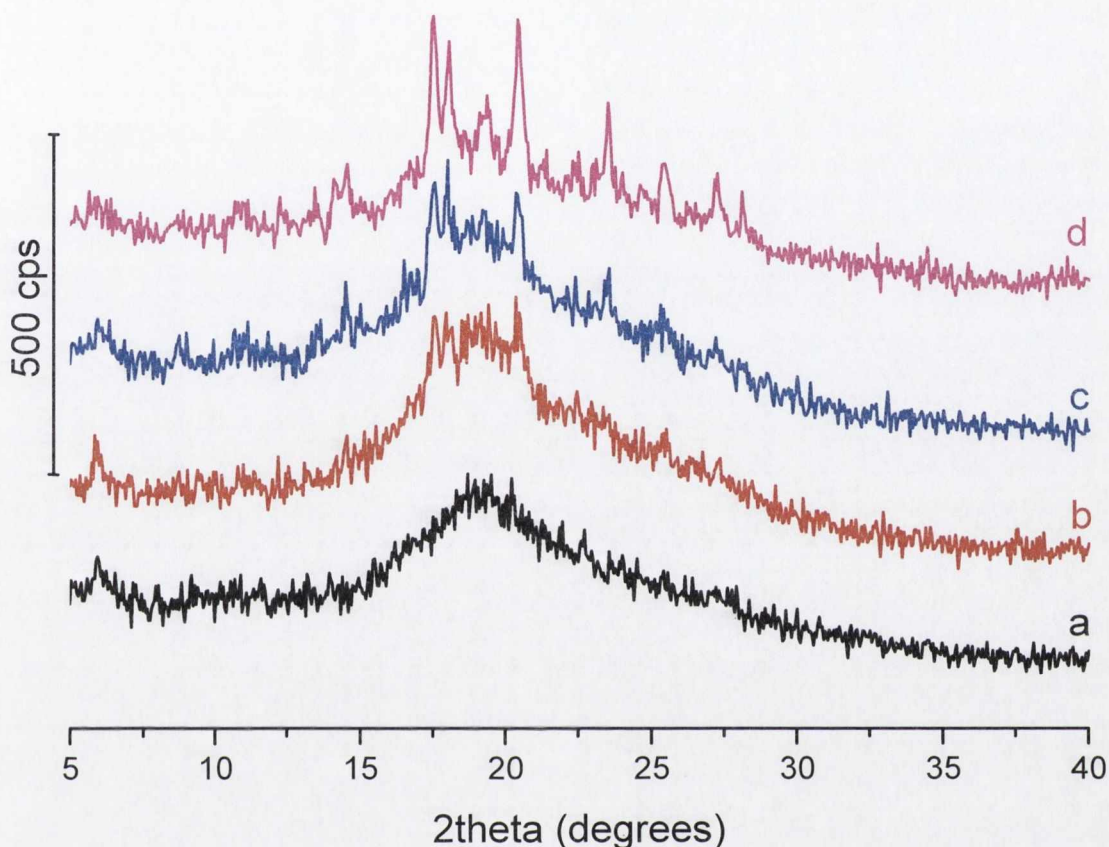


Figure 2.13. PXRD patterns of the remaining sample following DVS studies using water at 50°C of (a) spray dried microparticles, (b) stabilizer-free ITR NPs, (c) PEG stabilized NPs and (d) MPEG stabilized NPs.

2.4. Conclusions

The heat induced evaporative antisolvent nanoprecipitation (HIEAN) of ITR from acetone at 80 °C produces liquid crystalline NPs with the mean particle size less than 250 nm and 100% drug loading. These NPs demonstrated greater solubility and improved dissolution profile compared to the spray dried disordered ITR microparticles. Smaller NPs with lower polydispersity indices can be obtained when polyethylene glycol or its methoxylated derivative is included in the antisolvent phase as a stabilizer. Enhanced dissolution was associated with the use of MPEG as a stabilizer. In all samples, washing appears to improve the quality of the produced NPs. Centrifugation was found to be an essential step for the collection of NPs, otherwise a mixture of particles with different shapes and sizes were obtained.

The stabilizing polymer was in an amorphous state as evidenced by PXRD, DSC and DVS studies. Analysis indicates that there was no significant change in the solid state properties of ITR NPs when subjected to water vapor up to 90% RH at 25 °C with an increase in the crystallinity in the polymer stabilized samples if the process temperature was increased to 50 °C.

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CHAPTER III: Mesophase and Size Manipulation of Itraconazole Liquid Crystalline Nanoparticles Produced via Quasi Nanoemulsion Precipitation

3.1. Introduction

The advances in drug design have led to a radical change in the development of active pharmaceutical ingredients (APIs), however at a price of dramatically decreasing their biorelevant properties, mainly solubility. Those APIs are estimated to constitute 40% of the currently developed potential drugs, where low bioavailability is the undesirable consequence of the poor solubility. Nanotechnology has been established as a promising pathway to enhance dissolution and solubility of such substances (Dressman and Reppas, 2000). Itraconazole (ITR) is an anti-infective drug that suffers from the above drawbacks, however some attempts involving fabrication in the nanoscale have been found to boost its bioavailability (Miller et al., 2012, Matteucci et al. 2008).

While a few reports on amorphous and crystalline ITR nanoparticles (NPs) can be found in literature, liquid crystalline ITR NPs and, in general, liquid crystalline drug NPs were overlooked. A liquid crystal (LC) is an intermediate state between the crystalline and amorphous states with a combination of residual order and mobility thus known as mesophases (Mapesa et al. 2014). APIs in the LC form have been reported to possess enhanced solubility in comparison to their crystalline counterparts (Su 2011). Almost all types of supramolecular interactions, including van der Waals interaction, hydrogen bonds and π - π interactions, can be observed in LCs (Bisoyi et al. 2011). Molecules capable of forming mesophases are known as mesogens and can be classified according to the anisotropy of their shape into rod-like and disc-like (Tanaka et al. 2012) with the former exhibiting the highest molecular anisotropy. Thus nanosized liquid crystals possess the combinatorial effect of the nanomaterials' large surface area, the compromised molecular order of mesophases, which is expected to enhance solubility and dissolution, and advantageous stability on comparison to amorphous due to lower Gibbs free energy (Liversidge and Cundy, 1995).

ITR molecule is highly anisotropic with a rod like structure (Figure 3.1). Nematic and smectic liquid crystalline phases of ITR were previously achieved via controlled cooling of melted crystals (Mapesa et al. 2014, Six et al. 2001). However, a nematic phase can easily be misinterpreted as isotropic (amorphous) using X-ray diffraction due to similarity in diffraction patterns of both phases.

Here, tuning the LC phase in ITR NPs through controlling the quasi nano-emulsion precipitation process parameters, especially the solvent to antisolvent temperature difference ($\Delta T_{S:AS}$) is reported on. The $\Delta T_{S:AS}$ transpired to be a critical parameter associated with the nematic or smectic assembly with both phases confined in the

shell of nanospheres at room temperature which, to the best of found knowledge, has not been demonstrated before. The underlying mechanism of ITR NP formation was investigated and the impact of $\Delta T_{S:AS}$, the solvent:antisolvent viscosity ratio (ρ) and functional properties, such as miscibility and possibility of interactions, of the included polymer on the properties of the formed particles were examined. Different polymers were tested including poly(acrylic acid) (PAA), cellulose acetate phthalate (CAP) and Poloxamer 407 (P407), although the focus is mainly on P407 as it demonstrated the capability to markedly improve ITR dissolution (Sarnes et al. 2014, Badawi et al. 2011). The formation of ITR-P407 NPs at elevated temperature adds to the unique achievements of this work since it was reported to be infeasible before (Matteucci et al. 2006).

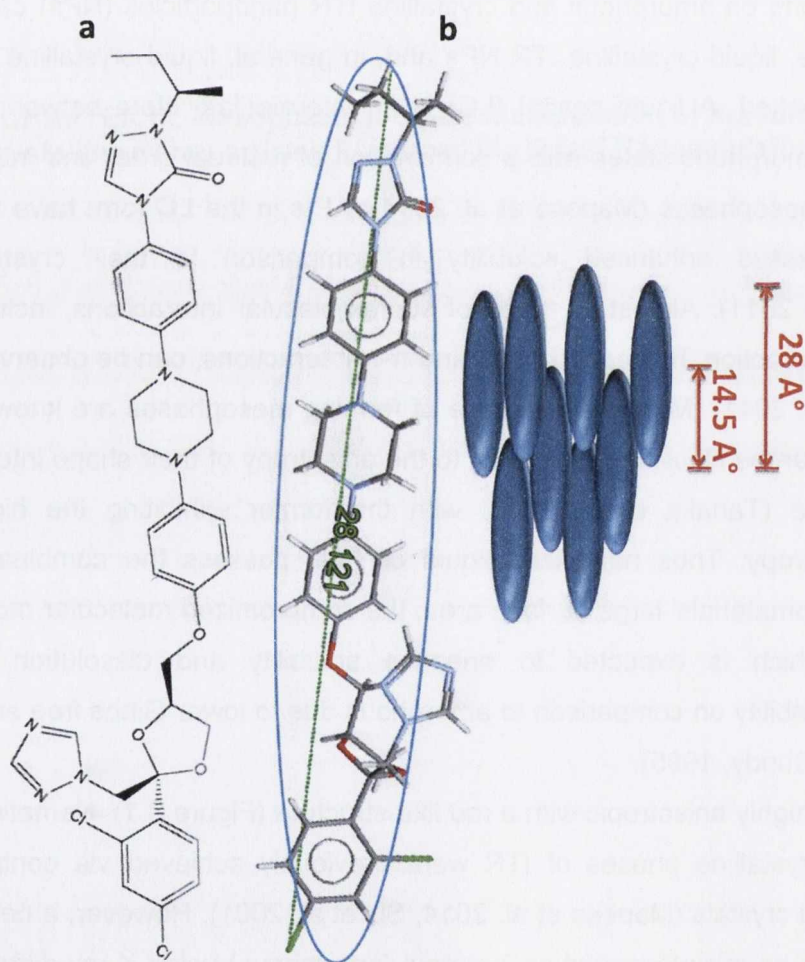


Figure 3.1. (a) Chemical structure of ITR (ChemBioDraw). (b) Schematic illustration of ITR molecule (Mercury[®] 3.5.1) with the molecular arrangement and interplanar spacing in the intercalated smectic A phase.

3.2. Materials and Methods

3.2.1. Materials

Itraconazole (ITR) was a gift from Neuland Laboratories Ltd. (Welding, Hamburg, Germany). Poloxamer 407 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol), P407) was purchased from BASF Corp. (Ludwigshafen, Germany). Acetone Chromasolv® HPLC grade was obtained from Sigma-Aldrich (Dorset, UK), while acetonitrile HPLC grade was purchased from Fisher Scientific (Loughborough, UK). Poly(acrylic acid) (PAA), as Carbopol 981 was obtained from BFGoodrich (Brecksville, OH, USA). Ethanol ($\geq 99.8\%$), employed in DVS experiments and cellulose acetate phthalate (CAP) were acquired from Sigma-Aldrich (Dublin, Ireland). All other chemicals were of analytical grade and used as supplied.

3.2.2. Determination of solubility of crystalline ITR

The solubility of crystalline ITR in acetone at 50 °C in the presence of P407 at different concentrations (1, 2, 3 and 4 mg/ml) was determined using a high performance liquid chromatography (HPLC) method as carried out before in Chapter 2 (Section 2.2.5).

3.2.3. Preparation of ITR NPs

ITR NPs were prepared as previously described in Chapter 2 with some modifications and details of experimental conditions are presented in Table 3.1. The modifications included changing the temperature of the antisolvent phase (water), thus creating a temperature gradient ($\Delta T_{S/AS}$). Solvent was acetone. PAA was used to tune the viscosity of the antisolvent phase. CAP was determined to be miscible with ITR based on calculated theoretical miscibility using Flory-Huggins interaction parameters (Thakral and Thakral 2013).

Table 3.1. Summary of the precipitation conditions and properties of NPs produced. The solvent:antisolvent (S:AS) v/v ratio was 1:10 in all experiments, based on results from Chapter 2 and the solvent phase was kept at 50 °C. T- temperature, $\Delta T_{S:AS}$ – temperature difference between solvent (S) and antisolvent (AS), ITR – itraconazole, P407 – Poloxamer 407, CAP - cellulose acetate phthalate, PAA - poly(acrylic acid) (as Carbopol®).

Sample	T_{AS} (°C)	$\Delta T_{S:AS}$ (°C)	ITR Concentration (mg/ml)	Polymer concentration in S phase (mg/ml)		Polymer concentration in AS phase (mg/ml)
				P407	CAP	PAA
F1	0.5	49.5	6.4	0	0	0
F2	0.5	49.5	7.9	1	0	0
F3	50	0	6.4	0	0	0
F4	50	0	7.9	1	0	0
F5	80	30	6.4	0	0	0
F6	80	30	7.9	1	0	0
F7	80	30	6.4	0	1	0
F8	80	30	6.4	0	0	0.033
F9	80	30	6.4	0	0	0.05
F10	80	30	6.4	0	0	0.1
F11	80	30	7.9	1	0	0.033
F12	80	30	6.4	0	1	0.033

3.2.4. Dynamic light scattering (DLS) and zeta potential (ZP)

The mean particle size and the polydispersity indices of NPs were measured using a Zetasizer Nano ZS series (Malvern Instruments, UK). The dispersions were placed in DTS1061 clear disposable zeta cells. All measurements were carried out at 25 °C with an equilibration time of 2 min. The analysis was performed in triplicate for each sample and the mean particle diameter along with the polydispersity index were recorded and corrected for viscosity of the continuous phase. Electrophoretic mobility values were measured by laser Doppler velocimetry (LDV) using DTS1061 cells and were converted to zeta potential values. Average zeta potential values of at least three batches were calculated and corrected for viscosity of the continuous phase (Umerska et al. 2012).

3.2.5. Residual acetone quantification

The residual acetone content in the continuous phase of ITR NP dispersions was determined by measuring ZP values of the 200 nm Nanosphere® standard (Malvern Instruments, UK) dispersed in acetone/water mixtures with varying acetone v/v ratios. A calibration curve (Figure 3.2) was constructed by plotting the ZP values of mixtures containing 10 µl the Nanosphere® standard dispersed in 500 µl of acetone/water mixtures against the acetone/water v/v ratio.

ITR dispersions prepared at the investigated ΔT_{SAS} were filtered using 0.1 µm filters (Sartorius Stedim, Germany). An aliquot of 500 µl of the filtrate was mixed with 10 µl of the Nanosphere® standard and the measured ZP values were used to calculate the acetone content using the calibration curve constructed earlier.

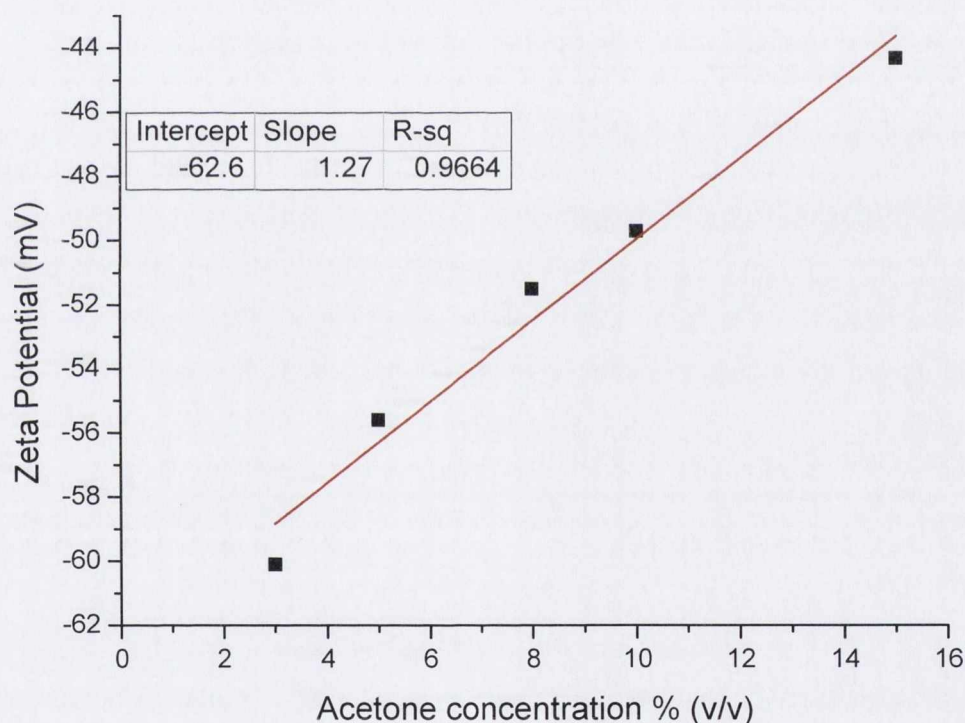


Figure 3.2. Calibration curve of zeta potential against acetone concentration.

3.2.6. NP morphology

3.2.6.1. Scanning electron microscopy (SEM)

A Zeiss Supra variable pressure field emission scanning electron microscope (Germany) equipped with a secondary electron detector and accelerating voltage of 5kV was used for the morphological examination of ITR nanoparticles. Aliquots of

the ITR nanodispersions were placed on a silicon chip 5*7 mm with (111) orientation, fixed on aluminum stubs and dried using nitrogen purge. For the samples collected after dynamic vapor sorption (DVS) experiments, powders were placed on carbon tabs fitted on aluminum stubs. All samples were sputter coated with gold-palladium under vacuum before analysis.

3.2.6.2. Transmission electron microscopy

Transmission electron microscopy (TEM) characterization of the NPs prepared using a different temperature of the antisolvent phase (0.5, 50 and 80 °C) was carried out to investigate the effect of ΔT_{SAS} on the thickness of shell of the NPs formed. TEM imaging was performed on a TEM Titan instrument (FEI Ltd, Hillsboro, OR). Centrifuged residues of ITR NPs were redispersed in water and mounted on Cu holey carbon-coated TEM grids and imaged at 300 kV.

3.2.7. Elucidation of the mechanism of NP formation

3.2.7.1. Viscosity measurements

A Vibro viscometer SV-10 (A&D Ltd., Japan) was used to measure the viscosity of deionized water and pure acetone at different temperatures employed in the precipitation studies. Each sample was subjected to a heating ramp to the required temperature using a thermostated water jacket followed by cooling to 0.5 °C. The average of the recorded viscosity values in the heating and cooling cycles at each temperature was used. The impact of the 1 mg/ml P407 and 1 mg/ml CAP on the viscosity of acetone was also studied using the same method. Similarly, a change in water viscosity at 80 °C upon the addition of different PAA concentrations was measured.

3.2.7.2. Visualisation of the Tyndall effect

The Tyndall effect, at the investigated temperatures, in a glass vial was examined before and after the injection of 0.1 ml acetone to 1 ml water as reported before (Wang et al. 2006a).

3.2.7.3. Investigation of micelle formation at 1 mg/ml P407

DLS was utilized to investigate if 1 mg/ml P407 is able to form micelles at the employed antisolvent temperatures. For this experiment, deionized water was ultrasonically degased for 10 min using a F15053 Fisherbrand sonicator (Elma, Germany). The degased water was used to prepare 1 mg/ml P407 solution, the solution was filtered using a 0.1 μ m membrane filter (Sartorius Stedim, Germany) and placed in a PCS8501 glass cuvette. The solution was heated *in situ* in a

Zetasizer Nano ZS series and size measurements were performed at 25, 50 and 80 °C.

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

WAXS measurements were performed over a 2θ range of 5-35° as described earlier in Chapter 2. Peak positioning and d-spacing measurements were performed using PDXL comprehensive analysis software (Rigaku, Japan). SAXS studies were performed using a Bruker D8 Discover with a monochromatic copper K α 1 source. Scanning with 666 s/point with effective 0.1 mm slits was applied (Arca et al. 2013). SAXS samples were analyzed by Dr. Karsten Fleischer, School of Physics and Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, Dublin 2, Ireland.

3.2.9. Differential scanning calorimetry (DSC)

A range of crystalline ITR/P407 physical mixtures prepared in different w/w ratios were examined by DSC as described in Chapter 2 and data were used to construct a calibration curve to quantify the P407 content in the NPs (Figure 3.3). For the purpose of P407 quantification, the samples were heated in an oven (Mettler U10, Germany) at 40 °C following dynamic vapor sorption analysis with ethanol to remove any remaining solvent. The dried samples were then subjected to DSC scans as described above and the enthalpy of P407 melting was used to quantify the P407 content.

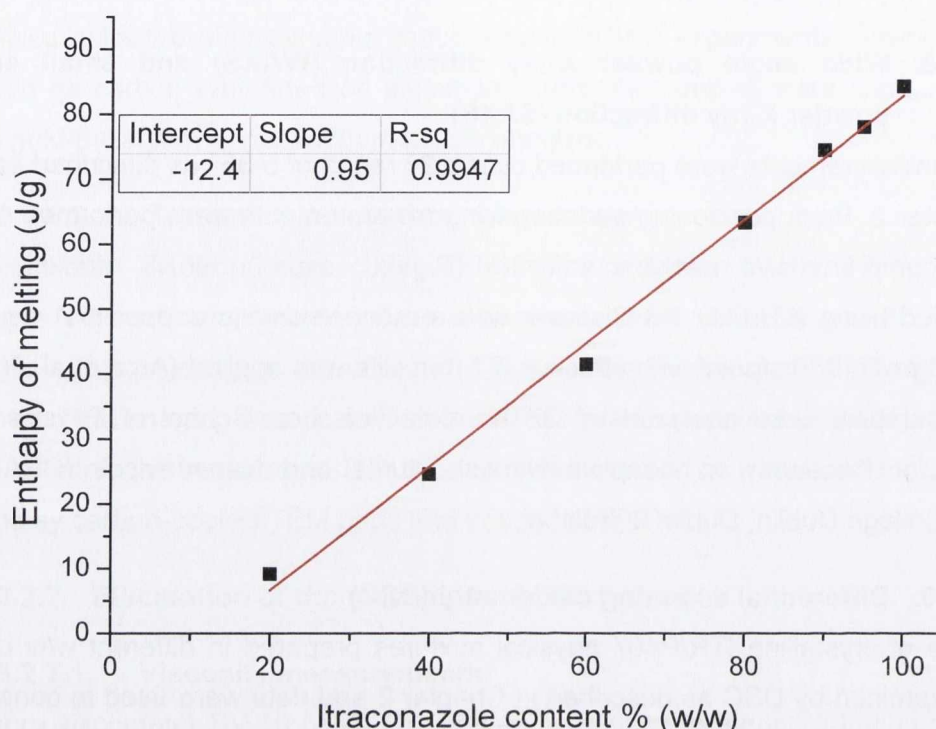


Figure 3.3. Calibration curve of enthalpy of melting of Poloxamer 407 against itraconazole content.

3.2.10. Attenuated total reflection Fourier transform infra-red spectroscopy (ATR-FTIR)

Infrared spectra were recorded on a PerkinElmer Spectrum One FT-IR Spectrometer and evaluated using Spectrum v5.0.1 software. Each spectrum was scanned in the range of 650–4000 cm^{-1} with a resolution of 4 cm^{-1} and minimum of four scans were collected and averaged in order to obtain good quality spectra. The spectra were normalized and background corrected using the following base points: 690, 909, 1307, 1596, 1780, 2507, 3029 and 4000 cm^{-1} . A second derivative, using the Savitzky–Golay algorithm with seven smoothing points, were calculated for each absorbance spectrum.

3.2.11. Determination of change in the degree of disorder by dynamic vapor sorption (DVS)

Freeze dried ITR NPs were subjected to DVS analysis as previously described in Chapter 2. Second sorption cycle isotherms were also determined where absence of a mass loss indicated full crystallization. The complete crystallization was further

confirmed using WAXS and DSC. The change in the degree of disorder in samples produced using different $\Delta T_{S:AS}$ compared to when $\Delta T_{S:AS} = 0$ °C was determined using Eqn. 3.1,

$$\% \text{ increase in disorder} = 100 \times \frac{\Delta m \times ms}{md} \times \frac{1}{\Delta m_{\Delta T=0}} \quad \text{Eqn. 3.1}$$

where Δm is the difference in the mass uptake (%) of the ITR NPs between the first and second sorption cycles at a system specific p/p_0 , ms is the sample mass in the DVS, md is the mass of the ITR in the overall sample mass, and $\Delta m_{\Delta T=0}$ is the difference in mass uptake between the first and second sorption cycles of the NPs precipitated when $\Delta T_{S:AS} = 0$ °C.

3.2.12. Statistical analysis

Statistical analyzes were performed via one way ANOVA with the Tukey comparison test, as specified in relevant sections, using Minitab Release 16. For all tests, $p \leq 0.05$ was used as the criterion to assess statistical significance.

3.2.13. Computational investigations

The full interaction map (FIM) for itraconazole (CSD refcode TEHZIP) was generated using Mercury 3.5.1 software. The carbonyl oxygen, C-Cl and aromatic C-H were used as the hydrogen bond (H-bond) acceptors, halogen bond and hydrophobic interactions probes, respectively. The propensities, a numerical representation of how much more likely an interaction is at a certain contour with respect to random chance (Wood et al. 2013), for the propped interactions along with the hotspots and short contacts were calculated.

3.3. Results and Discussion

3.3.1. Investigations into the formulation and process variables leading to ITR NPs

In an effort to bridge the gap between pharmaceutical nanotechnology and pharmaceutical liquid crystals, the impact of process parameter manipulation on the size and the mesophase of ITR NPs was probed. As a starting point, the effects of antisolvent temperature (T_{AS}) and polymer concentration in the solvent or antisolvent phase on the formation of NPs was investigated (Table 3.1) followed by solid state characterization and computational investigation. While an increase in ITR solubility with an increase in P407 concentration was observed, no complex formation between the two species in acetone was determined when the Higuchi and Connors relationship was applied (Higuchi and Connors 1965).

Higher concentrations of P407 in acetone (2, 3 and 4 mg/ml) resulted in the formation of NPs, however some of the produced NPs converted into more stable crystalline microparticles (Figure 3.4), thus in this work we have only focused on the 1 mg/ml P407 systems.

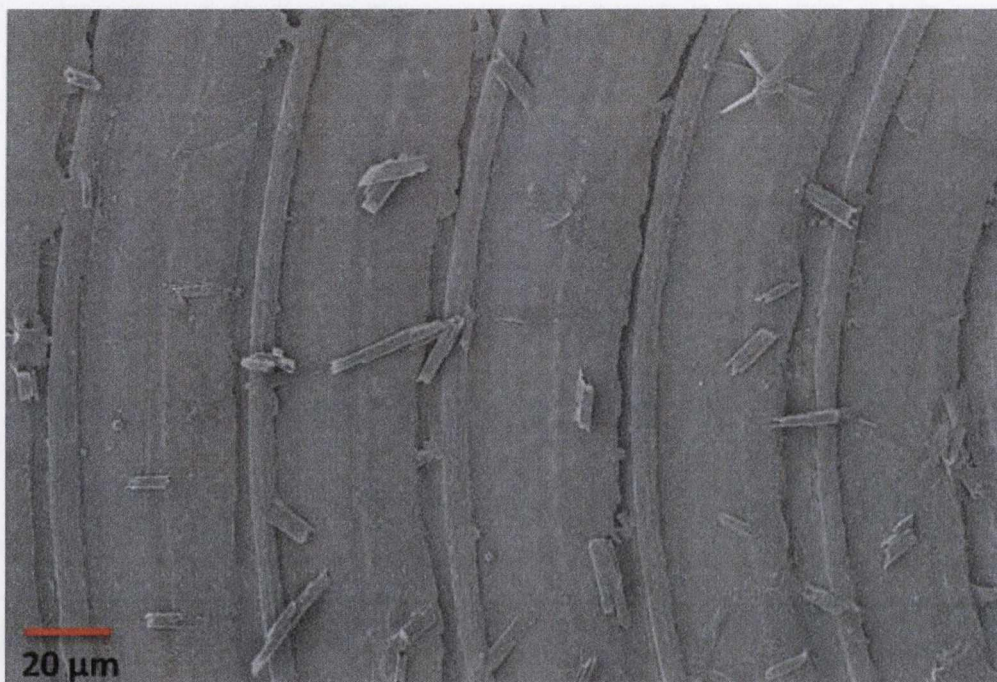


Figure 3.4. SE micrograph of itraconazole sample precipitated using 3 mg/ml Poloxamer 407 in the solvent phase.

SE micrographs (Figure 3.5) and hydrodynamic diameters (Figure 3.6) indicated that discrete NPs were formed for all formulations apart for F1, where aggregates with sizes of 521 ± 3 nm of particles smaller than 100 nm can be seen (Figure 3.5a).

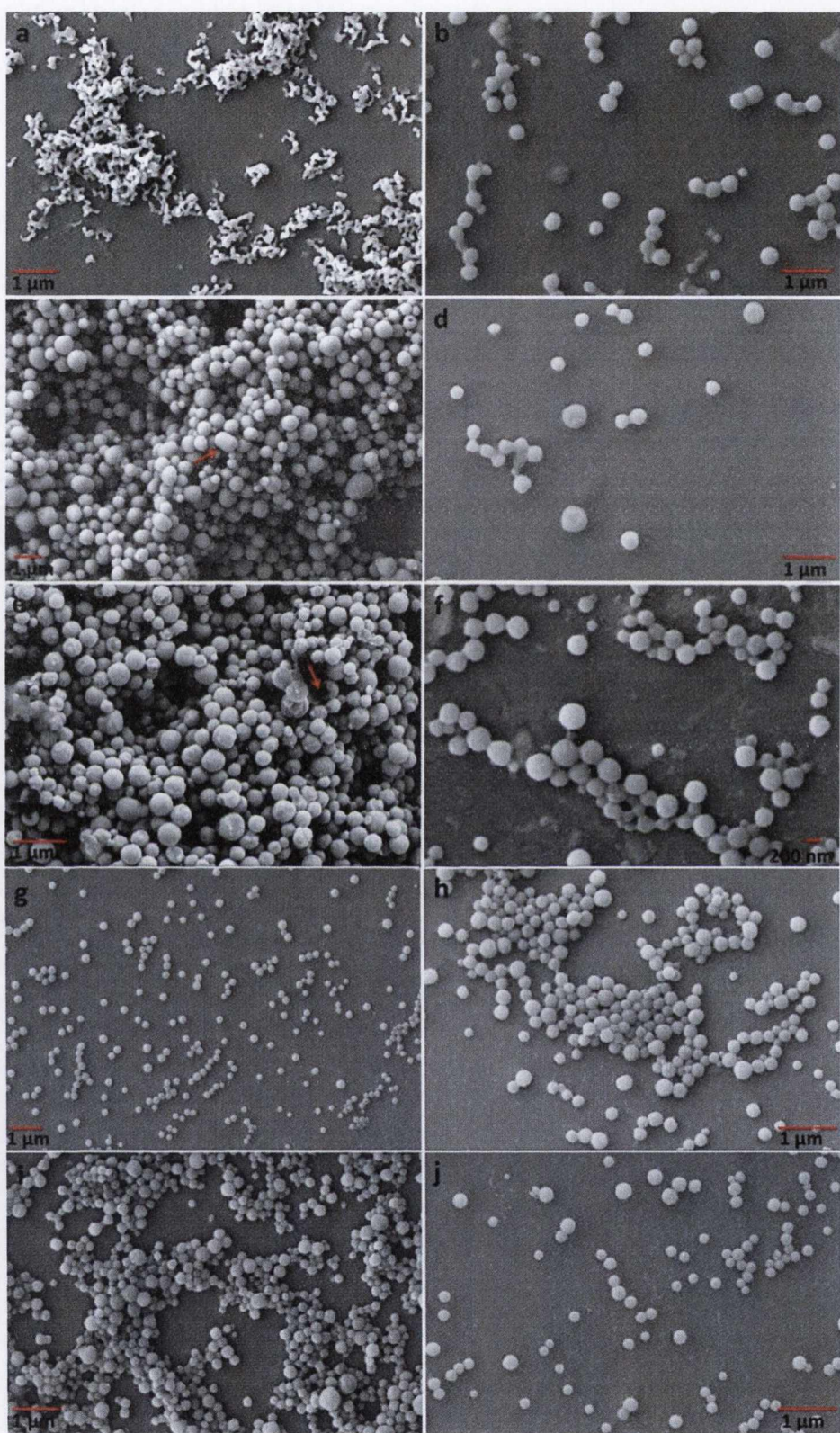


Figure 3.5. Scanning electron micrographs of (a) F1 nano-aggregates, (b) F2, (c) F3 with a deformed but non broken particle indicated by red arrow, (d) F4 (e) F5 showing hollow particle indicated by red arrow, (f) F6, (g) F7 with apparent homogenous size, (h) F8, (i) F11 and (j) F12 ITR NPs.

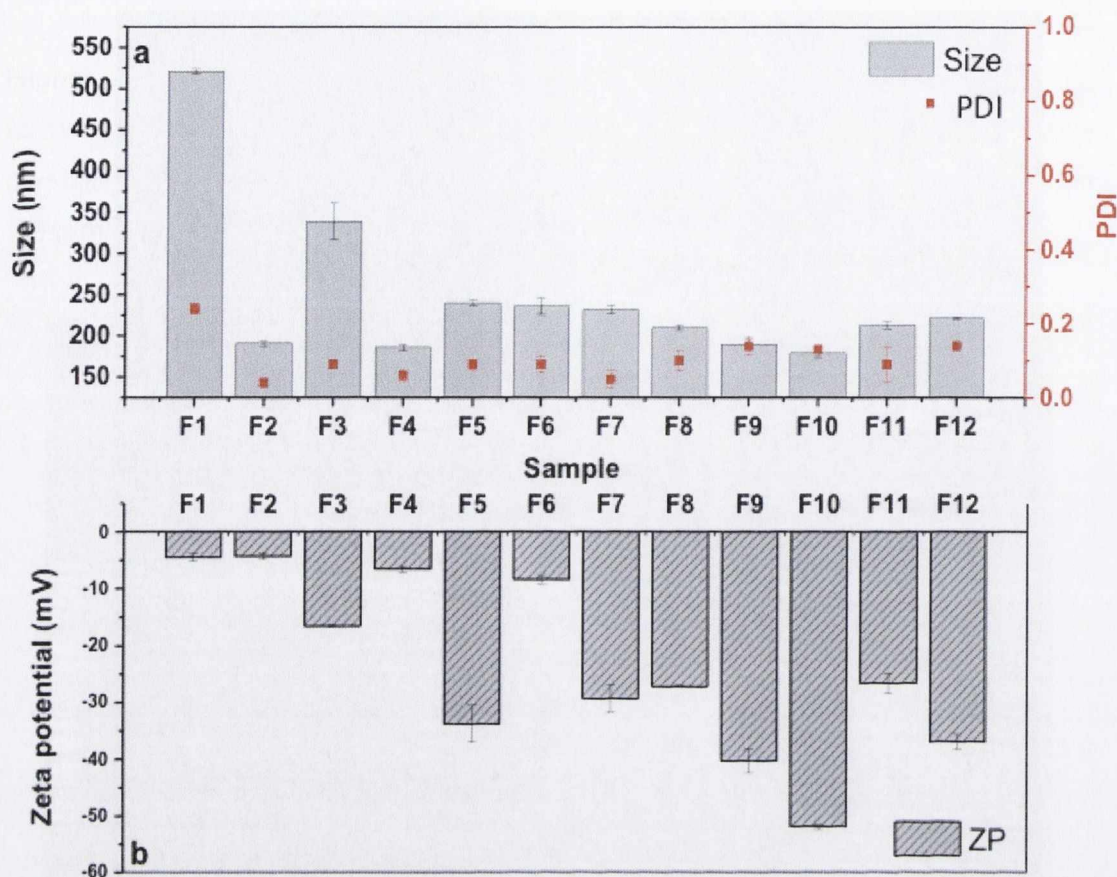


Figure 3.6. (a) Hydrodynamic particle size (grey bars) and polydispersity index (PDI, red squares) and (b) Zeta potential values (grey hashed bars) of samples F1 to F12.

Increasing viscosity of the antisolvent phase via the addition of PAA (F8) resulted in the production of smaller particles compared to F5 as can be inferred from the SEM micrographs (Figure 3.5h). The inclusion of 1 mg/ml CAP (F7), an ITR miscible polymer, in the solvent phase resulted in the formation of NPs with the same apparent morphology as their polymer free counterparts (F5). The low polydispersity index (PDI, Figure 3.6a) of those particles was confirmed by the high homogeneity of size as displayed in the SE micrograph (Figure 3.5g). Increasing viscosity of the antisolvent phase by the addition of 0.033 mg/ml PAA (system F12) resulted in the formation of smaller particles with greater polydispersity compared to F7. For P407, DLS investigation revealed that 1 mg/ml is below the critical micelle concentration (CMC) at the applied experimental conditions thus the micellar effect on NPs formation was ruled out.

The stabilizer-free NPs formed at 80 °C ($\Delta T_{SAS}=30$ °C, F5) did not fully redisperse in water following freeze drying. The relatively highly negative zeta potential (ZP) value

(-33.7 ± 3.2 mV, Figure 3.6b) indicates that NPs in this dispersion are electrostatically stabilized, which explains the poor redispersibility in water (Sun et al. 2011, Studart et al. 2007). In contrast, the surfactant nature of P407 suggests the presence of this polymer as a layer on the particle surface leading to a less negative ZP value for F6. This layer sterically stabilizes the NPs and shields their surface charge, which explains their good redispersibility in water (Vandervoort and Ludwig 2002). The ZP value of ITR NPs prepared when the AS temperature was $50\text{ }^{\circ}\text{C}$ ($\Delta T_{S:AS}=0\text{ }^{\circ}\text{C}$, F3) was -16.5 ± 0.6 mV, greater than -33.7 ± 3.2 mV of F5. The residual content of acetone in the AS phase was 6% v/v and this is expected to affect the solvation shell around the particles (Ketelson et al. 1996). The presence of acetone is believed to promote Ostwald ripening, where smaller particles dissolve and deposit on larger particles to achieve a more thermodynamically stable system (Voorhees 1985). Incorporation of P407 (F4) shifted the ZP to -6.5 ± 0.6 mV, when compared to F3. Despite the low negative charge on those particles, steric repulsion is expected to play a role in their stabilisation. For the sample fabricated using the AS phase at $0.5\text{ }^{\circ}\text{C}$ ($\Delta T_{S:AS}=49.5\text{ }^{\circ}\text{C}$, F1), the surface charge was very low with the acetone content of 9% v/v in the AS phase. It also can be noted that the ZP values with and without P407 (F1 and F2, respectively) were comparable. This can be attributed to the fact that without the inclusion of a stabilizer nanoaggregates, which are expected to possess lower surface charge (Li et al. 2008), were formed rather than discrete NPs.

The inclusion of PAA in the AS phase appeared to have a concentration-based effect on the particle size and ZP as can be seen in Figure 3.6b. The use of 0.033 mg/ml aqueous solution of PAA (F8) was found to result in a shift in ZP from -33.7 ± 3.2 mV in F5 to -27.2 ± 0.2 mV in F8, which was inconsistent with literature where PAA was found to render the surface of NPs more negative (Takeuchi et al. 2003).

A DSC scan of F8 (Figure 3.7) revealed an increase in the glass transition temperature (T_g) of ITR from 60 to $62\text{ }^{\circ}\text{C}$ using mid-point approach, which suggests the presence of considerable miscibility between ITR and PAA at the used concentration. Additionally, one of the endothermal (liquid crystalline) transitions (T_{LC1}) in ITR originally located at $74\text{ }^{\circ}\text{C}$, as described in Chapter 2, was almost invisible which indicates that the observed increase in T_g influenced the ability of ITR to undergo smectic A transition. This influence is most likely due to interaction of ITR with PAA owing to the acidic nature of the polymer and the presence of basic nitrogen atoms in ITR. The use of higher PAA concentrations (0.05 mg/ml in F9 and 0.1 mg/ml in F10) resulted in shifting the ZP towards more negative values

(-40.3 ± 2.2 and -51.9 ± 0.4 mV, respectively) compared to F8, which is an indication of concentration dependent miscibility of components in NPs.

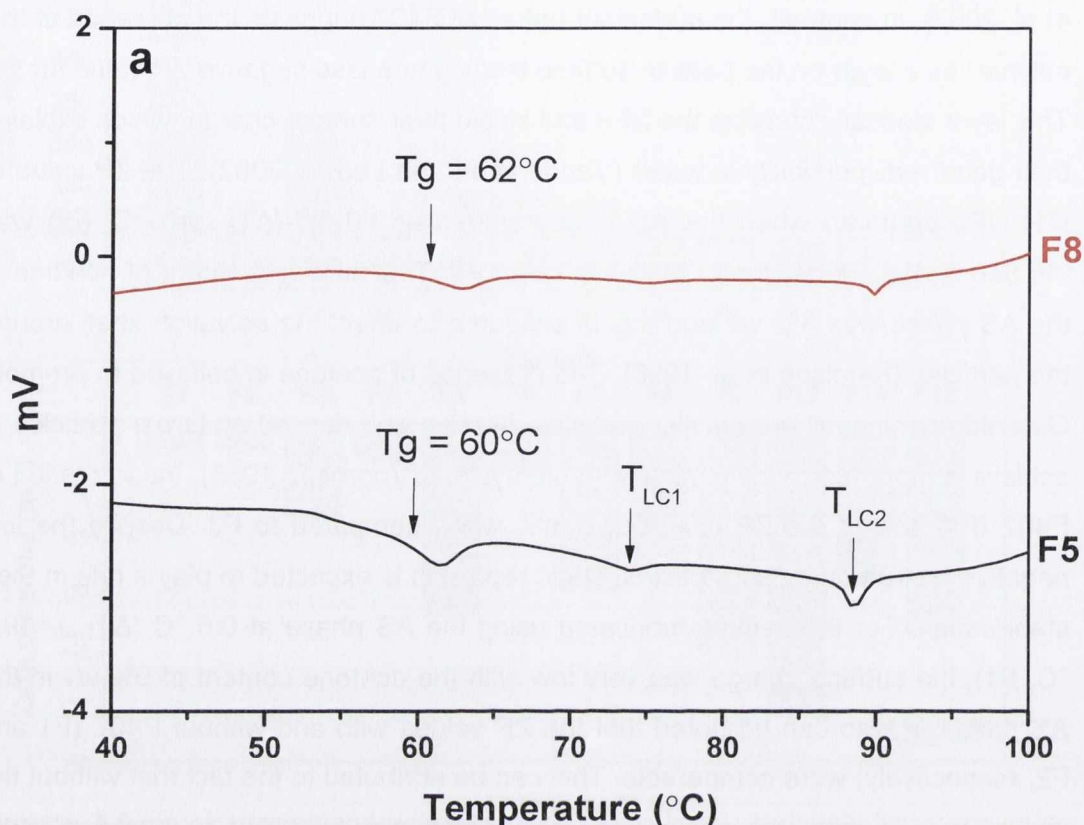


Figure 3.7. DSC thermograms of F5 and F8, T_g – glass transition, T_{LC1} and T_{LC2} – the first and second endothermic (liquid crystalline) transition, respectively. The T_g shifted from 60 to 62 °C upon the inclusion of 0.033 mg/ml PAA.

To further investigate the correlation between the PAA concentration in the AS phase and ZP, three additional dispersions made using the following concentration of PAA in water were examined, 0.025, 0.042 and 0.15 mg/ml, which had the following ZP values: -30.9 ± 0.2 , -34 ± 0.1 and -67.7 ± 2.76 mV, respectively. Figure 3.8 shows the outliers, at low PAA concentrations, for which deviations of ZP values were determined. The inclusion of CAP, a polymer that is miscible with ITR, had a similar effect on ZP to the low PAA concentration (F8).

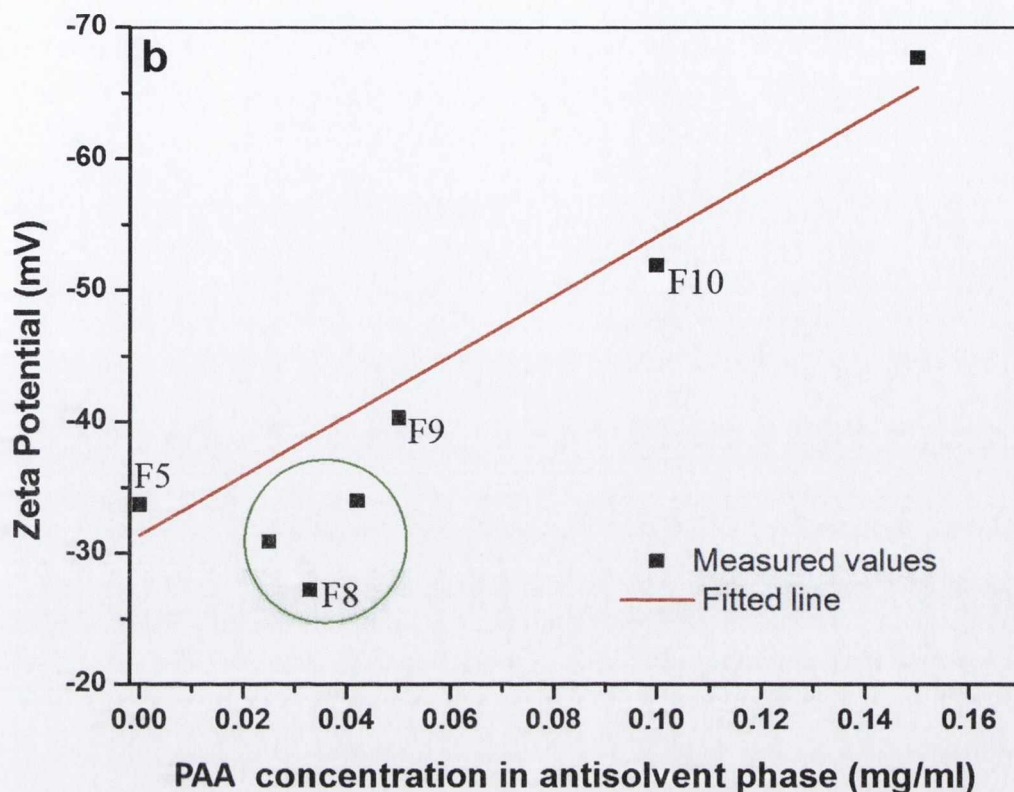


Figure 3.8. Zeta potential values of ITR NPs as a function of PAA concentration in the AS phase with the outliers enclosed in the green circle.

NPs obtained using a combination of 1 mg/ml P407 in the solvent phase and 0.033 mg/ml PAA in the AS phase (F11) had a comparable ZP value to F8 NPs. In contrast, when CAP was combined with 0.033 mg/ml PAA (F12), more negatively charged ITR NPs, compared to F8, were produced, which could be due to the favorable interaction of ITR with CAP where the cellulose-based backbone (DiNunzio et al. 2008) shields ITR from interaction with PAA, which in turn contributes to the shift of ZP towards more negative values.

3.3.2. Internal structure of ITR NPs

Figure 3.9 shows the TE micrographs of resultant NPs using different AS temperature. It is clear that the investigated particles were hollow. The effect of $\Delta T_{S:AS}$ on the shell diameter is illustrated with the thickest crust of about 120 nm achieved when $\Delta T_{S:AS}=0\text{ }^{\circ}\text{C}$ (F3, Figure 3.9b). With large $\Delta T_{S:AS}$, the shell thickness decreases significantly, where $\Delta T_{S:AS}=30\text{ }^{\circ}\text{C}$ was associated with around 30 nm NP shell with a blowhole (indicated by the red arrow in Figure 3.9c, which is consistent

with SE micrograph in Figure 3.5e). The $\Delta T_{S,AS}=49.5\text{ }^{\circ}\text{C}$ (F1) resulted in the formation of smaller particles/aggregates with the shell thickness of around 15 nm as shown in Figure 3.9a presenting a particle with a blowhole (red arrow).

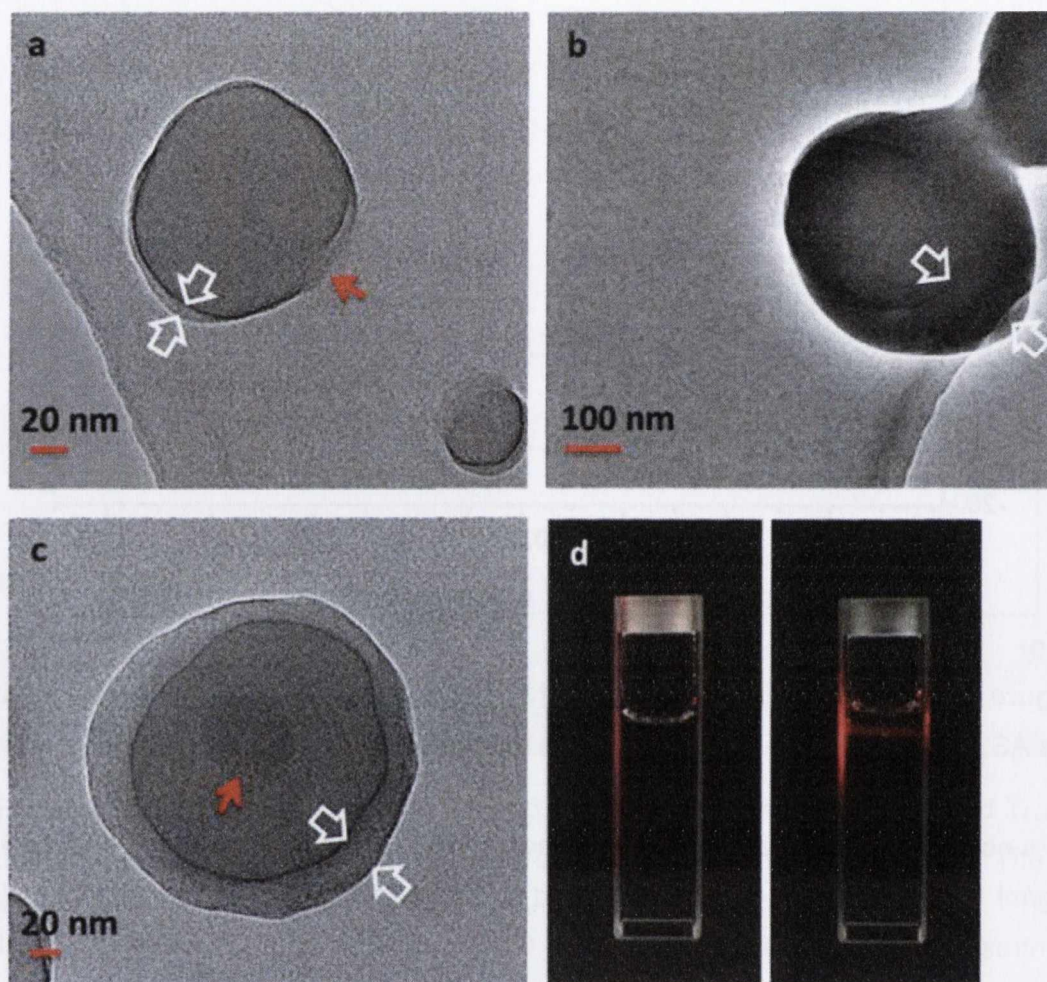


Figure 3.9. TEM images of a NP in (a) F1 with 15 nm thick shell and a blowhole (red arrow), (b) F3 showing the thickest shell of 120 nm and (c) F5 with a blowhole (red arrow) and 30 nm thick shell, and (d) Laser light scattering in pure water (left) and acetone:water 1:10 v/v mixture (right), 30 seconds after the addition of acetone to pure water. The Tyndall effect is clearly visible as the red pathway of laser light in the liquid.

3.3.3. Mechanism of NP formation

Quasi emulsion solvent diffusion (QESD) precipitation using the acetone-water system has been used in the past for the spherical crystallization of drug substances to improve their performance during formulation processes (Kachrimanis et al. 1998, Cui et al. 2003, Nocent et al. 2001, Re et al. 1999, Yang et al. 2003). Nevertheless, to the best of found knowledge, NPs formation using QESD has not been reported

before. The hypothesis is that ITR NPs are formed via solidification of droplets through counter-diffusion of solvent and antisolvent following the break up stage, which is confirmed by the hollow structure of the particles (Figure 3.9). The breakup of those droplets is controlled by deformation, which can be described using the Taylor's equation (Karam 1968):

$$D = \frac{Ga\mu}{\gamma} \left[\frac{19\rho+16}{16\rho+16} \right] \quad \text{Eqn.3.2}$$

where D - deformation of drop, G - shear rate, a - radius of liquid droplet, μ - viscosity of the continuous phase, ρ - viscosity ratio (μ'/μ) where μ' is viscosity of the solvent (dispersed) phase and γ - interfacial tension. The higher the D value, the easier the droplet breaks up.

To investigate the applicability of this mechanism to the systems at the nanoscale, a viscosity ratio between the solvent to antisolvent (ρ) was determined for each condition investigated (Table 3.2). For water at 0.5 °C, the ρ is 1:7. This ratio changed with an increase in the antisolvent phase temperature to 1:2 and 1:1.4 for the AS phase at 50 and 80 °C, respectively. Statistical analysis (ANOVA, $p \leq 0.05$) revealed that the viscosity values of pure acetone and the 1 mg/ml P407 solution in acetone were not significantly different. Therefore, the change in ρ upon the addition of 1 mg/ml P407 to acetone can be considered negligible. The addition of PAA to water at 80 °C was found to decrease ρ , compared to F5, to 1:1.7, 1:2 and 1:2.5 for 0.033, 0.05 and 0.1 mg/ml PAA, respectively. The Tyndall effect was observed in all systems for which the viscosity ratios were calculated (an example is shown in Figure 3.9d) confirming the generation of liquid segregation in the range of viscosity ratios (ρ) 1:7 to 1:1.4. These values are in agreement with those suggested by Karam et al. for a series of silicone fluids and PEGs (Karam 1968), however are inconsistent with those reported by Wang et al. for some polymorphic pharmaceuticals (Wang et al. 2006a, Wang and Kirwan 2006b). This discrepancy shows that the viscosity ratios necessary to form a quasi-emulsion are system specific and as described by Taylor's equation (eqn 3.2), droplet deformation occurs due to the interactive effect of various parameters including interfacial tension, shear rate and droplet radius.

Table 3.2. Summary of calculated solvent:antisolvent (S:AS) viscosity ratios of various conditions. T_S - temperature of solvent, T_{AS} - temperature of antisolvent

Solvent phase	Antisolvent phase	T_S	T_{AS}	S:AS viscosity ratio
Acetone	Water	50	0.5	1:7
Acetone	Water	50	50	1:2
Acetone	Water	50	80	1:1.4
1 mg/ml P407 in acetone	Water	50	0.5	1:7
1 mg/ml P407 in acetone	Water	50	50	1:2
1 mg/ml P407 in acetone	Water	50	80	1:1.4
Acetone	0.025 mg/ml PPA in water	50	80	1:1.6
Acetone	0.033 mg/ml PPA in water	50	80	1:1.7
Acetone	0.042 mg/ml PPA in water	50	80	1:1.8
Acetone	0.05 mg/ml PPA in water	50	80	1:2
Acetone	0.1 mg/ml PPA in water	50	80	1:2.5
Acetone	0.15 mg/ml PPA in water	50	80	1:2.9
1 mg/ml CAP in acetone	Water	50	80	1:1.4
1 mg/ml CAP in acetone	0.033 mg/ml PPA in water	50	80	1:1.7
1 mg/ml P407 in acetone	0.033 mg/ml PPA in water	50	80	1:1.7

Increasing viscosity of the antisolvent phase due to the addition of PAA (F8) resulted in the production of smaller particles compared to F5, which obeys the Taylor's equation. Furthermore, DLS data shows that the higher the concentration of PAA, the smaller the particle size of the precipitated NPs. Meanwhile, the higher the PAA concentration, the greater the PDI values (Figure 3.6a), which is also demonstrated in the SE micrographs. The polydispersed NPs can be explained by the non-uniform breakage of some droplets into smaller entities due to the relatively high viscosity ratio as reported for oil droplets before (Karam 1968), which results in a range of sizes formed. A combination of the high viscosity effect of PAA with the surfactant effect of P407 at 80 °C (Figure 3.5i, sample F11) did not result in any statistically significant impact on the particle size nor PDI (size = 213 ± 4 nm and PDI = 0.09 ± 0.05) when compared to F8.

Collectively, DLS, SEM and TEM data indicated a $\Delta T_{S:AS}$ dependent particle size of NPs with and without P407. Without the surfactant, an increase in the particle size with a decrease in $\Delta T_{S:AS}$ was noticed, where the largest particles (338 ± 21 nm by DLS) were obtained for $\Delta T_{S:AS} = 0$ °C. Temperature was reported to have no effect on

size of particles in the microscale (Yang et al. 2003), while it does herein by affecting the viscosity ratio. According to Taylor's equation (Eq. 3.2), during the precipitation of F3 ($T_{AS}=50\text{ }^{\circ}\text{C}$) without the addition of P407, interfacial tension is expected to be higher than that when P407 was employed, which results in a low D value and thus the formation of large droplets. Those large droplets cannot be further broken easily through deformation into smaller drops. Additionally, if deformation had occurred particles could have started the solidification process as it can clearly be seen in the SE micrograph (red arrow in Figure 3.5c) showing deformed but non-broken, solidified, peanut-shaped particles. That is to say, the interfacial tension effect dominates the viscosity ratio effect. In contrast, when a surface active agent such as P407 was included in the solvent phase (F4), smaller particles were observed (Figure 3.5d) even though the polymer has a negligible effect on viscosity. The ease of droplets breakage by lowering the interfacial tension was reported before (Karam 1968). Decreasing the interfacial tension facilitates the breakage of droplets into smaller globules with the aid of the high viscosity of the antisolvent phase at $0.5\text{ }^{\circ}\text{C}$ in F2 compared to F1.

The shell thickness of the formed particles and the presence of particle rupture is determined by $\Delta T_{S:AS}$. Heat transfer is reported to be semi-instantaneous, while mass transfer is the limiting step as it continues for longer following the heat transfer (Espitalier et al. 1997) and this leads to the continuation of mass transfer until a thicker crust is formed for the sample made at $\Delta T_{S:AS}=0$ (F3). The effect of $\Delta T_{S:AS}$ is accompanied by the difference in gas solubility in water at different temperatures (Wiebe and Gaddy 1940), a parameter disregarded before for the microspheres (Yang et al. 2003, Espitalier et al. 1997). When $\Delta T_{S:AS}=49.5\text{ }^{\circ}\text{C}$, the addition of the hot solvent to water or aqueous polymer solution results in gas bubbles generation as the net temperature of the mixture increases and consequently gives a rise to particle rupture, observed as blowholes, and terminates the solvent diffusion process. A similar scenario is expected when $\Delta T_{S:AS}=30\text{ }^{\circ}\text{C}$, except that the evaporated solvent is responsible for the gas bubbles.

The production of ITR NPs at high temperatures using P407 was not feasible when an ordinary supersaturation-based nucleation process was employed using evaporative precipitation into aqueous solution (EPAS) (Matteucci et al. 2006), where an elliptical conical nozzle was used to atomize an organic phase containing ITR and P407 into an aqueous phase at high temperature (Chen et al. 2004). According to the authors, in the EPAS process, as the organic solvent evaporates, the formed nuclei are forced to coagulate despite the presence of P407 (Matteucci

et al. 2006). In contrast, here, each nanodroplet solidifies as an individual nanoparticle (Figures 3.5f and 3.10), which then remains dispersed and stabilized by the polymer. Micellar interference is another possible explanation of the failure of the EPAS process to form ITR NPs, while the P407 concentration used herein was below the CMC.



Figure 3.10. SE micrograph of freeze dried ITR NPs precipitated using 1 mg/ml Poloxamer 407 in the solvent phase at 80°C.

3.3.4. Evaluation of the phase of matter of ITR NPs

Evaluation of the phase of matter of ITR NPs was accomplished using a group of techniques including wide and small angle X-ray scattering (WAXS and SAXS), as shown in Figure 3.11, and Fourier transform infra-red spectroscopy (FTIR) (Figure 3.14).

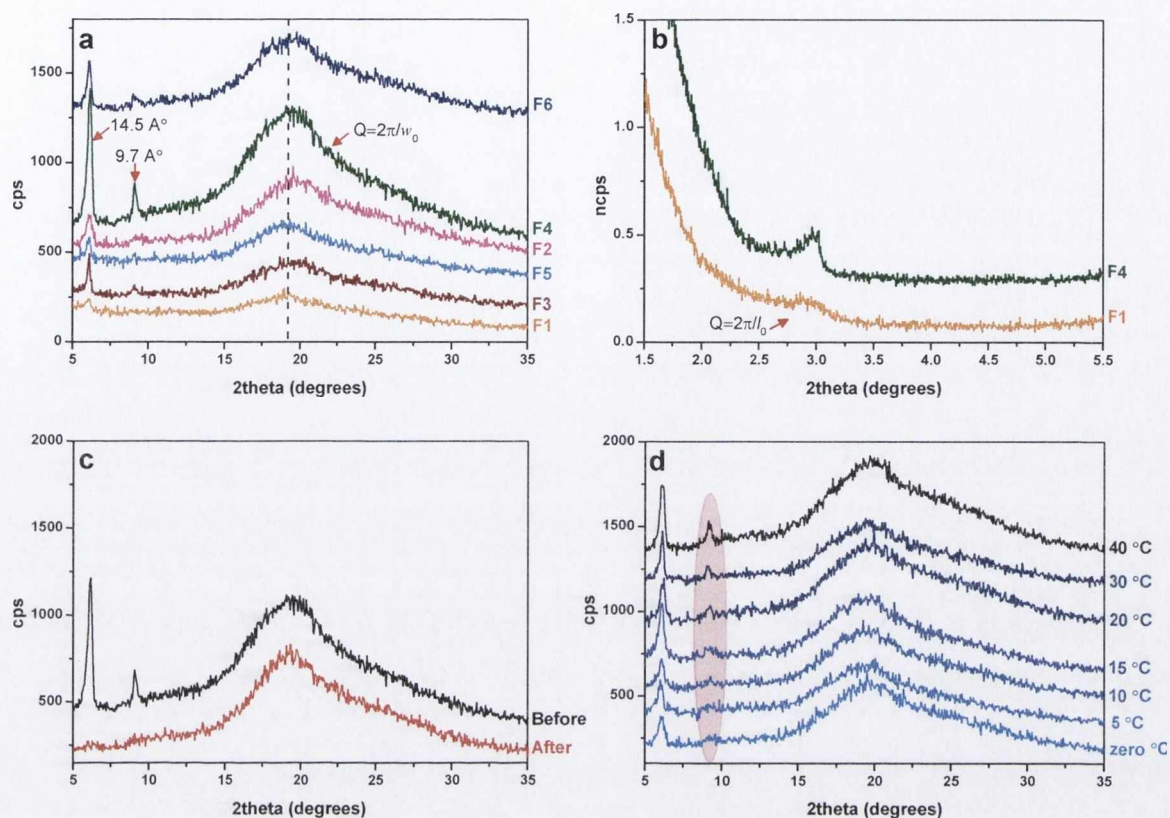


Figure 3.11. (a) WAXS diffractograms of F1 to F6 illustrating the highest peak intensities in case of F4, (b) SAXS diffractograms of the diffuse maxima at 3° 2θ in F1 and the increase in peak intensity in F4, (c) WAXS diffractograms of F4 before and after DSC scan from 25 to 65°C with both peaks disappeared following the DSC scan, (d) WAXS patterns of ITR NPs precipitated at a series of T_{AS} values zero, 5, 10, 15, 20, 30 and 40°C highlighting the increase in peak intensity (red background). cps=count per second and ncps=normalized count per second.

A previous X-ray diffraction study of bulk ITR LC phase using a $\text{CuK}\alpha 1$ source ($\lambda=1.5406\text{ \AA}$) revealed the presence of two diffuse maxima at around 7° and 18° 2θ at 82°C which was assigned for a chiral nematic phase (Six et al. 2001). Another interesting study using $\text{AgK}\alpha$ source ($\lambda=0.5608\text{ \AA}$) revealed the presence of a diffuse maxima at the low angle region, above and below the LC transition at 89°C , suggesting the presence of an additional ordering in the sample (Tarnacka et al. 2013). The latter cannot be compared with the diffraction patterns presented in this work as the X-ray source used has a different wavelength to the one used in this study. Moreover, the exact position of that maximum was not specified in the paper.

WAXS patterns of ITR nanoparticles F1-F6, Figure 3.11a, showed the presence of a characteristic liquid crystal peak at 6.05° 2θ , as described before in Chapter 2, and the absence of characteristic diffraction peaks of the crystalline starting materials, neither ITR nor P407 (Figure 3.12). The quasi diffraction peak at 6.05° 2θ corresponds to 14.5 Å interplanar d-spacing and indicates the presence of periodicity or a translational order (Zeng et al. 2004, Domenici 2007) since orientational order cannot be investigated using X-ray diffraction (Demus et al. 1998). In addition to this peak, a second quasi-Bragg peak was observed at 9.05° 2θ in F3, F4 and F6. This peak had the highest intensity when P407 was included in the solvent phase and precipitation performed at 50°C (F4). The 9.05° 2θ value corresponds to 9.7 Å d-spacing and suggests the presence of additional positional order in the particles precipitated at 50°C compared to other samples. The occurrence of this peak in ITR, to the best of found knowledge, has not been reported before.

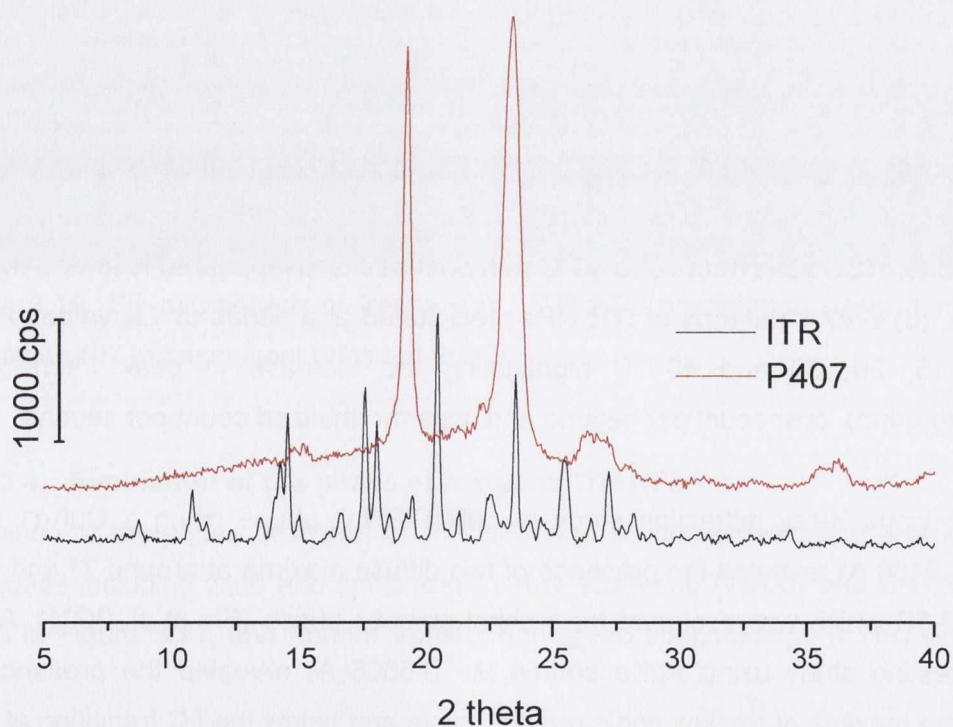


Figure 3.12. Powder X-ray diffractograms of itraconazole (ITR) starting material powder and Poloxamer 407 (P407) starting material powder.

The diffuse maxima in the wide angle position, in the same position described by Six et al. (2001), were similar in all samples and were in close agreement with the theoretical nematic phase maxima positions. The theoretical position (17° 2θ) was

calculated using the formula: $\sin\theta = \frac{Q\lambda}{4\pi}$ where Q is the diffraction vector and for the nematic and smectic phases is estimated to be around $\frac{2\pi}{w_o}$, where w_o is the molecule width. A low angle diffuse peak around 3° 2θ , corresponding to Q of $\frac{2\pi}{l_o}$, where l_o is the molecule length, was observed in F1 and is characteristic of a nematic phase (Figure 3.11b). The length and width of ITR molecule are 2.81 and 0.54 nm, respectively as calculated using Mercury[®] 3.5.1 software (from the crystal structure, CSD code: TEHZIP) which is typical for nematic mesogens (Demus et al. 1998).

In order to assess whether the peak at 6.05° 2θ is due to the presence of crystalline ITR in the particles or only due to some order in the liquid crystal phase, sample F4 was heated in the DSC furnace to 65°C , just above the T_g of ITR, and then characterized using WAXS. It was found that the peaks at 6.05° and 9.05° 2θ disappeared (Figure 3.11c) excluding the presence of crystalline ITR and suggesting hkl quasi-planes of 002 and 003, respectively. F4 WAXS pattern, with two pseudo-Brag peaks is typical for one dimensional smectic (Sm) stacking in the thick crust (Tanaka et al. 2012, Demus et al. 1998). In addition, the SAXS scan (Figure 3.11b) illustrates the sharpening of the 3° 2θ peak (hkl 001) with interplanar spacing of 28 Å in F4. This equally spaced X-ray diffraction pattern, with Q spacing of $2\pi/d$, is typical of SmA_1 arrangement. The intensity of the 001 quasi-Bragg peak in a perfectly ordered SmA phase is higher than that of the 002 peak, which is not the case here. In contrast, since the highest intensity peak corresponds to layer periodicity close to half the molecule length, intercalated $\text{SmA}_{1/2}$ is more likely to exist, facilitated by the curvature strain in the three dimensional spherical shape.

Meanwhile, the hardly visible peak at 6.05° 2θ in the F1 diffraction patterns excludes the presence of any layered structure and suggests some lamellar order or localized cybotactic, smectic-like, clusters in the nematic mesophase (Demus et al. 1998, Mcmillan 1972). The wide angle diffuse peak of the smectic phase was similar to that observed from the nematic phase, indicating that the lateral packing of the molecule remains liquid-like (Demus et al. 1998). The existence of the nematic and smectic phases at 25°C was surprising and has not been reported before. This unusual behavior can be attributed to the phase confinement in the shell of nanospheres, where reorientation is not feasible at room temperature.

To further assess the effect of $\Delta T_{\text{S,AS}}$ on the intensity of the peak at 9.05° 2θ , series of experiments using a range of $\Delta T_{\text{S,AS}}$ values, 10, 20, 30, 35, 40 and 45°C , were carried out and NPs were examined using WAXS (Figure 3.11d). In all experiments,

the solvent phase temperature was kept at 50 °C, while the temperature of the antisolvent phase was varied. The diffractograms of these systems revealed that the 9.05° 2θ peak intensity increased gradually with a decrease in $\Delta T_{S:AS}$ indicating an increase in order.

Espitalier et al. (1997) reported full crystallization inside ketoprofen quasi-emulsion micro-droplet, which was not observed in ITR NPs with and without polymers in all conditions investigated. This difference can be attributed to the effect of the droplet size (droplet in the nanometer-range in this work compared to micrometer-ranged ketoprofen droplets in their work) on the solidification process since nucleation has been reported to be more difficult in smaller drops (Davey et al. 1995). Kaminska et al. (2014) asserted ITR inability to form nematic and smectic phases in binary systems which was found to be possible in this study. ITR weight fraction used in their study was 0.833 while it was 0.95 herein which could be an indication that the formation of LC phases in the binary mixtures is concentration dependent.

A further insight into the changes at the molecular level that accompanied the liquid crystalline assembly can be acquired from the full interaction map (FIM), generated based on crystallographic data and infrared spectroscopy (Figure 3.13 and 3.14). As displayed in Figure 3.1a, there are no conventional hydrogen bond donating groups in the molecule but a number of available acceptor groups. The FIM of molecule (Figure 3.13) illustrates the preferred strength and directionality of interactions of the potential functional group and short contacts. The lack of conventional H-donor groups in the adjacent molecule, to satisfy the H-donor-H-acceptor interaction, suggests that H-bonding is not involved in the formation of either the crystalline or the liquid crystalline forms of ITR. In contrast, the presence of the $-CH\cdots\pi$ contact and the $\pi\cdots\pi$ stacking, which fit the hydrophobic preference contours in the map, is suggested to stabilize the crystalline form and, most likely, the liquid crystalline form. The blue "hotspots" (Figure 3.12) indicate the highest propensity for interaction of the C-Cl group with the nucleophilic nitrogen of the triazole ring (13.16) and with the carbonyl oxygen (10.94). Nevertheless, the latter hotspot does not satisfy the preferences for interaction of the nucleophilic carbonyl oxygen in the crystal structure and could be preferred in the intercalated smectic A assembly since it is restricted geometrically in the shell of a nanosphere. It should be also highlighted that the presence of weak halogen bond contacts ($C-Cl\cdots O$, between dichlorophenyl and dioxolane groups in a dimeric arrangement) was acknowledged for the first time by Lahtinen et al. (2013). FTIR investigations were focused in the following bands/regions: carbonyl $C=O$ stretching at around 1700 cm^{-1} , asymmetric

and symmetric O-C-O stretching at around 1230 and 1147 cm^{-1} , respectively, a 960-1080 cm^{-1} region comprising =C-H out of plane bending and C-N stretching vibrations as well as a 850-920 cm^{-1} region of triazole ring deformations and triazole =C-H out of plane bending absorptions (Cyr et al. 1996). Considering the various NP formulations, the greatest differences (Figure 3.12), however only in the triazole, mixed aromatic, C-N and C=O regions, were seen for the sample F4 ($T_{AS}=50\text{ }^{\circ}\text{C}$, with P407) with band shapes resembling those of crystalline ITR. Thus it is logical to assume that this sample had a higher degree of order compared with the rest of NPs, consistent with WAXS data (Figure 3.10a). Infrared data implies that the part of ITR molecule with the greatest mobility is that containing the triazolone ring.

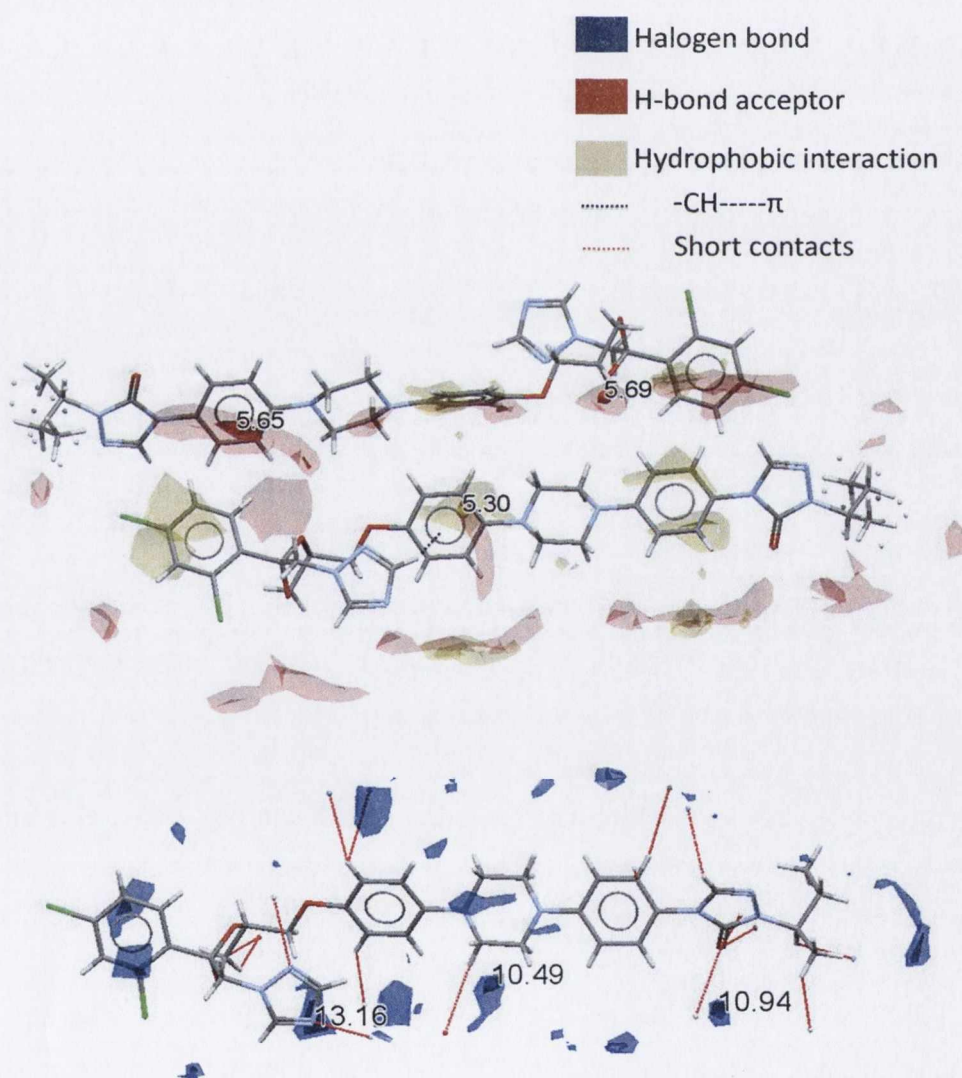


Figure 3.12. Full interaction map shown around a single ITR molecule in the asymmetric crystal unit (top) and isolated ITR molecule (bottom) with the numerical estimation of propensity for interaction.

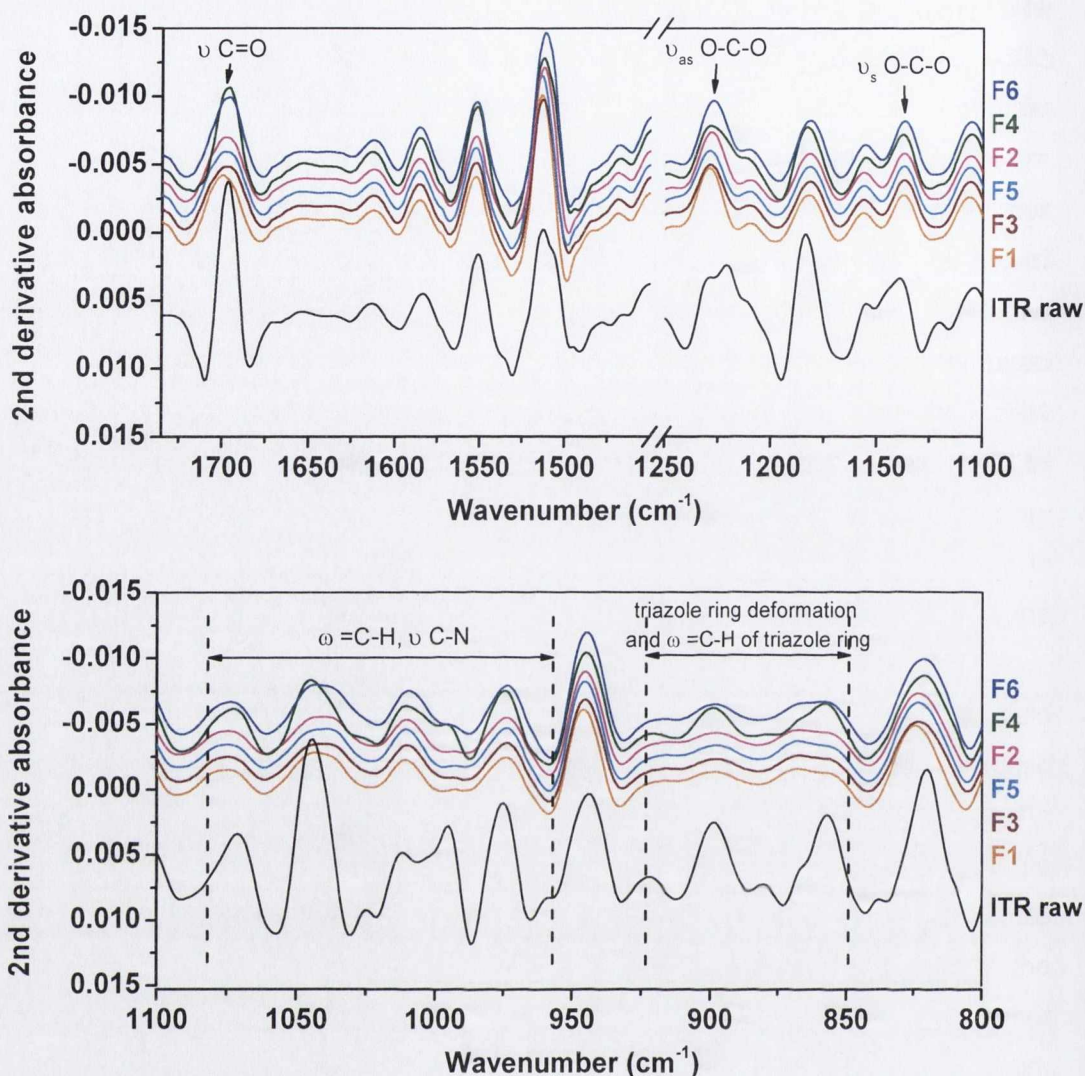


Figure 3.13. FTIR analysis (as 2nd derivative of absorbance) of ITR starting material powder (ITR raw) and F1 to F6. ω - out of plane bending, ν - stretching, ν_{as} - asymmetric stretching and ν_s -symmetric stretching.

The sorption-desorption isotherms of the investigated samples using ethanol as a probe solvent, shown in Figure 3.14, was used to confirm the subtle change in molecular order as a response to ΔT_{SAS} alteration. Pure drug NPs (F1, F3 and F5) were crystallizing at 70% partial pressure (p/p_0) of ethanolic vapor, while those stabilized with P407 (F2, F4 and F6) were found to crystallize at 50% p/p_0 . The enthalpies of melting obtained from the DSC analysis (Figure 3.3), suggest that the change in the P407 content with antisolvent temperature was not statistically significant, $6.76 \pm 1.15\%$ at $T_{AS}=0.5$ °C (F2), 6.43 ± 0.37 (F4) at $T_{AS}=50$ °C, and 5.19 ± 0.99 (F6) $T_{AS}=80$ °C and indicates a relatively high drug loading in these

samples. Crystallization of ITR NPs at lower p/p_0 of ethanolic vapor when a hydrophilic polymer is included has been reported by us in Chapter 2.

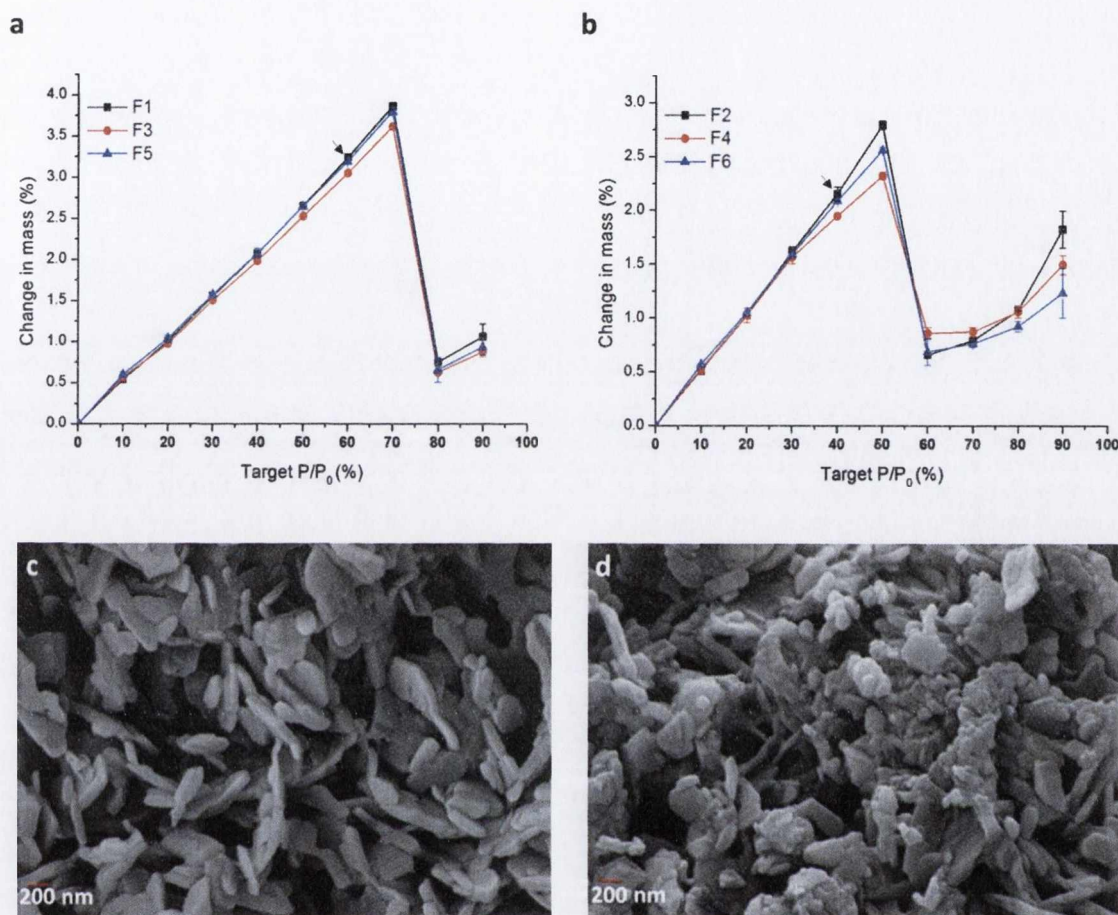


Figure 3.15. DVS sorption-desorption isotherm of (a) stabilizer-free ITR NPs, (b) P407 stabilized ITR NPs, sample codes according to Table 3.1. Experiments were carried out at 25 °C using ethanol as the probe solvent. Arrows indicate the system specific p/p_0 at which the change in mass was used for calculations using Eq.3.1. Scanning electron micrographs of (c) F3 and (d) F4 post-DVS analysis.

Here, crystallization of NPs at those low p/p_0 values is not only due to the hydrophilic nature of the polymer included, but also due to its known effect of inducing crystallization of ITR via increasing molecular mobility (Matteucci et al. 2008). The change in the degree of disorder of NPs with the change in $\Delta T_{S:AS}$ compared to $\Delta T_{S:AS}=0$ °C has been calculated using Eqn 3.1. In case of pure drug NPs, there was a statistically significant increase in the degree of disorder as the $\Delta T_{S:AS}$ increased, 5.39 ± 0.6 and $2.22 \pm 0.9\%$ for $\Delta T_{S:AS}=49.5$ and 30 °C, respectively, compared to $\Delta T_{S:AS}=0$ °C. The maximum disorder was observed when

$\Delta T_{S,AS}=49.5$ °C. This increase in the degree of disorder with larger $\Delta T_{S,AS}$ was higher when P407 was included (18.6 ± 0.3 and $13.5\pm7\%$ for $\Delta T_{S,AS}=49.5$ and 30 °C, respectively), which is consistent with the WAXS results. SEM investigations of post DVS powder (Figure 3.15c and d) revealed that NPs crystallized individually to form nanocrystalline aggregates, which had the same diffraction peaks as the starting material when examined using WAXS.

DVS moisture isotherm of a full cycle of sorption-desorption of the nematic (F1) and smectic (F3) are displayed in Figure 3.16. The isotherms revealed that, at 90% RH, the nematic LC NPs sorbed slightly more water ($2.49\pm0.03\%$) compared to their smectic counterparts ($2.38\pm0.01\%$) which indicate higher permeability of the nematic NPs. Hysteresis was observed in both isotherms, where the water content at each relative humidity point is higher in the desorption than sorption as reported in Chapter 2. The absence of a sharp mass loss in both isotherms, similar to those observed in Figure 3.16 excludes the presence of any crystallization and indicate that the LC NPs are stable under the investigated conditions.

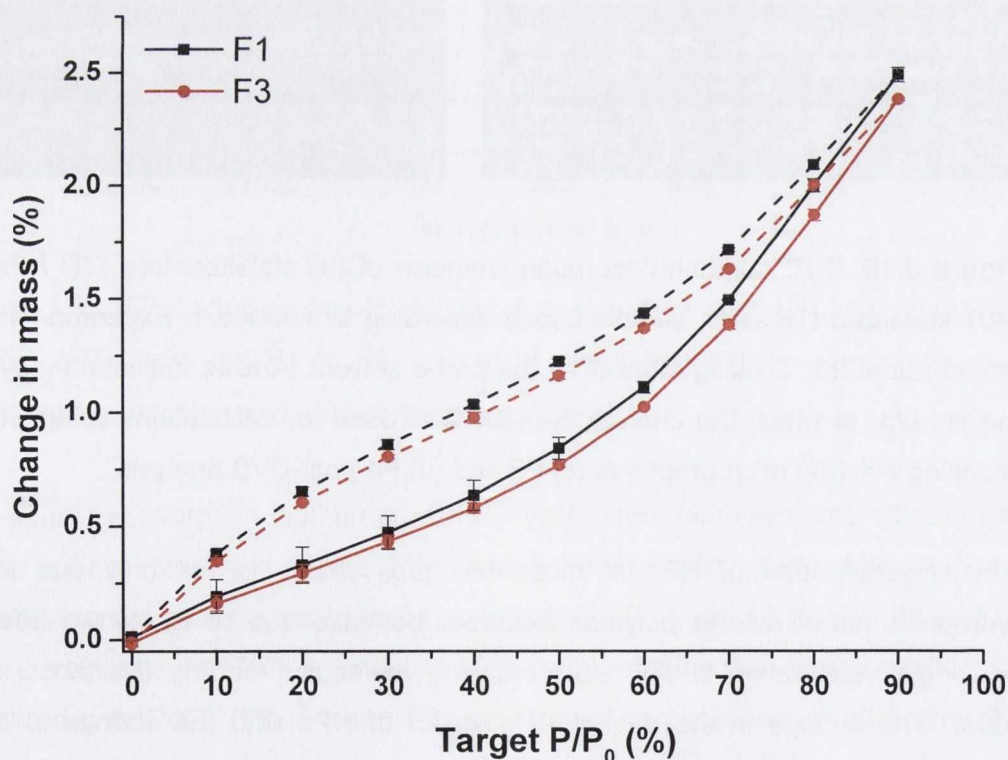


Figure 3.16. DVS moisture sorption-desorption isotherm of nematic (F1) and smectic (F3) LC NPs. Solid line representing sorption cycle while dashed line representing desorption cycle.

3.4. Conclusions

An investigation of the factors governing the quasi nanoemulsion precipitation of ITR revealed the ability to tune the properties of the produced nanoparticles/nanoaggregates via controlling the fabrication conditions. The higher the temperature difference between the solvent and antisolvent phases ($\Delta T_{S,AS}$), the thinner the particle shell thickness and the lower the molecular order with the nematic LC phase arrangement. The highest periodically ordered intercalated SmA mesosphere was observed when $\Delta T_{S,AS}=0$ °C with the inclusion of P407 in the antisolvent phase. Similarly, at a given temperature, the higher the solvent to antisolvent viscosity ratio, the smaller the nanoparticles produced. The ability to prepare P407 stabilized ITR NPs, at an elevated temperature, was demonstrated here while was not feasible before (Matteucci et al. 2006). It should be highlighted that to date a liquid crystalline phase of ITR has been only achieved via melting followed by cooling and here we demonstrate the feasibility of the use of a precipitation technique to accomplish this and the ability to confine the obtained phase in the nanosphere. This comprehensive study might help in the rational design of NPs made of other drugs.

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**CHAPTER IV: Crystal Habits of Itraconazole Microcrystals: Unusual
Isomorphic Intergrowths Induced via Tuning Recrystallization Conditions**

4.1. Introduction

Solid active pharmaceutical ingredients (APIs) are usually synthesized via crystallization from solution (Stoica et al. 2004). Crystallization is usually achieved by creating supersaturated conditions, with respect to the API, via cooling a hot solution, antisolvent precipitation or slow evaporation (Nagy et al. 2008, Shahidzadeh-Bonn et al. 2008). Crystalline materials exhibit a higher thermodynamic stability than their amorphous counterparts, thus encounter less difficulties during pharmaceutical manufacturing processes (Hancock and Parks 2000). Many drugs can exist in more than one polymorphic form, where the space-lattice arrangement of molecules is distinctive in each crystal form depending on the crystallization conditions used, such as the solvent nature, cooling and/or mixing rate, pressure and the inclusion of a co-solvent and other solutes (Haleblian 1975, Shell 1963). Crystalline materials can also be rendered in different crystal habits where the growth of one or more crystal faces is promoted or inhibited (Hartman and Bennema 1980).

In general, the area of pharmaceutical performance design via polymorphic manipulation is more extensively studied compared to crystal habit manipulation, although the latter has a surmised influence on the behavior of the final product including tablettability, wettability and dissolution properties (Modi et al. 2013, Tenho et al. 2007). Recently, it has been demonstrated for celecoxib that the crystal habit of this compound has a considerable impact on its intrinsic dissolution (Modi et al. 2014). While some reports on manipulating crystal habits are available for APIs, the investigation of regular isomorphic intergrowth of crystals remained primarily limited to inorganic materials (Purkayastha et al. 2008).

A regular intergrowth relation can occur between the same crystal species, which is usually identified as twinning or a parallel intergrowth. It can also be observed between crystals of different species and is referred to as an epitaxial growth. Twinning is an intergrowth between two crystals of the same species by a symmetry operation, which is not part of the single crystal. Twinning can occur during the process of crystallization, transformation and deformation and is referred to as growth twinning, transformation twinning and deformation twinning, respectively (Sunagawa 2005). Parallel intergrowth is different from twinning and usually adapted to reduce system energy. It occurs when two or more crystals of the same material are joined in a parallel arrangement with clearly visible parallel extinction (Jocelyn and Pidgeon 1974). Epitaxy describes the growth of a guest crystal with a certain orientation over a host crystal of different species (Sunagawa 2005). A

different type of intergrowth is observed for some APIs including aspirin, promethazine and felodipine and referred to as intergrowth polymorphism, in which the single crystal contains distinct domains of different polymorphs (Borodi et al. 2012, Mishra et al. 2014).

The presence of impurities, additives and other solvents, even at minute quantities, impact the crystallization process via altering the nucleation and growth rates and thus predetermine the resultant crystal polymorphic forms and/or their crystal habits (Yin et al. 2011, Wood 2001). Additives can be “tailor-made”, structurally related to the drug with the ability to incorporate into the crystal lattice (Weissbuch et al. 2003), or polymeric and surfactant additives with little or no structural similarity to the drug of interest and capable of selective adsorption at the crystal/solution interface (Tian et al. 2009). The ultimate crystal shape is governed by the growth kinetic of interfaces to allow maximum growth rate at a given degree of supersaturation that could be due to supercooling (Tiller 1964). Interrelations occurring between the solute and additives, at the molecular level, that could have kinetic, thermodynamic and molecular recognition origin, remain one of the not fully explored areas of chemistry and physics (Yin et al. 2011).

Itraconazole (ITR) is a Biopharmaceutics Classification System (BCS) class II triazole antifungal drug (Odds et al. 2000). Two crystalline forms have been reported in the scientific literature to date (Peeters et al. 1996, Werling et al. 2007) however, to the best of found knowledge, no crystal habit manipulation reports are available in the literature. Crystalline ITR has very low aqueous solubility (Peeters et al. 1996) and could benefit from modifying crystal habit and/or introducing crystal intergrowth. The former can increase the surface area of hydrophilic crystal facets, while the latter can introduce some crystal defects which could increase water penetrability. Herein, three different crystal habits (shapes) of ITR, each having different wettability and moisture sorption properties, thus exhibiting different functional properties relevant to the performance of pharmaceutical drug substances are reported on. This study expands the area of crystal habit manipulation beyond the classical simple morphology manipulation to the intricate crystal intergrowth behavior of a pharmaceutical API and the subsequent impact on its water sorption behavior. Water sorption properties of a crystalline API are of a great importance since this parameter has been reported to induce caking of powder and can adversely affect drug dissolution rate (Rohrs et al. 1999). In addition, moisture sorption can be correlated with the dissolution rate, as demonstrated recently by Puncochova and co-workers (2014). Twinning and dendritic intergrowth were

observed in this study for ITR crystals produced by manipulating antisolvent crystallization conditions. The recrystallization process was investigated by monitoring ITR concentration, temperature as well as the shape and the phase of matter of intermediates throughout the process. The effect of the crystallization conditions on the March-Dollase (MD) parameter, an indication of the preferred orientation and the crystal shape, was investigated. Finally, to complete the studies, computational investigation into the functionalities available on the facets of interest was performed using Mercury 3.5.1 software.

4.2. Experimental and computational methods

4.2.1. Materials

Itraconazole (ITR) was a gift from Neuland Laboratories Ltd. (Welding, Hamburg, Germany). Poloxamer 407 (P407) was purchased from BASF Corp. (Ludwigshafen, Germany). Acetone Chromasolv®, HPLC grade, was purchased from Sigma-Aldrich (Dorset, UK), while acetonitrile HPLC grade was obtained from Fisher Scientific (Loughborough, UK). All other chemicals were analytical grade and used as received.

4.2.2. Antisolvent crystallization of ITR

The solvent phase was either 6.4 or 7.9 mg/ml ITR in acetone (aprotic solvent) at 50 °C (87% of the saturation solubility of ITR in acetone as described in Chapters 2 and 3) with or without P407 (at a concentration of 1 mg/ml unless stated otherwise). An aliquot of this solvent phase was rapidly poured into the static thermostated antisolvent phase kept at 0.5, 50 or 80 °C (deionized water, protic solvent) using the following solvent to antisolvent ratios: 2:1, 1:2, 1:2.5 and 1:1 v/v as displayed in table 4.1. Conditions associated with the most unique crystal morphologies are displayed in Table 4.2. To ensure reproducibility, at least four replicates were performed. The formed crystals were centrifuged at 12,000 rpm (4 °C) for 20 min using an Eppendorf 5810R centrifuge (Hamburg, Germany). The supernatant was removed and the solid residue was thoroughly washed using deionized water and dried under nitrogen purge.

Table 4.1. Summary of crystallization conditions employed to manipulate the crystal habits of ITR crystals. P407 concentration was 1mg/ml.

S:AS (v/v) ratio	2:1		1:2		1:2.5		1:1	
Solvent phase (mg/ml)	6.4 ITR in acetone	7.9 ITR in acetone	6.4 ITR in acetone	7.9 ITR in acetone	6.4 ITR in acetone	7.9 ITR in acetone	6.4 ITR in acetone	7.9 ITR in acetone
Antisolvent temperature (°C)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	50	50	50	50	50	50	50	50
	80	80	80	80	80	80	80	80

Table 4.2. Summary of crystallization conditions associated with the most unique morphology types of ITR crystals. Three distinct isomorphic crystal habits of ITR, a twinned blade-shaped (CH_{tw}), a plate-shaped (CH_{pl}), and a flat sheet-shaped with dendritic ends (CH_{sh}), were obtained by controlling crystallization conditions.

Morphology type**	Antisolvent temperature (°C)	Solvent (S)	Antisolvent (AS)	S:AS (v/v) ratio
CH _{tw}	0.5	ITR (7.9 mg/ml) and P407 (1 mg/ml)	Deionized water	2:1
CH _{pl}	50	ITR (7.9 mg/ml) and P407 (1 mg/ml)	Deionized water	1:2
CH _{sh}	80	ITR (7.9 mg/ml) and P407 (8 mg/ml)*	Deionized water	1:1

* The only case where 8 mg/ml P407 was used.

** The abbreviations are described in Section 4.3.1 and Figure 4.1.

4.2.3. Characterization of ITR crystals

4.2.3.1. Particle size and morphology

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 crystals and process intermediates. The dried samples were sputter coated with a layer of gold/palladium under vacuum before analysis.

4.2.3.2. Powder X-ray diffraction

Reflection powder X-ray diffraction (PRXRD) measurements were performed using a Rigaku Miniflex II, a desktop X-ray diffractometer (Japan) operating in the Bragg-Brentano reflection mode, equipped with a Cu K α radiation X-ray source. The samples were front-loaded on a zero-background silicon sample holder without grinding, gently pressed using a glass slide and scanned over a 2θ range of 2–40°. The texture of the produced crystals was evaluated using the March-Dollase approach to quantitatively describe the preferred orientation. The calculations were performed for samples produced according to the method presented in Section 2.2 using 0.5 to 80 °C antisolvent crystallization temperature as well as the solvent composition ranging from 28% acetone (equivalent to a 1:2.5 v/v solvent:antisolvent ratio) to 66% acetone (equivalent to a 2:1 v/v solvent:antisolvent ratio) in the final media with and without the inclusion of 1 mg/ml P407 in the solvent phase. The sample CH_{sh} made by applying the conditions outlined in Table 4.2 was also analyzed. The March-Dollase parameter (MD) was calculated using Eqn. 4.1, as described by Zolotoyabko (2009).

$$MD = \left[\frac{\sin^2 \alpha}{(k/k_p)^2 / \varepsilon - \cos^2 \alpha} \right]^{1/3} \quad \text{Eqn. 4.1}$$

where α is the angle between two reciprocal lattice vectors H and h . H (HKL) is the preferably oriented plane and h (hkl) corresponds to an atomic plane, inclined by a small angle ($\cos \alpha \leq 1$) with respect to the plane H , with strongly suppressed diffraction peaks. k and k_p are the diffraction intensity ratios for the powder in question and a randomly oriented powder, respectively.

Transmission powder X-ray diffraction (PTXRD) experiments were carried out at room temperature using a Rigaku-Denki D/MAX RAPID II-R diffractometer (Rigaku Corporation, Japan) with a rotating anode Ag K α tube ($\lambda = 0.5608 \text{ \AA}$), an incident beam (002) graphite monochromator and an image plate in the Debye–Scherrer geometry as a detector. The pixel size was $100 \mu\text{m} \times 100 \mu\text{m}$. All samples were

placed inside glass capillaries (1.5 mm in diameter). The measurements were performed for the filled and empty capillaries and then the signal intensity of the empty capillary was subtracted. The beam width at the sample was 0.3 mm. The two-dimensional diffraction patterns were then converted into one-dimensional intensity data by integration around each ring using suitable software (Grzybowska ET AL. 2010). PTXRD samples were analyzed by Ms. Karolina Jurkiewicz (Institute of Physics, University of Silesia, Katowice, Poland).

4.2.3.3. Computational investigations

Powder patterns simulation and interaction maps were generated using Mercury 3.5.1[®] software (CSD refcode TEHZIP). The surface interaction maps were built using the Superstar approach (Verdonk et al. 2001). Molecules on the surface of interest were split into IsoStar central groups and appropriate 3D scatterplots were then superimposed on the molecules of interest followed by removal of any clashing data points. Normalized density maps were then generated and overlapping maps from the same probe were combined by multiplication followed by the generation of the final interaction map (Wood et al. 2013). Herein, carbonyl, uncharged NH and aromatic CH were employed to probe the interaction maps indicative of H-bond acceptor, H-bond donors and hydrophobic interactions, respectively. Mercury 3.5.1 was also utilized to visualize the accessible voids in a perfectly packed ITR crystal. Hirshfeld surface analysis was performed using CrystalExplorer (ver. 3.0) software (Spackman and McKinnon 2002).

4.2.3.4. Single crystal X-ray diffraction (SCXRD)

SCXRD was performed to verify the solid-state isomorphic form and to face-index the crystals. A specimen of CH_{tw}, approximate dimensions 0.020 mm x 0.040 mm x 0.100 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured at 100(2)K using a Bruker Apex Duo system equipped with an Oxford Cryosystems Cobra low temperature device and using a MiTeGen micromount. Bruker APEX2 software was used to correct for Lorentz and polarization effects. A total of 2743 frames were collected. The total exposure time was 42.67 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. This analysis was carried out in triplicate on separate crystals. SCXRD samples were analyzed by Dr. Brendan Twamley (School of Chemistry, TCD).

4.2.3.5. Differential scanning calorimetry (DSC)

Accurately weighed samples (3-5 mg) were placed in pin-holed aluminum pans. The DSC measurements were performed using a Mettler Toledo DSC 821e (Greifensee, Switzerland) and a nitrogen purge at a speed of 10 ml/min. A temperature range of 25-200 °C was applied with a heating rate of 10 °C/min.

4.2.3.6. Optical and polarized light microscopy

Optical and polarized light (PL) microscopy was performed with an Olympus BX53 polarizing optical microscope equipped with a U-POT cross polarizer and a Q IMAGING Fast 1394 camera. Samples were either dried before analysis or analyzed in the crystallization medium.

4.2.4. Crystal formation monitoring

4.2.4.1. Change in ITR concentration in the crystallization media

The change in the concentration of ITR in the solution upon the addition of the solvent phase to the antisolvent phase and throughout the crystallization process was monitored. For each preparation condition, as summarized in Table 4.2, an aliquot was withdrawn at predetermined time points (0, 2, 5, 10, 20 and 40 min) following the addition of the solvent phase to the antisolvent phase. The aliquot was filtered using 0.1 µm PTFE filter membrane (Whatman, UK), diluted with the mobile phase and ITR concentration was determined using a HPLC method as described before in Chapter 2. The net temperature of the crystallization media was measured simultaneously.

4.2.4.2. Morphology of crystallization process intermediates

The morphology of the intermediate phases was characterized using SEM and TEM as described above. For SEM, aliquots of the various liquids were placed directly on silicon chips 5*7 mm with (111) orientation fixed on aluminum stubs and then dried using nitrogen purge. Transmission electron microscopy (TEM) characterization of the crystallization process intermediates was carried out on a TEM Titan instrument (FEI Ltd, Hillsboro, OR, USA). An aliquot of the crystallization solution was drop casted on Cu holey carbon-coated TEM grids and imaged at 300 kV.

4.2.5. Contact angle measurement

Sessile drop contact angle can be measured on the surface of compressed disc or on a layer of powder adhered to an inert substrate (He et al. 2008, Overhoff et al. 2007). The former is the most widely used and the latter are used to avoid unintended crystal structure modification upon compaction. Here, the thin layer

method was adopted with some changes. A thin powder film prepared immediately following crystallization was used where the crystals were filtered under vacuum for 20 seconds. The produced film was then washed with deionized water under vacuum filtration for 1 min and carefully transferred to a glass slide and left to dry overnight. Advancing contact angle of double distilled water drops with the prepared films were calculated at ambient conditions using a Drop Shape analyzer (FTA 125, First Ten Angstrom, Virginia, USA). From the captured photos, the contact angle was calculated based on the Young-Laplace equation (Schonberg et al. 1995). The slope of the tangent to the drop at the liquid–solid–vapor interface line was calculated. All measurements were performed in triplicate under ambient conditions of 25 ± 2 °C.

4.2.6. Dynamic vapor sorption and mathematical modelling of moisture distribution

Moisture absorption/adsorption behavior characteristic of each sample was monitored using a DVS advantage-1 automated gravimetric vapor sorption analyzer (Surface Measurement Systems Ltd., London, UK). Samples were equilibrated at 0% RH until dry ($dm/dt \leq 0.002$ mg/min for at least 10 min) and the reference mass was recorded. Each sample was exposed to a relative humidity profile from 0% to 90% and back to 0% RH with 10% steps at 37 ± 0.1 °C.

The Young-Nelson model was fitted to the sorption-desorption isotherms. The model can differentiate between bound monolayer, externally adsorbed multilayer and internally absorbed water using the following equations (Bravo-Osuna et al. 2005),

$$M_s = A(\beta + \theta) + B\theta RH \quad \text{Eqn. 4.2}$$

$$M_d = A(\beta + \theta) + B\theta RH_{max} \quad \text{Eqn. 4.3}$$

Where M_s and M_d are the mass percentage of water sorbed and desorbed on the crystals at each %RH equilibrium. A and B are constants characteristic of each system. In the used model, θ is the fraction of the surface covered by at least one layer of water molecules and $A\theta$ is the mass of water in a complete adsorbed monolayer expressed as a fraction of the dry mass of the crystals. $A(\beta + \theta)$ is the total amount of adsorbed water and $A\beta$ is the mass of water which is adsorbed as a multilayer. B is the mass of absorbed water at 100% RH, thus $B\theta RH$ is the mass of the absorbed water when the monolayer coverage is θ and the relative humidity is RH.

θ and β can be expressed mathematically as:

$$\theta = \frac{RH}{RH + E(1 - RH)} \quad \text{Eqn. 4.4}$$

$$\beta = -\frac{ERH}{E - (E - 1)RH} + \frac{E^2}{(E - 1)} \ln \left[\frac{E - (E - 1)RH}{E} \right] - (E + 1) \ln(1 - RH) \quad \text{Eqn. 4.5}$$

Where E is equilibrium constant between monolayer water and the 'normally condensed' water adsorbed externally to the monolayer and is depending on the material. All data were fitted to Eqn. 4.2 and 4.3 using multiple linear regression and the sum of squares of the residuals between the experimental and the calculated values were used as the fitting criteria. The corresponding profiles of water adsorbed in monolayer ($A\theta$), multilayer ($A\beta$) and absorbed ($B\theta RH$) were constructed from the estimated values of A, B and E using the above equations.

4.2.7. Statistical analysis

Statistical analyzes were performed via one way ANOVA with the Tukey comparison test using Minitab Release 16. For all tests, $p \leq 0.05$ was used as the criterion to assess statistical significance.

4.3. Results and discussion

4.3.1. Crystal morphology

Crystallization of ITR was achieved via tuning the antisolvent crystallization conditions by controlling the percentage of the added solvent in the final volume (expressed as a solvent to antisolvent ratio), cooling temperature, polymer concentration and precipitation temperature (antisolvent temperature). Preparative conditions associated with the most characteristic shape types (habits) are summarized in Table 4.2. SE micrographs and polarized light (PL) microscope images (Figure 4.1) revealed a twinning behavior of ITR crystals (CH_{tw}), with a characteristic blade like shape of the principal (parent) crystals. Multiple or repeated twinning dominates the sample, where several daughter crystals are arranged in a radiating pattern as an example of interpenetration growth twinning. A different type of intergrowth was observed in the CH_{sh} sample, where each crystal is visible as a very thin, brittle sheet with branched ends. A similar plagioclase crystal shape was described as a tabular crystal with dendritic projections from the corners (Lofgren 1974). Thus, CH_{sh} crystals can be described as sheets with dendritic ends since they are too thin to be tabular (Sunagawa 2005).

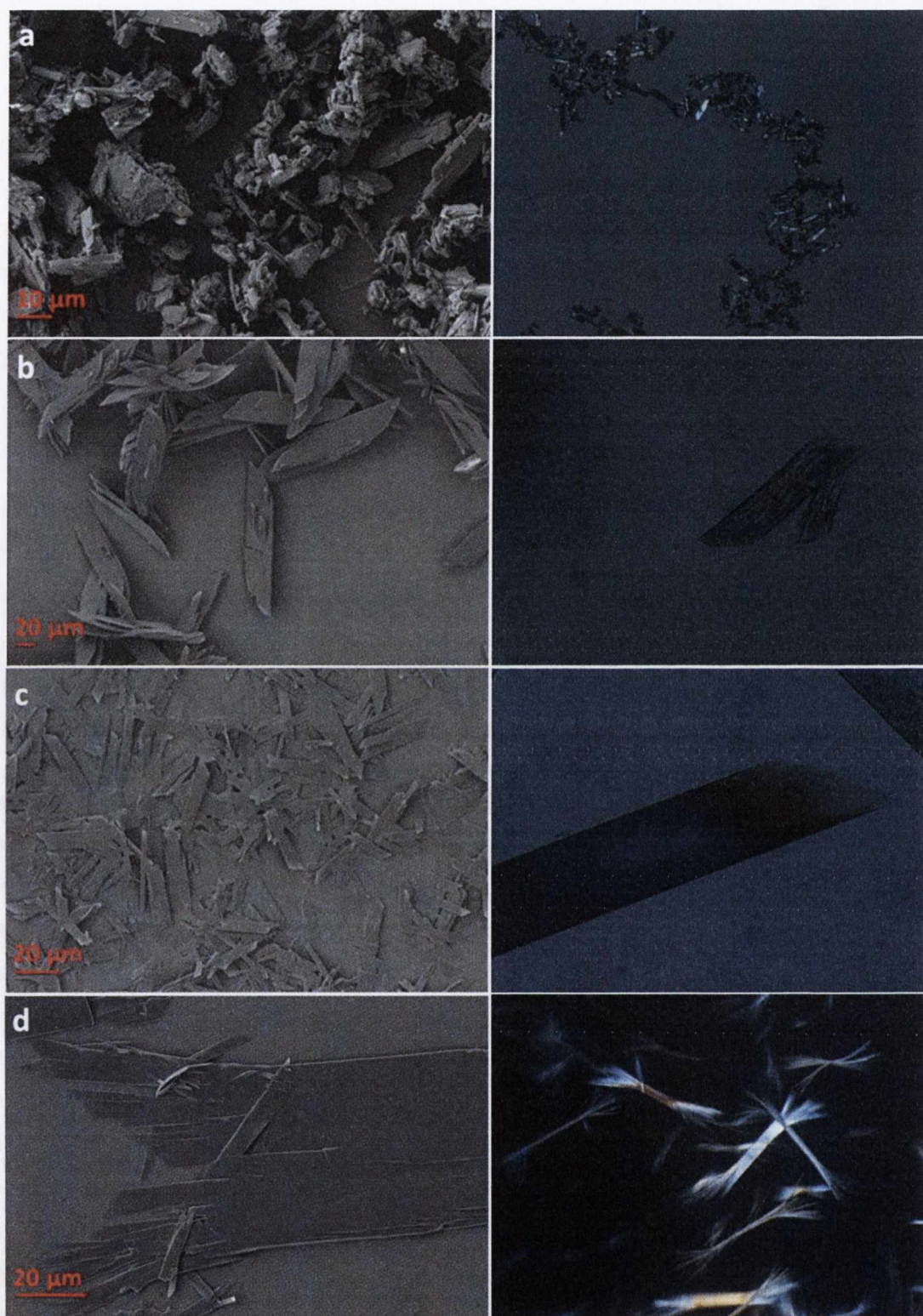


Figure 4.1. SE micrographs (left) and PL images (right) of: (a) as-received ITR, (b) CH_{tw}, (c) CH_{Pl} and (d) CH_{sh}. All PL imaging was performed on dried crystals except for CH_{sh}, where the crystals were imaged in the crystallization solution to avoid breakage of the characteristic dendritic ends.

The third crystal habit was a thin-plate like morphology (CH_{pl}) under the optical microscope and confirmed by SEM (Figure 4.1c). Birefringence was observed for all crystal habits under cross polarized light microscope (PLM), indicating their crystalline nature. Sloped ends were observed for CH_{tw} and CH_{pl} in SE micrographs and confirmed by PLM.

4.3.2. Powder X-ray diffraction

PRXRD diffractograms (Figure 4.2) confirmed the crystalline nature of all samples. Remarkable variations in relative intensities of some peaks were observed. To rule out any possible polymorphic crystallization, PRXRD patterns of all crystallized forms were compared with the simulated powder pattern, generated using Mercury 3.5.1 software (Figure 4.3), with the assumption of a random crystal orientation, which revealed that all peaks observed in the PRXRD patterns in Figure 4.2 are present in the simulated pattern displayed in Figure 4.3 (CSD refcode TEHZIP).

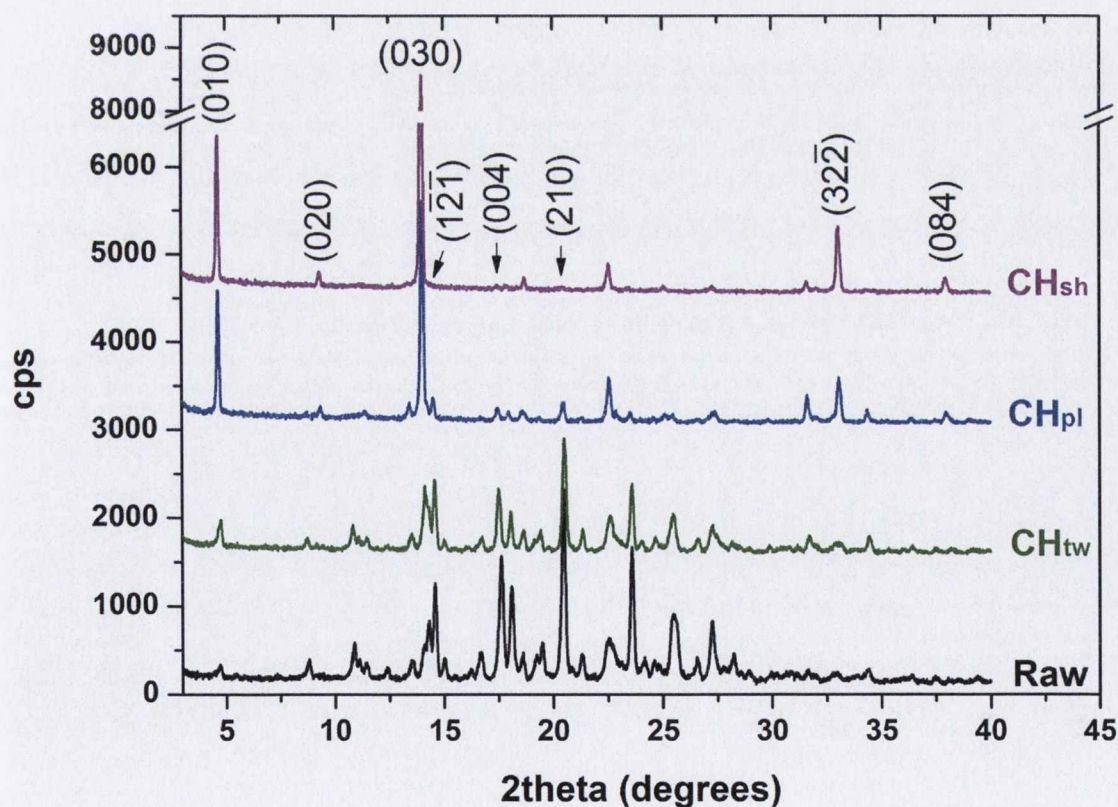


Figure 4.2. Overlay of PRXRD diffractograms of the three principal crystal habits (CH_{tw} , CH_{pl} and CH_{sh}) and the starting material (Raw). The Miller indices (hkl) of the affected facets/planes are labelled.

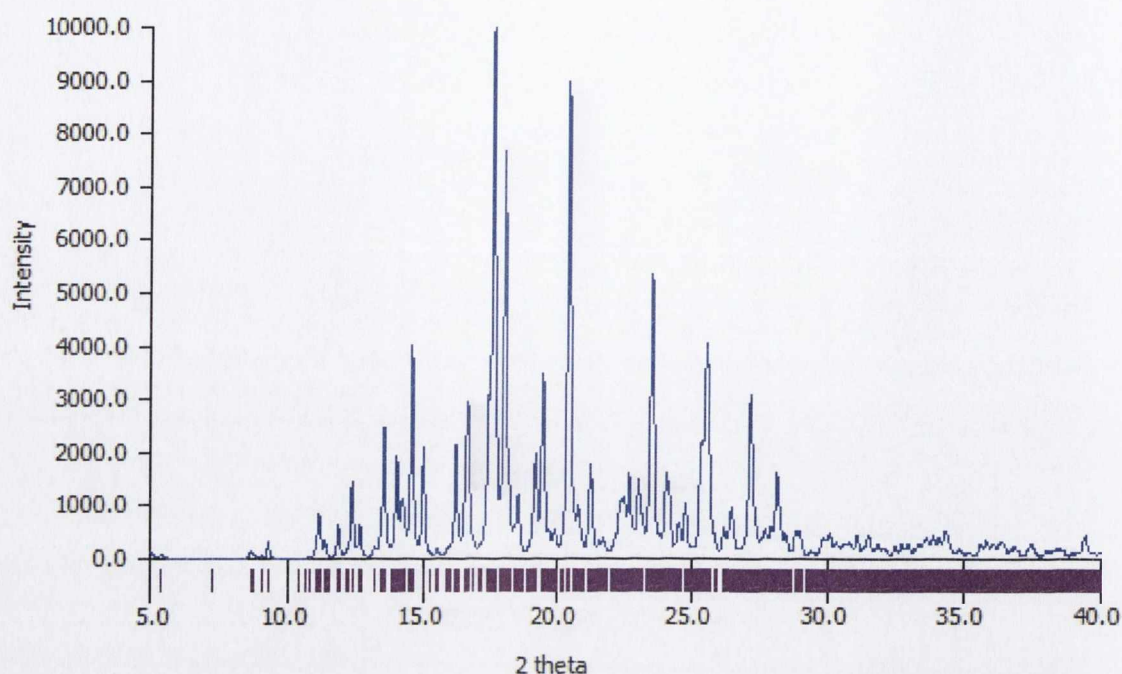


Figure 4.3. Simulated X-ray diffraction pattern of ITR generated using Mercury 3.5.1 software with the assumption of random orientation of crystals.

Moreover, one dimensional and two dimensional PTXRD patterns (Figure 4.4) were identical for the three samples further confirming the isomorphic phase of the ITR crystals. PTXRD also indicates that this type of twinning observed in CH_{tw} is simple contact twinning because the daughter crystals did not introduce any diffraction complications to the diffraction patterns (Heising and Kanatzidis 1999).

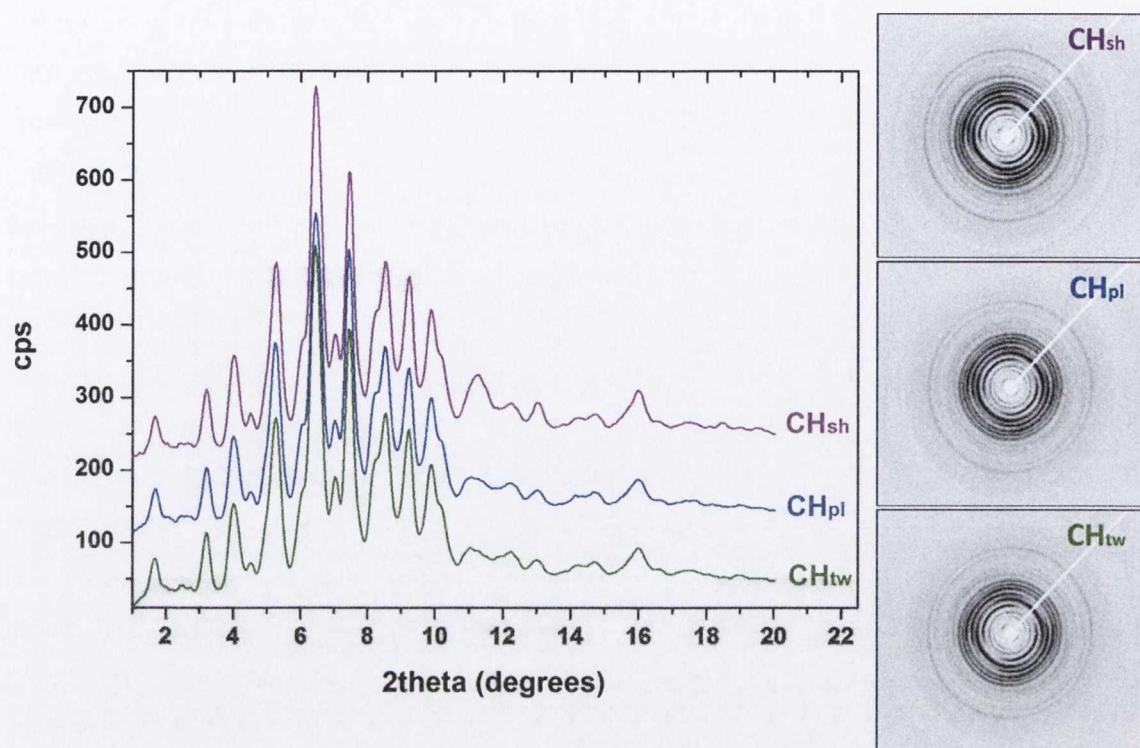


Figure 4.4. One dimensional PTXRD (left) and two dimensional diffraction rings of the three principal ITR crystal habits.

Preferred orientation was utilized to assign the large crystal facets/planes. For CH_{tw} , an increase, in comparison to the starting material, in the intensity of the diffraction peak corresponding to the (030) was observed. For CH_{pl} and CH_{sh} , the plane (030) was associated with the highest intensity indicating an enhanced diffraction originating from the (010) reflections. It should be highlighted that the intensity of the peak at $17.5^\circ 2\theta$, corresponding to the atomic plane (004), was reduced in all samples. Therefore, (030) and (004) were chosen as the lattice vectors H and h , respectively, to calculate the March-Dollase parameter (MD). The calculations were performed for samples manufactured using a range of recrystallization conditions, as described in Sections 4.2.2 and 4.2.3.2, including the three principal and characteristic crystal habits (conditions of the preparative process are presented in Table 4.2). Results are plotted as contour maps, separate for samples crystallized with and without P407 along with the simulated powder patterns in Figure 4.5. The MD parameter ($0 \leq MD \leq 1$) is an indication of the degree of the preferred orientation of the sample on the sample holder, which in turn is related to the crystal shape. A value of 0 is an indication of absolute preferred orientation, which is usually observed with flat anisotropic crystals, while a value of 1 is an indication of randomly oriented powder (zero preferred orientation) and indicates isotropic crystal

shapes (Tenho et al. 2007). The white regions in the plot (Figure 4.5) represent the conditions where a non-crystalline product was obtained and their location appears to be identical in the absence and presence of P407. The black regions, corresponding to the MD parameter below 0.4, was absent in the maps indicating that the CH_{sh} habit can only be obtained using 8 mg/ml P407 and cannot be obtained using any other of the investigated combination of conditions using 1 mg/ml P407 in acetone or just acetone. The CH_{pl} habit with the MD parameter less than 0.5 (dark blue regions) can only be obtained when a low solvent/antisolvent ratio is used with the antisolvent temperature above 45 °C and in the presence of P407. The cyan and green regions, with the MD parameters between 0.5 and 0.8, correspond to the conditions where twinning (habit CH_{tw}) is expected to take place. The polymer appears to be inducing twinning at high antisolvent temperatures and high solvent contents, however the same combinations of conditions (antisolvent temperature and solvent content) when no P407 was used resulted in almost isotropic crystals with the MD parameter ≥ 0.8 . P407 has surfactant properties, which have been reported to trigger twinning (Purkayastha et al. 2008). It is worth mentioning that the PRXRD pattern of ITR as supplied (raw material, Figure 4.2) was similar to the simulated powder patterns when the MD parameter is around 0.9, indicating that the starting material was not a fully isotropic powder, confirmed by the SE micrograph (Figure 4.1a), as some anisotropic crystals can be observed in the specimen. Moreover, the contour maps revealed that, in order to obtain fully isotropic faceted crystals, the solvent and antisolvent should be mixed in comparable volumes and the antisolvent temperature should be below 20 °C, in case where no polymer is added to the crystallization mix, and below 10 °C, if P407 is included in the solvent phase.

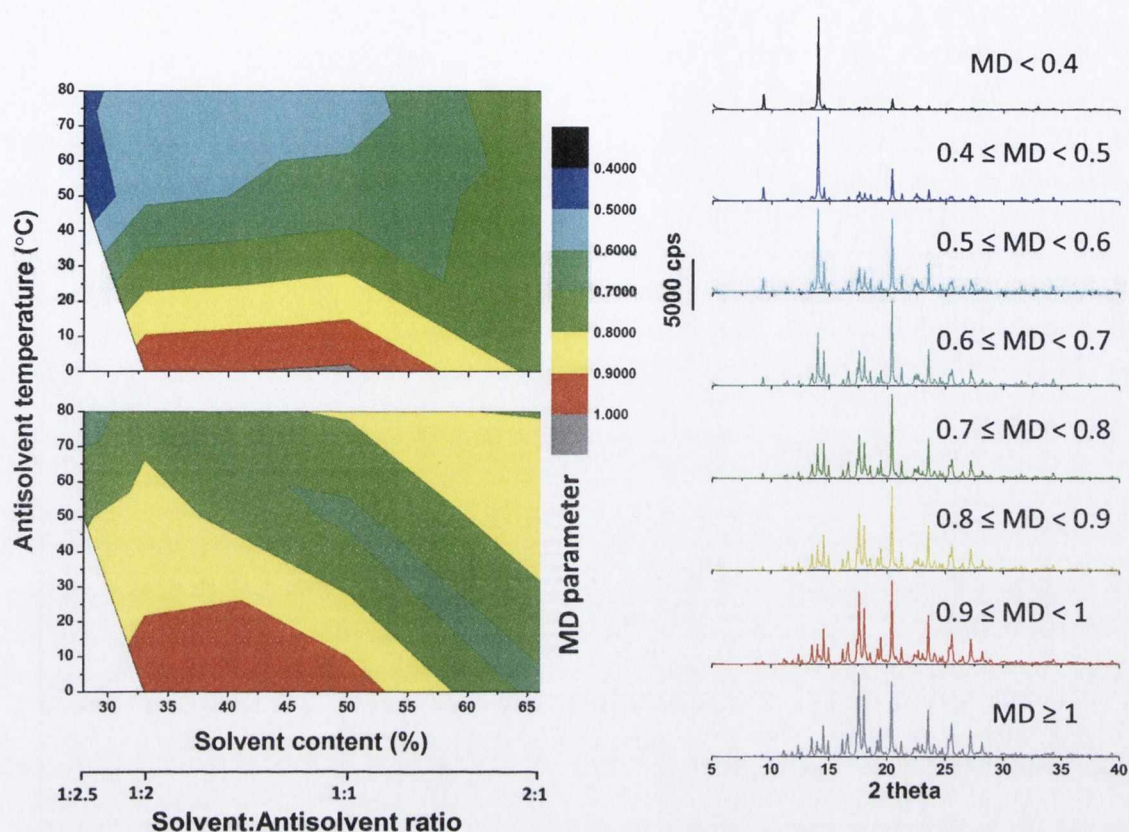


Figure 4.5. Contour maps (antisolvent temperature versus solvent content) of the MD parameter values for the crystallized ITR samples along with the calculated powder X-ray diffraction patterns for the different MD values. Upper map: data pertaining to the samples containing 1 mg/ml P407 in the solvent phase (1 mg/ml P407 in acetone) and lower map: data pertaining to the samples containing no polymer in the solvent phase (ITR in pure acetone).

4.3.3. Thermal analysis

The DSC investigation demonstrated subtle differences in the melting temperature of the different crystal habits (Figure 4.6). CH_{sh} exhibited the lowest melting point (162.9 °C, peak value 165.7 °C) among the crystalline samples, compared to the melting point of 166.1 °C of the starting material powder and CH_{tw} . The melting point appearing at a lower temperature could be attributed to the dendritic intergrowth in CH_{sh} , where the small protruding ends readily break to form fines. Subtle changes in melting points upon changing the crystal habit have been reported before for nifedipine and were attributed to the difference in the examined habits' crystal size (Kumar and Shastri 2014). Grinding pretreatment was avoided in this study to prevent unintended phase changes. The subtle variation in melting point of different

crystal habits could be partially attributed to the difference in crystal orientation in the bottom of the DSC pan which could impact thermal conductivity.

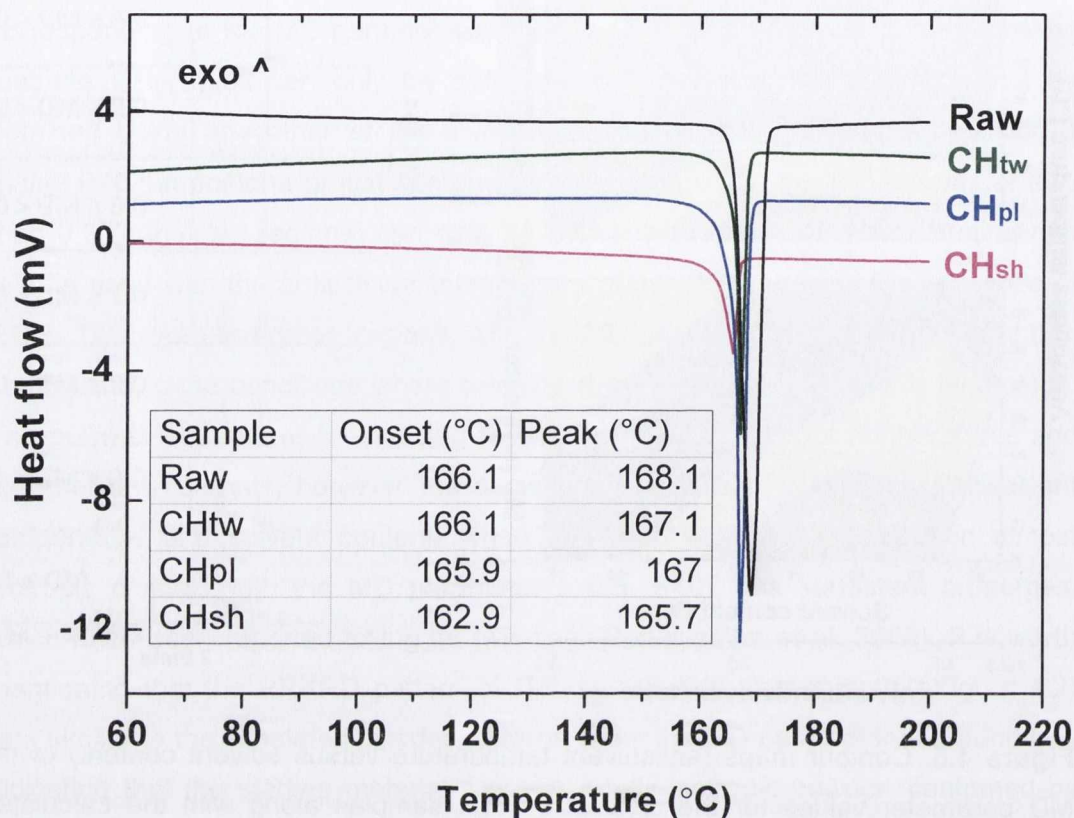


Figure 4.6. DSC thermograms of the three principal crystal habits (CH_{tw}, CH_{pl} and CH_{sh}) and the starting material of ITR (Raw).

4.3.4. Crystal formation process and intermediates investigation

Data pertaining to monitoring the crystallization process kinetics resulting in the principal ITR crystal habits is displayed in Figure 4.7. During crystallization of CH_{tw} a cloudy dispersion formed initially upon addition of the solvent phase to the antisolvent phase and nearly instantly disappeared (CH_{tw} images 1 and 2 in Figure 4.8). Monitoring the concentration of ITR in solution supports the visual observation as a spike in soluble ITR at around 2 minutes, net temperature 6.9±0.2 °C, indicates solubilisation of the initially formed cloudy dispersion. As the solution cooled further to 1.7±0.2 °C, ITR concentration in the solution continued to decrease indicating an initiation of the crystallization process as shown in Figure 4.7 (CH_{tw} image 3 in Figure 4.8).

In contrast, crystallization of CH_{pl} and CH_{sh} followed a different kinetics. Both crystallization mixtures became cloudy upon the addition of the solvent to the

antisolvent phase (CH_{pl} and CH_{sh} image 1 in Figure 4.8) and remained so until shiny crystals began to be noticeable (CH_{pl} and CH_{sh} image 2 in Figure 4.8). Throughout the sampling period, ITR available in the solution decreased gradually with similar concentration profiles for both CH_{pl} and CH_{sh} over the first 20 minutes of the crystallization process. Temperature profiles were different with a 14.3 and 28.2 °C fall in the temperature of the media for CH_{pl} and CH_{sh} , respectively, after first 2 minutes. For the next 3 minutes, the temperatures further decreased by 5.45 and 12.85 °C for the CH_{pl} and CH_{sh} crystallization media, respectively. For the following 20 minutes, a higher decrease in ITR concentration in solution was observed for CH_{sh} compared to CH_{pl} , while the temperature drop was comparable for both systems.

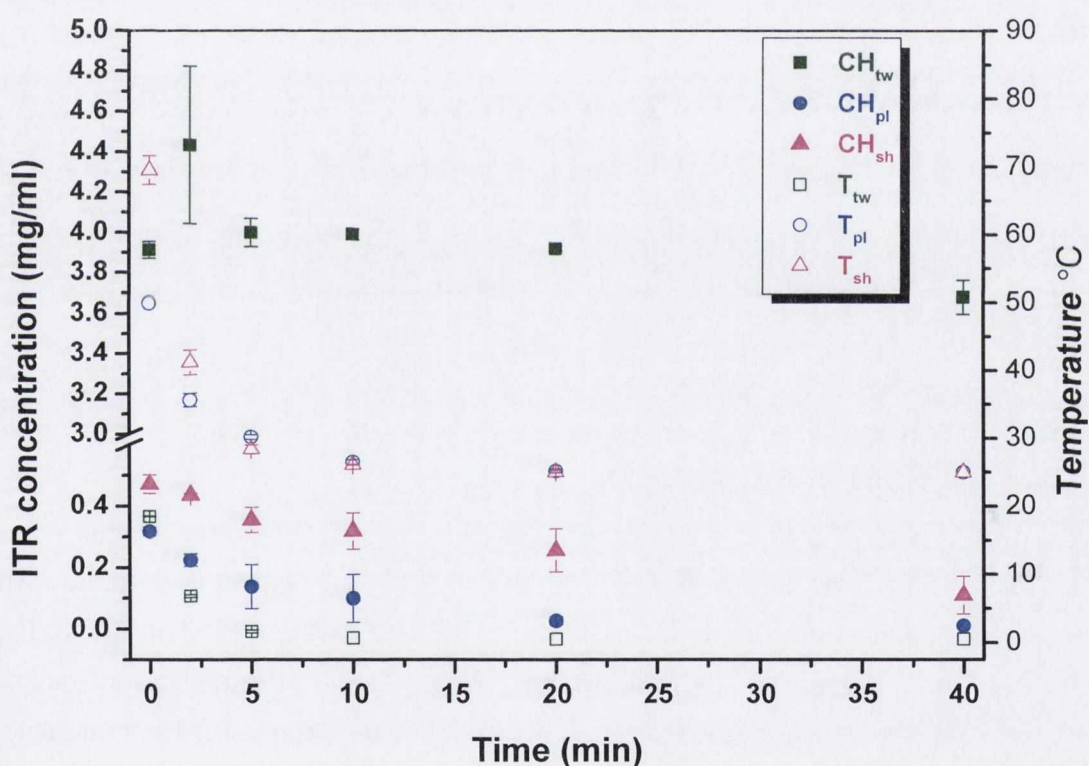


Figure 4.7. Concentration versus time (closed symbols) and temperature versus time (open symbols) profiles for the crystallization processes of the principal crystal forms of ITR.

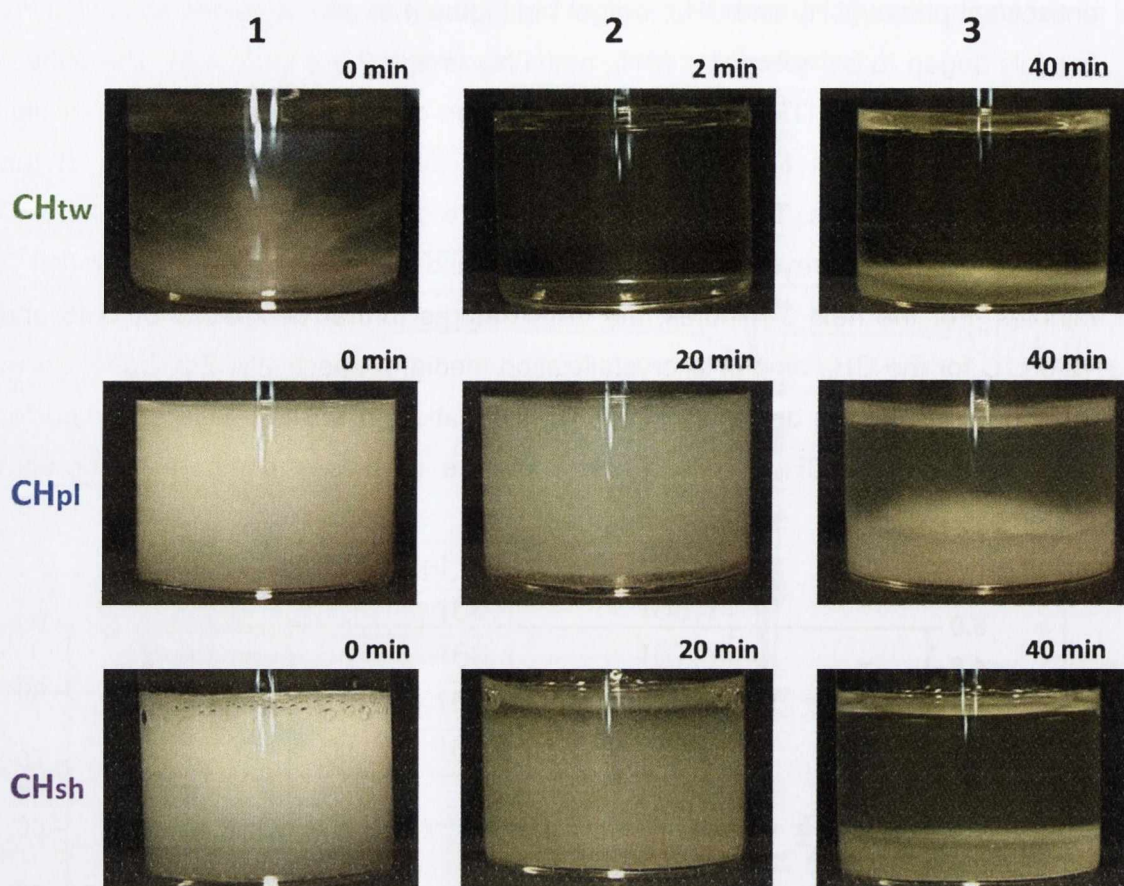


Figure 4.8. Photos taken using a 9MP 2-200 x digital microscope (Conrad Electronics) indicating the various stages of the crystallization process leading to the formation of the three different ITR crystal habits.

The various stages of the crystallization process can be interpreted using morphologies of the intermediate products observed by SEM, PLM and TEM (Figure 4.9). For CH_{pl} and CH_{sh} , as the solvent was added to the antisolvent, spherical particles with a wide size range (300 nm to 4 μ m) were formed (1st intermediate) as shown in Figure 4.9a, b and d. Those particles were liquid crystalline (LC) as concluded from their birefringent nature with Maltese crosses signifying the LC formation (Gharbi et al. 2013) (Figure 4.9d). The LC phase of ITR has been reported to possess higher solubility than the crystalline phase in Chapter 2. These particles underwent Ostwald ripening and, due to availability of soluble ITR, this phase transformed as explicitly presented by the particles representing steps 1, 2 and 3 in Figure 4.9a and Figure 4.9c. Those square shaped structures (2nd intermediate) continued to grow for the rest of the crystallization process and coexisted with the final crystals when the crystallization liquor was sampled later (Figure 4.10).

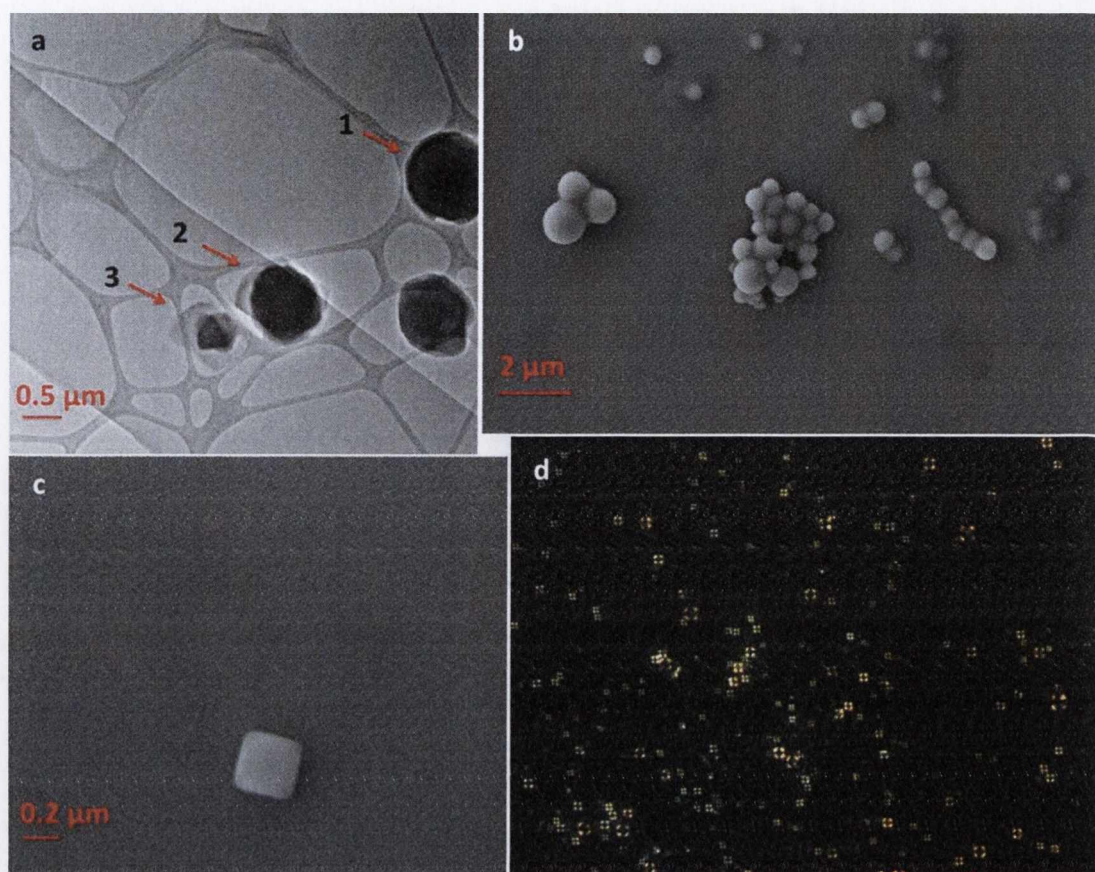


Figure 4.9. (a) TEM of CH_{pl} 1st intermediate presenting particles undergoing a transformation in three steps, (1) represents the initially formed liquid crystalline particles while (2) and (3) represent the transformation of those liquid crystalline particles to crystals via Ostwald ripening (b) SEM CH_{sh} 1st intermediate (c) 2nd intermediate and (d) PLM 1st intermediate (magnification of 500).

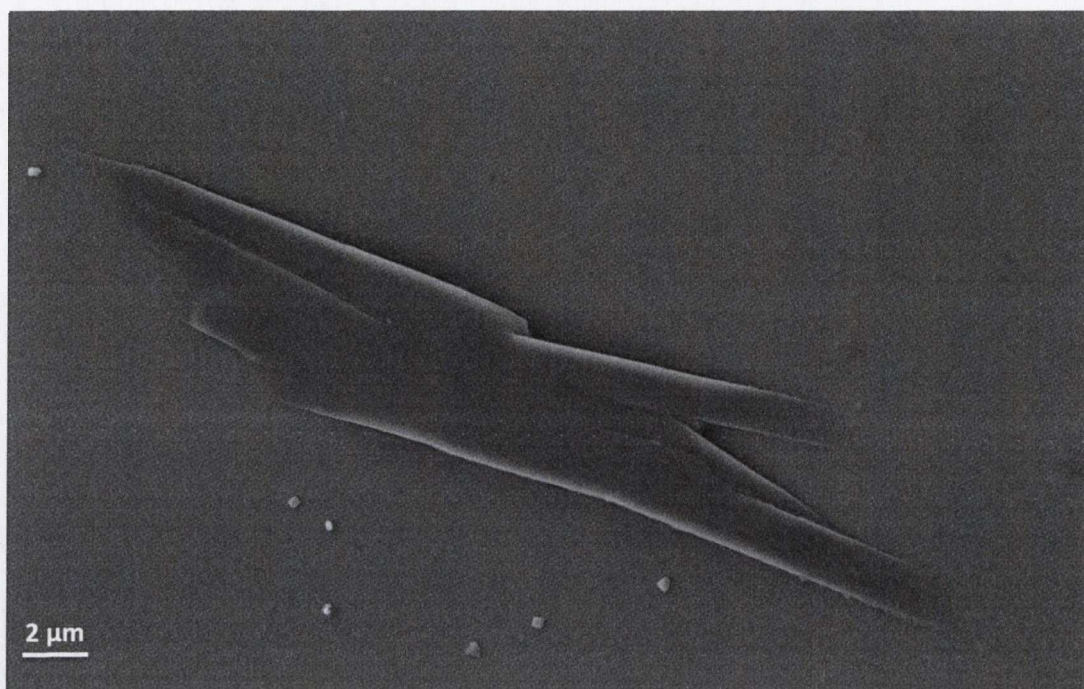


Figure 4.10. SE micrograph illustrating the coexistence of the square-shaped 2nd crystallization intermediate with the final sheet-like crystal (CH_{sh}).

In contrast, for the CH_{tw} , the LC intermediate formation was anticipated, however it could not be isolated as this phase readily solubilized due to the presence of a high content of acetone (solvent), which explains visually clearing the solution after two minutes from the mixing and the high concentration of soluble ITR detected in the crystallization mix (Figure 4.7 and Figure 4.8 CH_{tw} image 2). As the cooling process proceeded nucleation and subsequent crystallization continued. ITR was reported, in Chapter 3, to form stable liquid crystalline nanoparticles (LC NPs), in nematic as well as smectic phases, using a 1:10 v/v acetone:water system. It should be highlighted that a liquid-liquid phase separation (Ilevbare and Taylor 2013) and liquid-glass phase separation (Mosquera-Giraldo and Taylor 2015) have been reported before as intermediate stages occurring before crystallization and herein we observe a new phenomenon of a liquid-liquid crystal phase separation preceding crystallization of an API.

4.3.5. Computational investigations

Bravais, Friedel, Donnay and Harker (BFDH) simulations, a simplified representation of the thermodynamic shape, also known as “equilibrium” habit devoid of any interaction with the milieu, yielded ten unique facets along with their planes, % surface area and distance from the centre. The eight most morphologically important faces exhibiting the largest surface area are displayed in

Table 4.3. It should be highlighted that solvent interaction and temperature effect are not accounted for in the BFDH morphology.

Table 4.3. The eight most important faces bounding the crystal with their surface area and distance from the origin as measured for the BFDH morphology.

<i>hkl</i>	Relative face area (%)	Distance from the centre (Å)
(001)	17.45	20.16
(00$\bar{1}$)	17.45	20.16
(010)	15.0	19.00
(0$\bar{1}$0)	15.0	19.00
(011)	7.7	16.35
(0$\bar{1}$1)	7.7	16.35
(100)	5.2	8.47
($\bar{1}$00)	5.2	8.47

The BFDH model indicates that (001) and (010) are the most important faces since they constitute 17.45 and 15.0% of the external crystal surface, which further justify the use of (00 l) and (0 l 0) diffractions to calculate the MD parameter earlier. For the different crystal habits, crystal slicing was carried out using Mercury 3.5.1 software based on the PRXRD data to investigate the exposed functional groups on the affected faces.

For the three habits, as demonstrated earlier, the intensities of PRXRD peaks at 4.6 and 13.9 2 θ° , corresponding to the (010) and (030) planes, increased in the rank order: CH_{tw} < CH_{pl} < CH_{sh}. On the other hand, the intensity of the peak corresponding to (001) was the greatest for CH_{tw}, intermediate in CH_{pl} and this peak was almost invisible in CH_{sh}. This observation is in agreement with the face indexing (Figure 4.11) and preferred orientation investigations for CH_{tw}, where the (010) face was identified as the largest flat surface in the investigated crystals. SCXRD analysis and face indexing was only performed for CH_{tw}, as it was not feasible in the case of CH_{pl} and CH_{sh} due to the crystals being very thin and too fragile to be handled. It is well known that during a crystallization process, some facets will have a faster growing rate than others where the slow growing faces will have larger area in the final crystal. Therefore, additives or solvent molecules interacting with a specific facet will retard the growth of that facet and thus increase its relative area

(Addadi et al. 1985). Thus the growth of the (010) face is expected to be retarded during the crystal growth process, while the growth of (001) face is promoted.

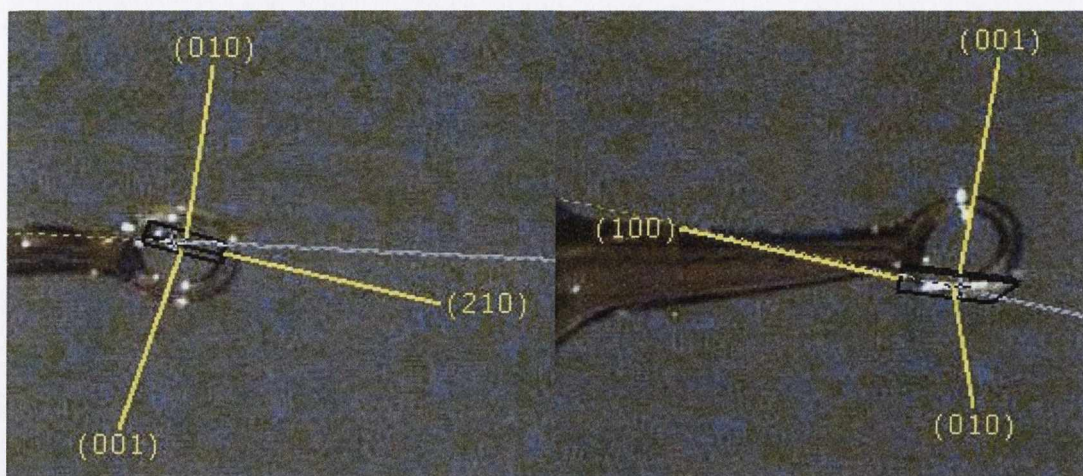


Figure 4.11. Face indexing of two specimens of CH_{tw} with approximate dimensions of $0.020 \times 0.040 \times 0.100$ mm placed on plastic holders. The (010) and (001) faces are consistent in both crystals.

Figure 4.12 illustrates the growth of both planes with the (010) face forming an angle of 73.175° with the (001) face which further supporting the above discussion (Wood et al. 2013). An investigation of the exposed functional groups on the surface of both faces, as indicated in Figures 4.12 and 4.13a, revealed the abundance of polar functional groups including O17, O14, N9, N10, N12, N40C and N41C of each asymmetric unit on the (010) face. In contrast, nonpolar groups dominate the (001) face including C18, C19, C20, C21, C25, C26, C31 and the chlorine attached to the aromatic ring. The rule of thumb is that polar additives yield crystal habits with higher relative surface area of polar faces than nonpolar counterparts (Myerson 2002).

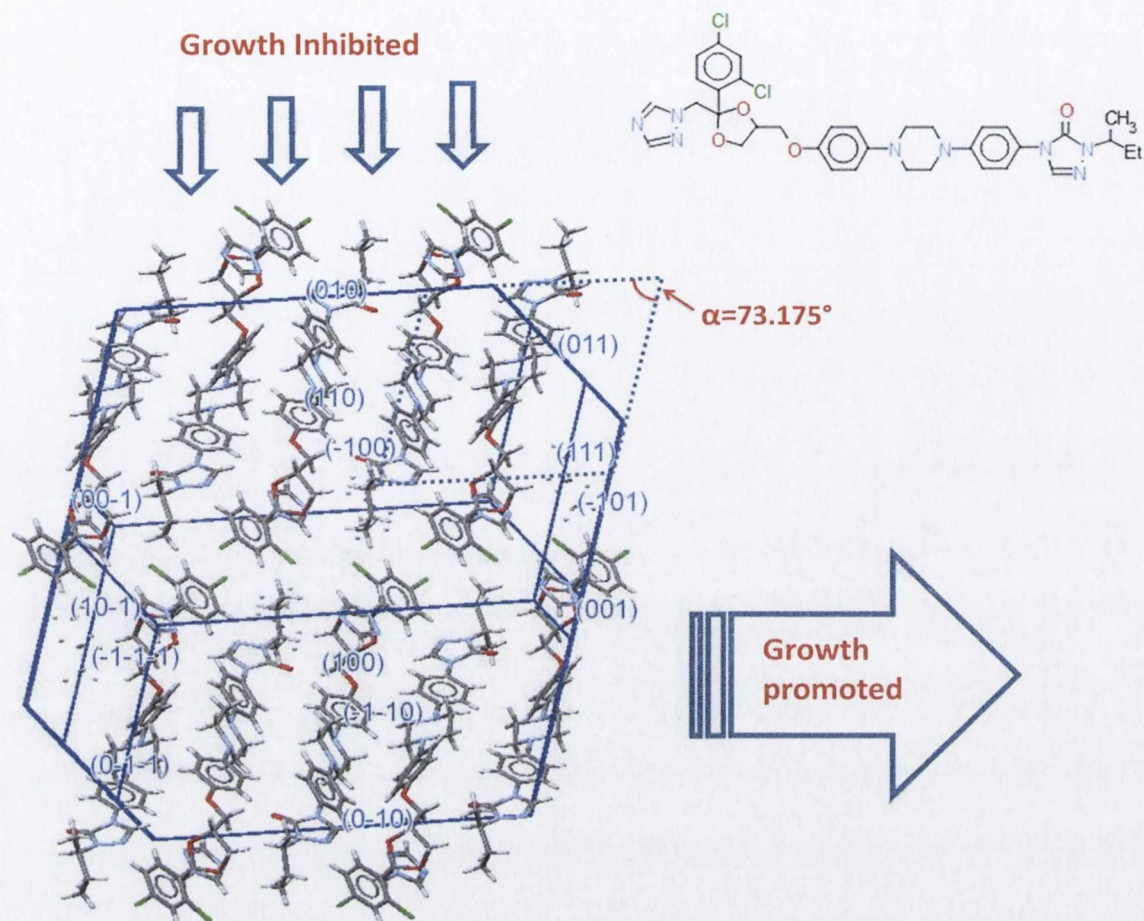


Figure 4.12. Predicted BFDH crystal morphology of ITR, filled with ITR molecules and showing the angle between the (010) and (001) facets to be 73.175° .

Kumar et al. observed a correlation between the polar:nonpolar proportional surface area of nifedipine crystals to the proportion of polar:nonpolar crystallizing solvents (Kumar and Shastri 2014). It should be highlighted that nifedipine is capable of donating hydrogen atoms to form conventional H-bonds and thus can interact with polar aprotic solvents while, based on the structural analysis carried out with Mercury 3.5.1, ITR cannot. This is further confirmed from the Hirshfeld surface analysis (Figure 4.13b) showing a limited number of red hot spots. The red spots correspond to d_{norm} reciprocal interactions stronger than van der Waals interactions such as hydrogen bonds and/or weaker short-range contacts. The 2D fingerprint plot displays 'wing' like shapes (red arrows in Figure 4.13c) characteristic of the weak donor/weak acceptor type ($\text{C-H}\cdots\pi$), while the absence of sharp features pointing to the left of the plot indicates the absence of any conventional strong H-bond donor/strong H-bond acceptor contacts (Spackman and McKinnon 2002).

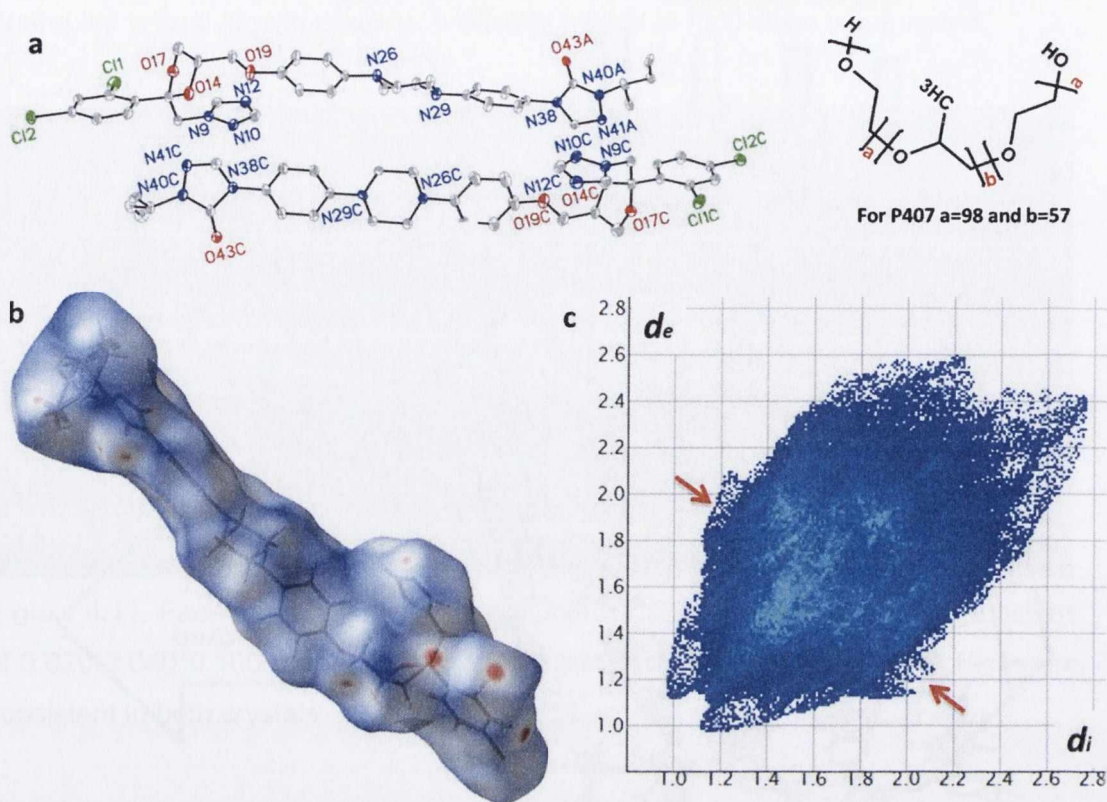


Figure 4.13. (a) Asymmetric unit of ITR (hydrogen atoms omitted and only non-carbon atoms labelled for clarity) and a chemical structure of P407 (ChemDraw®), (b) Hirshfeld surface of ITR, shown in the transparent mode and (c) 2D fingerprint plot of ITR showing the “wing” like features (red arrows) characteristic of C-H... π reciprocal interactions. d_i and d_e are the distance from the surface to the nearest atom interior to the surface and the distance from the surface to the nearest atom exterior to the surface, respectively.

To understand the possible type of interactions on the faces of question, a full interaction map was generated for those facets using Mercury 3.5.1 as demonstrated in Figure 4.14 with opacity level applied. The highly opaque contours are indicative of greater chances of the investigated type of interaction at a certain grid point to occur (Wood et al. 2013). The red hotspots, indicative of possible interactions with H-acceptors, observed in the surface mapping of both (010) and (001) faces are non-conventional hydrogen bonding donors due to the weak donor type (C-H...acceptor) interactions. ITR molecule can serve as a conventional H-bond acceptor only, thus it can interact, through H-bonds, with protic solvents only. The (010) plane shows more preferred interaction sites for H-bond donors than the (001) plane. Therefore, herein, in the case of a mixture of acetone (aprotic) and

water (protic), water is expected to interact with the polar surfaces and inhibit their growth thus the correlation of protic:aprotic crystallizing solvent proportion to the polar:nonpolar crystal surface area, could be applicable. Poloxamer has the ability to form hydrogen bonds as it is an H-bond donor and an H-bond acceptor, thus it may react with the (010) facet. The effect of P407 was clear in the contour map (Figure 4.5) as CH_{pl} and CH_{sh} were only observable upon the inclusion of P407 in the solvent phase.

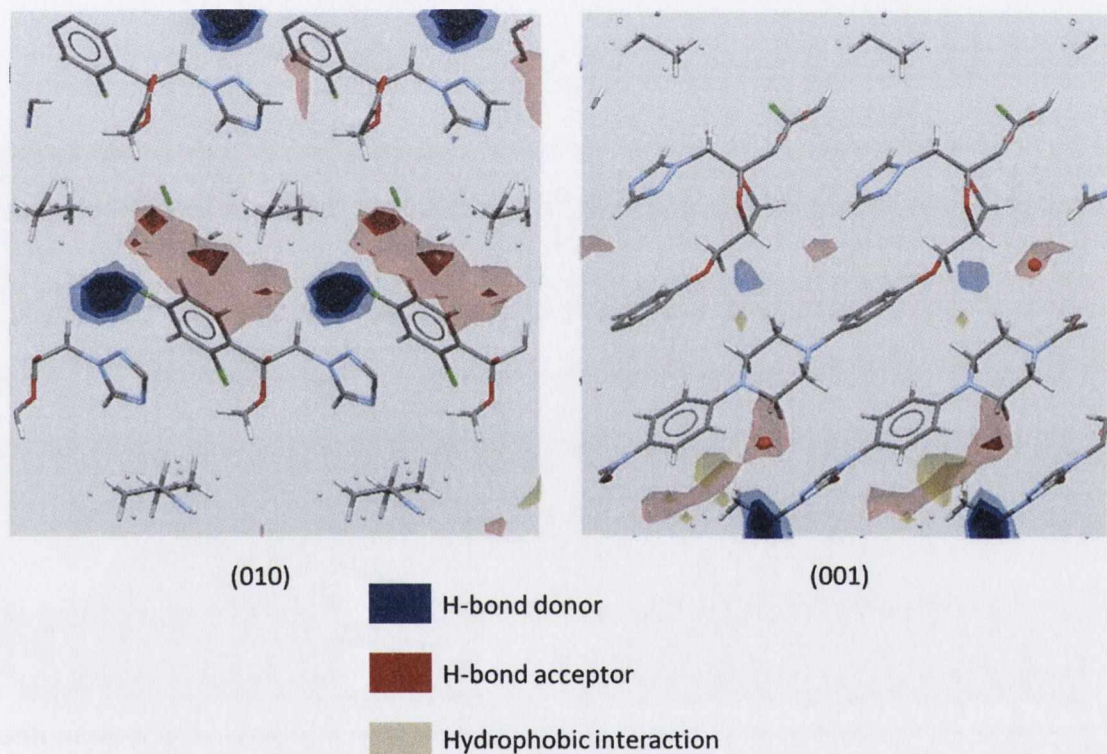


Figure 4.14. Packing diagram and interaction maps of (010) and (001) surfaces illustrating the possible hydrogen bond donor, hydrogen bond acceptor and hydrophobic interactions of functional groups on each surface. The higher the opacity of the contour, the greater the chance of the investigated interaction to take place.

4.3.6. Impact of crystal habit on the wetting and water sorption behavior of ITR

The advancing contact angle using deionized water reflected the wettability properties of the crystals. The starting material of “as received” ITR had the highest contact angle with the water droplets ($123 \pm 2^\circ$) indicating high hydrophobicity of the exposed surface. The measured contact angle was lower for CH_{tw} ($113 \pm 3^\circ$) followed

by CH_{pl} ($58\pm4^\circ$). CH_{sh} had the lowest angle of $48\pm2^\circ$ indicating the least hydrophobic surface (Figure 4.15).

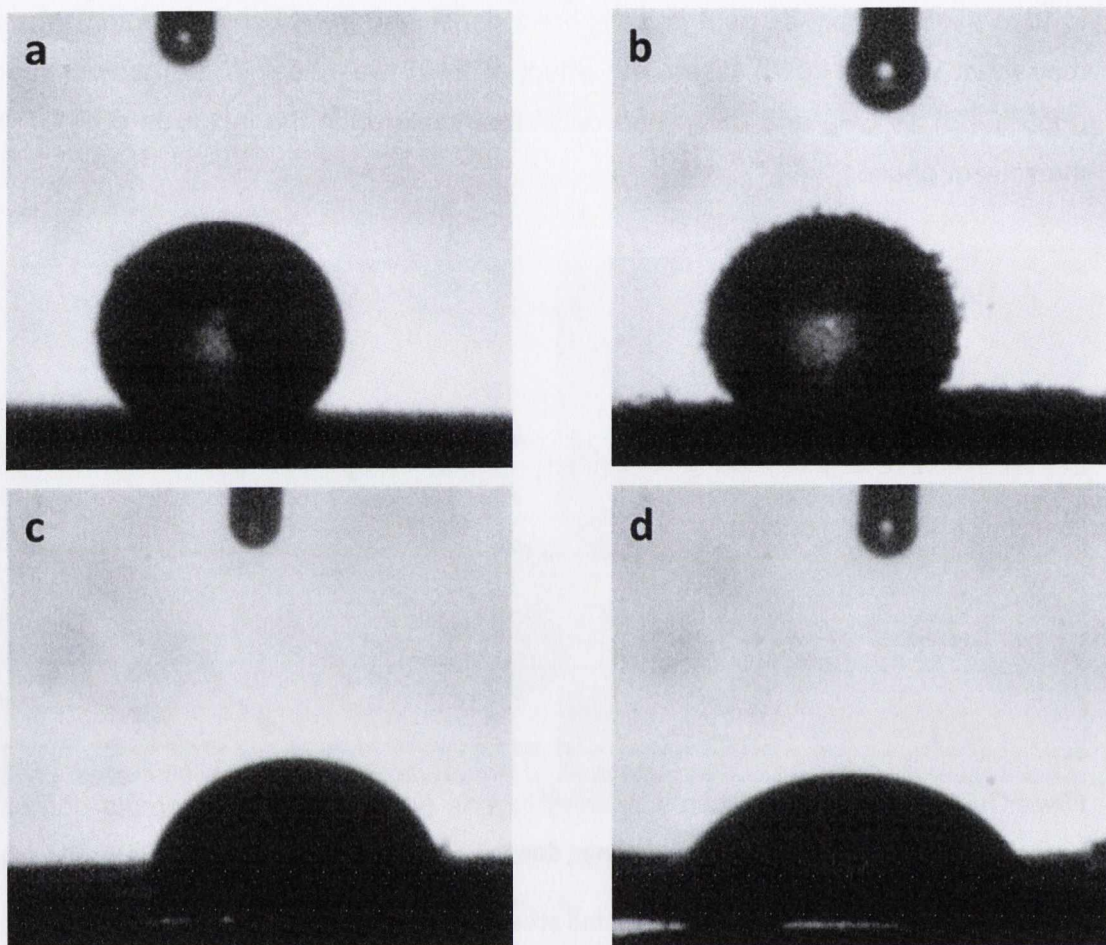


Figure 4.15. Contact angle measurement of: (a) the starting material and the three crystal habits: (b) CH_{tw}, (c) CH_{pl} and (d) CH_{sh}.

DVS was used to more fully investigate the effect of different habits on the interaction with water molecules. The correlation between the surface polarity and its ability to adsorb water has been demonstrated before for materials such as carbon nanotubes (Bradley et al. 2010). Nevertheless, the influence of surface polarity, due to the crystal habit manipulation, on water adsorption/absorption properties is not studied often. Therefore, it was of a great interest to investigate this effect especially that it has a direct impact on dissolution behavior and physical stability (Puri et al. 2010). When we only consider the total amount of sorbed water (Figure 4.16), there was no difference between the behavior of the three different habits.

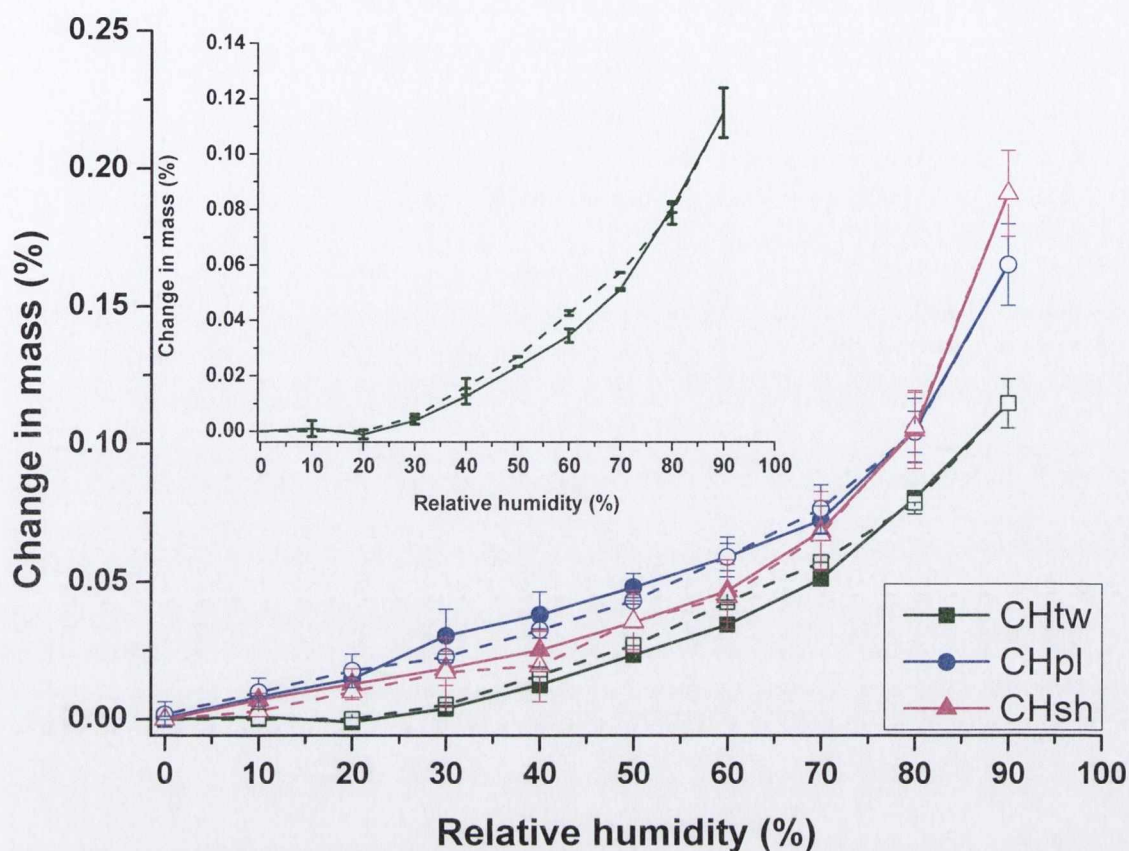


Figure 4.16. DVS sorption-desorption isotherms illustrating the total water uptake for the three principal ITR crystal habits. Closed symbols with solid line represents sorption cycle while open symbols with dashed line represents desorption cycle. CH_{tw} data is replotted in the inset to illustrate hysteresis.

Therefore, there was a need to establish a quantitative correlation between the distribution of water in the different systems and the % RH via fitting the DVS data with the Young and Nelson model (Eqns. 4.2 and 4.3). Using this model, the difference in water distribution within the three systems was demonstrated (Figure 4.17). In general, the model showed a good fitting for all crystal habits as inferred from the R-sq values.

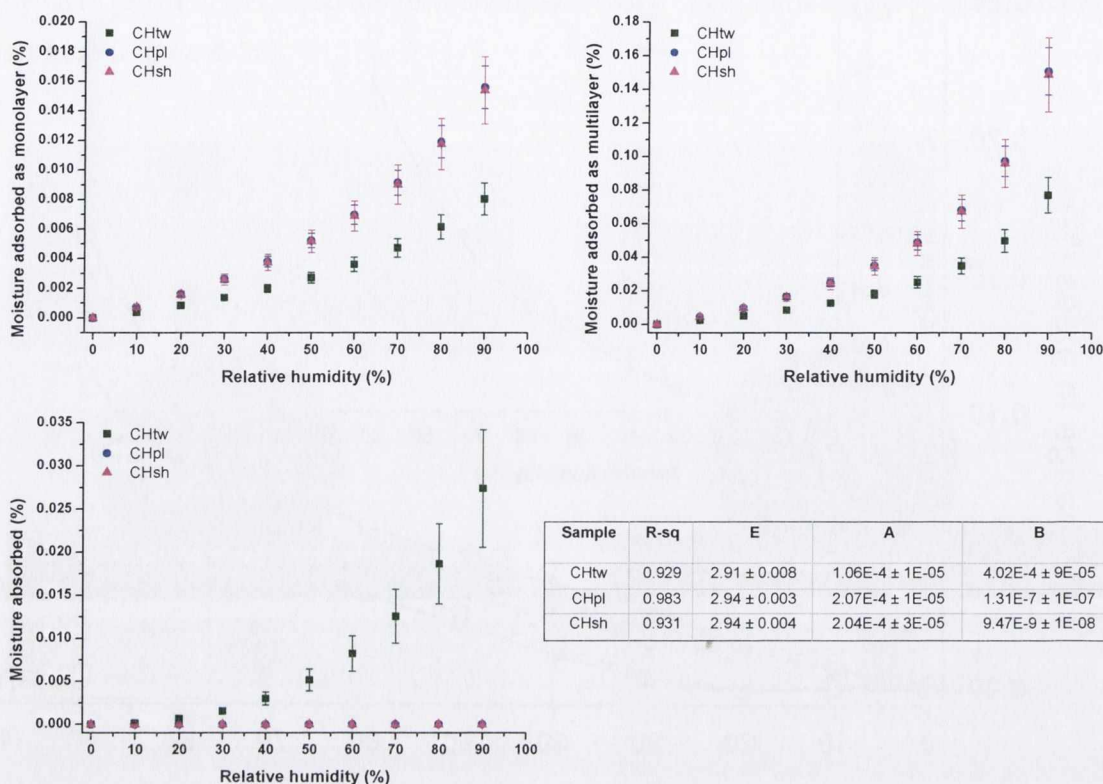


Figure 4.17. Moisture distribution for the three different habits as (a) adsorbed monolayer, (b) adsorbed multilayer and (c) internally absorbed and a summary table of the estimated parameters E, A and B based on the Young and Nelson model and fit correlation coefficients for all samples.

The amount of water adsorbed, as mono and multilayer, on the surface of CH_{pl} and CH_{sh} crystals was comparable and it was about twice that adsorbed on CH_{tw} crystals as shown in Figure 4.17a and b. This can be confirmed by the values of A parameter, which was comparable for CH_{pl} and CH_{sh} and was twice that of CH_{tw}. These findings are consistent with the wettability results and further indicate that more hydrophilic groups are exposed on the surfaces of CH_{pl} and CH_{sh} than on the CH_{tw} surfaces. The comparable moisture adsorption isotherms for CH_{pl} and CH_{sh}, even though CH_{sh} showed a slightly better wettability value, can be due to the fragility of CH_{sh} (Figure 4.18) crystals, which can lead to the exposure of other hydrophobic faces upon breakage. However, in the contact angle measurements only the wettability of (010) face is preferentially probed due to the preferred orientation effect. In contrast, the CH_{tw} sample absorbed statistically significantly ($p \leq 0.05$) more water ($0.027 \pm 0.006\%$) and exhibited a higher B parameter than the CH_{pl} and CH_{sh} samples, which practically did not absorb any moisture. Although the

amount absorbed was very small, it was a surprising behavior by otherwise highly hydrophobic ITR crystals. This minute water absorption could possibly be attributed to the presence of crystal packing defects due to the twinning boundary which could allow some water diffusion above 40% RH.



Figure 4.18. SE micrograph displaying the fragility of CH_{sh} as can be inferred from the presence of fines in the sample.

In a perfectly packed crystal, based on Mercury 3.5.1 calculations (Barbour 2006), ITR possesses lattice voids (Figure 4.19), which can fit a molecule with a maximum radius of approximately 1.16 Å. This value is smaller than the radius of a water molecule (1.5 Å) (Hermann 1972). In other words, there is no space available for a single water molecule in a defect-free crystal. The presence of some internally absorbed water can also be inferred from the hysteresis observed for CH_{tw} (Figure 4.16), where more water is being held during the desorption cycle than during sorption as previously demonstrated in Chapter 2 (York 1981).

This variation in moisture adsorption/absorption behaviour might be translated into variation in the dissolution profile of these crystal habits. CH_{tw} is expected to exhibit better water penetrability owing to the presence of twinning defects in the crystal. On the other hand, the presence of fines in the CH_{sh} is expected to increase the surface area of the crystals which in turn can increase dissolution according to Noyes-

Whitney equation (Eqn 1.1). However, the presence of those fines could expose more hydrophobic surfaces and reduce crystal wettability.

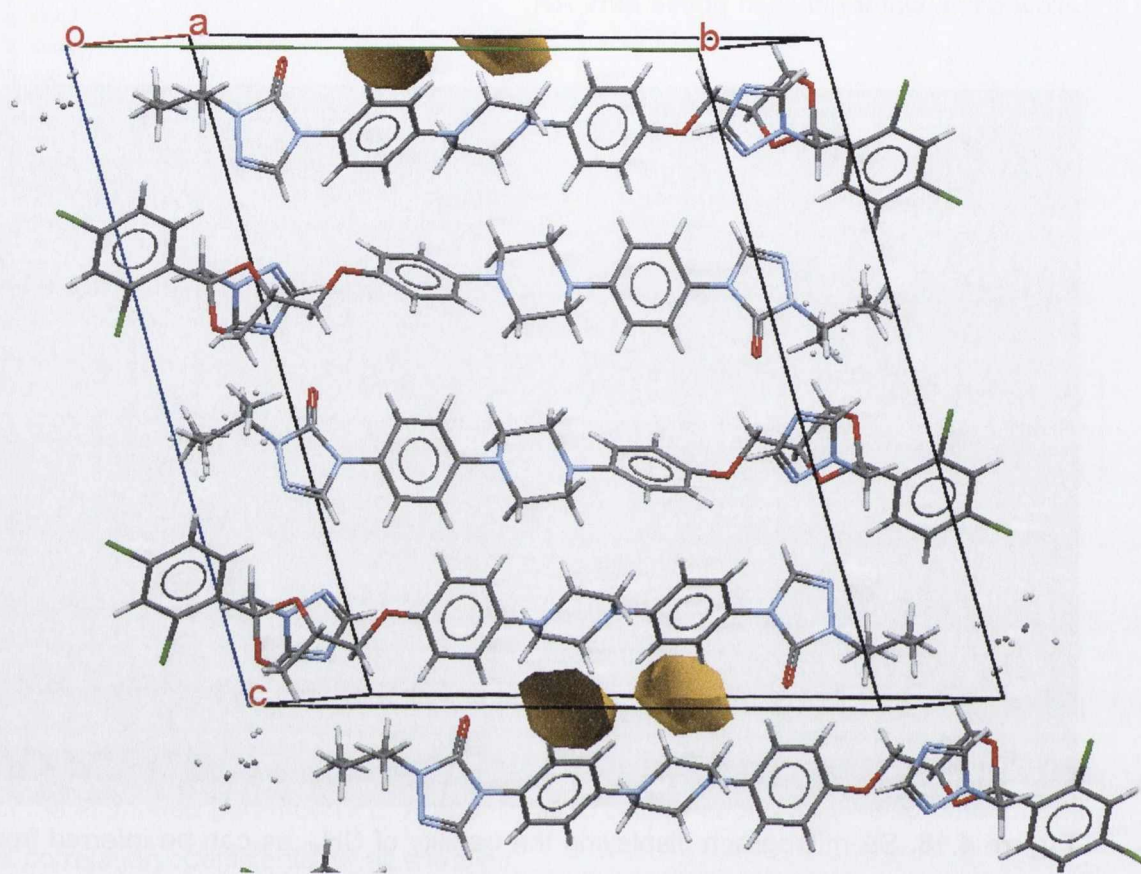


Figure 4.19. Packing arrangement of ITR in the unit cell showing the voids (yellow contact surface) generated using a probe radius of 1.16 Å.

PRXRD investigations of the three crystal habits following a full DVS cycle (sorption and desorption) (Figure 4.20) revealed that they are physically stable under the investigated conditions.

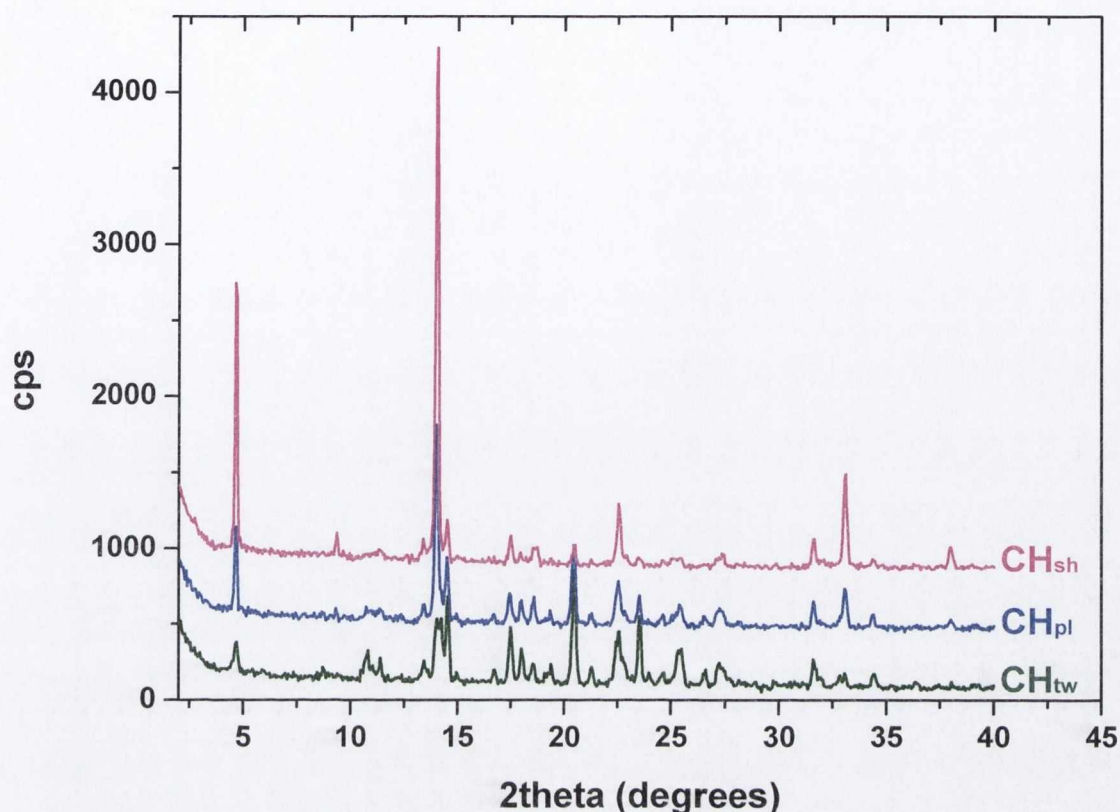


Figure 4.20. Overlay of PRXRD diffractograms of the three principal crystal habits following DVS analysis.

4.4. Conclusions

Itraconazole has been shown to form three distinct crystal habits/intergrowths when crystallized from a mixture of protic/aprotic solvents, in the presence of P407, due to the isotropic chemical functionalities on crystal facets, which interact differently with components of the crystallization mixture. A liquid-liquid crystalline phase separation was observed preceding the crystal growth phase. The shape of the final crystals is governed by the protic/aprotic solvent ratio, polymer concentration and crystallization temperature. The twinned blade-like CH_{tw} crystals exhibited highly hydrophobic surfaces and showed considerable permeability to water vapor due to the presence of twin boundary stacking defects. The CH_{pl} and CH_{sh} samples displayed comparable water adsorption/absorption behavior. These two habits were practically unable to absorb water although their surface can adsorb twice the amount of water molecules than CH_{tw}, which could be ascribed to the large 010 crystal facet surface area with the abundance of hydrophilic functionalities. This study expands investigations of the impact of crystal habit manipulation on API's

physicochemical properties beyond the well-known and established solubility improvement approaches, extensively studied in the literature.

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CHAPTER V: A Rare Case of Mesomorphic Behavior – Molecular Reorientation of Itraconazole Liquid Crystal Induced by a Hygrothermal Treatment

5.1. 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 (Muller-Goymann 2002). LCs can be categorized 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 (Six et al. 2001, Bunjes and Rades 2005). Other molecular arrangements including cubic and hexagonal columnar phases can also be observed in TLCs (Demus et al. 1998). 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 (Holmberg 2002). Some molecules, referred to as amphotropic, can exhibit both, TLC and LLC phases (Demus et al. 1998).

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 (Bunjes and Rades 2005, Shashidhar 1977). 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 (Bunjes and Rades 2005). Some pharmaceuticals have been reported to form LCs, including MK-571 (L-66071, a leukotriene D4 receptor antagonist), nafoxidine hydrochloride, palmitoyl propranolol hydrochloride, arsphenamine, penbutolol sulfate and cyclosporine (Bunjes and Rades 2005, Vadas et al. 1991). Among those, the first three exhibit the amphotropic behavior.

Water vapor 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 (Kontny et al. 1987, Giron et al. 2002). However, a few reports on a crystal to liquid crystal, MK-571 (Vadas et al. 1991) and trifluoperazine dihydrochloride (Chen 2009), and a liquid crystal to the isotropic phase transition (GNE068 (Chakravarty and Lubach 2013)) following the water vapor uptake are available in the literature for pharmaceuticals.

In addition to the amphotropic behavior, 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 (Demus et al. 1998, Kim et al. 2012, Tanaka et al. 2012). 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 (Kolbel et al. 2000). However, to the best of found 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. These transitions were assigned to the formation of the nematic and smectic A LC phases, respectively (Tarnacka et al. 2013, Six et al. 2001). The formation of ITR LC nanoparticles using antisolvent precipitation is described in Chapter 2. 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 as shown in Chapter 3. In Chapter 4 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 is described. The aim of this work was to demonstrate and characterize 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 behavior 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 crystallization offers a new approach of formulating pharmaceuticals into LCs, where feasible.

5.2. Materials and Methods

5.2.1. 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.

5.2.2. Production of ITR samples

Mesomorphous microparticles of ITR (ITR-N, median particle size of $3.1\ \mu\text{m}$ 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 $40\ ^\circ\text{C}$, outlet temperature of $34\ ^\circ\text{C}$, atomizing 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).

5.2.3. Investigation of ITR-N ordering using saturated salt solutions in an oven

ITR-N samples (50 mg) were equilibrated over solutions of saturated salts at $50\ ^\circ\text{C}$. Saturated solutions of salts were used to provide certain relative humidities as described by Nyqvist (1983), with slight modifications when necessary to achieve the desired values as shown in Table 5.1 below. The relative humidities were monitored using a Sensirion SHT75 sensor (Sensirion, Switzerland). The phase change following 1 hr incubation of ITR-N at the above conditions was examined using WAXS.

Table 5.1. Saturated salt solutions with the corresponding relative humidities at $50\ ^\circ\text{C}$.

Salt	NaCl:MgCl ₂ (1:2 w/w)	NaBr:KBr (1:1 w/w)	NaCl	K ₂ SO ₄
RH (%)	50%	60%	75%	95%

5.2.4. Dynamic vapor sorption

ITR samples were analyzed with a Dynamic Vapour Sorption (DVS) Advantage-1 automated gravimetric vapor sorption analyzer (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/p_0) 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 vapor 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 visualize 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 5.1) to enable the texture investigation using a glass cover slide as described later in Section 2.8.

DVS was utilized 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 $200 \text{ cm}^3/\text{min}$ for all experiments. The slope method was employed to calculate the diffusion coefficient (D_{ba}) (Yu et al. 2008).

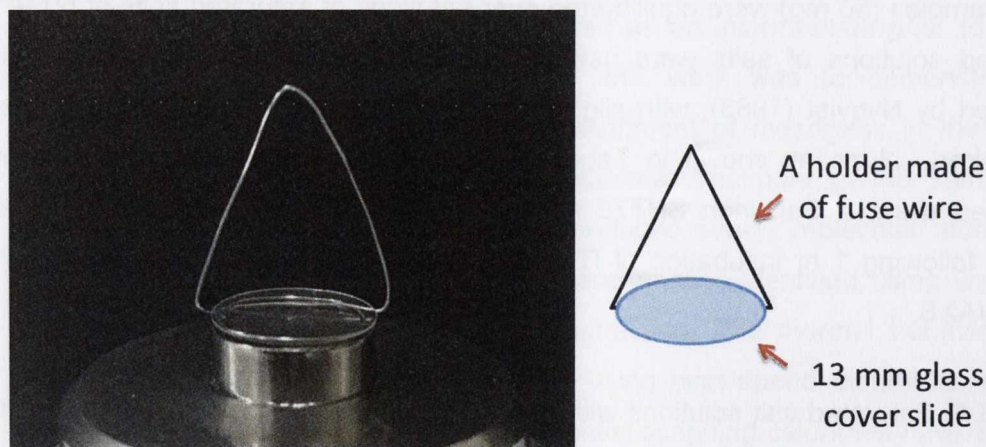


Figure 5.1. An in-house made cover slide holder composed of fuse wire, which was used to hold the cover slide for the hygrothermal treatment in DVS presented in Figure 5.16. Two holders were used, one for the sample and one for the reference, with masses of 44.1 mg and 42.5 mg, respectively.

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 (Burnett et al. 2004).

5.2.5. 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° or 5-35°, at 25 °C, as described previously in Chapters 3 and 4. 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 parallelized, monochromated Cu K α source (Goebel mirror, Ge(220) double bounce monochromator). Scanning with 666 s/point with effective 0.1 mm slits was applied as performed in Chapter 3. SAXS samples were analyzed by Dr. Karsten Fleischer, School of Physics and Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, Dublin 2, Ireland.

5.2.6. 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 aluminum stubs using carbon tabs. Samples were sputter-coated with a layer of gold/palladium under vacuum before analysis.

5.2.7. 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 aluminum pans. The heating rates employed were 100 and 300 °C/min. The temperature calibration was performed using pure indium. Presented results are the average of at least duplicate analyzes (Curtin et al. 2013).

5.2.8. Modulated Differential Scanning Calorimetry (MDSC)

Approximately 3-5 mg of a powdered sample was analyzed using a DSC Q200 (TA Instruments, UK) in a crimped Tzero aluminum 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 analyzed using Universal Analysis® (version 4.7A) software.

5.2.9. Polarized Light and Hot Stage Microscopy

Polarized light (PL) and hot stage (HS) microscopy were performed with an Olympus BX53 polarizing 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.

5.3. Results and discussion

ITR has been reported to form two distinct, bulk thermotropic LC phases (nematic and smectic) upon a thermal treatment (Tarnacka et al. 2013, Mapesa et al. 2014). In Chapter 3, the ability of ITR to form LC nanoparticles with nematic and smectic assemblies locked in the nanospheres is described. 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 changes.

5.3.1. Liquid crystalline phase assignment

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 5.2. It should be highlighted that using WAXS and conventional DSC alone, this liquid crystalline phase can be misinterpreted as amorphous. This is suggested by the fact that the values of heat of transitions obtained by integration of the melting endotherm at around 168 °C (Δh_{melt}) and under the crystallization peak shoulder at around 120 °C (Δh_{cryst}) were the same. A similar issue with the correct phase identification has been reported for spray dried cyclosporine and quenched ursodeoxycholic acid (Lechuga-Ballesteros et al. 2003). However, in the current case, the presence of a broad diffraction peak in addition at $3.00^\circ 2\theta$ recorded by SAXS, characteristic of a nematic arrangement of ITR molecules, and birefringence by PLM (as shown in Figures 5.3 and 5.4) supports this phase assignment of spray dried ITR material, further referred to as ITR-N. Samples referred to as peak (P), valley (V) and shoulder (S), based on the sorption kinetic profile (Figure 5.5).

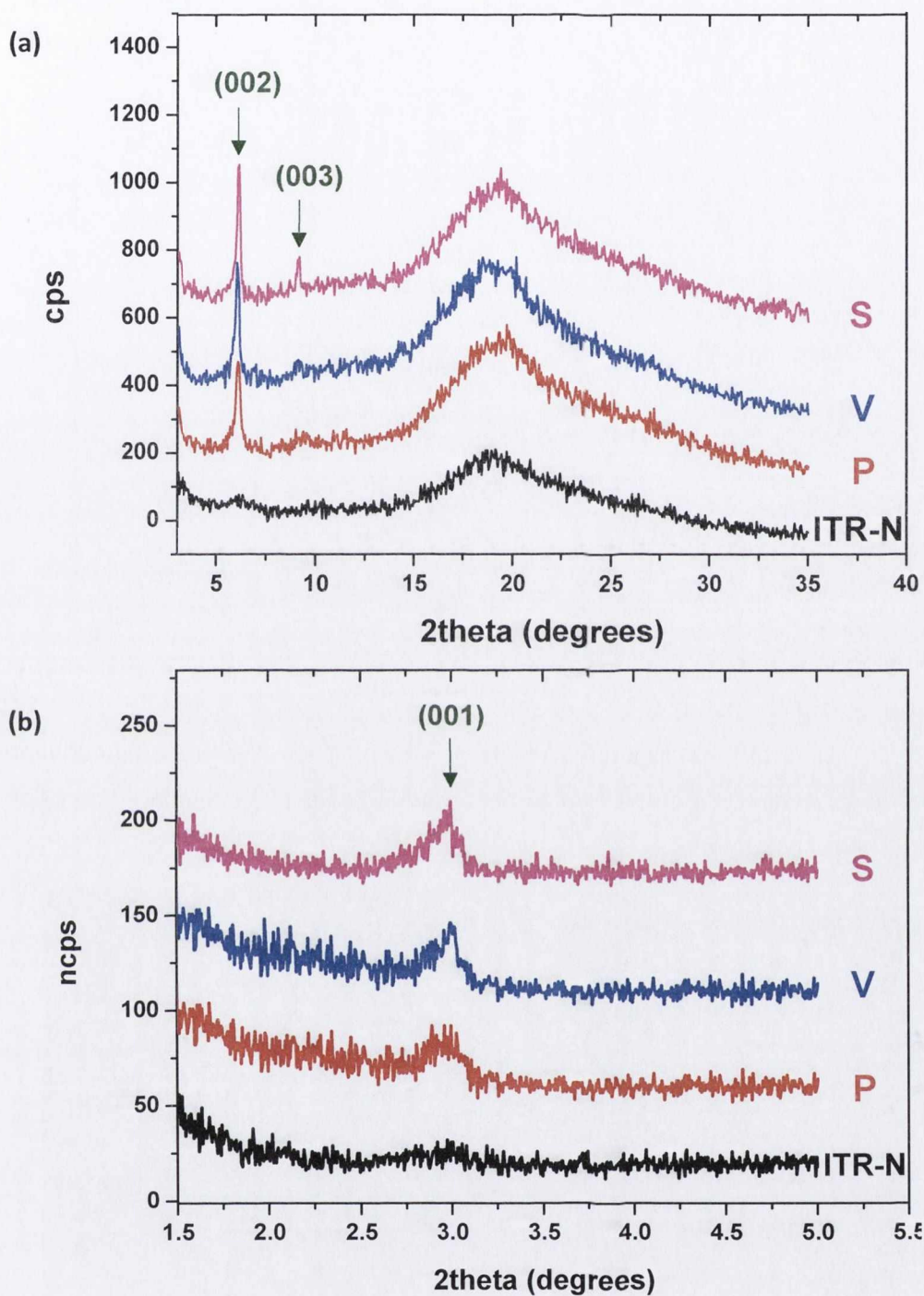


Figure 5.2. (a) WAXS diffractograms of ITR-N, P, V and S with the peak at 6.05° 2θ displaying the highest peak intensity in case of S, (b) SAXS diffractograms showing the diffuse maxima at 3.00° 2θ for ITR-N and the increase in peak intensity in P and S. Arrows indicate (001), (002) and (003) quasi-Bragg diffraction peaks of LC phases, symbols S, V and P represent samples of ITR-N at the different stages of hydrothermal treatment by DVS as described in text. cps – counts per second, ncps – normalized counts per second.

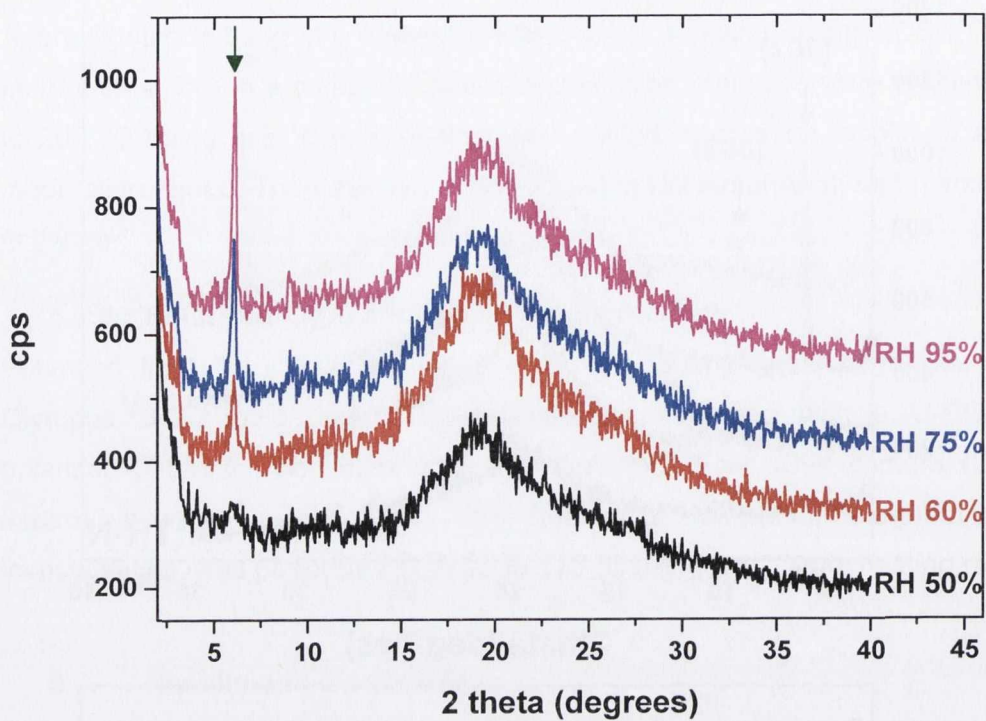


Figure 5.3. WAXS diffractograms illustrating phase changes following incubation of ITR-N at different RH for one hour. The arrow indicate a quasi-diffraction peak at 6.05° 2theta of smectic ITR, cps – counts per second.

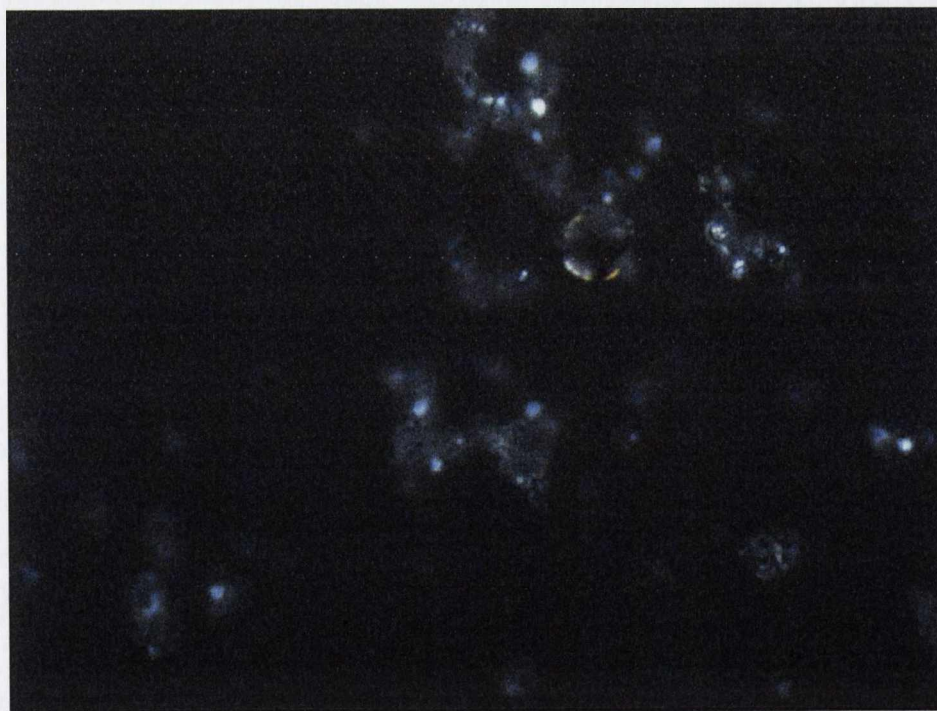


Figure 5.4. PLM of ITR-N at 25°C (as recovered from the spray dryer) (500 magnification).

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. Following storing ITR-N at the abovementioned conditions for one hour, samples were taken for WAXS investigation. WAXS results, as displayed in Figure 5.3, illustrate the absence of the characteristic peaks of crystalline ITR (Figure 5.4) 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 phase. The positions of quasi-Bragg peaks were different to those of any Bragg peaks characteristic of the crystalline sample (Figure 5.4).

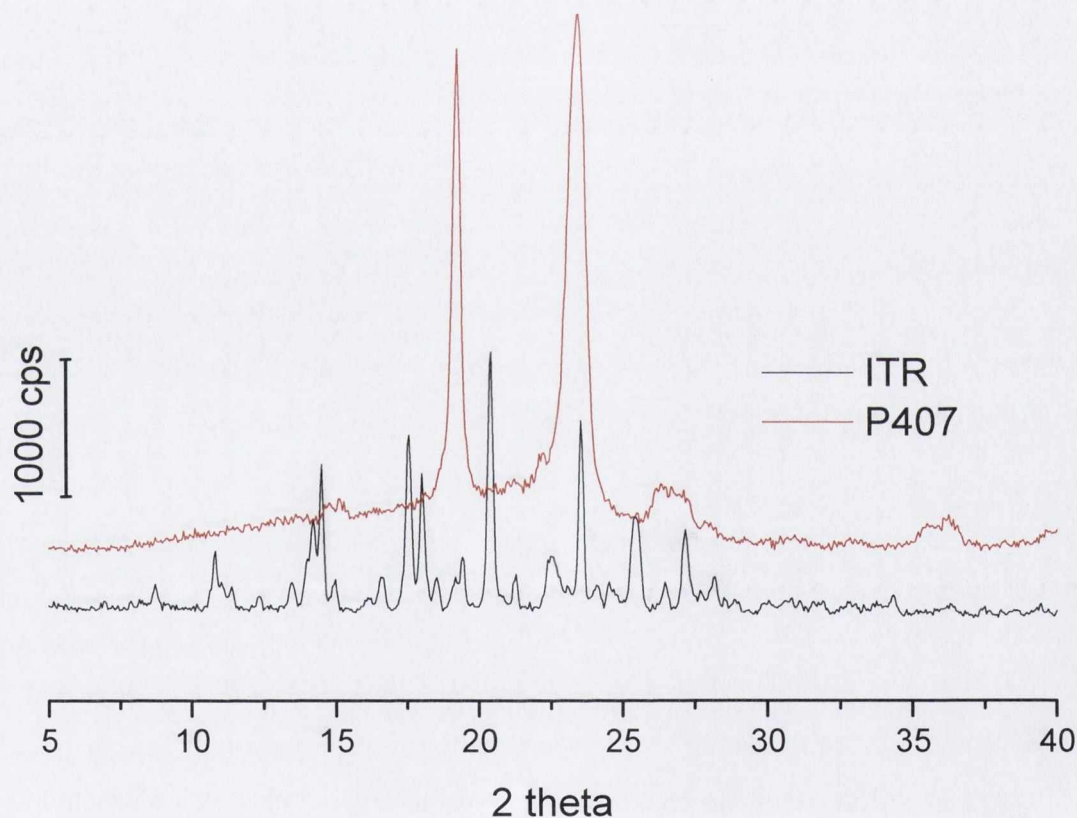


Figure 5.4. Powder X-ray diffractograms of itraconazole (ITR) starting material powder and Poloxamer 407 (P407) starting material powder.

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).

The kinetic data obtained during water sorption of ITR-N at 95% RH (constant humidity condition by DVS) and 50°C , presented in Figure 5.5, 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.

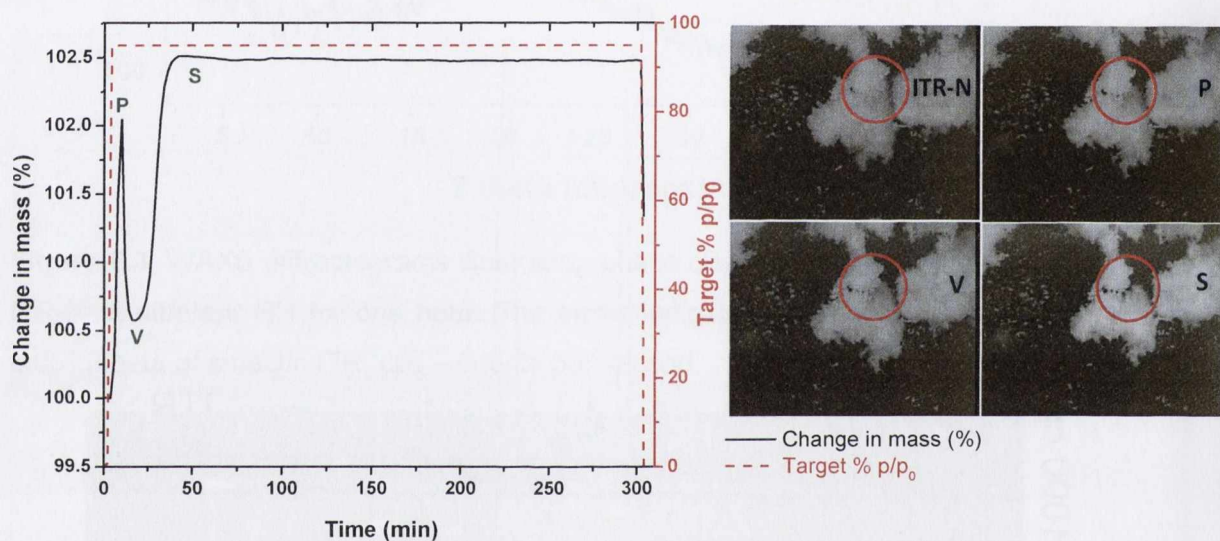


Figure 5.5. Water sorption kinetic profile for ITR-N at 95% RH (50°C) for 300 min by DVS displaying the major transitions of ITR-N to P, V and S samples (left) and the corresponding images captured during the run (right).

There was no further phase change observed when the sample was subjected to a second cycle in DVS (the upper profile in Figure 5.6). 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 5.6).

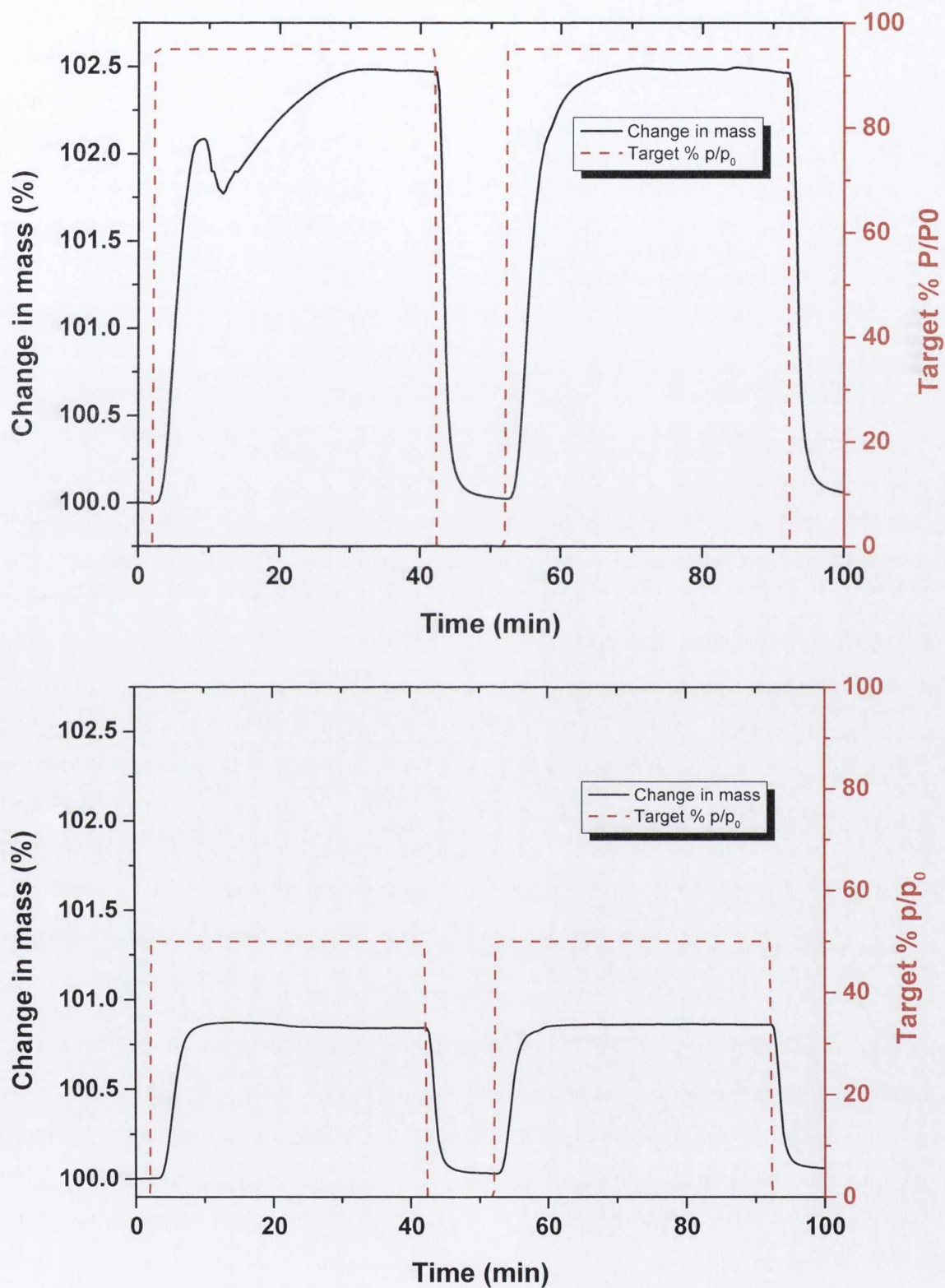


Figure 5.6. Kinetic profiles (by DVS at 50 °C) of two cycles of water sorption for ITR-N at 95% RH (upper panel) and 50% RH (lower panel).

WAXS and SAXS profiles characteristic of the three samples (P, V and S) are presented in Figure 5.2. 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 phase as discussed in Chapter 3. WAXS data is consistent with the visible changes to the ITR-N sample as seen in the captured video images presented in Figure 5.5. 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 behavior has not been reported before for mesophases and it is similar to the water expulsion observed during crystallization of amorphous materials (Burnett et al. 2004).

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 5.5 and an increase in order as can be seen from WAXS data (Figure 5.2a). The SAXS data is displayed in Figure 5.2b 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: $\theta = \frac{Q\lambda}{4\pi}$ (Demus et al. 1998). Here, Q is the diffraction vector and positioned at $\frac{2\pi}{l_o}$ where l_o is the molecule length and λ is the X-ray wavelength. The second diffuse maximum at Q of $\frac{2\pi}{w_o}$, where w_o is the molecule width (Demus et al. 1998), 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 5.2b) compared to that of ITR-N indicating a phase switching (Mcmillan 1972).

Similar phase changes were observed for ITR-P407, however they were followed by a crystallization event, which can be ascribed to the effect of P407 inducing crystallization of ITR (Chapter 3). The magnitude of the mass change during the transition from P to V was lower than that observed in the absence of P407 (Figure 5.7). 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 crystallization event was confirmed by WAXS (Figure 5.8) and it was associated with powder shrinkage as can be seen in Figure 5.7.

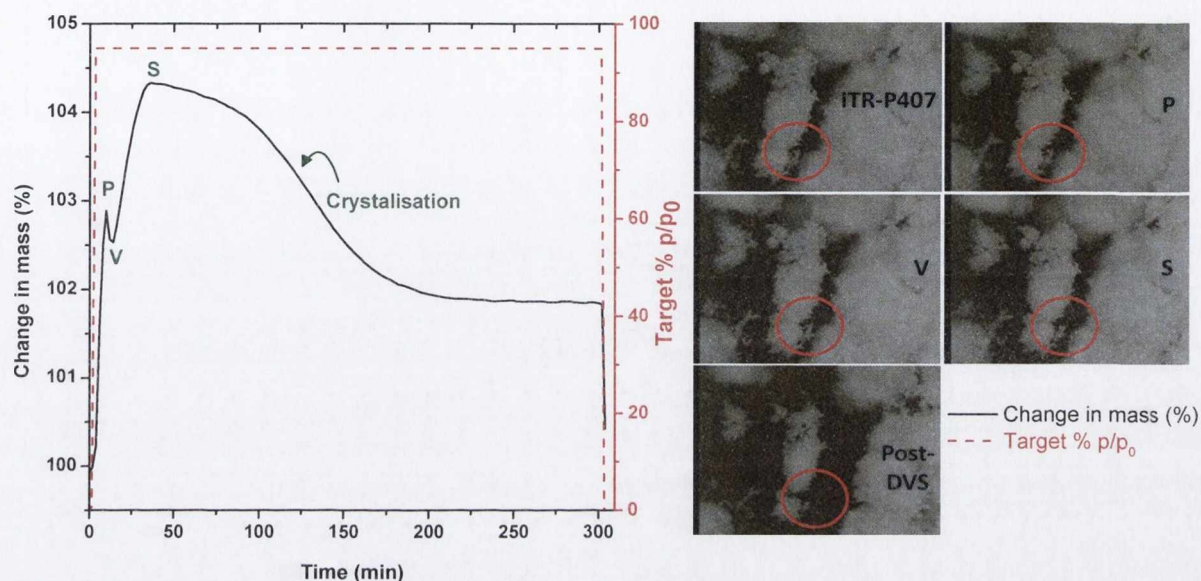


Figure 5.7. Water sorption kinetic profile for ITR-P407 at 95% RH (50 °C) for 300 min displaying the transitions of ITR-P407 to P, V and S samples (left) and the corresponding images captured during the run (right).

It should be emphasized that ITR-P407 also possesses a liquid crystalline assembly as can be inferred from WAXS and PLM results (Figures 5.8 and 5.9). These observations are in agreement with the current results of phase determination reported for ITR NPs containing P407. 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.

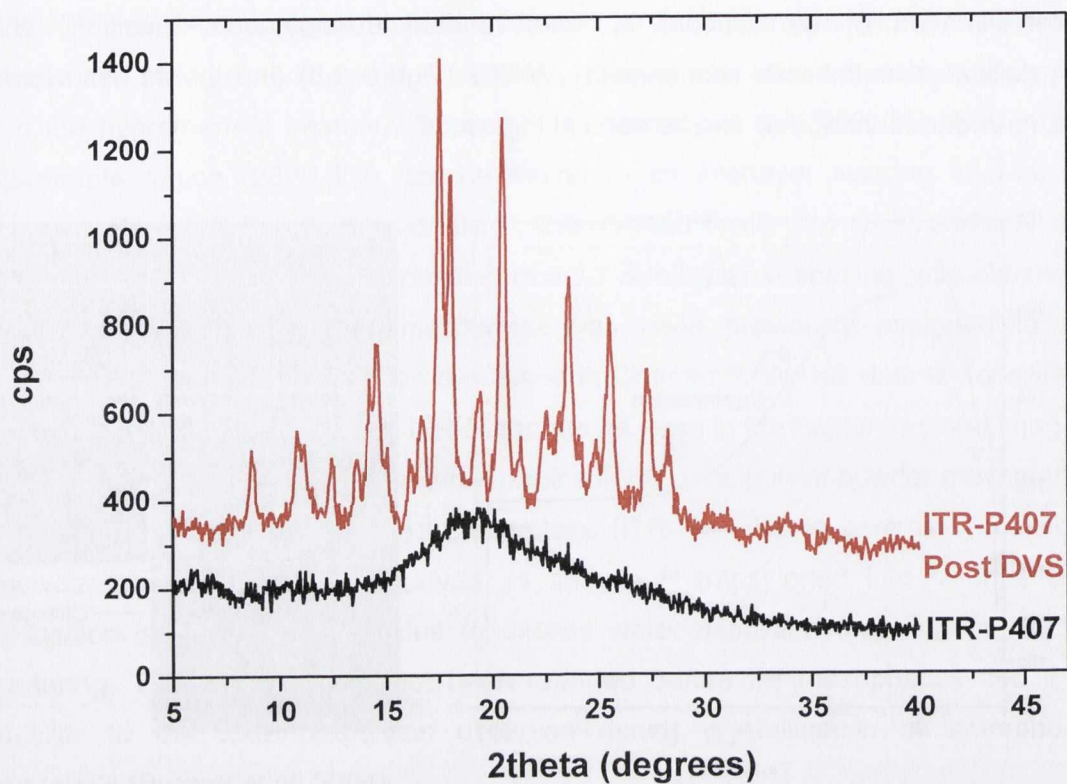


Figure 5.8. WAXS diffractograms of ITR-P407 and the same sample after 5 hr treatment in DVS at 50 °C and 95% RH. cps – counts per second



Figure 5.9. PLM of ITR-P407 at 25 °C (as recovered from the spray dryer) (500x magnification).

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 5.2), 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 5.2. 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 ± 3.52E-10	6.28E-10 ± 1.93E-11	4.90E-09 ± 6.79E-10

A step-wise kinetic sorption-desorption-resorption DVS profile (using 10% RH increments) at 50 °C (Figure 5.10) indicated the existence of a phase transformation at 60% RH in the sorption step and 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 5.10, and indicating reversibility of this transition. This reversible transition can be seen explicitly when 5% RH increments were used (inset in Figure 5.10). This reversible event is characterized 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 (Martin 2000).

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 5.6) indicating that the phase reversal is kinetically limited by the equilibration with the vapor phase at a certain RH.

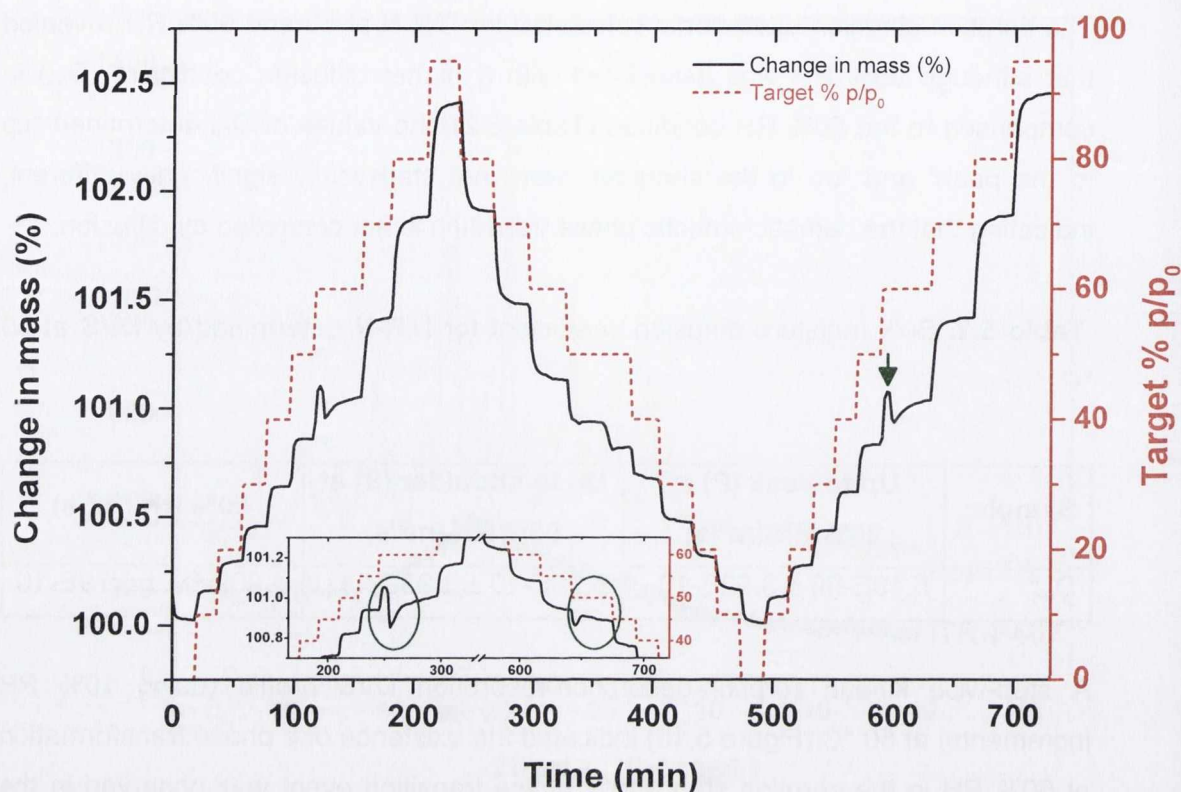


Figure 5.10. Water sorption kinetic profile (at 50 °C) for ITR-N obtained using a stepwise (10% RH) increase in humidity up to 95% RH and the reverse for desorption. The sample mass was allowed to reach equilibrium defined as $dm/dt \leq 0.002$ mg/min over 10 min before the RH environment was changed. 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 5.11). 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 5.12). 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.

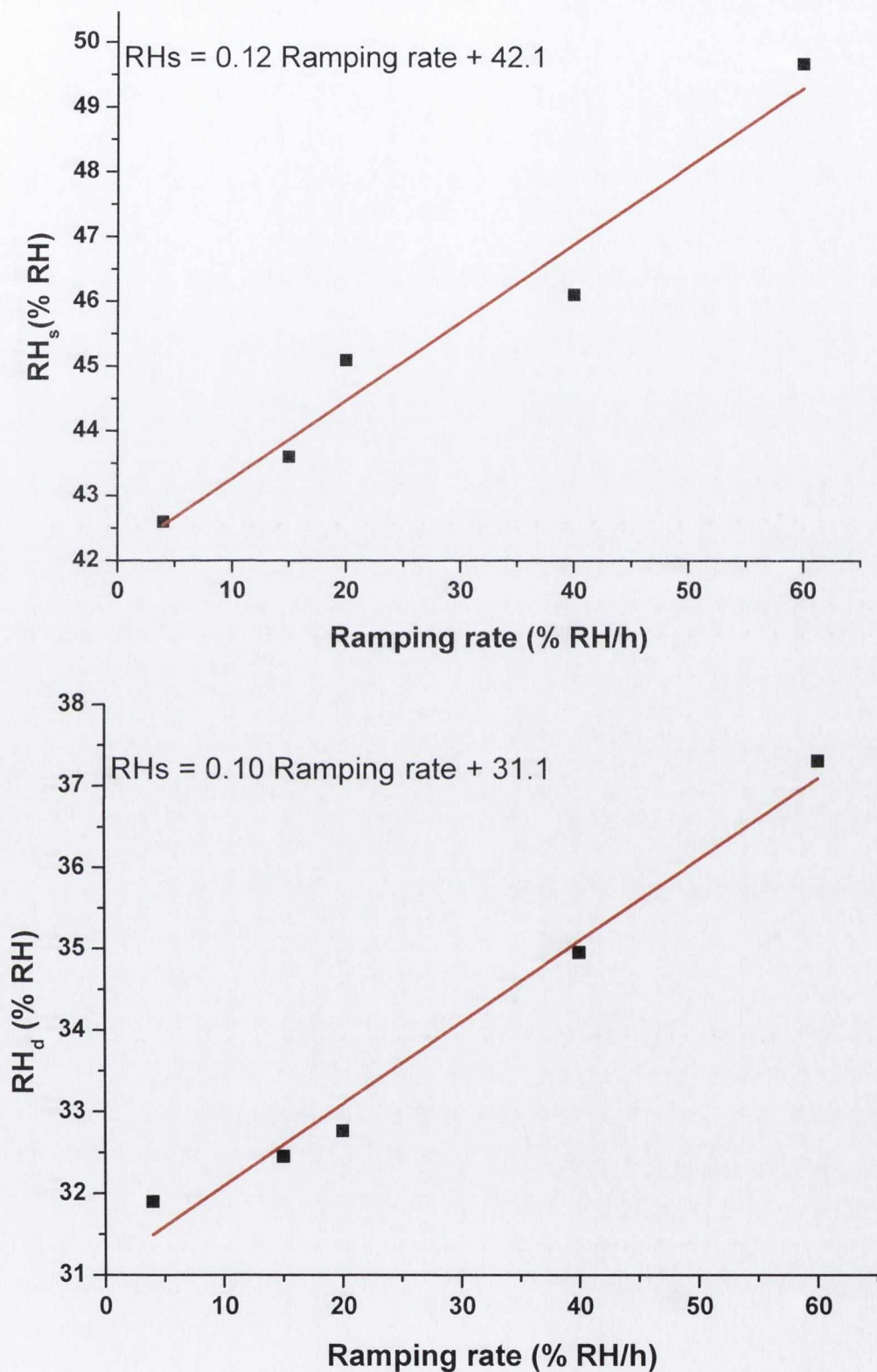


Figure 5.11. Critical relative humidity for ordering in the sorption cycle (RH_s) vs. relative humidity ramping rate (upper panel) and critical relative humidity for deordering in the desorption cycle (RH_d) vs. relative humidity ramping rate (lower panel) for ITR-N at 50 °C.

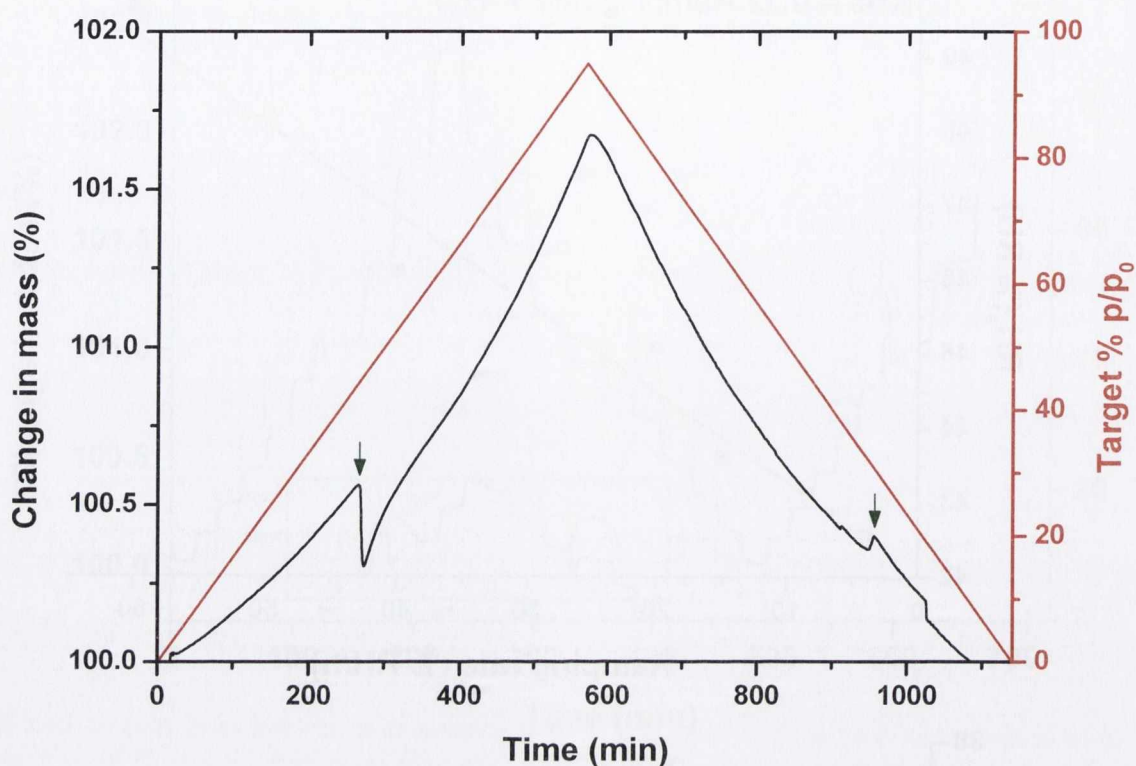


Figure 5.12. Kinetic profile of ITR-N sample upon changing the relative humidity from 0 to 95% at a ramping rate of 10% RH/h (50 °C).

5.3.2. Morphology investigation

SE micrographs of ITR-N and samples P, V and S are displayed in Figure 5.13. The ITR-N particles appear as collapsed spheres, which upon water sorption swell until the P state is achieved (Figure 5.13b). No observable changes were recorded for sample V (Figure 5.13c), 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 5.1). 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 5.13d, 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 5.13e).

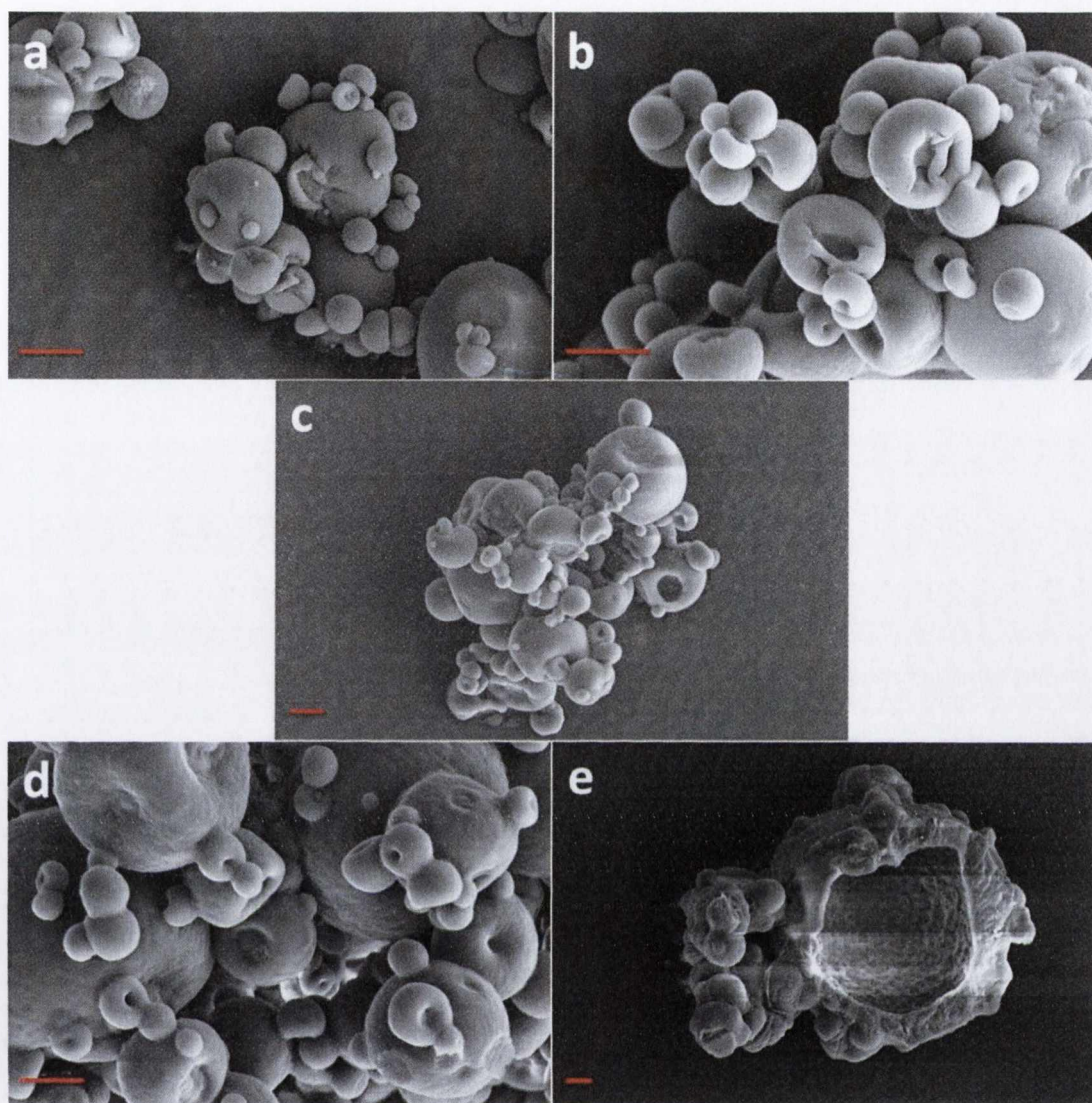


Figure 5.13. SE micrographs of (a) ITR-N, (b) sample P, (c) sample V (d) sample S and (e) sample post-DVS (DVS conditions as described in text). The red line corresponds to 2 μm .

Although the collapsed particles comprising this sample displayed a WAXS pattern similar to the original SD MPs (absence of smectic WAXS pattern) (Figure 5.14), 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 5.10) is presented in Figure 5.15.

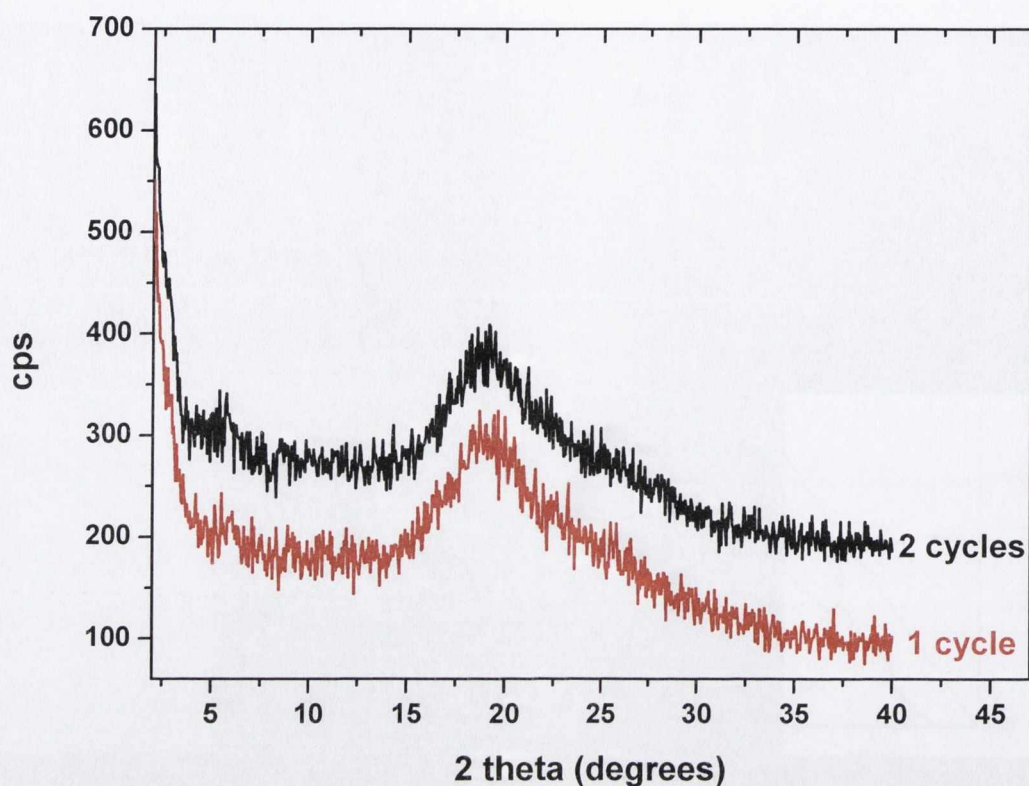


Figure 5.14. WAXS diffractograms of ITR MPs following one or two DVS cycles using dm/dt of ≤ 0.002 in DVS at 50 °C. cps – counts per second.

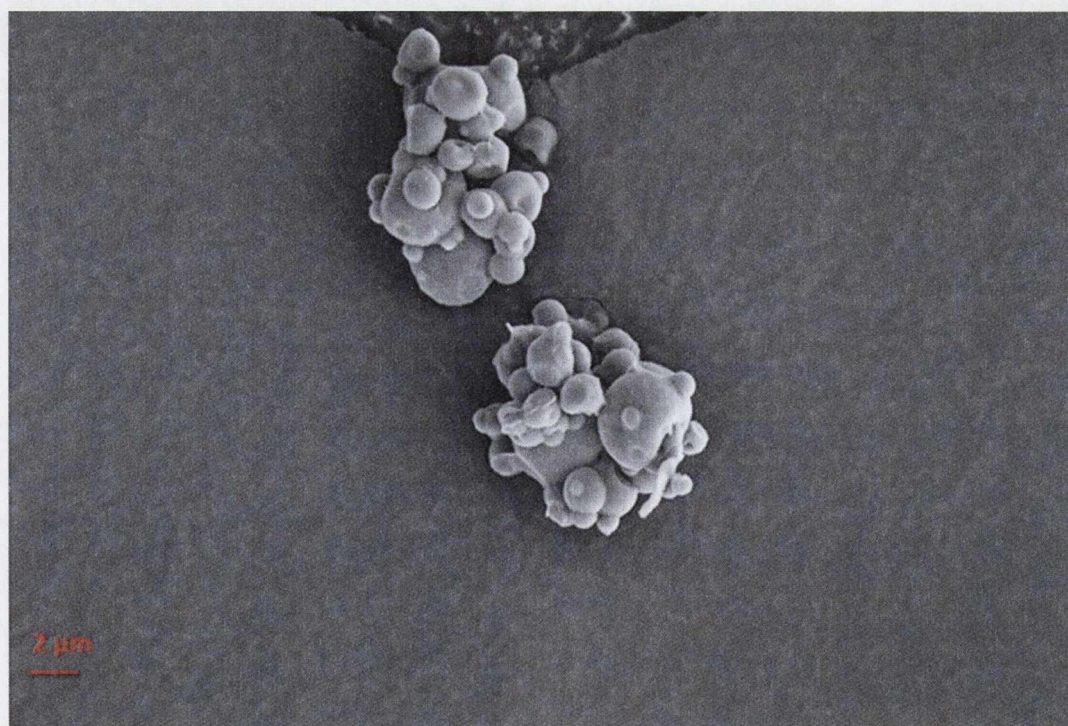


Figure 5.15. SE micrograph of ITR-N particles following resorption in DVS. Particles were collected at 90% RH in the second DVS sorption cycle, as shown in Figure 5.10.

5.3.3. Thermomicroscopic investigation

Figure 5.16 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 °C/min up to 110 °C. PL micrographs of the same area before (ITR-N) and after (sample S) exposure to 95% RH at 50 °C in DVS (Figure 5.16) revealed absence of any visible changes. However, the sample S exhibited a transformation upon heating at 70 °C and when the sample reached the first liquid crystalline transition, at 74 °C, particles started to merge with the neighboring small particles until larger, highly birefringent droplets formed at 88 °C. Those droplets underwent an endothermic phase transition (nematic to isotropic) 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 crystallization of the surrounding region.

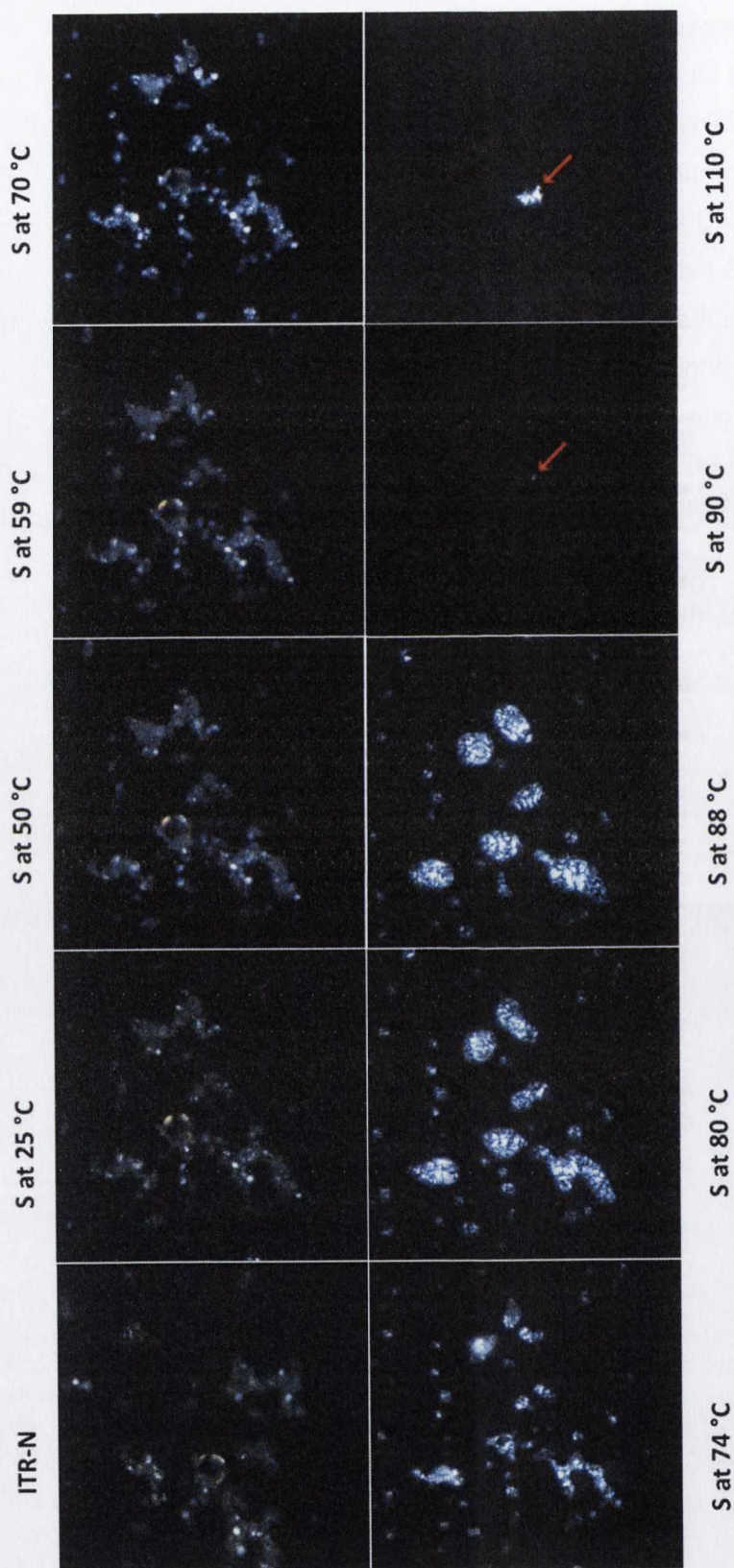


Figure 5.16 PL micrographs representing birefringence of nematic (ITR-N) and smectic (sample S) ITR TLC phases. The remaining micrographs illustrate the thermomicroscopic investigation of the sample S from 50 to 110 °C using HSM.

5.3.4. Thermal analysis

Results of thermal analysis using 100 and 300 °C/min scanning rates are displayed in Figures 5.17 and 5.18. Fast heating rates were used due to the increase in sensitivity of thermal events (Ford 2012). 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 5.17), 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 5.18). Modulated DSC thermograms (Figure 5.19) indicated that this is a non-reversing thermal event with a broad endotherm typical for desolvation process (Rabel et al. 1999).

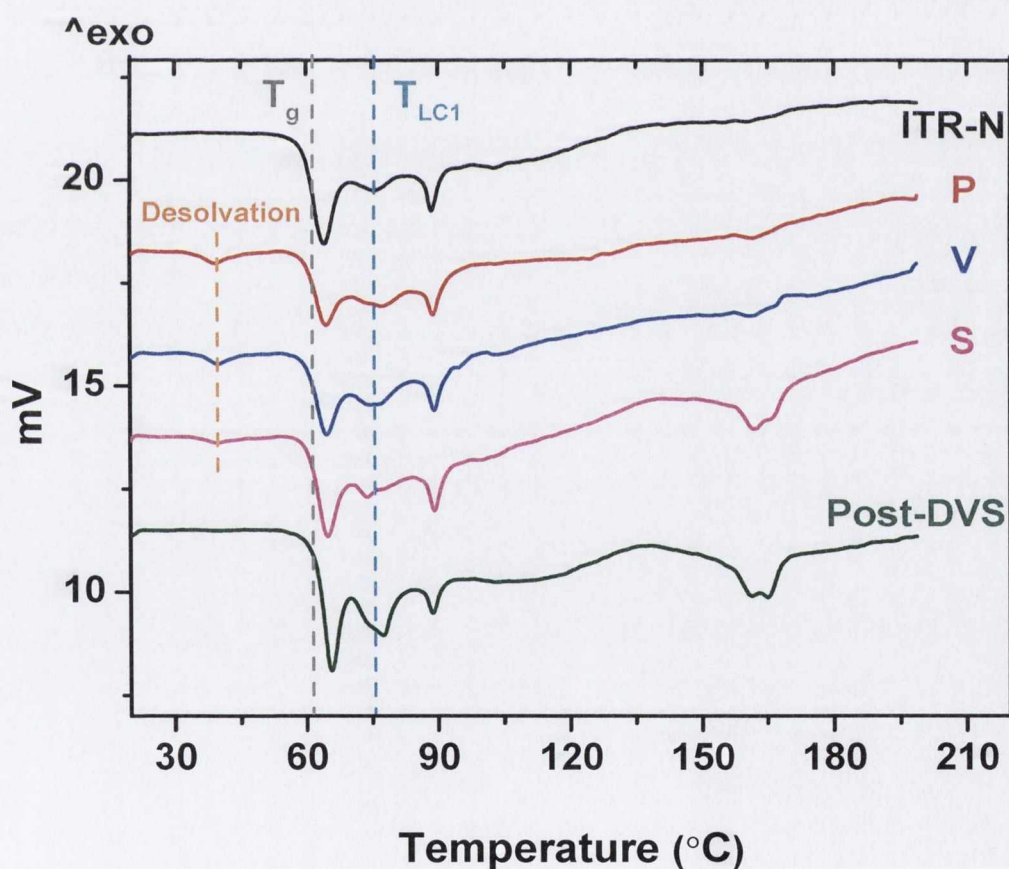


Figure 5.17. HSDSC scans of ITR-N, P, V, S and the sample following a DVS cycle (DVS conditions are described in text) at 100 °C/min (b). T_g – glass transition temperature, T_{LC1} – temperature of the first liquid crystalline transition. T_g broken line was added for visual guide and was positioned according to T_g of ITR-N.

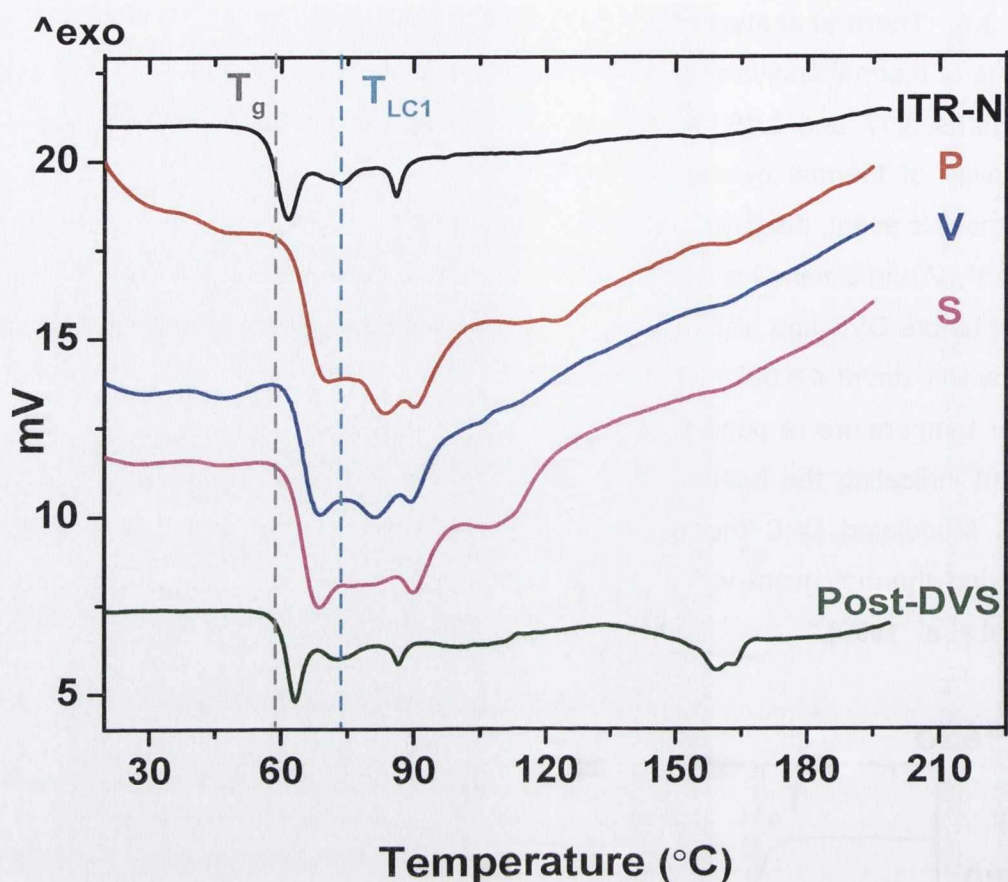


Figure 5.18. HSDSC scans of ITR-N, P, V, S and the sample following a DVS cycle (DVS conditions are described in text) at 300 °C/min (b). T_g – glass transition temperature, T_{LC1} – temperature of the first liquid crystalline transition. T_g broken line was added for visual guide and was positioned according to T_g of ITR-N.

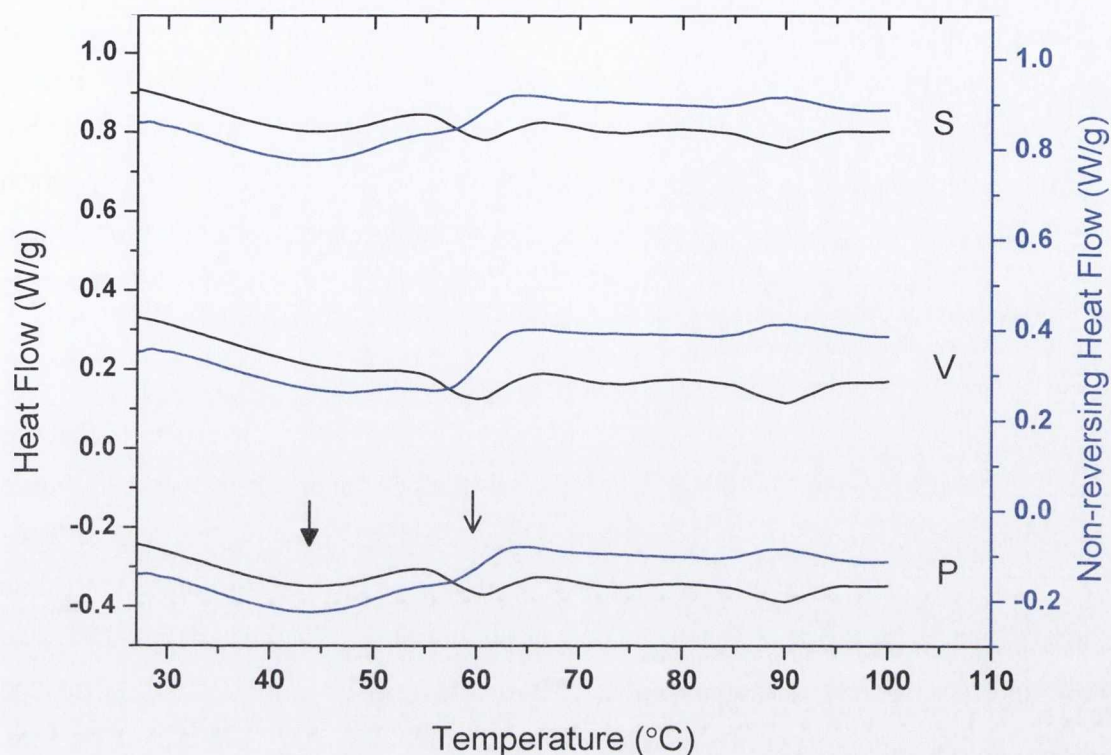


Figure 5.19. MDSC thermograms (total and non-reversing heat flow) of samples P, V and S. The non-reversing heat flow curves illustrate the loss of moisture represented as the broad endotherm at around 43 °C (closed arrow) and the enthalpic relaxation at around 60 °C (open arrow).

Glass transition temperatures (T_g s) varied depending on the scanning speed (Figures 5.17 and 5.18), which is in agreement with the observation reported by Lambert for polystyrene (Lambert 1969). The higher scanning rate (300 °C/min) was able to discriminate between the T_g s of the samples (Figure 5.18). 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 moisture (Liu et al. 2009). However, the increase in T_g in the samples P, V and S is attributed 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 regions (Zelezna and Hosney 1987).

Similarly, an increase in the T_g was observed upon immersing epoxy resins in water at elevated temperatures via promoting crosslinking and subsequently enhancing rigidity (Zhou and Lucas 1999). 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.

On the other hand, the lower heating rate of 100 °C/min was utilized to detect the effect of the hygrothermal history on physical destabilisation of the sample. Figure 5.17 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 (5.18). This clearly indicates that there was no sufficient time available to allow sample crystallization 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.

5.4. 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 °C (Six et al. 2001). This behavior is uncommon since, although it possesses some anisotropy, ITR molecule lacks terminal linear aliphatic chain(s) which is usually a common feature of TLCs, however it is not a prerequisite (Kolbel et al. 2000, Six et al. 2001). 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 complexes (Kolbel et al. 2000). This is the first account of this type of behavior for ITR 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 behavior is similar to the deliquescence/fluorescence typically exhibited by hygroscopic materials.

ITR is a hydrophobic molecule with very low aqueous solubility (Peeters et al. 1996). However, in Chapter 4, it is observed that ITR can interact with hydrogen-bond donors. The two faces of water have been reported by Chandler, where the reason for separation of water and oil in ambient conditions was determined to be due to the favorable hydrogen bonding between water molecules and not due to repulsion between oil and water molecules (Chandler 2002). 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 NPs with the smectic arrangement in an aqueous environment has also been observed as described in Chapter 3.

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.

5.5. 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 crystallization.

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CHAPTER VI: General Discussions, Key Findings and Future Recommendations

This Chapter provides a comprehensive discussion of achievements made by the research described in Chapters 2 to 5 followed by overall conclusions. At the end of this Chapter, future research recommendations are highlighted.

6.1. General discussions

As mentioned earlier in the introduction, the goal of this research project was to investigate unexplored and/or previously reported as infeasible formulation approaches, including crystal habit tuning, manipulating the state of matter of the drug and employing bottom up miniaturization techniques, to improve the pharmaceutical performance of ITR. Background information on ITR and the various reported techniques to enhance its pharmaceutical performance is summarized in Chapter 1.

In Chapter 2, the formation of 100% ITR nanoparticles using antisolvent precipitation technique from acetone (at 50 °C)/water (at 80 °C) (solvent:antisolvent) system was reported. The produced, stabilizer free, ITR NPs had diameters below 250 nm and possessed improved dissolution profiles compared to the spray dried ITR microparticles. The successful production of NPs, especially the stabilizer-free batch, was explained using the acetone induced phase separation and droplet formation according to the mechanism reported by Re and Biscans (1999). Minute drug-solvent droplets are produced upon injecting the solvent phase into the antisolvent phase. This process of droplet formation is promoted by the mechanical shear forces of injection and mixing (Re and Biscans 1999, Nocent et al. 2001, Yang et al. 2003). Each droplet then solidifies, due to the low aqueous solubility of ITR, to form a shell enclosing the solvent phase supersaturated with drug followed by solvent evaporation through the shell with the aid of the high temperature of the antisolvent phase (Figure 6.1).

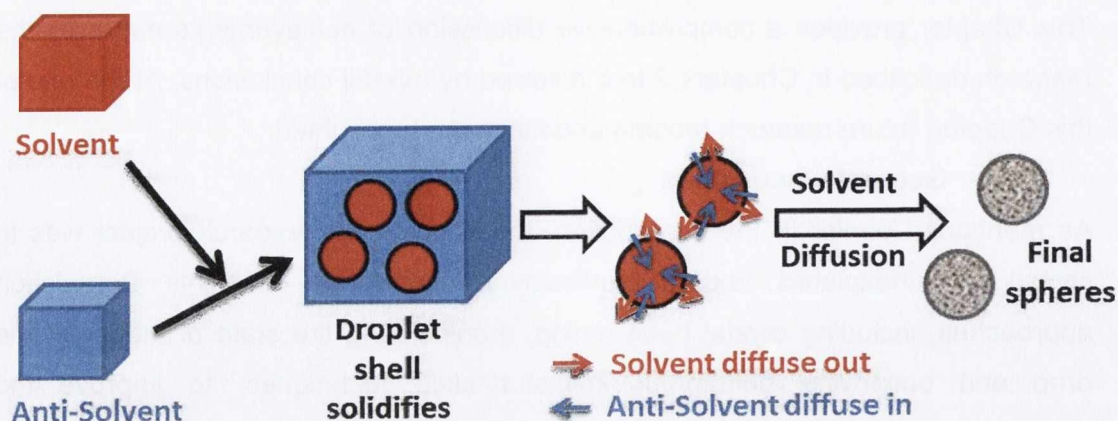


Figure 6.1. A schematic representation of the QESD method. Adapted from (Nocent et al. 2001).

The inclusion of polyethylene glycol or its methoxylated derivative, as a stabilizer, in the antisolvent phase resulted in statistically significantly smaller NPs, compared to those made of pure ITR, with lower polydispersity indices. The smallest particle size with the lowest polydispersity index (PDI) was observed with the use of the solvent:antisolvent v/v ratio of 1:10 for all samples. In addition, centrifugation was found to be an essential step before freeze drying to collect the NPs.

The thermal behaviors of the ITR NPs, without and with PEG or MPEG as stabilizers, were similar to that of the glassy ITR as described by Wang et al. (2004). The T_g s were all at approximately 60 °C followed by two endothermic transitions at 74 (T_{LC1}) and 90 °C (T_{LC2}) due to the formation of a smectic and nematic mesophases as described by Six et al. (2001). However, each ITR NPs sample underwent cold crystallization at a different temperature indicating that the presence of polymer influences the thermal stability of the drug with MPEG appearing to delay the crystallization event to 113 °C. Integration of the area under the crystallization exotherm and melting endotherm were utilized to measure crystallinity of ITR NPs using Eqn 2.1. According to this equation, the SD ITR MPs were assumed to be 100% amorphous and the stabilizer free NPs exhibited the highest crystalline content (24±1%), which contradicted the results of DVS studies using ethanol (Chapter 2). This inconsistency can be explained by the fact that the presence of hydrophobic polymers in ITRPEG and ITRMPEG NPs affected how these samples interacted with ethanol and water vapors. Upon close inspection of data on SD ITR MPs, the presence of the very broad hump at $6.05^\circ 2\theta$ by WAXS as well as the SAXS and PLM data (Figures 5.2 and 5.4) implied that this sample was not 100%

amorphous as assumed, but liquid crystalline. Unfortunately, Equation 2.1 is not designed to distinguish between the fully disordered (amorphous) and partially ordered (LC) phases. Thus, none of these two techniques, DSC and DVS, were deemed to accurately quantify the crystalline content. ITRMPEG NPs displayed the highest apparent solubility and extent of supersaturation compared to the ITR NPs, ITRPEG NPs and SD ITR MPs, which was correlated with the presence of the CH₃-group in MPEG possibly increasing the affinity of MPEG to the drug thus resulting in an improved NPs dissolution performance. Based on Whitney-Noyes equation (Eqn 1.1), it is considered that the improved dissolution profile is due to the augmented surface area associated with the nanosized particles and the enhanced solubility of the partially ordered LC phase compared to the tightly packed crystalline phase.

Chapter 3 further investigates tuning the properties of the produced NPs or nano-aggregates via manipulating the antisolvent process parameters. Solvent temperature was kept at 50 °C and the solvent:antisolvent v/v ratio was 1:10 in all experiments. The higher the temperature difference between the solvent and antisolvent phases ($\Delta T_{S:AS}$), the thinner the particle shell thickness and the lower the molecular order. The use of $\Delta T_{S:AS}$ of ~50 °C resulted in 15 nm particle shell thickness with the nematic liquid crystalline (LC) phase arrangement. The highest periodically ordered intercalated smectic A (SmA) mesosphere was associated with a shell thickness of 120 nm, observed when $\Delta T_{S:AS}$ = ~0 °C with the inclusion of P407 in the antisolvent phase. SAXS and WAXS diffractograms of SmA phase displayed three quasi Bragg peaks at 3°, 6.05° and 9.05° 2theta arising from the (001), (002) and (003) *hkl* quasi-planes, respectively. The peak at 6.05° 2theta was observed in the PXRD (WAXS) patterns (Figure 2.4) of all NPs, however it had different intensity for the different samples (Chapter 2). The greatest observed intensity was for the sample produced using PEG as a stabilizer (Figure 2.4d). In addition, PXRD patterns obtained from the remaining undissolved powders following solubility studies (Figure 2.6) showed an increase in the intensity of the peak at 6.05° 2theta. Similarly, Figure 5.3 in Chapter 5 illustrates an increase in this peak intensity upon exposure to moisture. Collectively, this peak intensity increases in the presence of moisture or a -OH rich polymers such as PEG and Poloxamer indicating that hydrogen bonding is involved in the formation of this layered liquid crystalline phase. As displayed in Figure 3.1a, there are no conventional hydrogen bond donating groups in the molecule of ITR, but a number of available acceptor groups which could form hydrogen bonds with the -OH rich polymers and/or moisture.

Additionally, Chapter 3 illustrates that at a given temperature, the higher the solvent viscosity in relation to the antisolvent viscosity, the smaller the NPs produced (Table 3.2), which is in agreement with Taylor's equation (Eqn 3.2) and confirming the postulated mechanism of NPs formation. The ability to prepare P407 stabilized ITR NPs, at an elevated temperature (80 °C), adds to the remarkable achievements of this work since it was demonstrated to be not feasible when an ordinary supersaturation-based nucleation process was employed using evaporative precipitation into aqueous solution (EPAS) (Matteucci et al. 2006). According to the authors, in the EPAS process, as the organic solvent evaporates, the formed nuclei are forced to coagulate despite the presence of P407 (Matteucci et al. 2006). In contrast, herein, each nanodroplet solidifies as an individual nanoparticle (Figures 3.5f and 3.9), which then remains dispersed and stabilized by P407. Micellar interference is a second possible explanation of the inability of the EPAS process to form ITR NPs, while the P407 concentration used here was measured to be below the CMC.

Increasing the solvent:antisolvent v/v ratio above 1:10 resulted in the formation of liquid crystalline particles with a wide size range (300 nm to 4 µm). These particles were observed to form even upon mixing larger volumes of the solvent and antisolvent in beakers instead of injecting the solvent to antisolvent using a syringe (Chapter 4). These particles are referred to as the 1st intermediate of the ITR crystallization process as shown in Figure 4.9a, b and d. These particles were LC as concluded from their birefringent nature with Maltese crosses signifying the LC formation (Gharbi et al. 2013) (Figure 4.9d). Since the LC phase of ITR is reported to possess higher solubility than its crystalline counterpart (Chapter 2), these particles underwent Ostwald ripening and, in the presence of solution supersaturated with ITR, this phase transformed gradually to a crystal. This evolution is explicitly presented in Figure 4.9a and Figure 4.9c by the steps 1, 2 and 3. Those square shaped structures, referred to as the 2nd intermediate, continued to grow for the rest of the crystallization process and coexisted with the final crystals upon sampling the crystallization liquor later (Figure 4.10). The formation of the initial cloudy phase can be referred to as liquid-liquid crystalline phase separation. Liquid-liquid phase separation (Ilevbare and Taylor 2013) and liquid-glass phase separation (Mosquera-Giraldo and Taylor 2015) have been reported before as intermediate stages occurring before crystallization and herein a new phenomenon of a liquid-liquid crystal phase separation preceding crystallization of an API is observed.

In addition, the role of the different parameters of antisolvent crystallization impacting on the ITR crystal habit was investigated and how this crystal habit manipulation, including crystal intergrowth, can affect crystal interactions with water molecules. Three distinct isomorphic crystal habits of ITR, a twinned blade-shaped (CH_{tw}), a plate-shaped (CH_{pl}), and a flat sheet-shaped with dendritic ends (CH_{sh}), were obtained by controlling the crystallization conditions. The shape of the final crystals is controlled by a combination of factors including the protic/aprotic solvent ratio, polymer concentration and crystallization temperature. The March–Dollase parameter was employed to quantitatively describe crystals' preferred orientation, where CH_{sh} exhibited the highest preferred orientation.

DVS enabled a full investigation of the effect of the different habits on the interaction with water molecules. DVS data was fitted using the Young and Nelson model (Eqs. 4.2 and 4.3). This model provided information on the difference in water distribution within the three systems (Figure 4.17). The twinned blade-like CH_{tw} crystals exhibited highly hydrophobic surfaces and were considerably permeable to water vapor due to the presence of twin boundary stacking defects. This can be inferred from the fact that in a perfectly packed crystal, based on Mercury 3.5.1 calculations (Barbour 2006), ITR possesses lattice voids (Figure 4.19), which can fit a molecule with a maximum radius of approximately 1.16 Å. This value is smaller than the radius of a single water molecule (1.5 Å) (Hermann 1972). That is to say, there is no space available for a single water molecule in a crystal free of defects. The CH_{pl} and CH_{sh} samples displayed comparable water adsorption/absorption behavior. However, they were practically unable to absorb water even though their surface can adsorb twice the amount of water molecules as CH_{tw} , which could be related to the large (010) crystal facet surface area with the abundance of hydrophilic functionalities.

In Chapter 5 the behavior of mesomorphous, nematic ITR microparticles (MPs), produced via spray drying, upon exposure to a range of humidity conditions at 50 °C is discussed. As mentioned earlier, the nematic phase of spray dried APIs can be easily misinterpreted as amorphous due to the absence of Bragg peaks in X-ray diffractograms using WAXS (Lechuga-Ballesteros et al. 2003). However, when PLM is used in combination with X-ray diffraction, it is plausible to assign nematic LC phase due to its birefringent nature under PLM. The spray dried ITR was assigned as being composed of the nematic phase using a combination of SAXS, WAXS and PLM. An increase in a long-range order, within the MPs, upon moisture sorption at 50 °C was observed. The resultant LC phase was assigned as an intercalated

smectic A, due to the presence of an intense diffraction peak at around $6.05^\circ 2\theta$ in the diffractograms, as also reported in Chapter 3. Based on the change in mass, representing the amount of sorbed water, accompanied the phase change, the smectic phase was found to be formed by a 1:1 mol/mol ITR/water complex. This is an example of rare thermotropic mesomorphism of a solvent complex (Kolbel et al. 2000) and the water presence was found to be necessary for the material to retain the smectic order. This observation confirms that the presence of extrinsic -OH groups is necessary for the formation of this phase. 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 depended on the hygrothermal conditions applied.

Thermal analysis of samples before and after moisture sorption was indicative of subtle differences between the samples. The presence of moisture, within the smectic phase, led to a significant decrease in molecular mobility, and subsequently an increase in the T_g of the samples, an observation counterintuitive to what typically is seen in disordered materials. This behavior highlights the ability of nematic ITR to accommodate stress conditions via molecular rearrangement. LC phases of ITR thus resist full crystallization, which rationalizes their importance in pharmaceutical industry.

6.2. Key findings of this work

- ITR NPs using an antisolvent phase at 80°C were successfully prepared in a one step process (HIEAN), which was reported to be not feasible before.
- The new NP manufacturing process (HIEAN) could reduce further solvent removal steps.
- The produced LC NPs exhibited improved dissolution profiles and higher extent of supersaturation compared to their micron sized counterpart.
- A new liquid crystalline phase of itraconazole, smectic A (SmA) in the intercalated arrangement was discovered with the presence of polymers containing -OH groups promoting the formation of this phase.
- The feasibility of manipulating the LC phase of ITR, nematic (N) or Sm A, in the NPs via controlling the temperature difference (ΔT_{SAS}) between the solvent and antisolvent phases was demonstrated.
- The feasibility of tuning the size of ITR NPs via changing the solvent :antisolvent viscosity ratio was demonstrated.

- A new phenomenon of liquid-liquid crystalline phase separation prior to ITR crystallization was observed.
- Three distinct crystal habits of ITR, including twinning and intergrowth, crystallizing from a mixture of protic/aprotic solvents, in the presence of P407, were reported.
- The effect of crystal shape, including twinning and intergrowth, on the distribution of water molecules, adsorption on the crystal vs absorption within the crystal, was investigated using the Young-Nelson model.
- The phase of spray dried ITR microparticles (MPs) was identified as a nematic phase (N-ITR).
- ITR molecules in the N-ITR MPs realigned into a SmA phase (a more ordered phase), upon moisture sorption in a range of humidity conditions at 50 °C.
- The SmA phase was formed of 1:1 mole/mole ITR complex with water and it is an intriguing example of rare thermotropic mesomorphism of a solvent complex.
- The 1:1 mole/mole ITR complex with water 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.

6.3. General Conclusions

In this thesis, successful production of liquid crystalline (LC) ITR NPs using acetone/water solvent to antisolvent system is reported for the first time. It has been demonstrated that the LC phase of the NPs is controlled by the temperature difference ($\Delta T_{S,AS}$) between the solvent and antisolvent phase. ITR NPs/nanoaggregates with nematic (N) LC arrangement are formed when $\Delta T_{S,AS}$ is around 50 °C. In contrast, ITR NPs with layered smectic arrangement (SmA) was observed when $\Delta T_{S,AS}$ is around zero °C with the presence of -OH groups rich polymer is favored. On the other hand, NPs size can be manipulated via changing the Solvent :antisolvent viscosity ratio. In general, the use of antisolvent phase at high temperature enabled the evaporation of the solvent phase, which could reduce further solvent removal steps, and produced ITR NPs with improved dissolution profile.

A higher ratio of solvent:antisolvent resulted in a liquid-liquid crystalline phase separation and ITR particle in the mesoscale were formed. Those particles

underwent ostwald ripening and ultimately crystallized. Based on the initial conditions, the final crystals' shape can be tuned. Three distinct crystal habits/intergrowths when crystallised from a mixture of protic/aprotic solvents, in the presence of P407, due to difference in chemical functionalities on crystal facets which will interact differently with components of the crystallisation mixture. The twinned blade-like CHtw crystals exhibited highly hydrophobic surfaces and showed considerable permeability to water vapour due to the presence of twin boundary stacking defects.

Nematic ITR MPs were successfully produced using spray drying. Those particles exhibited 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 dependent 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.

6.4. Recommendations for future research

- Chapters 2 and 3 presented the production of ITR NPs with subtle differences in molecular order within the particles. Although in Chapter 2 ITR NPs demonstrated higher dissolution compared to the MPs using the traditional overhead paddle dissolution test, this test cannot be used to examine whether the subtle differences in the LC arrangement can impact on their dissolution properties. This is due to the fact that, in addition to the LC phase difference, the particles were different in size. Therefore, a dissolution test that could eliminate the size effect is required. An attempt to use intrinsic dissolution failed because the LC phase of the particles changed upon compression during disc preparation. It would be very interesting to develop a method that can enable testing the effect of the LC phase on dissolution.
- In Chapter 4 the use of bulk dissolution test was not representative of the effect that each crystal habit had on dissolution of ITR. Singh et al. (2014) tracked the disappearance of slow-dissolving faces upon dissolution using

hot-stage microscopy. An attempt to reproduce the experiment failed due to the lack of the required resources. Therefore, establishing a test that can monitor the dissolution of each phase using different dissolution media would be of high interest.

- As only a limited number of polymers were investigated in this work, the effect of other polymers with various physicochemical and functional properties on the characteristics of the produced NPs using the acetone:water system would be a nice expansion of current studies.
- The study of effect of different polymers, other than P407, and a different solvent, other than acetone, on the crystal habit of ITR might lead to more intriguing ITR crystal shapes.
- A thorough investigation of the stability of different crystal habits and liquid crystalline phases presented in this work for the first time should be performed.
- ITR LC NPs and different crystal habits of ITR formulations can proceed to animal studies. In those studies, either bioavailability and/or efficacy can be examined. For bioavailability studies, CD[®] IGS Sprague-Dawley rat (DiNunzio et. al. 2008) is an example of the animal model, which can be used to quantify the drug levels in plasma usually over 24 hours. For aspergillosis and candidiasis survival studies, DBA/2N female mice (Abruzzo et. al. 1997) can be used were mice usually infected via injecting *Aspergillus fumigatus* MF5668 spore suspension into the lateral tail vein.

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