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Doctoral thesis

**Influence of different amorphization methods
on the supramolecular structure and related properties
of active pharmaceutical ingredients
capable of forming liquid crystalline phases**

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Abstract

Poor aqueous solubility remains one of the major limitations in the development of active pharmaceutical ingredients. Several strategies have been developed to overcome this problem. Among them, amorphization is considered a promising approach. Within the broad class of low-soluble active pharmaceutical ingredients, some compounds are capable of forming liquid crystalline structures. Nevertheless, the influence of different amorphization methods and variation in their processing parameters on the structure and physical properties of these compounds remains unclear. Based on this knowledge gap, this thesis aimed to investigate the relationships between the amorphization pathway, the resulting supramolecular structure, and the associated physical properties of selected liquid crystalline-forming pharmaceutical compounds. For this purpose, two model active pharmaceutical ingredients were selected: itraconazole, a well-known in literature compound that forms thermotropic liquid crystalline mesophases, and a less explored fenoprofen calcium, an ionic compound occurring in hydrated form (as dihydrate). Their different molecular character and structural organization allowed the role of the preparation route on the structure and properties to be examined in two complementary systems. The investigated samples of these substances were prepared using several amorphization methods, including vitrification, cryomilling, and solvent evaporation. Their structure and properties were characterized using complementary experimental techniques, including X-ray scattering, differential scanning calorimetry, broadband dielectric spectroscopy, and supporting thermal, spectroscopic, and dissolution studies. The results demonstrated that, in both selected model systems, the final non-crystalline structure of the liquid crystalline-forming compounds depends strongly on the amorphization route and the specific preparation conditions. The resulting structural differences were reflected in the physical behavior of the materials. Changes in the supramolecular organization affected their thermal response, molecular dynamics, physical stability, and dissolution kinetics. These findings establish a direct relationship between the amorphization pathway, the resulting supramolecular structure, and the physical properties of the material. Overall, amorphization of liquid crystalline-forming compounds should be regarded not as a simple crystalline-to-amorphous conversion, but as a route-dependent structural transformation. This provides a structural framework for understanding and designing non-crystalline pharmaceutical solids, in which the preparation pathway is a critical factor controlling material performance.

Streszczenie

Słaba rozpuszczalność substancji czynnych farmaceutycznie w wodzie pozostaje jednym z głównych ograniczeń ich rozwoju. W celu pokonania tego ograniczenia opracowano różne strategie, wśród których amorfizacja jest uznawana za szczególnie obiecującą. W szerokiej klasie substancji czynnych farmaceutycznie o niskiej rozpuszczalności, niektóre związki wykazują zdolność do tworzenia struktur ciekłokrystalicznych. Jednak wpływ metod amorfizacji oraz parametrów procesów amorfizacji na strukturę i właściwości fizyczne takich układów pozostaje niejasny. Dlatego celem niniejszej rozprawy było określenie zależności między ścieżką amorfizacji, powstającą strukturą supramolekularną oraz właściwościami fizycznymi wybranych układów farmaceutycznych zdolnych do tworzenia faz ciekłokrystalicznych. Jako modele wybrano dwie substancje czynne: itrakonazol, dobrze znany w literaturze związek tworzący termotropowe mezofazy ciekłokrystaliczne, oraz mniej zbadany fenoprofen wapnia, zjonizowany związek stosowany w formie uwodnionej (jako dihydrat). Ich odmienny charakter molekularny i organizacja strukturalna umożliwiły zbadanie efektu drogi amorfizacji na strukturę i właściwości w dwóch komplementarnych układach. Próbkę otrzymano metodami witrifikacji, kriomielenia oraz odparowania rozpuszczalnika. Ich strukturę i właściwości scharakteryzowano za pomocą komplementarnych technik eksperymentalnych, obejmujących rozpraszanie promieniowania rentgenowskiego, różnicową kalorymetrię skaningową, szerokopasmową spektroskopię dielektryczną oraz uzupełniające badania termiczne, spektroskopowe i rozpuszczania. Wyniki wykazały, że w obu modelowych układach końcowa niekryształiczna struktura związków zdolnych do tworzenia faz ciekłokrystalicznych silnie zależy od drogi amorfizacji i warunków przygotowania. Różnice strukturalne znalazły bezpośrednie odzwierciedlenie w zachowaniu fizycznym materiałów, wpływając na ich odpowiedź termiczną, dynamikę molekularną, stabilność fizyczną oraz kinetykę rozpuszczania. Wyniki te wskazują na bezpośredni związek między ścieżką amorfizacji, powstającą strukturą supramolekularną i właściwościami fizycznymi materiału. Amorfizacja związków zdolnych do tworzenia faz ciekłokrystalicznych nie powinna być zatem traktowana jako prosta przemiana fazy kryształicznej w amorficzną, lecz jako zależna od drogi przygotowania transformacja strukturalna. Stanowi to podstawę do zrozumienia i projektowania niekryształicznych stałych postaci farmaceutycznych, w których ścieżka przygotowania jest kluczowym czynnikiem kontrolującym właściwości materiału.

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1. Introduction – motivation and scope

1.1. Development of amorphous drugs

Historically, many drug molecules were derived from natural sources such as plants and microorganisms. These compounds generally exhibited physicochemical properties compatible with biological systems, although their aqueous solubility varied considerably [1]. However, this paradigm changed significantly with the evolution of modern drug discovery approaches. The major transition occurred in the late 1980s and early 1990s with the introduction of high-throughput screening, which enabled rapid evaluation of large libraries of synthetic compounds against specific biological targets [2]. Although high-throughput screening markedly increased the identification of pharmacologically active candidates, it primarily emphasized target binding affinity and did not explicitly account for key biopharmaceutical properties such as aqueous solubility [3]. As a consequence, subsequent medicinal chemistry optimization frequently favored increased molecular lipophilicity to enhance binding interactions, which in turn often reduced aqueous solubility [4]. This trend has led to a growing number of drug candidates characterized by poor dissolution in physiological media, commonly referred to as “brick dust” compounds, indicating high potency but limited solubility [5]. **It is estimated that more than 40% of approved drugs and up to 70–90% of compounds in development exhibit low aqueous solubility, making this issue one of the primary challenges in contemporary pharmaceutical research [6,7,8].**

In response to this problem, considerable effort has been directed toward improving the dissolution behavior of poorly soluble drugs. Established approaches include salt formation, use of cosolvents and surfactants, complexation with excipients such as cyclodextrins, lipid based formulations, particle size reduction by micronization or nanocrystal formation, crystal engineering strategies such as polymorph selection and cocrystal formation, and amorphization [9,10,11]. **Among solid-state strategies, amorphization is considered a promising approach that involves the loss of long-range order characteristic of the crystal lattice.** Consequently, this transformation releases the molecules from the constraints imposed by periodic packing, such that they are no longer fixed in a unit cell repeating arrangement. As a result, the system shifts to a higher thermodynamic energy state, reflected by an increase in Gibbs free energy relative to the corresponding crystalline form [12,13,14]. This increase in free energy provides a greater thermodynamic driving force for dissolution, thereby leading to enhanced apparent solubility and dissolution rate compared to the crystalline counterpart [13,14,15]. This physicochemical

basis was established in the foundational work of Hancock, B. C. and Zografi, G. [12], who defined **the pharmaceutical significance of the amorphous state in relation to solubility, stability, and molecular mobility**. Subsequently, this framework was further developed by Yu, L. [14], through a detailed analysis of the preparation, characterization, and stabilization of amorphous pharmaceutical solids. However, subsequent studies have demonstrated that the solubility advantage of amorphous drugs is not determined solely by thermodynamic factors, but is also strongly influenced by kinetic processes occurring during dissolution [15]. Quantitative experimental studies provide direct evidence of the magnitude of the solubility increase resulting from the amorphization of **pure active pharmaceutical ingredients (APIs)**. For example, the apparent solubility of amorphous glibenclamide is approximately 23 times higher than that of its crystalline form, while amorphous terfenadine and indomethacin exhibit increases by factors of about 10 and 5, respectively, when probed in deionized water at 25 °C [16]. **This enhanced apparent solubility of amorphous forms relative to their crystalline counterparts is also reflected in pharmaceutical industry, where amorphization has been implemented in marketed products** such as Kalydeco® (ivacaftor), Orkambi® (lumacaftor/ivacaftor), Epclusa® (sofosbuvir/velpatasvir), Venclexta® (venetoclax), Zepatier® (elbasvir/grazoprevir), and Lynparza® (olaparib), in which the API is formulated in an amorphous form [17,18]. **The presence of such products in clinical use indicates sustained interest in amorphous pharmaceutical systems and has driven the development of methods capable of converting crystalline drugs into disordered solid states.**

1.2. Methods of amorphization

Various amorphization methods of active pharmaceutical ingredients have been described in the literature, reflecting the **diversity of pathways by which long range crystalline order can be disrupted and converted into a non-crystalline solid state** [19,20]. These methods can be broadly classified into two categories based on their underlying mechanism [21]. The first category comprises **thermodynamically driven approaches**, in which an amorphous solid is formed through a liquid to solid transformation under conditions that suppress crystallization. The second category includes **kinetically driven approaches**, in which the progressive accumulation of structural defects within the crystal lattice leads to the loss of long range order. Both pathways are schematically illustrated in **Figure 1.1**.

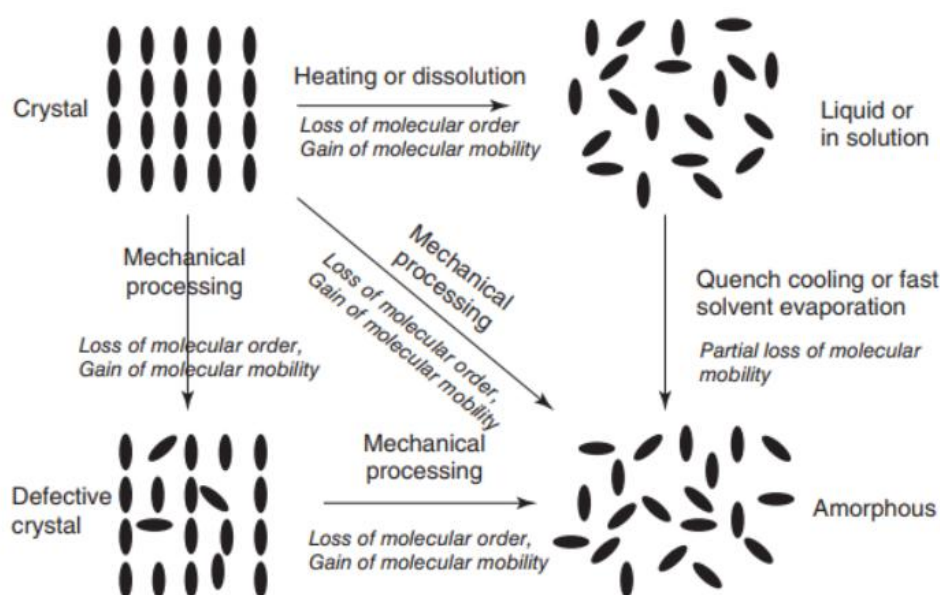


Figure 1.1 Schematic representation of two pathways for generating an amorphous solid structure from a crystalline state during pharmaceutical processing (adapted from [21]).

Although a wide range of amorphization techniques has been reported, **this PhD thesis focuses on three primary methods**. First, **vitrification**, achieved by rapid cooling of a molten API, represents the classical and most widely used route for producing amorphous pharmaceuticals. Second, **cryomilling** constitutes a kinetically-driven method, in which mechanochemical activation under cryogenic conditions induces structural disorder, resulting in a non-crystalline state. Finally, **solvent evaporation** represents another thermodynamically driven method, in which rapid solvent removal from a solution suppresses crystallization and results in the formation of an amorphous solid.

1.2.1. Vitrification

Vitrification, defined as the **rapid cooling of a melt to form a glass**, represents the most established thermodynamically driven method for producing amorphous pharmaceutical solids. In this approach, a crystalline API is first heated above its equilibrium melting temperature (T_m) to obtain a liquid state, after which it is cooled at a rate sufficient to suppress nucleation and crystal growth [14,22]. The cooling rate is a critical parameter controlling vitrification.

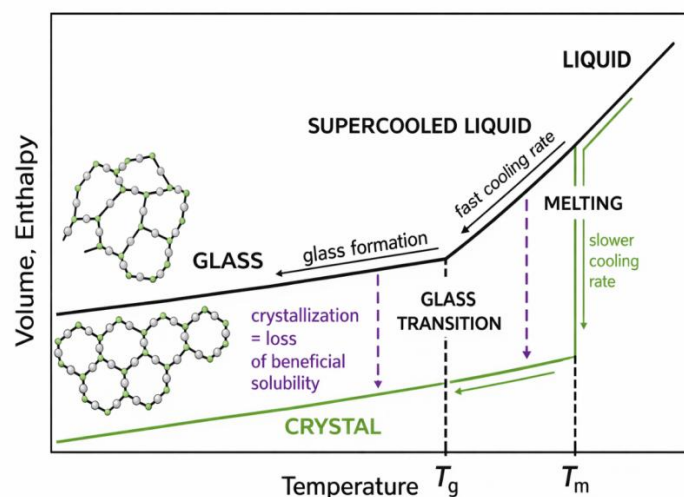


Figure 1.2 Schematic dependence of volume and enthalpy on temperature during vitrification (adapted from [24]).

During slow cooling, molecular mobility remains sufficiently high to allow structural rearrangement into a thermodynamically stable crystalline lattice. In contrast, rapid cooling reduces molecular mobility faster than crystallization can occur. As the temperature decreases, the structural relaxation time of the supercooled liquid increases sharply, and **the system falls out of equilibrium at the glass transition temperature (T_g), where molecular rearrangements become kinetically trapped, resulting in a disordered solid state characteristic of a glass** [12,14,23]. This process is illustrated in **Figure 1.2**, which shows the temperature dependence of volume and enthalpy for crystalline, liquid, supercooled liquid, and glassy states. Under slow cooling conditions, the system follows the equilibrium path and crystallizes at temperatures below T_m . In contrast, rapid cooling suppresses crystallization and leads to the formation of a supercooled liquid, which is subsequently arrested at T_g to form a glassy solid. The material obtained by vitrification therefore lacks the periodic long range molecular arrangement characteristic of crystals, and instead retains a frozen disordered configuration inherited from the supercooled liquid [24]. Despite its conceptual simplicity, vitrification is limited to APIs that are thermally stable in the molten state. Compounds that undergo thermal degradation, chemical transformation, or significant volatilization upon heating cannot be processed by this method, which restricts its applicability in pharmaceutical systems [22].

1.2.2. Mechanochemical amorphization

Mechanochemical amorphization refers to the mechanically driven loss of crystalline order, proceeding through non-equilibrium, kinetically controlled pathways rather than an equilibrium melt–solid transformation [21]. In contrast to thermodynamic routes such as vitrification, which involve melting followed by rapid cooling, mechanochemical amorphization occurs directly in the solid state without the formation of a liquid phase [22]. This process is generally described as a progressive accumulation of structural defects within the crystal lattice under mechanical stress. These defects include point defects (vacancies and interstitials), line defects (dislocations), and planar defects (such as stacking faults) [21,25]. As milling proceeds, the defect density increases, leading to lattice distortion and a gradual loss of long-range order. When a critical level of disorder is reached, the crystalline structure becomes unstable and transforms into an amorphous-like state [21,22,26]. However, the mechanism underlying this transformation remains a subject of discussion, particularly with respect to whether the loss of crystallinity arises from localized heating followed by rapid quenching or from a predominantly athermal process involving progressive defect accumulation and structural collapse [27]. Nevertheless, the review by Einfeld et al. [22] indicates that the outcome of mechanochemical activation is governed by the competition between mechanically induced disorder and temperature-dependent restorative processes. Within this framework, **amorphization occurs when the rate of disorder generation induced by repeated mechanical impacts exceeds the ability of the material to reorganize or recrystallize during this process** [22,28]. Under such conditions, **the resulting material exhibits characteristic features of an amorphous glass, including a detectable T_g , enthalpy recovery associated with physical aging, and altered molecular mobility** relative to the parent crystal [22,29,30]. A practical consequence of this kinetic competition was highlighted by Descamps and co-workers, who demonstrated that milling performed sufficiently below T_g promotes amorphization, whereas milling above T_g favors recrystallization or polymorphic transformation due to increased molecular mobility and enhanced structural reordering [31]. Accordingly, mechanochemical amorphization of pharmaceutical compounds is frequently conducted under cryogenic conditions, a process referred to as cryomilling.

Cryomilling

Cryomilling represents a specialized mechanochemical approach in which mechanical activation is performed at extremely low temperatures, typically by cooling the sample with

liquid nitrogen near $-196\text{ }^{\circ}\text{C}$ [17,25,32]. This technique is particularly suitable for thermosensitive pharmaceuticals, as it avoids the high temperatures that may otherwise induce melting or chemical degradation [17,19,33]. The efficiency of cryomilling is governed by two primary physical factors. First, brittle fragmentation occurs at cryogenic temperatures, where materials are processed below their T_g and therefore enter a brittle regime [24]. Under these conditions, plastic deformation is suppressed and particle fracture is favored, which accelerates the accumulation of lattice defects and promotes the collapse of the crystalline structure [24,17]. Second, suppression of recrystallization occurs because the extremely low processing temperature significantly reduces molecular mobility [33]. As a result, thermally activated relaxation processes that could repair lattice damage are inhibited, thereby facilitating a higher degree of amorphization compared to processing at ambient temperature [22].

1.2.3. Solvent evaporation

Another method used for processing thermosensitive drugs is solvent evaporation, in which amorphization is achieved without exposure to the high temperatures required for melt-based vitrification. **In this approach, the API is first molecularly dissolved in a volatile organic solvent.** The solvent must provide sufficient solubility to ensure complete molecular dispersion of the API, thereby eliminating long-range crystalline order [19,22,24,34]. **Subsequently, amorphization is achieved by removing the solvent at a rate sufficient to suppress nucleation and crystal growth.** During this process, solvent removal increases the API concentration, leading to a supersaturated state. In this state, nucleation and subsequent crystal growth can occur if molecular mobility is not kinetically restricted [35]. Therefore, the evaporation rate is the critical factor governing the final solid-state structure outcome [36]. It defines the time available for molecular rearrangement in the supersaturated state. Rapid solvent removal reduces this time window and thus suppresses nucleation and crystal growth [21,34]. In this sense, solvent evaporation represents a thermodynamically driven pathway in which crystallization is limited by kinetic constraints imposed during rapid drying. It is worth noting that this rate is governed by the physicochemical properties of the solvent, with key parameters including its boiling point and vapor pressure [17,20]. Solvents with low boiling points and high vapor pressure enable efficient solvent removal at temperatures well below the melting or degradation of the API [24,34].

Various techniques can be employed to remove the solvent. Spray drying is one of the most widely used methods, in which the drug solution is atomized into fine droplets and exposed to a hot drying gas. The large surface area of the droplets enables extremely rapid solvent evaporation, thereby minimizing the time available for crystallization and favoring formation of an amorphous solid [37]. Other solvent removal techniques include freeze drying and thin film drying, which differ in drying rate and thermal exposure but operate on the same principle of solvent removal under conditions that prevent crystallization. In this thesis, solvent removal was carried out by evaporation under reduced pressure. In this method, the solvent is removed by lowering the external pressure above the solution, which reduces the solvent boiling point and enables evaporation at temperatures well below its normal boiling temperature. As a result, solvent can be removed efficiently at relatively low temperatures, thereby reducing the risk of thermal degradation or a phase transition of the API [19,20].

1.3. Intermolecular structure of non-crystalline active pharmaceutical ingredients

Having established the amorphization methods used to disrupt the crystalline order of APIs, it is necessary to define the intermolecular organizations that can be produced by these methods. **In practice, the term “amorphous” is widely used to describe broad range of structures lacking long-range atomic/molecular order.** However, this definition remains imprecise [17,38]. In fact, non-crystalline pharmaceutical ingredients may encompass a broad range of structures with different degrees of intermolecular disorder. Therefore, the term “non-crystalline” is very often more appropriate in description of their intermolecular structures, as it defies standard 3D crystalline periodicity, which includes short-range and medium-range ordered systems, as well as liquid crystalline, plastic crystalline, and nano crystalline systems. A systematic structural classification can be established by considering the presence or **absence of translational (positional) and/or orientational (rotational) order** in space [17,38,39]. On this basis, structures can be classified along an order hierarchy from amorphous structures with short-range order, through structures exhibiting medium-range order, to crystalline structures with long-range order, as illustrated schematically in **Figure 1.3**.

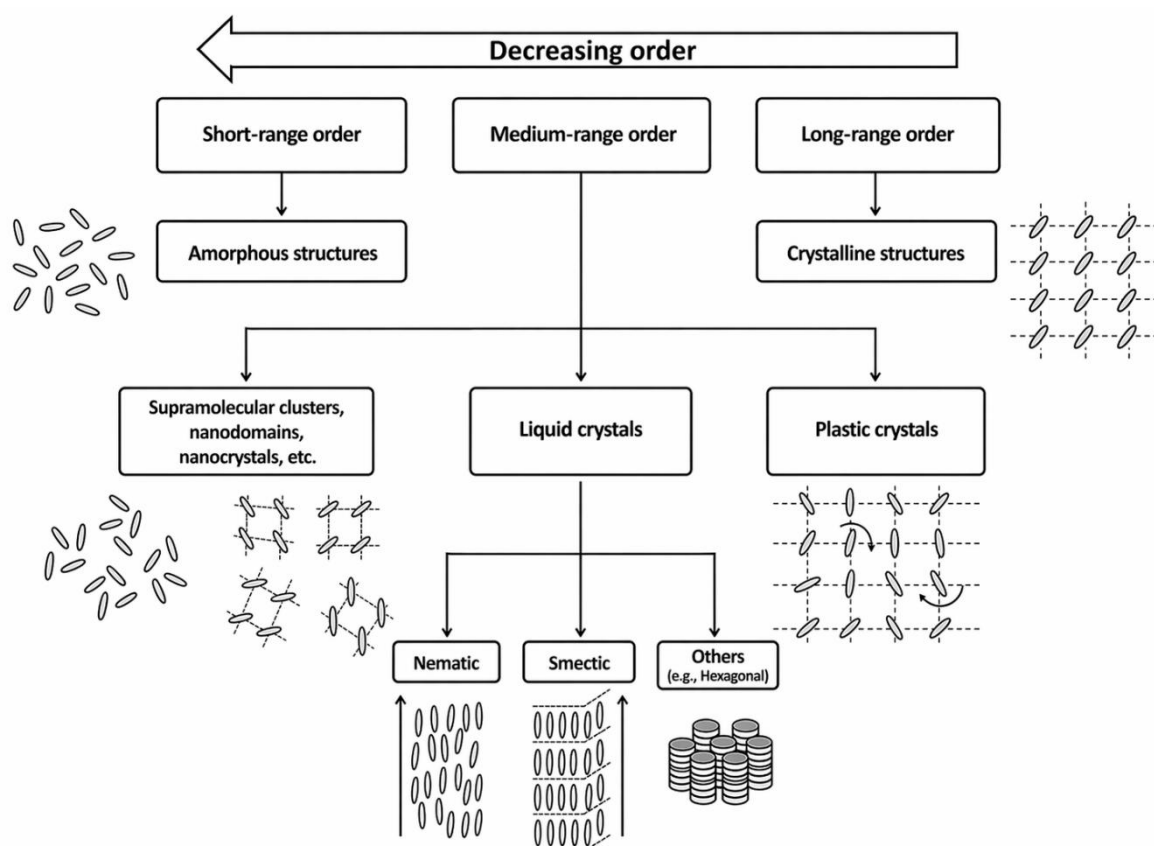


Figure 1.3 Schematic representation of structural order hierarchy of condensed matter (adapted from [40]).

1.3.1. Amorphous structures exhibiting only short-range order

The amorphous state represents the limiting case of structural disorder, in which translational, orientational, and conformational order are absent beyond the immediate local environment [38]. In the solid state, such as glass, amorphous materials correspond structurally to frozen liquids, in which molecules are randomly positioned and oriented without periodicity [17]. Consequently, structural correlations are restricted to short-range interactions involving nearest, up to a few next-nearest neighbors [39]. This local organization is commonly described in terms of random close packing, reflecting the absence of long-range periodicity. **In X-ray diffraction (XRD) pattern, this structural state is characterized by a broad diffuse halo** as presented in **Figure 1.4a**, which reflects the presence of short-range order and the absence of long-range periodicity [17].

Consistent with this structural description, pharmaceutical compounds such as olaparib ketoconazole, and repaglinide can form amorphous solids upon vitrification, characterized by the absence of detectable long-range order [41,42,43].

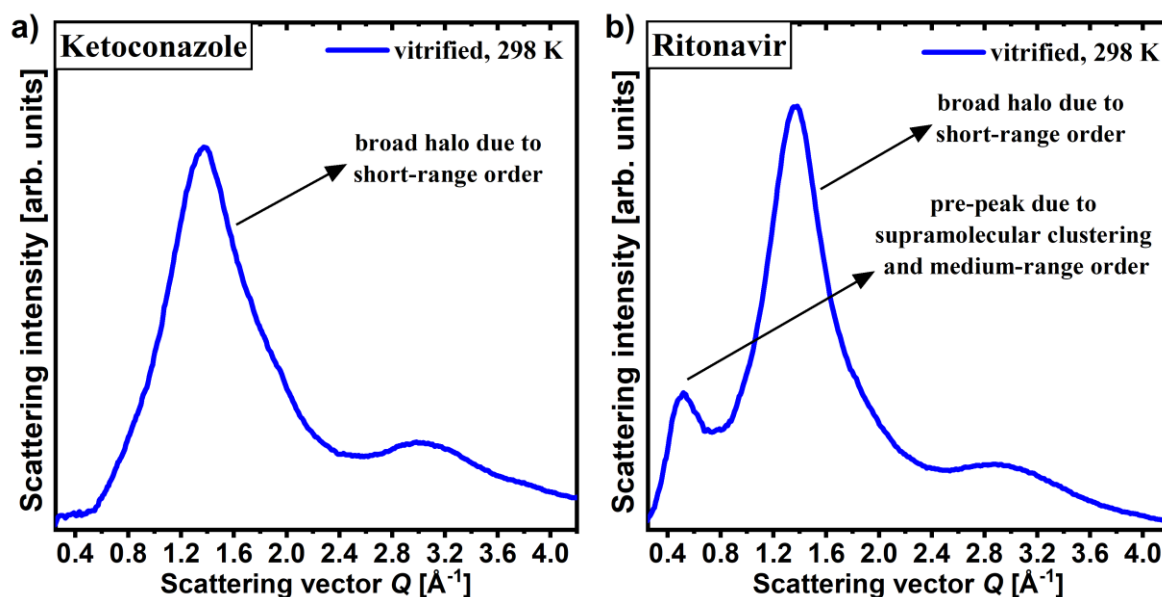


Figure 1.4 XRD patterns of vitrified pharmaceuticals showing different non-crystalline structural features: **a)** vitrified ketoconazole exhibiting an amorphous structure and **b)** vitrified ritonavir showing a diffraction feature associated with supramolecular clustering and formation of medium-range order.

1.3.2. Partially ordered structures

Between the crystalline and amorphous arrangements of molecules, a range of intermediate structures exists in which specific types of molecular order are partially retained [17,24,40]. These systems are characterized by **limited structural correlations**, which arise from the selective preservation or disruption of translational and/or orientational order [23,28,40]. Within this structural regime, **supramolecular clusters** represent assemblies of a limited number of molecules, usually stabilized by specific intermolecular interactions, that may act as pre-nucleation aggregates [25,42]. In diffraction data, **molecular clustering and the resulting medium-range order is commonly indicated by the appearance of a pre-peak in the small-angle scattering range, which is the low-scattering vector (Q) range, positioned before the main broad halo associated with short-range order** [25,42], as illustrated in **Figure 1.4b**. A more ordered form of structural organization can occur when crystalline packing is partially preserved within nanoscale domains. Nanocrystals represent this case, in which the molecular packing motif of a parent crystal is retained over finite regions, typically ranging from 1 to 100 nm [38]. Their diffraction pattern is characterized by a number of reflections, which are much broader than for the crystalline counterparts. The diffraction peaks become progressively broader as crystallite size decreases [38,39] and for very small

nanocrystals, coherently diffracting domains diminish, leading to wider, shallower, and harder-to-distinguish diffraction maxima. Nanocrystals often exhibit a high density of structural defects and disorder compared to their bulk counterparts, which frequently leads to substantial internal lattice strain, atomic rearrangement propagating from the surface to the core, and disruption on the long-range order.

For molecular compounds, the medium-range order and supramolecular clusters are usually stabilized by non-covalent intermolecular interactions, including hydrogen bonding, π - π stacking, van der Waals interactions, hydrophobic association, and electrostatic interactions [42]. A number of compounds forming such supramolecular clusters and medium-range order can be listed in previous studies of our research group [42,44,45,46,47] and include, among others, ibuprofen, ritonavir, nifedipine, curcumin. Moreover, partial order may also occur when translational and orientational orders are preserved, while conformational order is disrupted [39,48]. This case corresponds to conformationally disordered crystals, also termed *condis* crystals, in which different molecular conformers are randomly distributed within an otherwise ordered lattice [40]. However, direct pharmaceutical examples of such structures are less frequently reported [39,48].

1.3.3. Plastic crystalline structures

Plastic crystals, commonly known also as orientationally disordered crystals, **retain translational order, with molecular centers of mass arranged on a regular lattice, while orientational order is lost due to rotational mobility of the molecules** [39]. Thus, molecules occupy fixed lattice positions but possess rotational freedom, allowing dynamic reorientation about their lattice sites [39]. Plastic crystals are typically formed by near-spherical molecules. However, they can also occur for less symmetrical molecules, such as planar or chain-like species [24,25,40]. As a result of rotational averaging, these phases usually adopt simple, high-symmetry lattices, such as face-centered cubic or hexagonal structures [24,40]. **Their XRD patterns therefore contain a limited number of sharp Bragg reflections resulting from the ordered high-symmetry lattice, followed by strong, structured diffuse scattering, caused by the rotational disorder of the molecules** [40], as shown in **Figure 1.5a**. Examples of APIs exhibiting plastic crystalline structures are memantine, and adamantane [49,50]. It is worth to mention that the plastic crystalline phase may be vitrified and the orientational disorder can be "frozen", resulting in an orientational glass [25].

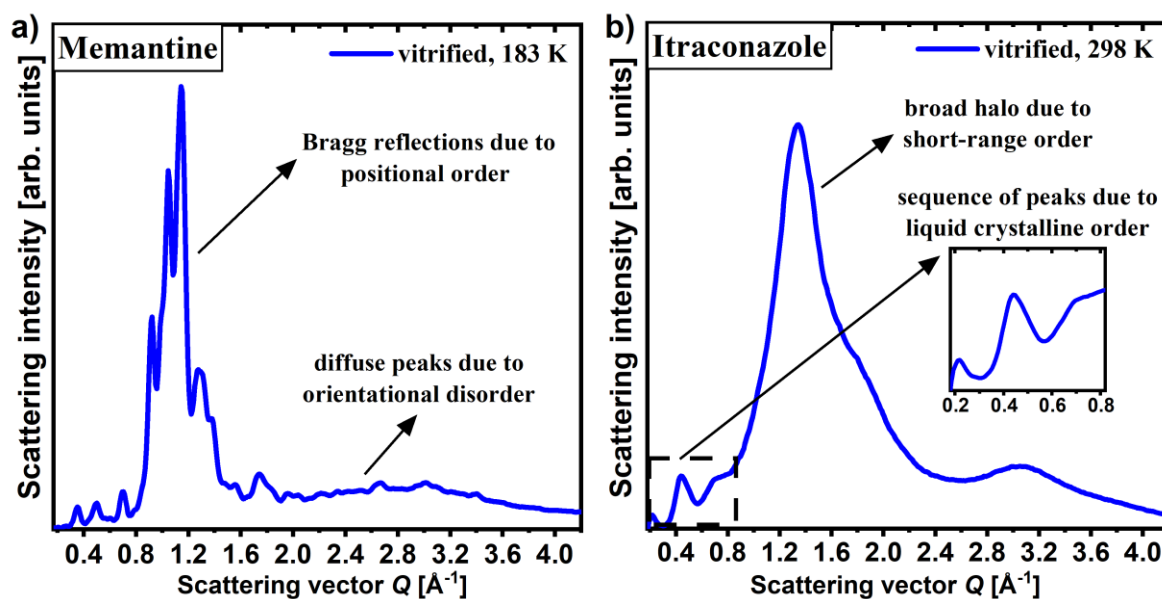


Figure 1.5 XRD patterns of vitrified pharmaceuticals showing different non-crystalline structural features: **a)** vitrified memantine displaying diffraction features characteristic of a plastic crystalline structure and **b)** vitrified itraconazole showing diffraction features characteristic of a liquid crystalline structure, with the inset presenting small-angle scattering data showing a sequence of peaks associated with liquid crystalline order.

1.3.4. Liquid crystalline structures

The structure of liquid crystalline (LC) mesophases is characterized by preserved orientational order along a common axis, termed the director, combined with a partial or complete loss of translational periodicity [25,39]. Accordingly, molecules in the LC structures exhibit directional alignment without full three-dimensional positional order [40]. In XRD data, LC order results in the formation of broad halo due to short-range (liquid-like) lateral correlations and a series of peaks in the lower- Q range, as shown in **Figure 1.5b**. These peaks arise from periodic correlations due to the LC orientational order, such as layer stacking in smectic structures or columnar stacking and organization into 2D hexagonal structures.

Liquid crystals are commonly classified into thermotropic and lyotropic types, depending on the variables governing their formation [24,40]. Lyotropic liquid crystals arise in multicomponent systems as a function of solvent concentration, typically through the self-assembly of amphiphilic or lipid-based molecules in the presence of a solvent, leading to mesophases such as lamellar, hexagonal, or cubic structures [51,52]. In contrast, thermotropic liquid crystals are compounds where molecules with direction-dependent

geometry, such as rod-like or disc-like structures, are able to form the LC mesophases in neat systems (without solvent), in response to temperature changes [51,39]. In these systems, one can distinguish **nematic phases**, which exhibit orientational order only, from **smectic phases**, where orientational order is coupled with one-dimensional layering [25,39]. Many pharmaceutical emulsifiers, such as soybean and egg yolk lecithin (phospholipids) combined with surfactants like sucrose monolaurate or polysorbate 80, can form smectic LC phases. Representative pharmaceutical compounds capable of forming such mesophases in their neat forms include vitamin A and itraconazole [53,54].

It is worth noting that, upon cooling below the T_g , vitrification of the thermotropic mesophases described above can kinetically arrest molecular mobility and preserve the characteristic intermolecular order of the parent mesophase in the glass, similarly to vitrification of a liquid, which arrests the liquid-like structure. As a result, a non-crystalline solid may be obtained that retains the LC order of the mesophase.

1.4. Structure of thermotropic liquid crystalline active pharmaceutical ingredients as a distinct class of non-crystalline systems

Compounds capable of forming LC structures have been reported across a wide range of pharmaceutical classes, including anti-inflammatory, antifungal, anticancer, and antibiotic agents [55]. However, APIs capable of forming LC organization are generally used as lyotropic liquid crystal systems, where the self-assembly is triggered by the presence of a solvent - most commonly water. These systems are highly valued in drug delivery for their ability to provide sustained release of bioactive agents. **In contrast, the uses of neat APIs in solid forms with LC structure, and the detailed knowledge of molecular organization within such structures, are not common.** Therefore, this dissertation focuses on the structure of APIs capable of forming thermotropic LC mesophases that can be frozen in a glass state after cooling to room temperature, since most of drugs is distributed and stored under such conditions. Within this framework, it is necessary to distinguish the principal structures of thermotropic mesophases and to define the basic intermolecular organization characteristic of each structure.

1.4.1. Nematic order

The nematic phase represents the least ordered thermotropic LC state where **molecules align their longitudinal axes along a common average direction** defined by the director vector ($\vec{n}$), thereby establishing a degree of **orientational order** (see **Figure 1.6**) [52]. However, their **centers of mass remain randomly distributed, and no long-range translational periodicity is present** [39]. From a perspective of studies of pharmaceutical compounds, the nematic phase is often the first mesophase to emerge upon cooling from the isotropic liquid (melt) [51], as reported for compounds such as vitamin A, posaconazole, and itraconazole [55,56,57].

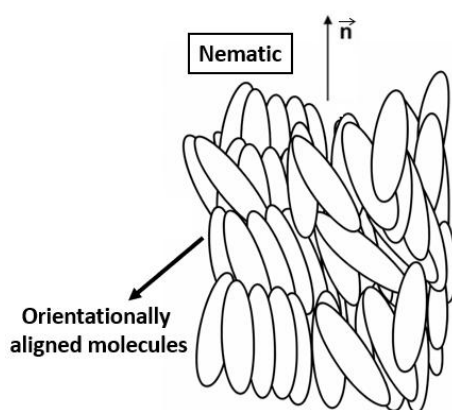


Figure 1.6 Schematic representation of nematic LC structure (adapted from [58]).

1.4.2. Smectic order

Smectic phases exhibit a higher degree of structural organization than nematic phases, as they combine preserved **orientational order with one-dimensional translational order** [52]. In these phases, molecules remain aligned along a common direction ($\vec{n}$), as in nematic structure, but they also **organize into diffuse, regularly spaced layers**, leading to a periodic arrangement along the layer normal ($\vec{k}$) (see **Figure 1.7**) [52]. However, within each layer, the molecular arrangement remains liquid-like and lacks long-range in-plane positional order [52]. Consequently, smectic phases retain partial translational organization while remaining structurally distinct from crystalline organization. Among the different smectic phases, the smectic-A phase is characterized by molecular axes oriented, on average, perpendicular to the layer planes [39,52]. The layer spacing is typically comparable to the molecular length, reflecting the absence of significant tilt [51,52]. In contrast, the smectic-C phase is defined by a collective tilt of the molecules with respect to the layer normal ($\vec{k}$) as illustrated

in **Figure 1.7b** [39,52]. From a pharmaceutical perspective, smectic organization has been reported for several APIs. For example, fenoprofen sodium and itraconazole can form smectic structures under specific thermal conditions [51,59].

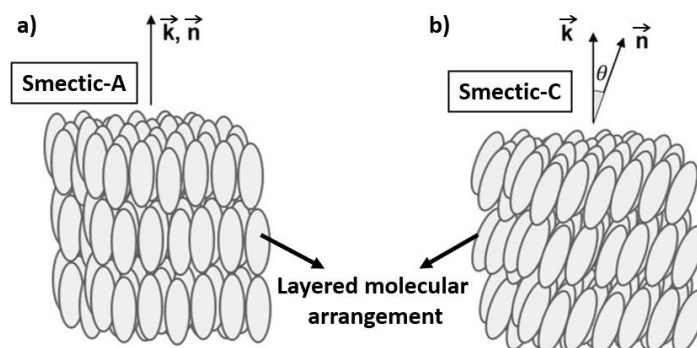


Figure 1.7 Schematic representation of smectic LC structures: **a)** smectic-A and **b)** smectic-C (adapted from [60]).

1.4.3. Hexagonal order

In addition to nematic and smectic organizations, a higher degree of structural ordering can also occur in thermotropic hexagonal phases, although the hexagonal mesophases are more frequently encountered as lyotropic LC phases in pharmaceutical contexts [24,40]. These phases are characterized by two-dimensional translational order combined with preserved orientational alignment [40]. Structurally, the molecular units, which may consist of discotic molecules or elongated aggregates, self-assemble into columns extending along a single direction ($\vec{n}$) (see **Figure 1.8**).

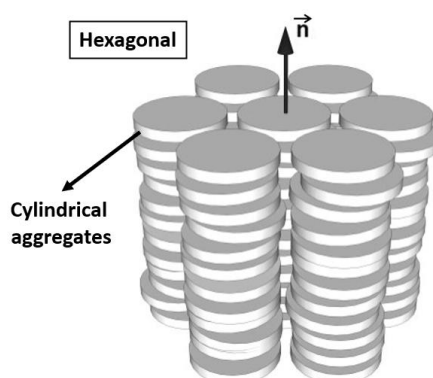


Figure 1.8 Schematic representation of hexagonal columnar LC organization (adapted from [52]).

The most common arrangement among the hexagonal mesophases is the hexagonal columnar phase (Col_h) [52] where columns are arranged on a two-dimensional hexagonal lattice, resulting in long-range positional correlations in the plane perpendicular to the column axis [51,52]. In contrast, along the column direction, positional order is absent and the structure remains dynamically disordered [52]. Consequently, molecules in these phases are organized into a two-dimensional hexagonal lattice while maintaining liquid-like translational disorder in the third spatial dimension [51,52]. From a pharmaceutical perspective, hexagonal organization has been reported in specific systems where intermolecular packing is strongly influenced by molecular shape or counterion effects [51]. For instance, fenoprofen calcium forms a reverse hexagonal phase at elevated temperatures, in contrast to its sodium salt analog, due to differences in ionic interactions that modify the packing constraints [51]. Similarly, disodium cromoglycate has been reported to exhibit hexagonal ordering in its self-assembled state [52]. These examples illustrate that, although less common than nematic or smectic phases for the thermotropic LC compounds, hexagonal mesophases can arise in pharmaceutical compounds when intermolecular interactions promote columnar aggregation and two-dimensional packing symmetry [51].

1.5. Research questions and objectives

The subject of this PhD thesis is the influence of different amorphization methods on the supramolecular structure and related properties of APIs capable of forming thermotropic LC mesophases. The first research question is **whether different amorphization methods, including vitrification, cryomilling, and solvent evaporation, together with a modification of their process parameters, such as evaporation rate, and milling conditions, influence the degree of LC order** in solid forms of these APIs. This question arises from a clear gap in the literature. Although the effect of amorphization techniques on the structure of amorphous APIs has been widely investigated [22,39,41,43,61], their impact on compounds capable of forming thermotropic LC phases remains untouched in scientific reports. Furthermore, it is necessary to consider a fundamental limitation of non-crystalline systems, including LC APIs. These systems occupy higher thermodynamic energy states, which results in their enhanced solubility and dissolution rate, compared to crystalline analogs, but simultaneously promotes physical instability and tending to crystallization [15]. As a result, they exhibit reduced physical stability and a progressive loss of dissolution advantage [25]. Accordingly, the second question is **whether variations in the internal structure induced by different amorphization methods affect the physical behavior of APIs exhibiting the LC structures,**

particularly their molecular mobility and thermodynamics, and whether these structure-dependent changes subsequently influence their physical stability and dissolution kinetics. The objective of this work is therefore to investigate the relationship between the amorphization pathway, the resulting supramolecular structure, and the associated thermal behavior, molecular dynamics, physical stability, and dissolution kinetics of selected pharmaceuticals. Two APIs capable of forming thermotropic LC structures were selected. The first is itraconazole, one of the most studied small-molecule thermotropic LC compounds, which is widely used as an antifungal API [62]. The second is fenoprofen calcium dihydrate, a less extensively studied API belonging to the class of nonsteroidal anti-inflammatory and analgesic drugs [63].

2. Materials and methods

2.1. Characteristics of the studied compounds

2.1.1. Itraconazole

Itraconazole (ITZ) is a widely recognized azole antifungal compound with the molecular formula $C_{35}H_{38}Cl_2N_8O_4$ and a molecular weight of 705.6 g/mol. The molecular structure of ITZ is presented in **Figure 2.1a**. Commercial ITZ is available as a crystalline powder in polymorphic form I (see **Figure 2.1c**), which crystallizes in the triclinic $P\bar{1}$ space group ($a = 8.58 \text{ \AA}$, $b = 20.09 \text{ \AA}$, $c = 20.89 \text{ \AA}$; $\alpha = 73.5^\circ$, $\beta = 88.96^\circ$, $\gamma = 79.29^\circ$) and forms the basis of Sporanox[®] capsules [64].

From previous studies it is known that upon melting at $T_m \approx 448 \text{ K}$, crystalline ITZ transforms into an isotropic liquid [65]. Subsequent cooling from T_m induces ITZ molecules to form nematic mesophase at $T_1 \approx 363 \text{ K}$, followed by a transition to smectic mesophase at $T_2 \approx 347 \text{ K}$ [65]. On further cooling, below the $T_g \approx 330 \text{ K}$, the smectic mesophase can be kinetically arrested in the glass solid state [65]. Upon heating from this glassy state, the same LC transitions are observed in reverse order [66], as shown in the thermogram in **Figure 2.2**. Several studies have investigated ITZ using different amorphization methods, motivated by the extremely low aqueous solubility of its crystalline form, reported to be approximately 1 ng/mL at pH 7.4 [67]. These studies demonstrated that the LC order in ITZ can be controlled by various processing parameters, including cooling rate, substrate temperature, deposition rate, precipitation temperature, and mechanical stress [59,68,69,70].

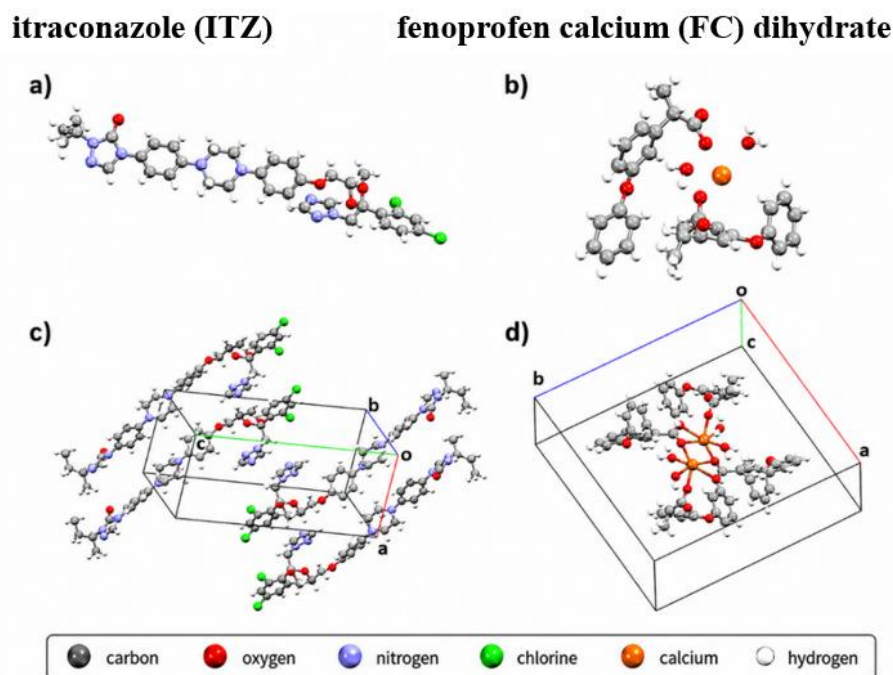


Figure 2.1 Molecular structures and crystalline lattice arrangements of (a, c) itraconazole and (b, d) fenoprofen calcium dihydrate.

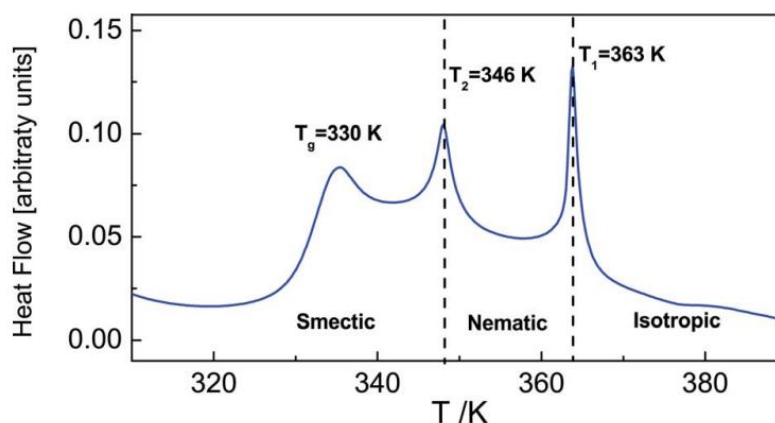


Figure 2.2 Differential scanning calorimetry thermogram obtained upon heating vitrified itraconazole, showing the glass transition and two endothermic transitions associated with LC ordering (adopted from [66]).

For example, it was demonstrated that the degree of smectic-to-nematic order frozen in the ITZ glass depends on the structure of the parent supercooled phase. By controlling the cooling rate, different degrees of smectic ordering can be obtained. In the X-ray scattering patterns shown in **Figure 2.3**, the slowly cooled ITZ glass exhibits distinct low- Q reflections at approximately $Q \approx 0.20$, 0.41 , and $0.62\text{ \AA}^{-1}$. In addition, the broad halo centered

at $Q \approx 1.3 \text{ \AA}^{-1}$ reflects short-range lateral intermolecular packing within the non-crystalline structure. With increasing cooling rate, the low- Q smectic reflections become progressively weaker and broader, indicating a reduction in smectic order. When the cooling rate exceeds 18 K/s, the nematic-to-smectic transition can be avoided, and the low- Q smectic reflections are largely suppressed. This allows a nematic order to be trapped in the glass, where orientational correlations are preserved but smectic layering is not fully developed [59].

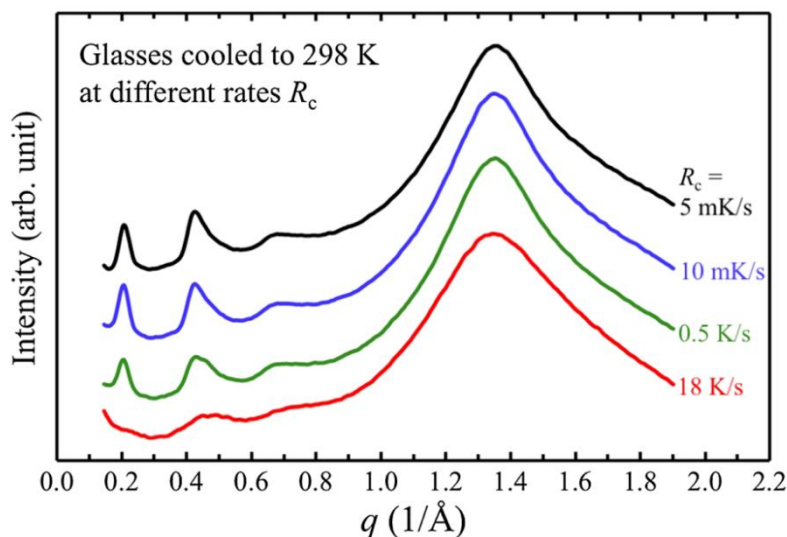


Figure 2.3 X-ray scattering patterns of ITZ glasses prepared by vitrification of liquid at different cooling rates (adopted from [59]).

Moreover, previous studies have shown that mechanical shear can modify nematic alignment in ITZ, thereby affecting both the smectic alignment formed upon cooling below T_2 and the smectic order trapped below T_g . Correspondingly, ITZ stressed in the liquid state exhibited higher smectic order than its non-stressed counterpart [68]. In turn, vapor deposition studies further showed that by manipulation of the substrate temperature it is possible to control the molecular orientation in ITZ glasses, with selected conditions producing high smectic order [69].

In addition to Form I, other ITZ polymorphs have been reported, mainly Forms II and III [64]. Form I is generally considered the most thermodynamically stable crystalline form, whereas Forms II and III are metastable [64]. In a comparative solubility study performed in 50% v/v aqueous ethanol the solubility of Form II was found to be approximately 2–3 times higher than that of Form I [64]. The saturation solubility of Form III could not be reliably determined because this form converted to Form I during the dissolution experiment. However, dissolution measurements showed that Form III initially dissolved faster, reaching a transient

concentration about seven times higher than Form I before decreasing due to conversion toward the stable crystalline form [64]. Therefore, although metastable ITZ polymorphs can show improved apparent solubility or faster initial dissolution, this advantage is limited by their tendency to undergo solid-state conversion [64].

Beyond polymorph control, several ITZ-loaded formulations have been developed to overcome the solubility limitation described above. Among them, amorphous solid dispersions (ASDs) are the most widely studied. In these systems, ITZ is dispersed in an amorphous form within a polymeric carrier to increase apparent solubility and maintain supersaturation. ASDs can be prepared by different procedures, including HME and spray drying. HME is a solvent-free melt-processing method, whereas spray drying is a solvent-based method in which a drug–polymer solution is rapidly dried to obtain amorphous particles. Other solubility enhancement strategies distinct from ASDs, including cyclodextrin complexation, self-emulsifying systems, and cocrystals, have also been reported. For instance, HPMCAS-based ASD tablets reached apparent ITZ concentrations of about 47 µg/mL after 60 min at pH 6.8 [71]. Hot-melt-extruded ITZ/Soluplus® ASDs showed almost complete drug release, compared with about 40% release from ITZ nanocrystals [72]. Spray-dried ITZ solid dispersions containing TPGS 1000 and PVPVA 64 showed much faster dissolution than crystalline ITZ in simulated gastric fluid, with selected formulations releasing more than 80% of ITZ within 10 min [73]. More recently, ITZ cocrystals were reported to reach solubility values of about 60 µg/mL in both 0.1 N HCl and phosphate buffer at pH 6.8 [74].

In experiments performed within this thesis, ITZ powder with a purity above 98% purchased from Angene (CAS Number 84625-61-6) as a racemic mixture comprising all four *cis* stereoisomers in a 1:1:1:1 ratio, crystallized in the polymorphic form I, was used.

2.1.2. Fenoprofen calcium

Fenoprofen calcium (FC) is a nonsteroidal anti-inflammatory, analgesic, and antipyretic drug marketed under the name Nalfon® [75]. In the crystalline state, FC exists as a dihydrate with the molecular formula $(C_{15}H_{13}O_2)_2 Ca \cdot 2H_2O$. As listed, FC dihydrate consists of two fenoprofen molecules and one calcium ion, associated with two water molecules. The corresponding molecular and coordination motif, including the Ca^{2+} center and coordinated water molecules, is shown in **Figure 2.1b**. The crystalline FC dihydrate adopts a monoclinic $P2_1/n$ unit cell ($a = 19.01 \text{ \AA}$, $b = 7.73 \text{ \AA}$, $c = 19.47 \text{ \AA}$; $\alpha = 90^\circ$, $\beta = 91.66^\circ$, $\gamma = 90^\circ$), where each Ca^{2+} ion is coordinated by six oxygen atoms from water molecules and carboxylate groups of fenoprofen anions, as illustrated in **Figure 2.1d** [76]. Only this one

crystalline form has been observed for FC dihydrate up to now and it is stable from 94% to less than 1% relative humidity (RH) at room temperature [77]. Aqueous solubility of this crystalline form is approximately 2.89 ± 0.2 mg/mL [75]. In contrast to ITZ, the structural investigation of FC remains limited. Previous studies demonstrated that open-air heating removes water from the crystalline lattice and transforms crystalline FC dihydrate into a thermotropic LC form [75,78].

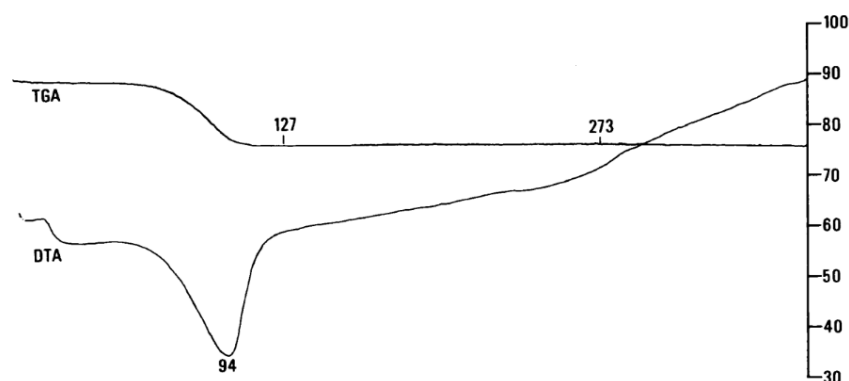


Figure 2.4 Differential thermal and thermogravimetric analysis for FC (adopted from [77]).

On one of the first FC thermograms, presented in **Figure 2.4**, it was claimed that this transition occurs near 94°C (367 K), where a large endotherm corresponding to a loss of water occurs, and it is accompanied by collapse of the crystal structure and partial liquification of the sample [79]. However, as supposed: “this does not appear to be a true melt”, the FC does not melt. Further studies revealed that this is a transition to an LC mesophase [78]. Zhu et al. further demonstrated that the dehydration of FC dihydrate is strongly dependent on the heating temperature. In isothermal TGA experiments, dehydration occurred at different rates at different temperatures, with a non-linear temperature dependence. This behavior was attributed to the presence of two distinct water environments in the FC dihydrate crystal structure [78]. This dehydration-driven transition is reflected in the X-ray scattering patterns, where the sharp reflections of crystalline FC dihydrate gradually disappear and are replaced by diffraction features characteristic of hexagonal LC ordering, first in the monohydrate and then in the anhydrous form, as illustrated in **Figure 2.5**. In both forms, the polar carboxylate groups of fenoprofen anions, coordinated with Ca^{2+} ions, point toward the interior of cylindrical

molecular aggregates, while the less polar aromatic regions are oriented outward (see **Figure 2.6**) [75].

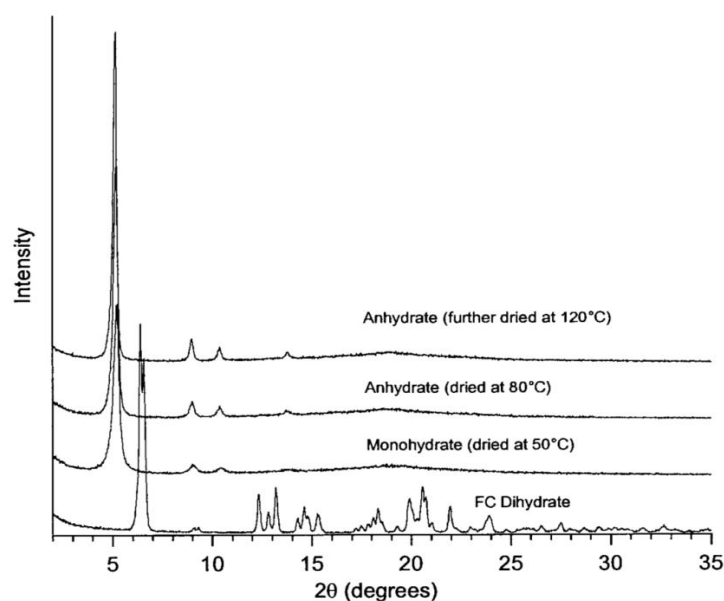


Figure 2.5 X-ray scattering patterns showing the dehydration-induced transformation of FC dihydrate into monohydrate and anhydrous forms with hexagonal LC ordering upon heating (adopted from [78]).

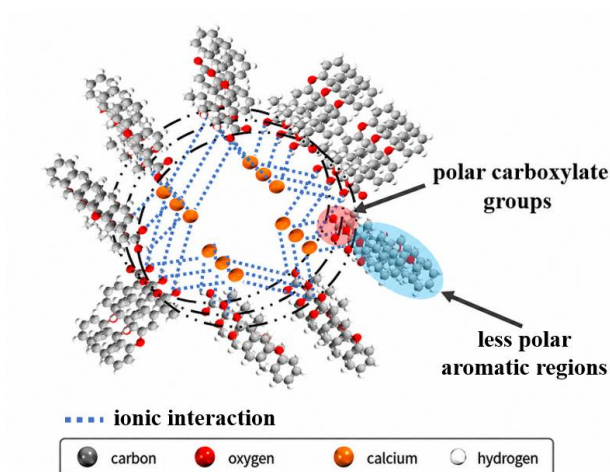


Figure 2.6 Schematic representation of FC cylindrical molecular aggregates.

Upon cooling, the anhydrous FC mesophase can be vitrified, producing a solid form that retains LC organization and shows higher solubility than the crystalline dihydrate [75,80]. The maximum solubility of the LC form was reported to be 5.0 ± 0.3 mg/mL for the pure drug and 6.9 ± 0.6 mg/mL in the tablet formulation [71]. Thus, compared with the crystalline dihydrate described above, vitrification of the LC mesophase increases the apparent solubility

of FC by approximately two-fold, with the larger increase observed in the tablet matrix. However, this vitrified FC form is sensitive to environmental conditions, particularly RH. Under high RH, rehydration promotes structural reorganization back to crystalline FC dihydrate [75]. In this work, polycrystalline FC dihydrate with a purity above 98% was purchased from Angene (CAS No. 71720-56-4). The compound was supplied as a racemic mixture containing the S- and R-enantiomers in a 1:1 ratio.

2.2. Procedures of sample preparation

2.2.1. Vitrification

Vitrification was used as the reference amorphization route in this work. Therefore, the structural order retained in the vitrified samples was considered the reference state for comparison with LC structures obtained by other amorphization methods. The **vitrified ITZ samples (V-ITZ)** were prepared by melting the crystalline powder at 440 K, followed by quenching the melt in air at a moderate cooling rate of approximately 10 K/s to 295 K (room temperature), resulting in glassy solids with a milky appearance. The **vitrified FC samples (V-FC)** were prepared from the crystalline dihydrate form by heating the material at 423 K for 1 h in an open-air capillary. According to the literature, this treatment leads to complete water removal and generates the anhydrous LC structure, as confirmed by thermogravimetric analysis presented in the results section. The samples were then cooled directly to 295 K at a rate of 10 K/s, yielding white solids.

2.2.2. Cryomilling

Cryomilled samples were prepared using a RETSCH CryoMill (model 207490001) equipped with a 50 mL zirconia grinding jar and a single zirconium oxide ball with a diameter of 2 cm. For each milling load, 100 mg of crystalline powder was used, and the instrument was pre-cooled with liquid nitrogen for 10 min before milling. **Cryomilled ITZ (CM-ITZ)** samples were prepared under different milling conditions to examine the effects of milling time and frequency on their structure. First, crystalline ITZ was milled for 1, 3, 12, 84, and 216 min using 12 min cycles separated by 3 min intervals at a frequency of 20 Hz. In a second set of experiments, the milling frequency was varied between 10 and 30 Hz, while the milling time was kept constant at 12 min. **Cryomilled FC (CM-FC)** was prepared using the same instrumental configuration but with a fixed milling protocol: crystalline FC was milled for a total of 270 min, divided into 18 cycles of 15 min

with 3 min intervals between cycles, at a frequency of 20 Hz. The resulting CM-ITZ and CM-FC samples were in the form of fine, white powders.

2.2.3. Solvent evaporation

Solvent-evaporated ITZ samples (SE-ITZ) were prepared by dissolving 20 mg portions of commercial crystalline ITZ in different volumes of dichloromethane (DCM). Volumes of 2, 1, 0.67, and 0.5 mL were used to obtain ITZ:DCM solutions with concentrations of 10, 20, 30, and 40 mg/mL, respectively. Each solution was mixed in a cylindrical borosilicate glass vial with a diameter of 2 cm and a height of 3 cm to ensure homogeneity. Solvent evaporation was then performed under reduced pressure at a rate of approximately 1 ± 0.02 mL/min using two-stage rotary vane CRVPro 6 pumps connected through a Schlenk-line, until only a minimal solvent volume remained. The evaporation rate was determined by weighing the solvent and measuring the time required for its evaporation. To remove residual solvent, the obtained materials were transferred to a vacuum desiccator and dried at 295 K and 4% RH for 12 h. The final solid samples showed a milky appearance with foam-like morphology and are denoted **SE-ITZ X, where X corresponds to the initial ITZ concentration in DCM**: SE-ITZ 10, SE-ITZ 20, SE-ITZ 30, and SE-ITZ 40. In addition, **SE-ITZ 20 CLF** was prepared from a 20 mg/mL ITZ solution in chloroform (CLF) using the same evaporation procedure. This method was applied only to ITZ because in this case suitable solvents with high ITZ solubility and relatively low boiling points were found, namely DCM and chloroform, with mole-fraction solubility of 3.27×10^{-2} and 5.04×10^{-2} M/M and boiling points of 313 and 334 K, respectively [81]. Comparable solvent conditions were not available for FC. Therefore, the solvent evaporation method was not applied to this compound in the present PhD work.

2.3. Characterization methods

2.3.1. X-ray scattering

X-ray scattering (including X-ray diffraction, wide-angle X-ray scattering and small-angle X-ray scattering) was employed as a primary characterization method of the structural organization of the prepared samples. In this technique, an incident X-ray beam interacts with the electron density of the material, and the scattered radiation is recorded as an intensity pattern [82]. Although the studied samples exhibiting liquid crystalline structures do not exhibit long-range crystalline order and their structure cannot be resolved using classical crystallography tools, X-ray scattering patterns can still provide essential information about

the internal structure of such systems. In crystallography, the classic equation for diffraction is Bragg's law:

$$n\lambda = 2d \sin \theta \quad (\text{Equation 2.1})$$

where: n represents the order of the reflection, λ is the wavelength of the incident beam, d is the spacing between the atomic planes, and 2θ is the scattering angle. Bragg's law defines the conditions required for waves (X-rays as well as neutrons or electrons) to constructively interfere when scattered by the parallel planes of a crystal lattice. When many scattered waves overlap perfectly "in-phase", their amplitudes add together, producing a reflection/peak. In non-crystalline structure, atoms/molecules lack three-dimensional periodic but are not completely random and may create local order. Because this ordering is spatially limited, the resulting interference peaks are much broader and less intense than the sharp Bragg peaks found in crystals. The position of a diffraction peak for a non-crystalline system is related to characteristic intermolecular or interatomic periodicity distance in the structure of the studied material, while its intensity reflects the strength of the corresponding structural correlations. In turn, the width and shape of the feature provide information about the spatial extent of this order.



Figure 2.7 Two-dimensional scattering pattern of V-ITZ sample, obtained using the laboratory diffractometer.

Sharp Bragg reflections indicate long-range periodic order, whereas broad diffuse features arise when structural correlations are limited to shorter distances, as observed in non-crystalline materials [83]. Therefore, X-ray scattering served as a key tool for evaluating both structure formation and the structural stability. Wide-angle X-ray scattering (WAXS) measurements were performed in the Institute of Physics, University of Silesia in Katowice, using a D/Max

Rapid II diffractometer (Rigaku Corporation, Japan) equipped with a rotating Ag anode operated at 60 kV and 200 mA. A graphite (002) monochromator was used for the incident beam, providing monochromatic Ag K_α radiation with a wavelength of $\lambda = 0.5608 \text{ \AA}$. The beam size on the sample was approximately 0.3 mm. The samples were packed and sealed in borosilicate glass capillaries and measured in transmission geometry. Two-dimensional scattering patterns were collected using a curved image plate detector (see **Figure 2.7**), corrected for the empty-capillary background, azimuthally integrated, and converted into one dimensional intensity profiles as a function of the scattering angle (2θ), which covered a range of approximately $1\text{--}160^\circ$. The diffraction data were then transformed to the scattering vector (Q) scale, covering approximately $0.17\text{--}20 \text{ \AA}^{-1}$ range. This transformation is useful because Q is expressed in reciprocal-space units and allows diffraction data collected with different X-ray wavelengths to be compared directly. The conversion was performed using the following formula:

$$Q = \frac{4\pi \sin \theta}{\lambda} \quad (\text{Equation 2.2})$$

One can substitute $\sin \theta$ from the Q 's definition into Bragg's law (assuming $n = 1$) and get the formula for d :

$$d = \frac{2\pi}{Q} \quad (\text{Equation 2.3})$$

This formula allows one to convert the position of a diffraction peak in "reciprocal space" (Q) into a physical distance in "real space" (d).

As discussed previously in the section 1.4. Structure of thermotropic liquid crystalline active pharmaceutical ingredients as a distinct class of non-crystalline systems, structures revealing LC order commonly exhibit diffraction reflections in the low- Q range – at Q values lower than the position of the amorphous halo. Due to the fact that for ITZ, the measuring range of the diffractometer was at the limit of the observed first low- Q reflection arising from the LC order, selected ITZ samples were additionally studied by small-angle X-ray scattering (SAXS). SAXS measurements were performed using a Xenocs Xeuss 2.0 SAXS system (Xenocs, France) located at the Joint Laboratory for SAXS studies, Faculty of Physics and Astronomy, Adam Mickiewicz University in Poznań. The instrument is equipped with a MetalJet D2 microfocus X-ray source with a liquid metal-jet gallium-alloy anode and a wavelength of $\lambda = 1.34 \text{ \AA}$. The SAXS data were collected for powder samples placed between two Kapton films, using a Pilatus3R 1M hybrid photon-counting detector.

Pair distribution function (PDF) and radial distribution function (RDF) analyses were also used in selected parts of this PhD work to obtain real-space structural information,

which may provide additional features of the local atomic/molecular structure, and is highly valuable especially for structural characterization of non-crystalline systems. The PDF/RDF data were derived from the WAXS data. The scattering data were corrected for polarization, absorption, and incoherent Compton scattering, then normalized to electron units and transformed into the structure factor, $S(Q)$, according to the following equation:

$$S(Q) = \frac{I(Q) - (\langle f^2 \rangle - \langle f \rangle^2)}{\langle f \rangle^2} \quad (\text{Equation 2.4})$$

where: $I(Q)$ is the coherently scattered intensity normalized to electron units, $\langle f^2 \rangle = \sum_{i=1}^n c_i f_i^2$, $\langle f \rangle = \sum_{i=1}^n c_i f_i$, and c_i and f_i are the concentration and atomic scattering factor of the i -th atomic species, respectively. The structure factor was then converted into the $PDF(r)$, by the sine Fourier transformation:

$$PDF(r) = \frac{2}{\pi} \int_0^{Q_{\max}} Q[S(Q) - 1] W(Q) \sin(Qr) dQ \quad (\text{Equation 2.5})$$

where: $Q_{\max}$ is the upper limit of Q achieved in the measurements (here: $20 \text{ \AA}^{-1}$). This broad experimentally accessible Q range supports the extraction of well-resolved real-space structural correlations from the WAXS data. The corresponding $RDF(r)$ was calculated from $PDF(r)$ using the following equation:

$$RDF(r) = \frac{PDF(r)}{4\pi\rho_0 r^2} \quad (\text{Equation 2.6})$$

where: ρ_0 is the atomic number density. A Lorch-type window function ($W(Q)$) was applied to reduce termination oscillations:

$$W(Q) = \frac{\sin\left(\frac{\pi Q}{Q_{\max}}\right)}{\frac{\pi Q}{Q_{\max}}}. \quad (\text{Equation 2.7})$$

2.3.2. Differential scanning calorimetry and thermogravimetry

Together with the structural characterization by X-ray scattering, differential scanning calorimetry (DSC) was used to examine the thermal behavior of the differently prepared samples. DSC records heat-flow changes in a sample during controlled heating or cooling. When the sample undergoes a thermal event, such as glass transition, LC transition, melting, or crystallization, the heat flow changes and appears as a feature in the DSC thermogram [84]. In this work, DSC was used to relate the structural differences identified by XRD to the thermal response of the prepared samples. Thus, DSC provided complementary information on how the different structures respond to temperature. These measurements were carried out with the help of Dr. Magdalena Tarnacka from the Institute of Physics, University of Silesia in Katowice using a Mettler-Toledo DSC/TGA apparatus (Mettler-Toledo International,

Switzerland) equipped with a liquid-nitrogen cooling accessory and an HSS8 ceramic heat-flux sensor containing 120 thermocouples. Temperature and enthalpy calibrations were performed using indium and zinc standards. The samples were prepared outside the apparatus in open aluminum crucibles with a volume of 40 μL , and measurements were performed using heating and cooling rates of 10 K/min. Additional DSC measurements for CM-ITZ at a high heating rate of 60 K/min were performed using a Netzsch DSC 214 Polyma in aluminum crucibles under a protective argon atmosphere. Thermogravimetric analysis (TGA) was performed using the same DSC/TGA apparatus to examine residual water, particularly in FC samples. For ITZ samples prepared by solvent evaporation, TGA was used to confirm the absence of trace residual solvent.

2.3.3. Broadband dielectric spectroscopy

Broadband dielectric spectroscopy (BDS) was used to examine molecular dynamics in selected samples and to assess how differences in their internal structure affect molecular mobility. This technique measures the complex dielectric permittivity of a material as a function of frequency, usually expressed through the real and imaginary components, $\epsilon'(\omega)$ and $\epsilon''(\omega)$; ω is the angular frequency [85]. The dielectric loss, $\epsilon''(\omega)$, is especially useful because molecular motions appear as relaxation peaks in this signal. In non-crystalline systems, the main relaxation process is the structural α -relaxation, which is associated with cooperative molecular rearrangements near the glass transition. Faster and more localized motions may appear as secondary relaxations, usually at higher frequencies than the α -relaxation at the same temperature [85,86,87]. The dielectric loss spectra can be fitted using empirical relaxation functions to extract the characteristic relaxation times of these processes. In this work, mainly the Havriliak–Negami function was used for this purpose. This fitting procedure allowed the structural α -relaxation time to be determined from the main relaxation peak. When faster secondary processes were resolved, their relaxation times were also extracted from the same fitting analysis. The measurements were performed with the help of Dr. Magdalena Tarnacka and Dr. Paulina Jesionek-Ratka in the Institute of Physics, University of Silesia in Katowice, using a Novocontrol Alpha dielectric spectrometer (Novocontrol Technologies GmbH & Co. KG, Germany) at ambient pressure. Temperature control was provided by a Quatro Cryosystem with a nitrogen-gas cryostat, ensuring temperature stability better than 0.1 K. Powder samples were placed between two stainless-steel electrodes separated by a thin Teflon spacer.

2.3.4. Fourier-transform infrared and Raman spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used to examine local molecular interactions in the prepared samples. In this technique, infrared radiation is absorbed when its energy matches the vibrational energy of molecular bonds, producing characteristic bands for specific functional groups [88,89]. The position, shape, and intensity of these bands depend on the local molecular environment. Therefore, changes in the intermolecular association, especially hydrogen bonding and other polar interactions, can shift, broaden, or modify the corresponding vibrational bands [90]. In this work, FTIR provided complementary information to the X-ray scattering techniques on the local structure and intermolecular interactions of the studied systems. These measurements were performed with the help of Dr. Barbara Hachuła in the Institute of Physics, University of Silesia in Katowice. FTIR spectra were recorded using a Nicolet iS50 spectrometer (Thermo Fisher Scientific, USA) equipped with an attenuated total reflectance (ATR) accessory. All measurements were performed in the spectral range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} , and each spectrum was obtained by averaging 16 scans. Temperature-dependent FTIR measurements were carried out using a diamond ATR crystal mounted in a Low Temperature Golden Gate™ accessory (Specac).

Raman spectroscopy was used to monitor in-situ structural changes of FC during ball milling. Raman spectroscopy technique is based on inelastic scattering of monochromatic laser light. When the laser interacts with molecular vibrations, part of the scattered light changes energy, producing Raman bands characteristic of specific bonds, functional groups, or intermolecular arrangements and interactions [91]. Therefore, changes in band position, intensity, or width can be used to get insight into the atomic- and molecular-scale structure and phase transitions. The in-situ configuration used in this work enabled Raman spectra to be recorded during milling without interrupting the process. Therefore, the structural evolution of FC during milling process was monitored continuously as a function of milling time. These measurements were performed during my internship at the Ruđer Bošković Institute in Zagreb, Croatia, with the help of Dr. Ivan Halasz. A modular Raman system was used, equipped with a PD-LD BlueBox red laser source operating at an excitation wavelength of 785 nm, a B&W-Tek BAC102 fiber-optic Raman probe, and an OceanOptics Maya2000Pro spectrometer (Ocean Optics Inc., USA). The Raman probe was positioned approximately 4 mm below the transparent PMMA reaction vessel. Time-resolved Raman spectra were collected automatically, while the jar contribution was subtracted in parallel using an in-house MATLAB code [92]. Each spectrum was obtained by averaging 59 individual

spectra, each collected with an integration time of 2500 ms. The laser power was set to 485 mW, and the laser wavelength was calibrated using the silicon Raman band at 520.5 cm⁻¹.

2.3.5. Molecular dynamics simulations

Molecular dynamics simulations (MDS) were used to study the structure of water molecules trapped within the LC structure of FC. In MD approach, atoms interact according to a force field, which defines the potential energy of the system. The forces derived from this potential are then used to solve Newton's equations of motion numerically, allowing the positions and velocities of atoms to be followed as a function of time [93]. Therefore, MDS were used here to analyze the organization and mobility of water molecules inside the LC structure, including their local environment and changes during structural rearrangement. The simulations and data analysis were performed with the help of Dr. Patryk Włodarczyk from the Institute of Non-Ferrous Metals, Łukasiewicz Research Network in Gliwice, using the GROMACS package. The starting model was built from the experimental CIF structure of crystalline FC dihydrate. Molecular interactions were described using the GAFF force field with AM1-BCC partial charges, while water was modeled using TIP3P. Electrostatic interactions were treated using the particle mesh Ewald method, and simulations were carried out with a time step of 0.5 fs. Temperature was controlled using a velocity-rescaling thermostat, whereas pressure was controlled in NPT simulations using an anisotropic Parrinello–Rahman barostat to account for the anisotropic nature of the system. The crystalline dihydrate was first equilibrated at 298 K. Then, to introduce structural perturbations analogous to mechanical processing, a slab geometry was generated by extending the simulation box along one direction and removing water molecules. The perturbed system was simulated at elevated temperature to allow structural relaxation under reduced confinement, then rehydrated to the monohydrate composition. Subsequent simulations were performed during stepwise cooling, in order to examine the evolution of water organization and dynamics. The MD-optimized configurations of molecules were employed to calculate the structure factor, $S(Q)$, using the TRAVIS software to enable comparison with $S(Q)$ computed based on the experimental scattering data.

3. Results and discussion for itraconazole

3.1. Influence of different processing methods on structural order

3.1.1. Cryomilling

The first results obtained during this PhD work concerned CM-ITZ, prepared by mechanical treatment under cryogenic conditions to disrupt the crystalline structure. In the initial experiment, crystalline ITZ was milled for 216 min at a milling frequency of 20 Hz, following the protocol described in the section 2.2. Procedures of sample preparation. The structure recovered after cryomilling was subsequently examined at room temperature (around 295 K in all experiments) using X-ray scattering, and the resulting diffraction data are presented in **Figure 3.1**. V-ITZ and crystalline ITZ were also measured as reference samples and their scattering patterns were set together in **Figure 3.1**.

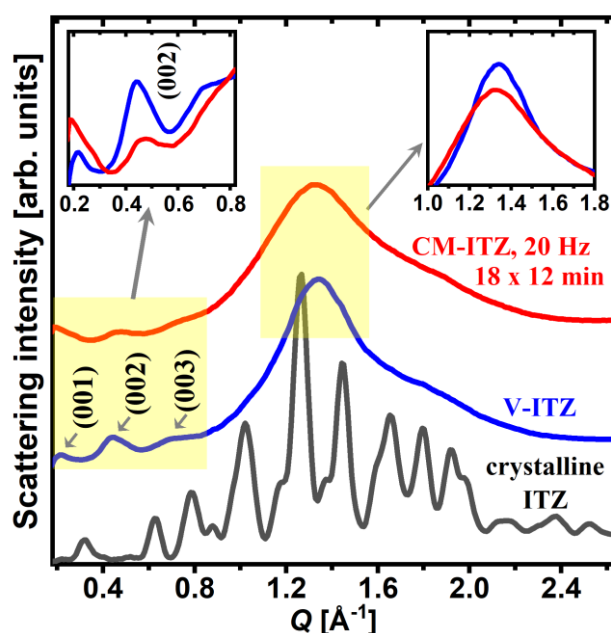


Figure 3.1 X-ray scattering patterns of crystalline ITZ, V-ITZ and CM-ITZ. The inserts in the left and right corners show superimposed patterns in the ranges of the low- Q reflections and the amorphous halo, respectively.

To enable a quantitative comparison of diffraction peak amplitudes, all patterns were rescaled to data derived for crystalline ITZ in the high- Q range (available in the WAXS technique), as illustrated in **Figure 3.2**. Since in the experimentally available Q range of 4-20 $\text{\AA}^{-1}$ there are almost no Bragg peaks, the arising scattering is mostly due to intramolecular ITZ structure, and the studied various ITZ systems are pure and chemically identical, so they should exhibit a similar scattering power in this range. This is indeed the case,

as can be seen in **Figure 3.2**. Therefore, differences in scattering intensity below $4 \text{ \AA}^{-1}$ highlight variations in the intermolecular structure and microstructure of the samples under probe. The same normalization procedure was applied to all X-ray scattering data discussed in this chapter.

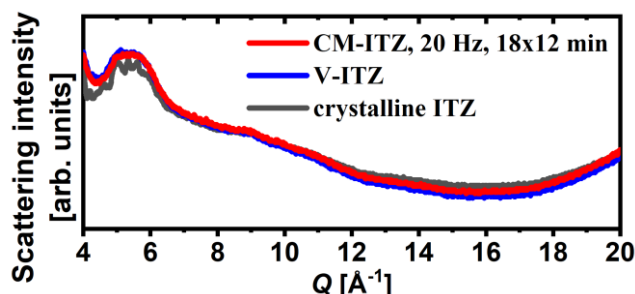


Figure 3.2 Normalization of the diffraction data in the high- Q range used for quantitative comparison.

The diffraction data for both V-ITZ and CM-ITZ show no Bragg peaks, confirming the loss of long-range crystalline order due to the applied vitrification and cryomilling procedures, respectively. Instead, both samples exhibit a broad halo centered at $Q \approx 1.33 \text{ \AA}^{-1}$, which reflects the short-range, nearest-neighbor molecular organization and corresponds to an average intermolecular distance $d \approx 4.7 \text{ \AA}$. In the low- Q region, V-ITZ is known to exhibit diffraction features characteristic of smectic LC order, with maxima located at $Q \approx 0.2, 0.4$, and $0.6 \text{ \AA}^{-1}$, assigned to the (001), (002), and (003) reflections, respectively, arising due to stacking of layers of ITZ molecules [59,69,94,95]. The (001) reflection at $Q \approx 0.2 \text{ \AA}^{-1}$ corresponds to $d \approx 31 \text{ \AA}$, which is close to the molecular length of ITZ [96]. This periodicity is characteristic of lamellar ordering, in which small assemblies of rod-like molecules are arranged in layers, with their long molecular axes oriented approximately perpendicular to the layer planes. In the presented results, only part of the (001) reflection at $Q \approx 0.21 \text{ \AA}^{-1}$ for V-ITZ could be recorded because the accessible low- Q range was limited by beam stopper to approximately $Q \approx 0.17 \text{ \AA}^{-1}$. In addition, this reflection cannot be analyzed straightforwardly for the milled samples, since it overlaps with a small-angle scattering contribution arising from the reduced particle size. The (002), and (003) reflections represent higher orders of diffraction from the fundamental (001) layer plane. The (003) reflection at $Q \approx 0.7 \text{ \AA}^{-1}$ is not affected by the low- Q limit, but its amplitude is much lower than that of the already very weak (001) reflection, because the (003) peak represents interference over longer distances compared to (001) and is much more sensitive to any structural disruptions

over these distances. Therefore, the (002) reflection at $Q \approx 0.45 \text{ \AA}^{-1}$ was used to analyse changes in the LC order of the studied ITZ species. For CM-ITZ, the (002) reflection becomes weaker and broader compared with the corresponding reflection of V-ITZ, as shown in the **left inset of Figure 3.1**. This behavior indicates that cryomilling of crystalline ITZ generates a lower degree of LC order than the smectic order preserved in V-ITZ. Since a similar diffraction pattern with almost identical intensities of the low- Q reflections relative to the amorphous halo was assigned by Teerakapibal et al. to nematic ITZ (shown in **Figure 2.3** with red line) [59], the CM-ITZ pattern was also interpreted as evidence for the formation of a nematic LC structure. This assignment is further supported by DSC results discussed later in this chapter. The reduced degree of the structural order in CM-ITZ compared to V-ITZ is also manifested in the amorphous halo, as shown in the **right inset of Figure 3.1**. The broadening and reduced intensity of the halo feature with a maximum located at $Q \approx 1.33 \text{ \AA}^{-1}$ indicate a lower degree of the short-range nearest-neighbor molecular ordering, compared with V-ITZ. Thus, **cryomilling not only destroys the crystalline structure of ITZ but also yields solid particles exhibiting nematic LC order — more disordered structure than the smectic arrangement trapped in the glass produced by vitrification. What is more, cryomilling also generates a greater structural disorder within the short-range intermolecular environment, which concerns the lateral organization of ITZ molecules.**

As discussed in the section 2.1. Characteristics of the studied compounds, Teerakapibal et al. demonstrated that the LC order of ITZ can be tuned during vitrification through the cooling rate [59]. Motivated by this result, the next step was to test whether mechanical treatment could be used to similarly control the structural organization of CM-ITZ. The milling conditions were therefore varied in two steps: first by changing the milling time at a fixed frequency, and then by changing the milling frequency at a fixed milling time.

A series of milling experiments was performed at a fixed frequency of 20 Hz. Crystalline ITZ was milled for 1, 3, and 12 min, and the milling duration was then extended using repeated 12 min cycles, namely $7 \times 12 \text{ min}$ and $18 \times 12 \text{ min}$. The WAXS measurements were performed on fresh samples, recovered from the milling jar, a few hours after milling was finished, to allow them to return to room temperature.

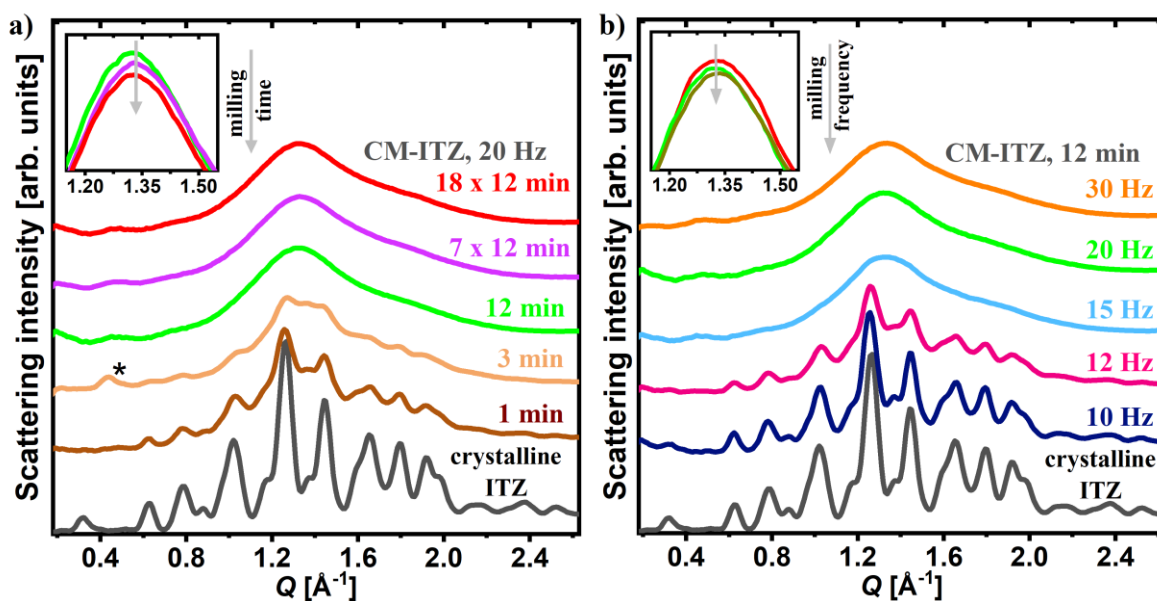


Figure 3.3 X-ray scattering patterns of CM-ITZ prepared under different cryomilling conditions: **a)** at a fixed milling frequency of 20 Hz with varying milling time, and **b)** at a fixed milling time of 12 min with varying milling frequency. The diffraction pattern of crystalline ITZ is included as a reference. The insets show magnified views of the halo peak.

The resulting X-ray scattering data are shown in **Figure 3.3a**. Compared with crystalline ITZ, even 1 min of milling already causes a clear disruption of the crystalline structure, as evidenced by the reduced Bragg peak intensities. After 3 min of milling, only a small residual contribution from the crystalline phase remains. At the same time, a distinct peak appears at $Q \approx 0.45 \text{ \AA}^{-1}$, as indicated by an **asterisk in Figure 3.3a**. Its position corresponds to the (002) reflection observed for V-ITZ, suggesting that smectic ordering is generated in part of the milled material, while the remaining fraction still retains crystalline order. For milling times longer than 3 min, the Bragg peaks disappear completely. The resulting diffraction patterns become similar to that of CM-ITZ obtained after prolonged milling, $18 \times 12 \text{ min}$. **Thus, it is demonstrated that milling at 20 Hz, even for a relatively short time of 12 min, is sufficient to destroy the crystalline structure, as well as the transitional smectic structure, and produce solid ITZ particles with exhibiting a nematic order.** The main difference between the samples milled longer than 3 min is observed in the short-range order region within the halo peak, as shown in the **inset of Figure 3.3a**. The decrease in the intensity of the halo at $Q \approx 1.33 \text{ \AA}^{-1}$ with increasing milling time indicates that **prolonged milling primarily disrupts the short-range order, while the nematic LC order remains stable**. The signature of a fraction with the smectic order in the sample milled for 3 min further motivated the variation of the second

fundamental milling parameter, namely, the milling frequency. This step was performed to determine whether a purely smectic order could be generated in the CM-ITZ under more suitable mechanical conditions.

Based on the previous results, it was established that 12 min of milling was already sufficient to obtain ITZ particles with nematic organization of molecules. Therefore, in the next step, the milling time was fixed at 12 min, and the milling frequency was varied. The resulting diffraction patterns from ITZ samples milled for 12 min at varied milling frequencies are presented in **Figure 3.3b**. At 10 Hz, the sample maintained its initial crystalline structure. At 12 Hz, a clear decrease in the intensity of the Bragg peaks was observed, indicating that, under these conditions, the crystalline order of ITZ starts to be reduced. Increasing the frequency to 15 Hz leads to an almost complete transformation of the structure, producing a diffraction pattern characteristic of nematic ordering of ITZ molecules. Only a weak residual contribution from the crystalline phase remains, close to the detection limit. Further increases in milling frequency do not produce substantial changes in the overall diffraction pattern. However, very subtle changes proceed within the halo peak. The amplitude of the halo associated with the lateral nearest-neighbor organization of ITZ molecules slightly decreases with increasing milling frequency. This behavior is similar to that observed when the milling time is increased. Thus, these data show that **increasing both milling time and frequency, when the nematic structure has already been generated, affects the short-range molecular organization, without changing the overall LC character of CM-ITZ**. The direct transition from a crystalline structure at 12 Hz to a nematic LC structure at 15 Hz, without the smectic structure, which is a transitional state between the crystalline and nematic phases on heating, shows that controlling the balance between smectic and nematic order in cryomilled ITZ by varying milling time and frequency is difficult. **Nevertheless, these results demonstrate that both crystalline and smectic structure in ITZ can be disrupted by cryomilling, leading to ITZ particles with nematic order**. This conclusion was further supported by the diffraction pattern obtained from cryomilled V-ITZ, denoted as CM-(V-ITZ). As shown in **Figure 3.4**, its diffraction pattern is similar to that of CM-ITZ, particularly within the (002) diffraction peak, which becomes weaker and broader than that reported for V-ITZ sample with frozen partial smectic order. The manifestation of weak bumps for CM-ITZ and CM-(V-ITZ) structures considered to be nematic, at positions corresponding to (001), (002), and (003) reflections due to layered structure, originates from residual periodic density modulation of along the long axis of the molecules.

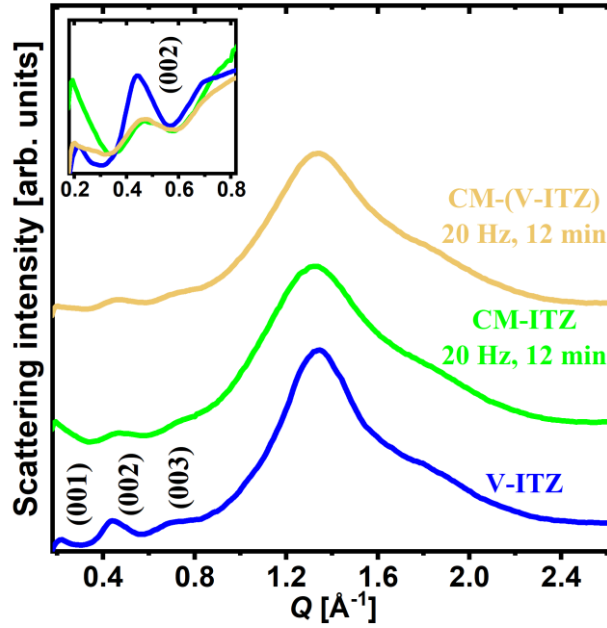


Figure 3.4 X-ray scattering patterns of CM-ITZ and CM-(V-ITZ) samples prepared by cryomilling for 12 min at 20 Hz, together with V-ITZ as a reference. The inset shows superimposed patterns the low- Q range.

It is worth to add that for selected ITZ samples, optical microscopy images were collected with the help of Dr. Maciej Zubko from the Institute of Materials Engineering, University of Silesia in Katowice. They showed similar average particle sizes for crystalline ITZ and V-ITZ, approximately 30 μm , as presented in **Figure 3.5**. After cryomilling, the particles became much smaller, with sizes close to the resolution limit of optical microscopy. Therefore, transmission electron microscopy (TEM) was additionally used to characterize the morphology of powders obtained by cryomilling. The resulting images were presented in **Figure 3.7**. For CM-ITZ obtained after 18×12 min of milling, TEM revealed submicrometric grains with an average size of approximately 0.5 μm . For CM-ITZ obtained after 12 min of milling, the grains were too large for reliable TEM size-distribution analysis, so an approximate size of 5 μm was adopted for them from the optical microscopy.

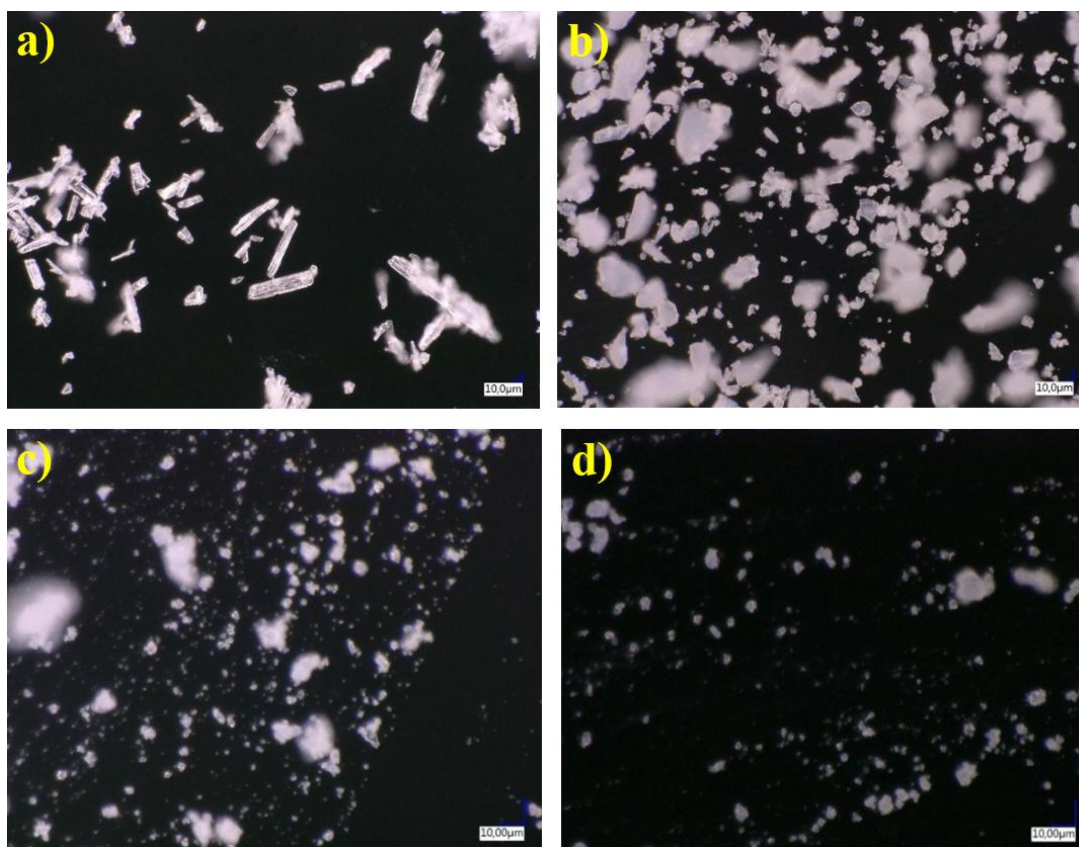


Figure 3.5 Optical microscopy images of **a)** crystalline ITZ, **b)** V-ITZ, and CM-ITZ obtained under different milling conditions: **c)** 12 min at 20 Hz and **d)** 18 × 12 min at 20 Hz.

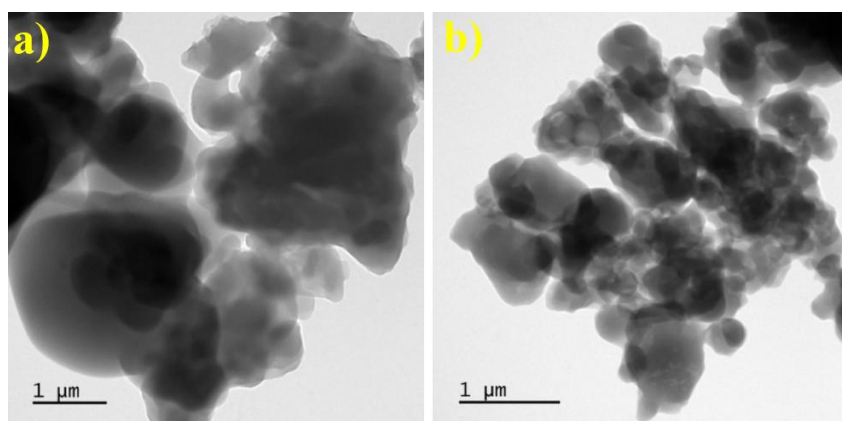


Figure 3.6 TEM images of CM-ITZ obtained using different milling conditions: **a)** 12 min at 20 Hz and **b)** 18 × 12 min at 20 Hz.

3.1.2. Solvent evaporation

Another set of experiments was performed using solvent evaporation as an alternative method for producing non-crystalline structures. Unlike cryomilling, solvent evaporation can be considered a more thermodynamically driven route toward vitrification. **Figure 3.7** shows the X-ray scattering data collected for V-ITZ and the SE-ITZ 20 sample. In the notation SE-ITZ 20, the number 20 refers to the initial concentration of ITZ in DCM before solvent evaporation. The absence of Bragg peaks in the SE-ITZ 20 pattern confirms that solvent evaporation procedure, like vitrification, induces the loss of long-range crystalline order of ITZ. Interestingly, the shape and amplitude of the main diffuse halo at $Q \approx 1.33 \text{ \AA}^{-1}$ are very similar for both samples, as shown in the **right inset of Figure 3.7a**, indicating that solvent evaporation and vitrification produce ITZ samples with comparable nearest-neighbor molecular organization. However, the main difference between the two samples appears in the low- Q region. As shown in the **left inset of Figure 3.7a**, SE-ITZ 20 exhibits a sharper and more intense (002) reflection (as well as the first- and third-order reflections) than V-ITZ, indicating a higher degree of the smectic order in the SE-ITZ sample.

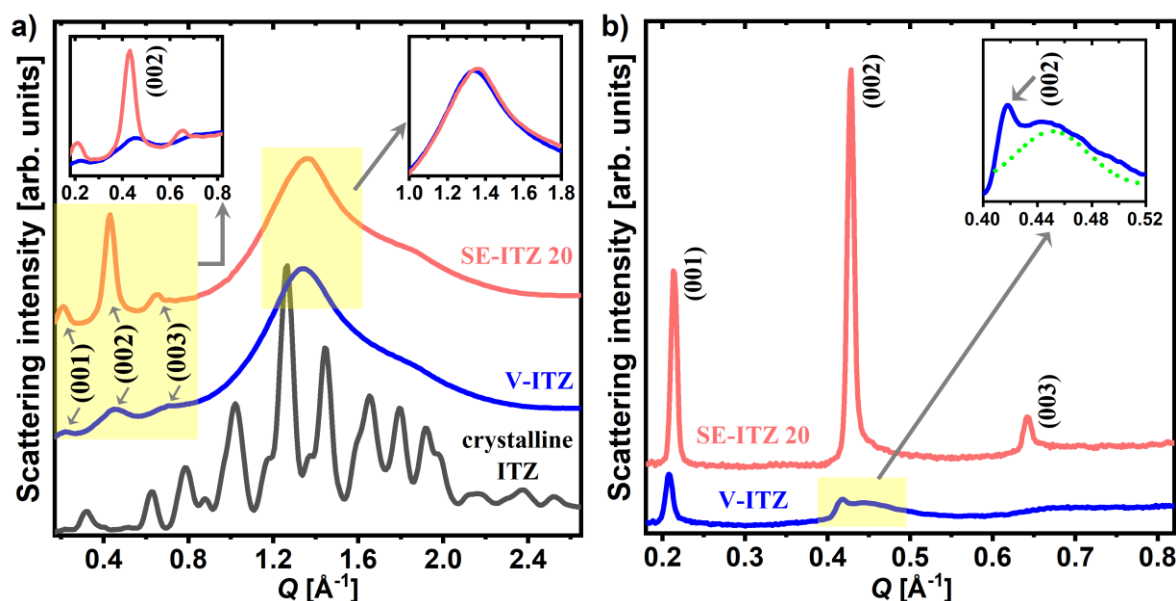


Figure 3.7 X-ray scattering data for SE-ITZ 20 and V-ITZ: **a)** WAXS patterns shown together with pattern of the crystalline ITZ, with insets presenting magnified views of the superimposed patterns in the low- Q region (left) and the main halo (right), and **b)** SAXS patterns with an inset showing a zoomed-in view of the (002) reflection for V-ITZ.

In order to get a deeper insight into the nature of the extraordinary smectic order induced by the solvent evaporation, SAXS measurements were performed. The SAXS data

for SE-ITZ 20 and V-ITZ samples are shown in **Figure 3.7b**. Compared with WAXS, the performed SAXS measurements provided better resolved and full range of scattering data within the (001), (002), and (003) reflections associated with the layered organization. In agreement with the WAXS analysis, SE-ITZ 20 shows sharper and more intense low- Q reflections than V-ITZ. Such behavior further supports the conclusion that the developed **solvent evaporation approach yields non-crystalline ITZ with a higher degree of smectic order than can be achieved by vitrification**. Moreover, the higher resolution of SAXS in the low- Q range clarifies that the apparent (002) peak observed for V-ITZ in the WAXS pattern at $Q \approx 0.44 \text{ \AA}^{-1}$ does not arise from a single contribution. Instead, this feature results from two unresolved overlapping components. The first is a sharper peak at $Q \approx 0.42 \text{ \AA}^{-1}$, indicated by an **arrow in the inset of Figure 3.7b**, corresponding to the (002) reflection arising from the smectic organization of ITZ molecules into layers. The second is a broader contribution at $Q \approx 0.44 \text{ \AA}^{-1}$, shown by the **green dotted** curve in the same inset. This broader component has been attributed in the literature to an excluded-volume effect [97,98]. Its overlap with the smectic contribution shifts the apparent (002) peak position of V-ITZ in the WAXS pattern toward higher Q values, opposite to the trend observed in SAXS, where the resolved smectic (002) reflection of V-ITZ appears at lower Q values than that of SE-ITZ 20. A further difference between V-ITZ and SE-ITZ 20 is visible in the relative intensities of the (001) and (002) reflections. For V-ITZ, the first-order (001) reflection is more intense than the (002) reflection, in agreement with previous studies of ITZ glasses [97,99]. In contrast, SE-ITZ 20 shows a stronger (002) reflection than the (001) reflection, which deviates from the typical diffraction response of smectic LC structures. Yet, in a recent study, Fatina et al. proposed a structural model to explain this effect quantitatively. According to their model, the relative intensities of the (001) and (002) reflections contain information on the density modulation perpendicular to the smectic layers and depend strongly on the intralayer molecular offset (see the **Figure 3.8**). Reducing the random molecular offsets increases the intensity of the (002) reflection ($I_{(002)}$) relative to the (001) reflection ($I_{(001)}$). For a perfectly arranged layered structure with no molecular offset along the direction perpendicular to the layers, the $\frac{I_{(002)}}{I_{(001)}}$ intensity ratio approaches approximately 2. The peak positions in the SAXS patterns support this interpretation. For SE-ITZ 20, the reflections are shifted toward higher Q values compared with V-ITZ, indicating a shorter interlayer spacing in the solvent-evaporated system of approximately 29 Å, which is possible

where there is no molecular offset. Together with the enhanced $\frac{I_{(002)}}{I_{(001)}}$ ratio, this behavior suggests that the performed solvent evaporation process promotes a more compact smectic organization in solid ITZ particles, with reduced intralayer molecular offsets, compared with the applied vitrification procedure.

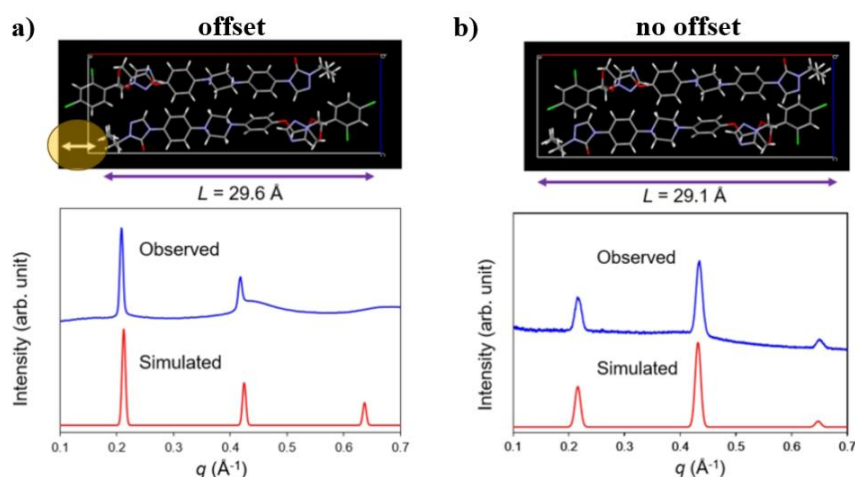


Figure 3.8 Molecular models of smectic ITZ layers and their corresponding low- Q diffraction intensities: **a)** arrangement with molecular offset and **b)** arrangement without molecular offset (adopted from [99]).

The results obtained in the previous section 3.1. Influence of different processing methods on the structural order of itraconazole, showed that the intermolecular structure of ITZ solid particles can be modified by controlling the milling parameters/conditions. This finding motivated a similar investigation for solvent evaporation method, where selected processing parameters were varied to examine their effect on the resulting internal structure. The first parameter considered was the initial concentration of ITZ in DCM. Following the procedure described in the section 2.2. Procedures of sample preparation, several SE-ITZ samples were prepared using different initial ITZ concentrations in DCM, namely SE-ITZ 10, SE-ITZ 20, SE-ITZ 30, and SE-ITZ 40. The WAXS patterns, collected immediately after sample preparation, are presented in **Figure 3.9**. All samples exhibit the same general scattering features previously discussed for SE-ITZ 20. However, the amplitude of the (002) reflection shows a clear dependence on the initial ITZ concentration, as evidenced in the **left inset of Figure 3.9**. The highest amplitude is observed for SE-ITZ 10, which is the sample prepared from the most diluted ITZ solution in DCM, and the peak amplitude gradually decreases with increasing ITZ concentration. **These results demonstrate that the degree of the smectic order in SE-ITZ solid particles can be tuned by adjusting the initial ITZ concentration**

before solvent evaporation. By contrast, the positions of the (002) maximum and the main diffuse halo remain essentially unaltered across the SE-ITZ series. Thus, **changing the initial ITZ concentration mainly affects the degree of smectic ordering, while the lateral packing and the spacing within and between smectic layers remain largely preserved.** Therefore, the nature of smectic structure formed by solvent evaporation appears to be essentially independent of the initial ITZ concentration in the ITZ:DCM solution.

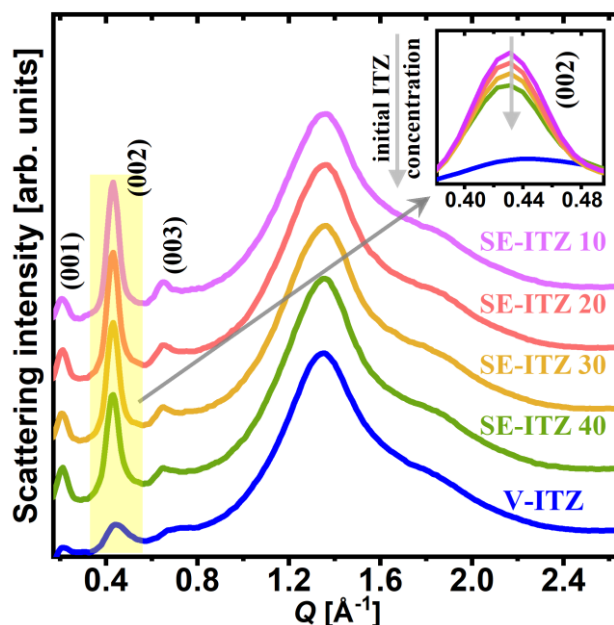


Figure 3.9 X-ray scattering patterns of V-ITZ and SE-ITZ samples prepared from different initial ITZ concentrations in DCM. The inset shows a magnified comparison of the (002) reflection for the SE-ITZ series and V-ITZ.

To characterize the smectic structures of SE-ITZ and V-ITZ quantitatively, the WAXS data were normalized to electron units using Equation. 2.4, described in section 2.3.1. X-ray scattering, and presented as the structure factor $S(Q)$ in **Figure 3.10a**. The concentration-dependent variation of the (002) peak amplitude observed in the diffraction patterns is also preserved in the $S(Q)$ representation, as shown in the **inset of Figure 3.10a**. Further, to evaluate the local structure and the coherence length of the structural correlations in the studied ITZ species, the $S(Q)$ data were then transformed into the pair distribution function $PDF(r)$ representation, using Equation. 2.5. At short distances, the first $PDF(r)$ peaks, which represent intramolecular and short-range intermolecular correlations, almost overlap for V-ITZ and the SE-ITZ samples, as shown in **Figure 3.11a**. This points out that **the local molecular structure within these materials shows no significant differences.**

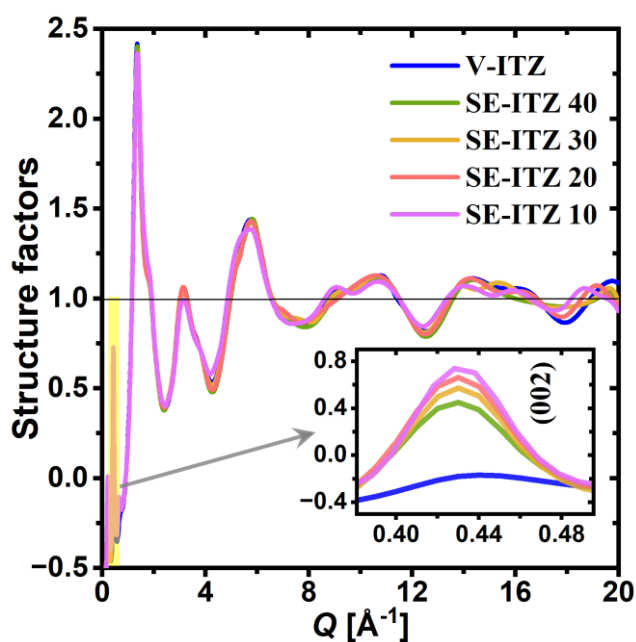


Figure 3.10 Structure factors of V-ITZ and SE-ITZ samples. The inset shows the concentration dependence of the (002) reflection.

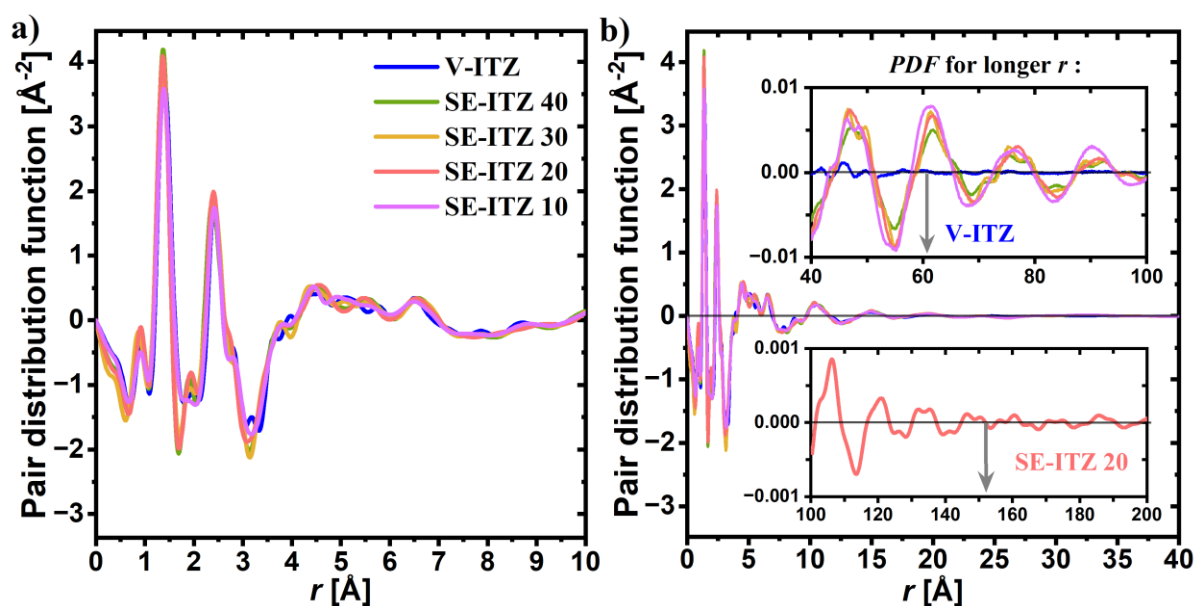


Figure 3.11 Pair distribution functions of V-ITZ and SE-ITZ samples: **a)** short-range intra- and intermolecular correlations and **b)** extended distances correlations. Insets highlight longer-range structural correlations for V-ITZ and SE-ITZ 20.

A clear difference in the $PDF(r)$ data for these samples appears at longer distances. For V-ITZ, the $PDF(r)$ oscillations are attenuated at approximately 60 Å, as shown in the **upper inset of Figure 3.11b**, whereas those of the SE-ITZ samples persist up

to approximately 150 Å. **These results demonstrate that the applied solvent evaporation processing resulted in the formation of longer-range structural correlations than vitrification.** Since all SE-ITZ materials show a similar attenuation of the $PDF(r)$, SE-ITZ 20 was selected as a representative example and shown in the **lower inset of Figure 3.11b.**

After examining the effect of the initial ITZ concentration in the DCM solvent on the structure in the recovered ITZ particles, the solvent evaporation rate was considered as another parameter that may control the organization of molecules during this process. To test this hypothesis, an in-situ X-ray scattering experiment was performed on an ITZ:DCM solution placed in a glass capillary. The capillary end was flame-sealed to prevent rapid outflow of the ITZ:DCM mixture during further heating. This configuration maintained the sample within the X-ray beam and provided slow evaporation conditions for in-situ monitoring. As shown in **Figure 3.12**, the freshly prepared ITZ:DCM solution with an ITZ concentration of 20 mg/mL showed only a broad halo at $Q \approx 1.6 \text{ \AA}^{-1}$, with no diffraction features characteristic of either crystalline or LC ordering. This evidences that ITZ was initially dispersed in DCM without detectable pre-existing structural order. The sample was then heated from 295 to 308 K to promote DCM evaporation. Under these conditions, the evaporation rate was estimated to be approximately $0.01 \pm 0.005 \text{ mL/min}$. After 30 min, the residual ITZ crystallized into the same polymorphic form as commercial crystalline ITZ, namely Form I. Similar experiments performed for ITZ:DCM solutions with different initial ITZ concentrations led to the same outcome, with **crystalline ITZ obtained after slow evaporation.** In turn, previous experiments using spray drying reported a relevant case where **extremely rapid solvent removal (typically $> 10 \text{ mL/min}$) was reported to produce ITZ particles with a more disordered — nematic molecular arrangement** [100]. In the present work, the SE-ITZ samples discussed above were prepared under reduced pressure, where the evaporation rate was lower than in spray drying technique, but much higher than in the capillary experiment, approximately $1 \pm 0.02 \text{ mL/min}$. **Under such evaporation conditions, ITZ did not crystallize but formed solid particles with smectic LC order.** These results show that **the evaporation rate is a key parameter for controlling the organization of ITZ molecules in the solid particles formed via solvation—solvent evaporation procedure. Slow evaporation promotes crystallization, faster evaporation result in smectic LC ordering, whereas extremely fast solvent removal leads to nematic ITZ organization.** Thus, increasing the evaporation rate shifts the final structure from crystalline order toward progressively more disordered LC organization.

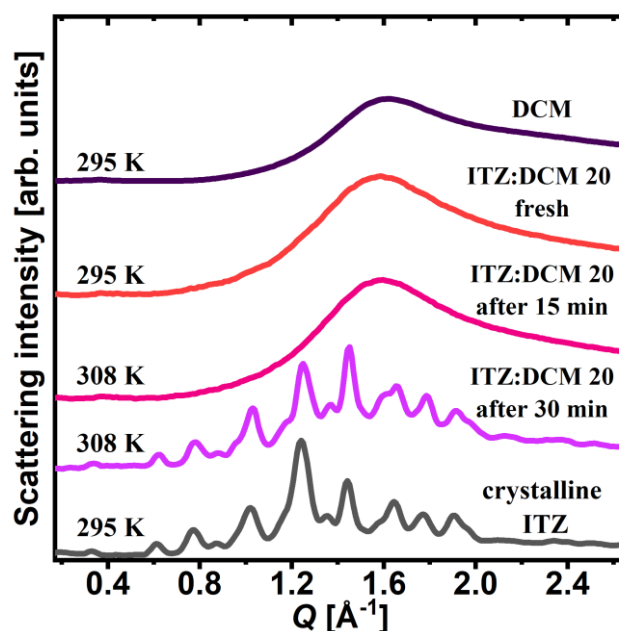


Figure 3.12 X-ray scattering patterns of the freshly prepared ITZ:DCM solution with an ITZ concentration of 20 mg/mL collected at 295 K, and of the same solution after 15 and 30 min at 308 K. The patterns of liquid DCM and crystalline ITZ collected at 295 K are included as references.

A further issue considered was whether the enhanced smectic ordering obtained by solvent evaporation from DCM is specific to this solvent or can also be generated using another solvent. To examine this point, CLF was selected as an alternative dissolving agent. This choice was based on the high solubility of ITZ in CLF, as noted in the section 2.2. Procedures of sample preparation. Following the same protocol used for SE-ITZ 20 prepared by evaporation from ITZ:DCM solution, an analogous sample was obtained from an ITZ:CLF solution containing 20 mg of ITZ per 1 mL of CLF. This sample is denoted as SE-ITZ 20 CLF. As presented in **Figure 3.13**, the X-ray scattering pattern of SE-ITZ 20 CLF confirms the formation of a non-crystalline structure with smectic LC order. However, the reflections associated with smectic ordering are much weaker than those observed for SE-ITZ 20 prepared from DCM. Nevertheless, they arise slightly more pronounced than for V-ITZ. Thus, it was demonstrated that the type of solvent is a crucial factor influencing the organization of molecules during the formation of solid particles from the dissolved ITZ as the solvent evaporates. **CLF solvent promotes the formation of the smectic domains in ITZ particles produced by this approach, as DCM, but the degree of the smectic order is lower than that obtained from DCM.**

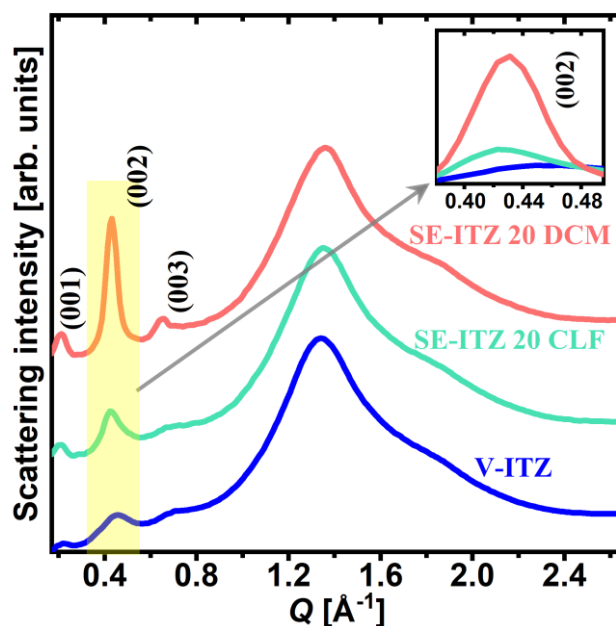


Figure 3.13 X-ray scattering patterns of freshly prepared SE-ITZ 20 samples obtained by solvent evaporation using DCM and CLF as solvents. The pattern of V-ITZ is included as a reference. The inset shows a magnified comparison of the (002) reflection.

Additional solvents, including tetrahydrofuran, acetonitrile, acetone, and methanol, were also tested. In contrast to DCM and CLF, evaporation of these solvents resulted in the recovery of the crystalline structure in ITZ. This behavior may be related to their lower ability to dissolve ITZ compared with chlorinated solvents. ITZ shows high solubility in DCM and CLF, with reported mole-fraction solubility of 3.27×10^{-2} and 5.04×10^{-2} , respectively, whereas its solubility in several non-chlorinated organic solvents is considerably lower [81]. Therefore, DCM and CLF can maintain ITZ in a homogeneous molecular solution before evaporation. In poorer solvents, partial dissolution or early precipitation may occur, preventing the final solid from developing from a fully dispersed molecular state. Sufficiently high ITZ solubility therefore appears important because it enables controlled structural organization during solvent evaporation and favors stabilization of the non-crystalline LC form. Nevertheless, the comparison between the DCM and CLF effects shows that solubility alone does not determine the final ITZ structure generated by this method. Although ITZ has higher solubility in CLF than in DCM [81], this does not lead to a higher degree of smectic order. A plausible explanation for the lower smectic ordering in SE-ITZ 20 prepared from CLF, compared with the corresponding DCM-derived sample, is the different solvent-removal pathway. CLF has a higher boiling point and lower volatility than DCM and is therefore expected to remain longer within the drying ITZ-rich regions under comparable

reduced-pressure conditions. The presence of residual CLF between the smectic ITZ domains may hinder their growth. **Thus, the obtained results demonstrate that the degree of structural order in SE-ITZ species also depends on the evaporation kinetics of the solvent.**

3.1.3. Structure of itraconazole in solid dispersions with polyvinylpyrrolidone polymers

As part of this doctoral work, I also contributed to studies on ITZ-based amorphous solid dispersion (ASD) systems conducted by Luiza Orszulak, a PhD candidate at the University of Silesia in Katowice. Her research broadly focuses on non-crystalline API:excipient binary systems. One of these studies examined the influence of polyvinylpyrrolidone (PVP) polymer topology on the LC order of ITZ in ITZ:PVP ASDs. Within this collaboration, I performed X-ray scattering analysis of these materials. It is worth to mention that PVP is a highly biocompatible and water-soluble polymer widely utilized as a pharmaceutical excipient. Different PVP architectures were investigated, including star-shaped PVP synthesized in our research group, denoted here as *star*PVP. **Figure 3.14** presents the X-ray scattering patterns of ITZ:*star*PVP systems, at different ITZ to *star*PVP weight ratios of 95:5, 90:10, and 85:15 w/w, prepared by vitrification. The data show that increasing the *star*PVP fraction in the mixture progressively disrupts the structural order in ITZ phase. In the low- Q region, the amplitudes of the reflections associated with LC ordering decrease with increasing polymer content, as shown in the **left inset in the Figure 3.14**. A similar amplitude's decrease is observed for the main halo, zoomed in the **right inset**, indicating a reduction in the degree of the short-range molecular order. **These results demonstrate that the LC order of ITZ in ITZ:*star*PVP binary systems can also be tuned by the ITZ:polymer fraction.** Increasing the *star*PVP content weakens both the longer-range LC correlations and the short-range molecular organization of ITZ. Thus, it was demonstrated that by manipulating the cryomilling and solvent evaporation parameters, it is possible to tune the structure of pure ITZ, while adjusting the polymer type and fraction extends this structural control to ITZ-based ASDs.

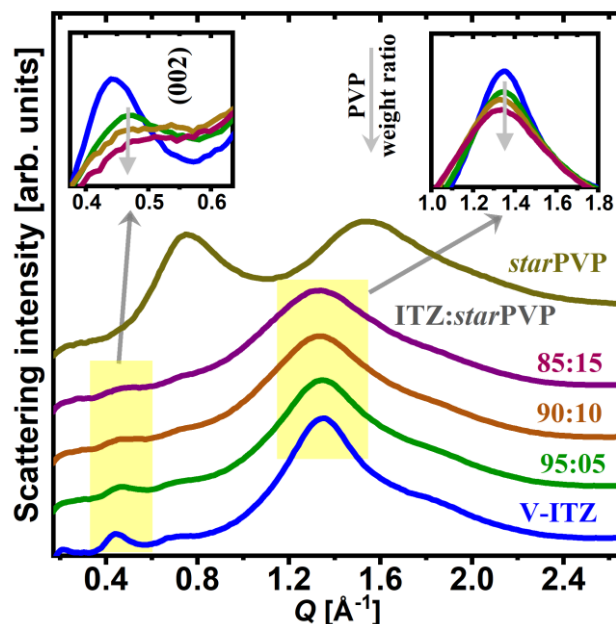


Figure 3.14 X-ray scattering patterns of V-ITZ, *starPVP*, and ITZ:*starPVP* binary systems at different ITZ to polymer weight ratios (95:5, 90:10, and 85:15), prepared by the same vitrification procedure as that applied for pure V-ITZ. The insets show magnified views of the (002) reflection and the main halo.

3.2. Influence of structural order on physical stability

Further analysis was performed to determine the physical stability of the different structural forms of ITZ obtained by the applied preparation methods. This issue is particularly important from the pharmaceutical industry's point of view, since the practical use of non-crystalline drug forms depends not only on their initial structural state, but also on their ability to resist crystallization during storage. Therefore, the physical stability of the prepared CM-ITZ and SE-ITZ samples was examined under controlled storage conditions and compared with V-ITZ. It is worth to add that the chemical stability of these samples was also verified by the nuclear magnetic resonance (NMR) technique. NMR analyses were performed by Luiza Orszulak and they revealed that the chemical structure of ITZ does not degrade when subjected to the treatments presented here.

The first part of the stability study concerned the cryomilled ITZ samples. Two representative materials were selected: CM-ITZ obtained after 12 min of milling (short-milled) and CM-ITZ obtained after prolonged milling for 18×12 min (long-milled). For comparison, V-ITZ was analyzed under the same conditions. All samples were stored in closed capillaries at ambient pressure and humidity, at a controlled temperature of 295 K, which is below the T_g of ITZ. **Figure 3.15** presents the temporal evolution of the X-ray

scattering patterns collected for these samples on a scale from days to months. **V-ITZ showed the lowest physical stability in such conditions, with the first signs of crystallization appearing after 40 days.** The short-milled CM-ITZ sample was more stable, with no clear crystallization detected up to 53 days. However, sharp Bragg peaks due to crystalline order became visible after 70 storage days. In contrast, **the long-milled CM-ITZ sample showed no evidence of crystallization even after 300 days of storage.** During this period, both the low- Q features and the main broad diffraction maximum remained essentially unchanged. This result indicates that the solid particles with nematic arrangement of ITZ molecules generated by prolonged cryomilling are highly stable at room temperature.

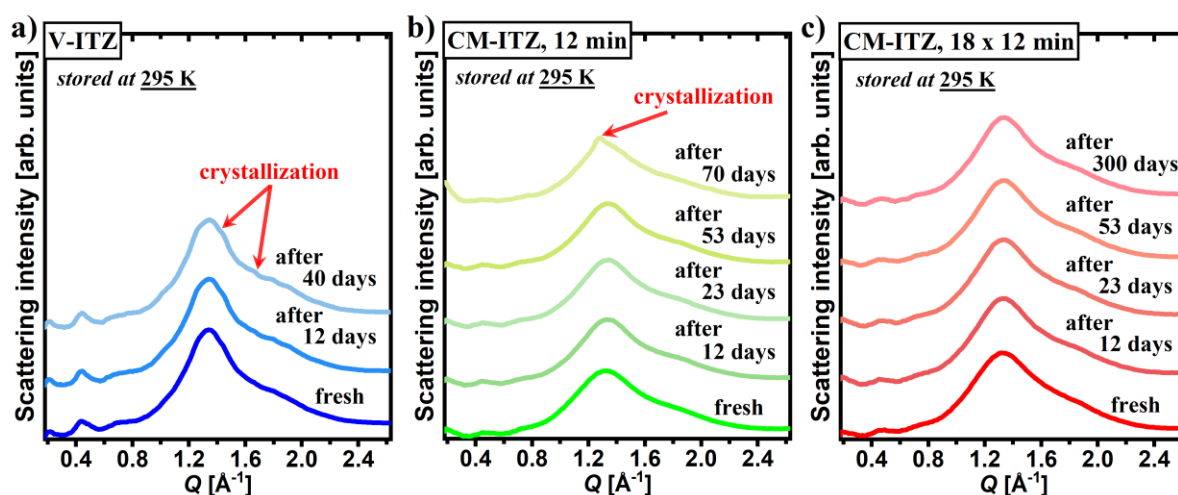


Figure 3.15 Temporal evolution of X-ray scattering patterns for ITZ samples stored at 295 K: a) V-ITZ, b) CM-ITZ 12 min, and c) CM-ITZ 18 × 12 min. Both CM-ITZ samples were milled at 20 Hz.

The high stability observed for the CM-ITZ samples at 295 K, particularly for the LC-ordered long-milled sample, motivated additional measurements at elevated temperature. Therefore, another set of samples was stored at 323 K in an oven and measured day by day in the diffractometer to assess whether the nematic order remains stable under conditions of increased molecular mobility, while still remaining below the reported T_g of ITZ. The corresponding X-ray scattering patterns are shown in **Figure 3.16**. Heating remarkably accelerated structural changes in all samples. **V-ITZ crystallized rapidly, with crystalline reflections already visible after 1 day** and becoming pronounced after 2 days. **The short-milled CM-ITZ sample showed improved stability relative to V-ITZ, but crystallization was observed after 2 days.** For the long-milled CM-ITZ sample, the

LC scattering profile was preserved up to 4 days, whereas clear crystalline reflections appeared after 5 days. Thus, although CM-ITZ samples exhibit improved physical stability compared with V-ITZ, it is temperature-dependent and progressively decreases when the material is stored at temperature closer to T_g .

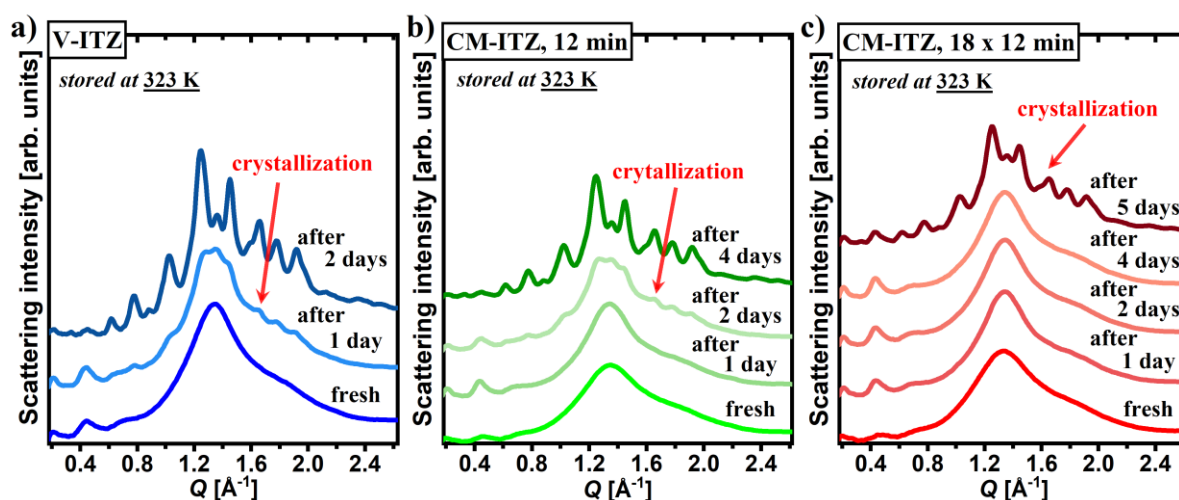


Figure 3.16 Temporal evolution of X-ray scattering patterns for ITZ samples stored at 323 K: a) V-ITZ, b) CM-ITZ 12 min, and c) CM-ITZ 18×12 min. The CM-ITZ samples were prepared by milling at 20 Hz.

Overall, the structural evolution of ITZ samples stored at 295 K and 323 K demonstrates that **their physical stability notably depends on the structural state obtained after preparation. The CM-ITZ samples, which exhibit nematic order, showed higher resistance to crystallization than V-ITZ, where a degree of smectic order is preserved.** This enhanced stability was more apparent for the long-milled CM-ITZ sample than for the short-milled one. This enhanced stability was more apparent for the long-milled CM-ITZ sample than for the short-milled one. This behavior can be related to the structural changes described in the section 3.1. Influence of different processing methods on the structural order of itraconazole. In that section, it was shown that prolonged milling introduces additional disorder at the short-range scale. Therefore, the higher stability of the long-milled CM-ITZ sample may be attributed to the increased lateral disorganization of molecules within the nematic structure. **Such reduced lateral coherence likely delays the rebuilding of structural order and, consequently, slows the onset of crystallization.**

The SE-ITZ samples provided a complementary system for examining the relationship between the degree of structural order and physical stability. In the previous paragraph,

the comparison between V-ITZ and CM-ITZ showed that transforming the LC structure from smectic to nematic increases the resistance of ITZ to crystallization. Moreover, prolonged milling further improved the stability of CM-ITZ by introducing additional disorder at the short-range scale within the nematic structure. In the SE-ITZ samples, the situation is different. Samples produced by the solvent evaporation preserve smectic order, where the degree of the smectic order varies, depending on the initial ITZ concentration in the ITZ:DCM solution. Simultaneously, all these SE-ITZ samples exhibit a similar short-range order. Therefore, selected SE-ITZ samples with different coherence lengths of the smectic order, namely SE-ITZ 40, SE-ITZ 30, and SE-ITZ 20, were monitored over time at room temperature by X-ray scattering. The corresponding temporal evolution of the scattering patterns is presented in **Figure 3.17**. **The results show a clear trend between the degree of the smectic order in the SE-ITZ samples and their physical stability. Specifically, lower smectic order is associated with higher stability.** SE-ITZ 20, which exhibited the highest smectic order, crystallized after only 7 days. SE-ITZ 30 showed signs of crystallization after 24 days, whereas SE-ITZ 40 displayed the highest stability among the studied SE-ITZ samples, remaining unchanged for at least 42 days.

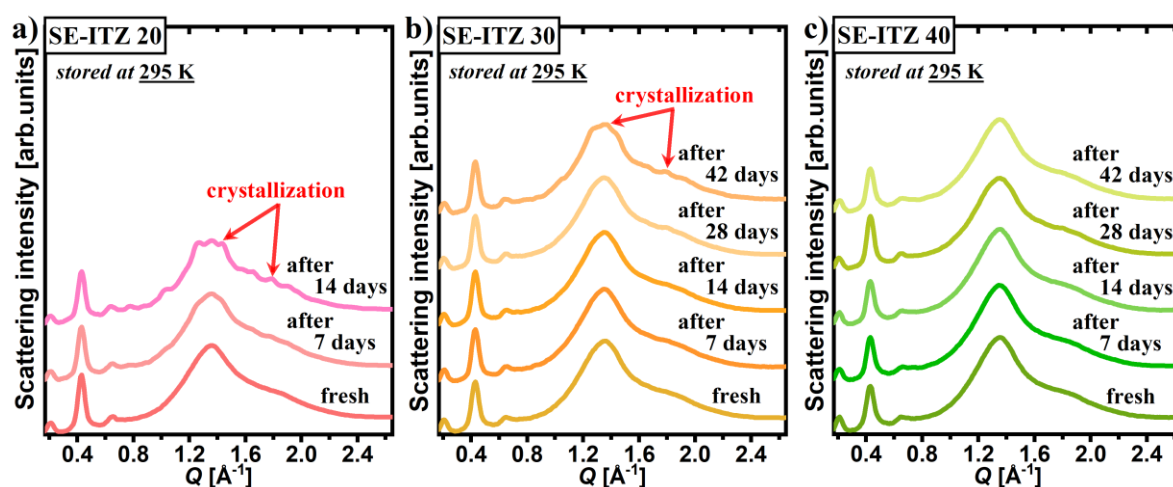


Figure 3.17 Temporal evolution of X-ray scattering patterns for SE-ITZ samples prepared with different initial ITZ concentrations: **a)** SE-ITZ 20, **b)** SE-ITZ 30, and **c)** SE-ITZ 40. All samples were stored at 295 K.

However, an important difference in physical stability appears when SE-ITZ 30 is compared directly with V-ITZ. As shown in **Figure 3.18**, both samples exhibit a similar timescale of physical stability at 295 K, since the first clear signs of crystallization in these samples appear after approximately 40 days. This behavior is noteworthy because SE-ITZ 30

initially shows a higher degree of smectic ordering than V-ITZ. Therefore, the physical stability cannot be explained only by the overall intensity of the smectic correlations. Instead, it also appears to depend on more subtle nearest-neighbor intermolecular correlations in the short-range scale. This interpretation is consistent with, demonstrated in the previous section 3.1. Influence of different processing methods on the structural order of itraconazole, the presence of molecular offsets between adjacent ITZ molecules within the smectic layers of V-ITZ, whereas such offsets were not observed in SE-ITZ. Thus, **the differences in the molecular packing inside the LC structure can affect the crystallization tendency**. This comparison shows that physical stability is controlled not only by the degree of medium-range LC order, but also by the nature and degree of the short-range molecular organization within the LC structure.

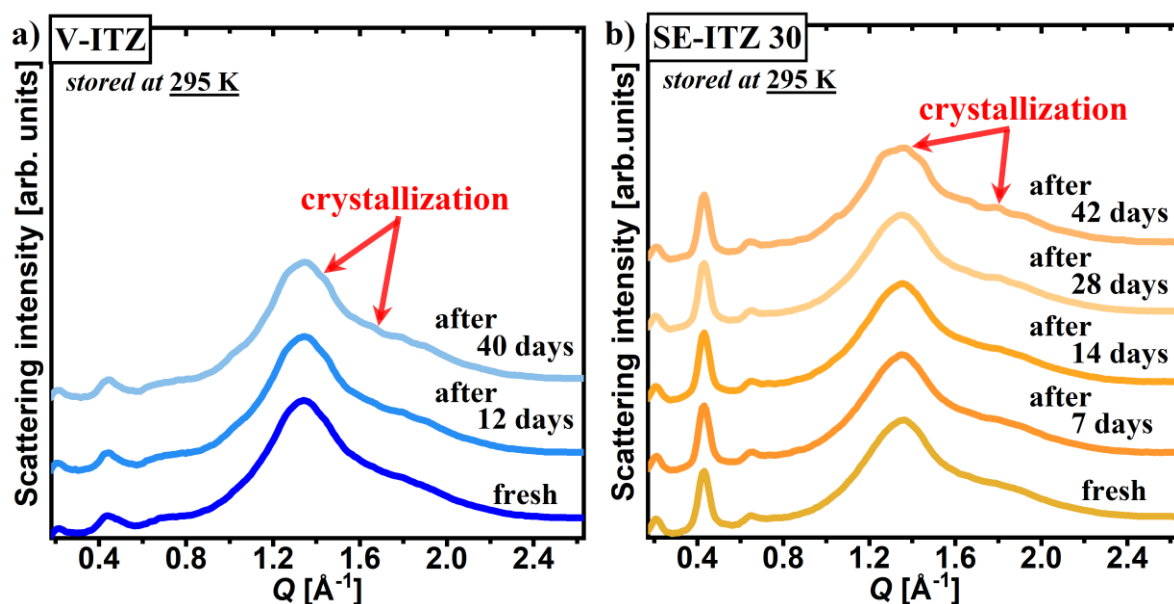


Figure 3.18 Temporal evolution of X-ray scattering patterns for **a)** V-ITZ and **b)** SE-ITZ 30 stored at 295 K in closed capillaries.

3.3. Thermal and molecular dynamics properties

The structural analysis demonstrated that vitrification, cryomilling, and solvent evaporation procedures resulted in distinct non-crystalline forms of ITZ with different degrees of molecular ordering. To determine whether these structural differences are accompanied by changes in thermal properties and molecular mobility, V-ITZ, CM-ITZ, and SE-ITZ samples were further analyzed by DSC and BDS techniques. The following section focuses on the thermal transitions and relaxation dynamics of these samples, providing a complementary view of how preparation-induced structural disorder affects the physical

properties of ITZ. To clearly describe the calorimetric response associated with LC transitions, the temperature of the transition from smectic (*Sm*) to nematic (*N*) mesophase is denoted as (T_{Sm-N}), whereas the temperature of the transition from nematic mesophase to the isotropic liquid (*I*) is denoted as (T_{N-I}).

The thermograms of fresh V-ITZ and CM-ITZ samples (short-milled (12 min) and long-milled (18x12 min)) recorded on heating from 300 to 450 K are shown in **Figure 3.19**. V-ITZ exhibits a heat-capacity step associated with the glass transition at $T_g \approx 330$ K, followed by two endothermic events corresponding to LC transitions. The first transition, observed at $T_{Sm-N} \approx 345$ K, is assigned to the transformation from the *Sm* to *N*, whereas the second transition, observed at $T_{N-I} \approx 362$ K, corresponds to the *N* to *I* transition. The determined temperatures are consistent with previously reported calorimetric data for V-ITZ, discussed in section 2.1. Characteristics of the studied compounds. For the CM-ITZ samples, the DSC curves also show thermal events related to LC transitions. However, their thermal response differs from that of V-ITZ. In addition to the transitions, heating above T_g leads to crystallization of the CM-ITZ samples, followed by melting near $T_m \approx 440$ K. It should also be noted that the short-milled CM-ITZ sample crystallizes at lower temperature than the long-milled sample, indicating its lower thermal stability under non-isothermal heating conditions. Particular attention was given to the weak thermal features observed in the T_g region. In the conventional DSC curves recorded at 10 K/min, this region is broad and poorly resolved, especially for the CM-ITZ samples. To clarify the nature of this region, temperature-modulated differential scanning calorimetry (TM-DSC) measurements were performed in the T_g region, as shown in the **inset of Figure 3.19a**. In TM-DSC, a small temperature modulation is superimposed on the linear heating ramp, allowing the heat-capacity response to be separated from overlapping thermal effects. The modulated signal suggests the presence of two partially resolved glass-transition contributions in both CM-ITZ samples, which may reflect different relaxation processes associated with motions around the long and short molecular axes of ITZ [59,101].

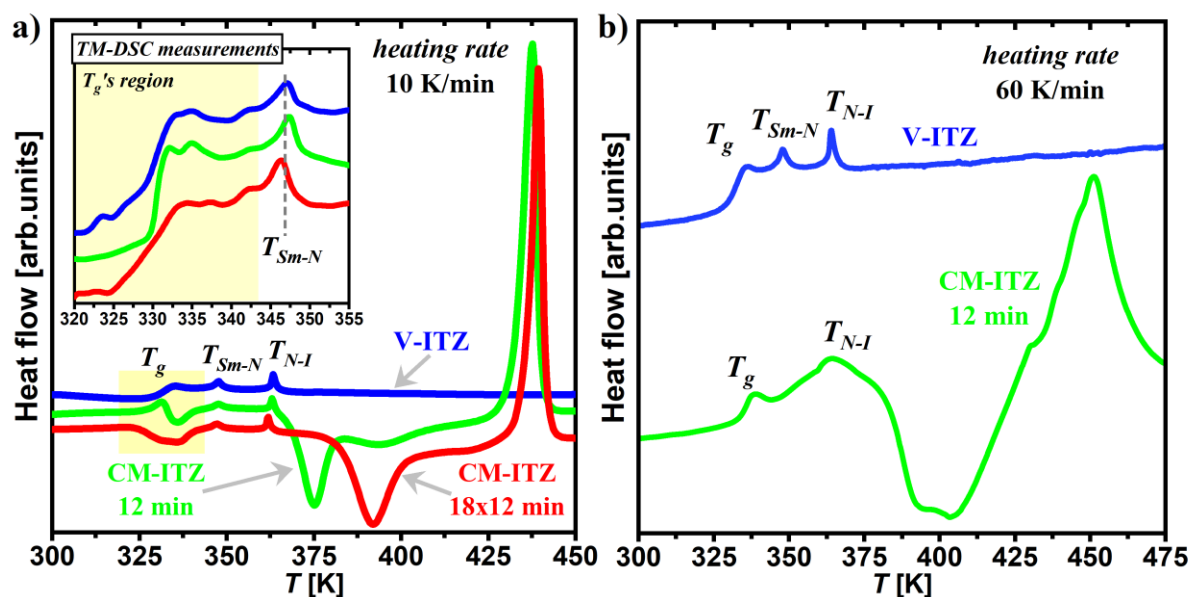


Figure 3.19 DSC thermograms of V-ITZ and CM-ITZ samples: **a)** measurements recorded at a heating rate of 10 K/min, with the inset showing TM-DSC data in the T_g region, **b)** measurements recorded at a heating rate of 60 K/min.

In the conventional DSC measurements recorded at 10 K/min, the $Sm - N$ transition was observed for the CM-ITZ samples. This behavior can be interpreted in the light of the results of physical stability studies for samples storage at 323 K, presented in the section 3.2. Influence of structural order on physical stability, which showed that CM-ITZ rebuilds the smectic order on storage at temperatures close to T_g . This supports the hypothesis that the observed transition may result from the formation of the Sm structure during DSC heating. To test this hypothesis, additional DSC measurements were performed for the short-milled sample at a higher heating rate of 60 K/min. Under these conditions, the time available for molecular rearrangement is strongly reduced. As shown in **Figure 3.19b**, the short-milled CM-ITZ sample exhibits only two main thermal events, assigned to the glass transition and the $N - I$ transition, whereas no distinct $Sm - N$ transition is detected. This behavior contrasts with the V-ITZ thermogram measured under the same conditions, where the $Sm - N$ transition remains visible. The absence of this transition for CM-ITZ at 60 K/min indicates that Sm order did not have sufficient time to form during rapid heating, in contrast to the experiment performed with the lower heating rate of 10 K/min. Thus, **these results support the conclusion that fresh CM-ITZ is characterized by N organization of ITZ molecules after cryomilling, without a contribution from Sm organization.** Finally, it is worth adding that, after heating CM-ITZ above T_m , the subsequent

thermogram resembled that obtained for V-ITZ, indicating that, after melting, the material's history was erased.

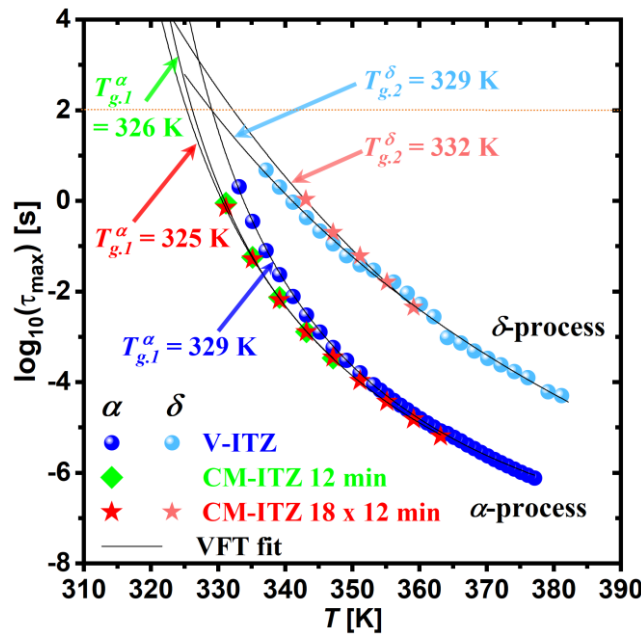


Figure 3.20 Relaxation map of V-ITZ and CM-ITZ samples showing the temperature dependence of the α - and δ -relaxation times.

The molecular dynamics of V-ITZ and CM-ITZ materials was investigated near T_g – defined as the temperature at which the relaxation time (τ) reaches 100 s. Here, the relaxation times of the α - and δ -processes are denoted as τ_α and τ_δ , respectively. As shown in **Figure 3.20**, the structural dynamics differ slightly between the CM-ITZ and V-ITZ samples. The α -relaxation, associated with molecular motions around the long molecular axis of ITZ, is faster in the CM-ITZ samples than in V-ITZ, as indicated by the shorter τ_α values. However, this difference becomes less pronounced at higher temperatures, especially near 363 K, corresponding to the $N - I$ transition. In this temperature region, the τ_α values of CM-ITZ samples tend to converge, although they do not fully overlap with those of V-ITZ. Dielectric spectra measured for V-ITZ and the long-milled CM-ITZ sample also allowed determination of τ_δ . This relaxation process is associated with orientational motions of ITZ molecules around the short molecular axis. The δ -mode dynamics of CM-ITZ is slightly slower than that of V-ITZ close to T_g . However, this difference is small, remains within the experimental uncertainty, and disappears at higher temperatures. Reliable τ_δ dependence could not be obtained for the short-milled CM-ITZ sample, since it crystallized rapidly at lower temperatures than the long-milled sample, consistent with the behavior observed in the DSC

thermograms presented in **Figure 3.19a**. Analysis of the $\tau = 100$ s criterion using the Vogel–Fulcher–Tammann (VFT) function also helped explain the two resolved glass-transition contributions observed for the CM-ITZ samples in DSC data. For V-ITZ, interpolation of both relaxation processes gives the same glass-transition temperature, $T_{g,1}^{\alpha} = T_{g,2}^{\delta} = 329$ K, which is consistent with the single T_g detected by DSC at approximately 330 K. In contrast, for the long-milled CM-ITZ sample, interpolation of the two relaxation processes gives different T_g values, $T_{g,1}^{\alpha} = 325$ K and $T_{g,2}^{\delta} = 332$ K. These values are close to those obtained from the calorimetric measurements, $T_{g,1} = 327$ K and $T_{g,2} = 331$ K, respectively. This indicates that, as suggested above, the two T_g contributions observed in the thermograms of CM-ITZ may originate from the kinetic arrest of different types of molecular motion in ITZ, namely, 1) motions around the long molecular axis, reflected by the α -process, and 2) motions around the short molecular axis, reflected by the δ -process.

After analyzing the thermal behavior of the V-ITZ and CM-ITZ species, the same calorimetric approach was applied to the SE-ITZ systems to determine how the LC structure derived by solvent-evaporation affects the thermal response of ITZ. Before DSC analysis, the residual DCM content was evaluated by TGA. As shown in **Figure 3.21**, for the SE-ITZ 20 sample, the mass loss estimated from the TGA curve close to 313 K is approximately 0.1 wt%, indicating that residual DCM is present only in trace amounts. The thermograms collected for freshly prepared SE-ITZ samples after DCM evaporation, together with their vitrified derivatives obtained from the second heating scans, are compared with thermogram of V-ITZ in **Figure 3.22a**. Similar to the behavior described above, V-ITZ and all SE-ITZ samples exhibit a heat-capacity step associated with the glass transition, followed by two endothermic events corresponding to the $Sm - N$ and $N - I$ transitions. These transitions occur at temperatures comparable to those observed for V-ITZ and CM-ITZ, indicating that the SE-ITZ systems retain the characteristic of the ITZ sequence of LC transitions.

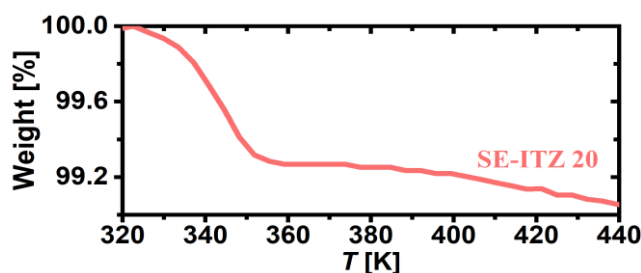


Figure 3.21 TGA curve of SE-ITZ 20 sample.

Despite this similarity, the SE-ITZ samples differ from V-ITZ in their high-temperature response. Above 400 K, the SE-ITZ materials undergo crystallization, followed by melting near $T_m \approx 440$ K, whereas V-ITZ does not show crystallization under the same conditions. In addition, the SE-ITZ samples show a slight decrease in T_g , with $\Delta T_g \approx -3$ K relative to V-ITZ. This shift may reflect structural differences generated during solvent evaporation. However, this effect is fully reversible after melting. The second heating scans, recorded after heating the SE-ITZ samples above T_m and subsequent vitrification, overlap with V-ITZ in both transition temperatures and peak areas. This confirms that the extensive smectic order produced during the solvent evaporation is disrupted upon melting, leading to a vitrified state comparable to V-ITZ.

It is also worth emphasizing that the thermograms of the freshly prepared SE-ITZ samples show a clear enthalpy-recovery overshoot in the T_g region. Such an overshoot is commonly observed in physically aged glasses and reflects structural relaxation toward a lower-enthalpy state [102].

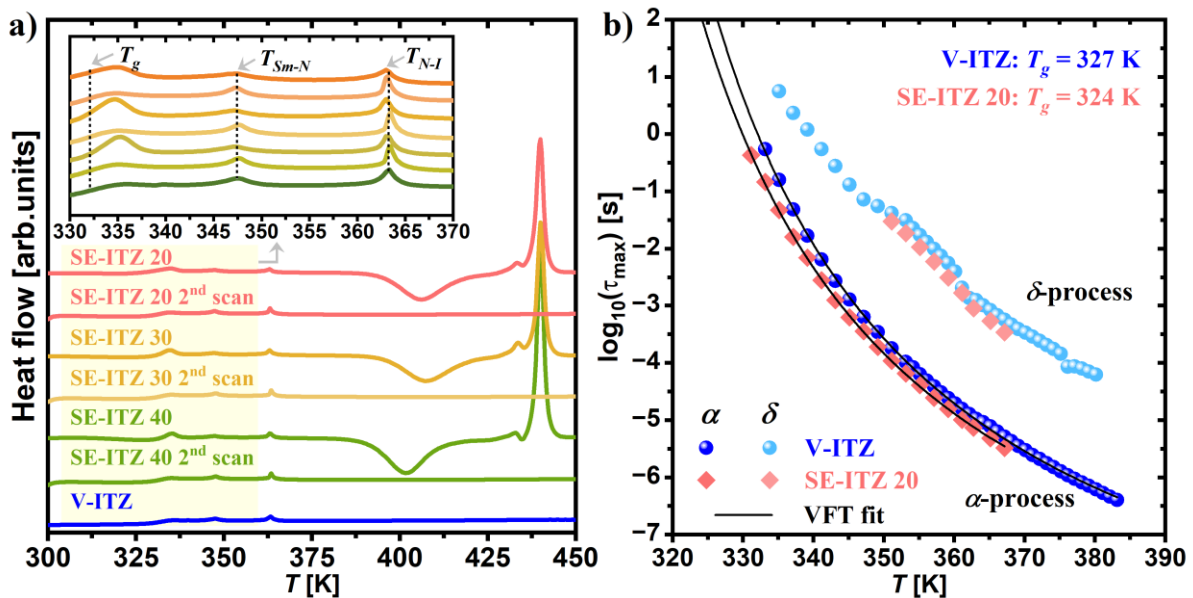


Figure 3.22 DSC thermograms and relaxation map of SE-ITZ samples compared with V-ITZ: **a)** shows the thermal response of freshly prepared SE-ITZ samples and their second heating scans, while **b)** presents the temperature dependence of the α - and δ -relaxation times for V-ITZ and SE-ITZ 20.

The molecular dynamics of V-ITZ and SE-ITZ were then investigated by dielectric spectroscopy in the temperature range slightly above T_g . This analysis was performed

to determine how the smectic order induced by solvent evaporation affects the relaxation behavior of ITZ relative to the vitrified form. The calculated temperature dependencies of τ are shown in **Figure 3.22b**. Clear differences in the structural dynamics are observed between V-ITZ and SE-ITZ close to T_g . **The SE-ITZ 20 sample exhibits shorter τ_α than V-ITZ, indicating faster α -relaxation dynamics.** This difference decreases at higher temperatures, especially near 363 K, corresponding to the $N - I$ transition. **Although the $\tau_\alpha(T)$ of SE-ITZ tends to approach that of V-ITZ in this temperature region, they do not fully overlap.** The dielectric data also show a difference in the T_g values derived using the same criterion ($\tau = 100$ s) applied above for the CM-ITZ samples. Based on this criterion, T_g values of approximately 327 and 324 K are obtained for V-ITZ and SE-ITZ 20, respectively. Thus, the solvent-evaporated sample exhibits a slightly lower T_g than the vitrified sample, consistent with the T_g behavior observed in the calorimetric data. In addition, the δ -process was analyzed at temperatures above T_g . **SE-ITZ 20 shows slightly faster δ -mode dynamics than V-ITZ.** However, this difference remains within the experimental uncertainty and disappears at higher temperatures. **These results indicate that the LC molecular organization produced by solvent-evaporation affects the molecular dynamics of the ITZ mainly near T_g ,** while the relaxation behavior becomes more similar to that of conventional glass at elevated temperatures.

3.4. Effect of structural order on dissolution rate

Motivated by the high physical stability of the CM-ITZ samples, dissolution experiments were performed to examine how the cryomilling-induced structural disorder affects the release behavior of ITZ. The dissolution rates were measured in 0.1 M HCl aqueous solution at pH = 1 and 310 K. Such conditions accurately simulate the highly acidic, warm conditions of the human stomach. Short- and long-milled CM-ITZ samples were compared with crystalline ITZ and V-ITZ as reference materials. The corresponding dissolution profiles are shown in **Figure 3.23a**. Crystalline ITZ showed very poor dissolution, with the dissolved concentration below 6 $\mu\text{g/mL}$ after 90 h. In contrast, conversion of the crystalline material into the more disordered V-ITZ form, (in which part of the smectic molecular alignment is kinetically preserved) enhanced dissolved fraction, reaching approximately 103 $\mu\text{g/mL}$ after 60 h. The concentration increased gradually to this maximum value and then decreased slightly to approximately 100 $\mu\text{g/mL}$ after 90 h. Both CM-ITZ samples exhibited improved dissolution profiles relative to crystalline ITZ and V-ITZ. For the short-milled sample,

the concentration increased to approximately 107 $\mu\text{g/mL}$ after 30 h and remained nearly constant until 90 h. **The long-milled sample reached the highest concentration of dissolved ITZ – approximately 110 $\mu\text{g/mL}$ after 30 h, followed by a gradual decrease to a level close to that observed for the short-milled sample.** These results indicate that the intermolecular disorder is the primary factor governing the dissolution behavior of ITZ. Crystalline ITZ and V-ITZ have nearly identical average particle sizes. Nevertheless, **V-ITZ reaches approximately 17 times higher concentration than the crystalline material.** Thus, in this case, the transition from a crystalline lattice to a disordered non-crystalline structure has a stronger effect on dissolution kinetics than particle size alone. **Particle size may influence the early stage of the dissolution profile, as the long-milled sample, which has the smallest average particle size, shows the steepest initial concentration increase.** However, at longer times, its profile becomes comparable to that of the short-milled sample. **Thus, after the initial dissolution stage, the internal molecular structure of ITZ appears to constitute the key factor influencing the dissolution curve of this API.**

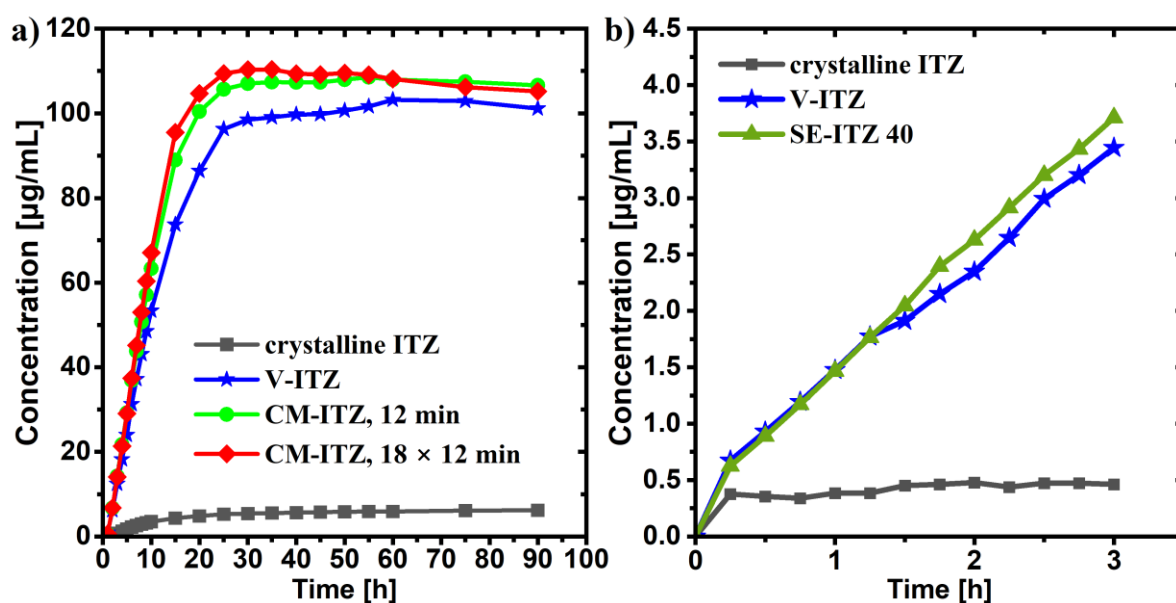


Figure 3.23 Dissolution profiles of ITZ samples in 0.1 M HCl aqueous solution at 310 K: **a)** long-time dissolution behavior of crystalline ITZ, V-ITZ, and CM-ITZ powders. **b)** short-time drug release from crystalline ITZ, V-ITZ, and SE-ITZ 40 powders.

To further assess the relation between the physical stability and dissolution behavior in the solvent-evaporated systems, separate drug release measurements were performed for SE-ITZ 40 under the same acidic medium used for the CM-ITZ dissolution study, i.e., 0.1 M

HCl aqueous solution at 310 K. This sample was selected because it showed the highest physical stability among the SE-ITZ systems during storage at 295 K. Since gastric emptying under fasting conditions usually occurs within approximately 2 h [103,104], the SE-ITZ 40 release experiment was limited to about 3 h. Crystalline ITZ and V-ITZ were used as reference materials. The corresponding profiles are shown in **Figure 3.23b**. After 3 h, SE-ITZ 40 reached a released ITZ concentration of approximately 37 $\mu\text{g/mL}$, whereas crystalline ITZ reached only 5 $\mu\text{g/mL}$. Thus, the released concentration from SE-ITZ 40 was approximately seven times higher than that from the crystalline reference. V-ITZ also showed strongly enhanced release relative to crystalline ITZ, reaching approximately 34 $\mu\text{g/mL}$ after 3 h. Therefore, both non-crystalline forms released substantially larger amounts of ITZ than the crystalline material under acidic conditions. However, the difference between SE-ITZ 40 and V-ITZ was small. This suggests that, within the time scale of this experiment, differences in the degree and correlation length of the frozen smectic order do not produce a pronounced change in the release profile. By analogy with the CM-ITZ dissolution results, where particle size mainly affected the initial dissolution stage, the short-time release of SE-ITZ 40 may also be influenced by particle morphology. Thus, the structural contribution associated with the different smectic ordering may be partly masked by morphology-dependent dissolution effects.

3.5. Summary of results for itraconazole

The obtained results demonstrated that the amorphization pathway strongly determines the final supramolecular organization of ITZ. The vitrification procedure leads to the characteristic smectic LC structure of ITZ, whereas high-energy milling can disrupt both crystalline and smectic order, forming ITZ solid particles with a more disordered nematic molecular organization. In contrast, by the solvent-evaporation method using DCM as the solvent, ITZ particles with extended smectic domains and a higher degree of layered order than for the conventional glass (vitrified sample) may be produced. Thus, these three preparation routes lead to distinct non-crystalline structures with different degrees of frozen liquid-crystalline molecular order. It was demonstrated that for CM-ITZ, increasing milling time or frequency also enhances disorder in the short-range intermolecular arrangement. Once the nematic structure is formed, further mechanical treatment does not destroy it completely, but it increases the lateral disorder between neighboring ITZ molecules. SE-ITZ samples show a kind of the opposite tendency. Namely, this route promotes smectic organization, and the degree of this order can be tuned by the initial ITZ concentration in DCM.

Evaporation of solvent from more diluted solutions results in the creation of stronger smectic intermolecular correlations. PDF analysis further showed that SE-ITZ exhibits much longer-range structural correlations than V-ITZ. The final structure of the solvent-evaporated ITZ depends on the evaporation kinetics: slow evaporation leads to crystallization, whereas faster evaporation under reduced pressure results in freezing of molecules into a layered LC organization. Moreover, the structure also depends on the solvent type: DCM provided a higher degree of the smectic order, while CLF resulted in a more disordered smectic structure, and the other tested solvents led to crystallization.

It was also revealed that the structural differences among the various LC organizations directly affect the physical stability of ITZ. ITZ amorphized by cryomilling shows markedly higher resistance to crystallization than the conventional glass obtained by vitrification. At room temperature — below the T_g of ITZ, the long-milled CM-ITZ remains non-crystalline for up to 300 days, whereas V-ITZ shows signatures of crystallization after about 40 days. The same trend is also observed at 323 K, where cryomilled samples remain more stable than V-ITZ, although the crystallization is accelerated in all cases. This demonstrates that the nematic organization produced by cryomilling, especially after prolonged milling, stabilizes the non-crystalline ITZ state more effectively than the smectic structure preserved in V-ITZ. The SE-ITZ series confirms that physical stability is also sensitive to the degree of the smectic ordering. Samples prepared from lower initial ITZ concentrations in DCM show stronger smectic order but lower stability, whereas samples prepared from higher concentrations show weaker smectic order and higher stability. Moreover, results for SE-ITZ 30 and V-ITZ showed that samples with varying degrees of smectic order can exhibit similar physical stability, as both systems crystallize on comparable timescales. This demonstrates that the physical stability of non-crystalline ITZ is not determined only by the overall degree of LC order. Other factors, such as the short-range molecular organization, molecular dynamics, and thermodynamic state of the material, may also play an important role in controlling crystallization tendency.

Further, the DSC and BDS results supported the distinction among the various non-crystalline ITZ forms. V-ITZ exhibits the characteristic thermal response of ITZ, with a glass transition near 330 K followed by the $Sm - N$ and $N - I$ transitions. CM-ITZ also shows LC-related thermal events, but its response differs from V-ITZ because heating above T_g promotes crystallization followed by melting near 440 K. The short-milled CM-ITZ crystallizes at lower temperature than the long-milled sample, confirming its lower thermal

stability during non-isothermal heating. In addition, TM-DSC revealed two partially resolved glass-transition contributions in CM-ITZ, whereas V-ITZ shows a single T_g response. Heating-rate-dependent DSC further confirmed the nematic nature of molecular organization in CM-ITZ, as the $Sm - N$ transition is not observed at high heating rates, indicating that this transition can arise from partial rebuilding of smectic order during slower heating.

It was shown that molecular dynamics also differ among the various non-crystalline ITZ forms, particularly near T_g . BDS measurements showed that the α -relaxation is faster in CM-ITZ than in V-ITZ close to T_g , while this difference becomes smaller at higher temperatures. The δ -relaxation of CM-ITZ was slightly slower than that of V-ITZ near T_g . In turn, the δ -relaxation in SE-ITZ was slightly faster than in V-ITZ, although this difference remains within experimental uncertainty and vanishes at higher temperature. For SE-ITZ, the α -relaxation is also faster than in V-ITZ near T_g , accompanied by a slightly lower dynamic T_g . Therefore, these observations demonstrate that the preparation-induced molecular organization in solid ITZ affects its dynamics below and near the glass-transition region, whereas the molecular relaxation behavior becomes more similar at elevated temperatures where all structures tend toward a single preferred thermodynamic equilibrium.

Dissolution measurements in acidic medium showed that all studied non-crystalline ITZ forms release substantially more drug than the crystalline form. This confirms that conversion from the crystalline state to a non-crystalline structure, even with signatures of different degrees of the liquid-crystalline order, strongly enhances ITZ dissolution. The samples obtained by cryomilling and characterized a disordered nematic structures show particularly interesting behavior from the pharmaceutical perspective, as they combine an enhanced dissolution profiles with improved physical stability. The sample produced by the solvent-evaporation method also shows strongly enhanced release compared with crystalline ITZ, but its short-time release profile remains close to that of V-ITZ. Thus, one can state that within the experimental time scale, differences in the degree and coherence length of the frozen smectic order have only a minor effect on ITZ release.

4. Results and discussion for fenoprofen calcium

4.1. Influence of different processing methods on structural order

The second pharmaceutical compound selected for study was FC, which is also a thermotropic liquid crystal, but a structurally distinct system from ITZ. FC is an ionic salt that crystallizes as a dihydrate with the chemical formula $(C_{15}H_{13}O_2)_2 Ca \cdot 2H_2O$. Therefore,

it provides a second model system for addressing the central questions of this thesis, namely whether different amorphization pathways can control the degree of LC order in APIs and whether the resulting structures govern their thermal behavior, molecular dynamics, physical stability, and dissolution properties. Here, vitrification and cryomilling were used to disrupt the crystalline structure of FC. The corresponding X-ray scattering data are presented in **Figure 4.1a**, together with the patterns of crystalline FC at room temperature and after heating at 373 K. The related schematic structural models are shown in **Figure 4.1b**. Crystalline FC, with its corresponding molecular and coordination motif schematically shown in **Figure 4.1b-i**, was subjected to gradual heating from 293 to 423 K. During this treatment, the sharp Bragg reflections of the dihydrate disappeared at approximately 373 K, indicating a thermally induced loss of crystalline order. This transition gave rise to a diffraction pattern characterized by a series of four reflections, followed by a broad halo at $Q \approx 1.35 \text{ \AA}^{-1}$. The positions of these reflections follow the ratio $1:\sqrt{3}:2:\sqrt{7}$, which is characteristic of a hexagonal lattice [71]. The reflections can be indexed as (10), (11), (20), and (21) of the two-dimensional hexagonal structure. This structural transition is consistent with previous studies of FC, where the appearance of 2D inverse hexagonal arrangement of FC molecules was assigned to a transformation into thermotropic LC mesophase [71]. In this organization, FC molecules form cylindrical aggregates in which the polar Ca^{2+} -carboxylate regions are directed toward the core, whereas the hydrophobic molecular moieties extend outward. The cylindrical channels form the hexagonal lattice. The structural model of such a single cylindrical aggregate is schematically illustrated in **Figure 4.1b-ii** whereas the schematic representation of cylindrical aggregates organized in the hexagonal structure was presented in **Figure 1.8**. Further heating to 423 K result in more intense series of (10), (11), (20), and (21) reflections (**Figure 4.1a**). **According to the literature, the formation of hexagonal mesophase observed upon heating of FC dihydrate is associated with the progressive removal of water from the crystalline lattice, and, finally, the formation of an anhydrous phase [71].** When this high-temperature mesophase is cooled rapidly to room temperature, **the anhydrous inverse hexagonal organization is preserved in the vitrified (glass-like) solid [51,71].** The corresponding structural model of the anhydrous V-FC, where the water was removed from the cylindrical nanochannels organized in the hexagonal lattice, is shown in **Figure 4.1b-iv**.

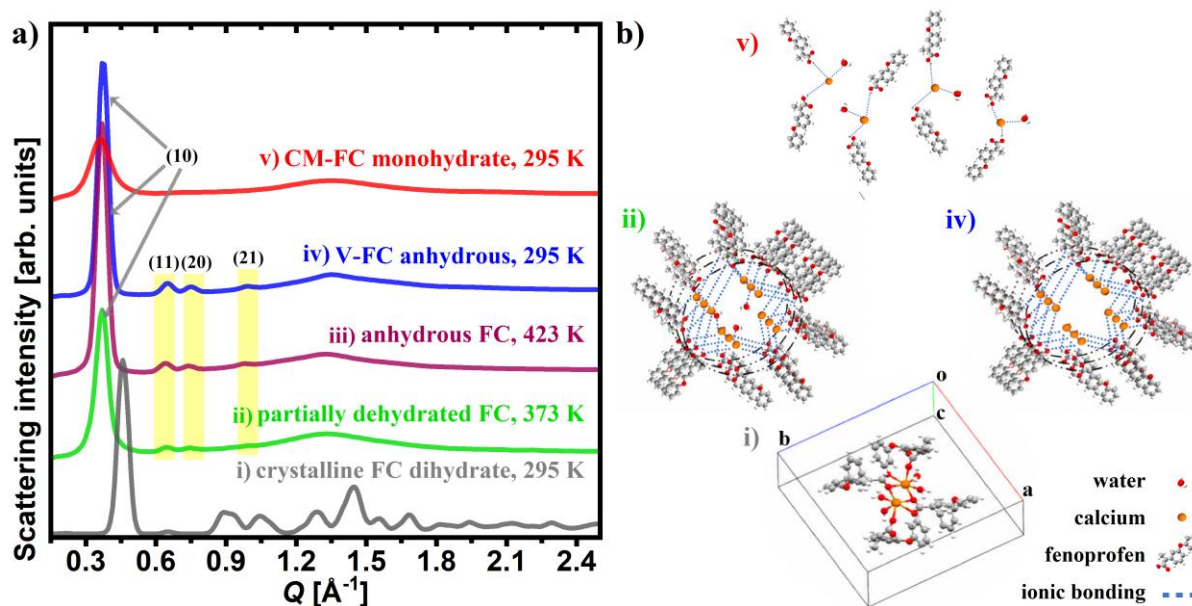


Figure 4.1 **a)** X-ray scattering patterns of FC in different structural states: i) crystalline dihydrate at 293 K, ii) partially dehydrated FC obtained by heating to 373 K, iii) anhydrous FC obtained by heating to 423 K, iv) anhydrous V-FC obtained by vitrification, and v) CM-FC monohydrate obtained by cryomilling. **b)** Schematic structural models corresponding to the states shown in panel a.

In light of the results obtained for cryomilled itraconazole (CM-ITZ), the same mechanical amorphization approach was applied to crystalline FC dihydrate using the cryomilling procedure described in section 2.2. Procedures of sample preparation. The freshly prepared CM-FC sample was examined by X-ray scattering, and the obtained XRD pattern is shown in **Figure 4.1a**. Compared with the heated and V-FC samples, which exhibit a well-defined hexagonal reflection sequence, CM-FC shows a markedly different scattering pattern. In the low- Q region, only a broad feature, close to the position of (10) reflection, remains before the broad amorphous halo, whereas the higher-order reflections, (11), (20), and (21), are not resolved. **Such diffraction pattern is typical for a nematic structure and indicates that molecules in CM-FC adopts a nematic LC order [105],** schematically presented in **Figure 4.1b-iv**. The low- Q feature reflects a **characteristic correlation distance (d_{cr}) of approximately 1.7 nm between FC molecules aligned along the director axis**, but without long-range positional periodicity. This distance was estimated from the position of the low- Q maximum using the Braggs' law (Equation 2.3): $d_{cr} = \frac{2\pi}{Q}$. The correlation length (l_{cr}) of approximately 7 nm, determined based on the full width at half

maximum (*FWHM*) of the low- peak using the formula: $l_{cr} = \frac{2\pi}{FWHM}$, reflects the range of structural correlations in this nematic structure along the director axis.

Because the structural transformations of FC were reported to be governed by dehydration of crystalline FC dihydrate [75], TGA was performed to quantify the water content in the studied samples and clarify the features of the nematic structure generated by cryomilling. The examples of TGA curves are shown in **Figure 4.2**. **The sample heated up to 373 K contains approximately 2 wt% residual water**, indicating substantial dehydration relative to **the initial crystalline dihydrate, which contains about 7 wt% water**. This value was estimated from the total mass loss associated with complete water removal at higher temperatures, before the chemical degradation of FC. By comparison, **CM-FC retains approximately 3.5 wt% water**, as shown in **Figure 4.2b**, corresponding to a stoichiometric monohydrate. Thus, the obtained results evidence that the applied cryomilling procedure not only lead to destroying of the crystalline order of FC dihydrate and formation of the structure with a nematic organization of molecules, but also lead to removal of one H₂O molecule per one FC molecule.

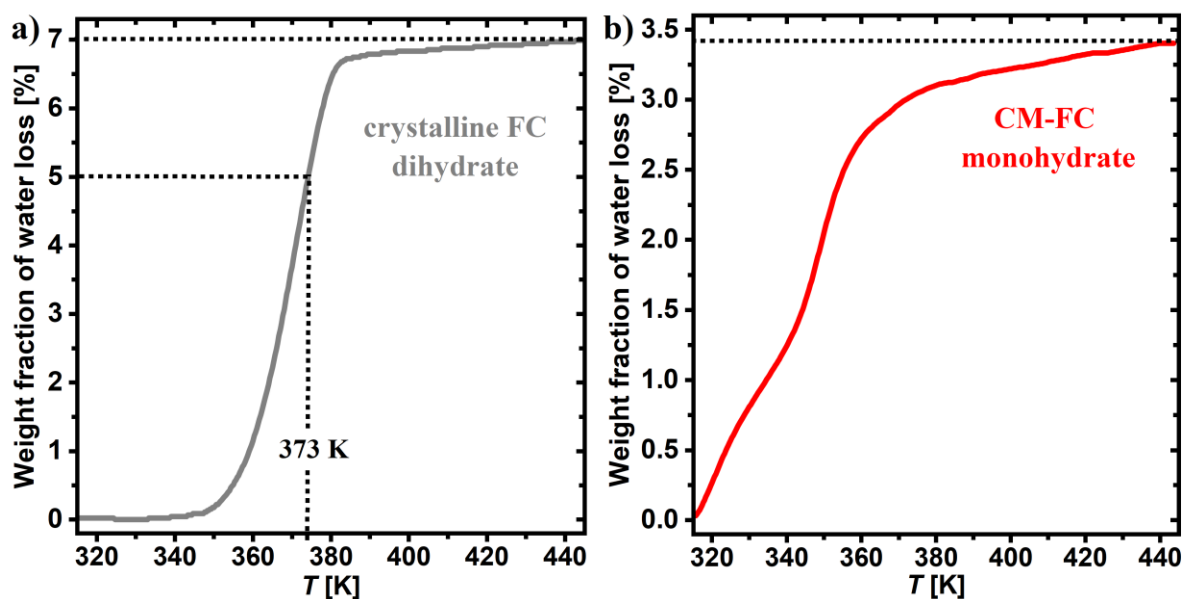


Figure 4.2 TGA curves of: **a)** crystalline FC (dihydrate) and **b)** CM-FC (monohydrate).

It should be noted that, before cryomilling, the milling chamber was filled with argon to eliminate atmospheric moisture and prevent water uptake during processing. Therefore, the water retained in the cryomilled FC originates from H₂O molecules intrinsically present within the initial crystalline structure. It is worth adding that a previous study in our research

group showed that cryomilling of crystalline bosentan monohydrate (which is not a thermotropic liquid crystal) also leads to sample dehydration [106]. In that case, nearly anhydrous amorphous particles were obtained.

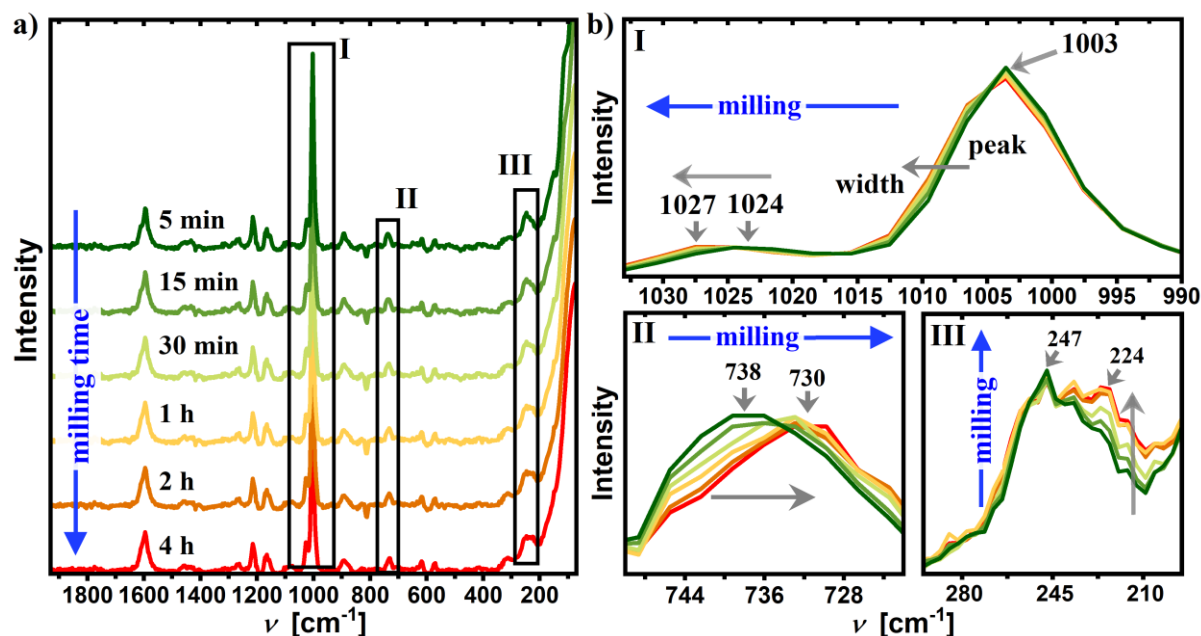


Figure 4.3 In-situ Raman spectra of FC collected as a function of time during the progress of milling: **a)** full spectral range and **b)** enlarged regions showing the evolution of selected bands.

To further examine whether the cryomilling-induced structural transformation in FC occurs gradually or through an abrupt dehydration event, an in-situ Raman spectroscopy experiment was performed. The evolution of the Raman spectra provides additional insight into the progression of structural changes in FC during the mechanical treatment. As shown in **Figure 4.3**, the Raman spectrum changes systematically with increasing milling time, indicating a gradual modification of the local molecular environment. The strong band near 1003 cm^{-1} gradually decreases in intensity and broadens over the milling time, which reflects the progressive loss of crystalline order during the mechanical treatment [107,108]. At the same time, the peak near 1027 cm^{-1} gradually shifts toward 1024 cm^{-1} . In the $745\text{--}728\text{ cm}^{-1}$ region, the spectral intensity progressively shifts from the band near 738 cm^{-1} toward the feature near 730 cm^{-1} . Clear changes are also observed in the low-frequency range between 280 and 200 cm^{-1} , where the bands near 247 and 224 cm^{-1} evolve in relative intensity and shape. A definitive assignment of these bands to specified functional groups would require dedicated vibrational calculations. However, the mid-frequency changes are most likely associated with vibrations

of the aromatic molecular framework, whereas the low-frequency region is expected to be sensitive to collective lattice motions and changes in intermolecular organization [108,109]. In the case of FC, these low-frequency changes may also reflect modifications in the Ca^{2+} –carboxylate coordination environment during milling. The continuous character of these spectral changes supports a gradual mechanically induced reorganization of FC during cryomilling, rather than an abrupt structural transition. It is worth clarifying that the sample recovered from the in-situ Raman experiment during milling was probed by XRD, which confirmed its nematic structure, and by TGA, which confirmed that it is a monohydrate.

4.2. Influence of structural order on physical stability

The different non-crystalline structures of solid FC produced by vitrification and cryomilling provided an opportunity to examine the relationship between the structural order and physical stability. To remind, it was established that vitrification leads to an anhydrous hexagonal organization of FC molecules, whereas cryomilling yields a more disordered (– nematic) and hydrated structure, with roughly one H_2O molecule per one FC molecule (monohydrate). Therefore, V-FC and CM-FC represent two solid structures with distinct LC order and different water content. Given this contrast, together with the importance of physical stability from a pharmaceutical materials perspective, sealed V-FC and CM-FC samples sealed in glass capillaries samples were stored under ambient laboratory conditions at 295 K and approximately 40% RH, and temporarily examined by the XRD method. The corresponding time-dependent X-ray scattering patterns are shown in **Figure 4.4**. **V-FC shows the first signs of crystallization after only 1 day of storage**, followed by a progressive increase in the intensity of Bragg reflections over the next several days. This sample, after a few days, returns to the same polymorphic form as the commercial crystalline dihydrate. In contrast, **CM-FC exhibits exceptional physical stability – the first fingerprint of crystallization is detected only after 810 days of storage. This corresponds to more than two years of stability under ambient (room) conditions**, showing that the nematic FC monohydrate generated by cryomilling is substantially more resistant to crystallization than the FC glass created by the vitrification of the dehydrated hexagonal mesophase.

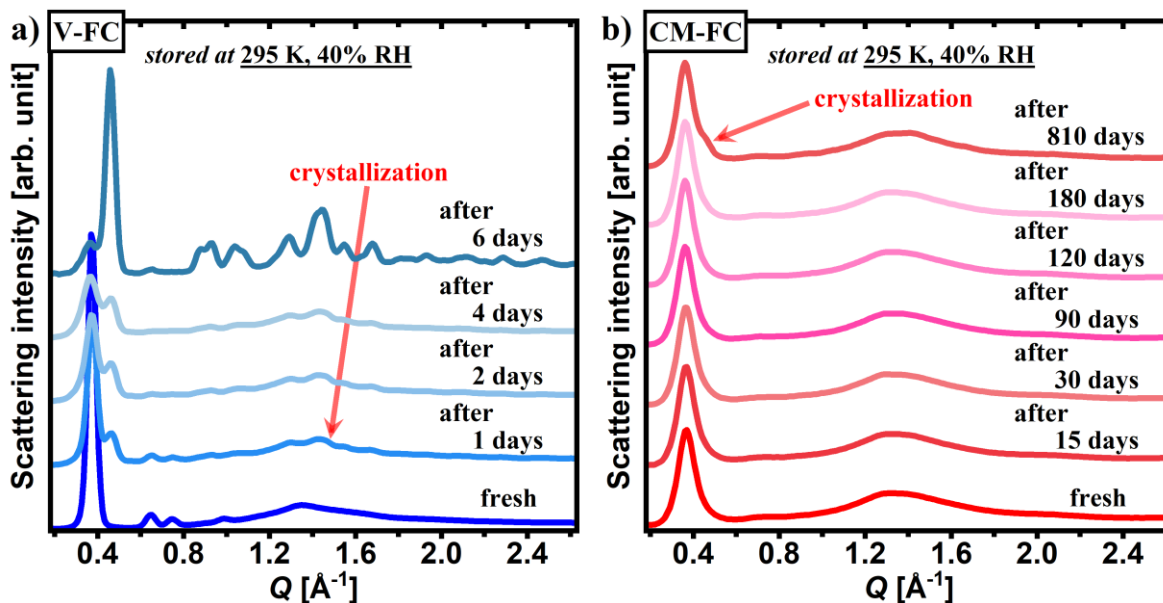


Figure 4.4. Temporal evolution of X-ray scattering patterns for FC samples stored at 295 K, 40% RH: **a)** V-FC, **b)** CM-FC

Interestingly, this result is consistent with the physical stability behavior observed for ITZ, where the cryomilled nematic samples also showed higher physical stability than their vitrified counterparts. This behavior is notable because cryomilling of crystalline pharmaceutical solids is generally expected to generate highly disordered or amorphous materials that are often less physically stable than amorphous forms prepared by vitrification or solvent-based methods [26,110].

4.3. Thermal properties

After the structural analyses, DSC measurements were performed to examine the thermal behavior of FC samples exhibiting different LC orders and hydration levels. The resulting thermograms are shown in **Figure 4.5**. Crystalline FC dihydrate was first heated from 130 to 420 K. The thermogram exhibits a pronounced endothermic peak at approximately 375 K. Based on the combined calorimetric, TGA, and X-ray scattering results, this thermal event was attributed to dehydration accompanied by the formation of the hexagonal mesophase. After heating to 420 K, where water was completely removed according to the TGA data, the sample was cooled directly to 130 K. No detectable thermal transition was observed during cooling. **The V-FC sample was then heated and showed a glass transition at approximately 312 K. Since this sample was anhydrous, this event was assigned to the FC matrix (the glass transition temperature of FC is denoted as T_g^{FC}).**

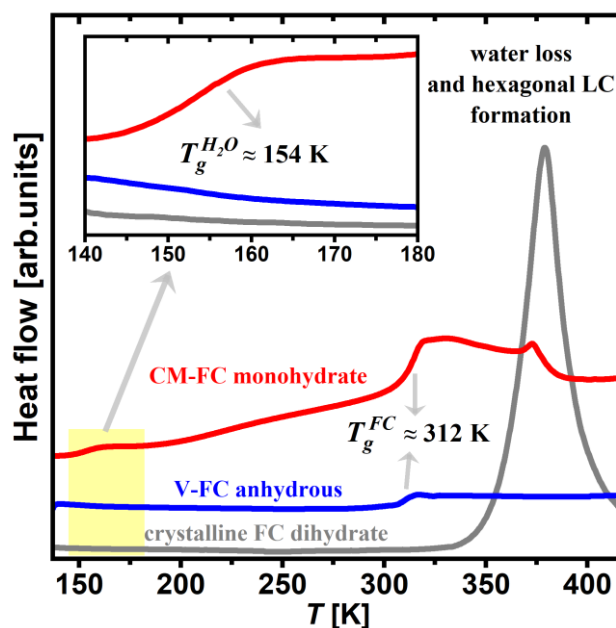


Figure 4.5 DSC thermograms of crystalline FC dihydrate, anhydrous V-FC, and CM-FC monohydrate.

The same heating protocol was then applied to CM-FC monohydrate. **In this case, two glass-like transitions are observed on the DSC thermogram. The first occurs at approximately 154 K, as shown in the inset of Figure 4.5. The second transition near 312 K coincides with the T_g^{FC} of V-FC and was therefore assigned to the FC matrix in CM-FC.** The similar T_g^{FC} for V-FC and CM-FC materials, despite the former being anhydrous whereas the latter contains approximately one H₂O molecule per one FC unit, is noteworthy, since water commonly acts as a plasticizer in non-crystalline pharmaceutical solids by increasing molecular mobility and lowering T_g [111]. **However, CM-FC does not show a pronounced decrease in T_g^{FC} relative to anhydrous V-FC. This suggests that the retained water is likely strongly incorporated into the local Ca²⁺–carboxylate ionic environment, where its contribution to matrix plasticization is limited.** This interpretation is consistent with reports showing that salt ions can bind water in hydration shells through strong ion-dipole interactions, thereby decreasing water mobility and reducing the plasticization effect [112]. Since CM-FC is composed of FC and water, **the glass-like transition at approximately 154 K can be attributed to water trapped within the FC matrix.** This assignment is supported by the thermogram of the anhydrous vitrified sample, where only the T_g^{FC} is observed. Upon further heating of CM-FC, an endothermic peak is detected near 375 K, similar to that observed for crystalline FC dihydrate due to the water

loss. However, its intensity is significantly reduced, reflecting the lower water content in CM-FC (denoted as $T_g^{H_2O}$) as compared to crystalline FC. Notably, the temperature of the glass-like transition – T_g of water in CM-FC is lower than the typical 170-200 K range reported T_g for H₂O trapped in nanoporous materials [113]. This unusually low $T_g^{H_2O}$, together with the absence of a plasticizing effect on the FC matrix, suggests that water in CM-FC behaves differently from water under conventional nanoporous confinement. This unusual behavior of trapped water in the LC structure of FC will be discussed further in section 4.5.

4.4. Effect of structural order on dissolution rate

Based on the exceptional physical stability of CM-FC, dissolution experiments were performed to verify whether this cryomilled nematic form may provide an advantage for the drug release. The measurements were carried out in FaSSIF medium at pH 6.5, which simulates fasted-state small-intestinal conditions [114]. This medium was selected in this case because FC is a weak acidic drug [115] and is expected to dissolve more efficiently under intestinal pH conditions than in strongly acidic gastric medium [116,117]. The dissolution behavior of CM-FC was compared with that of crystalline FC dihydrate and anhydrous V-FC. The corresponding release profiles are presented in **Figure 4.6. CM-FC shows the fastest release over the entire measurement time, reaching approximately 36% after 3 h. Crystalline FC dihydrate exhibits an intermediate release rate, reaching approximately 29% after the same time.** In contrast, V-FC anhydrous shows a relatively rapid release up to approximately 15 min of the experiment, after which the release rate slows markedly. As a result, **V-FC exhibits the lowest total release after 3 hours, reaching only approximately 22%.** This behavior suggests that the anhydrous FC hexagonal does not provide a sustained dissolution advantage in FaSSIF medium, possibly because of structural transformation during contact with the aqueous medium. Thus, CM-FC shows a favorable dissolution performance compared with the other structural forms, which can be attributed to the structural disorder introduced by cryomilling, together with the reduced particle size and higher effective surface area of the milled material.

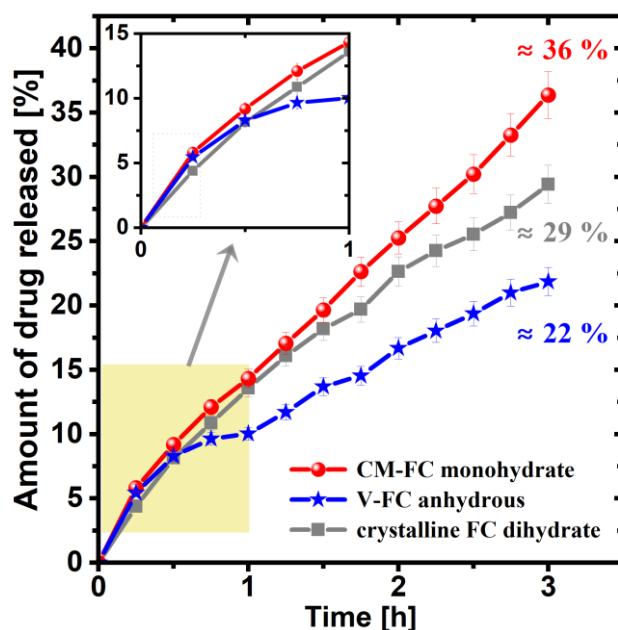


Figure 4.6 Dissolution profiles of crystalline FC dihydrate, anhydrous V-FC, and CM-FC monohydrate in FaSSiF medium.

4.5. Unusual behavior of water trapped in liquid crystalline fenoprofen calcium structure

The results presented in section 4.1. Influence of different processing methods on structural order, show that the structure of FC is strongly affected by its hydration level. In this system, changes in water content directly influence the supramolecular organization of the material and lead to the formation of different structural states. FC therefore provides an example of a molecular system in which water has an important structural role. This observation is consistent with the broader importance of water in many biological, geological, physical, and chemical systems. More generally, despite its simple molecular structure, water exhibits several anomalous properties that distinguish it from most simple liquids [118]. These include the density maximum near 277 K [119], the increase in density upon melting [120], and the anomalous pressure dependence of viscosity [121]. These properties are commonly attributed to the directional hydrogen-bond network of water [118,122], in which each molecule can form up to four hydrogen bonds and adopt different local structural arrangements [118]. The deeply supercooled region of the water phase diagram is especially important because it may provide insight into the microscopic origin of these anomalies [123,124]. However, direct experimental access to this region is strongly limited by crystallization. Liquid water crystallizes below the homogeneous nucleation temperature $T_H \approx 232$ K, whereas amorphous ice crystallizes upon heating at approximately $T_x \approx 150$ K

[124,125]. Consequently, the temperature interval between T_x and T_H is commonly referred to as “no man’s land,” because liquid water is extremely difficult to study in this range without crystallization [118].

Nanoscale confinement has become one of the main strategies for overcoming this limitation. By restricting the size of water domains, confinement can suppress cooperative ice growth and enable the study of water dynamics in temperature ranges that are inaccessible for bulk liquid water. Confined water has therefore been investigated in diverse systems, including silica pores [126], calcium silicate hydrates [127], metal–organic frameworks [128], proteins [129], hydrogels [130], and reverse micelles [131]. In most cases, however, water is introduced into a pre-existing confining matrix through hydration or pore filling. Consequently, several water populations may coexist, including strongly bound interfacial water, partially restricted water, and more bulk-like water located farther from the confining surface [129]. This heterogeneity can complicate the assignment of thermal and dynamical features to a specific water fraction.

The structure of CM-FC provides a unique, non-classical confinement case. Here, water is not introduced externally into a host matrix. Instead, it is already present in the parent FC dihydrate and is partially retained during cryomilling. The structural transformation induced by cryomilling traps the retained water within the nematic FC matrix. Based on the limited plasticization effect, the retained water is expected to be located mainly in the Ca^{2+} –carboxylate ionic domains, where its mobility is largely restrained.

To further investigate the nature of the water confined in CM-FC, a dielectric spectroscopy experiment was performed to probe its molecular dynamics over 273–193 K, where the relaxation process approaches the glass transition regime previously identified near 154 K. **The resulting relaxation map (Figure 4.7) shows a characteristic feature commonly reported for confined water, namely a fragile-to-strong dynamic crossover in the temperature dependence of the water’s relaxation times (τ_{H_2O})** [132,133]. Two distinct temperature-dependent regimes are observed, separated by a crossover temperature at $T_c \approx 226$ K. Fitting these regimes with the Arrhenius equation gives activation energies of $E_{a1} = 56.23$ kJ/mol above T_c and $E_{a2} = 38.49$ kJ/mol below T_c . In both temperature regimes, the Arrhenius behavior indicates a locally thermally activated relaxation process. Above T_c , the higher activation energy reflects a stronger temperature dependence of the τ_{H_2O} [134]. Upon cooling below T_c , the activation energy decreases, indicating that the relaxation becomes less sensitive to temperature [135]. To examine whether

this dynamic crossover is accompanied by changes in the local molecular environment of confined water, FTIR studies were performed with particular attention to the OH stretching region.

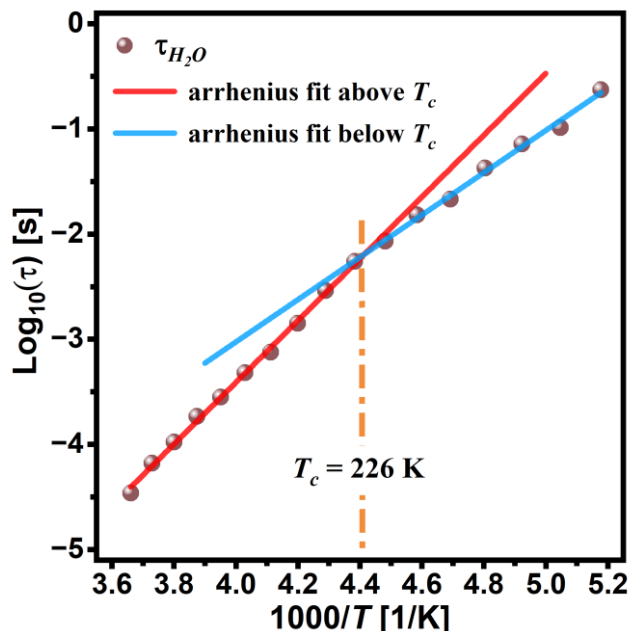


Figure 4.7 Relaxation map of water confined in FC matrix.

FTIR spectra in the OH and CH stretching regions were recorded during cooling from room temperature, 295 K, to 123 K, as shown in **Figure 4.8**. At 295 K, the OH stretching region exhibits three main components corresponding to different hydrogen-bonding environments of water. The band at approximately 3616 cm^{-1} is assigned to weakly or non-hydrogen-bonded OH groups. The broader bands centered at approximately 3324 and 3170 cm^{-1} arise from moderately and strongly hydrogen-bonded OH stretching modes, respectively. These contributions are associated with water–water interactions and water–polar carboxylate group interactions within the FC matrix. In particular, the band near 3170 cm^{-1} is mainly attributed to water interacting with polar FC groups, consistent with reports showing that carboxylate-rich environments promote strongly hydrogen-bonded water [136,137]. During cooling, the positions of the bands assigned to strongly hydrogen-bonded and free OH groups change only weakly, whereas the band corresponding to moderately hydrogen-bonded OH groups evolves more clearly. The temperature dependence of its band position, (denoted as ν_{OH}), is shown in the inset. Similar to what is observed for confined water in mesoporous silica [138,139], ν_{OH} from water in CM-FC exhibits a pronounced red-shift around 233 K during cooling, indicating a significant modification of the hydrogen-bond network.

This temperature is close to the dynamic crossover detected by BDS, $T_c \approx 226$ K, suggesting that the change in relaxation behavior is accompanied by a rearrangement of the local hydrogen-bonding environment of the retained water. Further cooling toward the estimated $T_g^{H_2O}$ of confined water results in a noticeable change in the OH band shape. The moderately and strongly hydrogen-bonded contributions progressively merge into a single broad band centered at approximately 3270 cm^{-1} . Below $T_g^{H_2O}$, the intensity in the region near 3232 cm^{-1} increases, while the amplitude of the free OH band is gradually suppressed, indicating a strengthening of the hydrogen-bond network. This behavior is consistent with previous studies of nanoscale confined water, where surface interactions were shown to promote orientational ordering of water molecules without inducing crystallization [140].

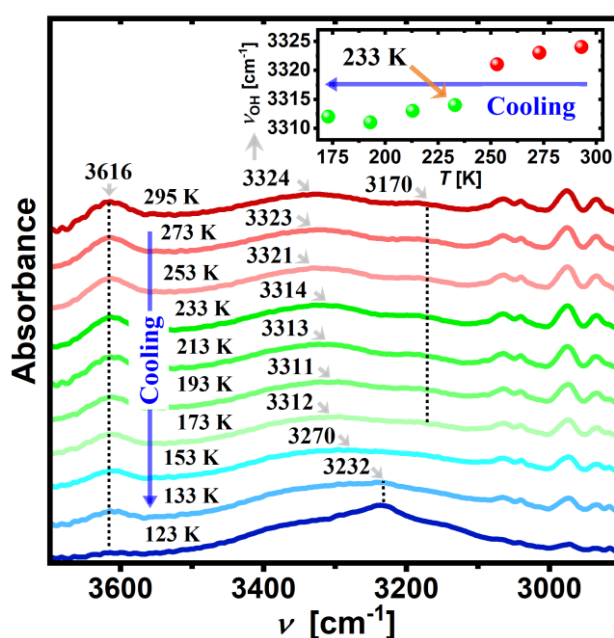


Figure 4.8 Temperature-dependent FTIR spectra for FC monohydrate, showing OH and CH stretching regions.

To examine the mechanism of the dynamic crossover in depth, MD simulations were performed. First, a disordered LC model of CM-FC monohydrate was constructed to reproduce the supramolecular structure obtained through cryomilling. To mimic the experimental system, a structural perturbation extending from the simulation box's surface toward the core was introduced, together with an adjustment of the water content to match the monohydrate composition. As shown in **Figure 4.9**, the good agreement between the simulated and experimental $S(Q)$ and $RDF(r)$ confirms that the model reliably captures

both intra- and intermolecular structural features of the FC monohydrate system. This agreement indicates that the confining environment experienced by water in the simulations is representative of the experimental CM-FC matrix, thereby providing a reliable basis for analyzing water organization and dynamics.

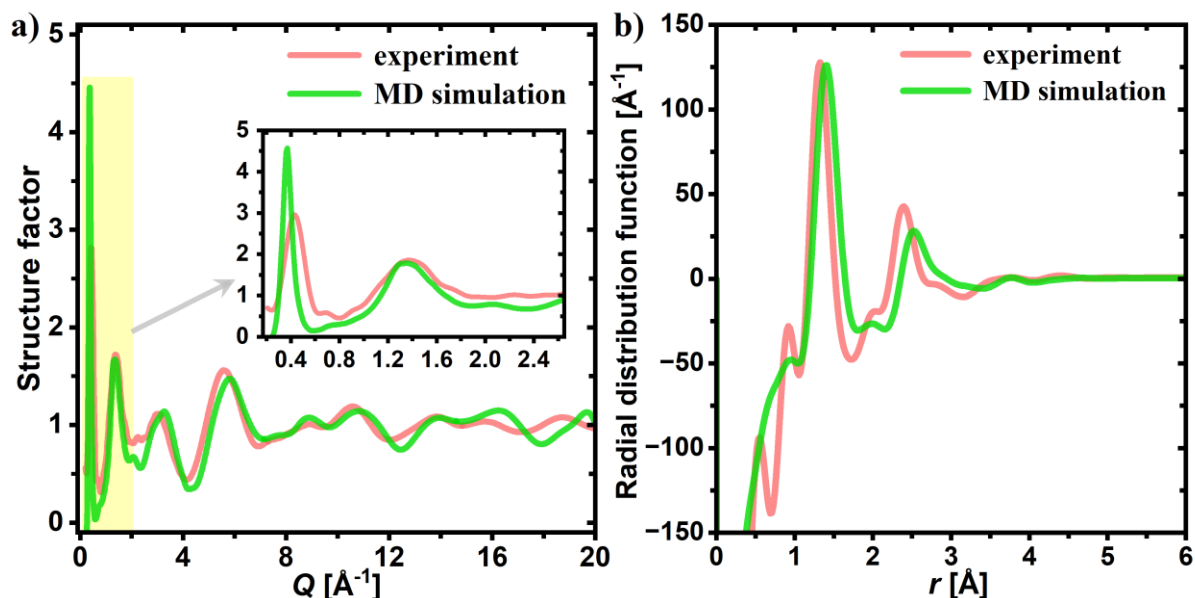


Figure 4.9 Experimental and MD model-based structural data for CM-FC monohydrate: **a)** structure factors, with the inset showing the low- Q peak and the broad amorphous halo, and **b)** radial distribution functions.

On this basis, cluster analysis was used to quantify the organization of water in CM-FC (**Figure 4.10**). The cluster-size distribution in panel a reveals pronounced structural homogeneity of the retained water. **Even at 295 K, approximately 70% of the water molecules exist as isolated molecules, referred to here as monomers. Dimers and other small clusters are a minority. This high monomer fraction shows that most H₂O molecules are spatially isolated from one another and are therefore strongly confined by the ionic environment dominated by Ca²⁺ ions.** This result is consistent with the Arrhenius-type behavior observed by dielectric spectroscopy both above and below T_c , **indicating predominantly local and non-cooperative dynamics. Upon cooling, the monomer fraction increases further to approximately 81%, while the fraction of dimers and larger clusters decreases. This trend indicates a progressive reduction of the already limited water–water connectivity.** This structural evolution is directly linked to the dynamics of H₂O molecules, which are primarily governed by their interactions with Ca²⁺ ions. The Ca²⁺–H₂O intermittent residence time, shown in **Figure 4.10b**, increases from approximately 0.4 ns at 295 K

to about 1.1 ns at 180 K. This increase demonstrates that H₂O molecules remain longer within the Ca²⁺ coordination environment at lower temperature. As a result, intermolecular rearrangement becomes increasingly hindered, and the water dynamics become slower and more localized. The temperature dependence of the cluster populations also reveals two regimes separated by a temperature close to the dynamic crossover temperature, T_c , identified by BDS. Above T_c , the fractions of monomers and clusters vary markedly with temperature, indicating that the water organization remains sensitive to thermal fluctuations and that minor transient H₂O–H₂O contacts still contribute to the relaxation process. Below T_c , these populations become nearly temperature-independent, showing that the retained water enters a more structurally constrained regime. This change is also reflected in the average cluster size (Figure 4.10c), which first vitally decreases with cooling and then shows a clear change in slope near T_c . Therefore, the crossover is associated with the suppression of residual intermolecular water connectivity.

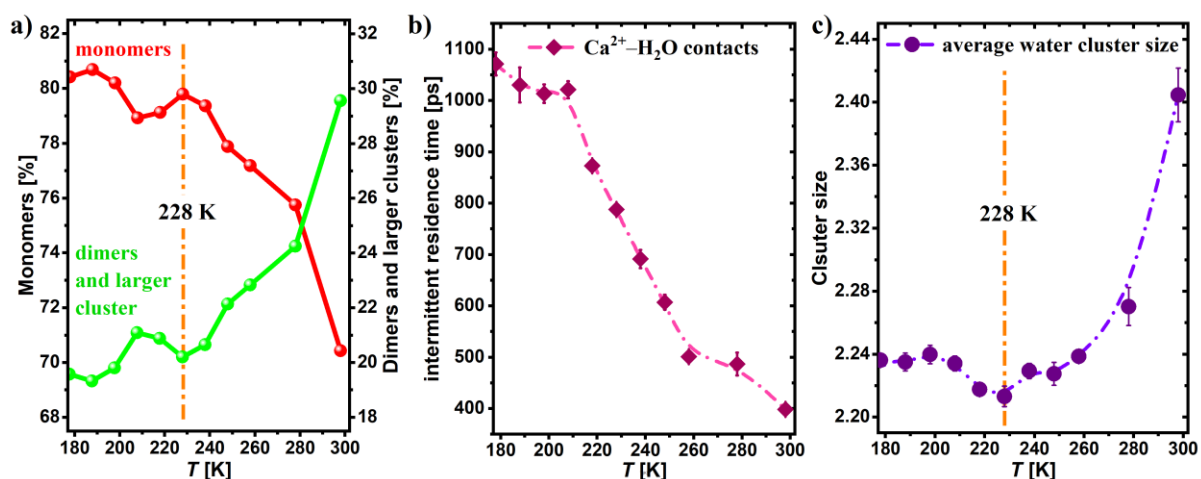


Figure 4.10 Temperature dependence of water organization in CM-FC monohydrate obtained from MD simulations: **a)** fractions of H₂O monomers and dimers/larger clusters, **b)** intermittent residence time of Ca²⁺–H₂O contacts, and **c)** average H₂O cluster size.

Overall, the MD results provided a microscopic explanation for the dielectric spectroscopy data. The two Arrhenius regimes arise from a change in the dominant relaxation mechanism of the confined water. Above T_c , the relaxation is influenced by some minor water clusters and residual intermolecular coupling. These cluster-assisted rearrangements lead to a stronger temperature dependence and therefore to the higher activation energy observed experimentally. Below T_c , the increased monomer fraction and the longer residence time in the Ca²⁺ coordination environment

indicate that water motion becomes mainly local and coordination-controlled. Consequently, the relaxation becomes less sensitive to temperature, which explains the lower activation energy at low temperatures. Based on this interpretation, the FTIR results, particularly the pronounced red-shift in the OH stretching band near T_c , can be attributed to the stabilization of localized hydrogen-bond configurations within the confined Ca^{2+} -carboxylate environment. Importantly, the cluster analysis showed that H_2O molecules in CM-FC do not aggregate into extended hydrogen-bonded clusters. Nevertheless, the retained water still exhibits a detectable glass-transition behavior and a clear dynamic crossover. Therefore, extended H_2O - H_2O connectivity is not a necessary condition for crossover behavior. In contrast, in CM-FC, the crossover arises from a change between two regimes of localized, coordination-controlled water dynamics under strong ionic confinement. This finding provides a new perspective on the apparently general character of crossover phenomena in confined water.

4.6. Summary of results for fenoprofen calcium

The results obtained for FC demonstrated that the supramolecular organization of this API is strongly determined by the applied amorphization pathway. Thermal treatment of crystalline FC dihydrate induced dehydration and transformed the crystalline phase into an inverse hexagonal LC mesophase. After rapid cooling, this hexagonal organization was preserved, leading to the formation of an anhydrous vitrified structure (V-FC). Cryomilling led to a different structural outcome. By cryomilling, it was possible to disrupt the crystalline order of the initial FC dihydrate and generate CM-FC structure, which exhibits a more disordered – nematic – LC organization, than the hexagonal structure of V-FC. At the same time, TGA confirmed that this mechanically induced transformation was accompanied by partial dehydration, resulting in a monohydrate state. Therefore, through vitrification and cryomilling, it was possible to generate two distinct non-crystalline FC forms: an anhydrous hexagonal LC structure and a monohydrate nematic LC structure. In-situ Raman spectroscopy further showed that this transformation proceeds through gradual changes in the molecular and intermolecular environment, rather than an abrupt structural transition. These differences in LC organization and hydration state directly affected the physical stability of FC. V-FC, preserving an ordered anhydrous hexagonal structure, crystallized rapidly, with the first Bragg reflections appearing after only 1 day of storage. In contrast, CM-FC remained stable for more than two years, with crystallization detected only after 810 days. This comparison shows that the nematic structure generated by cryomilling is substantially

more resistant to crystallization than the vitrified hexagonal structure. The same tendency was observed for ITZ, with cryomilled nematic structures being more stable than vitrified structures. Thus, for both investigated LC-forming APIs, cryomilling-induced nematic organization provides a highly stable non-crystalline state.

DSC measurements showed that the thermal behavior of FC reflects the combined effect of structural order and hydration state. Crystalline FC dihydrate exhibited a strong endothermic event near 375 K, assigned to dehydration coupled with the formation of the hexagonal mesophase. After dehydration and vitrification, anhydrous V-FC showed a glass transition of the FC matrix at approximately 312 K. CM-FC monohydrate exhibited the same FC matrix glass transition at nearly the same temperature, despite containing approximately one H₂O molecule per FC unit. This indicates that the retained water does not significantly plasticize the FC matrix. The most likely reason is that water molecules are strongly bound within Ca²⁺–carboxylate ionic domains, which limits their ability to increase the mobility of the surrounding FC molecules. In addition to the matrix glass transition, CM-FC showed a separate glass-like transition near 154 K, assigned to trapped water. The unusually low temperature of this transition suggests that water retained in CM-FC behaves differently from water confined in conventional nanoporous systems.

The favorable structural stability of CM-FC was also accompanied by improved dissolution behavior. In FaSSIF medium, CM-FC released approximately 36% of the drug after 3 h, exceeding both crystalline FC dihydrate and anhydrous V-FC. The crystalline form showed intermediate release, whereas V-FC displayed only an initially fast release followed by a strong slowdown, giving the lowest total release after 3 h. Therefore, the cryomilled nematic monohydrate provides the most favorable balance between physical stability and dissolution performance among the studied FC forms. This improvement can be related to the structural disorder introduced by cryomilling, together with particle-size reduction and the resulting increase in effective surface area.

The presence of retained water in CM-FC also made this material a non-classical system for studying water under strong confinement. In this case, water was not externally introduced externally into the host matrix, but was retained from the parent dihydrate during cryomilling and trapped within the nematic FC structure. BDS revealed two Arrhenius relaxation regimes separated by a dynamic crossover near 226 K, indicating a change in the relaxation mechanism of the confined water. FTIR measurements showed a pronounced change in the OH stretching region near the same temperature, linking this dynamic crossover to a rearrangement of the local hydrogen-bonding environment. MD simulations showed that most water

molecules in the CM-FC matrix exist isolated as monomers and are strongly confined within Ca^{2+} -carboxylate ionic domains. Upon cooling, H_2O - H_2O connectivity decreases further, while Ca^{2+} - H_2O residence times increase. As a result, the dynamics become increasingly localized and controlled by the ionic coordination environment. These results show that extended water-water hydrogen-bond connectivity is not required for dynamic crossover behavior. In CM-FC, the crossover arises from a transition between two regimes of localized, ion-controlled water dynamics under strong ionic confinement.

5. Conclusions

The results presented in this thesis demonstrate that, for APIs capable of forming LC phases, amorphization is a route-dependent process that can yield solid forms with distinct supramolecular organization and varying degrees of intermolecular order. For such compounds, disruption of long-range crystalline order by various methods may lead to structures strongly varying in the supramolecular organization and the degree of the intermolecular order, between the crystalline and amorphous states. The final structure depends strongly on the amorphization route and on the specific preparation conditions. Different degrees of short- and medium-range molecular order may be generated, leading to structurally distinct non-crystalline forms of the same API. This conclusion directly answers one of the central research questions posed in this thesis: whether different amorphization methods can lead to different supramolecular structures in APIs capable of forming LC order. The results provide a clear affirmative answer. This was first demonstrated for ITZ, where different preparation routes yielded non-crystalline forms with different types and degrees of LC organization. The same general conclusion was then supported by FC, an ionic and hydrated API. Therefore, APIs capable of forming LC order should not be expected to form equivalent non-crystalline LC structures after different amorphization-targeted treatments. Importantly, structural control is not determined only by the general amorphization method. Even changes in the processing parameters within a given preparation strategy, such as milling conditions, solvent type, evaporation conditions, or thermal history, can modify the resulting supramolecular order in the solid form of API below its glass transition temperature. Thus, the LC-forming APIs are highly sensitive to their preparation history. Even subtle, at first glance, differences in their degree of order may cause significant consequences for material behavior and properties, as is the case with various polymorphic forms among crystalline structures. Differences in the liquid crystalline organization of itraconazole and fenoprofen calcium compounds in the created solid forms were reflected

in their thermal behavior, molecular dynamics, physical stability, and dissolution kinetics. The presented results demonstrated direct relationships between the amorphization pathway, the resulting structure, and the physical properties of the APIs capable of forming liquid crystalline phases.

A particularly important result is that by cryomilling it is possible to generate nematic molecular organization in both ITZ and FC. Despite the expected increase in surface area caused by milling, these cryomilled forms showed higher physical stability compared with the LC structures obtained by other amorphization methods. This finding is significant because cryomilled amorphous APIs are often considered less physically stable than their vitrified counterparts (conventional glasses). In the case of the studied compounds, however, the nematic organization produced by cryomilling increased resistance to crystallization. Moreover, the cryomilled samples also showed the most favorable dissolution kinetics among the investigated solid forms. For ITZ, dissolution studies further showed that non-crystalline forms markedly improved drug release compared with the crystalline analogue, while differences between the prepared non-crystalline forms reflected the combined influence of supramolecular organization and sample morphology. In the case of FC, cryomilling also resulted in partial removal/partial retain of the structural water within the LC matrix, yielding an unique hydrated nematic organization of FC molecules where H₂O molecules are confined and coordinated by Ca²⁺ ions. Although H₂O molecules do not form extended clusters in such disordered matrix, signatures of the water glass transition and an anomalous crossover were observed. This indicates that hydrated thermotropic LC-forming molecular compounds may provide a novel class of systems for studying the properties of supercooled water under molecular confinement. These are the main conclusions of the thesis, which provide a clearer basis for understanding amorphized LC-forming pharmaceutical solids. These findings have broader significance for pharmaceutical materials science. In laboratory studies and formulation development, amorphization is often treated mainly as a strategy for improving solubility, dissolution, or bioavailability. However, the present results show that, for APIs capable of forming LC phases, the method used to obtain the non-crystalline form plays a decisive role not only in removing crystallinity, but also in defining the molecular organization that remains after processing. This shifts the interpretation of amorphization from a simple crystallinity-reduction step to a structural design strategy for controlling non-crystalline pharmaceutical solids. From an industrial perspective, different amorphization methods should therefore not be assumed to produce equivalent non-crystalline forms. Careful control of processing conditions is required, particularly

for LC-forming APIs, where subtle structural differences may lead to different thermal behavior, storage stability, molecular mobility, or dissolution response. The preparation history of such materials should therefore be treated as a critical formulation parameter.

Overall, this thesis provides a structural framework for understanding non-crystalline LC-forming APIs as route-dependent solid states with distinct molecular organizations and properties. This view is important for the rational design of pharmaceutical materials because it shows that material performance can be strongly influenced by the preparation pathway that defines the final molecular organization. Such an approach may support the development of more stable and better-performing non-crystalline pharmaceutical forms.

6. List of publications, patents, and conference presentations

6.1. List of papers directly related to the results of this thesis

- P1. **Lamrani, T.**, Jesionek, P., Tarnacka, M., Zakowiecki, D., Włodarczyk, P., Zubko, M., Ozimina-Kamińska, E., Kamiński, K., & Jurkiewicz, K. (2024). Generation of nematic order in itraconazole by cryo-milling. *Journal of Molecular Liquids*, 414, 126106.
DOI: 10.1016/j.molliq.2024.126106
- P2. **Lamrani, T.**, Orszulak, L., Tarnacka, M., Hachula, B., Nowakowska, K., Kaminski, K., & Jurkiewicz, K. (2025). Effect of solvent evaporation on the liquid-crystalline order of itraconazole. *Scientific Reports*, 15, 40129.
DOI: 10.1038/s41598-025-23979-9
This publication was distinguished in 2026 by the Committee of Crystallography, Polish Academy of Sciences, with the "Crystallographic Diamonds" award in the section: Crystallography in Materials Science and Technology.
- P3. **Lamrani, T.**, Włodarczyk, P., Hachula, B., Tarnacka, M., Halasz, I., Kamiński, K., & Jurkiewicz, K. Glass transition and dynamic crossover of water embedded in liquid-crystalline fenoprofen calcium monohydrate. – in preparation

6.2. Patent

- “Sposób otrzymywania itraconazolu w stabilnej fazie nematycznej”, Karolina Jurkiewicz, **Taoufik Lamrani**, Paulina Jesionek, Kamil Kamiński, Ewa Ozimina-Kamińska, Urząd Patentowy RP, 01.03.2024, numer P.447909

6.3. List of conference presentations

- C1. Zakopane School of Physics 2023, Zakopane, Poland, 23–27 May 2023, “The atomic-scale structure of pharmaceuticals amorphized by different methods”, oral presentation
- C2. YOUNG MULTIS – Multiscale Phenomena in Condensed Matter, online, 3–5 July 2023, “The influence of the various amorphization processes on the molecular-scale structure of ritonavir active pharmaceutical ingredient”, oral presentation
- C3. 15th International Conference on the Structure of Non-Crystalline Materials, Churchill College, United Kingdom, 15–19 July 2024, “Exploring cryomilling as a tool for modifying liquid-crystalline order”, oral presentation

- C4. Joint Meeting of PSRS Members and SOLARIS Centre Users, Cracow, Poland, 11–13 September 2024, “Studies of molecular order in itraconazole by X-ray diffraction”, oral presentation
- C5. MULTIS 2024 Conference, Cracow, Poland, 16–19 September 2024, “Modification of the liquid-crystalline order in itraconazole using mechanical stress, temperature variation, and solvation”, oral presentation
- C6. 10th International Discussion Meeting on Relaxations in Complex Systems, Barcelona, Spain, 20–25 July 2025, “Enhancing liquid-crystalline order of itraconazole via solvent evaporation”, oral presentation
- C7. XXV Conference on Applied Crystallography, Wisła, Poland, 7–10 September 2024, “Modulation of smectic liquid-crystalline order in itraconazole via solvent evaporation”, oral presentation

6.4. List of other papers

- O1. Jesionek, P., Hachuła, B., Heczko, D., **Lamrani, T.**, Jurkiewicz, K., Tarnacka, M., Książek, M., Kamiński, K., & Kamińska, E. (2023). Studies on the nature and pressure evolution of phase transitions in 1-adamantylamine and 1-adamantanol. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 299, 122794.
Doi: 10.1016/j.saa.2023.122794
- O2. Orszulak, L., **Lamrani, T.**, Tarnacka, M., Hachuła, B., Jurkiewicz, K., Ziola, P., Mrozek-Wilczkiewicz, A., Kamińska, E., & Kamiński, K. (2024). The impact of various poly(vinylpyrrolidone) polymers on the crystallization process of metronidazole. *Pharmaceutics*, 16(1), 136.
Doi: 10.3390/pharmaceutics16010136
- O3. Orszulak, L., **Lamrani, T.**, Bernat, R., Tarnacka, M., Żakowiecki, D., Jurkiewicz, K., Ziola, P., Mrozek-Wilczkiewicz, A., Zięba, A., Kamiński, K., & Kamińska, E. (2024). The influence of PVP polymer topology on the liquid-crystalline order of itraconazole in binary systems. *Molecular Pharmaceutics*, 21(6), 3027–3039.
Doi: 10.1021/acs.molpharmaceut.4c00215
- O4. Jurkiewicz, K., Liszka, B., Gancarz, P., Smykała, S., Zygałło, D., Nokielski, P., **Lamrani, T.**, Talik, E., Wrzałik, R., Walkowiak, M., & Ilavsky, J. (2024). Sucrose-based dense, pure, and highly-crystalline graphitic materials for lithium-ion batteries. *Advanced Functional Materials*, 34(51), 2410409.

Doi: 10.1002/adfm.202410409

- O5. Orszulak, L., Włodarczyk, P., Hachuła, B., **Lamrani, T.**, Jurkiewicz, K., Tarnacka, M., Hreczka, M., Kamiński, K., & Kamińska, E. (2025). Inhibition of naproxen crystallization by polymers: The role of topology and chain length of polyvinylpyrrolidone macromolecules. *European Journal of Pharmaceutics and Biopharmaceutics*, 207, 114581. Doi: 10.1016/j.ejpb.2024.114581
- O6. Jurkiewicz, K., Shabbir, M. J., **Lamrani, T.**, Liszka, B., Szubka, M., Hachuła, B., Smykała, S., & Ilavsky, J. (2025). Graphite crystals in catalytically-graphitized glass-like carbon. *Diamond and Related Materials*, 155, 112236. Doi: 10.1016/j.diamond.2025.112236
- O7. Jesionek-Ratka, P., Hachuła, B., Tarnacka, M., **Lamrani, T.**, Jurkiewicz, K., Kamiński, K., & Kamińska, E. (2025). Insights into the nature of phase transitions and relaxation dynamics in memantine: An active substance forming orientationally disordered crystals. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 341, 126427. Doi: 10.1016/j.saa.2025.126427
- O8. Orszulak, L., Minecka, A., Bernat, R., **Lamrani, T.**, Jurkiewicz, K., Hachuła, B., Tarnacka, M., Geppert-Rybczyńska, M., Zubko, M., Staniszevska, M., Smoleński, M., Dobosz, J., Garbacz, G., Kamiński, K., & Kamińska, E. (2025). Hydrophilic and amphiphilic macromolecules as modulators of the physical stability and bioavailability of piribedil: A study on binary mixtures and micellar systems. *Molecular Pharmaceutics*, 22(8), 4708–4730. Doi: 10.1021/acs.molpharmaceut.5c00276
- O9. Sudhakaran Nair Valsala Kumari, A. K., Hachuła, B., Tarnacka, M., Kamińska, E., Grelska, J., **Lamrani, T.**, Jurkiewicz, K., Włodarczyk, P., Grzybowska, K., & Kamiński, K. (2025). Specific interactions and mechanism of association in highly viscous aldehyde 3-phenylpropanal. *The Journal of Chemical Physics*, 163(10), 104505. Doi: 10.1063/5.0283944

6.5. Attached publications P1 and P2, the patent abstract, and the decision to grant the patent



Generation of nematic order in itraconazole by cryo-milling

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ABSTRACT

Mechanical milling is a well-known method of solid-state amorphization of crystalline materials. Here, it is revealed that cryo-milling of itraconazole, a model thermotropic liquid-crystalline active pharmaceutical ingredient, leads to a preferred nematic order of molecules in solid particles instead of isotropic disorder – usually observed for most molecules that do not form liquid-crystalline mesophases. While standard quenching of itraconazole isotropic liquid down to room temperature results in the formation of glass with some degree of smectic order, cryo-milling easily destroys not only crystalline order but also smectic organization of molecules into layers, omitting the melting. The cryo-milled itraconazole samples exhibit faster α -relaxation and slightly slower δ -mode above the glass transition temperature. They are also more physically stable, with the stability dependent on the grinding time, below this temperature, with respect to the ordinary glass. The explanation for this behavior is believed to be increased short-range intermolecular disorder. Moreover, the cryo-milled particles with nematic order are better soluble than ordinary glass. Therefore, this work can guide the formulation of stable pharmaceuticals with nematic order/disorder and enhanced solubility.

1. Introduction

The knowledge of how to control the molecular organization in materials is the prerequisite for their development. A flagship group of materials exhibiting a very close correlation between the internal molecular structure and properties are liquid crystals (LCs), which combine attributes commonly associated with crystalline solids (orientational order) and isotropic liquids (high molecular mobility). The molecular order in LCs can be facily switched as a response to external stimuli, such as electric or magnetic field, light, temperature, and pressure [1–6]. Even subtle differences in the local structure affect the properties of LCs and program their responsivity [7]. That is why LCs were nominated for the title of “versatile self-organized smart soft materials” [8]. The most recognizable industrial applications of LCs are flat-panel LC display devices. However, recent reports have demonstrated impressive new developments, i.e., LC-based windows, sensors and membranes [1–3,5,9]. The responsivity of LCs can also be useful in pharmacy to

optimize and control the pharmacokinetics, release and therapeutic efficacy of drugs [10].

Here we used itraconazole (ITZ) – one of the most studied small-molecular, thermotropic LCs, which is an active pharmaceutical ingredient (API) with antifungal properties. Upon cooling from the isotropic (*I*) liquid, ITZ transforms to a nematic (*N*) phase at temperature $T_{I-N} \sim 363$ K [11]. On further cooling, it converts to a smectic (*Sm*) phase at $T_{N-Sm} \sim 347$ K [11]. Moreover, below the glass transition temperature $T_g \sim 330$ K, the *Sm* state is trapped in the glass [11]. Upon reheating, these transitions reverse. Papers of Ediger’s group have demonstrated that the orientation of vapor-deposited ITZ molecules depends on temperature (*T*) of the substrate [12–14]. By deposition onto substrates below T_g , it is possible to control the spacing of the smectic-like layers and even make the molecules preferentially lie nearly flat in the plane of the substrate, which cannot be obtained by thermal annealing. Moreover, Teerakapibal et al. have shown that the degree of the LC order frozen in the glassy phase of ITZ may be tuned by the structure of the

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supercooled liquid from which it comes, and by increasing the cooling rate (R_c) above ~ 20 K/s it is possible to avoid the N to Sm order transition and maintain the N order in the glass [15].

Mechanical stress is an external factor already considered in the literature as a driving force to tune LC order in the fluid phases of compounds known to form LC mesophases. In recent papers, it was shown that oscillatory shear can induce changes in the alignment of molecules in N phase and enhance the resulting nascent Sm order [16]. On the other hand, hydrostatic compression of N and I phases was shown to reduce the molecular organization in the ensuing Sm phase [17]. Generally, mechanical treatment is a well-known approach to promote structural transformations also in the solid state [18]. However, up to now, it has been demonstrated that high-energy milling of solid materials induces only two main effects: polymorphic transition causing the generation of another crystal system or a partial or total amorphization leading to the formation of a disordered isotropic phase [19–21]. To the best of our knowledge, there are no literature reports demonstrating that the mechanical milling of compounds forming LC phases may lead to a preferred nematic order of molecules in solid particles instead of their amorphization.

Here, we applied cryo-milling to ITZ and induced a solid–solid transition of the crystalline order towards a non-crystalline phase. It turned out that the most stable ITZ structure, achieved by cryo-grinding with different times and frequencies, exhibited the nematic order. This type of molecular spatial organization was also formed when ITZ ordinary glass produced by melt-quenching was cryo-milled. The physical stability of the cryo-milled samples was studied, and it was discovered that by increasing milling time, the stability of the nematic order can be improved. In order to explain this phenomenon, we carried out thermal studies by differential scanning calorimetry, structural studies by X-ray diffraction and molecular dynamics studies by broadband dielectric spectroscopy. The size of the milled particles was estimated in microscopic observations. Based on the complementary results from various techniques, it was possible to propose a mechanism causing the observed properties of the cryo-milled ITZ and their changes with the milling parameters. Moreover, to verify the potential application of the cryo-milled ITZ in the pharmacy, solubility tests were performed.

2. Experimental section

2.1. Materials

Itraconazole (ITR) with a molecular formula of $C_{35}H_{38}Cl_2N_8O_4$, a molar mass of 705.64 g/mol, and a purity of $> 98\%$, was purchased from Angene (CAS Number 84625–61-6) as racemic mixture comprising all four *cis* stereoisomers in a 1:1:1:1 ratio. The ordinary glass (OG) of ITZ was prepared by melting the crystalline powder at 440 K followed by quenching of the melt in the air at a moderate cooling rate of ~ 10 K/s down to 295 K (room temperature). The glass was delicately grounded in an agate mortar and the obtained powder was used for further studies. For the milling of crystalline ITZ powder, a RETSCH 207490001 cryo-mill was employed. A zirconium oxide ball with a diameter of 2 cm was used in a 50 ml grinding jar of the same material. The amount of milling powder was 100 mg every load. The instrument was pre-cooled with liquid nitrogen for 10 min, followed by cryo-milling for varying durations: 1, 3, 12, 84 min (7x12 min), and 216 min (18x12 min) in 12 min cycles with 3 min intervals, all at 20 Hz frequency (f). Additionally, different frequencies of milling: 10, 12, 15, 20, and 30 Hz were applied with the same grinding time of 12 min in each case. The OG sample was also milled for 12 min at 20 Hz.

2.2. Methods

2.2.1. X-ray scattering (diffraction) measurements and data normalization

The wide-angle X-ray scattering (WAXS) measurements, which also partially cover the low-scattering vector range where ITZ exhibits re-

flections due to the liquid–crystalline order, were performed using a Rigaku D/Max Rapid II diffractometer equipped with a rotating Ag anode. A graphite (002) monochromator was used for the incident radiation and the average wavelength of the monochromatic K_α beam was $\lambda = 0.5608$ Å. Fresh samples (just after milling or melt-quenching) were packed and closed in borosilicate glass capillaries and measured in transmission Debye-Scherrer geometry. The X-ray scattering patterns were collected in a single shot using a 2D curved image plate detector. The reliable scattering angle (2θ) range in this experiment was around $1 - 130^\circ$ which corresponds to the scattering vector ($Q = 4\pi\sin(\theta)/\lambda$) range of around $0.17 - 20$ Å $^{-1}$. However, in the manuscript, only the Q range $0.17 - 3.25$ Å $^{-1}$ is presented, where the intermolecular structure contributes mostly. The high- Q range above ~ 4 Å $^{-1}$, which arises mostly from the intramolecular structure, was taken to normalize the scattering data (Fig. SM1 in Supplementary Material (SM)). Therefore, quantification of the low- Q signal can be made based on the rescaled/normalized data. For simplicity and understanding by a wide audience, the data were presented in the form of diffraction patterns and not normalized to the form factors. Nevertheless, rescaling the data to the same intensity at the high- Q range allowed us to assign the changes in the low- Q peaks to changes in the structure induced by milling and their further treatment. The background from an empty capillary was also measured and subtracted from the data collected for ITZ samples. The temperature during standard measurements was 295 K. The samples closed at capillaries and long-term stored at 295 and 323 K were measured using the same set-up alignment at 295 K. During measurements of fresh samples in the heating mode, the temperature was controlled by an Oxford Cryostem system with an accuracy of 5 K.

In order to assess long-term stability, selected short- (12 min) and long-milled (18x12 min) samples, together with ordinary glass, were stored in closed capillaries at 295 K as well as at 323 K on a heating plate, at ambient pressure and humidity. It should be mentioned that the chemical stability of the cryo-milled samples stored 1 week in such conditions was probed by 1H nuclear magnetic resonance (NMR) method and no signs of chemical decomposition were observed. Besides the physical stability studies at 323 K, X-ray diffraction measurements on heating for fresh samples and for samples long-term stored at 295 K were performed. Moreover, the X-ray scattering patterns were obtained by subjecting the samples to temperature increments at each 15-minute interval, commencing at room temperature up to the temperature where a pronounced recrystallization was registered.

2.2.2. Differential scanning calorimetry (DSC) and temperature-modulated differential scanning calorimetry (TM-DSC)

Calorimetric measurements of ITZ with a constant heating rate of 10 K/min were carried out by a Mettler-Toledo DSC apparatus equipped with liquid nitrogen cooling accessory and an HSS8 ceramic sensor (heat flux sensor with 120 thermocouples). Temperature and enthalpy calibrations were performed by using indium and zinc standards. The sample was prepared in an open aluminum crucible (40 μ L) outside the DSC apparatus and measured on heating from 300 K to 450 K. Using a stochastic TM-DSC technique implemented by Mettler-Toledo TOPEM®, the dynamic behavior of ITZ has been analyzed in the frequency range from 4 to 30 mHz in one single measurement ($T = 320 - 355$ K) at a heating rate of 0.5 K/min. In the experiment, the temperature amplitude of the pulses of 0.5 K was selected with a switching time range with minimum and maximum values of 15 and 30 s, respectively. DSC measurements at high heating rates (60–200 K/min) were performed in Al crucibles using a Netzsch DSC 214 Polyma, in a protective atmosphere of argon. Samples were initially cooled to 223 K, and after temperature stabilization, rapid runs to 473 K were performed with desired heating rates in the range of 60 – 200 K/min.

2.2.3. Broadband dielectric spectroscopy (BDS)

Isobaric measurements of the complex dielectric permittivity $\epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega)$ were carried out using a Novocontrol Alpha dielectric

spectrometer over the frequency range from 10^{-1} to 10^6 Hz at ambient pressure. The temperature was maintained with a Quatro Cryosystem using a nitrogen gas cryostat with control better than 0.1 K. The melt-quenched and milled samples (powder forms) of ITZ were placed between two stainless-steel electrodes. The molecular dynamics studies were performed in the temperature range from 203 K up to 379 K (melt-quenched sample) and from 243 K up to 367 K (cryo-milled samples). Time-dependent measurements were carried out at $T = 335$ K.

2.2.4. Microscopic observations

The as-received commercial crystalline powder, OG delicately grounded in a mortar, and both short- and long-milled ITZ samples were observed under a digital Keyence VHX-5000 microscope (magnifications of 500 – 1500) equipped with the Keyence image analysis software. This software was used for the analysis of the particle size in a group of at least 250 particles. The transmission electron microscopy (TEM) observations in the bright field on milled samples were performed using a JEOL high resolution (HRTEM) JEM 3010 microscope operating at a 300 kV accelerating voltage, equipped with a Gatan 2 k × 2 k Orius 833SC200D CCD camera. The powder samples were suspended in isopropanol, and the resulting materials, after dispersion in an ultrasonic bath, were deposited on a Cu grid with an amorphous carbon film standardized for TEM observations. Particle size analysis was carried out using the ImageJ open-source software.

2.2.5. Solubility tests

The dissolution rates of the examined ITZ samples were probed in 0.1 M hydrochloric acid solution (pH=1) at 310 K using a Pharma Test Apparatebau AG USP apparatus II PTWS 820D. Around 300 mg of each sample was placed in 300 mL of medium and stirred at 150 rpm for 90 min. The concentration of dissolved drug substance was determined by UV/Vis method at 255 nm using a PharmaTest AG T70 UV/VIS split-beam spectrophotometer equipped with flow-through quartz cuvettes with a path length of 10 mm. Measurements were taken every minute until the 10 min of the test, then every 5 min, and beyond 60 min every 15 min until the end of the test.

3. Results and discussion

In the first stage of the cryo-milling experiment, various frequencies (f) of milling for the same milling time (t) of 12 min were applied for the same portion of crystalline ITZ powder. The structure recovered from the cryo-mill was studied at room T (295 K) using the wide-angle X-ray scattering (WAXS) reaching also the small-angle X-ray scattering (SAXS) region above $\sim 0.17 \text{ \AA}^{-1}$, where diffraction peaks arising from the ITZ liquid-crystalline organization appear. As can be seen in Fig. 1a, for 10 Hz the sample maintained its initial crystalline structure, while for 15 Hz, only a small contribution from the Bragg peaks of the crystalline phase was observed at the detection limit. Further increasing of f did not result in significant changes in the diffraction pattern. The main maximum at scattering vector $Q \sim 1.33 \text{ \AA}^{-1}$ arises due to the nearest-neighbor organization of molecules with an average intermolecular distance $d = 2\pi/Q \sim 4.7 \text{ \AA}$. The arrangement of ITZ molecules in smectic layers is known to exhibit a sequence of diffraction peaks (first-, second- and third-order) at lower $Q \sim 0.2, 0.4$ and $0.6 \text{ \AA}^{-1}$ [13,14,15,22]. The $0.2 \text{ \AA}^{-1}$ peak position corresponds to ordering of molecules with a periodicity $d \sim 31 \text{ \AA}$ – roughly the length of ITZ molecule [23]. It is an indicator of a lamellar order in which small bunches of rod-like molecules are aligned end-on in layers along the common direction. Here, only part of the intensity of the first-order reflection at $Q \sim 0.21 \text{ \AA}^{-1}$ was measurable with attention to the limit of the experimental set-up. It would not be possible to analyze it simply anyway because for the milled samples, the first-order diffraction peak is affected by a small-angle scattering component due to the small size of the particles, which is difficult to subtract without a complex modelling of SAXS intensity. Collecting the scattering signal in such low- Q range on a dedicated SAXS

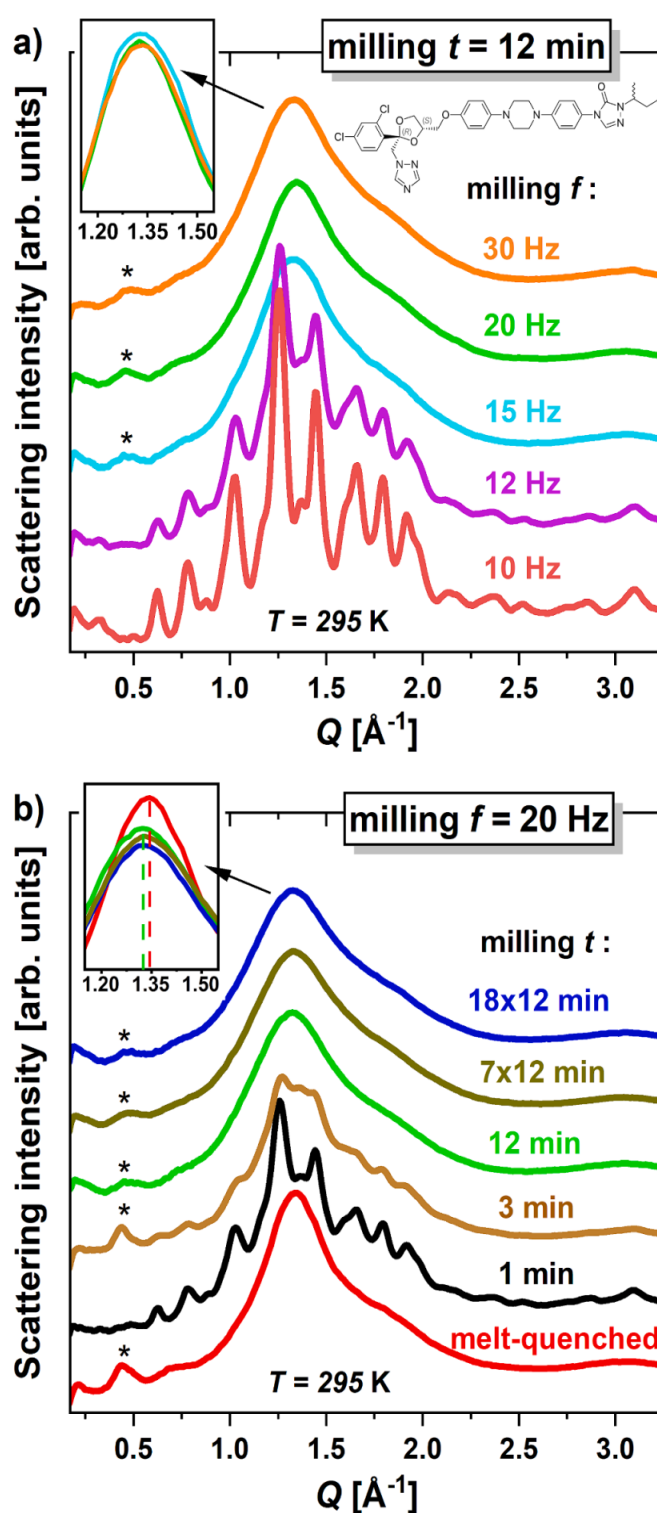


Fig. 1. X-ray scattering patterns collected for ITZ cryo-milled: a) for 12 min with different frequencies and b) with the frequency of 20 Hz for different milling times. X-ray scattering pattern of ordinary glass produced by melt-quenching was shown for comparison. The insets present zoom on the main peak maxima for selected diffraction patterns, which were superimposed on each other.

set-up, would not solve the problem of extraction of the first-order diffraction peak. In turn, the third peak, registered here at $Q \sim 0.7 \text{ \AA}^{-1}$, is very weak. Therefore, the second-order diffraction overtone at $Q \sim 0.45 \text{ \AA}^{-1}$ (marked with an asterisk in Fig. 1) was followed to analyze the LC order. Such a method was also applied for analysis of the degree of the LC order in ITZ vapour-deposited glasses [14]. It is worth mentioning that the scattering patterns were normalized to the high- Q

range (Fig. SM1). Therefore, we were able to quantitatively compare the changes in the scattering signal in the presented range $0.17\text{--}3.25\text{ \AA}^{-1}$ and assign them to structural changes induced by different milling and temperature conditions. It is also worth adding that other experimental techniques commonly used for the quantification of LC phases, such as polarization optical microscopy, fail in the characterization of the solid-state materials composed of a mixture of randomly distributed particles.

When comparing the scattering peak at $Q \sim 0.45\text{ \AA}^{-1}$ for the samples milled with $f > 12\text{ Hz}$ with the pattern of OG obtained by melt-quenching with a moderate $R_c \sim 10\text{ K/s}$ (Fig. 1b), it is clear that it has a much lower intensity. In fact, the diffractograms for these milled samples resemble that presented by Teerakapibal et al. for an extremely fast quenched ITZ ($R_c > 20\text{ K/s}$), which was free of Sm order [15]. In order to check whether it is possible to tune the degree of the LC order, as Teerakapibal et al. achieved by variation in R_c , we manipulated the milling t for the same milling $f = 20\text{ Hz}$. Even just 1 min of cryo-milling caused the destruction of the crystalline order in a significant fraction (Fig. 1b). Interestingly, after milling for $t = 3\text{ min}$, only a small contribution from the crystalline phase survived. However, there was also a clear peak at $Q \sim 0.45\text{ \AA}^{-1}$, resembling what was observed for the OG. It suggested that the Sm order was generated in such conditions in part of the material, the remainder still showed crystalline order. Further increasing t resulted in complete suppression of the crystalline order and, simultaneously, the disordering of the Sm phase to a lower-ordered N system. It was evidenced by the vanishing, broadening, and shifting of this low- Q peak toward higher Q . For all samples milled for $t > 3\text{ min}$ and $f = 20\text{ Hz}$, the Bragg peaks were completely lost and the diffraction patterns looked typical for pure N order, resembling the diffraction pattern obtained for N mesophase generated by supercooling of liquid down to $T_{I-N} \sim 363\text{ K}$ (Fig. SM2 in SM). Moreover, from the inset in Fig. 1b, one can see that also the main maximum broadened and lost intensity with respect to OG, so the nearest-neighbor structure was also disordered by the cryo-milling. However, it should be pointed out that for ITZ, cryo-milling does not lead to the generation of a totally disordered isotropic phase, as was previously the case with other materials [19,24]. Considerable elongation of milling from 1 to 18 cycles, each ongoing 12 min, did not affect the low- Q peak anymore. The nematic organization of molecules was very stable and not susceptible to further

mechanical stress. However, slightly stronger suppression of the main peak's amplitude for the longer-milled ($7 \times 12\text{ min}$ and $18 \times 12\text{ min}$) samples compared to the short-milled (12 min) one (inset in Fig. 1b) suggested that increasing milling t might cause a higher degree of the nearest-neighbor intermolecular disorder. The same behavior of the main diffraction maximum was observed when increasing the milling f (the inset in Fig. 1a). In order to verify whether there is any “frequency-time-milling superposition”, analogous to changing the degree of the LC order with deposition temperature and rate in vapor deposited ITZ, a quantitative analysis of the amplitude of the low- Q peak was performed [14]. Based on data presented in panel a) of Fig. 2, one can deduce that there is no “frequency-time superposition” referenced to changing the degree of the Sm/N order within the framework of the applied milling parameters. For the milling t of 12 min, we were not able to catch the Sm order, intermediate between the crystalline and N . After increasing the milling f to 15 Hz, the N order was directly created, while for milling with a slightly smaller f of 12 Hz, only the crystalline order was observed. Further increasing of the milling f did not result in any significant change of the analyzed low- Q peak in terms of its amplitude, but also width and position, within the resolution and statistics of the measurements. The bottom part of panel a) in Fig. 2 concerns the changes in the amplitude of the low- Q peak with increasing milling t for f of 20 Hz. In this case, it was possible to catch the structure with order between crystalline and Sm (for milling $t = 3\text{ min}$), where a slightly more intense peak at $Q \sim 0.45\text{ \AA}^{-1}$ was registered. For longer milling times, this peak only exhibited lower amplitude, characteristic of the N order, and it survived at the same level up to much longer milling times of 216 min probed in the experiment. To sum up this part, it seems to be challenging to control the degree of the Sm/N order in the cryo-milled ITZ by variation in the milling t and f . Our data revealed that the crystalline and Sm order in ITZ can be easily destroyed by cryo-milling, leading to a mechanically-stable N order. Interestingly, the damping of the Sm order toward the lower N order was also evidenced when the powdered OG sample was cryo-milled (Fig. SM3 in SM). A kind of “frequency-time-milling superposition” where the ratio of the Sm/N order changes gradually may happen for a narrow range of f and t , but we were not able to observe it for the milling parameters available in our experiment.

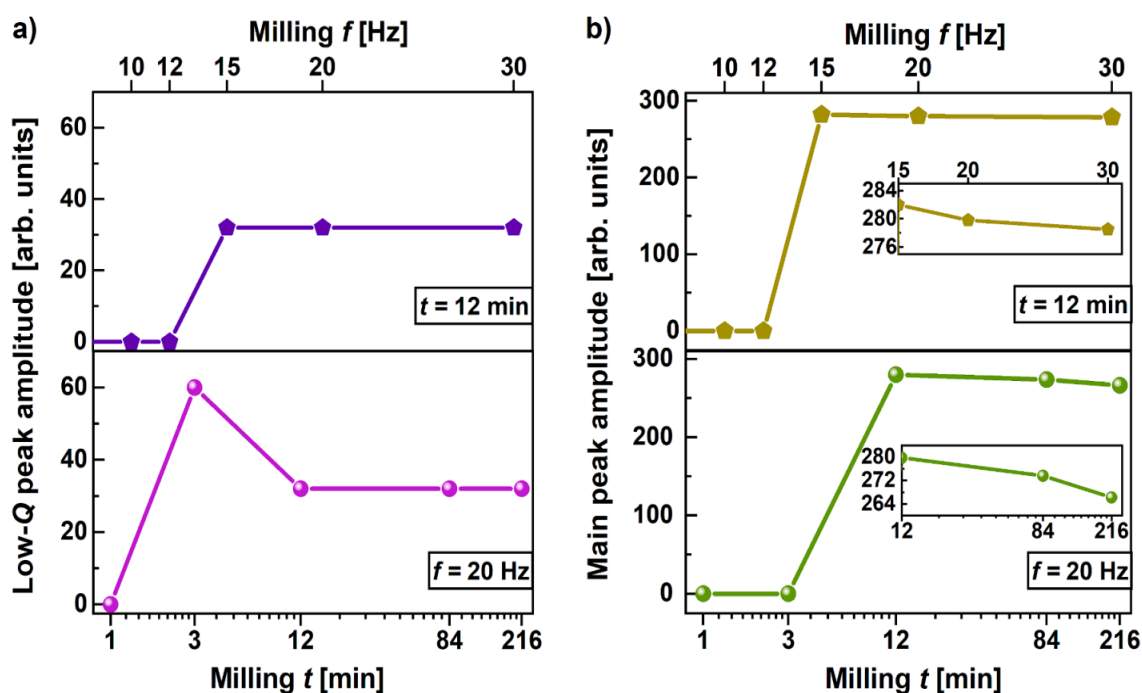


Fig. 2. a) Changes in the amplitude of the diffraction maximum located at $Q \sim 0.45\text{ \AA}^{-1}$ with increasing milling frequency for time of 12 min (upper graph) and with increasing milling time for frequency of 20 Hz (lower graph). Panel b) shows changes in the amplitude of the main diffraction maximum located at $Q \sim 1.33\text{ \AA}^{-1}$ within the same milling parameters.

The scenario was different when the amplitude of the main diffraction peak, considered as an indicator of the degree of the nearest-neighbor order (lateral organization of ITZ molecules), was analyzed. In panel b) of Fig. 2, it can be seen that with increasing both milling f and t , the amplitude slightly decreases, in agreement with the data presented in insets of Fig. 1. For the same milling t of 12 min, within the studied f range of 15 – 30 Hz, the changes in peak's amplitude are very subtle. For 20 Hz, the variation of the milling t within the studied range of 12 – 216 min causes more significant suppression of the short-range order.

The unusual ease of destruction of the crystalline order and, on the other hand, the exceptional resistance of the N order to prolonged mechanical milling, prompted us to probe the physical stability and recrystallization kinetics of the cryo-milled samples. First, the X-ray scattering patterns were obtained for samples subjected to temperature increments at 15-minute intervals, from 295 K up to recrystallization temperatures (Fig. SM4 in SM). OG and short-milled samples began to recrystallize at 348 K while the long-milled sample recrystallized later – at 353 K. Thus, these results indicated that the recrystallization rate of the cryo-milled powders may be attained through the control of milling time.

What is more, the data in Fig. SM4 in SM for lower temperatures suggested that the structural stability of the LC phases before the recrystallization may also be tuned in such a way. The comparison of the superimposed scattering patterns in the range of diffraction peaks related to the LC ordering for fresh OG and cryo-milled samples at 295 K

was presented in the inset of Fig. 3a. Significantly, at 323 K – just below T_g , the intensity of the peak at $Q \sim 0.45 \text{ \AA}^{-1}$ for all samples increased compared to 295 K (Fig. 3a). It indicated a rise in the degree of Sm/N order. Thus, it is clear that the ITZ samples prepared by cryo-milling are out-of-equilibrium solids at temperatures below the T_g , similar to metastable glass with frozen LC order. The amplitude of the $0.45 \text{ \AA}^{-1}$ peak for the short-milled sample at 323 K reached almost the maximum of that observed for OG. However, for the long-milled sample, this maximum only slightly increased up to around half of the amplitude for OG. It demonstrated that the rate of the N to Sm order transformation of the long-milled ITZ was slower than that of the short-milled one. The intensity of the main peak increased for all samples at 323 K, but it was still lower for cryo-milled samples than for OG, which means their nearest-neighbor structure still remained more disordered. Starting from 333 K – just above T_g , the low- Q peak for OG and short-milled ITZ started to vanish due to the thermotropic transformation of the Sm towards the N phase (Fig. 3b). The long-milled ITZ seemed to be more stable again – no big change compared to 323 K was detected. In turn, at $\sim 343 \text{ K}$ – in the vicinity of the Sm to N transition, the low- Q peaks for all three samples looked very similar and the structure of the cryo-milled samples differed from the OG only slightly in the region of the main peak (Fig. 3c).

The results obtained from the temperature-dependent X-ray scattering experiment indicated that the N order of the cryo-milled samples is more resistant to rebuilding the Sm order at elevated temperatures as

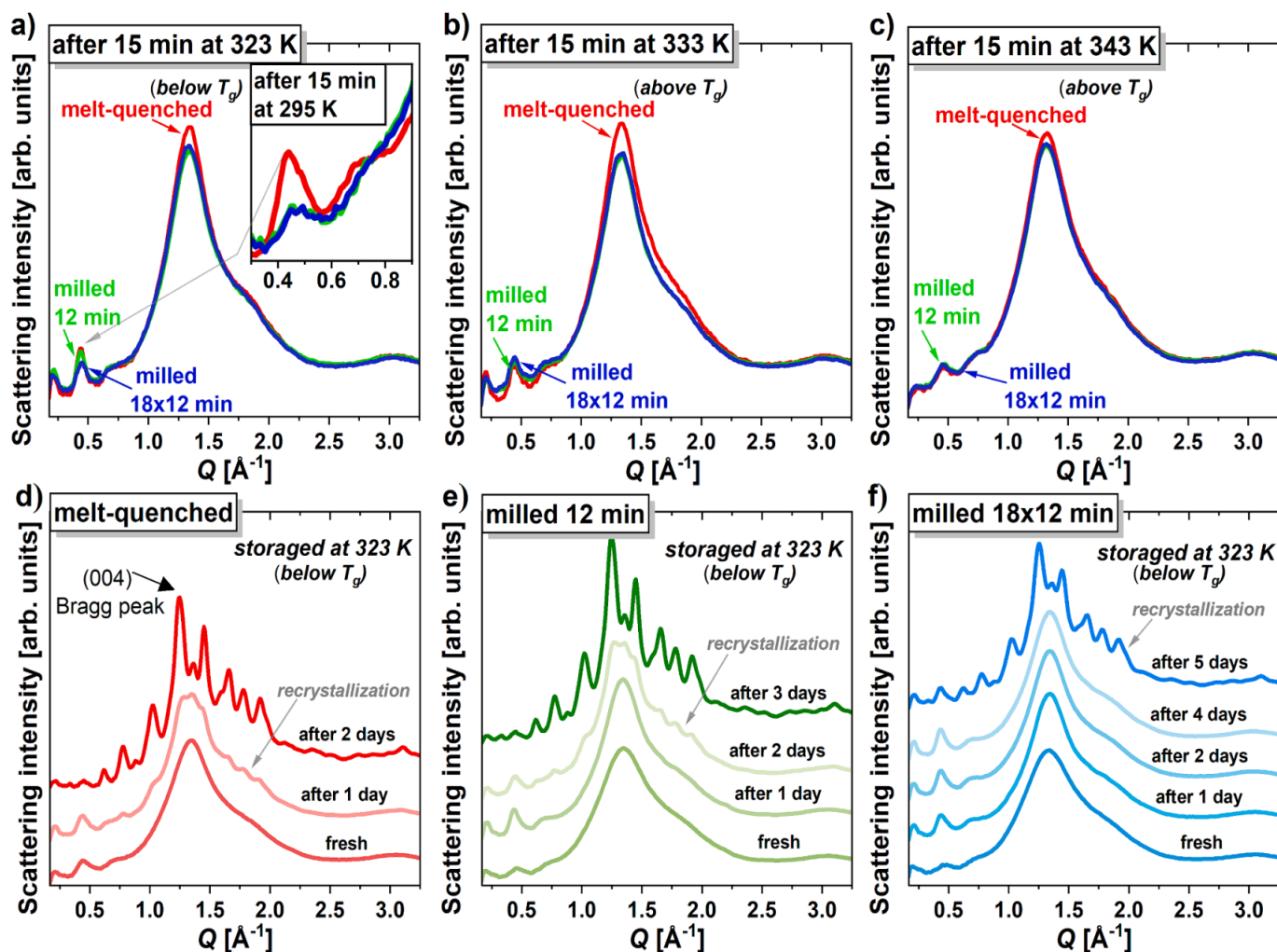


Fig. 3. Comparison between the superimposed X-ray scattering data for: ITZ glass produced by melt-quenching, short-milled (12 min) and long-milled (18x12 min), collected on heating at a) 323 K, b) 333 K, c) 343 K. Besides, the inset in panel a) shows the low- Q range for fresh samples at 295 K. Panels d), e), f) present temporal evolution of X-ray scattering patterns for OG produced by melt-quenching, short-milled (12 min), and long-milled (18x12 min) ITZ, respectively, stored at 323 K.

well as to the recrystallization than OG, especially in the case of the long-milled sample. Therefore, in the next step, we followed the physical stability at 323 K (below T_g) as a function of time. From Fig. 3d,e,f, it is clear that the OG started to recrystallize after 1 day at 323 K, the short-milled – after 2 days, and the long-milled – after 5 days. The recrystallization progress in time at this temperature was quantified by the amplitude of the (004) Bragg reflection of the starting crystalline form. The data are presented in Fig. 4. What is more, after 1 day at 323 K, the long-milled ITZ exhibited a much lower degree of the LC order than the short-milled. In fact, the amplitude of the peak at $Q \sim 0.43 \text{ \AA}^{-1}$ for the long-milled sample became equal only after 4 days to that for the short-milled phase after 1 day of such annealing below the T_g (Fig. SM5 in SM). For some reason, the reconstruction of the Sm order in the long-milled sample took longer.

Considering the possible application of cryo-milled ITZ in the pharmacy, we also probed its physical stability at room temperature (X-ray scattering data presented in Fig. SM6 in SM). As expected, the OG turned out to be the least stable – it started to recrystallize after 40 days from the preparation. The short-milled sample was more stable – up to 70 days. In turn, the long-milled ITZ did not reveal recrystallization even after 300 days from the preparation. During this period, the low- Q peaks as well as the main peak remained unaffected, proving the extraordinary stability of the N order obtained by cryo-milling. The obtained pattern of the longer thermodynamic stability of the cryo-grounded ITZ samples below T_g , compared to the melt-quenched one, is very surprising since usually cryo-milled crystalline solids result in amorphous forms less stable than those obtained by melt-quenching and dissolution techniques [24,25].

Looking for a comprehension of the better physical stability of the ITZ cryo-milled species (below T_g) compared to the glass obtained by standard melt-quenching and, what is more, significantly better stability of the long-milled sample compared to the short-milled one, it is worth to elaborate more on the structural features of these systems. The diffraction data presented in Figs. 1 and 2 show that the atomic-scale structure of ITZ with the nematic order is subjected to disordering

with increasing milling duration. The main difference between short-milled and long-milled samples is manifested within the main diffraction peak which arises due to the nearest-neighbor (lateral) organization of molecules. Therefore, one can deduce that the enhanced stability of the long-milled ITZ may be due to the lower degree of the short-range order fingerprinted by the damping of the main diffraction peak's amplitude. The higher lateral disorganization of the molecules in the nematic structure may delay the rebuilding of the Sm ordering and recrystallization. It should be mentioned that the increase in the nearest-neighbor intermolecular disorder with longer milling t may be the natural consequence of a smaller size of the particles in the milled powders and a higher concentration of defects on the surface. In order to quantify the average size of the studied samples, microscopy observations were performed. Fig. SM7 a,b,c,d in SM presents the optical microscopy images of as-received crystalline, OG, short-milled and long-milled ITZ species, respectively. The determined average particle sizes of the crystalline and melt-quenched samples were very similar, $\sim 35 \text{ }\mu\text{m}$. In turn, for both grounded samples, the obtained sizes $\sim 5 - 6 \text{ }\mu\text{m}$ were at the limit of the resolution of the optical microscope. Therefore, TEM observations were additionally undertaken and representative images are shown in Fig. SM8 a and b in SM. The established average size of the grains for the long-milled sample was $\sim 0.5 \text{ }\mu\text{m}$, while in the case of the short-milled powder, the grains were too big for reliable size distribution analysis. Therefore, the limit value of $5 \text{ }\mu\text{m}$ from optical images was adopted. A summary of the determined average particle sizes of the

Table 1
The average size of particles of the examined ITZ samples derived from the analysis of microscopic observations.

Sample	Particle size [μm]
crystalline	34 ± 3
melt-quenched	35 ± 3
short-milled	5 ± 3
long-milled	0.5 ± 0.2

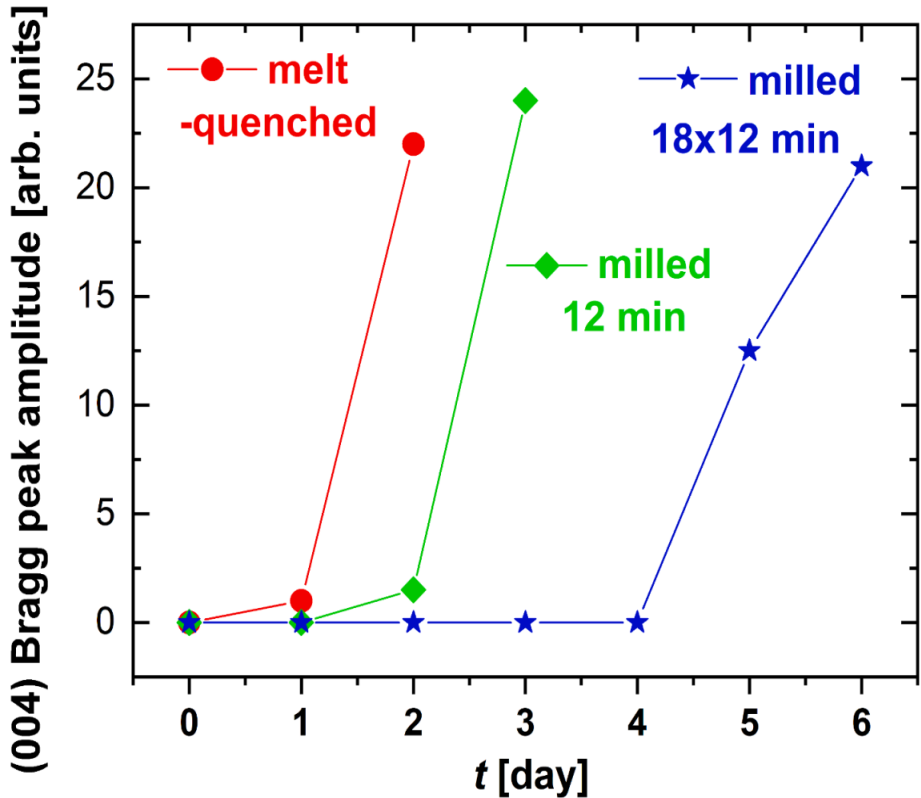


Fig. 4. Comparison of the recrystallization progress in time for the ITZ samples heated at 323 K: glass produced by melt-quenching, short-milled (12 min) and long-milled (18x12 min).

ITZ species is presented in Table 1.

As complementary, thermal and dynamical properties of the studied ITZ samples were characterized by differential scanning calorimetry and broadband dielectric spectroscopy. Representative thermograms of the systems revealed that melt-quenched ITZ exhibits one jump in the heat capacity related to the glass transition at T_g , followed by two endothermic peaks at $T_{Sm-N} = 345$ K and $T_{N-I} = 362$ K (Fig. 5a). This agrees perfectly with the literature data widely reported [15,16,17,22]. DSC curves recorded for the milled samples showed also two endothermic processes assigned to the LC phase transitions, but both systems recrystallized at higher T , which was further followed by melting at $T_m \sim 440$ K. However, the most interesting seems to be the presence of some additional exothermic events at T -range corresponding to T_g 's region of OG (the black box in Fig. 5a). Taking into account that the X-ray scattering data showed that the structure of the cryo-milled samples exhibit only N order at 295 K deep below T_g , and their annealing below T_g reconstructs gradually the Sm order, one can assume that those additional transitions indicate the reformation of the Sm layering upon heating. For a better insight into the nature of these thermal events, temperature modulated calorimetry measurements were carried out at T_g 's region (inset in Fig. 5a). The obtained curves revealed double glass transition for both milled samples, which might be related to the vitrification of motions occurring around long and short axis of ITZ. It should be mentioned that a similar argumentation was used to explain the presence of a double glass transition phenomenon in the thermograms observed by Teerakapibal et al. and by some of us for the rapid quenched and highly compressed ITZ [15,17]. It is worth adding that the TM-DSC curve of OG also demonstrates a signature of such double glass transition.

The hypothesis that the fresh cryo-milled ITZ samples with destroyed crystalline structure exhibit only N organization of molecules and recover the higher Sm order when annealed below the T_g was confirmed by DSC studies performed with much higher heating rates (≥ 60 K/min) limiting the effect of annealing. In such a case, the probed short-milled sample showed only two thermal events – related to the glass and the N - I phase transitions (Fig. 5b and Fig. SM9 in SM). The smectic order did not have time to rebuild (which is the case for the sample heated with a lower rate of 10 K/min), and, consequently, no peak related to the Sm - N transition was recorded. Finally, it is worth to add that the DSC curve recorded for the cryo-milled ITZ after heating above the respective T_m resembled the thermogram obtained for OG (Fig. SM10 in SM). So, the milled-induced suppression of the Sm order is a reversible effect.

The molecular dynamics of OG and cryo-milled phases was probed in

the vicinity of T_g determined as T at which structural (α) relaxation time (τ_α) equals 100 s. The dielectric spectra and the analysis method were presented in SM (Fig. SM11 and Fig. SM12). As illustrated in Fig. 6a, the structural dynamics, consisting of motions around the long molecular axis, clearly varied between the cryo-milled and melt-quenched samples close to $T_g - \tau_\alpha$ of the cryo-milled species was shorter than those of the melt-quenched ITZ [26]. However, this effect became less apparent at higher T around 363 K, which corresponds to the N - I phase transition. The τ_α of all studied systems converged around this T but it never reached τ_α of the melt-quenched sample. What is more, from the analysis of dielectric spectra measured for the melt-quenched and long-milled systems, we determined relaxation times of the δ -process (τ_δ), which is due to reorientational motions of ITZ molecules around the short axis.^{15,17} As can be seen in Fig. 6a, the dynamics of the δ -mode in the cryo-ground sample is slightly slower around the T_g with respect to the melt-quenched ITZ. However, the change in τ_δ is very small, within the experimental uncertainty, and vanishes at higher T . It is due to fast recovery of the dynamics as well as the structure of the cryo-milled samples above the T_g , as can be deduced from the X-ray diffraction and further dielectric investigations. As a consequence of that, the dynamics of the α - and δ -processes in cryoground and melt-quenched ITZ is almost the same at higher temperature region. Herein, it should be added that we were not able to obtain reliable $\tau_\delta(T)$ dependency for the short-milled ITZ since it recrystallized very fast at much lower T than the long-milled one (see BDS spectra presented in Fig. SM11b in SM). It is also worth to stress that the glass transition temperatures T_g^{BDS} determined from the $\tau_\alpha(T)$ and $\tau_\delta(T)$ dependencies for the long-milled sample ($T_{g,1}^{BDS} = 325$ K and $T_{g,2}^{BDS} = 332$ K, respectively) are similar to the glass transition temperatures T_g^{DSC} obtained from the calorimetric data ($T_{g,1}^{DSC} = 327$ K and $T_{g,2}^{DSC} = 331$ K, respectively). This indicates that, as suggested above as well as in Refs [15] and [17], both T_g 's observed in the thermograms of the examined ITZ samples might be due to vitrification of different kinds of ITZ molecular motions – around long (α -process) and short (δ -process) axis.

It should be noted that additionally we carried out annealing experiments on the short- and long-milled ITZ at 335 K near T_g . As can be observed in Fig. 6b, in both cases, the maximum of the α -process shifted towards lower f with t of annealing. However, even in the equilibrium state, it did not reach the maximum for the melt-quenched sample at the same T . This could be explained by the discrepancy in the nearest-neighbour order between the cryo-milled and vitrified phases that was never the same even at high T , as evidenced by the X-ray scattering

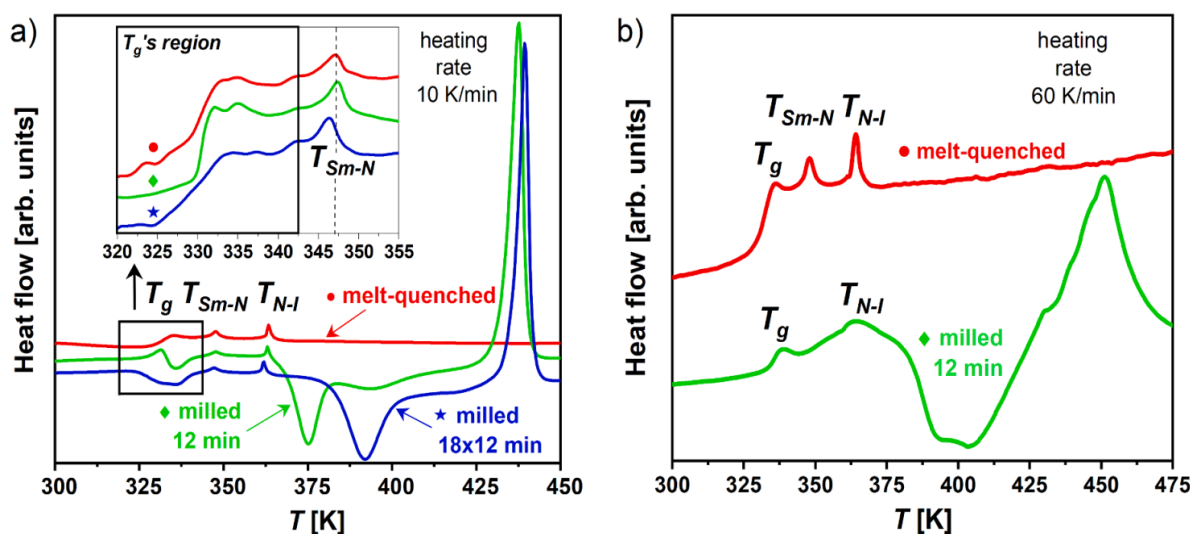


Fig. 5. a) DSC thermograms for ITZ glass produced by melt-quenching, short-milled (12 min), and long-milled (18x12 min), recorded during heating with a rate of 10 K/min. The inset shows TM-DSC curves recorded with the amplitude of the pulses of 0.5 K. Panel b) Comparison of DSC thermograms for ITZ glass and short-milled sample recorded during heating with a higher rate of 60 K/min.

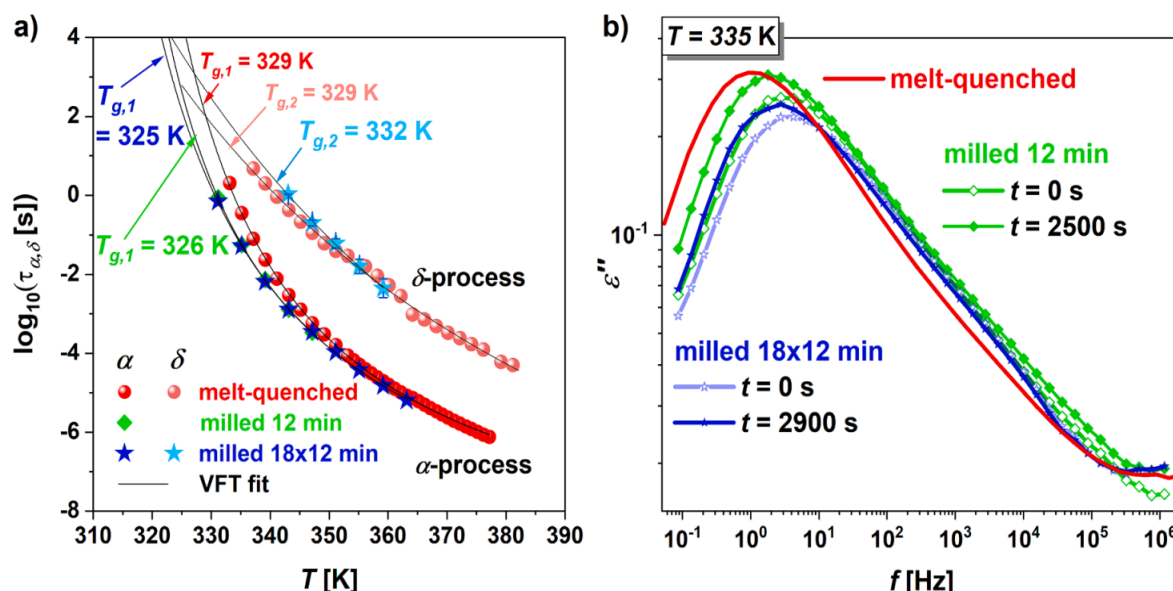


Fig. 6. a) Dependencies of $\log_{10}\tau_{\alpha}$ and $\log_{10}\tau_{\delta}$ versus T for the examined ITZ samples. Solid black lines represent Vogel-Fulcher-Tammann (VFT) fits. Panel b) shows the time evolution of dielectric loss spectra collected upon the annealing experiments for the short- and long-milled ITZ at 335 K.

investigations.

Finally, inspired by the very promising physical stability performance of the cryo-milled ITZ specimens, we found it interesting to verify their potential for use in therapy. Therefore, we performed dissolution rate tests for the short- and long-milled samples, as well as for the crystalline and OG references. The results are presented in Fig. 7. As can be seen, the solubility of the crystalline ITZ was very poor, below ~ 6 $\mu\text{g/ml}$ after 90 h. Transformation of the crystal to the more disordered OG with a degree of frozen Sm molecular alignment had a significant impact on the solubility – it increased up to ~ 103 $\mu\text{g/ml}$ at 60 h. The increase in solubility with time was gradual up to the maximum value, and then it was slightly decreasing. In the case of the short-milled sample, the increase was also gradual but up to a higher value of ~ 107 $\mu\text{g/ml}$ and within a shorter time of 30 h. After, the solubility was at the same high level up to 90 h. The long cryo-milled sample exhibited the highest solubility of ~ 110 $\mu\text{g/ml}$ at 30 h, which then gradually dropped to more or less the same level as for the short-milled sample. From the behavior of the presented dissolution rate curves, one can simply deduce that the most powerful factor controlling the solubility of ITZ is the degree of the intermolecular disorder. Crystalline and melt-quenched samples are characterized by practically identical average particle sizes, but the solubility of OG is 17 times higher. The particle size seems to affect the solubility slightly at the first stage of the

dissolution profile, before the stabilization level – the long-milled sample with the smallest average particle size has the steepest slope of the dissolution curve, but after a longer time, it behaves similarly to the short-milled sample with a slightly bigger average particle size. Then, the internal structure seems to play the main role, and the more disordered structures generated by milling reveal a slightly higher solubility level than the more ordered OG. These establishments are extremely important for the future design of better soluble APIs in liquid-crystalline formulations.

4. Conclusions

To sum up, our results demonstrated that cryo-milling effectively disrupts the crystalline and also the layered smectic order in ITZ, while the nematic order is much more resistant to mechanical treatment. Although extending the milling time does not destroy the N alignment of molecules, it does frustrate their nearest-neighbor organization. The uncovering of the subtle changes in the intermolecular structure and the qualification between the degree of the liquid-crystalline order in the solid state phases of the milled ITZ and melt-quenched glass were possible by the X-ray scattering and differential scanning calorimetry including temperature-modulated studies. Moreover, by using dielectric spectroscopy, we demonstrated that the intermolecular disorder translates into faster dynamics of cryo-milled species, which never matches that observed for the melt-quenched phase even at high T . By the analysis of the dynamical properties, we could also explain the origin of double glass transition for the cryo-milled systems as arising due to the vitrification of reorientational motions around the long (α -process) and short (δ -process) axis of ITZ molecules. Finally, we found that ITZ structures obtained by cryo-milling are much more physically stable as well as better soluble with respect to ordinary glass, and their stability increases with milling time, so it may be easily tuned. Importantly, we proposed the mechanism of this phenomenon to be related to the higher degree of the short-range intermolecular disorder, which was demonstrated to progress with a rise in the milling duration or frequency. Therefore, the obtained results may be useful for the development of formulations of ITZ with enhanced stability and solubility. We are convinced that the data presented herein open new fascinating ways to generate unique nanospatial organization of the molecules forming mesophases by mechanical treatment.

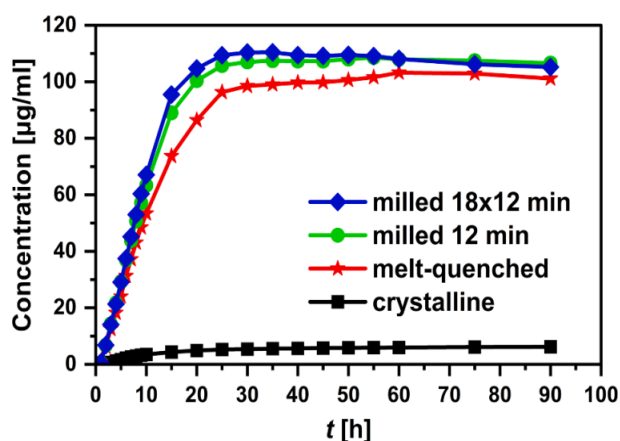


Fig. 7. Dissolution rate curves of various ITZ samples: crystalline, ordinary glass obtained by melt-quenching, short-cryo-milled (12 min) and long-cryo-milled (18x12 min).

CRedit authorship contribution statement

Taoufik Lamrani: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paulina Jesionek:** Visualization, Investigation. **Magdalena Tarnacka:** Visualization, Investigation. **Daniel Zakowiecki:** Visualization, Investigation. **Patryk Włodarczyk:** Investigation. **Maciej Zubko:** Investigation. **Ewa Kaminska:** Validation, Supervision, Project administration, Funding acquisition, Formal analysis. **Kamil Kaminski:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Karolina Jurkiewicz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary Material contains: a) Results of supporting X-ray diffraction studies. b) Microscopic images of ITZ powders. c) Results of supporting DSC measurements. d) Dielectric loss spectra. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2024.126106>.

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OPEN Effect of solvent evaporation on the liquid-crystalline order of itraconazole

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Solvent evaporation is widely used for the formation of amorphous active pharmaceutical ingredients. This study investigates the effect of solvent evaporation on the structure of itraconazole – an antifungal drug and a model thermotropic liquid crystal that exhibits nematic and smectic phases when supercooled from the melt, and when cooled below the glass transition temperature at ~330 K, it forms glass with frozen liquid-crystalline order. Using X-ray scattering, it is demonstrated that the solid form of itraconazole obtained at room temperature via evaporation of dichloromethane reveals a smectic order extending up to ~15 nm, which is about two times more than what is observed for itraconazole glass obtained by the conventional vitrification. Moreover, the degree of the smectic order may be tuned by adjusting the concentration of the drug in the solution or the choice of solvent. The obtained results are in agreement with previous reports, which indicated an enhancement of smectic order in the system of itraconazole with glycerol derived by evaporation from dichloromethane as compared to pure itraconazole glass [Fatina, C. et al. (2024). *Cryst. Growth & Des.*, 24(11), 4596–4603]. Nevertheless, the new insight has clearly shown that glycerol stabilizing the liquid-crystalline order in itraconazole is not required at all for its generation. This can be achieved with pure itraconazole by evaporation of solvent. Moreover, the presence of glycerol does not extend the long-term room temperature physical stability of the smectic structure in itraconazole. Our findings may be used for producing non-crystalline formulations offering improvements in drug release over the conventional crystalline form.

Keywords Itraconazole, Solvent-evaporation, Liquid-crystalline structure, Smectic order, Dichloromethane

Itraconazole (ITZ), a widely recognized azole antifungal agent, serves as an exemplary model for investigating liquid crystalline (LC) phenomena due to its ability to form distinct LC structures under various thermodynamic conditions and treatments¹. On the market, pure ITZ is available as a crystalline powder in the polymorphic form I^2 , while the most known pharmaceutical product based on ITZ is SporanoX³, which is available as both an oral solution and capsules where a solid dispersion formulation is utilized. Upon melting and subsequent cooling from the isotropic (I) liquid phase, ITZ undergoes a transition into a nematic (N) mesophase at temperature $T_{I-N} \sim 363$ K³. Further cooling below $T_{N-Sm} \sim 347$ K induces a transformation from the N into a smectic (Sm) mesophase³. When cooled down below its glass transition temperature $T_g \sim 330$ K, ITZ forms a glassy state that may preserve the molecular order of the Sm mesophase⁴. Importantly, Teerakapibal et al.⁴ demonstrated that Sm layering in ITZ can be frustrated by increasing the cooling rate during vitrification, enabling the formation of glasses with different degrees of the Sm -to- N order. What is more, for cooling rates exceeding ~20 K/s, the N to Sm transition may be avoided, resulting in formation of glass with only N order (with Sm order parameter = 0). In fact numerous reports have shown that different factors, besides cooling rate, such as deposition rate, precipitation temperature, substrate temperature or mechanical stressing, may tune the LC order of ITZ molecules^{5–8}. Vapor deposition experiments have established that depositing of ITZ at different substrate temperatures results in glasses characterized by wide range of molecular orientations, with some deposition conditions providing high levels of Sm order⁵. Further, it has been shown that shear can change the N alignment in this API, which indirectly can affect also the Sm alignment upon cooling down below

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T_N-Sm , as well as the Sm order trapped in the glass upon further cooling below T_g ⁶. The ITZ stressed in the liquid state exhibited a higher Sm order below T_g than its non-stressed counterpart. On the other hand, a recent study performed by our group demonstrated that mechanical stress in the form of vibrational or ball milling can disrupt the crystalline and Sm order of solid ITZ, resulting in particles with N organization, where, additionally, the lateral intermolecular order can be tuned by adjusting milling time and frequency⁷. Last but not least, microfluidic anti-solvent precipitation techniques were used to produce ITZ nanoparticles with Sm structures wherein the molecular arrangement can be precisely controlled by the precipitation temperature⁸.

Generally, various solvation approaches have been widely utilized to produce ITZ or its binary mixtures with a vast degree of structural disorder. The interest in this topic stems from the fact that crystalline ITZ exhibits extremely low water solubility (1 ng/ml at pH 7.4), which may account for its low oral bioavailability, while amorphization is one way to improve this important parameter. For instance, Mugheirbi et al. reported the formation of N order in ITZ particles produced by spray-drying of the API dissolved in dichloromethane (DCM)⁹. Moreover, they demonstrated unique N -to- Sm switching behavior of ITZ via moisture-triggered activation of molecular mobility below the T_g . It is worth to note that spray-drying is a very efficient solvent evaporation-based amorphization method, since it allows for extremely rapid solvent evaporation (SE), leading to the creation of amorphous-like particles. Amponsah-Efah et al. used SE from DCM to prepare mixtures of ITZ with glycerol (GLY). They found that the Sm -to- N phase transition was avoided upon heating when the GLY content was 5 wt% or higher¹⁰. Complementary studies by Rams-Baron et al. using dielectric spectroscopy revealed that the melt-quenched ITZ:GLY system obtained through DCM evaporation exhibits shorter structural relaxation times compared to the pure ITZ glass, indicating that GLY simultaneously stabilizes and fluidizes the Sm ITZ phase¹¹. In parallel, recent work by Fatina et al. demonstrated that trace GLY strongly enhances the LC order of vitrified ITZ:GLY system obtained via DCM evaporation compared to its pure vitrified ITZ. They stated that 5 wt% of GLY enhances the Sm layering of the ITZ host by reducing the random molecular offsets in each layer and creating more compact layers due to the formation of hydrogen bond cross-links between GLY and ITZ molecules within a monolayer. This was evidenced by the diffraction patterns where the second-order diffraction peak (002) due to the LC ordering was more intense than the first-order reflection (001) while the diffraction pattern of the pure vitrified ITZ is usually characterized by less intense (002) than (001) reflection¹².

The aim of this report is to show an alternative method for the formation of ITZ solid particles with a more extensive Sm order compared to that generated by vitrification – a straightforward solvent evaporation at room temperature without adding any other compound like GLY. The effect of the solvent evaporation process on the phase behaviour of the resulting ITZ solid form, the features of its molecular organization, and related physical stability, thermal properties, and molecular relaxation processes was investigated using X-ray scattering, differential scanning calorimetry, and broadband dielectric spectroscopy. The collected results were compared and discussed with reference to the corresponding data obtained for ITZ glass produced by vitrification, pointing out the differences between these two solid structures with trapped LC order. Moreover, infrared spectroscopy studies were performed in order to verify the possible interactions between the molecules of ITZ and solvent, which would bring closer the mechanism of the formation of the Sm order during the solvent evaporation.

Experimental section

Materials

Itraconazole (ITZ) with a molecular formula of $C_{35}H_{38}Cl_2N_8O_4$, a molar mass of 705.64 g/mol, and a purity of > 98%, was purchased from Angene (CAS Number 84625-61-6) as a 1:1:1:1 racemic mixture of four diastereomers (two enantiomeric pairs), each possessing three chiral centers. Dichloromethane (DCM, CAS Number 75-09-2) and chloroform (CLF, CAS Number 67-66-3), both with a purity $\geq$ 99%, were purchased from Karpinex. Glycerol (GLY, CAS Number 56-81-5), with a purity of 99.5%, was obtained from Sigma-Aldrich.

Methods

Sample preparation

The vitrified ITZ (V-ITZ), appearing as a milky, glass-like solid, was prepared by melting the crystalline powder at 440 K, followed by quenching of the melt at a cooling rate of ~ 1 K/s down to 295 K (room temperature). Such a procedure is further referred to in the manuscript as “vitrification” while the V-ITZ sample serves as a reference system with a Sm order frozen in a glassy state. Solvent-evaporated ITZ (SE-ITZ) samples, exhibiting a milky appearance with foam-like morphology, were obtained as follows: 20 mg portions of commercial crystalline ITZ were added to: 2, 1, 0.67, and, 0.5 ml of DCM, resulting in ITZ:DCM solutions of different concentrations: 10, 20, 30, and 40 mg/ml, respectively, which were then thoroughly mixed in a cylindrical borosilicate glass vial with a 2 cm diameter and a height of 3 cm to ensure homogeneity. The solutions then underwent solvent evaporation under reduced pressure at a rate of $\sim 1 \pm 0.02$ ml/min using two-stage rotary vane CRVPro 6 pumps connected via a Schlenk line until only a minimal volume of the solvent remained. The evaporation rate was calculated by weighting the solvent and measuring the time required for its evaporation that was observed. To ensure the complete removal of the residual solvent, the samples were subsequently transferred to a vacuum desiccator and subjected to drying at 293 K and 4% RH for 12 h. The solvent-evaporated materials obtained from this process are hereinafter referred to as SE-ITZ X (where X represents the initial ITZ concentration solved in DCM: 10, 20, 30, or 40 mg/ml): SE-ITZ 10, SE-ITZ 20, SE-ITZ 30, and SE-ITZ 40, respectively. The residual DCM content was evaluated using thermogravimetric analysis (TGA), and the results confirmed only trace amounts of solvent (< 0.1 wt%) in the studied materials, as shown in Fig. S11 in Supplementary Information. A similar procedure as described above was followed for preparing solvent-evaporated itraconazole with glycerol system SE-ITZ:GLY 20 from 20 mg/ml of solved ITZ:GLY in DCM, incorporating GLY at a concentration of 5% w/w refer to ITZ. Moreover, an alternative solvent was also utilized – CLF, and by applying the same methodology as described above, it was possible to obtain SE-ITZ 20 CLF sample from 20 mg/ml of solved ITZ in CLF. It is worth noting

that both compounds, DCM and CLF, were used due to high solubility of ITZ in these solvents (3.27×10^{-2} and 5.04×10^{-2} M/M, respectively)¹³, and their relatively low boiling points (313 K and 334 K, respectively) enabling their fast evaporation at room temperature under reduced pressure. Although DCM and CLF are not common solvents for pharmaceutical production, they allowed us to induce the *Sm* organization of ITZ molecules by the above-described solvent evaporation process, avoiding crystallization.

X-ray scattering

The wide-angle X-ray scattering (WAXS) measurements were conducted using a D/Max Rapid II diffractometer (Rigaku Corporation, Japan) equipped with a rotating Ag anode and accelerated using 60 kV voltage and 200 mA current. The WAXS data are standard X-ray diffraction (XRD) data collected at wide-angle range. A graphite (002) monochromator was employed for the incident radiation. The wavelength of the monochromatic Ag K_α beam was $\lambda = 0.5608$ Å and the size of the beam on the sample was 0.3 mm. Fresh SE-ITZ samples, prepared after being stored overnight in a vacuum chamber, were packed and sealed in borosilicate glass capillaries and subjected to measurements in transmission mode for one hour. The fresh V-ITZ sample, just after melting and cooling down to 295 K, was also measured using the same settings. The temperature T of 295 K was maintained throughout all measurements. X-ray scattering patterns were collected using a 2D curved image plate detector, corrected for background (empty capillary), azimuthally integrated, and converted into 1D functions of the scattering intensity versus the scattering angle (2θ). These were then transformed to the scale of the scattering vector $Q = 4\pi \sin(\theta)/\lambda$, covering a range of approximately 0.17 – 20 Å^{−1} and normalized with respect to the intensity at the high Q range of approximately 4 – 20 Å^{−1} where the scattering mostly from the ITZ intramolecular structure contributes to the scattering pattern (Fig. S12 in Supplementary Information). Our results indicated that the SE-ITZ and V-ITZ samples have the same intramolecular structure, as expected. Therefore, here, the diffraction patterns are presented in only the Q range of 0.17 – 2.65 Å^{−1}, where the intermolecular structure primarily contributes. Moreover, the WAXS data for selected ITZ samples were corrected for polarization, absorption, incoherent Compton scattering, normalized to the electron units, and transformed to the structure factor $S(Q)$ as follows:

$$S(Q) = \frac{I(Q) - \langle f^2 \rangle - \langle f \rangle^2}{\langle f \rangle^2}$$

where: $I(Q)$ is the coherently scattered intensity, normalized to electron units, $\langle f^2 \rangle = \sum_{i=1}^n c_i f_i^2$, $\langle f \rangle = \sum_{i=1}^n c_i f_i$, c_i and f_i are the concentration and the atomic scattering factor of the i -th atomic species, respectively, and n is the number of atomic species in the sample. The structure factor was then converted by the sine Fourier transform to the real space representation of diffraction data — the pair distribution function (PDF):

$$PDF(r) = G(r) = 4\pi r [\rho(r) - \rho_0] = \frac{2}{\pi} \int_0^{Q_{max}} Q [S(Q) - 1] W(Q) \sin(Qr) dQ,$$

where Q_{max} is the upper limit of Q achieved in the measurements (20 Å^{−1}). The window function

$W(Q) = \sin\left(\frac{\pi Q}{Q_{max}}\right)$ was used to minimize the termination oscillations. The range of PDF's oscillations

was used to quantitatively describe the spatial extent of structural order (the coherence length) in the studied *Sm* forms. Additionally, small-angle X-ray scattering (SAXS) studies for V-ITZ and SE-ITZ 20 samples were performed using a laboratory Xenocs Xeuss 2.0 SAXS (Xenocs, France) system located at the Joint Laboratory for SAXS studies at the Faculty of Physics and Astronomy, Adam Mickiewicz University. The system was equipped with a MetalJet D2 microfocus X-ray source ($\lambda = 1.34$ Å) with a liquid metal-jet anode (gallium alloy). SAXS data were collected for powder samples placed between two Kapton films at T of 295 K and recorded using a Pilatus3R 1 M hybrid photon counting detector in the Q range of approximately 0.05 – 1 Å^{−1}. The distance between the sample and the detector was approximately 580 mm.

Differential scanning calorimetry (DSC)

Calorimetric measurements of ITZ were carried out by Mettler-Toledo DSC apparatus (Mettler-Toledo International, Switzerland) equipped with a liquid nitrogen cooling accessory and an HSS8 ceramic sensor (heat flux sensor with 120 thermocouples). Temperature and enthalpy calibrations were performed by using indium and zinc standards. The sample was prepared in an open aluminum crucible (40 µl) outside the DSC apparatus. All samples were measured as followed: at first, they were heated from 325 to 450 K, cooled down to 325 K and then heated again to 450 K at a constant heating/cooling rate of 10 K/min.

Broadband dielectric spectroscopy (BDS)

Isobaric measurements of the complex dielectric permittivity $\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega)$, where ω is frequency, ε' and ε'' represent the real and imaginary parts of ε^* , were carried out for SE-ITZ 20 and V-ITZ samples using a Novocontrol Alpha dielectric spectrometer (Novocontrol Technologies GmbH & Co. KG, Germany) over the ω range of 10^{-1} – 10^6 Hz at ambient pressure. The temperature was maintained with a Quatro Cryosystem using a nitrogen gas cryostat with a control better than 0.1 K. Approximately 60 mg of each sample in the powder form was placed between two stainless-steel electrodes, separated by a thin Teflon spacer (diameter of 15 mm, gap of 0.1 mm). The dielectric spectra were investigated in T range of 333–385 K.

To obtain relaxation times (τ), the dielectric loss spectra were fitted using the Havriliak-Negami (HN) function¹⁴ with dc-conductivity (DC) term:

$$\varepsilon^*(\omega) = \frac{\sigma_{DC}}{\varepsilon_f \omega} + \varepsilon_\infty + \sum_{i=1}^2 \left(\frac{\Delta\varepsilon_i}{[1 + (i\omega\tau_{HN,i})^{\alpha_{HN,i}}]^{\beta_{HN,i}}} \right) \quad (1)$$

where σ_{DC} is the DC factor, ε_f is the permittivity of the free space, $\Delta\varepsilon$ is the dielectric strength, ω is the angular frequency ($\omega = 2\pi f$), τ_{HN} describes HN relaxation time and α_{HN} , β_{HN} are the shape parameters. The subscript i is referred to each process. The temperature dependences of the structural relaxation times were fitted using the Vogel-Fulcher-Tamman (VFT) function^{15–17}:

$$\tau = \tau_\infty \exp \left(\frac{B}{T - T_0} \right) \quad (2)$$

where, τ_∞ is the relaxation time in the limit of very high temperatures, T_0 is the so-called Vogel temperature, and B is the activation parameter. T_g of all examined samples were determined as the temperature at which structural relaxation time $\tau_\alpha = 100$ s, by extrapolating the VFT fits.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra for ITZ:DCM 20, ITZ:DCM 30, and ITZ:DCM 40 mixtures were measured using a Nicolet iS50 spectrometer (Thermo Fisher Scientific, USA) at a spectral resolution of 4 cm^{-1} with 16 scans. The spectra were recorded in ATR mode over the wavenumber (ν) range of $4000\text{--}400 \text{ cm}^{-1}$ at 295 K. During the measurements, the FTIR spectrometer was purged with liquid nitrogen to remove water vapour and carbon dioxide from the instrument effectively.

Drug release tests

To assess the aqueous release profiles of different ITZ forms, V-ITZ, SE-ITZ 40 and crystalline ITZ, their release kinetics in 0.1 M HCl water solution were measured. The details of the experiment and the registered release curves can be found in the Supplementary Information.

Results and discussion

Impact of evaporation rate on the resulting structure of solvent-evaporated itraconazole

Initially, in order to assess the structural behavior of the ITZ:DCM solution during DCM evaporation, the XRD experiment was conducted for the solution sealed in a glass capillary. As shown in Fig. 1, the XRD pattern of freshly prepared ITZ:DCM mixture of 20 mg/ml exhibited only one broad halo at $Q \sim 1.6 \text{ \AA}^{-1}$, with no evidence of LC or crystalline ordering, suggesting that ITZ was fully dispersed in DCM. The sample was subsequently heated from 295 up to 308 K to facilitate faster DCM evaporation. Notably, after 30 min, when DCM evaporated, the residual ITZ crystallized to the same polymorphic form (I) as the commercial reference ITZ. A number of similar experiments were performed for ITZ:DCM solutions with various ITZ concentrations. However, the resulting structure of ITZ obtained by these experiments was always crystalline. The solvent evaporation rate in these experiments was much slower ($\sim 0.01 \pm 0.005 \text{ ml/min}$) than in the case of the evaporation experiments performed under reduced pressure ($\sim 1 \pm 0.02 \text{ ml/min}$), as described in the Sample preparation section. We refer to these two evaporation rates below as “slower” and “faster”. The crystallization of ITZ occurred in less than 30 min, and the time resolution of the XRD measurements did not allow observing whether any intermediate LC phases were formed before crystallization.

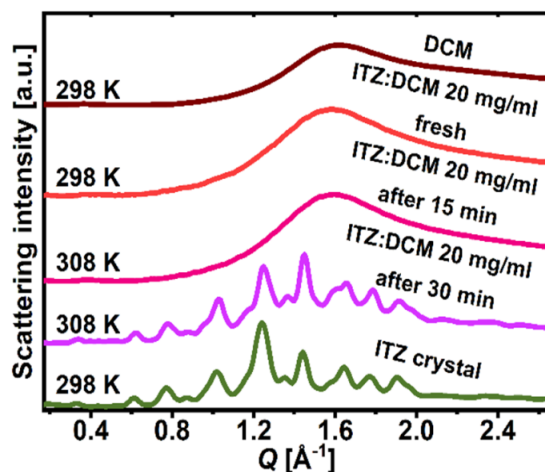


Fig. 1. XRD patterns of freshly prepared ITZ:DCM solution of 20 mg/ml, measured at 295 K, and the solution after 15 and 30 min at 308 K, compared with the patterns for pure crystalline ITZ and liquid DCM at 295 K.

Unfortunately, using this XRD setup it was not possible to track the structural changes in-situ during the solvent evaporation process under reduced pressure, which was used to prepare target SE-ITZ samples, as described in the Sample preparation section. Therefore, the structure of SE-ITZ samples was studied ex-situ after complete DCM removal. Their diffraction patterns, presented in Fig. 2, indicate that they are not characterized by either crystalline or amorphous structure but exhibit a *Sm* order manifested by the presence of (001), (002), and (003) reflections due to the layered organization. These findings underscore the influence of the solvent evaporation rate on the resulting organization of molecules in ITZ, as the slower DCM evaporation favored ITZ crystallization while the faster solvent removal resulted in the formation of *Sm* order. In contrast, it is worth to remind that ITZ particles obtained by spray drying, which involves extremely rapid solvent removal, exhibited a lower degree of structural order (namely, nematic order in this case)⁹ than the SE-ITZ samples studied here. Thus, it seems that using different solvent evaporation rate scales, ITZ can be obtained in solid crystalline, *Sm*, or *N* forms, while the higher the evaporation rate, the higher the resulting structural disorder of ITZ molecules.

Liquid crystalline order of the solvent-evaporated itraconazole

Here, it is presented that the DCM evaporation from the ITZ:DCM solution may lead to the formation of a LC order in ITZ, instead of its crystallization, when a faster evaporation rate (1 ml/min) is applied than that reported for XRD experiment in closed capillaries (0.01 ml/min), as described in the Sample preparation section. The diffraction patterns of such prepared SE-ITZ samples after complete DCM removal are presented in Fig. 2a. The XRD curves for all these samples exhibit a broad main peak at $Q \sim 1.35 \text{ \AA}^{-1}$, corresponding to the lateral packing of ITZ molecules. The main peak's position indicates an average intermolecular distance $d = 2\pi/Q \sim 4.65 \text{ \AA}$. Additional peaks in the low- Q region suggest the presence of LC order. Specifically, for ITZ molecules arranged in *Sm* layers, a series of diffraction peaks appear at $Q \sim 0.21, 0.43$, and $0.65 \text{ \AA}^{-1}$, consistent with previously reported patterns in the literature^{4,18}. These peaks indicate a LC periodic structure with a periodicity $d \sim 30 \text{ \AA}$ ¹⁹, which is approximately the length of ITZ molecule²⁰. Due to a limitation of the setup used for the WAXS measurements, the intensity of the first-order diffraction peak (001) from the layer organization of ITZ molecules is partially obscured by the beam stopper and cannot be taken into analysis. Additionally, the third peak, observed at $Q \sim 0.65 \text{ \AA}^{-1}$, is notably weaker. Therefore, the second-order diffraction overtone (002) at $Q \sim 0.43 \text{ \AA}^{-1}$ (inset in Fig. 2a) was analyzed to assess the features of the LC order in the SE-ITZ samples. Just to note, all diffraction data presented in Fig. 2a were scaled to the same background level for high Q -values ($4\text{--}20 \text{ \AA}^{-1}$), which depend mainly on the intramolecular structure, so one can compare the relative peak amplitudes at low Q -values ($<4 \text{ \AA}^{-1}$) arising mainly due to intermolecular structure. One can see that there is a clear trend in the (002) peak's amplitude as a function of ITZ concentration – it is the highest for ITZ derived from the most diluted solution in DCM (10 mg/ml) and subsequently decreases with the higher concentration of ITZ. Thus, this finding demonstrates that the degree of the *Sm* order of SE-ITZ can be tuned by adjusting the ITZ:DCM concentration in the solution prior to solvent evaporation. Generally, the greater the amplitude of the diffraction peaks, the smaller their full width at half maximum (FWHM), which indicates the increase in the correlation length within the *Sm* layering. A similar trend was also observed for the third-order (003) peak. On the other hand, the positions of the (002), (003) and the main diffraction peaks for SE-ITZ samples do not vary with ITZ concentration. This shows that the nature of the *Sm* as well as the lateral packing, including the molecular spacings between and within *Sm* layers in the SE-ITZ solid particles, is essentially the same regardless of ITZ concentration in the initial ITZ:DCM solution.

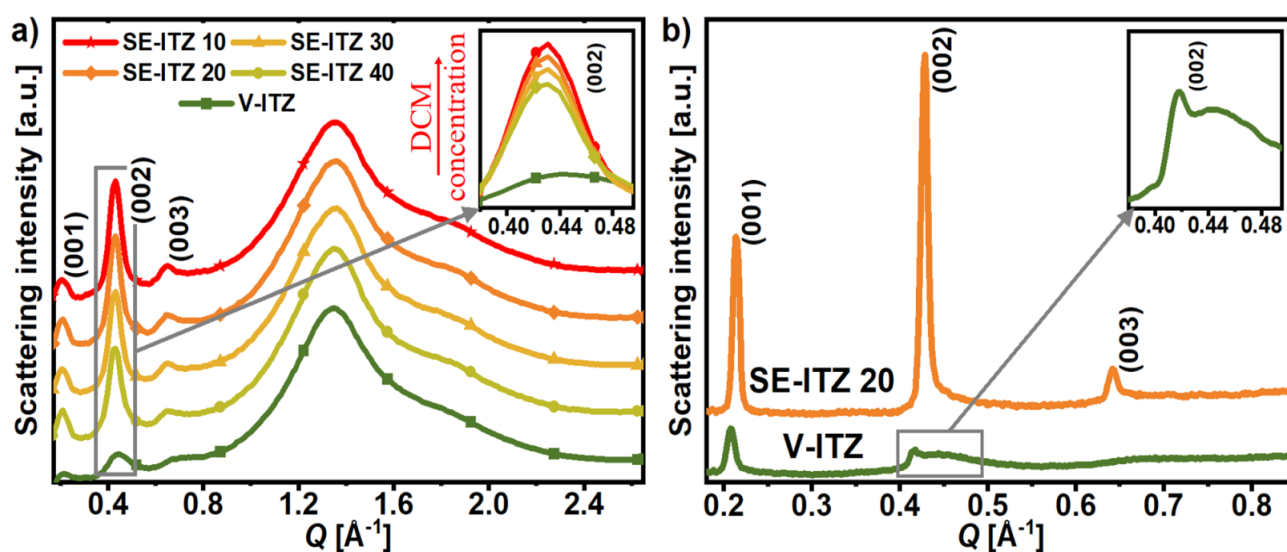


Fig. 2. (a) XRD patterns of V-ITZ and SE-ITZ samples prepared from ITZ:DCM mixtures of different concentrations (10, 20, 30, and 40 mg/ml). A comparison of the (002) reflection between SE-ITZ samples and V-ITZ is given as an inset to panel (a). (b) SAXS patterns of V-ITZ and SE-ITZ 20. The inset to panel (b) provides a zoomed-in view on the (002) peak for V-ITZ.

In order to gain deeper knowledge about the nature of the *Sm* order in the SE-ITZ materials, their diffraction patterns were then compared with the pattern of ITZ glass obtained by vitrification (V-ITZ), presented also in Fig. 2a. The (002) reflection for V-ITZ is much more damped and broader than those registered for SE-ITZs. What is more, the position of the (002) peak's maximum for V-ITZ is clearly shifted towards higher $Q \sim 0.45 \text{ \AA}^{-1}$ compared to that registered for SE-ITZs: $Q \sim 0.43 \text{ \AA}^{-1}$. Such Q positions correspond to interlayer distances $d \sim 28$ and $29 \text{ \AA}$, respectively. So, the average distance between *Sm* layers in V-ITZ is shorter than that of SE-ITZ. A smaller spacing between *Sm* layers can be due to a tilt of molecules in the layers from *Sm A* towards *Sm C* arrangement or due to a reduction of offsets of molecules in the direction perpendicular to layers, which promote a disorder in the layer-like organization. More insight into the organization of the ITZ molecules in the studied V-ITZ and SE-ITZ forms was obtained from the SAXS patterns collected for lower Q -values than the WAXS data, and allowed us to register (002), (003) as well as the (001) reflections due to the layered organization.

Figure 2b presents the comparison between the SAXS curves of SE-ITZ 20 and V-ITZ. The SAXS data differ from WAXS patterns presented in Fig. 2a in terms of the probed Q range and resolution. However, the most important finding revealed by WAXS diffraction patterns that SE-ITZ samples show much more intense and sharper (002) and (003) peaks than V-ITZ is also confirmed by the SAXS patterns. Nevertheless, the SAXS patterns provide some interesting features about the *Sm* structures of these two ITZ forms. First of all, the (002) peak of V-ITZ is comprised of two components: a more sharp feature at $Q \sim 0.42 \text{ \AA}^{-1}$ and a broader bump at $Q \sim 0.44 \text{ \AA}^{-1}$. In WAXS pattern, these two contributions merge in one peak at $Q \sim 0.43 \text{ \AA}^{-1}$. A similar two-component feature of the second-order reflection from ITZ layers was found by Fatina et al. and Yu et al. for ITZ glass prepared by melt-quenching^{12,21}. The sharper component was attributed to the *Sm* layering while the broader one to the excluded volume effect^{21,22}. The first order (001) peak for V-ITZ on the SAXS curve has a higher intensity than the (002) one. A similar behavior was also found in previous studies of ITZ glasses^{12,21}. In contrast, for SE-ITZ 20, one can see that the (002) peak on the SAXS curve is stronger than the (001) one, which deviates from the typical diffraction pattern for LC structures. Fatina et al. proposed a structural model to quantitatively explain such an effect. They showed that the relative intensities of (001) and (002) reflections in ITZ contain information on the density modulation perpendicular to the *Sm* layers and depend strongly on the intralayer molecular offset. A reduction of the random molecular offsets results in an increase in the intensity of the (002) peak compared to the (001) peak, and for the perfect arrangement of ITZ molecules with no offset in the direction perpendicular to the layers, the ratio of the (002)/(001) peaks' intensities is around 2. They observed that such a situation occurs in ITZ:GLY system obtained through DCM evaporation and proposed that no offset ordering of ITZ molecules in *Sm* layers is due to GLY cross-linking of ITZ by hydrogen bonds within a monolayer. Here, we reveal that a similar *Sm* structure to that reported by Fatina et al. for ITZ system containing 5% w/w of GLY may be achieved by evaporation of ITZ from the DCM solvent without the participation of GLY. Therefore, the formation of the ITZ *Sm* order with the reduced intralayer molecular offset seems to be the characteristic feature of the used SE method itself, not the effect of GLY. The correctness of the hypothesis about the reduced offsets of molecules in the SE-derived ITZ is supported by the behaviour of positions of the diffraction peaks in the SAXS pattern. For the SE-ITZ sample, the peaks are clearly shifted towards higher Q -values, compared to peaks for V-ITZ, indicating lower interlayer spacing in the SE-ITZ than in the ITZ glass.

In order to characterize the *Sm* structures of SE-ITZs and V-ITZ quantitatively, the WAXS data were normalized to electron units and presented as structure factors in Fig. 3a. As can be seen, the trend in the (002) peak's amplitude revealed in corresponding diffraction patterns in Fig. 2a is also maintained for the structure factor representation of the diffraction data. The ratio of the (002) peak's amplitude in the $S(Q)$ data for V-ITZ and SE-ITZ 20 is $\sim 1:3$. Further, to quantify the coherence length of the atomic correlations in the *Sm* structures of SE-ITZs and V-ITZ, the $S(Q)$ data were transformed to the atomic pair distribution functions, as described in the Methods section, and the PDFs were presented in Fig. 3b. As can be seen, the first peaks of the PDFs

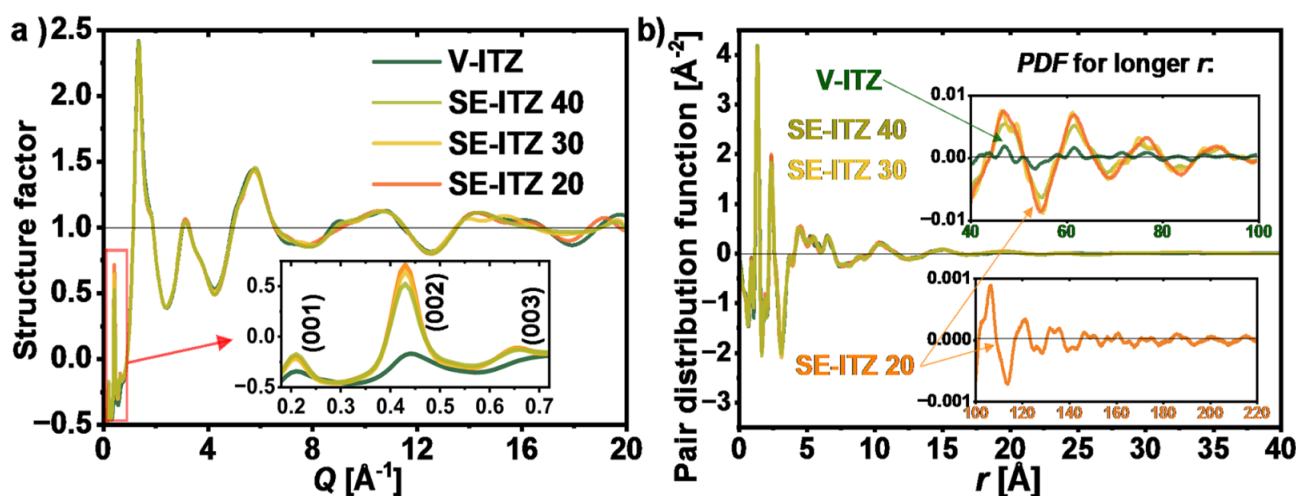


Fig. 3. (a) Structure factors and (b) pair distribution functions derived from WAXS data for V-ITZ and selected SE-ITZ samples. The insets in panel b) show the extent of structural correlations where PDF oscillations gone, which is $\sim 70 \text{ \AA}$ for V-ITZ and $\sim 150 \text{ \AA}$ for SE-ITZ 20.

for small distances, representing the intramolecular and short-range intermolecular correlations, almost overlap for V-ITZ and SE-ITZs. However, there is a clear difference in the extent of the *PDF*'s oscillations between V-ITZ and SE-ITZs. For the V-ITZ system, the structural correlations are completely attenuated at ~ 70 Å. On the other hand, the *PDF*'s oscillations for the SE-ITZ systems extend to about 150 Å, indicating longer-range ordering of these forms as compared to V-ITZ. Moreover, the differences in the coherence lengths between the different SE-ITZ samples were very small, reaching a few Å between SE-ITZ 20 and SE-ITZ 40, where the latter one exhibited the faster decay of the *PDF*'s oscillations, as visible in the upper inset to Fig. 3b.

The X-ray scattering data presented above were collected for fresh SE-ITZ samples – up to 2 days after the solvent evaporation procedure. Further, in order to verify whether the *Sm* order generated by the SE method is stable, the long-term physical stability of these materials was monitored, which is especially important for potential applications of APIs in pharmacy.

Physical stability of the solvent-evaporated itraconazole materials below the glass transition temperature

Figure 4 presents the temporal evolution of the WAXS patterns recorded weekly for SE-ITZ samples stored under room conditions ($T \sim 295$ K, ambient pressure and humidity) in sealed capillaries. It is worth mentioning that in one of the recent papers by our group⁷, it was demonstrated that V-ITZ may be stable in its *Sm* form in such conditions for up to approximately 5 weeks while ITZ samples generated by cryo-milling and exhibiting a lower structural order (*N*) than V-ITZ were better physically-stable and did not crystallize for at least 10 weeks when stored at analogous conditions. What is more, it was presented that the resistance to the crystallization of the cryo-milled ITZ samples increases with a lower degree of the short-range order (quantified by the main diffraction peak amplitude and FWHM) whereas the *N* order was comparable between these samples. Therefore, the diffraction patterns of the SE-ITZ samples with different coherence lengths of the *Sm* order were probed over time to explore the relationship between the features of their *Sm* structures and physical stability. As can be deduced from the results presented in Fig. 4, there is a trend between the amplitude of the second-order (002) diffraction peak for the fresh SE-ITZ samples and their physical stability. Namely, the lower the amplitude, the better the stability. Over time, the amplitude of the (002) peak minimally decreased for all SE samples. Then, SE-ITZ 20 underwent crystallization after just 1 week, SE-ITZ 30 showed signs of crystallization after 4 weeks, while SE-ITZ 40 revealed the best stability, among the studied samples, for at least 6 weeks. Additional diffraction measurements performed on SE-ITZ 40 sample stored 5 months still did not reveal crystallization, as presented in Fig. SI3, so SE-ITZ 40 material is characterized by at least two times longer physical stability than SE-ITZ 30 and V-ITZ, in the same conditions.

Due to the promising, from the pharmaceutical industry point of view, physical stability of the SE-ITZ 40, in order to further explore the potential of this ITZ form for pharmaceutical applications, we decided to test its drug release kinetics in 0.1 M HCl aqueous solution, simulating the acidic conditions of the stomach, and compare this solubility with that of crystalline as well as glass ITZ forms. It is widely accepted that fasting gastric emptying usually takes about 2 h^{23,24}. Therefore, in our study, we adopted a measurement duration of approximately 3 h. The registered drug released profiles are included in Supplementary Information as Fig. SI7, and they show that the amount of released of SE-ITZ 40 after 3 h reached ~ 0.037 mg/ml while that of crystalline ITZ was around 7 x lower (~ 0.005 mg/ml). Herein, it should be noted that the solubility of crystalline ITZ in an acidic environment is ~ 0.004 mg/ml at 297 K²⁵. In turn, our studies conducted at a temperature reflecting the human body (310 K) showed a slightly higher amount of dissolved and released active substance (~ 0.005 mg/ml), which is solely due to the temperature effect, and the amount of released API corresponds well with literature data. Moreover, the concentration of released of V-ITZ after 3 h was slightly lower (~ 0.034 mg/ml) than that of SE-ITZ 40. Thus, both non-crystalline, SE-ITZ and V-ITZ, show much better bioavailability than crystalline ITZ in 0.1 M HCl. However, the differences in release profiles due to the differences in the degree

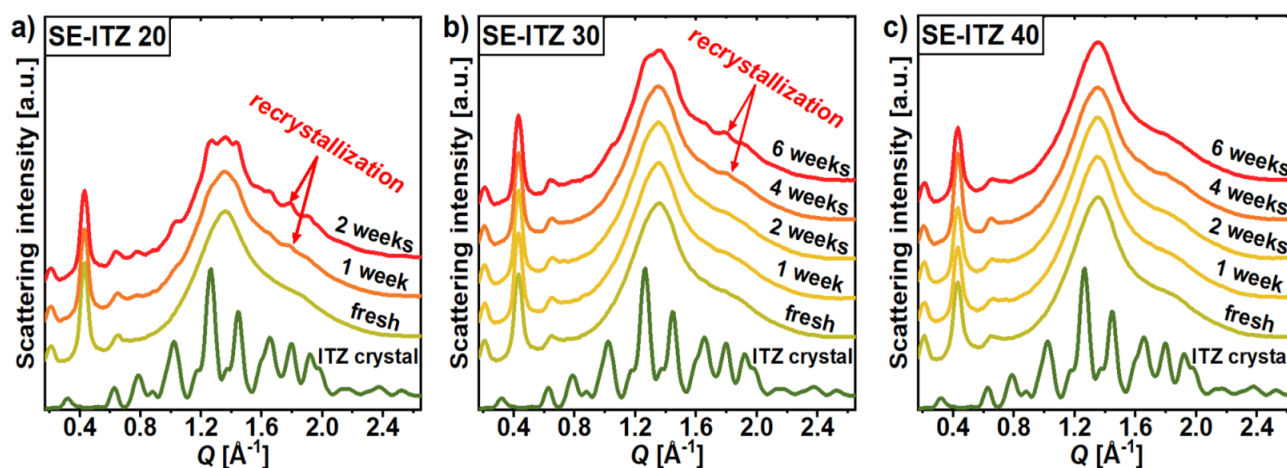


Fig. 4. Temporal evolution of XRD patterns for SE-ITZ samples prepared from different ITZ:DCM concentrations: (a) 20 mg/ml, (b) 30 mg/ml, and (c) 40 mg/ml, at room conditions.

and length of the Sm order between SE-ITZ and V-ITZ are negligible or may be blurred due to their distinct morphology, which is known to highly affect the solubility as well.

Additionally, to examine the influence of an elevated temperature, but still below T_g , on the SE-ITZ physical stability, WAXS measurements were conducted for SE-ITZ 30 stored at $T = 313$ K, ambient pressure and humidity. Just to remind, at room temperature, SE-ITZ 30 showed a similar stability as V-ITZ (approximately 4–5 weeks). As shown in Fig. SI4 in Supplementary Information, the amplitude of the second-order (002) diffraction peak of the SE-ITZ 30 at 313 K was gradually decreasing over the storage time of 14 h, trending toward the amplitude of the V-ITZ. However, the crystallization of the SE-ITZ 30 at 313 K was registered after 15 h, before the amplitude of its (002) peak merged with the amplitude of the V-ITZ. Therefore, there is no trend in the amplitude of the LC peaks and the physical stability of ITZ species produced by the SE method and vitrification, which may imply a different characteristic of their Sm order and related properties such as the molecular dynamics, which will be discussed further.

The effect of glycerol on the liquid-crystalline order of the solvent-evaporated itraconazole

As discussed in the Introduction, Fatina et al. demonstrated that GLY enhances the LC order of ITZ:GLY obtained via SE from DCM and vitrified as compared to pure vitrified ITZ¹². In order to elucidate further the effect of the presence of GLY in the ITZ:DCM mixture on the final LC order of ITZ obtained after DCM evaporation, the SE-ITZ:GLY 20 sample (where 20 indicates 20 mg/ml concentration of ITZ:GLY system in DCM and GLY concentration in ITZ:GLY system was 5% w/w) was prepared, as described in the Sample preparation section, and probed by XRD. The XRD data for this system were compared with the data obtained for SE-ITZ 20 sample. Position, shape and amplitude of the main diffraction peaks for these two freshly prepared systems were very similar, as can be seen in Fig. 5a. Nonetheless, the SE-ITZ:GLY 20 exhibited a slightly higher intensity of the (002) reflection than the SE-ITZ 20 (inset in Fig. 5a), beyond measurement uncertainty. This suggests that, indeed, GLY enhances the LC ordering in ITZ during the solvent evaporation.

Nevertheless, as demonstrated in Fig. 2a, a similar increase in the (002) peak's amplitude may be induced without involving GLY, by only reducing the ratio of ITZ to DCM in the solution from 20 down to 10 mg/ml. What is more, the XRD studies of the physical stability of the SE-ITZ:GLY 20 exhibited its very similar temporal stability to SE-ITZ 20, as shown in Fig. 5b. The crystallization of ITZ from the SE-ITZ:GLY 20 system started after one week from the preparation, i.e., after the same period as the SE-ITZ 20 sample, according to the XRD results presented in Fig. 4a. This suggests that incorporating GLY into the SE-ITZ structure does not improve its resistance to crystallization at room conditions, below the glass transition temperature.

Impact of solvent type on the structure of the solvent-evaporated itraconazole

To gain further insight into the mechanism of the formation of LC order in ITZ by the solvent evaporation process, an alternative solvent – CLF was employed. CLF was chosen, despite the fact that it is increasingly discouraged by the industry, the same as DCM²⁶. However, CLF exhibits the highest ITZ mole fraction solubility at room temperature, among the other solvents, of $\sim 5.04 \times 10^{-2}$, which is 1.54 times higher than that of DCM¹³, while it is difficult to find other solvents for ITZ that ensure its high solubility while having a relatively low boiling point, ensuring rapid evaporation of the solvent at around room temperature. Following the same preparation method of the SE-ITZ 20 system from the solution with DCM, SE-ITZ 20 CLF analog was prepared from the ITZ mixture with CLF, in the same ratio of 20 mg of ITZ per 1 ml of solvent. The XRD pattern in Fig. 6 shows that SE-ITZ 20 CLF exhibits much lower amplitudes of the diffraction peaks originating from the Sm order than SE-ITZ 20 DCM, but still, they are more intense than for the V-ITZ system. This suggests that DCM is not

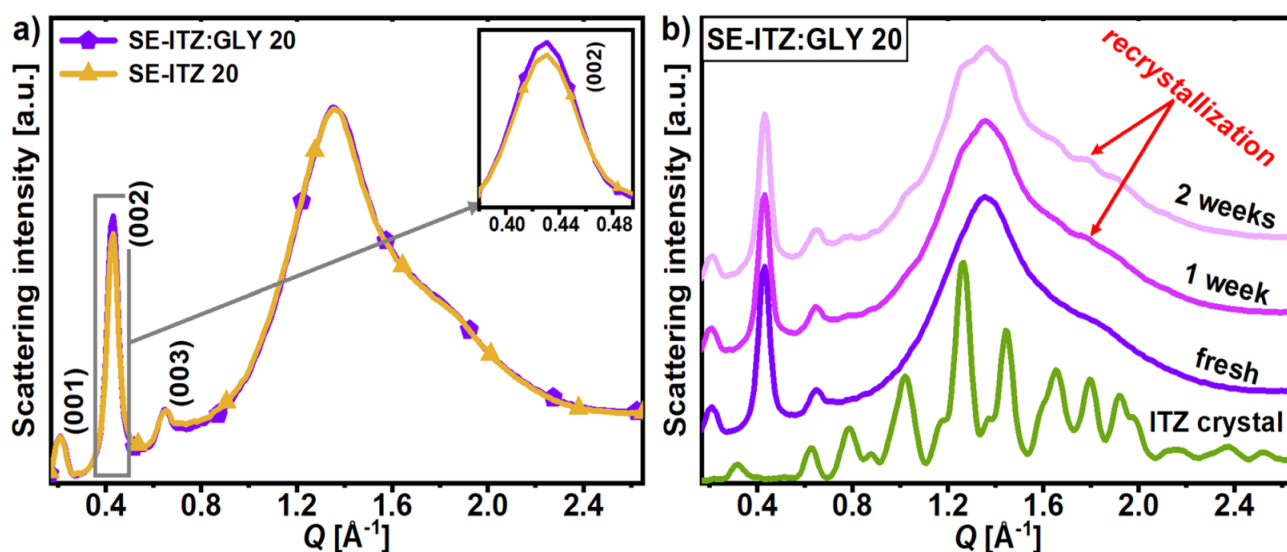


Fig. 5. (a) Comparison of XRD patterns for fresh SE-ITZ 20 and SE-ITZ:GLY 20 samples. The inset in panel (a) shows a zoom on the second-order (002) peak. (b) Temporal evolution of the XRD pattern for the SE-ITZ:GLY 20 at room temperature.

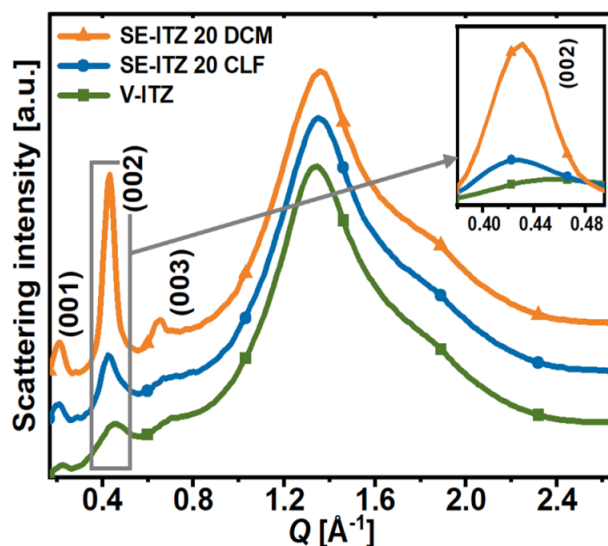


Fig. 6. XRD patterns of fresh SE-ITZ 20 samples prepared by solvent evaporation method using DCM (SE-ITZ 20 DCM) and CLF (SE-ITZ 20 CLF) as solvents. The patterns were compared to the data collected for vitrified ITZ (V-ITZ).

the only solvent capable of inducing an enhanced *Sm* ordering of ITZ, as compared to the ITZ glass obtained by vitrification. However, the question arises as to why DCM supports the formation of a much higher degree of the *Sm* organization in ITZ than CLF. In this context, other solvents, such as tetrahydrofuran, acetonitrile, acetone, and methanol, were also tested. However, due to lower ITZ solubility by these solvents compared to the chlorinated ones, such as DCM and CLF¹³, the resulting SE-ITZ systems exhibited a crystalline structure, regardless of the ITZ:DCM concentration used. Thus, a high solubility of ITZ in a solvent is one of the most crucial factors for the effective amorphization of ITZ, but its final structure and the degree of the LC order/its coherence length generated by the SE method may depend on other properties of the solvent, such as boiling point, external conditions or interactions between ITZ and solvent, which will be discussed further.

Thermal transitions of the solvent-evaporated itraconazole systems

To further investigate the thermal properties of the SE-ITZ systems, calorimetric measurements were performed. The collected thermograms for freshly prepared systems after DCM evaporation and their vitrified derivatives (2nd scans) are compared in **Fig. 7** with data for V-ITZ. As illustrated, V-ITZ and all SE-ITZ samples exhibit the glass transition, marked at the T_g by a heat capacity jump, and two distinct endothermic peaks at $T_{Sm-N} \sim 345$ K and $T_{N-I} \sim 362$ K, consistent with the well-known LC behavior of ITZ^{3,27,28}. So, the SE-ITZ systems preserve the sequence of LC transitions reported for the ITZ glass. However, the SE-ITZ materials undergo crystallization above 400 K, followed by melting at $T_m \sim 439$ K, in contrast to V-ITZ. One can also notice that SE-ITZ materials show a slight shift in T_g ($\Delta T_g \sim -3$ K) compared to V-ITZ, which may be due to the structural differences between these specimens, i.e., in the degree of the *Sm* ordering below T_g , in the size of the *Sm* domains, and/or in the lateral packing of molecules. However, this effect is fully reversible after melting, as all 2nd DSC scans (obtained after heating SE-ITZ above its melting point and vitrification) matched that of V-ITZ in terms of the temperatures as well as areas under the thermal events. This confirms that the extensive *Sm* order generated in the SE-ITZ systems is fully disrupted upon melting.

Herein, it is also worth to emphasize that in the thermograms registered for SE samples, one can easily detect enthalpy recovery peak (overshoot) at the T_g . Such a feature is observed in the aged glasses. Hence, it might be an indicator of the higher thermodynamic stability of the obtained materials and a higher order of the molecular arrangement in ITZ obtained via solvent evaporation as compared to the vitrified form. A similar finding was also reported for the cryo-milled ITZ⁷.

Differences in the molecular dynamics of the solvent-evaporated and vitrified itraconazole

Moreover, the molecular dynamics of V-ITZ and SE-ITZ systems were investigated by dielectric spectroscopy to verify how the *Sm* order induced by the SE method influences the relaxation processes of ITZ compared to its glass form obtained by vitrification. Figure 8a shows dielectric loss spectra obtained for SE-ITZ 20, which are composed of three relaxation processes: (i) a DC-process (related to the ion transport) at lower frequencies, (ii) a prominent structural α -relaxation process (reflecting rotations around the long molecular axis) at higher frequencies, that dominates the recorded spectra, and (iii) a δ -process (corresponding to molecular reorientation around the short axis), appearing at the ride side of the structural relaxation. As can be seen, upon heating, the amplitude of the α -relaxation peak decreases rapidly at higher temperatures ($T > 360$ K), indicating the crystallization process, which agrees with the outcomes of the calorimetric data for SE-ITZ, where crystallization was also observed (**Fig. 7**). Further, the temperature dependences of the relaxation times τ for the observed relaxation processes were determined, where τ_α and τ_δ refer to the relaxation times of the α and δ processes, respectively. The collected dielectric data were fitted using two Havriliak-Negami (HN) functions with

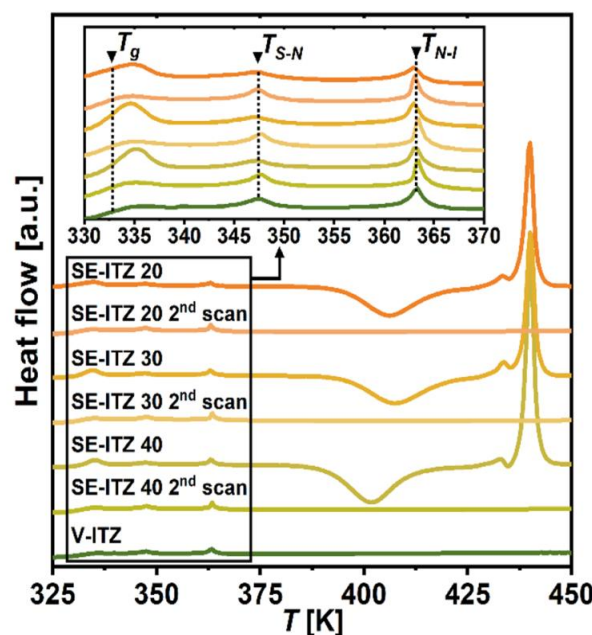


Fig. 7. DSC thermograms of SE-ITZ and V-ITZ samples recorded during heating at 10 K/min. The second scans performed on the SE-ITZ samples first heated up to 450 K and cooled down are also presented. The inset shows a zoomed range of the data in the region where the LC transitions occur.

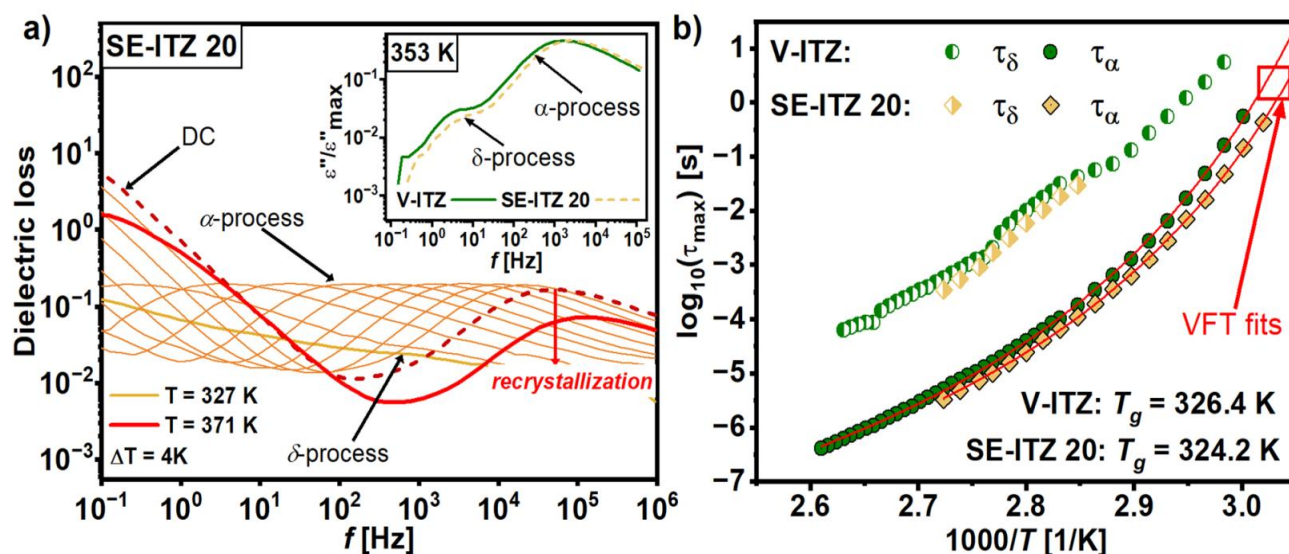


Fig. 8. (a) Dielectric loss spectra recorded for SE-ITZ 20 above T_g . As the inset in panel (a), the comparison of the position of the loss peaks obtained for V-ITZ and SE-ITZ is presented. (b) Relaxation maps of the SE-ITZ and V-ITZ samples.

the additional DC term²⁹ (see Methods). The calculated $\tau(T)$ dependencies are displayed in **Fig. 8b**. As one can see, there are significant differences in the structural dynamics between the V-ITZ and SE-ITZ near T_g . The SE-ITZ 20 sample exhibits shorter τ_α values compared to their vitrified counterpart. These differences diminish at higher temperatures ~ 363 K, upon approaching the N-I transition. However, even though the τ_α of the SE-ITZ converges to that of the V-ITZ, it never reaches it. Moreover, there is also a difference in the T_g of the V-ITZ and SE-ITZ systems derived from the dielectric data using the Vogel-Fulcher-Tamman (VFT) function^{15–17} (see Methods). Namely, $T_g \sim 326.4$ and 324.2 K for V-ITZ and SE-ITZ 20, respectively, which aligns well with the outcomes of the calorimetric data. Note that T_g was defined as T where $\tau_\alpha = 100$ s.

Additionally, the δ -process was analyzed at high temperatures (see **Fig. 8a**). As one can see, at lower temperatures, SE-ITZ 20 shows slightly faster δ -mode dynamics compared to the V-ITZ, though this difference falls within the experimental uncertainty and disappears at elevated temperatures. Thus, it is evident that the molecular dynamics of both relaxation processes, α and δ , for the SE-ITZ becomes nearly identical to that of its vitrified form at higher temperatures, where ITZ transforms to the liquid phase. The loss of structural differences in the liquid state for ITZ specimens obtained by the distinct methods transfers to the unification

of their molecular dynamics. In turn, our results clearly demonstrate that the differences in the LC molecular arrangement in those ITZ forms at lower temperatures yield difference in their molecular dynamics.

Intermolecular interactions of itraconazole with dichloromethane and discussion on the possible mechanism of the formation of the smectic order in the solvent-evaporated itraconazole

Seeking an explanation of the mechanism behind the formation of the superior *Sm* order in the DCM-evaporated ITZ, as compared to V-ITZ reference, FTIR spectroscopy studies were performed. More precisely, the aim of the FTIR analysis was to verify whether there is any interaction between the solvent and ITZ molecules in the ITZ:DCM mixtures. The spectra collected at 295 K for pure DCM, V-ITZ as well as fresh ITZ:DCM mixtures (with concentrations of 20, 30, and 40 mg/ml) are presented in **Fig. S18** in Supplementary Information. Itraconazole contains several functional groups capable of interacting with the solvent, including proton donor C–H groups ($3100\text{--}2700\text{ cm}^{-1}$), as well as proton acceptor groups such as C=O moieties (around 1700 cm^{-1}), aromatic C=C groups ($1600\text{--}1500\text{ cm}^{-1}$), triazole C=N groups ($1450\text{--}1330\text{ cm}^{-1}$), dioxolane C–O–C groups ($1100\text{--}1050\text{ cm}^{-1}$) and aromatic C–Cl groups (around 1100 cm^{-1})^{30,31}. Thus, the shifts in these characteristic bands can provide insight into which functional groups are engaged in interactions with DCM. Analyzing the spectra illustrated in **Fig. S18**, it can be observed that most of the bands characteristic of ITZ in the ITZ:DCM mixtures exhibit only minor shifts (by approximately $1\text{--}2\text{ cm}^{-1}$) compared to those in pure vitrified ITZ. The peaks that shift the most under the influence of solvent (by max. 6 cm^{-1}) are those of the aliphatic CH ($2936\text{--}2827\text{ cm}^{-1}$) and the C=O stretching ($1750\text{--}1650\text{ cm}^{-1}$) vibrations. In more detail, the ITZ signal located at 2936 cm^{-1} after the introduction of solvent (for the ITZ:DCM of 20 mg/ml concentration) is blue-shifted to 2941 cm^{-1} , and the addition of more ITZ to reach higher concentrations leads to the red-shift of this band (to 2938 and 2939 cm^{-1} for the mixtures of 30 and 40 mg/ml concentrations, respectively). An analogous effect is detected for the band appearing at 2878 cm^{-1} for V-ITZ. It is shifted to 2884 , 2883 , and 2882 cm^{-1} for the ITZ:DCM mixtures of 20, 30, and 40 mg/ml, respectively. In the case of the peak located at 2827 cm^{-1} for V-ITZ, it is blue-shifted for 20 mg/ml solution as compared to V-ITZ, and then the blue-shift gets more significant with the higher ITZ concentration. These detected C–H band shifts are probably related to weak hydrogen bonding interactions between ITZ and DCM molecules. Moreover, the C=O band of ITZ is blue-shifted from 1697 cm^{-1} for pure V-ITZ to 1702 cm^{-1} for ITZ:DCM mixtures with the high ITZ concentration, which can suggest van der Waals interactions between ITZ and DCM molecules. It should also be noted that the IR peak positions originating from DCM also exhibit insignificant shifts (by approximately $1\text{--}2\text{ cm}^{-1}$) in the ITZ:DCM mixtures, compared to those in neat DCM (**Fig. S18**), supporting the hypothesis that only weak interactions occur between ITZ and DCM. Moreover, **Fig. S19** presents the comparison of FTIR spectra between the V-ITZ and SE-ITZ samples (SE-ITZ 20, 30, and 40) prepared from the ITZ: DCM mixtures after complete evaporation of solvent, and demonstrates no quantitative differences between the shape and position of their IR bands. This suggests that the resulting SE-ITZ structures retain similar features of the intermolecular interactions as the structure of V-ITZ.

In summary, the FTIR spectroscopy studies excluded the formation of any strong interactions between ITZ and DCM, which would somehow support the *Sm* organization of ITZ molecules. It is consistent with the results of XRD studies presented in **Fig. 1**, which demonstrate that in the freshly prepared ITZ:DCM mixture, there is no sign of *Sm* order of ITZ. Therefore, the mechanism of *Sm* order formation in ITZ may be sought in the process of the DCM evaporation itself without any significant bonding between ITZ and solvent. In fact, the process of the formation of an orientational order in a substance that was solved is known as evaporation-induced self-assembly (EISA) and has gained significant attention in recent years^{32,33}. It may occur based on different mechanisms including the Marangoni flows, stick-and slip motion, and various deposition patterns near the contact line, giving rise to unique morphologies. Anisotropic materials, such as liquid crystalline compounds, tend to undergo self-assembly in solutions and the EISA is frequently utilized to assemble elongated particles mainly parallel to the contact line or along the evaporation flow. For example, when an aqueous solution of deoxyribonucleic acid (DNA) is evaporated on a substrate, the DNA chains extend linearly along the moving direction of the contact line due to the receding force³⁴. Another example are ZnO nanorods – it was demonstrated that they may create a nematic alignment by a simple solvent evaporation technique and concluded that the degree of alignment varies with nanorod length, concentration and solvent evaporation rate³⁵. High concentrations led to immediate aggregation of rods, less concentrated dispersions experienced stick-slip motion leading to thick stripes, while further decreasing the concentration resulted in thin parallel stripes oriented parallel to the evaporation line, and individual ZnO nanorods showed similar orientation. Moreover, it was demonstrated that high evaporation rates ($> 4\text{ }\mu\text{m/min}$) led to highly disordered thick stripes, whereas slow evaporation rates ($< 2\text{ }\mu\text{m/min}$) resulted in thin homogeneous stripes. We find an analogy in these outcomes to the ITZ:DCM systems, where for high ITZ concentrations in solvent the crystallization was observed, less concentrated dispersions led to the formation of the *Sm* organization, while decreasing the concentration enabled the growth of the *Sm* order. If the evaporation process is fast enough, the initial arrangement of molecules induced by the evaporation may be frozen. When a solution with a lower ITZ concentration is evaporated, the anisotropic ITZ molecules may be aligned due to the force induced by the evaporation flow, and, consequently, create a well-ordered structure that may be frozen in this state, as during the vitrification. However, with an increase in the ITZ concentration, there are fewer solvent molecules per each ITZ molecule, so their alignment may not be so effective, and the final frozen state after solvent evaporation may be characterized by a lower degree of the liquid-crystalline order. When it comes to evaporation rate, we found that very slow evaporation rates led to the ITZ crystallization, while faster rates resulted in the formation of the *Sm* domains. In addition, it is known from the literature that the extremely fast evaporation rate used in the spray-drying method causes the formation of more disordered ITZ particles with *N* alignment. Therefore, there is a certain dependence of the degree of structural order on the rate of solvent evaporation. Essentially, other literature

reports have already pointed out that the rate of solvent evaporation is a crucial parameter for controlling the alignment of liquid crystals^{29,36}. Since the IR studies did not indicate the formation of strong bonds between ITZ and DCM solvent, the hypothesis on the EISA-based mechanisms with a key role of the evaporation rate parameter seems quite plausible for the case of ITZ system. Moreover, it is worth mentioning the phenomenon of molecular recognition directed self-assembly, which is the ability of molecules to selectively bind to each other based on their shapes, chemical properties, and non-covalent interactions, resulting in the formation of ordered structures^{37,38}. Such events between ITZ and the applied solvent molecules in their mixtures cannot be ruled out, especially in the context of possible polar interactions between ITZ and the solvents. Although ITZ is a weakly polar molecule, it is composed of nitrogen-containing polar rings, and both solvents employed in this work for dissolution of ITZ, DCM and CLF, are polar. Therefore, weak polar interactions between ITZ and DCM/CLF may be the case, while it was demonstrated in literature on molecular recognition phenomena that such interactions may cause a polar order and stabilize molecular packing in the *Sm* structure^{39,40}. Last but not least, molecular recognition events play an important role during the crystallization of different polymorphic forms from solutions^{41–43}. For instance, a metastable form that persists in a solvent may be the consequence of specific solute – solvent interactions. In the case of ITZ, it was demonstrated that the *Sm* structures obtained in the SE experiments are metastable and tend to crystallize to polymorphic form I. It cannot be ruled out that this is the result of the molecular recognition phenomena. However, a detailed mechanism of the formation of *Sm* order in the solvent-evaporated itraconazole particles remains foggy. Understanding of this process and the role that the solvent plays requires considering the complex interplay between thermodynamic, kinetic, and molecular recognition factors.

Conclusions

In this study, we characterized the structures of solid particles of a model thermotropic liquid crystal – itraconazole, obtained by a simple solvent evaporation method. We found that these particles exhibit smectic organization of molecules, which may be tuned by the concentration of itraconazole in the solvent. The character of the smectic order and the size of the ordered domains in itraconazole derived by its solvation in dichloromethane, followed by solvent evaporation, differ significantly from the smectic arrangement in the glassy state formed by supercooling of liquid. Namely, the applied solvent evaporation procedure results in reduced molecular offsets perpendicular to the layers as well as in much bigger smectic domains compared to those observed in itraconazole glass obtained by the vitrification. The lower the concentration of itraconazole in the mixture with solvent, the higher the degree of the smectic order quantified as sharpening and increase in the amplitude of the diffraction peaks and long-range pair distribution function oscillations. However, the physical stability (the resistance to crystallization) of itraconazole obtained from mixtures with dichloromethane of different solute to solvent concentrations turned out to be inversely proportional to the higher degree of the smectic order. The enhanced smectic order in powders received from the solvent evaporation method, as compared to that of glass obtained by vitrification, is metastable and tends to revert back towards a more disordered smectic structure with the storage time. Moreover, the crystallization occurs faster for the solvent-evaporated systems characterized by a higher degree of smectic organization. After heating the solvent-evaporated itraconazole systems to the liquid state, the smectic organization is disrupted, and their structure, as well as thermal properties and molecular dynamics, unify and tend to resemble those reported for glass obtained by vitrification.

Although the mechanism of the formation of smectic order in the solvent evaporation process has not been revealed, it was found that there are no strong interactions between the solvent and the itraconazole in their solutions. Understanding of this process and the role that the solvent plays requires considering the complex interplay between thermodynamic, kinetic, and molecular recognition factors. However, from the application point, it was demonstrated that the particles of itraconazole with the smectic structure obtained from the solvent evaporation method are characterized by higher release in 0.1 M HCl aqueous solution than commercially available crystalline powder. This knowledge is crucial for the development of liquid-crystalline systems with tailored structure and properties for applications in electronics and pharmaceuticals.

Data availability

Research data supporting the results of the manuscript have been deposited in the RepOD repository: <https://repod.icm.edu.pl/api/access/datafile/70081>.

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Author contributions

T.L.: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. L.O.: Visualization, Investigation. M.T.: Visualization, Investigation. B.H.: Visualization, Investigation. K.N.: Investigation. K.K.: Review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. K.J.: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Competing interests

The authors declare no competing interests.

Additional information

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“Sposób otrzymywania itraconazolu w stabilnej fazie nematycznej”

Karolina Jurkiewicz, Taoufik Lamrani, Paulina Jesionek, Kamil Kamiński, Ewa Ozimina-Kamińska

Streszczenie

Przedmiotem wynalazku jest sposób otrzymywania itraconazolu w stabilnej fazie nematycznej mogący znaleźć zastosowanie w medycynie oraz przemyśle farmaceutycznym, zwłaszcza do opracowania formułacji preparatów przeciwgrzybiczych oraz zwiększenia efektywności terapii przeciwgrzybiczych. Potencjalnym obszarem zastosowań wynalazku może być również leczenie lub wspomaganie leczenia szerokiego spektrum schorzeń innych niż infekcje grzybicze – stanów zapalnych, chorób autoimmunologicznych i nowotworowych. W tego typu zastosowaniach spodziewana jest znacznie lepsza rozpuszczalność tej substancji aktywnej uzyskanej w stabilnej fazie nematycznej sposobem według wynalazku, w porównaniu do najbardziej aktualnie rozpowszechnionej komercyjnie postaci krystalicznej.

Abstract

The invention provides a method for obtaining itraconazole in a stable nematic phase, which could find applications in medicine and the pharmaceutical industry, particularly in the development of antifungal formulations and increasing the effectiveness of antifungal therapies. Potential applications of the invention also include treating or supporting the treatment of a wide range of conditions other than fungal infections, including inflammatory conditions, autoimmune diseases, and cancer. In these applications, the active substance obtained in the stable nematic phase, according to the invention, is expected to have significantly improved solubility compared to the currently most commercially available crystalline form.



Departament Biotechnologii i Chemii

Warszawa, 16.03.2026r.

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DECYZJA

Na podstawie art. 24 i art. 52 ustawy z dnia 30 czerwca 2000r. Prawo własności przemysłowej (Dz. U. z 2023 r. poz. 1170) Urząd Patentowy RP po rozpatrzeniu zgłoszenia oznaczonego numerem **P.447909** dokonanego w dniu **2024-03-01** udziela na rzecz:

UNIwersytet Śląski w Katowicach, Katowice, Polska
Śląski Uniwersytet Medyczny w Katowicach, Katowice, Polska
PATENTU

na wynalazek(i) pt.:

Sposób otrzymywania itrakonazolu w stabilnej fazie nematycznej

pod warunkiem uiszczenia opłaty w wysokości **480zł.** za I okres ochrony wynalazku(ów) rozpoczynający się w dniu **2024-03-01** i obejmujący 1-3 rok ochrony*.

Podstawa prawna: art. 224 ust. 1 ustawy Prawo własności przemysłowej oraz pkt II ppkt 1 tabeli opłat stanowiącej załącznik nr 1 do rozporządzenia Rady Ministrów z 8 września 2016 r. zmieniającego rozporządzenie w sprawie opłat związanych z ochroną wynalazków, wzorów użytkowych, wzorów przemysłowych, znaków towarowych, oznaczeń geograficznych i topografii układów scalonych (Dz. U. z 2001 r. Nr 90, poz. 1000, z 2004 r. Nr 35, poz. 309, z 2008 r. Nr 41, poz. 241 oraz z 2016 r. poz. 1623).

Urząd Patentowy RP wzywa do wniesienia tej opłaty w **ciągu trzech miesięcy od dnia doręczenia decyzji.**

W razie nieuiszczenia wskazanej opłaty w wyznaczonym terminie Urząd Patentowy RP, na podstawie art. 52 ust. 2 ustawy Prawo własności przemysłowej stwierdzi wygaśnięcie decyzji o udzieleniu patentu.

Od niniejszej decyzji stronie przysługuje wniosek o ponowne rozpatrzenie sprawy przez Urząd Patentowy RP w terminie dwóch miesięcy od dnia jej doręczenia. Opłata za wniosek o ponowne rozpatrzenie sprawy wynosi 100 złotych i jest uiszczana na konto Urzędu. Jeżeli strona nie chce skorzystać z prawa do wniesienia wniosku o ponowne rozpatrzenie sprawy, może wnieść za pośrednictwem Urzędu skargę do Wojewódzkiego Sądu Administracyjnego w Warszawie w terminie 30 dni od dnia doręczenia stronie przedmiotowej decyzji. Opłata za wpis od skargi wynosi 1000 złotych i jest uiszczana na konto Sądu. Strona może ubiegać się na podstawie przepisów ustawy Prawo o postępowaniu przed sądami administracyjnymi o zwolnienie od kosztów sądowych albo o przyznanie prawa pomocy.

Na podstawie art. 227 ustawy Prawo własności przemysłowej oraz pkt I ppkt 12 powołanej tabeli opłat Urząd Patentowy RP wzywa do wniesienia w terminie trzech miesięcy opłaty w wysokości **210zł** za publikację o udzieleniu patentu.

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*** Pouczenie**

Jeżeli w chwili wydania decyzji rozpoczął się kolejny okres ochrony wynalazku (kolejne lata), a Zgłaszający chce przedłużyć ochronę na te okresy (te lata), powinien łącznie z opłatą za I okres ochrony, wymienioną wyżej, wnieść opłatę za następne okresy - art. 224 ust. 1 ustawy Prawo własności przemysłowej.

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