**Cocrystal Formulations: Evaluation of the Impact of Excipients on Dissolution by Molecular Simulation and Experimental Approaches**

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**ABSTRACT.** Cocrystallization has matured into an established technique for fine-tuning the physicochemical properties of active pharmaceutical ingredients. This technique has been adopted by pharmaceutical drug companies, with increasing numbers of cocrystal-based drug products now entering the market. Surprisingly, however, studies into the formulation aspects of cocrystal-based drugs are relatively few and far between compared to the vast literature on their design, synthesis, and characterization. We herein report results of our investigations into cocrystal-excipient interactions in water that determine the dissolution properties of cocrystals in formulation by a combination of molecular dynamics (MD) simulation and experimental approaches. Two cocrystals of an antirheumatic drug, leflunomide (LEF) with 3-hydroxybenzoic acid (HBA) and 2-picolinic acid (PIC) were assessed in formulation with the frequently used excipients, lactose and dicalcium phosphate (DCP). For comparison, the dissolution of neat LEF formulations with these excipients was also evaluated. The parameters deduced from MD simulations, such as solvent accessible surface area, intermolecular hydrogen bonds among formulation ingredients and water, and interaction energy between an API or cocrystal and water were found to be essential indicators in the prediction of cocrystal formulation dissolution trends. It was found that the presence of lactose as an excipient improved dissolution of cocrystal formulation compared to the neat cocrystals, most notably for the LEF-PIC cocrystal. In contrast, DCP was seen to have a detrimental effect on the dissolution of cocrystal formulations, all exhibiting lower dissolution than their neat cocrystal counterparts and LEF. Careful analysis of these results revealed that the nature of excipient plays a significant role in the dissolution properties. While the improved dissolution of the lactose formulations was attributed to its hydrophilic nature, the ionic and hydrophobic nature of DCP was likely responsible for its detrimental effect. The results of the MD simulations were found to be in excellent agreement with the experimentally observed dissolution hierarchy.

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KEYWORDS: pharmaceutical cocrystals, leflunomide, excipients, formulations, molecular dynamics simulations, dissolution, interactions

1. **INTRODUCTION**

Over the past two decades, cocrystallization has emerged as a viable tool for the fine-tuning of materials’ properties without the need for chemical modification.[1](#_ENREF_1) Control over solid-state molecular packing, through application of crystal engineering strategies and wise selection of partner molecules (coformers), produces a solid-form that shows desirable physiognomic and chemical characteristics including, but not limited to, morphology, stability, solubility, dissolution rate, mechanical strength, and bioavailability.[2-8](#_ENREF_2) This technique has showcased far-reaching impact, particularly in the pharmaceutical industry through development of novel solid-forms of new and existing active pharmaceutical ingredients (APIs), which has paved the way for many current marketed cocrystal-based drugs.[9-14](#_ENREF_9) Ergo, optimization of the physicochemical properties of suitable cocrystals, and subsequent development of appropriate formulations are crucial for the progress of this research interest. The enormous amount of current literature on cocrystals has established the evolution of reliable cocrystal design strategies, characterization, scale up and application of such cocrystals in improvement of drug properties.[15-21](#_ENREF_15) However, successful development of a drug formulation mostly depends on selection of the right combination of excipients, and to select the true combination, in-depth knowledge of interactions between excipient and active ingredient is important. In the case of cocrystal formulations, understanding the behavior of a cocrystal in the presence of excipients would provide a vital knowledge on performance attributes of cocrystals.

There have been a few experimental attempts reporting the impact of certain excipients on the performance and developability of cocrystals.[22](#_ENREF_22) For example, the earliest study that shed light on the importance of exploring formulation concepts for cocrystals has been performed by Remenar et al.[23](#_ENREF_23) The authors found that using a combination of 2 % sodium lauryl sulfate (SLS) and polyvinylpyrrolidone (PVP) together with a celecoxib-nicotinamide cocrystal led to *in vitro* formation of incipient amorphous materials, metastable polymorphs, and submicron particles of the API which are associated with good oral bioavailability. Li et al. have studied the impact of hydroxypropylmethylcellulose (HPMC) on the phase transformation and dissolution of a carbamazepine (CBZ)-nicotinamide cocrystal.[24](#_ENREF_24) It was found that, at a lower percentage of HPMC in tablets, the release of CBZ from the cocrystal was nonlinear and declined overtime, which was attributed to conversion of the cocrystal into CBZ dihydrate. However, higher concentrations of HPMC helped to improve CBZ release from the cocrystal formulation, which was rationalized through dissolution of only the outer surface of the matrix when HPMC undergoes a process of disentanglement. Concerning a cocrystal of danazol and vanillin, studied by Childs et al., the synergistic use of a solubilizer (vitamin E-TPGS) and a precipitation inhibitor (hydroxypropylcellulose) resulted in a dramatic increase in the apparent solubility levels in *in-vitro* powder dissolution experiments which were very well reflected in high danazol plasma levels in *in-vivo* animal studies.[25](#_ENREF_25) The impact of polymer and surfactant concentration on the dissolution of a cocrystal has been investigated by Alhalaweh et al.[26](#_ENREF_26) Using an indomethacin-saccharin cocrystal as a model system, the authors observed different solubilization effects of SLS and PVP on indomethacin and saccharin, respectively, which was suggested to maintain drug supersaturation levels sufficiently long enough for absorption. In a recent study, Koranne et al. revealed that the nature of the excipient (whether neutral or ionic) has a significant impact on the stability of the cocrystal.[27](#_ENREF_27) Using the theophylline-glutaric acid cocrystal as a model system, the authors demonstrated that the use of ionizable and hygroscopic excipients, such as magnesium stearate, led to dissociation of the cocrystal, whereas neutral and non-hygroscopic crystalline excipients, e.g. lactose monohydrate prevented cocrystal dissociation. In another study dealing with three cocrystals of a hypothetical drug, Lipert et al. noted that, even though the neat cocrystals are more soluble than the drug, not all cocrystals have a solubility higher than the drug in the presence of a solubilizing agent, emphasizing the importance of greater care in choosing right formulation ingredients.[28](#_ENREF_28) Furthermore, in a 2017 study by Duggirala et al. it was found that caffeine-oxalic acid cocrystals, widely reported to be stable at diverse temperature and humidity conditions, dissociate in the presence of numerous pharmaceutical excipients.[29](#_ENREF_29) A mechanism for cocrystal dissociation proposed by the authors suggested cocrystal dissociation, in the presence of excipients, was a consequence of the water sorption followed by dissolution of the cocrystal and excipient, respectively, in the sorbed water. Regarding ionizable excipients, the authors found the driving force for dissociation involved a proton transfer from oxalic acid to the excipient, forming metal salts and caffeine hydrate. In an attempt to demonstrate potential applications of freeze-drying for the synthesis of cocrystals, Ogienko et al. prepared a solid dispersion of the cocrystal meloxicam-succinic acid in a solubilizing agent, polyethyleneglycol, by freeze-drying and found the dissolution rate of the solid dispersion to be significantly higher than the cocrystal prepared by liquid-assisted grinding and conventional freeze-drying.[30](#_ENREF_30) Most recently, in an extensive study on the impact of physical properties of excipients on the physical stability of the cocrystal, Duggirala et al. conducted binary cocrystal-excipient compatibility studies on a cocrystal of ertugliflozin and L-pyroglutamic acid.[31](#_ENREF_31) The study revealed that, among the various properties studied, pH and hygroscopic nature of the excipients play a greater role in the dissociation of the cocrystal in formulation. Using this knowledge of cocrystal dissociation in the presence of excipients and its propagating factors, the authors responded by coating the cocrystal particles with a pH modifier and hydrophobic silica. This strategy proved effective in mitigating cocrystal dissociation and the tablet formulations, thus prepared, were found to be stable at accelerated stability condition (40 °C/75 % RH), even after 4 weeks.

Inspection of the vast literature on pharmaceutical cocrystals reveals that, despite spanning over almost two decades and facilitating the successful commercialization of cocrystal based drug products, there has been little attention paid to the behavior of formulated cocrystals. In this regard, the aforementioned studies testify the need to gain further insights into the impact of different excipients on cocrystal properties. There have been a few studies reported which aim to develop the models using mathematical/numerical modeling techniques that predict melting properties, ideal mole fraction solubility and aqueous solubility product of pure cocrystals.[32-35](#_ENREF_32) However, these studies have mainly focused on mathematical model development for prediction of pure cocrystal properties and the impact of molecular-level interactions between cocrystal and excipients in formulations has not been considered. In this respect, a fundamental understanding of the types and nature of molecular interactions between cocrystal and excipients is essential in order to discern their effect on physicochemical properties such as solubility, dissolution rate, and stability. With the rapid growth of computational resources in the last 10-20 years, molecular simulation techniques have evolved as reliable tools for effective understanding of complex phenomena at a molecular level, as well as computational a priori design of novel materials preceding synthesis.[36](#_ENREF_36), [37](#_ENREF_37) Most notably, molecular dynamics (MD) simulations have been the subject of significant recent interest in the field of science and engineering.[38](#_ENREF_38) The MD method is a deterministic technique that integrates Newton’s equation of motion, by taking small time increments to predict new positions and velocities of atoms in a dynamic process. MD simulations have been extensively used to understand the solubility and miscibility phenomena of APIs in the presence of excipients[39-41](#_ENREF_39) and have also gained attention in elucidating excipient-assisted solubilization of water insoluble drugs.[42-47](#_ENREF_42) For example, in order to understand dissolution of APIs, Jha et al. performed a series of MD simulations, highlighting the molecular interactions between polymeric excipients and a poorly soluble API (phenytoin) in an aqueous medium.[48](#_ENREF_48) Again, with an objective to understand dissolution and precipitation of a cocrystal in the presence of excipients, molecular interactions between the surfaces of cocrystals and the polymeric excipients were investigated by Li and coworkers through combined experimental and MD simulation approaches.[49](#_ENREF_49) Though the study provided some useful insights into cocrystal dissolution, its simulations were unfortunately not able to capture the true experimental scenario as the simulation models were prepared in the absence of a dissolution medium, i.e. water, and hence the interactions of polymer and cocrystal with dissolution media were not taken into account. Therefore, in the study presented herein, extensive simulations have been performed to mimic the experimental scenario by constructing the simulation models both in the presence and absence of a dissolution medium. Additionally, the models were prepared as such in order to monitor the dissolution events during the simulations, something not considered in the above referenced example.

The objectives of this current study are to integrate MD simulations as a complement to experimental methods and gain a molecular level understanding of the interactions between an API (and cocrystal) and selected excipients and water. Such studies would be expected to streamline excipient screening and help in the selection of suitable excipients for development of cocrystal formulations. The knowledge gained from MD simulations was subsequently used to prepare prototype oral dosage formulations, such as tablets, and evaluated their performance. Towards this goal, the current work indicates a detailed underlying mechanism in molecular interactions among active (API or co-crystals), excipients and water, and identifies governing factors to explain hierarchy of the aqueous dissolution of formulations obtained experimentally.

The model cocrystal systems were chosen from a library of leflunomide (LEF) cocrystals reported in our own recent work.[50](#_ENREF_50) LEF is a poorly soluble (aqueous solubility < 40 mg L-1) antirheumatic drug used to slowdown progression of rheumatoid arthritis through inhibition of pyrimidine synthesis.[51](#_ENREF_51) Our extensive cocrystal screening, which was supplemented by a knowledge-based design approach, led to the discovery of cocrystals with pyrogallol, 3-hydroxybenzoic acid (HBA), 2-picolinic acid (PIC), and 2-aminopyridine.[50](#_ENREF_50) Evaluation of the physicochemical properties, such as solubility and dissolution rate revealed that these cocrystals showed improved behavior compared to the analogous physical mixtures, as well as the parent API. As described in the previous work, there was a degree of commensurate modulation found in the crystal structure of the LEF-pyrogallol cocrystal,[50](#_ENREF_50) therefore this cocrystal, and the non-pharmaceutical cocrystal with 2-aminopyridine, were not considered for the current study. Specifically, MD simulations were conducted with the parent LEF, LEF-HBA (1:1) and LEF-PIC (1:1) cocrystalsin the presence of two frequently used pharmaceutical excipients, lactose and dibasic calcium phosphate (CaHPO4, DCP). Lactose is a disaccharide consisting of the sugars D-galactose and D-glucose. Pharmaceutical applications of lactose as a tablet and capsule diluent, filler, binder, dry powder inhaler carrier and lyophilization aid have been well documented.[52](#_ENREF_52) Its high water solubility, low hygroscopicity, cost effectiveness, bland taste and compatibility with several active ingredients and excipients make it a widely used excipient in pharmaceutical formulations.[53](#_ENREF_53) Lactose has also been used as an effective excipient in direct compression tableting applications and is a key component of the LEF oral tablet formulations.[52](#_ENREF_52) DCP is a water insoluble inorganic substance which has been used in pharmaceutical tablets and capsules as a filler and diluent, as well as a source of calcium in nutritional supplements.[53](#_ENREF_53) Its non-hygroscopicity, associated with excellent compaction and flow properties, makes it an ideal excipient for direct compression processes.[54](#_ENREF_54) DCP has also been used as an alternative to lactose in the development of lactose free formulations. The choice of DCP as an excipient in the current study has been made to understand (a) the impact of an ionic and water-insoluble excipient on the dissolution of the cocrystal and (b) whether DCP has the similar or different effect on cocrystals so it may be used as an alternative to lactose in cocrystal based formulations. The key governing parameters that can be obtained from MD simulations, namely solvent accessible surface area (SASA) of a complex (API/cocrystal-excipient), number of hydrogen bonds between a complex and water, and interaction energy between a complex and water, are analyzed for prediction of dissolution trends of either pure API or formulations.

**2. MATERIALS AND METHODS**

**Simulation Models and Methods**

**Figure 1** represents the planar view of the crystal structure of the LEF and the cocrystals, along with the molecular structure of lactose and the phosphate ion (HPO42−). A 3D simulation model containing a LEF-PIC cocrystal and four molecules of lactose in water is also shown in **Figure 1**. The Optimized Potentials for Liquid Simulations all-atom (OPLS-AA) force field is used to describe the LEF, HBA, PIC, lactose and calcium ion.[55](#_ENREF_55) The parameter files were generated using the MKTOP tool[56](#_ENREF_56) and charges on the atoms were adopted from OPLS-AA force field. The bonded and non-bonded parameters for the HPO42− ionwere taken from OPLS-AA except the charges, which were adopted from the literature.[57](#_ENREF_57)The water was modeled by the simple point charge (SPC).[58](#_ENREF_58) The Lennard-Jones (LJ) and Coulombic potentials were used to describe the non-bonded interactions as

 (1)

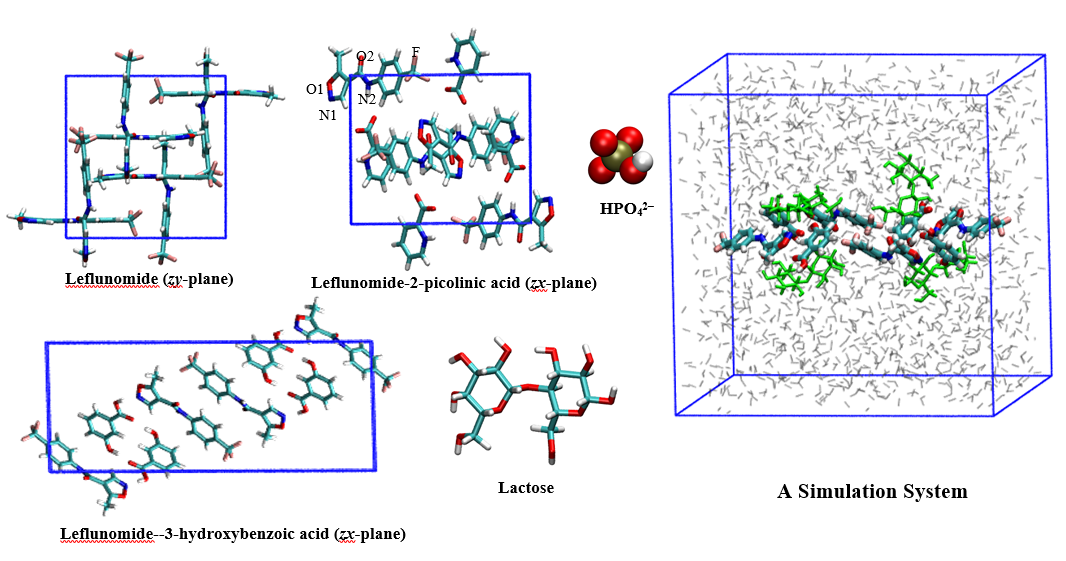
where *rij* is the distance between atoms *i* and *j, qi* is the atomic charge of atom *i*, *εij* and *σij* are the well depth and collision diameter, and *ε0* (8.8542 × 10-12 C2N-1m-2) is the permittivity of vacuum. The stretching, bending and torsional potentials were used to represent the bonded interactions as

 (2)

 (3)

 (4)

where ,  and *Cn* are the force constants; ,  and  are bond lengths, bond angles and torsional angles, respectively; ,  and  are the equilibrium values.



**Figure 1**. Planar view of the molecular structures of LEF and the cocrystals, molecular structures of lactose and HPO42−, and a model to represent simulation system. Color code: P, gold; F, light orange; O, red; N, blue; C, cyan; H, white, grey, water in the simulation system.

Four sets of simulations were performed. In the first set, the crystal structure of LEF and cocrystals was kept in the center of a large simulation box of dimensions ~5 nm × 5 nm × 5 nm and solvated with water in the absence of excipients. The solvated systems were simulated at room temperature using an isothermal and isochoric (NVT: during the simulations, number of molecules, volume and temperature of the system remains constant) MD scheme. In the second set of simulations, the crystal structure of LEF/cocrystals was kept in a simulation box of the same dimensions and four excipient molecules were randomly distributed within. This system was simulated at room temperature using the NVT MD scheme. The second set of simulations allow the excipients to interact with most favorable sites of LEF/cocrystals. Finally, in the third set of simulations, the output from second set was solvated with water and simulated again in the NVT MD scheme at room temperature. During the first 3 sets of simulations, the position of the atoms of the LEF/cocrystals were restrained by applying a force constant of 10000 kJ mol-1 nm-2. Finally, in order to evaluate dissolution behavior, in the fourth set of simulations position restraints were removed. As such, all atoms in the system were allowed to move during the simulation. Prior to the above simulations, crystal structures of the parent LEF crystal and LEF-HBA and LEF-PIC cocrystals were extended to 4 × 4 × 4, 4 × 8 × 2, and 4 × 4 × 4 unit cells and isobaric-isothermal MD simulations were performed at 1 bar. The calculated densities of the LEF and cocrystals, derived from the OPLS-AA model, are 1.49, 1.42 and 1.43 g cc-1 respectively, which are close to the experimental values of 1.47, 1.48, and 1.45 g cc-1.

Initially, using the steepest descent method, all four systems were subjected to energy minimization. The initial velocities of the atoms were generated by the Maxwell−Boltzmann distribution equation, followed by MD simulations. The velocity-rescaling scheme was adopted to control the temperature with a relaxation time of 0.1 ps. The equations of motion were integrated by the leap-frog algorithm and periodic boundary conditions were applied in all directions. A cutoff of 14 Å was used to calculate the LJ interactions, while the particle-mesh Ewald summation method was used to evaluate the Coulombic interactions with grid spacing of 1.2 Å. A time step of 1 fs was used and the trajectories were saved every 5 ps. The duration for the first 3 sets of simulations was 5 ns (last 3 ns trajectories were used for analysis) and for the fourth set it was 2.5 ns. The GROMACS v5.0.6 package was used to perform all the MD simulations.[59](#_ENREF_59)

**Experimental**

LEF was purchased from Biotain Pharma Co., Ltd., China. The coformers were purchased from Sigma-Aldrich, Singapore, and used as received. The excipients, lactose (α-lactose monohydrate, ≥99%) and DCP (98%) were purchased from Sigma Aldrich, Singapore and were used as received.

**Solid-State Grinding.** Solvent-drop grinding was used for preparation of bulk samples of the cocrystal powders. In a typical experiment, 2.7 g (10 mmol) of LEF, a stoichiometric amount of the coformer and 2 drops of methanol were added to stainless steel grinding jars (50 mL) with one 2 cm stainless steel grinding ball and ground at a rate of 20 Hz for 20 min using a Retsch Mixer Mill model MM301. Powder X-ray diffraction (PXRD) was used for the characterization of the resulting powders. These were compared with the reference PXRD patterns simulated from the crystal structure of the cocrystals (See Supporting Information, Figures S1-S2).

**Powder X-ray Diffraction (PXRD)**. PXRD data were collected using a Bruker D8 Advance powder X-ray diffractometer with Cu−Kα radiation (λ = 1.54060 Å) and an acceleration voltage of 35 kV, and current of 40 mA power. Using a continuous scanning mode, the samples were scanned in the 2θ range from 5° to 50° at a scan rate of 5° min−1.

**Tablet Preparation.** The excipients used in formulations were aligned with those used in the marketed LEF (ARAVA) such that each 100 mg of the tablet contains 20 mg of LEF (or a cocrystal that contains an equivalent amount of LEF, e.g. 30.2 mg of LEF-HBA cocrystal), 74.5 mg of lactose monohydrate/DCP – adjusted for cocrystal mass (e.g. 64.3 mg in LEF-3HBA cocrystal formulation), 5.0 mg of corn starch, and 0.5 mg of magnesium stearate (as a lubricant). The tablet samples for dissolution experiments were prepared by direct compression. Prior to tablet pressing, a formulation mixture of ca. 5 g each were thoroughly mixed using a laboratory powder mixing machine (Alphie powder mixer, Hexagon product development, Vadodara, Gujarat, India). For the tableting process, each mixed powder was then placed in a tablet press (Minipress II, Karnawati Engineering, India) and pressed at varying load to form 200 mg tablets. Hardness testing of the tablets was conducted using DT-YD-3 hardness tester (Guangzhou Raysky Scientific, China) and tablets of similar hardness were used in dissolution experiments.

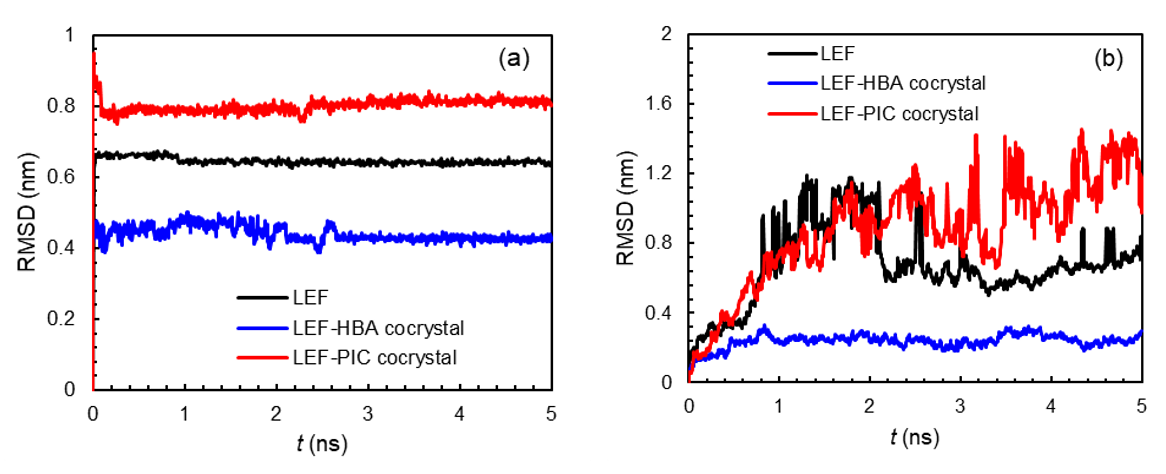
**Dissolution Studies.** The dissolution experiments on all samples (neat LEF, cocrystals, and their formulations) were conducted using an Agilent 708-DS dissolution sampling apparatus. As recommended by the US Food and Drug Administration, all the dissolution experiments were conducted in water as the dissolution medium (900 mL), with a rotation speed of 100 rpm at 37 °C.[60](#_ENREF_60) All the tablets were found to disintegrate instantly within a minute. 2 mL aliquots of sample from the vessel were withdrawn and filtering through a 45 μm syringe tip filter for analysis by HPLC. This was conducted at times of 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min. Total dissolution volume was maintained at 900 mL by replacing the samples with an equivalent amount of fresh dissolution medium. The dissolution experiments were run in triplicate (n=3).

**High Performance Liquid Chromatography (HPLC).** LEF concentration in the samples obtained from the dissolution experiments were quantified by HPLC. A ZORBAX ECLIPSE XDB-C18 column (4.6 mm x 250 mm x 5 μm) was used and was kept at a constant temperature of 37 °C throughout the analysis. A mobile phase consisting of acetonitrile and water in 50:50 (v/v) was delivered at a flow rate of 1 mL min−1 and 15 min run time. Samples of 50 μL of each are injected into HPLC. UV absorbance at 262 nm was used to quantify the LEF concentration. LEF was detected at a retention time of 7.4 min, while the retention times of the coformers HBA and PIC were detected at 2.8 and 2.2 min, respectively.

**Fourier Transform Infrared Spectroscopy (FT-IR).** A Frontier FT-IR/NIR Spectrometer (PIKE Technologies I, PerkinElmer) with a MIR TGS detector and a combined scan direction was used to collect transmission spectra of neat samples (without KBR). The samples were scanned over a range of 4000–650 cm-1 at a scan speed of 0.2 cm-1s-1 with a spectral resolution of 4 cm-1.

1. **Results and Discussion**
2. ***Lactose as an excipient***

The stability of the drug/cocrystal-excipient system was gauged by root mean square deviation (RMSD) with respect to the initial structure. **Figure 2a**, shows the RMSD of LEF, LEF-HBA, and LEF-PIC cocrystals with lactose as an excipient, without water. For each combination, the RMSD remains almost constant throughout the simulation. This reveals that lactose is strongly bonded to the molecular components and in the absence of water each complex is at a stable state. In contrast, in the presence of water, RMSDs are fluctuating throughout the simulation (**Figure 2b**), particularly in the case of LEF-PIC cocrystal. This is an indication of a comparatively higher dissolution of the LEF-PIC cocrystal-lactose complex. With higher dissolution in water, the complex would dissociate to a larger extent, thus RMSD would be expected to fluctuate more. Note that, even though the RMSD could be used to understand the dissolution behavior to a certain extent, it will not be possible to predict the dissolution hierarchy, as the RMSDs are calculated based on the deviation of the atoms in a molecule with respect to its initial structure.



**Figure 2.** Root mean-squared deviations (RMSDs) of complexes in the (a) absence of water and (b) presence of water.

To further elucidate the dissolution phenomena, the SASA of LEF and the cocrystals, and their combinations with lactose in water, were calculated with a probe of diameter 0.28 nm. Herein, SASA represents available surface area of LEF/cocrystal or their complex with the excipient to interact with water. **Table 1** lists the hydrophilic, hydrophobic and total SASAs of LEF/cocrystals and complexes. Since the volume of each component (API or cocrystal) is different, SASA is calculated per volume of components. Without lactose, the LEF-HBA cocrystal possesses the highest SASA compared to pure LEF and LEF-PIC cocrystal, indicating the higher water accessibility*.* However, in the presence of lactose, the highest SASA is observed for the LEF-PIC cocrystal-lactose complex. Interestingly, as lactose is introduced, each complex shows significant increase in total SASA, which is mainly contributed by the hydrophilic component. Owing to the hydrophilic nature of lactose, this contribution dominates when compared to the hydrophobic component. This indicates that the hydrophilic nature of each system is enhanced considerably in the presence of lactose, hence leading to higher dissolution of the complex in water. Among the three systems studied, the LEF-PIC cocrystal-lactose complex exhibits the highest total, as well as hydrophilic, SASAs and therefore suggests greater dissolution for the LEF-PIC cocrystal in the presence of lactose in water.

**Table 1.** Solvent accessible surface areas (SASAs) of LEF/cocrystals and complexes with lactose.

|  |  |  |  |
| --- | --- | --- | --- |
| **System** | **Hydrophilic (nm2)** | **Hydrophobic (nm2)** | **Total (nm2)** |
| without lactose | | | |
| LEF | 2.06 | 6.18 | 8.24 |
| LEF-HBA cocrystal | 2.69 | 7.23 | 9.93 |
| LEF-PIC cocrystal | 2.06 | 6.23 | 8.29 |
| with lactose | | | |
| LEF | 6.04 | 7.66 | 13.70 |
| LEF-HBA cocrystal | 6.40 | 8.85 | 15.25 |
| LEF-PIC cocrystal | 7.57 | 9.28 | 16.85 |

Hydrogen bonds play an important role in the solubility and dissolution of a solute in water.[47](#_ENREF_47), [61](#_ENREF_61) MD simulations have proved effective in the prediction of hydrogen bonds between a solute and bulk solvent, such as water, and hence aid in the prediction of the solubility/dissolution of a solid. We have calculated hydrogen bonds between molecular components of drug crystals and water, with and without lactose. Two geometrical criteria were implemented to calculate the hydrogen bonds (1) the distance (*r*) between a donor and an acceptor ≤ 3.5 Å and (2) the angle of hydrogen-donor-acceptor, *α* ≤ 30°.[62](#_ENREF_62) Two water molecules representing r and α, are shown in Table 2. Similar to SASA, the number of hydrogen bonds are estimated based on per volume of the components. As tabulated in **Table 2**, in the presence or absence of lactose, the number of hydrogen bonds between LEF/cocrystals or complexes and water follows the trend LEF < LEF-HBA cocrystal < LEF-PIC cocrystal, thereby representing the dissolution trend of pure LEF/cocrystals and complexes in water. It is worthwhile to note that the number of hydrogen bonds between any system and water in the presence of lactose (i.e. between complexes and water) is much higher compared to without lactose (i.e. between LEF/cocrystals and water), which is consistent with SASA. Besides, the number of hydrogen bonds between neat LEF and lactose is fewer than the number of hydrogen bonds between the cocrystal and lactose. Among this, the LEF-HBA cocrystal has the highest number of hydrogen bonds followed by the LEF-PIC cocrystal. This can be attributed to the fact that the –OH and –COOH groups of HBA act as both hydrogen bond donors and acceptors. In contrast, the –N+H and –COO‑ groups of PIC in the LEF-PIC cocrystal only act as hydrogen bond donor and acceptor, respectively, hence forming fewer hydrogen bonds with lactose.

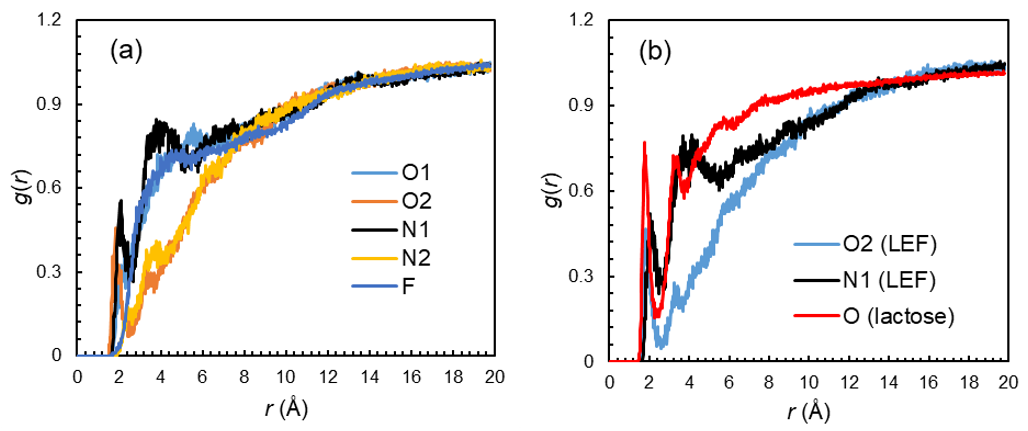
We further evaluate the interaction sites of water in the pure drug crystal and complex by radial distribution function *g*(*r*) as

 (5)

where *r* is the distance between atoms *i* and *j*, *Ni* and *Nj* are the numbers of atoms *i* and *j*,  is the number of atoms *j* around *i* within a shell from *r* to *r + Δr*, , respectively. **Figure 3a** shows the *g*(*r*) of the hydrogen (H) atom of water around the O1, O2, N1, N2 and F atoms (notations are shown in **Figure 1**) of LEF without lactose. Two prominent peaks are observed at *r* ~ 2.1 Å corresponding to the O2 and N1 atoms, indicating the most favorable sites for water. These peaks also indicate that LEF is able to form hydrogen bonds with water around these atoms. In the case of the LEF-lactose complex, the *g*(*r*) of the H atom of water around the most favorable atoms (O2 and N1) and all oxygen (O) atoms of lactose are plotted in **Figure 3b**. Along with two earlier peaks, a third peak is observed at a shorter distance (*r* ~ 1.8 Å) corresponding to the O atom of lactose. The peak height here is the highest, reflecting the most dominating interaction site for water. A similar phenomenon is also observed for the cocrystal-lactose complexes (See Supporting Information, Figures S4 and S5). All the oxygen atoms of the HBA in theLEF-HBA cocrystal and PIC in the LEF-PIC cocrystal are involved in hydrogen bond formation with water. The analysis identified the possible atoms which could form hydrogen bonds and suggests that the addition of lactose to pure drug/cocrystals enhances the dissolution of the complex by providing additional interaction sites for water.

**Table 2.** The number of hydrogen bonds between LEF/cocrystal and water with and without lactose.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **System** | **LEF** | **LEF-HBA cocrystal** | **LEF-PIC cocrystal** | **Geometrical description** |
| without lactose | | | |  |
| LEF/cocrystal-water | 8.27 | 13.63 | 15.55 |
| with lactose | | | |
| LEF/cocrystal-water | 32.56 | 38.53 | 44.59 |
| LEF/cocrystal-lactose | 0.15 | 3.28 | 2.29 |



**Figure 3.** The *g*(*r*) of the H atom of water around (a) the atoms of LEF and (b) the O2 and N1 atoms of LEF, and the O atom of lactose.

To quantify the strength of interactions between molecules, their energies in terms of electrostatic (Ecoul), Lenard-Jones (ELJ), and total (Etotal) are calculated for each molecule. Usually, attractive or favorable interaction is indicated by a negative value of the energy. A higher absolute value of energy is an indicator of stronger interaction among interacting molecules. Moreover, a positive value denotes repulsive or unfavorable interaction. The calculated interaction energy terms for LEF and cocrystal systems are negative, thus indicative of favorable interactions (**Table 3**). Both in pure LEF/cocrystals and complexes, the interaction energies with water are found to increase in the order of LEF < LEF-HBA cocrystal < LEF-PIC cocrystal. Compared to pure LEF/cocrystals, complexes with lactose possess much higher interaction energy. The observed trend is consistent with hydrogen bond analysis. The interaction energy terms Ecoul, ELJ, and Etotal for the LEF-PIC cocrystal-lactose complex are -713.7, -97.8 and -811.5 kJ mol-1, respectively. The contribution from electrostatic interaction is dominated /over Lenard-Jones in the total energy term due to the polar moiety of water strongly interacting with the hydrophilic lactose and ionic moieties of PIC in the LEF-PIC cocrystal. By attributing the highest interaction energy to water, the LEF-PIC cocrystal-lactose combination is predicted to have higher solubility/dissolution in water. Consistent with our prediction, it is reported that the interaction energy between drug and water plays an important role in elucidating the dissolution phenomena; specifically a stronger interaction between drug and water is considered to positively favor dissolution.[42](#_ENREF_42) The predicted dissolution trend in water by MD simulations is in direct agreement with the experimental observations (vide supra).

**Table 3.** LEF/cocrystals-water and complex-water interaction energies (kJ mol-1).

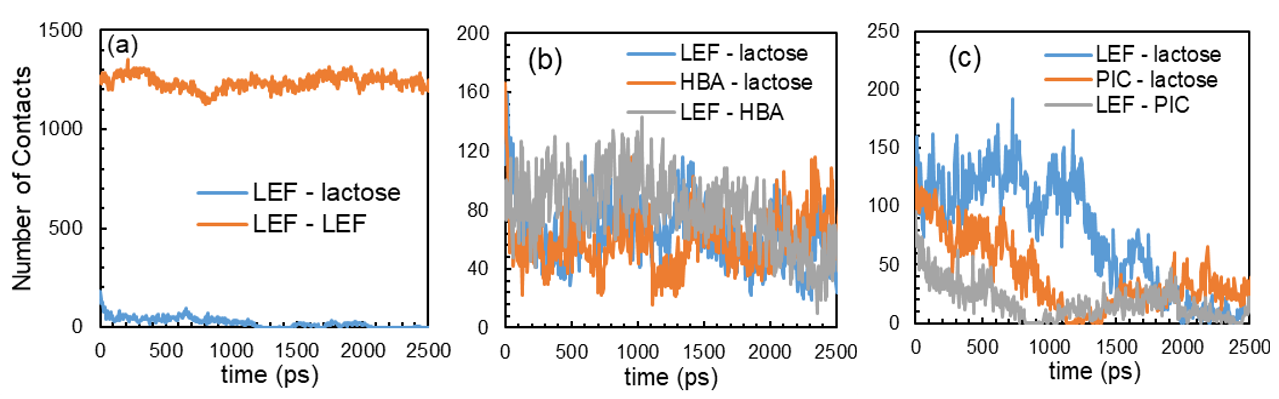
N2

O2

F

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **System** | **Ecoul** | | **ElJ** | | **ETotal** | |
| without lactose | | | | | | |
| LEF | -64.04 | | -63.01 | | -127.05 | |
| LEF-HBA cocrystal | -188.50 | | -97.82 | | -286.32 | |
| LEF-PIC cocrystal | -338.64 | | -69.59 | | -408.23 | |
| with lactose | | | | | | | |
| LEF | | -527.13 | | -80.05 | | -607.18 | |
| LEF-HBA cocrystal | | -543.22 | | -104.09 | | -647.31 | |
| LEF-PIC cocrystal | | -713.70 | | -97.84 | | -811.54 | |

While providing useful molecular-level insights, the above examined parameters (SASA, hydrogen bonds, and interaction energy) govern the dissolution hierarchy of the complexes in water. Nevertheless, to directly capture the dissolution event by MD simulations, additional simulations were conducted without applying position restraints on LEF/cocrystals. The dissolution event of the complexes in water were gauged by intermolecular contacts. Physically, a complex is considered to be dissolved completely in water if there are no, or almost no, intermolecular contacts remaining. **Figure 4** depicts the time evolution of intermolecular contacts during MD simulations. For the LEF-lactose complex, as time lapses, the number of contacts between LEF and lactose decreases; finally reaching ~ 0 after 1000 ps (**Figure 4a**). However, the number of LEF-LEF contacts remains almost constant throughout the simulation, which in turn reflects insignificant dissolution of LEF. Therefore, this complex is deemed to be in an undissolved state. For the LEF-HBA cocrystal-lactose complex, there is a slow decreasing trend in the number of intermolecular contacts with time (**Figure 4b**) despite a fluctuation in the plots. Notably, the intermolecular contacts between cocrystal and lactose (LEF-lactose and HBA-lactose), as well as those between LEF and HBA does not reach ~0, which indicates that this complex holds slow or reduced dissolution characteristics in water. On the other hand, in the LEF-PIC cocrystal-lactose complex (**Figure 4c**), all intermolecular contacts decrease in number with simulation time and reach almost zero after 2000 ps. This complex shows fast and complete dissolution in water, thereby suggesting best dissolving complex in water.



**Figure 4.** Time evolution intermolecular contacts in complexes in water (a) LEF-lactose, (b) LEF-HBA cocrystal-lactose, and (c) LEF-PIC cocrystal-lactose.

1. ***Dicalcium phosphate as an excipient***

MD simulations were performed to gauge the impact of DCP on the hierarchy of dissolution, as well as to gain atomic-level insights into trends in dissolution. **Table 4** lists the hydrophilic, hydrophobic, and total SASAs of LEF/cocrystals and complexes. Similar to lactose as an excipient, addition of DCP to LEF or cocrystal significantly increased SASA. The LEF-HBA cocrystal-DCP complex possesses the largest SASA, whereas in the case of lactose the LEF-PIC cocrystal-lactose complex showed the highest SASA. The hierarchy of both the number of hydrogen bonds and interaction energies between complexes and water follows the trend: LEF < LEF-PIC cocrystal < LEF-HBA cocrystal (**Tables 5** & **6**). A fewer number of hydrogen bonds and lower interaction energy for the LEF-PIC cocrystal-DCP complex in water as compared to the LEF-HBA cocrystal-DCP complex could be attributed to the ionic nature of PIC and DCP. The total interaction energy between the LEF-PIC cocrystal and DCP is the highest and the contribution from ionic interaction dominates (**Table 6**). The fact that the potential interaction sites of PIC are partially blocked by the Ca2+ and HPO42− ions of DCP means a fewer number of interactions between PIC and water, which would suggest lower dissolution of the LEF-PIC cocrystal-DCP complex than the LEF-HBA cocrystal-DCP complex. Analysis of the number of hydrogen bonds and interaction energy terms in greater detail further revealed that 8.11 out of the 24.20 hydrogen bonds and -127.20 kJ mol-1 of the -1239.84 kJ mol-1 interaction energy in the LEF-DCP complex were contributed by LEF. In comparison, the presence of PIC as an integral part of the cocrystal increases the number of hydrogen bonds by 29 % and interaction energy by 60 % in the LEF-PIC cocrystal-DCP complex. The greater number of hydrogen bonds and interaction energy in the LEF-PIC cocrystal-DCP complex than the LEF-DCP complex suggest higher dissolution of LEF-PIC cocrystal-DCP complex. Therefore, the dissolution trend in the presence of DCP follows the trend LEF < LEF-PIC cocrystal < LEF-HBA cocrystal, which is in good agreement with the experimentally observed dissolution trend (vide supra).

It is informative to analyze variation in the intermolecular contacts with respect to the simulation time. **Figure 5** depicts the time evolution of intermolecular contacts during MD simulations. With regard to the LEF-DCP complex, and similar to the LEF-lactose complex, over time the number of contacts between LEF and DCP decreases and reaches zero (**Figure 5a**), but LEF-LEF contacts remain almost constant throughout the simulation, suggesting LEF remains in an undissolved state. On the other hand, in the LEF-HBA cocrystal-DCP complex, all intermolecular interactions decrease with time and finally reach zero (**Figure 5b**), suggesting that the LEF molecules are free to form hydrogen bonds with water molecules thus making it the best dissolving solid. Comparatively, in the LEF-PIC cocrystal-DCP complex, there is a slower decrease in the number of intermolecular contacts between PIC and DCP over time that make this complex a slightly less or slower dissolving solid in water (**Figure 5c**).

**Table 4.** SASA of LEF/cocrystals and complexes with DCP.

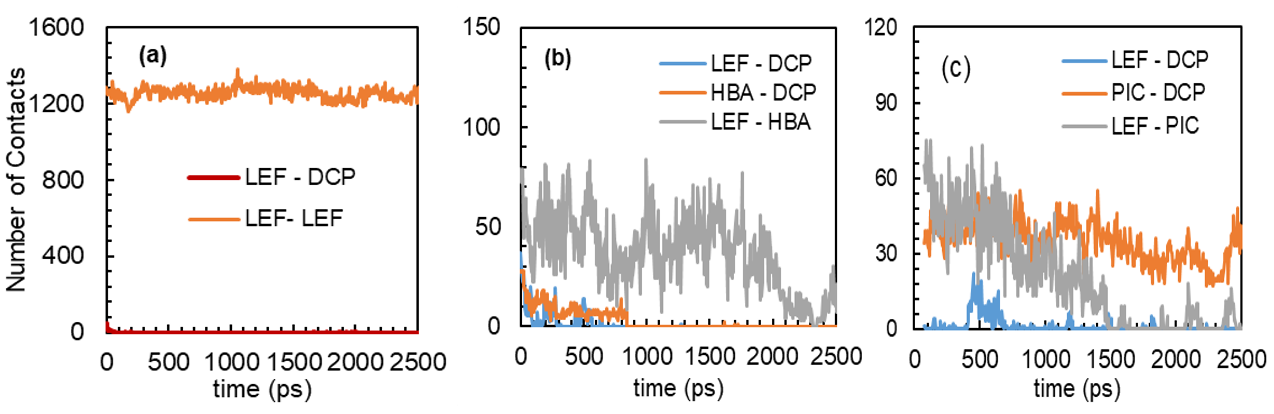
|  |  |  |  |
| --- | --- | --- | --- |
| **System** | **Hydrophilic (nm2)** | **Hydrophobic (nm2)** | **Total (nm2)** |
| LEF | 5.58 | 6.12 | 11.70 |
| LEF-HBA cocrystal | 7.76 | 7.42 | 15.18 |
| LEF-PIC cocrystal | 4.89 | 5.62 | 10.50 |

**Table 5.** The number of hydrogen bonds between LEF/cocrystals and complex with DCP.

|  |  |  |  |
| --- | --- | --- | --- |
| **System** | **LEF** | **LEF-HBA cocrystal** | **LEF-PIC cocrystal** |
| LEF/cocrystal-DCP | 00.00 | 00.00 | 01.97 |
| Complex-water | 24.20 | 34.71 | 29.77 |

**Table 6.** LEF/cocrystals-water and complex-water interaction energies (kJ mol-1).

|  |  |  |  |
| --- | --- | --- | --- |
| **System** | **Ecoul** | **ElJ** | **ETotal** |
| without DCP | | | |
| LEF | -1290.68 | 50.84 | -1239.84 |
| LEF-HBA cocrystal | -1467.37 | 16.77 | -1450.60 |
| LEF-PIC cocrystal | -1289.66 | 32.57 | -1257.09 |
| with DCP | | | |
| LEF | -0.02 | -0.02 | -0.04 |
| LEF-HBA cocrystal | -0.03 | -0.06 | -0.09 |
| LEF-PIC cocrystal | -71.46 | 2.25 | -69.21 |

****

**Figure 5.** Time evolution inter-molecular contacts in complexes (a) LEF, (b) LEF-HBA cocrystal, and (c) LEF-PIC cocrystal with DCP in water.

1. ***Experimental observations***

Dissolution is an important parameter that directly determines the bioavailability of a pharmaceutical active and therefore is a subject of great significance for pharmaceutical industry. In general, dissolution proceeds through a process of detachment of molecules from the solid (or crystal), which primarily depends on the breaking of solute-solute interactions and formation of new solute-solvent interactions. Addition of certain excipients or solubilizing agents help to improve the dissolution of solutes by way of increasing the number and strength of solute-solvent interactions. As described above, MD simulations provide a vital information on the molecular interactions (solute-solvent, solute-excipient, and excipient-solvent) and estimate dissolution trends in a family of solids. The MD simulations’ results presented above suggest that the use of lactose and DCP as excipients could potentially have differing impacts on the dissolution rate of all the solids studied herein. It was noted that while lactose would be expected to improve dissolution rate, a detrimental effect on the dissolution rate would be predicted from DCP.

Prior to the dissolution experiments, bulk samples of LEF and cocrystals were ground and sieved to achieve a uniform particle size of 53-90 μm. Microscopic analysis of the powdered samples using SEM revealed that all the samples adopt a broadly similar shape (see Supporting Information, Figure S6). These attributes negate the impact of particle size and shape on bulk dissolution. It has been reported that the choice of excipient and their compressional behavior may have a profound impact on the release of active from the formulations. In the current study, the impact of differing compressional behavior of excipients was mitigated by the use of tablets of the same hardness. This was achieved through assessing the hardness of test tablets of each formulation pressed at varying loads followed by choosing a pressing load that gave each tablet ~30 N hardness (see Supporting Information, Figure S3). Furthermore, during dissolution experiments all tablets were observed to completely disintegrate within 5-10s – showing that the compressional behavior of the excipients had little to no impact on the dissociation and thus release of LEF/cocrystals.

**Figure 6** shows the dissolution profiles of LEF and cocrystals in the presence of the chosen excipients and their comparison with dissolution profiles of LEF and cocrystals in neat form. It is evident that both the cocrystals show improved dissolution rate compared to dissolution rate of LEF in the absence of excipients (**Figure 6a)**. In addition, both cocrystals exhibited a similar dissolution rate for the first 60 min, with the LEF-PIC cocrystal showing a higher supersaturation level compared to both LEF and LEF-HBA cocrystal after 5 h. The addition of lactose as an excipient, which is soluble in water, improves the dissolution rate of all the three samples. However, it should be noted that while the initial dissolution of the formulations is greater than the neat samples (**Figure 6a)**, upon reaching plateau the cocrystal formulations exhibit a lower supersaturation level than the neat cocrystals (**Figure 6c)**. Therefore, the observed supersaturation level of LEF and cocrystal formulations with lactose at 5 h is in the order of LEF < LEF-HBA cocrystal < LEF-PIC cocrystal, which corroborates the dissolution trend that was predicted by the MD simulations.

The dissolution profile of the formulations with DCP are shown in **Figure 6b&c**. Comparison of the dissolution profiles of the formulations with those of the neat samples reveals that the presence of water-insoluble DCP has a detrimental effect on the dissolution rate. All three formulations displayed a lower dissolution rate than that of their respective neat samples. However, as with the neat samples, the cocrystal formulations show a higher dissolution rate than the LEF formulation (**Figure 6c)**. The supersaturation level observed at the end of the dissolution experiment (5 h) follows the trend LEF < LEF-PIC cocrystal < LEF-HBA cocrystal, which is once again in good agreement with the predicted hierarchy of dissolution from MD simulation results.

A comparative analysis of the dissolution trends of the formulations, neat cocrystals and LEF reveals that the solubility and the nature of the excipient, whether it is neutral or ionic, have a significant impact on the dissolution rate. While the water-soluble lactose improves the dissolution rate, the ionic and water-insoluble DCP has the reverse effect. In the case of lactose as an excipient, the hydrophilic nature and greater aqueous solubility of lactose facilitate the attraction of a greater number of water molecules towards the solute and contribute to the enhanced dissolution rate. On the other hand, the lower dissolution of formulations that contained DCP could be attributed to its hydrophobic nature. Furthermore, the ionic components of DCP potentially interact with the ionic components of the PIC coformer, blocking the hydration sites of the solute leading to an additional decrease in the dissolution of the LEF-PIC cocrystal in the formulation. It is worth noting that the computationally derived parameters, such as RMSDs, SASA and intermolecular interaction energies, point to the higher dissolution of the LEF-PIC and LEF-HBA cocrystal formulations, compared to LEF formulations, when formulated with lactose and DCP, respectively. This is in excellent agreement with the experimentally observed trends, thus emphasizing the significance of MD simulations in understanding the dissolution behavior of cocrystal formulations.

FT-IR has been extensively used for analysis of drug-excipient interactions. It has been established that the shifts in the vibrational frequencies determine the extent of interaction between drug and excipient, and an inference of a chemical reaction can be made from the disappearance of characteristic vibrational bands.[63](#_ENREF_63) In the present study, LEF, neat cocrystals and their formulations were analyzed by FT-IR and their characteristic peaks were compared (see Supporting Information, Figures S7–S9, Table S1). It was found that there is no discernible difference, in either frequency or intensity, in the characteristic peaks of LEF when compared with pure LEF and cocrystals and their formulations with lactose, thus indicating that there is no chemical interaction between LEF and lactose. However, the FT-IR spectra of all formulations with DCP show an absence, or greatly reduced intensity, of the peak characterizing the N–H stretching vibration seen in LEF (strong broad peak at ~3350 cm-1). Previous studies on physical characterization of DCP have asserted to its Lewis acidity,[31](#_ENREF_31), [64](#_ENREF_64) while LEF is basic in nature (pKa value of 10.8 at 23 °C).[65](#_ENREF_65) Therefore, there is a high likelihood of a strong interaction between DCP and LEF potentially weakening the amide N–H bond and hence reducing the intensity of, or removing, the N–H stretching vibration in the IR spectra of the DCP formulations. The interaction between LEF and DCP, particularly between the H atom of N–H in LEF and the O atoms of DCP in all the three formulations was also evidenced by MD simulations (see Supporting Information, Figure S10). Strong interactions between LEF and water insoluble DCP could contribute to the lower observed dissolution of the DCP formulations (**Figure 6**).

D:\Leflunamide\Formulations\Joe's DATA\Dissolution Plot_Revised.tif

**Figure 6**. Comparison of the dissolution profiles (a) lactose formulations vs. neat samples of cocrystals and LEF, (b) DCP formulations vs. neat samples of cocrystals and LEF, and (c) lactose and DCP formulations.

It has been reported that lactose intolerance affects 70 % of the world’s population, which is known to occur due to a deficiency of the enzyme lactase.[66](#_ENREF_66) In some pharmaceutical formulations DCP has been suggested as a potential alternative to lactose for development of lactose-free drug formulations. The results of the current study emphasize the distinct dissolution behavior of lactose and DCP formulations, which has been computationally predicted and experimentally validated. Therefore, it is essential to gain prior knowledge of the impact of different kinds of excipients on the performance of cocrystals. In this respect, MD simulations can be considered a reliable tool for gaining a molecular-level understanding of the interactions between components of the cocrystal/API and dissolution medium and aid in the prediction of dissolution hierarchies.

1. **Conclusions**

Pharmaceutical cocrystallization has evolved as a much sought-after technique for addressing the solid-state issues of active pharmaceutical ingredients. It has been established that the choice of a coformer plays a critical role in determining the properties of neat cocrystals. As cocrystals are being developed and increasingly marketed as novel drug products there is a heightened interest in studies concerning cocrystal-excipient interactions. A review of the current literature revealed varied effects of excipients on the stability, solubility, dissolution, and bioavailability of cocrystals.

We have shown that MD simulations provide vital information for understanding the nature, number and strength of cocrystal-excipient interactions and resultant prediction of dissolution trends in a family of cocrystal-excipient combinations. Calculated parameters such as SASA, hydrogen bonds between an API or cocrystal in formulations with water, interaction energy between an API or cocrystal in formulation and water are found to be essential to elucidate experimentally observed dissolution trends of pure LEF/cocrystals and formulations in water. For LEF/cocrystals or complexes, dissolution in water would be expected to increase as the values of these parameters increase. This finding is consistent with the literature wherein the dissolution performance of API-excipient complexes were explained by these parameters.[47](#_ENREF_47) We have previously shown that cocrystals of LEF demonstrate improved dissolution rate over LEF.[50](#_ENREF_50) However, in this study a similar effect has only been observed with respect to lactose as an excipient, which improved the dissolution at different rates for different cocrystals. DCP, on the other hand, has a detrimental effect resulting in lower dissolution of cocrystal formulations when compared to neat LEF and its cocrystals. The excellent correlation observed between the results of MD simulations and experimental observations validates the role of MD simulations in the selection of suitable excipients for development of novel cocrystal-based formulations. Furthermore, the ever-growing computational power and advances in force fields further contribute to reducing simulation time and help in the quest for suitable excipients for development of cocrystal-based formulations.

**ASSOCIATED CONTENT**

**Supporting Information**. PXRD analysis of the bulk neat cocrystals, hardness evaluation of formulation tablets, radial distribution function *g*(*r*) of water around the atoms of the LEF-HBA and LEF-PIC cocrystals with and without lactose, and SEM images of LEF and cocrystal powders, FT-IR spectra of LEF and cocrystals with and without excipients, FT-IR vibrational assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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**References**

1. Aitipamula, S.; Banerjee, R.; Bansal, A. K.; Biradha, K.; Cheney, M. L.; Choudhury, A. R.; Desiraju, G. R.; Dikundwar, A. G.; Dubey, R.; Duggirala, N.; Ghogale, P. P.; Ghosh, S.; Goswami, P. K.; Goud, N. R.; Jetti, R. R. K. R.; Karpinski, P.; Kaushik, P.; Kumar, D.; Kumar, V.; Moulton, B.; Mukherjee, A.; Mukherjee, G.; Myerson, A. S.; Puri, V.; Ramanan, A.; Rajamannar, T.; Reddy, C. M.; Rodriguez-Hornedo, N.; Rogers, R. D.; Row, T. N. G.; Sanphui, P.; Shan, N.; Shete, G.; Singh, A.; Sun, C. C.; Swift, J. A.; Thaimattam, R.; Thakur, T. S.; Kumar Thaper, R.; Thomas, S. P.; Tothadi, S.; Vangala, V. R.; Variankaval, N.; Vishweshwar, P.; Weyna, D. R.; Zaworotko, M. J., Polymorphs, Salts, and Cocrystals: What's in a Name? *Cryst. Growth Des.* **2012,** *12*, 2147-2152.

2. Desiraju, G. R., *Crystal Engineering: The Design of Organic Solids*. Elsevier: Amsterdam, 1989.

3. Bolla, G.; Nangia, A., Pharmaceutical cocrystals: walking the talk. *Chem. Commun.* **2016,** *52*, 8342-8360.

4. Duggirala, N. K.; Perry, M. L.; Almarsson, Ö.; Zaworotko, M. J., Pharmaceutical cocrystals: along the path to improved medicines. *Chem. Commun.* **2016,** *52*, 640-655.

5. Kale, D. P.; Zode, S. S.; Bansal, A. K., Challenges in Translational Development of Pharmaceutical Cocrystals. *J. Pharm. Sci.* **2017,** *106*, 457-470.

6. Thipparaboina, R.; Kumar, D.; Chavan, R. B.; Shastri, N. R., Multidrug co-crystals: towards the development of effective therapeutic hybrids. *Drug Discovery Today* **2016,** *21*, 481-490.

7. Thakur, T. S.; Thakuria, R., Crystalline Multicomponent Solids: An Alternative for Addressing the Hygroscopicity Issue in Pharmaceutical Materials. *Crystal Growth & Design* **2020,** *20*, 6245-6265.

8. Aitipamula, S.; Vangala, V. R., X-Ray Crystallography and its Role in Understanding the Physicochemical Properties of Pharmaceutical Cocrystals. *Journal of the Indian Institute of Science* **2017,** *97*, 227-243.

9. Shaikh, R.; Singh, R.; Walker, G. M.; Croker, D. M., Pharmaceutical Cocrystal Drug Products: An Outlook on Product Development. *Trends Pharmacol. Sci.* **2018,** *39*, 1033-1048.

10. Imamura, M.; Nakanishi, K.; Shiraki, R.; Onda, K.; Sasuga, D.; Yuda, M. Kotobuki Seiyaku Co. Ltd. Astellas Pharma Inc. Cocrystal of C-glycoside derivative and L-proline. US8097592B2.

11. Lapina, O. V.; Albert, E.; Badalov, P. R.; Shen, J. Gilead Sciences Inc. Solid forms of a bet inhibitor. US20180141939A1.

12. Feng, L.; Karpinski, P. H.; Sutton, P.; Liu, Y.; Hook, D. F.; Hu, B.; Blacklock, T. J.; Fanwick, P. E.; Prashad, M.; Godtfredsen, S.; Ziltener, C., LCZ696: a dual-acting sodium supramolecular complex. *Tetrahedron Lett.* **2012,** *53*, 275-276.

13. Bowles, P.; Brenek, S. J.; Caron, S.; Do, N. M.; Drexler, M. T.; Duan, S.; Dubé, P.; Hansen, E. C.; Jones, B. P.; Jones, K. N.; Ljubicic, T. A.; Makowski, T. W.; Mustakis, J.; Nelson, J. D.; Olivier, M.; Peng, Z.; Perfect, H. H.; Place, D. W.; Ragan, J. A.; Salisbury, J. J.; Stanchina, C. L.; Vanderplas, B. C.; Webster, M. E.; Weekly, R. M., Commercial Route Research and Development for SGLT2 Inhibitor Candidate Ertugliflozin. *Organic Process Research & Development* **2014,** *18*, 66-81.

14. https://[www.accessdata.fda.gov/drugsatfda\_docs/label/2019/209884s000lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2019/209884s000lbl.pdf)

15. Mapp, L. K.; Coles, S. J.; Aitipamula, S., Design of Cocrystals for Molecules with Limited Hydrogen Bonding Functionalities: Propyphenazone as a Model System. *Crystal Growth & Design* **2017,** *17*, 163-174.

16. Saha, S.; Desiraju, G. R., Acid···Amide Supramolecular Synthon in Cocrystals: From Spectroscopic Detection to Property Engineering. *J. Am. Chem. Soc.* **2018,** *140*, 6361-6373.

17. Devogelaer, J. J.; Brugman, S. J. T.; Meekes, H.; Tinnemans, P.; Vlieg, E.; de Gelder, R., Cocrystal design by network-based link prediction. *CrystEngComm* **2019,** *21*, 6875-6885.

18. Karimi-Jafari, M.; Padrela, L.; Walker, G. M.; Croker, D. M., Creating Cocrystals: A Review of Pharmaceutical Cocrystal Preparation Routes and Applications. *Crystal Growth & Design* **2018,** *18*, 6370-6387.

19. Malamatari, M.; Ross, S. A.; Douroumis, D.; Velaga, S. P., Experimental cocrystal screening and solution based scale-up cocrystallization methods. *Adv. Drug Del. Rev.* **2017,** *117*, 162-177.

20. Cerreia Vioglio, P.; Chierotti, M. R.; Gobetto, R., Pharmaceutical aspects of salt and cocrystal forms of APIs and characterization challenges. *Adv. Drug Del. Rev.* **2017,** *117*, 86-110.

21. Wood, P. A.; Feeder, N.; Furlow, M.; Galek, P. T. A.; Groom, C. R.; Pidcock, E., Knowledge-based approaches to co-crystal design. *CrystEngComm* **2014,** *16*, 5839-5848.

22. Bhardwaj, S.; Lipert, M.; Bak, A., Mitigating Cocrystal Physical Stability Liabilities in Preclinical Formulations. *J. Pharm. Sci.* **2017,** *106*, 31-38.

23. Remenar, J. F.; Peterson, M. L.; Stephens, P. W.; Zhang, Z.; Zimenkov, Y.; Hickey, M. B., Celecoxib:Nicotinamide Dissociation:  Using Excipients To Capture the Cocrystal's Potential. *Mol. Pharm.* **2007,** *4*, 386-400.

24. Li, M.; Qiu, S.; Lu, Y.; Wang, K.; Lai, X.; Rehan, M., Investigation of the Effect of Hydroxypropyl Methylcellulose on the Phase Transformation and Release Profiles of Carbamazepine-Nicotinamide Cocrystal. *Pharm. Res.* **2014,** *31*, 2312-2325.

25. Childs, S. L.; Kandi, P.; Lingireddy, S. R., Formulation of a Danazol Cocrystal with Controlled Supersaturation Plays an Essential Role in Improving Bioavailability. *Mol. Pharm.* **2013,** *10*, 3112-3127.

26. Alhalaweh, A.; Ali, H. R. H.; Velaga, S. P., Effects of Polymer and Surfactant on the Dissolution and Transformation Profiles of Cocrystals in Aqueous Media. *Crystal Growth & Design* **2014,** *14*, 643-648.

27. Koranne, S.; Sahoo, A.; Krzyzaniak, J. F.; Luthra, S.; Arora, K. K.; Suryanarayanan, R., Challenges in Transitioning Cocrystals from Bench to Bedside: Dissociation in Prototype Drug Product Environment. *Mol. Pharm.* **2018,** *15*, 3297-3307.

28. Lipert, M. P.; Roy, L.; Childs, S. L.; Rodriguez-Hornedo, N., Cocrystal Solubilization in Biorelevant Media and its Prediction from Drug Solubilization. *J. Pharm. Sci.* **2015,** *104*, 4153-4163.

29. Duggirala, N. K.; Vyas, A.; Krzyzaniak, J. F.; Arora, K. K.; Suryanarayanan, R., Mechanistic Insight into Caffeine–Oxalic Cocrystal Dissociation in Formulations: Role of Excipients. *Mol. Pharm.* **2017,** *14*, 3879-3887.

30. Ogienko, A. G.; Myz, S. A.; Ogienko, A. A.; Nefedov, A. A.; Stoporev, A. S.; Mel'gunov, M. S.; Yunoshev, A. S.; Shalhtshneider, T. P.; Boldyrev, V. V.; Boldyreva, E. V., Cryosynthesis of Co-Crystals of Poorly Water-Soluble Pharmaceutical Compounds and Their Solid Dispersions with Polymers. The "Meloxicam-Succinic Acid" System as a Case Study. *Crystal Growth & Design* **2018,** *18*, 7401-7409.

31. Duggirala, N. K.; LaCasse, S. M.; Zaworotko, M. J.; Krzyzaniak, J. F.; Arora, K. K., Pharmaceutical Cocrystals: Formulation Approaches to Develop Robust Drug Products. *Crystal Growth & Design* **2020,** *20*, 617-626.

32. Avdeef, A., Cocrystal solubility product analysis – Dual concentration-pH mass action model not dependent on explicit solubility equations. *Eur. J. Pharm. Sci.* **2017,** *110*, 2-18.

33. Avdeef, A., Cocrystal Solubility Product Prediction Using an in combo Model and Simulations to Improve Design of Experiments. *Pharm. Res.* **2018,** *35*, 40.

34. Avdeef, A.; Fuguet, E.; Llinàs, A.; Ràfols, C.; Bosch, E.; Völgyi, G.; Verbj , T.; Boldyreva, E.; Takács Novák, K., Equilibrium solubility measurement of ionizable drugs - consensus recommendations for improving data quality. *Admet and Dmpk* **2016,** *4*, 117-178.

35. Gamidi, R. K.; Rasmuson, Å. C., Analysis and Artificial Neural Network Prediction of Melting Properties and Ideal Mole fraction Solubility of Cocrystals. *Crystal Growth & Design* **2020,** *20*, 5745-5759.

36. Ganesan, A.; Coote, M. L.; Barakat, K., Molecular dynamics-driven drug discovery: leaping forward with confidence. *Drug Discovery Today* **2017,** *22*, 249-269.

37. Katiyar, R. S.; Jha, P. K., Molecular simulations in drug delivery: Opportunities and challenges. *WIREs Computational Molecular Science* **2018,** *8*, e1358.

38. Maginn, E. J., From discovery to data: What must happen for molecular simulation to become a mainstream chemical engineering tool. *AIChE Journal* **2009,** *55*, 1304-1310.

39. Gupta, J.; Nunes, C.; Vyas, S.; Jonnalagadda, S., Prediction of Solubility Parameters and Miscibility of Pharmaceutical Compounds by Molecular Dynamics Simulations. *The Journal of Physical Chemistry B* **2011,** *115*, 2014-2023.

40. Maus, M.; Wagner, K. G.; Kornherr, A.; Zifferer, G., Molecular dynamics simulations for drug dosage form development: thermal and solubility characteristics for hot-melt extrusion. *Molecular Simulation* **2008,** *34*, 1197-1207.

41. Yani, Y.; Kanaujia, P.; Chow, P. S.; Tan, R. B. H., Effect of API-Polymer Miscibility and Interaction on the Stabilization of Amorphous Solid Dispersion: A Molecular Simulation Study. *Industrial & Engineering Chemistry Research* **2017,** *56*, 12698-12707.

42. Gao, Y.; Olsen, K. W., Molecular Dynamics of Drug Crystal Dissolution: Simulation of Acetaminophen Form I in Water. *Mol. Pharm.* **2013,** *10*, 905-917.

43. Gao, Y.; Olsen, K. W., Unique Mechanism of Facile Polymorphic Conversion of Acetaminophen in Aqueous Medium. *Molecular Pharmaceutics* **2014,** *11*, 3056-3067.

44. Parks, C.; Koswara, A.; Tung, H.-H.; Nere, N. K.; Bordawekar, S.; Nagy, Z. K.; Ramkrishna, D., Nanocrystal Dissolution Kinetics and Solubility Increase Prediction from Molecular Dynamics: The Case of α-, β-, and γ-Glycine. *Molecular Pharmaceutics* **2017,** *14*, 1023-1032.

45. Stephenson, B. C.; Rangel-Yagui, C. O.; Junior, A. P.; Tavares, L. C.; Beers, K.; Blankschtein, D., Experimental and Theoretical Investigation of the Micellar-Assisted Solubilization of Ibuprofen in Aqueous Media. *Langmuir* **2006,** *22*, 1514-1525.

46. Hirano, A.; Kameda, T.; Arakawa, T.; Shiraki, K., Arginine-Assisted Solubilization System for Drug Substances: Solubility Experiment and Simulation. *The Journal of Physical Chemistry B* **2010,** *114*, 13455-13462.

47. Li, C.; Wang, J.-X.; Le, Y.; Chen, J.-F., Studies of Bicalutamide–Excipients Interaction by Combination of Molecular Docking and Molecular Dynamics Simulation. *Molecular Pharmaceutics* **2013,** *10*, 2362-2369.

48. Jha, P. K.; Larson, R. G., Assessing the Efficiency of Polymeric Excipients by Atomistic Molecular Dynamics Simulations. *Molecular Pharmaceutics* **2014,** *11*, 1676-1686.

49. Kirubakaran, P.; Wang, K.; Rosbottom, I.; Cross, R. B. M.; Li, M., Understanding the Effects of a Polymer on the Surface Dissolution of Pharmaceutical Cocrystals Using Combined Experimental and Molecular Dynamics Simulation Approaches. *Molecular Pharmaceutics* **2020,** *17*, 517-529.

50. Cadden, J.; Klooster, W. T.; Coles, S. J.; Aitipamula, S., Cocrystals of Leflunomide: Design, Structural, and Physicochemical Evaluation. *Crystal Growth & Design* **2019,** *19*, 3923-3933.

51. Rozman, B., Clinical pharmacokinetics of leflunomide. *Clin. Pharmacokinet.* **2002,** *41*, 421-430.

52. Hebbink, G. A.; Dickhoff, B. H. J., Chapter 5 - Application of lactose in the pharmaceutical industry. In *Lactose*, Paques, M.; Lindner, C., Eds. Academic Press: 2019; pp 175-229.

53. Rowe, R. C.; Sheskey, P. J.; Owen, S. C.; American Pharmacists, A., *Handbook of Pharmaceutical Excipients*. Pharmaceutical Press: 2006.

54. Doldán, C.; Souto, C.; Concheiro, A.; Martínez-Pacheco, R.; Gómez-Amoza, J. L., Dicalcium phosphate dihydrate and anhydrous dicalcium phosphate for direct compression: A comparative study. *Int. J. Pharm.* **1995,** *124*, 69-74.

55. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J., Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J. Am. Chem. Soc.* **1996,** *118*, 11225-11236.

56. Ribeiro, A. A.; Horta, B. A.; Alencastro, R. B. d., MKTOP: a program for automatic construction of molecular topologies. *Journal of the Brazilian Chemical Society* **2008,** *19*, 1433-1435.

57. Demichelis, R.; Garcia, N. A.; Raiteri, P.; Innocenti Malini, R.; Freeman, C. L.; Harding, J. H.; Gale, J. D., Simulation of Calcium Phosphate Species in Aqueous Solution: Force Field Derivation. *The Journal of Physical Chemistry B* **2018,** *122*, 1471-1483.

58. Berendsen, H. J.; Postma, J. P.; van Gunsteren, W. F.; Hermans, J., Interaction models for water in relation to protein hydration. In *Intermolecular forces*, Springer: 1981; pp 331-342.

59. Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E., GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J. Chem. Theory Comput.* **2008,** *4*, 435-447.

60. US FDA Dissolution Methods. https://[www.accessdata.fda.gov/scripts/cder/dissolution/dsp\_getallData.cfm](http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_getallData.cfm)

61. Matziari, M.; Dellis, D.; Dive, V.; Yiotakis, A.; Samios, J., Conformational and Solvation Studies via Computer Simulation of the Novel Large Scale Diastereoselectively Synthesized Phosphinic MMP Inhibitor RXP03 Diluted in Selected Solvents. *The Journal of Physical Chemistry B* **2010,** *114*, 421-428.

62. Luzar, A.; Chandler, D., Hydrogen-bond kinetic in liquid water. *Nature* **1996,** *379*, 55-57.

63. Liltorp, K.; Larsen, T. G.; Willumsen, B.; Holm, R., Solid state compatibility studies with tablet excipients using non thermal methods. *J. Pharm. Biomed. Anal.* **2011,** *55*, 424-428.

64. Takahashi, T.; Yamamoto, R., Studies on the Stability of Vitamin D2 Powder Preparations. IV. : Isomerization of Vitamin D2 and Related Compounds catalyzed by Surface Acid on Excipients, and Active Site for Catalytic Reactions. *J. Pharm. Soc. Jpn.* **1998,** *89*, 925-932.

65. Pharmacology review of leflunomide. https://[www.accessdata.fda.gov/drugsatfda\_docs/nda/98/20905\_ARAVA\_PHARMR\_P1.PDF](http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20905_ARAVA_PHARMR_P1.PDF)

66. Mill, D.; Dawson, J.; Johnson, J. L., Managing acute pain in patients who report lactose intolerance: the safety of an old excipient re-examined. *Therapeutic Advances in Drug Safety* **2018,** *9*, 227-235.