Investigating the Influence of Polymers on Supersaturated Flufenamic Acid Cocrystal Solutions
Minshan Guo, Ke Wang, Noel Anthony Hamill, Keith Lorimer, and Mingzhong Li

Mol. Pharmaceutics, Just Accepted Manuscript • DOI: 10.1021/acs.molpharmaceut.6b00612 • Publication Date (Web): 05 Aug 2016

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.
Investigating the Influence of Polymers on Supersaturated Flufenamic Acid Cocrystal Solutions

Minshan Guo\textsuperscript{1}, Ke Wang\textsuperscript{1}, Noel Hamill\textsuperscript{2}, Keith Lorimer\textsuperscript{2} and Mingzhong Li\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1}School of pharmacy, De Montfort University, Leicester, UK

\textsuperscript{2}Almac Science, Seagoe Industrial Estate, Craigavon, UK
Table of contents graphic
Abstract

The development of enabling formulations is a key stage when demonstrating the effectiveness of pharmaceutical cocrystals to maximize the oral bioavailability for poorly water soluble drugs. Inhibition of drug crystallization from a supersaturated cocrystal solution through a fundamental understanding of the nucleation and crystal growth is important. In this study, the influence of the three polymers of polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and a copolymer of N-vinly-2-pyrrodidone (60%) and vinyl acetate (40%) (PVP-VA) on the flufenamic acid (FFA) crystallization from three different supersaturated solutions of the pure FFA and two cocrystals of FFA-NIC CO and FFA-TP CO has been investigated by measuring nucleation induction times and desupersaturation rates in the presence and absence of seed crystals. It was found that the competition of intermolecular hydrogen bonding among drug/coformer, drug/polymer and coformer/polymer was a key factor responsible for maintaining supersaturation through nucleation inhibition and crystal growth modification in a cocrystal solution. The supersaturated cocrystal solutions with predissolved PEG demonstrated more effective stabilization in comparison to the pure FFA in the presence of the same polymer. In contrast, neither of the two cocrystal solutions, in the presence of PVP or PVP-VA, exhibited a better performance than the pure FFA with the same predissolved polymer. The study suggests that the selection of a polymeric excipient in a cocrystal formulation should not be solely dependent on the interplay of the parent drug and polymer without considering the coformer effects.

KeyWords: Cocrystal; polymers; Flufenamic Acid; crystal growth; nucleation; supersaturation.
Introduction
Development of supersaturating drug delivery systems to enhance oral bioavailability of poorly water soluble drugs has been of interest for many decades \(^1\). In these systems, two essential steps need to be considered: the drug in a high energy form, e.g., amorphous forms, crystalline salts or cocrystals, should dissolve rapidly to generate a high concentration above the saturation solubility and then this supersaturated solution must be maintained for a reasonable period to allow for significant absorption and eventually sufficient bioavailability. This has been referred to as a “spring and parachute” approach \(^2\). As a supersaturated drug solution is thermodynamically unstable and has the tendency to return to the equilibrium state through drug crystallization, extensive work has been carried out to delay the drug crystallization by inclusion of different excipients as effective crystallization inhibitors in formulations \(^3\). For example, significant progress has been made in amorphous solid dispersion formations by using polymeric crystallization inhibitors to maintain the solid drug in an amorphous state and also maintain the drug supersaturation after dissolution \(^4, 5\). It has been found that inhibition of the drug crystallization is a result of the polymers interfering in the nucleation and/or crystal growth stages of the more stable phase, through physical or chemical interactions between the drug and polymer excipients, such as; solution viscosity enhancement, non-specific hydrophobic drug-polymer interactions and specific drug-polymer intermolecular interactions through hydrogen bonding \(^6-12\).

Compared with amorphous solid forms, the crystalline forms of the drug substances are generally preferred in a formulation because of their thermodynamic stability and purity. Pharmaceutical cocrystals have therefore attracted significant attention over the last decade due to their ability to modulate the physicochemical properties of a drug compound to overcome any solubility limited bioavailability problem \(^13-16\). Similar to the amorphous solid forms, those
cocrystals with improved solubility and dissolution rates become thermodynamically unstable once dissolved due to supersaturation of the drug. This results in precipitation of stable solid phases of the parent drugs and reduction of the solubility advantage of the cocrystal\textsuperscript{17-19}. In order to achieve the full potential of cocrystals, rational strategies are required that identify the appropriate crystallization inhibitors of polymers and/or surfactants in cocrystal formulations\textsuperscript{20-25}. In comparison with the amorphous solid dispersion systems, in which the supersaturated solution behavior is determined by the ternary drug/polymer/solvent interaction, the complexity of a cocrystal supersaturated solution increases considerably due to inclusion of an additional component of a coformer. This can interfere with the drug molecule, polymeric excipients, and/or solvent, resulting in alteration of the inhibition ability of the polymers on the drug. It is not surprising that inclusions of excipients of polymers and surfactants in the indomethacin or carbamazepine cocrystal formulations have not shown effectiveness in capturing the enhanced solubility advantage\textsuperscript{21, 24}. Although research has demonstrated that a combination of a cocrystal of celecoxib-nicotinamide or danazol-vanillin with both a polymer and surfactant can provide an enhanced dissolution rate and a high oral bioavailability, there is no mechanistic understanding of how these additives interact with the drug molecules in solution\textsuperscript{22, 23}. Therefore, it is of huge importance to investigate the role of polymeric excipients as potential crystallization inhibitors for rational design of cocrystal formulation systems.

In this work, for the first time, a systematic investigation was conducted to explore the impact of different polymeric additives in cocrystal formulations to elucidate the molecular mechanism of polymer/drug/coformer interactions that affect the kinetics of nucleation and growth of the parent drug. In the study, Flufenamic acid form I (FFA I) was selected as a parent model drug along with two coformers of Nicotinamide (NIC) and Theophylline (TP). This was due to their
ability to form FFA-NIC cocrystals (FFA-NIC CO) and FFA-TP cocrystals (FFA-TP CO), both
of which display different physicochemical properties. FFA, a nonsteroidal anti-
flammatory drug (NSAID), has the problem of low bioavailability after oral administration due
to its low solubility. Among its nine reported polymorphs, FFA I (white color) and FFA III
(yellow color) have been used in the commercial solid dosage forms. Three chemically diverse
polymers including polyethylene glycol (PEG), polyvinylpyrrolidone (PVP) and copolymer of
vinyl pyrrolidone/vinyl acetate (PVP-VA) were selected because they have been widely used as
crystallization inhibitors in other supersaturating drug delivery systems. Among these
polymers, PEG is the most hydrophilic, containing a high percentage of hydrogen donors.
In comparison to PVP, more hydrophobic PVP-VA, containing 40% acetate side chains, was used
to investigate the specific intermolecular interaction with the drug and/or coformers. The
solubility parameter was calculated for comparison of the hydrophobicity of the model drug,
coformers and polymers. Chemical structures of the model drug, coformers and monomer units
of the polymers are shown in Table 1.

Equilibrium solubility tests were first carried out to evaluate the potential role of polymers in
changing the apparent FFA solubility in solution. A solvent shift method was then used to
generate an initial FFA supersaturation condition to study crystallization kinetics of both
nucleation and growth. Induction time determined by polarized light microscopy was used to
quantify the drug nucleation from a supersaturated solution in the absence and presence of
different pre-dissolved polymers. The impact of different polymers on growth was characterized
by measuring desupersaturation curves in the presence of the seeds of the pure FFA I crystals.
The overall impact of polymers on inhibiting FFA crystallization from a supersaturated solution
was characterized and evaluated by measuring the desupersaturation curves in the absence of the
crystal seeds. In order to quantify inhibition ability of polymers to prolong drug supersaturation, supersaturation parameters in different supersaturated solutions were calculated and compared.

The solid residues after the solubility and the desupersaturation experiments with and without seeds were examined by differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FTIR) and polarized light microscopy. To further explore the intermolecular interaction mechanisms among a polymer, drug and coformer, solution infrared spectra of the parent drug FFA I and coformers of NIC and TP in combination with different polymers were collected and compared.

Materials and methods

Materials

Flufenamic acid form I (FFA I), Nicotinamide (NIC) (≥99.5% purity) and Theophylline (TP) (≥99.5% purity) were purchased from Sigma-Aldrich (Dorset, UK). Poly (ethylene glycol) 4000 (PEG) was purchased from Sigma-Aldrich (Dorset, UK). Plasdone K-29/32 (PVP) and Plasdone S-630 (PVP-VA) were gifts from Ashland Inc. (Schaffhausen, Switzerland). Methanol (HPLC grade) and ethanol (lab grade) were purchased from Fisher Scientific UK (Loughborough, UK) and used as received. Double distilled water was generated from a Bi-Distiller (WSC044.MH3.7, Fistreem International Limited, Loughborough, UK) and used throughout the study.

Methods

Preparation of FFA–NIC and FFA–TP cocrystals

Flufenamic acid and Nicotinamide cocrystal (FFA-NIC CO) was prepared by a solvent evaporation method. A 1:1 equimolar mixture of FFA I and NIC was dissolved in acetonitrile
with stirring at 80°C. The solution was placed in a fume cabinet overnight for solvent evaporation. Flufenamic acid and Theophylline cocrystal (FFA-TP CO) was synthesized by a cooling crystallization method. A 1:1 molar ratio of FFA and TP was dissolved in a cosolvent (7:3 acetonitrile and water) with stirring at 90°C and then the solution was placed into an ice bath for 2 h until the crystals were separated out from the solution. Both FFA-NIC CO and FFA-TP CO were characterized and confirmed by DSC, FTIR and XRPD.

**Apparent equilibrium solubility determination**

The apparent equilibrium solubility of FFA I, FFA-NIC CO and FFA-TP CO was determined by suspending an excess amount of crystalline materials in small vials with 20mL of the cosolvent (1:4 ethanol and water) in the absence or presence of 0.2 mg/mL of a pre-dissolved polymer (PEG, PVP or PVP-VA). This mixture was kept at 37 ± 0.5°C with shaking (150RPM) for 24 h. The supernatant was separated from excess solids in solution by MSE Micro Centaur at 13000RPM for 1 min in a MSB 010.CX2.5 centrifuge (MSE Ltd, London, UK). Subsequently, the supernatant was diluted and the concentrations of FFA and coformers were determined using a high-performance liquid chromatography system (HPLC). The solid residues were retrieved from the tests, dried for 24h at ambient temperature and analyzed by DSC, FTIR and XRPD. The cosolvent of 1:4 ethanol and water was used in this study to increase the apparent FFA equilibrium solubility and thus avoid immediate crystallization of FFA in media through maintaining slower kinetics of nucleation and growth. All experiments were conducted in triplicate and data were reported as an average concentration in solution.

**Monitoring nucleation induction time using polarized light microscopy**
Nucleation induction times were determined from desupersaturation experiments monitored using polarized light microscopy. The FFA stock concentration of pure FFA I, FFA-NIC CO or FFA-TP CO dissolved in ethanol was 1 mg/mL. Different initial supersaturated solutions of 50, 100 and 200 µg/mL were generated by adding the appropriate amount of the stock solution into a small quartz cell filled with 0.5 mL of the cosolvent in the absence or presence of 0.2 mg/mL of different polymers. The FFA crystallization behavior from a supersaturated solution was monitored by a LEICA DM 750 polarizing microscope (Leica Microsystem Ltd, Milton Keynes, UK) with a 200x or 100x objective and recorded using a version 4.0 studio capture. Data collection commenced immediately after addition of the drug stock solution to the test medium. The induction time was determined by observing the onset of the FFA crystal formation.

Effect of polymers on the supersaturated FFA and cocystal solutions

In order to decouple the nucleation process, the inhibition effect of a polymer on the growth of FFA crystals was assessed from the seeded experiments by measuring the desupersaturation curve of a supersaturated solution of pure FFA, FFA-NIC CO or FFA-TP CO in the absence and presence of 0.2 mg/mL of a pre-dissolved polymer (PEG, PVP or PVP-VA). 50 mg of FFA I crystal seeds, which were slightly ground and sieved by a 60 (size) mesh sieve, were added to 50 mL of the cosolvent medium and allowed to equilibrate at 37°C for 24 h. A supersaturated solution was generated by adding 0.3 mL of a 5 mg/mL FFA stock solution of pure FFA, FFA-NIC CO or 0.6 mL of 2.5 mg/mL FFA-TP CO. The amount of ethanol added to the medium was small and had a negligible impact on the apparent FFA equilibrium solubility. 1 mL of each sample was withdrawn from the solution at six predetermined time intervals, i.e. 5, 15, 30, 60, 120 and 240 min. The supernatant was separated from excess solids by centrifugation at 13000 rpm for 1 min in a MSE Micro Centaur. The supernatant was diluted to determine the concentrations of FFA and coformer of NIC or TP by HPLC. In order to evaluate the overall inhibition effect of a polymer on FFA crystallization kinetics from a supersaturated solution, unseeded desupersaturation experiments were conducted. A supersaturated solution was generated by adding 20 mL
of 500 µg/mL FFA stock solution of pure FFA, FFA-NIC CO or FFA-TP CO to 80 mL of water, resulting in 100 µg/mL FFA in the cosolvent of 1:4 ethanol and water. The solid residues after the desupersaturation experiments were examined by DSC, FTIR and polarized light microscopy. Supersaturation parameters were calculated and compared for both seeded and unseeded experiments to quantify different polymer inhibition abilities.

All experiments were conducted in triplicate and data were reported as the average of the experiments.

**High Performance Liquid Chromatography (HPLC) analysis**

The sample concentration of FFA, NIC or TP in solution was determined by a Perkin Elmer series 200 HPLC system (PerkinElmer Ltd, Beaconsfield, UK). A HAISLL 100 C18 column (5 µm, 250 × 4.6 mm) (Higgins Analytical Inc., Mountain View, CA, USA) was used at ambient temperature. FFA was detected by UV absorbance detection at a wavelength of 286 nm. The mobile phase used consisted of 15% water (including 0.5% formic acid) and 85% methanol and the mobile phase flow rate was maintained at 1.5 mL/min. Both NIC and TP were detected by UV absorbance detection at a wavelength of 265 nm, the mobile phase was composed of 55% methanol and 45% water, and the mobile phase flow rate was kept at 1 mL/min. The injection volume was 20 µL.

**Differential Scanning Calorimetry (DSC)**

The melting point of solids was measured by a PerkinElmer Jade DSC (PerkinElmer Ltd., Beaconsfield, UK) controlled by Pyris Software. The temperature and heat flow of the instrument were calibrated using indium and zinc standards. A test sample (8-10mg) was analyzed in crimped aluminum pan with a pin-hole pierced lid. Measurements were carried out at a heating rate of 20°C/min under a nitrogen flow rate of 20mL/min.

**X-ray powder diffraction (XRPD)**
X-ray powder diffraction pattern of solids was recorded from 5° to 50° at a scanning rate of 0.3° (2θ) min⁻¹ by D2 PHASER diffractometer (Bruker UK Limited, Coventry, UK). Cu-Kβ radiation was used with a voltage of 30KV and current of 10 mA.

**Fourier transform infrared spectroscopy (FTIR)**

FTIR spectra of the solid samples were measured using an ALPHA interferometer (Bruker UK Limited, Coventry, UK) with a horizontal universal attenuated total reflectance (ATR) accessory. Samples were placed on the surface of the diamond ATR plate and the ATR assembly was clamped to ensure good contact.

The investigation of the intermolecular interaction among FFA, NIC, TP and polymers (PEG, PVP and PVP-VA) in solution was carried out by FTIR. Solution spectra were collected using the same spectrometer fitted with a transmission accessory and the Bruker 6500S Circular Aperture liquid cell with size of 32×3 mm CaF2 window. The path length was 0.05mm. Methanol was selected for the intermolecular interaction study of FFA, NIC and polymers, in which the concentrations of individual components were 50, 21.7 and 20 mg/mL respectively. A cosolvent of 1M HCl and methanol at a ratio of 1:6 was selected for the intermolecular interaction study of FFA, TP and polymers, in which the concentrations of individual components were 14.3, 9.14 and 20 mg/mL respectively.

In each measurement, 30 scans were collected per spectrum with a resolution of 2 cm⁻¹ in the spectral region of 400 to 4000 cm⁻¹ using OPUS software. All the spectral data were collected at an ambient temperature, between 20 to 23°C.

**Solubility parameter (SP), supersaturation ratio (SR) and supersaturation parameter (SSP)**

*Solubility parameter (SP)* is used to compare the relative hydrophobicity of polymers, FFA and coformers in solution. The *SP* of an organic compound is estimated by Fedors 34 as

\[
SP = \frac{\sum_i \Delta e_i}{\sqrt{\sum_i \Delta v_i}} = \sqrt{\frac{\Delta e_{TV}}{V}}
\]  

(1)
where the $\Delta e_i$ and $\Delta \nu_i$ are the additive atomic and group contributions to the energy of vaporization and molar volume, respectively. $\Delta E_V$ is the energy of vaporization at a given temperature and $V$ is the corresponding molar volume which is calculated from the known values of molecular weight and density.

The method is based on group additive constants; therefore it requires only knowledge of the structural formula of the compound. Based on the group contributions provided in the literature, $SP$ values of the polymers and drug compounds used in the study are shown in Table 1. Details of calculation of $SP$ for each compound can be found in Table S1 in the supplementary materials.

Supersaturation ratio ($SR$) in this study is defined as

$$SR = \frac{C}{C_{eq}}$$  \hspace{1cm} (2)

where $C$ is the solute concentration and $C_{eq}$ is the solute equilibrium solubility.

Supersaturation parameter ($SSP$) is used to evaluate the drug precipitation behavior from a supersaturated system in comparison to a reference system based on the work by Chen et al. Fig. 1 shows the desupersaturation curves of supersaturated drug systems, in which the initial drug concentration $C_0$ is higher than its equilibrium solubility $C_{eq}$. Line $C_0C_0(t)$ represents an ideal situation where the drug remains in the medium and no crystallization occurs over the time period of $t$. The curve $C_0C_R(t)$ is the desupersaturation curve of a reference system. An integration area of $A_{C_0C_R(t)C_0(t)}$ can be used to indicate the amount of drug precipitated from the solution over time $t$. For a supersaturated system with the desupersaturation curve of $C_0C_a(t)$, the integration area of $A_{C_0C_a(t)C_0(t)}$ is smaller than that of the reference system, indicating less drug precipitation. Compared with the reference system, a supersaturated system with the desupersaturation curve of $C_0C_a(t)$ has more precipitated drug solids because of a larger integration area of $A_{C_0C_b(t)C_0(t)}$. To compare the abilities of different systems on maintaining the drug supersaturation, $SSP$ is defined as

$$SSP = \frac{A_{C_0C_R(t)C_0(t)} - A_{C_0C_a(t)C_0(t)}}{A_{C_0C_R(t)C_0(t)}} \times 100\%$$ \hspace{1cm} (3)
where \( C(t) \) is the desupersaturation curve of an investigated system. \( SSP \) is a dimensionless parameter. A system with a positive \( SSP \) value has an increased ability to prolong drug supersaturation while a negative \( SSP \) value indicates less ability to maintain the supersaturated drug in solution.

**Results**

**Solid characterization of FFA I, NIC, TP, and FFA cocrystals**

Fig. 2(a) shows the XRPD patterns of individual components of FFA I, NIC, TP, FFA-NIC CO and FFA-TP CO. The significant characteristic diffraction peaks of FFA I are at \( 2\theta=7.1^\circ, \ 14.2^\circ, \ 21.4^\circ \) and \( 24.6^\circ \). Key characteristic diffraction peaks of NIC are at \( 2\theta=14.9^\circ \) and \( 23.5^\circ \). After co-evaporation of FFA and NIC in acetonitrile, the new materials of FFA-NIC CO have been formed, showing the characteristic diffraction peaks at \( 2\theta=6.7^\circ, \ 9.6^\circ, \ 16.2^\circ, \ 16.8^\circ \) and \( 21.9^\circ \), which are in agreement with those of published data \(^26\). The characteristic diffraction peaks of TP are at \( 2\theta=7.2^\circ, \ 12.7^\circ \) and \( 14.5^\circ \). Through the cooling crystallization method described in Section 2, FFA-TP CO was generated, indicated by the characteristic diffraction peaks at \( 2\theta=5.9^\circ, \ 11.3^\circ, \ 15.6^\circ \) and \( 26.8^\circ \) \(^27\).

The structures of FFA-NIC CO and FFA-TP CO have been confirmed by the measured IR spectra in Fig. 2(b) \(^26, \ 27\). FFA-NIC CO is formed through an acid-pyridine heterosynthon involving FFA and NIC molecules \(^26\). The IR spectrum of FFA I has peaks at 3318 cm\(^{-1}\) and 1651 cm\(^{-1}\), corresponding to N-H and C=O stretching frequencies \(^35\). The spectrum of NIC has 2 peaks at 3353 cm\(^{-1}\) and 1592 cm\(^{-1}\), corresponding to N-H and pyridine ring C=N stretching \(^36\). In the spectrum of FFA-NIC CO, the frequencies of N-H stretching and C=O stretching of FFA are shifted to 3324 cm\(^{-1}\) and 1660 cm\(^{-1}\) while the peaks of N-H stretching and pyridine ring C=N stretching of NIC shifted to 1608 cm\(^{-1}\) from 1592 cm\(^{-1}\) and to 3395 cm\(^{-1}\) from 3353 cm\(^{-1}\). FFA-TP CO is formed through an O-H···O hydrogen bond involving the carboxylic acid of FFA and unsaturated N atom of the imidazole ring of TP \(^27\). The IR spectrum of TP has peaks at 3119 cm\(^{-1}\), 1660 cm\(^{-1}\) and 1561 cm\(^{-1}\), corresponding to N-H, C=O and C=N stretching frequencies which are shifted to 3068cm\(^{-1}\), 1669 cm\(^{-1}\) and 1558 cm\(^{-1}\) respectively. In the spectrum of FFA-
TP CO, FFA’s N-H stretching and C=O stretching frequencies are shifted to 3280 cm\(^{-1}\) and 1647 cm\(^{-1}\) respectively. The summary of IR peak identities of FFA I, NIC, TP and cocrystals are shown in Table S2 in the supplementary materials.

The DSC curve in Fig. 2(c) shows that the melting point of FFA-NIC CO was 136.4 °C which was higher than both of the melting points of FFA I (133.5 °C) and NIC (128.1 °C). In contrast, the melting point of FFA-TP CO was 185.6 °C which was in the middle of the melting points of FFA I and the TP melting point at 272.8 °C.

**Apparent FFA equilibrium solubility of FFA I, FFA-NIC CO and FFA-TP CO in cosolvent in the absence and presence of different polymers**

Fig.3(a) demonstrates the apparent equilibrium solubility of FFA I, FFA-NIC CO and FFA-TP CO in cosolvent media in the absence or presence of predissolved polymers of PVP, PEG and PVP-VA at equilibrium after 24 h. In the absence of a polymer, the apparent FFA equilibrium solubility of FFA-NIC CO (41.9±2.1 µg/mL) was slightly higher than those of FFA I and FFA-TP CO which were comparable (36.0±0.5 µg/mL for FFA-TP CO and 36.8±2.1 µg/mL for the pure FFA I). In the presence of 200µg/mL polymer, PEG, PVP or PVP-VA, the apparent FFA equilibrium solubility of FFA I or FFA cocrystals does not change, indicating that none of the polymers changed the solution properties.

The solid residues collected after the solubility tests were analyzed by DSC in Fig.3(b). For pure FFA I, the resultant solid residues were the same as the starting materials after the solubility test in the absence or presence of polymers, indicated by identical DSC thermographs in Fig. 3(b). Following the solubility tests of FFA-NIC CO and FFA-TP CO in presence or absence of polymers, the solid residues formed were yellow FFA III crystals, indicating the cocrystals of
FFA-NIC CO or FFA-TP CO had transformed into FFA III. This was confirmed by DSC thermographs of the solid residues in Fig. 3(b), in which the same thermal events occurred as that of the pure FFA III. Under DSC heating conditions, FFA III melted at 123.1°C and recrystallized to FFA I which then melted at 134.4°C \(^\text{37}\). However, the morphologies of FFA III particles collected from the two cocrystal tests in Fig. 3(c) were significantly different. The FFA III crystals from FFA-NIC CO tests were needle-shaped, whereas those from the FFA-TP CO tests were rod/disc-shaped. FTIR data of the solid residues are shown in Fig. S1 in the supplementary materials.

**Effect of polymers on the nucleation induction time of FFA crystallization in solution**

Based on the measured equilibrium solubility of FFA I in Section 3.2, the initial supersaturated solutions of 50, 100 and 200 µg/mL were corresponding to the \(SR\) values of 1.36, 2.72 and 5.44 respectively. The nucleation induction times in Table 2 were based on the initial observation times of FFA crystals detectable by polarized light microscopy. Without a predissolved polymer in the cosolvent media, the precipitation of FFA from the pure FFA and two cocrystal solutions occurred rapidly at the low \(SR\) of 1.36. The induction times were significantly different in the presence of different polymers, PEG, PVP and PVP-VA. With predissolved PEG in solution, the induction times were increased slightly for all test solutions at the low \(SR\) of 1.36. No FFA crystals were found for all test solutions in the presence of 200 µg/mL of pre-dissolved PVP or PVP-VA at a \(SR\) 1.36, indicating that PVP or PVP-VA can completely inhibit the crystallization of FFA during the 30 min experiment. In order to differentiate the inhibition abilities of PVP and PVP-VA, the experiments were conducted with a higher initial degree of supersaturation \(SR\)
2.72. From the recorded images, it was observed that dense liquid particles appeared immediately in the experiments with the predissolved PVP or PVP-VA and then the formation of the crystal nuclei within the dense liquid clusters followed. It is in excellent agreement with the two-step mechanism of nucleation of crystals in solution. A video clip of FFA crystals nucleation from a supersaturated FFA-NIC CO solution in the presence of predissolved PVP-VA can be found in the supplementary materials. In the presence of pre-dissolved PVP in solution, the order of the induction times was $T_{FFA-TP CO} < T_{FFA-NIC CO} < T_{FFA}$. In contrast, PVP-VA can completely inhibit the crystallization of FFA from the three test solutions. Further tests were conducted at the supersaturation level of $SR = 5.44$ with predissolved PVP-VA. It was shown that the induction times were comparable for the two cocrystal solutions, with the longest induction time being 446 s for the pure FFA solution.

Fig.4 shows the images of a representative part of the quartz cell, demonstrating the morphology of the FFA crystals after tests. In cosolvent without a pre-dissolved polymer, the needle shape morphology of FFA crystals from both the FFA-NIC CO and pure FFA solution was similar. In contrast, the FFA crystals from the FFA-TP CO solution were significantly smaller and rod-shaped. In the presence of PEG in solution, the FFA crystals precipitated from the three test samples became smaller. In the presence of PVP or PVP-VA in solution, all crystals precipitated from three test solutions were a similar shape, lacking any distinctive crystal morphology.

**Effect of polymers on the FFA crystal growth in solution**

Due to the variation of the initial FFA concentrations in the seeded solutions, the desupersaturation curve is represented by the normalized value of $C_{norm}(t)$ which is the ratio of the measured FFA concentration via the initial FFA concentration in solution as
where \( C(t) \) is the measured FFA concentration at sampling time \( t \), \( C_0 \) is the initial FFA concentration in the seeded solution without adding the stock solution and \( C_{stock} \) is the FFA concentration of the stock solution.

Fig. 5 shows the desupersaturation curves of the different test samples. The gradient of a FFA desupersaturation curve is directly related to bulk growth rate of FFA crystals in solution. Without a polymer, the growth rate of FFA crystals of the FFA-NIC CO and FFA-TP CO solutions was slower than that of the pure FFA solution, indicating the coformer of NIC or TP can inhibit the growth of FFA crystals, with NIC being more effective at 12% of SSP. PEG can slightly reduce the growth rate of the FFA crystals in the pure FFA solution with 4% of SSP. In contrast, PEG reduced the inhibition ability of NIC for the growth of FFA crystals in FFA-NIC CO solution in which SSP was reduced to 1% from 12% shown in Fig. 5(c). Surprisingly, both PVP and PVP-VA were ineffective in inhibiting the growth of FFA crystals and instead accelerated the FFA crystal growth rates, indicating that the FFA concentrations in solution quickly decreased to the equilibrium solubility, shown in Figs. 5(c)-(d). With pre-dissolved PVP, the SSP dropped to -17% in the pure FFA solution, to -28% in the FFA-NIC CO solution and to -12% in the FFA-TP solution. In the presence of PVP-VA in solution, the crystal growth rates in cocrystal solutions SSP values, -27% in the FFA-NIC CO solution and -24% in FFA-TP solution, were faster than that of the pure FFA solution, SSP of -13%. DSC thermographs and images of the solids isolated from the experiments were exactly the same as that of initial seeds of FFA I, shown in Fig. S2 in the supplementary materials. However, when closely examining the FTIR data of the solids collected in Fig. 5(f), it was found that a shift of the carbonyl peak of FFA I at 1651 cm\(^{-1}\) was observed in all the experiments, suggesting that a coformer or polymer was integrated in the solids.
The overall polymer inhibition ability on maintaining FFA supersaturation

The overall effect of a polymer on inhibition of FFA crystallization from a supersaturated solution was evaluated by unseeded desupersaturation experiments in the absence or presence of 200 µg/mL of a pre-dissolved polymer of PEG, PVP or PVP-VA, as described in the previous Section. The initial FFA concentration was 100 µg/mL corresponding to SR=2.72.

Fig. 6 shows the desupersaturation curves of the different test samples. It can be seen that the FFA concentrations from different test samples decreased rapidly in the cosolvent media without a pre-dissolved polymer in Fig. 6(a). The FFA-NIC CO and FFA-TP CO solutions show a comparable performance in which the rate of desupersaturation was slower than that of the pure FFA solution. The FFA concentrations in all three test solutions were reduced to the same static level of 42 µg/mL within 2 h, which was slightly higher than its solubility. In the pre-dissolved PEG media, the decreasing rates of the supersaturated FFA concentrations in the FFA-NIC CO and FFA-TP CO solutions are significantly slower in comparison with that of the pure FFA solution, showing an increased SSP of 13.4% for FFA-NIC CO solution, 12.2% for FFA-TP and just 3.2% for the pure FFA solution in Fig. 6(b). Among the three solutions with pre-dissolved PVP, Fig. 6(c) demonstrates that PVP is the effectively inhibitor for the pure FFA solution as seen by a 15.9 % increase in inhibition of FFA. Compared with PEG, PVP has a reduced ability on maintaining FFA in either the FFA-NIC CO or FFA-TP CO solutions. PVP-VA pre-dissolved in solution can significantly reduce the rate of the FFA precipitation from both supersaturated FFA and FFA-NIC CO solutions, showing 27.4% and 26.4% increases of SSP values in Figs. (d)-(e). However, there is no difference between PVP and PVP-VA in maintaining the supersaturated FFA in FFA-TP CO solution in Fig. 6(e).
The solids precipitated from all of the experiments were yellow needle-shape FFA III crystals confirmed by the DSC results and images in Fig. S3 in the supplementary materials. The FTIR data showed that a shift of the carbonyl peak of FFA III at 1655 cm\(^{-1}\) was observed in all the experiments, suggesting that a coformer or polymer was coprecipitated in the solids in Fig. 6(f).

**IR spectroscopic investigation of intermolecular interactions among FFA, coformer and polymer in solution**

Fig. 7 shows the comparison of the solution IR spectra of individual components of FFA, NIC, TP and mixtures of FFA and coformers in the absence and presence of different polymers. In Fig. 7(a) a strong peak of FFA in methanol was found at 1686 cm\(^{-1}\), indicating C=O stretching \(^{35}\). When a component of NIC, PVP or PVP-VA was added in the solution, this FFA characteristic peak was shifted to a smaller wavelength number of 1684 cm\(^{-1}\), indicating an intermolecular interaction between them in solution. In contrast, there is no change in the FFA C=O peak in the PEG solution, suggesting no interaction between these two components. NIC can interact with FFA or PVP in solution, demonstrated by a change in the characteristic peak of NIC at 1625 cm\(^{-1}\), corresponding to N-H stretching \(^{36}\), to 1617 cm\(^{-1}\) in the presence of FFA and to 1631 cm\(^{-1}\) in the presence of PVP in Fig. 7(b). Surprisingly there is no interaction between NIC with PVP-VA or PEG in solution, confirmed by no change in the characteristic peak of NIC at 1625 cm\(^{-1}\). The IR characteristic peak of TP at 925 cm\(^{-1}\), corresponding to N-H symmetric stretching \(^{39}\), has been shifted to a lower wavenumber by inclusion of PVP or PVA-VA and to a higher wavenumber by adding PEG or FFA in solution, indicating TP can interact with any of components, FFA, PEG, PVP or PVP-VA in solution.
Discussion

Effect of a polymer on the apparent FFA equilibrium solubility of FFA I, FFA-NIC CO and FFA-TP CO in cosolvent

There is widespread acceptance that the crystalline nature of pharmaceutical cocrystals can offer advantages over amorphous materials to formulate drug compounds with limited solubility and bioavailability, due to superior thermodynamic stability and purity. Although significant advances in design and discovery have been made, little work has been conducted to formulate cocrystals into a drug product. Therefore, the behavior of a cocrystal in a formulated product is largely unknown. In order to offer the most desired in vivo performance with the highest bioavailability for many life-saving drugs with poor biopharmaceutical properties, a fundamental understanding of the critical factors that control the dissolution and absorption performance of a cocrystal formulated product is required. In this work, the focus was on understanding the parent drug crystallization kinetics from a supersaturated cocrystal solution in the presence of a polymeric excipient. It aimed to provide the mechanistic understanding of the properties of a polymer as a good inhibitor of crystallization for a given drug cocrystal. Two FFA cocrystals, FFA-NIC CO and FFA-TP CO, were chosen due to significant differences in their physicochemical properties. The low polymer concentration of 200 µg/mL used in the investigation was based on the rational consideration of a 500 mg tablet containing 250 mg of stabilizing polymer, in which 20% of the polymer was released in 250 mL of the GI tract at the beginning stage of dissolution. According to the equilibrium solubility results in Fig. 3(a), the FFA concentrations of FFA I, FFA-NIC CO or FFA-TP CO were constant in solution in the absence and presence of 200 µg/mL polymer of PEG, PVP or PVP-VA, indicating that the impact of a polymer on FFA crystallization in a supersaturated solution was not caused by a change in the level of supersaturation. Furthermore, due to the low molecular weight of the polymer used in the study, the viscosity of the 200 µg/mL polymer solution was essentially the same as that of the dissolution medium without a predissolved polymer. Therefore, the
interplay of API-coformer, API-polymer, and coformer-polymer elucidated in this study was not affected by the changes of the solution bulk properties of solubility and mass transport.

**Effect of intermolecular interactions of drug/coformer, drug/polymer and coformer/polymer on parent drug nucleation and growth kinetics in solution**

This study has clearly demonstrated that a cocrystal coformer can interfere with a polymer to alter its ability to maintain the parent drug superstation in solution. This property involves both nucleation and growth through competition of the intermolecular interactions of drug/coformer, drug/polymer and coformer/polymer in solution.

In the solid state, cocrystals are formed through hydrogen bonding between an API and coformer. Once the cocrystals are dissolved in solution, they could be regarded as completely separate individual molecules. For example, the US FDA has elected to classify cocrystals within their framework as dissociable “API-excipient” molecular complexes. However, in this study it was found that the hydrogen bonds between FFA and coformers, NIC or TP, were not broken completely, indicated by the changes in the their characteristic peaks of the solution spectra in Fig. 7(a). This API/coformer interaction in solution certainly affected the formation of nuclei by hindering the reorganization of a cluster of FFA molecules into its ordered structure. Therefore, the coformer of NIC or TP can be regarded as a nucleation inhibitor for FFA crystallization, generating slightly longer induction time (15 s for FFA-NIC CO solution and 24 s for FFA-TP CO solution) compared with the pure FFA supersaturated solution (9 s of induction time) in the absence of a polymer, as shown in Table 2.

The FFA molecule, shown in Table 1, has the very strong hydrogen bond donor of O-H combined with a middle strength acceptor of C=O, thus displaying higher hydrophobicity with a low value of $SP$ (18.62 MPa$^{1/2}$). Therefore, FFA self-association should be disrupted by a polymer with strong acceptor groups that can effectively compete with the FFA acceptor group C=O $^{40}$. Indeed, the formation of the hydrogen
bonding between the polymer of PVP or PVP-VA with FFA in solution was demonstrated by the IR spectroscopic investigation in Fig. 7(a), as both polymers (N-C=O in PVP and N-C=O and O-C=O in PVP-VA) have strong acceptors. This suggested that both PVP and PVP-VA were able to act as effective nucleation inhibitors, indicated by the significantly increased nucleation induction times at different degrees of supersaturation. A higher level of inhibition effectiveness of PVP-VA in comparison with PVP was due to the presence of carbonyl oxygens C=O on the side chain which contributed to a more hydrophobic nature and flexibility to interact with FFA molecules in solution. Therefore, evidence for a two-step mechanism of cocrystal nucleation was revealed in the presence of PVP-VA. The precipitated solids in Fig. 4 show a lack of birefringence under polarized light and no distinct particle morphology, indicating the amorphous nature of the particles was due to the integration of PVP or PVP-VA in the FFA crystal structure and/or rapid desupersaturation. The amorphous nature of the precipitated particles could be also related to liquid-liquid phase separation (LLPS) which was observed in amorphous solid dispersion systems (ASDs) in recent publications \(^{41,42}\). The high supersaturation generated by ASDs can lead to a two phase system wherein one phase is an initially nano-dimensioned drug-rich phase and the other is a drug-lean continuous aqueous phase. In those studies the stronger nucleation inhibitors PVP/PVP-VA allowed the system to reach supersaturation levels such that the system underwent LLPS. The excess drug then precipitated forming a dispersed, colloidal amorphous drug-rich phase which resulted in the absence of birefringence in the precipitated particles.

The ineffectiveness of PEG as a nucleation inhibitor was probably due to its structural rigidity in which the hydrogen acceptor, C-O-C, on the main chain had been prevented from interacting with FFA molecules in solution. Thus no change was observed in the characteristic peak of FFA in solution with the predissolved PEG (Fig. 7(a)). The limited inhibition ability of PEG may be due to the steric barrier for the formation of nuclei via the adsorption of the polymer on the surface of pre-nuclear clusters \(^{43}\). It has to be stressed that although all three polymers of PEG, PVP and PVP-VA interacted with FFA with different mechanisms, they were all integrated into the FFA crystal lattices, showing as a variation of the FFA III characteristic peak at 1655 cm\(^{-1}\), which corresponds to its C=O stretching frequency in Fig. 6(f). The
results are in good agreement with previous studies which have shown that multicomponent molecular complexes in solution lead to a metastable form precipitating preferentially. A change in the crystal morphologies, seen in Fig S3 in the supplementary materials, also supported this.

It was not surprising that the nucleation induction time was reduced for FFA-NIC CO solution in the presence of PVP compared to the pure FFA solution because the competition between NIC and FFA with PVP weakened the polymer inhibition ability. There was no interaction between NIC with PVP-VA in solution shown in Fig. 7(b). Therefore, the nucleation induction time from the FFA-NIC CO solution was almost the same as that of the pure FFA solution, in the presence of PVP-VA. As TP can interact with both polymers of PVP and PVP-VA in solution, the nucleation induction time reduced in the FFA-TP CO solution compared to the pure FFA solution in the presence of the polymers, as shown in Table 2. PEG inhibits FFA crystallization using a different mechanism in comparison with PVP or PVP-VA, for reasons outlined above. The nucleation induction time increased in both the FFA-NIC CO and FFA-TP CO solutions in the presence of PEG due to the accumulated inhibition effects of both the coformer and polymer on FFA.

In order to study the effectiveness of the polymers on inhibiting FFA crystal growth after nucleation, desupersaturation experiments were conducted including the addition of the FFA seeds. A low $SR$ of 1.27 (based on 36.6 µg/mL of the solubility of FFA I measured in this study) was used in the growth experiments to avoid secondary nucleation. It was observed that polymer effectiveness at reducing crystal growth rates was not found to have a similar impact on nucleation. In the nucleation induction time study, PVP and PVP-VA were effective nucleation inhibiting agents in the pure FFA solution. In contrast, they were poor at inhibiting growth and actually accelerated the growth of FFA crystal seeds, as seen by the negative values of $SSP$ in Fig. 5(e). It is known that the alteration of crystal growth by additives can be achieved through modifying the step speed or altering the step edge energy, which is classified as step pinning, incorporation, kink block, and step edge adsorption mechanisms. To occur, the additives must be adsorbed on the surface of the crystals to block active crystal growth sites. There are a number of interactive forces responsible for the adsorption of additive molecules on the solid surface including
electrostatic, hydrogen bonding and hydrophobic interactions. The electrostatic force was not considered because of the neutral natures of the solution and drug components used in this study.

The coformer molecules of NIC or TP in a cocrystal solution were most likely adsorbed on the FFA crystal surface due to hydrogen bonding attraction as the growth rate inhibitor. This led to moderately increased SSP values of 12% for FFA-NIC CO solution and 5% for FFA-TP CP solution, as shown in Fig. 5(e). In the pure FFA solution with the predissolved PEG, hydrogen bonding was not promoted between PEG and FFA as shown in the IR spectroscopic investigation in Fig. 7(a). Therefore, hydrophobic interaction was the main interactive force to drive PEG molecules to be adsorbed on the surfaces of the FFA crystal seeds. A large difference in their SP values in Table 1 suggests a weak interactive force between FFA and PEG in solution. It was not surprising that PEG was neither an effective FFA nucleation nor growth inhibitor. The decrease in the growth inhibition in the FFA-NIC CO solution in the predissolved PEG can most likely be the reduced NIC, being a more effective inhibitor in comparison to PEG, when adsorbed on the solid surface due to competition by PEG for the same adsorption sites. In the FFA-TP CO solution in the presence of PEG, there was no noticeable change in the extent of growth inhibition as both TP and PEG were equally effective on an individual basis shown in Fig. 5(e).

In the pure FFA solution in the presence of PVP or PVP-VA, acceleration of crystal growth occurred, indicated by the negative SSP values of -17% for PVP and -13% for PVP-VA. Similar phenomena were found in other studies when one or more surfactants were predissolved in the solution, in which it was believed that the adsorbed additives could lead to a decrease in interfacial tension to be favorable to growth. However, in this study the enhanced crystal growth was not likely to be caused by the reduced interfacial tension between the crystal and solution due to the polymer adsorption. It is known that strong intermolecular hydrogen bonding was occurring between FFA and PVP or PVP-VA in solution. When the polymer molecules were adsorbed on the surface of the FFA seeds, the bound FFA molecules were driven around the FFA seeds, leading to increase local supersaturation at the surface and contributing to the acceleration in crystal growth.
In the FFA-NIC CO solution with the predissolved PVP or PVP-VA, acceleration of crystal growth was enhanced in comparison to the pure FFA solution in the presence of the same polymer. In the FFA-TP CO solution with the predissolved PVP, acceleration of crystal growth was reduced in contrast to PVP-VA where growth was promoted. These results demonstrated that the combination of PVP or PVP-VA in the presence of either coformer (NIC or TP) can either enhance or reduce the rate of the crystal growth. Overall, the effect was to accelerate the growth. Thus rational selection of a polymer is required to enhance the inhibition ability in a cocrystal supersaturation solution.

The comparison of the overall desupersaturation profiles of three supersaturation solutions in the absence and presence of a polymer of PEG, PVP, or PVP-VA is given in Fig. 7. In the absence of a polymer, a cocrystal solution showed a better performance to maintain the FFA in solution in comparison to the pure FFA solution due to the enhanced combination effects of the nucleation and growth inhibition abilities of the coformers. In the predissolved PEG, a cocrystal solution showed an increased ability to maintain supersaturation for extended time periods, which was most likely due to the enhanced combination effects of the individual nucleation and growth inhibition abilities of the coformer and PEG. Clearly the polymer nucleation inhibition effect outweighed its growth acceleration ability for FFA in solution, indicating that the rate of desupersaturation was reduced dramatically in the presence of PVP or PVP-VA. The desupersaturation behavior of FFA cocrystal solutions in the presence of PVP or PVP-VA depends on different interaction mechanisms of the polymer and coformer and on the competition effect of the polymer and coformer for formation of hydrogen bonding with FFA molecules, in which PVP-VA was a good crystallization inhibitor for FFA-NIC CO solution.

It is worth noting that the study has shown the coformers and polymers have been integrated in the solid particles recovered from the seed and unseeded experiments based on the measured IR spectra. However, the IR data cannot quantify the relative proportion of co-former/polymer co-precipitated in the desupersaturated solids, which could be determined by solid state NMR or other techniques. In the meantime, more fundamental research is required to guide the selection of polymers in co-crystal formulation systems through understanding the parent drug crystallization kinetics.
Conclusions
Development of enabling formulations is a key stage when demonstrating the effectiveness of pharmaceutical cocrystals and is applied to maximize the oral bioavailability for poorly water soluble drugs. Inhibition of the drug crystallization from a supersaturated cocrystal solution through a fundamental understanding of the nucleation and crystal growth is important. In this study, the influence of the three polymers PEG, PVP and PVP-VA on the FFA crystallization in three different supersaturated solutions of the pure FFA and two cocrystals of FFA-NIC CO and FFA-TP CO has been investigated by measuring nucleation induction times and desupersaturation rates in the presence and absence of seed crystals. It was found that the competition of intermolecular hydrogen bonding among drug/coformer, drug/polymer and coformer/polymer was a key factor responsible for maintaining the supersaturation through nucleation inhibition and crystal growth modification in a cocrystal solution. The supersaturated cocrystal solutions with predissolved PEG demonstrated effectiveness at stabilizing supersaturated solution compared to pure FFA in the presence of the same polymer. In contrast, the two cocrystal solutions in the presence of PVP or PVP-VA did not perform as well as pure FFA with the same predissolved polymer. The study suggested that the selection of a polymeric excipient in a cocrystal formulation should not be solely dependent on the interplay of the parent drug and polymer without considering the coformer effects.

Associated Content
Supporting Information

1) Additional tables of SP values of FFA, NIC, TP, and polymers;
2) Summary of IR peak identities of FFA I, NIC, TP, FFA-NIC CO and FFA-TP CO;
3) Additional figures of the FTIR results of solid residues after the solubility testing of FFA, FFA-NIC CO and FFA-TP CO;
4) test results of solids collected after the seeded and unseeded desupersaturation experiments including DSC results and images.

Author Information
Corresponding author

*School of Pharmacy, De Montfort University, Leicester, LE1 9BH, UK. Tel: +44(0) 1122577132; E-mail address: mli@dmu.ac.uk (M. Li)

Acknowledgments
The authors would like to thank Dr. Simon Roberts from Ashland Specialty Ingredients for providing materials for this study.

References


15. Blagden, N.; Coles, S. J.; Berry, D. J., Pharmaceutical co-crystals - are we there yet? *CrystEngComm* 2014, 16 (26), 5753-5761.


40. Van Eerdenbrugh, B.; Taylor, L. S., An ab initio polymer selection methodology to prevent crystallization in amorphous solid dispersions by application of crystal engineering principles. *CrystEngComm* 2011, 13 (20), 6171-6178.


Table 1: Structure and SP values of FFA, NIC, TP, and polymers

<table>
<thead>
<tr>
<th></th>
<th>FFA</th>
<th>NIC</th>
<th>TP</th>
<th>PEG</th>
<th>PVP</th>
<th>PVP-VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular structure</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>SP (MPa(^{1/2}))</td>
<td>18.62</td>
<td>29.39</td>
<td>30.21</td>
<td>21.94</td>
<td>21.24</td>
<td>20.98</td>
</tr>
</tbody>
</table>

Table 2: Nucleation induction time

<table>
<thead>
<tr>
<th><img src="image" alt="Structure" /></th>
<th>Cosolvent</th>
<th>Cosolvent with predissolved PEG</th>
<th>Cosolvent with predissolved PVP</th>
<th>Cosolvent with predissolved PVP-VA</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>9±2 (sec)</td>
<td>176±37 (sec)</td>
<td>No crystal appeared</td>
<td>No crystal appeared</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>15±7 (sec)</td>
<td>288±172 (sec)</td>
<td>No crystal appeared</td>
<td>No crystal appeared</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>24±10 (sec)</td>
<td>218±161 (sec)</td>
<td>No crystal appeared</td>
<td>No crystal appeared</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>658±47 (sec)</td>
<td>555±93 (sec)</td>
<td>No crystal appeared</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>658±47 (sec)</td>
<td>510±166 (sec)</td>
<td>No crystal appeared</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>446±73 (sec)</td>
<td>392±93 (sec)</td>
<td>No crystal appeared</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>397±63 (sec)</td>
<td></td>
<td>No crystal appeared</td>
<td></td>
</tr>
</tbody>
</table>
Figure. 1: Illustration of supersaturation parameter
Figure 2: Characterization of solid samples: (a) XRPD patterns; (b) IR spectra; (c) DSC thermographs
(c)
Figure. 3: Solubility test results: (a) apparent equilibrium solubility; (b) DSC results of solid residues; (c) images of solid residues
<table>
<thead>
<tr>
<th></th>
<th>FFA</th>
<th>FFA-NIC CO</th>
<th>FFA-TP CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting materials</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Cosolvent</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Cosolvent with predissolved PEG</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>Cosolvent with predissolved PVP</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Cosolvent with predissolved PVP-VA</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
</tbody>
</table>

(c)
Figure 4: Images of FFA crystals after induction time tests

<table>
<thead>
<tr>
<th>S = 1.37</th>
<th>Cosolvent</th>
<th>Cosolvent with predissolved PEG</th>
<th>Cosolvent with predissolved PVP</th>
<th>Cosolvent with predissolved PVP-VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td><img src="image1.jpg" alt="Image" /></td>
<td><img src="image2.jpg" alt="Image" /></td>
<td><img src="image3.jpg" alt="Image" /></td>
<td><img src="image4.jpg" alt="Image" /></td>
</tr>
<tr>
<td>S = 2.74</td>
<td>FFA</td>
<td><img src="image5.jpg" alt="Image" /></td>
<td><img src="image6.jpg" alt="Image" /></td>
<td><img src="image7.jpg" alt="Image" /></td>
</tr>
<tr>
<td>FFA-NIC CO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA-TP CO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S=5.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA-NIC CO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA-TP CO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACS Paragon Plus Environment
Figure. 5: Seeded desupersaturation curves in the absence or presence of polymers: (a) cosolvent; (b) Cosolvent with predissolved PEG; (c) Cosolvent with predissolved PVP; (d) Cosolvent with predissolved PVP-VA; (e) Comparison of supersaturation parameters; (f) FTIR data of solids.
(f)
Figure. 6: Unseeded desupersaturation curves in the absence or presence of polymers: (a) cosolvent; (b) Cosolvent with predissolved PEG; (c) Cosolvent with predissolved PVP; (d) Cosolvent with predissolved PVP-VA; (e) Comparison of supersaturation parameters; (f) FTIR data of solids.
Figure. 7: IR spectroscopic investigation of molecular interaction in solution: (a) FFA interaction with NIC and polymers; (b) NIC interaction with FFA and polymers; (c) TP interaction with FFA and polymers