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Crystallisation inhibition fails against solution mediated solid-state transformation

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Overcoming the Common Ion Effect for Weakly Basic Drugs: Inhibiting the Crystallization of Clofazimine Hydrochloride in Simulated Gastrointestinal Media

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ABSTRACT

Bile salts, phospholipids, and digestive proteins are amphipathic compounds found naturally in the human gastrointestinal system. Therefore, it is important to consider their effects on the crystallization kinetics and solution behaviour of drugs intended for oral delivery. Supersaturating drug delivery systems that employ high energy solid forms and polymeric additives are often hailed as the gold standard for increasing drug concentration in the gastrointestinal system. However, the effects of amphiphilic compounds present in the gastrointestinal system on the crystallization behaviour of these systems are often overlooked. In this study, the effects of bile salts, phospholipids, mixtures of phospholipid and bile salts as well as digestive proteins on the crystallization kinetics of the antimicrobial agent clofazimine (CFZ) were evaluated. The crystallization inhibitory properties of these gastrointestinal amphiphiles were compared with commonly used synthetic polymers, and several of these amphipathic gastrointestinal compounds showed promise as crystallization inhibitors of clofazimine hydrochloride during induction time experiments. The best crystallization inhibitors from this induction time screening were then compared as solid physical mixtures in modified fasted state simulated gastric fluid (m-FaSSGF). Here it was found that heterogeneous nucleation of CFZ hydrochloride onto the dissolving surface of CFZ solid forms prevented these additives from inhibiting crystallization in this biorelevant media. This heterogeneous nucleation of CFZ hydrochloride was monitored in real time, using optical microscopic techniques.
INTRODUCTION

There is an ever increasing number of poorly water-soluble active pharmaceutical ingredients (APIs) coming through the drug development pipelines. This trend toward more hydrophobic drug substances has led to greater selectivity and potency of APIs, but at the expense of physiochemical properties such as solubility and dissolution rate [1], [2]. These properties, particularly intestinal solubility, are strongly correlated with the intestinal absorption of orally deliverable medicines and are therefore often the rate-limiting step in the absorption of these APIs. Factors which can impact the concentration of an API at the site of drug absorption, in particular, dissolution rate and crystallization kinetics can become tuneable parameters when designing oral dosage forms to optimize their bioavailability [3]. To improve the bioavailability of hydrophobic APIs, formulation scientists have developed various strategies to manipulate solubility in the gastrointestinal system. Briefly these include; (i) solubilising strategies (such as formulation with cosolvents and/or surfactants as well as inclusion into lipid-based formulations) [4], (ii) partial size reduction strategies (such as top down or bottom up manufacture of nanoparticles) [5], [6] and (iii) supersaturating drug delivery systems (SDDS), such as amorphous solids, crystalline salts, cocrystals, etc. [7]–[11]. Along with concentration in the gastrointestinal system, good permeability is a requirement for adequate intestinal absorption. Permeation through the gastrointestinal membranes is thought to be improved by increasing the concentration of molecularly dissolved drug [12], [13]. Thus formulations that produce supersaturated solutions of API have significant potential to enhance oral absorption.

However, such supersaturated solutions of API are inherently unstable and result in a loss of solution concentration through crystallization. Therefore maintaining supersaturation levels of an API in the human gastrointestinal system is essential to enhance the permeability of the API.
Crystallization from solution occurs via nucleation (formation of an initial solid) and growth (expansion of that solid). Most previous studies have focused on using polymeric additives, such as cellulose derivatives, to prolong supersaturation by inhibiting nucleation [14]–[17]. However, recently several studies have demonstrated that bile salts can also act as crystallization inhibitors for hydrophobic APIs [3], [18]–[21]. A recent systemic study of the effects of bile salts, phospholipids, and digestive peptides, on the solution concentration of the antibiotic agent clofazimine (CFZ), showed that the presence of these amphipathic compounds has a strong influence on solution behaviour i.e. stability and concentration [22]. These findings suggest that compounds present in the gastrointestinal system have solubilizing and/or crystallization inhibitory potential, which could benefit supersaturating drug delivery systems for hydrophobic APIs. In this study, we examined the effects of phospholipids, bile salts and the digestive protein pepsin on the crystallization behaviour of CFZ.

CFZ is a weakly basic drug, with a pKa of 8.511 [23]. In its free base form (CFZB), the API is practically insoluble in neutral, aqueous media with solubility reported to be < 0.01 mg/L [24]. CFZ is marketed as an oily suspension of the free base form of the API, called Lamprene® [25]. This formulation is usually administered as 50 mg capsules, which give erratic bioavailability, 45 – 60 %, following oral administration in the fasted state, although administration with food can improve bioavailability by reducing the time to reach peak plasma concentration from 12 h to 8 h [25]. The extent to which CFZ dissolves is pH dependent and can be calculated from experimentally derived solubility-pH studies [22]. Being weakly basic and ionisable, CFZ readily forms pharmaceutical salts with acidic coformers [11]. While CFZB exhibits very poor aqueous solubility, salt forms can increase solution concentration in water, and in biorelevant media [11]. However, in low pH media, solution concentration and solution stability of the protonated CFZH⁺
species is reduced through the common ion effect [22]. The concentrations of CFZ that can be
reached in biorelevant media are ~30 mg/L for modified-fasted state simulated gastric fluid (m-
FaSSGF - pH 1.6) and ~6 mg/L for modified-fasted state simulated intestinal fluid (m-FaSSIF -
pH 6.5). However, the solution concentration of CFZ in m-FaSSGF and m-FaSSIF is significantly
reduced in the absence of the phospholipids, bile salts and the digestive proteins present in these
buffers. The effect of these amphipathic gastrointestinal compounds on the solution behaviour of
CFZ is best described in previous studies [22], [26]. Briefly, CFZ molecules are taken up into bile
salt/phospholipid mixed micelles in m-FaSSIF, thus solubilising the neutral CFZ molecules. While
in m-FaSSGF, CFZ binds to the digestive enzyme pepsin, which acts as a molecular carrier for the
API and prevents the precipitation of CFZ hydrochloride from the low pH solution (common ion
effect) [22], [26].

The common ion effect ultimately drives protonated CFZ molecules in solution to crystallization
as a hydrochloric acid salt, CFZ hydrochloride. This phenomenon is not unique to CFZ, but rather
the common ion effect is a challenge encountered for many crystalline salt forms of weakly basic
APIs in low pH environments, where crystallisation occurs as a chloride salt, and for salt forms of
weakly acidic APIs in high pH environments, where crystallisation occurs as a sodium salt [8],
[27]–[29]. For example, the weakly basic API promethazine exhibits the common ion effect at low
pH, resulting in a reduction of solution concentration and crystallization as promethazine
hydrochloride [30]. Serajuddin et al. reported a similar finding for a sodium salt of an acid drug
(REV 3164; 7-chloro-5-propyl-1H,4H-[1,2,4]triazolo[4,3-a]quinoxaline-1,4-dione), where the
solubility of the drug in water decreased upon the addition of NaCl [31]. However, the common
ion effect could potentially be overcome by formulation with a crystallization inhibitor, which
could slow down the rate of precipitation from solution.
In this study, solid forms of CFZ were expected to dissolve in the low pH gastric environment and then precipitate from solution as CFZ hydrochloride, through the common ion effect. CFZ hydrochloride does not dissolve to any detectable extent in the presence of chloride ions, even at pH values where other salt forms of CFZ display solubility [11]. Such solid-state transformations of APIs into less soluble solid forms can compromise solution concentration in vivo and potentially bioavailability. The aim of this study was to develop a novel solid dose formulation of a CFZ salt, with a crystallization inhibitor, to prolong supersaturation in FaSSGF. A panel of potential crystallization inhibitors was screened through crystallization induction time experiments of CFZ hydrochloride where supersaturation was induced by increasing chloride concentration in the media, to mimic the common ion effect. Potential crystallization inhibitors screened included: the synthetic polymers PVP and HPMC, and the gastrointestinal (GI) components; bile acids, sodium taurocholate, and porcine bile extract, as well as lecithin (a phospholipid extract) and pepsin (a digestive enzyme), Figure 1. The crystallization inhibitory properties of these compounds in biorelevant dissolution media were then examined as solid physical mixtures with CFZB and CFZ phosphate.
**Figure 1:** Chemical structure of compounds involving in induction time experiments. Chemical structures of CFZB, CFZ hydrochloride, and CFZ phosphate are shown. *Porcine bile extract is primarily composed of glycine or taurine conjugates of hyodeoxycholic acid, but also contains various bile salts.*
MATERIALS AND METHODS:

Materials. Clofazimine (FI, triclinic polymorph, Cambridge Crystallographic Data Centre Ref code: CCDC 1135856) was purchased from Beijing Mesochem Technology Co., Ltd. Hydrochloric acid, orthophosphoric acid, methanol, tetrahydrofuran (THF), L-α-phosphatidylcholine (lecithin, from egg yolk), pepsin (from porcine gastric mucosa) were obtained from Sigma Aldrich (Ireland). Sodium chloride and sodium taurocholate hydrate (NaTc) were obtained from Fisher Scientific (Ireland). Hydroxypropyl methylcellulose (HPMC, 4000 mPaS, Harke Pharma) and polyvinylpyrrolidone K 30 (PVP, average Mw 40,000, Sigma Aldrich) were used as received from suppliers.

Methods.

Preparation of clofazimine salts. CFZ salts were prepared by recrystallization of CFZ and coformer in a 1:1 molar ratio from methanol, as previously reported [11], [22].

Solubility of clofazimine hydrochloride. The experimental setup for the solubility measurements consisted of a thermostatic water bath, equipped with a cooling unit, a magnetic stir plate and a submersible water pump to enhance circulation in the bath. An excess of clofazimine hydrochloride was added to solutions of water or water with additives dissolved to 0.005 %. These additives included NaTc, PBE, lecithin, PBE and lecithin (4:1), NaTc and lecithin (4:1), PVP, HPMC and pepsin (0.1 mg/mL rather than 0.005 %). These suspensions were then placed in the water bath at 37°C and stirred at 400 rpm for 24h. Following this, the solutions were filtered (using preheated syringes and syringe filters) and appropriately diluted for solubility measurement on a UV-vis spectrophotometer. All solubility measurements were carried out in triplicate.
**Measurement of crystallisation induction times.** Experimental induction time, $t_{ind}$, is defined as the sum of the time taken for a critical nuclei formation to occur (true nucleation time, $t_n$) and the time is taken for nuclei to grow to a detectable size, $t_g$ [32].

$$t_{ind} = t_n + t_g$$ (1)

Undersaturated solutions of CFZH$^+$ (40 mg/L) were prepared by dissolving CFZ hydrochloride, (Cambridge Crystallographic Data Centre Ref code: CCDC 1053718), or CFZ phosphate, (Cambridge Crystallographic Data Centre Ref code: CCDC 1559558), in water or in the presence of additives (0.005 % of either NaTc, PBE, lecithin, PBE and lecithin (4:1), NaTc and lecithin (4:1), PVP and HPMC or 0.1 mg/mL solutions of pepsin) in 250 mL Duran flasks, using ultra-pure (Milli-Q) water. These solutions were prepared at 37°C on a submersible magnetic stir plate at 400 rpm using PTFE coated magnetic stir bars (Bath A). 200 mL of this stock solution was filtered into 10 x 20 mL glass vials using either a PTFE or cellulose acetate syringe filter (25 mm, 0.2 µM). A magnetic stir bar was added to each vial before capping with a plastic screw cap, lined with PTFE. Vials, stir bars, screw caps, syringes, needles and syringe filters were preheated to 50°C prior to filtration. Solutions were then subjected to a second equilibrium period of 1 h at 60°C (Bath B); before crystallization experiments were conducted (solutions containing pepsin were stirred at 40°C due to the thermal instability of the protein). Following the second equilibrium period, vials were transferred to a third water bath (Bath C) which was set to 37°C and 800 rpm. Once added to Bath C, 0.5 mL of each solution was discarded, leaving a volume of 19.5 mL in each vial. Supersaturation was generated by pipetting 0.5 mL of 1 M NaCl (heated to 37°C) into each vial containing 19.5 mL of the stock solution while stirring at 800 rpm (resulting in a final concentration of NaCl of 25 mM). Crystallisation was detected using a high definition camcorder (Sony HDRXR520VE), similar to that reported previously by Mealey et al [33]. Bath C was fitted with a strip of white ultra-bright LED lights (12V IP65 Waterproof, Qbspring) to increase contrast.
and aid in the detection of crystals and the 10 vials were positioned directly in front of the light strip. Once nucleation occurred, a cloudy purple/black residue was observed in the solution along with a loss of the characteristic red colour of the CFZH\textsuperscript{+} solution species. Soon after nucleation, the solution in the vial would become completely colourless, Scheme 1. This process was repeated with a fresh stock solution in each case, giving a total of 20 data points for each additive investigated during induction time experiments.

**Data Analysis for induction time experiments.** Where necessary, differences in the average induction time data were compared by means of a \( t \)-test. A one-way between-subjects ANOVA test was conducted using OriginPro 8 SR1 and the ANOVA statistical analysis package to compare the data (Tukey HSD test). Differences were considered significant at the 0.05 level.

**Preparation of m-FaSSGF for dissolution experiments.**

Modified FaSSGF was prepared consisting of pepsin (0.1 mg/mL), sodium taurocholate (80 \( \mu \)M), lecithin (20 \( \mu \)M), sodium chloride (7.2 mM) and hydrochloric acid (25 mM), as previously published [11], [22]. Due to the instability of the protein, pepsin was only added to the solution 30 mins before dissolution experiments began.

**Dissolution experiments of CFZ solid forms and potential crystallisation inhibitors as physical mixtures.** Dissolution experiments were conducted in 100 mL Duran flasks containing PTFE stir bars and 100 mL of m-FaSSGF. Solutions were stirred at 150 rpm on a submersible stir plate in a water bath at 37°C. An excess of a CFZ solid form (20mg, particle size distribution of 63 - 90 microns) and 5 mg of either NaTc, PBE, PBE and lecithin (4:1), NaTc and lecithin (4:1), or HPMC was added to the dissolution medium and the flask was inverted several times. Samples were then withdrawn at predetermined time intervals using preheated (40 °C) 5 mL syringes and
hypodermic needles and then filtered using preheated (40 °C) PTFE or cellulose acetate syringe
filters (0.2 microns, 25 mm diameter, Fisher Scientific). CFZ concentration was then immediately
determined using a double beam UV-vis spectrophotometer (Shimadzu, UV-1800) at a detection
wavelength of 488 nm.

**Growing CFZB single crystals for optical microscopy experiments.** Single crystals of CFZ F I
were grown in THF by solvent evaporation crystallization. Saturated solutions of CFZ F I in THF
were prepared at 37°C in 20 mL glass vials containing an excess of solute (FI) and agitated at 800
rpm over a 24 h period. These saturated solutions were then filtered using preheated PTFE syringe
filters (25 mm, 0.2 µM) into clean, preheated vials. The solution was allowed to cool at to room
temperature and the top of the vial was covered with aluminium foil into which several pin holes
were made. Single crystals were harvested after cooling and evaporation of one week.

**Optical microscopy experiments to monitor solution-mediated transformation of CFZB into
CFZHCl.** An inverted light optical microscope (Olympus IX53) integrated with Olympus SC100
camera combined with a PC with image/video capture using Olympus Stream Essentials software was
used to monitor the transformation of CFZ F I (CFZB) into CFZ hydrochloride. Briefly single crystals
of CFZ F I were placed on a glass slide. A drop of 25 mM HCl was added onto the single crystal using
a 10 µL micropipette. Micrographs were then obtained at various time points following the addition of
the HCl to monitor the transformation.

**Slurry experiments to monitor solution-mediated transformation of CFZB into CFZHCl.** 50
mg of CFZ F I was added into 20 mL of 25 mM HCl in 25 mL glass vials. These vials contained
PTFE magnetic stir bars and were placed on a submersible magnetic stirring in a temperature
controlled water bath at 37°C and 400 rpm. Samples were removed, filtered and characterized via either SEM or PXRD at various time points.

**Scanning electron microscopy (SEM).** SEM imaging was performed using a JEOL CarryScope scanning electron microscope JCM-5700. Samples were mounted on aluminium stubs with carbon tape tabs and coated by an ultrathin gold layer prior to analysis, using a gold sputterer (EMITECH K55) and the particles were imaged at a voltage of 5 kV.

**Powder X-ray Diffraction (PXRD).** Reflection PXRD was performed using an Empyrean diffractometer (PANalytical, Phillips) with Cu Kα₁,₂ radiation (γ = 1.5406 Å) operating at 40 kV and 40 mA and at room temperature. Samples were scanned from 4º to 35º (2θ) with 0.0131º (2θ) step size and 48.195 seconds per step, on a flat stage that was spinning at 4 rpm.

**RESULTS AND DISCUSSION:**

The common ion effect is a problem for poorly soluble ionisable APIs because where these API form sodium or chloride salts, the common ion effect causes them to crystallize out of solution in the presence of Cl⁻ and Na⁺ ions [8], [31], [34]–[36]. The resulting chloride or sodium salt forms of the drug are practically insoluble in solutions that contain their respective counterions. This is a problem for oral delivery, as the gastrointestinal system has a high NaCl concentration. Furthermore, if alternative salt forms of these APIs are prepared, they will dissolve in the presence of these chloride or sodium ions, only to rapidly crystallize out of solution as the poorly soluble sodium or chloride salt forms. The common ion effect for CFZ in biorelevant media was previously highlighted in a recently published study by our group [11]. In this study, several salt forms of CFZ were prepared, of which CFZ phosphate showed the greatest improvement in solution behaviour but as described above, in FaSSGF, it crystallized from solution as CFZ hydrochloride.
Solution Mediated Transformation of CFZB (F I) into CFZ hydrochloride. When an excess of CFZB or any salt form of CFZ is added to low pH chloride media, e.g. FaSSGF, the residual solid remaining after the dissolution experiments is primarily the CFZ hydrochloride salt [11], [24]. This crystallization of CFZ from solution is driven by the common ion effect and results in the eventual conversion of the entire solid administered into CFZ hydrochloride. This solution-mediated transformation was monitored here over time by adding CFZB to 25 mM HCl and removing solid samples at various time points for analysis via PXRD, Figure 2. From this diffractogram, it can be seen that the characteristic peaks of CFZB lose intensity as the slurry experiment continues. This loss of the CFZB form occurs simultaneously with the formation and growth of CFZ hydrochloride peaks, with pure CFZ hydrochloride removed from the slurry following 24 h.
Figure 2: Solution mediated transformation of CFZB (F I) into CFZ hydrochloride. Comparison of the PXRD patterns obtained from samples of CFZB (F I) in 25 mM HCl at the various ageing time, with pure CFZB (F I) and the CFZ hydrochloride salt.
Screening the impact of polymers, bile salts and pepsin on the crystallization of CFZ hydrochloride in conditions of high chloride concentration. The driving force for the common ion effect for weakly basic ionisable APIs is an increase in counterion concentration, which shifts the solubility equilibrium of the dissociated reaction to favour crystallization of the solid salt form of the API. The use of synthetic polymers, and recently of bile salts, to inhibit the crystallization of hydrophobic APIs is well described in the literature [3], [14]–[17], [21], [37], [38]. Induction time experiments were employed here to identify compounds which could slow down this crystallization of CFZ hydrochloride from solution. The panel of potential crystallization inhibitors screened included PVP and HPMC, lecithin, NaTc, porcine bile extract and porcine pepsin, Figure 1. To mimic the common ion effect observed in the human gastric system, supersaturation was generated by titrating NaCl into aqueous solutions of CFZ hydrochloride, to a final sodium chloride concentration of 25 mM (Scheme 1). As previously mentioned CFZ hydrochloride does not dissolve to any detectable extent in 25 mM NaCl (detection limit 0.1 mg/L), thus traditional supersaturation ratios were not employed in this study. However, the level of supersaturation was maintained in the presence of the different additives, as the thermodynamic solubility of CFZHCl remained unchanged in the presence of 0.005 % additives (0.1 mg/mL for pepsin) compared to CFZHCl in deionised water only (supplementary information), the concentration of CFZH⁺ in solution at the beginning of the induction time experiments was always 40 mg/L and the amount of NaCl used to generate supersaturation remained constant.

During these experiments, induction times were detected by a loss of the characteristic deep red colour of aqueous solutions of CFZH⁺. Following crystallization of CFZ hydrochloride, the solution begins to lose colour, turning a pale cloudy pink, with specks of black CFZ hydrochloride salt forming in the solution, Scheme 1. The resulting induction time plots obtained for CFZ hydrochloride in the presence of these compounds are compared in Figure 3.
Scheme 1: Summary of induction time procedure; (1) Undersaturated solutions of CFZ solid forms (CFZ hydrochloride or CFZ phosphate) were prepared in the presence or absence of potential crystallization inhibitors; (2) 0.5 mL of 0.1 M NaCl was titrated into these solutions to induce supersaturation through the common ion effect; (3) these vials were stirred in front of a high definition camcorder to detect the time of crystal formation (induction time).
Figure 3: Summary of the induction time studies carried out for CFZ hydrochloride in the presence or absence of potential crystallization inhibitors. (a) % of solutions crystallized plotted versus induction time (log scale) and (b) average induction times compared as a bar chart.

The potential crystallization inhibitors screened here (Figure 1), had varying effects on the induction times of CFZ hydrochloride, Figure 3. In the presence of NaTc, PBE, NaTc and lecithin (4:1), PBE and lecithin (4:1) or HPMC, the average induction time of CFZ hydrochloride was
increased from 53 ± 16 s (in the absence of inhibitors) to values of 309 ± 87 s, 666 ± 246 s, 190 ± 75 s, 423 ± 156 s and 1955 ± 896 s respectively, Figure 3b. This indicates that, in the presence of these additives, the crystallization of CFZ hydrochloride from solution was slowed down. Conversely, in the presence of lecithin, PVP and pepsin, changes in the average induction time were not as obvious, with average induction times of 82.68 ± 31 s, 65 ± 25 s and 92 ± 36 s afforded respectively. Due to the partial overlap of these induction time plots (Figure 3a) and considering the stochastic nature of nucleation, statistical analysis was used to compare these average induction times. Here a significant difference in the presence of lecithin as well as in the presence of pepsin was observed at the p < 0.05 level, Supplementary Information. However, in the presence of PVP, there was no significant difference in average induction time compared to in its absence, Supplementary Information. This indicates that PVP does not have any effect of the crystallization of CFZ hydrochloride. The ability of the compounds screened here to inhibit the crystallisation of CFZ hydrochloride from solution followed the order: HPMC > PBE > PBE + lecithin > NaTc > NaTc + lecithin > pepsin > lecithin > PVP. Interestingly, each of the components of FaSSGF has an effect on the crystallization of CFZ hydrochloride from chlorine-rich media. This complements our previous study which shows how solution concentration and stability of CFZH+ at pH 1.6 (in 25 mM HCl) depends on the presence and concentration of NaTc, lecithin, and pepsin in the system [22]. These results explain that the underlying mechanism for this manipulation of solution behaviour was through interference with the crystallization kinetics of CFZ hydrochloride from these solutions, with the exception of pepsin. The interference of pepsin with the crystallization of CFZ is more likely through a protein-drug binding interaction, which is discussed in a previous study [26].
The observation that NaTc and porcine bile extract (a mixture of bile salts) can prolong the supersaturation of CFZH\(^+\), also correlates well with previous studies conducted by Chen et al., Li et al. and Lu et al. [3], [18]–[21], which showed how the crystallization of hydrophobic drugs can be slowed and in some cases prevented by the presence of NaTc and other bile acids [3], [18]–[21]. The enhanced induction time observed here in the presence of the bile salt mixture, PBE, compared to NaTc alone (Figure 3b) is consistent with a study by Lu et al., where it was noted that mixtures of different bile salts, namely cholate and deoxycholate, were more effective at preventing crystallization from solution compared to a solution of only taurocholate [21]. Likewise, Lu et al. observed that mixtures of bile salts and lecithin (either cholate and deoxycholate with lecithin, or cholate and chenodeoxycholate with lecithin or FaSSIF), showed a lower ability to prevent the nucleation of supersaturated solutions of telaprevir compared to systems with single bile salt or mixtures of bile present [21]. Here it was also observed that the solutions of a single bile salt (NaTc) are better at inhibiting the crystallization of CFZ hydrochloride compared to the combination of lecithin and NaTc (Figure 3b). A mixture of bile salts (PBE) is better at inhibiting the crystallization of CFZ hydrochloride compared to NaTc alone (Figure 3b) but similar to NaTc alone, the ability of PBE to inhibit nucleation is diminished in the presence of phospholipids (Figure 3b). In general, in the literature, bile salts are reportedly able to prolong the lifetime of supersaturated solutions of hydrophobic drugs, and this ability is somewhat diminished in the presence of phospholipids, through the formation of mixed micelles \textit{in situ}. Thus, the presence of phospholipids in the gastrointestinal system, and their prevalence to form mixed micelles with bile salts may limit the effectiveness of bile salt based crystallization inhibitors for supersaturating drug delivery systems \textit{in vivo}. 
Dissolution experiments of CFZ solid forms and potential crystallization inhibitors as physical mixtures in m-FaSSGF. The most promising crystallization inhibitors from the induction time screening (NaTc, PBE, NaTc + lecithin, PBE + lecithin and HPMC), were then analyzed as physical mixtures with the solid forms CFZB or CFZ phosphate in m-FaSSGF, to assess their effectiveness as solid dosage forms. From the resulting dissolution profiles (Supplementary Information), values for the initial dissolution rate ($D_{\text{initial}}$), area under the curve (AUC) and maximum solution concentration ($C_{\text{max}}$) were obtained, Figure 4. No improvement in $C_{\text{max}}$ was observed in the presence of any of the compounds added as physical mixtures, compared with the respective CFZ solid forms alone Figure 4a. However, AUC was marginally increased for CFZB in the presence of HPMC and for CFZ phosphate in the presence of PBE and lecithin (4:1), Figure 4b. This marginal increase in AUC is likely a result of the crystallization inhibitory properties of these compounds identified during induction time screening, Figure 3. For CFZB, $D_{\text{initial}}$ also remained largely unchanged, with a small increase in the presence of the additive of NaTc and lecithin (4:1), Figure 4c. For the faster dissolving phosphate salt, the addition of any of these additives significantly reduced $D_{\text{initial}}$ in m-FaSSGF, Figure 4c.
Figure 4: Summary of the solution behaviour of CFZB and CFZ phosphate in m-FaSSGF as solid physical mixtures with crystallization inhibitors. (a) impact on maximum solution concentration ($C_{\text{max}}$); (b) impact on area under the curve (AUC); (c) impact on initial dissolution rate ($D_{\text{initial}}$).
Monitoring of the heterogeneous nucleation of CFZ hydrochloride onto the dissolving surface of CFZB. Given that NaTc, PBE, NaTc + lecithin, PBE + lecithin and HPMC each prevented the crystallization of CFZ hydrochloride during the induction time studies (Figure 3), an improvement in solution behaviour was expected during the dissolution experiments of their physical mixtures. However, this was not observed, and none of the potential crystallization inhibitors provided a significant advantage as physical mixtures in m-FaSSGF compared to the respective solid forms of CFZ administered alone, (Supplementary Information). Upon further analysis, it became clear that the cause of this failure as physical mixtures was the result of a solution mediated solid-state transformation into the CFZ hydrochloride salt. When particles of CFZB were removed from solution at the end of dissolution experiments they were found to have a black colour, typical of the CFZ hydrochloride salt, while the inside of these black particles retained the bright red colour, characteristic of CFZB, Supplementary Information. This solution-mediated transformation of CFZB into CFZ hydrochloride was further investigated using SEM and optical microscopy techniques. CFZB and CFZ hydrochloride crystals are easily distinguishable by their respective morphologies using SEM. CFZB can be seen to have a flat block-like morphology, while the CFZ hydrochloride salt has a characteristic a thin flake-like morphology, Figure 5. When CFZB was added to 25 mM HCl, shaken for 15 mins, filtered and dried, the resulting dried crystals showed evidence of CFZB converting into CFZ hydrochloride through heterogeneous nucleation of CFZ hydrochloride occurring on the surface of the free base, Figure 5.
Figure 5: SEM images obtained from samples of CFZB extracted from 25 mM HCl, showing the conversion of CFZB into CFZ hydrochloride occurring on the surface. Pure CFZ hydrochloride is shown for reference.

Highlighted by a red border in Figure 5 is a region of the CFZB crystal, where circular arrangements of CFZ hydrochloride crystals have nucleated onto the surface of the free base. As CFZB dissolved in 25 mM HCl, the surface of the dissolving crystals appears to provide a template onto which the HCl salt can nucleate and grow. From these initial nucleation sites, CFZ hydrochloride then grows to expand and cover the entire surface of the CFZB crystals.
Figure 6: Microscopic analysis of CFZB conversion into CFZ hydrochloride; (1) single crystal of CFZB (FI), (2) 25 mM HCl was dropped onto the CFZB single crystal and (3) conversion into CFZ HCl monitored by the passage of polarized light through the crystal.

Following the addition of 25 mM HCl to the surface of the CFZB single crystal, small rough purple regions were seen to begin to form on the CFZB surface, Figure 6. These regions slowly grew to occupy more of the CFZB surface and within 1 h of exposure to the HCl solution, the entire surface of the crystal was coated in CFZ hydrochloride salt, Figure 6. This coating can be seen as a dark purple layer, which prevented the passage of light through the crystal. It is assumed that the 25 mM HCl added to the surface of CFZB dissolves a layer of CFZB. The high chloride concentration of the 25 mM HCl solution then provides a high driving force for CFZH\(^+\) in solution to crystallize as CFZ hydrochloride. The rough surface of the CFZB created by the dissolving free base provides a surface onto which the heterogeneous nucleation can occur. From these initial nucleation sites, CFZ hydrochloride expands to cover the entire surface of the CFZB crystal. Eventually, the entire sample, not only the surface, will be converted to the CFZ hydrochloride salt as was observed from the slurry experiments, Figure 2. The same is true for the solid phosphate salt exposed to 25 mM HCl (Supplementary Information). This heterogeneous nucleation of CFZ hydrochloride on crystals of CFZB explains why the crystallization inhibitory properties of NaTc, PBE, NaTc +
lecithin, PBE + lecithin and HPMC observed during induction time experiments are not seen when these compounds were added as physical mixtures. Although these compounds could successfully prevent primary nucleation in the induction time experiments, where everything was in solution, they appear unable to prevent this heterogeneous nucleation mechanism from occurring.

The vast majority of publications that deal with the development of supersaturating drug delivery systems of BCS class II APIs compare the results of their studies with existing formulations under sink conditions, generally to compare dissolution rates. Although the dissolution rates of BCS class II compounds are strongly correlated with their absorption rates in the gastrointestinal system, many APIs which exhibit poor solubility require high doses of the API. For example, in the case of CFZ, 50 mg of the API is administered as an oily emulsion in one dose. Supersaturated solutions of API are intrinsically unstable and the presence of some undissolved solid in the gastrointestinal system could provide a surface onto which heterogeneous nucleation could occur, thereby limiting the effectiveness of such systems. The results of this study would be very different had we chosen to compare CFZ salts and crystallization inhibitors under sink conditions, given that the crystallization inhibitors identified were able to prevent primary nucleation. This study highlights the importance of analysing the solid-state of the formulation during dissolution analysis, to uncover possible solid-state transformations or possible heterogeneous nucleation occurring in situ.
CONCLUSIONS

High ion concentrations in the GI tract and the common ion effect are limiting factors for the oral delivery of many ionizable drugs which exhibit very poor water solubility. This phenomenon ultimately results in a high driving force for the crystallization of an API from solution in either low pH or high pH media, depending upon the physiochemical properties of the API. Formulation of an API with a crystallization inhibitor should be sufficient to slow desupersaturation and temporarily prevent the crystallization of the poorly soluble salt. Such crystallization inhibitors are often included in supersaturating drug delivery systems as effective methods of increasing GI solubility, and consequently bioavailability, provided that supersaturation is maintained long enough for an increase in API absorption across the epithelium cells in the GI tract. In this study we have identified several compounds which are able to inhibit the crystallisation of CFZHCl to various extents; these include various amphipathic gastrointestinal compounds, such as NaTc, PBE, lecithin, pepsin and mixtures of lecithin and bile salts as well as the synthetic polymers HPMC and PVP. The ability of these compounds to stabilize supersaturated solutions of CFZ in biorelevant media is compromised by heterogeneous nucleation of the poorly soluble salt onto the dissolving supersaturating solid form of the API. In m-FaSSGF, a hydrochloric acid salt form of CFZ nucleates onto the dissolving solid form, coating the dissolving particles in an insoluble layer of the hydrochloric acid salt. Thus of the compounds screened a novel solid dose formulation of a CFZ salt, with a crystallization inhibitor, which might prolong supersaturation in FaSSGF was not developed. However, this study also demonstrated how components present in the human GI system have a natural tendency to impact the crystallisation kinetics and temporarily stabilize supersaturated solutions of the hydrophobic drug CFZ. The presence of bile salts and phospholipids in the human gastrointestinal system may indeed provide a viable option for
crystallisation inhibition in supersaturating drug delivery systems, but in the case of ionisable
drugs which exhibit poor solubility and are subject to the common ion effect, such as clofazimine,
solution mediated solid-state transformations can negate the crystallisation inhibition properties of
additives and/or endogenous components of the GI tract.
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ABBREVIATIONS:

API, active pharmaceutical ingredient; CFZ, clofazimine; CFZB, clofazimine free base; FaSSGF, fasted state simulated gastric fluid; m-FaSSGF, modified fasted state simulated gastric fluid; FaSSIF, fasted state simulated intestinal fluid; HPMC, hydroxypropyl methylcellulose; NaTc, sodium taurocholate; PBE, porcine bile extract; PTFE, polytetrafluoroethylene; PXRD, powder X-ray diffraction; PVP, polyvinylpyrrolidone; SEM, scanning electron microscopy; SDDS, supersaturating drug delivery systems; THF, tetrahydrofuran.

SUPPLEMENTARY INFORMATION:

Table of results from statistical analysis of average induction times; solubility of CFZHCl in the presence of crystallisation inhibitors; dissolution profiles from physical mixtures of CFZB or CFZ phosphate with additives; photographs of CFZB removed from dissolution media; SEM images of phosphate exposed to 25 mM HCl.
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Crystallization of Aqueous Suspensions of Celecoxib Amorphous Solid Dispersion Spray 
Overcoming the Common Ion Effect for Weakly Basic Drugs: Inhibiting the Crystallization of Clofazimine Hydrochloride in Simulated Gastrointestinal Media

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Synopsis

The presence of crystallisation inhibitors, for example, polymers, bile salts and phospholipids, in gastrointestinal media can inhibit crystallisation in supersaturating drug delivery systems. However, in the case of ionisable drugs which exhibit poor solubility and are subject to the common ion effect, such as clofazimine, solution mediated solid-state transformations can negate their crystallisation inhibition properties.