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Maintaining Supersaturation of Active Pharmaceutical Ingredient Solutions with Biologically Relevant Bile Salts.

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ABSTRACT

Currently, it is of interest to improve the oral absorption of poorly water soluble therapeutic agents using supersaturating formulations. Understanding crystallization kinetics of supersaturated drug solutions is central to the design and evaluation of such formulations. Bile salts have drawn increasing attention in this context as they serve important roles in biorelevant dissolution media, *in vivo*, and have been shown to slow down the crystallization of active pharmaceutical ingredients. The goal of this study was to evaluate the impact of bile salt monomers and micelles on the crystallization of telaprevir, a poorly water soluble drug, from aqueous solution. To better describe the crystallization driving force in the presence of the bile salts, a side-by-side diffusion cell was used to evaluate telaprevir mass flow rate, and hence solute activity, in the absence and presence of different bile salts. The effectiveness of monomeric and miceller bile salts as crystallization inhibitors was then evaluated by performing crystallization induction time experiments at constant, activity-based supersaturation. The six most abundant biologically relevant bile salts were investigated (sodium taurocholate, sodium taurodeoxycholate, sodium taurochenodeoxycholate, sodium glycocholate, sodium glycodeoxycholate, and sodium glycochenodeoxycholate). All six bile salts exhibited nucleation inhibition properties in both homogenous supersaturated telaprevir solutions and highly supersaturated telaprevir solutions containing a second phase. The ability to retard telaprevir nucleation, however, varied amongst the bile salts and also depended on the aggregation state. Monomeric bile salts were found to be effective crystallization inhibitors. At higher bile salt concentrations, trihydroxy bile salts showed better inhibition compared to dihydroxy bile salts. These results highlight the importance of considering the composition of the test medium used to evaluate product performance, in particular in the context of evaluating crystallization kinetics.

Key words: bile salts; crystallization; supersaturation

INTRODUCTION

With an increasing number of poorly water soluble compounds in pharmaceutical developmental pipelines,[1, 2] there has been considerable focus on formulation strategies that elevate compound solubility without decreasing the apparent permeability across the gastrointestinal (GI) membrane. In general, this involves utilization of a higher energy state of the compound,[3, 4] such as an amorphous solid dispersion (ASD) formulation. In this instance, a highly supersaturated solution is created upon dissolution, where the resulting concentration gradient then facilitates absorption of the drug across membranes. Due to its unstable thermodynamic nature, supersaturated solutions tend to crystallize and lose the enhanced solubility advantage. Thus, the key determinant dictating the success of these strategies is the ability to maintain supersaturation and avoid crystallization over biologically relevant timeframes, thus allowing drug transfer across the GI membrane and maximizing the extent of oral absorption.

Depending on the magnitude of supersaturation created upon dissolution, the two major kinetic events of crystallization, nucleation and crystal growth, will take place and consume the enhanced solution concentration generated by the employed formulation strategy. Additives are often used in commercial formulations to serve as crystallization inhibitors. A wide variety of structurally different polymers has been shown to stabilize both the amorphous state[5-7] and highly supersaturated solutions[5, 8-10] of poorly soluble drugs. Besides the commonly employed polymeric additives, bile salts, which are endogenous surfactants, have attracted the interest of researchers since they show potential as crystallization inhibitors.[11-13] Bile salts, the main product of cholesterol metabolism, form mixed micelles with phospholipids and phospholipid hydrolysis products and are present in the upper human intestine. Bile salt molecules consist of a rigid steroid

ring system, with hydroxyl groups distributed on one side, exhibiting facial polarity. Due to their unique molecular structure, bile salts exhibit step-wise aggregation,[14, 15] dissimilar to ordinary aliphatic surfactants, and show broader critical micelle concentration ranges.[16] The most abundant biologically relevant bile salts found in human intestinal fluids are sodium taurocholate (STC), sodium taurodeoxycholate (STDC), sodium taurochenodeoxycholate (STCDC), sodium glycocholate (SGC), sodium glycodeoxycholate (SGDC), and sodium glycochenodeoxycholate (SGCDC),[17] with reported critical micelle concentration (CMC) values in the range of 2 to 12mM.[16, 18] The trihydroxy bile salts are generally more water soluble and have higher CMC values than the dihydroxy bile salts. The total concentration and composition of bile salts in the GI tract, however, has been reported to vary to a great extent from person to person.[19]

Chen et al.[12] first demonstrated differences between bile salts and general aliphatic surfactants in terms of crystallization inhibition. At surfactant concentrations below the CMC, sodium taurocholate (STC) was noted to slow down solution crystallization of a group of structurally diverse active pharmaceutical ingredients (APIs), while sodium dodecyl sulfate (SDS) showed a tendency to promote solution crystallization. Two other bile salts, sodium glycocholate (SGC) and sodium glycodeoxycholate (SGDC), were also revealed to be effective crystallization inhibitors for celecoxib and nevirapine in monomer form. Recently, Li et al.[13] further explored the crystallization inhibitory ability of 13 bile salts at a concentration below their CMC in supersaturated solutions of celecoxib, nevirapine and flibanserin. Most bile salts studied slowed down crystallization of these fast crystallizing compounds, though the extent of inhibition varied amongst the bile salts, showing that bile salts are not interchangeable in the context of crystallization inhibition. These studies showed the crystallization inhibitory effects of monomeric bile salts towards a variety of APIs; however, bile salts exist in human intestinal fluid (HIF) as

mixed micelles which contain other biorelevant species such as lecithin, and there is limited information about how bile salt aggregation level differences impact the crystallization inhibition properties of bile salts. In addition, the stabilizing ability of bile salts in more complex solutions, that is highly supersaturated solutions in which liquid liquid or glass liquid phase separation (LLPS or GLPS) has occurred, has not yet been explored. These more complex solutions are of relevance in the context of ASDs which have been shown to dissolve to produce an amorphous drug-rich phase of sub-micron dimensions through the process of LLPS[20, 21] (or GLPS if the T_g of the water saturated amorphous drug is higher than temperature of the dissolution process[22]). Moreover, the mechanism for the anti-crystallization properties of bile salts has not been studied extensively. It is important to understand the mechanism and to determine if it is complementary to other anti-crystallization excipients such as polymers.

An inconsistency between the concentration-based supersaturation ratio and thermodynamic activity ratio, which is the fundamental driving force for crystallization, has been observed for some systems that contain solubilizing additives.[23] In a previous study,[24] we addressed this issue by scrutinizing the impact of the six most abundant biorelevant bile salts, in both monomeric and micellar form, on solution thermodynamics, and a supersaturation calibration method was developed. It was further noted that bile salts show different patterns of interaction with supersaturated telaprevir solutions, with trihydroxy bile salts having less effect on telaprevir solution thermodynamics than dihydroxy bile salts. Based on our knowledge of the impact of bile salts on telaprevir solution thermodynamics, herein, a systematic evaluation of the potential role of both monomeric and micellar bile salts as crystallization inhibitors was carried out by performing crystallization induction time experiments at the same activity-based supersaturation

in the presence of six taurine/glycine conjugated dihydroxy (STDC, SGDC, STCDC, and SGCDC) and trihydroxy (STC and SGC) bile salts.

MATERIALS

Telaprevir was obtained from Attix Pharmaceuticals (Toronto, Ontario, Canada) and ChemShuttle (CA). Hydroxypropyl methyl cellulose acetate succinate grade AS-MF (HPMCAS-MF) was obtained from Shin Etsu Chemical Co., Ltd (Tokyo, Japan). Sodium taurocholate (practical grade, MP Biomedicals, LLC, OH), sodium glycocholate (≥99%, Chem-Impex Int'l. Inc., IL), sodium taurodeoxycholate (≥97%, Chem-Impex Int'l. Inc., IL), sodium glycodeoxycholate (≥97%, Sigma, MO), sodium taurochenodeoxycholate (98%, Sinova Inc., MD) and sodium glycochenodeoxycholate (≥99%, Chem-Impex Int'l. Inc., IL) were used as received. Molecular structures of telaprevir and the six bile salts are shown in Figure 1. A regenerated cellulose membrane with a molecular weight cutoff (MWCO) of 6-8 kDa was acquired from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). The aqueous media used in all experiments was 50mM pH 6.5 sodium phosphate buffer.

METHODS

Supersaturation Determination.

To better design formulations with improved oral absorption, the fundamentals of crystallization kinetics must be considered. Solution crystallization is a thermodynamically favored event when a chemical potential difference between the solute and its crystalline state exists.[25] The driving force, supersaturation δ, can be quantified and expressed as the activity ratio of the solute to its equilibrium crystalline state:[26]

$$\ln \delta = \frac{\mu - \mu^{\circ}}{RT} = \ln \frac{a}{a^{\circ}} \quad (1)$$

where μ is the chemical potential of the solute, R is the ideal gas constant, T is temperature, and a is the solute activity. ° indicates the property at standard state (defined as solute in a solution in equilibrium with the stable crystalline form in this study). A side-by-side diffusion cell (PermeGear, Inc. Hellertown, PA) was used to evaluate the supersaturation level of telaprevir in the absence and presence of different bile salts (at 1.86 mM or 12 mM bile salt concentrations). The basic concepts and methods were as described in our previous study.[24] The mass flow rates of telaprevir, *F,* are directly proportional to solute activity. The ratio of solute mass flow rate in the solution of interest to the mass flow rate of the corresponding standard state system (solute in a solution in equilibrium with crystalline state in this study) yields the fundamental supersaturation:

$$\frac{F}{F^{\circ}} = \frac{a}{a^{\circ}} = \delta \quad (2)$$

With the mass flow rate data from our previous study[24] and equation (2), telaprevir supersaturation level in the absence and presence of bile salts can be determined from Figure 2. In the following crystallization study, two supersaturation levels were then chosen, δ = 11.5 which yields a homogeneous supersaturated solution where telaprevir is molecularly dissolved and δ = 28.8 which represents a high supersaturation condition where a glassy second phase of telaprevir is present since the amorphous solubility is just exceeded.[24]

Crystallization Induction Time Measurements.

Crystallization induction time measurements were used to evaluate bile salt anti-crystallization properties for supersaturated telaprevir solutions. Supersaturated telaprevir solutions were prepared by titrating concentrated methanolic telaprevir stock solution (12 or 20 mg/mL) to 50mL of 50 mM pH 6.5 sodium phosphate buffer with or without predissolved bile

salts or polymer, stirred at 300 rpm at 37 °C. Bile salts were present in solution at a concentration of 1.86 mM or 12 mM. Solutions with a higher bile salt concentration (18mM) for STC and SGC were as well prepared to further study the role of micellar trihydroxy bile salts. For comparative purposes, HPMCAS-MF was used at a concentration of 5μg/mL and 1mg/mL. The onset of telaprevir crystallization can be detected from a sudden decrease in the UV signal at a maximum absorption wavelength (270nm in this study) due to the consumption of telaprevir solution concentration when nucleation took place. Owing to the scattering effect from the newly form crystals, an increase in the UV signal at a non-absorbing wavelength (370nm in this study) can also be detected at the same instance. The time point at which crystallization is detected is then defined as the crystallization induction time t_{ind} (example plot shown in Figure 3). The change in UV signal was monitored using an SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber-optically coupled with a 1 cm or 0.5cm path length dip probe.

The measured crystallization induction time, t_{ind}, in this study is a combination of the nucleation induction time, t_n, the nucleation time for critical nucleus formation,[25] and a growth period, t_g, the time needed for crystals to grow to a detectable size:[10, 27, 28]

$$t_{ind} = t_n + t_g \tag{3}$$

Induction time measurements in this study were performed in triplicate, and the error bars in each figure represent standard deviation.

Surface Tension Measurements.

The critical micelle concentrations of STC and SGDC in the absence and presence of telaprevir were determined by measuring solution surface tension using a Processor Tensiometer K12 (Krüss, Germany) by the Wilhelmy plate method. All measurements were performed with

50mL of predissolved bile salt buffer solution at 37°C, and the temperature was maintained within ± 0.5 °C by a circulating waterbath. For measurements with telaprevir, methanolic telaprevir stock solution (12mg/mL) was titrated into the predissolved bile salt buffer solution to reach a telaprevir concentration of 60μg/mL, with thorough mixing. The accuracy of all measurements was within ±0.05 mN/m.

Zeta Potential Measurements.

The zeta potentials of the telaprevir-rich colloidal nanodroplets formed upon glass-liquid phase separation (GLPS) in solutions in the presence of monomeric bile salts were measured using a Nano-Zetasizer (Nano ZS, Malvern, Westborough, MA). Highly supersaturated solutions with telaprevir-rich nanodroplets (180 μg/mL) were obtained by titrating concentrated methanolic telaprevir stock solution (12 mg/mL) into 10mL of 50 mM pH 6.5 sodium phosphate buffer with 1.86mM bile salts, stirred at 300rpm at 37°C, until the solution became turbid indicating phase separation. Solutions were evaluated using an optical microscope under cross-polarized light, and no visible crystals could be observed. The zeta potential of the telaprevir drug-rich phase with no bile salt was also measured for reference.

RESULTS

Crystallization Induction Time - Effect of Bile Salt Concentration

Crystallization induction times were determined at constant supersaturation level to understand the inhibitory ability of different bile salt aggregation levels. In the absence of bile salts, homogeneous telaprevir supersaturated solutions ($\delta = 11.5$) crystallized within 30 minutes. Figure 4 shows that all six bile salts slow down crystallization of homogeneous supersaturated solutions, regardless of the bile salt aggregation levels. At 1.86mM bile salt concentration, bile salts maintain

solution supersaturation for over 16 hours and no observable crystallization events were detected. Besides the UV-vis spectroscopy method, solutions were also checked with an optical microscope under cross-polarized light and no visible crystals could be observed. As bile salt concentration increases to 12mM, trihydroxy bile salts (STC and SGC) and SGCDC maintain solution supersaturation over 16 hours, having comparable inhibitory ability to their monomeric form. Dihydroxy bile salts (STDC, SGDC, and STCDC) on the other hand, are less effective compared to their monomeric forms, delaying the occurrence of telaprevir crystallization to about 2 hours.

Meanwhile, highly supersaturated telaprevir solutions ($\delta = 28.8$) containing a drug-rich amorphous phase, crystallized much faster compared to the homogeneous supersaturated solutions. Figure 5 shows that the presence of STC, SGC, STDC, and SGDC slowed down crystallization of highly supersaturated solutions containing telaprevir-rich amorphous phase. Solutions containing STCDC and SGCDC cannot reach the same supersaturation level due to partitioning of bile salts into telaprevir-rich phase.[24] For the trihydroxy bile salts, STC and SGC, an increase in crystallization induction time was observed with increasing bile salt concentration. For dihydroxy bile salts, STDC and SGDC, an increase in bile salt concentration resulted in reduced crystallization induction time.

The CMCs of STC and SGDC, as representative trihydroxy and dihydroxy bile salts respectively, in the absence and presence of 60μg/mL telaprevir, were determined using surface tension measurements, and the results are summarized in Figure 6. Assuming the CMC values do not vary significantly amongst the trihydroxy and dihyhydroxy groups, and the concentration dependence of telaprevir is negligible, then it can be reasonably assumed based on these results that the majority of bile salts in solution at a concentration of 1.86mM are in monomeric form. In addition, micellar bile salts are most likely the dominant species at a bile salt concentration of

12mM, given that it is well above their CMCs. The concentration dependence of bile salt crystallization inhibitory effect was examined in more depth with STC, the only bile salt present in commercial biorelevant media, at concentrations below its CMC and at constant supersaturation level (δ = 11.5). Figure 7 shows that the crystallization induction time increased as STC concentration increased. With the presence of minimal STC (0.47mM, less than 0.025% (w/w)), the crystallization induction time changed from within 30 minutes to approximately 3 hours, revealing the potential of STC as a crystallization inhibitor for amorphous formulations. This prolonged induction time is of relevance in the context of small intestinal transit time which have been reported to be around 3-4 hours.[29]

Crystallization Induction Time - Effect of Drug Concentration and Solution Homogeneity

The impact of supersaturation level and solution homogeneity (single phase or containing amorphous nanodroplets) is summarized in Figure 8 and 9. Without any additives, the crystallization induction time decreased as drug concentration increased. This result is expected since both nucleation and crystal growth rates increase with higher crystallization driving force. However, these kinetic events appear to be more complicated as bile salts are introduced to the systems. Figure 8 shows the extent of bile salt retardation of telaprevir nucleation under constant bile salt concentration (1.86mM) as a function of telaprevir concentration. Overall, all six bile salts at monomeric levels are good crystallization inhibitors, as they all slow down telaprevir crystallization at δ = 11.5 (homogeneous solution) and the induction time is increased to more than 16 hours. Increasing the drug concentration to δ = 28.8, where a telaprevir-rich amorphous phase was created, generally results in a decrease in induction time, and in these systems the inhibitory ability varies considerably amongst the different bile salts. Monomeric dihydroxy bile salts (STDC and SGDC) are more effective than monomeric trihydroxy bile salts (STC and SGC), with SGDC

being the most effective crystallization inhibitor in the presence of the telaprevir-rich amorphous phase.

At 12mM bile salt concentration, the six bile salts slow down crystallization of homogeneous and heterogeneous (containing the telaprevir-rich amorphous phase) supersaturated solutions to different extents (Figure 9). The trihydroxy bile salts, STC and SGC, maintained supersaturation of the homogeneous solutions for over 16 hours, while their inhibitory effect decreased in the heterogeneous solutions with higher supersaturation. In contrast, the dihydroxy bile salts, STDC and SGDC, are less effective crystallization inhibitors but, interestingly, their inhibitory effect improved for the heterogeneous, more supersaturated solutions, in particular for SGDC. This is different from previous cases where crystallization induction times decreased as crystallization driving force increased, thus further mechanistic studies are warranted.

Zeta Potential of the Telaprevir-rich Droplets

Zeta potential measurements were conducted to understand the interactions of monomeric bile salts and the telaprevir-rich droplets formed when the concentration exceeds the amorphous solubility. Zeta potential, ζ, is a measurement of the electric potential difference, with contributions from both physically adsorbed and chemically absorbed ions, at interfaces in solution.[30] Since telaprevir is neutral in the buffer medium used in this study, the change in zeta potential upon addition of monomeric bile salt, which is negatively charged at the pH employed, serves as an indication of adsorption of bile salts at the telaprevir-rich droplet-water interface. Figure 10 summarizes the zeta potential values of telaprevir-rich droplets in the absence and presence of six monomeric level bile salts. The zeta potential of telaprevir-rich droplets in the absence of bile salts was -9.2 mV. A ~4-5 fold increase in the magnitude of zeta potential was observed with the addition of each of the six monomeric bile salts (1.86mM bile salt concentration), which implies

adsorption of bile salts at the telaprevir-rich droplet-water interface. A similar phenomena has been observed in a previous study with danazol, a poorly soluble API, in the presence of HPMCAS (a negatively charged polymer).[31]

DISCUSSION

Nucleation Inhibition Versus Growth Inhibition

Induction time measurement is a well-established and commonly employed method to quantify nucleation. Induction time, t_{ind}, by definition, is a combination of the time needed for nuclei formation and the time needed for nuclei to grow to a detectable size. It has been common practice to measure induction time for nucleation kinetic studies,[10, 27, 28] assuming the time required for critical nuclei formation is the dominant factor. As demonstrated in previous studies, it is possible to decouple the t_g term in equation 3 with proper experimental design and hence have a better understanding of nucleation kinetics.[32, 33] By determining seedless induction times and the seeded growth rates in the presence and absence of bile salts under similar experimental conditions, the relative nucleation rate in the presence and absence of the bile salt can be estimated:

$$\frac{J}{J_0} = \left(\frac{R_0}{R}\right)^3 \left(\frac{t_{u,0}}{t_u}\right)^4 \quad (4)$$

where J is the nucleation rate in the presence of bile salts, J_0 is the nucleation rate in the absence of bile salts, R_0/R is the mass growth rate in the absence of bile salts divided by the rate in the presence of bile salts, $t_{u,0}$ is the unseeded induction time in the absence of bile salts and t_u is the induction time in the presence of bile salts.

To confirm the assumption that t_n of equation 3 is the dominant factor, and not the effect of the bile salts on growth rates, selected additional kinetic studies (growth rate measurements)

were carried out with STC and SGC, the most commonly studied bile salts, and equation 4 was applied to decouple the nucleation and growth rates. From Figure 4, the induction time in the absence of bile salts was determined to be ~30 minutes and in the presence of STC or SGC the induction time was estimated to be more than 16 hours (960 minutes). From Figure 11, the mass growth rates in the absence and presence of bile salts were calculated by taking the slope of the concentration versus time data over the initial 10 minutes of the experiment. The mass growth rate of telaprevir without bile salts is 89.2 μg/min, and the mass growth rates in the presence of 1.86mM STC and SGC are 6.4 μg/min and 60.5 μg/min, respectively (telaprevir growth rate is slowed down by 14 fold and 1.5 fold, respectively). These values were used as input for equation 4, where J/J_0 was then estimated to be 2.5×10^{-3} for STC, and 2.9×10^{-6} for SGC. In other words, telaprevir nucleation rate is slowed down by at least 400 fold and 340,000 fold in the presence of 1.86mM STC and SGC, respectively. It is evident that the impact of bile salts on telaprevir nucleation rate is greater than the impact on telaprevir crystal growth rate. That is to say, the assumption of nucleation time t_n being the predominant factor in the induction time t_{ind} may be applied in this study, allowing evaluation of nucleation inhibition of bile salts with the measured crystallization induction time. Moreover, the preliminary results from Figure 11 reveal the potential of bile salts as crystal growth inhibitors, which is clearly an area for future study.

Mechanistic Understanding of Bile Salt Inhibition Properties

Bile salts are endogenous species in human gastrointestinal tract, and their solubilization and crystallization inhibition properties potentially play a direct role on API *in vivo* performance. Although there are at least six different bile salts in human intestinal fluid,[17] STC is still the only bile salt component in common current simulated fluid recipes,[34] and this possibly contributes to the observed discrepancy between some *in vivo* and *in vitro* tests noted in the literature.[35] Therefore,

having a better understanding of the impact of biologically relevant bile salts on crystallization kinetics of supersaturated API solutions is critical for formulation design. In supersaturated solutions, a longer crystallization induction time in the presence of additives could result from two factors: solubilization and/or intermolecular interactions that directly modify the nucleation process. For additives that solubilize the model compound, the increase in crystalline solubility leads to a decrease in supersaturation; therefore, a lower crystallization driving force exists in the presence of solubilizing additives under constant solution concentration.[23] Besides solubilization, additives can interfere with the nuclei formation process and thus slow down crystallization of the model compound. [10, 36-38] Compared to general amphiphilic surfactants, it has been noted that bile salts have a lower solubilization capacity.[11, 12, 39] That is to say, the delayed solution crystallization in the presence of bile salts is more likely to be a result of intermolecular interactions.

In this study, supersaturation levels in the presence of bile salts were carefully calibrated and all crystallization studies were performed at equivalent driving forces. Ruling out solubilization as a factor in the prolonged crystallization induction times enables better mechanistic understanding of bile salt inhibition properties. As summarized in Figures 4 and 5, the ability to retard telaprevir nucleation varies not only with structural differences amongst the bile salts, but also with their aggregation levels for the same crystallization driving forces. From Figure 4, it is evident that all monomeric bile salts in this study are effective crystallization inhibitors for telaprevir at the lower supersaturation employed. The good crystallization inhibitory ability of monomeric bile salts has been noted in other studies.[11-13] As bile salt concentration increased above the CMC, some structural dependence, mainly related to the number of hydroxyl groups on the steroid ring, was observed amongst the bile salts. For trihydroxy bile salts, STC and SGC, the ability to retard telaprevir nucleation persisted with increasing bile salt concentration,

even with the presence of micelles. An analogous nucleation inhibition behavior was reported in a previous study with another poorly soluble compound celecoxib.[11] In this study, a more than ten-fold increase in celecoxib crystallization induction time was observed in the presence of STC at concentrations below and above its CMC, suggesting that the presence of micellar STC does not promote celecoxib crystallization. Li *et al*.[13] made similar observations for celecoxib regarding the STC concentration dependent crystallization inhibitory effect. In the same study, STC was also noted to slow down solution crystallization of nevirapine, another poorly soluble compound, even at bile salt concentrations above CMC. It was suggested that the step-wise aggregation behavior of STC is a possible reason for higher variations in nucleation inhibition properties observed for nevirapine at STC concentrations above its CMC. The observation of the inhibitory impact of solutions containing both free and micellar bile salts on crystallization kinetics is opposite to that observed for surfactants with a chain-like hydrophobic tail, such as sodium dodecyl sulfate,[11, 39] Polysorbate 80,[11] and Poloxamer 188,[11] where enhanced crystallization was observed. It was proposed that STC has a different impact on crystallization than conventional surfactants because of the bulky hydrophobic group and rigid structure.[11]

While trihydroxy bile salts still demonstrate inhibitory ability for homogeneous supersaturated solutions as the bile salt concentration increased to 12mM, a notable reduction in the extent of retardation of telaprevir nucleation was observed for the dihydroxy bile salts (Figure 4). A similar trend was observed when the supersaturation level was elevated (Figure 5), where trihydroxy bile salts showed improved inhibitory ability at 12mM concentration, while the dihydroxy bile salts were less effective inhibitors when micelles were present. Trihydroxy bile salts have higher CMC values than dihydroxy bile salts, as noted from the literature[16, 40, 41] and confirmed by our experimental data (Figure 6). That is to say, there should be a higher percentage

of monomer, which we note is the effective crystallization inhibitory species (Figure 7), present at a 12mM bile salt concentration for trihydroxy bile salts than for dihydroxy bile salts, and correspondingly less aggregated bile salt. A higher concentration of the effective crystallization inhibitor, i.e. the monomer, at the same bile salt concentration is a possible contributing factor with regards to the persistent, or even improved, crystallization inhibitory ability of trihydroxy bile salts compared to dihydroxy bile salts. Moreover, Anwar et al.[38] commented in a molecular simulation study that amphiphilic additives can inhibit nucleation via influencing the packing of the emerging nuclei structure if the solute–additive interaction is relatively strong. This explanation might be relevant for SGCDC, the only dihydroxy bile salt that was shown to be effective in inhibiting telaprevir crystallization even in the presence of micelles (Figure 4), as it was noted previously to have stronger intermolecular interactions with telaprevir.[24] Alternatively, we can speculate that bile salt micelles promote crystallization by providing interfaces that act as heterogeneous nucleation sites. Therefore, the dihydroxy bile salt 12mM solutions, which contain more micellar species due to the lower CMC relative to the trihydroxy bile salts, may have both inhibitory and promoting species present. To further confirm our hypothesis of bile salt micelles negating the inhibitory impact of the monomers, induction time experiments were performed at δ=11.5 in the presence of 18mM of STC and SGC. The supersaturated solutions all crystallized within 16 hours; at 12mM bile salt concentration, no crystallization was observed within 16 hrs (Figure 4). Given that there are more micelles present in the solution at 18mM bile salt concentration, this observation supports our contention that micelles oppose the inhibitory effect of monomers and lead to faster crystallization kinetics at higher bile salt concentrations.

For a highly supersaturated solution in the presence of a drug-rich phase, crystallization kinetics are expected to be facilitated as there are more interfaces present, enabling heterogeneous

nucleation.[25] This impact of the drug-rich phase, which has been previously characterized to have a size of around 300nm,[42] on the crystallization kinetics can be readily seen by replotting the data shown in Figures 4 and 5 to enable a comparison of supersaturation level at equivalent bile salt concentrations, Figure 8 (monomer) and Figure 9 (micellar). Supersaturated solutions containing a telaprevir-rich phase and no inhibitors crystallized immediately. However, the presence of bile salts (both monomer and micellar levels) slowed down the crystallization kinetics (Figures 8 and 9), suggesting that bile salts interact with the drug-rich phase, as supported by zeta potential measurements (Figure 10). Zeta potential is commonly employed to evaluate colloidal stability,[43-45] and it is well known that adsorption of ionized species can change the measured zeta potential.[46-48] As shown in Figure 10, the highly supersaturated telaprevir solutions containing drug-rich droplets have a small negative zeta potential, hence a high tendency for aggregation. [43, 44] With the addition of 1.86mM bile salts, the zeta potential of these systems decreased to around -50mV, and can be considered to be electrostatically stabilized.[43, 44] From these results, it is evident that that all six bile salts interacted with the surface of the telaprevir-rich droplets as zeta-potential decreased significantly. This evidence of bile salt interaction with the drug-rich phase helps explain the observed inhibitory effect of bile salts on crystallization in these systems, since the presence of the bile salt at the particle-water interface can be expected to modify heterogeneous nucleation. However, it should be noted that the use of zeta-potential measurements in this work can only provide qualitative evidence of interfacial telaprevir-bile salt interactions, and is not a quantitative measure of the extent of absorption, as zeta-potential measurements have contributions from both physically adsorbed and chemically absorbed ions within the slip plane at the solid-solution interface. Hence, additional studies are needed to better understand and relate

the crystallization inhibitory effect amongst different bile salts in these complex two phase solutions.

To help summarize our observations on bile salt retardation of telaprevir nucleation kinetics, the various supersaturation and aggregation levels used in this study are shown in schematic form in Figure 12. Our experimental results suggest that 1) all of the monomeric bile salts evaluated are effective crystallization inhibitors, in particular in homogenous solutions of telaprevir (Figure 4, bottom left quadrant of Figure 12), 2) micelles of dihydroxy bile salts, with the exception of SGCDC, appear to negate the inhibitory impact of the monomers (Figure 4, top left quadrant Figure 12), 3) monomeric trihydroxy bile salts are less effective inhibitors in the presence of a drug-rich phase as compared to the dihydroxy bile salts studied (Figure 8, bottom right quadrant Figure 12), 4) in the presence of both micellar bile salt and a drug-rich phase, the pattern of inhibitory effects is complex, unpredictable and more work is clearly needed to investigate these systems (Figure 9, top right quadrant Figure 12). Moreover, these are probably the most relevant systems since micellar species are present *in vivo*, and many formulations have the potential to form a drug-rich phase following dissolution.[22] To the best of our knowledge, very limited understanding and exploration of the role of micellar bile salts in the context of solution crystallization exist in the literature. Given the fact that bile salts are present in the human GI tract in the form of mixed micelles, this study provides important insights into the potential role of a more biorelevent bile salt aggregation state as well as a foundation to study more complex bile salt forms, e.g., mixed micelles with lecithin.

It has been demonstrated that bile salts are not interchangeable from either a solution thermodynamics[24] or a crystallization inhibition standpoint,[13] and observations are of high significance in terms of selection of appropriate biorelevant dissolution media with predictive

capabilities of *in vivo* API formulation performance, in particular in terms of crystallization kinetics. Based on observations made in this study, STC may not be a good surrogate for the mixture of bile salts found *in vivo* in terms of understanding the crystallization tendency of a given system.

Potential as Crystallization Inhibitor – A Comparison with Other Additives

The potential of bile salts as crystallization inhibitors is of particular interest since they are endogenous species that share the advantages possessed by commonly employed formulation additives, such as surfactants and polymers. Surfactants solubilize hydrophobic molecules by incorporating them into micelles. This enhanced solubility is often achieved in the expense of drug membrane transport since less free drug (i.e. molecularly dissolved drug) is available in the presence of micelles for a given concentration.[3, 23] Polymers, on the other hand, at the concentrations typically employed, have negligible impact on API crystalline solubility[10, 31] and membrane transport rate.[31] Based on previous studies,[11-13, 24] bile salts are less effective solubilizing agents compared to conventional surfactants; however, these have been shown to promote crystallization of supersaturated solutions of poorly soluble compounds.[11, 39] In contrast, polymers are often used as crystallization inhibitors, and improve the bioavailability of APIs through stabilizing the drug in the ASDs and the resultant highly supersaturated solutions.[20, 49, 50] Bile salts also decrease solution crystallization kinetics of poorly water soluble APIs, even at concentrations above their critical micelle concentrations.[11-13] A comparison of the crystallization inhibitory effect of monomeric bile salts (1.86mM) and a commercially available polymer, HPMCAS-MF, at comparable supersaturation levels is provided in Figure 13. Mosquera-Giraldo *et al.,*[37] showed that HPMCAS-MF is one of the most effective inhibitors of telaprevir amongst the multiple polymers studied. In the context of our study, biologically relevant bile salts showed

comparable inhibition to HPMCAS-MF in homogenous solutions where all additives were extremely effective. In the presence of a drug-rich phase, HPMCAS-MF was better than the least effective bile salt, but not as effective as the best bile salt at inhibiting crystallization. The comparable inhibition ability of bile salts and polymers can also been seen by reviewing various literature studies on celecoxib crystallization.[10, 13, 50] More detailed experimental and molecular modeling studies of how bile salts disrupt the API crystallization process are required in order to understand how different bile salt-drug interactions contribute to the observed differences in nucleation retardation ability. Nevertheless, it is clear that understanding the anti-nucleation properties of bile salts is important in the context of improving API formulation design and testing approaches. In addition, bile salts are unique species from both a solubilization and crystallization inhibition standpoint.

CONCLUSIONS

In this study, the impact of six biologically relevant bile salts on telaprevir crystallization induction time was evaluated at different bile salt aggregation levels. By carefully calibrating the supersaturation to maintain the same crystallization driving force, differences in the inhibitory effects of the bile salts were noted. For telaprevir supersaturated solutions below the amorphous solubility, monomeric bile salts were extremely effective inhibitors. Interestingly, the presence of bile salt micelles opposed the monomeric inhibitory effect for some of the bile salts. Telaprevir solutions above the amorphous solubility also showed delayed crystallization in the presence of both monomeric and micellar bile salts, but had a much higher tendency to crystallize in general. Importantly, it was observed that the bile salts are not interchangeable with respect to their ability to inhibit crystallization. In addition, these biologically relevant bile salts, in monomeric form, showed a comparable nucleation inhibition ability to hydroxypropylmethyl cellulose acetate

succinate, a commonly employed crystallization inhibitor. Based on these observations, further studies of how bile salts impact the crystallization kinetics of drugs are essential in order to design biopredictive dissolution media and better predict *in vivo* behavior of supersaturating systems.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Merck & Co., Inc., Kenilworth, NJ, USA for providing research and financial support for this study. We would also like to acknowledge Sanjaykumar Patel, Anthony Leone, Ron Smith, Andre Hermans, Steve Wang, James DiNunzio and Filippos Kesisoglou for discussion and insight.

REFERENCES

(1) Thayer, A. M., Finding solutions. *Chem. Eng. News* **2010,** 88, (22), 13-18.
(2) Williams, H. D.; Trevaskis, N. L.; Charman, S. A.; Shanker, R. M.; Charman, W. N.; Pouton, C. W.; Porter, C. J. H., Strategies to Address Low Drug Solubility in Discovery and Development. *Pharmacol. Rev.* **2013,** 65, (1), 315-499.
(3) Miller, J. M.; Beig, A.; Carr, R. A.; Spence, J. K.; Dahan, A., A win-win solution in oral delivery of lipophilic drugs: supersaturation via amorphous solid dispersions increases apparent solubility without sacrifice of intestinal membrane permeability. *Mol. Pharm.* **2012,** 9, (7), 2009-16.
(4) Miller, J. M.; Beig, A.; Krieg, B. J.; Carr, R. A.; Borchardt, T. B.; Amidon, G. E.; Amidon, G. L.; Dahan, A., The solubility-permeability interplay: mechanistic modeling and predictive application of the impact of micellar solubilization on intestinal permeation. *Mol. Pharm.* **2011,** 8, (5), 1848-56.
(5) Chauhan, H.; Kuldipkumar, A.; Barder, T.; Medek, A.; Gu, C. H.; Atef, E., Correlation of inhibitory effects of polymers on indomethacin precipitation in solution and amorphous solid crystallization based on molecular interaction. *Pharm. Res.* **2014,** 31, (2), 500-15.
(6) Yokoi, Y.; Yonemochi, E.; Terada, K., Effects of sugar ester and hydroxypropyl methylcellulose on the physicochemical stability of amorphous cefditoren pivoxil in aqueous suspension. *Int. J. Pharm.* **2005,** 290, (1-2), 91-9.
(7) Broman, E., Khoo, C., & Taylor, L. S., A comparison of alternative polymer excipients and processing methods for making solid dispersions of a poorly water soluble drug. *Int. J. Pharm.* **2001,** 222, (1), 139-151.

(8) Warren, D. B.; Benameur, H.; Porter, C. J.; Pouton, C. W., Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: A mechanistic basis for utility. *J. Drug Target* **2010,** 18, (10), 704-31.
(9) Ozaki, S.; Kushida, I.; Yamashita, T.; Hasebe, T.; Shirai, O.; Kano, K., Inhibition of crystal nucleation and growth by water-soluble polymers and its impact on the supersaturation profiles of amorphous drugs. *J. Pharm. Sci.* **2013,** 102, (7), 2273-81.
(10) Ilevbare, G. A.; Liu, H.; Edgar, K. J.; Taylor, L. S., Maintaining Supersaturation in Aqueous Drug Solutions: Impact of Different Polymers on Induction Times. *Cryst. Growth Des.* **2013,** 13, (2), 740-751.
(11) Chen, J.; Ormes, J. D.; Higgins, J. D.; Taylor, L. S., Impact of surfactants on the crystallization of aqueous suspensions of celecoxib amorphous solid dispersion spray dried particles. *Mol. Pharm.* **2015,** 12, (2), 533-41.
(12) Chen, J.; Mosquera-Giraldo, L. I.; Ormes, J. D.; Higgins, J. D.; Taylor, L. S., Bile Salts as Crystallization Inhibitors of Supersaturated Solutions of Poorly Water-Soluble Compounds. *Cryst. Growth Des.* **2015,** 15, (6), 2593-2597.
(13) Li, N.; Mosquera-Giraldo, L. I.; Borca, C. H.; Ormes, J.; Lowinger, M.; Higgins, J.; Slipchenko, L. V.; Taylor, L. S., A Comparison of the Crystallization Inhibition Properties of Bile Salts. *Cryst. Growth Des.* **2016**.
(14) Mukerjee, P.; Cardinal, J. R., Solubilization as a method for studying self-association: Solubility of naphthalene in the bile salt sodium cholate and the complex pattern of its aggregation. *J. Pharm. Sci.* **1976,** 65, (6), 882-886.
(15) Mazer, N. A.; Carey, M. C.; Kwasnick, R. F.; Benedek, G. B., Quasielastic light scattering studies of aqueous biliary lipid systems. Size, shape, and thermodynamics of bile salt micelles. *Biochemistry* **1979,** 18, (14), 3064-3075.
(16) Reis, S.; Moutinho, C. G.; Matos, C.; de Castro, B.; Gameiro, P.; Lima, J. L., Noninvasive methods to determine the critical micelle concentration of some bile acid salts. *Anal. Biochem.* **2004,** 334, (1), 117-26.
(17) Riethorst, D.; Mols, R.; Duchateau, G.; Tack, J.; Brouwers, J.; Augustijns, P., Characterization of Human Duodenal Fluids in Fasted and Fed State Conditions. *J. Pharm. Sci.* **2015**.
(18) Natalini, B.; Sardella, R.; Gioiello, A.; Ianni, F.; Di Michele, A.; Marinozzi, M., Determination of bile salt critical micellization concentration on the road to drug discovery. *J. Pharm. Biomed. Anal.* **2014,** 87, 62-81.
(19) Hofmann, A. F.; Small, D. M., Detergent properties of bile salts: correlation with physiological function. *Annu. Rev. Med.* **1967,** 18, (1), 333-376.
(20) Jackson, M. J.; Kestur, U. S.; Hussain, M. A.; Taylor, L. S., Dissolution of Danazol Amorphous Solid Dispersions: Supersaturation and Phase Behavior as a Function of Drug Loading and Polymer Type. *Mol. Pharm.* **2016,** 13, (1), 223-31.
(21) Trasi, N. S.; Taylor, L. S., Dissolution performance of binary amorphous drug combinations--Impact of a second drug on the maximum achievable supersaturation. *Int. J. Pharm.* **2015,** 496, (2), 282-90.
(22) Taylor, L. S.; Zhang, G. G., Physical chemistry of supersaturated solutions and implications for oral absorption. *Adv. Drug Deliv. Rev.* **2016,** 101, 122-42.
(23) Raina, S. A.; Zhang, G. G.; Alonzo, D. E.; Wu, J.; Zhu, D.; Catron, N. D.; Gao, Y.; Taylor, L. S., Impact of Solubilizing Additives on Supersaturation and Membrane Transport of Drugs. *Pharm. Res.* **2015,** 32, (10), 3350-64.
(24) Lu, J.; Ormes, J. D.; Lowinger, M.; Xu, W.; Mitra, A.; Mann, A. K.; Litster, J. D.; Taylor, L. S., Impact of Endogenous Bile Salts on the Thermodynamics of Supersaturated Active Pharmaceutical Ingredient Solutions. *Cryst. Growth Des.* **2017,** 17, (3), 1264-1275.
(25) Mullin, J. W., Crystallization. *Butterworth-Heinemann.* **2001**.

(26) Mullin, J. W.; Söhnel, O., Expressions of supersaturation in crystallization studies. *Chem. Eng. Sci.* **1977,** 32, (7), 683-686.
(27) Söhnel, O., & Mullin, J. W., Interpretation of crystallization induction periods. *J. Colloid Interface Sci.* **1988,** 123, (1), 43-50.
(28) Kuldipkumar, A., Kwon, G. S., & Zhang, G. G., Determining the growth mechanism of tolazamide by induction time measurement. *Cryst. Growth Des.* **2007,** 7, (2), 234-242.
(29) Davis, S. S.; Hardy, J. G.; Fara, J. W., Transit of pharmaceutical dosage forms through the small intestine. *Gut* **1986,** 27, (8), 886-892.
(30) Tadros, T. F., Interfacial phenomena and colloid stability: basic principles (Vol. 1). *Walter de Gruyter GmbH & Co KG.* **2015**.
(31) Jackson, M. J.; Kestur, U. S.; Hussain, M. A.; Taylor, L. S., Characterization of Supersaturated Danazol Solutions - Impact of Polymers on Solution Properties and Phase Transitions. *Pharm. Res.* **2016,** 33, (5), 1276-88.
(32) Verdoes, D., Kashchiev, D., & Van Rosmalen, G. M., Determination of nucleation and growth rates from induction times in seeded and unseeded precipitation of calcium carbonate. *J. Cryst. Growth* **1992,** 118, (3), 401-413.
(33) Alonzo, D. E.; Raina, S.; Zhou, D.; Gao, Y.; Zhang, G. G. Z.; Taylor, L. S., Characterizing the Impact of Hydroxypropylmethyl Cellulose on the Growth and Nucleation Kinetics of Felodipine from Supersaturated Solutions. *Cryst. Growth Des.* **2012,** 12, (3), 1538-1547.
(34) Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. B., Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm. Res.* **2008,** 25, (7), 1663-76.
(35) Carlert, S.; Palsson, A.; Hanisch, G.; von Corswant, C.; Nilsson, C.; Lindfors, L.; Lennernas, H.; Abrahamsson, B., Predicting intestinal precipitation--a case example for a basic BCS class II drug. *Pharm. Res.* **2010,** 27, (10), 2119-30.
(36) Raghavan, S. L., Trividic, A., Davis, A. F., Hadgraft, J., Crystallization of hydrocortisone acetate: influence of polymers. *Int. J. Pharm.* **2001,** 212, (2), 213-221.
(37) Mosquera-Giraldo, L. I.; Borca, C. H.; Meng, X.; Edgar, K. J.; Slipchenko, L. V.; Taylor, L. S., Mechanistic Design of Chemically Diverse Polymers with Applications in Oral Drug Delivery. *Biomacromolecules* **2016**.
(38) Anwar, J.; Boateng, P. K.; Tamaki, R.; Odedra, S., Mode of action and design rules for additives that modulate crystal nucleation. *Angew. Chem., Int. Ed. Engl.* **2009,** 48, (9), 1596-600.
(39) Rodríguez-Hornedo, N.; Murphy, D., Surfactant-facilitated crystallization of dihydrate carbamazepine during dissolution of anhydrous polymorph. *J. Pharm. Sci.* **2004,** 93, (2), 449-460.
(40) Matsuoka, K.; Maeda, M.; Moroi, Y., Micelle formation of sodium glyco- and taurocholates and sodium glyco- and taurodeoxycholates and solubilization of cholesterol into their micelles. *Colloids Surf., B* **2003,** 32, (2), 87-95.
(41) Matsuoka, K.; Suzuki, M.; Honda, C.; Endo, K.; Moroi, Y., Micellization of conjugated chenodeoxy- and ursodeoxycholates and solubilization of cholesterol into their micelles: comparison with other four conjugated bile salts species. *Chem. Phys. Lipids* **2006,** 139, (1), 1-10.
(42) Mosquera-Giraldo, L. I.; Taylor, L. S., Glass-liquid phase separation in highly supersaturated aqueous solutions of telaprevir. *Mol. Pharm.* **2015,** 12, (2), 496-503.
(43) Missana, T.; Adell, A., On the Applicability of DLVO Theory to the Prediction of Clay Colloids Stability. *J. Colloid Interface Sci.* **2000,** 230, (1), 150-156.
(44) Patel, V. R.; Agrawal, Y. K., Nanosuspension: An approach to enhance solubility of drugs. *J. Adv. Pharm. Technol. Res.* **2011,** 2, (2), 81-7.
(45) Jacobs, C., & Müller, R. H., Production and characterization of a budesonide nanosuspension for pulmonary administration. *Pharm. Res.* **2002,** 19, (2), 189-194.

(46) Yang, Y. J.; Kelkar, A. V.; Zhu, X.; Bai, G.; Ng, H. T.; Corti, D. S.; Franses, E. I., Effect of sodium dodecylsulfate monomers and micelles on the stability of aqueous dispersions of titanium dioxide pigment nanoparticles against agglomeration and sedimentation. *J. Colloid Interface Sci.* **2015,** 450, 434-45.
(47) Keesom, W. H.; Zelenka, R. L.; Radke, C. J., A zeta-potential model for ionic surfactant adsorption on an ionogenic hydrophobic surface. *J. Colloid Interface Sci.* **1988,** 125, (2), 575-585.
(48) Brown, W.; Zhao, J., Adsorption of sodium dodecyl sulfate on polystyrene latex particles using dynamic light scattering and zeta potential measurements. *Macromolecules* **1993,** 26, (11), 2711-2715.
(49) Liu, H.; Taylor, L. S.; Edgar, K. J., The role of polymers in oral bioavailability enhancement; a review. *Polymer* **2015,** 77, 399-415.
(50) Xie, T.; Taylor, L. S., Dissolution Performance of High Drug Loading Celecoxib Amorphous Solid Dispersions Formulated with Polymer Combinations. *Pharm. Res.* **2016,** 33, (3), 739-750.

FIGURES

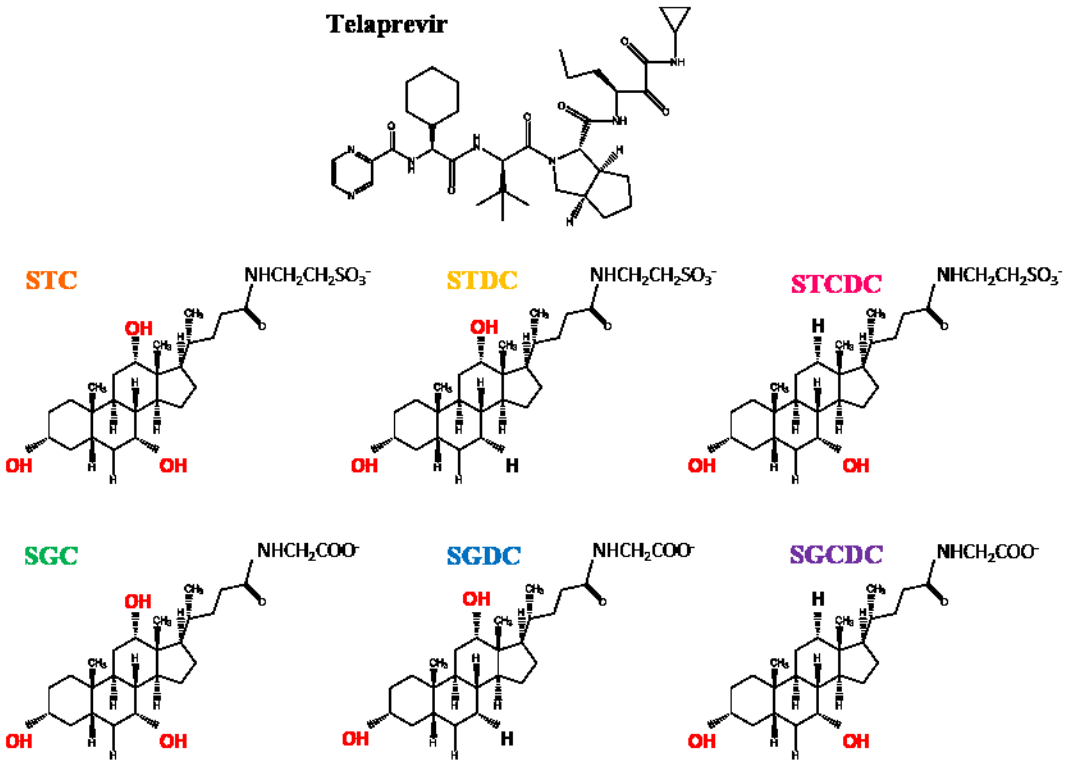

Figure 1. Molecular structures of telaprevir and bile salts.

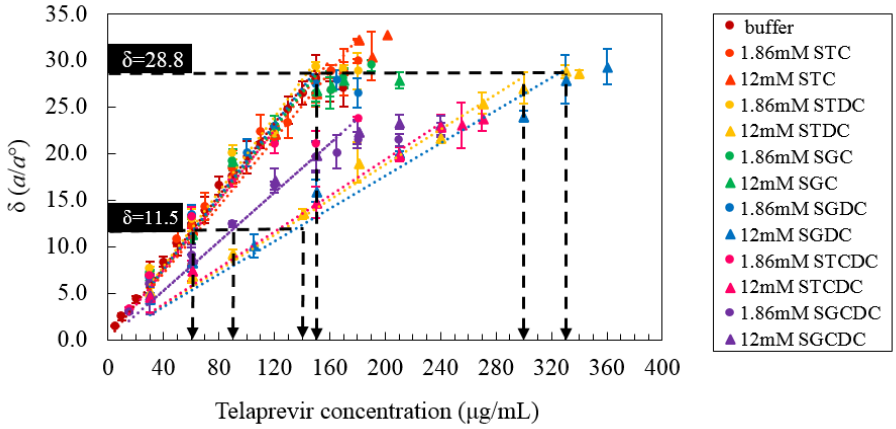

Figure 2. Telaprevir concentrations under constant supersaturation levels in the absence and presence of bile salts. The mass flow rate data from our previous study were used for determination of telaprevir supersaturation level in solutions containing different bile salts.[24]

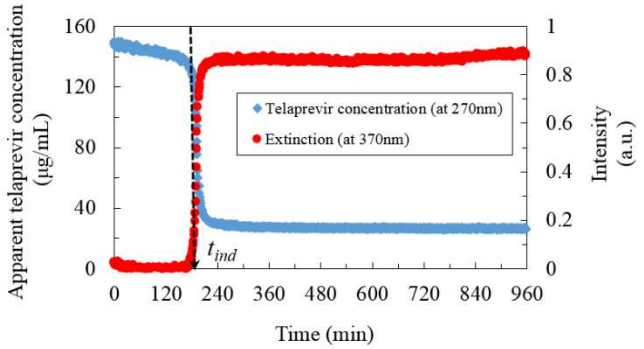

Figure 3. Crystallization induction time of a supersaturated telaprevir solution was determined as the time when a sudden increase in extinction signal and a sudden decrease in apparent concentration was observed.

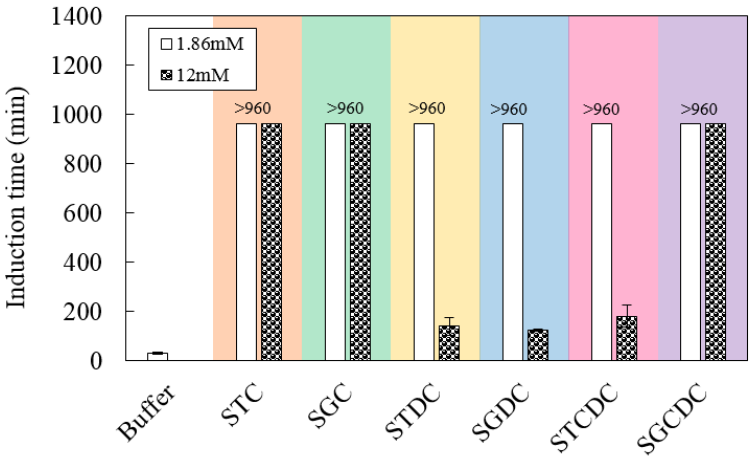

Figure 4. Induction time for telaprevir at supersaturation level of δ=11.5, in the absence and presence of predissolved 1.86mM and 12mM bile salts.

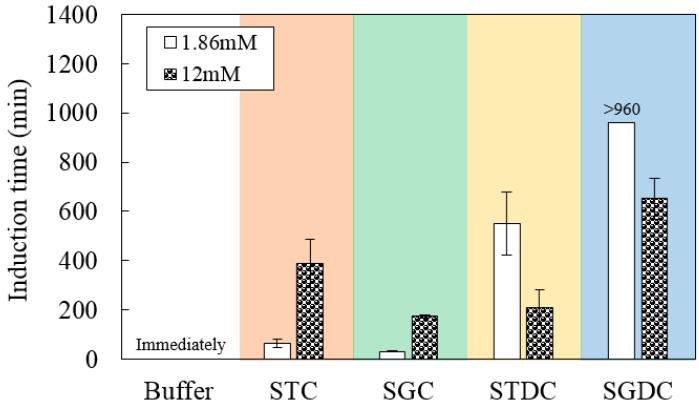

Figure 5. Induction time for telaprevir at supersaturation level of δ=28.8, in the absence and presence of predissolved 1.86mM and 12mM bile salts.

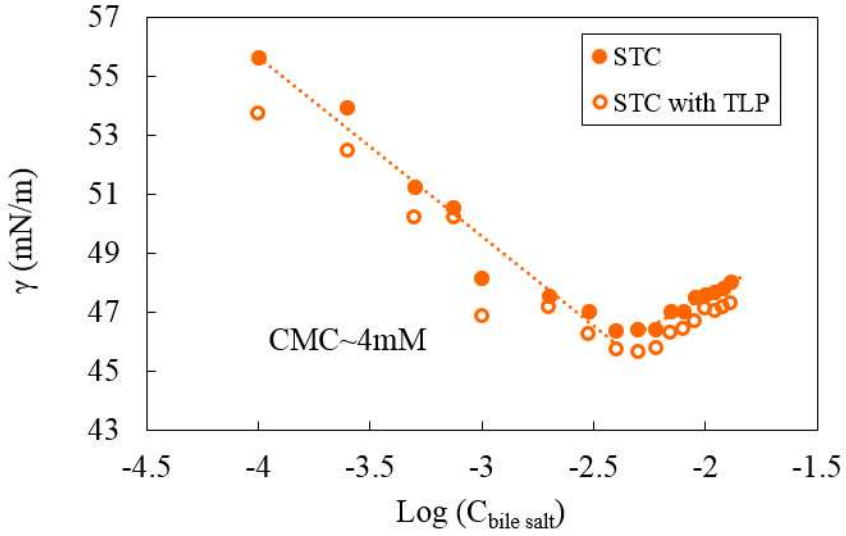

Figure 6a. CMC of STC in the absence and presence of telaprevir as determined by measuring solution surface tension as a function of STC concentration.

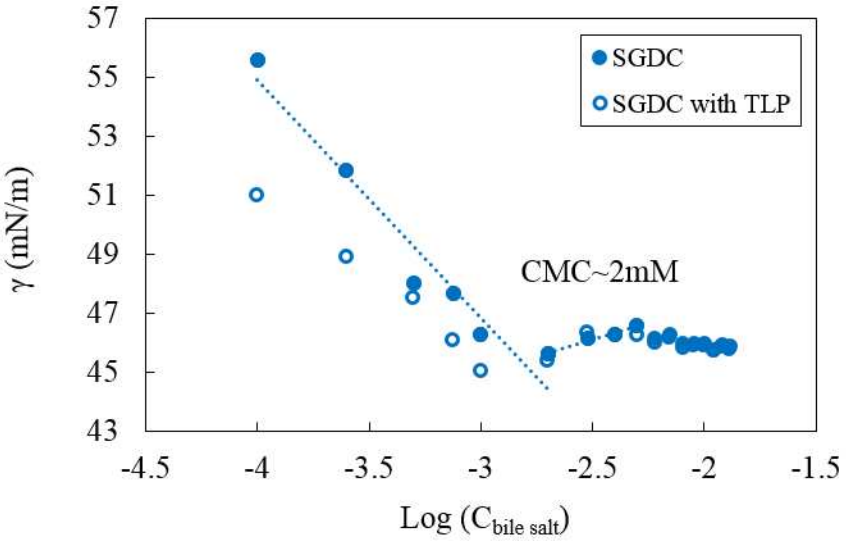

Figure 6b. CMC of SGDC in the absence and presence of telaprevir as determined by measuring solution surface tension as a function of SGDC concentration.

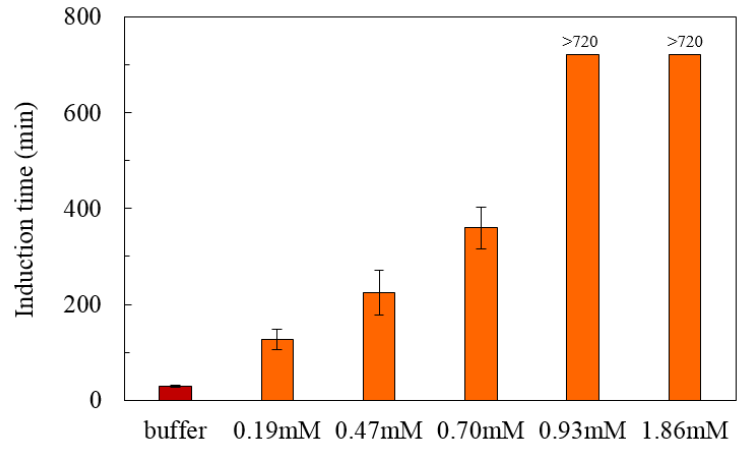

Figure 7. Effect of STC concentration on induction time with 60µg/mL telaprevir (δ=11.5).

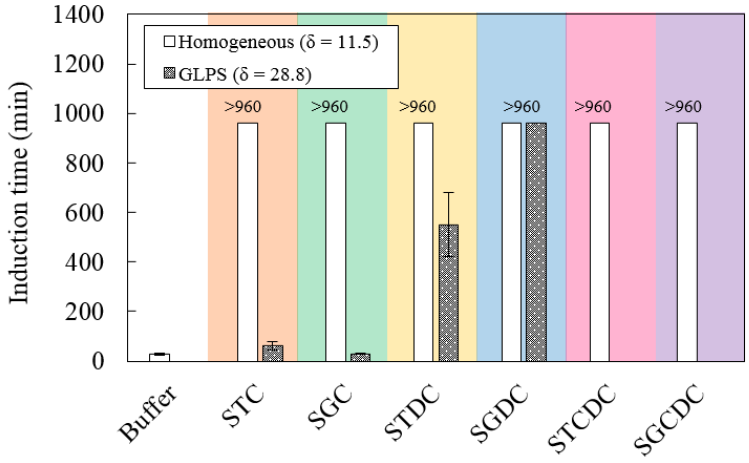

Figure 8. Induction time for telaprevir in the absence and presence of predissolved 1.86mM bile salts. Homogeneous solutions are at a concentration below the amorphous solubility of telaprevir, while GLPS refers to solutions just above the amorphous solubility of telaprevir which contain small (~300nm)[42] amorphous telaprevir particles. Induction time results for solutions containing STCDC and SGCDC at δ=28.8 were not reported as the same supersaturation level cannot be reached due to partitioning of these two bile salts into telaprevir-rich phase.[24]

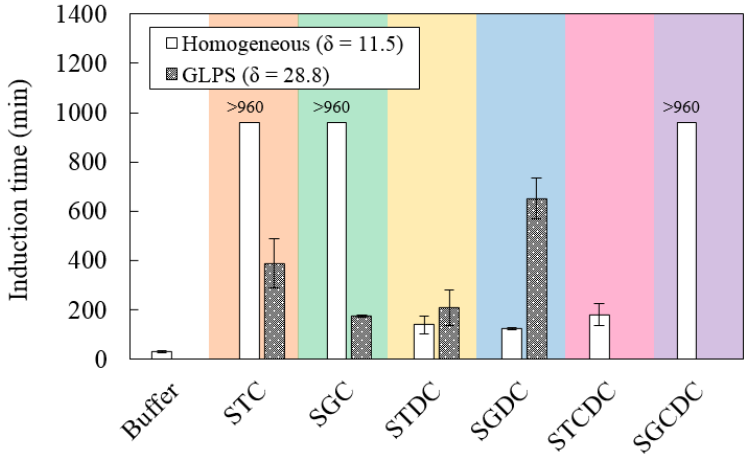

Figure 9. Induction time for telaprevir in the absence and presence of predissolved 12mM bile salts. Induction time results for solutions containing STCDC and SGCDC at δ=28.8 were not reported as the same supersaturation level cannot be reached due to partitioning of these two bile salts into telaprevir-rich phase.[24]

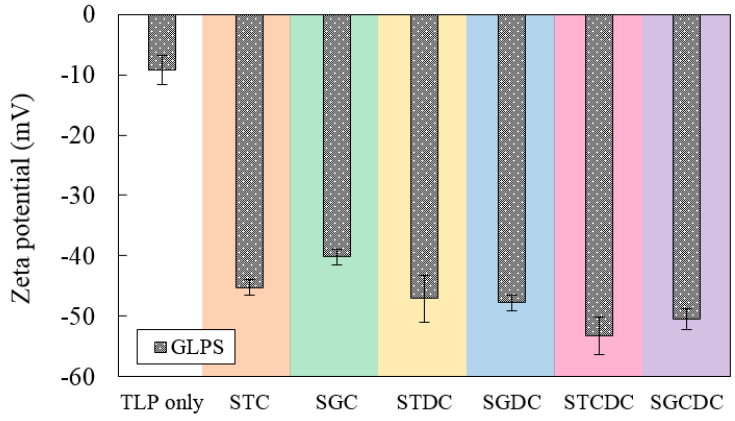

Figure 10. Zeta potential of highly supersaturated telaprevir solution in the absence and presence of 1.86mM bile salts.

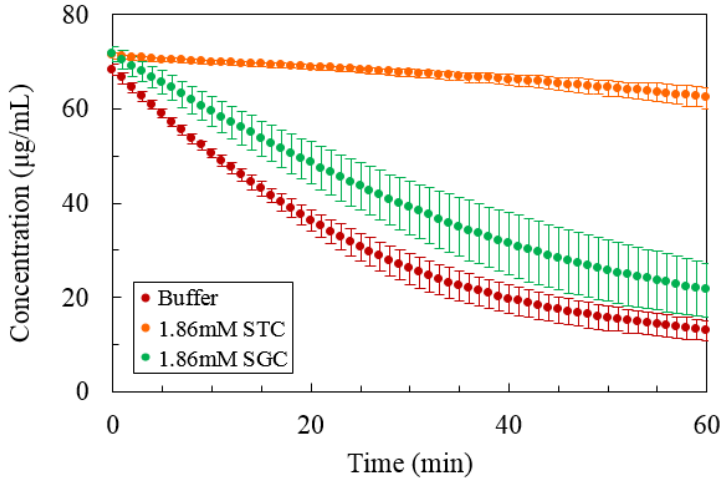

Figure 11. Desupersaturation profile of seeded telaprevir solution in the absence and presence of 1.86mM STC, and 1.86mM SGC. The slope of the initial desupersaturation profiles (t < 10min) was used to determine the mass growth rate.

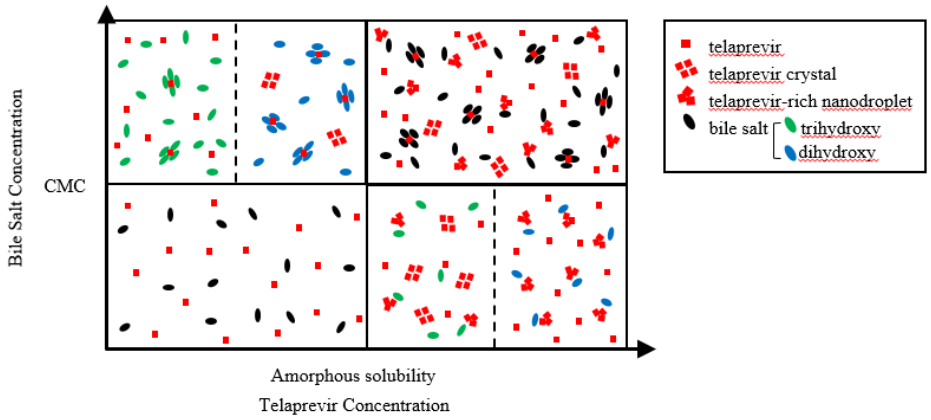

Figure 12. Schematic description of the competing effect of monomeric and aggregated bile salts for supersaturated telaprevir solutions above (containing amorphous drug-rich nanodroplets) and below (homogeneous solution) the amorphous solubility.

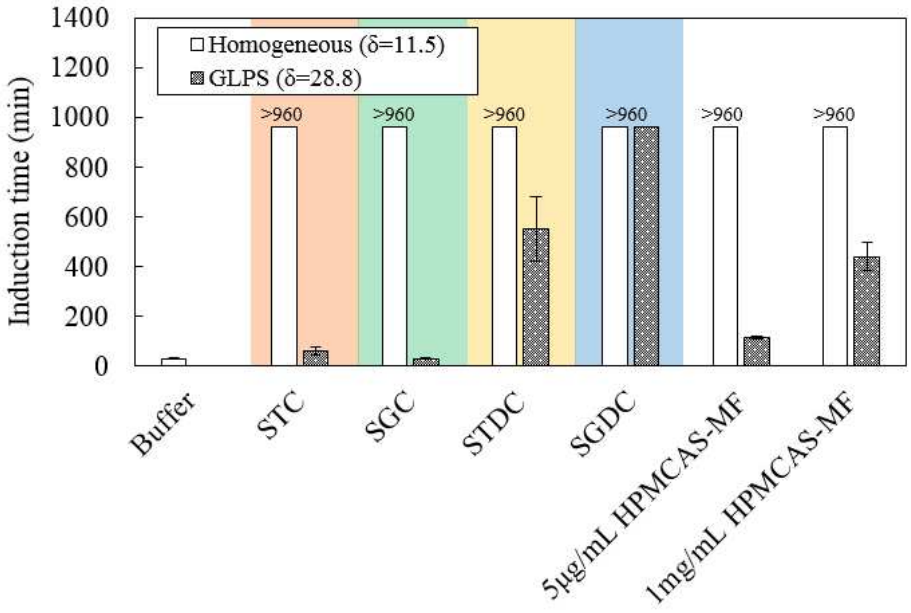

Figure 13. Induction time for telaprevir in the absence and presence of predissolved bile salts (at 1.86mM concentration) and the polymer, HMPCAS-MF (at 5μg/mL and 1mg/mL concentration).

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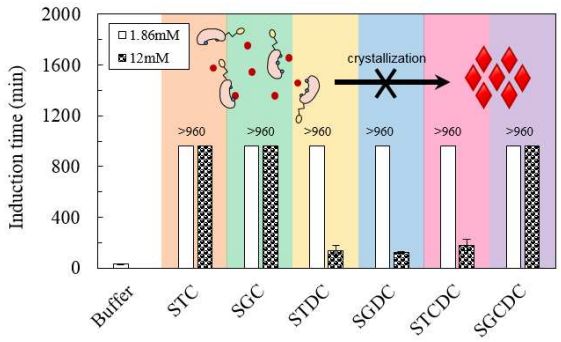
Maintaining Supersaturation of Active Pharmaceutical Ingredient Solutions with Biologically Relevant Bile Salts.

Jennifer Lu, James D. Ormes, Michael Lowinger, Amanda K.P. Mann, Wei Xu, James D. Litster, Lynne S. Taylor



Six biologically relevant bile salts were found to inhibit crystallization of telaprevir from supersaturated aqueous solutions. Monomeric bile salts were found to be more effective crystallization inhibitors than the corresponding micelles.