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Compositional Effect of Complex Biorelevant Media on the Crystallization Kinetics of an Active Pharmaceutical Ingredient

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ABSTRACT

Bile salts are endogenous surfactants present in the human gastrointestinal tract in the form of mixed micelles that also contain phospholipids. Due to the inevitable encounter of oral drug formulations with bile salts, it is important to understand the impact of bile salts on the crystallization tendency of poorly soluble compounds that form supersaturated solutions in vivo in order to maximize oral drug absorption. Although there has been an increasing number of studies focusing on the role of individual bile salts on drug crystallization, the effects of mixed micelles and biorelevant media composition on crystallization kinetics have only been studied to a limited extent. In this study, we evaluated the ability of binary and ternary bile salt combinations to maintain supersaturated aqueous solutions of telaprevir. Crystallization kinetics were also compared in more complex media that also contained the phospholipid, lecithin. These included fasted state simulated intestinal fluid (FaSSIF) (a widely used medium for formulation testing which contains a single bile salt, sodium taurocholate), and media that contained several endogenous bile salts. Finally, the combined effects of a polymer, hydroxypropylmethyl cellulose acetate succinate, and the testing media on crystallization kinetics were evaluated to provide insights into supersaturation formulation design. Solution bile salt composition was found to significantly influence crystallization kinetics. However, the presence of the polymer increased induction times sufficiently that differences between media were minimized. This study suggests that when evaluating the crystallization kinetics of systems with a propensity to undergo supersaturation in vivo, attention should be paid to selecting biorelevant media.

INTRODUCTION

Currently, oral delivery is the preferred drug administration route.[1] For oral administration, the solubility and permeability of the active pharmaceutical ingredient (API) are the two key determinants of drug absorption extent.[2] According to the biopharmaceutical classification system (BCS), a large number of marketed drugs, and the majority of drugs currently under development, are poorly soluble.[3] Solubility enhancement strategies have been developed for improving the bioavailability of poorly soluble compounds. Most of these formulation methods rely on generation of supersaturation in the gastrointestinal (GI) tract either through initial solubilization of the drug substance (cosolvent, lipid-based formulation) or by delivering a high energy solid state form of the drug substance (amorphous solid dispersion, cocrystal, salt).[4] The extent of supersaturation depends on the chemical potential difference between the solute in the solution and in its equilibrium state.[5] Supersaturation provides the driving force for drug membrane transport, enhancing the extent of drug absorption.[4, 6] However, the energy difference between the solute in supersaturated solutions and the equilibrium crystalline state makes crystallization thermodynamically favorable. Other factors, such as variation in the pH along the GI tract, can also impact the supersaturation of ionizable compounds. In vivo crystallization of a drug substance is expected to diminish the solubility enhancement gained from a supersaturating system.[7] In other words, in vivo drug precipitation kinetics play an important role in affecting the bioavailability of supersaturating formulations.

Stabilizing additives are often mixed with the drug substance to retard crystallization and maintain the solubility enhancement. Various polymers have been demonstrated as effective crystallization inhibitors for poorly soluble drugs,[8, 9] and are widely employed for this purpose in commercial formulations. Recently, it has been noted that bile salts, which are endogenous

surfactants, are, in many instances, effective crystallization inhibitors.[10-13] These observations are of particular interest due to the inevitable encounter of oral formulations with bile salts in biorelevant dissolution media and in vivo. Most studies to date have focused on the crystallization inhibitory ability of individual bile salts. These studies revealed the potential of monomeric bile salts as nucleation[11-13] and/or crystal growth,[14] inhibitors. Furthermore, it was noted that bile salts showed a comparable inhibition ability to polymers for poorly water soluble drugs.[13] Bile salts exist in the upper intestinal tract as mixed micelles that also contain phospholipids and cholesterol.[15] Consequently, it is also important to understand the impact of multicomponent bile salt micelles on drug crystallization kinetics.

In order to better predict drug in vivo oral performance, biorelevant media, intended to simulate the GI fluids, have been widely employed for product testing as an alternative to simple buffer solutions. Although various refinements have been made to the biorelevant medium composition, with the goal of improving in vitro/in vivo correlations, sodium taurocholate remains the only bile salt component used in many commercially available simulated human intestinal fluid recipes. However, it has been recently demonstrated that biologically relevant bile salts are not interchangeable from either a thermodynamic[16] or crystallization standpoint.[12, 13] Therefore, it is of interest to investigate whether the current biorelevant medium used for in vitro testing might be oversimplified in the context of supersaturating dosage forms. The goal of this study was to evaluate the impact of biorelevant testing media composition on drug crystallization kinetics from supersaturated solutions. Telaprevir was chosen as the model compound since the influence of individual biorelevant bile salts on this compound has been thoroughly studied from both a thermodynamic[16] and crystallization inhibition stand point[13, 14]. In addition, the effects of binary and ternary bile salt combinations, as well as bile salt-polymer systems on telaprevir crystallization,

were investigated in order to systematically evaluate the impact of multiple additives on crystallization kinetics. Findings from these studies provide important insights into the design and testing of supersaturating formulations.

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, STC (practical grade, MP Biomedicals, LLC, OH), sodium glycocholate, SGC (≥99%, Chem-Impex Int'l. Inc., IL), sodium taurodeoxycholate, STDC (≥97%, Chem-Impex Int'l. Inc., IL), sodium glycodeoxycholate, SGDC (≥97%, Sigma, MO), sodium taurochenodeoxycholate, STCDC (98%, Sinova Inc., MD) and sodium glycochenodeoxycholate, SGCDC (≥99%, Chem-Impex Int'l. Inc., IL) were used as received. FaSSIF instant powder (Biorelevant, London, United Kingdom) was used to prepare FaSSIF solutions as directed by the manufacturer. L-α-Phosphatidylcholine (lecithin, ≥99%) was obtained from Sigma-Aldrich (MO). A regenerated cellulose membrane with a molecular weight cutoff (MWCO) of 6-8k Da was acquired from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). The aqueous media used in all experiments was 50mM pH 6.5 sodium phosphate buffer. Mixed micelle with lecithin solutions were stirred for 2 days before experiments.

METHODS

Supersaturation Determination

For complex solutions where multiple additives are present, independent experiments are required to estimate the degree of supersaturation in the different solutions, and hence to design experiments with comparable crystallization driving forces.[17] In this study, a side-by-side diffusion cell

(PermeGear, Inc. Hellertown, PA) was used to evaluate the supersaturation level of the complex biorelevant media. The composition of media tested in this study is summarized in Table 1. With the previously established method,[13, 16] the supersaturation ratio, δ, of an solution of interest can be estimated from the ratio of the solute mass flow rate in the solution of interest (F) to the mass flow rate of the corresponding standard state system (F°), i.e. the solute in a solution in equilibrium with crystalline state:

$$\frac{F}{F^{\circ}} = \frac{a}{a^{\circ}} = \delta \qquad (1)$$

where a is the solute activity in the solution of interest, and a° is the solute activity at standard state. Using the mass flow rate data for supersaturated telaprevir solutions in buffer from our previous study[16] and equation (1), the determination of telaprevir supersaturation level in different media is summarized in Figure 2. For the binary/ternary additives as well as the multicomponent media studies, δ = 11.5 was chosen to study telaprevir crystallization tendency in a homogeneous supersaturated solution. To study the additional effect of a polymer, a supersaturation level of δ = 28.8 was chosen to represent a highly supersaturated solution where a glassy second phase of telaprevir is present.[16]

Induction Time Measurements

Crystallization induction time measurements were used to evaluate telaprevir crystallization tendency in different media. Supersaturated telaprevir solutions were prepared by the solvent-shift method. A small aliquot of concentrated methanolic telaprevir stock solution (12 mg/mL) was titrated into 50mL of the selected media, stirred at 300 rpm at 37 °C. The onset of telaprevir crystallization was detected from a sudden change in the UV signal at a maximum absorption wavelength (270nm in this study) and at a non-absorbing wavelength (370nm in this

study). The time point of the onset of crystallization is then defined as the crystallization induction time, t_{ind}. 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.

Due to the difficulty of accurately detecting the appearance of the first nucleus, it is common practice to present the measured crystallization induction time, t_{ind}, as a combination of the nucleation induction time, t_n, which is the nucleation time for critical nucleus formation, and a growth period, t_g, which is the time needed for crystals to grow to a detectable size:

$$t_{ind} = t_n + t_g \quad (3)$$

Mass Growth Rate Measurements

Mass growth rate measurements were performed to understand how bile salt mixtures impact bulk crystal growth. An in situ common history seeding method was employed to generate seeds for the mass growth rate experiments. The concept of the in situ common history seeding method was explained previously.[14] After seeding, a bile salt mixture (Table 1) was added to the solution and allowed to equilibrate prior to supersaturation regeneration. A small aliquot of concentrated methanolic telaprevir stock solution (12 mg/mL) was then titrated into the 50mL seeded solution to achieve a telaprevir supersaturation level of δ=7.9 based on the activity-based supersaturation calibration curves described previously.[13] During both the seeding and growth rate determination period, the solution was stirred at 300 rpm at 37 °C, and the concentration change for telaprevir solutions was monitored by a SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber optically coupled with a 1 cm or 0.5cm path length dip probe. Wavelength scans (200-450nm) were performed at 1 minute intervals. The mass crystal growth rate of telaprevir was then determined by estimating the rate of desupersaturation in the seeded solution. The initial slope of

the desupersaturation curve (first 10 minutes) after supersaturation regeneration was used as the desupersaturation rate, R, at a wavelength of 270 nm. SIMCA 13.0.3 software (Umetrics Inc., Umea Sweden) was used to take second derivatives of the UV spectrum for both calibration and sample acquisition to mitigate potential particle scattering effect caused by seed crystals and bile salt micelles. The standard curve was linear (R^2> 0.99) over the relevant concentration range. The effectiveness of bile salt mixture in inhibiting crystal growth, E_G, was estimated using the equation below:

$$E_G = \frac{R_0}{R} \tag{4}$$

where R_0 and R are the desupersaturation rate in the absence and presence of bile salt, respectively. E_G >1 means that the additive slows down telaprevir crystal growth. The higher the E_G value, the more effective the additive (bile salt or bile salt mixtures) is in slowing down crystal growth.

Crystal Morphology Characterization

Scanning electron microscopy (SEM) was used to evaluate the morphology of telaprevir crystals formed after determination of the induction time. Small aliquots (approximately 10μL) were extracted from the crystallized solution, deposited on glass coverslips, and placed into a vacuum oven for at least a week to remove any remaining liquid. The dried samples were sputter-coated with platinum for 60 seconds, and imaged with a FEI NOVA nanoSEM field emission SEM (Hillsboro, Oregon), using an Everhart−Thornley detector (ETD) and through-the-lens detector (TLD). The parameters were 5 kV accelerating voltage, about 5 mm working distance, beam spot size of 3, 30 μm aperture, and magnifications in the 5000−40000× range.

RESULTS AND DISCUSSION

Binary and Ternary Bile Salt Systems

The potential of individual bile salts to delay telaprevir crystallization was demonstrated in a previous study,[13] where various biorelevant bile salt monomers were all found to be extremely good inhibitors when in monomer form. Solutions containing micellar bile salts, however, showed different effects on induction times and growth rates depending on the type of bile salt used. Given that there are multiple bile salts present in vivo, it is important to understand how bile salt combinations impact crystallization. To begin to address this question, the impact of binary and ternary bile salt combinations on telaprevir crystallization was first evaluated. For binary additive systems, STDC and STCDC were mixed at a 1:1 molar ratio yielding a final molar concentration of 1.86mM (binary-L) or 3.72mM (binary-H); these concentrations were selected to yield solutions containing monomeric and micellar bile salts respectively. This bile salt combination was selected as STCDC was found to be an extremely effective growth inhibitor, while STDC was one of the better nucleation inhibitors for the conditions studied.[14] The mass flow rate data (Figure 2) show no significant change of telaprevir mass flow rate as a function of telaprevir concentration in the presence of the binary-L mixture, but a decrease in mass flow rate was observed for the binary-H system, consistent with the presence of solubilizing micelles in the latter system. Figure 3 shows the crystallization induction time of telaprevir at δ=11.5 in the absence and presence of bile salts. With the binary-L mixture, solution supersaturation was maintained over 16 hours. The inhibitory effect achieved with the binary-L mixture is comparable to the individual effects of monomeric STDC and STCDC. As the bile salt concentration increased to 3.72 mM (binary-H), the inhibitory ability of the bile salts is decreased in general and the additive mixture leads to a slightly prolonged (binary-H and 12mM STDC, $p<0.05$, statistically significant, t-test) or similar (binary-H and 12mM STCDC, $p>0.05$, not statistically significant, t-test) induction time as compared to the individual dihydroxy bile salts. The binary-L system appears to be a mixture of monomers, which

are extremely effective crystallization inhibitors, as observed previously for the single component systems,[13] while binary-H contains mixed micelles and is less effective at prolonging induction times. No evidence of synergistic interactions are observed when using a mixture of two bile salts; in general, the binary bile salt mixtures exert an analogous impact on crystallization as their single component counterparts.

According to our previous study, the impact of bile salts on telaprevir crystallization kinetics largely depends on the number and position of hydroxyl groups in their molecular structures.[14] Equal molar ratio combinations of STC and STDC and STCDC were chosen to study the impact of a ternary mixture at total bile salt concentrations of 1.86mM (ternary-L) and 5.58mM (ternary-H). STC was added to the mixture since it shows good inhibition of telaprevir crystallization even in the presence of micelles. Similar to the binary system, no significant change of telaprevir mass flow rate was observed in the presence of the ternary-L mixture while a decrease in mass flow rate was noted for the ternary-H system. The inhibition of telaprevir crystallization by the ternary mixture is compared against that observed for the single component bile salt solutions with results summarized in Figure 4. For individual bile salt systems, the trihydroxy bile salt, STC, is effective in inhibiting telaprevir crystallization over 16 hours in solutions in the absence and presence of STC micelles, while the presence the dihydroxy bile salt micelles strongly negates the inhibition effect of the corresponding monomers. For the ternary-L mixture of the three bile salts, no crystallization was observed over 16 hours at a supersaturation ratio δ=11.5. As bile salt concentration increases to 5.58 mM (ternary-H), the induction time of telaprevir supersaturated solutions at the same supersaturation level decreases, and the system shows a slightly improved inhibition ability to that observed for the corresponding dihydroxy bile salts micellar solutions (ternary-H and 12mM STDC, $p<0.05$, statistically significant, t-test; ternary-H and 12mM STCDC,

$p>0.05$, not statistically significant, t-test), but considerably lower than observed for STC alone. The mass flow rate and induction time results suggest that STC, STDC, and STCDC existed as a mixture of monomers at 1.86mM total bile salt concentration, while mixed micelles of the three bile salts were formed at 5.58mM total bile salt concentration. Thus, the ternary mixed micelle system appears to show an intermediate effect on the induction times relative to that observed for the single bile salt micelles solutions, and the inhibitory impact of STC is reduced by the presence of the other bile salts.

The impact of binary mixtures of bile salts on crystal growth was also evaluated by determining the seeded desupersaturation rates of telaprevir (Figure 5). It is assumed that bulk crystal growth is the dominating mechanism of desupersaturation, although it should be noted that secondary nucleation can occur at the supersaturation level ($\delta=7.9$) employed. The impact of the individual bile salts on telaprevir crystal growth was reported in a previous study.[14] Monomeric STDC and STCDC exhibit quite different extents of inhibition ability, with STCDC being an extremely effective growth inhibitor while STDC has a more modest impact. With the binary-L mixture, the bile salt combination shows an intermediate extent of inhibition as compared to the individual monomeric bile salts. As the bile salt concentration increased two-fold (binary-H), the inhibitory ability of the bile salt mixture decreased relative to that of the binary-L system.

There are different possible scenarios for interface adsorption in multi-additive systems. The different additives can be adsorbed independently or can compete for the adsorption sites,[18] or they can interact to form complexes and adsorb as the complex.[19] Supporting evidence for these scenarios can be found in studies of polymer/polymer and polymer/surfactant systems.[20, 21] Based on our experimental data, we can speculate that when bile salts are present in monomeric form in seeded solutions (binary-L), both bile salts are adsorbed. A different effectiveness of each bile salt

at the crystal surface in terms of preventing addition of solute molecules results in the observed intermediate inhibition effect relative to the individual bile salts. However, the situation is much more complex when the total bile salt concentration increases as mixed micelles form and these serve to counteract the inhibitory effect of the monomers.

Compositions of the Biorelevant Testing Media.

To better understand the crystallization inhibition properties of human bile and to compare to current FaSSIF recipes, we combined the six most abundant bile salts in human intestinal fluids with lecithin to mimic human bile composition. Two types of mixed micelle with lecithin solutions were investigated in this study, and their compositions are listed in Table 1. The total bile salt concentration is kept at 3mM, and the lecithin concentration at 0.75mM, for direct comparison with the FaSSIF recipe. For mixed micelles with lecithin solution 1 (MWL-1), the bile salt composition is modified from that reported in literature,[22] with cholate and chenodeoxycholate bile salts being the predominant species in terms of hydroxylation.[23, 24] A high degree of variability in bile salt composition in GI contents has been reported.[22, 25] In an attempt to evaluate the impact of variability in bile salt composition on crystallization kinetics, mixed micelles with lecithin solution 2 (MWL-2) was also prepared. In MWL-2, the recipe was adjusted so that cholate and deoxycholate bile salts are the predominant species. Deoxycholate bile salts appear to be more effective nucleation inhibitors for telaprevir.[14] It was thus of interest to determine if having a higher proportion of effective nucleation inhibitors in the mixed micelle solution would prolong supersaturation.

To investigate the impact of testing media on telaprevir crystallization tendency, crystallization induction times were determined at a constant supersaturation level (δ=11.5) with FaSSIF, as well as the MWL-1 and MWL-2 solutions. As shown in Figure 6, the crystallization

induction time of telaprevir is around 30 minutes in buffer and in FaSSIF solutions. In contrast, supersaturation was maintained in both of the mixed micelles with lecithin solutions and no crystallization was observed over 16 hours. According to our previous study,[14] it appears that several of the other biorelevant bile salts are better nucleation inhibitors than STC, the sole bile salt used in current biorelevant testing media. Furthermore, STC is a minor component of human bile and hence in our mixed micelle systems. The significant difference between telaprevir induction times in FaSSIF and in the mixed micelles with lecithin solutions further demonstrates that STC may not be a good surrogate for human bile composition. Our results are in general agreement with those of Li et al.[12] who reported a two fold increase in celecoxib induction time in mixed bile salt solutions compared to the STC only solution under constant total bile salt concentration and initial celecoxib concentration, although no lecithin was used in these studies. In addition, it is interesting that little difference was seen between MWL-1 and MWL-2, thus varying the composition of bile salts in terms of hydroxylation shows a minimal impact on telaprevir crystallization at the supersaturation level studied. Taken in concert, these observations suggest that the in vivo crystallization tendency of poorly soluble compounds may not be adequately predicted by experiments conducted in commonly used simulated biorelevant media. This may be of particular relevance for two stage dissolution tests used to evaluate the precipitation tendency of weakly basic compounds on transferring from the acidic stomach to the higher pH small intestine environment; for these experiments, samples are commonly diluted into simulated intestinal media containing STC and lecithin.[26-28] However, while the large differences in induction times in the different media are extremely interesting, it should be noted that our mixed micelles with lecithin recipe is also a simplified biorelevant testing medium. In addition, there are other variables in the gastrointestinal tract, such as dynamic fluid motion, concentration fluctuation

and food effect, which further complicate drug in vivo behavior. Although in vitro testing might not fully capture drug precipitation taking place in the GI tract, nevertheless, this study provides evidence of a significant impact of media selection on the propensity of supersaturated telaprevir solutions to crystallize.

Combined Effect of Polymer and Testing Media.

As polymers are used in some instances in formulations to prolong supersaturation, it is of interest to investigate whether bile salt and polymer combinations show synergistic effects on crystallization inhibition. In addition, given the observed impact of testing medium composition on telaprevir crystallization, an important question to be addressed is: does the presence of an effective polymeric crystallization inhibitor override the impact of testing media differences? HPMCAS-MF is one of the most potent polymeric inhibitors of telaprevir crystallization from aqueous solutions.[9] We therefore investigated the crystallization tendency of telaprevir at its amorphous solubility in different media containing 150 μg/mL predissolved HPMCAS-MF. Assuming that the polymer at the concentration employed has negligible effect on solution thermodynamics,[29] the supersaturation level at the amorphous solubility is δ=28.8.[13] In all experiments, the supersaturated solutions were observed to be turbid, indicating that phase separation (Glass-liquid phase separation, GLPS) had occurred i.e., the solution was at the amorphous solubility.[30] From Figure 7, it is apparent that a telaprevir solution at its amorphous solubility is highly unstable in buffer and crystallized immediately. With the addition of HPMCAS-MF, the supersaturated solution containing a telaprevir-rich second phase can be stabilized for more than 6 hours. At the same polymer concentration (150 μg/mL) and crystallization driving force (δ=28.8), there is no statistical difference observed for telaprevir crystallization tendency in different testing media (One-way analysis of variance, $F<F_{critical}$). This

is contradictory to our observations with systems in the absence of HPMCAS-MF at a lower supersaturation, where the composition of the testing medium plays an important role in dictating crystallization kinetics. The prolonged induction times observed in these systems is of relevance in the context of small intestinal transit times which have been reported to be around 3-4 hours.[31] That is, the solubility enhancement from formulations of telaprevir can likely be guaranteed over a biorelevant time frame with proper selection of the inhibitory excipient, regardless of fluctuations in media composition. Similar observations were made in a different study, where the effects of bile on the oral absorption of halofantrine administered to rats in vehicles consisting of the co-solvent polyethylene glycol 400 alone or in mixtures with the surfactant polysorbate 80 were studied.[32] It was suggested that the addition of exogenous surfactants above a certain concentration threshold rendered the bioavailability of the formulation more bile composition independent. So far, excipient selection still remains highly empirical, and extensive screenings are often required to obtain drug-excipient combinations that optimize formulation stability.[9, 33] In other words, the impact of media composition on API crystallization may not always be outweighed by the stabilizing effect of excipients in the drug formulation. More attention should be paid to testing media selection when evaluating performance of supersaturation formulations.

Phospholipids form mixed micelles with bile salt in vivo and solubilize poorly soluble drugs. Lecithin is the phospholipid mixture[34] that is added to commercial biorelevant media. It consists of poorly water soluble amphiphilic molecules that incorporate into bile salt micelles.[35] The role of lecithin in the presence of the polymer and bile salts was also evaluated, and the results are summarized in Figure 8. By comparing the induction time results for FaSSIF (3mM STC with 0.75mM lecithin) and 3mM STC, it is evident that the presence of lecithin decreases the inhibitory effect of STC on telaprevir crystallization. A similar trend is also observed by comparing the

induction time results of MWL-2 (3mM of mixed bile salts with 0.75mM lecithin) and M-2 (3mM of mixed bile salts) from Figure 8. Given that there has been some debate in the literature about the amount of lecithin that should be included in media formulations,[35, 36] this is an important observation. As shown in Figure 8, the induction time of supersaturated solutions at δ=28.8 increases with the addition of either 3mM of STC or 3mM of mixed bile salts (M-2) at constant polymer concentration (150 μg/mL of HPMCAS-MF). Moreover, the addition of mixed bile salts delays telaprevir crystallization longer than the addition of 3mM STC. That is, bile salts and HPMCAS-MF show a synergistic effect on crystallization inhibition of telaprevir. The synergistic effect is largely negated by the presence of lecithin; this can be seen by comparing the induction times for buffer alone and the FaSSIF medium (both with polymer) where very similar induction times are observed. In the recent update, FaSSIF-V2 was proposed with a lower lecithin concentration (0.2 mM) compared to FaSSIF-V1 (0.75 mM).[37] With the decreased amount of lecithin, a more pronounced media composition impact on telaprevir crystallization tendency is expected in solutions in the presence of HPMCAS-MF.

As well as influencing the crystallization induction times, the media composition also impacted the crystal morphology (Figure 9). Telaprevir crystals formed in buffer are bouquet-like aggregates of prismatic particles with smooth surfaces that radiate from a central origin (Figure 9(a)). The presence of HPMCAS-MF reduces the number of particles forming each aggregate and results in particles with a rough surface (Figure 9b). The crystals obtained from lecithin- containing mixed micelle solutions in the absence and presence of HPMCAS-MF are shown in Figures 9c, 9e and 9d, 9f, respectively. From MWL-1 and MWL-2, the crystals are similar in shape and surface smoothness to those obtained from buffer, although they are less aggregated and more individual particles can be seen. Adding HPMCAS-MF to the mixed micelle medium leads to crystals of

similar morphology and roughness to those obtained from buffer containing HPMCAS-MF (Figure 9b). A number of examples in the literature have demonstrated the impact of polymer on crystal morphology.[38-41] It was suggested in an early study that the polymer (PVP) formed a net-like structure on crystal surfaces, from which the drug (sulfathiazole) grew into finger-like protrusions leading to rough surfaces.[42] A similar roughening effect was observed with felodipine crystals in the presence of HPMCAS under atomic force microscopy, in which the roughening effect was suggested to be the result of HPMCAS pinning the growth steps of felodipine crystals.[43, 44]

Although no difference in induction times was observed for telaprevir in mixed micelle with lecithin solutions and FaSSIF solutions containing HPMCAS-MF, the resultant crystals show distinct morphology differences. The crystals obtained from MWL-1 and MWL-2 in the presence of HPMCAS-MF are rough aggregates, while the crystals obtained from FaSSIF in the presence of HPMCAS-MF are smooth and more plate-like (Figure 9h). In other words, additives clearly interact differently with the crystal surface leading to variations in the observed morphology. The effect of crystal morphology on in vitro dissolution rate has been demonstrated in the literature. Adhiyaman and Basu[45] revealed that the crystallization conditions as well as the medium where crystallization takes place have a major impact on the morphology of dipyridamole. It was noted that improved dissolution rate was achieved with the rod shaped crystals formed from benzene compared to needle shaped crystals produced using methanol. Nokhodchi et al.[46] also showed that phenytoin crystallized from different solvents led to distinct crystal morphology and differences in dissolution rate. Since morphology is known to influence dissolution kinetics, the redissolution behavior of precipitated systems may vary depending on the environment in which they form. This

is an area that has not been widely investigated, although it has been suggested that precipitate properties may influence bioavailability.[47]

CONCLUSION

In this study, the effect of additive mixtures of varying complexity, designed to contain components found in simulated and human intestinal fluids, on telaprevir crystallization tendency was investigated. Media composition was found to play a critical role in influencing crystallization kinetics, as evaluated through the study of nucleation-induction times. FaSSIF, a widely used biorelevant dissolution medium containing a single bile salt, was found to lead to shorter induction times as compared to an analogous medium containing several bile salts. This observation has potential implications for predicting in vivo precipitation tendency of compounds from in vitro tests using simulated media. Addition of a polymer was found to lead to synergistic effects between the polymer and biorelevant bile salts. In contrast, lecithin was found to reduce the bile salt inhibitory effect. These observations are of importance in the context of designing experiments to test precipitation potential, as well as for formulation design.

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TABLE

Table 1. Compositions of biorelevant media and testing media used in this study.

Biorelevant media	Bile Salts	Phospholipids (Lecithin)
fasted state simulated intestinal fluid (FaSSIF)	3mM STC	0.75mM
human jejunum fluid	1.5-5.3mM total concentration[25]	0.08-0.2mM[25]
human duodenum fluid	1.4-5.9mM total concentration[25]	0.09-1.81mM[25]
binary-L	1.86mM total concentration (50% STDC, 50% STCDC)	none
binary-H	3.72mM total concentration (50% STDC, 50% STCDC)	none
ternary-L	1.86mM total concentration (33.3% STC, 33.3% STDC, 33.3% STCDC)	none
ternary-H	5.58mM total concentration (33.3% STC, 33.3% STDC, 33.3% STCDC)	none
mixed micelle with lecithin solution 1 (MWL-1)	3mM total concentration (12% STC, 28% SGC, 6% STDC, 15% SGDC, 12% STCDC, 27% SGCDC)	0.75mM
mixed micelle with lecithin solution 2 (MWL-2)	3mM total concentration (12% STC, 28% SGC, 12% STDC, 27% SGDC, 6% STCDC, 15% SGCDC)	0.75mM
mixed micelle solution 2 (M-2)	3mM total concentration (12% STC, 28% SGC, 12% STDC, 27% SGDC, 6% STCDC, 15% SGCDC)	none

FIGURES

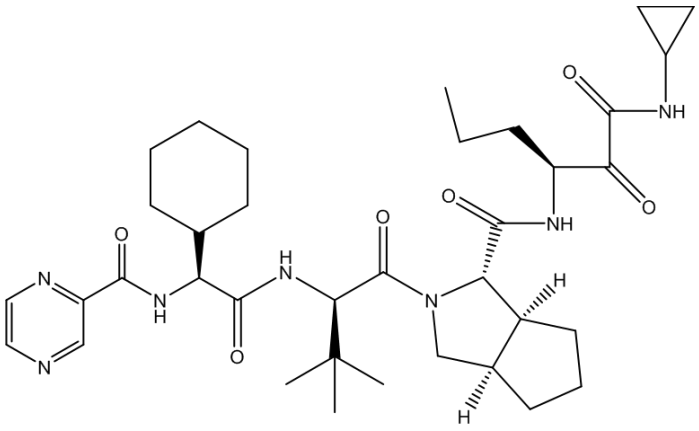

Figure 1. Molecular structure of telaprevir.

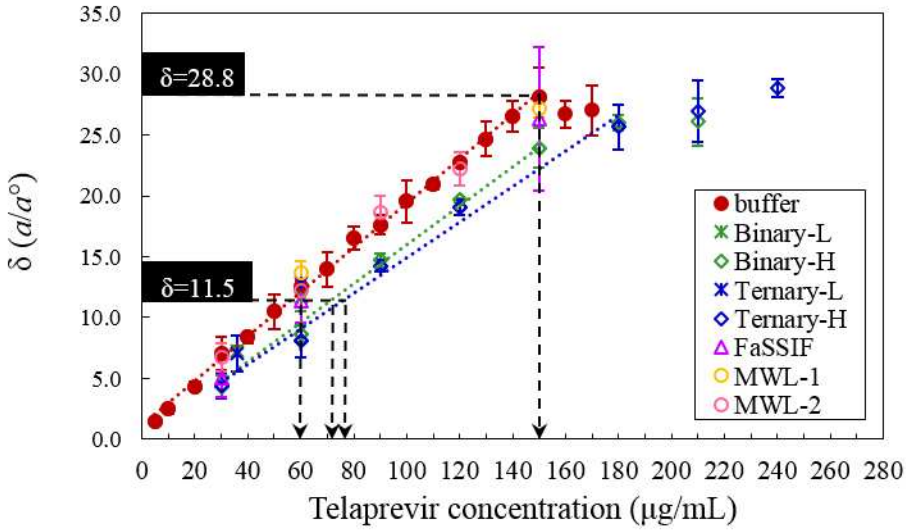

Figure 2. Telaprevir concentrations correspond to constant supersaturation levels in different media. The mass flow rate data of telaprevir in buffer solutions from our previous study were used for determination of telaprevir supersaturation level in different media.[16]

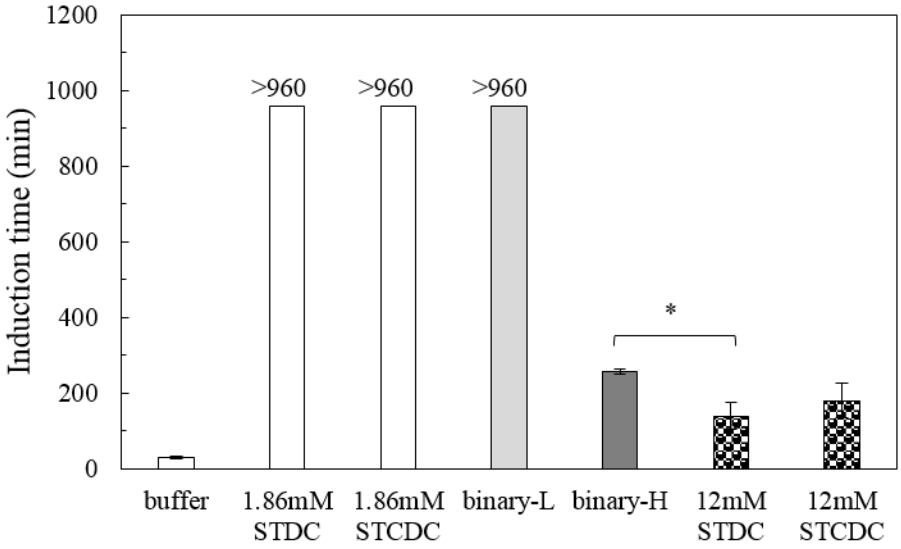

Figure 3. Induction times for telaprevir at a supersaturation level of δ=11.5, in the presence of predissolved bile salts and bile salt binary mixtures (Total bile salt concentrations for binary-L and binary-H mixtures are 1.86 mM and 3.72 mM, respectively). The * symbol indicates that the difference in induction time is statistically significant ($p < 0.05$) using t-test.

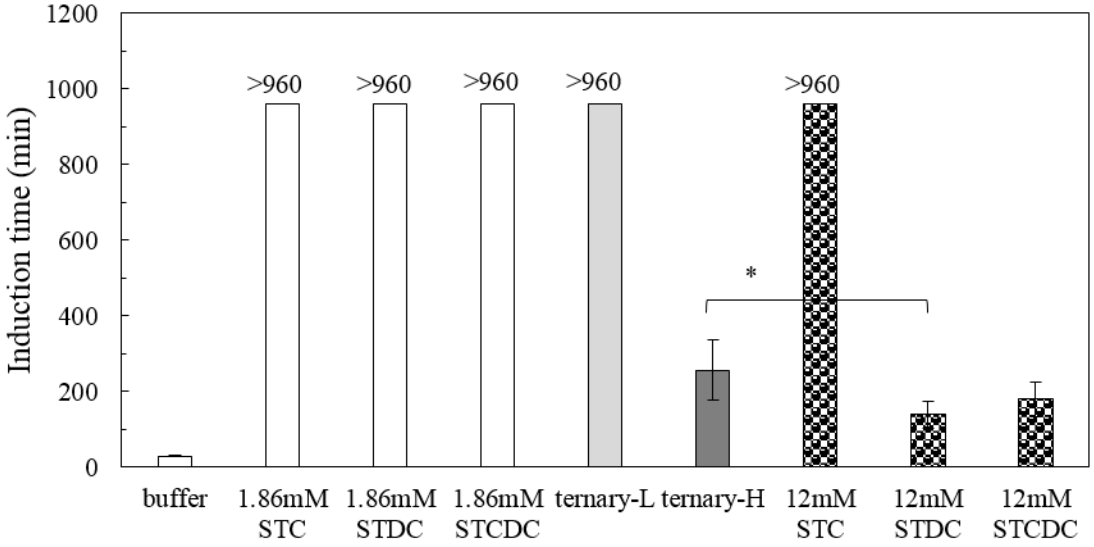

Figure 4. Induction time for telaprevir at a supersaturation level of δ=11.5, in the presence of predissolved bile salts and a ternary bile salt mixture. (Total bile salt concentrations for ternary-L and ternary-H mixtures are 1.86 mM and 5.58 mM, respectively). The * symbol indicates that the difference in induction time is statistically significant ($p < 0.05$) using t-test.

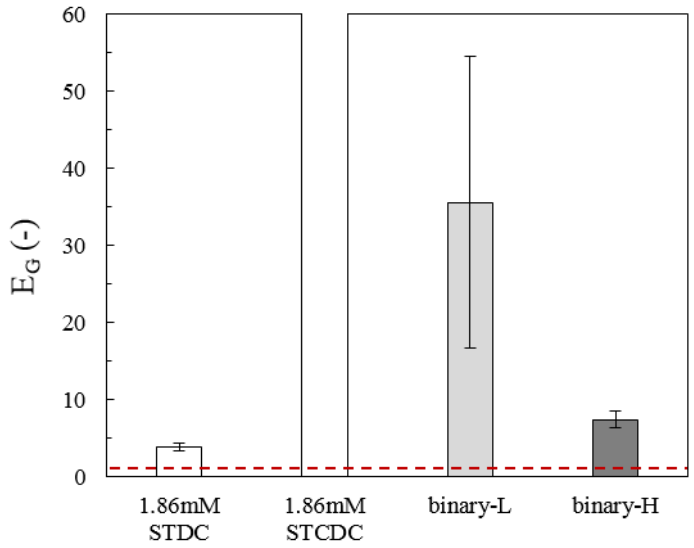

Figure 5. Effectiveness of bile salts and their binary mixtures as growth inhibitors of seeded telaprevir solutions at an initial supersaturation level δ=7.9. The red dashed lines represent the scenario of equivalent rates of bulk crystal growth in the absence and presence of bile salts (E_G=1).

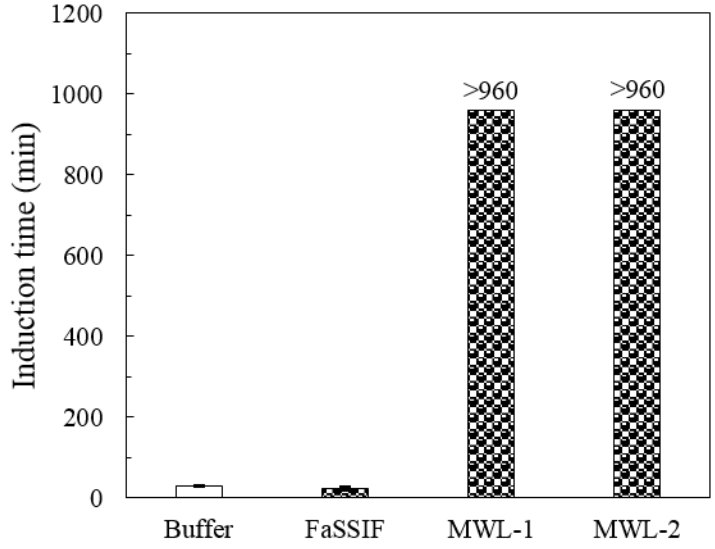

Figure 6. Induction times for telaprevir at a supersaturation level of δ=11.5, in various media namely buffer, FaSSIF and mixed micelles with lecithin solutions.

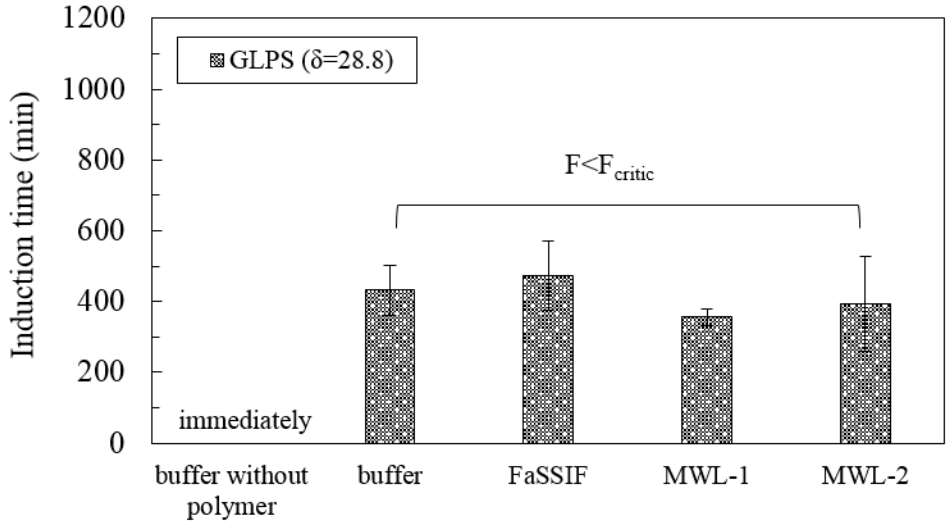

Figure 7. Induction times for telaprevir at a supersaturation level of δ=28.8 in different testing media with 150 μg/mL of HPMCAS-MF. GLPS refers to solutions just above the amorphous solubility of telaprevir which contains small glass-like amorphous telaprevir particles.

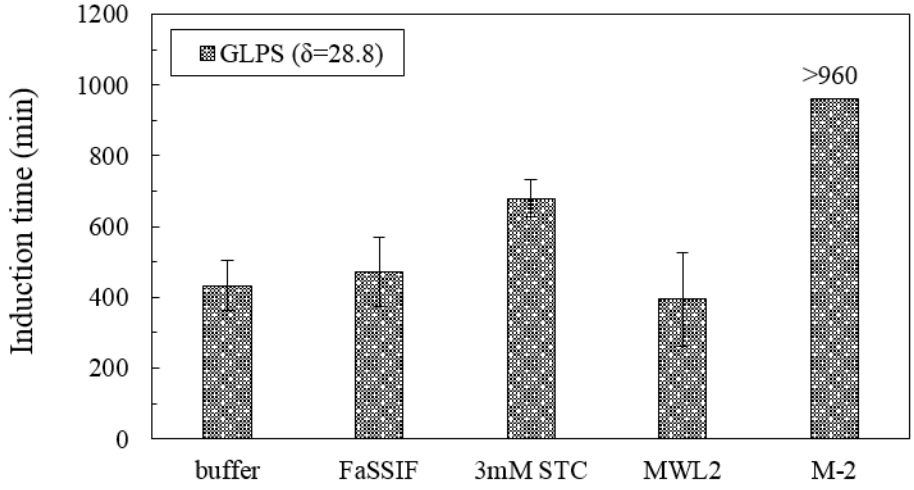

Figure 8. Impact of mixed bile salt micelles containing lecithin (FaSSIF and MWL-2) versus bile salt micelles with no lecithin (3mm STC and M-2) on telaprevir crystallization in solutions

containing 150 μg/mL of HPMCAS-MF. GLPS refers to solutions just above the amorphous solubility of telaprevir which contains small glass-like amorphous telaprevir particles.

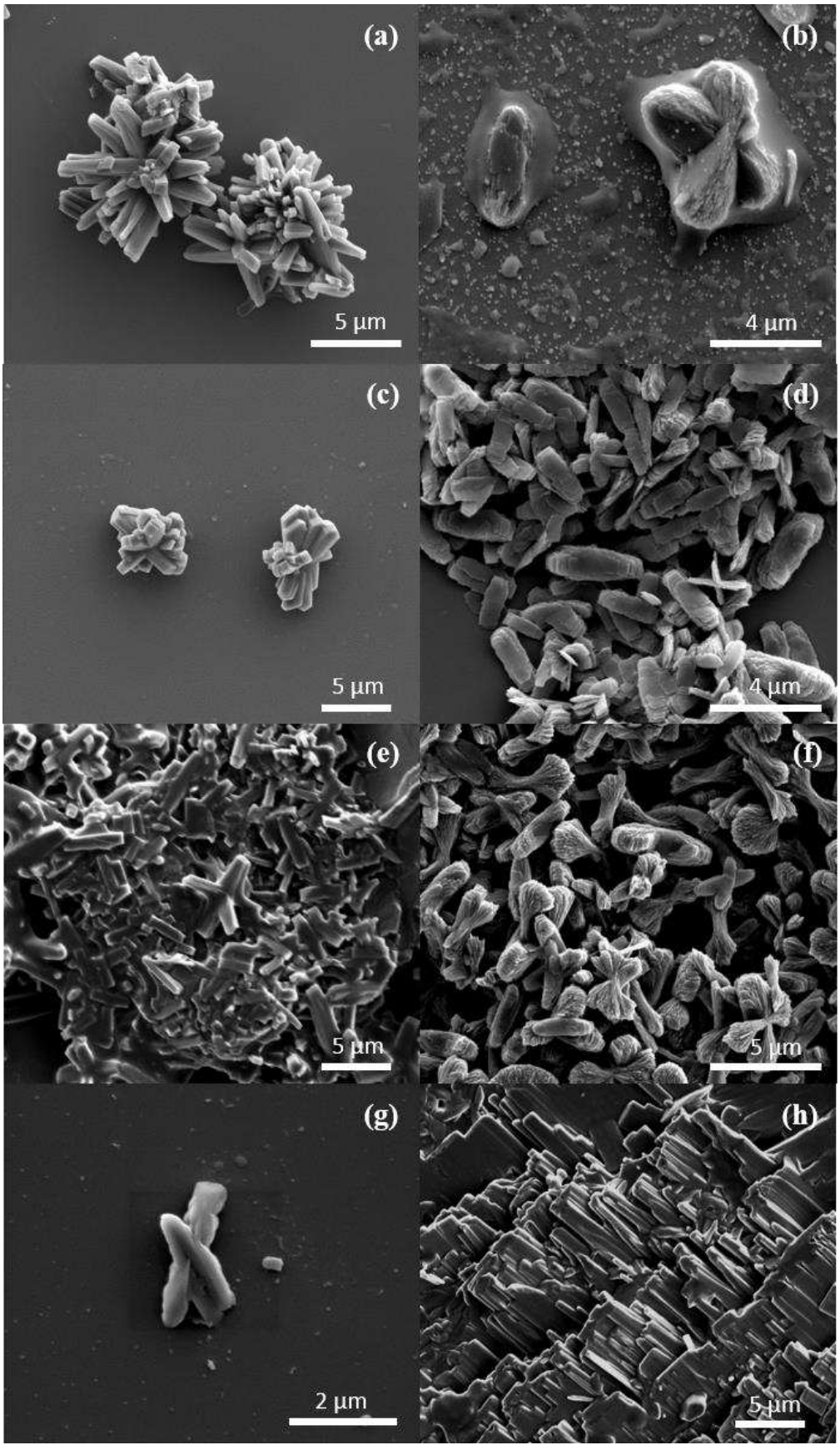
(a)
5 µm
(b)
4 µm
(c)
5 µm
(d)
4 µm
(e)
5 µm
(f)
5 µm
(g)
2 µm
(h)
5 µm

Figure 9. SEM images showing telaprevir crystals after crystallization induction time experiments (a) in buffer, (b) in the presence of predissolved 150 μg/mL HPMCAS-MF in buffer, (c) in mixed micelles with lecithin solution MWL-1, (d) in the presence of predissolved 150 μg/mL HPMCAS-MF in MWL-1, (e) in mixed micelles with lecithin solution MWL-2, (f) in the presence of predissolved 150 μg/mL HPMCAS-MF in MWL-2, (g) in FaSSIF, (h) in the presence of predissolved 150 μg/mL HPMCAS-MF in FaSSIF.

TOC Graphic

Bile salts impact crystal nucleation and growth of supersaturated solutions of poorly water soluble drugs.