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Lu, J., Ormes, J.D., Lowinger, M. et al. (5 more authors) (2017) Impact of Bile Salts on Solution Crystal Growth Rate and Residual Supersaturation of an Active Pharmaceutical Ingredient. Crystal Growth and Design, 17 (6). pp. 3528-3537. ISSN 1528-7483

https://doi.org/10.1021/acs.cgd.7b00464

Impact of Bile Salts on Solution Crystal Growth Rate and Residual Supersaturation of an Active Pharmaceutical Ingredient.

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ABSTRACT

Given the fact that amorphous solid dispersions (ASD) have been one of the most popular formulation strategies to address the issue of inadequate aqueous solubility for small molecules, it is of particular interest to control crystal growth from supersaturated solution in order to maximize the extent of oral absorption of therapeutic agents. Recently, bile salts have been shown to delay crystallization of poorly soluble compounds. In this study, an in-situ common history seeding method was proposed to provide seeds that better mimic crystals formed from supersaturated solutions. The effectiveness of both monomeric and miceller biorelevent bile salts on solution crystal growth and secondary nucleation of telaprevir was then examined. Most bile salts investigated in this study exhibit growth inhibition properties. The extent of growth inhibition, however, various with structural difference amongst the bile salts and their aggregation level.

INTRODUCTION

Controlled crystallization with additives has been of great interests to the pharmaceutical industry. Due to the increased number of poorly soluble compound in the drug developmental pipeline, contemporary formulation strategies often involve implementing a higher energy form of active pharmaceutical ingredients (APIs). Take amorphous solid dispersion for example, the amorphous solids and the dissolved solution improve the extent of oral absorption by providing drug transport with a higher concentration gradient across the membrane.[1-3] However, the inhered crystallization tendency of these high energy forms of API often imperils the stability and performance of the formulations.[4] Employing foreign components to interrupt the crystallization process thus become an answer to maintaining solubility advantages of these formulations.[5]

Solution crystallization consists of two major kinetic events: nucleation and crystal growth. Nucleation take place when a supersaturated system overcomes the free energy barrier for critical nucleus formation[6], following by a consumption of molecularly dissolved solute due to new crystalline matter formation. Current research mostly focus on the impact of additives on nucleation. For instance, polymers are well known nucleation inhibitors for APIs,[7-10] while surfactants on the other hands are found to promote nucleation[11]. Besides the commonly employed excipients in commercial formulation, bile salts, the endogenous surfactant exist in human intestinal fluids, has been shown to inhibit nucleation of a wide variety of structurally diverse APIs[11-13][cite manuscript#2]. Meanwhile, there has been rising interests in controlled crystal growth in the past decade. Owing to the instable nature of amorphous formulations, there's a high chance of crystalline seeds forming during formulation, manufacturing, storage or even upon drug administration. When crystal seeds presence, the lower energy barrier for kinetic

events such as secondary nucleation and crystal growth facilitates supersaturation consumption, thus deplete the enhanced solubility advantage. The ability to control crystal growth, therefore, ensure a longer oral absorption time frame as the consume rate of molecularly dissolved solute may be slowed down.

It is widely accepted that foreign species such as additives can alter crystal growth kinetics by adsorbing to growth sites and acting as a mechanical barrier.[6, 14] Polymeric additives are known as effective growth inhibitors for poorly soluble compounds. Some of the key factors found to affect the extent of growth inhibition are the hydrophobic interactions between polymer and drug[15-18], polymer conformation upon surface absoption,[19, 20] ability to form hydrogen bonding between polymer and drug,[21, 22] as well as seed preparation.[23] On the other hand, it has been demonstrated that surfactants can promote crystal growth, and negate the inhibitory impact of polymers.[23, 24] Thus far, the impact of bile salts on drug crystallization kinetics has not been explored in detail. However, this is an important area of research given the inevitable encounter of supersaturating dosage forms and these endogenous surfactants during *in vitro* dissolution testing and *in vivo*. In our previous study, it was observed that all biorelevant bile salts effectively slow down formation of crystals from both homogenous supersaturated telaprevir solutions and highly supersaturated telaprevir solutions containing a second phase. Monomeric bile salts were found to be effective inhibitors, while the presence of bile salt micelles opposed the monomeric inhibitory effect for some of bile salts[cite manuscript#2]. In addition, the preliminary results reveal the potential of bile salts as crystal growth inhibitors. This current study builds upon our knowledge of the impact of bile salts on telparevir solution thermodynamics[cite M1] and solution crystallization[cite M2], and a systematic evaluation of both monomeric and miceller bile salts as crystal growth inhibitors was carried out by

performing seeded desupersaturation 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 and Table 1. 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.

METHODS

In-situ Common History Seeding and Mass Growth Rate Measurements.

Crystal growth is sensitive to seed properties. To better mimic how nuclei grow when they first form in supersaturated solution, an in-situ common history seeding method was implemented to provide suitable seeds for telaprevir crystal growth rate measurements (Figure 2). Supersaturated telaprevir solutions were prepared by titrating concentrated methanolic telaprevir stock solution (12 mg/mL) to 50mL of 50 mM pH 6.5 sodium phosphate buffer to achieved

telaprevir concentration of 60μg/mL. The supersaturated solution was then stirred at 300 rpm at 37 °C for 6 hours to allow crystallization taking place. After fixed 6 hour seeding period, telaprevir solution concentration was observed to decrease back to it crystalline solubility, and supersaturation in solution was regenerated by adding methanolic telaprevir stock solution according to the activity-based supersaturation calibration curves shown in our previous study[cite manuscript#2] to achieve different initial supersaturation levels. The corresponding concentrations are listed in Table 2. For experiments with bile salts, bile salts (1.86mM or 12mM concentration) were added to the seed solution and allow to equilibrium prior to supersaturation regeneration. The crystal growth rate of telaprevir were then determined by measuring the rate of desupersaturation in the seeded solution. The initial slope of the desupersaturation curve (first 30 minutes for δ=2.7 and first 10 minutes for higher supersaturation levels) after supersaturation regeneration was determined as the desupersaturation rate *R*. The concentration change of telaprevir solution during the seeding process, as well as the crystal growth period were 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, at a wavelength of 270 nm. Wavelength scan (200-450nm) were perform at 1 minute interval for 16 hours. Second derivatives of the UV spectrum were taken using SIMCA 13.0.3 software (Umetrics Inc., Umea Sweden) for calibrations and sample acquisitions to mitigate potential particle scattering effect caused by seed crystals and bile salt micelles. The standard curve presented good linearity (R^2> 0.99) over the relevant concentration range. Solution turbidity was as well recorded by monitoring change in extinction at 370nm wavelength, where telaprevir have no absorbance, as an indication for secondary nucleation.[17]

Residual Supersaturation Determination.

A side-by-side diffusion cell (PermeGear, Inc. Hellertown, PA) was used to evaluate the residual supersaturation level of telaprevir crystallized solutions in the absence and presence of different bile salts (at 1.86 mM or 12 mM bile salt concentrations). The basic concepts were as described in the previous studies.[cite M#1 and M#2] The ratio of solute mass flow rate in the solution of interest (F) to the mass flow rate of the corresponding standard state system ($F°$), solute in a solution in equilibrium with crystalline state in this study, yields the fundamental supersaturation δ:

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

where a is the solute activity in the solution of interest, and $a°$ is the solute activity at standard state. With the mass flow rate data and the previously established calibration curve[cite manuscript#1], residual supersaturation level of telaprevir solution after 15 hour growth can be determined from Equation (1).

Seed Crystal Morphology Characterization.

The scanning electron microscopy (SEM) was used to reveal the morphology of telaprevir crystals prepared by the in-situ common history seeding method as well as the ones obtained from suppliers. For telaprevir crystals prepared by in-situ common history seeding method, small amount of liquid samples (approximately 10μL) were extracted from the seeded solution after 6 hour seeding time, and deposit on glass coverslips and placed into the vacuum oven for a week prior to observation 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 5000−30000× range.

Zeta Potential Measurements.

The zeta potentials of telaprevir seed crystals in solutions in the presence of monomeric bile salts were measured using a Nano-Zetasizer (Nano ZS, Malvern, Westborough, MA). Telaprevir seed crystals 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. The solutions were stirred at 300rpm at 37°C, allowing crystallization to take place, and the seed crystals were continuously stirred overnight for equilibrium. 1.86mM of bile salts were added to the stirred seed crystal solution 30 minutes prior to zeta potential measurements. The zeta potentials of telaprevir seed crystals with no bile salt were also measured for reference.

RESULTS AND DISCUSSIONS

Common History Seed Crystals and Crystal Growth rates

Seeding has been a crucial step to crystallization in the pharmaceutical industry as it strongly dictates the final product properties. It has been demonstrated that seed crystals prepared in different environments leads to different abilities to subsequently grow[23]. To prepared seeds that better imitate crystal growth after direct nucleation from supersaturated solutions, we proposed an in-situ common history seeding methods. The concept of common history seed was first introduced by White et al., in which the authors described common history (CH) seed as a batch of crystal that were nucleated at the same time and then experience the same growth history, i.e. under same temperature, same supersaturation and for the same length of time.[25] For

CH seed, it is reported that the shape of the crystal size distribution will be maintained throughout the crystallization process and as these seed crystal grows.[26, 27]

In this study, all seeds crystals were prepared by direct nucleation from telaprevir supersaturated solutions, with an initial concentration of 60μg/mL, at 37°C for 6 hours. After the fixed seeding period, telaprevir solution concentrations were observed to be around telaprevir crystalline solubility. That is, same seed loading (mass-wise) were provided with our proposed seeding method. Figure 3 shows the desupersaturation profile of telaprevir crystals in the absence of bile salts after supersaturation was regenerated. The desupersaturation rate under three different supersaturation levels is 5.4±0.8μg/min, 58.6±2.2μg/min and 89.2±7.6μg/min, respectively. The high reproducibility of the desupersaturation curves imply our good control over seed properties with the proposed method. The impact of preparation methods on seed properties is revealed in Figure 4. It should be noted that control experiment (in-situ seeding in water) was shown as well to confirm that the observed crystals were not from buffer salts. It is evident that the primary particle size of crystal obtained from supplier is larger than the ones prepared by the in-situ CH seeding method and the shape are different, which can potentially affect crystal growth behavior. In addition, we observed that telaprevir seeds prepared by the in-situ CH method tend to form bouquet-like aggregates, which is difficult to evaluate crystal growth rates by performing image analysis for individual crystals. Therefore, crystal growth rates in this study were determined by the monitoring the change in solution concentration, which is directly related to the mass deposition on existing seed crystals.[6]

Amongst the three supersaturation levels performed in this study, there was no observable change in turbidity measurement for experiments with initial supersaturation level δ=2.4. That is, there was no evidence of significant secondary nucleation for these systems, and

the desupersaturation can be assumed to be mainly due to the bulk crystal growth of the seed crystals. On the other hand, there were detectable increase in turbidity measurements for experiments at higher supersaturation levels (δ=7.9 and 12.5), indicating the occurrence of secondary nucleation. Therefore, crystal growth rates in this study were determined from the desupersaturation rates at supersaturation level δ=2.4. The desupersaturation rates at higher supersaturation levels were as well investigated to evaluate the impact of bile salts on secondary nucleation kinetics.

Crystal Growth and Desupersaturation Rates of Telaprevir in the Presence of Bile Salts

The impact of bile salts on telaprevir desupersaturation rate was investigated at three different initial supersaturation ratio. At supersaturation level δ=2.4, where crystal growth being the dominating kinetics, the effectiveness of bile salts in inhibiting crystal growth E_G was estimated using the following equation:

$$E_G = \frac{R_0}{R} \tag{2}$$

where R_0 and R are the initial desupersaturation rate in the absence and presence of bile salt, respectively. Bile salts with E_G >1 were considered to be effective crystal growth inhibitors. At higher supersaturation levels δ=7.9 and 12.5, where both crystal growth and secondary nucleation can take place, the effectiveness of bile salts in inhibiting the combined kinetics E_c was then estimated using the Equation (3). Bile salts with E_C >1 were considered to be effective inhibitors for the combined kinetics.

$$E_C = \frac{R_0}{R} \tag{3}$$

Figures 5 shows a comparison of effective E_G and E_c of bile salts at 1.86mM and 12mM concentration, respectively. As discussed in the previous studies,[cite M#1 and 2], the majority of bile salts in solution at a concentration of 1.86mM are in monomeric form, and micellar bile salts are most likely the dominant species at a bile salt concentration of 12mM. As shown in Figure 5(a), the biorelevant bile salts show different extent in inhibiting telaprevir crystal growth. Except for STDC showing minimal effectiveness, four out of five investigated bile salts are effective at 1.86mM concentration (monomer). The E_G value for monomeric STC, SGC, and SGDC ranges around 1.4-3.2, which is comparable to reported values of commercially available polymers to poorly soluble drugs.[17, 28] Interestingly, monomeric STCDC appears to be the extreme case, where no desupersaturation was observed during the experiments. In other words, monomeric STCDC blocked telaprevir crystal growth. Similar observations i.e. complete suppress of crystal growth are commonly encountered in crystallization with impurities or additives.[29-31] As bile salt concentration increased to 12mM, comparable crystal growth inhibition was observed for all investigated bile salts, except for STCDC. This observation suggested that the presence of bile salt micelles have minimal impact on crystal growth inhibition ability for monomeric STC, SGC, STDC, and SGDC. However, STCDC micelles show opposed effect to its monomers. At a much higher supersaturation level δ=12.5, similar patterns in terms of the extent of inhibition amongst the investigated bile salts were observed (Figure 5(b)). Monomeric STCDC remained the most effective inhibitor for combined kinetics of crystal growth and secondary nucleation (no desupersaturation was observed), yet the presence of micelles at 12mM concentration appeared to negate the inhibition effect. SGC, STDC, and SGDC show comparable inhibition towards the combined kinetics at both aggregation levels. An

increased in effectiveness of monomeric STC was observed compared to the growth only condition, suggesting impact on secondary nucleation.

To investigate the potential impact of monomeric bile salts on telaprevir secondary nucleation kinetics, the desupersaturation rate raito as a function of supersaturation is shown in Figure 7. For solutions with 1.86mM STC, the desupersaturation rate ratio increased with increasing supersaturation level. Meanwhile, desupersaturation was completely inhibited in the presence of monomeric STCDC at all supersaturation levels. These observations serve as evidence that monomeric STC and STCDC slow down telaprevir crystallization kinetics even when secondary nucleation takes place. This supposition is further supported by comparing the turbidity measurements at different supersaturation levels. While there appeared to be detectable secondary nucleation based on turbidity measurements for solutions in the absence of bile salts at higher supersaturation level (δ=7.9 and 12.5), there was no evidence for secondary nucleation for solutions in the presence of monomeric STC and STCDC. That is, these two bile salts appear to inhibit telaprevir secondary nucleation at 1.86mM concentration.

Zeta potential measurements, an indication of electric potential difference at interfaces in solution, were conducted to understand the interactions of monomeric bile salts and the telaprevir crystals. In the literature, it is well known that adsorption (both physically and chemically) of ionized species can changes the measured zeta potential.[32] [33] Telaprevir is neutral, and monomeric bile salt is negatively charged in the buffer medium used in this study. Therefore, the change in zeta potential upon addition of monomeric bile salt serves as a qualitative indication of adsorption of bile salts at the telaprevir crystal interface. Figure 6 summarizes the zeta potential values of telaprevir crystals in the absence and presence of six monomeric level bile salts. The zeta potential of telaprevir crystal in the absence of bile salts was -7.7 mV. A ~5-6 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), suggesting that bile salts interact with telaprevir crystal surfaces. Although no direct correlation was observed between zeta-potential data and the extent of inhibition ability of bile salts, these results serve as evidence for bile salt interactions on telaprevir crystal surface. The growth inhibition mechanism of bile is not well understood, and there are very limited information in the literature. Mithani[34] investigated the effect of STC on the crystal growth of dipyridamole using single crystal experiments. It was speculated that the adsorption of STC on the active growth sites leads to the growth inhibition of certain surface. With bulk crystal growth rate measurements and population balance modeling, Abbou Oucherif[35] showed that the growth mechanism of dipyridamole shifted from being mass transfer controlled to being a hybrid between mass transfer and surface integration controlled in the presence of 2.2mM STC. More detailed studies are indeed required to fully explain the inhibition mechanism of bile salts.

In order to further confirm the inhibition ability of bile salts, residual supersaturation of the telaprevir solutions (δ=12.5) after 15 hour of crystallization was determined. By comparing Table 3 and Figure 5, the residual supersaturaiton is directly related to the effectiveness of bile salts (E_C). With STC being the medium crystallization inhibitor, the majority of the initial provided supersaturation was consumed after 15 hours of crystallization. On the other hand, STCDC at 1.86mM concentration was able to maintain the supersaturation over 15 hours, while similar effect was not observed when bile salt concentration increased to 12mM. This observation further support our previous statement that monomeric STCDC is a highly effective inhibitor, for both crystal growth and secondary nucleation, for telaprevir. Since possible complexation can be form between SGCDC and telaprevir,[cite M#1] residual supersaturation

was used to evaluate the effectiveness of SGCDC instead of using the desupersaturation method. As shown in Table 3, SGCDC shows similar behavior to STCDC. At 1.86mM SGCDC concentration, supersaturation was maintained over 15 hours. However, similar inhibition effect was not observed as SGCDC concentration increased to 12mM. By summarizing the observations, it is evident that monomeric STCDC and SGCDC are highly effective inhibitor for telaprevir crystal growth and secondary nucleation inhibitors. In addition, micelles of STCDC and SGCDC appear to negate the inhibitory impact of the monomers.

Nucleation Inhibition Versus Growth Inhibition

For system with additives, it is feasible to decouple the crystal growth effect from the measured nucleation induction time with proper experimental design and hence have a better understanding of nucleation kinetics.[28, 36] In the previous study, we showed that unseeded supersaturated telaprevir solutions can be maintain to different extent in the presence of bile salts. However, we revealed that bile salts show different impact on crystal growth rates in the current study. That is, it is possible that the observed prolonged induction time was contributed by strong crystal growth inhibition. With the determining seedless induction times from the previous study[cite M#2] and the seeded desupersautration rates in the presence and absence of bile salts (Figure 5(b)) under similar experimental conditions, the relative nucleation rate in the presence and absence of the bile salt is estimated with the following equation:

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

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 desupersautration 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. The effectiveness of bile salts in inhibiting nucleation E_J was further defined as below, and bile salts with E_J >1 were considered to be effective nucleation inhibitors.

$$E_J = \frac{J_0}{J} \tag{5}$$

For example, the induction time in the absence of bile salts was determined to be ~30 minutes and in the presence of monomeric bile salts the induction time was found to be more than 16 hours (960 minutes was used in the calculation).[cite M#2] With the desupersautration rate ratios from Figure 5(b), E_J for monomeric bile salt, except STCDC and SGCDC, was then estimated to be around the range of 400-340,000, much higher than the estimated values of E_G (ranging between 1-3). That is to say, the ability of monomeric STC, SGC, STDC and SGDC to maintain supersaturation via growth inhibition is much weaker compared to their ability to inhibit nucleation. As for the extreme effective growth inhibitor, monomeric STCDC and SGCDC, there was no desupersautration observed during the experiments. By assuming E_G to be 100, the E_J value estimated with Equation (4) and (5) is minimal, indicating that the prolonged induction time we observed in the previous study can be due the strong growth inhibition by STCDC and SGCDC. In other words, it is possible that nucleation did take place, but the nuclei were not able to grow to a detectable size due to monomeric STCDC and SGCDC blocking crystal growth. It was reported in a previous nucleation induction time study that monomeric STCDC possesses the highest inhibitory effect for the three poorly soluble compounds, celecoxib, nevirapine, and flibanserin, compared to other 12 different bile salts.[13] Based on our observations, growth inhibition can be a possible contributing factor.

Similar analysis was performed for bile salts at 12 mM, and Figure 8 summarized the inhibition ability of biorelevant bile salts at different aggregation levels. In general, we can categorize bile salts with some structural dependence. For trihydroxy bile salt STC and SGC, they exhibit strong ability in inhibiting telaprevir nucleation. As bile salt concentration increased to 12mM, the presence of micellar bile salts does not appeared to show significant impact on the inhibition ability (Figure 8(a)). For STDC and SGDC, dihydroxy bile salt with the absence of hydroxyl group at R_3 position on the steroid ring system, these bile salts appeared to be strong nucleation inhibitors for telaprevir at monomeric form. However, micelles of these dihydroxy bile salts appears to negate the inhibitory impact of the monomers(Figure 8(b)), which is consistent to our observation in the previous study.[cite M#2] For STCDC (and SGCDC), dihydroxy bile salt with the absence of hydroxyl group at R_4 position on the steroid ring system, the monomeric form show extreme effectiveness in inhibiting telaprevir crystal growth. Similar to other dihydroxy bile salts, micelles of these dihydroxy bile salts appears to negate the inhibitory impact of the monomers(Figure 8(c)).

Importance of Bile Salt Inhibition Properties.

Due to the inevitable encounter of oral formulations with bile salts in biorelevant dissolution media and *in vivo*, understand the impact of bile salts on API crystallization has been a new area of research interest. For amorphous formulations, maintaining supersaturation for a biologically relevant time frame in the gastrointestinal tract is important for improved absorption. Although the composition varies from person to person, the bile salts investigated in this study are commonly found in human intestinal fluids.[37, 38] It was demonstrated in the previous studies that bile salt significantly delayed crystallization of poorly soluble compounds from solution.[12, 13][cite M#2] However, their impact on crystal growth rate has not been considered. The presence

of residual (seed) crystals is often unavoidable in an amorphous formulation due to its instable nature. The ability to control or slow down crystal growth significantly impact the degree of supersaturation available for absorption. Take telaprevir for example, supersaturation is possible to be maintained upon exposure *in vivo*, even with residual crystallinity present in the ASD, due to the crystal growth inhibition of the biorelevant bile salts. For polymer systems, it was reported that the extent of crystal growth inhibition can be improved through surface poisoning upon seed preparation.[23] If similar mechanism applied, larger impact of bile salts on drug crystallization is expected if bile salts are employed as excipient in amorphous formulations.

CONCLUSIONS

In this study, the impact of six biologically relevant bile salts on desupersautration rate of seeded telaprevir solutions was evaluated at different bile salt aggregation levels. With the proposed in-situ common history seeding method, differences in the inhibitory ability of the bile salts were revealed. Most bile salts investigated slowed down telaprevir crystal growth. Monomeric STCDC and SGCDC were extremely effective growth inhibitors for telaprevir, and solution supersaturation was able to be maintained over time. However, the presence of the bile salt micelles opposed their monomeric inhibitory effect. In addition, Monomeric STC and STCDC were found to inhibit secondary nucleation of telaprevir at higher supersaturation levels. These observations of the endogenous bile salts provide the foundation for further studies on how biorelevant bile salt mixture impact the crystallization kinetics of drugs, which are crucial for better biopredictive dissolution media design and better *in vivo* behavior prediction of supersaturating systems. Lastly, bile salts are not interchangeable from a crystallization inhibition standpoint.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Merck & Co., Inc. for providing research and financial support for this study. We would also like to acknowledge Anthony Leone, Ron Smith, Andre Hermans, Michael McNevin and Filippos Kesisoglou for discussion and insight.

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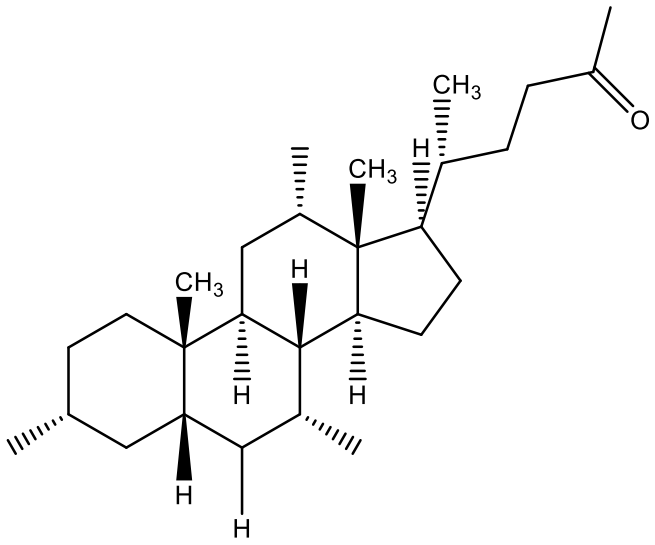
TABLES

Table 1. Chemical structure of bile salts.

R_5

R_4

R_1 R_3



Bile salt	Abbreviation	R1	R3	R4	R5
Sodium taurocholate	STC	OH	OH	OH	$NHCH_2CH_2SO_3^-$
Sodium taurodeoxycholate	STDC	OH	H	OH	$NHCH_2CH_2SO_3^-$
Sodium taurochenodeoxycholate	STCDC	OH	OH	H	$NHCH_2CH_2SO_3^-$
Sodium glycocholate	SGC	OH	OH	OH	$NHCH_2COO^-$
Sodium glycodeoxycholate	SGDC	OH	H	OH	$NHCH_2COO^-$
Sodium glycochenodeoxycholate	SGCDC	OH	OH	H	$NHCH_2COO^-$

Table 2. Supersaturation level for depsupersaturation experiments based on activity calibration. [citeM#1 and M#2]

Solution media	Supersaturation ratio δ	Regenerated concentration for growth (μg/mL)
Buffer only, trihydroxy bile salts, and 1.86mM dihydroxy bile salts	2.4	12.4
12mM dihydroxy bile salt		20.2
Buffer only, trihydroxy bile salts, and 1.86mM dihydroxy bile salts	12.5	65.2
12mM dihydroxy bile salt		145.2

Bile Salt	Regenerated concentration for growth (μg/mL)	Residual effective free drug concentration (μg/mL)
1.86mM STC	65.2	9.0±1.8
12mM STC	65.2	13.6±2.5
1.86mM STCDC	65.2	60.5±6.9
12mM STCDC	145.2	4.0±1.3
1.86mM SGCDC	95.2	91.7±6.5
12mM SGCDC	95.2	11.5±3.3

Table 3. Chemical structure of bile salts.

FIGURES

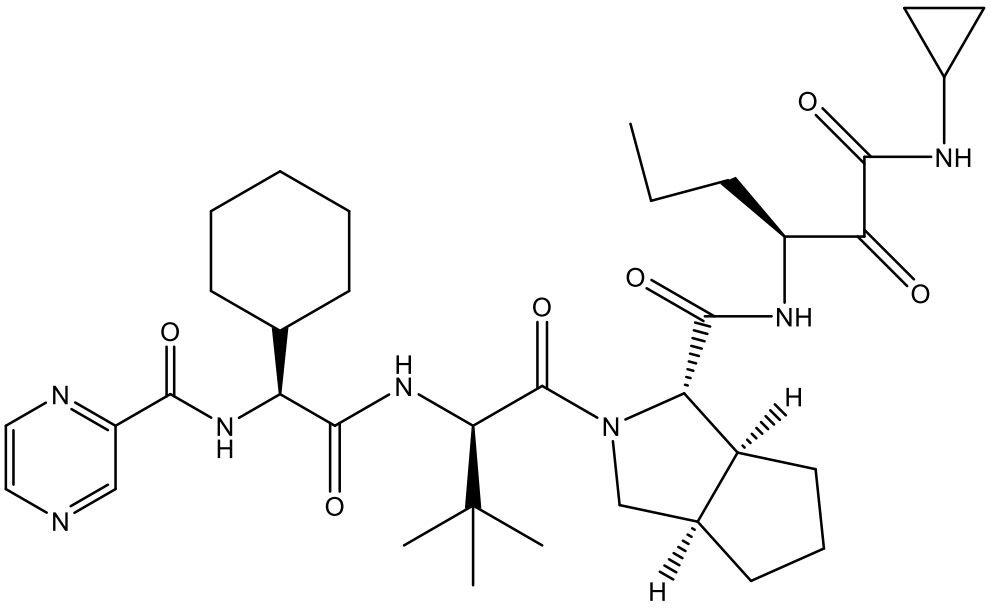


Figure 1. Molecular structure of telaprevir.

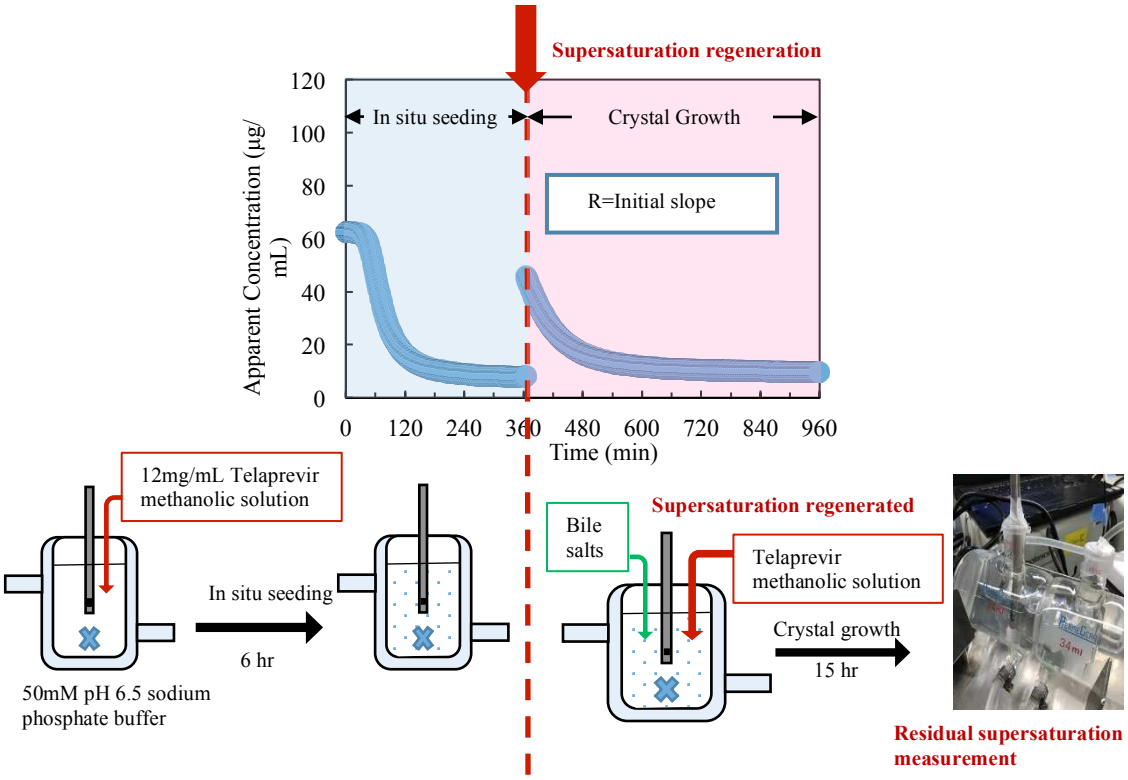


Figure 2. Experimental series for in-situ common history seeding method followed by desupersaturation rate and residual supersaturation measurements.

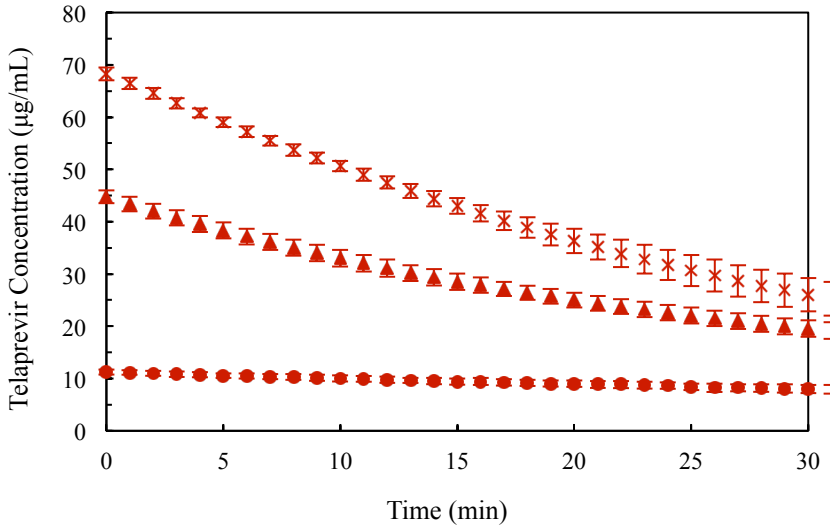
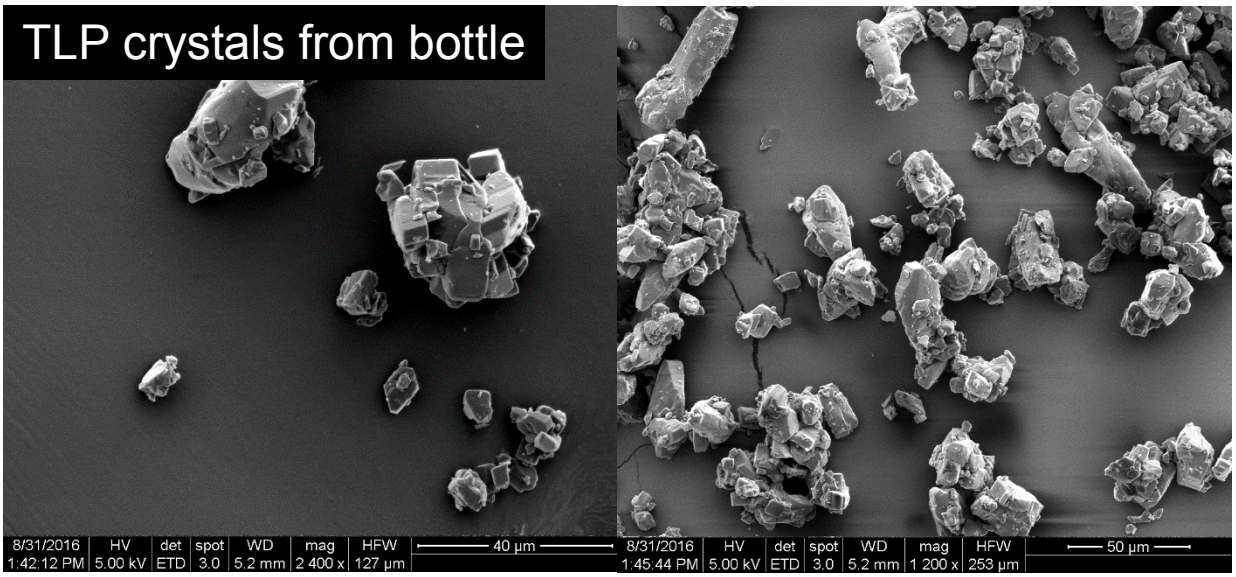


Figure 3. Rate of desupersaturation of telaprevir with seed crystals in the absence of bile salts at supersaturation ratios of 2.4 (red circles), 7.9(red triangles) and 12.5 (red crosses).



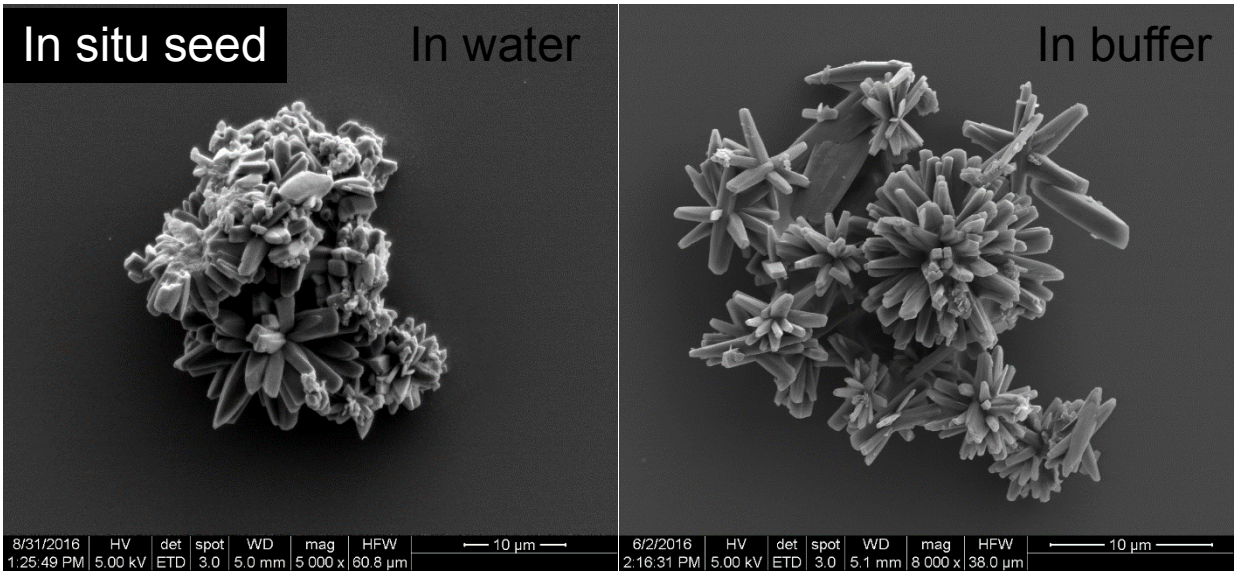
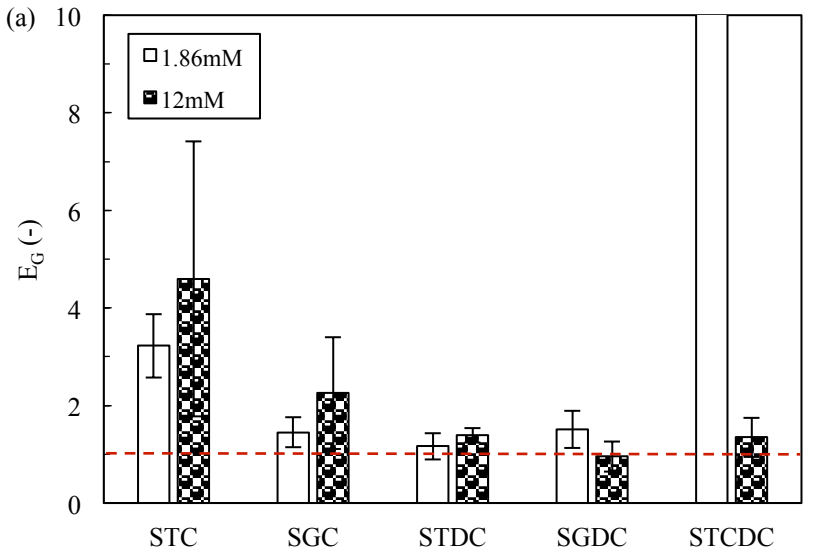


Figure 4. SEM images of telaprevir crystals obtained from suppliers (upper row), and prepared from the in-situ common history seeding method (bottom row).


(a)
10
8
6
4
2
0
E_G (-)
1.86mM
12mM
STC
SGC
STDC
SGDC
STCDC

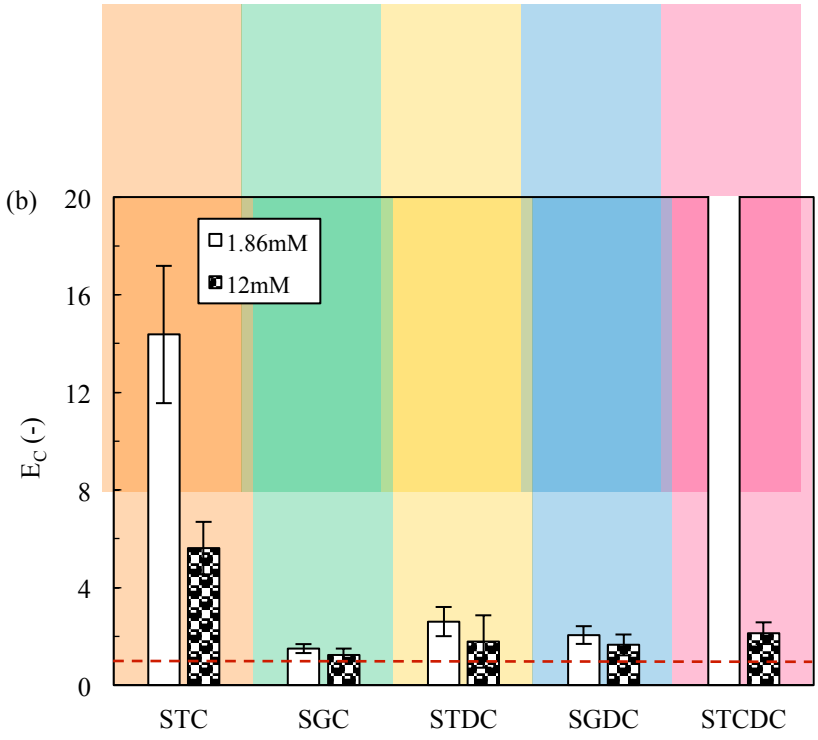

(b)
20
16
12
8
4
0
E_C (-)
1.86mM
12mM
STC
SGC
STDC
SGDC
STCDC

Figure 5. (a) Effectiveness as growth inhibitor of bile salts at an initial supersaturation level δ=2.4. (b) Effectiveness as crystallization inhibitor of bile salts at an initial supersaturation level δ=12.5.

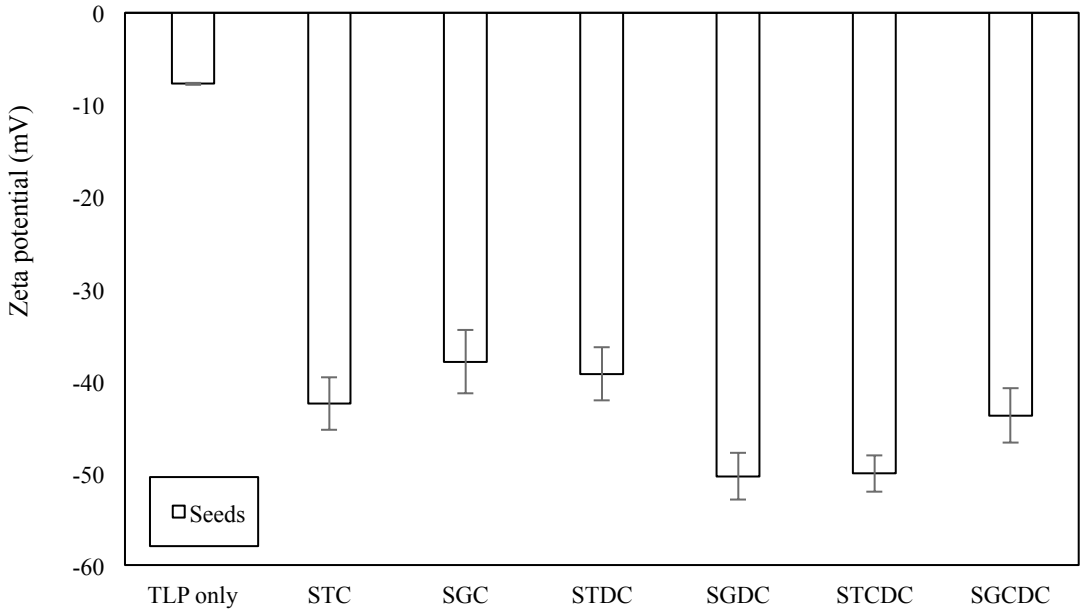


Figure 6. Zeta potential of telaprevir seed solution in the absence and presence of 1.86mM bile salts.

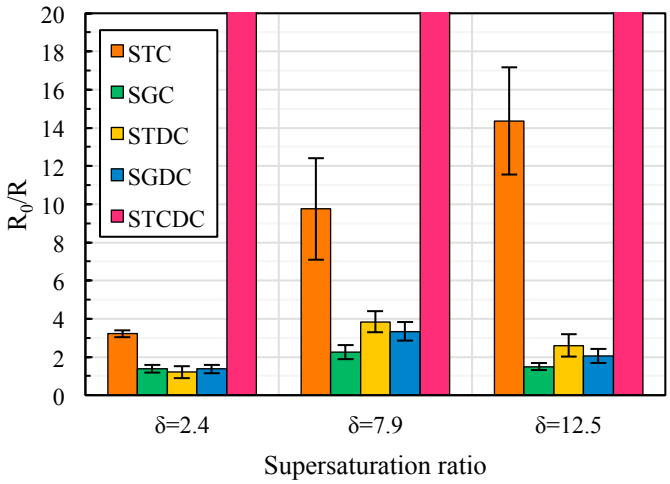
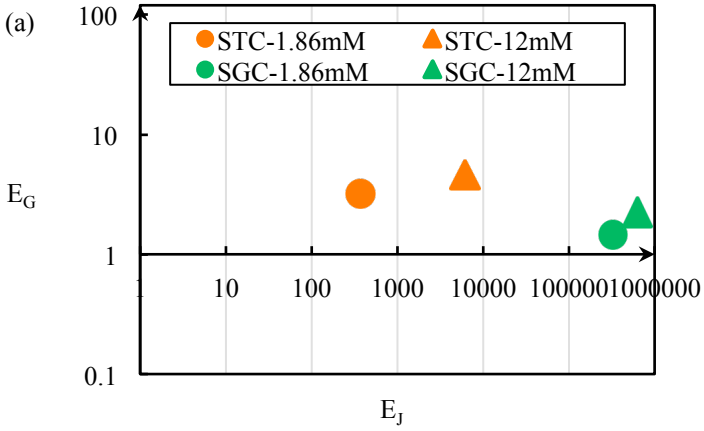


Figure 7. Desupersaturation rate ratio of telaprevir at differernt supersaturation ratios in the presence of 1.86mM bile salt.



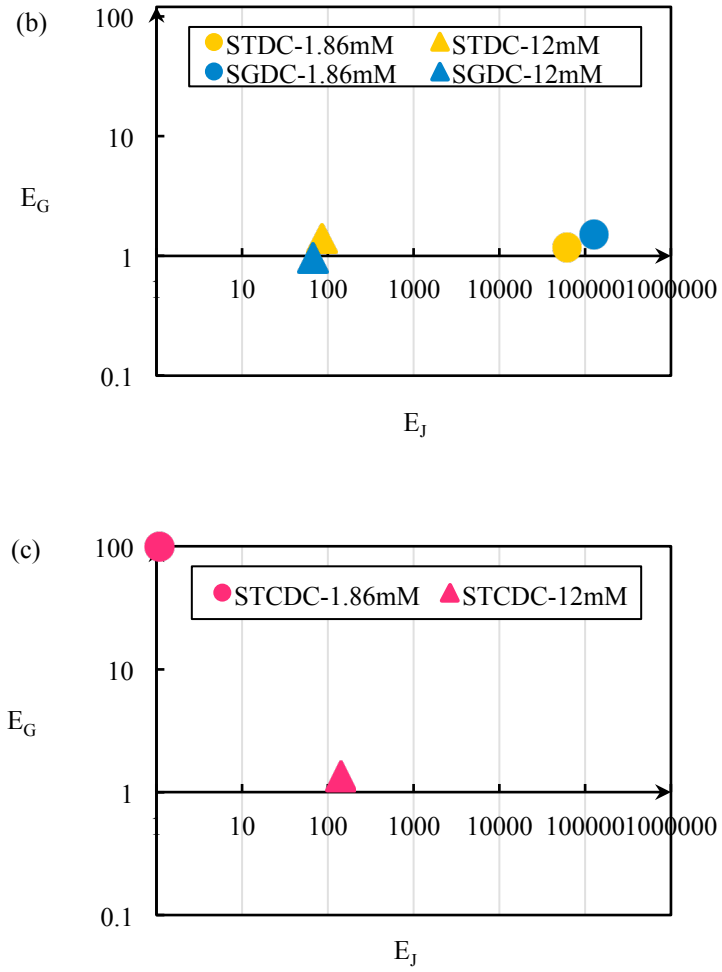


Figure 8. Effectiveness of bile salts as crystal growth inhibitor (E_G) and nucleation inhibitor (E_J) for telaprevir crystallization: (a) trihydroxy bile salt STC and SGC (b) dihydroxy bile salt STDC and SGDC (c) trihydroxy bile salt STCDC.