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Impact of Endogenous Bile Salts on the Thermodynamics of Supersaturated Active Pharmaceutical Ingredient Solutions.

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ABSTRACT

A variety of formulation strategies have been developed to mitigate the inadequate aqueous solubility of certain therapeutic agents. Amongst these, achieving supersaturation *in vivo* is a promising approach to improve the extent of oral absorption. Due to the thermodynamic instability of supersaturated solutions, inhibitors are needed to kinetically hinder crystallization. In addition to commonly used polymeric additives, bile salts, naturally present in the gastrointestinal tract, have been shown to exhibit crystallization inhibition properties. However, the impact of bile salts on solution thermodynamics is not well understood, although this knowledge is essential in order to explore the mechanism of crystallization inhibition. To better describe solution thermodynamics in the presence of bile salts, a side-by-side diffusion cell was used to evaluate solute flux for solutions of telaprevir in the absence and presence of the six most abundant bile salts in human intestinal fluid at various solute concentrations; flux measurements provide information about the solute thermodynamic activity and hence can provide an improved measurement of supersaturation in complex solutions. Trihydroxy bile salts had minimal impact on solution phase boundaries as well as solute flux, while micellar dihydroxy bile salts solubilized telaprevir leading to reduced solute flux across the membrane. An inconsistency between the concentration-based supersaturation ratio and that based on solute thermodynamic activity (the fundamental driving force for crystallization) was noted, suggesting that the activity-based supersaturation should be determined to better interpret any modification in crystallization kinetics in the presence of these additives. These findings indicate that bile salts are not interchangeable from a thermodynamic perspective, and provide a foundation for further studies evaluating the mechanism of crystallization inhibition.

INTRODUCTION

There are two key determinants to oral drug absorption: solubility and permeability. [1] The solubility of the drug in the gastrointestinal (GI) tract and the permeability of the drug through the GI membrane dictate the extent of oral drug absorption, and thus affect the bioavailability of the drug. Over the past few decades, there has been an increase in the number of poorly soluble drugs in the developmental pipeline.[2, 3] To tackle the issue of inadequate solubility, a wide variety of formulation strategies have been evaluated and applied, including salts, cosolvents, solubilization with lipids and surfactants, nanocrystals, and amorphous solid dispersions.[3] Recently, there has been increasing attention paid to the trade-off between solubility increase and apparent permeability decrease when some of these strategies are employed. Significant reduction in membrane mass transport was observed for systems in the presence of solubilizing additives due to a change in solute thermodynamic activity. [4] Miller et al.[5] described the advantages of implementing supersaturation strategies via amorphous solid dispersion, in which increased apparent solubility is achievable without the expense of reduced apparent membrane permeability.

Guzman et al.[6] proposed the "spring and parachute" approach to describe the concept of employing supersaturation as a strategy to improve oral absorption of poorly soluble drugs. A supersaturated drug solution can be generated with different formulation strategies (the spring). In the absence of rapid crystallization, the maximum achievable supersaturation of a compound is limited by its amorphous solubility,[7] above which phase separation occurs due to the formation of disordered nano-sized aggregates. However, once supersaturation is generated, drug molecules have a tendency to crystallize in order to reduce their chemical potential. With crystallization inhibitors (the parachute), the generated supersaturation can be kinetically maintained and controlled in order to increase drug absorption.[8] Polymeric additives, e.g., poly vinylpyrrolidone

(PVP) based polymers and cellulose derivatives, have been commonly used in commercial formulations to inhibit crystallization. Ilevbare et al.[9, 10] exploited the anti-nucleation and growth inhibition properties of a group of chemically and structurally diverse polymers. The relative hydrophobicity and intermolecular interactions between drugs and polymers were suggested to be important factors impacting crystallization kinetics. Besides polymeric additives, there has been increasing use of surfactants in amorphous solid dispersion formulations. Surfactants are often added to improve the processing properties of formulations or used as solubilizing agents to improve the drug solubility. Recently, the impact of surfactants on the crystallization kinetics of active pharmaceutical ingredients has attracted attention. It has been shown that surfactants can either enhance or inhibit nucleation[11, 12] and crystal growth,[13] and influence polymorphic transformations.[14] Furthermore, an inconsistency between the concentration-based supersaturation ratio and solute thermodynamic activity, which is the fundamental driving force for crystallization, has been observed for some systems when solubilizing additives such as surfactants are present.[4] A fundamental understanding of the impact of surfactants on supersaturation and crystallization kinetics is critical for formulation design and performance assessment of poorly soluble drugs.

Chen et al.[11] evaluated the impact of commonly used surfactants on the crystallization of celecoxib supersaturated solutions, and sodium taurocholate (STC), a member of the bile salt family, was found to inhibit crystallization. Bile salts, as biological surfactants, are the main product of cholesterol metabolism and form mixed micelles with lecithin and cholesterol *in vivo*. The 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) (Table 1).[15] Despite the fact that at least six different kinds of bile salts exist in the GI

milieu, STC is the only bile salt present in commercial Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF). The general structure of bile salts consists of a steroid ring system with hydroxyl groups distributed on one side, leading to facial polarity. Because of their unique molecular structure, the pattern of bile salt aggregation is not analogous to typical aliphatic surfactants. Previous studies have shown that the aggregation of bile salts is complex and step-wise,[16, 17] and the aggregation is found to occur over a relatively wide range of concentrations compared to other types of surfactants.[18] The broad critical micelle concentration (CMC) range has led to difficulties in characterizing the aggregation behavior, and bile salts are thought to solubilize solutes by different mechanisms as compared to traditional surfactants. Changing the functional groups on the bile salt scaffold impacts the CMC,[19] which in turn is expected to result in different solubilization abilities among different bile salts. The reported CMC values for biologically relevant bile salts are in the range of 2 to 12mM. [18, 20] In general, the effect of bile salts on supersaturated solutions containing poorly water soluble APIs has been studied to a limited extent. However, this is an important area of research given the increasing use of supersaturating dosage forms to improve the oral absorption of poorly water soluble compounds.

In order to better understand the impact of bile salts on supersaturated API solutions, two key questions were addressed in this study. First, how does a bile salt, at a concentration above and below the CMC, affect the thermodynamic properties of supersaturated solutions containing a model poorly water soluble API? Here, the interplay between solubilization and membrane transport is studied. Second, is sodium taurocholate a good surrogate for other bile salts in terms of impact on supersaturated solutions? Most *in vitro* studies of bile salt solutions focus on those containing sodium taurocholate. However, several different bile salts have been identified from human aspirates of intestinal fluid, and some of these are present in higher concentration than STC.

Hence it is imperative to understand if bile salts are interchangeable with respect to their impact on the thermodynamics of supersaturated solutions. In this work, we systematically evaluated the impact of six biologically relevant bile salts on supersaturated telaprevir solutions. Telaprevir is a poorly soluble drug with high glass transition temperature and large reported supersaturation window.[21] The thermodynamic properties of supersaturated telaprevir solutions, including the equilibrium solubility and the onset of glass-liquid phase separation, were determined in the absence and presence of four taurine/glycine conjugated dihydroxy (STDC, STCDC, SGDC and SGCDC) and trihydroxy (STC and SGC) bile salts. The impact of bile salts on supersaturated telaprevir solutions was evaluated in terms of their aggregation state and differences in molecular structure, respectively.

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

Crystalline Solubility Measurements.

The solubility of crystalline telaprevir in different media was determined by adding an excess amount of the drug to 15mL of 50 mM pH 6.5 phosphate buffer solution with or without bile salt. Bile salts were present in solution at a concentration of 1.86mM (monomer level) or a 12mM (micellar level). The solutions were stirred at 300rpm and equilibrated for 48 h in a water bath at 37 °C. Samples were then ultracentrifuged in an Optima L-100 XP ultracentrifuge equipped with a Swinging-Bucket Rotor SW 41 Ti (Beckman Coulter, Inc., Brea, CA) at 35000 rpm for 30 min at 37 °C. The supernatant obtained was diluted 2-fold with methanol, and the concentration of the supernatant was determined with a SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber optically coupled with a 2 cm path length dip probe at a wavelength of 270 nm. The standard curve presented good linearity ($R^2 > 0.99$) over the relevant concentration range.

UV/Vis Extinction Measurements.

UV extinction measurements were used to determine the onset of glass-liquid phase separation (GLPS) in supersaturated telaprevir solutions. A syringe pump (Harvard Apparatus, Holliston, MA) was used to add the predissolved telaprevir methanol stock solution (12 mg/mL) at 0.05 mL/min to 15 mL of 50 mM pH 6.5 phosphate buffer, with or without dissolved bile salts, stirred at 300 rpm at 37 °C. Bile salts were present in solution at a concentration of 1.86 mM (monomer level) or 12 mM (micellar level). The formation of a drug-rich phase in solution, i.e., the onset of glass-liquid phase separation, leads to light scattering and can be detected from an increase in the UV signal at a non-absorbing wavelength (370nm in this study). The change in the

signal at 370 nm was monitored using the SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber-optically coupled with a 1 cm path length dip probe.

Ultracentrifugation Method.

Pre-dissolved telaprevir methanol solution (12 mg/mL and 20 mg/mL) was added to 15 mL of 50 mM pH 6.5 phosphate buffer, with or without dissolved bile salts, and stirred at 300 rpm at 37 °C, to produce a solution with a concentration above the concentration where GLPS occurs. Bile salts were present in solution at a concentration of 1.86 mM or 12 mM. The resultant turbid solutions were then ultracentrifuged in an Optima L-100 XP ultracentrifuge equipped with a Swinging-Bucket Rotor SW 41 Ti (Beckman Coulter, Inc., Brea, CA) at 35000 rpm for 40 min at 37 °C. Once the supernatant was separated from the disperse drug-rich phase, the supernatant obtained was diluted 2-fold with methanol, and the concentration of telaprevir in the supernatant was measured using the SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber optically coupled with a 0.2 cm path length dip probe at a wavelength of 270 nm. The standard curve presented good linearity ($R^2 > 0.99$) over the relevant concentration range.

Diffusion Rate Measurements.

A side-by-side diffusion cell (PermeGear, Inc. Hellertown, PA), as depicted in Figure 2, was used to evaluate solute flux across membrane for solutions of telaprevir in the absence and presence of different bile salts at various solute concentrations. These measurements were then used to estimate the solute (telaprevir) thermodynamic activity in solutions containing bile salts. The 34 mL donor and receiver chambers were separated by a regenerated cellulose membrane with a molecular weight cut off (MWCO) of 6−8KDa, and connected with an orifice diameter of 30 mm. The membranes were hydrated in deionized water overnight before experiments. In each

experiment, both the donor and receiver chamber were filled with 32 mL of 50 mM pH 6.5 sodium phosphate buffer with 5 μg/mL HPMCAS-MF (to prevent crystallization). Control experiments in the absence of HPMCAS-MF were conducted. The mass flow rate results are consistent, indicating that the polymer does not affect solution thermodynamics at the concentration used. For systems with bile salts, an equal concentration of bile salt was added in both receiver and donor chambers, hence there was no driving force for bile salt diffusion. A methanolic stock solution of telaprevir (12 or 20 mg/mL) was added to the donor chamber to obtain the desired concentration. The concentration change in the receiver chamber was monitored by a SI Photonics UV/vis spectrometer (Tuscon, Arizona), fiber optically coupled with a 2 cm path length dip probe, at a wavelength of 270 nm. The standard curve presented good linearity (R^2> 0.99) over the relevant concentration range. The slope of the concentration versus time profile of the receiver chamber was obtained by linear regression, and used as the estimated mass flow rate. A typical example of changes in the UV signal as a function of time is shown in Figure S1, Supporting Information. The mass flow rate, *F,* of telaprevir molecules diffusing across the membrane*,* is a function of the diffusion coefficient *D*, the membrane cross-sectional area *S*, the solute thermodynamic activity a, the thickness of the membrane *h*, and the activity coefficient of telaprevir in the membrane, γ_m. The activity of telaprevir can be further expressed as a function of the activity coefficient of telaprevir, γ, and the telaprevir concentration *C* in the donor chamber.

$$F = \frac{dM}{dt} = \frac{DSa}{h\gamma_m} \quad (1)$$

$$a = C\gamma \quad (2)$$

In all experiments, the membrane cross-sectional area *S*, and the thickness of the membrane *h* are constant. The activity coefficient of telaprevir in the membrane γ_m and diffusion coefficient *D* are also assumed to be constants as well. Sink conditions were assumed, i.e. the thermodynamic

activity of telaprevir in the receiver compartment was considered negligible. This assumption is reasonable as the maximum concentration obtained in the receiver chamber was always less than one-half of the crystalline solubility. Membrane permeability was assumed to be unchanged for the different systems, hence the change in solute mass flow is assumed to be due to the change in solute activity.

RESULTS

Crystalline Solubility and the Onset of GLPS

The equilibrium crystalline solubility values for telaprevir in the absence and presence of six different bile salts in 50 mM pH 6.5 phosphate buffer are summarized in Table 2. In the presence of monomer level bile salts (1.86 mM bile salt concentration, about 0.1 w/w %), no significant solubilization was observed. On the other hand, the crystalline solubility of telaprevir increased about 2-fold in the presence of a micellar level of all of the dihydroxy bile salts (STDC, STCDC, SGDC, and SGCDC) while no significant solubilization was observed in the presence of a micellar level of either trihydroxy bile salt (STC and SGC). Surfactants are commonly used in commercial formulations of poorly soluble APIs, whereby more than ten-fold solubility enhancement can be readily achieved locally due to drug incorporation into surfactant micelles.[3, 22] However, the bile salts investigated in this study are not strong solubilizers of telaprevir at the concentrations employed, even when these concentrations are above their CMC.

Telaprevir is a compound with high glass transition temperature, and has been shown to undergo GLPS in highly supersaturated solutions.[21, 23] Figure 3 summarizes the telaprevir concentration where GLPS was observed in the absence and presence of six different bile salts. It

is apparent that the three approaches used to make these GLPS measurements, namely the UV extinction method, ultracentrifugation method and diffusion rate measurements give similar values for GLPS onset. The ultracentrifugation and diffusion rate approaches are likely to provide values close to the coexistence concentration of the continuous phase, while the UV extinction method, where the drug is added continually, might approach one of the spinodal decomposition points for phase separation.[24] However, given the good agreement between the three methods, it is apparent that the system does not undergo substantial supersaturation with respect to GLPS in agreement with previous observations.[25, 26] Figure 4 shows an example of the UV extinction experiment of telaprevir in 50mM pH 6.5 phosphate buffer, in which the onset of GLPS is indicated by increased light scattering due to the spontaneous formation of a drug-rich phase in the solution when the concentration exceeds a certain value. By subsequently separating the drug-rich dispersed phase from the continuous phase, using ultracentrifugation to pellet the drug-rich phase, the composition of the continuous phase can be determined, thereby yielding one of the binodal points (at 37°C).[24] Diffusion rate measurements were also implemented to determine the concentration of telaprevir in the continuous phase following GLPS. Figure 5a shows the concentration in the receiver chamber as a function of time, from which telaprevir mass flow rate for different donor chamber concentration levels (shown in Figure 5b) can be determined from the slope. The linearity of each concentration versus time plot in Figure 5a confirms the constant parameter assumption (the membrane cross-sectional area S, the thickness of the membrane h, the activity coefficient of telaprevir in the membrane γ_m and the diffusion coefficient D) in equation (1). As shown in Figure 5b, telaprevir mass flow rate across the cellulose membrane increases linearly as a function of donor chamber concentration. At donor concentrations above 150 μg/mL, the mass flow rate of telaprevir reaches a plateau. At these concentrations, solutions in the donor chamber were observed

to be turbid, indicating that phase separation had occurred. It has been shown in previous studies that the maximum mass flow rate of poorly soluble APIs is obtained with supersaturated solution at or above concentrations where LLPS/GLPS occurs.[3] Therefore, flux measurements can be used to determine the onset of LLPS/GLPS from the concentration where the maximum in mass flow rate is observed. Based on all of the implemented methods, the GLPS onset of telaprevir in 50 mM pH 6.5 phosphate buffer is about 150 μg/mL. This value is about 1.5 fold higher than the value reported in 100 mM pH 6.8 phosphate buffer.[21] Hence the effect of buffer ionic strength and pH on telaprevir GLPS onset concentration was investigated. Figure 6 summarizes the GLPS concentration of telaprevir in different media, determined using the UV/Vis extinction and centrifugation method. The GLPS concentration decreases with increased buffer salt concentration. This is due to the promoted aggregation of molecules via hydrophobic interactions in a medium of increased ionic strength, and similar pattern of behavior has been observed for supersaturated ritonavir solutions[7], as well as for other aggregation-based phenomena such as micelle formation[27].

The impact of bile salts on the concentration where telaprevir undergoes GLPS reveals some interesting patterns with regard to bile salt molecular structure (Figure 3). For trihydroxy bile salts (STC and SGC), the presence of both monomer and micellar level bile salts slightly increases the GLPS onset concentration; however, the aggregation state of bile salts does not appear to significantly affect the onset concentration of GLPS. For the dihydroxy bile salts, micellar levels of STDC and SGDC increased the GLPS onset concentration of telaprevir by a factor of 2. Thus, micellar level dihydroxy bile salts have a stronger interaction with telaprevir molecules, consistent with the crystalline solubility data. For solutions containing STCDC and SGCDC, the onset of GLPS was difficult to determine using the UV extinction method. Taking solutions containing SGCDC as an example, an immediate increase of light scattering (extinction)

upon the addition of pre-dissolved telaprevir methanol stock solution was seen. There was no visual change in solution turbidity until much higher concentrations were reached. Solutions were evaluated using an optical microscope with cross-polarized light, and no crystals/aggregates could be observed suggesting that the scattering species are of a size below the detection limit. We suspect complex formation between drug and bile salt, with some supporting evidence provided by the diffusion data. Therefore, only the ultracentrifugation and/or flux methods are reported for these systems. Solutions with STCDC or SGCDC at concentrations above the onset concentration determined by flux methods were observed to be visually turbid. For monomeric STCDC, the onset concentration of GLPS was slightly lower than for pure telaprevir, while the value was approximately doubled in the presence of micellar STCDC. SGCDC resulted in a small increase in the GLPS concentration in both monomeric and micellar form.

Impact of Bile Salts on Telaprevir Diffusion Rates

Mass flow rate of telaprevir solutions of different concentrations in the presence of bile salts were studied using a diffusion cell. The impact of bile salts on the mass flow rate can be again divided into three categories based on bile salt molecular structures. As shown in Figure 7a, the presence of either monomeric or micellar levels of trihydroxy bile salt (STC and SGC) did not have a major impact on the diffusion mass flow rate of a telaprevir solution of a given concentration. The impact of STC on the diffusion mass flow rate at an even higher bile salt concentration (18mM) was also tested (data not shown), and no significant change in mass flow rate was again observed. Maximum mass flow rates were achieved at concentrations at or above the GLPS concentration of telaprevir in the presence of the relevant bile salts. For STDC and SGDC, dihydroxy bile salt with the absence of hydroxyl group at R_3 position on the steroid ring system, no effect on mass flow rate was observed in the presence of monomeric bile salt (Figure

7b). On the other hand, a ~2-fold reduction in mass flow rate for a given telaprevir concentration was observed in the presence of micellar level bile salts. From equation 1, the decrease in mass flow rate indicates a decrease in telaprevir activity in the presence of micellar bile salt. A similar trend has been observed in a previous study with felodipine, a poorly soluble API, in the presence of Vitamin E TPGS (surfactant). At Vitamin E TPGS concentrations below the CMC, the mass flow rate of felodipine remained unchanged compared to the solution without Vitamin E TPGS. However, a major decrease in felodipine mass flow rate was observed with a concurrent enhanced equilibrium crystalline solubility at Vitamin E TPGS concentrations above CMC. [4] From both the equilibrium crystalline solubility and mass flow rate data, it is apparent that STDC and SGDC solubilize telaprevir by incorporation of the drug into bile salt micelles. For STCDC, a dihydroxy bile salt lacking a hydroxyl group at R_4 position on the steroid ring system, a similar trend is observed as for the aforementioned dihydroxy bile salts (Figure 7c). In contrast, the final dihydroxy bile salt studied (SGDC) showed a different pattern of behavior. For this system, mass flow rate was observed to decrease in the presence SGCDC, but no difference was observed between bile salt concentrations above and below CMC. Interestingly, the maximum observed mass flow rate decreases in the presence of both STCDC and SGCDC, indicating that the maximum achievable supersaturation decreases. The decreases in maximum achievable mass flow rate are statistically significant (t test, p value$<<$0.05). The maximum achievable mass flow rate is reduced by the presence of STCDC and SGCDC to about three fourths of the value in the absence of bile salt.

Raina et al. [4] mentioned a possible scenario where the maximum supersaturation of solute decreases in the presence of additives. Trasi et al.[28] and Alhalaweh et al. [29] have shown that the maximum achievable supersaturation of a poorly water-soluble compound can be reduced by the

presence of a second solute, if the two solutes are miscible in the liquid phase. In our case, the reduced maximum mass flow rate in the presence of STCDC and SGCDC suggests mixing of these two bile salts with the telaprevir drug-rich phase.

DISCUSSION

Solution thermodynamics play a critical role in the oral drug delivery of poorly water soluble drugs, especially for supersaturation strategies such as using amorphous solid dispersions. The extent of supersaturation is important since it influences membrane transport rate,[5, 30] as well as providing the driving force for crystallization. Hence, a better understanding of how components present in the solution impact drug solution thermodynamics is necessary, both to select appropriate additives that enhance the apparent solubility of the drug without affecting the apparent permeability, and to deconvolute mechanisms of solubility enhancement, namely solubilization versus supersaturation. In addition, the presence of various solubilizing components, such as bile salts, in human intestinal fluids further complicates the *in vivo* dissolution of drugs. In order to improve prediction of *in vivo* behavior, it is crucial to gain insights into solution thermodynamics of biologically relevant media.

Diffusion rate measurements across a membrane serve as a method to evaluate the thermodynamic activity of a solute in the presence of additives. This in turn enables determination of the level of supersaturation. Determining supersaturation is essential for understanding if additives alter crystallization kinetics by changing the supersaturation of the system, or via other effects. Fundamentally, supersaturation can be expressed in terms of the chemical potential difference between the solute in the solution of interest and in its equilibrium state:[31]

$$\ln \delta = \frac{\mu - \mu^{\circ}}{RT} = \ln \frac{a}{a^{\circ}} \quad (3)$$

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 (solute in a solution in equilibrium with crystalline state in this study). The maximum achievable supersaturation is limited by the amorphous solubility of the solute, above which liquid-liquid (or glass-liquid) phase separation occurs. Thus in the absence of crystallization, addition of further solute above this concentration leads to the formation of an amorphous solute-rich disperse phase.[24] Therefore, we can define the maximum achievable supersaturation ratio as:

$$\ln \delta_{Max} = \ln \frac{a_{amorphous}}{a_{crystalline}} \quad (4)$$

For a simple dilute system, it is reasonable to assume that the solute activity coefficient remains constant over the concentration range encompassing the crystalline and amorphous solubilities. Hence, combining equations 2 and 4, the maximum achievable supersaturation following LLPS/GLPS can be expressed in terms of amorphous to crystalline solubility ratio:

$$\ln \delta_{Max} = \ln \frac{C_{amorphous}}{C_{crystalline}} \quad (5)$$

However, in systems with additives or bile salts that interact with the solute of interest, the solute activity coefficient changes. The level of discrepancy between concentration-based supersaturation and activity-based supersaturation varies, depending on intermolecular interactions between solute, solvent and additives and how these vary as a function of supersaturation. In such instances, it may no longer be accurate to use concentration ratios to determine the supersaturation in the system. This concept has been discussed previously in the context of surfactant systems.[4]

Figure 8 summarizes the impact of the six bile salts on telaprevir mass flow rates. In our diffusion rate experiment setup, the solute mass flow rate is assumed to be directly proportional to solute activity (see equation 1). The ratio of solute mass flow rate in the solution of interest to the mass flow rate of its standard state (solute in a solution in equilibrium with crystalline state in this study) thus yields the fundamental supersaturation:

$$\frac{F}{F^{\circ}} = \frac{\frac{DS_m}{h\gamma_m}a}{\frac{DS_m}{h\gamma_m}a^{\circ}} = \frac{a}{a^{\circ}} = \delta \qquad (6)$$

It should be noted that F° should be constant for systems in the absence and presence of bile salts if our assumptions are reasonable (i.e. D, S_m, h and γ_m are constants). This was confirmed by extrapolating the telaprevir mass flow rate versus concentration data to a concentration corresponding to the crystalline solubility (values taken from Table 2) for systems in the absence and presence of bile salts. F° values are comparable within experimental error confirming that our assumptions appear reasonable.

Figure 9 shows the relationship between the fundamental supersaturation δ and the commonly used concentration-based supersaturation for telaprevir (below the GLPS concentration) in the absence of any bile salts. The curve has a slope very close to 1, indicating that the activity coefficient ratio is 1 and hence the activity coefficient of telaprevir in buffer is constant over the concentration range studied. Thus this system can be used as a calibration curve, using the measured mass flow rate value of a telaprevir solution where the solute activity coefficient is unknown (i.e. a solution containing bile salts), to determine the corresponding fundamental supersaturation. Knowing the extent of activity-based supersaturation is essential to evaluate crystallization kinetics in media containing bile salts, enabling experiments to be conducted at a comparable thermodynamic

driving force. In the literature, concentration-based supersaturations have long been employed in crystallization studies. [32, 33] However, from equations (2) and (3), the fundamental supersaturation and the concentration-based supersaturation are only equivalent when the solute activity coefficient ratio is unity in systems of interest. Figure 10 clearly shows the discrepancy between the fundamental supersaturation (a/a^o) and the concentration-based supersaturation (C/C^o) for telaprevir supersaturated solutions. The deviation between concentration-based supersaturation and activity-based supersaturation varies both with bile salt type as well as their aggregation state. Taking $\delta = 5.0$ as an example, the corresponding concentration-based supersaturation varies between 3.9 ~ 6.7 for the different additive systems. It is well know from theoretical considerations as well as experimental observations that crystallization kinetics, in particular nucleation rates, are highly dependent on the supersaturation level.[33] Thus the measurements presented herein provide an improved approach for the estimation of crystallization driving forces in solutions containing bile salts and other solubilizing additives, which in turn will enable better understanding of how additives modify crystallization kinetics.

Based on equations 1 and 2, the deviation of the slope of each curve (before the plateau region) from the slope of the calibration curve in Figure 8 represents the change in the activity coefficient of telaprevir in the presence of the corresponding bile salt.

$$\frac{\frac{dF}{dC}}{\left(\frac{dF}{dC}\right)_{drug\ in\ buffer}} = \frac{\gamma}{\gamma_{drug\ in\ buffer}} \tag{7}$$

Thus the variation of slopes between the curves for telaprevir in the presence of bile salts and the curve for telaprevir in the absence of any bile salts reveals the six bile salts in their monomeric and

micellar form uniquely impact solution thermodynamics. Bile salts are known to exhibit stepwise aggregation in solution, and the reported CMC values for the bile salts used in this study are between the range of 2-12mM.[18, 20] Therefore, assuming that telaprevir does not alter the CMC, at a bile salt concentration of 1.86mM, the solution will contain the monomeric form, while at 12mM, a mixture of micelles and monomers will be present. From Figure 8, it is evident that monomeric bile salts have minimal interaction with telaprevir, with the exception of SGCDC. This is apparent from the unaltered flux versus concentration profile for these systems. However, at concentrations where micelles are present, the slopes of the flux versus concentration plots are reduced, indicating that the activity-based supersaturation at a given telaprevir concentration is reduced. The dihydroxy bile salts have stronger interaction with telaprevir molecules than trihydroxy bile salts, reducing the supersaturation to a greater extent at a given telaprevir concentration, which also approximately correlates with the extent of solubilization of the crystalline form observed in this study (Table. 2). One possible explanation for the greater extent of interaction of the dihydroxy bile salts is the hydrophobicity of bile salts molecules. Trihydroxy bile salts are less hydrophobic and have higher CMCs than dihydroxy bile salts,[34] and hence there will be a lower extent of micellization for trihydroxy bile salts at a concentration of 12mM relative to for the dihydroxy bile salts. Hydrophobic drug molecules can be incorporated into bile salt aggregates.[35] Based on the Stokes–Einstein equation,[36] the diffusion coefficient of a particle is inversely proportional to its radius. That is, diffusion rate of a particle decreases with increasing particle size. The decrease in telaprevir mass flow rate in solutions containing dihydroxy bile salt micelles suggests that telaprevir molecules are incorporated into bile salt aggregates, forming larger particles in the solution. For trihydroxy bile salts, no comparable decrease in mass flow rate was observed even at concentrations higher than the reported CMCs (data not shown). This is consistent with a

previous solubilization study of bile salts,[37] where dihydroxy bile salts showed a greater extent of solubilization of various drugs as compared to trihydroxy bile salts. On the other hand, no impact of a different conjugation group at R_5 position on the steroid ring system is observed.

Using the activity calibration, in combination with the amorphous and crystalline solubility values, phase boundaries in the presence of bile salts are summarized in schematic form in Figure 11. In systems with the trihydroxy bile salts (STC and SGC), crystalline solubility and the onset concentration of GLPS are only marginally changed compared to the control (telaprevir only), thus these bile salts have minimal impact on either the concentration- or activity-based boundaries (Figure 11a). Figure 11b shows that micellar STDC and SGDC dihydroxy bile salts significantly increase the crystalline solubility and the onset concentration of GLPS, but the thermodynamic activity-based boundaries remained unchanged. In other words, the bile salts do not change the thermodynamic activity of either the crystalline or amorphous phases for the systems shown in Figures 11a-b, indicating that the composition of these phases are not altered in the presence of the bile salts. However, since STDC and SGDC do alter the crystalline and amorphous solubility values, the slopes of the flux versus telaprevir concentration profiles are altered, due to solubilization of the drug. Hence there is a difference between the activity and concentration-based regions of the schematic in terms of supersaturation. In Figure 11c, it is clear that not only did solubilization of telaprevir occur in the presence of micellar STCDC, but that the thermodynamic supersaturation window became narrower for both monomeric and micellar STCDC. This presumably stems from the mixing of STCDC into the telaprevir drug-rich phase upon GLPS, leading to a reduced activity of telaprevir in the drug-rich phase.[28, 29, 38, 39] For SGCDC (Figure 11d), the maximum achievable supersaturation also decreases in the presence of both monomeric and micellar bile salts, indicating mixing of SGCDC and the telaprevir drug-rich phase.

Thus, for the telaprevir solution system, we observed at least three types of impact on solution thermodynamics amongst the six biologically relevant bile salts. It is obvious that STC, the only bile salt component in commercial simulated fluids, is not an adequate surrogate for the entire bile salt family. As noted in the literature, SGC, STCDC and SGCDC are more abundant *in vivo* than STC,[15] hence *in vitro* testing with current simplified STC based FaSSIF simulated medias could potentially lead to inaccurate prediction of drug *in vivo* supersaturation and crystallization rates.

CONCLUSION

To maximize oral absorption of poorly soluble drugs, it is crucial to understand how endogenous bile salts impact drug solution thermodynamics. In this study, we have demonstrated that bile salts alter the thermodynamics of supersaturated telaprevir solutions. A new approach is proposed for better estimation of crystallization driving forces in solutions containing bile salts and other solubilizing additives. Furthermore, bile salts representative of the most prevalent species found in human intestinal fluids, show different patterns of interaction with supersaturated telaprevir solutions and hence are not interchangeable. Overall, trihydroxy bile salts have less effect on telaprevir solution thermodynamics than dihydroxy bile salts. These observations lay the framework for mechanistic studies into the impact of bile salts on crystallization kinetics as a function of the fundamental supersaturation.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

The Supporting Information related to mass flow rate determination is available free of charge on the ACS Publications website.

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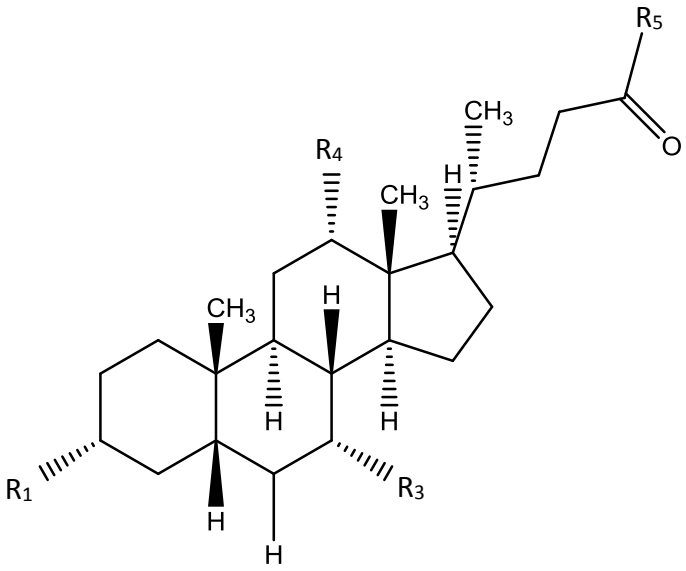
TABLES

Table 1. Chemical structure of bile salts.



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. Impact of bile salts on equilibrium crystalline solubility of telaprevir at 37 °C.

	Crystalline solubility of Telaprevir (μg/mL)					
Bile salt concentration	STC	STDC	STCDC	SGC	SGDC	SGCDC
12 mM	6.7±1.2	11.1±0.6	12.2±0.6	6.2±0.4	12.4±1.3	9.6±0.6
1.86 mM	6.0±0.9	5.6±0.3	6.8±0.7	5.5±0.4	6.9±0.8	5.7±0.8
0 mM	5.2±0.1					

FIGURES

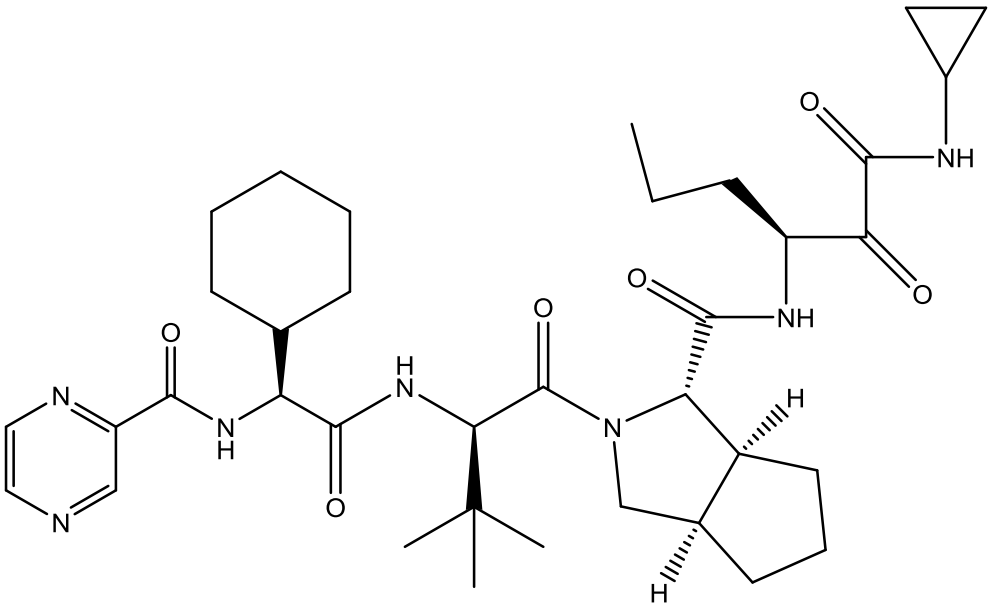


Figure 1. Molecular structure of telaprevir.

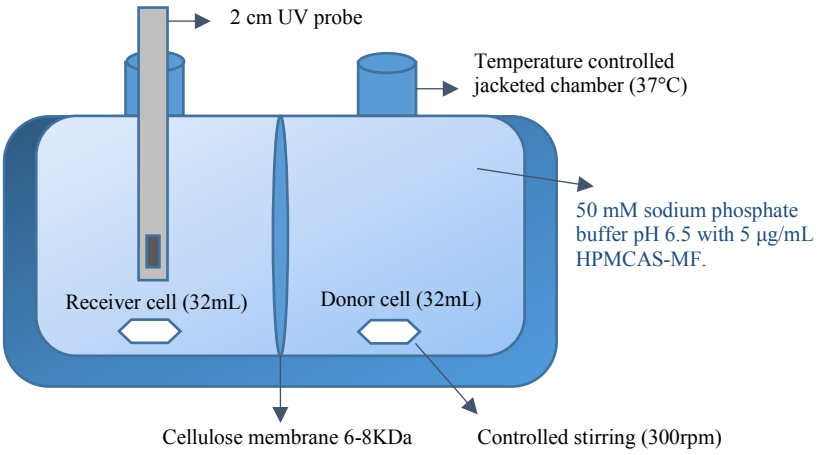


Figure 2. Side-by-side diffusion cell apparatus used for mass flow rate experiments.

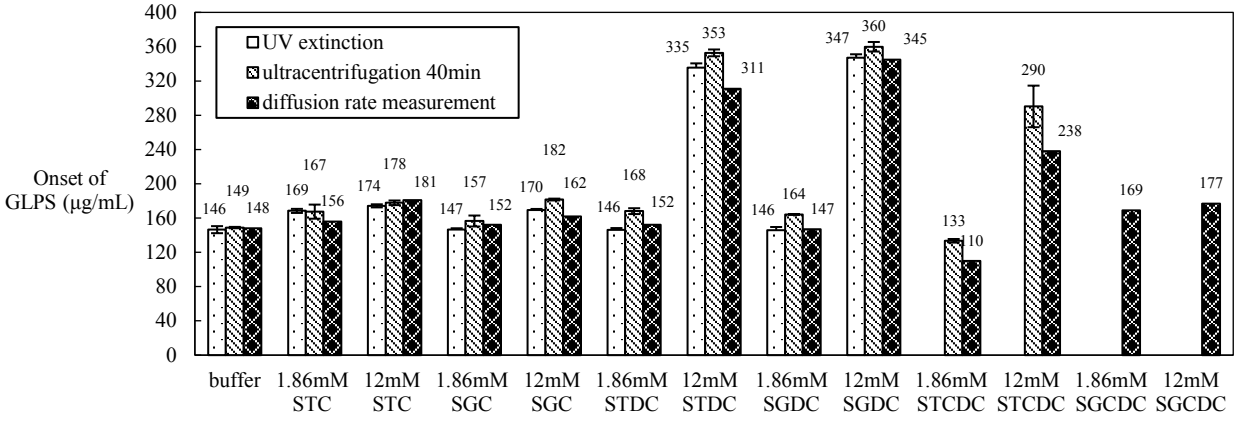


Figure 3. Onset concentration of GLPS in the presence of bile salts, n= 3 and error bars represent standard deviation.

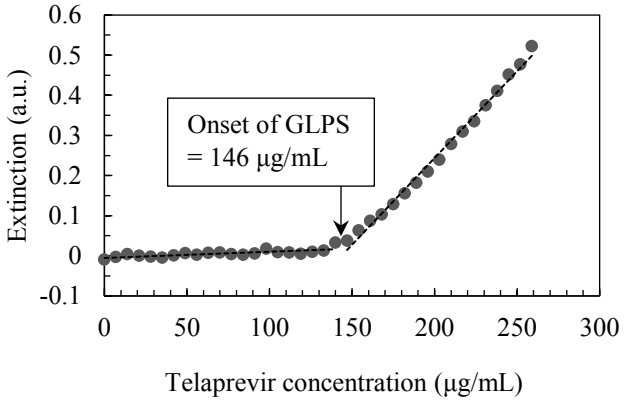


Figure 4. Determination of GLPS concentration of telaprevir with UV extinction method.

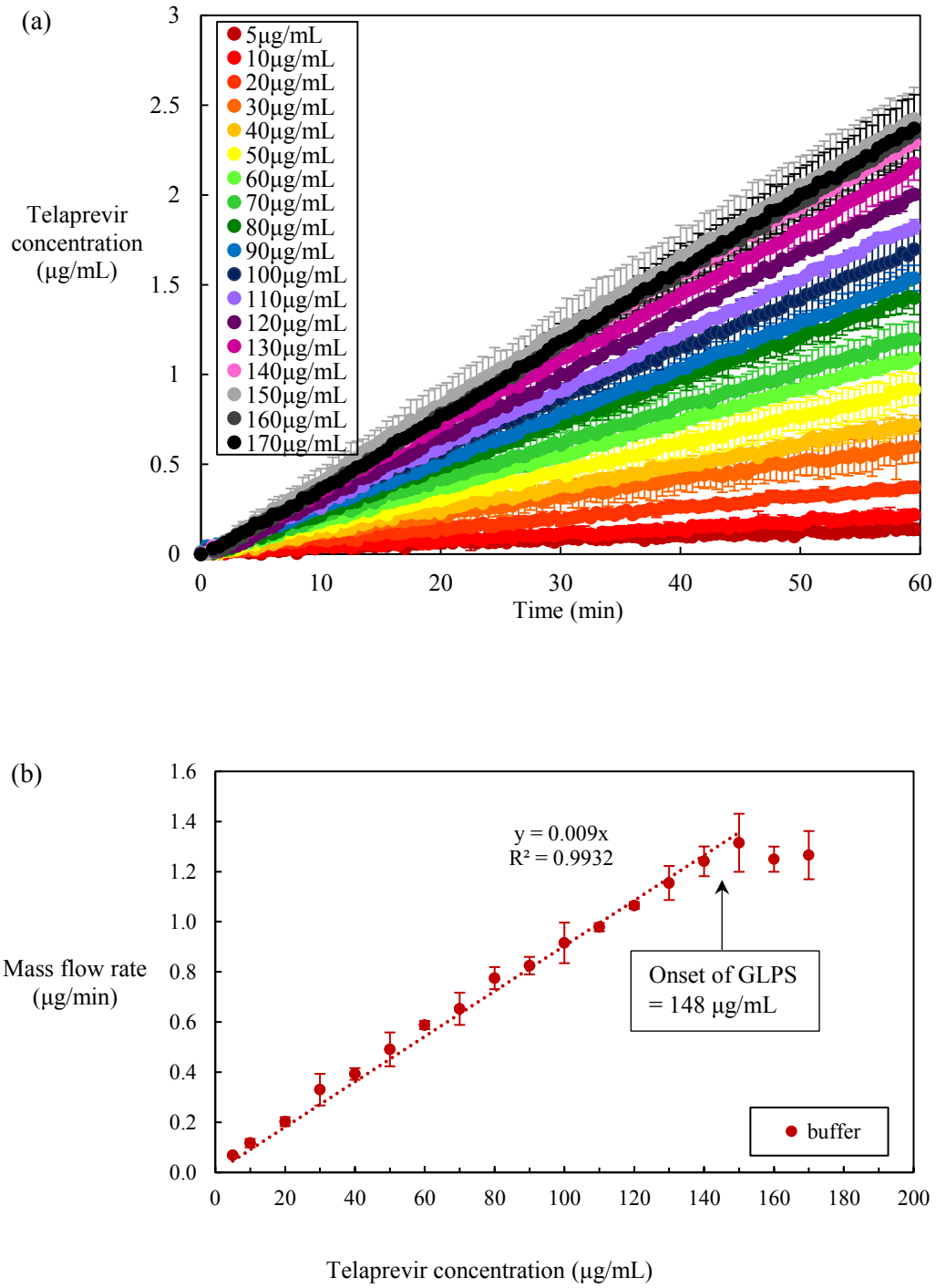


Figure 5. Diffusion cell results (a) concentration versus time profile for telaprevir in the receiver chamber, and (b) diffusion mass flow rate versus telaprevir concentration in buffer.

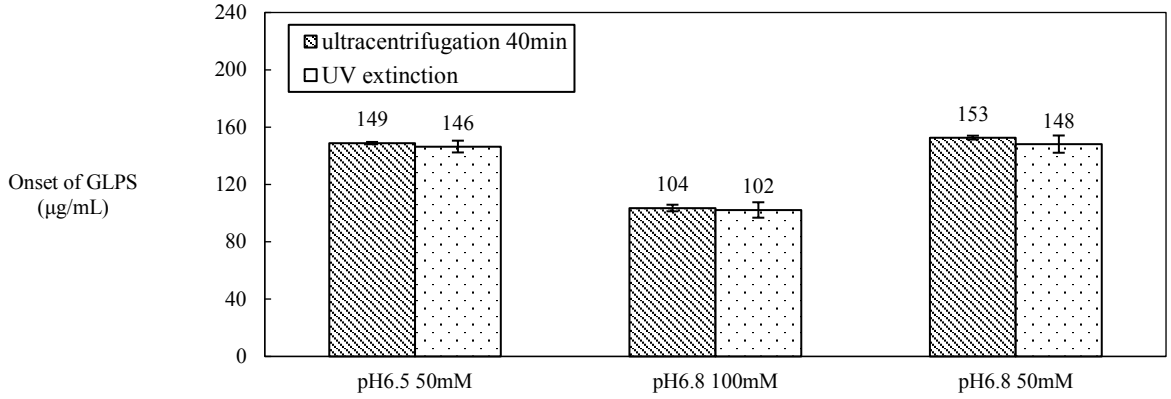
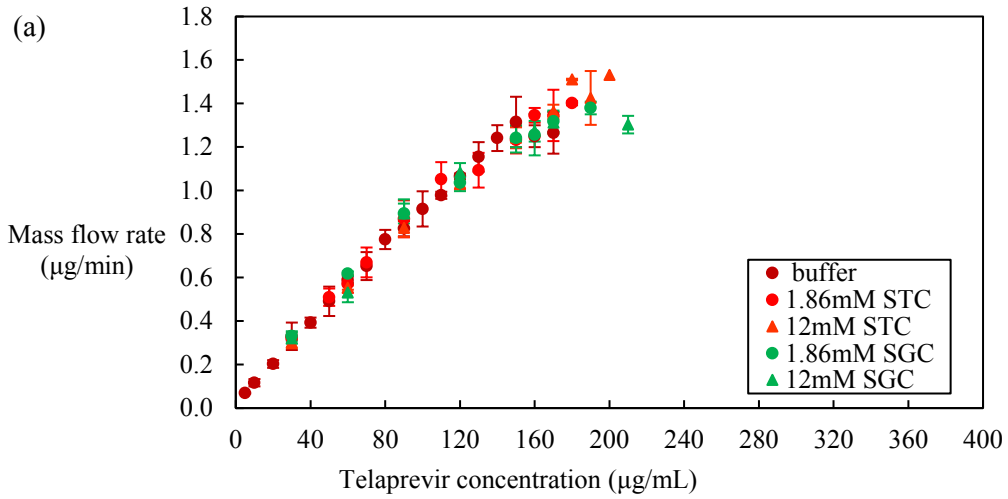
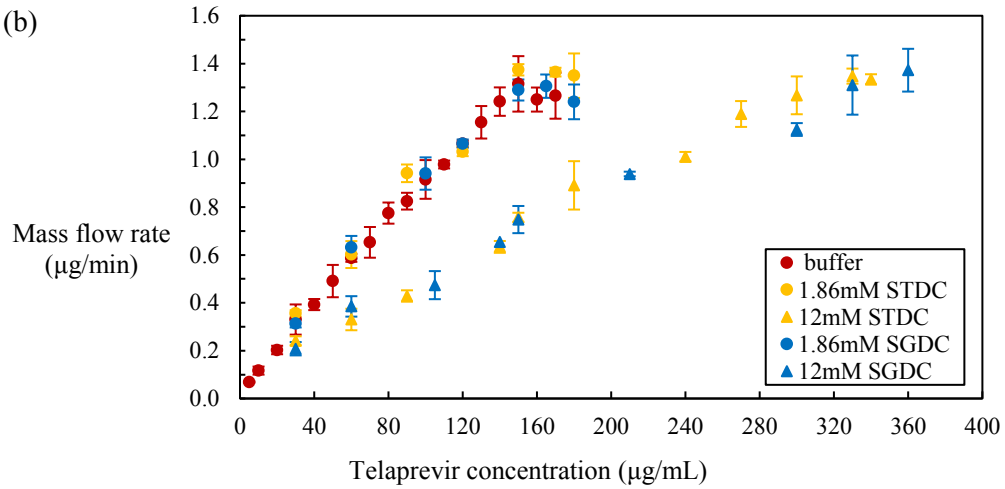


Figure 6. The effect of buffer ionic strength and pH on telaprevir GLPS onset concentration.

(a)


Mass flow rate (μg/min)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
40
80
120
160
200
240
280
320
360
400
Telaprevir concentration (μg/mL)
buffer
1.86mM STC
12mM STC
1.86mM SGC
12mM SGC

(b)


Mass flow rate (μg/min)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
40
80
120
160
200
240
280
320
360
400
Telaprevir concentration (μg/mL)
buffer
1.86mM STDC
12mM STDC
1.86mM SGDC
12mM SGDC

(c)

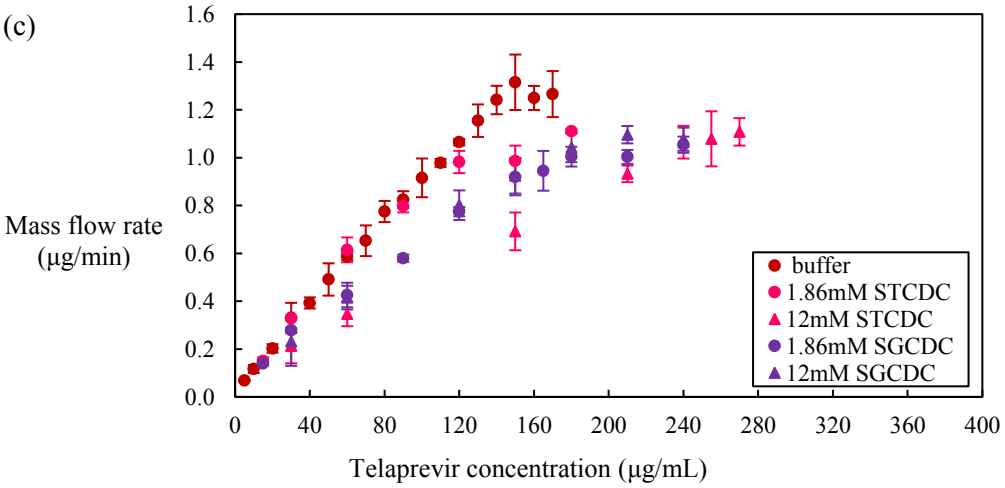

Mass flow rate (μg/min)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
40
80
120
160
200
240
280
320
360
400
Telaprevir concentration (μg/mL)
buffer
1.86mM STCDC
12mM STCDC
1.86mM SGCDC
12mM SGCDC

Figure 7c. Diffusion mass flow rate versus telaprevir concentration in the presence of (a) STC and SGC, (b) STDC and SGDC, and (c) STCDC and SGCDC.

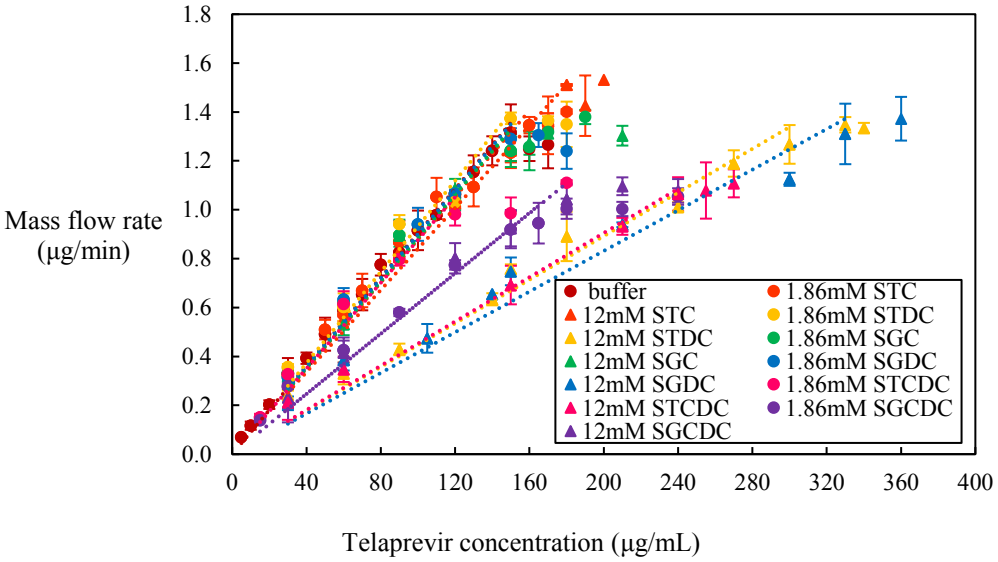


Figure 8. Diffusion mass flow rate versus telaprevir concentration in the presence of bile salts.

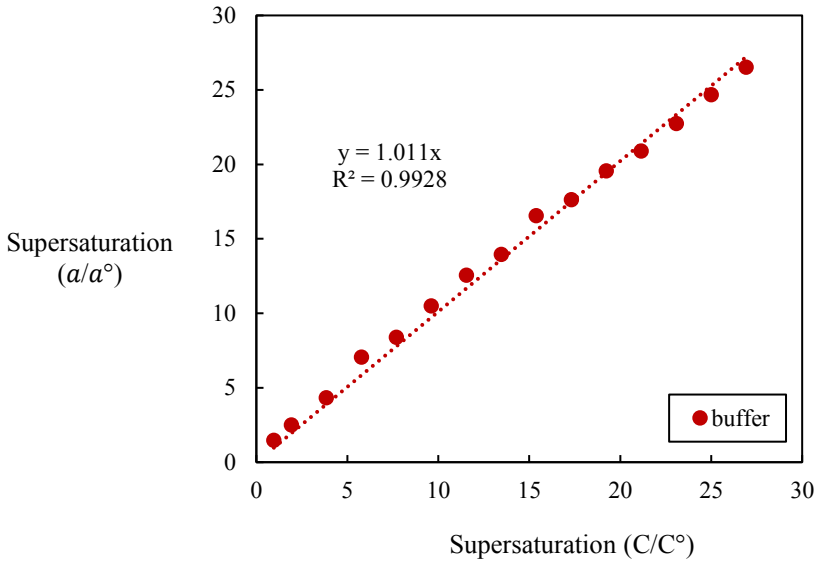


Figure 9. The relationship between concentration-based supersaturation and the fundamental supersaturation for telaprevir in the absence of bile salts.

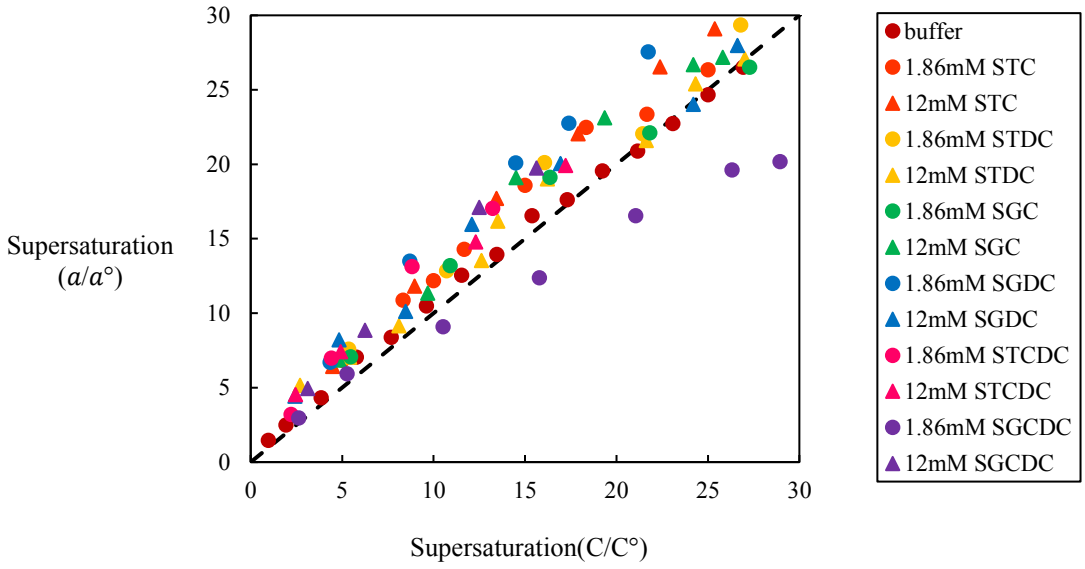
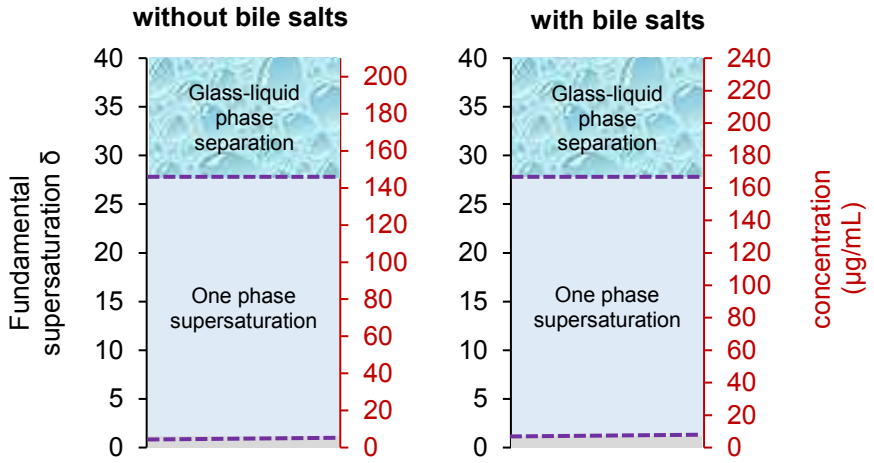
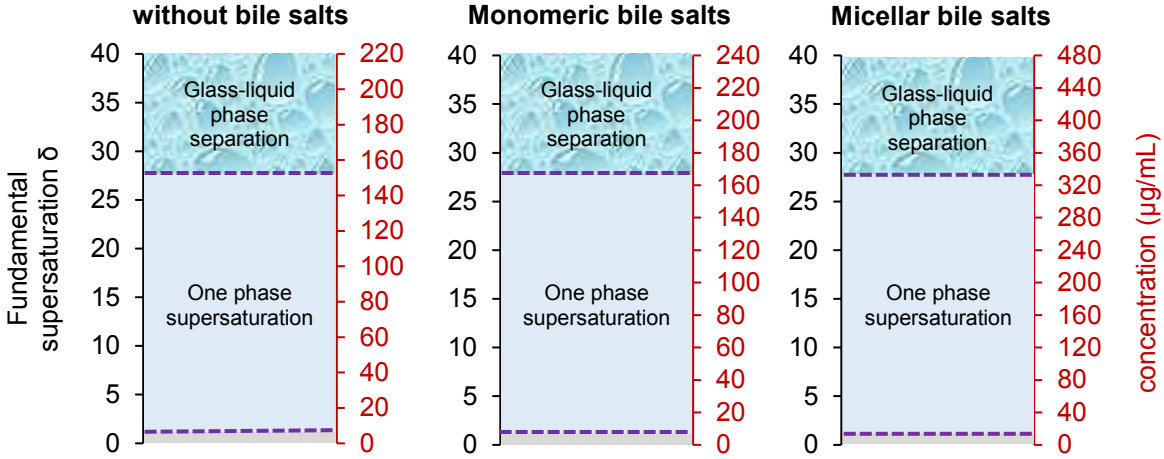


Figure 10. The relationship between concentration-based supersaturation and the fundamental supersaturation for telaprevir in the absence and presence of bile salts. The dotted line represents the theoretical curve with a slope of 1.

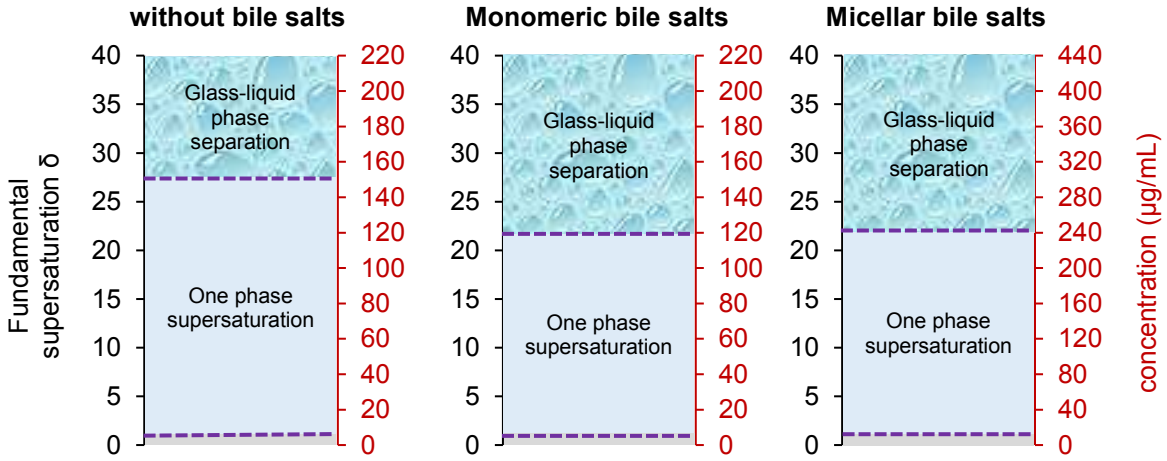
(a) Trihydroxy bile salts



(b) Dihydroxy bile salts STDC and SGDC



(c) Dihydroxy bile salts STCDC



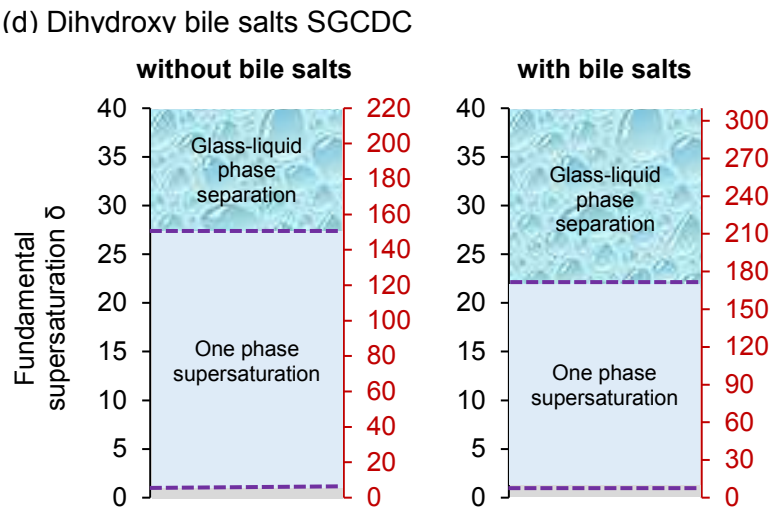


Figure 11d. Visual depiction of the impact of (a) trihydroxy bile salt, (b) dihydroxy bile salt STDC and SGDC, (c) dihydroxy bile salt STCDC, and (d) dihydroxy bile salt SGCDC on phase boundaries and solute thermodynamic activity for supersaturated solutions of telaprevir.