

Impact of Solubilizing Additives on Supersaturation and Membrane Transport of Drugs

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Received: 12 March 2015 / Accepted: 12 May 2015 / Published online: 28 May 2015
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ABSTRACT

Purpose Many enabling formulations give rise to supersaturated solutions wherein the solute possesses higher thermodynamic activity gradients than the solute in a saturated solution. Since flux across a membrane is driven by solute activity rather than concentration, understanding how solute thermodynamic activity varies with solution composition, particularly in the presence of solubilizing additives, is important in the context of passive absorption.

Methods In this study, a side-by-side diffusion cell was used to evaluate solute flux for solutions of nifedipine and felodipine in the absence and presence of different solubilizing additives at various solute concentrations.

Results At a given solute concentration above the equilibrium solubility, it was observed that the solubilizing additives could reduce the membrane flux, indicating that the extent of supersaturation can be reduced. However, the flux could be increased back to the same maximum value (which was determined by the concentration where liquid-liquid phase separation (LLPS) occurred) by increasing the total solute concentration. Qualitatively, the shape of the curves of solute flux through membrane as a function of total solute concentration is the same in the absence and presence of solubilizing additives. Quantitatively, however, LLPS occurs at higher solute

concentrations in the presence of solubilizing additives. Moreover, the ratios of the LLPS onset concentration and equilibrium solubility vary significantly in the absence and presence of additives.

Conclusions These findings clearly point out the flaws in using solute concentration in estimating solute activity or supersaturation, and reaffirm the use of flux measurements to understand supersaturated systems. Clear differentiation between solubilization and supersaturation, as well as thorough understanding of their respective impacts on membrane transport kinetics is important for the rational design of enabling formulations for poorly soluble compounds.

KEY WORDS membrane transport · solubilization · supersaturation · surfactants

ABBREVIATIONS

ASD	Amorphous solid dispersion
BSA	Bovine serum albumin
CMC	Critical micelle concentration
FaSSIF	Fasted state simulated intestinal fluid
GI	Gastrointestinal
HPMC	Hydroxypropylmethyl cellulose

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LLPS	Liquid-liquid phase separation
MWCO	Molecular weight cut off
TFA	Trifluoroacetic acid
UV	Ultraviolet

INTRODUCTION

A growing number of new drug molecules emerging from pharmaceutical pipelines exhibit low aqueous solubility and poor bioavailability profiles. Use of enabling formulations frequently improves oral drug absorption for systems with solubility-limited bioavailability (1–3). Amorphous solid dispersions (ASD), where the higher apparent solubility and dissolution rates of amorphous solids lead to supersaturated solutions (4,5) are a common formulation approach. Lipid based drug delivery systems (6), salts and weak bases (7) may also result in the formation of supersaturated solutions *in vivo*. From a thermodynamic perspective, the elevated solution concentrations obtained from supersaturated solutions are fundamentally different from those generated in the presence of solubilizing additives such as surfactants. Specifically, the chemical potential or thermodynamic activity of the solute in supersaturated solutions is higher than that in a saturated or subsaturated solution. Solubilizing additives increase concentration by increasing the equilibrium solubility rather than increasing chemical potential. This distinction is important because unlike solubilizing additives, supersaturated solutions will lead to an increase in flux across a membrane. This observation is supported by the studies of Corrigan *et al.* (8), Davis *et al.* (9), Pellett *et al.* (10), Frank *et al.* (11), Kumprakob *et al.* (12) and Santos *et al.* (13) who have all demonstrated enhancements in membrane transport with increased supersaturation in the presence of small amounts of polymers, added to prevent crystallization. In contrast, a solubilized solution at or below its equilibrium solubility may either have no impact or actually decrease the flux of the drug. This is because complexing agents, co-solvents, lipids and surfactants increase the thermodynamic or equilibrium crystalline solubility (11,14) of small molecules thereby increasing solution concentration but often lowering (or at best not altering) the solute thermodynamic activity. Since diffusive flux depends on activity and not concentration gradients (15), solubilizing additives can lead to lower diffusive flux. In many cases, the reduced flux has been interpreted as reduced permeability on account of solubilization. Malik, Canaham and Gouda also showed that surfactants such as dioctyl sodium sulfosuccinate and poloxalence decreased gastrointestinal (GI) absorption of phenolsulfonaphthalein in rats (16). Thus the mechanism by which elevated solution concentrations are generated is important in terms of the subsequent impact on passive absorption. Because of their enhanced flux, supersaturated solutions of poorly water soluble drugs and formulations strategies that lead to supersaturation are of great current interest.

Aqueous supersaturated solutions of poorly water-soluble drugs are complex because they are metastable and can crystallize. In addition, at very high supersaturations, a non-crystalline drug-rich may be formed (17–19). Formation of a disordered drug-rich phase has been widely observed during dissolution of amorphous solid dispersions (20–26). Certain polymers are believed to facilitate formation of this disordered phase by inhibiting crystallization and can stabilize the resultant drug-rich phase in the nano-sized regime (23,24). The underlying phenomenon that leads to the formation of the non-crystalline drug-rich phase is liquid-liquid phase separation (LLPS) (11,12,21). LLPS occurs in supersaturated solutions when a critical threshold supersaturation has been exceeded. This critical upper limit has been shown to occur at concentrations at or slightly above the estimated amorphous solubility (21,27). Following LLPS, one phase consists of drug-rich, initially nanosized droplets which are dispersed in a continuous drug-lean, aqueous phase. The latter phase has a solution concentration corresponding to that generated by dissolving an amorphous solid (where no crystallization occurs). The phases are in metastable equilibrium with each other and thus the nanosized drug-rich droplets can serve as reservoir, maintaining the supersaturation at a constant value as drug in the aqueous phase is removed by diffusion across a membrane. Therefore, it has been suggested that the formation of a LLPS system is likely to be beneficial for oral drug delivery (17,27).

In recent studies, it has been shown that the diffusive flux of two poorly water-soluble drugs, felodipine and nifedipine, increases linearly with increasing concentration and plateaus at the LLPS onset concentration (which corresponds to the amorphous solubility) (27). This suggests that there is a maximum increase in drug flux that can be obtained with a supersaturated solution that is dictated by the concentration at which LLPS occurs. This is because when a second phase forms *i.e.*, the drug-rich disperse phase, the chemical potential of the solute in each phase is the same, and is invariant. Thus when more drug is added to the system, the free drug concentration does not increase, instead more of the disperse phase is formed. The diffusion measurements have clearly demonstrated the link between diffusive flux and supersaturation, as well as provided a method to demonstrate that there is a maximum achievable flux.

Due to the variety of excipients that are often present in enabling formulations it is important to understand the impact of both solubilizing and non-solubilizing additives on the properties of supersaturated solutions, such as chemical potential and thermodynamic activities, in order to design and deliver effective enabling formulations. Additives such as polymers at low solution concentrations do not generally enhance equilibrium crystalline solubility of small molecules (28). Rather, they inhibit nucleation (5,29–32), and/or crystal growth (33–36) and thereby stabilize supersaturated solutions (29). In contrast, the other additives such as surfactants or complexing agents typically increase equilibrium solubility.

For many enabling formulations, a combination of supersaturation and solubilization can occur. For example, surfactants may be included in an amorphous solid dispersion formulation. Alternatively, a lipid formulation may lead to a supersaturated solution *in vivo* following lipid digestion. Therefore, it is important to expand studies on the mass transfer kinetics of supersaturated solutions to include the impact of solubilizing additives. In addition, the impact of additives on LLPS and the maximum flux observed has not been investigated to date. Thus, the goal of this study was to evaluate the interplay between changes in equilibrium crystalline solubility, thermodynamic activity, supersaturation and passive drug diffusion in the presence of solubilizing additives. Felodipine and nifedipine were used as model drug substances and a variety of solubilizing additives including surfactants were evaluated. Diffusion rates across a membrane were studied using a side-by-side diffusion cell as described previously (8,27). The impact of the additives on equilibrium solubility was determined and linked to the changes in the flux values of solutions containing different concentrations of the drug and additives.

MATERIALS

Model compounds, felodipine and nifedipine, were purchased from Attix Pharmaceuticals (Toronto, ON, Canada) and Euroasia (Mumbai, India) respectively. Hydroxypropylmethyl cellulose (HPMC) Pharmacoat grade 606 was obtained from ShinEtsu (Shin-Etsu Chemical Co., Ltd, Tokyo, Japan). The diffusion medium used in the majority of experiments comprised of 50 mM pH 6.8 phosphate buffer (ionic strength $\mu = 0.155$ M) with pre-dissolved polymer at a concentration of 100 $\mu\text{g}/\text{mL}$ and 1 mg/mL for felodipine and nifedipine respectively. FaSSiF was prepared by dissolving ready to use SiF powder (Biorelevant, Surrey, UK) in pH 6.8 phosphate buffer. Methyl alcohol was purchased from Pharmco Products, Inc., Brookfield, CT, USA. Molecular structures and selected physicochemical properties of the model compounds are shown in Fig. 1. Regenerated cellulose membrane with a molecular weight cut off (MWCO) of 6–8 K was obtained from Spectrum Laboratories, Inc. (Rancho

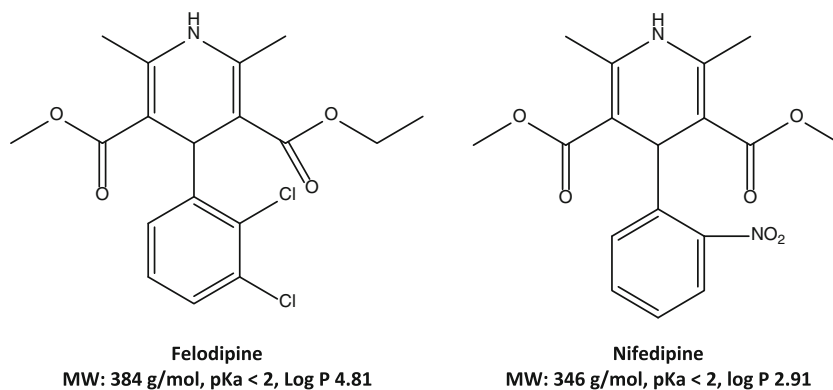
Dominguez, CA). Kolliphor®RH40 (Cremophor®RH40), Kolliphor® EL (Cremophor® EL) and Kolliphor® TPGS (Vit E TPGS) were purchased from BASF (BASF Corporation, NJ, USA). Bovine serum albumin (BSA), Polysorbate 20 (Tween® 20) and β -Cyclodextrin were obtained from the Sigma-Aldrich Co. LLC (St. Louis, MO, USA).

METHODS

Crystalline Solubility Measurements

An excess of crystalline felodipine and nifedipine was equilibrated in 20 mL scintillation vials with 50 mM pH 6.8 phosphate buffer in an agitating water bath (Dubnoff metallic shaking incubator, PGC Scientific, Palm Desert, CA) for 48 h at 37°C. Samples in triplicate were then ultracentrifuged to separate excess solid from the supernatant (which is saturated with the drug). An Optima L-100 XP ultracentrifuge equipped with Swinging-Bucket Rotor SW 41 Ti (Beckman Coulter, Inc., Brea, CA) was used and samples were centrifuged at 40,000 rpm (equivalent of $274,356 \times g$) for 15 min. The supernatant obtained was diluted 2-fold with acetonitrile: water acidified with 0.1% trifluoroacetic acid (TFA) in a 70:30 *v/v* ratio. This also served as mobile phase for chromatographic separations. One hundred μL samples were injected into an Agilent 1100 high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) and HPLC was performed with a Synergi Polar-RP analytical column (4.6×150 mm I.D., $4 \mu\text{m}$, 80 \AA) (Agilent Technologies, Santa Clara, CA) using acetonitrile: water acidified with 0.1% trifluoroacetic acid (TFA) (70:30) as mobile phase. Flow through the column was maintained at 1 mL/min, the column was heated to 40°C and detection was carried out using ultraviolet (UV) absorbance at a wavelength of 360 nm. A standard curve was prepared using samples dissolved in mobile phase with an injection volume of 100 μL covering a concentration range of 0.1–50 $\mu\text{g}/\text{mL}$. Standards were prepared in triplicate and injected. The standard curve exhibited good linearity ($R^2 > 0.998$) over the specified concentration range.

Fig. 1 Molecular structures of model compounds and key physicochemical parameters (37–39).



The equilibrium solubility of felodipine and nifedipine was also determined in the presence of 100 µg/mL and 1 mg/mL of HPMC in 50 mM pH 6.8 phosphate buffer ($\mu=0.155$ M) using the method outlined above. In addition, the equilibrium solubilities of felodipine and nifedipine were measured in the presence of three different concentrations of the various additives listed in Table I. Felodipine solubility was also determined in FaSSIF at pH 6.8.

Amorphous Solubility Estimations

Amorphous solubility (σ_a) can be estimated from a knowledge of the crystalline solubility (σ_c) and then by calculating the free energy difference between the crystal and supercooled liquid using the Hoffman equation as shown in Eq. 1. Although the Hoffman equation typically provides a reasonable approximation of the free energy difference between the amorphous and crystalline material (40), neither the impact of the degree of ionization of the molecules nor the reduced activity of the amorphous solid resulting from water sorption are taken into account. For the model systems studied herein, there is no ionization, therefore it is only necessary to modify Eq. 1 to account for moisture sorption effects, which can be done by using the approach developed by Bogner and coworkers (41) leading to Eq. 2.

$$\frac{\sigma_a}{\sigma_c} = \frac{\Delta G}{RT} = e^{-\frac{(\Delta H_f - \Delta T)(\frac{T}{T_m})}{RT}} \quad (1)$$

$$\frac{\sigma_a}{\sigma_c} = e^{-I(a_2)} \cdot \frac{\Delta G}{RT} \quad (2)$$

here ΔG is the free energy difference between amorphous and crystalline material, R is the ideal gas constant, T is the temperature of interest, T_m is the melting point of the API, ΔT is the difference in temperature between T_m and T , and $-I(a_2)$ is a term that accounts for the reduction in activity of the amorphous solid caused by absorbed water.

Enthalpy of fusion and melting point were measured using a TA Q2000 DSC (TA Instruments, New Castle, DE). Tin was used for temperature calibration, while cell constant and enthalpy calibrations were performed using indium. The crystalline sample in an aluminum pan with a pinhole in the lid

was heated at 10°C/min to determine the melting point and enthalpy of fusion (37). The reduction in the thermodynamic activity of the amorphous drug due to the presence of water was calculated using moisture sorption measurements obtained from a TA Q5000 automated gravimetric analysis system (TA Instruments, New Castle, DE). Amorphous material was placed in a platinum pan and a humidity ramp was carried out from 5 to 95% RH and the weight change at each relative humidity was used to determine the moisture sorption profile.

Determining Liquid-Liquid Phase Separation Onset

Ultraviolet (UV) extinction measurements (4,21,25) were used to evaluate the phase behavior of felodipine and nifedipine at different solution concentrations. Felodipine and nifedipine were dissolved in methanol to obtain a drug stock solution of 20 mg/mL. A 5 mL syringe containing this solution was loaded into a pulsating syringe pump (Harvard Apparatus, Holliston, MA). Stock solution was infused into a well-stirred 20 mL scintillation vial containing 50 mM pH 6.8 phosphate buffer ($\mu=0.155$ M) maintained at 37°C, with and without dissolved polymer. The polymer, HPMC, was added at 100 µg/mL and 1 mg/mL respectively for felodipine and nifedipine in the absence of surfactants. In the presence of surfactants or FaSSIF, HPMC was used at 1 mg/mL to inhibit crystallization of felodipine. Stock solutions of drugs were infused into the vials at a rate of 20 µL/min for felodipine and 40 µL/min for nifedipine. The system was stirred at 120–300 rpm whereby slower stirring rates were used for nifedipine systems where crystallization was observed at higher rates. UV extinction was monitored at a wavelength of 455 nm (neither compound nor the FaSSIF media absorb at this wavelength), and data were collected every 10 s. A sharp change in a plot of extinction *versus* time indicates the appearance of a new scattering phase which is the onset of LLPS. Since LLPS onset represents the amorphous solubility, amorphous to crystalline solubility ratios (σ_c/σ_a) were also calculated by dividing the LLPS onset concentration by the experimentally determined equilibrium crystalline solubility.

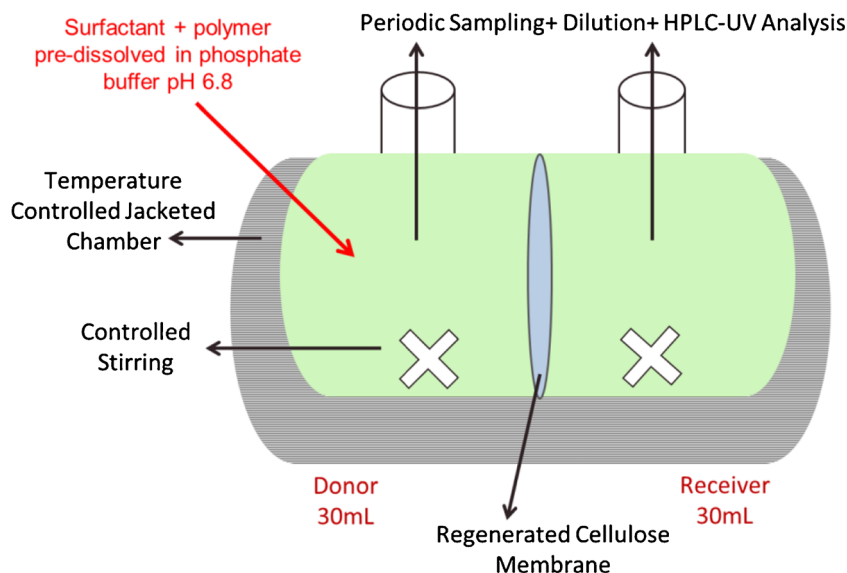
Diffusion Rate Measurements

The relationship between diffusion rate and donor solution concentration was evaluated using a side-by-side diffusion cell (PermeGear, Inc. Hellertown, PA) as depicted in Fig. 2. The donor and receiver chambers were separated by a regenerated cellulose membrane with a molecular weight cut off (MWCO) of 6–8 KDa and connected with an orifice of 30 mm diameter (surface area of 7.065 cm²). The temperature was maintained at 37°C using a circulating water bath. The donor and receiver compartments were filled with 30 mL of 50 mM pH 6.8

Table I Concentrations of Solubilizing Agents Used

Solubilizing additives	Function	CMC	C ₁	C ₂	C ₃
BSA	Complexing agent	NA	0.001%	0.1%	1%
Cyclodextrin		NA	0.001%	0.1%	1%
Cremophor EL	Surfactant	0.01%	0.001%	0.02%	0.1%
Cremophor RH40		0.03%	0.01%	0.05%	0.1%
Tween 20		0.009%	0.001%	0.02%	0.1%
Vit E TPGS		0.02%	0.004%	0.04%	0.1%

Fig. 2 Side-by-side diffusion cell set-up.



phosphate buffer with a pre-dissolved polymer, HPMC added to the donor compartment at a concentration of 100 $\mu\text{g}/\text{mL}$ for felodipine and 1 mg/mL for nifedipine. Drug stock solutions were prepared in methanol at 10, 20 and 30 mg/mL . Supersaturated solutions were generated in the donor compartment by addition of aliquots of methanolic drug stock such that less than 300 μL of methanol were introduced at any time to the 30 mL volume. The donor and receiver compartments were stirred using a cross-shaped magnetic stirrer rotating at around 300 RPM. In the experiments evaluating the additives, in addition to the polymer, the donor compartment contained various pre-dissolved additives at different concentrations. These concentrations are summarized in Table I.

Drug concentration in the donor and receiver chambers was monitored over time by withdrawing 200 μL aliquots and diluting with 100 μL of mobile phase. Samples were then analyzed using the HPLC method described above. Sink conditions were maintained in the receiver cell whereby the maximum solution concentration was always less than one-third of the crystalline solubility.

For flux measurements on suspensions, an excess amount of crystalline felodipine was equilibrated overnight at 37°C with 50 mM phosphate buffer pH 6.8 in an agitating water bath (Dubnoff metallic shaking incubator, PGC Scientific, Palm Desert, CA). A volume of 30 mL of buffer was equilibrated with approximately 500 mg felodipine. The entire sample was then added to the donor side of the diffusion cell. Similar felodipine suspensions were generated with Vit E TPGS pre-dissolved in 30 mL of 50 mM phosphate buffer pH 6.8.

Concentration *versus* time plots were generated for the receiver compartment and the slope of the linear region was estimated using linear regression. This slope represents the

flux, J , of drug molecules diffusing across the semipermeable membrane. Equation 3 (15,42) describes the flux, J , which is equivalent to the change in mass per unit time (dM/dt) which depends on the membrane cross sectional area, S , the diffusion coefficient (D), the solute thermodynamic activity (a), the activity co-efficient of the drug in the membrane, γ_m and the thickness of the membrane, h . For the given experimental set-up, S , D , γ_m and h are constants. The activity is given by Eq. 4 where γ is the activity coefficient of the drug in the donor solution and C is the drug concentration.

$$J = \frac{dM}{dt} = \frac{DSa}{h\gamma_m} \quad (3)$$

$$a = \gamma C \quad (4)$$

The slope of a plot of M versus t yields the flux, which is directly proportional to the solute activity since D , S , γ and h are all constants (41).

RESULTS

Equilibrium Solubility

The equilibrium crystalline solubility of felodipine and nifedipine was determined at 37°C in the absence and presence of HPMC pre-dissolved in pH 6.8, 50 mM phosphate buffer and also in FaSSIF for felodipine (see Table II). Solubilization was not observed for felodipine or nifedipine in the presence of HPMC at concentrations of 100 $\mu\text{g}/\text{mL}$ and 1 mg/mL respectively. In FaSSIF, the equilibrium solubility of felodipine increased approximately 40-fold relative to in buffer (Table II). Using the values for the crystalline solubility in phosphate buffer and FaSSIF, both with HPMC, the

Table II Impact of Additives and Media on the Solubility of Felodipine and Nifedipine at 37°C

API	Felodipine			Nifedipine
	Concentration ($\mu\text{g/mL}$)			
Media	Phosphate Buffer	FaSSIF	Phosphate buffer	
Equilibrium	–	1.2 \pm 0.0	40.8 \pm 5.0	10.0 \pm 0.0
Crystalline solubility	(w HPMC)	1.1 \pm 0.0	42.8 \pm 7.0	10.3 \pm 0.0
Amorphous solubility ^a	(w HPMC)	8.7 \pm 0.0	308.0 \pm 7.0	72.9 \pm 6.0
LLPS Onset	(w HPMC)	10.4 \pm 3.0	214.6 \pm 8.0	71.2 \pm 6.0

^a Estimated value based on Eqs. 1 and 2

$n = 3$; errors indicate one standard deviation

theoretical amorphous solubility of felodipine and nifedipine was estimated using Eqs. 1 and 2. These estimates assume that components of FaSSIF do not change the amorphous-to-crystalline solubility ratio. The LLPS concentration in buffer and FaSSIF was also determined experimentally using UV extinction measurements. In buffer containing HPMC, the experimentally determined LLPS concentration, which corresponds to the amorphous solubility, was in good agreement with the theoretically estimated value for both felodipine and nifedipine. For felodipine in FaSSIF, however, the experimentally determined amorphous solubility was much higher than in buffer reflecting the increased crystalline solubility, but was substantially lower than the estimated value.

To assess the impact of solubilizing additives on the degree of supersaturation, the equilibrium crystalline solubility of felodipine and nifedipine was measured in the presence of different concentrations of the additives. Three concentrations were selected for each of the four surfactants *viz.* Cremophor® RH 40, Cremophor® EL, Vit E TPGS and Tween® 20 such that one concentration (C_1) was below the critical micelle concentration (CMC) and the other two (C_2 and C_3) were above the CMC. Similarly, three concentrations were selected for the two complexing agents, BSA and β -cyclodextrin, such that C_1 gave little or no drug solubility enhancement and C_2 and C_3 provided varying degrees of solubility enhancement. The impact of the additives on the equilibrium solubility values of felodipine and nifedipine are summarized in Tables III and IV respectively. Overall, higher concentration of additives led to a higher solubility value.

Flux of Two Phase Systems

To determine the flux at a constant solute activity, suspensions of crystalline felodipine in different media as opposed to solutions were introduced into the donor chamber and the flux was monitored. In a suspension, there exists an equilibrium between the crystalline solid and dissolved drug. The thermodynamic activity of the dissolved solute is therefore equal to the activity of the drug in the crystal, and is independent of the

medium (and hence is independent of solute concentration) if the system remains in equilibrium. Furthermore, for a suspension, the supersaturation ratio, $S = C/C^*$, is 1, where C is the solution concentration and C^* is the crystal solubility in the same medium. Figure 3 compares the diffusive flux of felodipine suspensions in the absence and presence of Vit E TPGS. The respective solution concentrations of felodipine are approximately 1 $\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$. Also included in the plot is a solution with the same concentration as that produced by a saturated felodipine solution *i.e.*, 1 $\mu\text{g/mL}$. First, it can be seen that the flux of the saturated solution is the same as that of the suspension in buffer. This is expected since the concentration of the free drug or thermodynamic activity is the same for both systems. Second, within experimental error (which is quite large due to the low concentrations being detected), suspensions of felodipine, in the absence and presence of Vit E TPGS, yield the same flux, despite having significantly different solute concentration. The flux

Table III Equilibrium Crystalline Solubility of Felodipine in the Presence of Solubilizing Additives Pre-Dissolved in 50 mM Phosphate Buffer pH 6.8 with HPMC at 37°C

Solubilizing additive	Felodipine ($\mu\text{g/mL}$)		
	C_1^a	C_2^b	C_3^b
BSA	0.7 \pm 0.4	39.0 \pm 4.6	47.8 \pm 9.9
Cyclodextrin	0.7 \pm 0.3	36.8 \pm 6.3	48.2 \pm 13.6
Cremophor® RH40	0.7 \pm 0.4	36.0 \pm 5.1	45.2 \pm 3.4
Cremophor® EL	0.6 \pm 0.8	37.2 \pm 7.7	47.0 \pm 8.1
Vit E TPGS	0.9 \pm 0.9	39.3 \pm 8.5	48.2 \pm 6.2
Tween® 20	0.7 \pm 0.5	35.3 \pm 2.3	49.0 \pm 10.8

^a C_1 represents a concentration below critical micelle concentration (CMC) of pre-dissolved surfactant as outlined in Table I, or for the complexing agents, a concentration that causes minimal change in equilibrium solubility

^b C_2 and C_3 represent concentrations above critical micelle concentration (CMC) of pre-dissolved surfactant as outlined in Table I, or for the complexing agents, a concentration that causes appreciable changes in equilibrium solubility

$n = 3$; errors indicate one standard deviation

Table IV Equilibrium Crystalline Solubility of Nifedipine in the Presence of Solubilizing Additives Pre-Dissolved in 50 mM Phosphate Buffer pH 6.8 with HPMC at 37°C

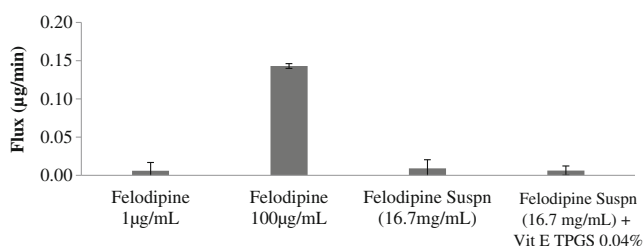
Solubilizing additive	Nifedipine ($\mu\text{g/mL}$)		
	C_1^a	C_2^b	C_3^b
BSA	18.0 \pm 7.7	53.4 \pm 3.9	86.0 \pm 20.5
Cyclodextrin	20.6 \pm 8.6	60.9 \pm 12.4	88.2 \pm 16.5
Cremophor® RH40	22.0 \pm 5.4	58.5 \pm 12.8	84.4 \pm 7.5
Cremophor® EL	18.9 \pm 7.7	59.8 \pm 8.2	85.1 \pm 18.3
Vit E TPGS	18.7 \pm 5.8	54.8 \pm 5.5	78.8 \pm 18.1
Tween® 20	16.3 \pm 8.1	53.5 \pm 8.9	90.7 \pm 12.0

^a C_1 represents a concentration below critical micelle concentration (CMC) of pre-dissolved surfactant as outline in Table I, or for the complexing agents, a concentration that causes minimal change in equilibrium solubility

^b C_2 and C_3 represent concentrations above critical micelle concentration (CMC) of pre-dissolved surfactant as outlined in Table I, or for the complexing agents, a concentration that causes appreciable changes in equilibrium solubility

$n = 3$; errors indicate one standard deviation

of a solution saturated with respect to the crystalline form is a commonly used reference point for diffusion experiments since the solute activity is controlled by the crystal. In our experiments since we are interested in supersaturated solutions, it is relevant to consider a second reference system which defines the maximum flux. Previous studies have shown that in the absence of solubilizing additives, flux increases linearly with supersaturation and reaches a maximum value beyond which a plateau is observed (27). This maximum value corresponds to the concentration where the system has undergone LLPS, and is dictated by the amorphous solubility; the flux cannot exceed this value due to phase separation of the system. The calculated amorphous solubility of felodipine in buffer is 8.7 $\mu\text{g/mL}$ (27) at 37°C (see Table II). At and above this concentration, felodipine is liquid-liquid phase separated and the solute thermodynamic activity approaches a constant (27). As a result, solutions with felodipine concentrations higher than 8.7 $\mu\text{g/mL}$ will possess the same solute thermodynamic activity and consequently give rise to the same flux (27). Therefore in buffer, felodipine solutions at concentrations above $\sim 9 \mu\text{g/mL}$ split into two phases. Thus in Fig. 3, a 100 $\mu\text{g/mL}$ solution of felodipine exhibits the maximum

**Fig. 3** Comparison of diffusive flux of felodipine suspensions at 37°C in the absence and presence of Vit E TPGS.

flux obtainable by an LLPS system. These experiments clearly show that the flux is controlled by solute thermodynamic activity rather than solute concentration.

Impact of Solubilizing Additives on Flux

Our next steps were to evaluate the flux of felodipine and nifedipine solutions of different concentrations in the presence and absence of solubilizing excipients. Figure 4a depicts concentration as a function of time plots for the donor compartment of the diffusion cell whereas Fig. 4b shows analogous plots for the receiver cell, for felodipine solutions at 37°C in the absence and presence of different Vit E TPGS concentrations. Figure 5 compares diffusive flux values with and without solubilizing additives added at three concentrations for both model drugs.

As discussed above, the maximum possible flux is dictated by the LLPS concentration. At 50 $\mu\text{g/mL}$ in buffer, felodipine is above the LLPS concentration and thus the flux is maximized. However, the presence of additives such as Vit E TPGS may impact the flux depending on the concentration of the additive and its impact on drug supersaturation. When the Vit E TPGS concentration is above the CMC where it enhances the equilibrium crystalline solubility of felodipine, there is a large decrease in felodipine flux (Figs. 4b and 5). In contrast, the flux of a 50 $\mu\text{g/mL}$ felodipine solution with only 0.004% Vit E TPGS (less than the CMC of 0.02% with no solubility enhancement, Table III), is unchanged relative to the solution with no additive (Fig. 5, see also overlapping slopes in Fig. 4b).

The impact of other additives on diffusive flux for felodipine and nifedipine at 37°C is summarized in Fig. 5a and 5b. In these experiments, the amount of felodipine and nifedipine that was added exceeded the LLPS concentration for the drug in buffer. In general, when additives are present at concentrations where the equilibrium solubility of the drug is increased, they lead to a large reduction in diffusive flux. However, when the additives are present at concentrations where there is little or no solubility enhancement of the drug, diffusive flux remains constant and identical to the maximum value seen for the liquid-liquid phase separated felodipine or nifedipine solutions.

It is apparent that increasing additive concentration leads to solubility enhancement and a subsequent decrease in flux. To evaluate if increasing the API concentration in the presence of an additive can lead to recovery in the diffusive flux, the felodipine:Vit E TPGS system was selected for further evaluation. By increasing the felodipine concentration on the donor side from 50 to 100 $\mu\text{g/mL}$ in the presence of 0.04% Vit E TPGS, it was observed that the flux increased slightly (see Fig. 6a). With 150 $\mu\text{g/mL}$ of felodipine on the donor side, the diffusive flux increases further but still remains just below the maximum value obtained from neat felodipine at 10 $\mu\text{g/}$

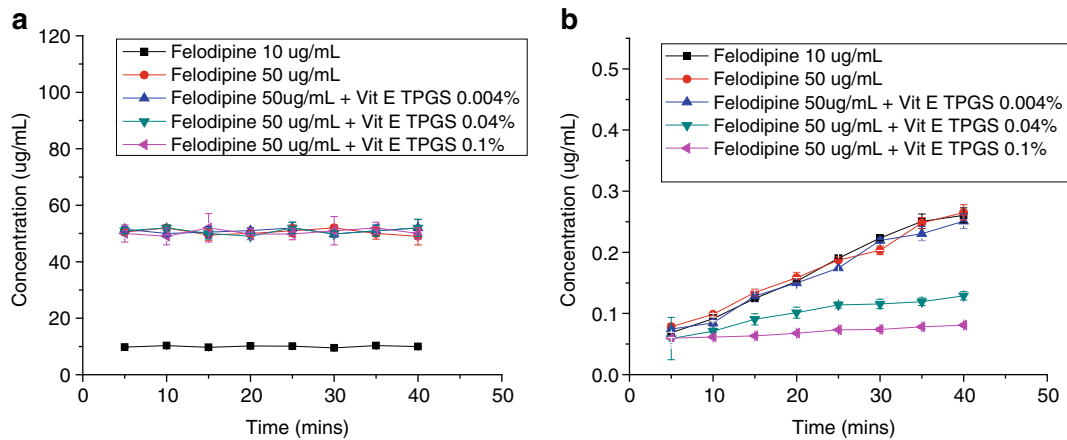


Fig. 4 Concentration versus time profiles for felodipine at 37°C in the donor cell (a) and receiver cell (b) of the diffusion cell in the absence and presence of different concentrations of solubilizing additive, Vit E TPGS.

mL and 50 $\mu\text{g/mL}$ in buffer. Increasing the felodipine concentration to 175, 200, 250, 300 and 350 $\mu\text{g/mL}$ led to an increase in flux. In fact, between 175 and 350 $\mu\text{g/mL}$, the flux appears to have reached a plateau. In summary, the same maximum flux value was observed in the presence of Vit E

TPGS as for buffer, but only at higher felodipine concentrations where LLPS had occurred.

Because solubility enhancing formulations may be tested in media (i.e., FaSSIF) that simulate gastrointestinal conditions, it is important to evaluate the impact of the media on the properties of supersaturated solutions. Figure 6b compares the diffusive flux of various concentrations of felodipine in buffer *versus* in FaSSIF. The diffusive flux of felodipine at 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ in phosphate buffer is identical because both systems are above the liquid-liquid phase separation point, which is the concentration at which maximum flux is achieved. However, a 50 $\mu\text{g/mL}$ solution of felodipine in FaSSIF has a much lower diffusive flux. Increasing the

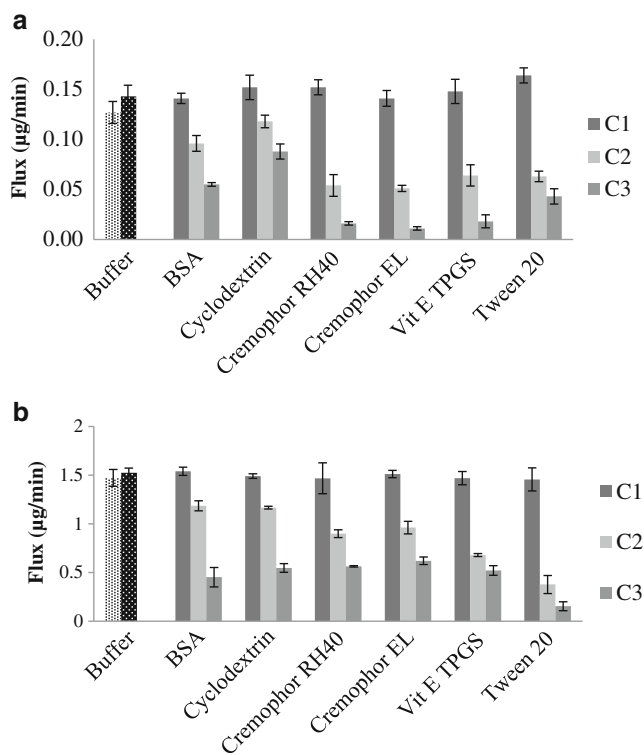


Fig. 5 Comparison of diffusive flux at 37°C in the absence and presence of solubilizing additives at various concentrations (see Table 1) of (a) felodipine and (b) nifedipine. The felodipine concentration in the donor cell was 50 $\mu\text{g/mL}$ in the presence of additives. The flux of neat felodipine is shown at two concentrations, 10 $\mu\text{g/mL}$ (▨) and 50 $\mu\text{g/mL}$ (▩). The nifedipine concentration in the donor cell was 100 $\mu\text{g/mL}$ in the presence of additives. The flux of neat nifedipine is shown at two concentrations, 72 $\mu\text{g/mL}$ (▨) and 100 $\mu\text{g/mL}$ (▩).

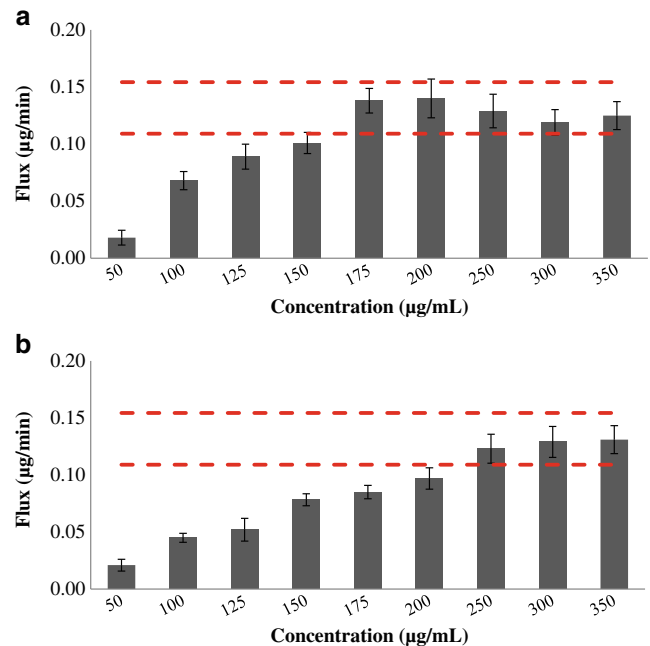


Fig. 6 Effect of (a) Vit E TPGS (0.04%) and (b) FaSSIF on diffusive flux of felodipine at 37°C. Red dotted lines indicate ± 1 standard deviation of average diffusive flux values of neat felodipine at 50 $\mu\text{g/mL}$ in buffer.

felodipine solution concentration on the donor side from 50 to 100 $\mu\text{g/mL}$ leads to some recovery in flux as compared to the buffer solution. This recovery is further enhanced with more concentrated felodipine solutions. At concentrations of 250 $\mu\text{g/mL}$ and above, the diffusive flux approaches a constant and this value is comparable to the maximum value observed for felodipine solutions in buffer that are above the LLPS concentration.

To study the effect that additives may have on amorphous solubility and the amorphous to crystalline solubility ratio (σ_a/σ_c), the amorphous to crystalline solubility ratios for felodipine were calculated in phosphate buffer, in the presence of Vit E TPGS at 0.04% in phosphate buffer and in FaSSIF, as presented in Table V. To calculate this ratio, the LLPS concentration determined using UV extinction measurements was divided by the experimentally determined crystalline solubility in the appropriate medium. Extinction experiments revealed that LLPS occurs at 8.7 $\mu\text{g/mL}$ in phosphate buffer, 177 $\mu\text{g/mL}$ with 0.04% Vit E TPGS and at 215 $\mu\text{g/mL}$ in FaSSIF. The calculation shows that even though equilibrium crystalline solubility increases almost 40 times, the amorphous-to-crystalline solubility ratio or “supersaturation” as calculated from concentration ratios decreases from approximately 9.2 in phosphate buffer (the reference point) to 4.5 in 0.04% Vit E TPGS and to 5.0 in the presence of FaSSIF (see Fig. 7). Thus, it is clearly possible to increase solution concentrations to such an extent that systems will undergo LLPS in the presence of additives. However, in the presence of additives, the amorphous-to-crystalline solubility ratio as calculated from total or apparent solution concentration may vary between different additives relative to simple buffer solutions.

DISCUSSION

Supersaturation and Thermodynamic Activity: Implications for Passive Drug Uptake

Poor passive drug uptake can have two main origins; low aqueous solubility, low membrane permeability or some combination thereof. If the equilibrium solubility is low, the amount of molecules in solution available to partition into

the membrane is correspondingly low. Hence membrane transport can be increased by formulations that generate supersaturated solutions, since the number of molecules in solution is higher than for a saturated solution. However, not all formulation approaches that increase the number of molecules in solution are effective since speciation (i.e., the amount of drug available for membrane partitioning) needs to be considered (43). For example, incorporation of the solute into surfactant micelle increases the equilibrium solubility (solubilization rather than supersaturation) and hence the achievable solution concentration, but does not increase the amount of “free drug” available to partition into the membrane. The amount of free drug corresponds to the solute thermodynamic activity, and this parameter, rather than total solution concentration, drives flux across the membrane (Eq. 3). In a supersaturated solution, the thermodynamic activity of solute molecules increases with the extent of supersaturation, which leads to a corresponding increase in membrane flux, as long as crystallization does not occur. As recently demonstrated, the increase in membrane transport with supersaturated solutions is not infinite; once the supersaturation exceeds a threshold value the solution spontaneously phase separates into two phases, a process known as liquid-liquid phase separation, and the flux reaches a maximum (27).

In oral delivery of supersaturating dosage forms, there is the potential for both solubilization and supersaturation. The solubilization results from the presence of endogenous solubilizing substances and digestion products present in the gastrointestinal (GI) tract as well as solubilizing additives added to the formulation, while the supersaturation is generated by the formulation strategy and/or intrinsic properties of the molecules (e.g., a weak base moving from the stomach to the small intestine). Given the dependence of passive transport on the solute thermodynamic activity rather than total concentration, it is thus important to deconvolute increases in solution concentration arising from solubilization *versus* those arising from supersaturation. This requires an estimation of the thermodynamic activity of the solute molecules in the presence of the additives, since it is this parameter (rather than the total solution concentration) that dictates the membrane transport rate (and hence passive absorption), the extent of supersaturation in the system (the driving force for crystallization), as well as the liquid-liquid phase separation behavior. This can be achieved by using the flux measurements to estimate the solute

Table V Comparison of Solubility Ratio of Amorphous to Crystalline in the Absence and Presence of Surfactants

	Phosphate buffer Concentration ($\mu\text{g/mL}$)	Vit E TPGS	FaSSIF
Equilibrium crystalline solubility	1.1 \pm 0.0	39.3 \pm 8.5	42.8 \pm 7.4
LLPS Onset (Amorphous Solubility)	10.4 \pm 2.7	177.3 \pm 7.5	214.6 \pm 7.5
σ_a/σ_c	9.2	4.5	5.0

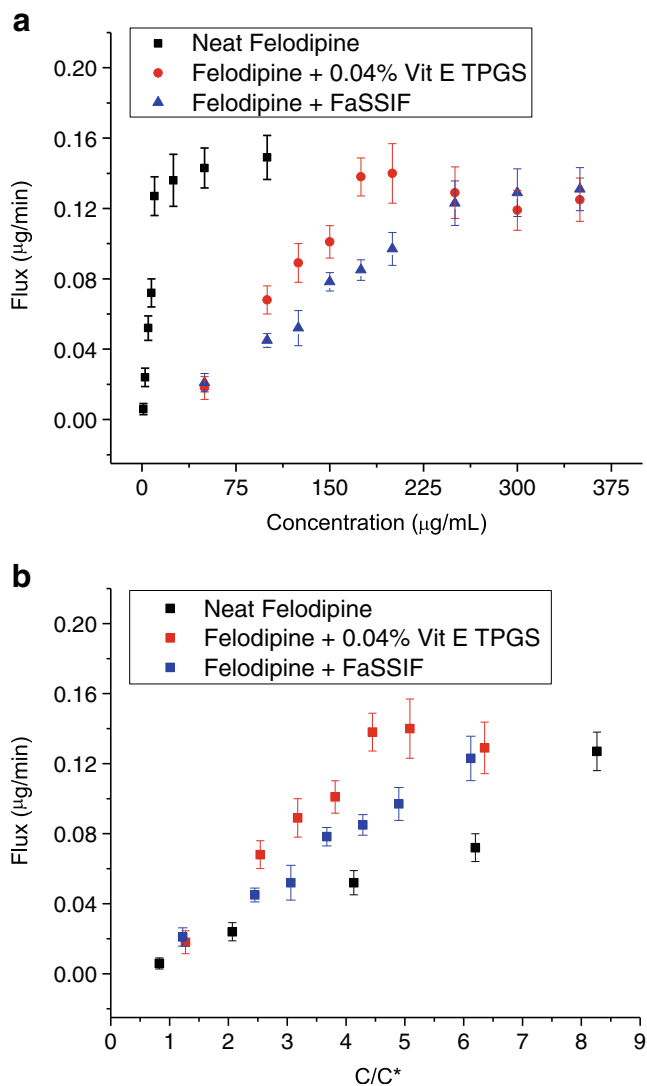


Fig. 7 Diffusive flux of felodipine in the presence of phosphate buffer, Vit E TPGS and FaSSiF at 37°C as a function of A) concentration and B) c/c^* (see Eq. 6).

thermodynamic activity and supersaturation rather than the total concentration in a given system.

Understanding Supersaturation in the Presence of Additives: Thermodynamic Activity versus Apparent Concentration

The supersaturation ratio (S) can be fundamentally expressed as (44):

$$\ln S = \frac{\mu - \mu^*}{RT} = \ln \frac{a}{a^*} = \ln \frac{\gamma c}{\gamma^* c^*} \quad (5)$$

where μ is the solute chemical potential, c is concentration, a is solute activity, γ is the solute activity

coefficient and $*$ is the property at saturation (i.e., for a solute in a solution in equilibrium with the crystal). For dilute solutions, it may be reasonable to assume that $\gamma/\gamma^* \sim 1$, and thus the supersaturation ratio, S can be expressed as the relative concentrations:

$$S = \frac{c}{c^*} \quad (6)$$

Because the flux is directly proportional to the solute activity, as shown in Eq. 3, it follows that supersaturation is also directly proportional to the flux. Thus a plot of flux versus c/c^* should be linear if $\gamma/\gamma^* \sim 1$. This has been found to be the case for supersaturated solutions of both felodipine and nifedipine in buffered aqueous solutions, up to the concentration where LLPS occurs, at which point the supersaturation reaches a maximum value (27). These and other studies (9) show that flux measurements can be used to evaluate the degree of supersaturation in a given system.

In the pharmaceutical literature, it is a common practice to define S based on a concentration ratio (Eq. 6), where the solubility of the crystalline form is measured in medium of interest, even in complex solutions which contain micelles (e.g., FaSSiF). This approach is used to account for the increase in equilibrium solubility that results from the solubilizing additive, which is exemplified by the data in Tables III and IV which show that the equilibrium solubility of both felodipine and nifedipine can be considerably increased in the presence of the additives. Thus, if a solubilizing excipient is added to a solution that would be supersaturated in the absence of that additive, the supersaturation would be reduced by a factor that depends on the magnitude of the solubilizing effect. In other words, the supersaturation is “consumed” to a certain extent by the solubilization of the drug resulting in a decrease in membrane transport. This effect is clearly shown for the systems tested herein, where the flux at a constant solute concentration is decreased in the presence of additives (Fig. 4). The change in the relationship between flux and solute concentration in the presence of additives can be seen in Fig. 7a, where it is apparent that in order to achieve the same flux when a solubilizing additive is present, the concentration of the solute needs to be increased. Thus it is readily apparent that a supersaturated solution can be generated in the presence of a solubilizing additive, but that the total concentration needs to be increased in order to achieve the same degree of supersaturation as in the absence of the solubilizing additive. This clearly needs to be taken into account when designing formulations so that desirable supersaturation (and hence flux) can be achieved.

The flux data can be used to accurately predict the degree of supersaturation in a given system without relying on the assumption that $\gamma/\gamma^* \sim 1$. As a result, the flux data can also be used to determine if Eq. 6 provides an accurate estimate of the supersaturation in a system containing solubilizing

additives. For Eq. 6 to provide an accurate estimate of supersaturation, the relative speciation (relative amount of free *versus* bound drug) in the supersaturated solution must be the same as in the saturated solution. For example, in the case of micellar solubilization and for a given surfactant concentration, if 50% of the drug molecules are solubilized in micelles in a saturated solution, then 50% of the drug molecules should be also solubilized in micelles in the supersaturated solution. This can be evaluated by plotting the flux data *versus* c/c^* , as calculated using Eq. 6, and the results are shown in Fig. 7b. For solutions containing surfactants, the flux is variable for a given value of c/c^* . If Eq. 6 was valid for these systems, the flux should have one value for a given c/c^* ratio regardless of the composition of the system where the data points for the different systems all lie on the same line. This observation points to a breakdown in the validity of the assumption inherent to Eq. 6, i.e., that $\gamma/\gamma^* \sim 1$ and indicates that $c/c^* \neq S$. This is most likely due to the fact that the micelles evaluated in this study have a variable capacity for incorporation of solute molecules that depends on the number of solute molecules already solubilized. Therefore, if the number of API molecules is increased while keeping the fraction of micelles constant (as in this study), the micelles will eventually lose the ability to solubilize the same fraction of molecules resulting in an increase in the relative number of free drug molecules present in the bulk solution. In other words, a fixed volume of micelles will solubilize a greater fraction of the API molecules at a lower drug concentration relative to that at a higher drug concentration. If a two phase model of surfactant solubilization is considered valid, this would correspond to a decrease in the surfactant: water partition coefficient as a function of increasing API concentration. These results demonstrate, for the first time, that it is difficult to accurately determine S using the concentration ratios as described in Eq. 6. However, flux measurements provide a much better indicator of the supersaturation in the presence of solubilizing components, since they directly reflect the thermodynamic activity of the solute molecules.

A similar argument can be made for complexing agents. Consider the impact of BSA, which is well known to bind with drugs and has been reported previously to impact solute thermodynamic activity and flux (45). As a result of this binding (46), the equilibrium solubility of felodipine is increased by a factor of around 35 when 0.1% BSA is added to the solution; this factor increases to about 42 when the BSA concentration is increased to 1%. If Eq. 6 is used to estimate S for a 50 $\mu\text{g}/\text{mL}$ solution of felodipine containing BSA, the resultant values are around 1.3 and 1.1 for BSA concentrations of 0.1 and 1% respectively. These supersaturation ratios are close to 1 therefore the flux would be expected to be similar to that seen for a saturated solution of felodipine. Figure 5 shows that while the flux values for felodipine are indeed reduced in the presence of BSA, they are still considerably higher than the flux obtained from a saturated solution (see Fig. 3). In fact, the flux levels for

the two BSA systems correspond to an S of 7.3 and 4.2 based on the reference flux data obtained for felodipine in buffer (1 $\mu\text{g}/\text{mL}$). Thus, the true supersaturation (determined from the flux data) is again much higher than that predicted from Eq. 6. Again, it is apparent that the assumption that $\gamma/\gamma^* \sim 1$ is not correct when a complexing agent such as BSA is present in solution. This can most likely be explained by considering the relationship between the fraction of bound protein (f) and the API concentration:

$$f = \frac{[API]}{K_d + [API]} \quad (7)$$

where K_d is the dissociation constant for the complex and $[API]$ is the molar concentration of the API. Equation 7 is often used to describe the fraction of bound protein as a function of API concentration for situations where drug concentration is in excess of the protein concentration (molar concentrations, valid for the 0.1% BSA system). Reference to Eq. 7 shows that as the drug concentration increases, the fraction of protein bound drug increases. At some point, as the drug concentration increases further, the fraction of bound protein will approach 1, and any additional drug added will be free in solution rather than bound to the protein since there are a finite number of binding sites. Thus it is clearly a gross oversimplification to estimate the supersaturation ratio (which is directly related to the amount of “free” drug in solution) by using Eq. 6 when complexing agents such as BSA and cyclodextrin are present. The flux data in this study suggests that the use of Eq. 6 will underestimate the true supersaturation for systems containing BSA and cyclodextrin.

Solubilization, Supersaturation, and Maximum Diffusive Flux

For solutions of a drug in buffer, it has been demonstrated that the maximum flux that can be achieved in a supersaturated solution is dictated by the concentration at which liquid-liquid phase separation (LLPS) occurs (21) whereby the solution concentration following LLPS is essentially the same as the amorphous solubility, predicted using Eq. 2 (20,21,27). However, the influence of solubilizing additives on both the maximum achievable flux and the tendency of the system to undergo LLPS has not been widely explored.

The impact of select additives on the solution thermodynamics, is summarized in Fig. 7a which shows a comparison of the flux as a function of added drug concentration for felodipine in buffer and in the presence of 0.04% VitE TPGS and FaSSIF. The most obvious difference between the systems is that in order to achieve the same flux in the presence of a surfactant, a higher felodipine concentration is required. This reflects the solubilization of the compound by the surfactant micelles and the corresponding reduction in supersaturation.

However, the overall pattern of behavior for the surfactant solutions is similar to that observed for the drug in buffer, in that, as the felodipine concentration increases, the flux reaches a maximum value which is similar in the surfactant solutions to that observed in buffer. In other words, by substantially increasing the felodipine concentration in solutions containing surfactants, the supersaturation is restored to a maximum value, and the maximum possible enhancement in membrane mass transport is achieved. The increase in concentration where the plateau is observed in the presence of the surfactants, indicates that the LLPS concentration is much higher when solubilizing additives are present. Independent measurements show that this is indeed the case with phase separation occurring at 177 and 215 $\mu\text{g}/\text{mL}$ for 0.04% VitE TPGS and FaSSiF respectively as compared to around 9 $\mu\text{g}/\text{mL}$ in buffer (Table V), in good agreement with the concentrations where the flux approaches a plateau values.

Although the maximum flux values obtained in the VitE TPGS system are similar (within experimental error) to those in buffer, the amorphous:crystalline solubility ratio is 4.5, which is approximately half the ratio observed in buffer; a similar difference was seen for FaSSiF solutions. Clearly, the concentration-based ratio in the presence of solubilizing additives under predicts the true (i.e., activity-based) supersaturation whereas the flux measurements provide a much better indication of the solute thermodynamics. While the “amorphous solubility advantage” is predicted to be much lower in surfactant containing media, the most relevant metric for gauging performance, i.e., the flux, is equivalent at the amorphous solubility. To summarize, felodipine undergoes LLPS at higher concentrations in the presence of additives that enhance its thermodynamic solubility. Upon liquid-liquid phase separation in the presence of solubilizing additives, which occurs at a higher concentration than in buffer, the flux values are similar to those in the absence of solubilizing additives (Fig. 5). These observations highlight the great complexity of supersaturating solutions and may explain the

difficulty of making *in vitro in vivo* correlations if the impact of solubilizing components present in dissolution media, employed to mimic *in vivo* conditions, is not considered.

The results obtained in this study clearly demonstrate that the presence of solubilizing additives changes both solution thermodynamics and phase boundaries. Figure 8 provides a schematic overview of changes in these properties for a representative poorly water soluble, small molecule, X, in the absence and presence of solubilizing additives. In Fig. 8a, the equilibrium crystalline solubility of drug X is 1 $\mu\text{g}/\text{mL}$ and the solution undergoes LLPS when the solute activity exceeds 10, which is the amorphous solubility (S_A). In the region between the crystalline and amorphous solubility, the solution is a one phase supersaturated solution. At concentrations above the amorphous solubility, either liquid-liquid phase separation or crystallization can occur. For systems that crystallize slowly, LLPS is typically observed, as for the systems in this investigation where crystallization is impeded by the presence of a polymer. At the crystalline solubility, the unit activity is 1. Figure 8b shows how these phase boundaries, solute concentration and thermodynamic activity change when solubilizing additives are present. In this case, the additive has increased equilibrium crystalline solubility 50 fold, as can be seen from the concentration axis. However, the thermodynamic activity of the saturated solution remains at 1, despite the concentration increase, since the crystalline solid dictates the activity. Because of solubilization by the additive, LLPS occurs at a much higher concentration however, the solute activity observed is identical to that in the absence of the excipient (this holds if the additives do not mix with the drug-rich phase). The ratio of the amorphous-to-crystalline solubility is not necessarily the same as for buffer, even though the activity difference remains the same. A third scenario as depicted in Fig. 8c is possible. For a given sufficiently high additive concentration or an additive of certain chemistry, partitioning of the additive into the drug-rich phase may reduce the activity of the drug and LLPS could occur at a lower supersaturation; we did not see evidence of this in the current study.

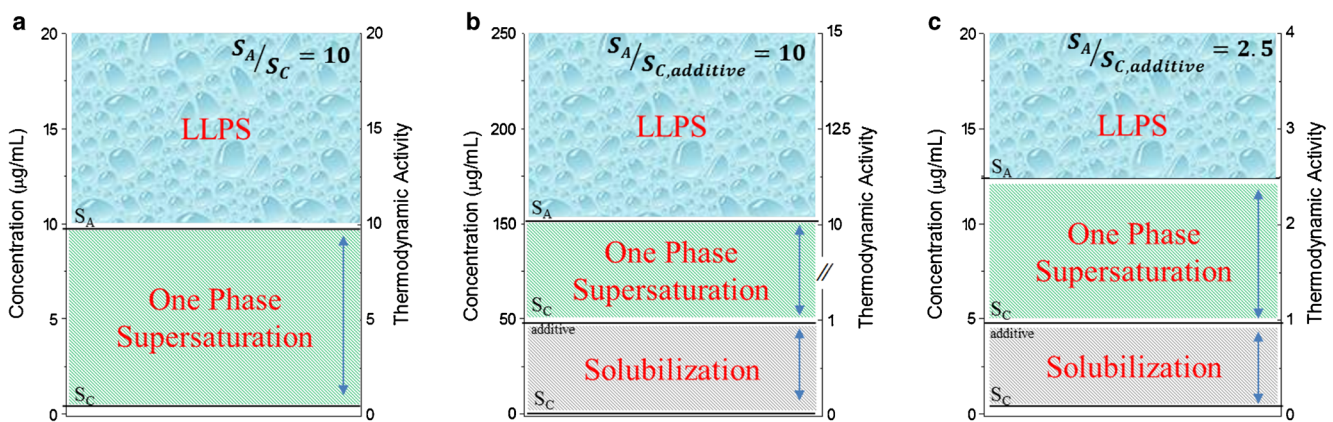
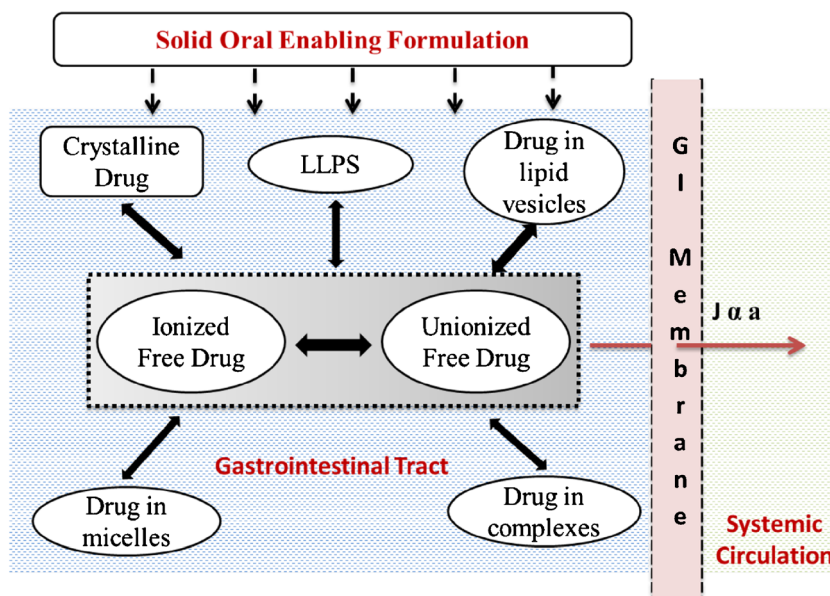


Fig. 8 Schematic illustration of additive effects on solubility, supersaturation and solute thermodynamic activity assuming a drug with crystalline solubility of 1 $\mu\text{g}/\text{mL}$ and amorphous solubility of 10 $\mu\text{g}/\text{mL}$ in pure buffer.

Fig. 9 Complex gastrointestinal equilibria and their implications on passive drug uptake.



In vivo, several different complex equilibria exist as summarized in Fig. 9. For ionizable drugs, there exists an equilibrium between ionized and unionized species that is dependent on the compound pKa and the local pH. Only unionized drug is thought to undergo passive absorption (47). In the presence of additives, the situation becomes much more complicated, whereby the drug can be incorporated into micelles in the case of surfactants, or form complexes with cyclodextrins. For supersaturated solutions, the situation is further complicated by the potential formation of new phases, either crystals or an amorphous precipitate. The total solution concentration present represents the sum of all of these various solubilized species, however, only the unionized, molecularly dissolved, free drug concentration is important for passive membrane transport kinetics (ignoring the often important kinetic effects of the dissolution process). The flux measurements described in this study thus represent an extremely useful approach to evaluate the free drug concentration in complex formulations enabling solubilization effects to be deconvoluted from supersaturation.

CONCLUSION

As formulation complexity increases to address the oral delivery of low aqueous solubility drug compounds, there is a concurrent need to develop better evaluation tools and thermodynamic understanding of the properties of these systems. In this study, we have demonstrated that flux measurements provide an excellent approach to deconvolute solubilization and supersaturation in supersaturated solutions containing solubilizing additives. For the first time, it has been shown that evaluating supersaturation using concentration based ratios may lead to erroneous conclusions, with this approach under predicting the actual

supersaturation for the systems evaluated in this study. Furthermore, it was found that the maximum membrane mass transport rate can still be achieved in the presence of solubilizing additives, by ensuring that the total concentration exceeds the liquid-liquid phase separation concentration. By understanding how phase boundaries move in the presence of solubilizing additives, together with analytical measurements that provide information about the solute thermodynamic activity rather than the total bulk concentration, greater insight into solubility enhancing formulations can be achieved.

ACKNOWLEDGMENTS AND DISCLOSURE

We would like to acknowledge AbbVie Inc. for providing research support for this project as well as 'The Lilly Endowment for the Optimization of Drug Manufacturing' grant for graduate student financial support.

Purdue University and AbbVie jointly participated in study design, research, data collection, analysis and interpretation of data, writing, reviewing, and approving the publication. Lynne S. Taylor is a professor at Purdue University, David A. Alonzo is an employee at Gilead Sciences, Inc., Donghua Zhu is an employee at Janssen China and Jianwei Wu is an employee of US FDA. They all have no additional conflicts of interest to report. Shweta A. Raina, Geoff G. Z. Zhang, Yi Gao, and Nathaniel D. Catron are employees of AbbVie and may own AbbVie stock.

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