Review Article

Theme: Develop Enabling Technologies for Delivering Poorly Water Soluble Drugs: Current Status and Future Perspectives
Guest Editors: Ping Gao and Lawrence Yu

Characterization of Supersaturatable Formulations for Improved Absorption of Poorly Soluble Drugs

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Abstract. With the increasing number of poorly water-soluble compounds in contemporary drug discovery pipelines, the concept of supersaturation as an effective formulation approach for enhancing bioavailability is gaining momentum. This is intended to design the formulation to yield significantly high intraluminal concentrations of the drug than the thermodynamic equilibrium solubility through achieving supersaturation and thus to enhance the intestinal absorption. The major challenges faced by scientists developing supersaturatable formulations include controlling the rate and degree of supersaturation with the application of polymeric precipitation inhibitor and maintenance of post-administration supersaturation. This review is intended to cover publications on this topic since April 2009. Scientific publications associated with characterization of supersaturatable systems and related preclinical and clinical pharmacokinetics (PK) studies are reviewed. Specifically, this review will address issues related to assessing the performance of supersaturatable systems including: (1) Diversified approaches for developing supersaturatable formulations, (2) meaningful *in vitro* test methods to evaluate supersaturatable formulations, and (3) *in vivo* PK study cases which have demonstrated direct relevance between the supersaturation state and the exposure observed in animal models and human subjects.

KEY WORDS: biorelevant *in vitro* testing; poorly water-soluble drugs; supersaturation.

INTRODUCTION

With the increasing number of poorly water-soluble compounds in contemporary drug discovery pipelines, the concept of supersaturation as an effective formulation approach for enhancing bioavailability is gaining momentum. This is intended to design the formulation to yield significantly high intraluminal concentrations of the drug than the thermodynamic equilibrium solubility through achieving supersaturation and thus to enhance the intestinal absorption that will ultimately increase the ability to provide clinically reproducible, safe, and efficacious response upon administration (1–3).

For this enhanced intestinal absorption to take place, supersaturation must be obtained and maintained in the gastrointestinal environment. *In vivo* induction of supersaturation can be achieved through various formulation approaches. Different approaches to induce supersaturation have recently been reviewed by Brewster *et al.* (3). The metastable state of supersaturation has to be sustained for a time period sufficiently

long in order to improve intestinal absorption. Maintenance of the supersaturated state has been the subject of research of various academic and industrial laboratories. It has been demonstrated that application of functional excipients (polymers, surfactants, *etc.*) can effectively minimize and/or delay drug precipitation in a highly supersaturated state and this stabilizes supersaturation as evidenced by appropriate *in vitro* tests (1–3). The awareness that supersaturation in the intraluminal environment could enhance intestinal absorption has urged implementation of high throughput precipitation screening methods to evaluate the supersaturation potential during lead selection/optimization and to guide excipient selection during formulation development. As it can be expected that the gastrointestinal environment induces drug precipitation *in vivo*, *in vitro* evaluation of supersaturation requires careful consideration of biorelevant test methods mimicking physiological environments.

Many active pharmaceutical ingredients are classified as weak acids or weak bases (and their corresponding salts) although a majority of ionizable drugs are classified as weak bases. For those compounds having a pK_a above the physiological pH range, solubility behavior within the gastrointestinal tract will be similar to that of neutral compounds. In such cases, formulations may be designed and tested in dissolution media of neutral pH. For weakly acidic compounds with pK_a in physiological pH range, precipitation is anticipated in the stomach typically in the fasted state (with a low pH). However, a greater

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solubility of the acid drug will be achieved in the gastrointestinal tract to facilitate absorption. Weak bases possessing low pK_a values (≤ 7) usually exhibit a substantial decrease in equilibrium solubility during intestinal transit experiencing environment of pH 5.5–7.5. For instance, a formulation containing a free base drug of such low pK_a (< 7) may provide sufficiently high dissolution rate in the acidic environment of the stomach. But this could lead to a high degree of supersaturation with a higher crystallization tendency during transit into the small intestine. Typically when a high dose is involved, as the free base reaches the primary site of absorption in the small intestine, the rate of nucleation and growth will be amplified, resulting in rapid precipitation of the drug.

Incorporating concentration-enhancing polymers to promote the degree and duration of supersaturation can be an effective method in formulation design of poorly soluble drugs (1–3). The presence of these polymers reduces precipitation rates by physical, chemical, or a combination thereof of interactions that can affect component solubility, solid–liquid boundary layer viscosity, molecular mobility, and interfacial solvation to thereby alter the nucleation and growth rates.

While supersaturatable formulation approaches including solid dispersions are being widely explored in pharmaceutical industry and have been demonstrated to improve oral absorption of poorly soluble drugs (4), there is still lack of fundamental understanding of such formulations regarding to how to achieve and sustain a supersaturated state in the light of drug–polymer interaction. Application of precipitation inhibition involving selection of a polymeric precipitation inhibitor (PPI) is still based on empirical approaches, and structure–activity relationships have not been established. It is understood that the kinetic solubility, the degree of supersaturation, and the rate at which supersaturation is generated affect the rate and mechanisms by which precipitation occurs. Development of supersaturatable formulations still primarily relies on tedious trial-by-error approach and *in vivo* screening in animal models. Rational design of supersaturatable formulations is of great interests and presents a challenge to pharmaceutical scientists.

Characterization of *in vitro* dissolution performance and, in particular, characterization of the supersaturated state with polymer-modulated interactions is desired to understand supersaturatable systems. It has been recognized that the metastable supersaturated state makes it difficult to reliably determine dissolution kinetics, degree of supersaturation, and precipitation kinetics (1–3). The major challenges faced by scientists developing supersaturatable formulations include controlling the rate and degree of supersaturation with the application of PPI and maintenance of post-administration supersaturation. Design and development of such systems desire rational selection of the most effective PPI and the polymer molecular weight grade, the optimal drug load, the ratio between drug and PPI, and analytical methodologies that warrant biorelevant dissolution method with *in vivo* relevance. Crystallization events in the gastrointestinal (GI) have been rarely explored as evidenced by the near absence of the subject from scientific literature. While studying crystallization *in vivo* is not practical for most development programs, the possibility has been explored by using biorelevant *in vitro* assays coupled with PK modeling.

This review is intended to cover publications after the excellent review by Brewster *et al.* (3) on this topic in April

2009. Scientific publications associated with characterization of supersaturatable systems and related preclinical and clinical PK studies are reviewed. Specifically, this review will address issues related to assessing the performance of supersaturatable systems including:

1. Diversified approaches for developing supersaturatable formulations
2. Meaningful *in vitro* test methods to evaluate supersaturatable formulations, and
3. *In vivo* PK study cases which have demonstrated direct relevance between the supersaturation state and the exposure observed in animal models and human subjects.

CHARACTERIZATION OF SUPERSATURATED STATE AND PRECIPITATION KINETICS *IN VITRO*

Biorelevant *In Vitro* Dissolution Tests

The most commonly used *in vitro* tests are United States Pharmacopeia (USP) compendial dissolution tests. The goal of such tests is to ensure complete release and dissolution of formulations under *sink* conditions. Such tests can be of enormous value for many drug products when the important parameters for evaluation are disintegration of the formulation and/or the rate of dissolution of different forms or particle sizes (5). However, the compendial dissolution tests do not adequately address some important changes (*e.g.*, pH change) and dynamic aspects that an oral formulation undergoes in GI luminal environment. A more physiologically relevant *in vitro* dissolution measurement is especially important for poorly water-soluble drugs formulated into supersaturatable systems where precipitation/crystallization of drug in GI tract is of a concern. Supersaturation and solution-mediated phase transformation of the drug may result from pH-induced precipitation (*e.g.*, free-base from salt/ionized form), precipitation from solution formulations (*e.g.*, cosolvents, lipids), or amorphous/metastable to stable polymorph/hydrate phase, transformations (*e.g.*, crystallization of an amorphous solid dispersion). Therefore, evaluating supersaturatable formulations with the use of biorelevant *in vitro* dissolution tests which can better represent *in vivo* conditions is more meaningful and highly desirable.

Various noncompendial dissolution methods have been developed to assess supersaturation under physiologically relevant conditions. Augustijns *et al.* (6) explored the use of human intestinal fluids as *in vitro* test medium for supersaturation. The authors revealed that the extent to which precipitation inhibition could be obtained appeared to be compound and excipient dependent. Experiments using intestinal fluids from volunteers in the fasted or fed state evidenced that the nutritional state did not significantly affect the extent of excipient-mediated precipitation inhibition. The usefulness of simple simulated intestinal fluids representative for the fasted or fed state as dissolution media to predict excipient-mediated precipitation inhibition in human intestinal fluids appeared to be limited. However, the aqueous buffer or simulated intestinal fluids could readily determine the absence of supersaturation stabilization by a given excipient.

Artificial stomach and duodena model (ASD) was developed to mimic the process of gastric emptying and potential drug precipitation in the intestinal compartment in biorelevant manner (7). In this method, a formulation is dispersed in a stomach chamber (20–70 mL) and transferred at a controlled rate to the duodenum chamber and mixed with simulated intestinal fluid (SIF). During this process, fresh gastric fluid and SIF were continuously infused into each chamber. By measuring drug concentration–time profile in the duodena compartment, the dynamic process of drug dissolution, precipitation, and recrystallization can be examined. The *in vivo* relevance of ASD dissolution profiles is based on the assumption that the concentration of dissolved drug in the simulated duodenum is proportional to its bioavailability. The use of ASD system for the evaluation of supersaturation-based formulations has been reported (8). Miller et al (9) recently reported a dual pH–dilution test (or a simplified ASD method based on the same concept) for evaluating amorphous solid dispersions of drug candidate with poor aqueous solubility. The test involves a serious dilution of the formulations using biorelevant dissolution media (pH 4 HCl and FaSSIF), physiologically based dilution factors and transit time simulating the rat GI transit (from stomach to duodenum, jejunum, ileum, cecum, and colon). *In vitro* drug concentration–time profiles obtained from the pH–dilution method were further used as inputs for PBPK modeling using GastroPlus® to predict *in vivo* oral plasma concentration profiles.

In vitro tests that mimic both drug dissolution and adsorption processes *in vivo* such as a biphasic test (10,11) and a cell-based membrane dissolution test method have also been reported. The biphasic test system involves a nonsink aqueous phase and an organic phase acting as a sink. Drug products are introduced in the aqueous phase *via* USP IV flow cells which are coupled with USP II vessels. The simultaneous drug dissolution in the aqueous phase and permeation into the organic phase mimic drug dissolution and adsorption processes *in vivo*. The hypothesis for this biphasic system is that free drug concentration in the aqueous phase is the driving force for drug partitioning into the organic phase and the drug concentration accumulated in the organic phase is related to the extent of absorption. The biphasic system has been used in evaluating supersaturation-based lipid (10) as well as amorphous solid dispersion formulations (11) and demonstrated differentiation and discrimination power over key formulation attributes and relevance to *in vivo* exposure. To further mimic drug permeation through GI tract, Yamashita et al. (12) has applied a Caco-2 cell-based dissolution system. The system contains a dissolution chamber and receiving chamber separated by a monolayer of Caco-2 cell, allowing the evaluation of permeation under conditions very close to that of human GI membrane. Due to the constraints of the Caco-2 cell setting, only a small portion of a drug dose can be evaluated.

SEDDS/Lipid Formulations

Zhang et al. (13) investigated the effect of polymers on drug precipitation from self-emulsifying drug delivery systems (SEDDS) formulations of carbamazepine (CBZ) when in contact with water. Their results show that 2% PVP K90 effectively prevented drug precipitation from a SEDDS

formulation for over 24 h (1 g of S-SEDDS in 50 mL 0.1 N HCl at 50 rpm mixing). Addition of 2% PVP K30 or PVP K60 in these formulations also prolonged drug precipitation to 4 h, while hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), and carboxymethyl cellulose (CMC)–Na showed no benefit on preventing drug from precipitation.

Shi et al. (10) utilized a biphasic *in vitro* release test method to evaluate three distinctly different celecoxib (CEB) formulations including SEDDS formulation, a solution formulation, and a marketed capsule formulation. Release profiles of CEB observed in the aqueous phase of the biphasic test from the solution and S-SEDDS formulations were comparable. However, their corresponding drug–concentration profiles in the octanol phase differed significantly and this was presumably attributed to different free drug concentrations in the aqueous phase of these two formulations. Due to the high level of surfactant in the solution formulation, CEB was mostly associated with surfactant micelles in the dissolution medium, resulting in a less amount of free CEB in the aqueous phase. In contrast, a significant lower level of surfactant was utilized in the S-SEDDS formulation which led to the generation of highly supersaturated state of CEB. It is worth noting that CEB release profiles in the aqueous phase exhibited little relevance to the pharmacokinetic observations (e.g., $C_{\max}$ and area under the curve (AUC)). However, a rank-order correlation was obtained between *in vitro* drug release profiles in the octanol phase and *in vivo* PK exposures and $C_{\max}$ values among the three CEB formulations. As the biphasic test permits a quick partition of drug into the organic phase, the amount of drug in the organic phase represents the amount of drug accumulated in systemic circulation *in vivo*.

Amorphous Solids

William et al. (14) proposed a mechanism regarding how supersaturation levels are maintained upon dissolution of amorphous particles as illustrated in Fig. 1. Amorphous particles may dissolve to form metastable highly supersaturated solutions. Drug may precipitate from these metastable solutions to lower the free energy depending upon the rate of nucleation to form particle embryos followed by growth *via* either condensation (Fig. 1a) or coagulation (Fig. 1b and c). The growing particles may crystallize as less soluble crystalline form than the amorphous form. Condensation of dissolved molecules onto these crystals will deplete supersaturation. The rate of growth by condensation is directly proportional to the excess surface area of undissolved particles. Growth by coagulation is dependent on the number of particles for a given stability ratio. The authors evaluated supersaturation of itraconazole (ITZ) particles of low to high surface areas when in contact with aqueous medium. To mimic the pH transition from stomach to intestine, the ITZ/hydroxypropylmethyl cellulose particles were exposed to pH 1.2 medium then shifted to pH 6.8 medium (Fig. 2). A slow decrease in supersaturation was observed with the medium surface area particles. While the high surface area particles showed fast dissolution and high supersaturation followed by a rapid precipitation with supersaturation reduced from 12 to 4 in only 20 min. The study showed that medium surface particles offered an optimum balance between favorable rapid dissolution and unfavorable nucleation

Fig. 1. Mechanisms for depletion of supersaturation in solution where m is mass of drug in solution; A total particle surface area, C drug solution concentration, C_{sat} drug solubility, N_{part} number of drug particles per volume, k_r rate constant, N_{emb} number of embryos per volume, A_{sp} specific surface area (area/mass), and d_{part} diameter of the particle. Reprinted with permission from Michal E. Matteucci (14)

and growth out of solution. This work revealed that the dissolution rate of the drug from related formulations is a critically important factor in dictating the generation and duration of the supersaturated state. A rapid dissolution that generates a high degree of supersaturation may not be optimal to sustain the supersaturation state since it may induce rapid crystallization.

Herbig *et al.* (15) reported their study on 41 polymeric and small molecules with respect to their ability to inhibit the precipitation of nine model compounds. Drug release from

solid dispersions was evaluated using a syringe/filter test and a microcentrifuge test. Hydroxypropylmethylcellulose acetate succinate (HPMCAS) was found most effective in maintaining supersaturation of those model compounds. The degree of achievable drug supersaturation increased with increasing HPMCAS content in the solid dispersions. Other polymers including HPMCP-50, HPMC K100, PVP, and PVA also showed good performance in maintaining drug supersaturation. While hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), and poly(ethyleneimine) (PEI) were found to perform poorly, no inhibition of precipitation was observed with poly(acrylic acid), CMC, pluronics, and sodium alginate.

Using a simple *in vitro* pH dilution test method, Miller *et al.* (9) evaluated the dissolution profiles of amorphous solid dispersions of a weak base (pK_a 5.3), hydrophilic model compound (MW ~450 Da, log D 4.6) with poor intrinsic aqueous solubility (0.11 $\mu\text{g/mL}$). The pH dilution test was also capable of discriminating the solubility/dissolution performance of different amorphous solid dispersions. Their results showed that the solid dispersion formulations were capable of yielding supersaturation with duration of several hours in FaSSIF. For example, at about 7 h, amorphous solid dispersion suspension A generated a supersaturation ratio of 247, while the amorphous solid dispersion suspension B only produced a supersaturation ratio of 60. Microscopic examination revealed phase separation between excipients of polymer and surfactant in the solid dispersion suspension B, and this may cause a greater tendency of drug crystallization and precipitation.

Mechanism on Polymeric Precipitation Inhibitor

Augustijns *et al.* (16) investigated excipient-mediated precipitation inhibition upon induction of supersaturation of five poorly water-soluble drugs (etravirine, ritonavir, loviride, danazol, and fenofibrate) in aspirated human intestinal fluids

Fig. 2. Supersaturation of ITZ formulations of Sporanox capsule and 4:1 ITZ/HPMC in media with pH shift from pH 1.2 (2 h) to pH 6.8 at a dose of 350 $\mu\text{g/mL}$ based on pH 1.2 volume. Reprinted with permission from Michal E. Matteucci (14)

at both the fasted and fed state (FaHIF and FeHIF) and compared with those in simple aqueous buffer, FaSSiF, and FeSSiF. To study polymer effect on precipitation inhibition, supersaturation was induced in test media in the presence of 0.05% (w/v) predissolved polymers (HPMCAS, HPMC-E5, HPMC-E50, HPMC-E4M, HPMC-P, and PVP) at degree of supersaturation (DS) of 20 using a solvent shift method. As shown in Fig. 3, polymer effects on supersaturation stabilization appeared to be compound dependent. Etravirine,

loviride, and danazol were sensitive to excipient-mediated stabilization of supersaturation. In contrast, these excipients showed insignificant or essentially no effect on ritonavir and fenofibrate. Cellulosic polymers such as HPMCAS and HPMCAS showed significant precipitation inhibition, whereas PVP K25 appeared to have no stabilizing effect. In general, the authors reported that excipient-mediated precipitation inhibition was less pronounced in HIF compared to simple aqueous buffer or FaSSiF/FeSSiF.

Fig. 3. Excipient gain factors of HPMCAS (open bars), HPMC-E5 (gray bars), and PVP (black bars) for etravirine, ritonavir, loviride, danazol, and fenofibrate, in difference media. The excipient gain factor was calculated as the ratio of the $AUC_{120 \text{ min}}$ of the DS-time profile in the presence of excipient to the $AUC_{120 \text{ min}}$ of the DS-time profile in the absence of excipient; mean $\pm$ SD ($n=3$). Bars indicated with asterisk on top represent a significant increase in AUC ($p < 0.05$); NA not available. Reprinted with permission from Jan Bevernage *et al.* (16)

Fig. 4. Schematic illustration of the competition between dissolution and crystallization via solid or solution state of amorphous systems. Reprinted with permission from David E. Alonzo (20)

Pouton *et al.* (17) studied danazol and its precipitation behavior with 53 polymers in a supersaturated solution. Low polymer concentration range of 0.001–0.1% (w/v, in pH 6.5 phosphate buffer) was used in this study based on physiological consideration. Cellulose-based polymers including HPMCAS (LF, MF, HF), HPC, HXF, MHEC, CAP, gum Arabic, and Eudragit E100 showed superior precipitation inhibition effects. Effective inhibition on precipitation was also observed with HPMC (E4M, 904, 606, K4M, K200M, CP), HEC (250GF, 30000 S), MC, PEOX, and PVP (10, 40, 360).

William *et al.* (18) investigated various cellulosic polymers including HPMC and HPMCAS (HF, MF, and LF) on the stabilization of ITZ in supersaturated solution using a nonsink *in vitro* dissolution test. They concluded that stabilization of the drug in supersaturated state was related to molecular weight and the degree of hydrophobic substitution of HPMCAS with a rank order of HF>MF≈LF, indicating that stabilization was achieved through a combination of steric hindrance and hydrophobic interaction, supplemented by the amphiphilic nature and ionization state of the polymer.

Hawley *et al.* (19) proposed that precipitation of free base drugs upon contact with aqueous media could be induced by two mechanisms: (1) surface-free base growth via salt hydrolysis and (2) bulk-free base precipitation from solution. Surface precipitation is considered the most damaging since it results in a coating of free base on the surface of the salt, reducing or eliminating its dissolution advantage over the free base form of the compound. The authors reported that the dissolution of the PNU-243672A at pH 4 was very slow and it was converted to free based during dissolution experiment. The solid blend of PNU-243672A and citric acid, however, dissolved completely without conversion. This approach allows modification of the surface pH of the dissolving granules, enabling pH-independent dissolution.

Taylor *et al.* (20) proposed two mechanisms that can negate the dissolution advantage of amorphous solids by either crystallization of the amorphous solid on contact with the dissolution medium or through rapid crystallization of the supersaturated solution as shown in Fig. 4. Polymer additives can retard both of these crystallization routes, leading to the generation of supersaturated solutions. Using felodipine as a model compound, the authors reported that PVP was a poor crystallization inhibitor. In contrast, both HPMC and HPMCAS were able to inhibit the crystallization of felodipine and maintain the drug solubility above 7 and 10 µg/mL for at least 4 h, respectively. Indomethacin concentration in the solution upon initial dissolution from amorphous solid decreased rapidly due to solution-mediated crystallization on contact with dissolution medium. Addition of a small amount of either PVP or HPMC at 250 µg/mL in the dissolution medium effectively inhibited the

Fig. 5. Release of fenofibrate in FaSSIF (n=3). The slower the release rate from the silica material, the higher the degree and longer the duration of supersaturation. The dashed line represents the thermodynamic solubility of fenofibrate in FaSSIF. Reprinted with permission from Michiel Van Speybroeck *et al.* (25)

Fig. 6. Plasma concentration–time profiles of fenofibric acid in the fasted state ($n=3$, left panel) and fed state ($n=3$, right panel). Reprinted with permission from Michiel Van Speybroeck *et al.* (25)

crystallization of drug from supersaturated state for at least 4 h. In another study of the same model drug by the same group, felodipine and related amorphous solid dispersions (21), the authors revealed that the amount of polymer relative to drug has a significant impact on the dissolution behavior of ASDs during dissolution. At low (10%) to moderate drug loading (50%), the solubility of the drug obtained from solid dispersions was similar to that of pure amorphous drug.

Using ritonavir (RTV) as a model drug, Talor *et al.* (22) evaluated the effect of 34 polymers, including a series of novel cellulose derivatives, on the solution crystal growth of RTV. This study assessed the key polymer properties inhibiting crystal growth of RTV. The general effectiveness of the cellulose derivatives (in particular the novel polymers) relative to the synthetic polymers was attributed to the following: (1) hydrophobicity—the effective polymers have a moderate level of hydrophobicity; (2) rigidity of polymer structure—the semirigid cellulose polymers are more effective than the semiflexible synthetic polymers of similar hydrophobicity; and (3) amphiphilicity of the novel cellulose-based polymers—the cellulose polymers containing more ionizable carboxylic acids are better inhibitors relative to neutral or less ionized cellulose polymers. These factors are likely to promote adsorption onto RTV crystal surfaces. Multivariate analysis indicated that hydrophobicity is the most important polymer property, impacting its ability to inhibit crystal growth. No significant correlation is found between the attributes of polymers including M_n , monomer

M_w , DP, HBA, or T_g values and their ability to inhibit crystal growth from solution.

Anderson *et al.* (23) recently proposed a drug crystal growth kinetic model from supersaturated aqueous suspension using indomethacin as a model compound. The crystal growth kinetics of indomethacin in the presence of seed crystals in 50 mM phosphate buffer (pH 2.15) was found to follow first-order crystal growth model and to be bulk diffusion controlled process. The apparent indomethacin solubility after crystal growth at DS of 6 was approximately 55% higher as compared to before crystal growth, which attributed to the deposition of a higher energy (metastable) indomethacin form on the seed crystals.

A recent publication by Ghosh *et al.* (24) reported adsorption of HPMC polymer on the surface of the nuclei of the drug resulted in crystal growth inhibition as observed by scanning electron microscopy study. When HPMC was replaced by PVP K-30, crystal growth was inhibited to some extent but not completely. It was concluded that HPMC interacted more strongly with the drug compared to PVP K-30 and gave a better surface coverage. HPMC polymer adsorbed onto drug crystals due to interaction of the hydrophobic (methoxyl) and hydrophilic (hydroxypropyl) groups with the drug and provided steric stabilization. The high affinity of HPMC on the drug molecule can be explained due to its open chain like structure whereas PVP K-30 has more compact or coil shaped structure.

Fig. 7. Correlations of increased absorption and intraluminal concentrations *in vivo* and the increased concentration *in vitro*. Reprinted with permission from Ryusuke Takano *et al.* (28)

Fig. 8. Comparison between the observed and simulated oral absorption of FTI-2600 crystalline free base and HCl salt. **a** The mean plasma concentration time profile and fitting curves of the crystalline free base (filled circle) and HCl salt (empty square). **b** The mean F_a time profiles and fitting curves of the crystalline-free base (filled circle) and HCl salt (empty square). **c** The simulated intraluminal drug concentration of FTI-2600 in dogs after oral administration of the crystalline-free base and the HCl salt. Reprinted with permission from Ryusuke Takano *et al.* (28)

BIO-AVAILABILITY IMPROVEMENT ASSOCIATED WITH SUPERSTATION-BASED FORMULATION

GI factors play a significant role in determining bio-performance of supersaturatable formulations *in vivo*. These

factors include gastric emptying rate, pH variation in different regions (*e.g.*, stomach *vs.* small intestine), the presence or absence of food, the level of bile salts, and residence times in the region, *etc.* These factors certainly affect the rates of dissolution and generate a “local super supersaturatable state” that may be highly un-uniform with induced nucleation and precipitation. The GI fluid volume and composition coupled with pH variation and the level of bile salts have been demonstrated to affect the apparent solubility of drug in each region and thus impact the *in vivo* dissolution and ultimately the product performance. Common species used to evaluate drug formulations are rat, dogs, and monkeys. The utilization of animal models to assess bioavailability of supersaturatable formulations is of importance in formulation development with implication to oral absorption in humans. However, the complexity of physiological factors associated with human subjects significantly increase when species differences are considered in the preclinical *in vivo* PK screening studies.

In Vivo Study in Animal Models

A study was reported by Augustijns *et al.* (25) on the relationship among the drug release rate, the rate of achieving supersaturation, and corresponding biopharmaceutical performance from mesoporous formulations of fenofibrate. As shown in Fig. 5, an *in vitro* dissolution profile clearly illustrates that a decrease in the fenofibrate release rate led to an improvement of the supersaturation profile. The relevance to the *in vivo* situation of the aforementioned effects is demonstrated by the PK profiles in rats shown in Fig. 6. The authors emphasized the qualitative agreement between the *in vitro* release experiments conducted under supersaturating conditions and the *in vivo* data. As PK profiles in the fasted state shown in Fig. 6 (left panel), FFB:SBA-15-A exhibits a significantly lower exposure than those of FFB:SBA-15-B and FFB:MCM-41. This indicates that a short-lasting supersaturation due to rapid release from FFB:SBA-15-A did not yield optimal *in vivo* performance. Similarly, FFB:MCM-41 exhibits both the highest AUC and highest $C_{\max}$ in the rats in fasted state, indicating that the slower release rate resulted in a more sustained supersaturation in intestinal media (as demonstrated *in vitro*). As these authors concluded that the decrease in drug release rate is accompanied by a decrease of the supersaturation rate and it is beneficial to the absorption of fenofibrate. These data suggest that it is essential not to “dump” the entire drug load instantaneously, but instead release it in a gradual fashion such that absorption can take place while the drug is being released.

William *et al.* (18) reported oral bioavailability of amorphous solid dispersions of ITZ with several cellulosic polymers including HPMC and HPMCAS (HF, MF, and LF). Oral bioavailability of ITZ formulations in rats demonstrated superior performance from amorphous solid dispersion containing HPMCAS over other polymers. These results showed that production of amorphous solid dispersions containing appropriate polymers can improve oral bioavailability. As *in vivo* results for solid dispersions displayed erratic absorption which may be attributed to the variability of gastrointestinal pH in the animals, formulation must be developed to minimize variability associated with natural variations in subject gastrointestinal physiology.

Fig. 9. Observed steady state plasma profiles of F1 formulation in humans under normal and achlorhydric conditions at a dose of 400 mg BID. Reprinted with permission from Amitava Mitra *et al.* (30)

A recent publication by William *et al.* (26) reported high *in vitro* supersaturation of ITZ from flocculated nanoparticle dispersions at pH 6.8, relative to that of a commercial product, Sporanox. Greater *in vivo* bioavailability in rats was correlated directly to the higher *in vitro* AUC at pH 6.8 for the flocculated nanoparticle dispersions relative to Sporanox. According to the authors, the *in vitro* pH shift dissolution test is useful in evaluating the solid dispersions with relevance to *in vivo* bioavailability of ITZ.

Miller *et al.* (9) examined an Abbott compound which is a weak base (pK_a 5.3) with molecular weight of approximately 450 Da. The compound is lipophilic with $\log D=4.6$ at pH 7.4 and exhibits very poor intrinsic aqueous solubility of 0.11 $\mu\text{g/mL}$. The pH dilution test was also capable of discriminating the solubility/dissolution performance of different amorphous solid dispersions. The *in vitro* drug concentration–time profiles of these formulations were used as solubility inputs for PK modeling using GastroPlus software. As the authors described predictions of plasma concentration–time profiles are in good agreement with PK data observed, confirming that the supersaturated state is primary driving force for improved bioperformance.

Ranzani *et al.* (27) investigated *in vitro* dissolution of solid solutions prepared by hot-melt extrusion (HME) with various polymers. Supersaturation dissolution study demonstrated that HME formulations of CB-1, a poorly soluble drug, prepared from Eudragit E and Kollidon VA64 increased drug solubility between 30- and 35-fold, respectively comparing to the drug substance. The formulation containing the drug and Eudragit E ($w/w=10:90$) was evaluated in male Wistar–Hannover rats. Approximately threefold increase of CB-1 absorption was observed from the HME formulation as compared to the crystalline drug suspension.

Zhang *et al.* (13) reported their work on a supersaturatable self-microemulsifying drug delivery system (S-SMEDDS) of CBZ and evaluation of drug precipitation behavior, dissolution rate *in vitro* and particle size distribution on their relative bioavailability in beagle dogs. The PK results showed that the presence of a small amount of PVP effectively sustained the supersaturated state by retarding precipitation kinetics. The

mean particle size of S-SMEDDS formulation after dispersion was about 33.7 nm and the release rate from S-SMEDDS was significantly higher than the commercial tablet *in vitro*. Relative exposure of CBZ from S-SMEDDS increased nearly five times compared to the market tablet at a dose of 200 mg.

Yamashita *et al.* (28) examined a BCS II drug, FTI-2600 (MW, 447.5; ClogP, 3.18; pK_a , 2.9, 5.1). To clarify the contribution of supersaturation on improving drug absorption, *in vivo* intraluminal concentration of FTI-2600 in dogs after oral administration was estimated from the pharmacokinetics data using a physiologically based model as described in Fig. 7. The intraluminal drug concentration in dogs from free base and HCl salt were determined and these results (Fig. 8) provide clear evidence that not only the increase in the dissolution rate, but also the supersaturation phenomenon, improved the solubility limited absorption of FTI-2600. These results indicate that a salt form of free base drug can induce supersaturation and improve oral bioavailability as compared to its neutral counterpart.

In Vivo Studies in Humans

Sperry *et al.* (29) reported a study of PK clinical evaluation of BCS II drug, LCX (free base) with two pK_a s in the range of 4–6. Clinical evaluation suggested the patient physiological conditions may have a significant effect on the supersaturated state *in vivo* and therefore the observed variability of exposure. They observed that the majority of patients were taking a proton pump inhibitor, which dramatically increases the stomach pH, and the oral exposure in the patients without taking PPI was the highest, strongly supports that stomach pH plays a role in inducing supersaturation and thus drug absorption. The authors hypothesized that once the drug was dissolved in the stomach at low pH, it maintained supersaturation as moving into the intestine, resulting in high exposure. Variability of the stomach pH in human subjects as a result of administering proton pump inhibitor is attributed to cause changes of the level of supersaturation in small intestines, thus resulting in high variability in oral exposure.

Similarly, Mitra *et al.* (30) reported that poor absorption of weakly basic drug A in patients with reduced gastric acidity can lead to loss of efficacy of the therapeutic agent. The F1 formulation containing a drug A dosed in beagle dogs under normal (pentagastrin pretreated) and high (famotidine pretreated) gastric pH conditions. This set of data showed high sensitivity of the gastric pH condition at dose=10 mg/kg. The observed steady state plasma profiles of the F1 formulation in patients with and without PPI are shown in Fig. 9. The significantly lower exposure from the F1 formulation in achlorhydria condition as compared to normal stomach conditions is in agreement with pH induced precipitation of the drug. As these authors reported, a citric acid containing formulation (F2) was selected and evaluated in humans and was confirmed to be successful in overcoming the achlorhydria effect. High variability was also observed in the non-PPI cohorts across the dose range, this was likely due to high variability in the stomach pH in gastric cancer patients. An acidifier co-existing with the weakly basic drug can effectively increase the dissolution rate of the drug due to a lower degree of supersaturation in that microenvironment of formulation and facilitated to the generation of supersaturated state in GI tract. The use of acidic excipient enabled a more robust formulation less susceptible to variance in gastric pH, improved the observed variability, and achieved adequate exposure under high gastric pH conditions.

CONCLUSIONS

Supersaturable formulations are designed to generate a supersaturated drug solution *in vivo* upon contact with GI fluids and maintain sufficiently long supersaturated state with the use of polymeric precipitation inhibitor. Supersaturated state generated from appropriate supersaturable formulations has been demonstrated to significantly improve intestinal absorption of poorly soluble drugs.

In this review, we attempted to summarize important formulation factors that affect the therapeutic performance of supersaturable formulations from an *in vitro* and *in vivo* perspective. As we learned from these excellent scientific publications, it is of critical importance to gain understanding of the physiological factors associated with the GI tract and their relevance to absorption of weakly acidic and basic drugs. Therefore, we consider it necessary to apply appropriate biorelevant dissolution tests to examine these systems and acquire knowledge of the interplay between formulation and physiological factors. In addition, with the assistance of theoretical modeling and simulation approaches for PK profile, understanding of supersaturation and its impact on improvement of oral absorption *in vivo* will be enforced. The selection of a predictive animal model for testing the bioavailability of formulations should be made on a case-by-case basis, where the anticipated physiological rate-determining factors will dictate the selection of the species that is most analogous to humans for testing the specific compound. We anticipate that the advancement of characterization of supersaturated state by appropriate means and establishing their relevance to oral absorption *in vivo* will greatly facilitate the development and commercialization of supersaturable formulations.

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