Review

Biorelevant test for supersaturable formulation

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ABSTRACT

Supersaturable formulation can generate supersaturation after dissolution, providing kinetic advantage in vivo. However, the supersaturation may precipitate before being absorbed, which makes it difficult to ensure and predict its in vivo performance. The traditional USP method is typically for Quality Control (QC) purpose and cannot be used to predict the formulation in vivo performance. Therefore, there is generally a lack of a predictive biorelevant testing method. In this review, different types of supersaturable formulations are described, including amorphous dispersions, polymorphs, salts/co-crystals, weak base and supersaturable solubilized formulations. Different kinds of in vitro dissolution methods for supersaturable formulations are also reviewed and discussed. Most of the methods take the physiology of gastrointestinal (GI) track into consideration, allowing reasonable prediction of the in vivo performance of supersaturable formulation. However, absorbing drug from GI track into blood stream is a complicate process, which can be affected by different in vivo processes such as transporter and metabolism. These factors cannot be captured by the in vitro testing. Thus, combining in vitro biorelevant dissolution methods with physiology-based pharmacokinetic modeling is a better way for the product development of supersaturable formulation.

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1. Introduction

Supersaturable formulations are designed to generate a supersaturated drug solution in vivo and can maintain a prolonged supersaturated state before absorption, which provides improved pharmacokinetics. Supersaturable formulations have been demonstrated to significantly improve bioavailability of poorly soluble drugs. However, supersaturated drug solution may precipitate before absorption, causing product variability in absorption and inaccurate prediction of its in vivo performance. Traditional USP method is normally for QC purpose and cannot be used to predict the formulation performance. Therefore, a suitable biorelevant test method is needed in order to predict the in vivo performance of supersaturable formulations [1,2].

2. Supersaturable formulation

The degree of supersaturation can be described as Supersaturation ratio (S) or Supersaturation index (σ) [3], which was calculated as Eq. (1):

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Where, \( C_t \) is the drug concentration at time \( t \), \( C_{eq} \) is the equilibrium concentration. A solution is defined as unsaturated \((S_t < 1 (\sigma < 0))\), saturated \((S_t = 1 (\sigma = 0))\) or supersaturated \((S_t > 1 (\sigma > 0))\) based on the value of \( S_t \) or \( \sigma \).

Several types of formulations can generate supersaturation in vivo, such as amorphous/metastable polymorphs, weak bases, crystal salts/co-crystals and supersaturable solubilized forms.

### 2.1. Amorphous/metastable polymorphs

Amorphous or metastable polymorph has high free energy, resulting in a higher apparent solubility than stable crystalline forms. Thus they can form supersaturated drug solution after being dissolved. Amorphous solid dispersion, with the enhanced bioavailability, has been under extensive research over the last decade, this brought us several commercial products such as Kaletra, Intelicence, Sporanox and most recently Kalydeco (Table 1).

### 2.2. Weak base

The intake of weakly basic drugs may result in supersaturation in the small intestine. Due to the intrinsic pH shift in the gastrointestinal lumen in fasting state conditions (pH 1.5–2 in the stomach vs. pH 5–7 in the intestine), the solubility of weak bases (ionized form) in the stomach typically exceeds their solubility in the intestine (unionized form). Hence, when the drug solution of poorly water-soluble weak base formed in the stomach transit to the intestine, the solution may reach supersaturation and increase flux across the intestinal mucosa. This phenomenon can be further augmented by using pH-sensitive polymers such as HPMCAS, Eudragit L100-55 etc.

### 2.3. Crystal salt/co-crystal

Salt is an ionic compound that results from the neutralization reaction of an acid and a base. Salt formation is the most common and effective method of increasing solubility and dissolution rates of acidic and basic drugs. Co-crystals are solids that are crystalline materials composed of two or more molecules in the same crystal lattice and are governed by nonionic interactions. The host active molecule interacts with the suitable guest compound (typically with high solubility), which may enhance the solubility and dissolution rate to some extent. When the dissolved salt or co-crystal concentration exceeds the equilibrium solubility of free form or host crystalline, supersaturation could happen.

### 2.4. Supersaturable solubilized formulation

Some poor water soluble drugs are solubilized in a mixture of cosolvents or oils in order to improve the bioavailability, which is called solubilized formulation. The solubilized formulations are divided into two categories, supersaturable or true solubilized based on the capacity of supersaturation formation [4,5]. Lipid or cosolvent formulations are commonly used for making supersaturable formulations. In general, drugs can be completely dissolved in various oils such as triglycerides and mixed glycerides or cosolvent. Once such formulation is exposed to aqueous environment and solubilization capacity is insufficient, a supersaturated state is generated. On the other hand, the true solubilized formulation such as the formulation with cyclodextrin or surfactant will not generate supersaturation after dilution since the drug is completely dissolved in the cyclodextrin ring or the micelluar surfactant. The true solubility formulation have less free drug, resulting in lower bioavailability in comparison with the supersaturable solubilized formulation [5].

### 3. Factors affecting in vivo performance of supersaturable formulation

Several factors can affect the in vivo performance of supersaturable formulation, including formulation factors, such as supersaturation and precipitation, and physiological factors.

| Brand name | Compound         | Polymer          | Technology used                  | Dosage form | Dosage (mg) | FDA approval |
|------------|------------------|------------------|----------------------------------|-------------|-------------|-------------|
| Sporanox   | Itraconazole     | HPMC             | Spray layering                   | Capsule     | 100 mg      | 1992        |
| Prograf    | Tacrolimus       | HPMC             | Spray drying/fluid bed           | Capsule     | 0.5, 1, 2 mg | 1994        |
| Kaletra    | Lopinavir/ritonavir | PVP/VA             | Melt extrusion                   | Tablet      | 200/50 mg   | 2005        |
|             |                  |                  |                                  |             | 100/25 mg   |             |
| Cesamet    | Naboline          | PVP              | Spray drying                     | Capsule     | 1–2 mg      | 2006        |
| Nimotop    | Nimodipine       | PEG              | Spray drying/fluid bed           | Capsule     | 30 mg       | 2006        |
| Implanon   | Etonogestrel     | EVA              | Melt extrusion                   | Rod         | 68 mg       | 2006        |
| Fenoglide  | Fenofibrate      | PEG              | Melt extrusion                   | Tablet      | 40, 120 mg  | 2007        |
| Intelicence| Etravirine       | HPMC             | Spray drying                     | Tablet      | 100 mg      | 2008        |
| Afnitor    | Everolimus       | HPMC             | Oven drying                      | Tablet      | 5, 10 mg    | 2009        |
| Norvir     | Ritonavir        | PVP/VA           | Melt extrusion                   | Tablet      | 100 mg      | 2010        |
| Incivek    | Telaprevir       | HPMC-AS          | Spray drying                     | Tablet      | 375 mg      | 2011        |
| Zelboraf   | Vemurafenib      | HPMC-AS          | Anti-solvent precipitation       | Tablet      | 240 mg      | 2011        |
| Kalydeco   | Ivacafor         | HPMC-AS          | Spray drying                     | tablets     | 150 mg      | 2012        |
3.1 **Formulation factors**

3.1.1 **Supersaturation**

After supersaturable formulation was taken into the GI tract, it will be dissolved and forms supersaturation with different drug species, including free drug, nanoaggregates, drug/polymer nanostructures, etc. as shown in Fig. 1 [6]. Only free drug can be absorbed, while the drugs in nanoaggregates and drug/polymer nanostructures can serve as reservoirs and exchange into free drug quickly before it can be absorbed.

Recently, Taylor et al. [7] found that the high supersaturation generated by dissolving amorphous solid dispersion lead to a two-phase system, called liquid–liquid phase separation (LLPS). LLPS contains both drug-rich phase and drug-lean continuous aqueous phase. There is an upper limit in the passive member transport indicated by the LLPS concentration. A linear increase in the flux was observed in the side-by-side diffusion cell experiment below LLPS concentration while the flux remained constant above LLPS concentration (Fig. 2). On the contrary, the supersaturated system with subsequent crystallization has less thermodynamic activity and low bioavailability. Compared with solubility enhancement generated by micellar surfactant or cyclodextrins, the supersaturation has higher drug chemical potential and can exhibit higher permeation rate.

Since only free drug can be absorbed, it is important to have an accurate estimate of the free drug concentration. Different approaches were used to determine the free drug concentration of supersaturation. Friesen et al. [6] perform dissolution test in microcentrifuge and determine the free drug level in the supersaturation after ultracentrifugation. This method is simple and very commonly used. Dong et al. [8] ran dissolution in the syringes coupled with a filter, and then the free drug was obtained by filtration. However, this method would allow small particles to pass through the filter and overestimate the amount of drug in solution. Recently, Shah et al. [9] proposed Pulsatile Microdialysis Method (PMD) to evaluate the free drug in the supersaturation. PMD showed more sensitive and accurate outcomes, and this method provides a means to collect filtered assayable samples in a matter of seconds. Due to the absence of small particles, the drug concentration determined by this PMD was significantly lower than those obtained by sampling and filtering. However, PMD is slightly more complicated.

3.1.2 **Precipitation**

The drug precipitation from supersaturation is actually the process of crystallization. The crystallization process includes nucleation and crystal growth [10–12]. Nucleation is the first step for drug crystallization, forming either a new thermodynamic phase or a new structure via self-assembly or self-organization. The nucleation rate \( J \) can be described by Eq. (2).

\[
J = A \exp \left[ \frac{-16\gamma^2\nu^2 \times \phi}{3(kT)^4(\ln S)^2} \right]
\]

Where:

![Diagram](image-url)

**Fig. 1 – Species that formed when amorphous formulation are added to aqueous solutions simulating duodenal and intestinal contents [6].**
A: the probability of intermolecular collision in the solution
ν: the molecular volume of the solute
γ: interfacial tension
T: absolute temperature
S: degree of supersaturation (C/C_{eq})
Φ: heterogeneous nucleation factor (0 < Φ < 1)

Crystal growth is a major stage of crystallization process, which is adding new molecules into the characteristic arrangement of a crystalline Bravais lattice. The crystal growth rate (dr/dt) can be described by Eq. (3).

\[ \frac{dr}{dt} = \frac{DvN_A(C - C_{eq})}{r + D/k^+} \]  

Where:
D: diffusion coefficient of the molecule
k + : the surface integration factor
N_A: the Avogadro constant
(C-C_{eq}): the difference between the bulk concentration and the concentration in the solution directly next to the cluster surface

If r ≥ D/k + , the process is diffusion-controlled while if r ≤ D/k + , the process is controlled by the surface integration.

In order to inhibit the precipitation, the following strategies can be used, base on the nucleation and crystal growth mechanism:
- Reducing the degree of supersaturation (S or C-C_{eq})
- Increasing the viscosity (D)
- Increasing the cluster-liquid interfacial energy (γ)
- Changing the adsorption layer at the crystal medium interface (K+)
- Changing the level of solvation at the crystal-liquid interface (K+)

The different excipients including surfactant, polymer (HPMC, PVP, HPMC-AS) and cyclodextran have been used to inhibit the precipitation of supersaturation. Xu et al. [13] have discussed this topic recently in their review. The main mechanism is to increase the viscosity of media and/or change the interface between crystal/cluster and media.

The solid state of precipitate can also have an effect on the bioavailability. Amorphous or metastable precipitate can further improve the bioavailability while stable crystal precipitate cannot. Psachoulas et al. [14] observed the difference of ketoconazole precipitate between in vitro and in vivo, the precipitate was crystalline in vitro while it was amorphous in vivo. The solid state characteristics of the precipitate may play an important role in terms of re-dissolution of the precipitate within the gut lumen, which can influence the ultimate rate and extent of absorption.

3.2. Physiological factors

Physiological factors are very critical for the in vivo performance of supersaturable formulation, which includes the pH values and the transition time of GI tract, stomach and intestine media, fasting/fed condition, and the permeability of the intestine at different segments.

For acidic and basic drugs, the pH values and transit time of the GI tract are very important. They can affect the drug solu-
used Mini-scale Dissolution Tests evaluate the precipitation tried to identify the mechanism behind the used two-step dissolution to evaluate supersaturable formulation more accurately, the designed dissolution media for the formulation in vivo. Stomach media has the pH value from 1 to 3.5 under the fasting condition and from 4.3 to 5.4 under the fed condition, while pH of intestine media is between 5 and 7 as shown in Table 2. Beside the pH difference, intestine media has bile salt and lecithin while stomach media has not. There are also differences between intestine media under fasting and fed condition, as shown in Table 2. In the fed state, there is more bile salt (sodium taurocholate) in the intestine media. The solubility of poor water soluble drug can be increased and no supersaturation/precipitation occurs, resulting in high bioavailability in the fed state. The biorelevant media are widely used in the dissolution method.

The permeability of the intestine is the most important factor for absorption, which is different at different segments of intestine. In some cases, the drug can only be absorbed in the upper GI, such as weak acidic drug, the supersaturable formulation can dramatically increase the bioavailability. In other case, if the permeability rate of intestine is low, and the supersaturation state cannot last long enough for absorption, the bioavailability would not be improved. Different approaches were used to mimic the permeability in the dissolution, including biphasic dissolution, caco-2 monolayer and absorption compartment.

Therefore, in order to predict the in vivo performance of supersaturable formulation more accurately, the designed dissolution method should consider both those formulation and physiological factors mentioned above.

### 4. Biorelevant dissolution

Different dissolution methods have been used to characterize the supersaturable formulations. They are discussed here based on the compartments, from simple to complex.

#### 4.1. One compartment

The simplest method is to use one compartment dissolution vessel to characterize the supersaturable formulation. Non-sink condition dissolution is one of the most commonly used dissolution. Hisada et al. used Mini-scale Dissolution Tests (paddle method with 50 ml media) to evaluate the dissolution and precipitation of salt, cocrystal, and solvate formulations, and to clarify their effects on enhancing oral absorption.

Qian et al. [18] tried to identify the mechanism behind the unexpected good bio-performance of amorphous solid dispersions with HPMC-AS compared with PVP-VA solid dispersion. Both sink and non-sink dissolution cannot be correlated with the in vivo performance. By doing 4h precipitation experiment, they found that HPMC-AS was more effective in prolonging drug supersaturation than PVP-VA in aqueous solution. Recently, Kambayashi et al. [19] evaluate the precipitation kinetics of two representative weak base drugs, dipyridamole and ketoconazole in the FaSSIF-V2.

Two step dissolution is another commonly used dissolution method to evaluate supersaturation of weak basic drug. Matteucci et al. [20] used two-step dissolution to evaluate supersaturated solutions from dissolution of amorphous itraconazole microparticles. The particles were exposed to pH 1.2 medium then shifted to pH 6.8 medium by adding concentrated high pH media in the same vessel. This pH shift simulated the transition from stomach to intestines. They found that the ability to generate and sustain high supersaturation at pH 6.8 can be increased by minimizing undissolved excess surface area, which may be expected to be beneficial for raising bioavailability by gastrointestinal delivery. This work revealed that the dissolution rate of the drug from supersaturable formulations is an important factor in dictating the generation and duration of the supersaturated state. A rapid dissolution that generates a high degree of supersaturation may

### Table 2 – The pH values and the transit time at different segments of the human GI tract [15].

| Anatomical site | Fasting condition | Fed condition |
|----------------|------------------|-------------|
|                | pH               | Time (h)    | pH               | Time (h)    |
| Stomach        | 1-3.5            | 0.25        | 4.3-5.4          | 1           |
| Duodenum       | 5-7              | 0.26        | 5.4              | 0.26        |
| Jejunum        | 6-7              | 1.70        | 5.4-6            | 1.7         |
| Ileum          | 6.6-7.4          | 1.30        | 6.6-7.4          | 1.3         |
| Cecum          | 6.4              | 4.50        | 6.4              | 4.5         |
| Colon          | 6.8              | 13.50       | 6.8              | 13.5        |

### Table 3 – Composition of FaSSIF and FeSSIF [16].

|                | Fasted state simulated intestinal fluid(FaSSIF) | Fed state simulated intestinal fluid(FeSSIF) |
|----------------|-----------------------------------------------|-------------------------------------------|
| pH             | 6.5                                           | 5                                         |
| Osmolality     | 270 ± 10 m osmol                              | 635 ± 10 m osmol                          |
| Sodium taurocholate | 3 mM                                        | 15 mM                                     |
| Lecithin       | 0.75 mM                                       | 3.75 mM                                   |
| KH₂PO₄         | 3.9g                                          | 8.65g                                     |
| KCl            | 7.7g                                          | 15.2g                                     |
| NaOH           | qs                                            | NaOH qs                                   |
| Deionized water| qs                                            | pH 5.0                                    |
| qs             | 1 liter                                       | qs                                        |

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induce rapid crystallization and may not be optimal to sustain the supersaturation state.

In order to mimic the in vivo absorptive sink condition, biphasic dissolution can be used in the one compartment vessel. To predict the in vivo absorption behavior of oral modified release dosage forms containing pH-dependent poorly soluble drugs, Heigoldt U et al. [21] developed a modified USP apparatus 2, combining biphasic dissolution with a pH-gradient in the aqueous dissolution medium as shown in Fig. 3. Quasi sink conditions in the aqueous phase were introduced by the removal of dissolved drug via distribution to an organic phase. The results indicated that dissolution testing using the biphasic approach enabled an improved prediction of the in vivo behavior and bioavailability of the modified release formulations compared to conventional dissolution testing at pH 1, pH 5.5, or pH 6.8.

4.2. Two compartments

The main purpose of using two compartments in the dissolution is to mimic the pH shift and/or establish the in vivo absorptive sink condition.

To evaluate the supersaturation of a weak base, a transfer model can be used to mimic pH shift instead of two step dissolution in one vessel. The transfer model can easily control the dissolution media and transfer rate. Kostewicz et al. [22] originally presented a transfer model that applies two compartments to simulate the stomach and intestine, respectively (Fig. 4) [23]. In the experimental set-up, a drug solution is placed in a simulated gastric fluid compartment (donor phase), then pumped into the simulated intestinal compartment (acceptor phase) at a constant rate which simulates the gastric emptying. This method was modified by others and it has been very popular. Carino and coworkers [24,25] developed the artificial stomach-duodenum (ASD) apparatus that is a two-compartment dissolution system consisting of chambers representing the stomach and duodenum. In ASD, the duodenal chamber is a mixing chamber filled with pH 6.5 dissolution media, and the acidic dissolution media from the gastric chamber constantly enters into the duodenal chamber. The multi-compartment dissolution method that will be discussed later typically has the component of a transfer model.

Besides biphasic dissolution, dissolution/permeation (D/P) system is another way to mimic the in vivo absorptive sink condition (Fig. 5). In D/P system, one compartment is for the drug dissolution and another compartment is for the permeated drug, the caco-2 monolayer is between them. The absorptive sink is directly determined by the Caco-2 permeability for the drug. The permeated amount accounts for formulation-related effects on apical concentrations as well as on permeability. If the drug permeability is low and the supersaturation generated by formulation cannot be maintained long enough, the permeated amount will be low and its in vivo performance may not be good. The D/P system is good for in vitro-in vivo correlations. A correlation between the absorbed fraction in human and the permeated amount in the D/P system has been established for poorly water soluble drugs [26,27]. However, the use of Caco-2 cells in the D/P system has some disadvantages, including limited size (not final formulation), and compatibility issues between dissolution media and monolayer integrity.

Another possible way to achieve absorptive sink condition is a flow-through cell (FTC) method (USP 4 apparatus). Fig. 6 A and B depicts the open and closed operating mode of an FTC [23]. The open system benefits from a constant medium supply whereby the absorptive sink conditions can be achieved and
a differential curve type is obtained by changing flow rate and/or media. In contrast, operating in the closed mode results in a cumulative curve and sink condition cannot be guaranteed. One outstanding advantage of FTC is the possibility of running pH gradients by altering the medium composition, which mimics the physiological conditions of the gastrointestinal tract. Thybo et al. used FTC to evaluate probucol solid dispersion [28]. Tajiri T et al. [29] used open operating mode of an FTC to evaluate solid dispersion matrix of indomethacin. They found that the open mode can discriminate supersaturable formulation (indomethacin solid dispersion) and a variety of testing options make it useful in revealing the drug release mechanism from the matrix and the critical factors of FTC.

In order to assure sink condition in closed operating mode of FTC, Shi et al. [30] employed a biphasic system with FTC to evaluate the release of the poorly soluble drug celecoxib from three formulations (the commercial Celebrex capsule, a solution containing co-solvent and surfactant, and a supersaturable self-emulsifying drug delivery system (S-SEDDS)) (Fig. 7). The

Fig. 5 – Schematic illustration of the dissolution/permeation system (D/P system). Caco-2 monolayer was mounted between the apical and basal chambers [26].

Fig. 6 – Schematic illustration of flow-through cell apparatus (FTC) (A: open-loop configuration; B: closed-loop configuration) [23].

Fig. 7 – Schematic diagram of biphasic test system [30].
biphasic system in the USP 2 apparatus contains an additional octanol layer to create an absorptive sink, which enabled discrimination among the three formulations. Compared with biphasic system in one compartment, which is only suitable for tablets or capsules, this new design can be used for different dosage forms. Shi et al. [30] showed that the data from this new dissolution method was well correlated with the in vivo celecoxib bioavailability, while release profiles in monophasic systems under both sink and non-sink conditions were not. The result demonstrates that an absorptive sink condition is needed for the dissolution in order to evaluate the supersaturable formulations more accurately.

4.3. Multi-compartments

In order to predict the in vivo performance with higher accuracy, multi-compartment dissolution was developed. Multi-compartment dissolution is typically the modified transfer model. It is more suitable for weak basic drug, in which pH shift is critical.

To predict the concentrations and potential precipitation of highly permeable, lipophilic weak bases in the fasting upper small intestine, Psachoulias et al. [14] setup a three-compartment in vitro methodology (Fig. 8). Depending on the dosage form administered in the in vivo studies, a solution or a suspension was placed in the gastric compartment. A medium simulating the luminal environment (FaSSIF-V2plus) was initially placed in the duodenal compartment. Concentrated FaSSIF-V2plus was placed in the reservoir compartment to maintain relevant bile salt and lecithin concentrations following dilution by the gastric medium. The methodology also incorporated a first order gastric emptying rate, which is similar to the kinetics of gastric emptying under fasting conditions [31]. These factors were considered in the methodology so that it is more consistent with the in vivo GI situation, therefore providing a more accurate prediction. Psachoulias et al. [14] used this methodology to successfully evaluate two other lipophilic weak bases, AZD and SB, using previously collected plasma data in humans.

Gu et al. [32] developed four-compartment dissolution method. Different from three-compartment methodology proposed by Psachoulias et al. [14], the modified system includes an “absorption” compartment, additional to a “gastric” compartment, an “intestinal” compartment and a reservoir vessel (Fig. 9). This method simulates the absorptive sink by the continuous transfer of dissolved drug from the intestinal compartment to the ‘absorption’ compartment. By adjusting the flow rate between the intestine and absorption compartments, various permeability values can be simulated. For both dipyridamole and cinnarizine, the in vitro dissolution using the multi-compartment system was able to predict the pH effect on oral exposure. The results from the multi-compartment system are more closely correlated with the in vivo data, compared with that from the conventional dissolution test.

Based on artificial stomach-duodenum (ASD) model, Amidon group developed gastrointestinal simulator (GIS), which consists of gastric, duodenal, and jejunal chambers (Fig. 10) [33–36]. Compared to ASD, the GIS has one extra compartment as a jejunal chamber. Because drug residence time in the human
Jejunum and ileum is much longer than the transit time of the human duodenum (~30 min), most drugs will be absorbed along the small intestine. Therefore, drug concentration in the jejunal chamber was also considered important for in vitro dissolution to predict drug absorption. The duodenal fluid has a broad pH range and a wide range of buffer capacity, while the jejunal fluid has a more stable pH and a certain range of buffer capacity. Thus, it will be beneficial to have an extra chamber with more stabilized pH. Amidon group [33–36] revealed that GIS is a practical tool to assess dissolution properties and to improve IVIVC of supersaturable formulation, especially for BCS class IIb drugs.

The TNO Simulated Gastro-intestinal Tract Model 1 (TIM-1) is a multi-compartmental, dynamic, computer controlled system that simulates the human upper GI tract [37] (Fig. 11). It was developed at TNO Nutrition and Food Research (Zeist, The Netherlands). The TIM-1 system simulates the physiological conditions of GI tract, including the gastric and intestinal pH values, gastric emptying and intestinal transit times, body temperature, and the composition of the GI fluids. Dickinson et al. [38] demonstrated that it is possible to study formulations delivering poorly soluble weak basic compounds (AZD8055) using TIM-1. They compared TIM-1 performance data with exposure data from the phase 1 clinical study and confirmed that TIM-1 system was able to show that AZD8055 exposure increase in an approximately dose proportional manner and not be limited by the solubility or dissolution. It was also shown that TIM-1 can predict the performance of a BCS class II compound in both fasting and fed conditions. However, this method is complicate and not widely used, and although TIM-1 incorporate most of physiological conditions of GI tract, some in vivo processes such as active transportation, efflux and intestinal transport...
wall metabolism are not modeled mechanistically by the system.

5. **PBPK modeling combined with dissolution data**

Although biorelevant dissolution tests with different compartments are useful for qualitatively and/or quantitatively prediction of the in vivo drug performance, the dissolution release test cannot incorporate all processes that may affect the in vivo performance. The pros and cons of different biorelevant dissolution tests have been summarized in Table 4. The most complex multi-compartment system, TIM-1, capture most of the physiological factors, but some in vivo processes such as active transportation, efflux and intestinal wall metabolism are still missing in the system. In addition, the experimental system does not provide an opportunity for the scientist to try multiple variables quickly, which is often critical in reaching the final conclusion of most biorelevant method and absorption mechanism for a given compound. Taking all these factors in consideration, it is better to combine the in vitro dissolution test results with physiologically based pharmacokinetic (PBPK) models.

Dressman group combined PBPK modeling with dissolution data to evaluate the in vivo performance of different supersaturable formulation successfully [39–41]. For example, Kambayashi et al. [39] used non-sink dissolution method to characterize both the dissolution and precipitation kinetics of dantrolene salt, and combined PBPK to predict its oral pharmacokinetic profile. Berlin et al. [40] use both biorelevant dissolution and transfer methods to investigate the dissolution, supersaturation and precipitation behavior of marketed cinnarizine tablets under fasting and fed state conditions and coupled with PBPK models to predict the in vivo performance of these cinnarizine formulations. They found that under fasting conditions, plasma profiles could be accurately predicted only when supersaturation and precipitation as well as dissolution (transfer method) were taken into account. Berlin et al. [41] also revealed that pre-absorptive and absorptive factors (dissolution, supersaturation, precipitation) had less impact on atazanavir bioavailability compared to post-absorptive parameters (P-gp mediated efflux). From the PBPK models, it was concluded that further enhancement of the formulation would bring little improvement in the pharmacokinetic response to atazanavir. Tsume et al. [33] from Amidon group also combined Gastrointestinal Simulator (GIS) with PBPK models to predict in vivo dissolution of a weak base drug, dasatinib, and found that the dissolution with mGIS (pH 1.2 SGF/pH 6.5 SIF) has a better correlation with clinical data. Gao et al. [42] combined the pH dilution method, similar to precipitation method, with PBPK to get better prediction of in vivo performance.

Therefore, in the future, the results from the in vitro supersaturation and precipitation tests should be incorporated with

### Table 4 – The pros and cons of different biorelevant dissolution tests.

| Compartment        | Dissolution method                                                                 | Pros                                                                                     | Cons                                                                                          |
|--------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| One compartment    | Mini-scale dissolution, non-sink condition [17]                                   | Simple, suitable for salt, cocystal, amorphous and supersaturable solubilized formulation | Not easy to accurately determine the free drug of supersaturation                             |
|                    | Precipitation [18,19]                                                              | For a given molecule, study the precipitation inhibition effect of polymer, suitable for polymer screening | Cannot evaluate the dissolution of formulation                                                 |
|                    | Two-step dissolution [20]                                                          | Evaluate both dissolution and precipitation, more suitable for weak basic drug or salts    | Only used for ranking order the formulation, cannot accurately predict in vivo performance    |
|                    | Biphasic dissolution [21]                                                          | The simple system to mimic the in vivo absorptive sink condition                          | Not easy to operate as research or QC method, sink condition is not perfect                   |
| Two compartment    | Transfer model [22–25]                                                             | Easily control the dissolution media and transfer rate; a good model to evaluate weak base compounds; it is a foundation for the multi-compartment dissolution method | Only used for ranking order formulation, cannot accurately predict in vivo performance          |
|                    | Dissolution/Permeation (D/P) System [26,27]                                        | Better mimic the in vivo absorptive sink condition using caco-2 monolayer. Good system for in vitro-in vivo correlations. | Limited size (not final formulation), and compatibility issues between dissolution media and monolayer integrity |
|                    | Flow-through cell (FTC) method [28,29]                                            | Another way to achieve absorptive sink condition, easy to run pH gradients by altering the medium composition | Need high volume of media                                                                      |
|                    | FTC plus Biphasic [30]                                                             | Assure absorptive sink condition in closed operating mode of FTC, less volume of media is needed | Sink condition is not perfect                                                                 |
| Multiple compartment| Three-compartment [14], four-compartment [32], gastrointestinal simulator (GIS) [33–36], TIM-1 [37] | Have a better prediction on the in vivo performance, especially suitable for the weak base compound | Complex, high cost, not easy for routine lab screening                                           |
physiologically based pharmacokinetic modeling (PBPK). The combination can lead to more accurate predictions of plasma levels, improving the mechanistic understanding of the absorption and helping find out the import parameters in the overall absorption process.

6. Conclusion

In this review, different supersaturable formulations are described, including amorphous, polymorph, salt, co-crystal, weak base and supersaturable solubilized formulation. The methods to characterize supersaturation and precipitation were summarized. Different kinds of in vitro dissolution method were also reviewed and discussed in terms of compartments (from simple to complex) for supersaturable formulations. Most of the methods have considered the physiology of GI track and can reasonably predict the in vivo performance of supersaturable formulation. However, the process of oral drug absorption into the blood is complicated. This process can be affected by different factors such as transporter and metabolism. The combination of the in vitro dissolution method with physiology-based pharmacokinetic modeling is a better tool for the supersaturable formulation development.

REFERENCES

[1] Newman A, Knipp G, Zografi G. Assessing the performance of amorphous solid dispersions. J Pharm Sci 2012;101:1355–1377.

[2] Gao P, Shi Y. Characterization of supersaturatable formulations for improved absorption of poorly soluble drugs. AAPS J 2012;14:703–713.

[3] Bevernage J, Brouwers J, Brewster ME, et al. Evaluation of gastrointestinal drug supersaturation and precipitation: strategies and issues. Int J Pharm 2013;453:25–35.

[4] Gao P, Morozowich W. Development of supersaturatable SEDDS (S-SEDDS) formulations for improving the oral absorption of poorly soluble drugs. Expert Opin Drug Deliv 2005;3:97–110.

[5] Frank KJ, Rosenblatt KM, Westedt U, et al. Amorphous solid dispersion enhances permeation of poorly soluble ABT-102 true supersaturation vs apparen soluble enhancement. Int J Pharm 2012;437:288–293.

[6] Friesen DT, Shanks R, Crew M, et al. Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: an overview. Mol Pharm 2008;5:1003–1019.

[7] Raina SA, Zhang GG, Alonso DE, et al. Enhancements and limits in drug membrane transport using supersaturated solutions of poorly water soluble drugs. J Pharm Sci 2014;103:2736–2748.

[8] Dong WY, Maincent P, Bodmeier R. In vitro and in vivo evaluation of carbamazepine-loaded enteric microparticles. Int J Pharm 2007;331:84–92.

[9] Shah KB, Patel PG, Khairuzzaman A, et al. An improved method for the characterization of supersaturation and precipitation of poorly soluble drugs using pulsatile microdialysis (PMD). Int J Pharm 2014;468:64–74.

[10] Kashchiev D, van Rosmalen GM. Review: nucleation in solutions revisited. Cryst Res Technol 2003;38:555–574.

[11] Lindfors L, Forssén S, Westergren J, et al. Nucleation and crystal growth in supersaturated solutions of a model drug. J Colloid Interface Sci 2008;325:404–413.

[12] Brouwers J, Brewster ME, Augustijns P. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? J Pharm Sci 2009;98:2549–2572.

[13] Xu S, Dui WG. Drug precipitation inhibitors in supersaturable formulations. Int J Pharm 2015;453:36–43.

[14] Psachoulias D, Vertzoni M, Butler J, et al. An in vitro methodology for forecasting luminal concentrations and precipitation of highly permeable lipophilic weak bases in the fasting upper small intestine. Pharm Res 2012;29:3486–3498.

[15] Hong W, Kinam P. Introduction and overview of oral controlled release formulation design. In: Hong W, Kinam P, editors. Oral controlled release formulation design and drug delivery: theory to practice. John Wiley & Sons, Inc; 2010. p. P1–P19.

[16] Galia E, Nicolaides E, Hörter D, et al. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res 1998;15:696–705.

[17] Hisada N, Takano R, Takata N, et al. Characterizing the dissolution profiles of supersaturable salts, cocrysats, and solvates to enhance in vivo oral absorption. Eur J Pharm Biopharm 2016;109:192–199.

[18] Qian F, Wang J, Hartley R, et al. Solution behavior of PVP-VA and HPMC-AS-based amorphous solid dispersions and their bioavailability implications. Pharm Res 2012;29:2766–2776.

[19] Kambayashi A, Yasuji T, Dressman JB. Prediction of the precipitation profiles of weak base drugs in the small intestine using a simplified transfer (“dumping”) model coupled with in silico modeling and simulation approach. Eur J Pharm Biopharm 2016;103:95–103.

[20] Matteucci ME, Fagiuoli JC, Miller MA, et al. Highly supersaturated solutions from dissolution of amorphous itraconazole microparticles at pH 6.8. Mol Pharm 2009;6:375–385.

[21] Heigoldt U, Sommer F, Daniels R, et al. Predicting in vivo absorption behavior of oral modified release dosage forms containing pH-dependent poorly soluble drugs using a novel pH-adjusted biphasic in vitro dissolution test. Eur J Pharm Biopharm 2010;76:105–111.

[22] Kostewicz ES, Wunderlich M, Brauns U, et al. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. J Pharm Pharmacol 2004;56:43–51.

[23] Kostewicz ES, Abrahamsson B, Brewster M, et al. In vitro models for the prediction of in vivo performance of oral dosage forms. Eur J Pharm Sci 2014;57:342–366.

[24] Carino SR, Sperry DC, Hawley M. Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach-duodenum model. J Pharm Sci 2006;95:116–125.

[25] Carino SR, Sperry DC, Hawley M. Relative bioavailability of three different solid forms of PNU-141659 as determined with the artificial stomach duodenum model. J Pharm Sci 2010;99:3923–3930.

[26] Kataoka M, Masaoka Y, Yamazaki Y, et al. In vitro system to evaluate oral absorption of poorly, water-soluble drugs: simultaneous analysis on dissolution and permeation of drugs. Pharm Res 2003;20:1674–1680.

[27] Kataoka M, Sugano K, Mathews C, et al. Application of dissolution/permeation system for evaluation of formulation effect on oral absorption of poorly water-soluble drugs in drug development. Pharm Res 2012;29:1485–1494.

[28] Thybo P, Pedersen BL, Hovgaard L, et al. Characterization and physical stability of spray dried solid dispersions of probucol and PVP-K30. Pharm Dev Technol 2008;10:375–386.
[29] Tajiri T, Morita S, Sakamoto R, et al. Developing dissolution testing methodologies for extended-release oral dosage forms with supersaturating properties. Case example: solid dispersion matrix of indomethacin. Int J Pharm 2015;490:368–374.

[30] Shi Y, Gao P, Gong Y, et al. Application of a biphasic test for characterization of in vitro drug release of immediate release formulations of celecoxib and its relevance to in vivo absorption. Mol Pharm 2010;7:1458–1465.

[31] Moore JG, Christian PE, Brown JA, et al. Influence of meal weight and caloric content on gastric emptying of meals in man. Dig Dis Sci 1984;29:513–519.

[32] Gu CH, Rao D, Gandhi RB, et al. Using a novel multicompartment dissolution system to predict the effect of gastric pH on the oral absorption of weak bases with poor intrinsic solubility. J Pharm Sci 2005;94:199–208.

[33] Tsume Y, Takeuchi S, Matsui K, et al. In vitro dissolution methodology, mini-gastrointestinal simulator(mGIS), predicts better in vivo dissolution of a weak base drug, dasatinib. Eur J Pharm Sci 2015;76:203–212.

[34] Takeuchi S, Tsume Y, Amidon GE, et al. Evaluation of a three compartment in vitro gastrointestinal simulator dissolution apparatus to predict in vivo dissolution. J Pharm Sci 2014;103:3416–3422.

[35] Matsui K, Tsume Y, Amidon GE, et al. In vitro dissolution of fluconazole and dipyridamole in gastrointestinal simulator (GIS), predicting in vivo dissolution and drug-drug interaction caused by acid-reducing agents. Mol Pharm 2015;12:2418–2428.

[36] Matsui K, Tsume Y, Amidon GE, et al. The evaluation of in vitro drug dissolution of commercially available oral dosage forms for Itraconazole in gastrointestinal simulator with biorelevant media. J Pharm Sci 2016;105:2804–2814.

[37] Minekus M, Marteau P, Havenaar R, et al. A multicompartamental dynamic computer-controlled model simulating the stomach and small intestine. Altern Lab Anim 1995;23:197–209.

[38] Dickinson PA, Abu Rmaileh R, Ashworth L, et al. An investigation into the utility of a multicompartamental, dynamic, system of the upper gastrointestinal tract to support formulation development and establish bioequivalence of poorly soluble drugs. AAPS J 2012;14:196–205.

[39] Kambayashi A, Dressman JB. An in vitro-in silico-in vivo approach to predicting the oral pharmacokinetic profile of salts of weak acids: case example dantrolene. Eur J Pharm Biopharm 2013;84:200–207.

[40] Berlin M, Przyklenk KH, Richtberg A, et al. Prediction of oral absorption of cinnarizine – a highly supersaturating poorly soluble weak base with borderline permeability. Eur J Pharm Biopharm 2014;88:795–806.

[41] Berlin M, Ruff A, Kesisoglou F, et al. Advances and challenges in PBPK modeling – analysis of factors contributing to the oral absorption of atazanavir, a poorly soluble weak base. Eur J Pharm Biopharm 2015;93:267–280.

[42] Gao Y, Carr RA, Spence JK, et al. A pH-dilution method for estimation of biorelevant drug solubility along the gastrointestinal tract: application to physiologically based pharmacokinetic modeling. Mol Pharm 2010;7:1516–1526.