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Review

Biorelevant test for supersaturable formulation

Enxian Lu *, *Shoufeng Li*, *Zhongqin Wang*

Shanghai Aucta Pharmaceuticals Co., Ltd., No. 3377 Kangxin Road. SIMZ Pudong, Shanghai 201318, China

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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.

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_t) or Supersaturation index (σ) [3], which was calculated as Eq. (1):

* *Corresponding author.* Shanghai Aucta Pharmaceuticals Co., Ltd., No. 3377 Kangxin Road. SIMZ Pudong, Shanghai 201318, China. Fax: +86-21-58585607.
E-mail address: enxian.lu@auctapharma.com (E. Lu).
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Table 1 – Summary of FDA approved amorphous solid dispersion products in U.S.

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 100/25 mg	2005
Cesamet	Nabilone	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
Intelence	Etravirine	HPMC	Spray drying	Tablet	100 mg	2008
Afinitor	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	Ivacaftor	HPMC-AS	Spray drying	tablets	150 mg	2012

$$S_t = \frac{C_t}{C_{eq}} \quad \sigma = S_t - 1 = \frac{C_t - C_{eq}}{C_{eq}} \tag{1}$$

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 σ.

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, Intelence, 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/cocrystal*

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.

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 micelluar 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).

$$J = A\exp\left[-\frac{16\pi\gamma^3 v^2 \times \Phi}{3(kT)^3(\ln S)^2}\right] \tag{2}$$

Where:

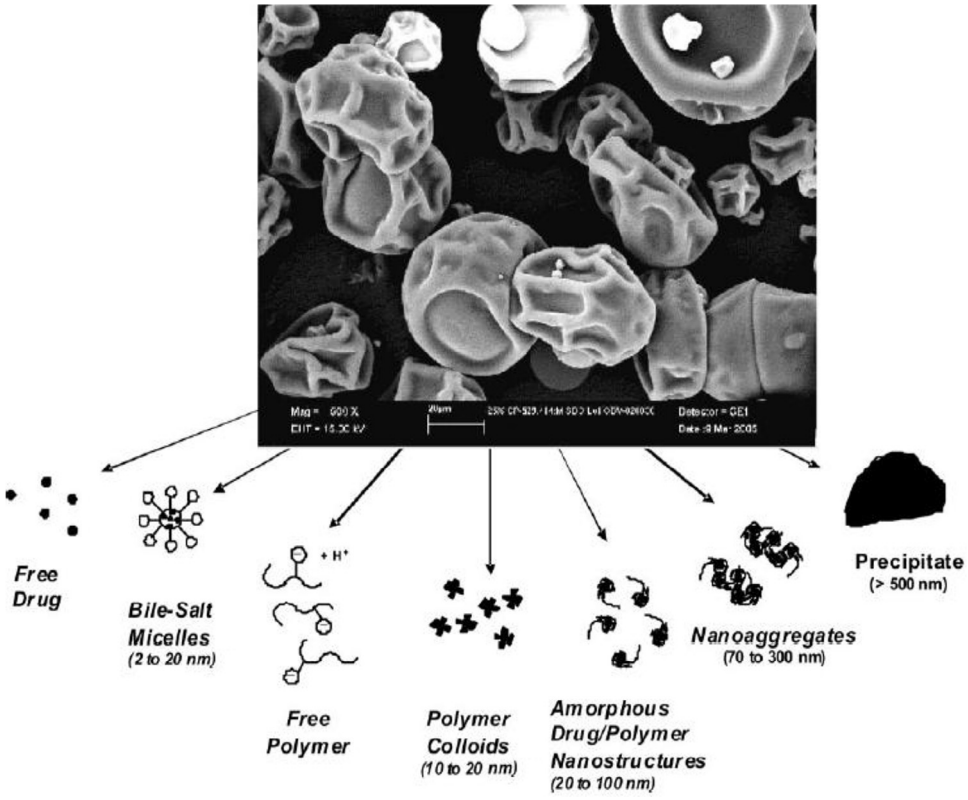

Fig. 1 – Species that formed when amorphous formulation are added to aqueous solutions simulating duodenal and intestinal contents [6].

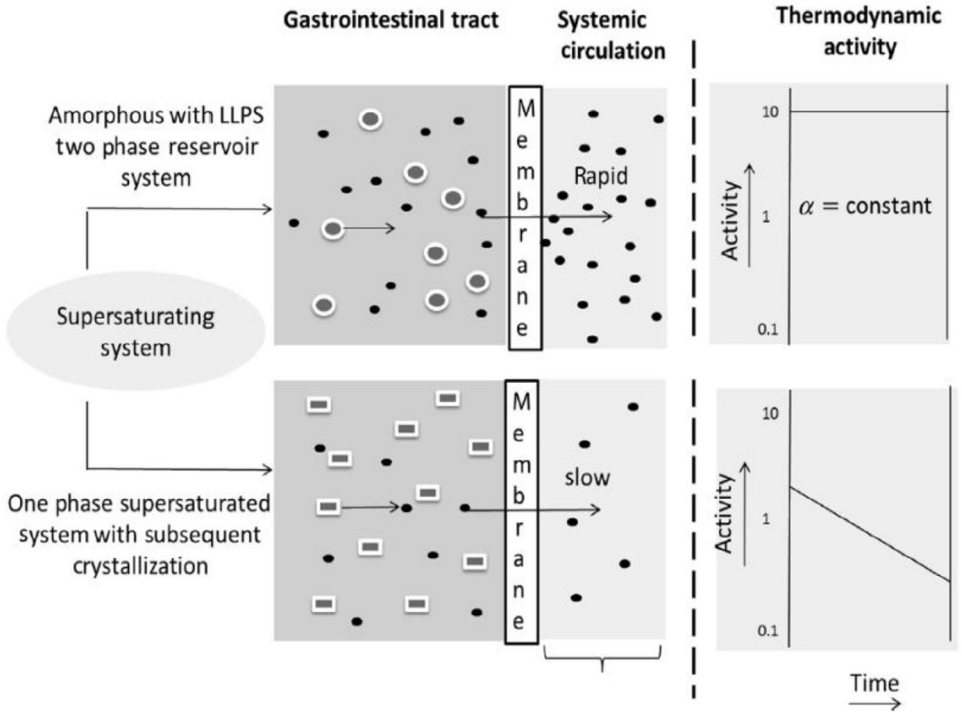

Fig. 2 – Comparison of passive diffusion and drug uptake of supersaturated systems that have undergone LLPS versus crystallization [7].

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_t/C_{eq})
Φ: heterogeneous nucleation factor ($0 < \Phi < 1$)

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).

$$\frac{dr}{dt} = \frac{D\nu N_A}{r + D/k_+}(C - C_{eq}) \quad (3)$$

Where:

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

If $r \geq D/k+$, the process is diffusion-controlled while if $r \leq D/k+$, 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 $C\text{-}C_{eq}$)
- 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. Psachoulias 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-

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	Transit Time (h)	pH	Transit 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

bility and bioavailability. Table 2 shows the pH values and transit time under both fasting and fed condition [15]. The weak base has a high solubility in stomach due to low pH environment and forms supersaturation in the intestine. Moreover, in the fed condition, the stomach pH is high and the drug can stay longer in stomach in comparison with the fasting condition.

Stomach and intestine media are also critical since they are the 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 3 [16]. 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. [17] 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 3 – Composition of FaSSIF and FeSSIF [16].

Fasted state simulated intestinal fluid(FaSSIF)			Fed state simulated intestinal fluid(FeSSIF)		
pH		6.5	pH		5
osmolality		270 ± 10 m osmol	Osmolality		635 ± 10 m osmol
Sodium taurocholate		3 mM	Sodium taurocholate		15 mM
Lecithin		0.75 mM	Lecithin		3.75 mM
KH_2PO_4		3.9g	Acetic acid		8.65g
KCl		7.7g	KCl		15.2g
NaOH	qs	pH 6.5	NaOH	qs	pH 5.0
Deionized water	qs	1 liter	Deionized water	qs	1 liter

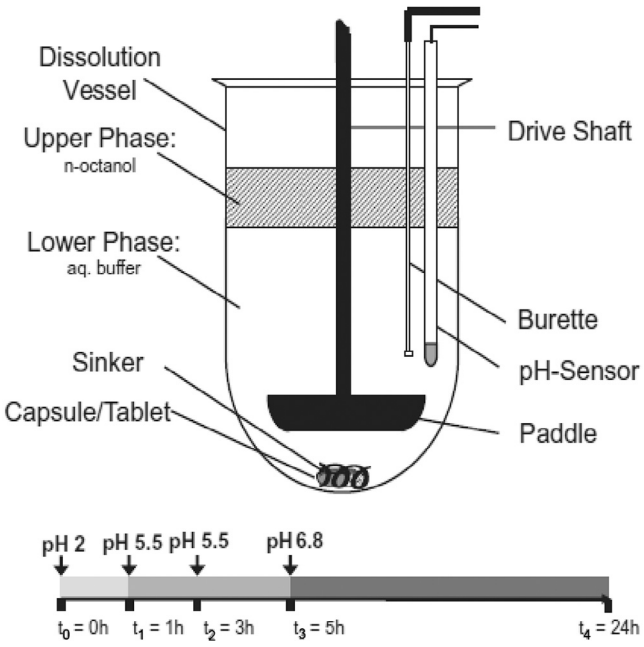

Fig. 3 – Schematic diagram of a pH-adjusted biphasic dissolution apparatus comprising two immiscible phases (aqueous and n-octanol) and a pH-controller [21].

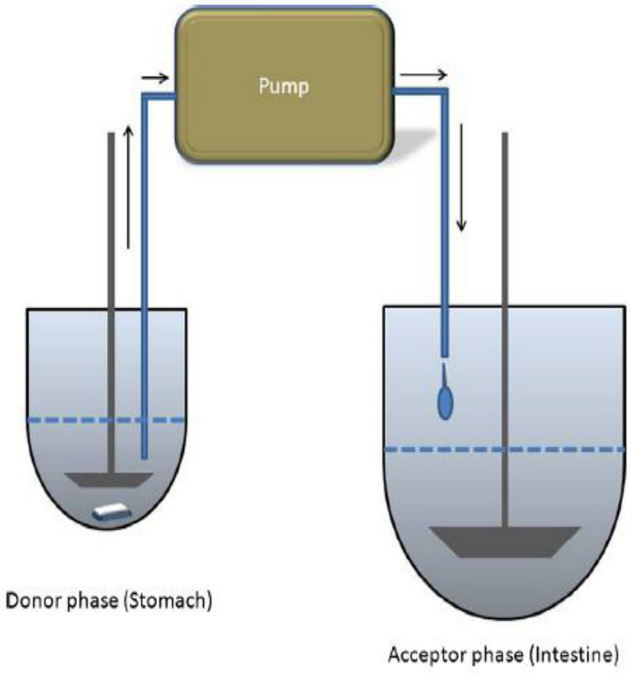

Fig. 4 – Transfer model for prediction of intestinal precipitation [23].

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

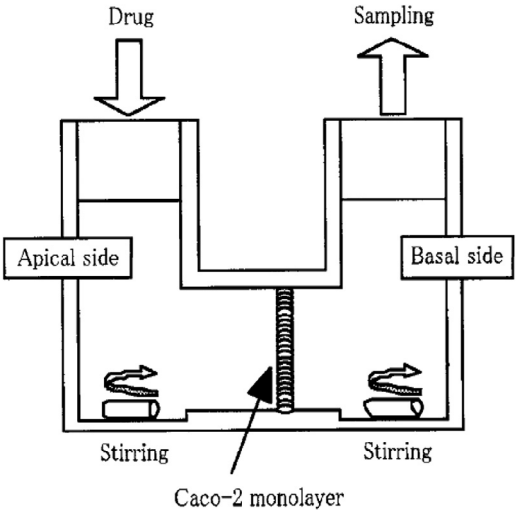

Fig. 5 – Schematic illustration of the dissolution/ permeation system (D/P system). Caco-2 monolayer was mounted between the apical and basal chambers [26] .

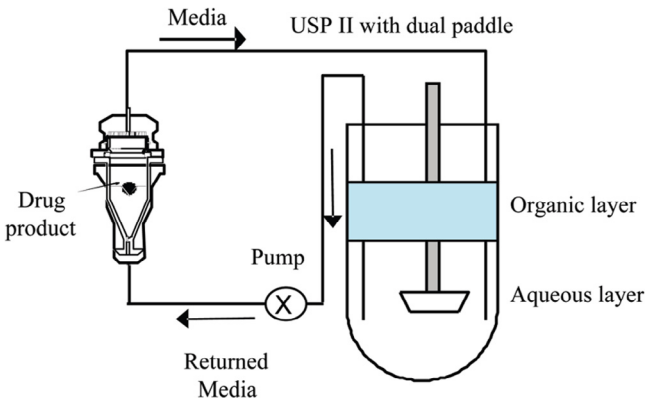

Fig. 7 – Schematic diagram of biphasic test system [30].

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

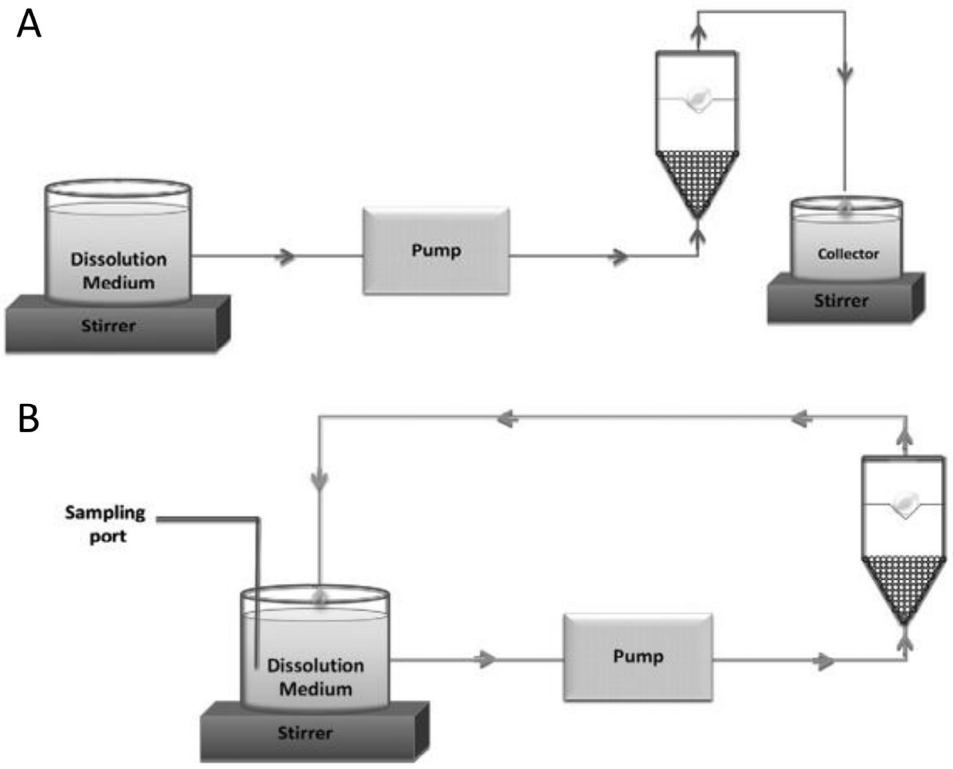

Fig. 6 – Schematic illustration of flow-through cell apparatus (FTC) (A: open-loop configuration; B: closed-loop configuration) [23].

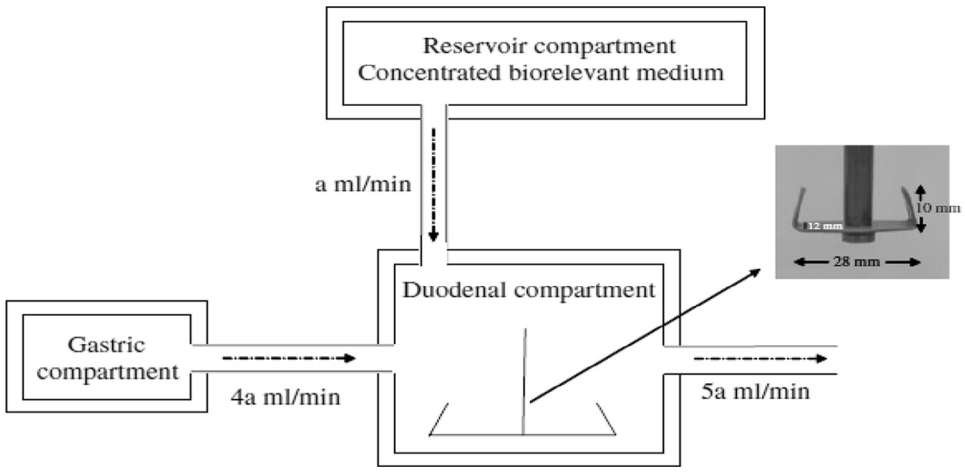

Fig. 8 – Scheme of the three compartment setup and photograph of the paddle used for agitating contents of the duodenal compartment [14].

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 methodology. 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

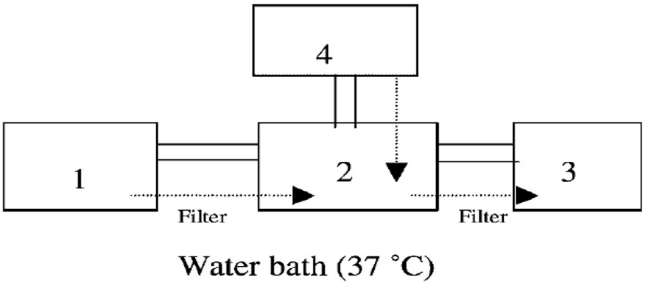

Fig. 9 – Scheme of multicompartment dissolution system. Vessel 1: "gastric" compartment, simulating the stomach conditions; Vessel 2: "intestinal" compartment, simulating the intestinal conditions; Vessel 3: "absorption" compartment, simulating absorption; Vessel 4:reservoir vessel, containing the dissolution medium identical to that in Vessel 2 [32].

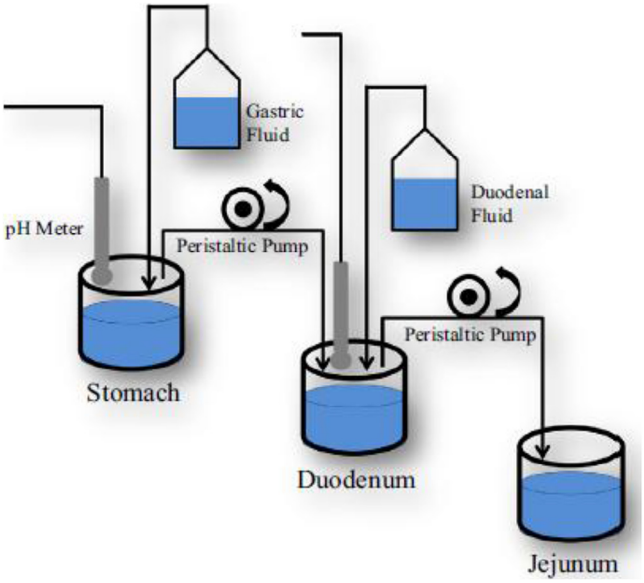

Fig. 10 – The diagram of Gastrointestinal Simulator (GIS) [33].

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

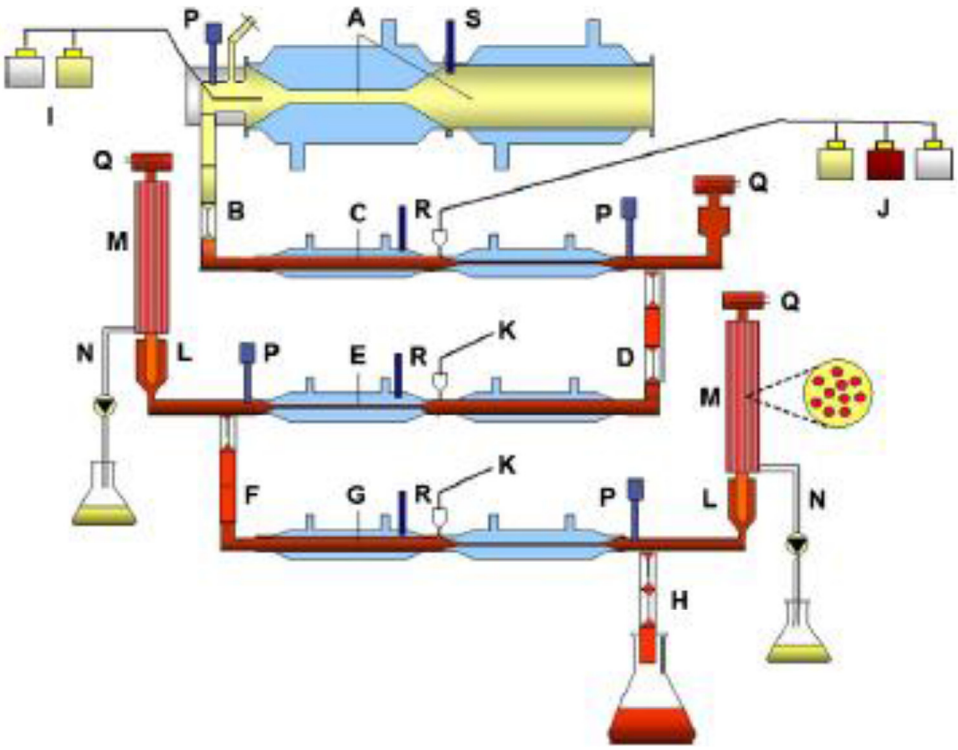

Fig. 11 – TIM-1 system (A. Stomach compartment; B. Pyloric sphincter; C. Duodenum compartment; D. Peristaltic valve; E. Jejunum compartment; F. Peristaltic valve; G. Ileum compartment; H. Ileo-caecal sphincter; I. Stomach secretion; J. Duodenum secretion; K. Jejunum/ileum secretion; L. Pre-filter; M. Semi-permeable membrane; N. Filtrate pump; P. pH electrodes; Q. Level sensors; R. Temperature sensor; S. Pressure sensor) [23].

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, cocrystal, 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 by 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.

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