International Journal of Pharmaceutics 393 (2010) 1–16

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

In vitro methods to assess drug precipitation

Wei-Guo Dai*

Johnson & Johnson Pharmaceutical Research & Development, LLC., 145 King of Prussia Road, Radnor, PA 19087, USA

ARTICLE INFO

Article history:
Received 25 October 2009
Received in revised form 13 March 2010
Accepted 16 March 2010
Available online 27 March 2010

Keywords:
Drug precipitation
In vitro precipitation assay
Intravenous formulation
Oral formulation

ABSTRACT

Drug precipitation *in vivo* is often an undesirable outcome after administration of a drug formulation into a human body. It may reduce drug concentration for immediate action, leading to a delayed or reduced efficacy. There are few practical *in vivo* assays available for evaluation of drug precipitation. Effective and efficient *in vitro* precipitation screening assays are highly desirable. In recent years, *in vitro* assays for assessment of drug precipitation potential have become available. The aim of this article is to provide the reader with a brief review of such *in vitro* precipitation screening assays for intravenous and oral formulations.

Contents

1. Introduction

Drug precipitation *in vivo* is often an undesirable outcome after administration of a drug formulation into a human body. It is a process in which a drug solute precipitates *in vivo* when the solubilization capacity of the formulation for the drug has decreased. Drugs may precipitate *in vivo* due to sharp pH change, formulation dilution with body fluids, or digestion of solubilizing excipients in formulations (Kaukonen et al., 2004b; Porter et al., 2007; Schroeder and DeLuca, 1974). Such precipitation often reduces drug concentration in aqueous phase needed for immediate action, leading to a delayed or reduced efficacy (Hoener and Benet, 2002). In addition, drug precipitation in the vein after intravenous administration may produce severe adverse venous irritation/inflammation (phlebitis)

* Correspondence address: Tel.: +1 610 240 8192; fax: +1 610 889 4677.
E-mail address: wdai3@its.jnj.com.

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.03.040

(Falchuk et al., 1985; Turco, 1975). Therefore, it is critical to assess drug precipitation *in vivo* during formulation screening and development.

While *in vivo* studies provide the most direct measurement of drug precipitation after administration, they are expensive and labor extensive. Very often, observation and quantitative measurement of drug precipitation *in vivo* pose a great technical challenge, particularly for oral formulations. As a result, there are few in vivo precipitation studies reported (Buck et al., 1985; Davio et al., 1991; Kovach et al., 1985; Lieber et al., 1986; Powis and Kovach, 1983; Tsukamoto et al., 1985). In contrast, *in vitro* studies for assessment of drug precipitation potential are inexpensive and rapid. The study results can be helpful in screening early formulations in preparation for preclinical studies and initial clinical trials. It is highly desirable to develop effective and efficient *in vitro* precipitation screening assays that correlate with *in vivo* precipitation.

In recent years, *in vitro* drug precipitation assays for formulation screening and development have become available. These assays enable the assessment of drug precipitation potential for various solubility-enhancing formulations. The aim of this article is to provide the reader with a brief review of such *in vitro* precipitation screening assays for intravenous and oral formulations.

2. Precipitation assays for intravenous formulations

2.1. Introduction

Intravenous formulations by injection or infusion administration have been widely used due to their rapid and excellent bioavailability. They typically require drugs to be solubilized in a low-viscosity aqueous solution. This may pose a great challenge for poorly water-soluble drugs. In order to formulate drugs in an injectable aqueous formulation, various solubilization techniques are often used such as changing pH of an aqueous formulation, and using water-miscible cosolvents, complexing agents and surfactants (Liu, 2000; Strickley, 2004).

However, drugs solubilized by these techniques in intravenous formulations could be subject to precipitation when injected or diluted with bloodstream (Pfeifle et al., 1981; Schroeder and DeLuca, 1974; Surakitbanharn et al., 1994). Formulation pH that is adjusted to solubilize drugs in intravenous formulations may change upon dilution with bloodstream, thereby leading to a reduced drug aqueous solubility if drug solubility is pH-dependent. Also concentrations of water-miscible cosolvents in intravenous formulations may drop quickly upon dilution and they are no longer able to maintain drug solubilized in formulations. Furthermore, extensive dilution of formulations in bloodstream may reduce surfactant concentrations below their critical micellar concentrations, leading to a drug precipitation (Yalkowsky, 2000).

Drug precipitation after intravenous administration often reduces drug concentration in the aqueous phase, and will further decrease drug bioavailability if precipitates cannot redissolve *in vivo*. A delayed drug efficacy can also occur if drug precipitates are embedded into vein wall for slow redissolution. As a result, drug precipitation can result in an uneven, delayed or reduced efficacy (Yalkowsky et al., 1998).

In addition to an altered bioavailability, drug precipitates in the vein after intravenous administration of formulations lead to severe phlebitis (Falchuk et al., 1985; Turco, 1975), or pulmonary embolism (Taniguchi et al., 1996, 1998). Such venous inflammation might be caused by mechanical abrasion when irregularly shaped drug particles produced on precipitation scratch vein wall through bloodstream (Avis et al., 1986). It can be also resulted from chemical irritation when drug precipitates become embedded into vein wall and, and cells are exposed to drug precipitates for extended period of time (Hecker et al., 1984). Both mechanical and chemical irritation caused by drug precipitates within the vein is one of the key factors in determining the duration and severity of undesirable phlebitis (Schroeder and DeLuca, 1974; Turco, 1975). Therefore, avoidance of drug precipitation is one of the most critical considerations in intravenous formulation development.

Ideally, drug precipitation is evaluated *in vivo* after dosing of intravenous formulations. Such attempts have been reported in the literature. For example, Buck et al. (1985) infused bisantrene in 5% dextrose formulations into the marginal ear vein of a rabbit, and then examined drug precipitates in the excised ear vein (Powis and Kovach, 1983). Using this *in vivo* method, they found bisantrene precipitates both in the vein and embedded in the inner wall of the vein. In addition to rabbit model, intravascular precipitation of bisantrene in the arteriolar and capillary bed of calves was reported after intravenous administration of formulations (Buck et al., 1985; Kovach et al., 1985; Lieber et al., 1986; Tsukamoto et al., 1985). Furthermore, Davio et al. (1991) infused ditekiren formulations through indwelling catheters in the right internal jugular vein of monkey and reported intravascular ditekiren precipitation in monkeys during a 30-day infusion study. These *in vivo* studies confirmed qualitatively *in vivo* precipitation of the tested formulations in animal models. These assays only provide the direct observation and qualitative measurement of drug precipitation *in vivo* after intravenous administration of formulations, and typically they are not able to quantitate drug precipitates accurately. In addition, these assays are labor extensive and time consuming. In contrast to the inherent difficulty of performing *in vivo* studies, *in vitro* drug precipitation can be measured and quantitated. Several *in vitro* assays have been developed to provide a simple, rapid and quantitative measurement of drug precipitation for intravenous formulations.

2.2. Static precipitation assays

Static *in vitro* assays have been developed to evaluate drug precipitation of intravenous formulations upon dilution. The first drug precipitation study for intravenous formulations was described by Schroeder and DeLuca (1974). They mixed 10 mL of human plasma with a varying amount of a diphenylhydantoin formulation solution at 1 mg/mL (from 0.157 mL to 1 mL), depending on the dilution factors. The mixture was filtered through a 0.45 μm membrane and the amount of the precipitates was quantitated by gravimetric analysis. In their studies they noticed drug precipitation immediately once the formulation was diluted with human plasma. Also the amount of diphenylhydantoin precipitates increased when dilution ratio of plasma to drug formulation decreased. Similar approaches have been also described to investigate *in vitro* precipitation of intravenous formulations containing ditekiren (Greenfield et al., 1991), aminosteroid antioxidant and phenytoin (Cox et al., 1991) and diphenylhydantoin (Markowsky et al., 1991).

In addition to this one-step dilution assay, a static serial dilution assay has been described to evaluate drug precipitation of intravenous formulation upon dilution (Alvarez-Nunez and Yalkowsky, 1999; Dannenfelser et al., 1996; Li et al., 1999, 1998). In the assay, drug formulation is diluted in a one-to-one ratio with dilution media such as normal saline or human plasma, and the mixture is agitated for approximately 3 s. If no precipitate is found visually after mixing, this mixture is further diluted with dilution media and agitated. The dilution, agitation and evaluation of precipitation are repeated ten times. The rate and the amount of precipitation are either qualitatively examined by visual observation, or quantitatively determined by measuring the amount of precipitate in the filtrate after a filtration step. This assay has been used to evaluate *in vitro* precipitation of intravenous formulations of flavopiridol (Dannenfelser et al., 1996; Li et al., 1999, 1998), phenytoin (Alvarez-Nunez and Yalkowsky, 1999), and diazepam (Li et al., 1998). The

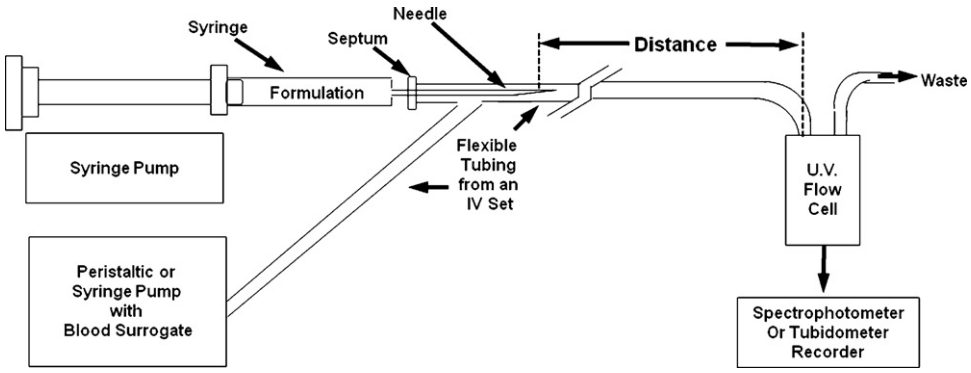


Fig. 1. Dynamic *in vitro* apparatus for evaluating precipitation upon injection, reproduced with permission (Johnson et al., 2003).

study results suggest that this assay seems to be most effective in quantifying the amount of precipitation and more descriptive of the formation and redissolution of the precipitate than the other static assays (Li et al., 1998).

Also, a dropwise addition assay has been reported (Li et al., 1998). Unlike the serial dilution assay that involves serial dilution of formulations with a dilution medium, this assay evaluates the potential of a formulation to precipitate by a serial addition of formulations into a fixed volume of dilution medium. Typically a formulation is added sequentially to a testing dilution media (typical 1:10 dilution ratio). If no precipitate is found visually after mixing, another aliquot of formulation is added. This procedure is repeated until a precipitate is found visually. The dropwise addition assay can be also performed without agitation until a precipitate formed does not redissolve in 30 s. The potential of drug precipitation upon dilution is measured by the minimum volume ratio of formulation to dilution media that would produce drug precipitates. Li et al. used the assay to measure the rate and the amount of precipitation of flavopiridol and diazepam formulations. They found that this assay was rapid and simple mean of assessing the potential of a formulation to precipitate upon dilution (Li et al., 1998).

The static precipitation assays utilize simple mixing or dilution of drug formulations with testing media, and then examine or measure amount of drug precipitates produced after dilution. The assays provide a rapid and direct observation and measurement of drug precipitation for intravenous formulations upon dilution. They also enable to study drug precipitation at various dilution media and dilution ratios. However, these assays are static, and may not represent dynamic scenarios where intravenous formulations are injected or infused continuously into the flowing bloodstream.

2.3. Dynamic precipitation assays

In order to simulate fluid dynamics at an injection site, Yalkowsky et al. have developed a dynamic *in vitro* assay to assess drug precipitation for intravenous formulation upon dilution (Fig. 1) (Johnson et al., 2003; Yalkowsky and Valvani, 1977;

Table 1
In vitro precipitation of twenty-one marketed intravenous products at different injection rates.[a].

Drug	Phlebitis reported	Average opacity ranked	Opacity by injection rate[b]		
			1 mL/min	5 mL/min	10 mL/min
Prop2[c]	Yes[d]	4.02	4.26	4.31	3.48
Prop1[c]	Yes[d]	2.67	4.43	1.67	1.91
Valium	Yes	1.70	0.67	2.1	2.35
Dilantin	Yes	1.196	1.87	1.05	0.67
Cordarone	Yes	0.315	0.24	0.32	0.39
Nafcillin	Yes	0.186	0.020	0.22	0.32
Phytonadione	Yes	0.027	0.000	0.030	0.060
Lidocaine	Yes	0.019	0.010	0.020	0.030
Ciprofloxacin	Yes	0.006	0.0090	0.0067	0.0023
Acyclovir	Yes[e]	0.005	0.0035	0.0050	0.0056
Ofloxacin	Yes	0.003[f]	0.0025	0.0035	0.0020
Dobutamine	Yes	0.003[f]	0.0044	0.0013	0.0019
Dopamine	No	0.003[f]	0.0	0.0027	0.0054
Epinephrine	No	0.003[f]	0.0020	0.0029	0.0042
Atropine sulfate	No	0.001	0.0019	0.0010	0.0014
Bretylium tosylate	No	0.001	0.0019	0.0010	0.0014
Diltiazem	No	0.001	0.0	0.0023	0.0010
Normal saline	No	0.0008	0.00040	0.00082	0.0013
Dextrose, 5%	No	0.0	0.0	0.0	0.0
Furosemide	No	0.0	0.0	0.0	0.0
Sterile water	No	0.0	0.0	0.0	0.0

[a] All reports of phlebitis were obtained from the Physcians' Desk Reference, except proprietary formulations and Acyclovir.
All injectables were prepared for testing according to the manufacturers' instructions.
[b] The ISPB flow rate was 5 mL/min for all injections.
[c] Proprietary formulations.
[d] Company report.
[e] Arndt, K.A., 1988. J. Am. Acad. Dermatol. 18, 188–190.
[f] Designates delineating opacity value for determining phlebitic potential.
Reproduced with permission (Johnson et al., 2003).

Table 2
Formulation-diluent ratios which cause precipitation in four *in vitro* precipitation methods.

Formulations	Formulation-diluent ratios which cause precipitation			
	Static serial dilution	Dynamic injection	Dropwise with stirring	Dropwise without stirring
Flavopiridol	0.03	0.1	0.1	0.3
Diazepam	0.03	0.1	0.07	0.19

Reproduced with permission (Li et al., 1998).

Yalkowsky et al., 1983). A dilution medium is pumped through tubing by a peristaltic pump to mimic *in vivo* dynamic scenario, while a tested formulation is injected by a syringe pump into dilution media at a specified rate. Amount of drug precipitates is quantitated turbidimeterically using a spectrophotometer. Similar dynamic *in vitro* assays have been also reported for evaluation of precipitation of intravenous formulations containing bropirimine (Irwin and Iqbal, 1992), ditekiren (Davio et al., 1991) and phenytoin (Cox et al., 1991).

Using the dynamic assay, Yalkowsky et al. investigated precipitation of intravenous formulations containing amiodarone HCl (Ward and Yalkowsky, 1993a,c), phenytoin (Alvarez-Nunez and Yalkowsky, 1999), flavopiridol (Li et al., 1998), diazepam (Li et al., 1998), and vancomycin (Johnson and Yalkowsky, 2006) at various experimental conditions. They were able to establish the correlation of precipitation and the degree of dilution, and found that the amount of drug precipitates was inversely proportional to the injection rate. However, a study result with twenty-one marketed intravenous products indicates that the correlation of injection rate with drug precipitation appears to be drug dependent; the formulations of Valium, Cordarone, Nafcillin, and Phytonadione produced less precipitates at a lower injection rate, while for intravenous formulations of Dobutamine and Dilantin, the opposite trend was observed (Table 1) (Johnson et al., 2003). How the injection rate influences drug precipitation is not well-understood yet. One hypothesis is that entire injected dose exhibits a plug-like behavior if the injection rate of formulations is higher than that of dilution flow. Therefore, precipitation occurs mostly at the edges of the plug, and the center of the plug remains relatively precipitation free upon dilution (Ward and Yalkowsky, 1993c; Yalkowsky et al., 1998). In contrast, for some formulations a slow injection into a constant flowing bloodstream may produce a rapid dilution. There may not be sufficient time for nucleation before the drug becomes soluble (Johnson et al., 2003).

2.4. Static vs. dynamic precipitation assays

One of the approaches to evaluate static and dynamic precipitation assays is to compare drug precipitation results from both assays. Alvarez et al. measured precipitates of diphenylhydantoin formulations upon dilution with isotonic Sorenson's phosphate buffer using both static and dynamic assays. They found that drug precipitation results from both assays were comparable (Alvarez-Nunez and Yalkowsky, 1999). Li et al. also reported the similar conclusion for flavopiridol and diazepam formulations. They measured drug precipitation using four precipitation methods (static serial dilution, dynamic injection, dropwise addition stirring, and without stirring). The precipitation for flavopiridol and diazepam formulations followed similar trends in all methods and four *in vitro* methods were comparable and complementary (Table 2) (Li et al., 1998).

However, other studies showed that dynamic precipitation assays generated different results compared with static assays. Cox et al. (1991) examined precipitation of a 21-aminosteroid antioxidant using both static and dynamic assays. They found that all liquid clear formulations of tested compounds showed minimal precipitates in the static assay, but produced a significant amount of drug precipitates in the dynamic assay. Whether drug precipitation pattern is comparable from static and dynamic assays may be dependent on the specific drugs or formulations.

Another approach to evaluate static and dynamic assays is to compare their *in vitro*/*in vivo* correlations. Davio et al. (1991) studied *in vivo* precipitation of ditekiren formulation in a 14-day infusion study in the cynomolgus monkeys. They found that precipitation results from the dynamic injection assay, but not static assay, correlated with *in vivo* precipitation results in monkeys. The intravenous formulations that did not show precipitation in the static assay did produce significant drug emboli in the heart and lungs of the monkeys. Their results were in agreement with the precipitation study of ditekren formulations by others (Greenfield et al., 1991). These studies suggest that the dynamic assays seem to be more predictive of *in vivo* precipitation than the static assays.

In addition to direct measurement of drug precipitates *in vivo*, phlebitis has been used as an indicator of *in vivo* drug precipitation to evaluate the predictive *in vivo* performance of precipitation assays. Yalkowsky et al. studied precipitation of amiodarone formulations by the dynamic assay, and then examined *in vivo* phlebitis and hemolysis in rabbits after intravenous dosing of formulations (Ward and Yalkowsky, 1993a,b,c; White and Yalkowsky, 1991). Their results showed that drug precipitation at different injection rates determined by the dynamic assay was in agreement with *in vivo* observation of phlebitis (Yalkowsky et al., 1998). Furthermore, validation studies of the dynamic assay using twenty-one marketed intravenous products suggested that the *in vitro* drug precipitation results from the dynamic assay correlated with literature reports on phlebitis occurrence caused by *in vivo* drug precipitation. These study results support the possibility of using the dynamic assay for predicting *in vivo* drug precipitation, and potential phlebitis caused by drug precipitates (Johnson et al., 2003).

The results of *in vitro*/*in vivo* correlation study seem to agree that dynamic assays give results that are more meaningful and correlate better with *in vivo* data than static assays. Nevertheless, the simulation in the dynamic assays is far away from the complicated scenarios *in vivo*, and very importantly, evaluation of static and dynamic assays is often conducted with a few model drugs. The conclusions need rigorous validations using considerably large number of drugs in different intravenous formulations. Furthermore, if phlebitis is used as an indicator of *in vivo* drug precipitation to evaluate *in vivo* predictive performance of the precipitation assays, it is important that occurrence and degree of phlebitis be quantitated accurately, which could be a challenge with current techniques available.

In addition, the static and dynamic precipitation assays described in the literature usually measure drug precipitates visually or turbidimeterically. Visual observation of drug precipitation is subjective, whereas turbidimeterical measurement of opacity is a crude measure of drug precipitation; therefore these assays are often semiquantitative. Furthermore, opacity measurement in turbidimeterical methods is based on particles or light scattering, and any factors that result in turbidity or light scattering could interfere with data. Thereby these assays provide a quick evaluation of drug precipitation, but there is a lack of specificity for the compound of interest. The quantitative measurement of drug concentration

using HPLC-UV can address this issue but would also increase the time required for each measurement.

It should also be noted that the *in vitro* precipitation assays for intravenous formulations typically focus on the measurement of the extent of drug precipitation and treat precipitates as non-dissolvable. The *in vitro* precipitation studies usually do not provide further investigation of solid state properties of precipitated materials. However, drug precipitates may redissolve in the blood stream, depending on chemical natures and solid state properties of drug precipitates such as amorphous or polymorphic form, and particle size. It would be important to further investigate solid state properties of such precipitated materials using *in vitro* precipitation assays to gain understanding of the nature of drug precipitates and mechanism of precipitation.

3. Precipitation assays for oral formulations

3.1. Introduction

Oral dosage formulations are often used in commercial products due to patient convenience, good stability, and low cost. They can be the form of liquid, semi-solid, and solid. Drugs formulated in oral formulations may precipitate *in vivo* when the formulations are extensively diluted by gastrointestinal tract fluid, or subject to a pH change in the gastrointestinal tract. For example, an oral formulation containing a solubility-dependent basic drug may dissolve completely in the stomach, but drug would precipitate in the intestine due to a sharp pH increase which leads to a low aqueous drug solubility (Kostewicz et al., 2004).

Drug precipitation is one of the several dominant factors leading to low oral bioavailability of poorly water-soluble compounds (Hoener and Benet, 2002). Such insufficient bioavailability of these compounds may result in delays in development or cause them to be dropped from the pipeline (Prentis et al., 1988). As a result, avoidance of drug precipitation is one of the most important considerations for oral formulation screening and development (Pouton, 2000). Since it has been a significant technical challenge to develop an *in vivo* assay that enable to observe and quantitate drug precipitation in the gastrointestinal tract after oral dosing, drug precipitation potential for oral formulations is often evaluated using *in vitro* assays.

3.2. USP dissolution methods with a basket (USP I) or a paddle (USP II)

Drug dissolution/precipitation in oral formulations is often evaluated using USP I or II dissolution methods. Typically, oral formulation solutions or solids (tablets or capsules) are introduced into a USP bath maintained at 37 °C. An appropriate testing medium in the bath is stirred with a basket or a paddle under a selected stirring rate. Drug concentration with time, a measurement of drug dissolution/precipitation, is measured by a UV or HPLC method.

USP I/II dissolution methods have been extensively used to study drug dissolution/precipitation of oral formulations under different testing parameters and conditions including testing media (Corrigan et al., 2003; Galia et al., 1998; Nicolaides et al., 1999; Stippler et al., 2004; Tang et al., 2001), stirring rate (Carvalho-Silva et al., 2004; Qureshi, 2004, 2006; Rost and Quist, 2003), and hydrodynamics in the bath (Bai and Armenante, 2008; Bai et al., 2007; Baxter et al., 2005; Kukura et al., 2004; Mirza et al., 2005). The correlation between *in vitro* dissolution/precipitation and *in vivo* absorption results has been reported in the literature. In addition, USP dissolution methods have demonstrated excellent robustness as quality control methods, and the dissolution testing has become one of the most important FDA required *in vitro* tests for oral formulation screening and development (Shah, 2005; Uppoor, 2001).

Although pH and media composition in USP I/II dissolution methods can be adjusted by adding or exchanging buffers, for routine testing the fixed pH and media are usually used in these methods. In contrast, drug dosage after oral dosing is exposed to a varying dissolution environment in the gastrointestinal track. Drugs with pH-dependent solubility may undergo dissolution, precipitation, and redissolution processes throughout the gastrointestinal tract because of the dramatic changes in solubility as pH changes. For example, a weak base with poor intrinsic solubility may dissolve completely in the stomach but precipitate in the intestine (Kostewicz et al., 2004). Such precipitation may not be obvious in the typical USP I/II dissolution methods at a constant pH level unless additional buffers are added to change pH and media composition during testing.

3.3. USP dissolution methods with flow-through cells (FTC)

In order to simulate more closely the pH gradient in the gastrointestinal tract, a USP dissolution method using FTC has been developed to evaluate drug dissolution/precipitation for oral solid dosage forms. A tablet is typically horizontally positioned in the sample holders in the cells. A dissolution medium at 37 °C flows through the cells. The pH and composition of the testing medium can be changed to more closely mimic scenarios in the gastrointestinal tract during each run. Precipitation is evaluated by drug concentration assayed by a UV spectrophotometer.

USP dissolution FTC methods have been used to evaluate drug dissolution/precipitation of various oral solid dosage forms (Emara et al., 2000; Moeller and Wirbitzki, 1990, 1993; Nicklasson et al., 1987; Perng et al., 2003; Phillips et al., 1989; Qureshi et al., 1994; Sunesen et al., 2005; Wennergren et al., 1989). Compared with USP I/II dissolution methods, FTC methods have the advantage of allowing a change in the dissolution medium during each run, which allows for better simulation of the pH gradient and fluids associated with transit throughout the gastrointestinal tract (Thoma and Ziegler, 1998). Also, they simulate *in vivo* hydrodynamics more closely than the USP I/II dissolution methods (Perng et al., 2003; Qureshi et al., 1994), and demonstrate good *in vitro*/*in vivo* correlations (Derendorf et al., 1983; Ikegami et al., 2003; Qureshi et al., 1994; Sunesen et al., 2005). Another advantage is that FTC methods at low flow rates are more sensitive to detect differences in the disintegration properties than other USP dissolution methods (Wennergren et al., 1989). Furthermore, they require relatively smaller sample holdup volume.

3.4. Modified USP dissolution methods with multicompartments

In order to specifically evaluate drug precipitation of oral solid dosage formulations, modified USP dissolution methods with multicompartments have been reported. Unlike the conventional USP methods with only one compartment, the modified methods feature multiple compartments that aim to more closely mimic different regions in the gastrointestinal tract. One of the methods, described by Kostewicz et al. (2004) contains two compartments that simulate stomach and intestine, respectively. To simulate the transfer from the stomach into the intestine, a drug solution in simulated gastric fluid compartment is continuously pumped into a simulated intestinal fluid compartment, and drug precipitation in the acceptor medium is evaluated via concentration–time measurement (Fig. 2).

Using this two-compartment model, Kostewicz et al. examined the *in vitro* precipitation of three poorly soluble weakly basic drugs, dipyridamole and two NMEs (BIBU 104 XX and BIMT 17 BS). They found that all three formulations had a potential of drug precipitation with time in the acceptor medium. Since the solubility of dipyridamole, BIMT 17 BS and BIBU 104 XX in fed state simulated

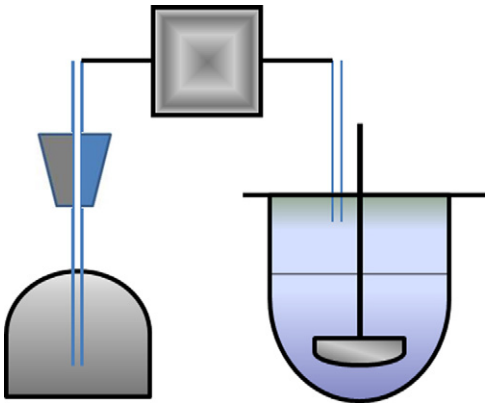


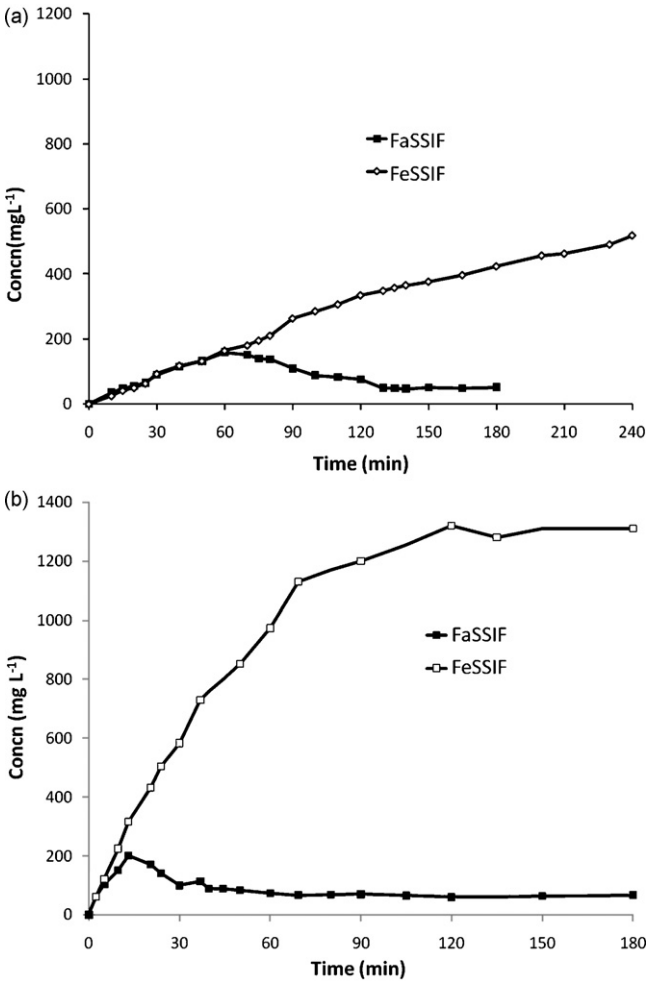
Fig. 2. Two-compartment model for evaluation of drug precipitation. An appropriate amount of drug powder was completely dissolved in SFG_{fast} (donor phase). A peristaltic pump was used to transfer the donor phase into a dissolution vessel containing 500 mL of either FaSSIF or FeSSIF as the acceptor phase. The acceptor phase media was maintained at 37 ± 0.5 °C, reproduced with permission (Kostewicz et al., 2004).

intestinal fluid (FeSSIF) was much higher than those in fasted state simulated intestinal fluid (FaSSIF), drug precipitation was highly dependent on the testing media; precipitations occurred for all three compounds in FaSSIF, whereas no drug precipitation was found in FeSSIF (Fig. 3). The study results suggest that this assay can be used to specifically evaluate and predict the precipitation profiles of the tested drugs at various conditions, and has a reasonable prediction of *in vivo* behavior of the tested poorly soluble weak basic compounds (Kostewicz et al., 2004).

In addition to the two-compartment model, a multicompartment model has been reported (Gu et al., 2005). The model contains 4 compartments. "Gastric", "intestinal" and "absorption" compartments simulate stomach conditions, intestinal conditions, and absorption, respectively, and the fourth compartment serves as a reservoir. All compartment are maintained at 37 °C in a water bath, and pH in the compartment is controlled by a pH stat. Gu et al. used this 4-compartment USP method to examine the effect of gastric pH on drug precipitation and possible *in vivo* exposure of two poorly soluble weak basic drugs, dipyridamol and cinnarizine. Their study results demonstrate that this method is able to predict drug precipitation potential in the simulated intestinal media (Fig. 4), and has a reasonable correlation with *in vivo* exposure of the tested drugs (Gu et al., 2005).

The multicompartmental USP dissolution methods exhibit advantages over the single compartmental methods. They not only allow directly evaluating drug precipitation behavior, determining the contribution of dissolution and precipitation as a possible cause for low oral absorption, but also enable estimating the precipitation potential to diagnose whether precipitation is the leading contributor to such a poor bioavailability (Gu et al., 2005). Also, using multicompartments to more closely simulate *in vivo* scenarios, the methods provide more realistic prediction of precipitation of compounds in the gastrointestinal tract (Gu et al., 2005; Kostewicz et al., 2004). Furthermore, they are more efficient than conventional dissolution methods. For example, in order to study precipitation of a poorly water-soluble weak basic compound in stomach and intestine, two dissolution tests with different dissolution media are needed with a conventional USP method, whereas the multicompartmental methods enable to estimate drug precipitation potential in one study (Kostewicz et al., 2004; Gu et al., 2005).

These modified USP methods utilize multicompartments to more closely simulate *in vivo* scenarios. However, drug dissolution, precipitation and absorption *in vivo* is far more complicated, and not well understood yet. For example, drug precipitation *in vivo* is influenced by many critical factors such as hydrodynamics, gastrointestinal fluid composition and pH, and empting rate. The modified USP methods need further improvement by incorporating these factors for better prediction of *in vivo* performance. Another limitation is that drug precipitation is often evaluated without considering the absorption of drug. If a drug is absorbed fast, or drug precipitates observed in these modified USP methods redissolve in the gastrointestinal tract and reabsorbed, such methods would overestimate drug precipitation potential.



Solubility of Dipyridamole, BIMT 17 BS and BIBU 104 XX in FaSSIF and FeSSIF**

	Solubility (mg/mL)	
	FaSSIF	FeSSIF
Dipyridamole	25	400
BIMT 17 BS	33.3	250
BIBU 104 XX	4.6	25

Fig. 3. Measured dipyridamole concentration in FaSSIF ($n = 3 \pm$ s.d.) and FeSSIF ($n = 1$) using a paddle speed of 75 rev min^{-1} at transfer rate, (a) 0.5 mL min^{-1}, (b) 4 mL min^{-1}, reproduced and adapted with permission (Kostewicz et al., 2004). **Solubility data is adapted with permission (Kostewicz et al., 2002).

3.5. Lipolysis assay for lipid-based formulations

In recent years lipid-based formulations have been receiving considerable interests for enhancing solubility and improving absorption of poorly water-soluble compounds. These formulations typically contain lipid-based oils, surfactants, and hydrophilic surfactants. Unlike conventional formulations in which a drug usually dissolves in the aqueous media upon dilution, lipid-based formulations usually form a water-immiscible oily solution upon

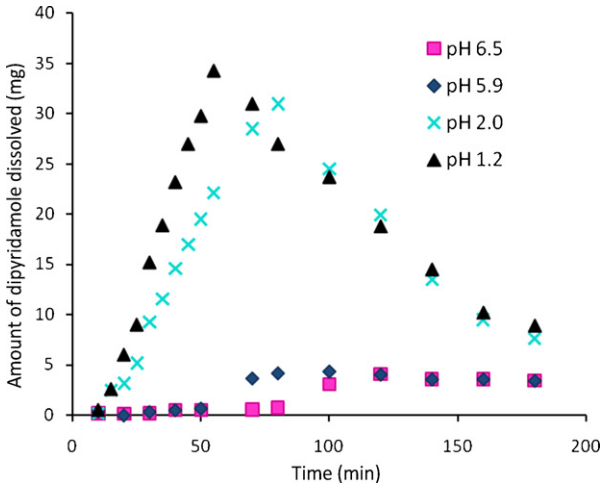


Fig. 4. Amount of dipyridamole dissolved in the "intestinal" compartment during dissolution at different pHs (50 mg), reproduced and adapted with permission (Gu et al., 2005).

dilution. The oily solution could have a large size of oil droplets that would be treated as insoluble drug precipitates if conventional precipitation assays are used. Lipids in the formulations, however, can be digested in the intestine. Subsequent to lipolysis their digested products of lipids can interact with endogenous solubilizing species including bile salts, phospholipids and cholesterol mixed micelles to form a colloidal species for solubilization of drugs, thereby *in vivo* drug precipitation would not occur. On the other hand, some lipid-based formulations are able to solubilize hydrophobic drugs and do not produce any drug precipitates in conventional precipitation assays. If the dietary lipids in the lipid-based formulations, however, are digested and dispersed in the gastrointestinal tract, lipids may lose solubilization capability after lipolysis process, leading to drug precipitation *in vivo* (Kaukonen et al., 2004a,b; Porter et al., 2004a,b). As a result, conventional precipitation assays based on particle size of the dispersion may not be appropriate for predicting *in vivo* precipitation of a lipid-based formulation because solubilization status of drug in such formulations will vary as a function of time and the simultaneous lipid-digestion process *in vivo*.

Recognizing that lipid digestibility is an essential determinant of drug solubilization/precipitation after oral dosing of lipid-based formulations, *in vitro* lipid lipolysis assays that are more reflective of the gastrointestinal environment have been developed to better predict *in vivo* drug solubilization/precipitation of lipid-based formulations (Christensen et al., 2004; Dahan and Hoffman, 2006, 2007; MacGregor et al., 1997; Porter et al., 2004a,b; Reymond and Sucker, 1988; Sek et al., 2002; Zangenberg et al., 2001a,b).

Fig. 5 shows a typical *in vitro* lipolysis assay (Porter et al., 2007). Lipolysis experiments are conducted in a dissolution vessel with a digestion medium containing bile salts, phospholipids, and dietary lipids. After a formulation is well-dispersed in the medium, lipase/co-lipase enzymes are added into the medium. Subsequent to lipolysis, pH of the medium is maintained by a computer-controlled pump. The medium is withdrawn at pre-set time interval. After centrifugation, samples are separated into three phases, an aqueous phase containing dissolved drugs along with lipolysis products, a lipid phase containing drug remaining in the lipid, and the sediment containing insoluble drug precipitates. The *in vitro* lipolysis assays control temperature, enzymes, and pH to simulate *in vivo* conditions and the precipitated drug can be quantified by analyzing the pellet in sediments during centrifugation (Christensen et al., 2004; Cuine Jean et al., 2008; Cuine et al., 2007).

In vitro lipolysis assays have been becoming well-accepted tools in assessing drug solubilization and precipitation for lipid-based formulations. Considerable studies have been conducted on lipid formulations with different fatty acid chain lengths and the degrees of unsaturation, and the different lipid classes. The study results have been well summarized in excellent reviews (Dahan and Hoffman, 2008; Dressman et al., 2007; Fatouros and Mullertz, 2007, 2008; Grove and Mullertz, 2007; Hauss, 2007; Jannin et al., 2008; Mullertz, 2007; Porter and Charman, 2001; Porter et al., 2008a,b, 2007; Pouton, 1999, 2000; Pouton and Porter, 2008; Sek, 2007; Vasanthavada and Serajuddin, 2007).

In vitro/*in vivo* correlation of lipolysis assays for lipid formulations has been reported. Porter et al. used the lipolysis assay to assess solubilization/precipitation of several lipid-based formulations of danazol and compared their *in vitro* precipitation profiles with bioavailability in beagle dog studies (Cuine Jean et al., 2008; Cuine et al., 2007; Porter et al., 2004b). They found that less precipitation formulations in the lipolysis assay had significantly higher oral absorption than those with significant *in vitro* drug precipitation. A rank-order correlation was also observed between the patterns of solubilization/precipitation obtained in the *in vitro* lipolysis assays and oral bioavailability for the tested lipid-based danazol formulations. Similar *in vitro*/*in vivo* correlation of lipolysis assays has been also reported for several lipid-based formulations of drugs such as atovaquone (Sek et al., 2006), halofantrine base (Porter et al., 2004b), progesterone and vitamin D3 (Dahan and Hoffman, 2006), dexamethasone and griseofulvin (Dahan and Hoffman, 2007). These studies demonstrate the potential utility of *in vitro* lipolysis assays in assessing and ranking order *in vivo* performance of lipid-based formulations.

Despite the reasonable *in vitro*/*in vivo* correlation reported in the literature, these study results are obtained with a small number of model drugs and lipid formulations. It would be desirable to test a variety of drugs, and considerably larger number of lipid formulations with different lipid classes, fatty acid chain lengths and the degrees of unsaturation (Porter et al., 2008a). In addition, when lymphatic transport is a significant route of absorption, the *in vitro* lipolysis data may not correlate well with *in vivo* absorption (Dahan and Hoffman, 2006).

4. High-throughput/miniaturized precipitation assays

4.1. Introduction

In vitro assays have been developed to evaluate drug precipitation for intravenous and oral formulations. However, these assays are usually in bench-scale and running in batch mode. They are typically time consuming and labor intensive, and require relatively large quantities of materials. Often, the number and type of formulations that can be tested is limited by availability of compounds, particularly for lead compounds in discovery and early development. Another concern is the high media cost when biorelevant media with high levels of bile salt and egg phosphatidylcholine are used for oral formulation testing (Dressman et al., 1998; Galia et al., 1998; Vertzoni et al., 2004).

In recent years, *in vitro* precipitation screening assays that are rapid, inexpensive, minimally labor intensive, and require only small quantities of a compound have become available. This review focuses on such assays that allow rapidly evaluating drug precipitation with limited quantities of compounds and small volumes of testing media.

4.2. SFinX™, AquanSFinX™ and Fast™ platforms

Three high-throughput formulation screening platforms (SFinX™, AquanSFinX™ and Fast™) have been developed at TransForm Pharmaceuticals (Lexington, MA) to rapidly evaluate

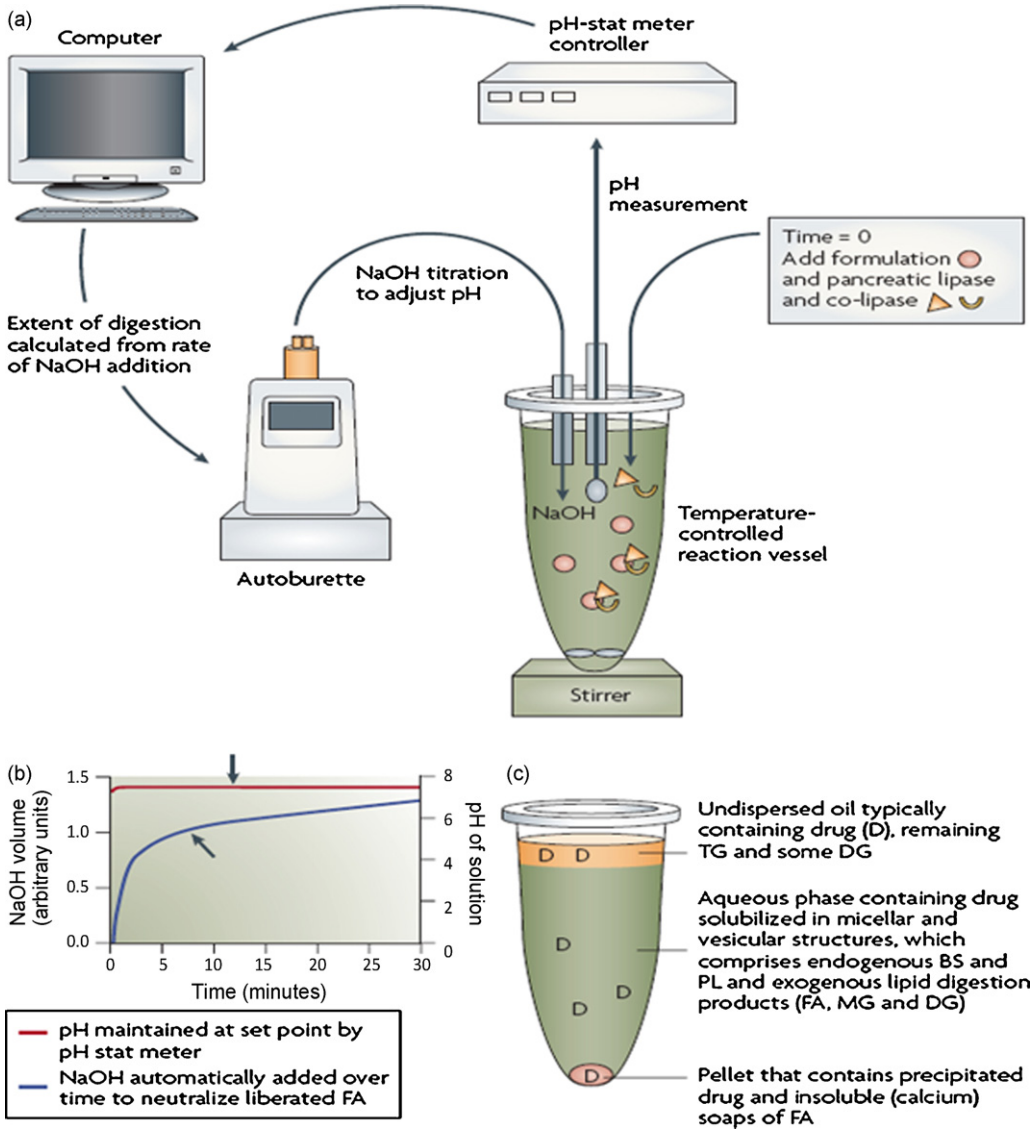


Fig. 5. Lipid-digestion model for *in vitro* assessment of lipidic formulations, reproduced with permission (Porter et al., 2007).

drug precipitation or solubilization using small amounts of compounds (Gardner, 2005; Gardner et al., 2004a,b). In the screening platforms, excipients are dispensed into each well of 96- or 384-well plates by liquid-handling systems, whereas drugs are introduced to each well of the well plates as a solid or as a solution. The plates with drug formulations are incubated at defined temperatures for a specified period of time. Drug precipitation or solubilization in the tested formulations is determined and rank-ordered by optical imaging techniques or various spectroscopic methods. Fast™ is designed mainly for evaluating drug precipitation/solubilization for intravenous formulations, whereas SFinX™/AquanSFinX™ are for oral formulations.

These high-throughput screening platforms have been used to evaluate drug precipitation/solubilization in the development of solubility-enhancing formulations for poorly water-soluble compounds. For example, in order to identify a Cremophor El-free paclitaxel formulation for intravenous administration Chen et al. used Fast™ platform to screen drug precipitation profiles of a total of about 9880 combinations of 12 excipients. Through extensive screening, the lead Cremophor El-free formulation dissolved at least 6 mg/mL paclitaxel in its concentrated state, and was able to keep paclitaxel in solution at 1.3 mg/mL for 48 h upon dilution of the concentrate into normal saline. It was also well tolerated in rat studies (Chen et al., 2003). In a separate study, they used Fast™ platform to evaluate propofol precipitation profiles of about 8000 formulations, and developed an intravenous lipid-free formulation of propofol that improved stability and antimicrobial activity over the marketed product (Chen et al., 2005). In addition to intravenous formulations, the platforms have been used to evaluate drug precipitation or solubilization of oral dosage formulations to improve solubility and oral bioavailability of poorly water-soluble drugs such as celecoxib (Guzman et al., 2007) and fenofibrate (Ratanabanangkoon et al., 2008). The study results demonstrate the feasibility of these high-throughput assays that enable to rapidly screen drug precipitation or solubilization to improve the quality of drug formulations or identify alternative formulations that are superior to existing products (Gardner et al., 2004b).

These fully automated and integrated screening platforms enable a rapid screening of drug precipitation of a considerably large number of formulations. SFinX™ and Fast™ can screen ~2500 and 4000–5000 formulations per platform per day, respectively (Gardner et al., 2004a). A potential technical challenge to apply theses assays is to screen drug precipitation in highly viscous,

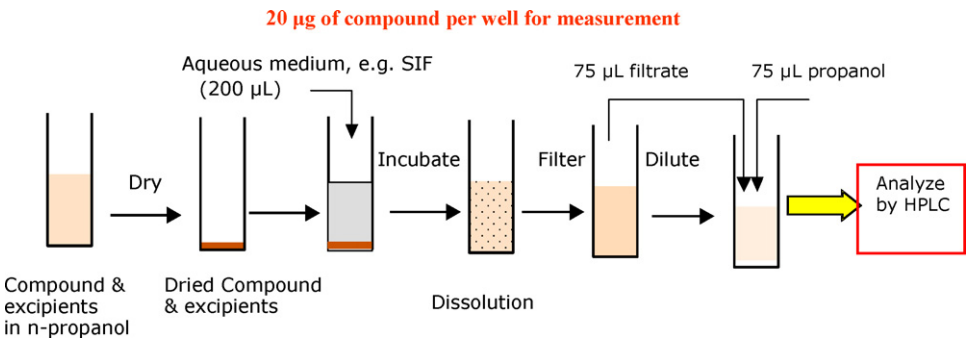


Fig. 6. Schematic diagram illustrating formulations screening process workflow, reproduced with permission (Dai et al., 2007a).

semi-solid, and solid excipients because of the technical difficulty of dispensing and mixing those excipients. In some cases, a heating and/or special dispensing system for viscous excipients may be required (Alsenz and Kansy, 2007), which would further increase the complexity and cost of these screening assays. Thereby these screening platforms are well suited for rapid screen of drug precipitation in low-viscosity formulation vehicles unless special heating and dispensing system for viscous excipients are developed and incorporated into the platforms.

4.3. Microscreening precipitation assay

To avoid the difficulty of dispensing, heating, and mixing viscous, semi-solid, and solid excipients, Dai et al. (2008a,b, 2007a,b) have developed an *in vitro* microscreening precipitation assay that uses a 96-well microtiter plate to evaluate drug precipitation kinetics of formulations with milligram quantities of compounds and milliliter volumes of biorelevant media (Mansky et al., 2007).

Fig. 6 illustrates the process flow of the microscreening precipitation assay (Dai et al., 2007a). Compound and excipients are first dissolved separately in selected solvents such as n-propanol. The solutions are then dispensed into each well of a 96-well microtiter plate by a TECAN robot according to experimental design. After mixing, the 96-well microtiter plate with solutions is placed in a centrifugal vacuum evaporator to remove solvent, which typically leaves neat formulation with 10–40 µg compound and 0.4 mg excipient at the bottom of each well. Milliliter volume of biorelevant (SIF, FaSSIF, or FeSSIF, depending on experimental design) is added to each well of the plate, leading to an approximately 250-fold or 500-fold dilution of excipient. The plates are incubated at room temperature for drug precipitation. At preset desirable time intervals, the diluted formulations are then filtered through a 96-well plate filter under the vacuum, and kinetic drug precipitation profiles are obtained by measuring compound concentration in the filtrate using UV plate readers or HPLC analysis.

A typical precipitation plate map is showed in Table 3. With one 96-well microtiter plate, the precipitation kinetics profiles of six formulations can be evaluated with duplicate measurements at four preset incubation time points and two dilution factors (for example, 250-fold and 500-fold). A typical experiment begins with a rational design of experiments process template. Once the experimental design is complete, TECAN robot follows specific programs (written using Gemini software) to carry out the experiments.

Dai et al. (2007a,b) have used the microscreening precipitation assay to evaluate drug precipitation of micelle formulations, self-emulsifying formulations (Mansky et al., 2007), solid solu-

Table 3
In vitro precipitation 96-well plate map.

	1	2	3	4	5	6	7	8	9	10	11	12
A	F1 - 50 - 1		F2 - 50 - 1		F3 - 50 - 1		F4 - 50 - 1		F5 - 50 - 1		F6 - 50 - 1	
B	F1 - 50 - 2		F2 - 50 - 2		F3 - 50 - 2		F4 - 50 - 2		F5 - 50 - 2		F6 - 50 - 2	
C	F1 - 50 - 4		F2 - 50 - 4		F3 - 50 - 4		F4 - 50 - 4		F5 - 50 - 4		F6 - 50 - 4	
D	F1 - 50 - 24		F2 - 50 - 24		F3 - 50 - 24		F4 - 50 - 24		F5 - 50 - 24		F6 - 50 - 24	
E	F1 - 250 - 1		F2 - 250 - 1		F3 - 250 - 1		F4 - 250 - 1		F5 - 250 - 1		F6 - 250 - 1	
F	F1 - 250 - 2		F2 - 250 - 2		F3 - 250 - 2		F4 - 250 - 2		F5 - 250 - 2		F6 - 250 - 2	
G	F1 - 250 - 4		F2 - 250 - 4		F3 - 250 - 4		F4 - 250 - 4		F5 - 250 - 4		F6 - 250 - 4	
H	F1 - 250 - 24		F2 - 250 - 24		F3 - 250 - 24		F4 - 250 - 24		F5 - 250 - 24		F6 - 250 - 24	

The first letter with a digit identifies the formulation. The digits between two dashes indicate dilution factor and the last one or two digits represent the incubation time (h). Therefore, F1-250-1 denotes Formulation 1 at a 250-fold dilution for 1 h of incubation time.
Reproduced with permission (Dai et al., 2007b).

tion/solid dispersion, and complexation formulations (Dai et al., 2008a,b). They found that the precipitation kinetics of formulations in the initial hours measured by the microscreening assay correlated to those determined by the conventional USP method in all three biorelevent media (SIF, FeSSIF and FaSSIF). The pharmacokinetic (PK) results in rat (Dai et al., 2007a) and dog (Dai et al., 2007b) studies showed that no-precipitation formulations in the microscreening testing had a significantly improved bioavailability compared with those which exhibited drug precipitation in the *in vitro* assay. In particular, the assay is useful in assessing precipitation potential of weak basic drugs that are solubilized in the acidic gastric fluid and are very likely to precipitate after the solution empties from the stomach into the small intestine (Dai et al., 2008b).

In order to further investigate the *in vitro*/*in vivo* correlation of the microsscreening assay, Dai et al. (2007b) first identified three formulations that showed distinct precipitation kinetics (fast precipitation, slow precipitation, and no precipitation) in SIF for a poorly water-soluble compound (Fig. 7). They then measured the *in vitro* precipitation profiles of these three formulations in SIF, FaSSIF, and FeSSIF, and compared the precipitation profiles with *in vivo* absorption at the fasted and fed states in canine PK studies. The PK results showed that the fast-precipitation formulation in the microscreening assay had the lowest oral bioavailability (Table 4), and the *in vitro* precipitation profile in FeSSIF correlated with absorption *in vivo* in canine PK study (Fig. 8). The results demonstrate the potential of the microscreening precipitation assay as a predictor of *in vivo* performance.

The microscreening precipitation assay has several advantages. This assay can be used to directly evaluate drug precipitation after the formulation is diluted with an aqueous medium. Drug precipitation potential can be assessed under various conditions such as testing biomedia containing different compositions and pH, dilution ratios, and stirring conditions.

In addition, unlike other assays that require dispensing solid compounds and neat pharmaceutical excipients, the microscreening assay uses solvent-casting approach to avoid the use of expensive powder-dispenser and the difficulty of handling viscous, semisolid, and solid excipients as well. The assay thus simplifies the equipment and procedures, and can be adapted in the labs without a significant capital investment. This assay is suitable for liquid and viscous, semi-solid and solid formulations, an advantage over the precipitation assays that only work low-viscosity pharmaceutical vehicles due to their limited dispensing capability.

Other advantages of the microscreening assay over existing manual methods are amount samples required, speed and scope. Solvent casting allows accurately dispensing and mixing small quantities of drug and excipients, thereby bringing an advantage in screening precipitation for a new molecular entity with limited availability. Also use 96-well plate format in parallel processing produces an acceptable sample throughput. Moreover, the microscreening assay is applicable to evaluate drug precipitation of various formulations such as micelle, microemulsion, lipid and supersaturatable lipid formulation, complexation, and solid solu-

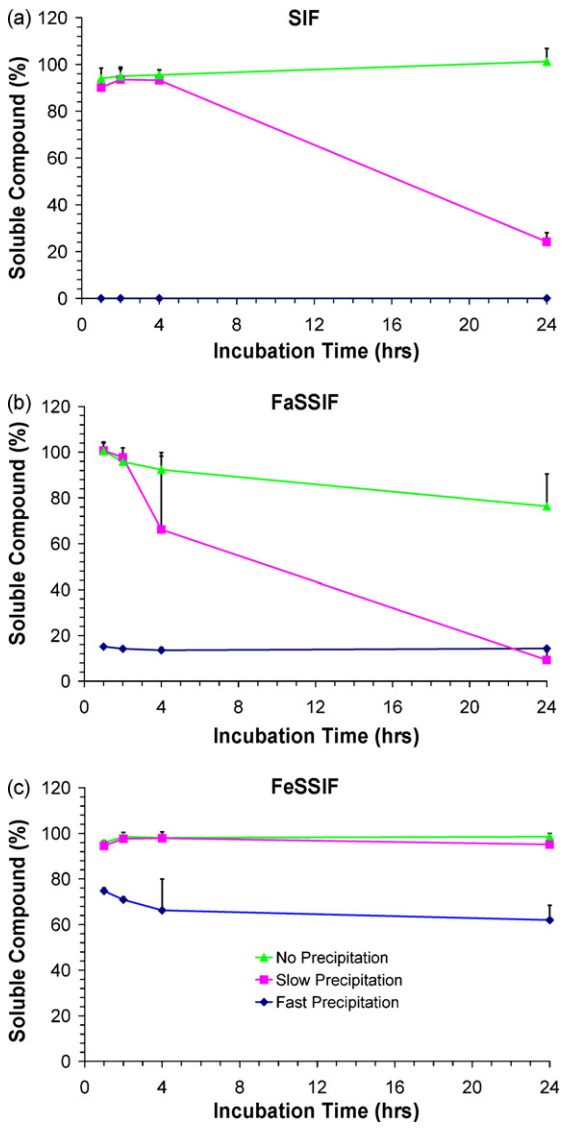
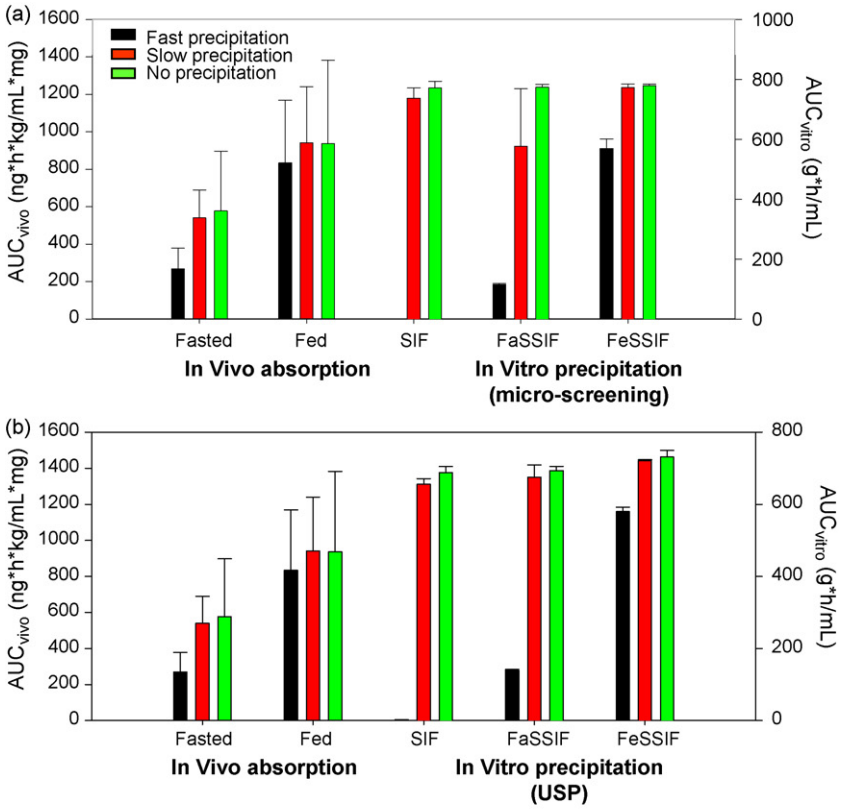


Fig. 7. *In vitro* precipitation kinetics of three formulations containing JNJ-25894934 in (a) SIF, (b) FaSSIF, and (c) FeSSIF identified by the precipitation screening method. Error bars represent standard deviations of four measurements, reproduced with permission (Dai et al., 2007b).

Table 4
Summary of dog pharmacokinetic studies of the compound following iv dosing and oral dosing of all three formulations.

Formulation	Feed state	$AUC_{0-\infty}$/dose (ng h kg/mL/mg)[a]	% BA[b]	C_{max}/dose (ng kg/mL/mg)[a]	T_{max} (h)	BA ratio[c] (fed/fasted)
IV	Fasted	4486.9 ± 682.6[d]	100	3338.0 ± 514.8[c]	0.0 ± 0.0	NA
Fast precipitation	Fasted	1060.8 ± 122.3	27.0 ± 7.5	125.6 ± 30.5	3.0 ± 2.0	2.3 ± 0.8
	Fed	2572.7 ± 730.2	57.2 ± 13.7	288.5 ± 105.1	2.2 ± 1.1	
Slow precipitation	Fasted	1809.0 ± 454.8	40.4 ± 8.8	229.1 ± 63.9	2.4 ± 0.9	1.6 ± 0.1
	Fed	2831.5 ± 646.4	63.1 ± 11.5	366.9 ± 155.8	1.8 ± 1.3	
No precipitation	Fasted	1704.9 ± 655.6	38.1 ± 14.4	238.7 ± 148.4	2.8 ± 1.1	1.8 ± 0.3
	Fed	2938.5 ± 775.8	65.7 ± 16.1	332.7 ± 108.0	2.2 ± 2.2	

[a] For all three formulations, oral dosing at 3 mg/kg ($n = 5$).
[b] % BA relative to iv dosing.
[c] BA ratio (fed/Fasted) in each animal was first calculated. Overall BA ratio (fed/fasted) was the average of individual BA ratio (fed/fasted) in 5 animals.
[d] iv dose corrected to 3 mg/kg ($n = 5$). Reproduced with permission (Dai et al., 2007b).



In vitro precipitation AUC (μg*h/mL)	*In-vivo* oral absorption AUC (ng*h*kg/mL*mg)	Correlation Coefficient (R^2)
Precipitation method using a 96-well plate		
SIF	Fasted State	0.83
SIF	Fed State	0.44
FaSSIF	Fasted State	0.89
FaSSIF	Fed State	0.54
FeSSIF	Fasted State	0.93
FeSSIF	Fed State	0.97
USP method		
SIF	Fasted State	0.83
SIF	Fed State	0.44
FaSSIF	Fasted State	0.93
FaSSIF	Fed State	0.59
FeSSIF	Fasted State	0.89
FeSSIF	Fed State	0.99

Fig. 8. Correlation of *in vitro* precipitation kinetics with *in-vivo* oral absorption in the dogs. *In vitro* precipitation AUC (μg h/mL) of all three formulations containing JNJ-25894934 was calculated from the initial 4-h time point of the precipitation profiles. *In-vivo* oral absorption $AUC_{0\text{-}4}$ (ng h kg/mL mg) was calculated from the individual plasma concentration of compound vs. time profiles up to 4 h in the dog PK studies, reproduced with permission (Dai et al., 2007b).

tion/solid dispersion using small quantities of compounds (Dai et al., 2008b), something that would be difficult to do manually.

Despite some advantages, solvent casting could bring a concern that drug may change its polymorph in the excipient matrix after solvent removal. In addition, like any other *in vitro* precipitation assays described above, the microscreening precipitation assay focuses on measurement of drug precipitates upon dilution and does not consider drug absorption. For example, drug precipitates observed in the microscreening assay could redissolve in the gastrointestinal tract and reabsorbed in body. Furthermore, the potential of drug precipitation would be reduced significantly if drug is absorbed fast *in vivo*. As a result, the assay could overestimate the potential of drug precipitation *in vivo* without taking drug absorption into account. Further method improvement needs take these considerations.

4.4. Other assays

A number of high-throughput/miniaturized assays have been developed to screen or measure drug solubility in pharmaceutical vehicles, particularly for compound screening during drug discovery and preclinical study (Alsenz and Kansy, 2007). These solubility

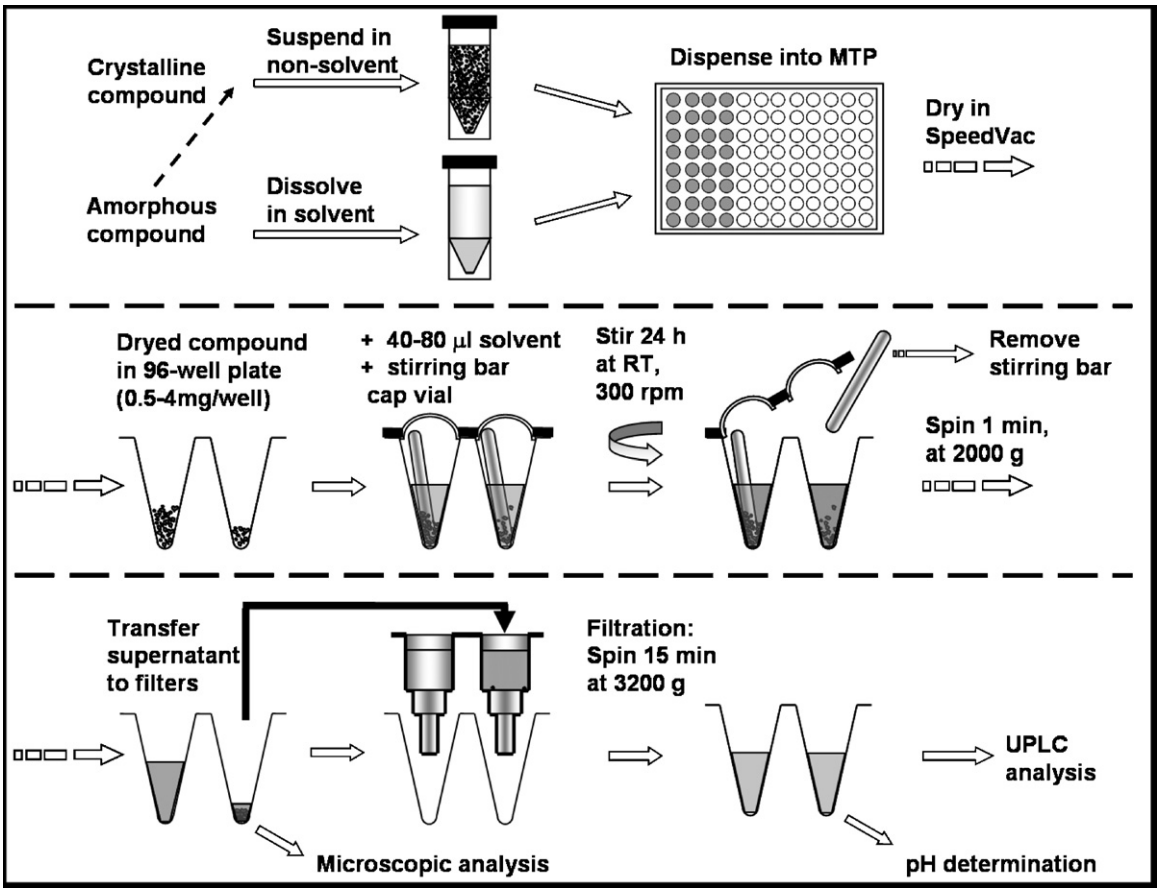


Fig. 9. Schematic diagram illustrating the partially automated solubility screening. (PASS) process. Crystalline compound suspended in heptane or amorphous compound dissolved in a solvent is dispensed into microtiter plates and the vehicle is removed in a SpeedVac. Forty to eighty microliters of solvent and a stir bar are added, wells are capped, and probes are stirred for 24 h at room temperature. After removal of the stir bars and centrifugation, residual solid in the supernatant is removed by centrifugation through syringe filters. Compound in the filtrate is quantified by UPLC analysis, the pH of the filtrate is determined and residual solid is analyzed by microscopic inspection, reproduced with permission (Alsenz et al., 2007).

screening assays can be potentially applicable to evaluating drug precipitation in the pharmaceutical vehicles. For example, Alsenz et al. (2007) have reported a partially automated solubility screening (PASS) assay for screening drug solubility in liquid pharmaceutical vehicles (Fig. 9). In the assay, drug is first dissolved or dispersed in heptane at 25 mg/mL, and the solution or suspension is then dispensed into 96-well plates using pipettes. Following removal of heptane by a vacuum centrifuge, liquid pharmaceutical vehicles are added into the 96-well plate. After 24-h incubation, the solution is filtered, and drug solubility or precipitation in the testing vehicles is determined via concentration quantified by UV-ultra performance liquid chromatography analysis. Using PASS assay, Alsenz et al. screened solubilization of 42 compounds in 18 different pharmaceutical acceptable vehicles. With a robotic liquid-handling system, they achieved a throughput of 45 samples per hour and >600 solubility measurements per week.

In addition to high-throughput feature, some assays allow for the simultaneous, small-scale screening of drug solubilization/precipitation and detection of drug solid or precipitate forms and crystallinity in excipients in a single assay, and therefore, they enables to investigate correlation of drug solubility with solid-state properties of drug precipitate in a high-throughput workflow (Seadeek et al., 2007; Sugano et al., 2006; Wyttenbach et al., 2007). In these assays, a compound and a liquid pharmaceutical vehicle are dispensed into a 96-well filter plate. After mixing drug-vehicle slurries for a period of time, residual drug precipitate or solid is separated from saturated solutions by centrifugation of filter plate (Fig. 10). The drug solids in the plate are

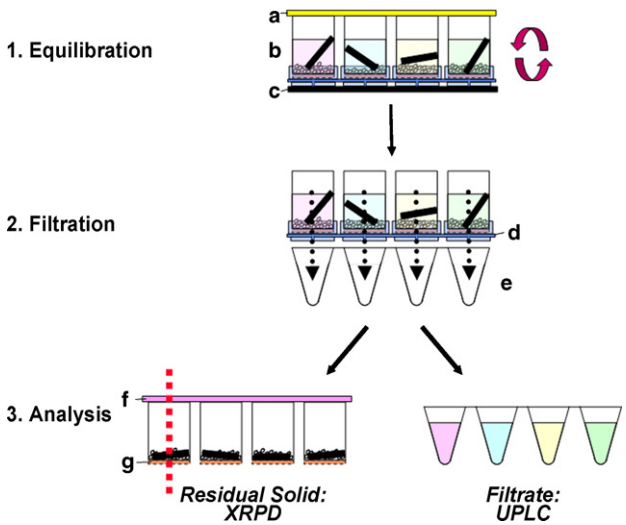


Fig. 10. Schematic representation of the experimental procedure of the solubility and residual solid screening (SORESOS) assay. Experiments are performed in 96-well MultiScreen\Solubility Filter Plates (b) sealed with an ELASi septum sheet (a, top) an adhesive aluminum foil (c, bottom). Drug-vehicle slurries are mixed by head-over-head rotation in the presence of stirring bars, seals are removed, and filtration is performed by centrifugation. Filtrates are collected in a 96-well polypropylene plate (e). After filtration, the plate is sealed by adhesive acetate foil (f), the underdrain support (d) of the filter plate is removed, and residual solid on the filter (g) of the plate is directly analyzed by HT transmission XRPD with vertical beam geometry (dashed line), reproduced with permission (Wyttenbach et al., 2007).

directly analyzed by X-ray powder diffraction (XRPD) (Seadeek et al., 2007; Wyttenbach et al., 2007) or by an automated polarized light microscopy analysis (Sugano et al., 2006). In addition to increasing sample throughput, other approaches focus on use of miniaturization to reduce drug consumption (Chen and Venkatesh, 2004).

These screening assays utilize high-throughput automation and parallel processing or miniaturization, thereby they provide a fast screen of drug solubilization/precipitation in pharmaceutical vehicles with milligram quantities of a compound. However, all the pharmaceutical vehicles reported in the assays are typically aqueous buffers, surfactant solutions, and low-viscosity lipids, organic solvent and oils. These assays are potentially applicable to screening drug precipitations in low-viscosity formulations unless special heating and dispensing system for viscous excipients are incorporated into the assays (Alsenz and Kansy, 2007). Also, a relatively large amount of a compound is required for testing if compound is dispensed using powder dispersal due to the difficulty of dispensing a small amount of drug power accurately in a high-throughput work flow.

Also, it should be pointed out that high-throughput/miniaturized precipitation assays need to be validated during method development. Several studies have demonstrated correlation results between high-throughput/miniaturized assays and bench-scale precipitation assays (Alsenz et al., 2007; Chen and Venkatesh, 2004; Dai et al., 2007a,b; Mansky et al., 2007; Wyttenbach et al., 2007). In fact, such high-throughput precipitation assays are often used to rapidly screen out formulations with the highest precipitation potential, then conventional bench-scale precipitation methods are applied to further optimize or refine lead formulation choices for preclinical testing or initial clinical assessment (Chen et al., 2005, 2003; Dai et al., 2007a, 2008b). Generally speaking, these new assays are more suited for screening solubility-enhancing formulations in drug discovery or early drug development because they usually do not focus on the long-term stability of such formulations.

5. Future perspective

To date, considerable investigations have been undertaken in an attempt to develop appropriate *in vitro* precipitation screening assays that mimic precipitation phenomena *in vivo* after administration of formulations. Such mimicking in the assays primarily focuses on selection of representative media simulating the fasted state and fed state conditions in the human intestinal fluid (Dressman and Reppas, 2000; Galia et al., 1998; Kostewicz et al., 2004, 2002; Persson et al., 2005), and on simulating lyosis of lipids *in vivo* pertinent to lipid-based formulations (Dahan and Hoffman, 2008; Porter and Charman, 2001; Sek, 2007). These *in vitro* assays have been used to assess precipitation potential of pharmaceutical formulations, and achieved successes to some extent in terms of *in vitro*/*in vivo* correlation. However, such mimicking is far away from the highly complicated *in vivo* scenarios. For example, current assays emphasize solubilization and dispersion of drugs in test media, but they typically fail to describe the interaction of formulations with the gastrointestinal tract environment, which is critical to establish predictive performance of screening assays. Therefore *in vitro* results do not always correlate with drug absorption *in vivo*. Undoubtedly, there is a need for further development of more predictive *in vitro* precipitation assays.

One important consideration that should be incorporated into future assays is hydrodynamic fluid flow *in vivo*. Drug dissolution and precipitation are significantly influenced by hydrodynamic conditions (Bai and Armenante, 2008; Bai et al., 2007; Baxter et al., 2005; D'Arcy et al., 2005; Kukura et al., 2004; Mirza et al., 2005; Qureshi, 2004, 2006). However, such hydrodynamic effect is often overlooked in the *in vitro* precipitation assays. Several studies have aimed to simulate hydrodynamic conditions (D'Arcy et al., 2005, 2006; McCarthy et al., 2004, 2003; Scholz et al., 2002, 2003), but simulating *in vivo* hydrodynamic conditions still remains an obstacle due to the complexity of the fluid flow and the heterogeneous chaos *in vivo*.

Other critical parameters in future *in vitro* precipitation assays should reflect re-absorption of drug precipitates. Current *in vitro* precipitation assays define drug precipitates based on particle size of the dispersion and treat precipitates as non-absorbable. Drug precipitates, however, could redissolve in the gastrointestinal tract and get reabsorbed in body. For example, depending on precipitation conditions such as media, pH, hydrodynamic flow, and excipients used in the formulations, drug precipitates produced during precipitation process could be amorphous or polymorphic forms and exist in different morphologies/sizes (Dai et al., 2008a; Gao et al., 2009; Simmons, 1993). The different solid forms of precipitates may have the changed solubility in the gastrointestinal fluids, and a small size of amorphous precipitate can redissolve fast (Dai et al., 2008a; Kim et al., 2008; Serajuddin et al., 1988). Therefore it is likely that drug precipitates with improved solubility could be re-solubilized *in vivo* for the absorption. It would be interesting to look into the nature of drug precipitates and how the morphology and size of the precipitates affect *in vivo* absorption using *in vitro* precipitation assays.

In addition, drug absorption rate can impact the assessment of drug precipitation potential in the gastrointestinal tract. For example, current precipitation assays may overestimate drug precipitation potential if drug is absorbed at a fast rate. A few assays using fiberglass dialysis (Blanquet et al., 2004) or the caco-2 cell membrane (Kobayashi et al., 2001) have been described to simulate the absorption step. Such *in vitro* assays would be useful in estimating potential for drug precipitation in gastrointestinal tract since removal of drug in these systems would provide a more realistic time-course of drug supersaturation level. It would be great that these assays are validated for routine testing.

Another interesting attempt that could contribute to the development of *in vitro* precipitation assays includes computational modeling. Investigations have been undertaken in an attempt to develop mechanistically-based mathematical models that are predictive of *in vivo* precipitation potential (Bolger et al., 2002; Cammarn and Sakr, 2000; Johnson, 2003, 2007; Lu et al., 1993; Narazaki et al., 2007a,b; Surakitbanharn et al., 1994). With more understanding of drug fate *in vivo* and more experimental data available, it could be possible in the future to estimate drug precipitation potential *in vivo* after dose administration using theoretical models.

Acknowledgements

The author gratefully thanks Dr. Bingxing Shi for his great assistance in reproducing figures, Dr. Jochem Alsenz and Dr. Nicole Wyttenbach for providing their original figures for this review article.

References

Alsenz, J., Kansy, M., 2007. High throughput solubility measurement in drug discovery and development. Adv. Drug Deliv. Rev. 59, 546–567.
Alsenz, J., Meister, E., Haenel, E., 2007. Development of a partially automated solubility screening (PASS) assay for early drug development. J. Pharm. Sci. 96, 1748–1762.
Alvarez-Nunez, F.A., Yalkowsky, S.H., 1999. Buffer capacity and precipitation control of pH solubilized phenytoin formulations. Int. J. Pharm. 185, 45–49.
Avis, K.E., Lachman, L., Lieberman, H.A. (Eds.), 1986. Pharmaceutical Dosage Forms: Parenter. Med. Vol. 2.

Bai, G., Armenante, P.M., 2008. Velocity distribution and shear rate variability resulting from changes in the impeller location in the USP dissolution testing apparatus II. Pharm. Res. 25, 320–336.
Bai, G., Armenante, P.M., Plank, R.V., Gentzler, M., Ford, K., Harmon, P., 2007. Hydrodynamic investigation of USP dissolution test apparatus II. J. Pharm. Sci. 96, 2327–2349.
Baxter, J.L., Kukura, J., Muzzio, F.J., 2005. Hydrodynamics-induced variability in the USP apparatus II dissolution test. Int. J. Pharm. 292, 17–28.
Blanquet, S., Zeijdner, E., Beyssac, E., Meunier, J.-P., Denis, S., Havenaar, R., Alric, M., 2004. A dynamic artificial gastrointestinal system for studying the behavior of orally administered drug dosage forms under various physiological conditions. Pharm. Res. 21, 585–591.
Bolger, M.B., Gilman, T.M., Fraczkiewicz, R., Steere, B., Woltosz, W.S., 2002. Predicting drug absorption by computational methods. Cell Cult. Models Biol. Barriers, 353–377.
Buck, M., Tsukamoto, T., Kvols, L.K., Kovach, J.S., 1985. Pharmacology studies of bisantrene, a poorly soluble anticancer drug, formulated in a lipid emulsion. Recent Adv. Chemother., Proc. Int. Congr. Chemother., 14th: Anticancer Sect. 1, 540–541.
Cammarn, S.R., Sakr, A., 2000. Predicting dissolution via hydrodynamics: salicylic acid tablets in flow through cell dissolution. Int. J. Pharm. 201, 199–209.
Carvalho-Silva, B., Moreira-Campos, L.M., Nunan, E.A., Vianna-Soares, C.D., Araujo-Alves, B.L., Cesar, I.C., Pianetti, G.A., 2004. Optimization and statistical evaluation of dissolution tests for indinavir sulfate capsules. Farmaco 59, 921–927.
Chen, H., Zhang, Z., Almarsson, O., Marier, J.-F., Berkovitz, D., Gardner, C.R., 2005. A novel, lipid-free nanodispersion formulation of propofol and its characterization. Pharm. Res. 22, 356–361.
Chen, H., Zhang, Z., McNulty, C., Olbert, C., Yoon, H.J., Lee, J.W., Kim, S.C., Seo, M.H., Oh, H.S., Lemmo, A.V., Ellis, S.J., Heimlich, K., 2003. A high-throughput combinatorial approach for the discovery of a cremophor EL-free paclitaxel formulation. Pharm. Res. 20, 1302–1308.
Chen, X.-Q., Venkatesh, S., 2004. Miniature device for aqueous and non-aqueous solubility measurements during drug discovery. Pharm. Res. 21, 1758–1761.
Christensen, J.O., Schultz, K., Mollgaard, B., Kristensen, H.G., Mullertz, A., 2004. Solubilization of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols. Eur. J. Pharm. Sci. 23, 287–296.
Corrigan, O.I., Devlin, Y., Butler, J., 2003. Influence of dissolution medium buffer composition on ketoprofen release from ER products and in vitro-in vivo correlation. Int. J. Pharm. 254, 147–154.
Cox, J.W., Sage, G.P., Wynalda, M.A., Ulrich, R.G., Larson, P.G., Su, C.C., 1991. Plasma compatibility of injectables: comparison of intravenous U-74006F, a 21-aminosteroid antioxidant, with Dilantin brand of parenteral phenytoin. J. Pharm. Sci. 80, 371–375.
Cuine Jean, F., McEvoy Claire, L., Charman William, N., Pouton Colin, W., Edwards Glenn, A., Benameur, H., Porter Christopher, J.H., 2008. Evaluation of the impact of surfactant digestion on the bioavailability of danazol after oral administration of lipidic self-emulsifying formulations to dogs. J. Pharm. Sci. 97, 995–1012.
Cuine, J.F., Charman, W.N., Pouton, C.W., Edwards, G.A., Porter, C.J.H., 2007. Increasing the proportional content of surfactant (cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. Pharm. Res. 24, 748–757.
D'Arcy, D.M., Corrigan, O.I., Healy, A.M., 2005. Hydrodynamic simulation (computational fluid dynamics) of asymmetrically positioned tablets in the paddle dissolution apparatus: impact on dissolution rate and variability. J. Pharm. Pharmacol. 57, 1243–1250.
D'Arcy, D.M., Corrigan, O.I., Healy, A.M., 2006. Evaluation of hydrodynamics in the basket dissolution apparatus using computational fluid dynamics-dissolution rate implications. Eur. J. Pharm. Sci. 27, 259–267.
Dahan, A., Hoffman, A., 2006. Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats. Pharm. Res. 23, 2165–2174.
Dahan, A., Hoffman, A., 2007. The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats. Eur. J. Pharm. Biopharm. 67, 96–105.
Dahan, A., Hoffman, A., 2008. Rationalizing the selection of oral lipid based drug delivery systems by an in vitro dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. J. Controll. Rel. 129, 1–10.
Dai, W.-G., Dong, L.C., Li, S., Deng, Z., 2008a. Combination of Pluronic/Vitamin E TPGS as a potential inhibitor of drug precipitation. Int. J. Pharm. 355, 31–37.
Dai, W.-G., Dong, L.C., Li, S., Pollock-Dove, C., Chen, J., Mansky, P., Eichenbaum, G., 2007a. Parallel screening approach to identify solubility-enhancing formulations for improved bioavailability of a poorly water-soluble compound using milligram quantities of material. Int. J. Pharm. 336, 1–11.
Dai, W.-G., Dong, L.C., Shi, X., Nguyen, J., Evans, J., Xu, Y., Creasey, A.A., 2007b. Evaluation of drug precipitation of solubility-enhancing liquid formulations using milligram quantities of a new molecular entity (NME). J. Pharm. Sci. 96 (11), 2957–2969.
Dai, W.-G., Pollock-Dove, C., Dong, L.C., Li, S., 2008b. Advanced screening assays to rapidly identify solubility-enhancing formulations: high-throughput, miniaturization and automation. Adv. Drug Deliv. Rev. 60, 657–672.
Dannenfelser, R.-M., Surakitbanharn, Y., Tabibi, S.E., Yalkowsky, S.H., 1996. Parenteral formulation of flavopiridol (NSC-649890). PDA J. Pharm. Sci. Technol. 50, 356–359.
Davio, S.R., McShane, M.M., Kakuk, T.J., Zaya, R.M., Cole, S.L., 1991. Precipitation of the renin inhibitor Ditekiren upon iv infusion; in vitro studies and their relationship to in vivo precipitation in the cynomolgus monkey. Pharm. Res. 8, 80–83.
Derendorf, H., Drehsen, G., Rohdewald, P., 1983. In vivo-in vitro correlations of salicylate saliva levels and continuous flow cell dissolution rates. Int. J. Pharm. 15, 167–175.
Dressman, J., Schamp, K., Beltz, K., Alsenz, J., 2007. Characterizing release from lipid-based formulations. Drugs Pharm. Sci. 170, 241–255.
Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15, 11–22.
Dressman, J.B., Reppas, C., 2000. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. Eur. J. Pharm. Sci. 11, S73–S80.
Emara, L.H., El-Menshawi, B.S., Estefan, M.Y., 2000. In vitro-in vivo correlation and comparative bioavailability of vincamine in prolonged-release preparations. Drug Dev. Ind. Pharm. 26, 243–251.
Falchuk, K.H., Peterson, L., McNeil, B.J., 1985. Microparticulate-induced phlebitis. Its prevention by in-line filtration. New England J. Med. 312, 78–82.
Fatouros, D.G., Mullertz, A., 2007. Using in vitro dynamic lipolysis modeling as a tool for exploring IVIVC relationships for oral lipid-based formulations. Drugs Pharm. Sci. 170, 257–271.
Fatouros, D.G., Mullertz, A., 2008. In vitro lipid digestion models in design of drug delivery systems for enhancing oral bioavailability. Expert Opin. Drug Metab. Toxicol. 4, 65–76.
Galia, E., Nicolaides, E., Horter, D., Lobenberg, R., Reppas, C., Dressman, J.B., 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm. Res. 15, 698–705.
Gao, P., Akrami, A., Alvarez, F., Hu, J., Li, L., Ma, C., Surapaneni, S., 2009. Characterization and optimization of AMG 517 supersaturatable self-emulsifying drug delivery system (S-SEDDS) for improved oral absorption. J. Pharm. Sci. 98, 516–528.
Gardner, C., 2005. Drug candidate selection: the role of physical chemistry and material science in form and formulation. Bull. Tech. Gattefosse, 9–18.
Gardner, C.R., Almarsson, O., Chen, H., Morissette, S., Peterson, M., Zhang, Z., Wang, S., Lemmo, A., Gonzalez-Zugasti, J., Monagle, J., Marchionna, J., Ellis, S., McNulty, C., Johnson, A., Levinson, D., Cima, M., 2004a. Application of high throughput technologies to drug substance and drug product development. Comput. Chem. Eng. 28, 943–953.
Gardner, C.R., Walsh, C.T., Almarsson, O., 2004b. Drugs as materials: valuing physical form in drug discovery. Nat. Rev. Drug Discov. 3, 926–934.
Greenfield, J.C., Loux, S.J., Sood, V.K., Jenkins, K.M., Davio, S.R., 1991. In vitro evaluation of the plasma and blood compatibility of a parenteral formulation for ditekiren, a novel renin inhibitor pseudopeptide. Pharm. Res., 475–479.
Grove, M., Mullertz, A., 2007. Liquid self-microemulsifying drug delivery systems. Drugs Pharm. Sci. 170, 107–127.
Gu, C.-H., Rao, D., Gandhi, R.B., Hilden, J., Raghavan, K., 2005. 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. 94, 199–208.
Guzman, H.R., Tawa, M., Zhang, Z., Ratanabanangkoon, P., Shaw, P., Gardner, C.R., Chen, H., Moreau, J.-P., Almarsson, O., Remenar, J.F., 2007. Combined use of crystalline salt forms and precipitation inhibitors to improve oral absorption of celecoxib from solid oral formulations. J. Pharm. Sci. 96, 2686–2702.
Hauss, D.J., 2007. Oral lipid-based formulations. Adv. Drug Deliv. Rev. 59, 667–676.
Hecker, J.F., Fisk, G.C., Lewis, G.B., 1984. Phlebitis and extravasation ("tissuing") with intravenous infusions. Med. J. Aust. 140, 658–660.
Hoener, B.-A., Benet, L.Z., 2002. Factors influencing drug absorption and drug availability. Drugs Pharm. Sci. 121, 93–117.
Ikegami, K., Tagawa, K., Kobayashi, M., Osawa, T., 2003. Prediction of in vivo drug release behavior of controlled-release multiple-unit dosage forms in dogs using a flow-through type dissolution test method. Int. J. Pharm. 258, 31–43.
Irwin, W.J., Iqbal, M., 1992. Bropirimine formulation: the dynamic testing of injections. Int. J. Pharm. 83, 241–249.
Jannin, V., Musakhanian, J., Marchaud, D., 2008. Approaches for the development of solid and semi-solid lipid-based formulations. Adv. Drug Deliv. Rev. 60, 734–746.
Johnson, J.L.H., Yalkowsky, S.H., 2006. Reformulation of a new vancomycin analog: an example of the importance of buffer species and strength. AAPS PharmSciTech 7, E5.
Johnson, J.L.H., He, Y., Yalkowsky, S.H., 2003. Prediction of precipitation-induced phlebitis: a statistical validation of an in vitro model. J. Pharm. Sci. 92, 1574–1581.
Johnson, K.C., 2003. Dissolution and absorption modeling: model expansion to simulate the effects of precipitation, water absorption, longitudinally changing intestinal permeability, and controlled release on drug absorption. Drug Dev. Ind. Pharm. 29, 833–842.
Johnson, K.C., 2007. Dissolution: fundamentals of in vitro release and the biopharmaceutics classification system. Drugs Pharm. Sci. 165, 1–28.
Kaukonen, A.M., Boyd, B.J., Charman, W.N., Porter, C.J.H., 2004a. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. Pharm. Res. 21, 254–260.
Kaukonen, A.M., Boyd, B.J., Porter, C.J.H., Charman, W.N., 2004b. Drug solubilization behavior during in vitro digestion of simple triglyceride lipid solution formulations. Pharm. Res. 21, 245–253.
Kim, M.-S., Jin, S.-J., Kim, J.-S., Park, H.J., Song, H.-S., Neubert, R.H.H., Hwang, S.-J., 2008. Preparation, characterization and in vivo evaluation of amorphous ator-

vastatin calcium nanoparticles using supercritical antisolvent (SAS) process. Eur. J. Pharm. Biopharm. 69, 454–465.
Kobayashi, M., Sada, N., Sugawara, M., Iseki, K., Miyazaki, K., 2001. Development of a new system for prediction of drug absorption that takes into account drug dissolution and pH change in the gastro-intestinal tract. Int. J. Pharm. 221, 87–94.
Kostewicz, E.S., Wunderlich, M., Brauns, U., Becker, R., Bock, T., Dressman Jennifer, B., 2004. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. J. Pharm. Pharmacol. 56, 43–51.
Kostewicz, E.S., Brauns, U., Becker, R., Dressman, J.B., 2002. Forecasting the oral absorption behavior of poorly soluble weak bases using solubility and dissolution studies in biorelevant media. Pharm. Res. 19, 345–349.
Kovach, J.S., Buck, M., Tsukamoto, T., Odegaard, A., Lieber, M.M., 1985. Regional targeting of bisantrene by directed intravascular precipitation. Cancer Chemother. Pharmacol. 15, 192–195.
Kukura, J., Baxter, J.L., Muzzio, F.J., 2004. Shear distribution and variability in the USP Apparatus 2 under turbulent conditions. Int. J. Pharm. 279, 9–17.
Li, P., Patel, H., Tabibi, S.E., Vishnuvajjala, R., Yalkowsky, S.H., 1999. Evaluation of intravenous flavopiridol formulations. PDA J. Pharm. Sci. Technol. 53, 137–140.
Li, P., Vishnuvajjala, R., Tabibi, S.E., Yalkowsky, S.H., 1998. Evaluation of in vitro precipitation methods. J. Pharm. Sci. 87, 196–199.
Lieber, M.M., Welch, T.J., Johnson, C.M., Farrow, G.M., Buck, M., Kovach, J.S., 1986. Directed intravascular precipitation of bisantrene for pelvic malignant lesions: preclinical studies. Mayo Clin. Proc. Mayo Clinic 61, 173–179.
Liu, R., 2000. Water-Insoluble Drug Formation. Interpharm Press, Buffalo Grove.
Lu, A.T.K., Frisella, M.E., Johnson, K.C., 1993. Dissolution modeling: factors affecting the dissolution rates of polydisperse powders. Pharm. Res. 10, 1308–1314.
MacGregor, K.J., Embleton, J.K., Lacy, J.E., Perry, E.A., Solomon, L.J., Seager, H., Pouton, C.W., 1997. Influence of lipolysis on drug absorption from the gastrointestinal tract. Adv. Drug Deliv. Rev. 25, 33–46.
Mansky, P., Dai, W.-G., Li, S., Pollock-Dove, C., Daehne, K., Dong, L., Eichenbaum, G., 2007. Screening method to identify preclinical liquid and semi-solid formulations for low solubility compounds: miniaturization and automation of solvent casting and dissolution testing. J. Pharm. Sci. 96, 1548–1563.
Markowsky, S.J., Kohls, P.R., Ehresman, D., Leppik, I., 1991. Compatibility and pH variability of four injectable phenytoin sodium products. Am. J. Hosp. Pharm. 48, 510–514.
McCarthy, L.G., Bradley, G., Sexton James, C., Corrigan Owen, I., Healy Anne, M., 2004. Computational fluid dynamics modeling of the paddle dissolution apparatus: agitation rate, mixing patterns, and fluid velocities. AAPS PharmSciTech 5, e31.
McCarthy, L.G., Kosiol, C., Healy Anne, M., Bradley, G., Sexton James, C., Corrigan Owen, I., 2003. Simulating the hydrodynamic conditions in the United States Pharmacopeia paddle dissolution apparatus. AAPS PharmSciTech 4, E22.
Mirza, T., Joshi, Y., Liu, Q., Vivilecchia, R., 2005. Evaluation of dissolution hydrodynamics in the USP, Peak and flat-bottom vessels using different solubility drugs. Dissolution Technol. 12, 11–16.
Moeller, H., Wirbitzki, E., 1990. Special cases of dissolution testing using the flow-through system. S.T.P. Pharma. 6, 657–662.
Moeller, H., Wirbitzki, E., 1993. Regulatory aspects of modified release dosage forms: special cases of dissolution testing using the flow-through system. Boll. Chim. Farm 132, 105–115.
Mullertz, A., 2007. Lipid-based drug delivery systems: choosing the right in vitro tools. Am. Pharm. Rev. 10, pp. 102, 104, 106–110.
Narazaki, R., Sanghvi, R., Yalkowsky, S.H., 2007a. Estimation of drug precipitation upon dilution of pH-controlled formulations. Mol. Pharmaceutics 4, 550–555.
Narazaki, R., Sanghvi, R., Yalkowsky, S.H., 2007b. Estimation of drug precipitation upon dilution of pH-cosolvent solubilized formulations. Chem. Pharm. Bull. 55, 1203–1206.
Nicklasson, M., Wennergren, B., Linberg, J., Persson, C., Ahlgren, R., Palm, B., Pettersson, A., Wenngren, L., 1987. A collaborative in vitro dissolution study using the flow-through method. Int. J. Pharm. 37, 195–202.
Nicolaides, E., Galia, E., Efthymiopoulos, C., Dressman, J.B., Reppas, C., 1999. Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data. Pharm. Res. 16, 1876–1882.
Perng, C.-Y., Kearney, A.S., Palepu, N.R., Smith, B.R., Azzarano, L.M., 2003. Assessment of oral bioavailability enhancing approaches for SB-247083 using flow-through cell dissolution testing as one of the screens. Int. J. Pharm. 250, 147–156.
Persson, E.M., Gustafsson, A.-S., Carlsson, A.S., Nilsson, R.G., Knutson, L., Forsell, P., Hanisch, G., Lennernaes, H., Abrahamsson, B., 2005. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. Pharm. Res. 22, 2141–2151.
Pfeifle, C.E., Adler, D.S., Gannaway, W.L., 1981. Phenytoin sodium solubility in three intravenous solutions. Am. J. Hosp. Pharm. 38, 358–362.
Phillips, J.G., Chen, Y., Wakeling, I.N., 1989. A flow-through dissolution approach to in vivo/in vitro correlation of adinazolam release from sustained-release formulations. Drug Dev. Ind. Pharm. 15, 2177–2195.
Porter, C.J.H., Charman, W.N., 2001. In vitro assessment of oral lipid based formulations. Bull. Tech. Gattefosse 94, 119–139.
Porter, C.J.H., Kaukonen, A.M., Boyd, B.J., Edwards, G.A., Charman, W.N., 2004a. susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation. Pharm. Res. 21, 1405–1412.
Porter, C.J.H., Kaukonen, A.M., Taillardat-Bertschinger, A., Boyd, B.J., O'Connor, J.M., Edwards, G.A., Charman, W.N., 2004b. Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine. J. Pharm. Sci. 93, 1110–1121.
Porter, C.J.H., Pouton, C.W., Cuine, J.F., Charman, W.N., 2008a. Enhancing intestinal drug solubilization using lipid-based delivery systems. Adv. Drug Deliv. Rev. 60, 673–691.
Porter, C.J.H., Trevaskis, N.L., Charman, W.N., 2007. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat. Rev. Drug Discov. 6, 231–248.
Porter, C.J.H., Wasan, K.M., Constantinides, P., 2008b. Lipid-based systems for the enhanced delivery of poorly water soluble drugs. Adv. Drug Deliv. Rev. 60, 615–616.
Pouton, C.W., 1999. Key issues when formulating hydrophobic drugs with lipids. Bull. Tech. Gattefosse 92, 41–50.
Pouton, C.W., 2000. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. Eur. J. Pharm. Sci. 11, S93–S98.
Pouton, C.W., Porter, C.J.H., 2008. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv. Drug Deliv. Rev. 60, 625–637.
Powis, G., Kovach, J.S., 1983. Disposition of bisantrene in humans and rabbits: evidence for intravascular deposition of drug as a cause of phlebitis. Cancer Res. 43, 925–929.
Prentis, R.A., Lis, Y., Walker, S.R., 1988. Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985). Br. J. Clin. Pharmacol. 25, 387–396.
Qureshi, S.A., 2004. Improved drug dissolution and product characterization using a crescent-shaped spindle. J. Pharm. Pharmacol. 56, 1135–1141.
Qureshi, S.A., 2006. Comparative impact of stirring and shearing in drug dissolution testing with USP paddle and crescent-shaped spindles. Dissolution Technol. 13, 25–30.
Qureshi, S.A., Caille, G., Brien, R., Piccirilli, G., Yu, V., McGilveray, I.J., 1994. Application of flow-through dissolution method for the evaluation of oral formulations of nifedipine. Drug Dev. Ind. Pharm. 20, 1869–1882.
Ratanabanangkoon, P., Guzman, H., Almarsson, O., Berkovitz, D., Tokarcyzk, S., Straughn, A.B., Chen, H., 2008. A high-throughput approach towards a novel formulation of fenofibrate in omega-3 oil. Eur. J. Pharm. Sci. 33, 351–360.
Reymond, J.P., Sucker, H., 1988. In vitro model for ciclosporin intestinal absorption in lipid vehicles. Pharm. Res. 5, 673–676.
Rost, M., Quist, P.O., 2003. Dissolution of USP prednisone calibrator tablets. Effects of stirring conditions and particle size distribution. J. Pharm. Biomed. Anal. 31, 1129–1143.
Scholz, A., Abrahamsson, B., Diebold, S.M., Kostewicz, E., Polentarutti, B.I., Ungell, A.-L., Dressman, J.B., 2002. Influence of hydrodynamics and particle size on the absorption of felodipine in labradors. Pharm. Res. 19, 42–46.
Scholz, A., Kostewicz, E., Abrahamsson, B., Dressman, J.B., 2003. Can the USP paddle method be used to represent in-vivo hydrodynamics? J. Pharm. Pharmacol. 55, 443–451.
Schroeder, H.G., DeLuca, P.P., 1974. A study on the in vitro precipitation of poorly soluble drugs from nonaqueous vehicles in human plasma. Bull. Parenter. Drug Assoc. 28, 1–14.
Seadeek, C., Ando, H., Bhattachar, S.N., Heimbach, T., Sonnenberg, J.L., Blackburn, A.C., 2007. Automated approach to couple solubility with final pH and crystallinity for pharmaceutical discovery compounds. J. Pharm. Biomed. Anal. 43, 1660–1666.
Sek, L., 2007. Use of in vitro lipid digestion models for assessing oral lipid formulations. Am. Pharm. Rev. 10, pp. 54, 56–58.
Sek, L., Boyd, B.J., Charman, W.N., Porter, C.J.H., 2006. Examination of the impact of a range of pluronic surfactants on the in-vitro solubilisation behaviour and oral bioavailability of lipidic formulations of atovaquone. J. Pharm. Pharmacol. 58, 809–820.
Sek, L., Porter, C.J.H., Kaukonen, A.M., Charman, W.N., 2002. Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. J. Pharm. Pharmacol. 54, 29–41.
Serajuddin, A.T.M., Sheen, P.C., Mufson, D., Bernstein, D.F., Augustine, M.A., 1988. Physicochemical basis of increased bioavailability of a poorly water-soluble drug following oral administration as organic solutions. J. Pharm. Sci. 77, 325–329.
Shah, V.P., 2005. The role of dissolution testing in the regulation of pharmaceuticals: the FDA perspective. Pharm. Dissolution Testing, 81–96.
Simmons, D.M., 1993. Characterization of the precipitates of poorly-water-soluble drugs and determination of the solubilities in human gastrointestinal fluids by microscopy. Drug Dev. Ind. Pharm. 19, 1103–1112.
Stippler, E., Kopp, S., Dressman, J.B., 2004. Comparison of US Pharmacopeia simulated intestinal fluid TS and phosphate standard buffer pH 6.8, TS of the International Pharmacopoeia with respect to their use in vitro dissolution testing. Dissolution Technol. 11, 6–10.
Strickley, R.G., 2004. Solubilizing excipients in oral and injectable formulations. Pharm. Res. 21, 201–230.
Sugano, K., Kato, T., Suzuki, K., Keiko, K., Sujaku, T., Mano, T., 2006. High throughput solubility measurement with automated polarized light microscopy analysis. J. Pharm. Sci. 95, 2115–2122.
Sunesen, V.H., Pedersen, B.L., Kristensen, H.G., Muellertz, A., 2005. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. Eur. J. Pharm. Sci. 24, 305–313.
Surakitbanharn, Y., Simamora, P., Ward, G.H., Yalkowsky, S.H., 1994. Precipitation of pH solubilized phenytoin. Int. J. Pharm. 109, 27–33.
Tang, L., Khan, S.U., Muhammad, N.A., 2001. Evaluation and selection of bio-relevant dissolution media for a poorly water-soluble new chemical entity. Pharm. Dev. Technol. 6, 531–540.

Taniguchi, T., Yamamoto, K., Kobayashi, T., 1996. The precipitate formed by thiopentone and vecuronium. Can. J. Anaesth. 43, 511–513.
Taniguchi, T., Yamamoto, K., Kobayashi, T., 1998. Precipitate formed by thiopentone and vecuronium causes pulmonary embolism. Can. J. Anaesth. 45, 347–351.
Thoma, K., Ziegler, I., 1998. Development of an automated flow-trough dissolution system for poorly soluble drugs with poor chemical stability in dissolution media. Pharmazie 53, 784–790.
Tsukamoto, T., Buck, M., Odegaard, A., Lieber, M., Kovach, J.S., 1985. Regional localization of an anticancer drug, bisantrene, by intentional intravascular precipitation. Recent Adv. Chemother., Proc. Int. Congr. Chemother., 14th: Anticancer Sect. 1, 538–539.
Turco, S.J., 1975. Phlebitis associated with intravenous drug administration. Bull Parenter. Drug Assoc. 29, 89–97.
Uppoor, V.R.S., 2001. Regulatory perspectives on in vitro (dissolution)/in vivo (bioavailability) correlations. J. Control. Rel. 72, 127–132.
Vasanthavada, M., Serajuddin, A.T.M., 2007. Lipid-based self-emulsifying solid dispersions. Drugs Pharm. Sci. 170, 149–183.
Vertzoni, M., Fotaki, N., Kostewicz, E., Stippler, E., Leuner, C., Nicolaides, E., Dressman, J., Reppas, C., 2004. Dissolution media simulating the intralumenal composition of the small intestine: Physiological issues and practical aspects. J. Pharm. Pharmacol. 56, 453–462.
Ward, G.H., Yalkowsky, S.H., 1993a. Studies in phlebitis. IV: injection rate and amiodarone-induced phlebitis. J. Parenter. Sci. Technol. 47, 40–43.
Ward, G.H., Yalkowsky, S.H., 1993b. Studies in phlebitis. V: Hemolysis as a model for phlebitis. J. Parenter. Sci. Technol. 47, 44–46.
Ward, G.H., Yalkowsky, S.H., 1993c. Studies in phlebitis. VI: dilution-induced precipitation of amiodarone HCl. J. Parenter. Sci. Technol. 47, 161–165.
Wennergren, B., Lindberg, J., Nicklasson, M., Nilsson, G., Nyberg, G., Ahlgren, R., Persson, C., Palm, B., 1989. A collaborative in vitro dissolution study: comparing the flow-through method with the USP paddle method using USP prednisone calibrator tablets. Int. J. Pharm. 53, 35–41.
White, M., Yalkowsky, S.H., 1991. Studies in phlebitis. III. Evaluation of diazepam and phenytoin. Pharm. Res. 8, 1341–1342.
Wyttenbach, N., Alsenz, J., Grassmann, O., 2007. miniaturized assay for solubility and residual solid screening (SORESOS) in early drug development. Pharm. Res. 24, 888–898.
Yalkowsky, S.H., 2000. Solubility and Solubilization in Aqueous Media. Oxford University Press.
Yalkowsky, S.H., Krzyzaniak, J.F., Ward, G.H., 1998. Formulation-related problems associated with intravenous drug delivery. J. Pharm. Sci. 87, 787–796.
Yalkowsky, S.H., Valvani, S.C., 1977. Precipitation of solubilized drugs due to injection or dilution. Drug Intell. Clin. Pharm. 11, 417–419.
Yalkowsky, S.H., Valvani, S.C., Johnson, B.W., 1983. In vitro method for detecting precipitation of parenteral formulations after injection. J. Pharm. Sci. 72, 1014–1017.
Zangenberg, N.H., Mullertz, A., Gjelstrup Kristensen, H., Hovgaard, L., 2001a. A dynamic in vitro lipolysis model II: evaluation of the model. Eur. J. Pharm. Sci. 14, 237–244.
Zangenberg, N.H., Mullertz, A., Kristensen, H.G., Hovgaard, L., 2001b. A dynamic in vitro lipolysis model I. Controlling the rate of lipolysis by continuous addition of calcium. Eur. J. Pharm. Sci. 14, 115–122.