

**Development of discriminative and predictive  
dissolution tests for immediate release oral  
dosage forms of poorly soluble drugs**

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by

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***To my family***

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## LIST OF ABBREVIATIONS

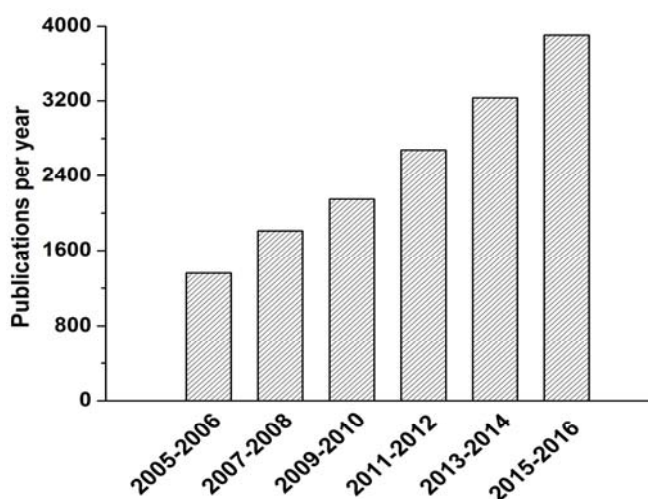
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
ASD	Artificial stomach duodenal
ASD	Amorphous solid dispersions
AUC	Area under the curve
BCS	Biopharmaceutical classification system
BDDCS	Biopharmaceutical drug disposition classification system
$C_{max}$	Peak plasma drug concentration
CR	Controlled release
CV	Coefficient of variation
DCS	Developability classification system
DGM	Dynamic gastric model
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
DS	Degree of supersaturation
ER	Extended release
EtOH	Ethanol
FaSSGF	Fasted state simulated gastric fluid
FaSSIF	Fasted state simulated intestinal fluid
FeSSGF	Fed state simulated gastric fluid
FeSSIF	Fed state simulated intestinal fluid
FDA	Food and drug administration
FTIR	Fourier transformed infrared
GI	Gastrointestinal
HCl	Hydrochloric acid
HGF	Human gastric fluid
HP $\beta$ CD	Hydroxypropyl- $\beta$ -cyclodextrin
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
HPMCAS	Hydroxypropyl methylcellulose acetate succinate
IDR	Intrinsic dissolution rate
IR	Immediate release

IVIVC	<i>In vitro-in vivo</i> correlation
IVIVR	<i>In vitro-in vivo</i> relationship
LBDDS	Lipid-based drug delivery system
MAD	Maximum absorbable dose
MTDSC	Modulated-temperature differential scanning calorimetry
NaOH	Sodium hydroxide
PEG 400	Polyethylene glycol 400
PK	Pharmacokinetic
PL	Phospholipids
PM	Physical mixtures
PVPVA 64	Polyvinyl pyrrolidone-vinyl acetate copolymer
QC	Quality control
R <sup>2</sup>	Coefficient of determination
SD	Solid dispersion
SD	Standard deviation
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SIWV	Small intestinal water volume
SITT	Small intestinal transit time
SLS	Sodium lauryl sulfate
T <sub>g</sub>	Glass transition temperature
TGA	Thermogravimetric analysis
TIM-1	TNO intestinal model
T <sub>max</sub>	Time to reach C <sub>max</sub>
TPGS	D- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate
USP	United states pharmacopeia
UV	Ultraviolet
vs.	Versus
XRPD	X-ray powder diffraction

# **1. GENERAL INTRODUCTION**

## 1.1. Significance and challenge of poorly soluble drugs

Oral formulation has been the most popular due to low costs, good therapy compliance and patient convenience. Approximately more than 50% of new drugs approved by the Food and Drug Administration (FDA) from 2012 to 2015 are orally administered (Mullard, 2013, 2014, 2015, 2016). With a dramatic increase in poorly water-soluble drug candidates (approximately up to 70%) in drug discovery by means of combinatorial chemistry and high throughput screening (Ku and Dulin, 2012), the improvement of oral bioavailability has become one of the greatest challenges since the first step in oral absorption for drug compound is to dissolve in the gastrointestinal (GI) fluids. Statistically, a pronounced growth in the number of researches concerning poorly aqueous soluble drugs for oral administration has been observed (Fig. 1.1).



**Fig. 1.1.** Increase in the number of publications per year concerning poorly aqueous soluble drugs for oral administration from 2005 to 2016. *Source:* Web of Science (accessed: April 6<sup>th</sup> 2017).

### 1.1.1. Scientific foundation

Oral bioavailability represents the fraction of drug absorbed through intestinal membrane into the bloodstream. Numerous complex factors influence the process of drug absorption, but a simple conceptual approach to understanding the key factors for drug absorption can be expressed by the maximum absorbable dose (MAD) (Eq. 1.1) (Johnson and Swindell, 1996):



$$MAD = S \times Ka \times SIWV \times SITT \quad (1.1)$$

where  $S$  is the drug solubility at intestinal pH,  $Ka$  is the intestinal absorption rate constant,  $SIWV$  is the small intestinal water volume available for drug dissolution (~250 ml), and  $SITT$  is the small intestinal transit time (3~4.5 h).

Based on Eq. 1.1, an integrated absorption model was proposed by considering transit flow, dissolution and permeation as three major processes of absorption. It introduced effective human intestinal permeability ( $P_{eff}$ ) and effective intestinal surface area ( $A$ ) to replace absorption rate constant and fluid volume (Eq. 1.2) (Lawrence, 1999):

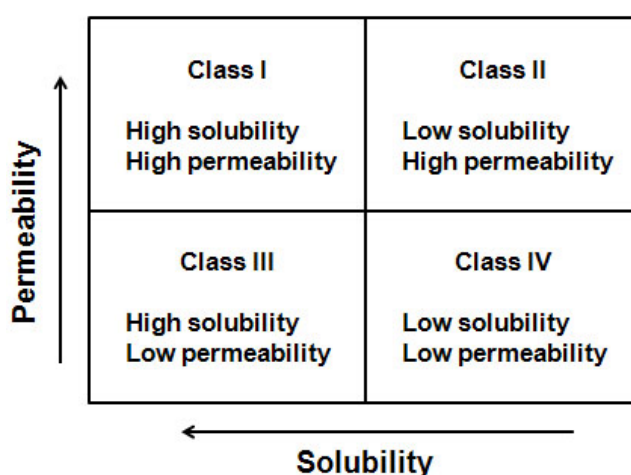
$$MAD = P_{eff} \times S \times A \times SITT \quad (1.2)$$

This model could estimate the fraction of dose absorbed and determine the potential causes (dissolution-, solubility- or permeability-limited absorption) for poor oral bioavailability.

#### *1.1.1.1. Biopharmaceutics classification system*

Biopharmaceutics Classification System (BCS) developed by Amidon et al. in 1995 indicated that solubility and permeability play a fundamental role in controlling the rate and extent of oral drug absorption (Amidon et al., 1995). The BCS is a scientific framework that provides a basis for predicting the oral absorption of drugs. It has been adopted by FDA to facilitate biowaivers of *in vivo* bioequivalence testing (Food and Drug Administration, 2000). Drug substances are categorized into four classes based on solubility and intestinal permeability (Fig. 1.2). According to the recent FDA guidance (Food and Drug Administration, 2015), a drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1.0-6.8. High permeability is defined as the intestinal absorption of 85% or higher of an administered dose in human. Conversely, if more than 250 ml is required to

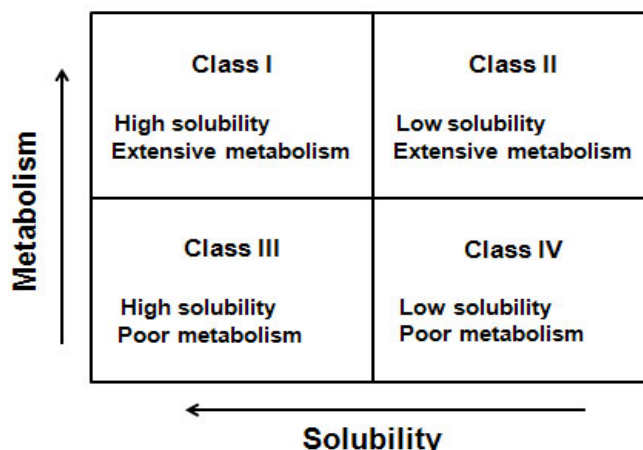
dissolve the drug, or absorption is less than 85%, it is regarded as the poorly soluble or poorly permeable, respectively. BCS has been a useful tool not only for waivers for *in vivo* bioequivalence studies but also for formulation strategies in early drug development (Kawabata et al., 2011; Ku, 2008).



**Fig. 1.2.** The Biopharmaceutics Classification System as defined by Amidon et al. (Amidon et al., 1995).

#### 1.1.1.2. Biopharmaceutics drug disposition classification system

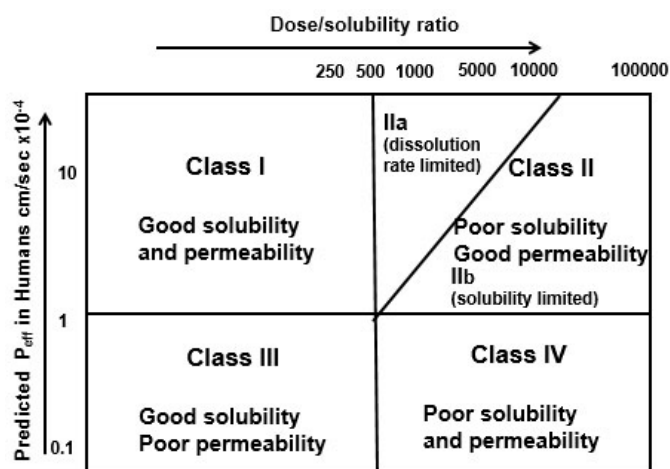
In 2005, Wu and Benet recognized an apparent correlation between the intestinal permeability rate and the extent of metabolism and proposed the Biopharmaceutics Drug Disposition Classification System (BDDCS) (Wu and Benet, 2005). Drug substances are categorized into four classes in terms of solubility and the extent of metabolism (Fig. 1.3). A drug substance is considered as extensive metabolism when metabolism is determined to be  $\geq 70\%$  of an oral dose in human. They noted that the major route of elimination in humans for highly permeable drugs was via metabolism, whereas renal and biliary excretion of unchanged drug were for poorly permeable drugs. BDDCS can be used as a tool to predict drug disposition and potential drug-drug interaction as well as transporter-enzyme interplay.



**Fig. 1.3.** The Biopharmaceutics Drug Disposition Classification System where major route of elimination serves as the permeability criteria (Wu and Benet, 2005).

*1.1.1.3. Developability classification system*

In 2010, Butler and Dressman proposed the Developability Classification System (DCS) which categorizes drugs based on dose/solubility ratio, dissolution rate and/or permeability (Fig. 1.4) (Butler and Dressman, 2010). DCS provides a better means of assessing the development risks in the context of quality by design. The concept of solubility limited absorbable dose is used to divide class II into IIa (dissolution-limited) and IIb (solubility-limited), which provides more useful information for appropriate formulation strategies. To improve oral drug absorption, IIa drugs can be formulated via particle size reduction, while IIb drugs need solubilization techniques (e.g., solid dispersions, lipid formulations).



**Fig. 1.4.** The Developability Classification System which aims at addressing issues in product development (Butler and Dressman, 2010).

#### 1.1.1.4. BCS sub-classification

In 2014, Tsume et al. proposed a sub-classification extension for BCS class II and IV drugs dependent on actual solubility and pKa including a (acids, pKa < ~5), b (bases, pKa > ~5) and c (neutral drugs, pKa < 0 or > 8) subclasses (Table 1.1) (Tsume et al., 2014). Based on BCS sub-classification, they further suggested the importance and potential in developing an *in vitro* predictive dissolution methodology for the *in vivo* performance of the product. Especially for BCS IIa, IIb, and IIc drug substances, it is advised to use a separate gastric compartment in the dissolution test and to transfer gastric contents into the intestinal compartment with an absorption compartment in a physiologically relevant manner.

**Table 1.1.** Solubility and permeability characteristics of drug substances based on BCS sub-classification (Tsume et al., 2014).

BCS sub-classification	Solubility at pH 2	Solubility at pH 6.5	Permeability
I	High	High	High
IIa	Low	High	High
IIb	High	Low	High
IIc	Low	Low	High
III	High	High	Low
Iva	Low	High	Low
IVb	High	Low	Low
IVc	Low	Low	Low

a with a pKa < 5; b with a pKa > 6; c with no pKa or pKa < 0 or > 8.

#### 1.1.2. Formulation strategies for dissolution enhancement

According to BCS and its extensions, poorly soluble drugs (class II) have attracted extensive interest due to the big challenge of formulation development and the potential to establish *in vitro-in vivo* relationship. To overcome low solubility, various formulation approaches have been developed such as particle size reduction (Chaumeil, 1998; Liversidge and Cundy, 1995), salt formulation (Serajuddin, 2007), polymorphs (Singhal and Curatolo, 2004), cocrystals (Thakuria et al., 2013), prodrugs (Rautio et al., 2008), cosolvents (Millard et al.,

2002), complexation (Brewster and Loftsson, 2007), nanonization (Chen et al., 2011; Shegokar and Müller, 2010), lipid-based formulations (Kohli et al., 2010; Mu et al., 2013), and solid dispersions (Vasconcelos et al., 2007; Vo et al., 2013).

These strategies ideally enhanced drug solubility and *in vitro* dissolution, but not all cases improved bioavailability (Newman et al., 2012; Park, 2014). The potential reasons for this inconsistency have raised concern. Buckley et al. reviewed the enabling formulation approaches with respect to the solubility and permeability of poorly soluble drugs and discussed their interplay (Buckley et al., 2013). The solubilization of poorly soluble drugs is achieved by their incorporation in colloidal species (emulsified oil, mixed micelles, etc.) or complexing agents. This approach not only is limited by drug itself with very poor aqueous solubility, but also impairs the free fraction of drug molecules for uptake across the intestinal membrane. However, supersaturatable formulations could enhance the free drug concentration available for absorption and have no effect on drug permeability. Miller et al. revealed a trade-off between solubility increase and permeability decrease for solubilizing excipients, whereas a win-win via amorphous solid dispersions increased apparent solubility without the expense of intestinal permeability (Miller et al., 2012). Furthermore, Ueda et al. investigated differences in permeation behaviors of supersaturated and solubilized solutions of carbamazepine and correlated with different molecular states by nuclear magnetic resonance measurements (Ueda et al., 2012). Carbamazepine was solubilized in the hydrophobic core of poloxamer 407 and reduced permeation due to the size of the polymer micelles. However, drug was self-associated in supersaturated solution with hydroxypropyl methylcellulose acetate succinate (HPMCAS) with weaker interaction, which did not affect the permeation behavior of carbamazepine.

The influence of various enabling formulation approaches on dissolution, solubility and permeability for poorly soluble drugs was summarized in Table 1.2.

**Table 1.2.** The formulation strategies for poorly soluble drugs and their influence on dissolution, solubility and permeability.

Formulation approaches	Dissolution	Solubility	Permeability
● Particle size reduction			
Micronization	+		
Nanonization	+	(+)	
● Crystal modifications			
Polymorphs	+	+	
Salts	+	+	
Co-crystals	+	+	
● Complexation/solubilization			
Use of surfactants	+	+	- (Katneni et al., 2006; Miller et al., 2011)
Use of cyclodextrins	+	+	- (Dahan et al., 2010; Miller et al., 2012b)
● Cosolvents	+	+	- (Beig et al., 2012)
● Amorphous dispersions	+	+	<b>N</b> (Miller et al., 2012a)
● Lipid-based formulations	+	+	- with surfactants (Fischer et al., 2011)

+, positive effect; -, inhibiting effect; **N**, no effect.

## 1.2. Significance of dissolution testing

Dissolution testing is routinely used in quality control (QC) and research & development. Dissolution research caused concern in the 1950s since the importance was realized to be associated with drug bioavailability. Edwards firstly described the relationship between drug dissolution rate and absorption (Edwards, 1951). The close link between *in vitro* dissolution and *in vivo* performance is based on the fact that a drug substance must be first released from the product and dissolved in the fluids of the GI tract before absorption. Therefore, dissolution testing is considered as a strong indicator of drug absorption. Noticeably, when dissolution tests do not have adequate discrimination for the candidate formulations which actually perform differently between *in vitro* and *in vivo* studies, the predictive results may be completely misleading. Thus, a discriminative and predictive *in vitro* dissolution test is highly attractive to facilitate pharmaceutical research by reducing a time consuming and costly process.

### 1.2.1. Dissolution theory

The first theory on dissolution process was proposed by Noyes and Whitney in 1897 (Noyes and Whitney, 1897). They noted that the dissolution rate was proportional to the difference between the concentration of the solution and the saturation solubility, as indicated by Eq. 1.3.

$$\frac{dW}{dt} = k(C_s - C) \quad (1.3)$$

where  $dW/dt$  is the rate of dissolution,  $k$  is the dissolution rate constant,  $C_s$  is the saturation solubility of the drug and  $C$  is the concentration of the drug in the dissolution medium at time  $t$ . The mechanism of dissolution is described that drug molecule diffuses from a saturated layer formed around the solid surface to the bulk solution. Clearly, the concentration gradient ( $C_s - C$ ) is the main driving force behind drug dissolution. When sink conditions exist,  $C_s \gg C$ , the Eq. 1.3 becomes

$$\frac{dW}{dt} = kC_s \quad (1.4)$$

In 1904, the dissolution model was further modified by Nernst and Brunner based on Fick's second law of diffusion (Brunner, 1903), which is known as the Nernst-Brunner equation (Eq. 1.5).

$$\frac{dW}{dt} = \frac{AD}{h}(C_s - C) \quad (1.5)$$

where  $A$  is the surface area of the dissolving solid,  $D$  is the diffusion coefficient of the drug and  $h$  is the thickness of diffusion layer. It is clear from Eq. 1.5 the dissolution rate is improved by increasing the available surface area, reducing the diffusion layer thickness, increasing drug solubility.

### 1.2.2. Factors affecting dissolution

The factors influencing dissolution can be understood by examining the Nernst-Brunner equation. The mainly potential possibilities for enhancing dissolution are to increase the surface area by reducing particle size and/or by improving wetting, to decrease the thickness of diffusion layer by decreasing particle size, to maintain sink conditions, and to improve drug solubility under physiological conditions by solubility-enabling approaches (e.g., solubilization and supersaturation). These parameters are greatly influenced not only by the physicochemical properties of the drug, but also by the physiological conditions in the GI tract (Table 1.3). Ideally, a desirable *in vitro* dissolution test can be sensitive to capture the variables that influence release behavior and be predictive for *in vivo* performance. Such variables could be resulted from the characteristics of active pharmaceutical ingredients (APIs) (e.g., particle size, solubility, pKa and polymorphic form), formulation compositions (e.g., dose strength, excipient type, grade and level), and manufacturing processes (e.g., granulation, tableting and coating).

**Table 1.3.** The physicochemical and physiological factors affecting drug dissolution (Dressman and Reppas, 2000; Hörter and Dressman, 2001; Mudie et al., 2010).

Parameter	Physicochemical factor	Physiological factor
Surface area, $A$	Particle size, shape, wettability	Native surfactants
Diffusion coefficient, $D$	Molecular size	Fluid viscosity
Diffusion layer thickness, $h$	Particle size, diffusion coefficient	Motility patterns, flow rates
Saturated solubility, $C_s$	Hydrophilicity, crystal structure, pKa, solubilization	Buffer capacity, pH, bile salts, food components,
Concentration of drug in solution, $C$	Dose, intrinsic solubility	Permeability

### 1.2.3. Compendial dissolution testing

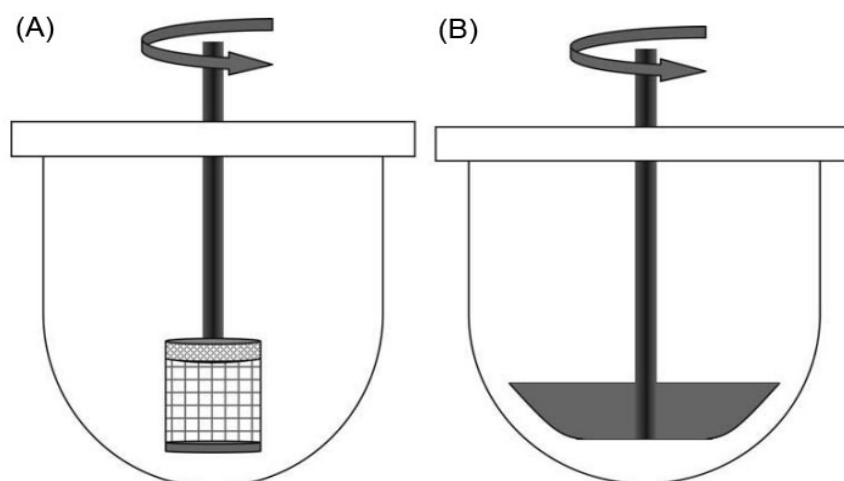
Seven apparatuses for dissolution testing are described in the General Chapters <711> and <724> of United States Pharmacopeia (USP) (USP, 2011; USP, 2001). They are rotating basket (Apparatus 1), paddle method (Apparatus 2),



reciprocating cylinder (Apparatus 3), flow-through cell (Apparatus 4), paddle over disk (Apparatus 5), cylinder (Apparatus 5), and reciprocating holder (Apparatus 7), respectively. The first four compendial apparatuses (USP 1-4) are commonly used to test oral dosage forms at 37 °C, while the other three apparatuses (USP 5-7) are designed mainly for transdermal delivery systems at 32 °C.

### **USP 1 and USP 2 apparatuses**

The basket and paddle apparatuses (Fig. 1.5) were adopted by USP in 1968 and 1978, respectively. They are the most widely used as the first choice for *in vitro* dissolution testing of oral solid dosage forms due to the simplicity, robustness, standardization and broad experience, which makes them the perfect tools for quality control. Apparatus 1 employs a rotating shaft attached a wire-mesh basket with dosage form held inside, and the common agitation is 50-100 rpm. Apparatus 2 uses a paddle with a common rotation speed of 50-75 rpm, and the dosage form is dropped directly into the dissolution vessel. If necessary, a sinker can be used to avoid dosage form floating. For both Apparatus 1 and 2, the typical medium volume is recommended to be 500-1000 ml which can maintain sink conditions for most drug substances. The common dissolution media are summarized in Table 1.4.

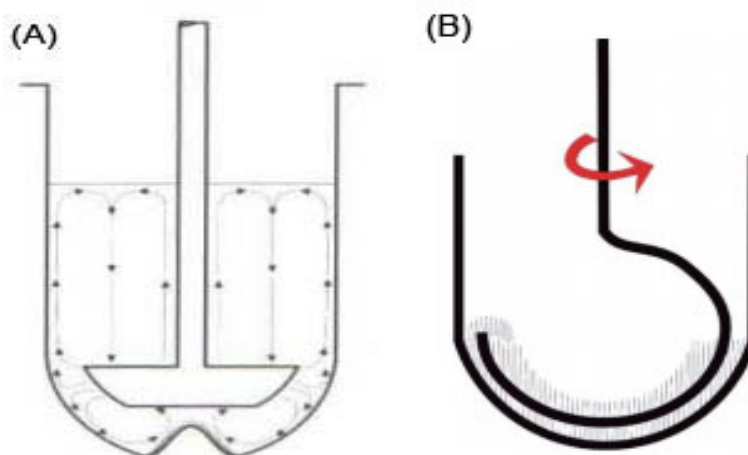


**Fig. 1.5.** Schematic illustration of (A) USP1 and (B) USP 2 apparatuses (Qiu et al., 2009).

**Table 1.4.** Common media used in dissolution testing (Qiu et al., 2009).

pH	Medium	Comments
-	Purified water	
1-3	Hydrochloric acid	Between 0.1 and 0.001N
1.2	Stimulated gastric fluid (SGF)	With or without enzyme
4.1-5.5	Acetate buffer	50 mM
5.8-8.0	Phosphate buffer	50 mM
6.8	Simulated intestinal fluid (SIF)	With or without pancreatin

However, these apparatuses also have their drawbacks. It is difficult to simulate pH changes in the GI tract due to the use of a single dissolution medium. The results obtained by Apparatus 1 or 2 are sensitive to the variability, including shaft wobble, centering, location, and the extent of degassing, etc. The non-homogeneity of the flow due to the presence of a cone under the paddle is another focused issue. Some studies proposed the solutions, including a PEAK vessel (Fig. 1.6A) and a crescent-shaped spindle (Fig. 1.6B), to eliminate the unstirred cone (Beckett et al., 1996; Qureshi, 2004).

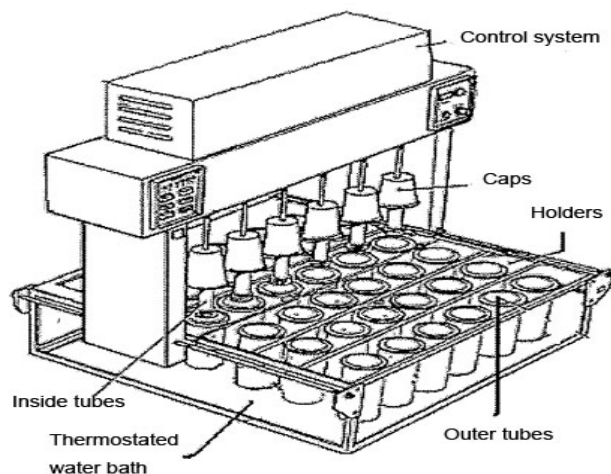


**Fig. 1.6.** Schematic representation of (A) PEAK vessel and (B) the crescent-shaped spindle (Beckett et al., 1996; Qureshi, 2004).

### **USP 3 apparatus**

Reciprocating cylinder (Fig. 1.7) was developed based on the above-mentioned physical factors potentially affecting the performance of USP 1 and 2, and added into the USP in 1991. Apparatus 3 is originally used for extended-release

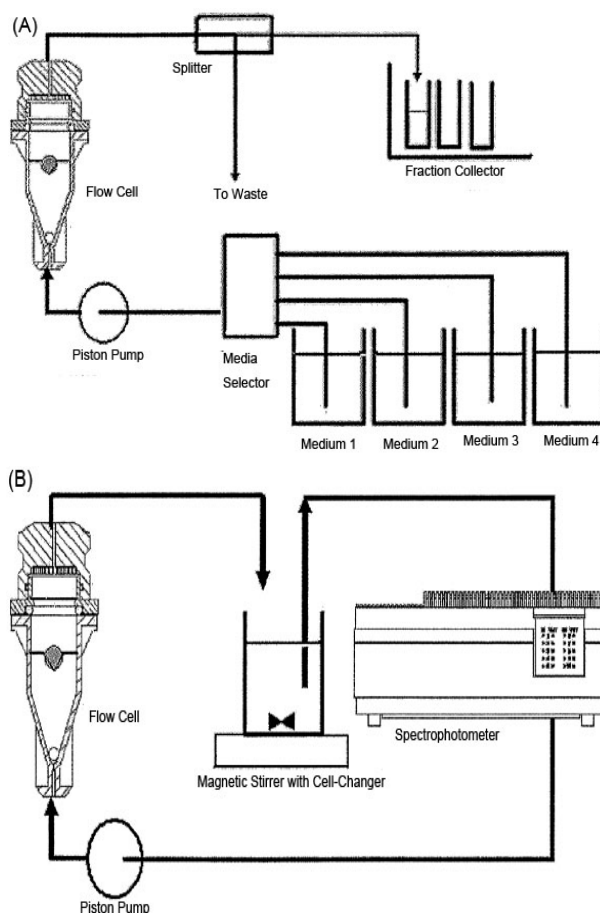
products which are placed in the cylinder. It offers the advantages of mimicking the GI transit conditions due to the free use of dissolution media at different pH and avoiding the core formation encountered with Apparatus 2 (Dressman and Krämer, 2005). However, it may be difficult to generate sink conditions due to a small volume of dissolution medium used (~250 ml).



**Fig. 1.7.** Schematic illustration of USP 3 apparatus (Dressman and Krämer, 2005).

### ***USP 4 apparatus***

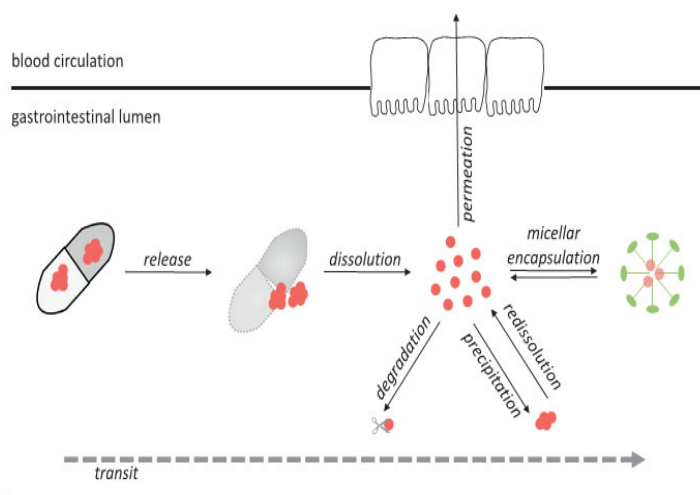
The flow-through cell was primarily developed for controlled release products and adopted by USP in 1990 (Dressman and Krämer, 2005). It is applicable not only for tablets and granules, but also for some specific dosage forms (e.g., suppositories, soft-gelatin capsules, semisolids, and implants). The dissolution medium flows through a cell which holds the dosage form. There are two operated modes: open loop and closed loop systems (Fig. 1.8). In an open system, drug dissolved is instantaneously transferred along the flow of the dissolution medium and the fresh dissolution medium is continuously passing through the cell. Conversely, a fixed volume of medium is recycled in a closed system. The apparatus offers the advantage of changing the media with pH gradients throughout one test and providing the biorelevant hydrodynamics to mimic intestinal flow.



**Fig. 1.8.** Schematic illustration of USP 4 apparatus: (A) open loop mode and (B) closed loop mode (Dressman and Krämer, 2005).

#### 1.2.4. Biorelevant dissolution testing

Drug absorption is an extremely complex process which greatly depends on the physiological conditions in the GI tract and the interplay between multiple factors (Fig. 1.9). A lack of a biorelevant dissolution system often leads to disconnect with the data from *in vivo* results. To bridge the gap between *in vitro* dissolution and *in vivo* absorption, more and more attentions have been paid to improve biorelevant conditions of *in vitro* methods. Several good reviews provided relatively comprehensive information of human GI physiological parameters that influence drug dissolution and oral absorption (Bergström et al., 2014; DeSesso and Jacobson, 2001; McConnell et al., 2008; Mudie et al., 2010).



**Fig. 1.9.** Schematic representation of intraluminal processes determining intestinal absorption (Brouwers and Augustijns, 2014).

### 1.2.4.1. Biorelevant dissolution media

Compendial dissolution media in the USP are widely applied in routine dissolution testing for QC purpose due to the simple composition with primarily considering pH. Through the detailed characterization of the GI fluids, biorelevant dissolution media have been developed by means of the simulation of pH, osmolality, surface tension, buffer capacity, natural surfactants (bile salts and phospholipids), enzyme activity, and the presence of food ingredients (Tables 1.5 and 1.6). For example, fasted state simulated gastric fluid (FaSSGF), fed state simulated gastric fluid (FeSSGF), fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were developed to simulate the environment in the stomach and intestine under fasted and fed conditions, respectively. Many studies showed biorelevant media had much higher predictive power for *in vivo* behavior of the test formulations than simple compendial media (Jinno et al., 2006; Kostewicz et al., 2002; Nicolaidis et al., 2001; Sunesen et al., 2005).

## General introduction

**Table 1.5.** Fasted and fed state simulated gastric fluids.

Fluid name	FaSSGF <sup>a</sup>	FaSSGF-V2 <sup>b</sup>	FeSSGF <sup>c</sup>
Year	2005	2007	2008
pH	1.6	1.6	5.0
Buffer type	Hydrochloric acid	Hydrochloric acid	Acetate
Buffer capacity (mM/pH)	-	-	25
Long life milk buffer ratio	-	-	1:1
Osmolality (mOsmol/kg)	120.7	186.9	400
Bile salt ( $\mu$ M)	80	80	-
Phospholipid ( $\mu$ M)	20	20	-
Pepsin (mg/ml)	0.1	0.1	-

a (Vertzoni et al., 2005); b (Vertzoni et al., 2007); c (Jantratid et al., 2008).

**Table 1.6.** Fasted and fed state simulated intestinal fluids.

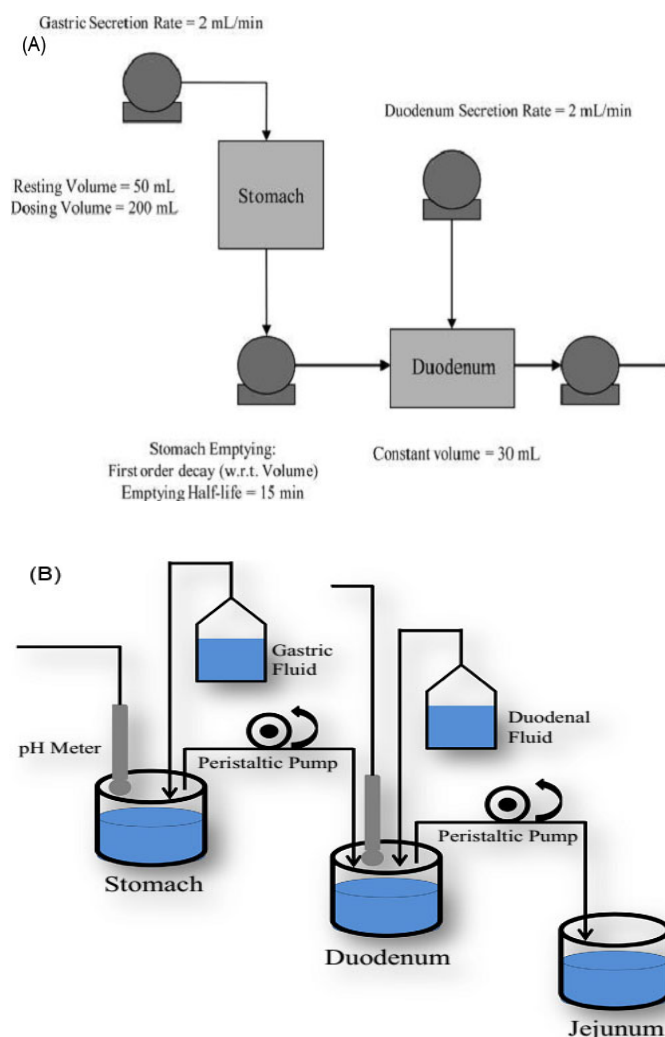
Fluid name	FaSSIF <sup>a</sup>	FaSSIF-V2 <sup>b</sup>	FeSSIF <sup>a</sup>	FeSSIF-V2 <sup>b</sup>
Year	1998	2008	1998	2008
pH	6.5	6.5	5.0	5.8
Buffer type	phosphate	maleate	acetate	maleate
Buffer capacity (mM/pH)	10	10	75	25
Osmolality (mOsmol/kg)	270	180	635	390
Bile salt ( $\mu$ M)	3	3	15	10
Phospholipid ( $\mu$ M)	0.75	0.2	3.75	2
Surface tension (mN/m)	45.5	-	46.3	40.45
Fluid name	FaSSIF <sup>a</sup>	FaSSIF-V2 <sup>b</sup>	FeSSIF <sup>a</sup>	FeSSIF-V2 <sup>b</sup>

a (Galia et al., 1998); b (Jantratid et al., 2008).

Recently, several attempts have been reported to simplify the complex composition of biorelevant media due to the high cost and limited stability. Lehto et al. identified that 0.1% SLS medium was the best to predict *in vivo* plasma profiles using compartmental absorption and transit model and considered it as a surrogate for FaSSIF to evaluate drug dissolution (Lehto et al., 2011). Clarysse et al. also investigated the use of a simpler medium with D- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate (TPGS) to correlate with the solubilizing capacity of human intestinal fluids. Comparable results were obtained from 0.1% TPGS for the fasted state and 2% TPGS for the fed state (Clarysse et al., 2011).

### 1.2.4.2. Multicompartment dissolution models

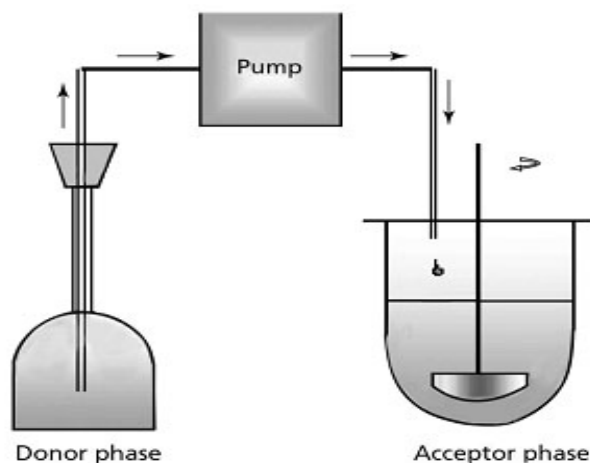
Poorly soluble drugs may occur the precipitation *in vivo* due to numerous factors associated either with the physicochemical properties of an API itself (e.g., weak bases) or with the characteristic of a formulation (e.g., supersaturation formulation). Weakly basic drugs can quickly dissolve at gastric pH, but not at intestinal pH following a supersaturation-precipitation process which greatly affects drug bioavailability. To evaluate the potential precipitation in the intestine, some multicompartment dissolution models have been developed to simulate a dynamic transit from gastric to intestinal conditions. These systems are typically composed of a stomach compartment and a duodenum compartment, allowing the dissolution medium to transfer from one to the other one controlled by gastric emptying rates in a biorelevant manner. The biorelevance of results is based on the assumption that drug concentration in the duodenum compartment is proportional to its bioavailability (Carino et al., 2010). A typical exemplar is the artificial stomach duodenal model (ASD) which was first proposed for evaluation of antacids (Vatier et al., 1992). Carino et al. employed the ASD model (Fig. 1.10A) to predict the relative bioavailability of carbamazepine crystal forms by simulating the fasted and fed states in dog (Carino et al., 2006). The rank order of *in vitro* AUC from duodenum chamber was in agreement with *in vivo* AUC for the three carbamazepine crystal forms. Tsume et al. developed a mini-Gastrointestinal Simulator (Fig. 1.10B) by the addition of an extra jejunum chamber (Tsume et al., 2015). Compared to the ASD model, it offered the advantage of more stable pH environment in the jejunum chamber to evaluate *in vivo* dissolution of weakly basic drugs. Dasatinib dissolution was reduced when the gastric pH was elevated, which was consistent with the results with *in silico* simulation.



**Fig. 1.10.** Diagram of (A) ASD model (Polster et al., 2010) and (B) mini-Gastrointestinal Simulator (Tsume et al., 2015).

Besides the ASD model, another transfer model (Fig. 1.11) was described by Kostewicz et al. (Kostewicz et al., 2004). Similarly, gastric medium was transferred from a donor phase to an acceptor phase with FaSSIF or FeSSIF to test the precipitation of three weakly basic drugs via concentration-time measurements. Compared to hydrodynamics, gastric emptying rates could have a more important effect on the precipitation kinetics. Moreover, precipitation for all three drugs was observed under simulated fasted state conditions but not fed state. Based on this transfer model, Klein et al. proposed a miniaturized transfer model with a mini-paddle apparatus for early formulation screening (Klein et al., 2012).





**Fig. 1.11.** Transfer model for prediction of intestinal precipitation (Kostewicz et al., 2004).

Although the multicompartiment dissolution model offers the advantages of evaluating the effect of gastric emptying and drug precipitation in the intestine, it lacks the consideration for drug removal by absorption across the intestinal membrane. In addition, since the model does not mimic the lower GI region, it may be limited for the effective evaluation of controlled-release formulations.

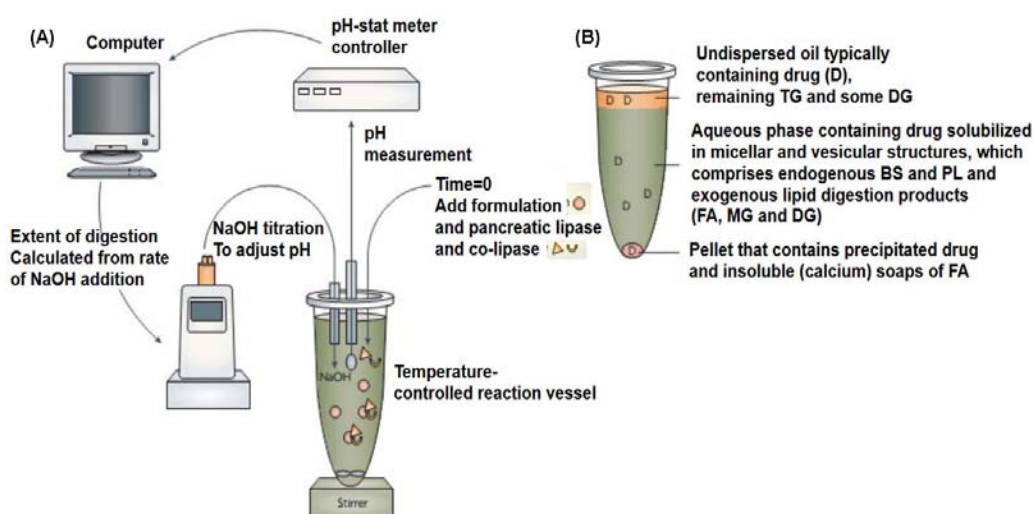
#### 1.2.4.3. Digestion models

The digestion process in the GI tract also plays a critical role in evaluating the performance of dosage forms, especially for lipid-based drug delivery systems (LBDDS) with the digestible excipients.

##### 1.2.4.3.1. *In vitro* lipolysis

*In vitro* lipolysis has become an important technique to better understand how LBDDS promote drug delivery. The activity of gastric lipase can account for approximately 10-25% of the total lipid digestion, so most studies using the *in vitro* lipolysis model have focused on the intestinal step (Thomas et al., 2012). It is typically carried out in a thermo-controlled reaction vessel containing the intestinal digestion medium (Fig. 1.12A). The lipolysis is followed by the titration with NaOH, since free fatty acids produced by enzymatic hydrolysis lead to decrease pH. Additionally, fatty acids potentially inhibit the activity of the

pancreatic lipase, so the addition of calcium can avoid this problem by precipitating the fatty acids. It is very important to precisely monitor the addition of NaOH and calcium. During *in vitro* lipolysis, samples are withdrawn and analyzed following ultracentrifugation to separate the sample in two or more phases (Fig. 1.12B). Some studies have demonstrated the rank order correlations between the results from *in vitro* lipolysis and *in vivo* observations (Cuiné et al., 2007; Fatouros et al., 2008; Porter et al., 2004).

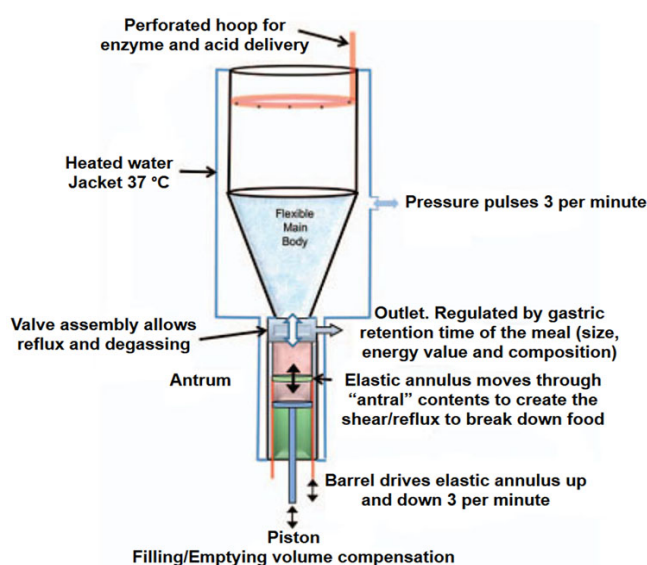


**Fig. 1.12.** Schematic illustration of (A) the *in vitro* lipolysis models and (B) the lipolysis medium after ultracentrifugation (Porter et al., 2007).

#### 1.2.4.3.2. Dynamic gastric models

The dynamic gastric model (DGM) (Fig. 1.13) was developed based on the observation concerning the gastric processing of complex meals by echo-planar magnetic resonance imaging studies (Marciani et al., 2001a; Marciani et al., 2000; Marciani et al., 2001b; Marciani et al., 2004). The DGM is expected to provide an accurate *in vitro* simulation of gastric mixing, shear rates and forces, peristalsis and gastric emptying, and food digestion (Wickham et al., 2009). A limited number of studies about DGM have been reported (Mercuri et al., 2008; Mercuri et al., 2011; Vardakou et al., 2011). Mercuri et al. used the DGM to evaluate the dynamic digestion of a self-emulsifying drug delivery system formulation (Mercuri et al., 2008). Results indicated that the DGM provided a

more accurate simulation of digestion for this lipid-based formulation compared to the conventional USP 2 apparatus. A recent study investigated the effect of gastric conditions on the emulsification process and the fate of the emulsion in the stomach using the DGM (Mercuri et al., 2011). Clearly, the main disadvantage of this model is its inaccessibility for common research, but its ability to replicate gastric forces and meal processing should be promising.



**Fig. 1.13.** Diagram of dynamic gastric model (Wickham et al., 2012).

#### 1.2.4.3.3. TNO intestinal models

The TNO intestinal model (TIM-1) system was developed in TNO Nutrition and Food Research (Zeist, The Netherlands) as a multicompartmental, dynamic and computer-controlled system that simulated the human upper GI tract (Minekus et al., 1995). To date, this *in vitro* system allows the closest simulation of *in vivo* dynamic physiological and digestive processes in the human stomach and small intestine, including pH values, body temperature, gastric emptying, peristaltic mixing and transit, gastric and intestinal secretion, and absorption of small molecules (e.g., nutrients, drugs) (Blanquet et al., 2004). This system consists of four interconnected compartments: stomach, duodenum, jejunum, and ileum (Fig. 1.14). The absorption phase is simulated in TIM-1 by the use of a dialysis membrane, so it is only suitable for drugs by passive absorption. Brouwers et al.

used TIM-1 to evaluate the food-dependent disintegration of immediate release fosamprenavir tablets (Brouwers et al., 2011). Disintegration and dissolution of tablets were significantly postponed in the fed state compared to in the fasted state, which resulted in a lag in the appearance of bioaccessible fosamprenavir. These results were consistent with the observed postprandial delay for fosamprenavir tablet disintegration in the stomach of healthy volunteers. Another study demonstrated that TIM-1 system could support formulation development of poorly soluble drugs by testing various polymorphic forms and formulations of AZD8055 compared to exposure data from the phase 1 clinical study (Dickinson et al., 2012). AZD8055 exposure would increase in a dose-dependent manner and not be limited by solubility or dissolution.

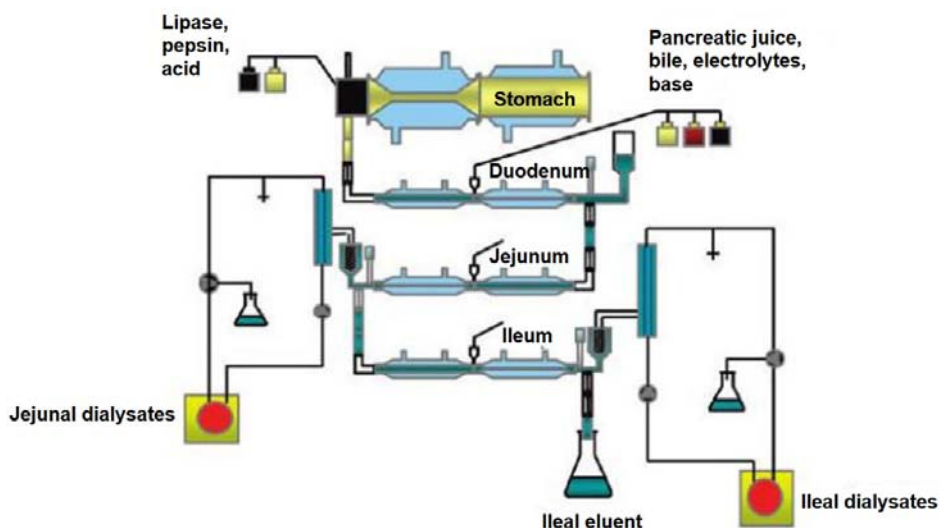


Fig. 1.14. Scheme of TIM-1 system (McAllister, 2010).

Although the TIM-1 system offers some advantages over *in vivo* studies such as accuracy, reproducibility and relatively easy manipulation, its application is limited for routine dissolution testing due to high complexity and often low effectiveness with respect to costs and time.

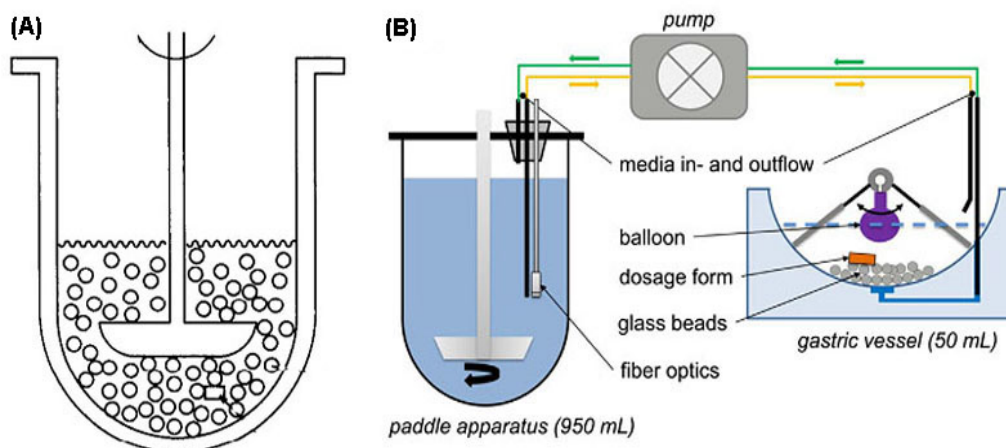
#### 1.2.4.4. Physical stress models

With the development of *in vivo* imaging techniques, an increasing insight has been gained into gastric motility. The *in vivo* heterogeneous hydrodynamics

conditions cannot be reflected in one relatively simple model like USP 1 and 2. The influence of gastric physical forces on drug release and absorption has also gained much attention, especially for extended-release formulations. On the one hand, the unexpected destruction or erosion may result in dose-dumping. On the other hand, inefficient or lagged erosion could influence drug release from the products. For instance, HPMC matrix systems have big differences between *in vitro* and *in vivo* performance due to the variability in erosion caused by different hydrodynamics and mechanic forces. As aforementioned DGM and TIM systems, they are capable of mimicking not only *in vivo* digestion process, but also gastric motility. The following introduction of these systems is designed to only mimic gastric mechanical forces.

#### 1.2.4.4.1. Beads-based methods

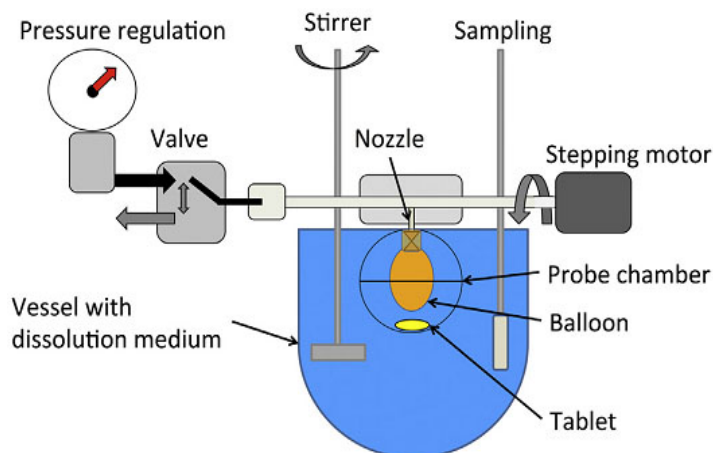
A paddle-beads method (Fig. 1.15A) was proposed by Aoki et al. by using some polystyrene beads (diameter 6.35 mm, specific gravity 1.05 g/cm<sup>3</sup>) to introduce a mechanical impact force (Aoki et al., 1992). It was assumed that the motion of the beads in the dissolution vessel reflected the *in vivo* GI mobility (Aoki et al., 1993). Mehuys et al. investigated the influence of mechanical stress on drug release from the matrix-in-cylinder system by the paddle-beads method (Mehuys et al., 2004). The protective effect of the EC-pipe for matrix erosion was confirmed and the observation of *in vivo* burst release in dogs was also noticed in the paddle-beads dissolution test. Recently, Koziolok et al. developed a fed stomach model with glass beads to mimic gastric conditions after food intake (Fig. 1.15B) (Koziolok et al., 2014).



**Fig. 1.15.** Schematic diagram of (A) the paddle-beads apparatus (Aoki et al., 1992) and (B) the fed stomach model (Koziolek et al., 2014).

#### 1.2.4.4.2. Stress test device

Garbacz et al. developed a novel stress test device (Fig. 1.16) to mimic the peristaltic movement in human GI tract (Garbacz et al., 2008). This stress test device provides sequences of agitation including movement and pressure fluctuations alternated with static phases as they typically occur *in vivo*. Based on the observed multiple peaks of plasma concentration from the extended release (ER) tablets of diclofenac sodium in the clinical trial, drug release from ER tablets was extremely variable and dependent on the applied mechanical stress in the stress test device, whereas a continuous and non-fluctuating release was observed in the conventional USP II method. The data suggested that the characteristic plasma peaks were most probably caused by sensitivity to physical stress in the GI tract. Other studies with the stress test apparatus were conducted with different ER formulations (Garbacz et al., 2009a; Garbacz et al., 2009b).

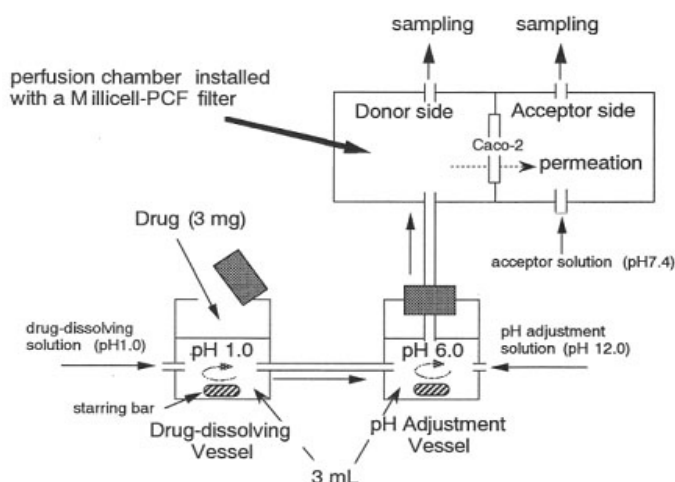


**Fig. 1.16.** Schematic diagram of stress test device (Kostewicz et al., 2014).

#### 1.2.4.5. Dissolution-permeability models

In view of BCS, permeability is also a critical parameter for drug absorption. There has been a marked increase in investigations related with permeability assays for drug delivery systems. *In vitro* models to facilitate permeability studies, including both cellular-based and non-cellular-based, were reviewed by Buckley et al. (Buckley et al., 2012). In particular, the combined dissolution and permeation models take into account drug dissolution prior to membrane permeation, which can assess both the dissolution and permeation processes in a simultaneous manner. Ginski et al. developed the first model which consisted of a donor and a receiver compartments separated by a Caco-2 monolayer to predict drug absorption (Ginski et al., 1999). This model predicted the rate-limiting step of absorption for fast and slow dissolving formulations and excipient effects on dissolution and intestinal permeation kinetics. Kobayashi et al. proposed a dissolution-permeation model with pH-gradient media to mimic pH changes along the GI tract (Fig. 1.17) (Kobayashi et al., 2001). Similar systems with Caco-2 cells or artificial membranes or isolated rat intestines were employed to investigate the dissolution-absorption relationships for poorly soluble drugs and obtain the consistent results with *in vivo* study (Fliszar and Foster, 2008; Hou et al., 2012; Li et al., 2011; Sugawara et al., 2005). Recently, Bevernage et al. pointed out the important impact of an absorptive sink on the interplay between supersaturation, absorption and precipitation (Bevernage et

al., 2012). Drug precipitation was significantly reduced with supersaturation induction in an absorption environment with Caco-2 cells compared to a non-absorption environment. Furthermore, the effect of a precipitation inhibitor using hydroxypropyl methylcellulose (HPMC) was overestimated in a non-absorption environment, which suggested ignoring permeation is unfavorable for prediction of the impact of supersaturation on absorption.



**Fig. 1.17.** Diagram of the dissolution-permeation system (Kobayashi et al., 2001).

The dissolution-permeation models seem promising for the prediction of poorly soluble drugs, but their use in drug development is limited due to drug retention within the barrier, *in vivo* irrelevant hydrodynamics, incompatible use of biorelevant media for Caco-2 cells, cell variability and integrity, relatively high cost, and insufficient concentrations of dissolved drug.

#### 1.2.4.6. Biphasic dissolution models

BCS II drugs usually undergo slow dissolution in the GI fluids under non-sink conditions, but rapidly permeate by the intestinal membrane acting as a perfect sink. Therefore, to better simulate *in vivo* conditions, an absorptive phase has been considered to introduce into *in vitro* dissolution systems.

Biphasic dissolution test could be suitable for BCS II drugs due to poor aqueous solubility. Sink conditions are usually maintained in the dissolution tests by



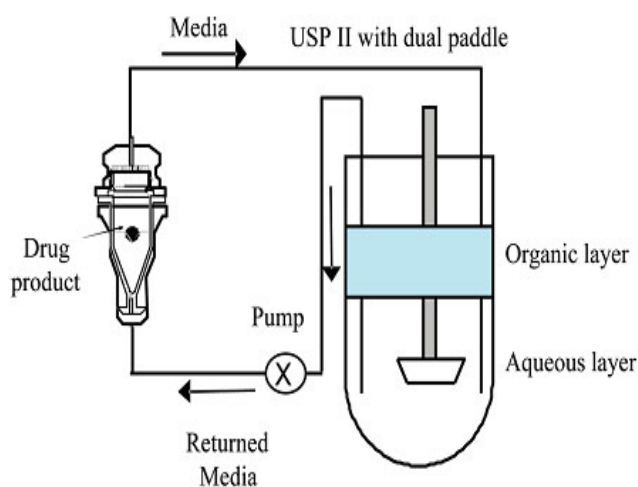
means of a large volume of dissolution medium, surfactants or cosolvents, but they may often lead to lack discrimination for different formulations and have no physiological relevance (Phillips et al., 2012a, 2012b). Biphase dissolution tests consist of two immiscible aqueous and organic phases, which can maintain sink conditions due to a continuous partitioning into organic phase. The drug initially dissolves in the aqueous medium and the organic phase mimics GI membrane that continuously removes the dissolved drug from the lower aqueous phase. Thus, the dissolution-partition process between two phases is analogous to drug dissolution and absorption from GI membrane. There are several available organic solvents used as the organic phase, such as chloroform (Hoa and Kinget, 1996), 1-octanol (Grundy et al., 1997a; Phillips et al., 2012b), a mixture of nonanol and cyclohexane (1:1) (Vangani et al., 2009), and decanol (Box et al., 2016). Octanol is the most common and biorelevant one, because it can mimic the biological membrane in terms of its structure combining lipophilic chains and hydrophilic groups (Fig. 1.18). And similar solubility parameters are very close to those of biological membranes (Panchagnula and Thomas, 2000; Smith et al., 1975). In addition, it has some advantageous physical/chemical properties: practically insoluble in water (0.05 g/100g H<sub>2</sub>O), lower density than water (0.827 g/cm<sup>3</sup> at 20 °C), low volatility (no evaporation at 37 °C), and relatively low viscosity (easy sampling) (Grundy et al., 1997a).



**Fig. 1.18.** The structure of 1-octanol (from PubChem).

A two-phase system was firstly proposed to maintain sink conditions in 1961 (Levy, 1966). Unfortunately, not much interest has been observed with this test. Previous studies have reported the development of a biphase dissolution system (Gibaldi and Feldman, 1967; Heigoldt et al., 2010; Vangani et al., 2009) and their correlation to *in vivo* absorption for different dosage forms, including

immediate release (Shi et al., 2010), modified release (Heigoldt et al., 2010), lipid (Kinet and Degreef, 1994) and amorphous formulations (Thiry et al., 2016). Heigoldt et al. developed a pH-adjusted biphasic dissolution test to predict drug release from modified release dosage forms with pH-dependent poorly soluble drugs (Heigoldt et al., 2010). Compared to the conventional dissolution testing at constant pH, the pH-adjusted biphasic test enabled an improved prediction of the *in vivo* behavior of modified release formulations. Vangani et al. presented a two-phase dissolution system by combining a biphasic media in USP 2 apparatus and a USP 4 apparatus (Vangani et al., 2009). Shi et al. employed this biphasic system (Fig. 1.19) to evaluate drug release from three immediate release formulations (the commercial Celebrex<sup>®</sup> capsule, a solution formulation containing cosolvents and surfactant, and a supersaturated self-emulsifying drug delivery system) (Shi et al., 2010). The single phase dissolution systems under sink and non-sink conditions did not successfully predict their *in vivo* performance, but the release profiles of these three formulations from the biphasic system were correlated with *in vivo* data. Recently, Thiry et al. tested three itraconazole formulations in different conditions (different USP apparatuses, sink or non-sink conditions, and biorelevant or non-biorelevant media) (Thiry et al., 2016). The biphasic test was predictive for these three formulations with the same ranking of *in vivo* results.



**Fig. 1.19.** Schematic diagram of the biphasic dissolution system (Shi et al., 2010).

The biphasic system is greatly dependent on the partition coefficients of drugs between the aqueous medium and the organic solvent, so it is particularly useful for lipophilic drugs as an absorptive sink. Undoubtedly, the biphasic test cannot account for drug-drug and drug-food interactions associated with the intestinal transporters or enzymes.

### **1.3. *In vitro-in vivo* correlation and *in vitro-in vivo* relationship**

Establishing a correlation between *in vitro* dissolution profiles and *in vivo* data has great interest and benefits in pharmaceutical research (Dokoumetzidis and Macheras, 2006; Kostewicz et al., 2014). It is highly desirable that the *in vivo* performance of candidate formulations could be predicted based on *in vitro* release data. However, the predictive power of dissolution tests is often poor (Dokoumetzidis and Macheras, 2008; Park, 2014). Ideally, changes in dissolution *in vivo* should be reflected by the corresponding *in vitro* release. A suitable dissolution test as a surrogate for *in vivo* absorption is highly attractive in the early stage of formulation development to reduce the high costs of animal and clinical studies.

There are two types of predictions for *in vitro* and *in vivo* data known as *in vitro-in vivo* correlation (IVIVC) and *in vitro-in vivo* relationship (IVIVR). IVIVC/IVIVR is very useful in understanding and identifying how the critical factors of formulations (e.g., excipient types, binders) and manufacturing processes (e.g., compression force, coating) behave and influence drug performance.

According to the definition proposed by FDA, *in vitro-in vivo* correlation is defined as a predictive mathematical model describing the relationship between an *in vitro* property of the dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response (usually  $C_{max}$ , AUC or amount of drug absorbed) (FDA, 1997). IVIVC has been categorized into four levels: Level A, B, C and multiple Level C. A level A represents a point-to-point relationship between *in vitro* dissolution and *in vivo* input rate of the drug from the dosage form, which is usually estimated by comparison of the fraction of drug absorbed

to the fraction of drug dissolved. Generally, this level of correlation is linear and is the most informative and very useful from a regulatory viewpoint. For level B, the mean *in vivo* dissolution time or the mean residence time is compared to the mean *in vitro* dissolution time by using statistical moment analysis. Since it does not uniquely reflect the actual *in vivo* plasma level curves and similar mean residence time values can be produced by different plasma curves, this is least useful for regulatory purposes. A level C is a single point relationship between a dissolution parameter (e.g.,  $t_{50\%}$  or percent dissolved in 4 h) and a pharmacokinetic parameter (e.g., AUC,  $C_{max}$  or  $T_{max}$ ). This level correlation does not reflect the complete shape the plasma concentration time curve, but it can be useful for pilot formulations in the early stages of formulation development, including selecting the appropriate excipients and optimizing manufacturing processes etc. A multiple level C relates one or several pharmacokinetic parameters (e.g., AUC or  $C_{max}$ ) to the amount of drug dissolved at several time points of the dissolution profiles. If a multiple level C is achievable, then a level A correlation is likely as well.

IVIVR is a broad term containing qualitative (a rank-order relationship) and even semi-quantitative associations between *in vitro* and *in vivo* data (Brown et al., 2004). In early development, IVIVR is likely used to qualitatively evaluate changes in formulation compositions and manufacturing variables by comparing dissolution data and early animal studies.

### **1.4. Objectives**

The main aim of this work was to explore a discriminative and predictive dissolution test for formulation and process changes within immediate release oral dosage forms for BCS II drugs. Biphasec dissolution system can simulate drug dissolution and absorption in the GI tract, but also its setup and handling are relatively simple and cost effective, and drug release and partitioning are detected simultaneously within a single vessel. There is still great potential to explore the biphasec dissolution models. Therefore, biphasec dissolution test was

used to evaluate its potential to discriminate different variables such as formulation compositions (e.g., different cosolvents, crystal forms and polymers) and manufacturing processes (e.g., different granulation methods). The results were compared to conventional dissolution tests under sink and non-sink conditions. A further objective was to establish IVIVC/IVIVR based on *in vivo* data obtained from the literatures or performed. The specific goals included as follows:

- a) To discriminate the effect of different cosolvents on drug absorption;
- b) To discriminate and predict different carbamazepine polymorphic forms;
- c) To discriminate and correlate with *in vivo* pharmacokinetic studies for differently formulated racecadotril granules;
- d) To discriminate and predict itraconazole amorphous solid dispersions prepared with different polymers.

## **2. MATERIALS AND METHODS**

## 2.1. Materials

**Drugs:** Racecadotril (Allphamed Pharbil Arzneimittel GmbH, Göttingen, Germany), carbamazepine and itraconazole (BASF AG, Ludwigshafen, Germany).

**Polymers:** Hydroxypropyl methylcellulose (HPMC, Methocel<sup>®</sup> E5, Colorcon Ltd., Dartford Kent, UK), aminoalkyl methacrylate copolymer E (Eudragit<sup>®</sup> EPO, Evonik Industries AG, Darmstadt, Germany), polyvinyl pyrrolidone vinyl acetate copolymer (PVPVA, Kollidon<sup>®</sup> VA 64, BASF SE, Ludwigshafen, Germany), pregelatinized maize starch (Lycatab PGS, Roquette, Lestrem, France), polyethylene glycol 400 (PEG 400, Roth GmbH & Co., Karlsruhe, Germany).

**Solvents:** 1-Octanol, ethanol 96% (v/v) (EtOH) (Sigma Aldrich Chemie GmbH, Steinheim, Germany), methanol (Merck, Darmstadt, Germany), acetonitrile (HPLC-grade, Honeywell/Burdick & Jackson, Muskegon, USA), dimethyl sulfoxide (DMSO), methylene chloride, decanol (Roth GmbH & Co., Karlsruhe, Germany).

**Other chemicals:** Thiorphan (Santa Cruz Biotechnology, CA, USA), lactose (Granulac<sup>®</sup> 200, Meggle AG, Wasserburg, Germany), cellactose (Cellactose<sup>®</sup> 80, Meggle GmbH, Wasserburg, Germany), hard gelatin capsules (size 0) (Capsugel, Bornem, Belgium), hypromellose capsule (size 00 and 3) (Quali-V<sup>®</sup>, Shionogi Qualicaps Co., Ltd., Whitsett, NC), sodium chloride (NaCl), sodium hydroxide (NaOH), sodium lauryl sulfate (SLS), hydrochloric acid (HCl) (Carl Roth GmbH & Co., Karlsruhe, Germany), sodium phosphate monobasic monohydrate (Merck KGaA, Darmstadt, Germany).

## **2.2. Evaluation of a discriminative biphasic dissolution test for different cosolvents**

### **2.2.1. Solubility measurements**

Equilibrium solubility of carbamazepine was determined by adding an excess of carbamazepine powder to 10 ml 50 mM phosphate buffer pH 6.8 with the increasing concentrations of PEG 400 (0, 5, 10 and 50%, v/v) and EtOH (0, 5 and 10%, v/v) (n = 3). The samples were incubated at 37 °C for 48 h in a horizontal shaker (GFL® 3033, GFL Gesellschaft für Labortechnik, Burgwedel, Germany). Saturated solutions were filtered with 0.22 µm membrane filters. Drug concentrations were detected by UV spectrophotometer at  $285 \pm 1$  nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany).

### **2.2.2. Viscosity measurements**

Viscosities of PEG 400 and EtOH solutions with aforementioned different concentrations were measured at 25 °C by using a rotational viscometer (MCR 302, Anton Paar GmbH, Graz, Austria) at varying shear rates ( $0.1-100 \text{ s}^{-1}$ ) fitted with a double gas measuring system DG27 (n = 3). All samples exhibited Newtonian flow property, so the average of viscosities was calculated.

### **2.2.3. Determination of partition coefficients**

Partition coefficients ( $\log K_P$ ) of carbamazepine in between octanol and aqueous solutions containing different concentrations of PEG 400 and EtOH were determined using shake flask (n = 3). Octanol and different aqueous solutions were pre-saturated for 24 h prior to use. 5 ml PEG 400 or EtOH solutions was added in the glass vials following the addition of 20 µl drug stock solution in methanol, and subsequently 5 ml octanol was added. After equilibrium through incubation in a horizontal shaker at 37 °C, samples were withdrawn from both phases and centrifuged at 16,000 rpm for 15 min for phase separation. The amount of carbamazepine was measured by UV-spectrophotometry.



#### **2.2.4. Biphasic dissolution test**

The effect of different cosolvent systems on drug absorption was evaluated using a biphasic dissolution test in a USP 2 apparatus (Vankel® VK 700, Vankel Industries, Edison, NJ, USA) (n = 3). 1 ml stock solution of carbamazepine in methanol was added into 300 ml phosphate buffer pH 6.8 with different concentrations of PEG 400 (0, 10 and 50%, v/v) and EtOH (0 and 10%, v/v) to achieve 50% saturated solubility of carbamazepine in phosphate buffer pH 6.8. 50 ml pre-saturated octanol was subsequently added as an absorptive compartment. The test was performed at 50 rpm and 37 °C. Samples were collected at predetermined time points from both the aqueous and organic phases and centrifuged at 16,000 rpm for 15 min (Biofuge 13/Haemo, Heraeus Instruments, Osterode, Germany). Drug concentrations in the two phases were determined by UV-spectrophotometry at 285 nm and 286 nm, respectively.

Various experimental parameters, including volume ratios of the aqueous and organic phases (30:10, 30:5 and 40:5) and rotation speeds (50 and 75 rpm), were investigated and optimized for good discrimination and correlation with previously reported data from the literature.

### **2.3. Evaluation of a discriminative biphasic dissolution test for estimating the bioavailability of carbamazepine polymorphic forms**

#### **2.3.1. Preparation of carbamazepine polymorphic forms**

Carbamazepine as received was identified to be form III. Form I was obtained by heating form III at 170 °C for 2 h (Kobayashi et al., 2000). Dihydrate form was prepared by suspending form III in distilled water and stirring with a magnetic stirrer for 24 h at room temperature, followed by filtration and drying at room temperature (Kobayashi et al., 2000). The carbamazepine dihydrate was stored at room temperature under 58% relative humidity to maintain the hydrate form (Mcmahon et al., 1996). The sample was gently ground using mortar and pestle,

and then sieved fraction (44-74  $\mu\text{m}$ ) was used.

### **2.3.2. Characterization of the polymorphic forms**

#### *2.3.2.1. X-ray powder diffraction (XRPD)*

Three carbamazepine crystal forms were analyzed by X-ray diffraction using Philips PW 1830 X-ray generator with a copper anode (Cu K $\alpha$  radiation,  $\lambda = 0.15418$  nm, 40 kV, 20 mA) fixed with a Philips PW 1710 diffractometer control unit (Philips Industrial & Electro-acoustic Systems Divisions, Almelo, The Netherlands). The scattered radiation was measured with a vertical goniometer (Philips PW 1820, Philips Industrial & Electro-acoustic Systems Division, Almelo, The Netherlands). Patterns were obtained over the range of 4-40° (2 $\theta$ ) at a step scan of 0.02° (2 $\theta$ ) at room temperature.

#### *2.3.2.2. Optical microscopy*

Microscopic observations were performed on samples suspended in silicone oil using polarized light microscope (Axioscope, Carl Zeiss Jena GmbH, Jena, Germany) equipped with image analysis software (Easy Measure, Inteq Informationstechnik GmbH, Berlin, Germany).

#### *2.3.2.3. Differential scanning calorimetry (DSC)*

DSC studies were performed by Mettler DSC 821<sup>e</sup> (Mettler Toledo, Giessen, Germany) and data were analyzed using the STAR<sup>®</sup> Software (Mettler Toledo, Giessen, Germany). Samples (approx. 5mg) were weighed into 40  $\mu\text{l}$  perforated aluminum pans and heated from 40 to 220 °C at a heating rate of 10 °C/min under a nitrogen atmosphere.

#### *2.3.2.4. Thermogravimetric analysis (TGA)*

Thermogravimetric analyses were conducted on a Mettler<sup>®</sup> TC 15 (Mettler, Giessen, Germany) and data were analyzed using the STAR<sup>®</sup> Software (Mettler Toledo, Giessen, Germany). Samples (5-8 mg) were weighed into aluminum

pans with two pinholes in their lid and heated from 40 to 220 °C at a heating rate of 10 °C/min with nitrogen flow rate of 100 ml/min. Loss of mass was recorded by a microbalance (Mettler® TG 50, Mettler, Giessen, Germany).

### 2.3.3. Intrinsic dissolution rate

Intrinsic dissolution rates of anhydrous and dihydrated carbamazepine were measured by the stationary disk method in a USP II apparatus. A compact of 8 mm diameter was prepared by compressing 150 mg drug with flat-faced round punches at a compression force of 300 kg/cm<sup>2</sup> for 3 min by a manual Sirius tablet press (Sirius Analytical Instruments Ltd., Forest Row, UK). XRPD revealed no polymorphic conversion after compression. Compacts were placed into a tablet disc holder with one side closed. The tablet holder was then placed at the bottom of the dissolution vessel.

The intrinsic dissolution test was performed in 200 ml 50 mM phosphate buffer pH 6.8 at 100 rpm for 120 min at 37 °C (n = 3). Aliquots of 5 ml were collected through 0.22 µm filters at predetermined time intervals and replaced with fresh medium. Drug concentrations were analyzed by UV spectrophotometer at 285 nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany).

During the intrinsic dissolution of the carbamazepine polymorphics, the slope of the dissolution profile was not completely linear due to the transformation from the anhydrous form to the dihydrate on the surface of the compacts (Kobayashi et al., 2000; Murphy et al., 2002). Therefore, the intrinsic dissolution rate was calculated from the slope of the initial linear part (up to 10 min) from the plot of cumulative amount dissolved per surface unit of the compact vs. time by Eq. 2.1 (Zakeri-Milani et al., 2009):

$$IDR = \frac{dC}{dt} \times \frac{V}{S} = \frac{DC_s}{h} \quad (2.1)$$

where  $IDR$  is the intrinsic dissolution rate ( $\mu\text{g}/\text{min}/\text{cm}^2$ ),  $dC$  is the change in drug concentration ( $\mu\text{g}/\text{ml}$ ),  $dt$  is the change in time (min),  $V$  is the volume of dissolution medium (ml),  $S$  is the surface area of the compact ( $\text{cm}^2$ ),  $D$  is diffusion coefficient ( $\text{cm}^2/\text{s}$ ),  $C_s$  is the solubility ( $\mu\text{g}/\text{ml}$ ), and  $h$  is the boundary layer thickness (cm).

### **2.3.4. Solubility of carbamazepine polymorphic forms**

The equilibrium solubility of the dihydrate form was determined by adding 20 mg carbamazepine dihydrate to 10 ml 50 mM phosphate buffer pH 6.8 and phosphate buffer pH 6.8 saturated with octanol, or 1 g dihydrate to 10 ml 1-octanol and 1% w/v SLS ( $n = 3$ ). The samples were incubated at 37 °C for 72 h using a horizontal shaker (GFL<sup>®</sup> 3033, GFL Gesellschaft für Labortechnik, Burgwedel, Germany). Saturated solutions were filtered through 0.22  $\mu\text{m}$  membrane filters. The drug concentration was detected by UV-spectrometry at  $285 \pm 2$  nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany). The equilibrium solubility method is not suitable to determine the solubility of anhydrous forms in aqueous solutions due to phase transformation to the dihydrate. Thus, the solubility of anhydrous carbamazepine in phosphate buffer pH 6.8 was estimated based on the intrinsic dissolution rates of different crystal forms and the equilibrium solubility of carbamazepine dihydrate by Eq. 2.1 (Murphy et al., 2002).

### **2.3.5. *In vitro* dissolution tests**

#### **2.3.5.1. Preparation of carbamazepine formulations**

Formulations were prepared by mixing 200 mg carbamazepine with lactose monohydrate at a weight ratio of 1:2. Powder blends were filled in size 0 hard gelatin capsules.

### *2.3.5.2. Conventional dissolution test under sink conditions*

Dissolution studies were performed in 900 ml water containing 1% w/v SLS at 75 rpm and 37 °C in a USP II apparatus (VK 7010, Vankel Technology Group, Cary, USA) according to the USP method (n = 3). Samples were collected at predetermined time points and assayed using UV-spectrophotometer at 287 nm.

### *2.3.5.3. Single phase dissolution test under non-sink conditions*

Single phase dissolution tests were carried out using a UPS II apparatus with 400 ml phosphate buffer (50 mM, pH 6.8) at 50 rpm under non-sink conditions (n = 3). Samples were collected at predetermined time intervals assayed by UV.

### *2.3.5.4. Biphasic dissolution test*

Carbamazepine dissolution from three polymorphic forms was determined using a biphasic dissolution test in a USP II apparatus (Vankel® VK 700, Vankel Industries, Edison, NJ, USA). 400 ml phosphate buffer (50 mM, pH 6.8) (lower aqueous phase) was equilibrated with 100 ml 1-octanol (upper organic phase) at 50 rpm and 37 °C for 1 h prior to the test (n = 3). Samples were withdrawn from both the aqueous and octanol phases at predetermined time points and centrifuged at 16,000 rpm for 15 min (Biofuge 13/Haemo, Heraeus Instruments, Osterode, Germany). Drug concentrations in the aqueous and organic phases were measured by UV-spectrometry at 285 nm and 286 nm, respectively.

### **2.3.6. Statistical analysis**

All data were expressed as mean ± standard deviation (SD). The results were compared by one-way analysis of variance (ANOVA) and  $p < 0.05$  was considered as statistically significant.

## **2.4. Evaluation of a discriminative biphasic dissolution test and correlation with *in vivo* pharmacokinetic studies for differently formulated racecadotril granules**

### **2.4.1. Preparation of granule formulations**

Three granule formulations of racecadotril with the same quantitative composition were prepared by different manufacturing techniques. 10 g drug powder was passed through a 250  $\mu\text{m}$  sieve and physically mixed with 8 g pregelatinized starch and 4 g lactose.

*Dry Granulation:* Powder blends were compressed into tablet slugs to a hardness of  $40 \pm 10$  N using a single punch tablet press EK0 (Korsch Pressen GmbH, Berlin, Germany). The slugs were crushed in a dry granulator (Erweka TG 2S attached to an Erweka AR 400 motor, Erweka GmbH, Frankfurt, Germany) to obtain granules using an 800  $\mu\text{m}$  mesh sieve.

*Wet Granulation:* Drug and diluents (lactose and pregelatinized starch) were mixed for 5 min in a mixer torque rheometer (Caleva Ltd., Dorset, UK) and granulated with 6.4 g water or 8.1% (w/w) Lycatab PGS binder solution. The wet mass was forced through a 1 mm sieve and then dried overnight at 50 °C. The dried granules were passed through an 800  $\mu\text{m}$  mesh sieve.

### **2.4.2. Determination of drug content**

50 mg granules were dispersed in 100 ml 80% (v/v) methanol aqueous solution. The samples were sonicated for 30 min, then placed in a shaker overnight. The solution was filtered through a 0.22  $\mu\text{m}$  membrane filter and drug amount was determined by UV spectrometer at 232 nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany). Each formulation was analyzed in triplicate.

### **2.4.3. Solubility measurements of racecadotril**

20 mg racecadotril was added to 10 ml aqueous media (50 mM phosphate buffer pH 6.8 with or without 0.75% w/v SLS and phosphate buffer pH 6.8 saturated

with octanol), and 1 g racecadotril was added to 10 ml 1-octanol. The solubility test was performed at 37 °C and 80 rpm for 48 h using a horizontal shaker (GFL® 3033, GFL Gesellschaft für Labortechnik, Burgwedel, Germany) (n = 3). Saturated solutions were filtered through 0.22 µm membrane filters. The drug concentration was measured by UV spectrophotometry at 232 nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany).

#### **2.4.4. Conventional dissolution test under sink conditions**

Racecadotril release from three granules was determined in the USP II apparatus (900 ml 50 mM phosphate buffer pH 6.8 containing 0.75% w/v SLS (Garala et al., 2013), 75 rpm, 37 °C, sink conditions, n = 3) (VK 7010, Vankel Technology Group, Cary, USA). Samples were taken at predetermined time points and detected UV-spectrophotometrically at 232 nm.

#### **2.4.5. Single phase dissolution test under non-sink conditions**

Single phase dissolution tests were carried out using the USP rotating paddle method (500 ml 50 mM phosphate buffer pH 6.8, 75 rpm, 37 °C, non-sink conditions, n = 3) (VK 7010, Vankel Technology Group, Cary, USA). Samples were collected at predetermined intervals and used for UV assaying.

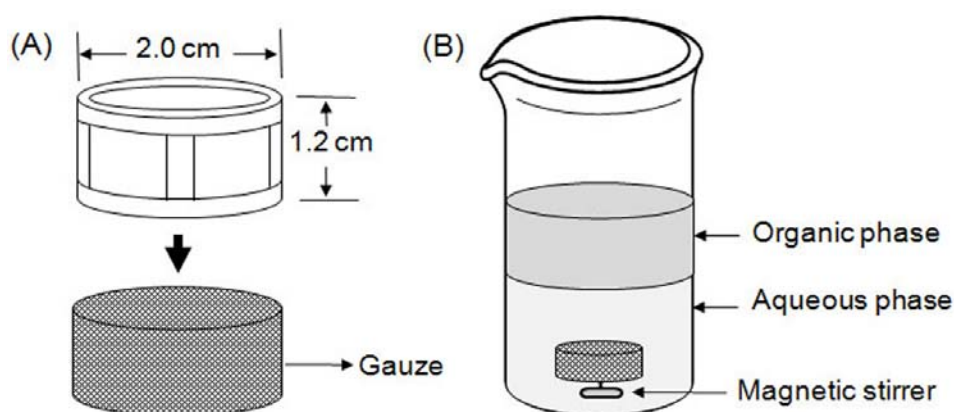
#### **2.4.6. Biphasic dissolution test**

Racecadotril granules were placed in a hollow plastic cylindrical holder (2.0 × 1.2 cm) and wrapped with one-layer gauze to avoid granules floating (Fig. 2.1A). This device was tied to a magnetic stirrer. The biphasic tests were performed in 250 ml glass beakers with 100 ml phosphate buffer (50 mM, pH 6.8) as the lower aqueous phase and 20 ml octanol as the upper organic phase at 150 rpm and 37 °C (Fig. 2.1B). These two media were saturated with each other prior to use. Samples were withdrawn from both the aqueous and octanol phases at predetermined time points. The samples from the octanol phase were centrifuged at 15,000 rpm for 15 min (5417R, Eppendorf, Germany) and the

supernatants were measured by UV-spectrometry at 232 nm (Lambda 900UV, PerkinElmer, USA).

Meanwhile, the amount of racecadotril in the aqueous phase was quantified according to a validated HPLC method with slight modifications (Prabu et al., 2007). The analysis was done by a HPLC system (Agilent Infinity 1260 series, Agilent Technologies, Santa Clara, CA, USA) equipped with an Ultimate XB-C18 column (250 × 4.6 mm, 5 μm, 120 Å pore size, Welch Materials, Maryland, USA) (mobile phase: phosphate buffer (1 mM, pH 3.5): acetonitrile 40:60, v/v; flow rate: 1.0 ml/min; UV detection at 210 nm). Calibration curves from 0.5 to 40.0 μg/ml were prepared freshly prior to analysis and the correlation coefficients were more than 0.999. The accuracy and the precision were obtained by measuring three different concentrations of racecadotril (0.5, 4.0 and 40.0 μg/ml) ranging from 98.5 to 100.1%, and 0.5 to 2.7% CV, respectively.

Partition rates were calculated with linear regression according to the amount partitioned into the octanol phase over time.



**Fig. 2.1.** Schematic diagram: (A) hollow plastic cylindrical holder and (B) biphasic system.

Various experimental parameters including agitation rates (100, 150 and 200 rpm), volume ratios of the aqueous and organic phases (10:2, 10:5 and 10:8), and interfacial areas between the two phases (20, 30 and 40 cm<sup>2</sup>) were investigated and optimized for establishing a good *in vitro* and *in vivo* correlation.



#### **2.4.7. Contact angle measurements**

Compacts (diameter: 13 mm, weight: 300 mg) were prepared by direct compression using a hydraulic press (P/N 25.011, Specac, Orpington, England) at a compression force of 3 ton for 1 min. Measurements were performed with a contact angle goniometer (G1, Krüss GmbH, Hamburg, Germany) by the sessile drop method at room temperature (n = 4). 10 µl phosphate buffer pH 6.8 was applied onto the compressed tablets with a microsyringe. The contact angle was measured 15 seconds after drop formation.

#### **2.4.8. *In vivo* studies**

##### *2.4.8.1. Animal experiments*

Animal studies were approved by the local ethical committee at Third Military Medical University, Chongqing, and performed in accordance with guidelines of experimental animal care. Male Sprague-Dawley rats weighing 250-300 g were fasted for 12 h before drug administration. Each formulation was dispersed in deionized water prior to dosing and administered by oral gavage at a dose of 30 mg/kg of racecadotril (n = 4). Blood samples were withdrawn from retro orbital choroid plexus under mild anesthesia at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12 and 24 h after dosing and placed into heparin pretreated tubes. The blood samples were centrifuged at 5,000 g for 10 min and plasma was stored at -20 °C until further analysis.

##### *2.4.8.2. Analysis of plasma concentration*

Plasma concentration of thiorphan, an active metabolite of racecadotril, was determined according to a validated HPLC method with slight modifications (Xu et al., 2008). Samples were analyzed using the Agilent HPLC system equipped with an Ultimate XB-C18 column (250 × 4.6 mm, 5 µm, 120 Å) maintained at 37 °C. The mobile phase was a mixture of 50 mM phosphate buffer (pH 2.6) and acetonitrile (60:40, v/v) at the flow rate of 0.4 ml/min. The UV detector was set at 210 nm. Prior to HPLC analysis, 0.1 ml of each plasma sample was vortex mixed

with 0.2 ml acetonitrile for 2 min and then centrifuged at 5,000 g for 10 min. The supernatant was transferred into a new tube and evaporated at 40 °C under a stream of nitrogen. The sediment was then dissolved in 100 µl mobile phase. The injection volume was 20 µl. Method validation was conducted according to the FDA guidance (FDA, 2001). Linearity was demonstrated from 0.05 to 4.00 µg/ml ( $R^2 \geq 0.999$ ). The lower limit of quantification was 0.05 µg/ml. The mean recovery of thiorphan in plasma was more than 93%. The inter- and intra-day variations of the three different concentrations of thiorphan (0.05, 0.40, and 4.00 µg/ml) were less than 10%.

### *2.4.8.3. Pharmacokinetic analysis*

The pharmacokinetic (PK) parameters, including the area under the plasma concentration-time curve from 0 to 24 h ( $AUC_{0-24h}$ ), the peak plasma concentration of drug ( $C_{max}$ ) and the time to reach maximum plasma concentration ( $T_{max}$ ), after administration of racecadotril formulations in Sprague-Dawley rats were determined using a one-compartmental analysis by a freely available add-in program for Microsoft Excel, PKSolver (Anitha et al., 2014; Childs et al., 2013; Zhang et al., 2010).

### **2.4.9. Statistical analysis**

All data were expressed as mean  $\pm$  standard deviation (SD). The results were compared by one-way analysis of variance (ANOVA) and  $p < 0.05$  was considered as statistically significant.

## **2.5. Evaluation of a discriminative biphasic dissolution test for estimating the bioavailability of itraconazole amorphous solid dispersions prepared with different polymers**

### **2.5.1. Preparation of itraconazole solid dispersions**

Solid dispersions (SD) were prepared with itraconazole and Eudragit® EPO or

Eudragit® EPO-PVPVA 64 (70/30, w/w) or HPMC at a weight ratio of 4:6 (Six et al., 2005) by solvent casting. 0.4 g itraconazole and 0.6 g polymer were dissolved in 40 ml methylene chloride for Eudragit® EPO and Eudragit® EPO/PVPVA 64 or a mixture of methylene chloride and ethanol (80/20, v/v) for HPMC (Verreck et al., 2003), respectively. The solutions were poured onto Teflon® plates and left to dry at room temperature for 24 h. Afterwards, the resulting film was removed from the Teflon® plates and milled for 1 min using a ball mill (Retsch MM 2000 small ball mill, Retsch GmbH, Haan, Germany) under cryogenic conditions. The final powders were passed through a 355 µm sieve. The formulations (100 mg dose) were prepared by mixing with an equivalent amount of cellactose and filled into hypromellose capsule size 00 for the following single phase dissolution tests (sink or non-sink, single pH or pH-gradient) using a sinker. Additionally, 2 mg dose with an equivalent amount cellactose was filled into hypromellose capsule size 3 for the biphasic dissolution test in Sirius® inForm.

### **2.5.2. Preparation of physical mixtures**

Physical mixtures (PM) were prepared by mixing itraconazole and the polymer (40/60, w/w) in a mortar and pestle for 10 min.

### **2.5.3. Characterization of itraconazole formulations**

#### *2.5.3.1. X-ray powder diffraction (XRPD)*

X-ray powder diffraction was performed using a Philips PW 1830 X-ray generator with a copper anode (Cu K $\alpha$  radiation,  $\lambda = 0.15418$  nm, 40 kV, 20 mA) fixed with a Philips PW 1710 diffractometer control unit (Philips Industrial & Electro-acoustic Systems Divisions, Almelo, The Netherlands). The scattered radiation was measured with a vertical goniometer (Philips PW 1820, Philips Industrial & Electro-acoustic Systems Division, Almelo, The Netherlands). Patterns were collected in the angular range of 4-40° (2 $\theta$ ) at a step scan of 0.02° (2 $\theta$ ) at room temperature.

### 2.5.3.2. *Differential scanning calorimetry (DSC)*

Thermal analysis was conducted using a DSC (DSC 6000, PerkinElmer LAS GmbH, Rodgau, Germany) and data were analyzed by Pyris software (PerkinElmer LAS GmbH, Rodgau, Germany). Samples were weighed to  $6 \pm 2$  mg in 30  $\mu$ l perforated and covered aluminum pans. The samples were heated from 25 to 200 °C with a heating rate of 20 °C/min, and afterwards cooled with a cooling rate of 20 °C/min to room temperature. A second heating cycle was then applied on the sample starting at room temperature up to 200 °C with a heating rate of 20 °C/min. Nitrogen was used at a flow rate 40 ml/min.

### 2.5.3.3. *Fourier transform infrared spectroscopy (FTIR)*

Samples were analyzed using an Excalibur 3100 FTIR spectrophotometer (Varian Inc., Palo Alto, USA) from 600 to 4000  $\text{cm}^{-1}$  in transmission mode equipped with a horizontal ATR accessory with a single reflection diamond crystal (Pike Miracle, Pike Technologies, Madison, USA) and Varian software (Resolution Pro 4.0). Each spectrum was obtained at a resolution of 4  $\text{cm}^{-1}$  and average of 16 scans.

## 2.5.4. Solubility of itraconazole

### 2.5.4.1. *Equilibrium solubility*

20 mg crystalline itraconazole was added in 10 ml different media (0.1 N HCl, 0.1 N HCl with 0.7% w/v SLS, 0.1 N HCl with 0.3 mg/ml polymer concentration, gastric buffer pH 2.0, phosphate buffer pH 6.8, gastric buffer pH 2.0 saturated with decanol and decanol). Drug solubility of physical mixtures was determined in 0.1 N HCl. Samples were incubated for 24 h at 37 °C using a horizontal shaker (GFL® 3033, GFL Gesellschaft für Labortechnik, Burgwedel, Germany) ( $n = 3$ ). Saturated solutions were filtered through a 0.45  $\mu$ m membrane filter and used for UV assaying (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany).

### *2.5.4.2. Kinetic solubility*

Kinetic solubility of solid dispersions was evaluated by adding samples equivalent to 100 mg itraconazole into 100 ml 0.1 N HCl. Crystalline itraconazole was tested as a control. The test was carried out at 37 °C and 100 rpm for 7 days using a horizontal shaker (GFL® 3033, GFL Gesellschaft für Labortechnik, Burgwedel, Germany) (n = 3). Samples (2ml) were taken at given time points, immediately filtered through 0.45 µm filters and diluted with methanol to avoid precipitation. Drug concentration was determined by UV-spectrometry.

### **2.5.5. Effect of different polymers on supersaturation stabilization and precipitation inhibition**

#### *2.5.5.1. Evaluation of supersaturation stabilization and precipitation inhibition by solvent shift method*

The solvent shift method is popular and simple to assess precipitation inhibition (Bevernage et al., 2013b). 10 ml DMSO stock solution of itraconazole (5 mg/ml) was added to 500 ml 0.1 N HCl with or without predissolved polymers (Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC) at a final concentration of 0.3 mg/ml in the USP II apparatus at 100 rpm and 37 °C (n = 3). An initial drug solution concentration of 100 µg/ml was induced and the final DMSO concentration in 0.1 N HCl did not exceeded 2% (v/v) which had no significant influence on drug solubility (Bevernage et al., 2011; Yamashita et al., 2011). Samples (3 ml) were taken at 10, 20, 30, 60, 120, 180, 240 min and filtered through 0.45 µm filters. The fresh medium was replaced at each time point. The filtrate was diluted with methanol and assayed by UV spectrophotometer at 257 nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany).

#### *2.5.5.2. Evaluation of supersaturation stabilization and precipitation inhibition by pH-shift method*

Due to pH-dependent solubility of itraconazole, the effect of different polymers on precipitation inhibition was also evaluated using a pH-shift method. 10 ml

concentrated solution of itraconazole in DMSO (5 mg/ml) was added to 500 ml 0.1 N HCl with or without predissolved polymers (Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC) at a final concentration of 0.3 mg/ml in the USP II apparatus at 100 rpm and 37 °C (n = 3). An initial drug solution concentration of 100 µg/ml was induced. After 1 h, 166.7 ml 0.2 M tribasic sodium phosphate was added and adjusted the pH of medium to 6.8 ± 0.05. Samples (3 ml) were withdrawn at 10, 20, 30, 60, 70, 80, 90, 120, 180, and 240 min and the fresh medium was replaced. The samples were filtered and diluted with methanol for the acidic media and ethanol for neutral media prior to UV assays.

### *2.5.5.3. Viscosity measurements*

Viscosity of polymer solutions (Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC) in 0.1 N HCl with a concentration of 0.3 mg/ml was measured at 25 °C by using a rotational viscometer (MCR 302, Anton Paar GmbH, Graz, Austria) at varying shear rates (0.1-100 s<sup>-1</sup>) fitted with a double gas measuring system DG27 (n = 3). All samples exhibited Newtonian flow property, so the average of viscosities was calculated.

### **2.5.6. *In vitro* dissolution tests**

#### *2.5.6.1. Conventional USP II dissolution test under sink conditions*

Dissolution testing was performed in a USP II apparatus (900 ml 0.1 N HCl with 0.7% w/v SLS, sink conditions, 100 rpm, 37 °C, n = 3) (VK 7010, Vankel Technology Group, Cary, USA). Samples were taken at given time points and detected by UV-spectrometry at 259 nm (Agilent 8453, Agilent Technologies GmbH, Waldbronn, Germany).

#### *2.5.6.2. Conventional USP II dissolution test under non-sink conditions*

Drug release from solid dispersions was evaluated using USP rotating paddle method (500 ml 0.1 N HCl, non-sink conditions, 100 rpm, 37 °C, n = 3) (VK 7010, Vankel Technology Group, Cary, USA). Physical mixtures were also tested.

Samples (5 ml) were collected at predetermined intervals and replaced with fresh dissolution medium, and immediately filtered through 0.45  $\mu\text{m}$  membrane filters. The filtrates were diluted with methanol and measured UV-spectrophotometrically at 257 nm.

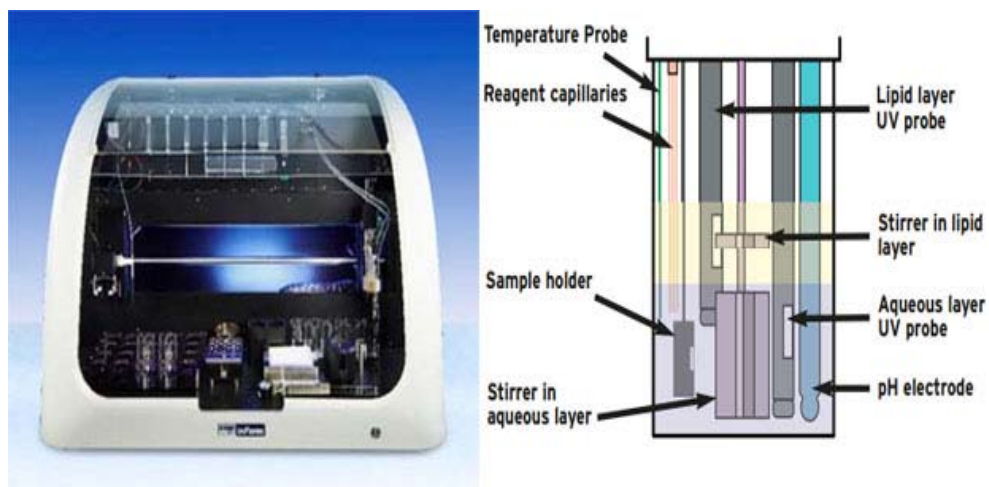
### *2.5.6.3. Conventional USP II pH-gradient dissolution test under non-sink conditions*

To evaluate the effect of pH shift, itraconazole release was also assessed according to the USP method A enteric dissolution test in a USP II apparatus. All solid dispersions were first tested in 750 ml 0.1 N HCl for 2 h followed by a pH adjustment to  $6.8 \pm 0.05$  by the addition of 250 ml 0.2 M tribasic sodium phosphate ( $n = 3$ ). Samples (5 ml) were withdrawn at 10, 20, 30, 45, 60, 120, 135, 150, 180, 210 and 240 min and filtered immediately. The acidic filtrates were diluted with methanol, while the neutral ones were diluted with ethanol. The drug concentrations were determined by UV-spectrophotometry at  $256 \pm 1$  nm. To better clarify the pharmaceutical performance, the area under the dissolution curve for the acid phase ( $\text{AUC}_{\text{acid}}$ ), neutral phase ( $\text{AUC}_{\text{neutral}}$ ), and total release ( $\text{AUC}_{\text{total}}$ ) was calculated by the linear trapezoidal method.

### *2.5.6.4. A pH-gradient biphasic dissolution test*

A pH-gradient biphasic dissolution test was performed using Sirius<sup>®</sup> inForm platform (Fig. 2.2) (Sirius Analytical Instruments Ltd, Forest Row, U.K.). Each formulation containing 2 mg itraconazole with a small sinker was added manually into 40 ml dissolution medium automatically adjusted to pH 2.0 at 37 °C, and then 30 ml pre-saturated decanol was added as the organic phase. The rotating speed was set to 100 rpm. After 2 h, the aqueous medium was automatically adjusted to pH 6.8 ( $n = 3$ ). Drug concentrations were determined between intervals using two *in-situ* fiber-optic UV probes immersed separately in the aqueous and organic phases. Prior to the dissolution test, the molecular extinction coefficients of itraconazole in the two media were determined, which

was used to convert the UV absorption data to dissolved drug amount.



**Fig. 2.2.** Sirius inForm instruments and probe set used in biphasic dissolution studies (adapted from Sirius Analytical).



### **3. RESULTS AND DISCUSSION**

### **3.1. Evaluation of a discriminative biphasic dissolution test for different cosolvents**

#### **3.1.1. Introduction**

The undesirably oral bioavailability resulting from low aqueous solubility of drug substances remains a great challenge in pharmaceutical research and development. A variety of solubility-enhancing approaches have been developed to deal with this problem, including cosolvents, cyclodextrins, surfactants, pH modification, self-emulsification, liposomes and amorphization (Buckley et al., 2013; Kawabata et al., 2011). However, some studies have reported that a consistent degree of enhancement between bioavailability and *in vitro* solubility was rarely achieved (Araya et al., 2005; Barakat, 2010; Mellaerts et al., 2008). Even unexpectedly, some cases have proven the failure of improving drug absorption (Wong et al., 2006). Many factors could influence *in vivo* drug performance, including food, gastric emptying, and metabolism etc.

According to the Biopharmaceutics Classification System, solubility and permeability play a fundamental role in controlling drug absorption (Amidon et al., 1995). These two key parameters have been comprehensively investigated separately, but the interrelation and interplay between them are often neglected (Dahan and Miller, 2012). Therefore, the interest in considering the interplay between solubility and permeability for solubilization strategies has arisen. Some studies have proven a tradeoff between solubility and permeability for some solubilization approaches, such as surfactants (Miller et al., 2011) and cyclodextrins (Beig et al., 2013b; Dahan et al., 2010). Increasing concentrations of these solubilizers showed an increase in drug solubility, but a decrease in drug permeability, which was attributed to reduced free drug concentration available across the membrane due to micellar association or complexation.

Cosolvents are of great interest as the simplest and most common solubilizing agents for poorly soluble drugs, especially in the pre-formulation stage. They are added into an aqueous system as water-miscible solvents. Cosolvency is

explained to reduce the ability of water to squeeze out hydrophobic compounds by disrupting its self-association and thereby to increase drug solubility. Another perspective is to reduce the polarity of aqueous system and make it more non-polar to facilitate solubilization by more closely matching the polarity of the drug (Millard et al., 2002).

To predict the solubilization ability of cosolvents, the log-linear model as one of the most common approach was proposed by Yalkowsky and coworkers (Millard et al., 2002; Yalkowsky and Rubino, 1985; Yalkowsky et al., 1972). It describes an exponential increase in aqueous solubility of non-polar compounds with a linear increase in cosolvent concentration, as described by Eq. 3.1:

$$\log S_{mix} = \log S_w + \sigma f_c \quad (3.1)$$

where  $S_{mix}$  is drug solubility in the cosolvent-water mixture,  $S_w$  is the solubility in water,  $\sigma$  is the cosolvency power, and  $f_c$  is the volume fraction concentration of the cosolvent in the aqueous mixture.

Also, a linear relationship between  $\sigma$  and partition coefficient was demonstrated by Valvani et al. (Valvani et al., 1981) (Eq. 3.2):

$$\sigma = s \log K_{ow} + t \quad (3.2)$$

where  $s$  and  $t$  are cosolvent constants and are independent of the solute,  $\log K_{ow}$  is the octanol-water partition coefficient of the solute.

The effect of cosolvents on drug permeability has been studied by Caco-2 cells, artificial membranes, and *in situ* perfusion models. Yamashita et al. investigated the effect of several additives on drug permeability by Caco-2 cell model (Yamashita et al., 2000). PEG 400 and DMSO reduced the permeability of dexamethasone with increasing their concentrations (0, 2, 5 and 10%, v/v), whereas ethanol had no significantly concentration-dependent change in permeability. In another study, Riad et al. studied the effect of varying PEG 400

concentrations (0, 10 and 50%, v/v) in aqueous solutions on intestinal permeability of carbamazepine using an *in situ* perfusion technique in rabbits (Riad and Sawchuk, 1991). The decreased permeability was observed with the increase of PEG 400 concentrations in both the duodenojejunal and colon segments, which may result from a decrease in the thermodynamic activity of carbamazepine with increasing PEG 400 concentrations.

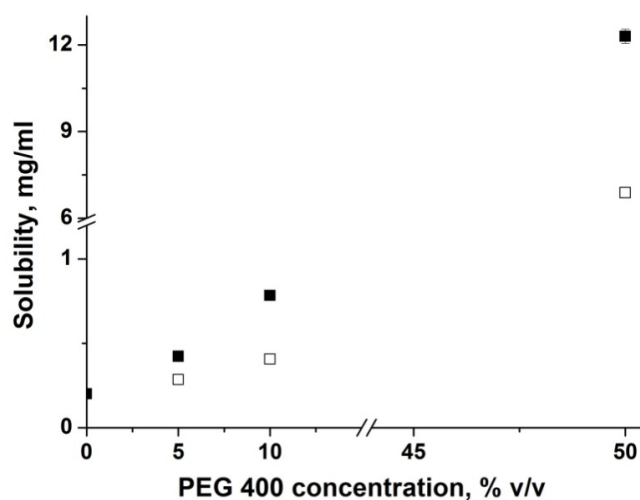
Biphasic dissolution models could be suitable for BCS II drugs, since the presence of octanol phase mimicking the biological membrane makes the dissolution-partition kinetics to resemble *in vivo* absorption. Biphasic dissolution tests have been used to investigate different dosage forms, including immediate release (Shi et al., 2010), modified release (Heigoldt et al., 2010), lipid (Kinget and Degreef, 1994) and amorphous formulations (Thiry et al., 2016). However, there is still a lack in studies to discriminate between different cosolvent systems by the biphasic dissolution test.

Therefore, the objective of this study was to evaluate the potential of biphasic dissolution system to discriminate the effect of different cosolvents on drug absorption based on the previously relevant reports about cosolvents acting on drug permeability (Riad and Sawchuk, 1991; Yamashita et al., 2000). PEG 400 and EtOH were chosen as the model cosolvents systems due to their widespread use in pharmaceutical formulations. Additionally, based on the different behaviors of PEG 400 and EtOH on solubilization and partitioning, a combined EtOH-PEG 400 cosolvent system was also investigated compared to single EtOH and PEG 400 at the same concentration. The mixed cosolvent system was hypothesized to simultaneously improve drug solubility and partitioning through compensation for the low solubilization in EtOH and the low partitioning for PEG 400.

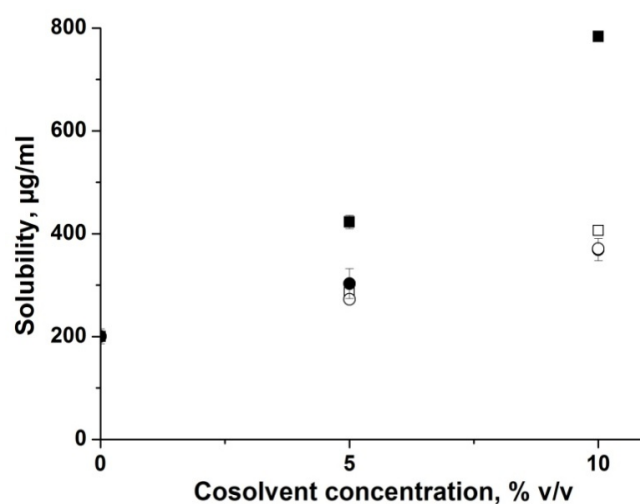
### 3.1.2. Solubility measurements of carbamazepine

Carbamazepine solubility in PEG 400 and EtOH solutions with different concentrations was estimated by the log-linear model according to Eqs. 3.1 and 3.2. The cosolvent constants ( $s$  and  $t$ ) of PEG 400 and EtOH were obtained from the literature (Millard et al., 2002). Drug solubility increased with increasing concentrations of PEG 400 and EtOH, respectively (Figs. 3.1 and 3.2). The estimated drug solubility in various PEG 400 solutions was lower than the tested one at 37 °C, while the estimated solubility in EtOH solutions was similar with the tested one. Millard et al. also reported that the best prediction using the log-linear model was obtained for EtOH and propylene glycol cosolvent systems, but glycerin and PEG 400 were reasonably estimated (Millard et al., 2002).

For the tested drug solubility, a pronounced increase was observed from 0.20 mg/ml with 0% PEG 400 to 12.30 mg/ml with 50% PEG 400 (Fig. 3.1). Compared to EtOH, PEG 400 had higher solubilization capacity at the same concentration (Fig. 3.2). Carbamazepine solubility increased from 0.20 mg/ml without cosolvents to 0.78 mg/ml with 10% PEG 400, but only up to 0.37 mg/ml with 10% EtOH. The increased solubility of carbamazepine by cosolvents was mainly attributed to the self-association of cosolvents through hydrogen bonding mediated by water molecules and alteration of the water structure (Jain and Yalkowsky, 2007). Moreover, PEG 400 is more polar than EtOH (Millard et al., 2002), so PEG 400 could have more favorable interaction with water and thus exhibit higher solubilization ability compared to EtOH.



**Fig. 3.1.** Solubility of carbamazepine in different PEG 400 concentrations: (■) tested solubility at 37 °C, and (□) estimated solubility by the log-linear model (mean ± SD; n = 3).



**Fig. 3.2.** Solubility of carbamazepine in different cosolvent systems with various concentrations: (■) tested solubility with PEG 400 at 37 °C, (□) estimated solubility with PEG 400 by the log-linear model, (●) tested solubility with EtOH at 37 °C, and (○) estimated solubility with EtOH by the log-linear model (mean ± SD; n = 3).

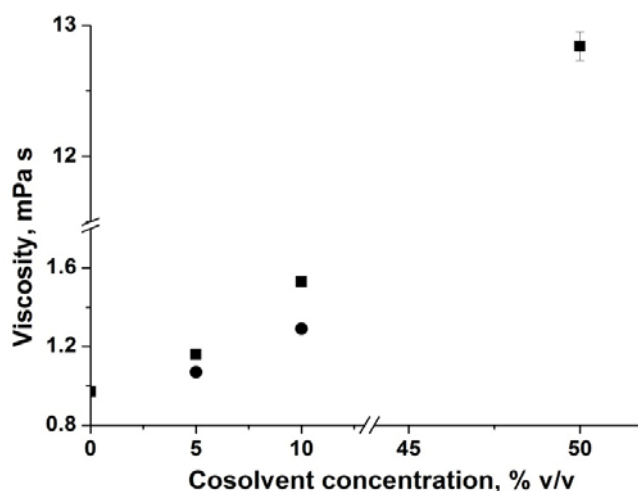
### 3.1.3. Viscosity of different cosolvent systems

Drug diffusivity was associated with the viscosity of different cosolvent solutions measured by a rotational viscometer. According to the Stokes-Einstein equation (Eq. 3.3), it describes an inverse relationship between viscosity and diffusion coefficient.

$$D = \frac{k * T}{6\pi\eta r} \quad (3.3)$$

where  $D$  is the diffusion coefficient,  $k$  is the Boltzmann constant,  $T$  is the temperature,  $\eta$  is the viscosity of the medium and  $r$  is the radius of the drug molecule.

PEG 400 showed an increase in viscosity with increasing concentration ( $0.97 \pm 0.02$ ,  $1.16 \pm 0.02$ ,  $1.53 \pm 0.01$  and  $12.84 \pm 0.11$  mPa s for 0, 5, 10 and 50% PEG 400 respectively), while EtOH had a lower increase ( $0.97 \pm 0.02$ ,  $1.07 \pm 0.01$  and  $1.29 \pm 0.02$  mPa s for 0, 5 and 10% EtOH respectively) (Fig. 3.3). This indicated that drug diffusivity in the cosolvent systems could decrease with increasing PEG 400 and EtOH concentrations, but PEG 400 had stronger inhibiting effect than EtOH at the same concentration.

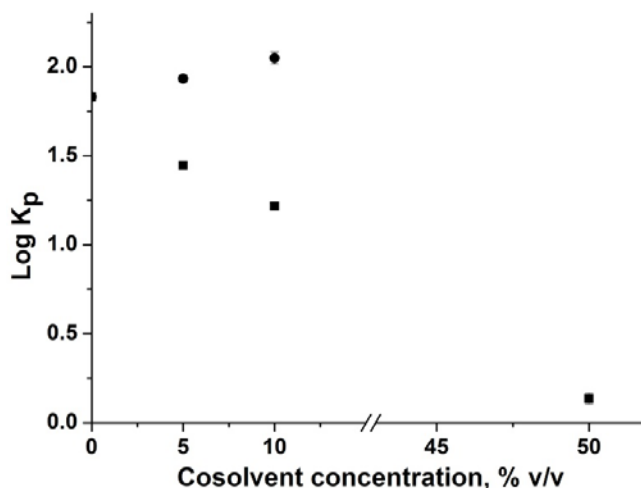


**Fig. 3.3.** Viscosity of different cosolvent systems with various concentrations at 25 °C: (■) PEG 400 and (●) EtOH (mean  $\pm$  SD;  $n = 3$ ).

#### 3.1.4. Determination of partition coefficient ( $\log K_p$ )

Partition coefficients of carbamazepine in different cosolvent systems were tested to assess drug affinity with PEG 400 and EtOH. PEG 400 and EtOH showed different influences on carbamazepine  $\log K_p$  (Fig. 3.4). An obvious decrease in  $\log K_p$  was observed with increasing PEG 400 concentrations ( $1.83 \pm 0.03$ ,  $1.45 \pm 0.01$ ,  $1.22 \pm 0.01$  and  $0.13 \pm 0.03$  for 0, 5, 10, and 50% PEG 400 respectively). In contrast, EtOH showed an increase in  $\log K_p$  with increasing concentration ( $1.83 \pm 0.03$ ,  $1.93 \pm 0.02$  and  $2.05 \pm 0.03$  for 0, 5 and 10% EtOH

respectively). This indicated that carbamazepine had stronger affinity with PEG 400 than EtOH.

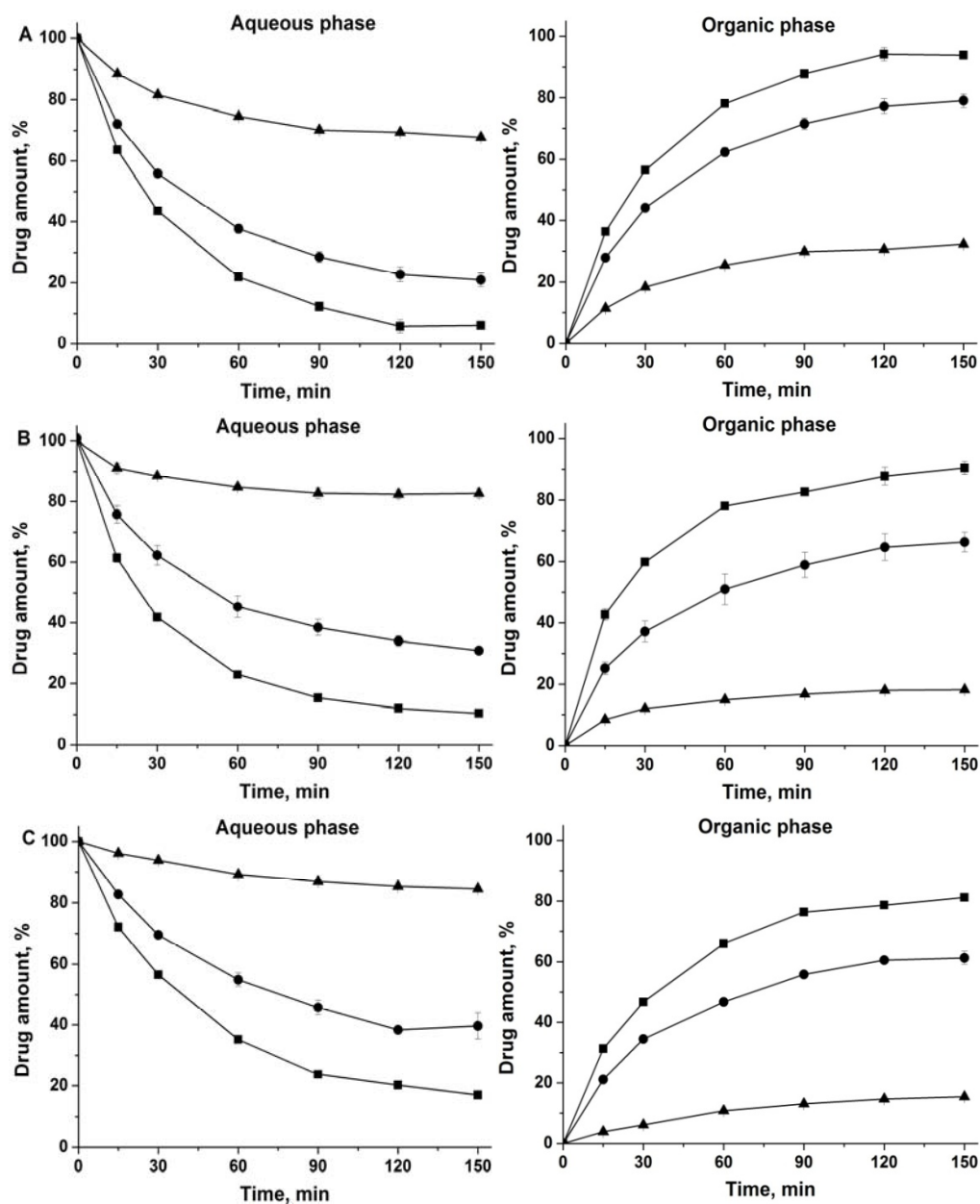


**Fig. 3.4.** Partition coefficients of carbamazepine in the different cosolvent systems: (■) PEG 400 and (●) EtOH (mean  $\pm$  SD;  $n = 3$ ).

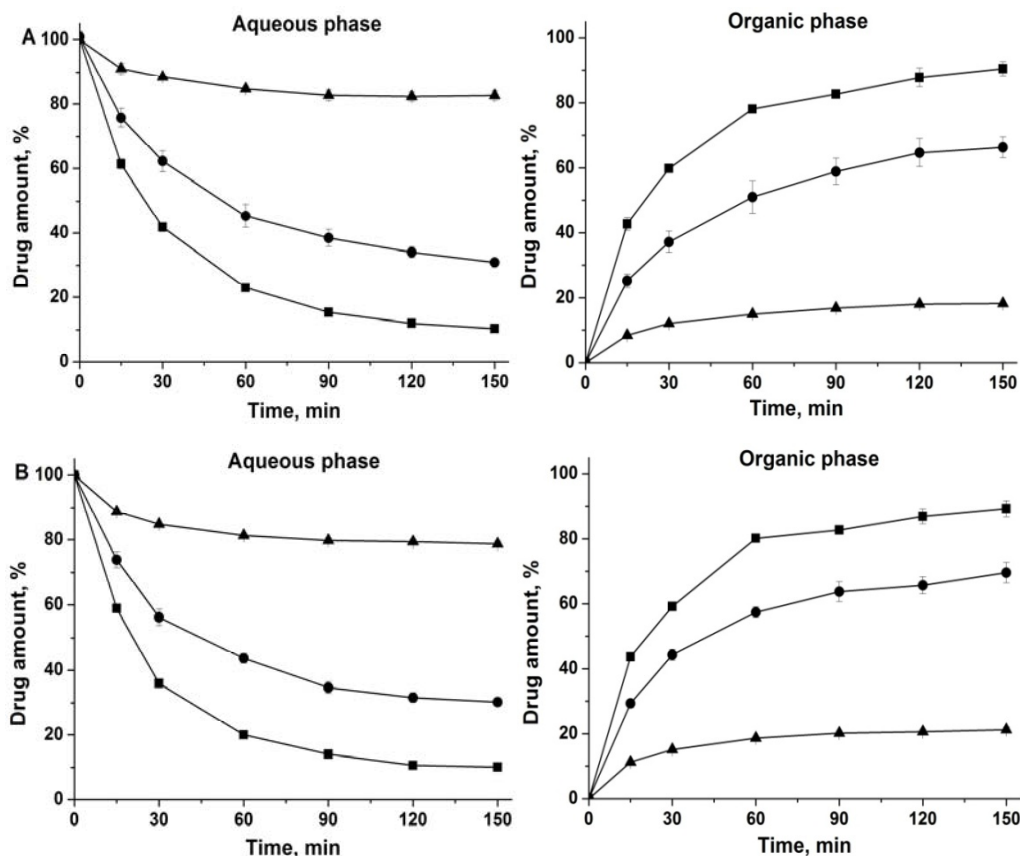
### 3.1.5. Effect of PEG 400 with different concentrations on drug absorption

Firstly, the influences of different volume ratios between the two phases and rotation speeds were investigated to achieve good discrimination for three PEG 400 concentrations (0, 10 and 50%, v/v). Compared with the volume ratio of 30:5 (Fig. 3.5B), increasing proportion of octanol (30:10) improved drug partitioning into the organic phase from all PEG 400 solutions (Fig. 3.5A), while increasing proportion of aqueous solution (40:5) slightly reduced drug partitioning (Fig. 3.5C). The volume ratio of 30:5 exhibited a pronounced discriminative power for the three different PEG 400 concentrations. The rotation speeds of 50 and 75 rpm showed no obvious difference in drug partitioning between 0 and 50% PEG 400 (Fig. 3.6). Optimized parameters for the biphasic test were a volume ratio of 30:5 and a rotation speed of 50 rpm.





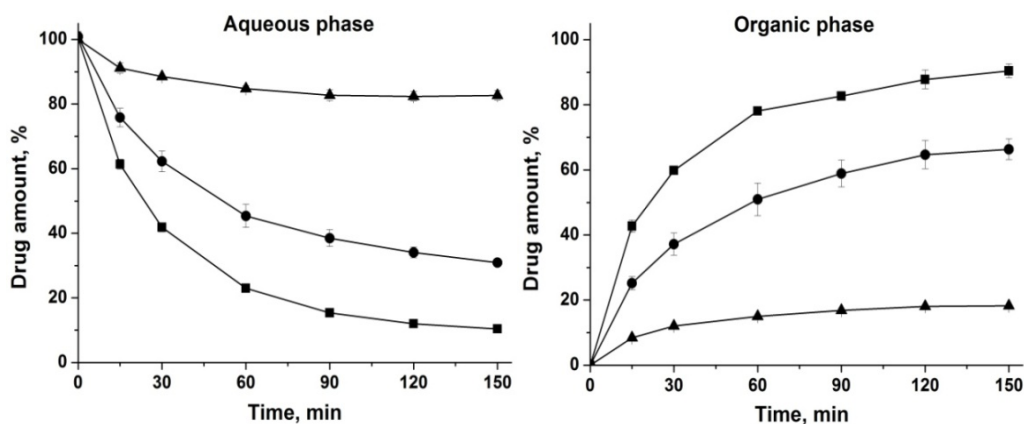
**Fig. 3.5.** The influence of different volume ratios of aqueous and organic phases (rotation speed of 50 rpm) on discrimination of various concentrations of PEG 400 in the biphasic test: (A) 30: 10, (B) 30:5, and (C) 40:5: (■) 0% PEG 400, (●) 10% PEG 400, and (▲) 50% PEG 400 (mean  $\pm$  SD; n = 3).



**Fig. 3.6.** The influence of different rotation speeds (with a constant volume ratio of 30:5) on discrimination of various concentrations of PEG 400 in the biphasic test: (A) 50 rpm and (B) 75 rpm: (■) 0% PEG 400, (●) 10% PEG 400, and (▲) 50% PEG 400 (mean  $\pm$  SD;  $n = 3$ ).

The effect of different PEG 400 concentrations on drug absorption was evaluated by the biphasic dissolution test with the optimized parameters. Carbamazepine amount in the aqueous phase remarkably reduced with decreasing PEG 400 concentrations due to the continuous drug partitioning from the aqueous to the organic phase. Correspondingly, drug amount increased significantly in the organic phase with a rank order of 0% PEG 400 > 10% PEG 400 > 50% PEG 400 (Fig. 3.7). It indicated that increasing PEG 400 concentrations induced an inhibiting effect on drug partitioning, although higher PEG 400 concentration pronouncedly improved carbamazepine solubility. As discussed above, higher PEG 400 concentration showed higher viscosity and lower partition coefficient, suggesting a decrease in drug diffusion and an increase in drug affinity with PEG 400 aqueous solution. Therefore, the inhibiting effect with higher PEG 400 concentration on drug absorption could be attributed

to stronger drug affinity and lower diffusivity, and further reduction of driving force for drug partitioning.

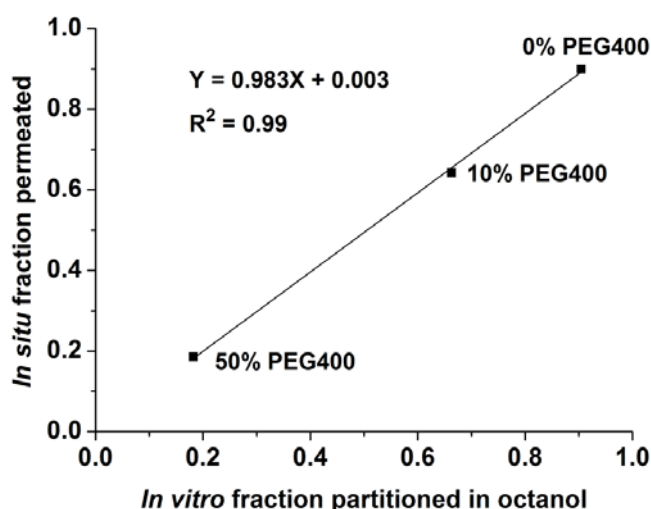


**Fig. 3.7.** Different behaviors of various PEG 400 concentrations in the biphasic dissolution test with optimized parameters: (■) 0% PEG 400, (●) 10% PEG 400, and (▲) 50% PEG 400 (mean  $\pm$  SD; n = 3).

Similar findings were also observed in previous studies (Beig et al., 2012; Miller et al., 2012; Riad and Sawchuk, 1991). Miller et al. studied the solubility-permeability interplay of cosolvents-based systems with various levels of propylene glycol and PEG 400 for the lipophilic drug progesterone (Miller et al., 2012). Both cosolvents increased progesterone solubility with increasing their levels, but decreased drug permeability in the single-pass rat jejunal perfusion studies. Beig et al. investigated the influence of various PEG 400 levels on the solubility-permeability interplay for carbamazepine (Beig et al., 2012). Similarly, decreased carbamazepine permeability with increased solubility was observed in both PAMPA and rat perfusion models. Both reported that a trade-off existed between solubility increase and permeability decrease when using cosolvent-based systems for the solubilization of lipophilic drugs. The solubility-permeability interplay also existed for cyclodextrin- and surfactant-based systems (Beig et al., 2013a; Beig et al., 2013b; Miller et al., 2011). The complexation or micellization involved solubilization could lead to a decreased free fraction of drug, and thus lower permeability and absorption. Cosolvents, irrespective of the free fraction of drug, may be associated with the

drug thermodynamic activity at different cosolvent levels, and further reduce the driving force for drug permeability (Beig et al., 2012).

In another study, the decreased carbamazepine permeability with increasing PEG 400 concentrations from 0% to 50% (v/v) was identified using *in situ* perfusion in rabbit intestinal regions (Riad and Sawchuk, 1991). Given the available data, therefore in our study, an excellent rank order correlation between *in vitro* fraction partitioned in the octanol phase of the biphasic test and *in situ* fraction permeated in rabbits ( $R^2 = 0.99$ ) was obtained (Fig. 3.8).

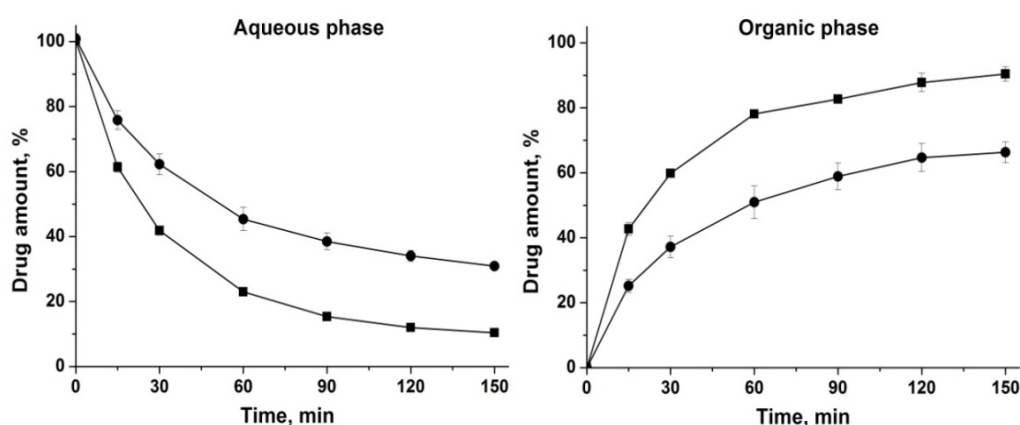


**Fig. 3.8.** Relationship of *in vitro* fraction partitioned in the organic phase of the biphasic test plotted against *in situ* fraction permeated in rabbits found in the literature (Riad and Sawchuk, 1991).

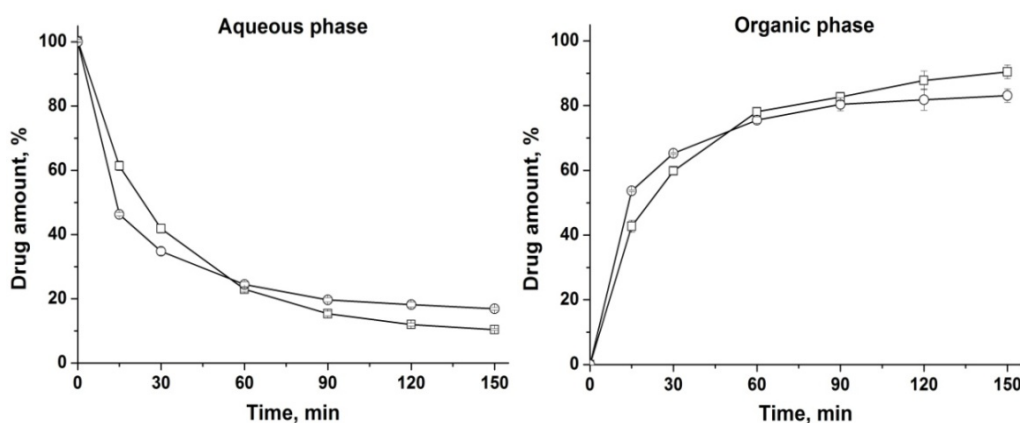
### 3.1.6. Effect of PEG 400 and EtOH on drug absorption

The effect of PEG 400 and EtOH on carbamazepine absorption was evaluated by the biphasic dissolution model. Interestingly, a pronounced difference was observed for PEG 400, but not for EtOH. Carbamazepine amount in the organic phase apparently decreased with increasing PEG 400 concentrations from 0% to 10% (Fig. 3.9), whereas EtOH had no effect (Fig. 3.10). Since PEG 400 exhibited lower partition coefficient and higher viscosity, the difference in biphasic behavior was attributed to the stronger drug affinity and lower drug diffusivity for PEG 400 than EtOH, which could reduce the driving force for drug partitioning. The different behavior of PEG 400 and EtOH were in good

agreement with the previous study about dexamethasone permeability to Caco-2 monolayers (Yamashita et al., 2000). PEG 400, but not EtOH, reduced drug permeability in a concentration-dependent manner from 0% to 10%. Therefore, solubilization is an important parameter in the development of cosolvent-based formulations, but the effect of cosolvent on drug absorption reflected by drug partitioning should also be considered.



**Fig. 3.9.** Different behaviors of PEG 400 with various concentrations in the biphasic dissolution system: (■) 0% PEG 400 and (●) 10% PEG 400 (mean  $\pm$  SD; n = 3).



**Fig. 3.10.** Different behaviors of EtOH with various concentrations in the biphasic dissolution system: (□) 0% EtOH and (○) 10% EtOH (mean  $\pm$  SD; n = 3).

### 3.1.7. Effect of the mixed EtOH-PEG 400 cosolvent system on drug absorption

PEG 400 markedly increased carbamazepine solubility and decreased drug partitioning in the concentration range from 0% to 10%, while EtOH had a lower solubilization ability and no effect on carbamazepine partitioning. Thus, it was

presumed the mixed EtOH-PEG 400 cosolvent system could compensate for the deficiency in lower solubilization for EtOH and lower partitioning for PEG 400. In a further study, 10% EtOH, 10% PEG 400 and 10% EtOH-PEG 400 (1:1) were investigated by solubility, viscosity, partition coefficient and biphasic test for confirming this hypothesis.

Carbamazepine solubility increased in all 10% cosolvent solutions compared to that in blank phosphate buffer pH 6.8 (Table 3.1). 10% EtOH-PEG 400 exhibited the stronger solubilization for carbamazepine than 10% EtOH, but lower capacity than 10% PEG 400. This indicated that the mixed EtOH-PEG 400 cosolvent could enhance carbamazepine solubility and compensate for the low solubilization with only EtOH.

**Table 3.1.** Solubility of carbamazepine in the single and mixed cosolvent systems at 37 °C (mean ± SD; n = 3).

Cosolvent systems	Solubility (µg/ml)
Phosphate buffer pH 6.8	200.2 ± 14.8
10% EtOH	368.9 ± 21.5
10% EtOH-PEG 400 (1:1, v/v)	654.1 ± 21.7
10% PEG 400	783.6 ± 5.1

As mentioned above, the viscosity of the aqueous solution was related with drug diffusivity. The viscosity of blank phosphate buffer pH 6.8 was the lowest. 10% EtOH-PEG 400 had higher than 10% EtOH, but lower viscosity than 10% PEG 400 (Table 3.2). This suggested that carbamazepine diffusivity in the different cosolvent systems was a ranking of 10% EtOH > 10% EtOH-PEG 400 > 10% PEG 400.

**Table 3.2.** Viscosity of the single and mixed cosolvent systems (mean ± SD; n = 3).

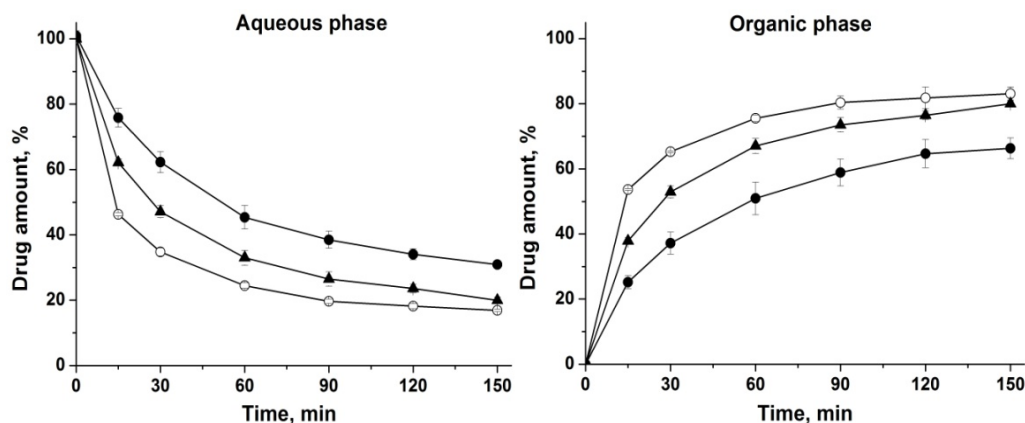
Cosolvent systems	Viscosity (mPa s)
Phosphate buffer pH 6.8	0.97 ± 0.02
10% EtOH	1.29 ± 0.02
10% EtOH-PEG 400 (1:1, v/v)	1.41 ± 0.01
10% PEG 400	1.53 ± 0.01

Partition coefficient of carbamazepine in different cosolvent systems was also tested to compare drug affinity. The highest log  $K_p$  was observed in 10% EtOH. Interestingly, the partition coefficient in 10% EtOH-PEG 400 was lower than in 10% EtOH, but higher than that in 10% PEG 400 (Table 3.3), indicating drug affinity with a rank order of 10% EtOH < 10% EtOH-PEG 400 < 10% PEG 400.

**Table 3.3.** Partition coefficient of carbamazepine in the single and mixed cosolvent systems (mean  $\pm$  SD; n = 3).

Cosolvent systems	Partition coefficient
Phosphate buffer pH 6.8	1.83 $\pm$ 0.03
10% EtOH	2.05 $\pm$ 0.03
10% EtOH-PEG 400 (1:1, v/v)	1.62 $\pm$ 0.03
10% PEG 400	1.22 $\pm$ 0.01

Different influences of the single and mixed cosolvent systems on carbamazepine absorption were observed in the biphasic test (Fig. 3.11). Carbamazepine showed a pronounced decreased with a rank order of 10% EtOH > 10% EtOH-PEG 400 > 10% PEG 400 in the aqueous phase. The drug amount partitioning into the organic phase for 10% EtOH-PEG 400 was lower than 10% EtOH, but higher compared to 10% PEG 400. This was attributed to the weaker drug affinity and higher drug diffusivity for 10% EtOH-PEG 400 than PEG 400, which could increase the driving force for drug partitioning. As hypothesized above, the mixed EtOH-PEG 400 cosolvent system not only enhanced drug solubilization, but also lowered the inhibiting effect on drug partitioning compared to the single EtOH and PEG 400. The biphasic dissolution model could discriminate the effect of different cosolvents on drug absorption reflected by drug partitioning. Moreover, the mixed cosolvent system could be considered as an optimal strategy for the compensation for the tradeoff between solubility and permeability in the development of cosolvent formulations.



**Fig. 3.11.** Different behavior of pure and mixed cosolvents in the biphasic dissolution system: (○) 10% EtOH, (▲) 10% EtOH-PEG 400 (1:1), and (●) 10% PEG 400 (mean  $\pm$  SD;  $n = 3$ ).

### 3.1.8. Conclusions

Both cosolvents increased carbamazepine solubility in a concentration-dependent manner, PEG 400 had stronger solubilization capacity than EtOH. However, PEG 400 exhibited a pronounced inhibition of drug partitioning with increasing concentrations, while EtOH had no effect. The biphasic dissolution model successfully discriminated different influences of PEG 400 and EtOH with different concentrations on drug absorption reflected by drug partitioning, which was consistent with previously published studies. The different performances of the two cosolvents could be associated with drug affinity and diffusivity. The solubility-permeability interplay should be taken into consideration when designing cosolvent-based formulations. Moreover, the mixed EtOH-PEG 400 cosolvent system outperformed single EtOH and PEG 400 by enhancing carbamazepine solubility to compensate for low solubilization for EtOH and decreasing the inhibiting effect on drug partitioning to compensate for low drug absorption for PEG 400. This optimal strategy could be considered in the development of cosolvents formulations. This work demonstrates that the biphasic dissolution model has the great potential to discriminate between cosolvent-based formulations.



## **3.2. Evaluation of a discriminative biphasic dissolution test for estimating the bioavailability of carbamazepine polymorphic forms**

### **3.2.1. Introduction**

Drug polymorphism has attracted great interest due to the marked effects on drug properties and performances such as solubility, dissolution rates and bioavailability (Datta and Grant, 2004). Approximately more than half of drug compounds surveyed were polymorphic (Santos et al., 2014; Stahly, 2007). Important cases involving polymorphic drugs were summarized over the past 25 years (Lee et al., 2011). For example, Norvir<sup>®</sup> (ritonavir) was withdrawn from the market due to its transformation to a thermodynamically more stable form during storage and consequently a slower drug dissolution and absorption (Chemburkar et al., 2000; Huang and Tong, 2004).

A thermodynamically stable polymorph is preferable in the final dosage form, with an absence of phase conversion during storage and thus potentially changes in bioavailability. However, the most stable and thus least soluble form may result in a too low bioavailability. Thus, metastable forms may be desirable to achieve a better therapeutic benefit.

Various manufacturing processes and excipients have a strong impact on the solid-state forms of formulated drugs (Nair et al., 2002; Singhal and Curatolo, 2004; Zhang et al., 2004). Therefore, it is essential to select an optimal form for further formulation development. Besides the identification and characterization of polymorphic transformation in dosage forms, it is important to evaluate *in vitro* release behavior and link it with *in vivo* performance.

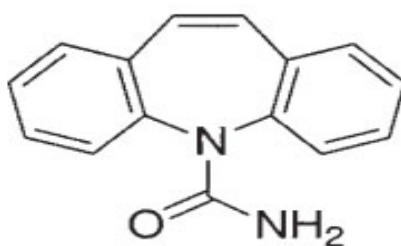
A clear relationship between *in vitro* dissolution and *in vivo* parameters is often difficult to be established (Aguilar and Zelmer, 1969; El-Zein et al., 1998; Horter and Dressman, 2001; Swanepoel et al., 2003). For example, identical *in vivo* plasma profiles of two polymorphs of mefenamic acid were observed, regardless of the higher solubility and higher dissolution rate for polymorph II compared to

polymorph I (Aguiar and Zelmer, 1969). Carbamazepine solid dispersions with different carriers (PEG 6000), coprecipitates with phospholipids (PL), and complexes with hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD) were prepared to improve oral bioavailability (El-Zein et al., 1998). X-ray diffraction identified different polymorphic forms in these formulations. The bioavailability ranked in the order of HP $\beta$ CD > PL > PEG 6000; this was inconsistent with *in vitro* release with PEG 6000 > HP $\beta$ CD > PL.

Several studies tried to evaluate a potential relationship between *in vitro* and *in vivo* behavior for polymorphic drugs by an artificial stomach-duodenal model (Carino et al., 2006) and an intrinsic dissolution-permeation model (Zhou et al., 2014). Compared with these biorelevant models, biphasic dissolution system could be potentially suitable for this case, because it can simulate drug dissolution and absorption in the GI tract by the implementation of an immiscible organic phase acting as an absorptive sink over the aqueous solution. Moreover, the setup and handling of biphasic tests are relatively simple and cost effective, and drug release and partitioning are detected simultaneously within a single vessel (Locher et al., 2016). Previous studies have reported that the biphasic dissolution model was potentially capable to discriminate between formulation parameters, such as dose strengths (Grundy et al., 1997), effect of excipients (Shi et al., 2010), drug precipitations (Heigoldt et al., 2010; Locher et al., 2016), drug loadings (Pestieau et al., 2017), and drug particle sizes (Al Durdunji et al., 2016; Pestieau et al., 2016). A pH-adjusted biphasic dissolution system was used to differentiate between four modified release formulations prepared with two weakly basic drugs (dipyridamole and BIMT 17) and to compare it with conventional dissolution tests at constant pH (Heigoldt et al., 2010). The drug release profiles obtained from different dissolution media at constant pH were inconsistent with *in vivo* performance, whereas biphasic test provided a qualitative prediction with respect to *in vivo* absorption. Different dissolution systems (sink vs. non-sink, and monophasic vs. biphasic) were investigated in order to discriminate three different fenofibrate formulations (Pestieau et al.,

2016). Only the biphasic test was discriminative and biorelevant for their performance. However, there is still a lack in studies to discriminate different crystal forms of BCS II drugs within the formulations by the biphasic dissolution model.

The objective was to evaluate the potential of the biphasic dissolution model to discriminate between different polymorphic forms of a lipophilic drug and to compare the results with conventional dissolution tests under sink and non-sink conditions. Carbamazepine (Fig. 3.12) was selected as a model polymorphic BCS II drug. It has four polymorphs and a dihydrate form as well as other solvates (Grzesiak et al., 2003; Harris et al., 2005). In particular, the bioavailability of three carbamazepine crystal forms (form I, form III and dihydrate) in dogs was in the ranking of form III > form I > dihydrate (Kobayashi et al., 2000). The *in vitro* dissolution rates did not properly reflect the *in vivo* performance. Thus, these three polymorphic forms of carbamazepine were selected in this study to assess the discriminative power of different dissolution systems and their correlation with the *in vivo* performance.



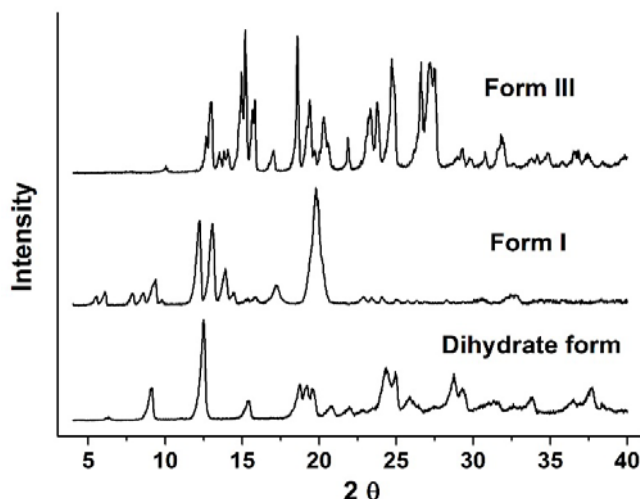
**Fig. 3.12.** Chemical structure of carbamazepine.

### 3.2.2. Characterization of carbamazepine polymorphic forms

#### 3.2.2.1. X-ray powder diffraction (XRPD)

Three carbamazepine polymorphic forms were distinguished by XRPD (Fig. 3.13). Form III was identified with characteristic peaks at  $2\theta = 15.2, 15.8, 17.0, 27.2$  and  $27.5^\circ$ , form I at  $2\theta = 6.1, 9.4$  and  $19.8^\circ$  and the dihydrate form at  $2\theta =$

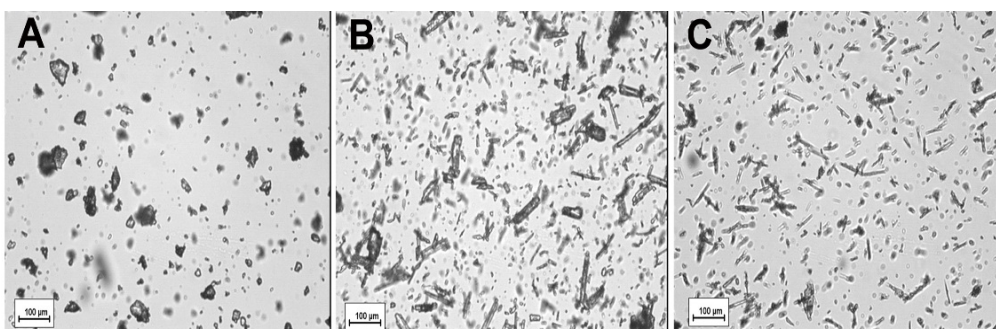
8.9, 18.9 and 19.46°. These X-ray diffractograms were in agreement with previous studies (Grzesiak et al., 2003; Kobayashi et al., 2000; Rustichelli et al., 2000).



**Fig. 3.13.** X-ray powder diffraction patterns of three carbamazepine polymorphic forms.

#### 3.2.2.2. Optical microscopy

Form III of carbamazepine appeared as prism-like particles (Fig. 3.14A), while form I and the dihydrate exhibited needle-like crystals (Figs. 3.14B and C). The differences of morphology for these three carbamazepine forms are likely associated with the packing of the carboxamide dimer units (Grzesiak et al., 2003).



**Fig. 3.14.** Photomicrographs of three carbamazepine polymorphic forms (magnification 20x): (A) form III, (B) form I and (C) dihydrate form.

### 3.2.2.3. Thermal analysis (DSC and TGA)

The DSC curve of form III showed the first endothermic peak at 174 °C, followed by one exotherm and a sharp endothermic peak at 191 °C (Fig. 3.15), which indicated the typical thermal behavior of form III with the melting of form III, crystallization and melting of form I, respectively. Only one endothermic melting peak was observed for form I at 191 °C. Compared to the two anhydrous forms, the dihydrate form exhibited a broad endotherm over the range of 50-80 °C due to loss of water and an endothermic melting peak at 191 °C. A weight loss of 13.1% was observed on the TGA curve of the dihydrate, no loss was recorded for forms III and I (Fig. 3.16). These results were consistent with previous reports (Kobayashi et al., 2000; McMahon et al., 1996).

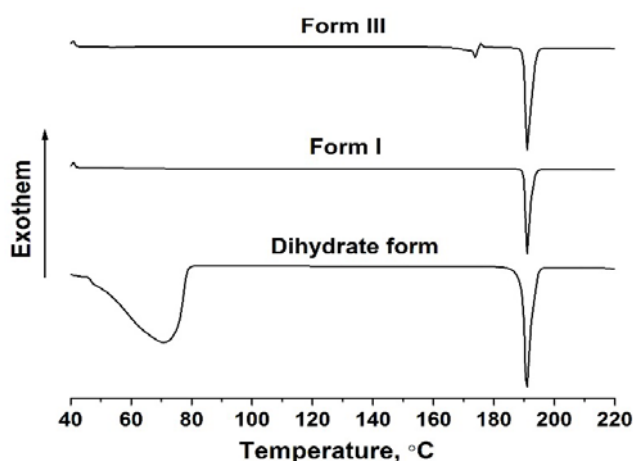


Fig. 3.15. DSC thermograms of three carbamazepine polymorphic forms.

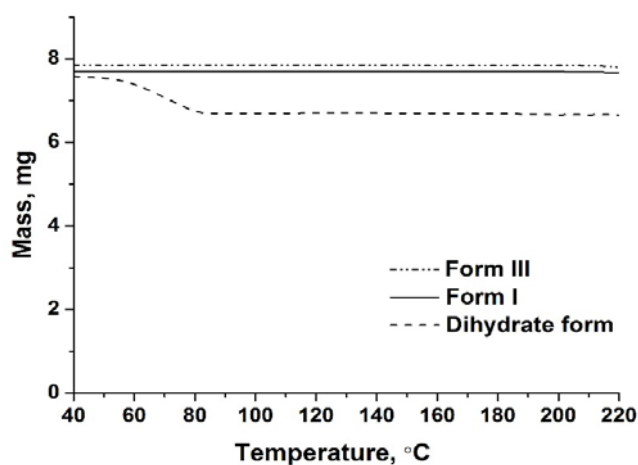
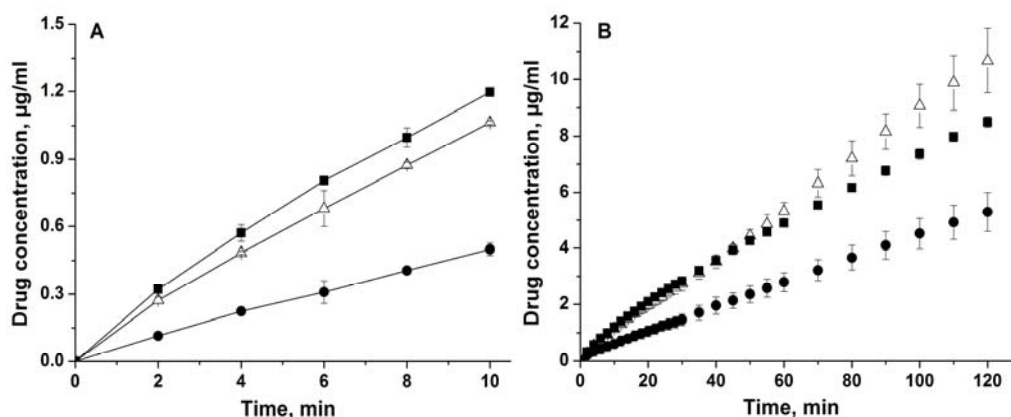


Fig. 3.16. TGA thermograms of three carbamazepine polymorphic forms.

### 3.2.3. Intrinsic dissolution rate

The intrinsic dissolution rates of the carbamazepine crystal forms had the rank order of form I > form III > dihydrate form (Table 3.4). Carbamazepine dihydrate is the most stable form in the aqueous medium, it exhibited the lowest intrinsic dissolution rate of  $18.6 \mu\text{g}/\text{min}/\text{cm}^2$ . For anhydrous carbamazepine, form I had a slightly higher intrinsic dissolution rate initially than form III ( $43.2 \pm 1.0 \mu\text{g}/\text{min}/\text{cm}^2$  vs.  $39.6 \pm 0.9 \mu\text{g}/\text{min}/\text{cm}^2$ ) (Fig. 3.17A), but subsequently it showed a slower dissolution due to the faster conversion to dihydrate compared to form III (Fig. 3.17B). Form I converted faster to the dihydrate than form III as shown by Fourier Transform Raman spectroscopy (Tian et al., 2006). The growth of carbamazepine dihydrate on the surface of anhydrous carbamazepine particles resulted in decreasing dissolution rates (Sehic et al., 2010; Tian et al., 2006).



**Fig. 3.17.** Intrinsic dissolution profiles of three carbamazepine polymorphic forms in pH 6.8 phosphate buffer: (A) up to 10 min and (B) up to 120 min: (■) form I, (△) form III, and (●) dihydrate form (mean  $\pm$  SD;  $n = 3$ ).

### 3.2.4. Solubility of carbamazepine polymorphic forms

The anhydrous forms of carbamazepine undergo a phase transformation to the dihydrate in aqueous conditions, thus the solubility of anhydrous form was estimated based on the intrinsic dissolution rates using Eq. 2.1. The dissolution rate constant should be same for these three crystal forms, since constant hydrodynamic conditions and surface areas were maintained. The

carbamazepine solubilities of form I, form III and dihydrate form were 503.9, 462.0 and 216.3 µg/ml, respectively (Table 3.4). The rank order of solubility of three carbamazepine polymorphic forms was in accordance with intrinsic dissolution rates.

**Table 3.4.** Intrinsic dissolution rates and solubility of three carbamazepine polymorphic forms in phosphate buffer pH 6.8 (mean ± SD; n = 3).

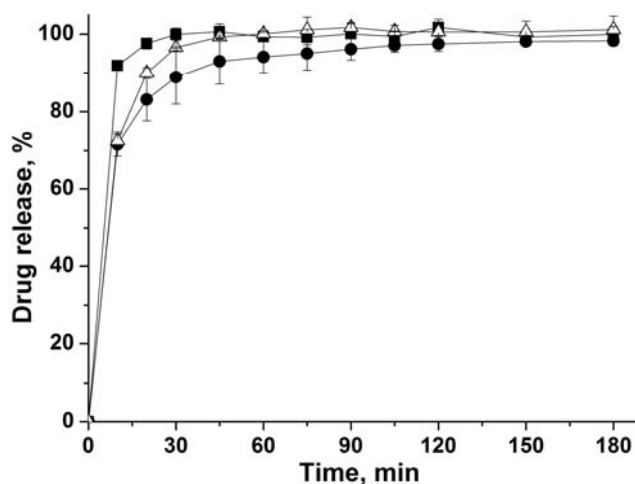
Polymorphic form	IDR (µg/min/cm <sup>2</sup> )	Solubility (µg/ml)
Form I	43.2 ± 1.0 <sup>b</sup>	503.9 ± 11.7*
Form III	39.6 ± 0.9 <sup>ab</sup>	462.0 ± 11.2*
Dihydrate form	18.6 ± 0.6 <sup>a</sup>	216.3 ± 3.7

a  $p < 0.05$  vs. form I; b  $p < 0.05$  vs. dihydrate form.

\* estimated by Eq. 2.1.

### 3.2.5. Conventional dissolution tests under sink and non-sink conditions

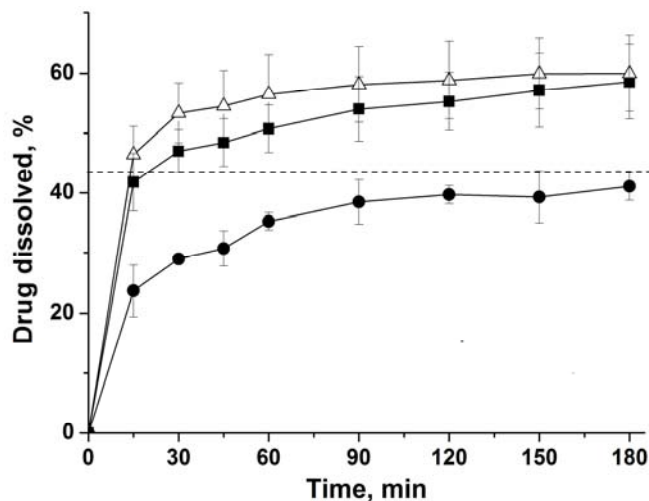
The solubility of carbamazepine dihydrate was 2.5 mg/ml in 1% w/v aqueous SLS solution. The dihydrate form had the lowest solubility and therefore sample equivalent to 200 mg drug was used to maintain sink conditions (< 10% of drug solubility in 900 ml medium). All three polymorphic forms of carbamazepine had similar dissolution profiles in the compendial USP II dissolution test, and they dissolved more than 80% at 20 min (Fig. 3.18). Different dissolution media were investigated to predict *in vitro-in vivo* correlation for carbamazepine immediate release (IR) and controlled release (CR) tablets (Kovačević et al., 2008). The results suggested that 1% SLS may be considered as a universal biorelevant dissolution medium for both the IR and CR carbamazepine tablets. However, 1% SLS appeared to be unsuitable to predict the performance of the carbamazepine polymorphs in this study. In fact, compendial dissolution test lacked discrimination between these carbamazepine crystal forms.



**Fig. 3.18.** Dissolution profiles of three carbamazepine polymorphic forms obtained with the USP II dissolution test (900 ml water containing 1% SLS): (■) form I, (△) form III, and (●) dihydrate form (mean  $\pm$  SD; n = 3).

BCS II drugs undergo a slower dissolution under non-sink conditions in the GI tract due to their low water solubility. The intrinsic dissolution rates of three polymorphic forms were below  $1 \text{ mg/min/cm}^2$ , which indicates the absorption of carbamazepine is dissolution rate-limited (Zakeri-Milani et al., 2009). A volume of 300-500 ml is recommended to be of physiological relevance (Klein, 2010). Therefore, drug dissolution from carbamazepine polymorphic forms was assessed in 400 ml phosphate buffer pH 6.8 under non-sink conditions. The drug release was much slower compared to sink conditions (Fig. 3.19). The two anhydrous forms had almost similar dissolution profiles, while the dihydrate form had the slowest dissolution approaching its solubility in the medium. Under non-sink conditions, a difference was observed between the anhydrous and dihydrate forms, but it still lacked relevance to the reported *in vivo* performance of these three carbamazepine polymorphic forms (Kobayashi et al., 2000).

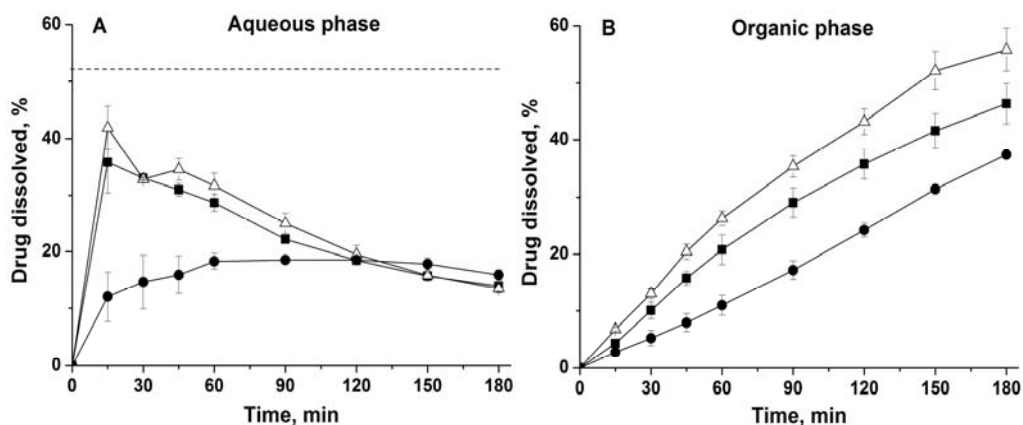




**Fig. 3.19.** Dissolution profiles of three carbamazepine polymorphic forms in the single phase dissolution test under non-sink conditions (400 ml pH 6.8 phosphate buffer) (dashed line represents the carbamazepine solubility of dihydrate form): ( $\Delta$ ) form III, ( $\blacksquare$ ) form I, and ( $\bullet$ ) dihydrate form (mean  $\pm$  SD;  $n = 3$ ).

### 3.2.6. Biphasic dissolution test

Each sample equivalent to 200 mg drug was evaluated in the biphasic dissolution test under sink conditions ( $< 20\%$  of drug solubility ( $C_s = 12.2$  mg/ml) in 100 ml octanol). Similar dissolution profiles were observed between anhydrous forms III and I in the aqueous phase, while the dissolution of the dihydrate was lower in the first 60 min and then approached the two anhydrous forms (Fig. 3.20A). The corresponding dissolution profiles observed in the organic phase, however, differed with a ranking of form III  $>$  form I  $>$  dihydrate form (Fig. 3.20B). This rank order correlated with *in vivo* AUC ( $9.10 \pm 1.00$ ,  $6.33 \pm 2.39$  and  $4.39 \pm 1.30$   $\mu\text{g h/ml}$ ) for forms III and I and the dihydrate, respectively (Kobayashi et al., 2000). The three carbamazepine polymorphic forms were thus discriminated well in the organic phase of the biphasic dissolution test.



**Fig. 3.20.** Dissolution profiles of three carbamazepine polymorphic forms from the (A) aqueous and (B) organic phases in the biphasic dissolution test (dashed line represents carbamazepine solubility of dihydrate form in phosphate buffer pH 6.8 saturated with octanol): ( $\Delta$ ) form III, ( $\blacksquare$ ) form I, and ( $\bullet$ ) dihydrate form (mean  $\pm$  SD;  $n = 3$ ).

By comparison with the single phase dissolution test conducted in the same setup of the biphasic test only without octanol phase, similar dissolution behavior of three carbamazepine crystal forms was also observed in the aqueous phase of the biphasic test, indicating non-sink conditions may exist in the aqueous phase of biphasic dissolution test. The octanol phase was the important part for the discrimination of carbamazepine polymorphisms in the biphasic test.

In this study, different dissolution behavior of these three carbamazepine polymorphic forms observed in the different dissolution systems (sink or non-sink, monophasic or biphasic) could be attributed to the interplay between dissolution and solution-mediated phase transformation of anhydrous forms. The compendial dissolution test lacked discrimination, which might be attributed to dramatically enhanced dissolution rates due to the presence of 1% SLS. A difference was observed in the single phase dissolution test under non-sink conditions, which may result from slow dissolution dominated by solubility in phosphate buffer pH 6.8. Compared with the single phase dissolution tests, the biphasic dissolution system was discriminative for the three carbamazepine crystal forms. Similar dissolution behaviors were observed between the aqueous phase in the biphasic test and the single phase dissolution test under non-sink conditions. However, derived from a continuous concentration gradient between

two phases, the differences of dissolution kinetics induced by dissolution and solution-mediated phase transformation in the aqueous buffer could be magnified by the presence of organic phase following a dissolution-partition process. First, form I converted faster to dihydrate than form III, which caused the decrease in dissolution rate. Second, the extensive dihydrate growth lead to an increased particle size and reduced the effective surface area, and impeded the surface of anhydrous forms in contact with the buffer solution (Lehto et al., 2009; Tian et al., 2006). Therefore, the faster transformation of form I than form III resulted in a decrease of the available drug concentration in aqueous solution for partitioning, as expressed by different partition rates with the rank order of form III > form I > dihydrate form ( $p < 0.05$ ) (Table 3.5) in the biphasic test.

**Table 3.5.** Partition rates of three carbamazepine polymorphic forms in the biphasic test (mean $\pm$ SD; n = 3).

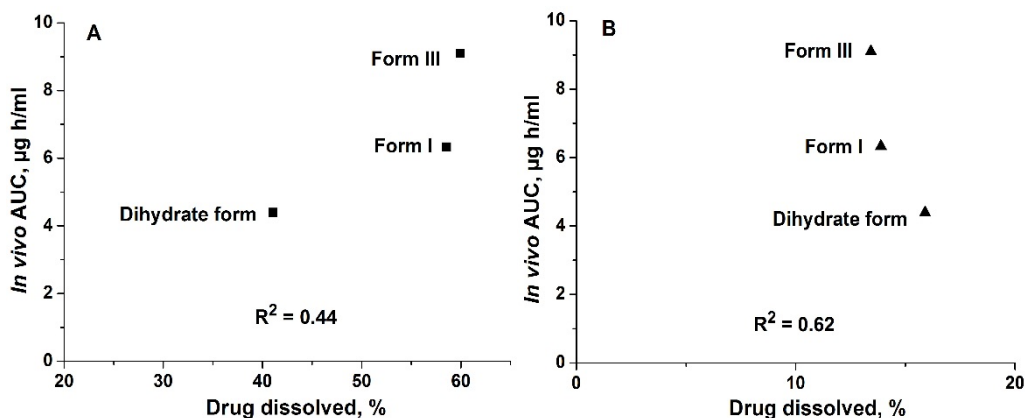
Polymorphic forms	Partition rate (mg/h)
Form III	37.7 $\pm$ 2.4 <sup>b</sup>
Form I	31.4 $\pm$ 2.1 <sup>ab</sup>
Dihydrate form	25.3 $\pm$ 0.1 <sup>a</sup>

a  $p < 0.05$  vs. form III; b  $p < 0.05$  vs. dihydrate form.

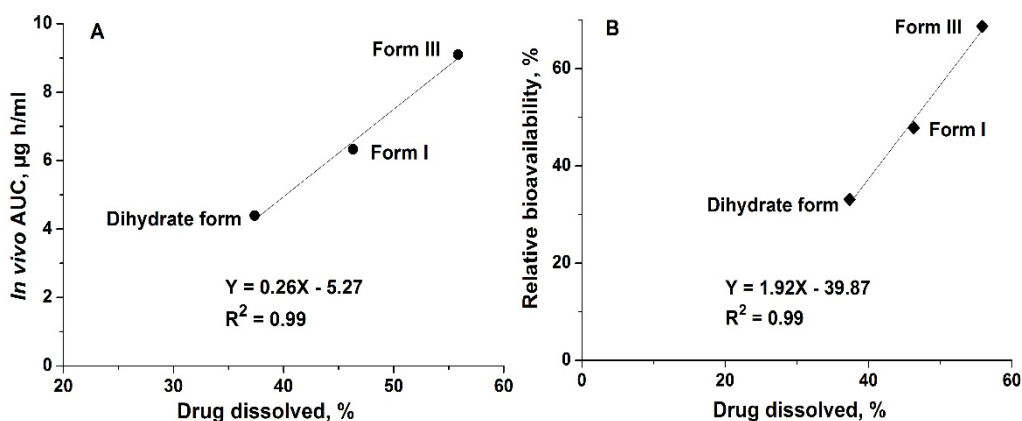
### 3.2.7. *In vitro-in vivo* correlation

Carbamazepine dissolution from the three crystal forms in the biphasic dissolution test correlated with the previously reported *in vivo* performance. Next, the potential of establishing a relationship between *in vitro* and *in vivo* dissolution was explored. A level C IVIVC was tested using the percent drug dissolved at 3 h in the single phase dissolution test under non-sink conditions and in both the aqueous and organic phases of the biphasic test vs. an *in vivo* parameter (AUC or relative bioavailability (Kobayashi et al., 2000)). Poor correlations were obtained between the percent drug dissolved in the single phase dissolution test under non-sink conditions or in the aqueous phase of biphasic test and the *in vivo* AUC (Fig. 3.21). In contrast, excellent linear correlations between the percent drug dissolved in the organic phase at 3 h and the *in vivo* AUC ( $R^2 =$

0.99) (Fig. 3.22A) or the relative bioavailability ( $R^2 = 0.99$ ) (Fig. 3.22B) were obtained. The biphasic test thus reflected both *in vitro* and *in vivo* dissolution kinetics of different carbamazepine polymorphic forms and the organic phase could serve as an indicator for predicting *in vivo* performance.



**Fig. 3.21.** Relationships of mean *in vitro* % dissolved at 3 h in (A) the single phase dissolution test under non-sink conditions and (B) the aqueous phase of the biphasic dissolution test plotted against mean *in vivo* AUC obtained from the literature (Kobayashi et al., 2000).



**Fig. 3.22.** Relationship of mean *in vitro* % dissolved at 3 h in the organic phase of the biphasic test plotted against (A) mean *in vivo* AUC or (B) relative bioavailability obtained from the literature (Kobayashi et al., 2000).

With polymorphic BCS II drugs, not only solubility, but also solution-mediated phase transformation plays a critical role in drug dissolution and absorption. The two anhydrous forms of carbamazepine were better absorbed than the dihydrate due to their higher solubility and faster dissolution rates. Nevertheless, the discrepancy between solubility and bioavailability for these two anhydrous forms

can be attributed to different transformation rates as identified in the intrinsic dissolution test. The faster transformation of form I to dihydrate than form III affected its dissolution kinetics and decreased the available dissolved drug for absorption (Kobayashi et al., 2000), which could be the main reason for lower bioavailability of form I observed *in vivo*.

Biphasic dissolution tests have been used to investigate different formulations with BCS II drugs, but there is no publication to discriminate the formulations with different crystal forms of BCS II drugs. A relationship between *in vitro* dissolution and *in vivo* absorption is likely to obtain for BCS II drugs when *in vitro* dissolution could reflect *in vivo* rate-limiting process. For polymorphic drugs, the interplay between dissolution and solution-mediated phase transformation may dominate the whole release kinetics. In this study, three carbamazepine crystal forms were successfully discriminated via the biphasic system and were correlated well with their *in vivo* performance.

### **3.2.8. Conclusions**

Conventional dissolution test under sink conditions lacked discrimination for these three carbamazepine polymorphic forms. A difference between the anhydrous and dihydrate forms was observed in the single phase dissolution test under non-sink conditions, as well as in the aqueous phase of biphasic test, but no meaningful IVIVC was obtained. In contrast, the biphasic dissolution system showed its sensitive discriminative power for these different polymorphic forms. An excellent IVIVC between *in vitro* dissolution in the organic phase of biphasic test and *in vivo* data was established. The biphasic system outperformed the conventional single phase dissolution tests by reflecting the available drug concentration affected by solution-mediated phase transformation. The biphasic model has great potential to discriminate between different crystal forms of BCS II drugs in the early development of formulations.

### **3.3. Evaluation of a discriminative biphasic dissolution test and correlation with *in vivo* pharmacokinetic studies for differently formulated racecadotril granules**

#### **3.3.1. Introduction**

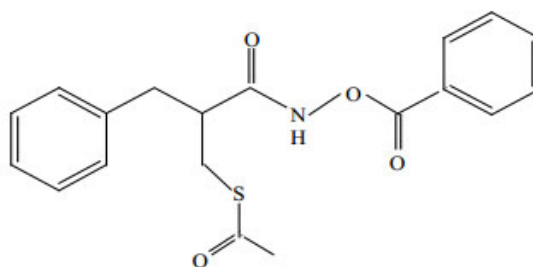
Tablets are the most popular oral dosage forms. They are preferably prepared by direct compression, however, dry and wet granulation are also widely used to improve the physical properties (particle size, wettability, density, porosity, mechanical strength, flowability and compactability) of the drug:excipient blend and the tablets. Granulation is a process of size enlargement that agglomerates small particles together into larger ones (Parikh, 2016). Dry granulation (slugging or roller compaction) is performed using a tablet press or roller compactor for the first compaction to produce slugs or ribbons without water, and afterwards milled and sized. Whereas, wet granulation is conducted by agglomerating powder particles with a granulation fluid or binder followed by drying the granules produced. Drug release rates are greatly influenced by different formulation compositions and manufacturing processes. Zhang et al. reported that different matrix tablets were prepared by dry blending, wet granulation, partial melt granulation, and melt granulation. Different dissolution profiles were obtained due to the formation of different matrix structures (Zhang et al., 2001). Juang and Storey investigated the effects of compositional and processing differences on drug dissolution from controlled release gel extrusion module tablets (Juang and Storey, 2003). Tablets prepared by wet granulation released faster than those prepared by direct compression due to different swelling properties. Liu et al. used two viscosity grades of HPMC (50 and 4000 cP) in varying ratios with water to prepare diclofenac sodium matrix tablets by a wet granulation method (Liu et al., 1995). The larger amount of high viscosity grade HPMC resulted in a slower release rate, which was in agreement with *in vivo* investigation. The aforementioned cases showed that differences of different granule formulations induced by composition and process variables could be reflected in the

conventional dissolution tests. However, the release from granule formulations prepared by dry and wet granulation investigated in our study could not be discriminated by the conventional dissolution tests.

In particular, biphasic dissolution tests could be suitable for BCS II drugs due to poor aqueous solubility. Biphasic dissolution tests consist of immiscible aqueous and organic phases, which can maintain sink conditions due to a continuous partitioning into organic phase. The drug initially dissolves in the aqueous medium and the organic phase mimics GI membrane that continuously removes the dissolved drug from the lower aqueous phase. Thus, the dissolution-partition process between two phases is analogous to drug dissolution and absorption from GI membrane. A two-phase system was firstly proposed to maintain sink conditions in 1961 (Wurster and Polli, 1961). Unfortunately, not much interest has been observed with this test.

Previous studies have reported the development of biphasic dissolution systems (Gibaldi and Feldman, 1967; Heigoldt et al., 2010; Vangani et al., 2009) and their correlation to *in vivo* absorption for different dosage forms, including immediate release (Shi et al., 2010), modified release (Heigoldt et al., 2010), lipid (Kinget and Degreef, 1994) and amorphous formulations (Thiry et al., 2016). The biphasic dissolution model ideally also enables the evaluation of various formulation factors such as dose strength (Grundy et al., 1997), excipient effects (Shi et al., 2010; Thiry et al., 2016), drug precipitation (Frank et al., 2014; Heigoldt et al., 2010) and particle size (Al Durdunji et al., 2016) on the *in vivo* performance of poorly soluble drugs. Shi et al. applied a biphasic system combining USP 4 and USP 2 methods to discriminate three celecoxib formulations (a Celebrex<sup>®</sup> capsule, a drug solution containing surfactant and a self-emulsifying drug delivery system) and to obtain a rank-order relationship between the amount of drug in the organic phase and *in vivo* pharmacokinetic parameters (Shi et al., 2010). The different release kinetics observed in the biphasic model were attributed to the effect of surfactant within the formulations on the free drug concentration. In another study, three fenofibrate formulations

(pure API capsule, micronized formulation and self-emulsifying lipid-based formulation) were discriminated by a biphasic dissolution test, whereas single phase dissolution tests (sink, non-sink and biorelevant) were not suitable (Pestieau et al., 2016). However, there is still a lack in studies to discriminate between minor formulation and process changes within the same dosage forms. Therefore, the objective was to develop a biphasic dissolution system and to evaluate its potential to discriminate three granules of racecadotril (Fig. 3.23) (model BCS II drug) with only minor differences induced by manufacturing variables and to compare the results to conventional dissolution tests under sink and non-sink conditions. A further objective was to establish an *in vitro-in vivo* correlation based on *in vivo* data obtained from studies in rats.



**Fig. 3.23.** Chemical structure of racecadotril.

### 3.3.2. Drug content of granule formulations

The drug content of each racecadotril granule formulation prepared by dry granulation, wet granulation without or with binder was  $45.4 \pm 0.2$ ,  $44.8 \pm 0.7$ , and  $44.6 \pm 0.2\%$  (w/w) (Table 3.6), respectively. This indicated that they were compositionally equivalent.

**Table 3.6.** Drug content of racecadotril granule formulations (mean  $\pm$  SD; n = 3).

Granule formulation	Drug content (% w/w)
Dry granulation	$45.4 \pm 0.2$
Wet granulation without binder	$44.8 \pm 0.7$
Wet granulation with binder	$44.6 \pm 0.2$



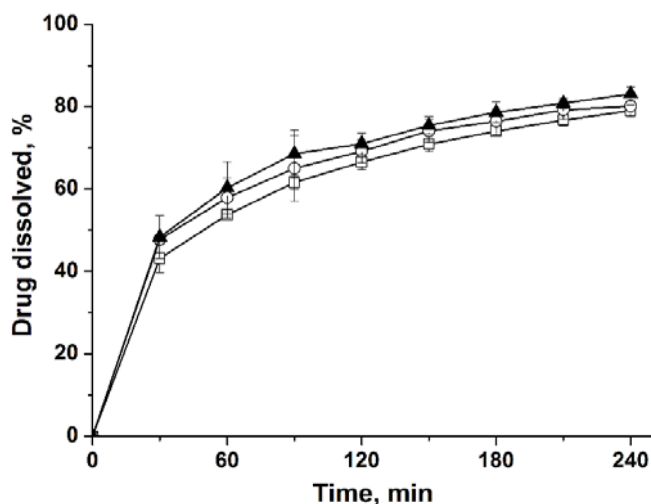
### 3.3.3. Conventional single phase USP II dissolution tests under sink and non-sink conditions

The solubility of racecadotril was determined in different dissolution media at 37 °C (Table 3.7).

**Table 3.7.** Solubility of racecadotril in different dissolution media (mean  $\pm$  SD; n = 3).

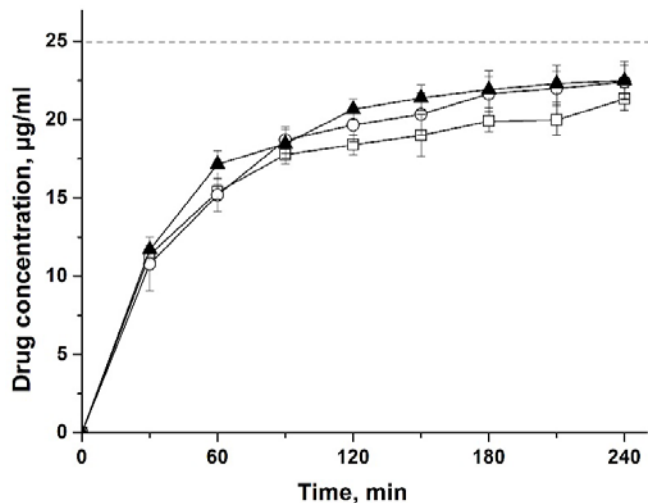
Media	Solubility
50 mM phosphate buffer pH 6.8 with 0.75% SLS	587.8 $\pm$ 31.1 $\mu$ g/ml
50 mM phosphate buffer pH 6.8	24.6 $\pm$ 0.8 $\mu$ g/ml
50 mM phosphate buffer pH 6.8 saturated with octanol	30.2 $\pm$ 0.2 $\mu$ g/ml
1-Octanol	29.8 $\pm$ 0.6 mg/ml

The solubility of racecadotril is 587.8  $\mu$ g/ml in phosphate buffer pH 6.8 with 0.75% SLS. A sample equivalent to 100 mg drug was set as sink conditions (< 20% of drug solubility in 900 ml medium). All three granule formulations had similar dissolution profiles in the conventional USP II dissolution test (Fig. 3.24). Although a surfactant was used in the dissolution medium to maintain sink conditions, it lacked discriminative power for these formulations.



**Fig. 3.24.** Dissolution profiles of racecadotril granules under sink conditions (900 mL phosphate buffer pH 6.8 containing 0.75% SLS): ( $\blacktriangle$ ) wet granulation with binder, ( $\circ$ ) wet granulation without binder, and ( $\square$ ) dry granulation (mean  $\pm$  SD; n = 3).

BCS II drugs have a slow dissolution under non-sink conditions in the GI tract, but rapidly permeate intestinal membranes. Previous studies demonstrated that drug release under non-sink conditions could potentially predict well *in vivo* performance (Gu et al., 2004; Tang et al., 2001). The small intestine is the primary site of absorption for racecadotril by oral administration (Singh and Narayan, 2008) and a volume of 500 ml is recommended to be of physiological relevance (Klein, 2010). Therefore, drug dissolution with the same dose (100 mg) was assessed in phosphate buffer pH 6.8 under non-sink conditions. Drug release only reached approx. 11% at 4 h, which was much lower compared to the dissolution under sink conditions. However, discrimination between dissolution profiles for three racecadotril granules was not observed under non-sink conditions as well as under sink conditions (Fig. 3.25). Moreover, their concentrations were close to the drug solubility in the medium. This indicated that racecadotril release from the three granules was limited by its solubility.

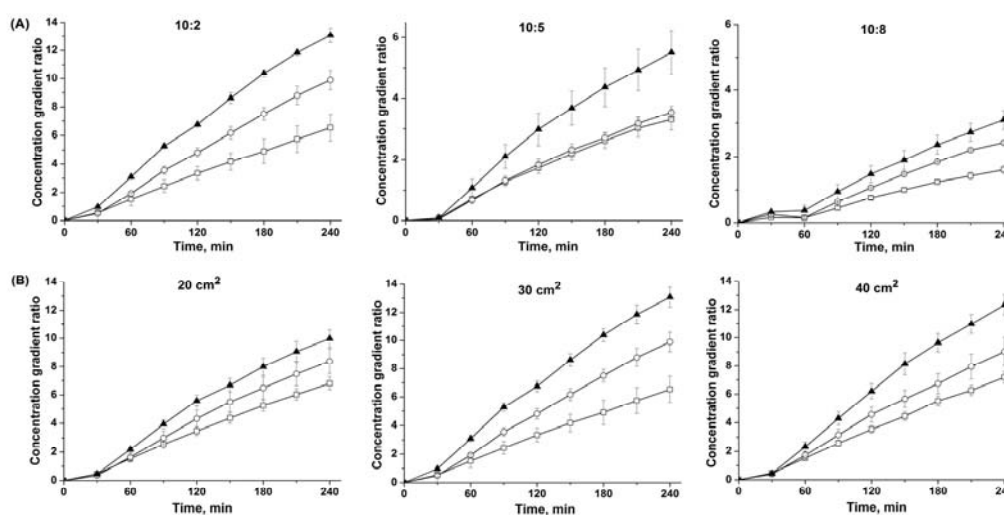


**Fig. 3.25.** Dissolution profiles of racecadotril granules under non-sink conditions (phosphate buffer pH 6.8) (dashed line represents the solubility of racecadotril): (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean  $\pm$  SD; n = 3).

#### 3.3.4. Biphasic dissolution test

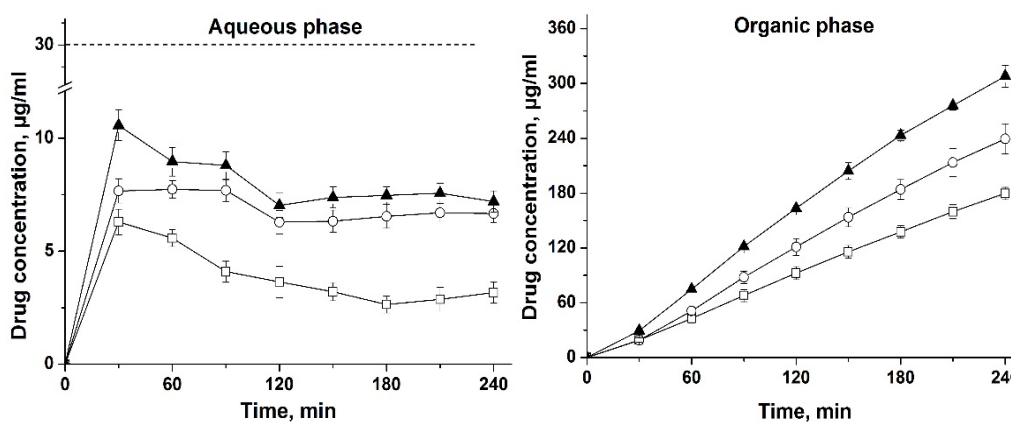
Various experimental parameters were investigated to achieve discrimination with the biphasic dissolution test for the three racecadotril granule formulations. The rotation speed was set at 150 rpm. A higher speed (200 rpm) resulted in a

vortex and emulsification at the interface disrupting the two-phase system, whereas a lower speed (100 rpm) generated insufficient hydrodynamics for drug partitioning. Additionally, the influence of different volume ratios and interfacial areas between the two phases was investigated. According to the Noyes-Whitney equation (Noyes and Whitney, 1897), a concentration gradient is considered as the main driving force for drug dissolution process. In a biphasic system two concentration gradients exist for a continuous dissolution-partition process. One gradient is between drug concentrations in the aqueous and organic phases, and the other one between drug dissolved and drug solubility in the aqueous buffer. Thus, the ratio of these two concentration gradients was used to assess the effect of different experimental parameters. A volume ratio of 10:2 and an interfacial area of 30 cm<sup>2</sup> generated the largest concentration changes for the three racecadotril granules (Fig. 3.26), indicating a pronounced discriminative power. The different effect caused by different parameters could be attributed to the changes in hydrodynamics and partition rates of the drug. Optimized parameters for the biphasic test were a rotation speed of 150 rpm, a volume ratio of 10:2 and an interfacial area of 30 cm<sup>2</sup>.



**Fig. 3.26.** The influence of (A) different volume ratios of aqueous and organic phases (constant interfacial area of 30 cm<sup>2</sup>) and (B) different interfacial areas between two phases (with a constant volume ratio of 10:2) on discriminating three granule formulations in the biphasic test: (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean±SD; n = 3).

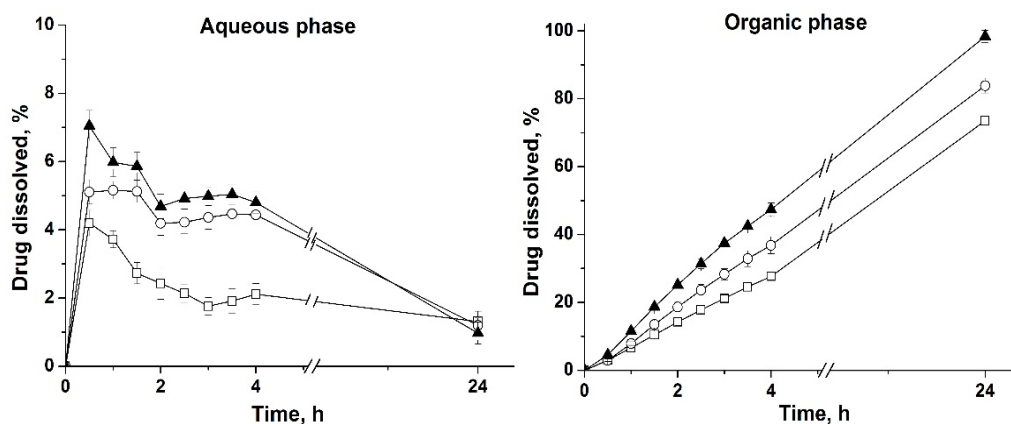
Each granule formulation (containing 15 mg drug) was evaluated with the optimized parameters in the biphasic test, which maintained sink conditions ( $< 10\%$  of drug solubility ( $C_s = 29.8 \text{ mg/ml}$ ) in 20 ml octanol). The three racecadotril granules showed slight differences in the dissolution in the aqueous phase. The drug concentrations of granules prepared by wet granulation with and without binder were approx.  $11 \text{ }\mu\text{g/ml}$  and  $7 \text{ }\mu\text{g/ml}$  respectively, and were reduced to  $6 \text{ }\mu\text{g/ml}$  at 4 h due to the continuous partitioning of drug from the aqueous to the organic phase. The concentration of granules prepared by dry granulation increased to  $6 \text{ }\mu\text{g/ml}$  and decreased to  $3 \text{ }\mu\text{g/ml}$ . In contrast, the corresponding concentration profiles in the organic phase differed with a ranking of granules prepared by wet granulation with binder  $>$  wet granulation without binder  $>$  dry granulation (Fig. 3.27) and the released drug concentrations reached around 310, 240 and  $180 \text{ }\mu\text{g/ml}$  at 4 h, respectively. The three racecadotril granules were thus discriminated well in the organic phase of the biphasic dissolution test.



**Fig. 3.27.** Dissolution profiles of racecadotril granules determined from the two phases in the biphasic dissolution test at 4 h (dashed line represents the solubility of racecadotril in phosphate buffer pH 6.8 saturated with octanol): (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean  $\pm$  SD;  $n = 3$ ).

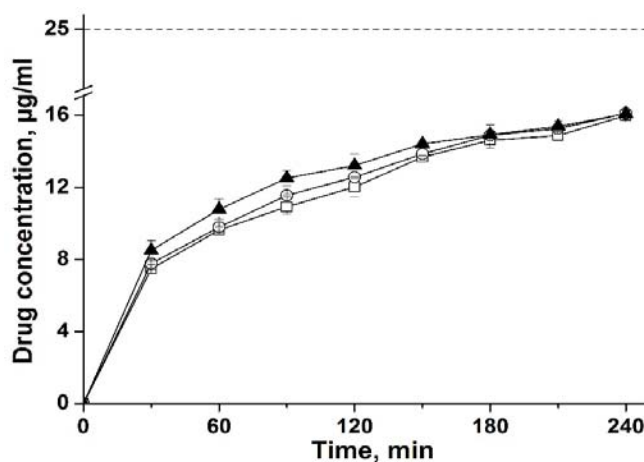
To investigate the mass balance of drug amount in the biphasic system, the biphasic dissolution test was further continued to 24 h. Concerning the total amount of racecadotril within both aqueous and organic phases, 100% release of racecadotril was reached from granules prepared by wet granulation with binder in accordance with the high solubility of racecadotril in octanol. However,

since the granules prepared by dry granulation and wet granulation without binder released slower, only around 75% and 85% were reached at 24 h, respectively (Fig. 3.28). This could be attributed to the different wettability of these granules.



**Fig. 3.28.** Dissolution profiles of racecadotril granules determined from the two phases in the biphasic dissolution test at 24 h: (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean  $\pm$  SD; n = 3).

The dissolution test was conducted in the same setup of the biphasic test but without octanol phase to identify the role of the organic phase. Similar release profiles of the three granules were identified (Fig. 3.29). This suggests that the discrimination of racecadotril granules in the biphasic test resulted from the presence of the organic phase.



**Fig. 3.29.** Dissolution profiles of racecadotril granules in the same setup of biphasic test without organic phase (dashed line represents the solubility of racecadotril in phosphate buffer pH 6.8): (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean  $\pm$  SD; n = 3).

The differences of dissolution behaviors observed in the biphasic test could be attributed to a different wettability of the granules which was caused by the different manufacturing techniques. The wettability of the granules was determined by contact angle measurements (Table 3.8). The contact angle of tablets prepared with granules formulated by dry granulation, wet granulation without or with binder was  $77.4 \pm 0.9$ ,  $70.4 \pm 0.9$  and  $65.4 \pm 0.9^\circ$  respectively, indicating a significant increase in wettability from wettability from granules prepared by dry granulation to granules prepared by wet granulation with binder ( $p < 0.05$ ). The results suggested that wet granulation resulted in better wettability than dry granulation, and binders further improved wettability. The hydrophobic drug was exposed on the surface of the granule made by dry granulation, whereas diluents/binders partially coated the drug during wet granulation thus improving its wettability. Lycatab PGS, a pregelatinized starch, functions as a binder to aid in interparticle bond formation during granulation. This binder improved drug dissolution by forming a hydrophilic layer on the surface of the water-insoluble drug particles.

**Table 3.8.** Contact angle values of pH 6.8 phosphate buffer on compacts prepared from three different granules (mean  $\pm$  SD; n = 4).

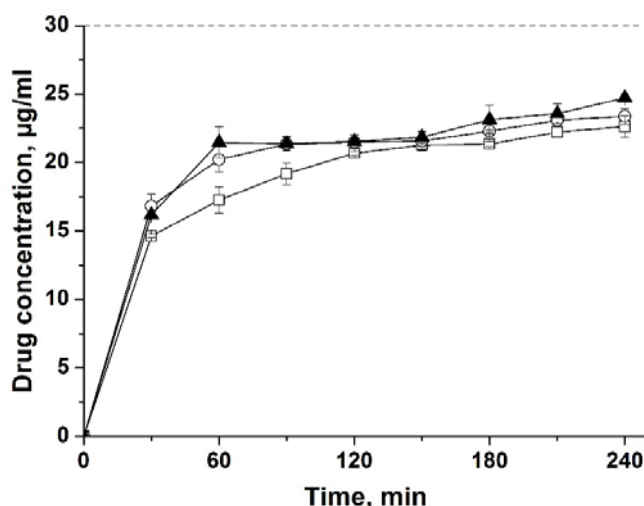
Granule formulation	Contact angle ( $^\circ$ )
Dry granulation	$77.44 \pm 0.87^b$
Wet granulation without binder	$70.38 \pm 0.90^{a,b}$
Wet granulation with binder	$65.44 \pm 0.86^a$

a  $p < 0.05$  vs. granules prepared by dry granulation; b  $p < 0.05$  vs. granules prepared by wet granulation with binder.

Similar results were observed in previous studies (Cai et al., 2013; Juang and Storey, 2003; Zhang et al., 2001). Tablets made by wet granulation released faster than tablets made by dry granulation (Juang and Storey, 2003; Zhang et al., 2001). Cai et al. demonstrated granules with a polymeric binder of poorly soluble drugs dissolved faster than the respective granules without binder (Cai et al., 2013). This is due to the fact that the granule properties were affected by

different solid bridges formed with or without a binder. The interparticle bridges for granules without binder were formed by drug dissolved during granulation, so drug solubility dominated granule strength.

In this study, different release behaviors of the three granules observed in the different dissolution systems could be attributed to the substantially different wetting conditions. Single phase dissolution tests under sink and non-sink conditions lacked the discrimination, which may result from undetected differences in wettability for these granule formulations by the presence of a wetting surfactant or the absence of surfactant, respectively. However, in the biphasic test, minor amounts of octanol dissolved in the aqueous phase acting as cosolvents could potentially contribute to the wetting process. Slightly higher drug dissolution was observed initially in the single phase of phosphate buffer pH 6.8 saturated with octanol compared to the blank phosphate buffer pH 6.8 (approx. 16  $\mu\text{g/ml}$  vs. 11  $\mu\text{g/ml}$  at 30 min, and 20  $\mu\text{g/ml}$  vs. 15  $\mu\text{g/ml}$  at 60 min), although no discrimination of the release profiles of three granules was observed (Fig. 3.30). Furthermore, derived from a continuous concentration gradient between two phases, the differences of dissolution kinetics induced by wettability in the aqueous buffer could be magnified by the presence of organic phase following the dynamic processes of dissolution and partition. Thus, more drug dissolved in the aqueous phase resulted in more drug partitioning into the organic phase, as shown by different partition rates with a rank order of granules prepared by dry granulation < wet granulation without binder < wet granulation with binder ( $p < 0.05$ ) (Table 3.9).



**Fig. 3.30.** Dissolution profiles of racecadotril granules under non-sink conditions in USP II apparatus (phosphate buffer pH 6.8 saturated with octanol) (dashed line represents the solubility of racecadotril): (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean  $\pm$  SD;  $n = 3$ ).

**Table 3.9.** Partition rates of racecadotril into the organic phase (mean  $\pm$  SD;  $n = 3$ ).

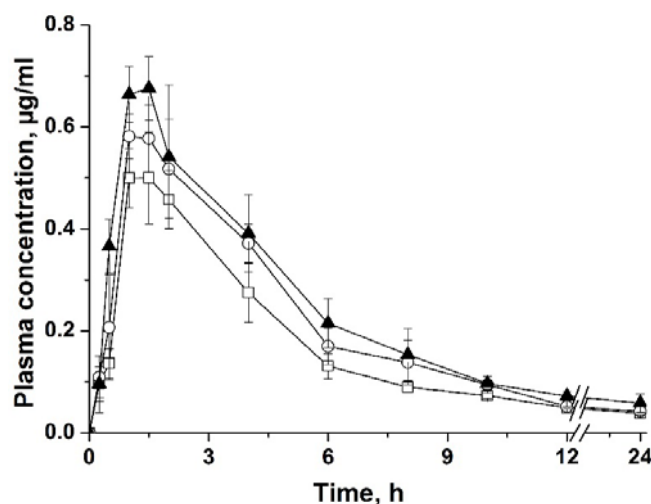
Granule formulation	Partition rate (mg/h)
Dry granulation	$0.92 \pm 0.02^b$
Wet granulation without binder	$1.27 \pm 0.08^{a,b}$
Wet granulation with binder	$1.60 \pm 0.06^a$

$a p < 0.05$  vs. granules prepared by dry granulation;  $b p < 0.05$  vs. granules prepared by wet granulation with binder.

### 3.3.5. *In vivo* studies

To assess *in vivo* PK performance of three racecadotril granules, a non-crossover study in rats was conducted. The plasma concentration-time profiles are shown in Fig. 3.31, and the PK parameters in Table 3.10. Noticeable differences in the  $AUC_{0-24h}$  values of the three racecadotril granules were observed ( $p < 0.05$ ). The rank order of release observed in the biphasic dissolution test correlated with the AUC values of granules prepared dry granulation, wet granulation without and with binder. The same rank order was observed with the mean  $C_{max}$  values. The lowest AUC and  $C_{max}$  for granules prepared by dry granulation probably resulted from poor wettability, since high wettability could improve drug dissolution and prevent particle aggregation when exposed to the gastrointestinal medium.





**Fig. 3.31.** Plasma concentration-time profiles of three racecadotril granules in rats after oral administration: (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean  $\pm$  SD;  $n = 4$ ).

**Table 3.10.** Pharmacokinetic parameters for racecadotril granules in rats after oral administration (dose of 30 mg/kg) (mean  $\pm$  SD;  $n = 4$ ).

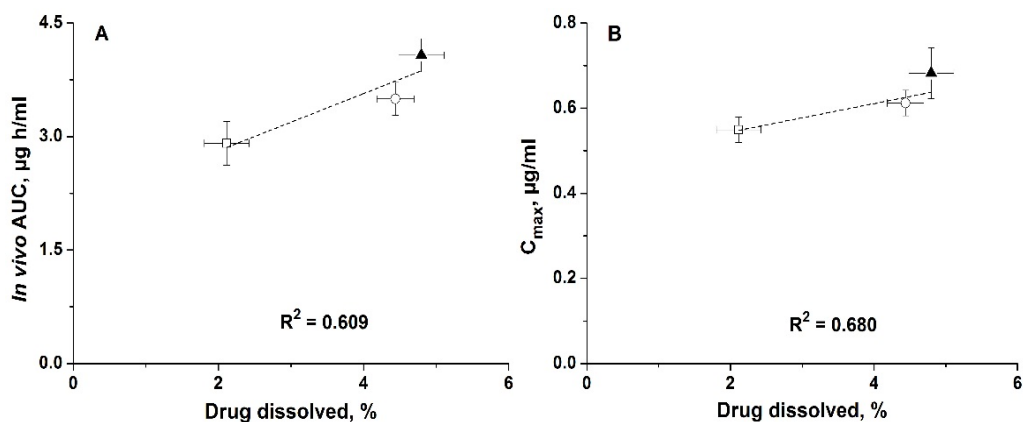
Granule formulation	AUC <sub>0-24h</sub> ( $\mu\text{g h/mL}$ )	C <sub>max</sub> ( $\mu\text{g/mL}$ )	T <sub>max</sub> (h)
Dry granulation	2.85 $\pm$ 0.29 <sup>b</sup>	0.56 $\pm$ 0.03 <sup>b</sup>	1.17 $\pm$ 0.29
Wet granulation without binder	3.50 $\pm$ 0.22 <sup>ab</sup>	0.61 $\pm$ 0.03	1.33 $\pm$ 0.28
Wet granulation with binder	4.07 $\pm$ 0.22 <sup>a</sup>	0.68 $\pm$ 0.06	1.33 $\pm$ 0.28

a  $p < 0.05$  vs. granules prepared by dry granulation; b  $p < 0.05$  vs. granules prepared by wet granulation with binder.

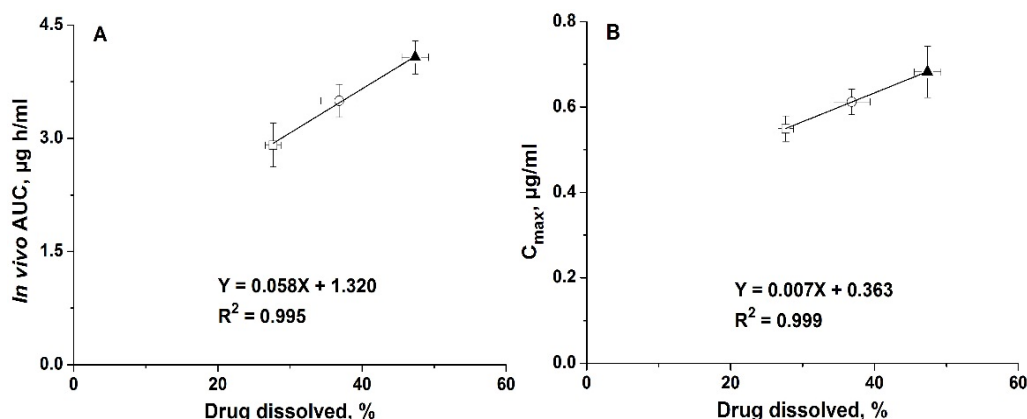
### 3.3.6. *In vitro-in vivo* correlation

Three racecadotril granules were discriminated by the biphasic dissolution test as well as by the *in vivo* performance. Next, the potential of establishing a relationship between the *in vitro* and *in vivo* release was explored. A level C IVIVC was tested using the percent dissolved in both aqueous and organic phases in the biphasic test at 4 h vs. an *in vivo* parameter (AUC or C<sub>max</sub>). No meaningful IVIVC was obtained between percent dissolved in the aqueous phase at 4 h and AUC<sub>0-24h</sub> or C<sub>max</sub> (Fig. 3.32). However, excellent linear relationships between the percent dissolved in the organic phase at 4 h and *in vivo* AUC<sub>0-24h</sub> ( $R^2 = 0.999$ ) (Fig. 3.33A) and *in vivo* C<sub>max</sub> ( $R^2 = 0.999$ ) (Fig. 3.33B) were obtained. This indicates that the biphasic dissolution system could sensitively discriminate similar dosage forms with only minor differences and the

release profiles from the organic phase could serve as an indicator for *in vivo* drug absorption.



**Fig. 3.32.** Relationship of *in vitro* % dissolved in the aqueous phase of the biphasic test at 4 h plotted against (A) *in vivo* AUC values or (B)  $C_{max}$ : (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean±SD; n = 3 for *in vitro* biphasic dissolution test, n = 4 for *in vivo* rat studies).



**Fig. 3.33.** Relationship of *in vitro* % dissolved in the organic phase of the biphasic test at 4 h plotted against (A) *in vivo* AUC values or (B)  $C_{max}$ : (▲) wet granulation with binder, (○) wet granulation without binder, and (□) dry granulation (mean±SD; n = 3 for *in vitro* biphasic dissolution test, n = 4 for *in vivo* rat studies).

Biphasic dissolution tests have been used to investigate different types of dosage forms. However, there is no publication to discriminate between same dosage forms with only minor formulation and process changes. A relationship between *in vitro* dissolution and *in vivo* absorption is likely to be obtained for BCS II drugs when *in vitro* dissolution could reflect the *in vivo* rate-limiting process. Thereby, if the release mechanism behind the effect on drug dissolution

is known, it is expected to predict *in vivo* performance based on *in vitro* behaviors concerning formulation/manufacturing factors having great influence for the *in vivo* dissolution (Dickinson et al., 2008). More recently, work by Mudie et al. has provided further insight into the mechanistic analysis of the kinetics of drug partitioning from the aqueous to the organic phase of a two-phase dissolution apparatus (Mudie et al., 2012). In this study, three differently formulated racecadotril granules were discriminated in the biphasic system by correlating their different wettability and their different performances in *in vivo* studies.

### **3.3.7. Conclusions**

Different dissolution systems providing different dissolution conditions (sink or non-sink) and different wetting environments were investigated to evaluate racecadotril release from three differently formulated granules. Similar release profiles of the three granules were observed in the single phase dissolution tests under sink and non-sink conditions. In contrast, the biphasic dissolution system had sensitive discriminative power for these different formulations with respect to different wettability induced by various process variables. An excellent linear correlation between *in vitro* release obtained from the organic phase of biphasic test and *in vivo* data was obtained. This biphasic system showed superiority to single phase dissolution tests by capturing the minor differences of dissolution kinetics when the same dosage forms are studied. This work demonstrates that the biphasic model is a good tool to discriminate between only minor formulation and process changes during early development of formulations with BCS II drugs.

### **3.4. Evaluation of a discriminative biphasic dissolution test for estimating the bioavailability of itraconazole amorphous solid dispersions prepared with different polymers**

#### **3.4.1. Introduction**

Poor aqueous solubility and slow dissolution rate of APIs have become one of the biggest challenges in the pharmaceutical development due to the dramatic increase of poorly water-soluble drug candidates emerging in combinatorial chemistry and high throughput screening (Vo et al., 2013). Solid dispersions are one of the most promising strategies to develop such drug substances. The use of solid dispersions has many advantages in improving drug release such as reduced particle size (Leuner and Dressman, 2000), increased wettability (Ghebremeskel et al., 2007; Karavas et al., 2006), higher porosity (Ghaderi et al., 1999), and drug amorphization (Lloyd et al., 1999; Taylor and Zografi, 1997). Melting and solvent evaporation are two major preparation methods for solid dispersions. The techniques as hot-melt extrusion, melt agglomeration, spray drying, vacuum drying, freeze drying, electrostatic spinning, and co-precipitation are often used (Vasconcelos et al., 2007; Vo et al., 2013).

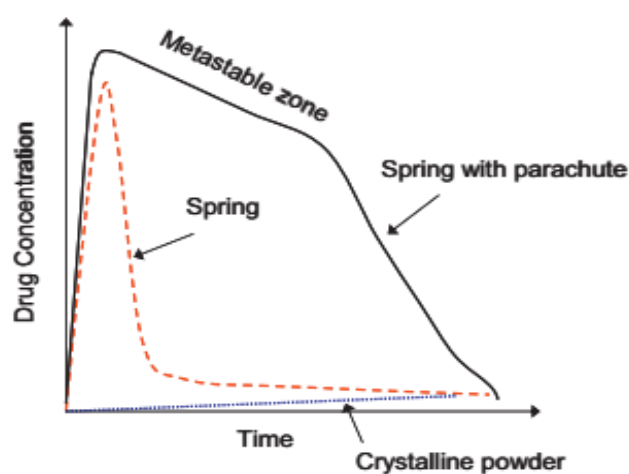
The concept of generation and maintenance of metastable supersaturated state was described as “spring and parachute approach” (Fig. 3.34) (Guzmán et al., 2007). A thermodynamically unstable, supersaturated drug solution is usually generated from a higher energy form of the drug (“spring”). A variety of formulation strategies may induce the generation of supersaturated solutions, including cosolvent systems, lipid-based formulations, cocrystals, salt forms, and amorphous forms. To benefit from the supersaturated concentration for increasing drug absorption, an extension of supersaturation and precipitation inhibition should be considered (“parachute”). Polymers have been used to inhibit or retard such precipitation by hydrogen bonding, hydrophobic interactions and polymer rigidity, polymer molecular weight and steric hindrance, and increased solution viscosity (Xu and Dai, 2013). *In vitro* evaluation of

supersaturation involves determination of drug concentration expressed relative to the equilibrium solubility as the degree of supersaturation ( $DS$ ) (Eq. 3.4) or the supersaturation index ( $\sigma$ ) (Eq. 3.5).

$$DS = \frac{C}{C_{eq}} \quad (3.4)$$

$$\sigma = DS - 1 = \frac{C - C_{eq}}{C_{eq}} \quad (3.5)$$

where  $C$  is the drug concentration in the dissolution medium at time  $t$ , and  $C_{eq}$  is the equilibrium solubility of the drug in the test medium. The saturation extent is assessed as subsaturated ( $DS < 1$ ,  $\sigma < 0$ ), saturated ( $DS = 1$ ,  $\sigma = 0$ ) and supersaturated ( $DS > 1$ ,  $\sigma > 0$ ) (Bevernage et al., 2013a; Brouwers et al., 2009).



**Fig. 3.34.** Schematic drug concentration-time profiles illustrating the spring and parachute approach of supersaturating drug delivery systems (Xu and Dai, 2013).

Next to the essential physicochemical characterization of solid dispersions, *in vitro* dissolution testing is crucial to evaluate the pharmaceutical performance. However, poor *in vitro-in vivo* correlations have been caused widespread attention (Augustijns and Brewster, 2012; Newman et al., 2012; Van den Mooter, 2012). Sarnes et al. prepared solid nanocrystal formulations of itraconazole by

freeze drying and granulation and compared with commercial product (Sporanox<sup>®</sup>) by performing *in vitro* and *in vivo* studies (Sarnes et al., 2014). Freeze dried nanocrystalline powders had significantly faster release than Sporanox<sup>®</sup> *in vitro*. However, the opposite performance was observed in *in vivo* absorption profiles of these two formulations. Qian et al. investigated amorphous solid dispersions of BMS-A prepared with PVPVA and HPMCAS by spray drying and evaluated their *in vitro* dissolution and *in vivo* performance (Qian et al., 2012). Higher drug release was achieved from the PVPVA dispersion in *in vitro* dissolution test, whereas higher oral bioavailability from HPMCAS dispersion was observed from *in vivo* studies. This finding was attributed to BMS-A recrystallization within the undissolved dispersion due to hydrophilicity and faster PVPVA dissolution rate.

Therefore, a selection of a suitable dissolution test to evaluate *in vitro* dissolution of solid dispersions is crucial to obtain a correlation with *in vivo* performance. Biphasic dissolution test could be a potential test for drug release study from solid dispersions. Briefly, drug first dissolves in the aqueous medium and partitions into the immiscible organic phase mimicking drug dissolution and absorption processes in the GI tract. Several studies reported that the biphasic dissolution test could potentially discriminate different supersaturation formulations (Pestieau et al., 2016; Sarode et al., 2014; Thiry et al., 2016). Sarode et al. investigated different amorphous solid dispersions containing felodipine and itraconazole prepared by hot melt mixing using various polymers and evaluated their release behaviors in the single and biphasic dissolution tests (Sarode et al., 2014). The influence of polymers on the dissolution, supersaturation and crystallization as well as the implication for oral bioavailability were reflected in the partitioning performance of drug substances in the biphasic test. Three different fenofibrate formulations (pure API, a micronized formulation and a self-emulsifying lipid-based formulation) were evaluated by different dissolution tests (sink, non-sink and biorelevant) (Pestieau et al., 2016). The biphasic test was considered as the most suitable *in vitro*

evaluation tool for the development of a future fenofibrate lipid-based formulations. Thiry et al. investigated different dissolution systems (different USP apparatuses and media) to test three itraconazole formulations (pure drug, extrudates prepared with Soluplus<sup>®</sup>, and commercial product Sporanox<sup>®</sup>) (Thiry et al., 2016). Only the test in acidic medium under non-sink conditions and the biphasic test showed a ranking was consistent with the reported *in vivo* performance. Although the indirect correlations with *in vivo* performance were observed in aforementioned studies, there is still a lack in studies to discriminate different solid dispersions and establish a relationship between *in vitro* and *in vivo* data.

The objective of this study was to evaluate the potential of the biphasic dissolution model to discriminate between different amorphous solid dispersions and to correlate the result with the published *in vivo* data. Itraconazole (Fig. 3.35), a weakly basic ( $pK_a = 3.7$ ) and highly lipophilic ( $\log P = 6.2$ ), has poor oral bioavailability due to poor aqueous solubility at  $\sim 4 \mu\text{g/ml}$  at pH 1 and  $\sim 1 \text{ ng/ml}$  at neutral pH (DiNunzio et al., 2008; Peeters et al., 2002). Many studies reported that solid dispersions significantly improved oral bioavailability of itraconazole (DiNunzio et al., 2008; Miller et al., 2008c; Miller et al., 2007; Piao et al., 2014). In particular, Six et al. reported that three solid dispersions of itraconazole were prepared with Eudragit<sup>®</sup> EPO, a mixture of Eudragit<sup>®</sup> EPO and PVPVA 64, and HPMC by hot-melt extrusion (Six et al., 2005). The oral bioavailability followed a ranking of HPMC > Eudragit<sup>®</sup> EPO > Eudragit<sup>®</sup> EPO-PVPVA 64, but the *in vitro* dissolution profiles were not consistent with *in vivo* performance. Interestingly, these three polymers have various backbones such as methacrylate (Eudragit<sup>®</sup> EPO), vinyl (PVPVA 64), and cellulose (HPMC) as well as different ionic properties. Eudragit<sup>®</sup> EPO is cationic, while PVPVA 64 and HPMC are non-ionic (Fig. 3.36). Therefore, these three itraconazole formulations were selected in this study to assess the discriminative power of different dissolution tests and to establish their correlation with the reported *in vivo* performance.

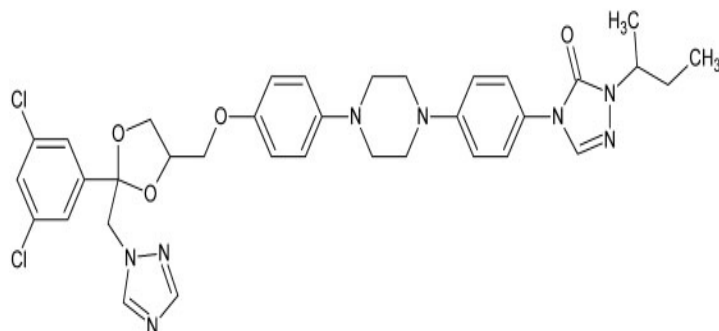


Fig. 3.35. Chemical structure of itraconazole.

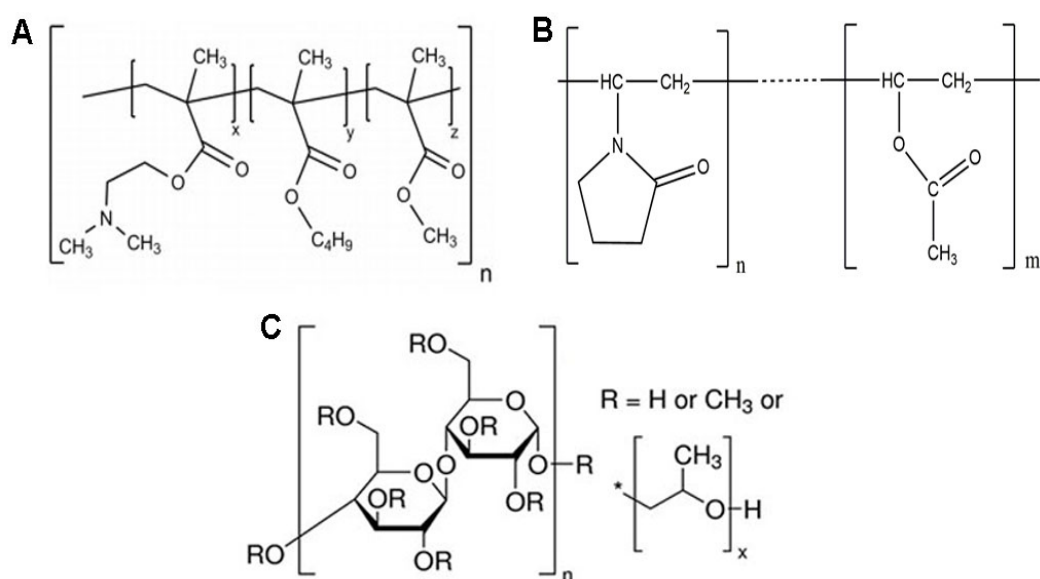
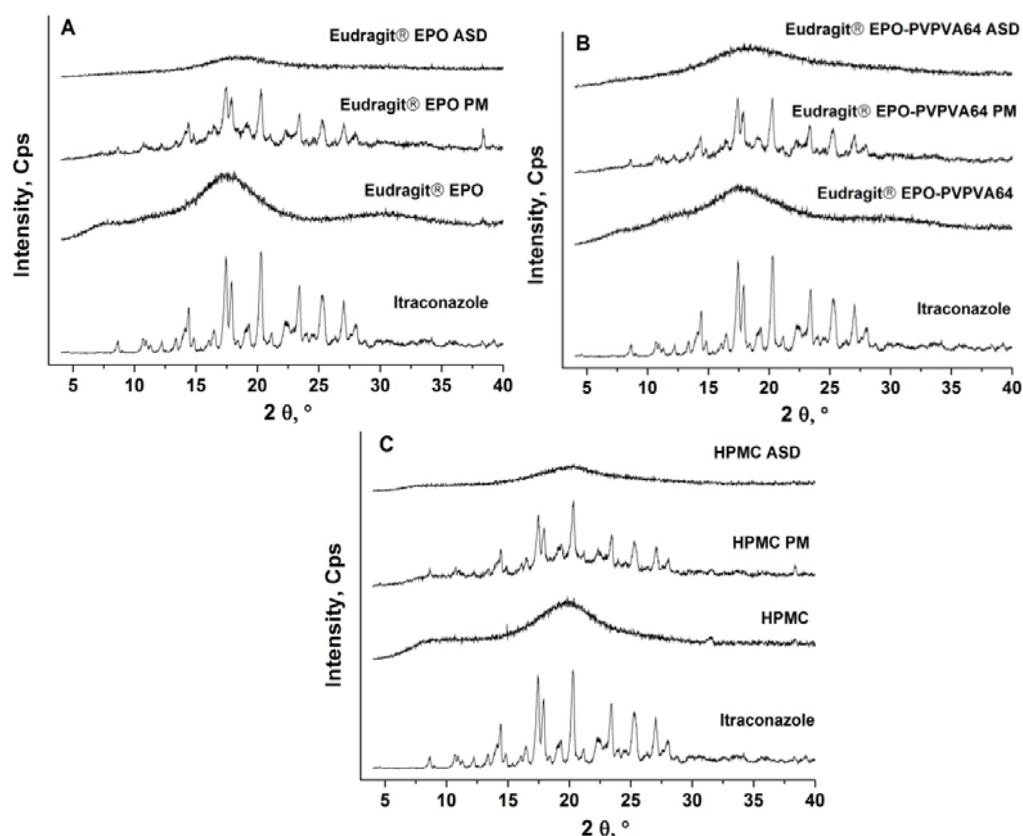


Fig. 3.36. Chemical structures of different polymers: (A) Eudragit® EPO, (B) PVPVA 64, and (C) HPMC.

### 3.4.2. Physical characterization of itraconazole formulations

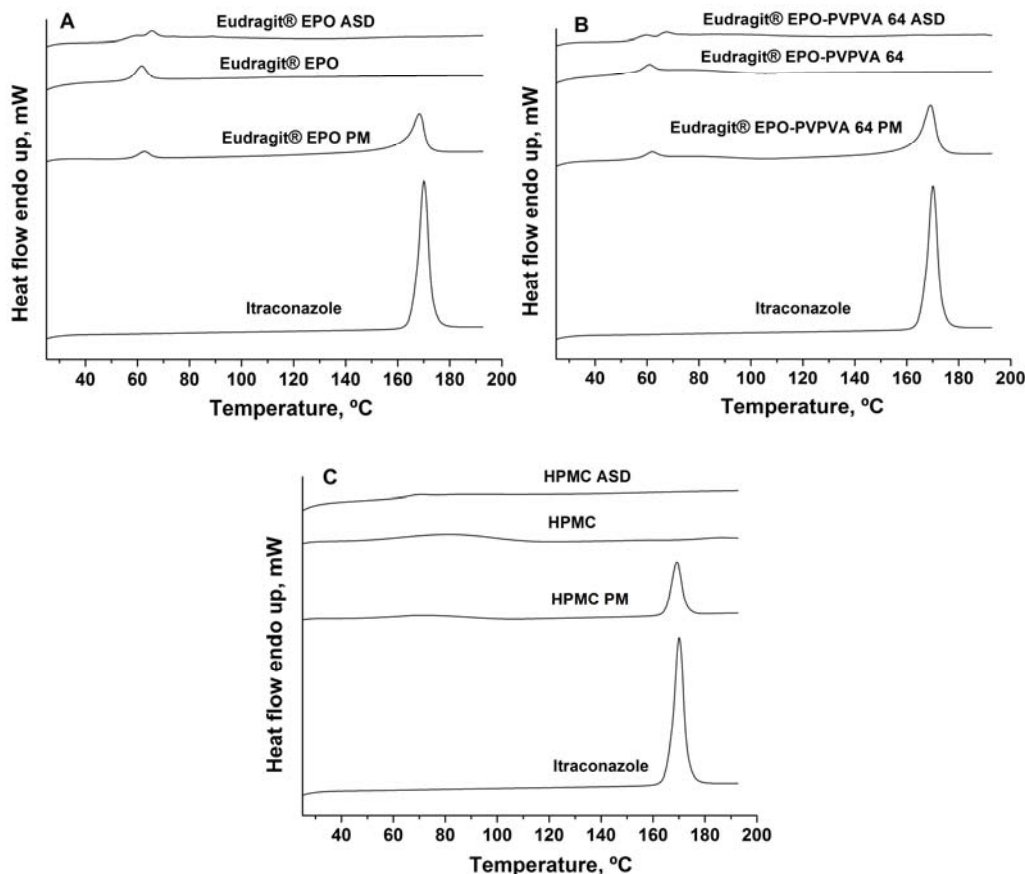
To evaluate the solid state of itraconazole formulations, XRPD was performed. The diffraction profile of itraconazole showed characteristic crystalline peaks at  $2\theta = 14.4, 17.4, 20.3, 23.4$  and  $25.3^\circ$  as well as in physical mixtures with polymers, while the milled solid dispersions exhibited amorphous characteristic with the absence of crystalline peaks (Fig. 3.37).





**Fig. 3.37.** X-ray powder diffraction patterns of pure drug, polymers, physical mixtures (PM) and amorphous solid dispersions (ASD): (A) Eudragit® EPO, (B) Eudragit® EPO-PVPVA 64, and (C) HPMC.

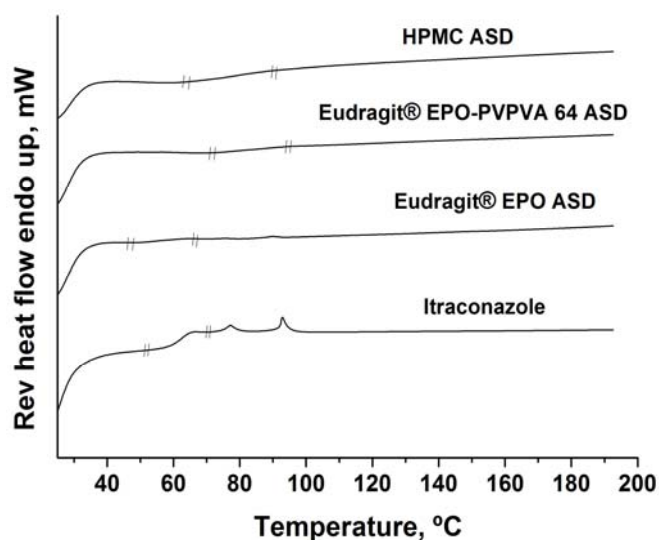
The DSC thermograms of pure drug and physical mixtures showed an endothermic melting peak of crystalline itraconazole at 170 °C, but it was not observed in the solid dispersions with Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC (Fig. 3.38). DSC analysis suggested these three solid dispersions were amorphous and further confirmed the results obtained by XRPD.



**Fig. 3.38.** DSC thermograms of pure drug, polymers, physical mixtures and amorphous solid dispersions: (A) Eudragit® EPO, (B) Eudragit® EPO-PVPVA 64, and (C) HPMC.

The miscibility between drug and different polymers in solid dispersions was assessed in the reversing heat flow, where more than a single  $T_g$  reflects immiscibility. Amorphous itraconazole formed by crystalline drug during analysis exhibited a glass transition at 60 °C and two endothermic peaks at 77 and 92 °C in the profile of reversing heat flow, respectively (Fig. 3.39). The appearance of two endothermic transitions represented the formation of a mesophase (Six et al., 2001). The thermogram of the solid dispersions with Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC showed a single  $T_g$  at 56, 84 and 73 °C respectively, indicating that itraconazole was miscible with each polymer. However, these results were not completely consistent with previous studies. Phase separation was observed in itraconazole/Eudragit® EPO and itraconazole/Eudragit® EPO-PVPVA 64 solid dispersions (Six et al., 2003b; Six et al., 2004). The modulated-temperature differential scanning calorimetry

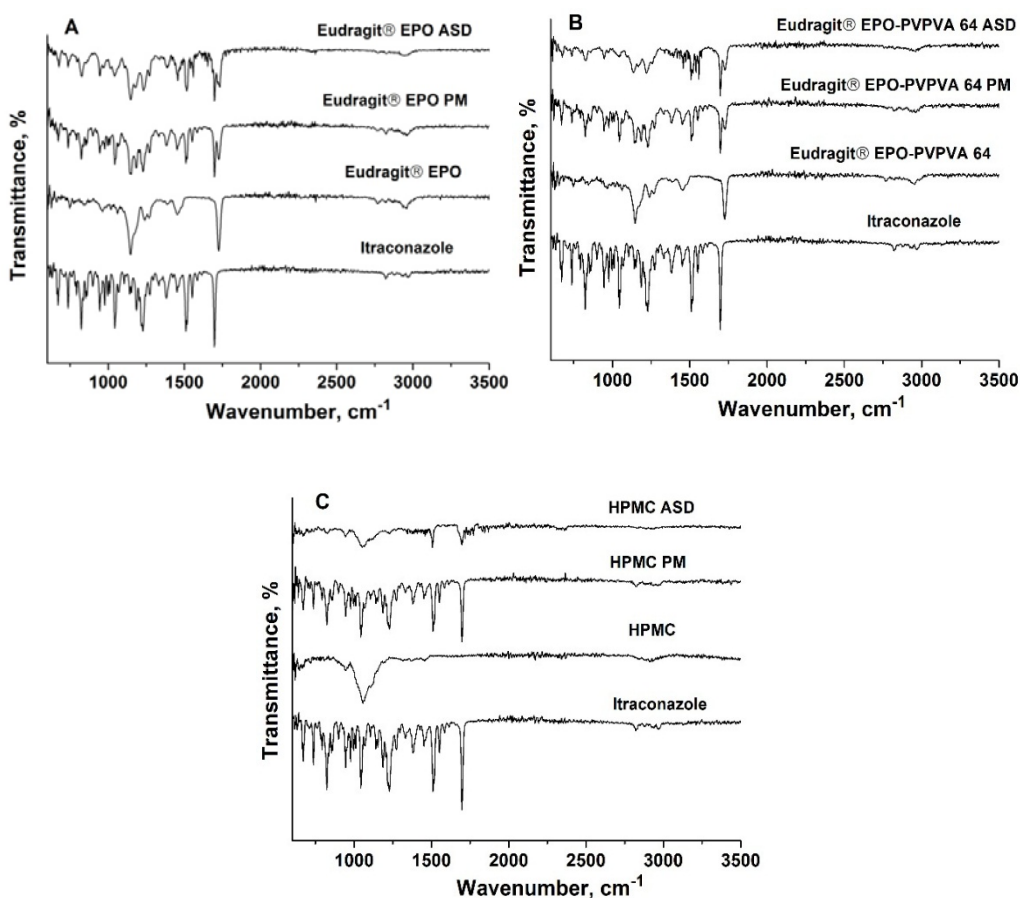
(MTDSC) revealed one  $T_g$  of glassy itraconazole at 59 °C and one originating from the drug-polymer mixture at 46 °C in itraconazole/Eudragit® EPO formulation, while in itraconazole/Eudragit® EPO-PVPVA 64 solid dispersions two  $T_g$ s at 48 and 80 °C were observed for an itraconazole-Eudragit® EPO phase and an itraconazole-PVPVA 64 phase, respectively. This discrepancy could be explained by different methods used to analyze the solid dispersions. The MTDSC is generally more sensitive to distinguish the consecutive glass transitions compared to the conventional DSC mode used in this study. For instance, Eudragit® EPO and itraconazole had very close glass transition temperatures (54 vs. 59 °C), which complicated the detection of phase separation (Janssens et al., 2010). Another probable reason resulted from different preparation methods for these solid dispersions (solvent casting vs. hot-melt extrusion).



**Fig. 3.39.** DSC analysis of crystalline itraconazole and different amorphous solid dispersions.

To investigate the potential interactions between drug and polymers, FTIR analysis was performed on the pure drug, physical mixtures and solid dispersions. The FTIR spectrum of itraconazole exhibited the characteristic peaks at 1383  $\text{cm}^{-1}$  (C-N stretch), 1450  $\text{cm}^{-1}$  and 1510  $\text{cm}^{-1}$  (aromatic C=C stretch), and 1697  $\text{cm}^{-1}$  (C=O stretch), while the polymers showed different FTIR

spectra (Fig. 3.40). The FTIR spectra of the physical mixtures as well as of the solid dispersions with Eudragit® EPO and Eudragit® EPO-PVPVA 64 showed no change in the characteristic peaks of itraconazole, indicating no interaction between itraconazole and each polymer (Figs. 3.40A and B). The lack of molecular interaction between drug and polymers could be explained by the fact that both, polymer and drug, are hydrogen acceptors (Janssens et al., 2010; Song et al., 2013). However, a strong decrease in the intensities of C=O, C=C, and C-N bonds and a broadening of the C=O stretch were observed in the FTIR spectrum of HPMC solid dispersion (Fig. 3.40C), suggesting the occurrence of intermolecular interactions between itraconazole and HPMC. HPMC with numerous hydrogen bond donors (i.e., free hydroxyl groups) would interact with multiple itraconazole hydrogen bond acceptors (Miller et al., 2008a).



**Fig. 3.40.** FTIR spectra of drug, polymers, physical mixtures and amorphous solid dispersions: (A) Eudragit® EPO, (B) Eudragit® EPO-PVPVA 64, and (C) HPMC.

### 3.4.3. Solubility of itraconazole

The solubility of crystalline itraconazole was determined in different dissolution media at 37 °C (Table 3.11). The solubility of itraconazole in 0.1 N HCl was 7.3 µg/ml. With increasing pH, itraconazole solubility in gastric buffer pH 2.0 and phosphate buffer pH 6.8 were not detected by UV spectroscopy, while it increased to 2.2 µg/ml in gastric buffer pH 2.0 saturated with decanol due to the cosolvent property of small amount of decanol dissolved. Additionally, the solubility of itraconazole was substantially enhanced to 566.2 µg/ml and 259.4 µg/ml in 0.1 N HCl with 0.7% SLS and decanol, respectively.

**Table 3.11.** Solubility of itraconazole in different dissolution media (mean ± SD; n = 3).

Media	Solubility (µg/ml)
0.1 N HCl	7.3 ± 0.3
0.1 N HCl with 0.7% w/v SLS	566.2 ± 5.7
Gastric buffer pH 2.0	ND
Gastric buffer pH 2.0 saturated with decanol	2.2 ± 0.2
Phosphate buffer pH 6.8	ND
Decanol	259.4 ± 3.8

ND, Not detected by UV.

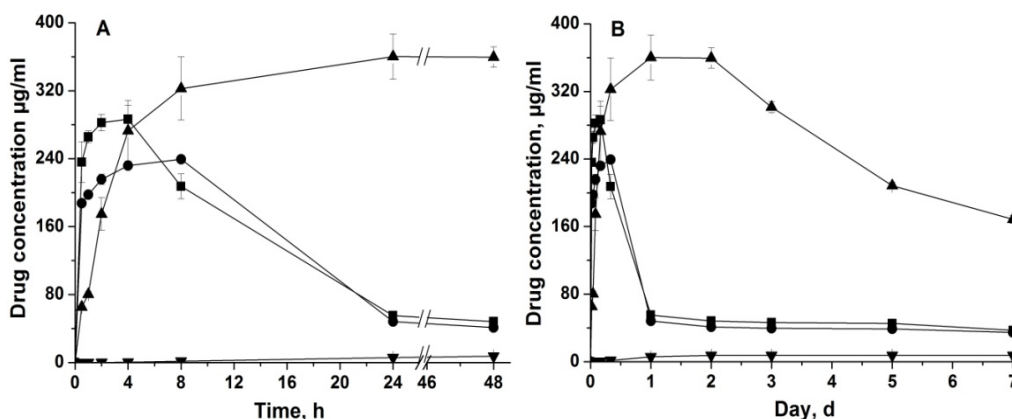
To examine the solubilizing power of Eudragit® EPO, Eudragit® EPO- PVPVA 64 and HPMC, drug solubility in 0.1 N HCl containing 0.3 mg/ml of each polymer increased approx. 1.5, 1.5 and 1.8 times respectively compared to the drug solubility in 0.1 N HCl (Table 3.12). Polymer concentration of 0.3 mg/ml was selected to be identical to those used in the acidic dissolution test under non-sink conditions. The solubility of physical mixtures in 0.1 N HCl increased 1.4-1.7 times compared to the pure drug, and was similar to the ones determined in 0.1 N HCl with 0.3 mg/ml polymer concentration.

## Results and discussion

**Table 3.12.** Solubility of itraconazole in 0.3 mg/ml polymer solutions (w/v) and physical mixtures in 0.1 N HCl at 37 °C (mean  $\pm$  SD; n = 3).

Sample	Solubility ( $\mu\text{g/ml}$ )
<i>Polymer concentration of 0.3 mg/ml</i>	
Eudragit <sup>®</sup> EPO	11.2 $\pm$ 0.7
Eudragit <sup>®</sup> EPO-PVPVA 64	10.8 $\pm$ 0.4
HPMC	13.4 $\pm$ 0.3
<i>Physical mixtures</i>	
Eudragit <sup>®</sup> EPO	10.0 $\pm$ 0.2
Eudragit <sup>®</sup> EPO- PVPVA 64	10.3 $\pm$ 0.1
HPMC	12.4 $\pm$ 0.1

To investigate supersaturation behavior of itraconazole solid dispersions, kinetic solubility was determined. The solid dispersion with Eudragit<sup>®</sup> EPO had the fastest drug release initially, reaching the maximum drug concentration of 286.3  $\mu\text{g/ml}$  at 4 h followed by a dramatic decrease in drug concentration to 48.2  $\mu\text{g/ml}$  at 48 h (Fig. 3.41A). Eudragit<sup>®</sup> EPO-PVPVA 64 had a similar profile reaching maximum concentration of 239.3  $\mu\text{g/ml}$  at 8 h following by a remarked reduction to 41.2  $\mu\text{g/ml}$  at 48 h. In contrast, HPMC exhibited the slowest release within the first 2 h, reaching the maximum drug concentration of 360.1  $\mu\text{g/ml}$  at 24 h following a constant supersaturation up to 48 h. The maximum degree of supersaturation reached approx. 49-, 39-, and 33-fold equilibrium solubility of crystalline itraconazole in 0.1 N HCl for HPMC, Eudragit<sup>®</sup> EPO and Eudragit<sup>®</sup> EPO-PVPVA 64 dispersions, respectively. Moreover, HPMC showed the best performance for supersaturation stabilization with a gradual decrease to 168.4  $\mu\text{g/ml}$  at 7 d compared to the other two formulations (Fig. 3.41B). It could be attributed to different mechanisms of stabilization of supersaturated solutions derived from different properties and backbone structures of polymers.

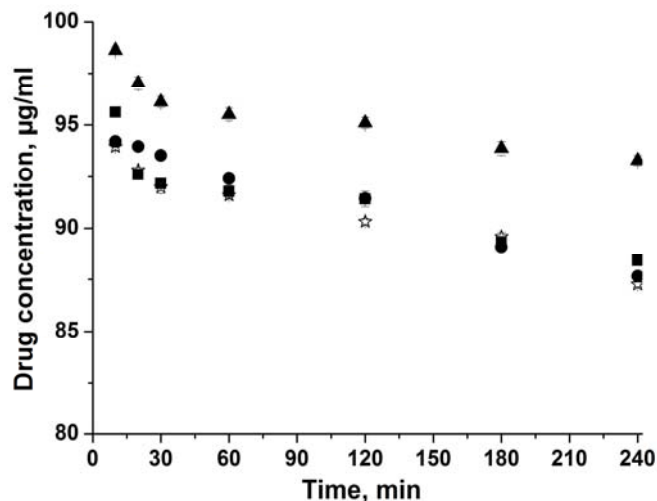


**Fig. 3.41.** Kinetic solubility profiles of itraconazole solid dispersions in 0.1 N HCl for (A) up to 48 h and (B) up to 7 d: (■) Eudragit® EPO, (●) Eudragit® EPO-PVPVA 64, (▲) HPMC, and (▼) crystalline drug (mean  $\pm$  SD; n = 3).

### 3.4.4. Effect of different polymers on supersaturation stabilization and precipitation inhibition

#### 3.4.4.1. Evaluation of supersaturation stabilization and precipitation inhibition by solvent shift method

To evaluate the ability of Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC to inhibit precipitation of itraconazole from a supersaturated solution, the solvent shift method in 0.1 N HCl was applied. Polymers were predissolved in the medium at the concentration of 0.3 mg/ml, identical to the one produced by total dissolution of solid dispersions in the acidic non-sink dissolution test, followed by addition of itraconazole stock solution in DMSO corresponding to an initial degree of superstation around 14 (100 µg/ml). No significant difference was observed between these polymer solutions for anti-precipitation performance in 0.1 N HCl, achieving 93, 89 and 88 µg/ml at 4 h for HPMC, Eudragit® EPO-PVPVA 64 and Eudragit® EPO, respectively (Fig. 3.42). Moreover, the result obtained in 0.1 N HCl without any polymer seemed to be unfavorable since high degree of supersaturation maintained.



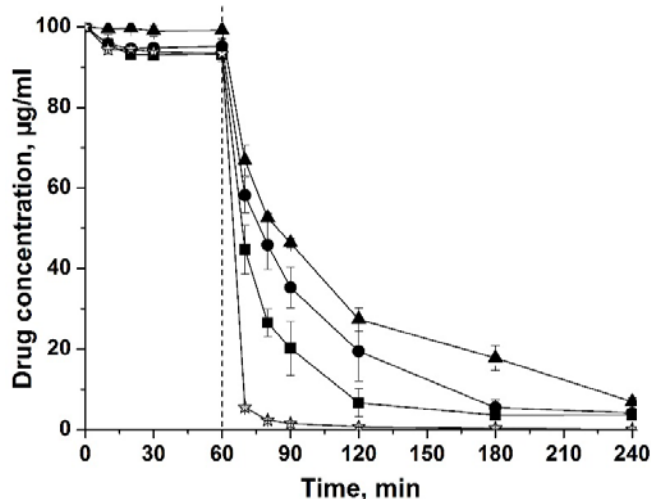
**Fig. 3.42.** Effect of inhibition of different polymers (0.3 mg/ml) on precipitation of a supersaturated solution of itraconazole (100 µg/ml) in 500 ml 0.1 N HCl: (▲) HPMC, (●) Eudragit® EPO-PVPVA 64, (■) Eudragit® EPO, and (☆) without any polymer (mean ± SD; n = 3).

Similar results were also reported in other studies. Bevernage et al. evaluated the excipient mediated precipitation inhibition of five poorly soluble drugs (loviride, glibenclamide, itraconazole, danazol, and etravirine) in human gastric fluids (HGFs), SGF and FaSSGF using a solvent shift method (Bevernage et al., 2012). The supersaturation factors for itraconazole were around 10 and 12 in HGF and FaSSGF respectively, whereas it reached around 17 in SGF without polymer. Similarly, SGF significantly overestimated the supersaturation stability for glibenclamide and danazol, indicating that FaSSGF was preferred for biorelevant evaluation of supersaturation behavior closing with HGF, rather than SGF. Speybroeck et al. reported that no itraconazole precipitation was observed in SGF irrespective of the presence of HPMC (Van Speybroeck et al., 2010). Therefore, solvent shift method in 0.1 N HCl was not suitable to evaluate anti-precipitant for itraconazole.



### 3.4.4.2. Evaluation of supersaturation stabilization and precipitation inhibition by pH-shift method

The anti-precipitation performance was also evaluated by pH-shift method since precipitation/crystallization inhibition of polymers for weakly basic drugs in the intestine dominated *in vivo* drug absorption. As discussed above, no pronounced difference was observed in the acidic phase of each polymer solution. However, they performed differently at neutral pH (Fig. 3.43). Drug precipitated rapidly in the buffer with the absence of polymer after pH change. HPMC exhibited the best ability for precipitation inhibition, whereas Eudragit® EPO had poor performance since it is insoluble in the neutral buffer. Eudragit® EPO-PVPVA 64 showed better precipitation inhibition than Eudragit® EPO due to pH-independent property of PVPVA 64. Compared to a solvent shift, a pH-shift method may be considered more biorelevant for evaluation of weakly basic drugs (Bevernage et al., 2013).



**Fig. 3.43.** Effect of different polymers (0.3 mg/ml) on precipitation inhibition of a saturated solution of itraconazole (100 µg/ml) by a pH-shift method (500 ml 0.1 N HCl for 1 h followed by a pH adjustment to  $6.8 \pm 0.05$  with addition of 166.7 ml 0.2 M tribasic sodium phosphate solution) (dashed vertical line represents the pH change): (▲) HPMC, (●) Eudragit® EPO-PVPVA 64, (■) Eudragit® EPO, and (☆) without any polymer (mean  $\pm$  SD; n = 3).

#### 3.4.4.3. Viscosity of polymer solutions

Solution viscosity is also one of the factors affecting precipitation inhibition for polymers (Xu and Dai, 2013). The supersaturation stabilization of different polymers could be attributed to a relatively high local viscosity. Due to the pH-dependent solubility of Eudragit® EPO, the viscosity of polymer solutions in 0.1 N HCl was used to assess the influence of supersaturation stabilization. Although HPMC E5 has low viscosity, it still showed significantly higher viscosity than Eudragit® EPO and Eudragit® EPO-PVPVA 64 at the same concentration in 0.1 N HCl ( $p < 0.05$ ) (Table 3.13). Miller et al. reported that higher viscosity of polymers exhibited better supersaturation stabilization, which was mostly attributed to a greater steric hindrance to prevent recrystallization (Miller et al., 2008b). Thus, the higher viscosity of HPMC solution could be one of the reasons for maintaining supersaturation.

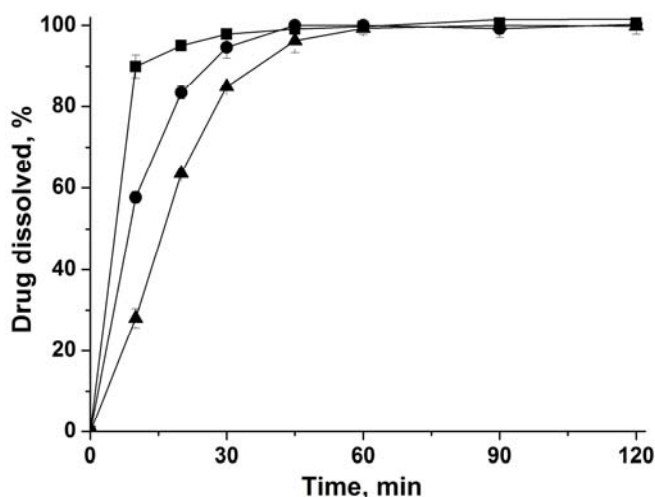
**Table 3.13.** Viscosity of different polymer solutions with a concentration of 0.3 mg/ml in 0.1 N HCl (\*,  $p < 0.05$ ) (mean  $\pm$  SD;  $n = 3$ ).

Polymer solutions	Viscosity (mPa s)
Eudragit® EPO	0.93 $\pm$ 0.01 <sup>a</sup>
Eudragit® EPO-PVPVA 64	0.93 $\pm$ 0.02 <sup>a</sup>
HPMC	1.05 $\pm$ 0.02

a  $p < 0.05$  vs. HPMC.

#### 3.4.5. Conventional single phase USP II dissolution tests under sink and non-sink conditions

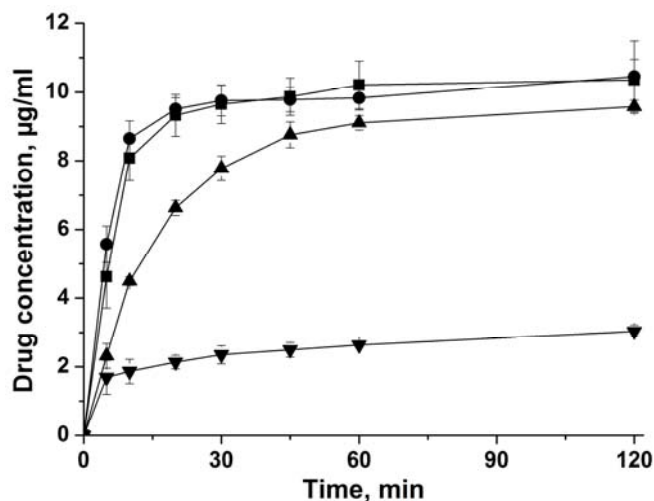
The solubility of itraconazole in 0.1 N HCl with 0.7% SLS was 566.2  $\mu$ g/ml. The drug release of a sample equivalent to 100 mg drug was evaluated under sink conditions ( $< 20\%$  of drug solubility in 900 ml medium). All solid dispersions released more than 80% within 30 min, although the dissolution rates showed differences at initial times with a rank order of Eudragit® EPO  $>$  Eudragit® EPO-PVPVA 64  $>$  HPMC (Fig. 3.44). SLS improved drug dissolution and solubility, however the results were not consistent with the reported *in vivo* performance (Six et al., 2005).



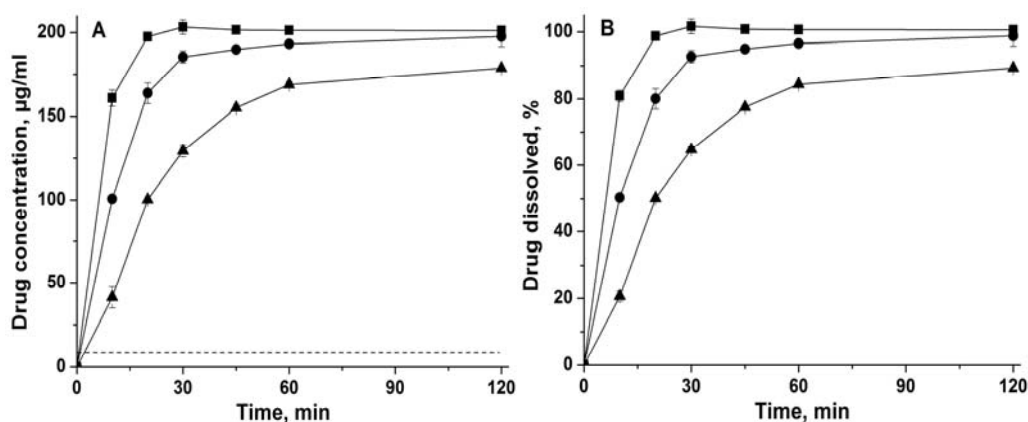
**Fig. 3.44.** Dissolution profiles of itraconazole solid dispersions under sink conditions (900 ml 0.1 N HCl containing 0.7% SLS): (■) Eudragit® EPO, (●) Eudragit® EPO-PVPVA 64, and (▲) HPMC (mean  $\pm$  SD; n = 3).

To obtain a comparable *in vitro* dissolution performance with the reported solid dispersions prepared by hot-melt extrusion and establish a correlation between *in vitro* and published *in vivo* data (Six et al., 2005), the release from solid dispersions prepared by solvent casting, physical mixtures and pure drug was evaluated in the same dissolution conditions (500 ml 0.1 N HCl, at drug dose of 100 mg, non-sink conditions). Physical mixtures enhanced drug dissolution slightly compared to pure drug, reaching concentrations close to drug solubility in these physical mixtures ( $10.0 \pm 0.2$ ,  $10.3 \pm 0.1$  and  $12.4 \pm 0.1$   $\mu\text{g/ml}$  for Eudragit® EPO, Eudragit® EPO-PVPVA 64 and HPMC respectively) (Fig. 3.45). Solid dispersions improved drug release dramatically with the rank order of Eudragit® EPO > Eudragit® EPO-PVPVA 64 > HPMC (Fig. 3.46). The degree of supersaturation for Eudragit® EPO and Eudragit® EPO-PVPVA 64 was 27-fold and 25-fold for HPMC (Fig. 3.46A). The solid dispersions containing Eudragit® EPO and Eudragit® EPO-PVPVA 64 had similar release rates, with  $t_{80}$  of 10 and 20 min (Fig. 3.46B). The release from the solid dispersion with HPMC was remarkably slower due to the slower dissolution kinetics of HPMC compared to Eudragit® EPO and PVPVA 64. Another possibility could be that HPMC swelled in the dissolution medium, resulting in the formation of a viscous hydrogel, which retarded drug diffusion. The release profiles were in agreement with previously

reported results (Six et al., 2005), however, the *in vitro* dissolution behavior was not consistent with *in vivo* performance.



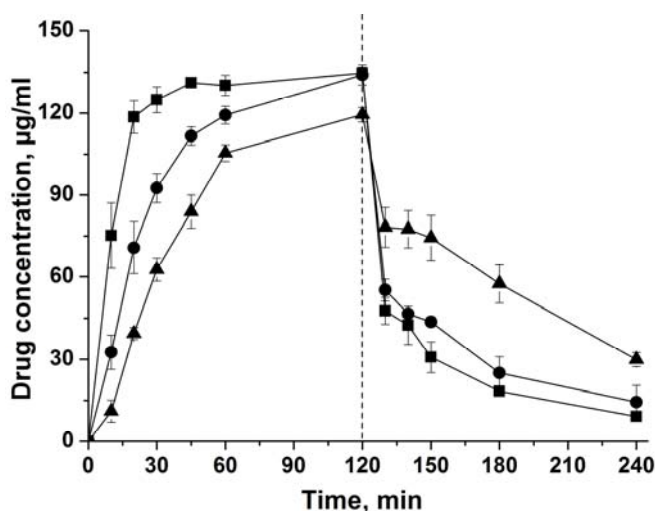
**Fig. 3.45.** Dissolution profiles of itraconazole and physical mixtures under non-sink conditions (500 ml 0.1 N HCl): (■) Eudragit® EPO, (●) Eudragit® EPO-PVPVA 64, (▲) HPMC and (▼) pure drug (mean  $\pm$  SD; n = 3).



**Fig. 3.46.** Dissolution profiles of itraconazole solid dispersions under non-sink conditions (500 ml 0.1 N HCl): (A) drug concentration and (B) % drug dissolved (dashed line represents the solubility of crystalline itraconazole in 0.1 N HCl): (■) Eudragit® EPO, (●) Eudragit® EPO-PVPVA 64, and (▲) HPMC (mean  $\pm$  SD; n = 3).

Due to the pH-dependent solubility of itraconazole, dissolution test with a pH change could closely mimic the transition throughout the GI tract. Thus, solid dispersions were evaluated by a pH-gradient dissolution test. As previously discussed, solid dispersions with Eudragit® EPO and Eudragit® EPO-PVPVA 64 showed faster release and a greater degree of supersaturation than the one of

HPMC in 0.1 N HCl for the first 2 h (Fig. 3.47). However, after pH change, drug concentration of itraconazole dramatically decreased, which was attributed to immediate precipitation due to a much lower solubility at neutral pH (~1 ng/ml) (Peeters et al., 2002). Although solid dispersion with Eudragit<sup>®</sup> EPO showed the highest supersaturation degree in 0.1 N HCl, it exhibited fast precipitation from 134.7 µg/ml to 9.1 µg/ml at 4 h in pH 6.8 buffer (Fig. 3.47) due to the insolubility of Eudragit<sup>®</sup> EPO above pH 5. The formulation with Eudragit<sup>®</sup> EPO-PVPVA 64 also showed the similar precipitation in the neutral medium. Conversely, the solid dispersion with HPMC exhibited the highest stabilization against drug precipitation, resulting in drug concentration change from 119.5 µg/ml to 30.0 µg/ml at 4 h after pH change. The precipitation inhibition of HPMC could result from its interaction with itraconazole identified by FTIR and the higher viscosity of HPMC solution. Some studies also reported that the stabilizing effect of HPMC on supersaturated itraconazole solutions could be attributed to intermolecular interaction and/or steric hindrance (Miller et al., 2008b; Miller et al., 2007).



**Fig. 3.47.** Dissolution profiles of itraconazole solid dispersions under pH-gradient conditions (750 ml 0.1 N HCl for 2 h followed by a pH adjustment to  $6.8 \pm 0.05$  with addition of 250 ml 0.2 M tribasic sodium phosphate solution) (dashed vertical line represents the pH change): (■) Eudragit<sup>®</sup> EPO, (●) Eudragit<sup>®</sup> EPO-PVPVA 64, and (▲) HPMC (mean  $\pm$  SD; n = 3).

Furthermore, the area under the concentration-time profile for the acid phase ( $AUC_{acid}$ ), neutral phase ( $AUC_{neutral}$ ), and total dissolution test ( $AUC_{total}$ ) was calculated to quantitatively evaluate the dissolution results. The *in vitro* AUC of these three solid dispersions in the acid phase ranked as Eudragit<sup>®</sup> EPO > Eudragit<sup>®</sup> EPO-PVPVA 64 > HPMC, where solid dispersions with Eudragit<sup>®</sup> EPO showed 17.6% and 44.1% greater mean  $AUC_{acid}$  values than Eudragit<sup>®</sup> EPO-PVP-VA 64 and HPMC (Table 3.14), respectively. However, solid dispersions with HPMC exhibited 72.8% and 106.1% greater mean  $AUC_{neutral}$  values than Eudragit<sup>®</sup> EPO-PVPVA 64 and Eudragit<sup>®</sup> EPO in the neutral phase. Finally, solid dispersion with Eudragit<sup>®</sup> EPO exhibited 9.0% and 4.4% greater mean  $AUC_{total}$  values than Eudragit<sup>®</sup> EPO-PVPVA 64 and HPMC from the total release profiles, respectively. Itraconazole, as a weakly basic drug, is soluble at low pH of stomach, but rapid precipitation occurs in the intestine due to its poor solubility at neutral pH, which greatly influences drug absorption. Thereby, the  $AUC_{neutral}$  values of these formulations showed a certain relevance with the reported *in vivo* absorption with a ranking of solid dispersions with HPMC > Eudragit<sup>®</sup> EPO-PVPVA 64 ~ Eudragit<sup>®</sup> EPO (Six et al., 2005).

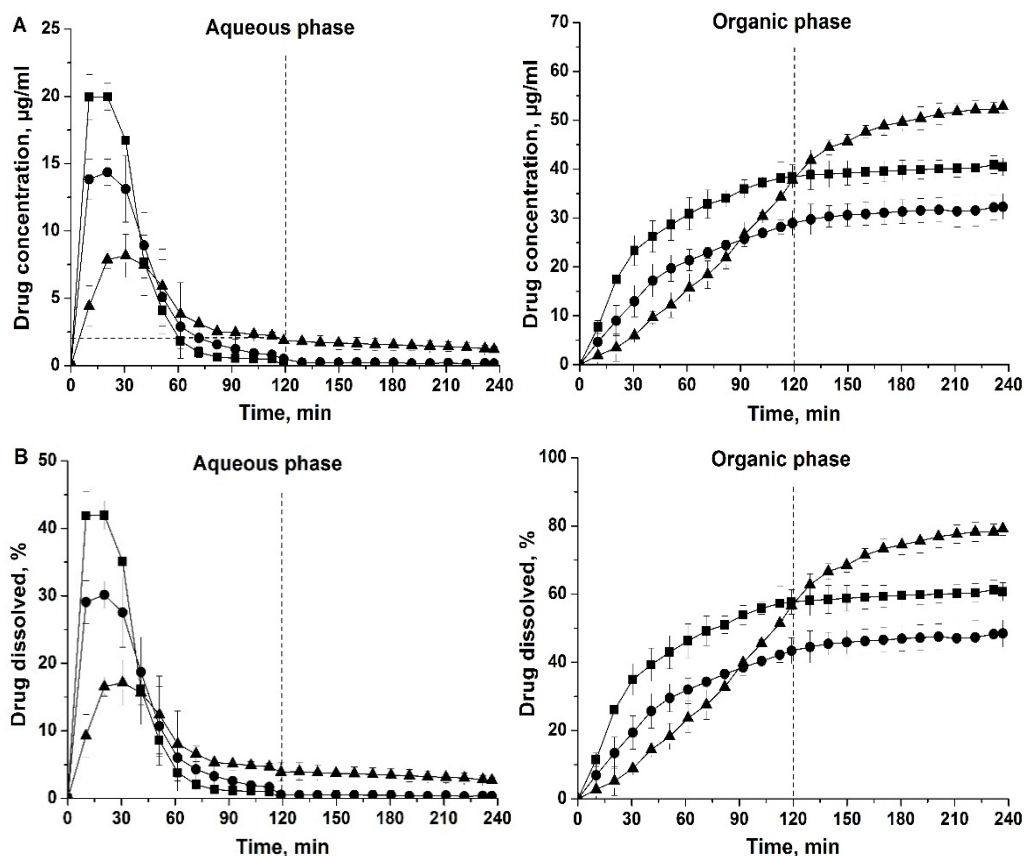
**Table 3.14.** *In vitro* area under the dissolution curve values for the acid phase, neutral phase and total dissolution test for itraconazole solid dispersions (mean  $\pm$  SD; n = 3).

Formulation	<i>In vitro</i> $AUC_{acid}$ ( $\mu\text{g}\cdot\text{h}/\text{ml}$ )	<i>In vitro</i> $AUC_{neutral}$ ( $\mu\text{g}\cdot\text{h}/\text{ml}$ )	<i>In vitro</i> $AUC_{total}$ ( $\mu\text{g}\cdot\text{h}/\text{ml}$ )
Eudragit <sup>®</sup> EPO	242.2 $\pm$ 7.5	57.6 $\pm$ 1.7	299.5 $\pm$ 12.4
Eudragit <sup>®</sup> EPO- PVPVA 64	206.0 $\pm$ 6.8	68.7 $\pm$ 6.5	274.7 $\pm$ 13.2
HPMC	168.1 $\pm$ 0.8	118.7 $\pm$ 5.4	286.8 $\pm$ 4.7

#### 3.4.6. A pH-gradient biphasic dissolution test

As discussed above, a pH-shift played an important role in evaluating drug release of itraconazole formulations. Therefore, a pH-gradient biphasic dissolution test mimicking drug dissolution, transit and absorption in the stomach and small intestine was used to assess the performance of the three

itraconazole solid dispersions. A sample equivalent to 2 mg drug was evaluated to maintain sink conditions ( $< 30\%$  of drug solubility ( $C_s = 259.4 \mu\text{g/ml}$ ) in 30 ml decanol). Three formulations showed different extent of supersaturation at initial times with 9.1-, 6.5- and 3.7-fold higher than solubility of crystalline drug for Eudragit<sup>®</sup> EPO, Eudragit<sup>®</sup> EPO-PVPVA 64 and HPMC in the aqueous phase, respectively, but subsequently the drug concentrations dropped dramatically (Fig. 3.48A). However, the corresponding release profiles in the organic phase differed with a rank order of solid dispersion with HPMC  $>$  Eudragit<sup>®</sup> EPO  $>$  Eudragit<sup>®</sup> EPO-PVPVA 64 reaching  $79.2 \pm 1.9$ ,  $60.7 \pm 2.7$  and  $48.5 \pm 3.9\%$  at 4 h (Fig. 3.48B), respectively. Eudragit<sup>®</sup> EPO and Eudragit<sup>®</sup> EPO-PVPVA 64 formulations maintained a relatively constant itraconazole concentrations in organic phase up to  $40.4 \pm 1.8$  and  $32.3 \pm 2.6 \mu\text{g/ml}$  at 4 h, respectively. This could be a result of the fast drug precipitation in the aqueous phase after pH change and Eudragit<sup>®</sup> EPO had poor ability for precipitation inhibition at neutral pH and therefore dramatically reducing drug concentration in the aqueous phase, which resulted in little available drug concentration partitioning into the organic phase. In contrast, HPMC formulation exhibited the slowest initial release in the acid phase, however the highest drug concentration of  $52.8 \pm 1.3 \mu\text{g/ml}$  at 4 h was achieved in the organic phase. The slower dissolution rate of HPMC itself contributed to the prevention of drug recrystallization within the undissolved formulation. Other factors attributing to its best ability of precipitation inhibition were the strong interaction with itraconazole and higher local viscosity. Thus, these three formulations were discriminated in the organic phase of the pH-gradient biphasic dissolution test. The ranking of release profiles from these formulations in the organic phase correlated with the reported *in vivo* AUC ( $1405.6 \pm 778.2$ ,  $1054.0 \pm 583.9$  and  $928.9 \pm 355.7 \text{ ng h/ml}$ ) for HPMC, Eudragit<sup>®</sup> EPO and Eudragit<sup>®</sup> EPO-PVPVA 64 respectively (Six et al., 2005). Thus, these solid dispersions were discriminated in the organic phase of the pH-gradient biphasic dissolution test.



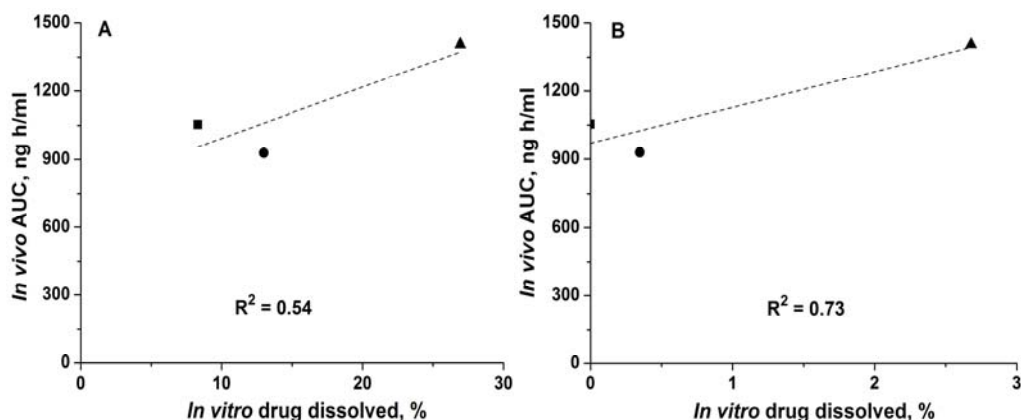
**Fig. 3.48.** Dissolution profiles of itraconazole solid dispersions determined from the two phases in the pH-gradient biphasic dissolution test in Sirius inForm (gastric buffer pH 2 for 2 h followed by a pH adjustment to  $6.8 \pm 0.05$ ) (dashed horizontal line represents drug solubility in gastric buffer pH 2.0 saturated with decanol and dashed vertical line represents the pH change): (A) drug concentration and (B) % drug release: (■) Eudragit® EPO, (●) Eudragit® EPO-PVPVA 64, and (▲) HPMC (mean  $\pm$  SD; n = 3).

### 3.4.7. *In vitro-in vivo* relationship

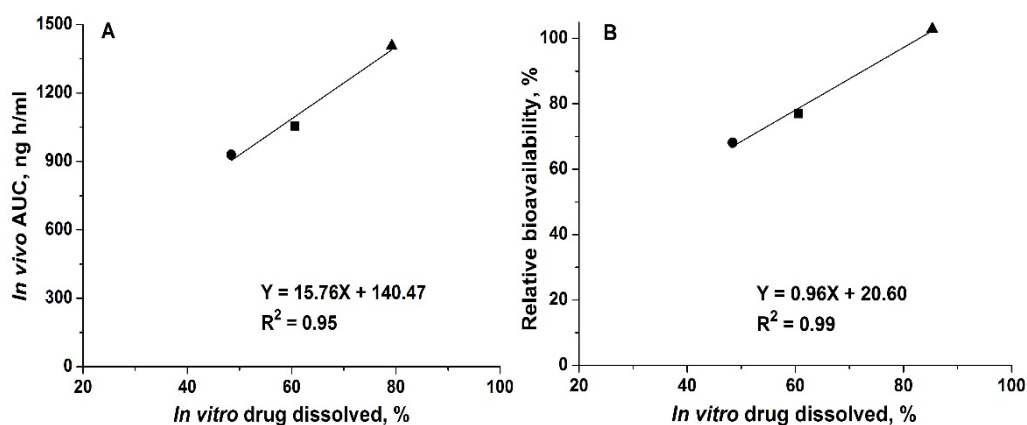
The release profiles of these three itraconazole solid dispersions in the organic phase of the pH-gradient biphasic dissolution test correlated with the reported *in vivo* performance. Next, the potential to establish a relationship between *in vitro* dissolution and *in vivo* performance was explored. Poor correlations were obtained between the percent drug dissolved at 4 h in the conventional pH-gradient dissolution test under non-sink conditions or in the aqueous phase of the pH-gradient biphasic test and the reported *in vivo* AUC (Fig.3.49). Conversely, good linear correlations between the percent dissolved in the organic phase at 4 h and the reported *in vivo* AUC ( $R^2 = 0.95$ ) (Fig. 3.50A) and the reported relative bioavailability ( $R^2 = 0.99$ ) (Fig. 3.50B) were obtained. This



indicates that the pH-gradient biphasic dissolution test could discriminate different amorphous solid dispersions of itraconazole with various polymers exhibiting different effects on drug supersaturation degree and precipitation. The organic phase played a critical role in predicting *in vivo* performance.



**Fig. 3.49.** Relationship of mean *in vitro* % dissolved at 4 h in (A) the single phase pH-gradient dissolution test under supersaturated conditions and (B) the aqueous phase of the pH-gradient biphasic test plotted against mean *in vivo* AUC values obtained from the literature (Six et al., 2005): (■) Eudragit® EPO, (●) Eudragit® EPO-PVPVA 64, and (▲) HPMC.



**Fig. 3.50.** Relationship of mean *in vitro* % dissolved in the organic phase of the pH-gradient biphasic test at 4 h plotted against (A) mean *in vivo* AUC values or (B) mean relative bioavailability obtained from the literature (Six et al., 2005): (●) Eudragit® EPO-PVPVA 64, (■) Eudragit® EPO, and (▲) HPMC.

### 3.4.8. Conclusions

Three itraconazole amorphous solid dispersions prepared with different polymers were evaluated in different *in vitro* dissolution systems (sink vs. non-sink, single-pH vs. pH-gradient, monophasic vs. biphasic). Conventional dissolution tests under sink and non-sink conditions showed poor prediction of *in vivo* behavior, while the conventional pH-gradient dissolution test under non-sink conditions exhibited a certain relevance with *in vivo* performance. Conversely, the pH-gradient biphasic dissolution test discriminated the three solid dispersions with respect to different supersaturation and precipitation performances induced by various properties of polymers and correlated with the *in vivo* performance obtained from the literature. An excellent correlation between *in vitro* drug release in the organic phase of the biphasic test and reported *in vivo* data was obtained. This study demonstrated that the pH-gradient biphasic model has great potential to discriminate between supersaturation formulations with different polymers during early formulation development of BCS II drugs with pH-dependent solubility.

## **4. SUMMARY**

A suitable dissolution test as a surrogate for *in vivo* absorption is highly attractive in the early stage of formulation development. Ideally, changes in dissolution *in vivo* should be reflected by the corresponding *in vitro* release. However, conventional dissolution tests have limitations to address this need due to the lack of biorelevance. The purpose of this work was to explore a discriminative dissolution test for manufacturing/ formulation changes within formulations of poorly soluble drugs.

### **Evaluation of a discriminative biphasic dissolution test for different cosolvents**

PEG 400 and EtOH increased carbamazepine solubility in a concentration-dependent manner, where PEG 400 had stronger solubilization capacity than EtOH. However, PEG 400 had an inhibiting effect on drug absorption with increasing concentrations, while EtOH had no effect. The biphasic dissolution model discriminated different influences of PEG 400 and EtOH on drug absorption reflected by drug partitioning, which was in good agreement with other studies. The different performances of the two cosolvents could be associated with drug affinity and diffusivity. Therefore, the solubility-permeability interplay should be taken into consideration when designing cosolvent-based formulations. Furthermore, the mixed EtOH-PEG 400 cosolvent system was superior to single EtOH and PEG 400 by enhancing carbamazepine solubility to compensate for low solubilization in EtOH, and by decreasing the inhibiting effect on drug partitioning to compensate for low drug absorption caused by PEG 400. This optimal strategy could be considered in the development of cosolvents formulations. The biphasic dissolution model has the potential to discriminate between cosolvent-based formulations with BCS II drugs.

### **Evaluation of a discriminative biphasic dissolution test for estimating the bioavailability of carbamazepine polymorphic forms**

Three crystal forms (forms I and III, and the dihydrate) of carbamazepine (BCS II) were prepared and characterized. A biphasic dissolution system (phosphate buffer pH 6.8 and octanol) was used to evaluate the dissolution of the three polymorphic forms and to compare it with conventional single phase dissolution tests performed under sink and non-sink conditions. Similar dissolution profiles of the three polymorphic forms were observed in the conventional dissolution test under sink conditions. Although a difference in dissolution was seen in the single phase dissolution test under non-sink conditions as well as in the aqueous phase of the biphasic test, little relevance to *in vivo* data obtained from the literature was observed. In contrast, the biphasic dissolution system could discriminate between the different polymorphic forms in the octanol phase with a ranking of form III > form I > dihydrate form. This was in agreement with the published *in vivo* performance. The dissolved drug available for oral absorption, which was dominated by dissolution and solution-mediated phase transformation, could be reflected in the biphasic dissolution test. Moreover, a good correlation was established between *in vitro* dissolution in the octanol phase of the biphasic test and *in vivo* pharmacokinetic data ( $R^2 = 0.99$ ). The biphasic dissolution method is a valuable tool to discriminate between different crystal forms in the formulations of poorly soluble drugs.

### **Evaluation of a discriminative biphasic dissolution test and correlation with *in vivo* pharmacokinetic studies for differently formulated racecadotril granules**

Three granule formulations of racecadotril (BCS II) were formulated with equivalent composition but prepared with different manufacturing processes (dry granulation, wet granulation with or without binder). *In vitro* release of the formulations was investigated using a biphasic dissolution system (phosphate buffer pH 6.8 and octanol) and compared to the conventional single phase USP

II dissolution test performed under sink and non-sink conditions. The effect of different volume ratios and interfacial areas in the biphasic test was investigated and optimized to establish a discriminative dissolution test and an *in vitro-in vivo* correlation. *In vivo* studies with each granule formulation were performed in rats. Interestingly, the granule formulations exhibited pronouncedly different behavior in the various dissolution systems depending on different wetting and dissolution conditions. Similar release profiles between the three granule formulations were observed in the non-discriminating single phase dissolution tests under sink and non-sink conditions. In contrast, biphasic dissolution system showed remarkable discrimination between the granule formulations in the octanol phase with a rank order of release from granules prepared by wet granulation with binder > wet granulation without binder > dry granulation. This release order correlated well with the wettability of these granules. The same rank order as in the biphasic dissolution test was observed with *in vivo* AUC<sub>0-24h</sub> values of the three granules, being  $4.07 \pm 0.22$ ,  $3.50 \pm 0.22$ , and  $2.85 \pm 0.29$   $\mu\text{g h/ml}$  ( $p < 0.05$ ) respectively. An excellent correlation was also established between *in vitro* release in the octanol phase of the biphasic test and *in vivo* data ( $R^2 = 0.999$ ). Compared to conventional dissolution methods, the biphasic method is a good tool to discriminate between only minor formulation and process changes within the same dosage form for poorly soluble drugs.

### **Evaluation of a discriminative biphasic dissolution test for estimating the bioavailability of itraconazole amorphous solid dispersions prepared with different polymers**

Three amorphous solid dispersions of itraconazole (BCS II) were prepared with different polymers (Eudragit® EPO, Eudragit® EPO-PVPVA 64, and HPMC). *In vitro* release of these three solid dispersions was evaluated by a pH-gradient biphasic dissolution test and compared to conventional single phase dissolution test performed under sink and non-sink conditions. Conventional dissolution tests in 0.1 N HCl under sink and non-sink conditions showed poor prediction of

*in vivo* behavior, while the conventional pH-gradient dissolution test under non-sink conditions exhibited a certain relevance with *in vivo* performance. In contrast, the pH-gradient biphasic dissolution test discriminated these three solid dispersions with a ranking of HPMC > Eudragit® EPO > Eudragit® EPO-PVPVA 64 in the organic phase and correlated well with the published *in vivo* performance. Solid dispersion with HPMC showed the best performance due to its superior supersaturation maintenance and precipitation inhibition induced by interaction between HPMC and itraconazole. An excellent correlation between *in vitro* release obtained from the organic phase of the biphasic test and reported *in vivo* data was obtained ( $R^2 \geq 0.95$ ). This study demonstrates that the pH-gradient biphasic model is a potential tool to discriminate between supersaturation formulations with different polymers during early development of formulations with pH-dependent BCS II drugs.

In conclusion, this entire work indicated that compared to conventional dissolution methods, the biphasic dissolution system provides great potential to discriminate between manufacturing/formulation changes within formulations for BCS II drugs during the early formulation development.

## **5. ZUSAMMENFASSUNG**



Im frühen Stadium der Formulierungsentwicklung ist ein geeigneter Auflösungstest als Ersatz der *in vivo* Absorption sehr attraktiv. Idealerweise sollten Änderungen in der *in vivo* Auflösung durch den entsprechenden *in vitro* Freisetzungstest reflektiert werden. Allerdings haben die konventionellen Auflösungstests eine mangelhafte Biorelevanz. Das Ziel dieser Arbeit war es, einen diskriminierenden Auflösungstest für Herstellungs-/Formulierungsänderungen in Formulierungen von schwer löslichen Arzneimitteln zu erforschen.

### **Entwicklung eines diskriminierenden zweiphasigen Auflösungstests für verschiedene Zusatzlösemittel**

Die Carbamazepinlöslichkeit erhöhte sich konzentrationsabhängig mit Zugabe von PEG 400 und EtOH, wobei PEG 400 eine stärkere Löslichkeitsvermittlung als EtOH aufwies. Allerdings hatte PEG 400 mit zunehmender Konzentration einen hemmenden Effekt auf die Arzneistoffabsorption, während EtOH keinen solchen Effekt hatte. Der zweiphasige Auflösungstest konnte die verschiedenen Einflüsse von PEG 400 und EtOH auf die Arzneistoffabsorption unterscheiden, was sich in der unterschiedlichen Arzneimittelverteilung widerspiegelte und in guter Übereinstimmung mit anderen Studien war. Die unterschiedlichen Ergebnisse der beiden Zusatzlösemittel konnten mit der Arzneistoffaffinität und der Diffusivität assoziiert werden. Das Löslichkeits-Permeabilitäts- Zusammenspiel sollte daher bei der Entwicklung von Zusatzlösemittel basierten Formulierungen berücksichtigt werden. Weiterhin war eine EtOH-PEG 400 Zusatzlösemittelmischung reinem EtOH und PEG 400 überlegen. Es verbesserte die geringere Löslichkeitsvermittlung von EtOH hinsichtlich der Carbamazepinlöslichkeit und verringerte den hemmenden Effekt auf die Arzneistoffverteilung, wodurch die geringe Arzneistoffabsorption von PEG 400 kompensiert wurde. Diese optimale Strategie könnte bei der Entwicklung von Zusatzlösemittel basierten Formulierungen in Betracht gezogen werden. Der zweiphasige Auflösungstest hat das Potenzial, zwischen Zusatzlösemittel basierten Formulierungen mit BCS II Arzneistoffen zu unterscheiden.

### **Entwicklung eines diskriminierenden zweiphasigen Auflösungstests zur Abschätzung der Bioverfügbarkeit von polymorphen Carbamazepinformen**

Es wurden drei Kristallformen (Form I und III und das Dihydrat) von Carbamazepin (BCS II) hergestellt und charakterisiert. Ein zweiphasiges Auflösungssystem (Phosphatpuffer pH 6,8 und Octanol) wurde verwendet, um die Auflösung der drei polymorphen Formen zu beurteilen und mit den Ergebnissen eines konventionellen einphasigen Auflösungsstes unter Sink- und Nicht-Sink-Bedingungen zu vergleichen. Im konventionellen Auflösungsstest wurden ähnliche Auflösungsprofile unter Sink-Bedingungen beobachtet. Obwohl ein Unterschied in der Auflösung im einphasigen Auflösungsstest unter Nicht-Sink-Bedingungen sowie in der wässrigen Phase des zweiphasigen Tests gesehen wurde, wurde eine geringe Korrelation mit *in vivo* Daten aus der Literatur erhalten. Im Gegensatz dazu konnte das zweiphasige Auflösungssystem in der Octanolphase zwischen den verschiedenen polymorphen Formen unterscheiden mit der Rangordnung Form III > Form I > Dihydratform. Dies war in Übereinstimmung mit veröffentlichten *in vivo* Ergebnissen. Die orale Bioverfügbarkeit dieses Arzneistoffes ist durch Auflösung und lösungsvermittelte Phasenumwandlung dominiert, was im zweiphasigen Auflösungsstest wiedergespiegelt werden konnte. Darüber hinaus wurde eine gute Korrelation zwischen der *in vitro* Auflösung in der Octanolphase des zweiphasigen Tests und den pharmakokinetischen *in vivo* Daten ( $R^2 = 0,99$ ) erhalten. Der zweiphasige Auflösungsstest ist ein wertvolles Werkzeug, um zwischen verschiedenen Kristallformen in Formulierungen mit schwer löslichen Arzneistoffen zu unterscheiden.

### **Entwicklung eines diskriminierenden zweiphasigen Auflösungstests und Korrelation mit pharmakokinetischen *in vivo* Studien für unterschiedlich formuliertes Racecadotril Granulat**

Es wurden drei Granulatformulierungen von Racecadotril (BCS II) mit gleichwertiger Zusammensetzung, aber mit verschiedenen Herstellungsverfahren (Trockengranulierung, Nassgranulierung mit oder ohne Bindemittel) formuliert. Die *in vitro* Freisetzung der Formulierungen wurde unter Verwendung eines zweiphasigen

Auflösungssysteme (Phosphatpuffer pH 6,8 und Octanol) untersucht und mit dem herkömmlichen einphasigen USP II Auflösungstest unter Sink- und Nicht-Sink-Bedingungen verglichen. Es wurde der Effekt unterschiedlicher Volumenverhältnisse und Grenzflächen im zweiphasigen Test untersucht und optimiert, um einen diskriminierenden Auflösungstest und eine bestmögliche *in vitro-in vivo* Korrelation zu erhalten. Es wurden *in vivo* Studien mit Ratten mit jeder Granulat-Formulierung durchgeführt. Interessanterweise zeigten die Granulatformulierungen in den verschiedenen Auflösungssystemen, in Abhängigkeit von unterschiedlichen Benetzungs- und Auflösungsbedingungen, ein unterschiedliches Verhalten. Es wurden ähnliche Freisetzungprofile zwischen den drei Granulatformulierungen in den nicht diskriminierenden einphasigen Auflösungstests unter Sink- und Nicht-Sink-Bedingungen beobachtet. Im Gegensatz dazu zeigte der zweiphasige Auflösungstest eine bemerkenswerte Diskriminierung zwischen den Granulatformulierungen in der Octanolphase mit einer Rangordnung der Freisetzung aus dem Granulat, hergestellt durch Nassgranulierung mit Bindemittel > Nassgranulierung ohne Bindemittel > Trockengranulierung. Diese Rangordnung korrelierte gut mit der Benetzbarkeit dieser Granulate. Die gleiche Rangordnung wie im zweiphasigen Auflösungstest wurde bei *in vivo*  $AUC_{0-24h}$  Werten der drei Granulate beobachtet, welche  $4,07 \pm 0,22$ ,  $3,50 \pm 0,22$  und  $2,85 \pm 0,29$   $\mu\text{g h/ml}$  ( $p < 0,05$ ) betragen. Eine ausgezeichnete Korrelation wurde auch zwischen der *in vitro* Freisetzung in der Octanolphase des zweiphasigen Auflösungstestes und den *in vivo* Daten ( $R^2 = 0,999$ ) festgestellt. Im Vergleich zu herkömmlichen Auflösungstests ist der zweiphasige Auflösungstest ein gutes Werkzeug, um zwischen geringen Formulierungs- und Prozessänderungen innerhalb der gleichen Dosierungsform für schlecht lösliche Arzneistoffe zu unterscheiden.

### **Entwicklung eines diskriminierenden zweiphasigen Auflösungstests zur Abschätzung der Bioverfügbarkeit von amorphen festen Dispersionen von Itraconazol, die mit verschiedenen Polymeren hergestellt wurden**

Drei amorphe feste Dispersionen von Itraconazol (BCS II) wurden mit verschiedenen

Polymere (Eudragit® EPO, Eudragit® EPO-PVPVA 64 und HPMC) hergestellt. Die *in vitro* Freisetzung dieser drei festen Dispersionen wurde mit einem zweiphasigen Auflösungstest mit pH Gradienten bestimmt und mit dem herkömmlichen einphasigen Auflösungstest verglichen. Beide Auflösungstests wurden unter Sink- und Nicht-Sink Bedingungen durchgeführt. Die konventionellen Auflösungstests in 0,1 N HCl unter Sink- und Nicht-Sink Bedingungen zeigten eine schlechte Vorhersage des *in vivo* Verhaltens, während der herkömmliche Auflösungstest mit pH Gradienten unter Nicht-Sink Bedingungen eine gewisse Relevanz für das *in vivo* Verhalten aufwies. Im Gegensatz dazu konnte der zweiphasige Auflösungstest mit pH Gradienten diese drei festen Dispersionen mit einer Rangfolge von HPMC > Eudragit® EPO > Eudragit® EPO-PVPVA 64 in der organischen Phase und korrelierte gut mit dem veröffentlichten *in vivo* Verhalten. Die feste Dispersion mit HPMC zeigte die beste Leistung aufgrund ihrer überlegenen Aufrechterhaltung der Übersättigung und Hemmung der Ausfällung, die durch die Wechselwirkung zwischen HPMC und Itraconazol hervorgerufen wurde. Es wurde eine ausgezeichnete Korrelation zwischen der *in vitro* Freisetzung, die aus der organischen Phase des zweiphasigen Auflösungstests erhalten wurde, und den berichteten *in vivo* Daten erhalten ( $R^2 \geq 0,95$ ). Diese Studie zeigt, dass der zweiphasige Auflösungstest mit pH Gradienten ein potentes Werkzeug ist, um zwischen Übersättigungsformulierungen mit verschiedenen Polymeren während der frühen Entwicklung von Formulierungen mit pH-abhängigen BCS II Arzneimitteln zu unterscheiden.

Zusammenfassend zeigte die gesamte Arbeit, dass im Vergleich zu herkömmlichen Auflösungstests der zweiphasige Auflösungstest ein großes Potenzial bietet, zwischen Herstellungs-/ Formulierungsänderungen innerhalb von Formulierungen für BCS II Arzneistoffe während der frühen Formulierungsentwicklung zu unterscheiden.

## **6. REFERENCES**

### REFERENCES

- Aguiar, A.J., Zelmer, J.E., 1969. Dissolution behavior of polymorphs of chloramphenicol palmitate and mefenamic acid. *J Pharm Sci* 58, 983-987.
- Al Durdunji, A., AlKhatib, H.S., Al-Ghazawi, M., 2016. Development of a biphasic dissolution test for Deferasirox dispersible tablets and its application in establishing an in vitro-in vivo correlation. *Eur J Pharm Biopharm* 102, 9-18.
- Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res* 12, 413-420.
- Anitha, A., Sreeranganathan, M., Chennazhi, K.P., Lakshmanan, V.K., Jayakumar, R., 2014. In vitro combinatorial anticancer effects of 5-fluorouracil and curcumin loaded N, O-carboxymethyl chitosan nanoparticles toward colon cancer and in vivo pharmacokinetic studies. *Eur J Pharm Biopharm* 88, 238-251.
- Aoki, S., Ando, H., Tatsuishi, K., Uesugi, K., Ozawa, H., 1993. Determination of the mechanical impact force in the in vitro dissolution test and evaluation of the correlation between in vivo and in vitro release. *Int J Pharm* 95, 67-75.
- Aoki, S., Uesugi, K., Tatsuishi, K., Ozawa, H., Kayano, M., 1992. Evaluation of the correlation between in vivo and in vitro release of phenylpropanolamine HCl from controlled-release tablets. *Int J Pharm* 85, 65-73.
- Araya, H., Tomita, M., Hayashi, M., 2005. The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds. *Int J Pharm* 305, 61-74.
- Augustijns, P., Brewster, M.E., 2012. Supersaturating drug delivery systems: fast is not necessarily good enough. *J Pharm Sci* 101, 7-9.
- Barakat, N.S., 2010. Enhanced oral bioavailability of etodolac by self-emulsifying systems: in-vitro and in-vivo evaluation. *J Pharm Pharmacol* 62, 173-180.
- Beckett, A.H., Quach, T.T., Kurs, G., 1996. Improved hydrodynamics for USP apparatus 2. *Dissolut Technol* 3, 7-10.

## References

---

- Beig, A., Agbaria, R., Dahan, A., 2013a. Oral delivery of lipophilic drugs: The tradeoff between solubility increase and permeability decrease when using cyclodextrin-based formulations. *Plos One* 8, e68237.
- Beig, A., Miller, J.M., Dahan, A., 2012. Accounting for the solubility-permeability interplay in oral formulation development for poor water solubility drugs: the effect of PEG-400 on carbamazepine absorption. *Eur J Pharm Biopharm* 81, 386-391.
- Beig, A., Miller, J.M., Dahan, A., 2013b. The interaction of nifedipine with selected cyclodextrins and the subsequent solubility-permeability trade-off. *Eur J Pharm Biopharm* 85, 1293-1299.
- Bergström, C.A., Holm, R., Jørgensen, S.A., Andersson, S.B., Artursson, P., Beato, S., Borde, A., Box, K., Brewster, M., Dressman, J., 2014. Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. *Eur J Pharm Sci* 57, 173-199.
- Bevernage, J., Brouwers, J., Annaert, P., Augustijns, P., 2012. Drug precipitation-permeation interplay: supersaturation in an absorptive environment. *Eur J Pharm Biopharm* 82, 424-428.
- Bevernage, J., Brouwers, J., Brewster, M.E., Augustijns, P., 2013. Evaluation of gastrointestinal drug supersaturation and precipitation: strategies and issues. *Int J Pharm* 453, 25-35.
- Bevernage, J., Forier, T., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2011. Excipient-mediated supersaturation stabilization in human intestinal fluids. *Mol Pharm* 8, 564-570.
- Bevernage, J., Hens, B., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2012. Supersaturation in human gastric fluids. *Eur J Pharm Biopharm* 81, 184-189.
- Blanquet, S., Zejdner, 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.
- Box, K., Mole, J., Watson, H., Taylor, Robert., 2016. Biphasic dissolution studies of poorly water soluble drugs. AAPS Annual Meeting and Exposition, November 2016, Denver, USA.

## References

---

- Brewster, M.E., Loftsson, T., 2007. Cyclodextrins as pharmaceutical solubilizers. *Adv Drug Deliver Rev* 59, 645-666.
- Brouwers, J., Anneveld, B., Goudappel, G.J., Duchateau, G., Annaert, P., Augustijns, P., Zeijdner, E., 2011. Food-dependent disintegration of immediate release fosamprenavir tablets: in vitro evaluation using magnetic resonance imaging and a dynamic gastrointestinal system. *Eur J Pharm Biopharm* 77, 313-319.
- Brouwers, J., Augustijns, P., 2014. Resolving intraluminal drug and formulation behavior: gastrointestinal concentration profiling in humans. *Eur J Pharm Sci* 61, 2-10.
- Brouwers, J., Brewster, M.E., Augustijns, P., 2009. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? *J Pharm Sci* 98, 2549-2572.
- Brown, C.K., Chokshi, H.P., Nickerson, B., Reed, R.A., Rohrs, B.R., Shah, P.A., 2004. Dissolution testing of poorly soluble compounds. *Pharm Technol* 28, 56-43.
- Brunner, E., 1903. *Reaktionsgeschwindigkeit in heterogenen Systemen*. Georg-Augusts-Universität, Gottingen.
- Buckley, S.T., Fischer, S.M., Fricker, G., Brandl, M., 2012. In vitro models to evaluate the permeability of poorly soluble drug entities: challenges and perspectives. *Eur J Pharm Sci* 45, 235-250.
- Buckley, S.T., Frank, K.J., Fricker, G., Brandl, M., 2013. Biopharmaceutical classification of poorly soluble drugs with respect to “enabling formulations”. *Eur J Pharm Sci* 50, 8-16.
- Butler, J.M., Dressman, J.B., 2010. The developability classification system: application of biopharmaceutics concepts to formulation development. *J Pharm Sci* 99, 4940-4954.
- Cai, L., Farber, L., Zhang, D., Li, F., Farabaugh, J., 2013. A new methodology for high drug loading wet granulation formulation development. *Int J Pharm* 441, 790-800.
- Carino, S.R., Sperry, D.C., Hawley, M., 2006. Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach-duodenum model. *J Pharm Sci* 95, 116-125.
- Carino, S.R., Sperry, D.C., Hawley, M., 2010. Relative bioavailability of three different solid forms of PNU-141659 as determined with the artificial stomach-duodenum model. *J Pharm Sci* 99, 3923-3930.



## References

---

- Chaumeil, J., 1998. Micronization: a method of improving the bioavailability of poorly soluble drugs. *Method Find Exp Clin* 20, 211-216.
- Chemburkar, S.R., Bauer, J., Deming, K., Spiwek, H., Patel, K., Morris, J., Henry, R., Spanton, S., Dziki, W., Porter, W., 2000. Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development. *Org Process Res Dev* 4, 413-417.
- Chen, H., Khemtong, C., Yang, X., Chang, X., Gao, J., 2011. Nanonization strategies for poorly water-soluble drugs. *Drug Discov Today* 16, 354-360.
- Chieng, N., Rades, T., Aaltonen, J., 2011. An overview of recent studies on the analysis of pharmaceutical polymorphs. *J Pharmaceut Biomed* 55, 618-644.
- Childs, S.L., Kandi, P., Lingireddy, S.R., 2013. Formulation of a danazol cocrystal with controlled supersaturation plays an essential role in improving bioavailability. *Mol Pharm* 10, 3112-3127.
- Clarysse, S., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2011. Intestinal drug solubility estimation based on simulated intestinal fluids: comparison with solubility in human intestinal fluids. *Eur J Pharm Sci* 43, 260-269.
- Cuiné, J.F., Charman, W.N., Pouton, C.W., Edwards, G.A., Porter, C.J., 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.
- Dahan, A., Miller, J.M., 2012. The solubility-permeability interplay and its implications in formulation design and development for poorly soluble drugs. *AAPS J* 14, 244-251.
- Dahan, A., Miller, J.M., Hoffman, A., Amidon, G.E., Amidon, G.L., 2010. The solubility-permeability interplay in using cyclodextrins as pharmaceutical solubilizers: mechanistic modeling and application to progesterone. *J Pharm Sci* 99, 2739-2749.
- Datta, S., Grant, D.J., 2004. Crystal structures of drugs: advances in determination, prediction and engineering. *Nat Rev Drug Discov* 3, 42-57.
- DeSesso, J., Jacobson, C., 2001. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. *Food Chem Toxicol* 39, 209-228.

## References

---

- Dickinson, P.A., Rmaileh, R.A., Ashworth, L., Barker, R.A., Burke, W.M., Patterson, C.M., Stainforth, N., Yasin, M., 2012. An investigation into the utility of a multi-compartmental, dynamic, system of the upper gastrointestinal tract to support formulation development and establish bioequivalence of poorly soluble drugs. *AAPS J* 14, 196-205.
- Dickinson, P.A., Lee, W.W., Stott, P.W., Townsend, A.I., Smart, J.P., Ghahramani, P., Hammett, T., Billett, L., Behn, S., Gibb, R.C., 2008. Clinical relevance of dissolution testing in quality by design. *AAPS J* 10, 380-390
- DiNunzio, J.C., Miller, D.A., Yang, W., McGinity, J.W., Williams III, R.O., 2008. Amorphous compositions using concentration enhancing polymers for improved bioavailability of itraconazole. *Mol Pharm* 5, 968-980.
- Dokoumetzidis, A., Macheras, P., 2006. A century of dissolution research: from Noyes and Whitney to the biopharmaceutics classification system. *Int J Pharm* 321, 1-11.
- Dokoumetzidis, A., Macheras, P., 2008. IVIVC of controlled release formulations: physiological–dynamical reasons for their failure. *J Control Release* 129, 76-78.
- Dressman, J.B., Krämer, J., 2005. *Pharmaceutical dissolution testing*. Taylor & Francis Group, Boca Raton, FL, pp 18-19.
- Dressman, J.B., Reppas, C., 2000. In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. *Eur J Pharm Sci* 11, S73-S80.
- Edwards, L., 1951. The dissolution and diffusion of aspirin in aqueous media. *Transactions of the Faraday Society* 47, 1191-1210.
- El-Zein, H., Riad, L., El-Bary, A.A., 1998. Enhancement of carbamazepine dissolution: in vitro and in vivo evaluation. *Int J Pharm* 168, 209-220.
- Farber, L., Tardos, G.I., Michaels, J.N., 2005. Micro-mechanical properties of drying material bridges of pharmaceutical excipients. *Int J Pharm* 306, 41-55.
- Fatouros, D.G., Nielsen, F.S., Douroumis, D., Hadjileontiadis, L.J., Mullertz, A., 2008. In vitro-in vivo correlations of self-emulsifying drug delivery systems combining the dynamic lipolysis model and neuro-fuzzy networks. *Eur J Pharm Biopharm* 69, 887-898.
- FDA, 1997. *Guidance for Industry: Extended release oral dosage forms: Development, evaluation, and application of in vitro/in vivo correlations*. Food and Drug Administration, Rockville, MD.

## References

---

- FDA, 2000. Guidance for industry: waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Food and Drug Administration, Rockville, MD.
- FDA, 2001. Guidance for Industry: Bioanalytical Method Validation. Food and Drug Administration, Rockville, MD.
- FDA, 2015. Guidance for industry, waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. Food and Drug Administration, Rockville, MD.
- Fischer, S.M., Flaten, G.E., Hagesæther, E., Fricker, G., Brandl, M., 2011. In-vitro permeability of poorly water soluble drugs in the phospholipid vesicle-based permeation assay: the influence of nonionic surfactants. *J Pharm Pharmacol* 63, 1022-1030.
- Fliszar, K.A., Foster, N., 2008. Examination of metformin hydrochloride in a continuous dissolution/HDM system. *Int J Pharm* 351, 127-132.
- Frank, K.J., Locher, K., Zecevic, D.E., Fleth, J., Wagner, K.G., 2014. In vivo predictive mini-scale dissolution for weak bases: Advantages of pH-shift in combination with an absorptive compartment. *Eur J Pharm Sci* 61, 32-39.
- Galia, E., Nicolaidis, E., Hörter, D., Löbenberg, R., Reppas, C., Dressman, J., 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm Res* 15, 698-705.
- Garala, K.C., Patel, J.M., Dhingani, A.P., Dharamsi, A.T., 2013. Quality by design (QbD) approach for developing agglomerates containing racecadotril and loperamide hydrochloride by crystallo-co-agglomeration. *Powder Technol* 247, 128-146.
- Garbacz, G., Blume, H., Weitschies, W., 2009a. Investigation of the dissolution characteristics of nifedipine extended-release formulations using USP apparatus 2 and a novel dissolution apparatus. *Dissolut Technol* 16, 7-13.
- Garbacz, G., Golke, B., Wedemeyer, R.S., Axell, M., Söderlind, E., Abrahamsson, B., Weitschies, W., 2009b. Comparison of dissolution profiles obtained from nifedipine extended release once a day products using different dissolution test apparatuses. *Eur J Pharm Sci* 38, 147-155.

## References

---

- Garbacz, G., Wedemeyer, R.S., Nagel, S., Giessmann, T., Mönnikes, H., Wilson, C.G., Siegmund, W., Weitschies, W., 2008. Irregular absorption profiles observed from diclofenac extended release tablets can be predicted using a dissolution test apparatus that mimics in vivo physical stresses. *Eur J Pharm Biopharm* 70, 421-428.
- Gibaldi, M., Feldman, S., 1967. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. *J Pharm Sci* 56, 1238-1242.
- Ginski, M.J., Taneja, R., Polli, J.E., 1999. Prediction of dissolution-absorption relationships from a continuous dissolution/Caco-2 system. *AAPS PharmSci* 1, 27-38.
- Ghaderi, R., Artursson, P., Carlfors, J., 1999. Preparation of biodegradable microparticles using solution-enhanced dispersion by supercritical fluids (SEDS). *Pharm Res* 16, 676-681.
- Ghebremeskel, A.N., Vemavarapu, C., Lodaya, M., 2007. Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble API: selection of polymer-surfactant combinations using solubility parameters and testing the processability. *Int J Pharm* 328, 119-129.
- Grundy, J.S., Anderson, K.E., Rogers, J.A., Foster, R.T., 1997a. Studies on dissolution testing of the nifedipine gastrointestinal therapeutic system. I. Description of a two-phase in vitro dissolution test. *J Control Release* 48, 1-8.
- Grundy, J.S., Anderson, K.E., Rogers, J.A., Foster, R.T., 1997b. Studies on dissolution testing of the nifedipine gastrointestinal therapeutic system. II. Improved in vitro-in vivo correlation using a two-phase dissolution test. *J Control Release* 48, 9-17.
- Grzesiak, A.L., Lang, M., Kim, K., Matzger, A.J., 2003. Comparison of the four anhydrous polymorphs of carbamazepine and the crystal structure of form I. *J Pharm Sci* 92, 2260-2271.
- Gu, C.H., Gandhi, R.B., Tay, L.K., Zhou, S., Raghavan, K., 2004. Importance of using physiologically relevant volume of dissolution medium to correlate the oral exposure of formulations of BMS-480188 mesylate. *Int J Pharm* 269, 195-202.

## References

---

- Guzmán, H.R., Tawa, M., Zhang, Z., Ratanabanangkoon, P., Shaw, P., Gardner, C.R., Chen, H., Moreau, J.P., Almarsson, Ö., 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.
- Harris, R.K., Ghi, P.Y., Puschmann, H., Apperley, D.C., Griesser, U.J., Hammond, R.B., Ma, C., Roberts, K.J., Pearce, G.J., Yates, J.R., 2005. Structural studies of the polymorphs of carbamazepine, its dihydrate, and two solvates. *Org Process Res Dev* 9, 902-910.
- Hörter, D., Dressman, J., 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv Drug Deliver Rev* 46, 75-87.
- Harris, R.K., Ghi, P.Y., Puschmann, H., Apperley, D.C., Griesser, U.J., Hammond, R.B., Ma, C., Roberts, K.J., Pearce, G.J., Yates, J.R., 2005. Structural studies of the polymorphs of carbamazepine, its dihydrate, and two solvates. *Org Process Res Dev* 9, 902-910.
- Heigoldt, U., Sommer, F., Daniels, R., Wagner, K.G., 2010. Predicting in vivo absorption behavior of oral modified release dosage forms containing pH-dependent poorly soluble drugs using a novel pH-adjusted biphasic in vitro dissolution test. *Eur J Pharm Biopharm* 76, 105-111.
- Hoa, N.T., Kinget, R., 1996. Design and evaluation of two-phase partition-dissolution method and its use in evaluating artemisinin tablets. *J Pharm Sci* 85, 1060-1063.
- Horter, D., Dressman, J.B., 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv Drug Deliver Rev* 46, 75-87.
- Hou, J., He, X., Xu, X., Shi, X., Xu, Y., Liu, C., 2012. Application of an in vitro DDASS to evaluate oral absorption of two chemicals simultaneously: establishment of a level A in vitro-in vivo correlation. *Drug Dev Ind Pharm* 38, 1305-1312.
- Huang, L.F., Tong, W.Q., 2004. Impact of solid state properties on developability assessment of drug candidates. *Adv Drug Deliver Rev* 56, 321-334.
- Jain, P., Yalkowsky, S.H., 2007. Solubilization of poorly soluble compounds using 2-pyrrolidone. *Int J Pharm* 342, 1-5.
- Janssens, S., De Zeure, A., Paudel, A., Van Humbeeck, J., Rombaut, P., Van den Mooter, G., 2010. Influence of preparation methods on solid state supersaturation of amorphous solid dispersions: a case study with itraconazole and eudragit E100. *Pharm Res* 27, 775-785.

## References

---

- Jantratid, E., Janssen, N., Reppas, C., Dressman, J.B., 2008. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm Res* 25, 1663-1676.
- Jinno, J.i., Kamada, N., Miyake, M., Yamada, K., Mukai, T., Odomi, M., Toguchi, H., Liversidge, G.G., Higaki, K., Kimura, T., 2006. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. *J Control Release* 111, 56-64.
- Johnson, K.C., Swindell, A.C., 1996. Guidance in the setting of drug particle size specifications to minimize variability in absorption. *Pharm Res* 13, 1795-1798.
- Juang, R.H., Storey, D., 2003. Correlation of characteristics of gel extrusion module (GEM) tablet formulation and drug dissolution rate. *J Control Release* 89, 375-385.
- Kahela, P., Aaltonen, R., Lewing, E., Anttila, M., Kristoffersson, E., 1983. Pharmacokinetics and dissolution of two crystalline forms of carbamazepine. *Int J Pharm* 14, 103-112.
- Karavas, E., Ktistis, G., Xenakis, A., Georgarakis, E., 2006. Effect of hydrogen bonding interactions on the release mechanism of felodipine from nanodispersions with polyvinylpyrrolidone. *Eur J Pharm Biopharm* 63, 103-114.
- Katneni, K., Charman, S.A., Porter, C.J., 2006. Permeability assessment of poorly water-soluble compounds under solubilizing conditions: The reciprocal permeability approach. *J Pharm Sci* 95, 2170-2185.
- Kawabata, Y., Wada, K., Nakatani, M., Yamada, S., Onoue, S., 2011. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications. *Int J Pharm* 420, 1-10.
- Kinget, R., Degreef, H., 1994. Absorption characteristics of novel 8-Mop semi-solid-lipid-matrix formulations in vitro in vivo correlation. *Int J Pharm* 110, 65-73.
- Klein, S., 2010. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. *AAPS J* 12, 397-406.
- Klein, S., Buchanan, N.L., Buchanan, C.M., 2012. Miniaturized transfer models to predict the precipitation of poorly soluble weak bases upon entry into the small intestine. *AAPS Pharmscitech* 13, 1230-1235.

## References

---

- Knopp, M.M., Nguyen, J.H., Becker, C., Francke, N.M., Jørgensen, E.B., Holm, P., Holm, R., Mu, H., Rades, T., Langguth, P., 2016. Influence of polymer molecular weight on in vitro dissolution behavior and in vivo performance of celecoxib: PVP amorphous solid dispersions. *Eur J Pharm Biopharm* 101, 145-151.
- 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.
- Kobayashi, Y., Ito, S., Itai, S., Yamamoto, K., 2000. Physicochemical properties and bioavailability of carbamazepine polymorphs and dihydrate. *Int J Pharm* 193, 137-146.
- Kohli, K., Chopra, S., Dhar, D., Arora, S., Khar, R.K., 2010. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. *Drug Discov Today* 15, 958-965.
- Kostewicz, E.S., Abrahamsson, B., Brewster, M., Brouwers, J., Butler, J., Carlert, S., Dickinson, P.A., Dressman, J., Holm, R., Klein, S., Mann, J., McAllister, M., Minekus, M., Muenster, U., Mullertz, A., Verwei, M., Vertzoni, M., Weitschies, W., Augustijns, P., 2014. In vitro models for the prediction of in vivo performance of oral dosage forms. *Eur J Pharm Sci* 57, 342-366.
- 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.
- Kostewicz, E.S., Wunderlich, M., Brauns, U., Becker, R., Bock, T., Dressman, J.B., 2004. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. *J Pharm Pharmacol* 56, 43-51.
- Kovačević, I., Parojcic, J., Homšek, I., Tubić-Grozdaniš, M., Langguth, P., 2008. Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. *Mol Pharm* 6, 40-47.
- Koziolek, M., Gorke, K., Neumann, M., Garbacz, G., Weitschies, W., 2014. Development of a bio-relevant dissolution test device simulating mechanical aspects present in the fed stomach. *Eur J Pharm Sci* 57, 250-256.
- Ku, M.S., 2008. Use of the biopharmaceutical classification system in early drug development. *AAPS J* 10, 208-212.

## References

---

- Ku, M.S., Dulin, W., 2012. A biopharmaceutical classification-based right-first-time formulation approach to reduce human pharmacokinetic variability and project cycle time from first-in-human to clinical proof-of-concept. *Pharm Dev Technol* 17, 285-302.
- Lake, O., Olling, M., Barends, D., 1999. In vitro/in vivo correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers. *Eur J Pharm Biopharm* 48, 13-19.
- Lawrence, X.Y., 1999. An integrated model for determining causes of poor oral drug absorption. *Pharm Res* 16, 1883-1887.
- Lee, A.Y., Erdemir, D., Myerson, A.S., 2011. Crystal polymorphism in chemical process development. *Annu Rev Chem Biomol* 2, 259-280.
- Lehto, P., Aaltonen, J., Tenho, M., Rantanen, J., Hirvonen, J., Tanninen, V.P., Peltonen, L., 2009. Solvent-mediated solid phase transformations of carbamazepine: Effects of simulated intestinal fluid and fasted state simulated intestinal fluid. *J Pharm Sci* 98, 985-996.
- Lehto, P., Kortejärvi, H., Liimatainen, A., Ojala, K., Kangas, H., Hirvonen, J., Tanninen, V.P., Peltonen, L., 2011. Use of conventional surfactant media as surrogates for FaSSIF in simulating in vivo dissolution of BCS class II drugs. *Eur J Pharm Biopharm* 78, 531-538.
- Leuner, C., Dressman, J., 2000. Improving drug solubility for oral delivery using solid dispersions. *Eur J Pharm Biopharm* 50, 47-60.
- Levy, G., 1966. Papers Presented Before the Industrial Pharmacy Section, American Pharmaceutical Association: Dallas Meeting, Dallas, USA, pp. 233-252.
- Li, Z.Q., He, X., Gao, X., Xu, Y.Y., Wang, Y.F., Gu, H., Ji, R.F., Sun, S.J., 2011. Study on dissolution and absorption of four dosage forms of isosorbide mononitrate: Level A in vitro-in vivo correlation. *Eur J Pharm Biopharm* 79, 364-371.
- Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2012. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliver Rev* 64, 4-17.
- Liu, C.H., Kao, Y.H., Chen, S.C., Sokoloski, T.D., Sheu, M.T., 1995. In-vitro and in vivo studies of the diclofenac sodium controlled-release matrix tablets. *J Pharm Pharmacol* 47, 360-364.



## References

---

- Liversidge, G.G., Cundy, K.C., 1995. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. *Int J Pharm* 125, 91-97.
- Lloyd, G., Craig, D., Smith, A., 1999. A calorimetric investigation into the interaction between paracetamol and polyethylene glycol 4000 in physical mixes and solid dispersions. *Eur J Pharm Biopharm* 48, 59-65.
- Locher, K., Borghardt, J.M., Frank, K.J., Kloft, C., Wagner, K.G., 2016. Evolution of a mini-scale biphasic dissolution model: Impact of model parameters on partitioning of dissolved API and modelling of in vivo-relevant kinetics. *Eur J Pharm Biopharm* 105, 166-175.
- Marciani, L., Gowland, P.A., Fillery-Travis, A., Manoj, P., Wright, J., Smith, A., Young, P., Moore, R., Spiller, R.C., 2001a. Assessment of antral grinding of a model solid meal with echo-planar imaging. *Am J Physiol-Gastr L* 280, G844-G849.
- Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., Al-Sahab, S., Bush, D., Wright, J., Fillery-Travis, A.J., 2000. Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. *J Nutr* 130, 122-127.
- Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., Fillery-Travis, A.J., 2001b. Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *Am J Physiol-Gastr L* 280, G1227-G1233.
- Marciani, L., Wickham, M., Hills, B., Wright, J., Bush, D., Faulks, R., Fillery-Travis, A., Spiller, R., Gowl, P., 2004. Intragastric oil-in-water emulsion fat fraction measured using inversion recovery echo-planar magnetic resonance imaging. *J Food Sci* 69, E290-E296.
- McAllister, M., 2010. Dynamic dissolution: a step closer to predictive dissolution testing? *Mol Pharm* 7, 1374-1387.
- McConnell, E.L., Fadda, H.M., Basit, A.W., 2008. Gut instincts: explorations in intestinal physiology and drug delivery. *Int J Pharm* 364, 213-226.
- Mcmahon, L.E., Timmins, P., Williams, A.C., York, P., 1996. Characterization of dihydrates prepared from carbamazepine polymorphs. *J Pharm Sci* 85, 1064-1069.

## References

---

- Mehuys, E., Vervaet, C., Gielen, I., Van Bree, H., Remon, J.P., 2004. In vitro and in vivo evaluation of a matrix-in-cylinder system for sustained drug delivery. *J Control Release* 96, 261-271.
- Mellaerts, R., Mols, R., Jammaer, J.A., Aerts, C.A., Annaert, P., Van Humbeeck, J., Van den Mooter, G., Augustijns, P., Martens, J.A., 2008. Increasing the oral bioavailability of the poorly water soluble drug itraconazole with ordered mesoporous silica. *Eur J Pharm Biopharm* 69, 223-230.
- Mercuri, A., Lo Curto, A., Wickham, M., Craig, D., Barker, S., 2008. Dynamic gastric model (DGM): a novel in vitro apparatus to assess the impact of gastric digestion on the droplet size of self-emulsifying drug-delivery systems. *J Pharm Pharmacol* 60, A2.
- Mercuri, A., Passalacqua, A., Wickham, M.S., Faulks, R.M., Craig, D.Q., Barker, S.A., 2011. The effect of composition and gastric conditions on the self-emulsification process of ibuprofen-loaded self-emulsifying drug delivery systems: a microscopic and dynamic gastric model study. *Pharm Res* 28, 1540-1551.
- Meyer, M.C., Straughn, A.B., Jarvi, E.J., Wood, G.C., Pelsor, F.R., Shah, V.P., 1992. The bioequivalence of carbamazepine tablets with a history of clinical failures. *Pharm Res* 9, 1612-1616.
- Millard, J.W., Alvarez-Nunez, F., Yalkowsky, S.H., 2002. Solubilization by cosolvents: Establishing useful constants for the log-linear model. *Int J Pharm* 245, 153-166.
- Miller, D.A., DiNunzio, J.C., Yang, W., McGinity, J.W., Williams III, R.O., 2008a. Enhanced in vivo absorption of itraconazole via stabilization of supersaturation following acidic-to-neutral pH transition. *Drug Dev Ind Pharm* 34, 890-902.
- Miller, D.A., DiNunzio, J.C., Yang, W., McGinity, J.W., Williams III, R.O., 2008b. Targeted intestinal delivery of supersaturated itraconazole for improved oral absorption. *Pharm Res* 25, 1450-1459.
- Miller, D.A., McConville, J.T., Yang, W., Williams, R.O., McGinity, J.W., 2007. Hot-melt extrusion for enhanced delivery of drug particles. *J Pharm Sci* 96, 361-376.

## References

---

- Miller, J.M., Beig, A., Carr, R.A., Spence, J.K., Dahan, A., 2012a. A win-win solution in oral delivery of lipophilic drugs: supersaturation via amorphous solid dispersions increases apparent solubility without sacrifice of intestinal membrane permeability. *Mol Pharm* 9, 2009-2016.
- Miller, J.M., Beig, A., Carr, R.A., Webster, G.K., Dahan, A., 2012b. The solubility-permeability interplay when using cosolvents for solubilization: revising the way we use solubility-enabling formulations. *Mol Pharm* 9, 581-590.
- Miller, J.M., Beig, A., Krieg, B.J., Carr, R.A., Borchardt, T.B., Amidon, G.E., Amidon, G.L., Dahan, A., 2011. The solubility-permeability interplay: mechanistic modeling and predictive application of the impact of micellar solubilization on intestinal permeation. *Mol Pharm* 8, 1848-1856.
- Minekus, M., Marteau, P., Havenaar, R., Huis in 't Veld, J.H.H., 1995. Multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Atla* 23, 197-209.
- Mu, H., Holm, R., Müllertz, A., 2013. Lipid-based formulations for oral administration of poorly water-soluble drugs. *Int J Pharm* 453, 215-224.
- Mudie, D.M., Amidon, G.L., Amidon, G.E., 2010. Physiological parameters for oral delivery and in vitro testing. *Mol Pharm* 7, 1388-1405.
- Mudie, D.M., Shi, Y., Ping, H., Gao, P., Amidon, G.L., Amidon, G.E., 2012. Mechanistic analysis of solute transport in an in vitro physiological two-phase dissolution apparatus. *Biopharm Drug Dispos* 33, 378-402.
- Mullard, A., 2013. 2012 FDA drug approvals. *Nat Rev Drug Discov* 12, 87-90.
- Mullard, A., 2014. 2013 FDA drug approvals. *Nat Rev Drug Discov* 13, 85-89.
- Mullard, A., 2015. 2014 FDA drug approvals. *Nat Rev Drug Discov* 14, 77-81.
- Mullard, A., 2016. 2015 FDA drug approvals. *Nat Rev Drug Discov* 15, 73-76.
- Murphy, D., Rodríguez-Cintrón, F., Langevin, B., Kelly, R.C., Rodríguez-Hornedo, N., 2002. Solution-mediated phase transformation of anhydrous to dihydrate carbamazepine and the effect of lattice disorder. *Int J Pharm* 246, 121-134.
- Nair, R., Gonen, S., Hoag, S.W., 2002. Influence of polyethylene glycol and povidone on the polymorphic transformation and solubility of carbamazepine. *Int J Pharm* 240, 11-22.

## References

---

- Newman, A., Knipp, G., Zografi, G., 2012. Assessing the performance of amorphous solid dispersions. *J Pharm Sci* 101, 1355-1377.
- Nicolaides, E., Symillides, M., Dressman, J.B., Reppas, C., 2001. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. *Pharm Res* 18, 380-388.
- Noyes, A.A., Whitney, W.R., 1897. The rate of solution of solid substances in their own solutions. *J Am Chem Soc* 19, 930-934.
- Panchagnula, R., Thomas, N.S., 2000. Biopharmaceutics and pharmacokinetics in drug research. *Int J Pharm* 201, 131-150.
- Parikh, D.M., 2016. Handbook of pharmaceutical granulation technology. CRC Press.
- Park, K., 2014. Absence of in vivo-in vitro correlation in per-oral drug delivery. *J Control Release* 180, 150.
- Peeters, J., Neeskens, P., Tollenaere, J.P., Van Remoortere, P., Brewster, M.E., 2002. Characterization of the interaction of 2-hydroxypropyl- $\beta$ -cyclodextrin with itraconazole at pH 2, 4, and 7. *J Pharm Sci* 91, 1414-1422.
- Pestieau, A., Krier, F., Brouwers, A., Streel, B., Evrard, B., 2016. Selection of a discriminant and biorelevant in vitro dissolution test for the development of fenofibrate self-emulsifying lipid-based formulations. *Eur J Pharm Sci* 92, 212-219.
- Pestieau, A., Lebrun, S., Cahay, B., Brouwers, A., Streel, B., Cardot, J.M., Evrard, B., 2017. Evaluation of different in vitro dissolution tests based on level A in vitro-in vivo correlations for fenofibrate self-emulsifying lipid-based formulations. *Eur J Pharm Biopharm* 112, 18-29.
- Phillips, D.J., Pygall, S.R., Cooper, V.B., Mann, J.C., 2012a. Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. *J Pharm Pharmacol* 64, 1549-1559.
- Phillips, D.J., Pygall, S.R., Cooper, V.B., Mann, J.C., 2012b. Toward biorelevant dissolution: application of a biphasic dissolution model as a discriminating tool for HPMC matrices containing a model BCS class II drug. *Dissolut Technol* 19, 25-34.

## References

---

- Piao, Z.Z., Choe, J.S., Oh, K.T., Rhee, Y.S., Lee, B.J., 2014. Formulation and in vivo human bioavailability of dissolving tablets containing a self-nanoemulsifying itraconazole solid dispersion without precipitation in simulated gastrointestinal fluid. *Eur J Pharm Sci* 51, 67-74.
- Polster, C.S., Atassi, F., Wu, S.J., Sperry, D.C., 2010. Use of artificial stomach-duodenum model for investigation of dosing fluid effect on clinical trial variability. *Mol Pharm* 7, 1533-1538.
- Porter, C.J., Kaukonen, A.M., Boyd, B.J., Edwards, G.A., Charman, W.N., 2004. 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., 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.
- Prabu, S.L., Singh, T., Joseph, A., Kumar, C.D., Shirwaikar, A., 2007. Determination of racecadotril by HPLC in capsules. *Indian J Pharm Sci* 69, 819.
- Qian, F., Wang, J., Hartley, R., Tao, J., Haddadin, R., Mathias, N., Hussain, M., 2012. Solution behavior of PVP-VA and HPMC-AS-based amorphous solid dispersions and their bioavailability implications. *Pharm Res* 29, 2766-2776.
- Qiu, Y., Chen, Y., Zhang, G.G., Liu, L., Porter, W., 2009. Developing solid oral dosage forms: pharmaceutical theory & practice. Academic press, San Diego.
- Qureshi, S.A., 2004. A new crescent-shaped spindle for drug dissolution testing-but why a new spindle? *Dissolut Technol* 11, 13-21.
- Rautio, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Järvinen, T., Savolainen, J., 2008. Prodrugs: design and clinical applications. *Nat Rev Drug Discov* 7, 255-270.
- Riad, L.E., Sawchuk, R.J., 1991. Effect of polyethylene glycol 400 on the intestinal permeability of carbamazepine in the rabbit. *Pharm Res* 8, 491-497.
- Rustichelli, C., Gamberini, G., Ferioli, V., Gamberini, M.C., Ficarra, R., Tommasini, S., 2000. Solid-state study of polymorphic drugs: carbamazepine. *J Pharm Biomed Anal* 23, 41-54.

## References

---

- Sarnes, A., Kovalainen, M., Häkkinen, M.R., Laaksonen, T., Laru, J., Kiesvaara, J., Ilkka, J., Oksala, O., Rönkkö, S., Järvinen, K., 2014. Nanocrystal-based per-oral itraconazole delivery: superior in vitro dissolution enhancement versus Sporanox® is not realized in in vivo drug absorption. *J Control Release* 180, 109-116.
- Santos, O.M.M., Reis, M.E.D., Jacon, J.T., Lino, M.E.d.S., Simões, J.S., Doriguetto, A.C., 2014. Polymorphism: an evaluation of the potential risk to the quality of drug products from the Farmácia Popular Rede Própria. *Brazilian J Pharm Sci* 50, 1-24.
- Sarode, A.L., Wang, P., Obara, S., Worthen, D.R., 2014. Supersaturation, nucleation, and crystal growth during single- and biphasic dissolution of amorphous solid dispersions: polymer effects and implications for oral bioavailability enhancement of poorly water soluble drugs. *Eur J Pharm Biopharm* 86, 351-360.
- Šehić, S., Betz, G., Hadžidedić, Š., El-Arini, S.K., Leuenberger, H., 2010. Investigation of intrinsic dissolution behavior of different carbamazepine samples. *Int J Pharm* 386, 77-90.
- Serajuddin, A.T., 2007. Salt formation to improve drug solubility. *Adv Drug Deliver Rev* 59, 603-616.
- Shah, V.P., Konecny, J.J., Everett, R.L., McCullough, B., Noorizadeh, A.C., Skelly, J.P., 1989. In vitro dissolution profile of water-insoluble drug dosage forms in the presence of surfactants. *Pharm Res* 6, 612-618.
- Shegokar, R., Müller, R.H., 2010. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. *Int J Pharm* 399, 129-139.
- Shi, Y., Gao, P., Gong, Y., Ping, H., 2010. Application of a biphasic test for characterization of in vitro drug release of immediate release formulations of celecoxib and its relevance to in vivo absorption. *Mol Pharm* 7, 1458-1465.
- Singh, N., Narayan, S., 2008. Racecadotril: A novel antidiarrheal. *Medical Journal Armed Forces India* 64, 361-362.
- Singhal, D., Curatolo, W., 2004. Drug polymorphism and dosage form design: a practical perspective. *Adv Drug Deliver Rev* 56, 335-347.
- Sinswat, P., Gao, X., Yacaman, M.J., Williams Iii, R.O., Johnston, K.P., 2005. Stabilizer choice for rapid dissolving high potency itraconazole particles formed by evaporative precipitation into aqueous solution. *Int J Pharm* 302, 113-124.

## References

---

- Six, K., Berghmans, H., Leuner, C., Dressman, J., Van Werde, K., Mullens, J., Benoist, L., Thimon, M., Meublât, L., Verreck, G., 2003a. Characterization of solid dispersions of itraconazole and hydroxypropylmethylcellulose prepared by melt extrusion, part II. *Pharm Res* 20, 1047-1054.
- Six, K., Daems, T., de Hoon, J., Van Hecken, A., Depre, M., Bouche, M.P., Prinsen, P., Verreck, G., Peeters, J., Brewster, M.E., Van den Mooter, G., 2005. Clinical study of solid dispersions of itraconazole prepared by hot-stage extrusion. *Eur J Pharm Sci* 24, 179-186.
- Six, K., Murphy, J., Weuts, I., Craig, D.Q., Verreck, G., Peeters, J., Brewster, M., Van den Mooter, G., 2003b. Identification of phase separation in solid dispersions of itraconazole and Eudragit® E100 using microthermal analysis. *Pharm Res* 20, 135-138.
- Six, K., Verreck, G., Peeters, J., Binnemans, K., Berghmans, H., Augustijns, P., Kinget, R., Van den Mooter, G., 2001. Investigation of thermal properties of glassy itraconazole: identification of a monotropic mesophase. *Thermochim Acta* 376, 175-181.
- Six, K., Verreck, G., Peeters, J., Brewster, M., Mooter, G.V.d., 2004. Increased physical stability and improved dissolution properties of itraconazole, a class II drug, by solid dispersions that combine fast-and slow-dissolving polymers. *J Pharm Sci* 93, 124-131.
- Smith, R.N., Hansch, C., Ames, M.M., 1975. Selection of a reference partitioning system for drug design work. *J Pharm Sci* 64, 599-606.
- Song, Y., Wang, L., Yang, P., Wenslow, R.M., Tan, B., Zhang, H., Deng, Z., 2013. Physicochemical characterization of felodipine Kollidon VA64 amorphous solid dispersions prepared by hot-melt extrusion. *J Pharm Sci* 102, 1915-1923.
- Stahly, G.P., 2007. Diversity in single-and multiple-component crystals. The search for and prevalence of polymorphs and cocrystals. *Cryst Growth Des* 7, 1007-1026.
- Sugawara, M., Kadomura, S., He, X., Takekuma, Y., Kohri, N., Miyazaki, K., 2005. The use of an in vitro dissolution and absorption system to evaluate oral absorption of two weak bases in pH-independent controlled-release formulations. *Eur J Pharm Sci* 26, 1-8.
- Sunesen, V.H., Pedersen, B.L., Kristensen, H.G., Müllertz, 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.

## References

---

- Swanepoel, E., Liebenberg, W., de Villiers, M.M., 2003. Quality evaluation of generic drugs by dissolution test: changing the USP dissolution medium to distinguish between active and non-active mebendazole polymorphs. *Eur J Pharm Biopharm* 55, 345-349.
- 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.
- Taylor, L.S., Zografi, G., 1997. Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharm Res* 14, 1691-1698.
- Thakuria, R., Delori, A., Jones, W., Lipert, M.P., Roy, L., Rodríguez-Hornedo, N., 2013. Pharmaceutical cocrystals and poorly soluble drugs. *Int J Pharm* 453, 101-125.
- Thiry, J., Broze, G., Pestieau, A., Tatton, A.S., Baumans, F., Damblon, C., Krier, F., Evrard, B., 2016. Investigation of a suitable in vitro dissolution test for itraconazole-based solid dispersions. *Eur J Pharm Sci* 85, 94-105.
- Thomas, N., Holm, R., Rades, T., Müllertz, A., 2012. Characterising lipid lipolysis and its implication in lipid-based formulation development. *AAPS J* 14, 860-871.
- Tian, F., Zeitler, J.A., Strachan, C.J., Saville, D.J., Gordon, K.C., Rades, T., 2006. Characterizing the conversion kinetics of carbamazepine polymorphs to the dihydrate in aqueous suspension using Raman spectroscopy. *J Pharmaceut Biomed* 40, 271-280.
- Tsume, Y., Mudie, D.M., Langguth, P., Amidon, G.E., Amidon, G.L., 2014. The Biopharmaceutics Classification System: subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC. *Eur J Pharm Sci* 57, 152-163.
- Tsume, Y., Takeuchi, S., Matsui, K., Amidon, G.E., Amidon, G.L., 2015. In vitro dissolution methodology, mini-Gastrointestinal Simulator (mGIS), predicts better in vivo dissolution of a weak base drug, dasatinib. *Eur J Pharm Sci* 76, 203-212.
- Ueda, K., Higashi, K., Limwikrant, W., Sekine, S., Horie, T., Yamamoto, K., Moribe, K., 2012. Mechanistic differences in permeation behavior of supersaturated and solubilized solutions of carbamazepine revealed by nuclear magnetic resonance measurements. *Mol Pharm* 9, 3023-3033.
- USP, G.C., 2011. <711> Dissolution. United States Pharmacopeia.



## References

---

- USP, U.P., 2001. <724> Drug Release, Rockville, MD: United States Pharmacopeial Convention.
- Valvani, S., Yalkowsky, S.H., Roseman, T., 1981. Solubility and partitioning IV: Aqueous solubility and octanol-water partition coefficients of liquid nonelectrolytes. *J Pharm Sci* 70, 502-507.
- Van den Mooter, G., 2012. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug Discov Today* 9, e79-e85.
- Vangani, S., Li, X., Zhou, P., Del-Barrio, M.A., Chiu, R., Cauchon, N., Gao, P., Medina, C., Jasti, B., 2009. Dissolution of poorly water-soluble drugs in biphasic media using USP 4 and fiber optic system. *Clin Res Regul Aff* 26, 8-19.
- Van Speybroeck, M., Mols, R., Mellaerts, R., Do Thi, T., Martens, J.A., Van Humbeeck, J., Annaert, P., Van den Mooter, G., Augustijns, P., 2010. Combined use of ordered mesoporous silica and precipitation inhibitors for improved oral absorption of the poorly soluble weak base itraconazole. *Eur J Pharm Biopharm* 75, 354-365.
- Vardakou, M., Mercuri, A., Barker, S.A., Craig, D.Q., Faulks, R.M., Wickham, M.S., 2011. Achieving antral grinding forces in biorelevant in vitro models: comparing the USP dissolution apparatus II and the dynamic gastric model with human in vivo data. *AAPS Pharmscitech* 12, 620-626.
- Vasconcelos, T., Sarmiento, B., Costa, P., 2007. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. *Drug Discov Today* 12, 1068-1075.
- Vatier, J., MalikovaSekera, E., Vitre, M., Mignon, M., 1992. An artificial stomach-duodenum model for the in vitro evaluation of antacids. *Aliment Pharm Therap* 6, 447-458.
- Verreck, G., Six, K., Van den Mooter, G., Baert, L., Peeters, J., Brewster, M.E., 2003. Characterization of solid dispersions of itraconazole and hydroxypropylmethylcellulose prepared by melt extrusion-part I. *Int J Pharm* 251, 165-174.
- Vertzoni, M., Dressman, J., Butler, J., Hempenstall, J., Reppas, C., 2005. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. *Eur J Pharm Biopharm* 60, 413-417.
- Vertzoni, M., Pastelli, E., Psachoulias, D., Kalantzi, L., Reppas, C., 2007. Estimation of intragastric solubility of drugs: in what medium? *Pharm Res* 24, 909-917.

## References

---

- Vo, C.L., Park, C., Lee, B.J., 2013. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. *Eur J Pharm Biopharm* 85, 799-813.
- Wickham, M., Faulks, R., Mann, J., Mandalari, G., 2012. The design, operation, and application of a dynamic gastric model. *Dissolution Technol* 19, 15-22.
- Wickham, M., Faulks, R., Mills, C., 2009. In vitro digestion methods for assessing the effect of food structure on allergen breakdown. *Mol Nutr Food Res* 53, 952-958.
- Wong, S.M., Kellaway, I.W., Murdan, S., 2006. Fast-dissolving microparticles fail to show improved oral bioavailability. *J Pharm Pharmacol* 58, 1319-1326.
- Wu, C.Y., Benet, L.Z., 2005. Predicting drug disposition via application of BCS: Transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res* 22, 11-23.
- Wurster, D.E., Polli, G.P., 1961. Investigation of drug release from solids IV. Influence of adsorption on the dissolution rate. *J Pharm Sci* 50, 403-406.
- Xu, F., Yang, L., Xu, G., 2008. A rapid and validated HPLC method to quantify racecadotril metabolite, thiorphan, in human plasma using solid-phase extraction. *J Chromatogr B* 861, 130-135.
- Xu, S., Dai, W.G., 2013. Drug precipitation inhibitors in supersaturable formulations. *Int J Pharm* 453, 36-43.
- Yalkowsky, S., Rubino, J.T., 1985. Solubilization by cosolvents I: organic solutes in propylene glycol-water mixtures. *J Pharm Sci* 74, 416-421.
- Yalkowsky, S.H., Flynn, G., Amidon, G., 1972. Solubility of nonelectrolytes in polar solvents. *J Pharm Sci* 61, 983-984.
- Yamashita, S., Furubayashi, T., Kataoka, M., Sakane, T., Sezaki, H., Tokuda, H., 2000. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. *Eur J Pharm Sci* 10, 195-204.
- Yamashita, T., Ozaki, S., Kushida, I., 2011. Solvent shift method for anti-precipitant screening of poorly soluble drugs using biorelevant medium and dimethyl sulfoxide. *Int J Pharm* 419, 170-174.

## References

---

- Zakeri-Milani, P., Barzegar-Jalali, M., Azimi, M., Valizadeh, H., 2009. Biopharmaceutical classification of drugs using intrinsic dissolution rate (IDR) and rat intestinal permeability. *Eur J Pharm Biopharm* 73, 102-106.
- Zhang, G.G., Law, D., Schmitt, E.A., Qiu, Y., 2004. Phase transformation considerations during process development and manufacture of solid oral dosage forms. *Adv Drug Deliver Rev* 56, 371-390.
- Zhang, Y.E., Tchao, R., Schwartz, J.B., 2001. Effect of processing methods and heat treatment on the formation of wax matrix tablets for sustained drug release. *Pharm Dev Technol* 6, 131-144.
- Zhang, Y., Huo, M., Zhou, J., Xie, S., 2010. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput Meth Prog Bio* 99, 306-314.
- Zhou, Y., Chu, W., Lei, M., Li, J., Du, W., Zhao, C., 2014. Application of a continuous intrinsic dissolution-permeation system for relative bioavailability estimation of polymorphic drugs. *Int J Pharm* 473, 250-25.

## **7. PUBLICATIONS & PRESENTATIONS**

**Research publications:**

- **J. Deng**, S. Staufenbiel, S.L. Hao, B.C. Wang, A. Dashevskiy, R. Bodmeier. Development of a discriminative biphasic in vitro dissolution test and correlation with in vivo pharmacokinetic studies for differently formulated racecadotril granules. *J. Control. Release.* 255 (2017) 202-209 (DOI: 10.1016/j.jconrel.2017.04.034).
- **J. Deng**, S. Staufenbiel, R. Bodmeier. Evaluation of a biphasic in vitro dissolution test for estimating the bioavailability of carbamazepine polymorphic forms. *Eur. J. Pharm. Sci.* 105 (2017) 64-70 (DOI: 10.1016/j.ejps.2017.05.013).
- **J. Deng**, S. Staufenbiel, R. Bodmeier. Evaluation of a discriminative and predictive biphasic dissolution test for itraconazole amorphous solid dispersions prepared with different polymers. (In Preparation).

**Poster presentations:**

- **J. Deng**, R. Bodmeier. Discriminating the relative bioavailability of carbamazepine polymorphs by biphasic dissolution test. PBP world meeting, April 2016, Glasgow, United Kingdom, poster 177.
- **J. Deng**, S. Staufenbiel, S. L. Hao, B. C. Wang, R. Bodmeier. A biphasic dissolution test to discriminate three racecadotril granules with poor IVIVC. AAPS Annual Meeting and Exposition, November 2016, Denver, USA, poster 11W0830.

## **8. CURRICULUM VITAE**

For reasons of data protection,  
the curriculum vitae is not published in the electronic version.