European Journal of Pharmaceutics and Biopharmaceutics 74 (2010) 298–303

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Methodology for phase selection of a weak basic drug candidate, utilizing kinetic solubility profiles in bio-relevant media

Shuntaro Furukawa *, Chenhua Zhao, Yasuhiro Ohki

Banyu Pharmaceutical Co., Ltd., Ibaraki, Japan

ARTICLE INFO

Article history:
Received 9 January 2009
Accepted in revised form 30 September 2009
Available online 6 October 2009

Keywords:
Phase selection
Dissolution
Bio-relevant media
Pharmacokinetics
Kinetic solubility

ABSTRACT

We aimed to develop a phase selection methodology for a weak basic active pharmaceutical ingredient (API) that would require less than 10 mg of the API and monitor the real-time kinetic solubility of the API in two bio-relevant media. Three sets of kinetic solubility measurements were conducted for free form I and the disulfate salt of an API (compound A) in order to determine the better API phase for further development of the compound. Tests consisted of solid API dissolution in both simulated gastric fluid (SGF) and fasted-state intestinal fluid (FaSSIF), and precipitation kinetics by injection of liquid state API into FaSSIF. All dissolution tests were conducted above the saturated concentrations in order to determine the compounds' thermodynamic and kinetic solubility to trace the API's phase transitions during dissolution. The pharmacokinetic profiles of compound A following oral administration of two API phases were evaluated in dogs. Results of the three sets of kinetic solubility measurements showed different kinetic solubility profiles for the two API phases under gastrointestinal conditions, indicating that the disulfate salt is preferred over free form I due to its superior kinetic solubility profile. This conclusion is consistent with the bioavailability results obtained in dogs.

1. Introduction

In the pharmaceutical industry, many drug candidates with high biological activity towards their target receptor have been rapidly obtained using in vitro high throughput screening (HTS) or combinatorial chemistry. However, not all these drug candidates are developed into drugs, as many have poor physicochemical properties such as low solubility, high lipophilicity and high molecular weight. These factors greatly impact their bioavailability. Therefore, there has been much effort to form salts or co-crystals, as well as to reduce the particle size of active pharmaceutical ingredients (APIs) [1–3]. Traditionally, the rank ordering of the in vivo absorption of all these API phases (i.e., the API, its salts, and its co-crystals) has been predicted by in vitro measurements, such as the dissolution test utilizing United States Pharmacopeia (USP) standards (Apparatus II, paddles). It is important to evaluate whether a given API phase has sufficient bioavailability to support its further development. However, dissolution tests using an USP II apparatus require a large amount of API and thus are not practical in the early phase of drug discovery when the supply of the API is quite limited. Dissolution tests are usually conducted under sink conditions (meaning a volume of dissolution media that is 3–10 times greater than the volume at the saturation point of the drug), and only dissolution rates of each API phase or formulation are compared. It is almost impossible to assess the actual condition of the orally administrated drug in the gastrointestinal tract.

Bio-relevant solubility values are increasingly required in early phases of drug discovery to predict qualitatively the bioavailability and absorption of orally administered drugs. These values, along with software simulations, are sometimes used to predict pharmacokinetic (PK) performance. HTS and shake-flask methods have been reported to provide bio-relevant solubility data [4,5], but most methods are intended to measure the thermodynamic solubility of the API (i.e., the most thermodynamically stable form of the API in a given medium). While these solubility data are very useful for preventing unsuitable compounds from entering the development process, it is doubtful whether they are useful for predicting PK performance. Of course, solubility data are very useful to predict PK performance when the solubility of the compound is high enough not to result in its precipitation in the gastrointestinal tract. In order to understand the kinetic solubility of a compound, a large number of data points are required because the solubility profile tends to be complex and not linear. Inaccurate data may lead to unreliable in vitro–in vivo correlations (IVIVC) or in vitro–in vivo relationships (IVIVR). If the compound is a weak base, its solubility is significantly enhanced in the stomach (low pH

* Corresponding author. API Analytical Research, Analytical Science, PreClinical Development, Banyu Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan. Tel.: +81 29 877 2000; fax: +81 29 877 2173.
E-mail addresses: shuntaro_furukawa@merck.com, shuntaro.furukawa@jp.astellas.com (S. Furukawa).

0939-6411/$ - see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.ejpb.2009.09.012

region), while its solubility will decrease as the compound moves to the higher pH gastrointestinal tract. This can cause the formation of a supersaturated solution and precipitation of the API [6]. When the API is in the form of a salt or co-crystal, which is often more soluble than the most stable thermodynamic form, similar phenomena could occur in the gastrointestinal tract. It is important to evaluate kinetic behavior using bio-relevant media because different dissolution media may lead to different kinetic solubility profiles [7]. Amphiphilic components in bio-relevant media such as bile salts and lecithin could act as anti-precipitation agents or form complexes with the API; these phenomena would not be observed in inorganic buffers. There is a high possibility that the in vitro thermodynamic solubility would not represent the solubility transition of the orally administrated API, resulting in the failure of the PK prediction.

The present study aimed to achieve a new API phase selection for the weak basic compounds using <10 mg API and monitor its real-time kinetic solubility in two bio-relevant media, simulated gastric fluid (SGF) and fasted-state intestinal fluid (FaSSIF), under saturation conditions. In this study, free form I and the disulfate salt of a developmental compound were compared in order to choose the better API phase for further drug development. The performance of the μDISS ProfilerPLUS™ is equivalent to that of the USP apparatus II but allows both smaller scale dissolution measurements and real-time solubility monitoring [8]. Three sets of dissolution tests were conducted for two API phases (free form I and disulfate salt) of a Merck developmental compound in order to obtain the kinetic solubility profile of both API phases in bio-relevant conditions. The tests consist of solid API dissolution in both SGF and FaSSIF, and precipitation kinetics of liquid state API in FaSSIF. These in vitro results were compared with in vivo results obtained in dogs.

2. Materials and methods

2.1. Materials

Merck developmental compound A was used as the model compound in this study (Merck & Co., Tsukuba, Banyu, Japan). Compound A has a molecular weight of 442.5, is practically water insoluble at physiological pH and is a zwitterion with two pK_a values [pK_{a1} = 3.9 (base), pK_{a2} = 7.1 (acid)] in the pH range of 2.5–11. Since compound A has only one pK_a at the pH values of SGF and FaSSIF, it acts as a weak basic compound. Three API phases (free form I, free form II, and disulfate salt) were identified by crystal screening. Although only one basic pK_a value has been reported for compound A, its disulfate salt has been identified, demonstrating that a weak, basic, non-titratable site might exist. Free form I is expected to be the most stable free form; free form II was identified as a metastable form of free form I by thermal analysis (data not shown).

HPLC grade acetonitrile (MeCN) was provided by Wako Pure Chemical Industries (Osaka, Japan). Water was from a MilliQ system from Millipore Corporation (USA). Phosphoric acid (85 w/w%; d = 1.10) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Potassium hydroxide solution (45, w/w%; d = 1.450) was obtained from Furuka (Steinheim, Netherlands).

Sodium taurocholate and lecithin were purchased from Spectrum Chemical (New Brunswick, NJ, USA) and Alfa Aesar (Ward Hill, MA, USA), respectively. Sodium chloride, potassium dihydrogen phosphate, 5 N hydrochloride solution, potassium chloride and 1 N sodium hydroxide solution were supplied by Wako Pure Chemical.

2.2. Preparation of bio-relevant media

SGF and FaSSIF were prepared following the procedure listed in Table 1 [9,10].

Table 1
Composition of SGF and FaSSIF.

Ingredient	SGF	FaSSIF
pH	1.8	6.5
Osmolality (mOsm)		270 ± 10
HCl	3.36 mL of 5 N HCl (0.0175 N)	
NaCl	2.0 g (34.2 mM)	
Na taurocholate		1.61 g (3 mM)
Lecithin		0.56 g (0.75 mM)
KH_2PO_4		3.9 g
KCl		7.7 g
NaOH		qs pH 6.5
Deionized water	1 L	1 L

2.3. Solubility measurement

An excess of free form I was added to 1.0 mL of bio-relevant media (Table 1), and the suspension was shaken at room temperature (25 ± 0.5 °C) for 24 and 72 h on a mechanical shaker (SI-300C, AS ONE) to determine its equilibrium solubility. Suspensions of SGF and FaSSIF were filtered through PTFE membranes (0.20 μm), and the filtrates were diluted 10 and 1.1 times, respectively, with a mixture of KH_2PO_4/KOH buffer (pH 7.0; 15 mM) and MeCN (50:50, v/v).

Standard solutions (0.0002–0.1 mg/mL) of free form I were prepared by serial dilution using a mixture of the above buffer and MeCN solutions. The solubilities of the samples were analyzed using a high performance liquid chromatography (HPLC) system (HP1100, Agilent, USA) comprised a UV detector, a column compartment, a pump, a thermostatted automatic injector, and a degasser. The UV detector was set at 210 nm. Samples (3, 15, or 50 μL) were fractionated on an ODS column (Eclipse plus C18; 4.6 × 50 mm, 1.8 μm; Agilent) with a gradient mixture of KH_2PO_4/KOH buffer (pH 7.0) and MeCN (from 90:10 to 10:90 v/v for 5 min; equilibrium time, 4 min) at 1.5 mL/min at 40 °C.

2.4. Kinetic solubility measurements with the μDISS ProfilerPLUS™

The μDISS ProfilerPLUS™ (pION Inc., MA, USA) instrument used in kinetic solubility measurements employs eight photodiode array (PDA) spectrophotometers, each with its own dedicated fiber optic dip probe, center-positioned in the vial holding the rotation disk carrier and 15 mL media. Stirring speed of a magnetic stir for this instrument was set at 150 ± 2 rpm, and the temperature was controlled at 25 ± 0.5 °C. Concentration measurements were performed directly by in situ fiber optic dip probes in the dissolution media, and the processed results were plotted in real time. Interference due to background turbidity is eliminated by a spectral second derivative method. Spectral scans (200–420 nm) of all eight channels take less than 1 s.

The dissolutions in SGF and FaSSIF of solid API were conducted by dispersing API powder in 0.4 mL dissolution medium and rapidly transferring to the dissolution vial filled with 14.6 mL of the same medium. The precipitation kinetics of liquid state API in FaSSIF were conducted by injecting a DMSO solution (10 mg/mL) of the free form of compound A using a micropipette. The API loading of solid samples was chosen based on two considerations here. First, the API loading for each vial should be at least 10-fold higher than the thermodynamic solubility in order to trace polymorph or phase changes during the dissolution test. Second, each aliquot of drug should be >1 mg for ease of handling. Data were collected at predetermined time points: 24 spectra were collected at 5-s intervals, and then three spectra were collected at 1-min intervals, five spectra at 5 min and finally five spectra after 10 min. Extra measurements were obtained for the dissolution of the disulfate salt in SGF (every 120 min for 600 min). After the 80-min measure-

Table 2
API amounts and concentrations used for the in vitro bio-relevant dissolution tests.

		Solid API dissolution	Precipitation kinetics of liquid state API	
Phase	MW	SGF (concentration of API)	FaSSIF (concentration of API)	
Free form I	443	2.0 mg → 15 mL (0.13 mg/mL)	1.0 mg → 15 mL (0.067 mg/mL)	100 μL of 10 mg/mL DMSO solution → 15 mL (0.067 mg/mL)
Disulfate salt	627	3.0 mg → 15 mL (0.13 mg/mL)	1.5 mg → 15 mL (0.067 mg/mL)	

ment, the dissolution sample of the disulfate salt in FaSSIF was measured again after 72 h. The concentrations of the solutions were calculated using an appropriate wavelength range; UV absorbances were <1.0 absorbance unit. The exact amount of sample loading used is listed in Table 2.

Calibration curves for the concentration determinations were obtained by adding a small amount of a DMSO solution of the sample to each bio-relevant medium. At least four concentrations were used for the calibration curves. The amount of DMSO was ⩽1% of the total dissolution volume.

2.5. Bioavailability studies in beagle dogs

Compound A was orally administered to male beagle dogs (body weights, 7–9 kg) as the free form or disulfate salt in dry filled capsules (DFC) at 4 mg API/kg. Three dogs received 40 mg of compound, followed by 20 mL water administered by syringe. Dogs were fed at 12:00 PM the day before the study, food bowls were removed at 4:00 PM, and the dogs were fasted overnight. Certificated dog diet (350 g) was made available 4 h after dosing. Dogs were given free access to water throughout the study. Blood samples were drawn pre-dose and 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose. Plasma was separated by centrifugation and kept frozen (−70 °C) until analysis. One portion of plasma (0.5–0.7 mL) from the API-administrated group was used to evaluate compound A concentration using LC-MS/MS.

These in vivo experiments were approved by the Banyu Institutional Animal Care and Use Committee.

2.6. X-ray powder diffraction (XRPD) and polarized microscope measurements

X-ray powder diffractograms were recorded on a Bruker AXS D8 advance X-ray solutions at 35 kV and 40 mA using CuK_α radiation. The dry samples of free form I and disulfate salt were placed on the non-refractive substrate plate and measured using a start angle of 4° 2θ and an end angle of 40° 2θ. The suspension samples used for solubility and dissolution studies were filtered through a PTFE membrane (0.20 μm), and its remained materials were placed on the non-refractive substrate plate and measured it in the wet state.

At 2 min time point of each dissolution test, an aliquot of suspension (2 μL) from the dissolution test was withdrawn using a micropipette, and its polarization was measured using a NIKON E600 POL (Tokyo, Japan) polarizing microscope.

3. Results and discussion

3.1. Solubility measurements

Equilibrium solubility values for compound A in SGF and FaSSIF selected for in vitro dissolution studies were 0.013 and 0.0004 mg/mL, respectively. Overall, no shifts in pH were observed during these measurements. All insoluble residues were measured by XRPD to confirm whether a form change had occurred. No form conversion was observed for solubility samples in SGF and FaSSIF, suggesting that free form I is the most thermodynamically stable form.

3.2. Real-time kinetic solubility measurements (API dissolution study)

3.2.1. Kinetic solubility in SGF

As illustrated in Fig. 1, free form I gradually dissolved in SGF and reached a concentration (0.011 mg/mL) close to its equilibrium solubility (0.013 mg/mL) in 80 min. In contrast, the disulfate salt reached a solubility of 0.11 mg/mL. The disulfate salt measurements were continued for 600 min to examine any crystalline form conversion. Precipitation was observed after 300 min, resulting in a rapid decrease in concentration of compound A from 0.11 to 0.003 mg/mL. The XRPD pattern of the precipitate after 600 min was measured. Although the concentration of the sulfate salt at 600 min was slightly higher than the equilibrium solubility of compound A, the crystal form was confirmed to be the same as free form I, demonstrating that crystal form conversion was incomplete after 600 min.

The crystalline form change was also followed using a polarization microscope. The dissolution suspensions were sampled after 2 min, and both free form I and the disulfate salt were observed to

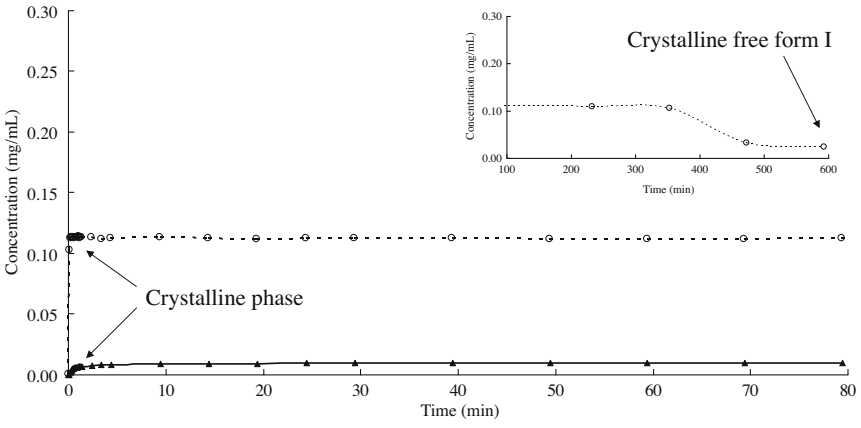

Fig. 1. In vitro dissolution profiles of free form I and the disulfate salt in SGF. Free form I (▲) and disulfate salt (○).

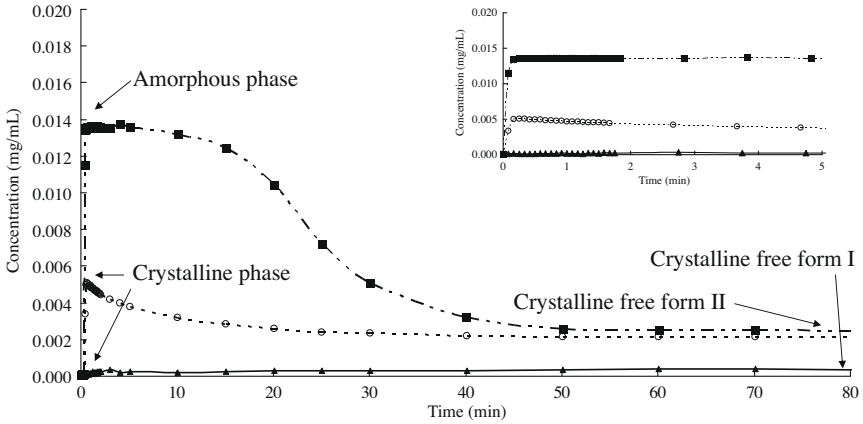

Fig. 2. In vitro dissolution profiles of free form I and disulfate salt, and precipitation kinetics using a DMSO solution of compound A in FaSSIF. Free form I (▲); disulfate salt (○) and DMSO solution of compound A (■).

be present in crystalline form. These results indicate that the kinetic solubility profile can serve to detect API phase or crystalline form conversion, which directly influence the solubility of the compound.

3.2.2. Kinetics of solubilization in FaSSIF

The dissolution profile of compound A in FaSSIF is illustrated in Fig. 2. The dissolution of free form I in FaSSIF was very similar to its dissolution behavior in SGF. The dissolution of the disulfate salt quickly increased to reach a concentration of 0.005 mg/mL in the first 0.3 min, then decreased to 0.002 mg/mL, which is 5-fold higher than the thermodynamic solubility of compound A (0.0004 mg/mL). The XRPD pattern of the insoluble residue after 90 min dissolution of the disulfate salt showed a crystalline form different from free form I, identified as a metastable form (free form II). After 72 h, the concentration was 0.0007 mg/mL and the crystalline form was free form I. After 2 min dissolution, the suspension was studied by polarization microscopy; both free form I and the disulfate salt were present as their crystalline forms.

In order to investigate the potential supersaturation and precipitation characteristics of compound A, a DMSO/compound A solution (10 mg/mL) was injected into FaSSIF. The FaSSIF immediately became cloudy. The suspension was sampled after 2 min, and polarization microscopy showed an amorphous phase. The concentration of compound A remained fairly constant around 0.014 mg/mL for 10 min then gradually decreased to 0.002 mg/mL during the dissolution experiment. XRPD confirmed that the crystalline form in the suspension after 80 min was free form II. These results suggest that both the disulfate salt and the liquid phase of compound A do not convert to free form I directly in FaSSIF, but to a metastable state, such as free form II or an amorphous form that retains higher solubility than the thermodynamic solubility of compound A. Finally, these forms convert to more thermodynamically stable phases, following the Ostwald Rule of Stages [11,12].

3.3. Bioavailability studies in dogs

In order to compare the actual bioavailability of the different phases of compound A, an in vivo study was conducted. The plasma concentration–time profile and the pharmacokinetic parameters of compound A resulting from the oral administration of DFC in beagles are presented in Fig. 3. The pharmacokinetic parameters, $AUC_{0-\infty}$ (μM h), C_{max} (μM) and T_{max} (h) for free form I were 8.14 ± 3.65, 0.913 ± 0.369 and 2.7 ± 1.2, respectively. While $AUC_{0-\infty}$ (μM h), C_{max} (μM), and T_{max} (h) for the disulfate salt were 33.8 ± 33.6, 4.08 ± 3.70, and 2.0 ± 0.0, respectively. In the fasted condition compared with the DFC of free form I, the DFC of the disulfate salt significantly increased

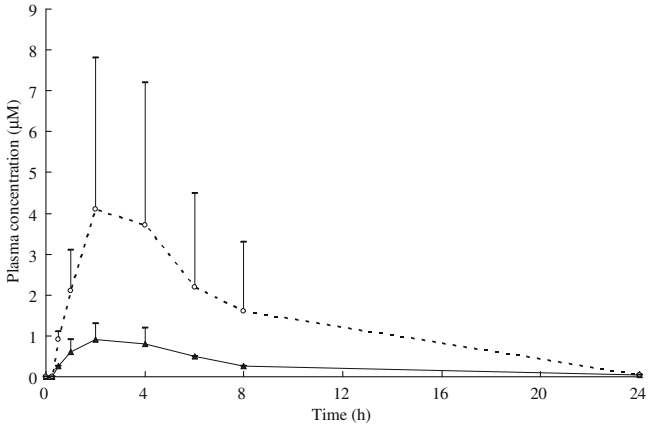

Fig. 3. Plasma concentration–time profiles of compound A after oral administration of DFC at a dose of 40 mg/body in beagle dogs. Results are expressed as the mean, with the bar showing SD values of three experiments. Free form I (▲) and disulfate salt (○).

the maximum plasma concentration (C_{max}) and the area under curve (AUC) of compound A by 4.5 and 4.2 times, respectively. However, there was little difference between the T_{max} of the two API phases. These results indicate that salt formation could improve the solubility of compound A in the gastrointestinal tract, leading to improved bioavailability of compound A.

3.4. New methodology for API selection

The present study demonstrates a set of three kinetic solubility measurements for two API phases of compound A using a μDiss ProfilerPLUS™ under the saturated conditions. The tests required a total of only 7 mg API (active component basis) and provided a precise understanding of the improved kinetic solubility profile induced by salt formation of compound A.

The tests were composed of solid API dissolution in both SGF and FaSSIF, and a precipitation kinetics study involving the injection of liquid state API into FaSSIF. The API/DMSO solution was injected into FaSSIF directly in order to examine the supersaturation and precipitation phenomena that is trying to mimic the transition of weak basic APIs from the stomach (low pH) to the small intestine (neutral pH). This study also examined the differences in the kinetic solubility profile of the solid and liquid phase samples in FaSSIF.

The dissolution results in SGF showed that the disulfate salt is superior to free form I in terms of both faster dissolution and high-

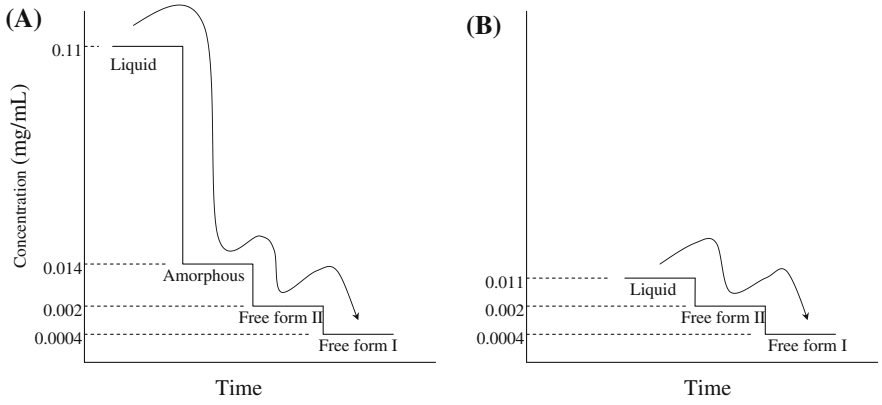

Fig. 4. Scheme showing the expected phase conversion for the two phases of compound A in the small intestine following oral administration. The concentration of each start point corresponds to the compound's kinetic solubility after 60 min in SGF. Keys: (A) phase conversion of SGF-treated disulfate salt in the small intestine and (B) phase conversion of SGF-treated free form I in the small intestine.

er concentration of compound A, ascribed to the formation of a supersaturated solution. Supersaturation persisted long enough to diminish the possibility of precipitation in the stomach, since it has been observed that gastric emptying occurs in less than 300 min in fed humans [13]. No other API phase formed in the SGF, such as an HCl salt which sometimes shows lower solubility than the free forms in SGF due to the common ion effect. In this case, it is little more complicated to judge the effect of API phase modification at higher exposure levels. However, such information is important for determining the PK profile and cannot be obtained from dissolution tests under sink conditions.

The dissolution results in FaSSIF also show that the disulfate salt is superior to free form I, and the concentration of the disulfate salt of compound A was higher than that of free form I throughout the dissolution experiment. The metastable form of the free form (free form II) was induced by the higher solubility of the disulfate salt in FaSSIF. Furthermore, dissolution in FaSSIF resulted in amorphous phase formation at concentrations above 0.014 mg/mL.

The combined dissolution results in SGF and FaSSIF indicate that the disulfate salt is the better API phase than free form I in terms of improved in vivo kinetic solubility profile. Compound A could form an amorphous phase in FaSSIF at concentrations above 0.014 mg/mL. The highest concentrations of free form I and the disulfate salt in the dissolution test in SGF were 0.011 and 0.11 mg/mL, respectively. It is remarkable that free form I does not form an amorphous phase in FaSSIF given that its solubility in SGF is lower than the solubility of the amorphous phase in FaSSIF (Fig. 4). The total quantity of dissolved disulfate salt is much larger than that of free form I. This information could not be obtained from traditional dissolution tests conducted under sink conditions because these tests can only discriminate differences in dissolution rates. Finally, in vitro evaluation using the kinetic solubility profile in bio-relevant media showed high consensus with bioavailability results obtained in animals. The pH and composition of SGF and FaSSIF used in this study were prepared to mimic human's gastrointestinal tract. The dogs were known to have higher gastric pH than that of human. Even if the gastric pH is high, the solubility of the disulfate salt is much higher than that of free form I and can form an amorphous phase in the intestine of dog leading to the conjecture that both API phases could have been subject to the same phase change as in the in vitro tests. We could therefore use the in vitro kinetic solubility profile to predict an API's behavior in the gastrointestinal tract.

3.5. Application of the kinetic solubility profile

The dissolution test results described earlier were performed using a total of only 7 mg API (active component basis) yet provided very useful information, including a precise understanding of the API phase selection. Real-time solubility can be monitored with a μDiss ProfilerPLUS™, a useful tool for in silico evaluation of APIs in conjunction with PK simulation software (not conducted in the present investigation) [14]. If there is no information about the kinetic solubility profile of an API in aqueous media, solubility data from just one or two time points might result in an inaccurate PK prediction. In addition, knowledge of the physicochemical nature of a compound in bio-relevant media is useful for formulation development, which follows API phase selection. Based on this information, the PK profile of the compound could be improved by modifying the surfactants or excipients in the formulation.

4. Conclusion

A set of three kinetic solubility measurements for API phase selection aimed at drug development were demonstrated utilizing a μDiss ProfilerPLUS™. This approach enabled real-time concentration monitoring and required only 1/60 (15 mL vs. 900 m) the API compared to a traditional USP apparatus II. The results demonstrate that the quality of measurements does not suffer when using the μDiss ProfilerPLUS™ and that this method can support drug discovery at the lead optimization stage, when the amount of API is quite limited. The dissolution results under saturated conditions exhibited an API phase-related solubility transition not obtained from dissolution tests under sink conditions. The combination of dissolution results in SGF and FaSSIF showed advantages of the disulfate salt over free form I, consistent with their bioavailability in dogs. The approach demonstrated in this study is likely applicable to API phase selection of basic drugs in general. Real-time concentration data for each phase in different bio-relevant media may help to improve in vitro–in vivo correlation models.

Acknowledgments

The authors would like to acknowledge the PreClinical development group for their helpful comments and suggestions; particular thanks go to Pharmaceutical Research & Development group.

References

[1] H.-S. Gwak, J.-S. Choi, H.-K. Choi, Enhanced bioavailability of piroxicam via salt formation with ethanolamines, Int. J. Pharm. 297 (2005) 156–161.
[2] H.-K. Han, H.-K. Choi, Improved absorption of meloxicam via salt formation with ethanolamines, Eur. J. Pharm. Biopharm. 65 (2007) 99–103.
[3] J. Jinno, N. Kamada, M. Miyake, K. Yamada, T. Mukai, M. Odomi, H. Toguchi, G.G. Liversidge, K. Higaki, T. Kimura, Effect of particle size reduction on

dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs, J. Control. Release 111 (2006) 56–64.
[4] B. Bard, S. Martel, P.-A. Carrupt, High throughput UV method for the estimation of thermodynamic solubility and the determination of the solubility in biorelevant media, Eur. J. Pharm. Sci. 33 (2008) 230–240.
[5] E.S. Kostewicz, U. Brauns, R. Becker, J.B. Dressman, Forecasting the oral absorption behavior of poorly soluble weak bases using solubility and dissolution studies in biorelevant media, Pharm. Res. 19 (3) (2002) 345–349.
[6] E.S. Kostewicz, M. Wunderlich, U. Brauns, R. Becker, T. Bock, J.B. Dressman, Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine, J. Pharm. Pharmacol. 56 (1) (2004) 43–51.
[7] D. Wagner, N. Glube, N. Berntsen, W. Tremel, P. Langguth, Different dissolution media lead to different crystal structures of Talinolol with impact on its dissolution and solubility, Drug Dev. Ind. Pharm. 29 (8) (2003) 891–902.
[8] A. Avdeef, O. Tsinman, Miniaturized rotating disk intrinsic dissolution rate measurement: effects of buffer capacity in comparisons to traditional Wood's apparatus, Pharm. Res. 25 (11) (2008) 2613–2627.
[9] E. Galia, E. Nicolaides, D. Hörter, R. Löbenberg, C. Reppas, J.B. Dressman, Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs, Pharm. Res. 15 (5) (1998) 698–705.
[10] D. Ilardia-Arana, H.G. Kristensen, A. Müllertz, Biorelevant dissolution media: aggregation of amphiphiles and solubility of estradiol, J. Pharm. Sci. 95 (2) (2006) 248–255.
[11] J. Nyvlt, The Ostwald rule of stages, Cryst. Res. Technol. 30 (4) (1995) 443–449.
[12] T.P. Feenstra, P.L. DeBruyn, The Ostwald rule of stages in precipitation from highly supersaturated solutions: a model and its application to the formation of the nonstoichiometric amorphous calcium phosphate precursor phase, J. Colloid Interface Sci. 84 (1) (1981) 66–72.
[13] V.C. Ibekwe, H.M. Fadda, E.L. McConnell, M.K. Khela, D.F. Evans, A.W. Basit, Interplay between intestinal pH, transit time and feed status on the *in vivo* performance of pH responsive Ileo-colonic release systems, Pharm. Res. 25 (8) (2008) 1828–1835.
[14] B.-M. Lue, F.S. Nielsen, T. Magnussen, H.M. Schou, K. Kristensen, L.O. Jacobsen, A. Müllertz, Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound, Eur. J. Pharm. Biopharm. 69 (2008) 648–657.