

Comparison of the Solubility and Dissolution of Drugs in Fasted-State Biorelevant Media (FaSSIF and FaSSIF-V2)

Mathew Leigh*, Bastian Kloefer, and Michael Schaich

biorelevant.com, Capital Business Centre, 22 Carlton Road, Croydon, Surrey, CR2 0BS, United Kingdom

ABSTRACT

The equilibrium solubility values and dissolution profiles in each of the media (FaSSIF or FaSSIF-V2) produced using methylene chloride and the corresponding SIF Powder are equivalent for the drugs tested representing acid, basic, and neutral compounds. Therefore, it is practical to switch from the more labor-intensive solvent-evaporation method to the use of standardized instant powders for preparing biorelevant media without affecting the solubility and dissolution results.

Testing in the two versions of FaSSIF media with different bile salt/lecithin ratios will give an indication on how the drug and formulation could be affected in vivo by the variations in bile salt/lecithin ratio within the range found in human small intestinal fluids.

INTRODUCTION

Biorelevant media are artificial in vitro media designed to share physicochemical properties with their corresponding fluids found in vivo in terms of osmolarity, pH, buffer capacity, and surfactant content, for example. Specifically, intestinal biorelevant media are widely used to mimic the properties of gastrointestinal fluids for in vitro solubility and dissolution studies. These media can also be used to simulate gastrointestinal stability and to perform permeability studies (1). Biorelevant media simulate the gastrointestinal fluids by containing the physiological surfactants, bile salt and lecithin. These media are particularly important for the development of poorly soluble drugs, because these surfactants significantly increase solubility.

Biorelevant intestinal media were first proposed by Dressman et al. (2) at the University of Frankfurt in collaboration with Reppas from the University of Athens who identified the deficiencies of standard pharmacopeial media. These two groups also demonstrated the clear advantage of biorelevant media over standard conventional media for studying the solubility and dissolution of poorly soluble compounds.

Biorelevant intestinal media simulate intestinal fluids secreted under fasted- (FaSSIF) or fed-state conditions (FeSSIF). Food intake alters the physicochemical properties and composition of intestinal fluids for digestion. That is why separate intestinal media are defined for fasted- and fed-state conditions. The two states of media enable formulation scientists to obtain solubility values and dissolution profiles of drugs and their formulations in vitro under both fasted and fed states. This can help detect food effects and optimize formulation performance using

standard pharmacopeial dissolution test apparatus (e.g., USP Apparatus 2 and 4).

There are biorelevant media representing fluids found along all regions in the gastrointestinal tract. However, simulated upper small intestinal media are the most commonly used, particularly for immediate-release drugs, because they simulate the fluids in the intestinal region from which most drugs are absorbed.

FaSSIF and FaSSIF-V2 are the two most frequently used compositions for biorelevant intestinal media reflecting fasted-state conditions. The main difference between FaSSIF and FaSSIF-V2 is the reduced amount of lecithin in FaSSIF-V2 (Table 1) as compared with FaSSIF. The level of lecithin in both versions falls within the range typically found in aspirates sampled from healthy human volunteers.

The purpose of this study was to evaluate the equilibrium solubility of four poorly water soluble drug substances (danazol, dipyrindamole, ketoconazole, miconazole) and the drug dissolution from five drug products containing drugs with various pK_a values under fasted-state conditions (i.e., without food interference) using the FaSSIF and FaSSIF-V2 media. The drug products contained acidic (mefenamic acid), basic (ketoconazole), or neutral (danazol, metoprolol, paracetamol) compounds. In addition, the solubility and drug dissolution behaviors were compared in media made from commercially available instant powders (SIF Powder Original and SIF Powder FaSSIF-V2 from biorelevant.com, UK) and prepared from methylene chloride as described by Marques (3).

MATERIALS AND METHODS

Materials

Drugs Used for Solubility Testing

Danazol was purchased from Jai Radhe (Ahmedabad, India). Dipyrindamole was from Sigma Aldrich Chemie GmbH

*Corresponding author.

Table 1. Composition and Properties of the Biorelevant Media FaSSIF and FaSSIF-V2

	FaSSIF	FaSSIF-V2
SIF Powder Product	SIF Powder Original	SIF Powder FaSSIF-V2
Sodium taurocholate	3.5	3.5
Lecithin	0.75	0.2
Buffer Composition		
Sodium chloride	105.85	68.62
Sodium hydroxide (pellets)	10.5	34.8
Sodium dihydrogen phosphate	28.65	-
Maleic acid	-	19.12
Properties		
pH	6.5	6.5
Osmolality (mOsm/kg)	270 ± 10	180 ± 10
Buffer Capacity (mmol/L/pH)	12	10
Surface Tension	54 ^a	54.3 ^b

^a Data from ref 5.

^b Data from ref 4.

(Buchs, Switzerland). Ketoconazole was from Nicholas Piramal India Ltd. (Gujarat, India), and miconazole was purchased from Silfavit srl (Milan, Italy). All drugs were either USP or Ph.Eur. grade.

Drug Products Used for Dissolution Testing

Danazol capsules (danazol 100 mg) from Mylan, UK; metoprolol tablets (Lopresor 100 mg) from Daiichi-Sankyo, Switzerland; mefenamic acid capsules (Mefenamin 250 mg) from Pfizer, Switzerland; ketoconazole tablets (Nizoral 200 mg) from Janssen Cilag, UK; and paracetamol tablets (paracetamol 500 mg) from Boots Pharmaceutical, UK, were purchased from local pharmacies.

Methods for Making FaSSIF and FaSSIF-V2

SIF Powders

The desired biorelevant medium was made by dissolving the appropriate amount of SIF Powder Original or SIF Powder FaSSIF-V2 in the recommended blank buffer. For FaSSIF, SIF Powder Original was added to a phosphate

buffer with a pH of 6.5. For FaSSIF-V2, SIF Powder FaSSIF-V2 was added to a maleate buffer with a pH of 6.5 corresponding to the recipe used by Jantratid (4).

Preparation Using Methylene Chloride (MC)

FaSSIF was prepared using methylene chloride (MC) as described by Marques (3). The taurocholate was dissolved in about 500 mL of the blank buffer. After the taurocholate dissolved completely, a freshly prepared solution of lecithin in methylene chloride was added. From the resultant emulsion, the methylene chloride was removed using a rotary evaporator (40 °C for 15 min at 250 mbar followed by 15 min at 100 mbar). Particular care was taken to ensure that all MC had been removed. Finally, the dispersion was made up to a volume of 2 L with the appropriate buffer.

Equilibrium Solubility Measurements

The equilibrium solubility of each of the four drug substances was determined after incubating an excess of the individual drug in biorelevant media in a sealed glass vial (Wheaton 6-mL short sample vial, Sigma Aldrich) for 24 h at 37 °C while stirring the suspension using a magnetic stirrer (8 × 2 mm) at 600 rpm. The 24-h time point was assumed to have reached equilibrium solubility, at which time the suspension was filtered through a 0.22-μm PVDF filter (Carl Roth, Switzerland) and drug content determined by HPLC. To reduce any potential adsorption effects on the filter, the first 200 μL of filtrate was discarded. The equilibrium solubility of each drug was determined in triplicate ($n = 3$). Where required to prevent drug precipitation, the filtrate was diluted with a suitable organic solvent.

Method for Drug Dissolution

The dissolution profile of each product was determined in USP Apparatus 2 (Pharmatest PTWS300) according to the sampling schedule shown in Table 2. All studies used 500 mL of FaSSIF or FaSSIF-V2 made with the respective SIF Powders (biorelevant.com, UK) or FaSSIF or FaSSIF-V2 prepared using methylene chloride and were carried out at 37 ± 0.5 °C. All media were degassed using the Inert Gas Purge Technique as described by Degenhardt et al. (6). Dissolution in each medium was carried out in triplicate ($n = 3$). All samples were removed from the middle of the vessel using a 2-mL syringe. To reduce any potential adsorption effects on the filter, the first 200 μL of filtrate was discarded.

Table 2. Sampling Schedules for Immediate-Release Drug Products

Drug Product	Formulation	Speed (rpm)	Sampling time points (min)
Boots own brand paracetamol	500-mg tablet	100	5, 10, 15, 20, 30, 60
Lopresor	100-mg tablet	100	5, 10, 15, 20, 30, 60
Mefenamin	250-mg capsule	50	5, 10, 15, 20, 30, 60
Danazol (generic)	100-mg capsule	100	5, 10, 20, 30, 60, 90
Nizoral	200-mg tablet	100	5, 10, 15, 20, 30, 60, 120

Table 3. HPLC Gradient Profile

Time (min)	Eluent B (%)
0	10
4.0	95
6.0	95
6.1	10
8.5	10

Table 4. Detection Wavelengths

Drug	Wavelength (nm)
Danazol	285
Dipyridamole	285
Ketoconazole	220
Mefenamic acid	280
Metoprolol tartrate	222
Miconazole	225
Paracetamol	245

HPLC Method

The following HPLC method was used:

Instrument: 1200 Series HPLC, Agilent
 Eluent A: water + 0.1 % formic acid
 Eluent B: acetonitrile + 0.1 % formic acid
 Column: SunFire C18, 50 × 4.6 mm, 3.5 μm
 Column temperature: 40 °C
 Flow rate: 1.2 mL/min
 Standard solution diluent: acetonitrile/water 3:1 v/v

An HPLC gradient method was used for drug analysis. The conditions are given in Table 3. The detection wavelengths are summarized in Table 4.

RESULTS**Equilibrium Solubility Results**

The solubility values for the five drug substances in FaSSiF and FaSSiF-V2 made from the respective instant SIF Powders and made from MC are compared in Table 5.

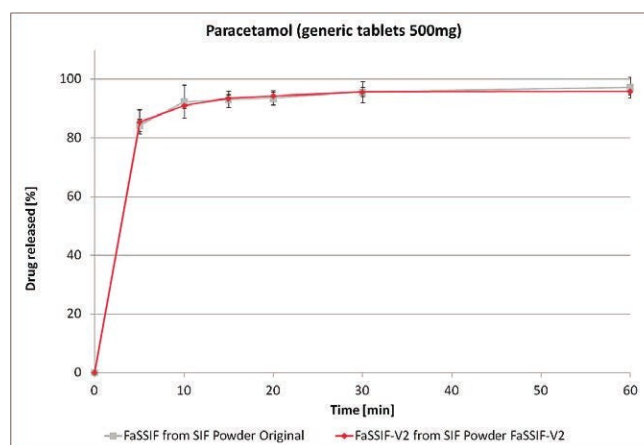


Figure 1. Comparison of the dissolution profiles of generic paracetamol tablets in FaSSiF and FaSSiF-V2 from the respective SIF powders.

In general, the method of preparation did not affect the equilibrium drug solubility for a given medium. The equilibrium solubilities of the four drugs in FaSSiF were generally higher than in FaSSiF-V2.

Dissolution Results

The similarity factor (f_2) proposed by the FDA for comparison of dissolution profiles (7) was employed to compare the similarity between the two preparation methods. Values of f_2 greater than 50 indicate that the two dissolution profiles in the graph are similar and that the media preparation methods differ by less than 10% (Table 6).

All f_2 values resulting from the comparison of dissolution profiles generated in FaSSiF and FaSSiF-V2 made from instant SIF Powders and prepared from methylene chloride were at least 82. This exceeded the criterion for similarity of $f_2 = 50$ by a wide margin. An f_2 value for ketoconazole tablets in FaSSiF-V2 media could not be calculated because the drug content was below the limit of detection in the FaSSiF-V2 media made from SIF Powder or methylene chloride.

The dissolution profiles of the five drug products in FaSSiF and FaSSiF-V2 prepared from instant powders are presented in Figures 1–5. The dissolution profiles for mefenamic acid, ketoconazole, and danazol in FaSSiF and

Table 5. Drug Solubility in FaSSiF and FaSSiF-V2^a

Drug	Drug Solubility (μg/mL)			
	FaSSiF from SIF Powder Original	FaSSiF prepared by MC	FaSSiF-V2 from SIF Powder-V2	FaSSiF-V2 prepared by MC
Dipyridamole	14.4 (0.3)	15.8 (0.2)	10.1 (2.0)	9.6 (1.2)
Miconazole	100 (20)	100 (20)	17.4 (0.2)	17.2 (1.3)
Danazol	8.2 (0.7)	8.1 (0.0)	2.6 (0.2)	2.7 (0.1)
Ketoconazole	29.5 (1.1)	27.9 (0.0)	6.5 (1.2)	5.3 (2.6)

^aStandard deviations shown in parentheses.

Table 6. Comparison of Drug Product Dissolution Profiles in FaSSIF and FaSSIF-V2 Using f_2 Values

Drug Product	Formulation	f_2 Value	
		FaSSIF (SIF Powder Original and MC)	FaSSIF-V2 (SIF Powder FaSSIF-V2 and MC)
Paracetamol	500-mg tablet	89.0	82.2
Metoprolol tartrate	100-mg tablet	97.4	92.2
Mefenamic acid	250-mg capsule	94.6	99.6
Danazol	100-mg capsule	94.4	95.1
Ketoconazole	200-mg tablet	98.3	NA

FaSSIF-V2 made from the respective SIF Powders and from methylene chloride are provided because these poorly soluble drugs are more likely to be affected by the preparation method.

The release of paracetamol from the generic immediate-release formulation (Boots Pharmaceuticals, UK) was complete within about 20 min in both FaSSIF and FaSSIF-V2 prepared from the respective instant SIF Powders. The corresponding dissolution profiles in these media prepared with methylene chloride are not shown, as they were the same as those in the media prepared with the SIF Powders. The high water solubility of paracetamol is reflected by a complete dissolution profile within 20 min. The bile salt and lecithin are unlikely to influence the dissolution of this highly soluble drug from its formulation.

Metoprolol tartrate release from Lopresor 100-mg tablets was slightly slower compared with paracetamol, but the dissolution of this salt was complete within 30 min in FaSSIF and FaSSIF-V2, again reflecting the high water solubility of this drug as was found with paracetamol. All dissolution profiles were similar to one another according to the f_2 calculation. The corresponding profiles in these media prepared from methylene chloride are not shown, as they were the same as those in the media prepared with the SIF Powders.

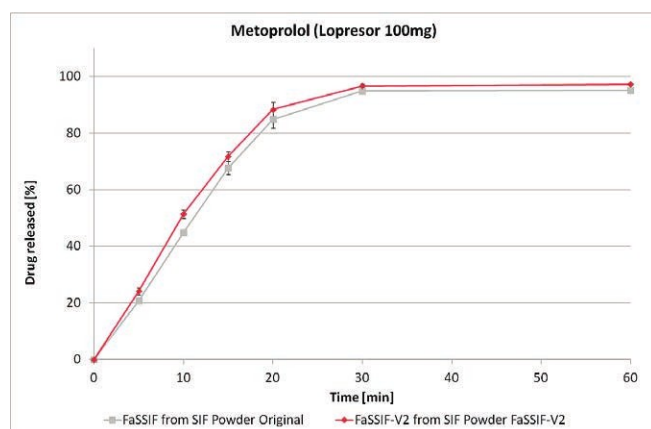


Figure 2. Comparison of the dissolution profiles of metoprolol from Lopresor tablets in FaSSIF and FaSSIF-V2 produced from the respective SIF powders.

The dissolution of mefenamic acid from Mefenamin capsules in FaSSIF and FaSSIF-V2 plateaued at about 5.5% within approximately 10 min in FaSSIF and 20 min in FaSSIF-V2, indicating a slightly faster dissolution rate in FaSSIF.

The dissolution of the mefenamic acid from the Mefenamin capsules in FaSSIF phosphate buffer (without the mixed micelles) was somewhat lower than in FaSSIF and reached a lower plateau value (about 4%). The method of making FaSSIF or FaSSIF-V2 from either the respective SIF Powder or methylene chloride did not affect the dissolution profile of the mefenamic acid from the capsules.

As can be seen from Figure 4, there was a significant difference between the dissolution rates of ketoconazole from Nizoral tablets in FaSSIF and FaSSIF-V2. In FaSSIF, the amount of drug released reached a plateau value of 4% within 30 min. In contrast, less than 1% was released in FaSSIF-V2 even after 120 min. The method of making FaSSIF or FaSSIF-V2 from either the respective SIF Powder or methylene chloride did not affect the dissolution profile of the ketoconazole from the tablets.

The dissolution rate and the amount of dissolved danazol (a neutral compound) from generic danazol capsules

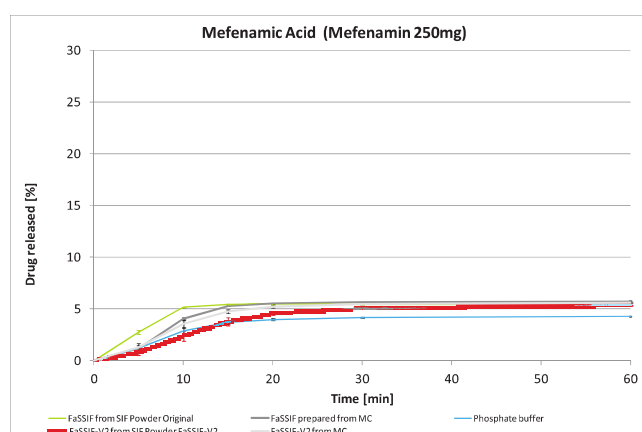


Figure 3. Comparison of the dissolution profiles of mefenamic acid from Mefenamin capsules in FaSSIF and FaSSIF-V2 made from the respective SIF powders, FaSSIF phosphate buffer, and FaSSIF and FaSSIF-V2 prepared from MC.

were higher in FaSSIF than in FaSSIF-V2. The amount of drug released in FaSSIF was about 3% after 10 min, whereas the amount of drug released in FaSSIF-V2 was less than 1% even after 60 min. The method of making FaSSIF or FaSSIF-V2 from either the respective SIF Powder or methylene chloride did not affect the dissolution profile of danazol from the capsules.

DISCUSSION

Equilibrium Solubility

Influence of Preparation Method on Solubility

From Table 5 it can be seen that the method of making FaSSIF does not significantly affect the equilibrium solubility of the four drugs tested. The solubility results for SIF Powder Original were equivalent to those for FaSSIF prepared using methylene chloride. Similarly, solubility results for SIF Powder FaSSIF-V2 were equivalent to those for FaSSIF-V2 prepared using methylene chloride.

Comparison of Drug Solubility in FaSSIF and FaSSIF-V2

The findings confirm the trend that lipophilic drugs with low solubility show higher solubility in FaSSIF than in FaSSIF-V2. For dipyridamole, the difference was only 1.5-fold. This difference may be exaggerated if the drug is more lipophilic. For the other drugs miconazole and ketoconazole (basic) and danazol (neutral), solubility was three to five times higher. The solubility of the drugs in the two fasted-state media with different bile salt and lecithin ratios (within the range found in human aspirates) may reflect how the variations in surfactant ratios occurring in vivo influence drug solubility.

Dissolution

Comparison of the Preparation Methods for FaSSIF and FaSSIF-V2 Using Instant SIF Powder and Methylene Chloride

As demonstrated in Table 6, the f_2 values for all drug products evaluated in the media were at least 82. The high values strongly indicate very close similarity of the dissolution profiles for drug products tested in the media made from either preparation method. Similar f_2 values prove that the two methods can be used interchangeably in preparing biorelevant media for dissolution as well as solubility experiments.

Comparison of Drug Release in FaSSIF and FaSSIF-V2

For the drug products paracetamol and metoprolol tartrate, which are more water soluble, there was no difference between the release rates in the two versions of fasted-state biorelevant media. This was to be expected since the volume of the media used (500 mL) was more than sufficient to provide sink conditions for dissolution in both cases.

The low level of mefenamic acid dissolved after 60 min reflects the low water solubility of this drug in small intestinal fluid. The total amount of drug released after 60 min was about the same in both tested biorelevant

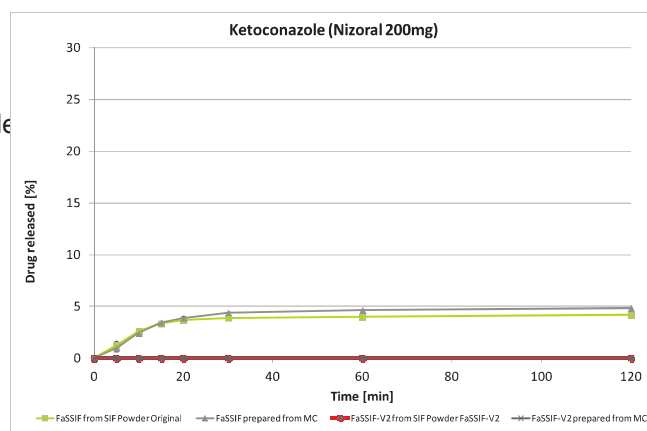


Figure 4. Comparison of the dissolution profiles of ketoconazole from Nizoral tablets in FaSSIF and FaSSIF-V2 made from the respective SIF powders, and FaSSIF and FaSSIF-V2 prepared from MC.

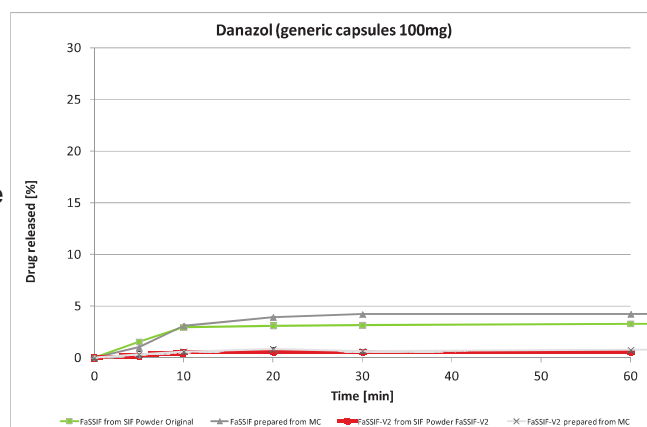


Figure 5. Comparison of the dissolution profiles of danazol from generic danazol capsules in FaSSIF and FaSSIF-V2 made from the respective SIF powders and prepared from MC.

media. However, the release in FaSSIF was faster than in FaSSIF-V2. After 10 min, mefenamic acid reached equilibrium solubility in FaSSIF, whereas in FaSSIF-V2, equilibrium was reached only after 30 min. The higher amount of lecithin in FaSSIF seemed to have a significant influence on the dissolution kinetics of this compound. It is likely that the higher level of lecithin in FaSSIF (compared with FaSSIF-V2) is more favorable for solubilization of mefenamic acid molecules. Since both media have similar surface tension, the effect of wettability is less likely to be the reason.

To understand the contribution of the phosphate buffer to drug dissolution, a control dissolution experiment in the FaSSIF buffer (without taurocholate and lecithin) was carried out. The results indicate that the solubility contribution (due to mixed micelles) is about 2% on an absolute basis. To understand the relative contributions of pH solubility and mixed micelles to dissolution, it is informative to

check dissolution (or at least determine the solubility) in the buffer as a control.

The dissolution profiles of ketoconazole, which is a basic drug, in small intestinal media should be interpreted with caution, as the experiments did not take into account the dissolution process of the drug in gastric media, which has an acidic pH of about 1.6, before exposure in FaSSIF or FaSSIF-V2. The transit through the stomach (acidic pH) is likely to increase solubility and potentially maintain the drug at a higher concentration before it reaches the small intestine. The present studies more closely represent dissolution where there is no previous contact with acidic stomach fluids that can arise in certain disease states (e.g., achlorhydria) or in individuals on medications that raise the stomach pH (e.g., proton pump inhibitors or H₂ antagonist). If there is a potential for supersaturation with basic drugs as identified by Kostewicz (8), two-stage dissolution (i.e., dissolution in FaSSGF prior to transferring to FaSSIF) may be informative.

The differences in the release profiles of danazol in the two intestinal media (FaSSIF and FaSSIF-V2) may be explained by the lipophilic nature of the compound and its relative affinity towards bile salt–lecithin mixed micelles. The type and arrangement of mixed micelles as suggested previously will be different because of the somewhat higher amount of lecithin in FaSSIF as compared with FaSSIF-V2. The ratio of the plateaus reached in the dissolution studies correlate well with the ratios of danazol solubilities in FaSSIF and FaSSIF-V2.

Further Considerations for Testing in FaSSIF or FaSSIF-V2

The main difference between FaSSIF and FaSSIF-V2 lies in the ability to solubilize drugs that are lipophilic and not ionized at intestinal pH. This is due to the differences in the mixed micelle populations formed using higher (FaSSIF) and lower (FaSSIF-V2) concentrations of lecithin. There was a trend, also observed by Söderlind et al. (9), for FaSSIF-V2 to provide lower drug solubility than FaSSIF.

In contrast, for the acidic drug mefenamic acid, no significant difference was observed, presumably because pH plays a more important role in the solubility for a drug that is ionized at intestinal pH. To gain a more complete picture of the range of solubilities that can be achieved in vivo, it may be advantageous to determine the solubility in both FaSSIF and FaSSIF-V2 fasted-state media.

Usage in Preclinical Development

Understanding Drug Solubility

For early stage development compounds where purity, amorphous content, and crystalline content may not be fully controlled, it is advisable to ensure that solubility comparisons are made with the same version. This would also facilitate meaningful comparisons with different drugs within libraries over time. As development progresses and synthetic pathways for a drug improve, drug

purity is likely to be affected. Further changes can occur in chemical (salt or co-crystal) or physical (polymorphic or amorphous content) form. For these reasons, solubility in the biorelevant media should be measured again. This is particularly important before pharmacokinetic testing because the solubility change detected in vitro may directly impact in vivo solubility and dissolution and hence the drug's relative bioavailability.

Support Selection of Formulation Strategy

Conventionally, nonbiological synthetic surfactants (e.g., sodium lauryl sulfate) have been used to simulate gastrointestinal fluids. These media often had to be used retrospectively (i.e., their performance can only be judged after in vivo studies) and then modified in hindsight because solubilization properties of the synthetic surfactants differed from the natural surfactants found in vivo. In contrast, because biorelevant media use surfactants that are present in vivo, biorelevant media can support the selection of the formulation strategy for a new poorly soluble drug prospectively (i.e., prior to in vivo studies). Dissolution testing in biorelevant media composed of bile salt and lecithin can provide an insight into how a drug actually dissolves in fluids that are found in vivo. Dissolution media for simulating in vivo drug dissolution and solubility should be confined more strictly to biorelevant media composed of bile salt and lecithin surfactants within the range found in the gastrointestinal regions.

Usage in Late Stage (Clinical) Development

The following usages apply to the development of generic formulations of known water-insoluble drugs as well as NCEs.

Optimization of Formulation

Once the formulation approach is identified, the media can be used during the formulation optimization of a poorly soluble drug. Biorelevant media, unlike synthetic surfactants, are product independent in vitro media that contain bile salts and lecithin in proportions similar to those found in the small intestines.

Derisking Bioequivalence

Clinical development and clinical testing typically require greater precision. This means that for a specific compound, more attention has to be given to the selection and reproducibility of the biorelevant intestinal media particularly for lipophilic, poorly soluble drugs. Once the release data in the biorelevant media have been examined against in vivo bioavailability, a particularly important routine application of biorelevant media is in dissolution testing to derisk bioequivalence bridging studies. These studies often have to be carried out when a change in formulation is made during clinical studies. This lowers the risk of expensive clinical failures because a new formulation is not bioequivalent to its predecessor.

CONCLUSIONS

Evaluating solubility and dissolution profiles of insoluble drugs and drug products in fasted-state media can greatly streamline development. These two parameters can help identify issues early on that may later occur in vivo. Biorelevant media compositions simulate physiological compositions and are therefore drug product-independent media. This means that the media containing bile salt and lecithin in proportions within the range found in small intestinal fluids do not require modification (e.g., retrospective adjustment of surfactant level) to benchmark in vitro dissolution of formulations of insoluble drugs.

The solubility values and dissolution profiles in each of the media (FaSSiF or FaSiF-V2) produced using methylene chloride and the corresponding SiF Powder are equivalent. Therefore, it is practical to switch from the more labor-intensive solvent evaporation to the use of standardized instant powders for preparing biorelevant media without affecting the solubility and dissolution results. The results show that biorelevant media made from standardized SiF Powders and prepared using methylene chloride may be used interchangeably for solubility and dissolution tests. The drug substances used for the dissolution tests were representative of acid, basic, and neutral compounds.

Testing in the two versions of FaSSiF media with different bile salt/lecithin ratios will give an indication of how the drug and formulation could be affected in vivo by the variation in bile salt/lecithin ratio within the range found in the small intestinal fluids.

REFERENCES

1. Frank, K. J.; Westedt, U.; Rosenblatt, K. M.; Hölig, P.; Rosenberg, J.; Mägerlein, M.; Brandl, M.; Fricker, G. Impact of FaSSiF on the solubility and dissolution-/permeation rate of a poorly water-soluble compound. *Eur. J. Pharm. Sci.* **2012**, 47 (1), 16–20.
2. Galia, E.; Nicolaides, E.; Hörter, D.; Löbenberg, R.; Reppas, C.; Dressman, J. B. Evaluation of Various Dissolution Media for Predicting In Vivo Performance of Class I and II Drugs. *Pharm. Res.* **1998**, 15 (5), 698–705.
3. Marques, M. Dissolution Media Simulating Fasted and Fed States. *Dissolution Technol.* **2004**, 11 (2), 16.
4. Jantratid, E.; Janssen, N.; Reppas, C.; Dressman, J. B. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. *Pharm. Res.* **2008**, 25 (7), 1663–1676.
5. Klein, S. The Use of Biorelevant Dissolution Media to Forecast the In Vivo Performance of a Drug. *AAPS J.* **2010**, 12 (3), 397–406.
6. Degenhardt, O. S.; Waters, B.; Rebelo-Cameirao, A.; Meyer, A.; Brunner, H.; Tötl, N. Comparison of the Effectiveness of Various Deaeration Techniques. *Dissolution Technol.* **2004**, 11 (1), 6–11.
7. *Dissolution Testing of Immediate Release Solid Oral Dosage Forms*; Guidance for Industry; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), U.S. Government Printing Office: Washington, DC, 1997.
8. Kostewicz, E.; Wunderlich, M.; Brauns, U.; Becker, R.; Bock, T.; Dressman, J. B. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. *J. Pharm. Pharmacol.* **2004**, 56 (1), 43–51.
9. Söderlind, E.; Karlsson, E.; Carlsson, A.; Kong, R.; Lenz, A.; Lindborg, S.; Sheng, J. Simulating Fasted Human Intestinal Fluids: Understanding the Roles of Lecithin and Bile Acids. *Mol. Pharm.* **2010**, 7 (5), 1498–1507.

