European Journal of Pharmaceutics and Biopharmaceutics 78 (2011) 531–538

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Use of conventional surfactant media as surrogates for FaSSIF in simulating *in vivo* dissolution of BCS class II drugs

Paula Lehto [a,b], Hanna Kortejärvi [b], Anni Liimatainen [b], Krista Ojala [b], Heli Kangas [b], Jouni Hirvonen [a], Veli Pekka Tanninen [b], Leena Peltonen [a,*]

[a] Division of Pharmaceutical Technology, University of Helsinki, Helsinki, Finland
[b] Orion Corporation, Orion Pharma, Espoo, Finland

ARTICLE INFO

Article history:
Received 22 December 2009
Accepted in revised form 9 February 2011
Available online 15 February 2011

Keywords:
Biorelevant media
Compartmental absorption and transit (CAT) model
Fasted state simulated intestinal fluid (FaSSIF)
Dissolution
In vitro–in vivo correlation (IVIVC)
Pharmacokinetic simulation

ABSTRACT

The usefulness of selected conventional surfactant media to enhance dissolution of BCS class II drugs similarly to fasted state simulated intestinal fluid (FaSSIF) and to predict the absorption of drugs *in vivo* was evaluated. Dissolution behavior of danazol (Danol®), spironolactone (Spiridon®) and N74 (phase I compound) was compared between FaSSIF, containing physiological levels of sodium taurocholate (STC) and lecithin, and dissolution media containing various concentrations of anionic surfactant, sodium lauryl sulfate (SLS) or non-ionic surfactant, polysorbate (Tween) 80. Although these media differed largely in their solubilization ability, micelle size, diffusivity and surface tension, similar dissolution enhancing levels were achieved between FaSSIF and drug-specific concentrations of conventional surfactants. The dissolution enhancement was shown, however, to be important only for danazol and N74, molecules that are characterized by high hydrophobicity. An *in vivo* pharmacokinetic dog study was carried out with N74. Comparison of observed plasma profiles with simulated profiles obtained using compartmental absorption and transit model (CAT) indicated that 0.1% SLS medium was the best to predict *in vivo* plasma profiles and pharmacokinetic parameters (C_{max} and *AUC*). This study demonstrates the potential of substituting FaSSIF with more simple and cost-effective conventional surfactant media. Use of *in vivo* prognostic amounts of synthetic surfactants in dissolution testing could largely assist in industrial drug development as well as in quality control purposes.

1. Introduction

Today, the discovery of drug substances with increasing lipophilicity and resultant poor aqueous solubility is a more and more common problem in the development of orally administered new formulations [1]. The majority of registered drugs fall into Biopharmaceutics Classification System (BCS) class II (high permeability, low solubility) or IV (low permeability, low solubility) [2,3]. For the BCS class II drugs, the oral absorption is predominantly limited by the solubility and/or dissolution in gastrointestinal (GI) tract. In such case, it would be of value to have an *in vitro* dissolution test that could be used to predict the *in vivo* behavior during the various steps in formulation development.

Whereas compendial dissolution media described in the US pharmacopeia [4] are well suited for quality control purposes (QC), they often fail to achieve *in vitro–in vivo* correlation (*IVIVC*), because they primarily consider the effects of pH. Another notable factor controlling the dissolution of lipophilic drugs in GI tract is the presence of solubilizing agents [3]. Biorelevant media simulating the fasted and fed states in the small intestine have been developed and utilized to increase the *in vivo* predictability [5,6]. These simplified media, named as fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF), contain the most important physiological amphiphiles, bile salts (BS) and lecithin (LC) and consider the pH, buffer capacity and osmolality of the gut lumen. To better reflect the solubilization capacity in the fed state, digestion products, such as fatty acids and monoglycerides, have additionally been included in the media [7–10].

Although the use of biologically relevant media has provided improvements in seeking *IVIVC* for BCS class II formulations, the high cost and complexity of these media limit their extensive use for industrial applications and more simple media are needed. An ideal solution would be got if BS/LC mixtures could be substituted with synthetic surfactant(s) exhibiting similar wetting and solubilization behavior [11]. The amounts of synthetic surfactants recommended by drug authorities are based on QC dissolution purposes of poorly water-soluble drugs and may not reflect the conditions *in vivo* [4,12]. Despite many speculations that matching the physicochemical properties of aliphatic surfactants with those

* Corresponding author. Division of Pharmaceutical Technology, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland. Tel.: +358 9 19159159; fax: +358 9 19159144.
E-mail address: leena.peltonen@helsinki.fi (L. Peltonen).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.02.007

of uniquely structured BS/LC mixtures may be difficult, no studies were found in literature where those would have been systematically compared with respect to dissolution enhancement.

The present study aims at evaluating the usefulness of synthetic surfactants for their ability to enhance drug dissolution similarly to FaSSIF and predict drug absorption *in vivo*. Dissolution behavior of three BCS class II drug formulations (Danol®, Spiridon® and N74 capsule (compound under phase I development)) was investigated in FaSSIF, in a medium containing biologically relevant levels of physiological amphiphiles (sodium taurocholate (STC) and lecithin (LC)) and in a medium containing various concentrations of two conventional detergents, anionic sodium lauryl sulfate (SLS) and non-ionic polysorbate (Tween) 80. Differences between the media were evaluated with regard to solubilization, micelle size, diffusivity and surface tension. Absorption of N74 was also evaluated in fasted dogs. The pharmacokinetic, solubility and dissolution data were combined with GI tract physiology in compartmental absorption and transit (CAT) [13] model to compare the simulated plasma profiles against the observed profile.

2. Materials and methods

2.1. Raw materials and drug products

Spironolactone (Ph.Eur.) (Pharmacia Ltd., United Kingdom), Danazol (USP XXVIII) (Yangzhou Pharmaceutical Co., Ltd., China) and N74 (phase I drug molecule) (Orion Pharma, Orion Corporation, Finland) were used as active substances in the studies. Spiridon® and Danol® tablets were purchased from Orion Pharma (Orion Corporation, Finland) and Sanofi-Synthelabo (UK), respectively. Ten milligrams of N74 was filled into hard gelatine capsules (size 0, Coni-Snap, Capsugel, USA). Sodium taurocholate (STC) (from ox, S9875 grade) and egg phosphatidylcholine (lecithin) (Lipoid E PCS, n.l.t. 96% pure) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and Lipoid GmbH (Ludwigshafen, Germany), respectively. Sodium lauryl sulfate (96% pure), Tween 80 (per sintesi) and sodium-based phosphate buffer pH 6.8 (Ph. Eur.) were supplied by Fluka (Bucfs, Switzerland), Merck-Schicherdt (Hohenbrunn, Germany) and FF Chemicals (Finland), respectively. Characteristics of the studied drug substances are summarized in Table 1.

2.2. Dissolution media

2.2.1. Composition of dissolution media

Fasted state simulated intestinal fluid (FaSSIF) [15], containing 3 mM of sodium taurocholate (STC) and 0.75 mM of lecithin, was used for the simulation of surfactant content in the intestine. Since all the studied drugs were unionized in physiological pH range (pH 1–7), dissolution tests were carried out only in one pH (pH 6.8 of small intestine [16]). Concentrations of 0.02, 0.1, 0.2, and 0.014, 0.035 and 0.07 (w/v (phosphate buffer pH 6.8)%) were chosen for SLS and Tween 80 media, respectively, based on critical micelle concentration (CMC) measurements. The studied surfactant and surfactant concentration were decided drug-specifically.

Table 1
Characteristics of drug substances and products studied.

	Danazol	N74[b]	Spironolactone[b]
MW	337.5 g/mol[a]	353.35 g/mol	416.6 g/mol
$pK_{a\ (acid/base)}$	Unionized in pH 1–7	Unionized in pH 1–7	Unionized in pH 1–7
Log *P* (octanol/water)	4.21; 3.91[a]	3.1	2.8
BCS classification	II (LS/HP[c]) [b]	II (LS/HP[c])	II (LS/HP[c])
Product	Danol® 100 mg	N74 10 mg	Spiridon® 25 mg
Formulation	IR-tablet	IR-capsule (phase I substance)	IR-tablet
Marketing license	Sanofi-Synthelabo[e]		Orion Pharma

[a] [14].
[b] Data provided by Orion Pharma.
[c] LS/HP = Low solubility/high permeability.

2.2.2. Critical micelle concentration (CMC) determination and surface tension measurements

The CMCs were determined by measuring surface tension change with surfactant concentration (37 °C, 0.1 M NaCl). The liquid/air surface tensions were measured with wire probes using Du Noüy maximum pull force method (Delta-8 automatic multichannel micro tensiometer with eight parallel balances, Kibron Inc., Finland). Calibration was verified using deionized distilled water before the measurements. Measurements were taken in 96-well plates, and 3–6 separate aliquots of each solution with a volume of 50 μl per sample were measured.

2.2.3. Micelle characterization

The hydrodynamic sizes ($d(H)$) of the micelles in each media were determined at 37 °C solutions by dynamic light scattering (DLS) measurements (Zetasizer Nano ZS, Malvern Instruments Ltd., UK) at detection angle of 173° and wavelength of 633 nm. All studied samples were dust-cleaned using membrane filters (Millipore) with a pore diameter of 0.22 μm. Translational diffusion coefficients (D) obtained from autocorrelation of the collected photons were converted to hydrodynamic sizes of particles using Stokes–Einstein relation (Eq. (1)):

$$d(H) = \frac{kT}{3\pi\eta D} \quad (1)$$

where $d(H)$ is hydrodynamic diameter, D is translational diffusion coefficient, k is Boltzmann's constant, T is absolute temperature and η is viscosity. The micelle sizes were presented as intensity distributions.

2.3. Solubility and dissolution experiments

2.3.1. Solubility determination

Excess amount of drug powder ($n = 3$) was added into 25 ml of different media at pH 6.8. The samples were shaken gently in a heating chamber at 37 °C for 3 h. The clear solution above the precipitate was collected and centrifuged at 2500 rpm for 5 min (37 °C). The supernatant was filtered through a 0.45-μm filter (Agilent). The filtrate was kept overnight at 37 °C, in case of supersaturation. If a precipitate was to be found, then centrifugation and filtering were repeated.

2.3.2. Flow-through dissolution rate study

A Sotax® flow-through dissolution tester (Sotax, Basel, Switzerland) was used. The system was equipped with Ø22.6-mm cells that were maintained at 37 ± 0.5 °C and connected to a piston pump (model CY7-50, Sotax). Drug formulations were positioned on a bed of glass beads (1 g) in testing chambers during the experiments. Flow rate of 8 ml/min was used since velocities (2 cm/min using Ø 22.6 mm cells) close to those reported to exist in GI tract (1.5 cm/min) were achieved [7]. The apparatus was used as an open loop, and the fractions were collected at 15-min intervals during the first hour, 30-min intervals during the second hour and 1-hour intervals after that. Before analysis, the sample solutions were filtered through filters chosen in each case for their lack of absorption of the compound in question.

2.3.3. Assay

Samples from solubility and dissolution experiments were analyzed by a reverse-phase HPLC (Agilent 1100) assay using a UV detector. Quantification was done using external standards. *Danazol:* 100-μl samples were injected onto Lichrospher RP-18 (125 × 4.0 mm, 5 μm) column. The flow rate of the mobile phase (acetonitrile/water (50:50, v:v) was 1.0 ml/min. Danazol was detected at 280 nm with a retention time of 4.9 min. *N74:* The mobile phase comprised of 50:50 water/acetonitrile. Twenty-five-microliter samples were injected onto Symmetry C8 (4.6 × 75 mm, 3.5 μm) column (45 °C) and N74 was detected at 268 nm. Flow rate of 1.0 ml/min resulted in a retention time of 2.7 min. *Spironolactone*: The mobile phase consisted of tetrahydrofurane/water (21.5:78.5, v/v); 10-μl samples were injected onto column (75 × 4.6 mm Zorbax exlipse XBD-phenyl column (3.5 μm)) kept at 45 °C. The flow rate of 2.2 ml/min resulted in a retention time of 9 min. Detection wavelength was 250 nm.

2.4. In vivo canine pharmacokinetics

The pharmacokinetics of N74 was studied with four male and four female beagle dogs ranging in body weight from 6 to 10 kg. They were fasted overnight until 2 h after dosing, but were allowed free access to water. Intravenous injection, oral solution and oral capsule were administered for each dog with a dose level of 1 mg/kg from body weight. A 7-day washout period between each treatment was used. Blood samples were collected at 5, 10, 15, 30 and 45 min and 1, 2, 3, 5 and 8 h after dosing. The N74 concentrations in the plasma were analyzed by an LC–MS/MS method provided by Orion Pharma (Orion Corporation, Finland).

The protocol was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee of NOTOX (DEC NOTOX 00-34) as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures described in the protocol are based on the following guidelines: ICH Harmonized Tripartite Guideline, Note for Guidance on Pharmacokinetics: The assessment of systemic exposure in toxicity studies, 27 October 1994.

Pharmacokinetic *i.v.* data were shown to follow best the pharmacokinetic two-compartment model, which was used to solve the basic pharmacokinetic parameters, such as volume of distribution (V_{ss}), elimination rate constant (K_{el}) and distribution rate constants (K_{12}, K_{21}). Dissolution ($K_{d(in\ vivo)}$) and apparent absorption rate (K_{app}) constants were calculated by means of mean residence times (*MRT*) resolved from *i.v.*, oral solution and capsule data by trapezoidal rule. Oral bioavailability (*BA*) of N74 derived from oral solution vs. *i.v.* data was utilized to transform apparent absorption rate constant to absorption rate constant (K_a). Obtained rate constants were further used for plasma profile simulations (Table 2).

Table 2
Physiological and pharmacokinetic parameters used in CAT model.

CAT model parameters	Value
Physiological parameters	
Gastric emptying rate constant of liquid ($K_{ge(l)}$) (h^{-1})	10[a]
Gastric emptying rate constant from capsule (K_{ge}) (h^{-1})	0.63 [18]
Intestinal transit rate constant (K_t) (h^{-1})	Low 3.7 [18], high 2.3 [19]
Liquid volume in stomach (ml)	500 [20]
Liquid volume in intestine (ml)	200 [20]
Pharmacokinetic parameters	
Absorption rate constant (K_a) (h^{-1})	2.2
Distribution constants (K_{12}, K_{21}) (h^{-1})	0.3, 0.82
Elimination rate constant (K_{el}) (h^{-1})	1.45
Volume of distribution (V_{ss}) (l)	7.46
Bioavailability (BA) of oral solution	0.69
Relative bioavailability of capsule vs. oral solution	0.29

[a] Defined by simulations.

2.5. Simulation of plasma profiles

Simulated plasma profiles of N74 were obtained using a compartmental absorption and transit model (CAT) first described by Yu et al. [13] (software STELLA® 8.1, isee systems, Lebanon, USA) and further developed by Kortejärvi et al. [17] to describe gastric emptying of liquids and solids in humans. In this study, the physiological part of the model was adapted to correspond to canine GI tract conditions. Gastric emptying and intestinal transit rates and liquid volumes in canine GI tract were taken from literature. Parameters used in simulations with corresponding references are summarized in Table 2. Since somewhat varying small intestinal transit times were found, simulations were performed with both low and high values. The simulation model (Fig. 1) was based on the assumptions of negligible gastric and colonic absorption, simultaneous solid and liquid emptying from the stomach, similar transit and distribution in the intestine and passive permeability to central compartment. The same first-order absorption and dissolution rate constants were used for each of the seven intestinal compartments.

Dissolution rate constants (K_d) calculated from the *in vitro* dissolution data obtained in the different media were used in this

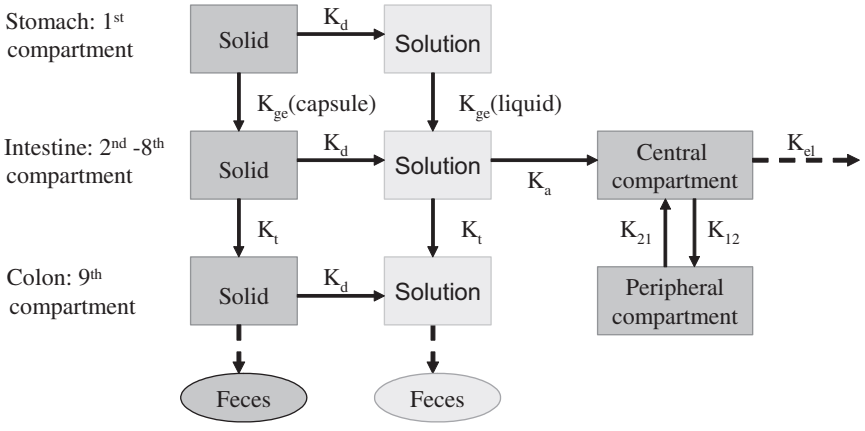

Fig. 1. The structure of CAT simulation model consisting nine compartments: stomach, seven-compartmental small intestine and colon. Rate constants for gastric emptying (K_{ge}) (for capsule and solution), dissolution (K_d), intestinal transit and distribution (K_t), absorption (K_a), distribution (K_{12}, K_{21}) and elimination (K_{el}) are illustrated in figure. In this model, absorption is only allowed from small intestine.

study as input parameters for simulations. Fractional dissolution rate is defined as (Eq. (2)) [21]:

$$k(t) = \frac{dDP(t)/dt}{1 - DP(t)} \quad (2)$$

In this study, first-order models with plateau correction, b, were fitted to each dissolution profile (Eq. (3)):

$$DP(t) = b(1 - e^{-at}) \quad (3)$$

Eq. (4) gives the fractional dissolution rate for first-order model, which is constant over time:

$$k(t) = ab \quad (4)$$

where $k(t) = K_d$.

Measured equilibrium solubilities were used to account for solubility-limited dissolution according to the Noyes–Whitney equation. In order to predict *in vivo* plasma profiles based on the *in vitro* data, scale factor of 0.29 (corresponding to the relative BA of capsule vs. solution) was used to correct the plasma concentration levels.

2.6. Statistical dissolution profile comparison

Flow-through dissolution profiles were evaluated using t-test to compare the dissolution efficiencies (DE%) (Eq. (5)) [22]:

$$\mathrm{DE}\%(T) = \frac{\int_0^T DP(t)dt}{DP_{100\%} \cdot T} \cdot 100\% \quad (5)$$

where $DP(t)$ is per cent of drug dissolved at time t, $DP_{100\%}$ symbolizes 100% dissolution and the integral represents the area under dissolution curve between time 0 and T. The per cents of DE were calculated using the trapezoidal method at 180 min. All model fittings in this study were performed with SAS software, version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results and discussion

3.1. Properties of the dissolution media

Critical micelle concentrations (CMC), surface tensions, micelle sizes and diffusivities of the studied dissolution media are compiled in Table 3. In all the media used for dissolution studies, the CMC was exceeded, indicating solubilization capability of drug molecules. In the presence of lecithin, the CMC of STC dropped from ~5.0 to ~0.4–0.6 mM STC (25 °C; STC/lecithin ratio 4:1; 0.1 M NaCl). This behavior was expected due to more effective solubilization capacity of the mixed micelles [23]. Moreover, whereas SLS and Tween 80 gave precise CMC values, the CMC of STC was not as evident. Difficulty to define the exact CMC value is related to the observed fact that bile aggregates gradually grow in size with increasing concentration over wide concentration range, while the conventional amphiphiles with long alkyl chains increase their aggregation number over narrow concentration range around the CMC resulting in more evident CMC [24]. Overall, direct comparison of the obtained CMCs to literature values is difficult, as CMC is dependent on purity and ionic/non-ionic character of surfactant used, pH, temperature and counterion concentration.

Surface tension comparisons were utilized to indicate the wetting ability of drug surfaces in the studied media (Table 3). A high surface tension (~72 mN/m) obtained for plain phosphate buffer corresponds to that of water (72.8 mN/m) (Table 3) [14]. Significantly lower surface tensions (~34–46 mN/m) and, thus, better wetting properties were revealed with each media containing the surfactants. Compared to surface tension of ~34 mN/m prevailing in human intestinal fluids (HIF) [16], SLS gave the closest values (~32 mN/m). The surface tension for FaSSIF (~45 mN/m) remained at a much higher level, but was closer to HIF than reported for originally published composition of FaSSIF [14]. This margin was mainly attributed to differences in purity and origin of the used bile salt and lecithin materials [25].

For STC/lecithin mixed micelles, DLS measurements gave hydrodynamic micellar radii (R_h) of 91 nm (Table 3). This was five-fold and 18-fold larger compared to R_h obtained for Tween (~5 nm) and SLS (~17 nm) micelles, respectively. Correspondingly, the diffusivity of micelles decreased in the order SLS > Tween ≫ STC/lecithin. Although all the media were characterized by single particle size, significantly higher deviations in R_h values were observed with FaSSIF (only particles assembling >1% of the volume were considered significant). The observed large STC/lecithin aggregates were in line with a recent study showing particles in the vesicular size range [7]. In another study, similar STC/lecithin ratio (4:1) produced species with much smaller sizes (~5 nm) [26]. It is generally presumed that larger mixed disk structures are present only with higher lecithin ratios (>0.6). However, the sensitivity of forming species to processes related to manufacture of the physiologically based media (*e.g.* diluting of the concentrated micellar solution) have recently been proposed as a source for deviating results [27]. In the cases of SLS and Tween 80 media, no progressive increases in the R_h were observed with increasing concentration, indicating that although large lamellar and liquid crystalline structures are known to exist in higher conventional detergent concentrations [25], in this concentration range principally typical, spherically shaped simple micelles existed. Moreover, no significant changes in particle sizes were observed upon saturation of the media with the studied drug substances (results not shown).

3.2. In vitro dissolution

Fig. 2a–c shows the equilibrium solubility values of danazol, N74 and spironolactone in the plain buffer, FaSSIF and conven-

Table 3
Characterization of dissolution media with regard to CMC, surface tension, size of colloidal species (R_h) and diffusion coefficient determined by dynamic light scattering, D_{DLS}.

Media	CMC (n = 3, mean value)	Surface tension ± s.d. (mN/m)	Micellar size R_h ± s.d. (nm)	D_{DLS} (cm^2/s)
Buffer	–	71.7 ± 0.1	–	–
STC	*CMC 0.4 mM*	45.8 ± 1.2	–	–
FaSSIF	Above CMC (3 mM STC)	45.5 ± 0.2	91 ± 10	0.69×10^{-6}
SLS	*CMC 0.8 mM*	32.6 ± 0.4	–	–
SLS 0.02%	Below CMC (0.7 mM)	36.5 ± 0.3	5.0 ± 0.7	12.5×10^{-6}
SLS 0.1%	Above CMC (3.5 mM)	32.3 ± 0.7	5.2 ± 0.8	12.1×10^{-6}
SLS 0.2%	Above CMC (6.9 mM)	32.4 ± 0.5	4.9 ± 0.4	12.8×10^{-6}
Tween 80	*CMC 0.038 mM*	45.7 ± 0.1	–	–
Tween 0.014%	Above CMC (0.11 mM)	46.0 ± 0.1	16.7 ± 1.8	3.75×10^{-6}
Tween 0.035%	Above CMC (0.27 mM)	45.7 ± 0.1	16.3 ± 2.4	3.85×10^{-6}
Tween 0.07%	Above CMC (0.53 mM)	45.8 ± 0.2	16.6 ± 1.2	3.78×10^{-6}

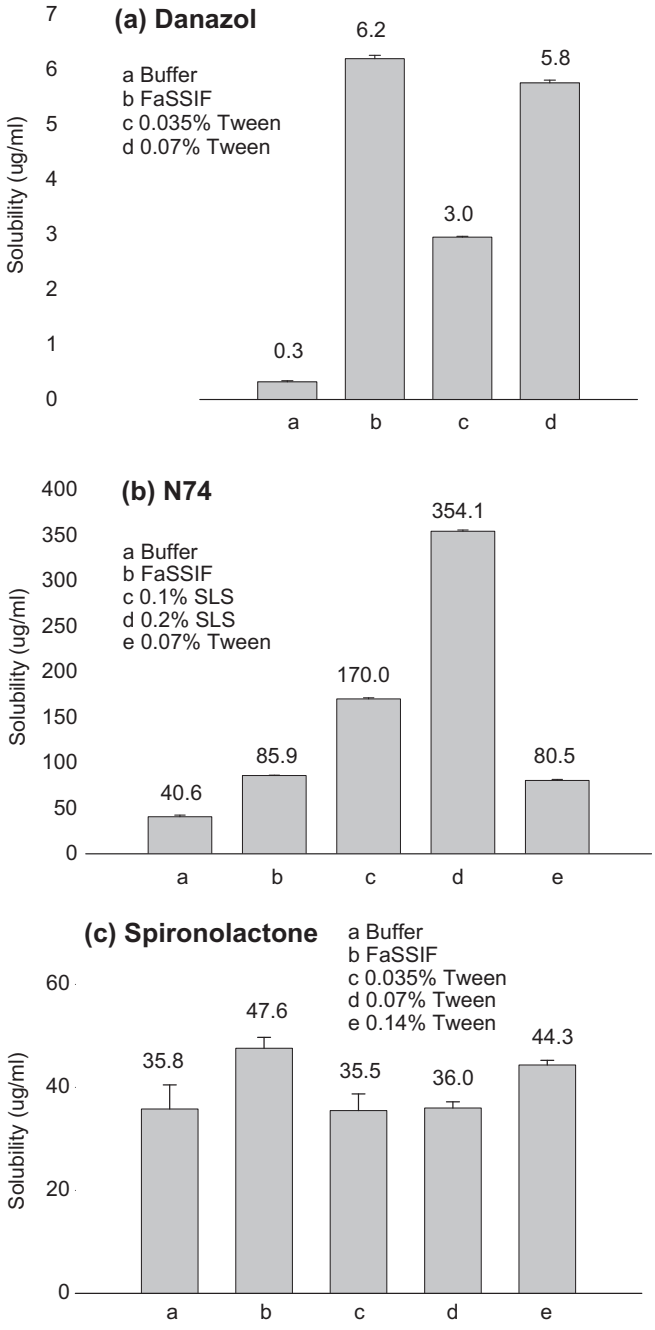

Fig. 2. Saturation solubilities of (a) danazol, (b) N74 and (c) spironolactone in different dissolution media at pH 6.8 (mean ± s.d., $n = 3$).

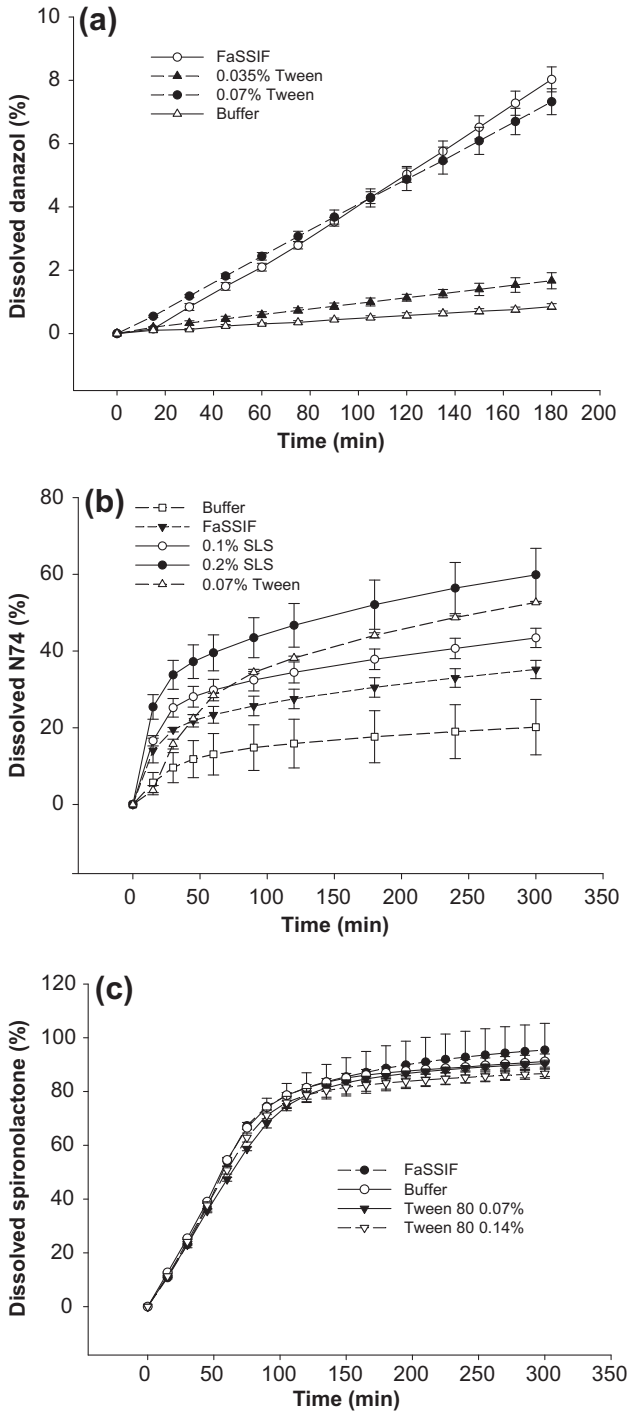

Fig. 3. Dissolution profiles of (a) Danol® 100-mg tablets, (b) N74 10-mg capsules and (c) Spiridon® 25-mg tablets in different dissolution media at pH 6.8 (mean ± s.d., $n \geqslant 3$).

tional surfactant media (pH 6.8). Dissolution profiles in the corresponding media are depicted in Fig. 3a–c. Due to the very low aqueous solubility characteristics, the dissolution of danazol tablet in buffer without added surfactant was ~0.85% within 3 h; however, this was increased ninefold in FaSSIF (Figs. 2a and 3a). More than an order of magnitude higher aqueous solubility of N74 contributed to higher per cents dissolved (~20% in 3 h), but dissolution was enhanced only modestly in FaSSIF (twofold) (Figs. 2b and 3b). Despite the almost equal solubility with N74, spironolactone exhibited much more complete dissolution (~90% in 3 h), implying much better wetting characteristics (Figs. 2c and 3c). Moreover, the dissolution of spironolactone showed no discrimination between the buffer and FaSSIF, or any other tested surfactant media. Observed trend in dissolution enhancement (danazol ≫ N74 > spironolactone) was clearly related to the magnitude of solubilization by surfactants, driven by hydrophobicity of drug (Table 1, log *P* values). This agrees well with previous studies, which, using large data sets, have shown correlation between increasing octanol–water partition coefficient (log *P*) and increasing affinity to bile salt micelles [11].

For more detailed comparison of dissolution behavior between FaSSIF and conventional surfactant media, dissolution efficiencies (DE%) were calculated (Table 4). For danazol, 0.07% Tween 80 concentration (w/v) gave equal DE% with FaSSIF. Considering the

Table 4
Dissolution efficiencies of Danol® tablets, Spiridon® capsules and N74 capsules measured up to 180 min in different dissolution media (pH 6.8).

(DE) (%) mean ± s.d.							
	Buffer	FaSSIF	0.035% Tween 80	0.07% Tween 80	0.14% Tween 80	0.1% SLS	0.2% SLS
N74	13.3 ± 2.5	23.8 ± 0.6	–	29.7 ± 0.2	–	29.9 ± 2.5	40.6 ± 2.0
Danol	0.4 ± 0.0	3.7 ± 0.2	0.9 ± 0.1	3.7 ± 0.2	–	–	–
Spiridon	60.6 ± 0.2	60.8 ± 2.3	–	57.5 ± 0.1	58.2 ± 0.2	–	–

similar solubility in these two media, it could have been assumed that the significantly lower diffusivity of large STC/lecithin mixed micelles (Table 3) would have resulted in lower rate of dissolution. Hence, these results indicate that the payload of danazol molecules per micelle was greater in FaSSIF, compensating the poor effective diffusive transport through the boundary layer [13]. In the case of less lipophilic N74, 0.07% Tween 80 and 0.1% SLS were the closest, but still overestimated the DE% in FaSSIF. It should be noted that, initially, the Tween 80 medium showed a less steep slope, indicating slower wetting process (Fig. 3b). The fact that higher solubilization capacity (Fig. 3a), better wetting ability (according to the lowest surface tension) (Table 3) and higher diffusivity (Table 3) of 0.1% SLS compared to 0.07% Tween 80 and FaSSIF media did not result in superior DE% implies lower payload of N74 molecules per SLS micelle.

Overall, the dissolution profiles that plateaued when clearly less than 100% was dissolved (danazol ~1–8%, N74 ~20–60% and spironolactone ~90%) evidence the absence of true sink conditions (<30% of saturation solubility) [4]. The non-sink conditions were likely caused by the low flow rate (8 ml/min). Although being physiologically relevant, this flow rate was probably not high enough to prevent the possible sedimentation of particles, especially the more hydrophobic ones (such as danazol and N74), on the lower sides of the cell (due to its vertical position) [28]. The non-sink conditions can be understood from aqueous dose/solubility ratios (*D*:*S*), which are 333, 1.2 and 0.7 l (Table 1) for danazol, N74 and spironolactone, respectively. Sink conditions could have been attained, at least in the cases of N74 and spironolactone, by increasing the flow rate. However, non-sink dissolution is considered acceptable, if it is shown to be more discriminating from *in vivo* perspective [29]. In addition to volume of luminal fluids (fasted 140–400 ml, fed 1.5 l [30,31]), the maintenance of sink conditions depends on permeability. At least in the case of danazol, it was obvious that even the high permeability characteristics (Table 1) were not able to maintain the sink conditions in fasted or fed states *in vivo*.

3.3. Simulated vs. observed plasma profiles of N74

In vitro solubility and dissolution data of N74 were further utilized for simulation of *in vivo* plasma profiles (using the CAT model). Fig. 4 shows the mean observed plasma profile obtained from a pharmacokinetic dog study and simulated profiles obtained using dissolution rate constants (K_d) in buffer, FaSSIF, SLS and Tween 80 media as input parameters. The predictability of the simulation model was evaluated by comparing simulated (using *in vivo* K_d and solubility in FaSSIF) versus observed plasma profiles and prediction errors for *AUC* and C_{max} values (Table 5). The predicted *AUC* and C_{max} were shown to be sensitive for intestinal transit rate. Although both high and low transit rate gave very good predictions of C_{max}, the high transit rate resulted as superior prediction of *AUC* (PE 13% (K_t, 2.3) vs. 22% (K_t, 3.7)). Hence, the reported faster small intestinal transit seems to better describe the dogs used for this study [32]

As a consequence of the above-described observations, the prediction ability of different *in vitro* dissolution media is discussed only from the high intestinal transit rate point of view. From the media studied, 0.1% SLS resulted in the closest prediction of the *in vivo* observed plasma profile (PE 1.05% and 0.87% for C_{max} and *AUC*, respectively) (Fig. 4 and Table 5). The prediction errors obtained from other media were significantly higher for either C_{max} (11–41%) or *AUC* (11–34%). The fact that FaSSIF, which is designed on the basis of human GI liquids, gave lower C_{max} and *AUC* than observed *in vivo* could be related to physiological differences between human and canine GI tract. For example, dogs have reportedly higher small intestinal bile salt levels, which increase the solubilization capacity and may ultimately lead to higher amounts absorbed [33]. Thus, against absorption data in humans, FaSSIF would probably correlate better.

The observed high variation between dogs was likely related to variable physiological conditions in canine GI tract, such as reported fluctuating small intestinal transit times [18,19] or volume of liquids (due to free access to water during the study). Such variation complicated the definition of representative *in vivo*

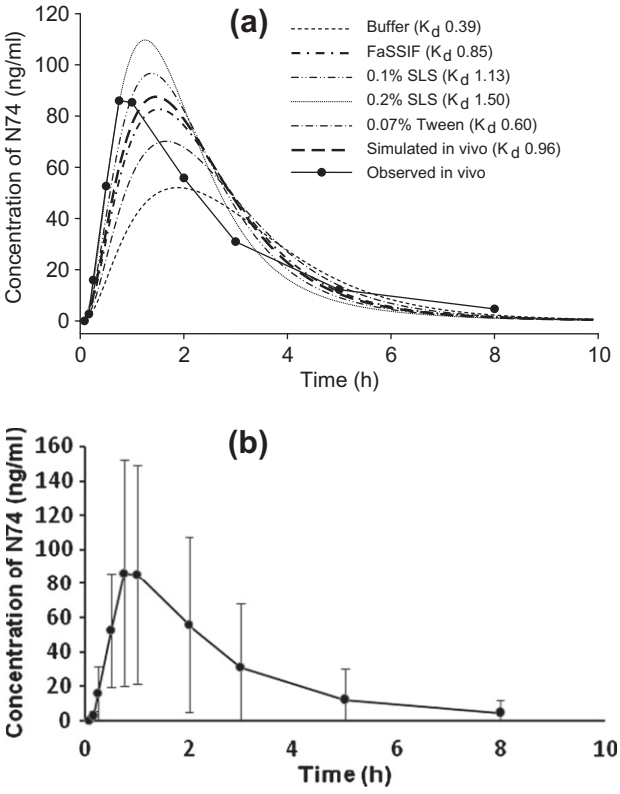

Fig. 4. Observed versus simulated plasma profiles of N74 are shown in Fig. 4a. Dissolution rate constants (K_d) used as input parameters in simulations are illustrated in figure. Simulated profile using *in vivo* K_d is shown in figure as an indicator of the predictability of the model. *In vivo* results with SD are shown in Fig. 4b (n = 8).

Table 5
Ratios of *AUC* and C_{max} values (simulated/observed *in vivo*) of N74 with prediction errors (PE%).

	C_{max} (ng/ml) Simulated/observed (PE%)		AUC (h ng/ml) Simulated/observed (PE%)	
	K_t, 2.3 (high)	K_t, 3.7 (low)	K_t, 2.3 (high)	K_t, 3.7 (low)
Simulated *in vivo*	0.95 (5)	0.98 (2)	0.85 (13)	0.78 (22)
Phosphate buffer	0.56 (41)	0.55 (45)	0.66 (34)	0.55 (45)
FaSSIF	0.89 (11)	0.89 (11)	0.83 (17)	0.75 (26)
0.1% SLS	1.05 (5)	1.04 (4)	0.87 (13)	0.80 (20)
0.2% SLS	1.19 (19)	1.18 (18)	0.89 (11)	0.84 (16)
0.07% Tween 80	0.76 (24)	0.89 (25)	0.78 (22)	0.68 (32)

dissolution and physiological GI parameters for the simulation model. This was presumably one, if not the main, reason for the slight inaccuracy in prediction of T_{max}. Another uncertainty in the simulations is related to observed non-sink *in vitro* dissolution conditions. Since the solubility limitation *in vivo* was already taken into account in the simulation model (by using the Noyes–Whitney equation), the use of non-sink dissolution data as input function may have unnecessarily 'increased the solubility limitation on dissolution', leading to lower simulated amounts absorbed. To validate the simulation model, dissolution data from different formulations of N74 would be needed. Overall, the utilized absorption plasma profile simulations that consider the input phase as the result of processes taking place in various compartments (*e.g.*, stomach, small intestine, colon) give much more precise estimation of the input profile than the traditional convolution approach, which usually succeeds only when the absorption rate is determined by the product (*e.g.* extended-release drug products) [13]. For construction of a simulation model to guide the early-phase formulation development and selection of drug products for human *in vivo* studies, canine pharmacokinetic data are increasingly exploited. Therefore, to increase the prediction accuracy, future efforts will be directed to transform the used advanced nine-compartment CAT model [13,17] to better correspond to GI physiology in dogs.

Currently, typical amounts of conventional surfactants in dissolution testing monographs of poorly soluble compounds range from 0.5% up to 2.0% [4]. On the basis of this study, those amounts can be considered to grossly overestimate the solubility and dissolution from *in vivo* point of view. The use of more biorelevant amounts of conventional surfactants in dissolution media will not only be beneficial for predicting absorption during various steps of drug product development, but it could also raise the quality control dissolution testing into a more meaningful level.

4. Conclusions

With the aim to simulate *in vivo* dissolution behavior of N74 (BCS class II drug), this study demonstrates the utility of simple and cost-effective conventional surfactant media in substituting more complex, physiologically based dissolution medium, FaSSIF. Similar dissolution enhancement was shown to be achievable with very different combinations of properties (such as solubilization, wettability, diffusivity) between the media, underlining the importance of understanding surfactant-facilitated dissolution as a whole. Although the biorelevant concentrations of SLS and Tween 80 found in this study can be used as good starting point, it is obvious due to specificity of drug–surfactant interactions that these amounts has to be judged against *in vivo* dissolution data specifically for each drug. By using biorelevant amounts of synthetic surfactants, the *in vivo* prognostic dissolution testing could be extended from research and development purposes to a meaningful quality control tool.

Acknowledgement

The study was sponsored by Orion Pharma.

References

[1] S. Stegman, F. Leveiller, D. Franchi, H. de Jong, H. Linden, When poor solubility becomes an issue: from early stage to proof of concept, Eur. J. Pharm. Sci. 31 (2007) 249–261.
[2] G.L. Amidon, H. Lennernäs, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability, Pharm. Res. 12 (1995) 413–420.
[3] J.H. Porter, W.N. Charman, In vitro assessment of oral lipid based formulations, Adv. Drug Deliv. Rev. 50 (2001) 127–147.
[4] USP XXXI, *<711> Dissolution and Monographs*. The United States Pharmacopeial Convention, Rockville, 2008.
[5] 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 class II drugs, Pharm. Res. 15 (1998) 698–705.
[6] E. Nicolaides, M. Symillides, J.B. Dressman, C. Reppas, Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration, Pharm. Res. 18 (2001) 380–388.
[7] V.H. Sunesen, B.L. Pedersen, H.G. Kristensen, A. Müllertz, 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 (2005) 305–313.
[8] H. Wei, R. Löbenberg, Biorelevant dissolution media as a predictive tool for glyburide a class II drug, Eur. J. Pharm. Sci. 29 (2006) 45–52.
[9] B.-M. Lue, F.S. Nielsen, T. Magnussen, H.M. Schou, K. Kristensen, L.O. Jacobseb, 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.
[10] E. Jantratid, N. Janssen, H. Chokshi, K. Tang, J.B. Dressman, Designing biorelevant dissolution tests for lipid formulations: case example – lipid suspension of RZ-50, Eur. J. Pharm. Biopharm. 69 (2008) 776–785.
[11] T.S. Wiedman, W. Liang, L. Kamel, Solubilization of drugs by physiological mixtures of bile salts, Pharm. Res. 19 (2002) 1203–1208.
[12] Center for Drug Evaluation and Research (CDER), FDA Guidance for Industry; Dissolution Testing of Immediate Release Solid Oral Dosage Forms, Rockville, 1997.
[13] L.X. Yu, J.R. Crison, G.L. Amidon, Compartmental transit and dispersion model analysis of small intestinal transit flow in humans, Int. J. Pharm. 140 (1996) 111–118.
[14] E. Galia, J. Horton, J.B. Dressman, Albendazole generics – a comparative in vitro study, Pharm. Res. 16 (1999) 1871–1875.
[15] M. Marques, Dissolution media simulating fasted and fed states, Dissolution Tech. 4 (2004) 14.
[16] B.L. Pedersen, H. Bronsted, H. Lennernäs, F.N. Kristensen, A. Müllertz, H.G. Kristensen, Dissolution of hydrocortisone in human and simulated intestinal fluids, Pharm. Res. 17 (2000) 183–189.
[17] H. Kortejärvi, A. Urtti, M. Yliperttula, Pharmacokinetic simulation of biowaiver criteria: the effects of gastric emptying, dissolution, absorption and elimination rates, Eur. J. Pharm. Sci. 30 (2007) 155–166.
[18] J.B. Dressman, K. Yamada, in: P. Welling, F.L. Tse (Eds.), Animal Models for Oral Drug Absorption, Pharmaceutical Bioequivalence, Dekker, New York, 1991, p. 256.
[19] J.D.R. Schulze, E.E. Peters, A.W. Vickers, J.S. Staton, M.D. Coffin, G.E. Parsons, A.W. Basit, Excipients effects on gastrointestinal transit and drug absorption in beagle dogs, Int. J. Pharm. 300 (2005) 67–75.
[20] A.C. Andersson, in: A.C. Andersson, L.S. Good (Eds.), Digestive System in Beagle Dogs as Experimental Animal, Iowa State University Press, Ames, IA, 1970, pp. 226–231.
[21] P. Lánský, P. Weiss P, Classification of dissolution profiles in terms of fractional dissolution rate and a novel measure of heterogeneity, J. Pharm. Sci. 8 (2003) 1632–1647.
[22] C.S. Khan, C.T. Rhodes, The concept of dissolution efficiency, J. Pharm. Pharmacol. 27 (1975) 48–49.
[23] L.J. Naylor, V. Bakatselou, J.B. Dressman, Comparison of the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems, Pharm. Res. 10 (1993) 865–870.
[24] K. Matsuoka, M. Maeda, Y. Moroi, Micelle formation of sodium glycol-and taurocholates and solubilization of cholesterol into their micelles, Colloids Surf. B: Biointerfaces 32 (2003) 87–95.
[25] S.H. Yalkowsky, in: S.H. Yalkowsky (Ed.), Solubility and Solubilization in Aqueous Media, Oxford University Press, New York, 1999, pp. 262–266.
[26] G.A. Kossena, B.J. Boyd, C.J. Porter, W.N. Charman, Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs, J. Pharm. Sci. 92 (2003) 634–648.
[27] D. Ilardia-Arana, H.G. Kristensen, A. Müllertz, Biorelevant dissolution media: aggregation of amphiphiles and solubility of estradiol, J. Pharm. Sci. 2 (2005) 248–2006.

[28] E. Nicolaides, J.M. Hempenstall, C. Reppas, Biorelevant dissolution tests with the flow-through apparatus?, Dissolution Tech 7 (2000) 8–15.
[29] USP–NF (Pharmacopeial Forum), The dissolution procedure: development and validation (1092), in process revision, The United States Pharmacopeial Convention, Rockville, vol. 31, 2005, pp. 1463–1475.
[30] R.L. Dillard, H. Eastman, J.S. Fodtran, Volume–flow relationship during the transport of fluid through the human small intestine, Gastroenterology 49 (1965) 58–66.
[31] J.B. Dressman, G.L. Amidon, C. Reppas, V.P. Shah, Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms, Pharm. Res. 15 (1998) 11–22.
[32] J.B. Dressman, Comparison of canine and human gastrointestinal physiology, Pharm. Res. 3 (1986) 23–131.
[33] A.S. Carlsson, E.S. Kostewicz, G. Hanisch, K. Krumkuhler, R.G. Nilsson, L. Loefgren, B. Abrahamsson, Is the dog a suitable model for bioavailability studies of poorly soluble drugs? In: Congress abstract in AAPS Annual Meeting and Exposition. Springer, Berlin, 2002.