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European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 648–657
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound

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Received 19 November 2006; accepted in revised form 17 December 2007
Available online 25 December 2007

Abstract

The usefulness of selected biorelevant dissolution media (BDM) to predict in vivo drug absorption was studied. Dissolution profiles of solid formulations of a poorly soluble model compound were compared in BDM simulating fasted and two levels of fed state. A non-physiologically relevant medium containing the cationic surfactant, cetrimide, was also investigated. All the media studied were capable of differentiating between the formulations employed, with formulation A consistently ranking high and formulations C and D ranking low. An in vivo dog study was carried out and an attempt was made to obtain a level A correlation between the plasma absorption curves and in vitro dissolution curves, using non-linear regression software. The in vitro–in vivo correlation (IVIVC) models developed indicated that fed state media (BDM 3) containing high levels of both bile salts (BS) and lipolysis products (LP) were best able to predict in vivo pharmacokinetic parameters (C_{max} and AUC) with prediction errors lower than 10%. Overall, design and use of appropriate media for in vitro dissolution is extremely important. This study demonstrates the potential of physiologically relevant media containing both BS and LP for use in formulation and early drug development.

Keywords: Biorelevant media; Solubility; Dissolution; Poorly soluble; In vivo–in vitro correlation (IVIVC)

1. Introduction

Characterized by their high permeability and poor water solubility [1], BCS Class II compounds have an in vivo absorption limited primarily by drug dissolution in the gastrointestinal (GI) tract. During early drug development, it would be extremely useful to have a predictive in vitro dissolution test that correlates with in vivo absorption. Such a test could be used when screening new formulations as well as changes in existing formulations with regard to their impact on bioavailability (BA) [2]. BCS Class IV compounds are also characterized by low water solubility, but with limited permeability across the gut wall. Correlation of in vivo data with dissolution testing is therefore even less likely than for Class II compounds [1], though the appearance of a correlation could prove just as useful in formulation development.

Correlations between in vitro dissolution tests and in vivo absorption may be more likely when physiologically relevant conditions are employed [3]. Media simulating the fasted (FaSSIF) and post-prandial (FeSSIF) states of the small intestine have been used extensively in vitro towards predicting bioavailability of poorly soluble drugs [2,4]. These media consider different bile salt (BS)/lecithin (PC)

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doi:10.1016/j.ejpb.2007.12.013

concentrations, pH and osmolality. Nevertheless, resulting solubility and dissolution profiles may still be underestimated since fats and their digestion products are not considered. Taking this into account, biorelevant dissolution media (BDM) containing varying levels of BS/PC and lipid digestion products in the form of fatty acids (FA)/monoglycerides (MG) have been developed [5]. Overall, it is thought that BDM may be able to more accurately represent the physiological conditions present in vivo compared with more simple media [6,7].

The present study aims primarily to evaluate the usefulness of selected BDM for their ability to predict drug absorption in vivo. As such, 5 solid formulations of a poorly soluble model compound under development for the treatment of Type II diabetes (NNC 25-0926, hemibenzathine) were evaluated using BDM containing different levels of BS/PC and FA/MG. The BDM compositions were adjusted to reflect a range of possible in vivo surfactant levels while also taking into consideration the high intestinal BS levels found in the dog [8,9]. The absorption of two of the solid formulations was evaluated in fed dogs and an in vitro–in vivo correlation (IVIVC) model was developed using GloboMax PDx-IVIVC™ software. In this way, it was possible to investigate the effectiveness of BDM for use in in vitro dissolution testing. Dissolution profiles in BDM and resulting IVIVC models were further compared with profiles obtained using the non-physiologically relevant surfactant, cetrimide (CET).

2. Materials and methods

2.1. Materials

Trisma® maleate (mono-[tris-(hydroxymethyl)-aminomethane] maleate, reagent grade), oleic acid (95%), *o*-phosphoric acid (85%) and crude ox bile extract (72.8% bile salts as determined by means of colorimetric determination of the total amount of 3α-hydroxy bile acids by an enzymatic kit (Enzabile®)) were from Sigma–Aldrich (St. Louis, MO, USA). Sodium azide and sodium chloride (ACS) were from Merck (Darmstadt, Germany). Egg lecithin (E PC S, >96%) was obtained from Lipoid (Ludwigshafen, Germany) and glycerol monooleate (RYLO MG 19 PHARMA) was supplied by Danisco A/S (Grindsted, Denmark). Ethanol (99.9%) as well as HPLC grade methanol and acetonitrile were purchased from VWR (Albertslund, Denmark). Water was obtained from a Milli-Q (Millipore, Milford, MA, USA) water purification system.

2.2. Model compound and solid formulations

The poorly soluble model compound, NNC 25-0926 (>96%, Novo Nordisk A/S, Måløv, Denmark), is an acid that was used in the form of a hemibenzathine salt (API·1/2 hemibenzathine unit). Table 1 lists selected physico-chemical properties for this compound. Apparent permeability in Caco-2 cells was evaluated as low and NNC 25-0926 is therefore believed to be a class IV compound according to the BCS.

Table 1
Physicochemical properties of the model compound

Property	NNC 25-0926, hemibenzathine
Salt form	Hemibenzathine salt
pK_a value	3.5
Log P value (unionized form)	6.9
Intrinsic water solubility (C_s)	0.4 μg/mL
Appearance	Off-white powder

Five solid experimental formulations (A–E) of the model compound NNC 25-0926, hemibenzathine, intended for oral administration were supplied by Novo Nordisk A/S (Måløv, Denmark). The formulations differed by composition and manufacturing process as described in Table 2. Formulations A–D were tablets compressed from granulate that was either produced by wet granulation (Wet), melt granulation (Melt) or a combination of the two (Wet/Melt). Formulation E was a control, containing NNC 25-0926 powder.

2.3. Methods

2.3.1. Dissolution media preparation

The BDM were prepared by weighing out appropriate amounts of crude bile salt, lecithin, oleic acid, glycerol monooleate, trisma® maleate, sodium azide and sodium chloride to a volumetric flask. Next, Milli-Q water was added to the volumetric flask and its contents were stirred at 37 °C overnight to ensure proper solubilization of media components. Media were then adjusted to a pH of 6.5. Overall, media were stable and present as a single phase. Table 3 shows an overview of the final concentrations of each component present in the BDM. Varying sodium chloride concentrations were employed to maintain a constant calculated osmolarity of 270 mM (as in a fasted state).

For comparison the dissolution media (CET) employed during the development of the formulations were used. This contained an artificial surfactant 1.0% (w/v) of the

Table 2
Excipient composition of NNC 25-0926, hemibenzathine formulations

Formulation	A	B	C	D	E
Manufacturing process	Wet	Melt	Wet/Melt	Melt	Capsule
Excipients					
Starch 1500	x	x	x	x	
Copovidone VA 64	x		x		
Croscarmellose sodium	x	x	x	x	
Water	x		x		
Magnesium stearate	x	x	x		
Talcum	x	x	x		
PEG 6000		x	x	x	
Mannitol				x	
Vitamin E TPGS				x	
NNC 25-0926 substance	x	x	x	x	x

Table 3
Composition of biorelevant media

Component	Media 1	Media 2 (mM)	Media 3
Bile salts	5	15	20
Lecithin	1.25	3.75	5
Oleic acid	0	5	20
Glycerol monooleate	0	2.5	10
Trisma maleate	100	100	100
Sodium azide	3	3	3
Sodium chloride	76.4	65.1	59.5

cationic surfactant cetrimide in an unbuffered aqueous solution containing 0.01 N HCl.

2.3.2. Solubility studies

Following preparation of each BDM, triplicate samples of approximately 10 mL were incubated with an excess of compound (approximately 100 mg) in centrifuge tubes at 37 °C with constant end-over-end rotation. The solubility of the compound in BDM was defined as the average solubility following a minimum of 3 samplings within an initial 24 h period, with less than 10% relative standard deviation (RSD) between them. Sampling was restricted to this time frame in order to circumvent compound degradation known to occur over longer periods, also seen following HPLC analysis. Centrifuge tubes were vortexed prior to the resumption of incubation. Media samples were prepared for HPLC analysis as described in Section 2.3.4.

2.3.3. Paddle dissolution studies

The dissolution profiles of the NNC 25-0926 solid formulations were examined in BDM, using an Erweka DT 70 (Biolab A/S, Denmark) apparatus. In all cases, paddle speed and temperature were set to 50 rpm and 37 °C, respectively, and the dissolution volume was held constant at 500 mL. Formulations A–E were examined in media 1 and 3. In addition, formulations A and D were examined in media 2. Dissolution profiles in biorelevant media were monitored up to 24 h (data not shown).

In order to remove the oxygen present in the BDM, helium gas was bubbled through it for 20–45 min per L prior to dissolution. Sinkers were used as necessary. At predefined time intervals, 5 mL samples were removed from each vessel and replaced with a similar volume of fresh media. Samples were subsequently prepared for HPLC analysis.

CET dissolution profiles were evaluated in a dissolution apparatus (VanKel DT8 Dissolution Tester) employing a 900 mL dissolution volume, paddle speed of 75 rpm and temperature of 37 °C. Unlike the BDM, these media were not sparged with helium. Sampling was automated and dissolution profiles were monitored using online UV detection over a 2 h period. Formulations A–E were investigated in these media.

Selected parameters of interest included $D_{120\text{min}}$ and DR, described as the percent dissolution at $t = 120$ min and the initial dissolution rate defined as the initial slope of the dissolution curve (%/min), respectively.

2.3.4. Sample preparation

BDM samples were centrifuged (Heraeus Labofuge 400R, Holm and Halby, Denmark) at 4500 rpm for 15 min at 37 °C, followed by the transfer of approximately 1.1 mL of media into Eppendorf tubes. Eppendorf tubes were further centrifuged (Heraeus Biofuge 15, Bie & Berntsen, Denmark) at 15,000 rpm for 15 min at room temperature, followed by the removal of 1 mL of media for dilution, in preparation for HPLC analysis. Verification of the sample preparation method confirmed precipitation was not likely during this second centrifugation step. Dilutions were made in ethanol (1:10–1:20) and then in an acetonitrile/water mixture (50:50, v/v, 1:10–1:25). The resulting clear monophasic solutions were subjected to quantification by HPLC analysis.

2.3.5. HPLC method

Analyses were carried out on a Hewlett Packard HPLC system (series 1100, Palo Alto, CA) equipped with an autosampler, quaternary pump and variable wavelength detector with integration by HP ChemStation software (Rev. A.06.01).

NNC 25-0926, hemibenzathine concentrations were quantified in samples using a Hichrom RBP column (5 μm, 250 × 4.6 mm) fitted with a precolumn, at a flow rate of 1 mL/min. The injection volume was 25 μL, the isocratic mobile phase consisted of an acetonitrile/phosphoric acid buffer (80:20, v/v) mixture and detection was carried out at 252 nm. Phosphoric acid buffer was prepared by adjusting water to a pH of 2.5 using phosphoric acid. Column temperature was maintained at 35 °C throughout the analysis.

Stock solutions (0.6 mg/mL) were prepared by weighing out 30 mg of the NNC 25-0926, hemibenzathine salt in duplicate, and dissolving each in a volumetric flask using 10 mL of methanol, 10 mL of acetonitrile and 30 mL of an acetonitrile/water (50:50, v/v) mixture. Reference solutions were prepared from these stock solutions by diluting 2 mL of the stock solution to a final volume of 25 mL using the acetonitrile/water (50:50, v/v) mixture.

Seven standards were prepared from each reference solution, with one set of reference standards run before and one set run after the samples of interest. Standards were clear monophasic solutions and were linear ($r^2 \geqslant 0.99$) within the concentration range of 0.25–25 μg/mL employed in the study. Standards and samples were run in duplicate.

2.3.6. In vivo bioavailability study

Eight healthy Beagle dogs, 4 females and 4 males, aged 15–66 months, with body weights between 13 and 19 kg, and fasted overnight entered the in vivo study. The dogs were acclimatized for at least 2 weeks before commencement of dosing. The animals were housed according to Novo Nordisk A/S standard procedures and the experiments were performed according to guidelines from The

Danish Animal Experiments Council, The Danish Ministry of Justice.

The study was conducted as a cross-over design with 3 groups consisting of 3, 3 and 2 dogs per group, respectively. Each dog was dosed with a single dose of NNC 25-0926, hemibenzathine as an oral solution, tablet (formulation A) and capsule (formulation D), respectively. The oral solution consisted of 15 mg/mL NNC 25-0926 dissolved in 13% Vitamin E TPGS and 1.5% Povidone K-12 PF in water. The animals received 3 doses of approximately 20 mg/kg of NNC 25-0926 calculated as a free acid, with a wash period of 3–4 days between each dose formulation (each animal served as its own control). The tablets were administered with approximately 10–20 mL of water. Approximately 15–30 min before dosing, the dogs were fed a standard dog diet (Leo Pharma A/S, Denmark) in accordance with standard procedures. The dogs had free access to water pre-dosing and approximately 3 h post posing.

Blood samples were drawn from the cephalic vein of each animal according to the following scheme: 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10 and 12 h after dosing. NNC 25-0926 was analyzed in plasma by a validated HTLC/MS/MS (High Turbulence Liquid Chromatography Tandem Mass Spectrometry) assay using a deuterium and ^{13}C labeled analogue of NNC 25-0926 as internal standard.

The plasma concentration of NNC 25-0926 was calculated using the peak area ratio of NNC 25-0926 to internal standard. A calibration graph based on blank plasma spiked with NNC 25-0926 in the concentration range 1.00–250 ng/mL and performance was assured by co-assaying quality control samples.

2.3.7. In vivo–in vitro correlation

In vitro–in vivo correlation (IVIVC) was performed using the PDx-IVIVC™ software (GloboMax® LLC, Slough, Berkshire, UK). Level A IVIVCs, correlating the entire in vitro and in vivo profiles, were obtained using a two-stage approach. Correlations were performed on mean data. In the first step, a unit impulse response (UIR) was determined for the oral solution using a maximum of 4 exponentials, allowing for lag time and weighting of data. The determined UIRs were employed in numerical deconvolution of the plasma concentration versus time curves for the two solid formulations, resulting in cumulative percentage absorbed versus time profiles. In the second step, percentage absorbed was related to percentage dissolved in vitro by fitting a nonlinear regression model Eq. (1) to the data through an iterative process that minimizes the sum of squared deviations between observed and predicted values.

$$x_{vivo}(t) = a_1 + a_2 \cdot x_{vitro}(-b_1 + b_2 \cdot t) \quad (1)$$

In Eq. (1), $x_{vivo}(t)$ is the percentage absorbed in vivo at time (t) and $x_{vitro}(t)$ is the percentage dissolved in vitro at time (t). The parameter a_1 allows for a difference between initial percentage dissolved and percentage absorbed, a_2 allows for a difference between percentage dissolved and percentage absorbed, b_1 allows for a time shift between percentage dissolved and percentage absorbed and b_2 allows for time scaling between percentage dissolved and percentage absorbed. The aim was to obtain the simplest model possible without losing predictability. Because the BA study was a single dose study and the formulations were immediate release, the parameters a_1 and b_1 were fixed to zero.

Model predictability was validated internally by comparison of prediction errors for pharmacokinetic parameters (C_{max}, AUC and T_{max}) derived from mean observed and predicted in vivo data. For a reasonable IVIVC, regulatory guidelines state prediction errors for C_{max} and AUC should not exceed 10% [10,11].

3. Results

BDM have been developed in order to simulate fluids present in the GI tract under a range of dosing conditions [5,29]. In this study, three different BDM were evaluated and compared following in vitro dissolution of selected NNC 25-0926, hemibenzathine formulations.

3.1. Solubility studies

Equilibrium solubility values for the poorly soluble salt in 3 BDM selected for in vitro dissolution studies are shown in Table 4. In BDM 1, which only contained bile salts, the solubility increased 1575 times compared with the intrinsic solubility, to 0.63 mg/mL. Further addition of bile salts and lipolysis products increased the solubility up to 6.54 mg/mL. Overall, no shifts in pH were observed during equilibrium solubility determinations.

3.2. Paddle dissolution studies

Fig. 1 shows dissolution profiles for the 5 formulations (A–E) in BDM 1, over a 2 h period. Under the conditions employed, in vitro dissolution using BDM 1 was found to be discriminatory between the different formulations of NNC 25-0926, hemibenzathine. Initially, formulations B and E showed the steepest slopes while formulation C

Table 4
Solubility of NNC 25-0926, hemibenzathine in selected BDM

Biorelevant media	Equilibrium solubility[a] (mg/ml)	Sink condition limits[b]
1	0.6	23
2	1.2	45
3	6.5	235

[a] Equilibrium solubility was defined as the average of at least 3 samplings with <10% RSD between them.

[b] Calculated as 30% of the maximum amount of salt to dissolve in a 500 mL volume, expressed as a percentage of the amount dosed (average of 416.7 mg salt).

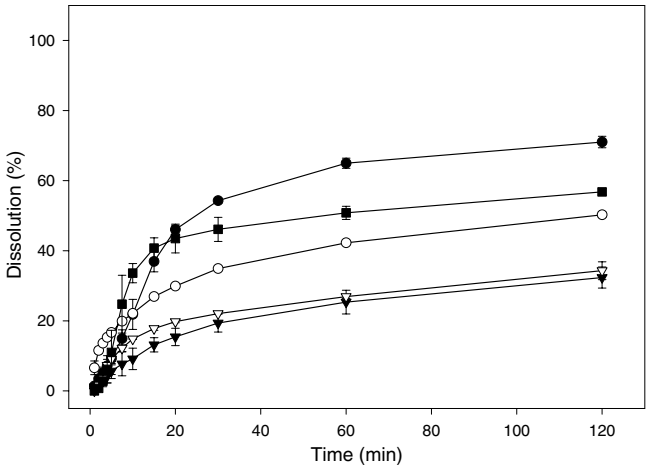

Fig. 1. In vitro dissolution profiles for 5 formulations, (●) A, (○) B, (▼) C, (▽) D and (■) E, of the model compound NNC 25-0926, hemibenzathine ($n = 3$) in BDM 1.

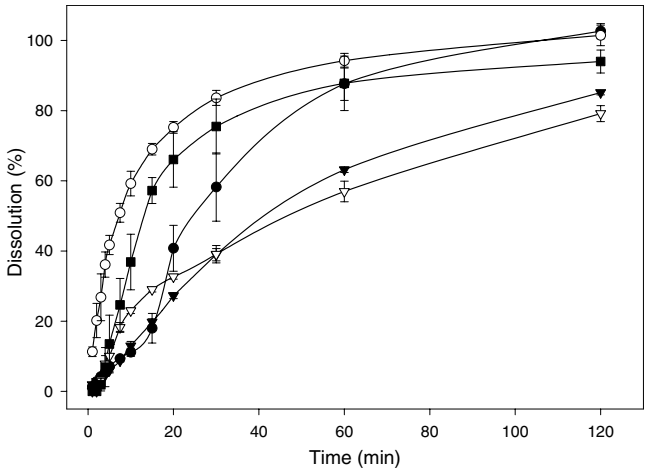

Fig. 2. In vitro dissolution profiles for 5 formulations, (●) A, (○) B, (▼) C, (▽) D and (■) E, of the model compound NNC 25-0926, hemibenzathine ($n \geqslant 3$) in BDM 3.

was least steep (Table 5). Over time, the % dissolution of formulation A surpassed that of E and resulted in the highest percent dissolution at 120min. ($D_{120\text{min}} = 71.0\%$), while formulations C ($D_{120\text{min}} = 32.4\%$) and D ($D_{120\text{min}} = 34.3\%$) had the lowest.

Dissolution profiles of all 5 formulations (A–E) are shown in BDM 3 (Fig. 2). Although formulations B and E initially exhibited the highest slopes in BDM 3, formulations A and B had the highest % dissolution values ($D_{120\text{min}} \cong 100\%$) following a 2 h period. Formulation D exhibited the lowest % dissolution value ($D_{120\text{min}} = 79.2\%$). Overall, it was also possible to discriminate between formulations in BDM 3.

In general, similar dissolution rates were observed in media 1 and 3, with % dissolution values in BDM 3 plateauing at a higher level. Only formulation B showed substantially higher initial dissolution in BDM 3 while the dissolution rate of formulation A decreased the most (Table 5).

Fig. 3 illustrates the dissolution profiles of the 5 formulations (A–E) in CET, a cationic surfactant, over a 2 h period. Dissolution in this medium yielded profiles for individual formulations that were differentiable from each other. Overall, formulation A appeared to have the fastest and most complete dissolution ($D_{120\text{min}} = 99.2\%$). In contrast, formulation C appeared to have the slowest and least

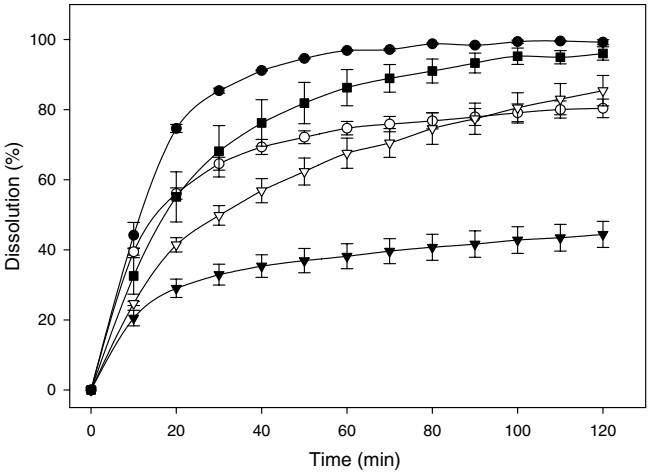

Fig. 3. In vitro dissolution profiles for 5 formulations, (●) A, (○) B, (▼) C, (▽) D and (■) E, of the model compound NNC 25-0926, hemibenzathine ($n \geqslant 3$) in 0.01 N HCl + 1.0% cetrimide.

Table 5
Comparison of 5 formulations of NNC 25-0926, hemibenzathine in selected dissolution media

Formulation	Media 1[a,c]		Media 3[a]		CET[b]	
	$D_{120\text{min}}$[d] (%)	DR[e] (%/min)	$D_{120\ \text{min}}$[d] (%)	DR[e] (%/min)	$D_{120\text{min}}$[d] (%)	DR[e](%/min)
A	71.0 (±1.6)	2.45	102.7 (±2.2)	1.41	99.2 (±0.7)	4.42
B	50.2 (±0.8)	5.93	101.4 (±2.9)	7.68	80.4 (±2.6)	3.94
C	32.4 (±3.0)	1.05	85.2 (±0.7)	1.32	44.4 (±3.7)	2.05
D	34.3 (±2.5)	3.20	79.2 (±2.3)	2.95	85.4 (±4.3)	2.47
E	56.8 (±0.8)	5.65	94.0 (±3.3)	5.07	96.0 (±1.9)	3.26

[a] Biorelevant media: dissolution volume 500 mL, stirring rate 50 rpm.
[b] CET: 0.01 N HCl + 1.0% cetrimide: dissolution volume 900 mL, stirring rate 75 rpm.
[c] Note the absence of sink conditions throughout dissolution in media 1.
[d] $D_{120\ \text{min}}$: percent dissolution at $t = 120$ min.
[e] DR: dissolution rate defined as the initial slope (%/min) of the dissolution curve.

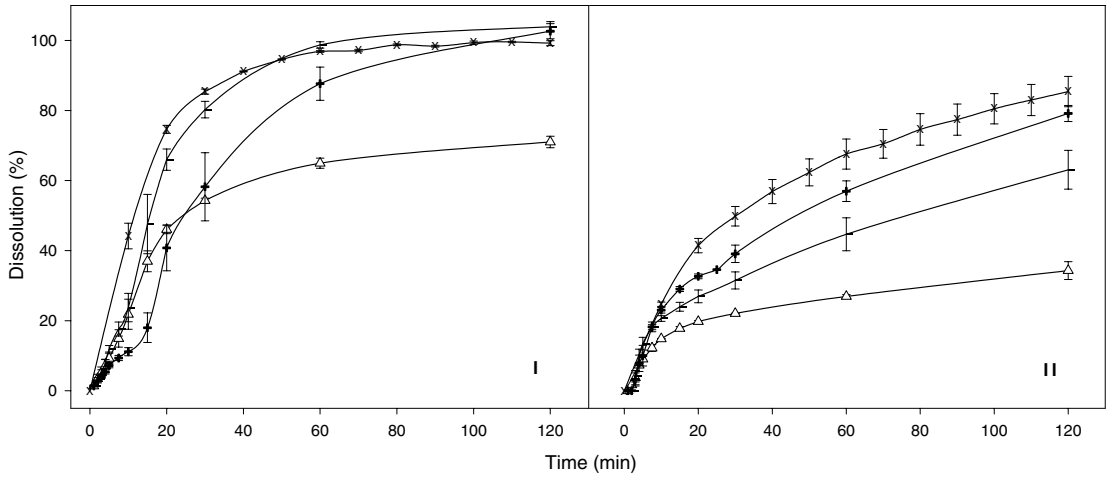

Fig. 4. Comparison of dissolution profiles of formulations (I) A and (II) D, in (△) BDM 1 ($n = 3$), (−) BDM 2 ($n = 3$), (+) BDM 3 ($n \geqslant 3$) and (X) CET ($n = 3$).

complete dissolution ($D_{120\text{min}} = 44.4\%$) of the 5 formulations investigated (Table 5).

Dissolution values ($D_{120\ \text{min}}$) of all formulations were compared in BDM 1, 3 and CET (Table 5). Formulation A ranked the highest in all media investigated. In addition, while the rank order of formulations B and E varied somewhat, $D_{120\ \text{min}}$ values for formulations C and D ranked low following dissolution in all media. Dissolution rates (DR) were also compared (Table 5). Although these rates varied a lot according to the experimental data, they were useful in the comparison of both formulations and media.

Formulations A and D were further examined in BDM 2 (Table 3 and Fig. 4). Both formulations exhibited very similar dissolution profiles to start; with formulation A (Fig. 4, I) reaching 100% dissolution after approximately 60 min. In contrast, the % dissolution of formulation D (Fig. 4, II) was still increasing steadily after 120 min, and reached a similar level of dissolution only after close to 23 h (data not shown).

All things considered, Fig. 4 shows a comparison of the dissolution profiles of formulations A (I) and D (II) in selected BDM as well as CET. Overall, it was clear that the NNC 25-0926, hemibenzathine in formulation A dissolved faster and to a much greater extent than the NNC 25-0926, hemibenzathine from formulation D in all dissolution media investigated.

3.3. In vivo–in vitro correlation

The mean plasma concentration-time profiles arising from administration of an oral solution as well as formulations A and D to fed dogs are depicted in Fig. 5.

The plasma profiles for formulations A and D were deconvoluted using the plasma profile for the oral solution as 100% input. Numerical deconvolution was performed on mean data allowing for lag time and weighting of data. The deconvoluted plasma profiles are depicted in Fig. 5 (insert)

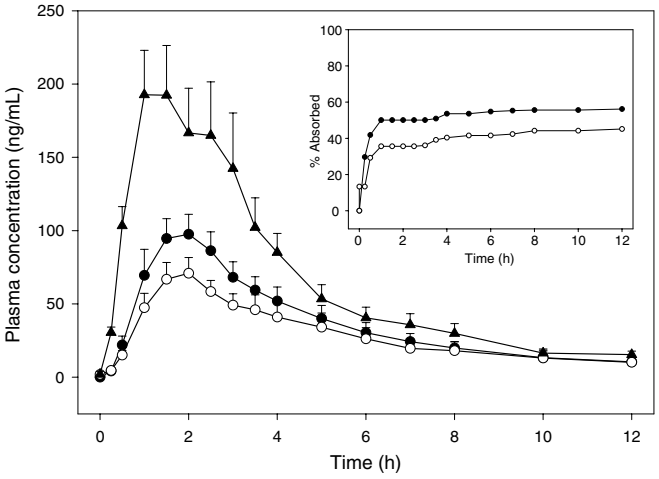

Fig. 5. Plasma concentration versus time profiles of the model compound NNC 25-0926, hemibenzathine following administration of an (▲) oral solution, (●) formulation A and (○) formulation D to beagle dogs fed 15–30 min prior to administration (mean + SE, $n = 8$). The inset depicts the deconvoluted plasma profiles for formulations A and D.

and the final unit impulse response model consisted of 3 exponentials and lag time (data not shown).

IVIVC models were developed for each of the dissolution media employed. The estimated model parameters for each are presented in Table 6. The models obtained were evaluated using an internal validation whereby predicted plasma profiles were compared with the actual plasma profiles. The actual and predicted plasma profiles obtained in the internal validation for each of the four IVIVC models are depicted in Fig. 6. The calculated prediction errors (PE) for $C_{\max}$ and AUC as well as $T_{\max}$ ratios for the two formulations, obtained from the internal validation, are also presented in Table 6.

The IVIVC model using cetrimide resulted in a very good prediction of $C_{\max}$ and AUC for formulation A; however, it did not predict the form of the plasma level curve or estimate $T_{\max}$ properly in the case of formulation D. BDM

Table 6
IVIVC model parameters and internal validation

IVIVC model	Model parameters			Formulation	Internal validation		
	a_2	b_2	T		C_{max} PE (%)	AUC PE (%)	T_{max} ratio
Media 1	0.827	1.111	∞	A	12.6	10.7	1
				D	28.9	31.8	1
Media 2	0.543	1.244	∞	A	10.4	6.5	1
				D	12.3	17.7	1
Media 3	0.512	1.776	∞	A	3.8	0.8	1
				D	9.5	2.4	1
Cetrimide	0.489	0.833	∞	A	5.7	6.6	1
				D	0.2	14.9	1.25

a_2, b_2 and T are the parameters from Eq. (1).
PE, Prediction error.
∞, infinitive.

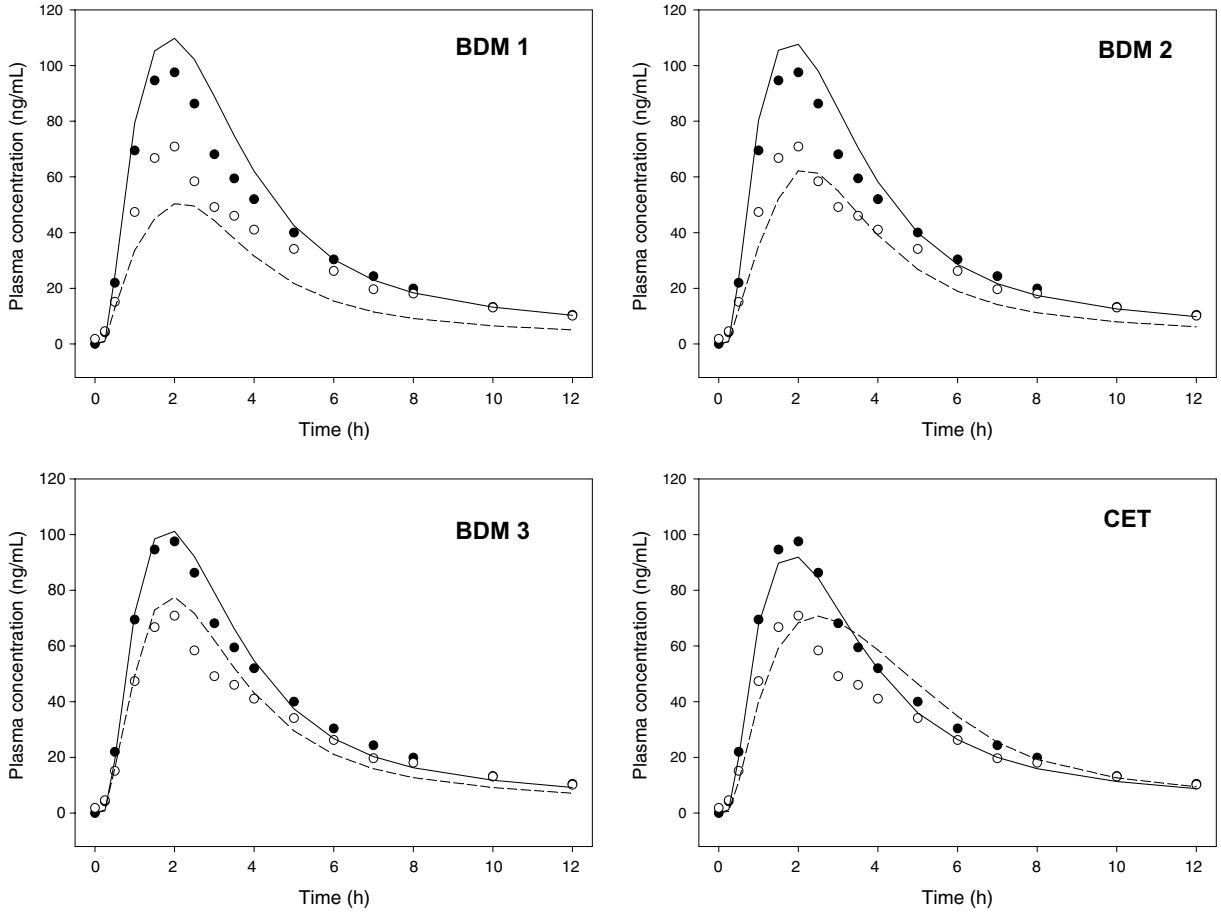

Fig. 6. Predicted and observed plasma concentration versus time profiles from internal validation. (●) observed formulation A (solid line) predicted formulation A, (○) observed formulation D and (dashed line) predicted formulation D. Prediction based on IVIVC with (I) BDM 1, (II) BDM 2, (III) BDM 3, (IV) Cetrimide.

1, as would be expected when fasted state media are used to predict fed state performance, did not result in acceptable prediction errors. BDM 2 did a better job of predicting curve shape but the error was still unacceptable. BDM 3 resulted in the best prediction of C_{max} and AUC with prediction errors below 10% (0.8% to 9.5%) and a T_{max} ratio of 1.

4. Discussion

4.1. Solubility studies

Human GI fluids have been characterized in both the fasted and post-prandial states. In the fasted state, mean BS levels were reported from 2.0 to 6.4 mM [3,12–16]. Por-

ter and Charman [15] quoted post-prandial BS levels around 10–20 mM, but with a rather high variability. Specifically, BS levels ranging from 5.2 to 37.0 mM have been reported in the literature [12,15–17]. This high degree of variability was attributed to differences in sampling and transit times as well as the type of fed state induced. BS levels measured in fasted dogs were found to be similar to levels measured in fasted humans [31]. Moreover, the intestinal fluids of fed state dogs reportedly had similar or higher BS levels than humans in the post prandial state, ranging from 8.0 to 18.0 mM [16,31].

In this study, equilibrium solubility was investigated in 3 BDM meant to mimic possible surfactant concentrations in the fasted and fed states (Table 3). These media contained increasing concentrations of amphiphiles, in the form of both bile salts (5–20 mM BS) and lipolysis products (0–20 mM FA). With an intrinsic water solubility of 0.4 μg/ml, the incorporation of increasing concentrations of amphiphiles into each subsequent media served to increase the equilibrium solubility of NNC 25-0926, hemibenzathine substantially (Table 4). Possible mechanisms for this include the formation of different colloidal phases, such as mixed micelles and vesicles in the media. Moreover, the determined solubility was in accordance with other solubility values previously determined in resembling media [29].

4.2. Paddle dissolution studies

Regarding poorly soluble drugs, medium selection for use in dissolution is often difficult because of problems in achieving sink conditions [18]. According to FIP and other guidelines, sink conditions prevail when <30% of the maximum amount of compound to solubilize in the volume of BDM employed is present [19,20]. Solubility results of NNC 25-0926, hemibenzathine are depicted in Table 4. Due to the low solubility of NNC 25-0926 in BDM 1 and 2, sink conditions were only achieved throughout the entire dissolution process in BDM 3.

In this study, sink conditions could have been attained by increasing the dissolution volume; however this was not considered necessary as non-sink dissolution is reportedly acceptable if shown to be more discriminating [20]. Tang et al. [21] highlighted this when they obtained better IVIVC under non-sink conditions. Furthermore, the maintenance of these conditions in vivo depends on the permeability of the GI mucosa as well as the composition and volume of luminal fluids [22]. A lack of sink conditions in vivo could be considered in this case due to NNC 25-0926, hemibenzathine's permeability, which was evaluated to be low in a Caco-2 cell assay (data not shown).

All 5 formulations (A–E) of NNC 25-0926, hemibenzathine had dissolution profiles in BDM 1 that plateaued somewhere between 60 and 80% (Fig. 1). This was likely due to the absence of true sink conditions throughout most of the dissolution process. Formulations tested under sink conditions (BDM 3) were more likely to undergo complete dissolution within a shorter time-frame. And while in vitro dissolution-testing using BDM 3 was discriminatory between formulations (A–E), differences were less prominent compared with results obtained using BDM 1. This was to be expected since inclusion of larger quantities of surfactants into the BDM increased its capacity for solubilization and reduced the differences between the formulations [13,22,23].

Fig. 3 illustrates the profiles of 5 formulations (A–E) in a cationic surfactant, CET. While the surfactant itself may not be physiologically relevant, this dissolution method is currently being used in industry to characterize formulations of poorly soluble compounds. Notable differences compared with studies carried out in BDM include rotation speed (75 rpm) and dissolution volume (900 mL) in addition to the maintenance of sink conditions throughout the complete dissolution process. A comparison of formulations using either cationic surfactant or selected BDM (Table 5) reveals that while rank order was not always the same, common traits did exist. Rank order results at 60 min for media simulating the fasted state (BDM 1) were similar to those obtained using CET (Figs. 1 and 3). Regarding synthetic surfactants, Shah et al. [26] recommended using media containing the lowest amount of surfactant needed to achieve 75–80% drug release in a reasonable amount of time (i.e. 60–90 min), based on compendial methods for IR dosage forms. Overall, dissolution results from both CET and BDM 3 conform to these recommendations.

No dissolution studies of IR formulations of class IV compounds using cetrimide as a surfactant were located in the literature; however, commonly used artificial surfactants include SLS, polysorbate and lauryldimethylamine oxide [20,24]. Artificial surfactants differ from each other in their wetting properties and solubilization capacity towards specific drugs. As a result, more investigations are needed before recommendations can be made as to the most appropriate surfactants and concentrations for simulation of in vivo conditions [25].

Formulations A and D were examined in a third BDM (Table 3, BDM 2) containing intermediate levels of surfactants. As stated earlier, an increased solubilization capacity due to increasing quantities of surfactants was expected to result in increasing % dissolution values. This trend was observed for formulation D. In contrast, formulation A did not follow this trend as the dissolution profile associated with BDM 2 appeared more favorable than that of BDM 3. Lastly, profiles for both formulations A and D (Fig. 4) show that dissolution occurs faster in CET compared with the BDM investigated in this study.

Dissolution profile differences (within a single medium) are often the result of multiple factors, including differences in manufacturing processes, excipient composition and particle size [27]. Varied excipient compositions (Table 2) for NNC 25-0926, hemibenzathine formulations along with manufacturing differences are responsible for the differing dissolution profiles. Yet as evidenced by this investigation, media composition also plays a large role in determining

the extent and rate of dissolution. Anwar et al. [28] reported longer disintegration times in media simulating fed state while Bertocchi et al., [27] reported improved penetration of surfactant-containing media into tablets.

4.3. In vivo–in vitro correlation

BDM 1, 2 and 3 differ in terms of their concentrations of BS/PC and LP, and were selected in order to represent fasted state and two levels of the post-prandial state with respect to solubilization capacity. Osmolality and pH were both kept constant in order to isolate and evaluate surfactant effects. The overall goal was to pursue a possible IVIVC using data from a study conducted in dogs fed prior to administration of the formulations.

Given its poor permeability and intrinsic solubility in the pH range relevant for the GI tract, the compound NNC 25-0926, hemibenzathine could be categorized as class IV according to the BCS. Remarkably it was still possible to obtain a good level A IVIVC using BDM.

The CET medium was included as more simple and well-defined medium, being used as the standard dissolution model in the development of the formulations. Cetrimide gave a fair prediction of the C_{max}, but the AUC PE was >10% for formulation D while the T_{max} prediction ratio was 1.25 (Table 6). Thus, this IVIVC model was inferior to the IVIVC models developed using media 3 under the conditions evaluated (Fig. 6).

The results indicate that BDM 2 and especially BDM 3 correlate better with the in vivo situation as compared to BDM 1 (Table 6). This suggests that BDM simulating the solubilization capacity in the fed state are useful in predicting in vivo performance of formulations in fed dogs. According to Hörter and Dressman [23], effective prediction of in vivo data requires similarities between physiological conditions and conditions employed in vitro. As dogs reportedly have high intestinal BS levels [8,9], it was not surprising to note that BDM 3 resulted in superior correlation.

IVIVC for a poorly soluble drug (class II) using fed state BDM has been reported using a flow-through apparatus [29]. This fed state BDM consisted of 18.8 mM BS, 3.75 mM PC, 30 mM FA and 4 mM MG. Moreover, this fed state could only be simulated using a fed state media containing MG and FA (LP) components. In another instance, Souliman et al. [30] compared two in vitro models (paddle dissolution, TNO intestinal model) using a class I substance and found that the best IVIVC existed using an artificial digestive system (TNO). Thus, development of improved IVIVCs is possible using various models and fluids meant to simulate physiological conditions. In the present study, focus was put on investigating simulated GI fluids (BDM) for their ability to influence dissolution, and consequently absorption/bioavailability of a poorly soluble drug compound.

While fed dogs are generally regarded as good models for studies on dissolution in the small intestine [16], physiological differences between species should not be forgotten. Dogs reportedly experience slower gastric emptying, faster small intestinal transit and higher and more variable intestinal pH [32]. Major bile acid type also differs between humans and dogs, with glycocholic acid (35–50%) predominating in humans and taurocholic acid (75%) being most common in dogs [33]. All these factors can influence the degree of solubilization and ultimately, absorption of the compound in vivo and should be kept in mind when attempting IVIVCs.

In this study, formulations and media compositions were somewhat limited. However, this study shows that the fed state medium (BDM 3) containing high concentrations of BS/PC and LP was superior to a fasted state medium (BDM 1), when investigating IVIVCs in fed dogs. Based on the IVIVC (Table 6), BDM 3 was also shown to correlate better than the non-physiologically relevant surfactant, CET. It can therefore be concluded that employing biorelevant dissolution media containing LP in the form of FA/MG has the potential to significantly improve the quality of IVIVCs.

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