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Dissolution media simulating the intralumenal composition of the small intestine: physiological issues and practical aspects

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Abstract

The objective of this study was to test various aspects of dissolution media simulating the intralumenal composition of the small intestine, including the suitability of the osmolality-adjusting agents and of the buffers, the substitution of crude sodium taurocholate (from ox bile) for pure sodium taurocholate and the substitution of partially hydrolysed soybean phosphatidylcholine for egg phosphatidylcholine. It was concluded that biorelevant media should contain sodium as the major cation species to better reflect the physiology. However, the use of non-physiologically relevant buffers is inevitable, especially for simulation of the fed state in the small intestine. The buffers used may affect the solubility product of weakly basic compounds with pK_a(s) higher than about 5, the solubility of extremely highly lipophilic compounds due to salting in/out properties of the anion of the buffer and the stability of the dissolving compound. It is prudent in relevant situations to run an additional dissolution test in a modified fed state simulated intestinal fluid (FeSSIF) (or fasted state simulated intestinal fluid (FaSSIF), where applicable) containing alternative buffer species. Although a mixture of bile salts is physiologically more relevant than pure sodium taurocholate, this issue seems to be of practical importance in only a few cases. Adequate simulations in these cases will probably require the use of a number of pure substances and could substantially increase the cost of the test. Finally, unless the drug is extremely lipophilic (ca. logP > 5), egg phosphatidylcholine can be substituted by partially hydrolysed soybean phosphatidylcholine.

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Introduction

Some years ago, it was proposed that biorelevant media could be used to enhance the predictive capability of dissolution testing with regard to the oral absorption of drugs and drug products (Dressman et al 1998). At that time, compositions were proposed for biorelevant media that could simulate the average fasted and fed upper small intestinal lumen by taking into account the most important factors affecting dissolution/release. Although fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) represent a simplification of the lumenal composition, they have been shown to predict the in-vivo dissolution process (Galia et al 1998) and the absorption characteristics of poorly soluble, lipophilic weak acids, weak bases and non-ionisable compounds (Nicolaides et al 1999, 2001; Kostewicz et al 2002) well and are widely used in the pharmaceutical industry to evaluate new drugs and dosage forms. To further improve the predictive capability of FaSSIF and FeSSIF, not only will it be necessary to consider how the physiology can be more nearly simulated, but practical aspects such as cost, ease of preparation and validation of the media will also need to be taken into account.

The major physiological issues with regard to the composition of FaSSIF and FeSSIF include selection of osmolality-adjusting agents and of buffer species, use of mixture of bile salts (rather than pure sodium taurocholate) and addition of further components to more nearly match lumenal composition in the fed state (e.g. product(s) of lipid digestion).

Practical issues with respect to the use of biorelevant dissolution tests include ease of preparation and reproducibility of FaSSIF and FeSSIF and the high direct costs of the materials used (principally bile salts and egg phosphatidylcholine). For these reasons,

together with the need to use HPLC analysis for most drugs and formulations, biorelevant dissolution media are not used for routine quality control purposes. For research purposes, it would also be of interest to lower the time required for manufacture and associated labour and material costs, especially when using FeSSIF (because of the high bile salt and egg phosphatidylcholine concentrations) or open dissolution systems such as the flow-through apparatus (because of the large volumes of media typically used in these experiments). Some potential approaches to reducing the costs of their manufacture without substantially affecting the physiological relevance of FaSSIF and FeSSIF include substitution of sodium taurocholate with a crude mixture of bile salts, finding alternative sources of phosphatidylcholine and using partially hydrolysed material (i.e. phosphatidylcholine containing small amounts of lysophosphatidylcho line).

In these studies, three specific aspects of FaSSIF and FeSSIF were addressed by using various drugs and dosage forms: the suitability of the osmolality adjusting agents and of the buffers; the substitution of crude sodium taurocholate (from ox bile) for pure sodium taurocholate and the substitution of partially hydrolysed soybean phosphatidylcholine for egg phosphatidylcholine.

Materials and Methods

Drugs and dosage forms

The drugs and the dosage forms tested in this study are presented in Table 1.

Substitution of sodium for potassium buffers

Dissolution profiles of Aktren Forte, Dolo-Puren 400T, Indomethacin AL 50, Arilin 250, Metronidazol Artesan and Clont tablets, and dissolution profiles of Aktren Spezial, Indo von ct, and Indomet-ratiopharm 50 capsules were obtained in US Pharmacopeia's (USP's) simulated intestinal fluid without pancreatin (The United States Pharmacopeia, 2000) and in pH 6.8 buffer of The International Pharmacopeia (Pharm. Int.) (1994). Both are pH 6.8 phosphate buffers, and they have identical osmolalities ($114\,mOsmol\,kg^{-1}$) and buffer capacities ($18.5\,mEq\,L^{-1}\,\Delta pH^{-1}$). However, in the USP medium, potassium (50 mM) dominates over sodium (22 mM) whereas in the Pharm. Int. buffer, sodium (49.7 mM) dominates over potassium (25 mM) (The United States Pharmacopeia, 2000; The International Pharmacopoeia, 1994).

To maintain the osmolality at the desired level, the substitution of sodium for potassium in FaSSIF and FeSSIF was made on an equimolar basis. The revised standard compositions of FaSSIF and FeSSIF are given in Table 2.

Substitution of buffer components in FaSSIF and FeSSIF

The physicochemical characteristics and the composition of FaSSIF containing maleic anhydride (FaSSIFm) and FeSSIF containing citrates (FeSSIFc) are included in Table 2. Dissolution experiments with Romozin, crystalline troglitazone tablets, GR253035X tablets and Wellvone, dissolution experiments with micronised felodipine powder and release experiments with felodipine from the hydrophilic matrix formulation were performed in FaSSIF, FaSSIFm, FeSSIF and FeSSIFc.

Substitution of crude for pure sodium taurocholate in FeSSIF

For all studies, purified sodium taurocholate (>97% pure) was purchased from Sigma-Aldrich Chemie GmbH (cat. no. T4009).

Three different lots of crude sodium taurocholate from ox bile were tested; two lots from Sigma-Aldrich (cat. no. T0750; lot no. 048H0684 (~35% taurocholic acid) and lot no. 050K0720 (~15% taurocholic acid)) and one from Fluka, Switzerland (cat. no. 86340; lot no. 386645/134499 (~32% taurocholic acid)). Apart from taurocholic acid, all three lots contain also a mixture of other components (for the Sigma-Aldrich lots these included glycocholic, cholic, deoxycholic and other bile acids). Using Enzabile (Nycomed Pharma AS, Norway) and a procedure that is recommended by the manufacturer of the kit for assaying 3-alpha-hydroxy bile salts in non-protein based media, the exact contents of the crude sodium taurocholate lots in 3-alpha-hydroxy bile salts were measured to be $85.8 \pm 2.1\%$ ($n = 6$) and $72.0 \pm 1.9\%$ ($n = 3$) for Sigma-Aldrich lots 048H0684 and 050K0720, respectively, and $87.9 \pm 2.2\%$ ($n = 4$) for the Fluka lot. Based on these analyses it was possible to prepare FeSSIF media using crude sodium taurocholate material that contained a molar amount of 3a-hydroxy bile salts very similar to the molar amount of sodium taurocholate used in the revised standard FeSSIF (Table 2). Acetates (or citrates) and egg phosphatidylcholine molar concentrations, as well as pH and osmolality of FeSSIF made with crude material, were also identical to that of the revised standard FeSSIF (Table 2).

Dissolution profiles of Romozin, crystalline troglitazone tablets, Nizoral, GR253035X tablets, Levopraid and Wellvone, as well as the release profile of L-sulpiride from an osmotic pump and the release profiles of isosorbide mono-5-nitrate (ISMN) from an osmotic pump and from Imdur, were obtained in FeSSIF (Table 2) and in FeSSIF prepared with crude sodium taurocholate.

Substitution of partially hydrolysed soybean phosphatidylcholine for egg phosphatidylcholine

For all studies, egg phosphatidyl choline (Lipoid E PC, >98% pure) was donated by Lipoid GmbH (Ludwigshafen, Germany). Major fatty acids in Lipoid E PC are palmitic (16:0, ~32 mol%), stearic (18:0, ~12 mol%), oleic (18:1, ~31 mol%), linoleic (18:2, ~15 mol%) and arachidonic (20:4, ~3 mol%). Soybean partially hydrolysed phosphatidylcholine was also from Lipoid GmbH (Lipoid S 100, >94% phosphatidylcholine and 3% lysophosphatidylcholine) and contained palmitic (~14 mol%), stearic

Table 1 The drugs and dosage forms tested in this study.

Drugs	Ionization properties	log P	Dosage forms	References
Immediate-release products				
Atovaquone	Non-ionizable	5.1	Wellvone, 250 mg/tablet, lot E96L1596 (GSK, UK)	Nicolaides et al 1999
Felodipine	Non-ionizable	4.5[a]	Micronized felodipine powder, 10 mg/dose, batch 41688-01 (AZ, Sweden)	—
GR253035X ($C_{22}H_{17}N_2O_3FS$)	$pK_a = 5.1$ Alkaline	2.8	Tablets, 100 mg/tablet, batch F97/005B (GSK, UK)	GSK data on file
Ibuprofen	$pK_a = 4.4$ Acidic	4.0	Aktren Forte, 400 mg/tab, lot IT10521 (Bayer Vital, Germany) Dolo-Puren 400T, 400 mg/tab, lot 4954301 (Isis Puren Arzn., Germany) Aktren Spezial, 400 mg/caps, lot GTADJ1 (Bayer Vital, Germany)	Avdeef et al 1998
Indometacin	$pK_a = 4.5$ Acidic	4.2[a]	Indomethacin AL 50, 50 mg/tab, lot 01804 (Aliud, Germany) Indo 50 von ct, 50 mg/caps, lot B19469 (ct-Arzneimittel, Germany) Indomet-ratiopharm 50, 50 mg/caps, lot B30513 (ratiopharm, Germany)	The Merck Index, 1989
Ketoconazole	$pK_{a1} = 6.5/pK_{a2} = 2.9$ Both alkaline	4.3	Nizoral, 200 mg/tab, lot 98F05/497 (Janssen-Cilag, Germany)	Galia et al 1998
L-Sulpiride	$pK_{a1} = 9.0/pK_{a2} = 10.2$ Both alkaline	0.65	Levopraid, 100 mg/tab, lot 001 (Ravizza, Italy)	Pitre et al 1988
Metronidazole	Non-ionizable	0.0[a]	Arilin 250, 250 mg/tab, lot 101011 (Dr A. Wolff, Germany) Clont, 250 mg/tab, lot CCLPV1 (Bayer Vital, Germany) Metronidazol Artesan, 250 mg/tab, lot 01005 (Cassela-Med, Germany)	—
Troglitazone	$pK_{a1} = 6.1/pK_{a2} = 12.0$ Both acidic	2.7	Romozin, 200 mg/tab, lot E97L2287 (GSK, UK) Crystalline troglitazone tablets, 200 mg/tab, batch GWPD0157/22A (GSK, UK)	Nicolaides et al 1999
Extended-release products				
Felodipine	Non-ionizable	4.5[a]	Hydrophilic matrix tablet, 10 mg/tab, batch H0573 (AZ, Sweden)	—
Isosorbide mono-5-nitrate (ISMN)	Non-ionizable	−0.28[a]	Imdur, 60 mg/tab, lots AE6139 and ZL6104 (AZ, Sweden) Osmotic pump, 60 mg/tab, lot IS-01240299 (Lavipharm, Greece)	—
L-Sulpiride	$pK_{a1} = 9.0/pK_{a2} = 10.2$ Both alkaline	0.65	Osmotic pump, 110 mg/tab, lot 01171199 (Lavipharm, Greece)	Pitre et al 1988

[a]Estimated from http://esc.syrres.com/interkow/kowdemo.htm

(~6 mol%), oleic (~6 mol%) and linoleic acid (~72 mol%). Estimated average molecular weights are 763 g mol^{-1} and 792 g mol^{-1} for the Lipoid E PC and the Lipoid S 100 product, respectively. Since the addition of phospholipids was done on a gravimetric basis, FaSSIF and FeSSIF prepared with Lipoid S 100 product contained slightly less phospholipids than the numbers shown in Table 2 (i.e. they contained 0.70 mM and 3.51 mM, respectively).

Dissolution profiles of Romozin, GR253035X tablets, Levopraid and Wellvone, as well as the release profile of L-sulpiride from an osmotic pump formulation, and the release profiles of ISMN from an osmotic pump and from Imdur, were obtained in FaSSIF and FeSSIF prepared with egg phosphatidylcholine and with partially hydrolysed soybean phosphatidylcholine.

Dissolution/release tests

Dissolution/release tests were performed with the USP 24 Apparatus II, using a Distek (model 2100B; Distek, New Brunswick, NJ), an Erweka (models DT6R and DT 80; Erweka, Heusenstamm, Germany) or a Vankel (model

Table 2 Physicochemical characteristics and composition (mM) of revised standard FaSSIF and FeSSIF and modified solutions, FaSSIFm and FeSSIFc, respectively[a].

Fasted state simulated intestinal fluids				**Fed state simulated intestinal fluids**			
pH		6.5		pH		5.0	
Osmolality[b] (mOsmol kg^{-1})		270 ± 10		Osmolality[b] (mOsmol kg^{-1})		635 ± 10	
Buffer capacity[c] (mmol L^{-1} ΔpH^{-1})		12		Buffer capacity[c] (mmol L^{-1} ΔpH^{-1})		76	
FaSSIF		FaSSIFm		FeSSIF		FeSSIFc	
Sodium taurocholate	3	Sodium taurocholate	3	Sodium taurocholate	15	Sodium taurocholate	15
Egg phosphatidylcholine	0.75	Egg phosphatidylcholine	0.75	Egg phosphatidylcholine	3.75	Egg phosphatidylcholine	3.75
Sodium dihydrogen phosphate	28.66	Maleic anhydride	25.01	Acetic acid	144	Citric acid	84
Sodium hydroxide	~13.8	Sodium hydroxide	~45	Sodium hydroxide	~101	Sodium hydroxide	~200
Sodium chloride	106	Sodium chloride	109	Sodium chloride	173	Sodium chloride	206

[a]In all cases media were freshly made and used. [b]Measured with an automatic semi-micro osmometer (Model A0300; Knauer GmbH, Berlin, Germany). [c]By titrating the medium with 0.1 M HCl.

7010, Cary, NC) apparatus. Standard conditions employed for all experiments were 500 mL of dissolution medium at 37 °C, a stirring rate of 100 rev min^{-1} and triplicate determinations (except from experiments in the USP and in the Pharm. Int. buffers where 75 rev min^{-1} were used with six replications per experiment). Samples (3–5 mL) were removed (with sample replacement in all but in the USP and Pharm. Int. buffers) using a 5-mL Fortuna Optima syringe fitted with stainless tubing to facilitate representative sampling. Samples were filtered through a regenerated cellulose membrane filter (Durapore, Millipore or Titan) or a PTFE filter (Minisart SRP25, Sartorius) with 0.45-μm pore size, discarding the first millilitre. Felodipine powder was sprinkled directly onto the surface of the dissolution medium at the start of the experiment. To avoid sticking to the vessel, the hydrophilic matrix tablet of felodipine was placed in a stationary basket above the rotating paddle. The basket was attached to a rod that was fixed directly onto the lid of the vessel (Wingstrand et al 1990).

Assay methods

Assay of ibuprofen, indometacin and metronidazole in USP and Pharm. Int. buffers was performed according to the USP methods (The United States Pharmacopeia, 2000) by measuring the absorbance at 221, 265 and 319 nm, respectively. For all other drugs, HPLC with UV detection methods were used. Assay methods of troglitazone, ketoconazole, atovaquone, felodipine and ISMN have been previously described (Agbaba et al 1994; Galia et al 1998; Nicolaides et al 1999; van der Lee et al 2000).

For the GR252035X assay, a Hypersil ODS C-18 (250 × 4.6 mm i.d., 5 μm) was used: the mobile phase consisted of ammonium formate 0.05 M and acetonitrile (40:60 v/v), the flow rate was 1.5 mL min^{-1} and the detection wavelength was 260 nm. The L-sulpiride assay method was a modification of a previously published assay method (El Walily et al 1999); a Hypersil BDS CPS (250 × 4.6 mm i.d., 5 μm) column was used; mobile phase consisted of acetonitrile, water and triethylamine (75:25:0.02) and its pH was adjusted to 7.0 with phosphoric acid; the flow rate was 1.5 mL min^{-1} and the detection wavelength was 243 nm.

Dissolution profile comparisons

Profile comparisons were performed with the use of $f_{1,area}$ (Vertzoni et al 2003) assuming the USP's buffer and the revised standard FaSSIF and FeSSIF compositions (Table 2) as reference.

Evaluation of $f_{1,area}$ was considered up to the time corresponding to the first experimental datum after 85% of the plateau level of the reference data set (Vertzoni et al 2003). In cases where this datum point was not observed within 6 h of experimentation, $f_{1,area}$ was evaluated up to 6 h, to reflect the maximum physiologically reasonable small intestinal residence period (Vertzoni et al 2003). Since the coefficient of variation of data points at every sampling time was, in all cases, less than 15%, $f_{1,area}$ was evaluated from mean data sets.

The average difference of cumulative dissolution data sets from various batches of the same product is usually considered to be about 10% (e.g. Tsong et al 1996). In this study, therefore, a 15% average difference of a test from a reference data set ($f_{1,area} = 0.15$) was set as the limit for identifying differences that would matter for the prediction of the in-vivo dissolution of the product.

In cases where the dissolution process of both sets of profiles-to-be-compared was complete within less than 30 min, profile comparisons were not performed.

Results

Substitution of sodium for potassium in buffers

Drug dissolution profiles from Aktren Forte, Dolo-Puren 400T and Clont tablets were complete within 20 min in both

the USP and Pharm. Int. media (data not shown). The $f_{1,area}$ values for the mean drug dissolution profiles from Indomethacin AL50, Arilin 250 and Metronidazol Artesan immediate release tablets, and from Aktren Spezial, Indo 50 von ct and Indomet-ratiopharm 50 immediate-release capsules obtained in the USP and the Pharm. Int. phosphate buffers (data not shown) were clearly below 0.15 (range: $0.01 \leqslant f_{1,area} \leqslant 0.11$), indicating that at a pH similar to that of the fasted small intestine the cation does not affect the dissolution process of ionized lipophilic weak acids and hydrophilic non-ionizable compounds.

Substitution of buffer components in FaSSIF and FeSSIF

Figure 1 shows the dissolution profiles of Romozin, crystalline troglitazone tablets, GR253035X tablets, Wellvone and micronised felodipine powder, and the release profiles of felodipine from the hydrophilic matrix tablet in FaSSIF, FaSSIFm, FeSSIF and FeSSIFc (Table 2).

Dissolution profiles of Romozin and crystalline troglitazone tablets in FaSSIFm and in FeSSIF were not obtained because of the presence of various degradation products in FaSSIFm and a single degradation product in FeSSIF. Half-lives estimated from separate concentration (nominal initial concentration: 11 $\mu g\,mL^{-1}$) versus time data for troglitazone in these media were 7.3 ± 0.3 h and 0.82 ± 0.03 h in FaSSIFm and FeSSIF, respectively (data not shown). Using standards, and by comparing HPLC-UV chromatograms, it was concluded that the degradation product in FeSSIF (that was also present in FaSSIFm) was a quinone that is known to be formed from an intermediate radical of troglitazone (Fu et al 1996). In our studies the identification of the quinone was additionally confirmed from its mass spectrum (data not shown). In aqueous solutions troglitazone and citrates compete for scavenging reactive oxygen species (Floyd et al 1990; Inoue et al 1997) and, therefore, troglitazone is more stable in the presence of citrates. On the other hand, maleates are known to enhance the formation of reactive oxygen species (Gstraunthaler et al 1983) and, therefore, they increase troglitazone's potential for oxidation.

With GR253035X, the dissolution profile in FaSSIFm was 21% lower than in FaSSIF whereas the dissolution profile in FeSSIFc was 30% lower than in FeSSIF. Since no extra peaks were observed on the assay chromatograms, these differences are most likely related to differences in the solubility product of this compound in media with different anions. With Wellvone, dissolution profiles of atovaquone in FaSSIF and FaSSIFm were very low

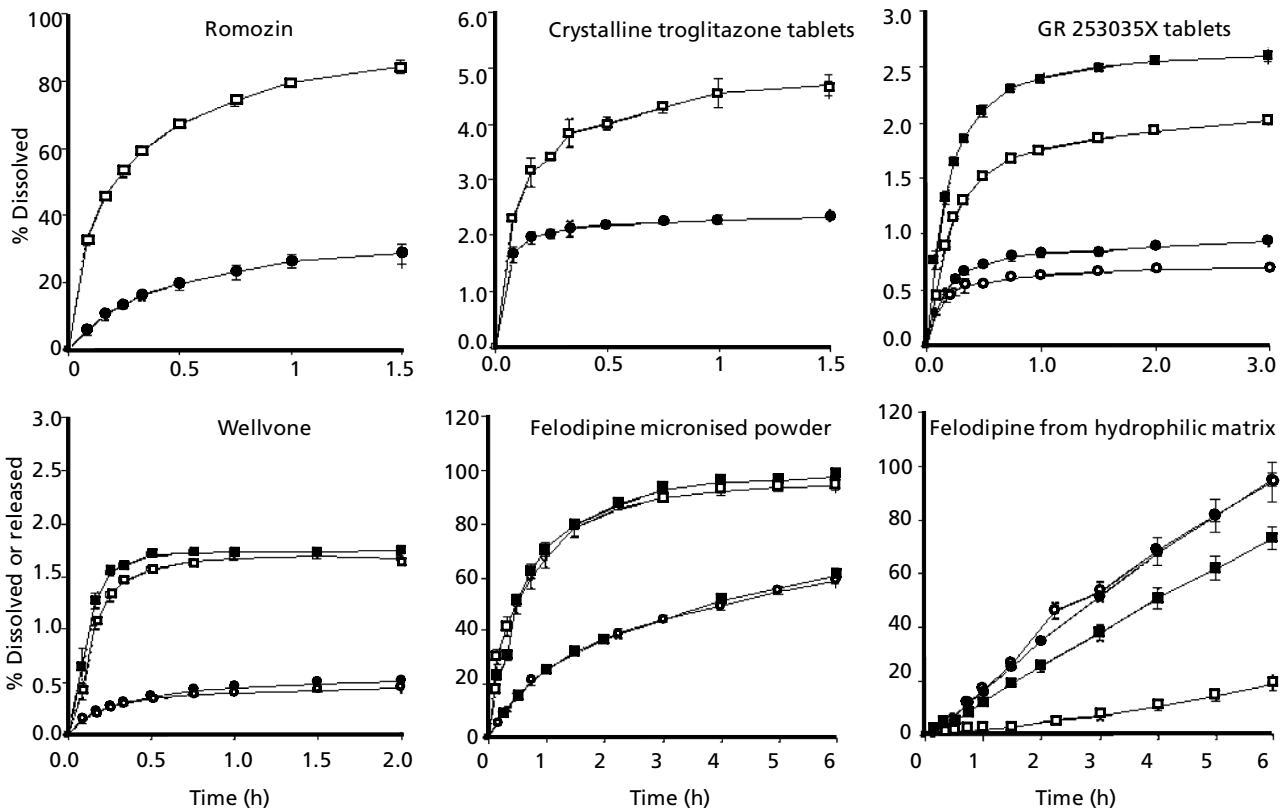

Figure 1 Mean ± s.d. cumulative % drug dissolved vs time profiles from Romozin, crystalline troglitazone tablets, GR253035X tablets, Wellvone and micronised felodipine powder, and mean ± s.d. cumulative % released vs time profiles of felodipine from a hydrophilic matrix tablet. ●, FaSSIF; ○, FaSSIFm; ■, FeSSIF; □, FeSSIFc. Please note that the y-axis scale may vary with the drug/dosage form under consideration.

(Nicolaides et al 1999) and similar ($f_{1,area}=0.07$), but dissolution in FeSSIFc was 21% lower than in FeSSIF. Buffer substitution had no effect on the dissolution of felodipine from powder ($f_{1,area}=0.05$ and 0.04 for the FaSSIF/FaSSIFm and the FeSSIF/FeSSIFc comparison, respectively). With felodipine's hydrophilic matrix formulation, the drug release profile in FaSSIFm was not different to FaSSIF ($f_{1,area}=0.02$), but the release profile in FeSSIFc was 79% lower than in FeSSIF (Figure 1). Since no ionisation-related mechanism could be considered, the decreased dissolution/release profiles of atovaquone or felodipine in FeSSIFc can only be attributed to the salting-out properties of citrates (Leontidis 2002); salting-out ions compete for water at various interfaces, leading to dehydration of the surface. In contrast, salting-in ions, such as acetates (Leontidis 2002), may easily lose their water or serve themselves as solvating species at an interface. It is obvious that salting-out effects will be more apparent with very lipophilic compounds (e.g. atovaquone) or with products for which hydration is a prerequisite for efficient release process (e.g. hydrophilic matrix formulation of felodipine) and, respectively, will lead to decreased solubility or decreased cloud point of the polymer used in the matrix formulation. With separate experiments it was confirmed that citrates indeed decreased the cloud point of HPMC (the polymer in the tested felodipine matrix formulation); cloud point estimates of 0.25% (w/w) HPMC solutions in FeSSIF and FeSSIFc at 50% transmission were 47.4 ± 0.08 and $40.6 \pm 0.07\,^{\circ}C$, respectively.

Substitution of crude for pure sodium taurocholate in FeSSIF

With Sigma-Aldrich's lot no. 050K0720, it was impossible to prepare FeSSIF solutions because of continuous precipitation of an unknown material (that was shown not to include any 3a-hydroxy-bile salt). Figure 2 shows the cumulative data sets of five immediate-release tablets and three extended-release products in FeSSIF (Table 2) and in FeSSIF made with crude taurocholate from ox bile using the Sigma-Aldrich lot no. 048H0684 and the Fluka lot. Experiments were run under conditions simulating the lumenal environment of the fed small intestine in an attempt to observe the strongest possible effect (bile salt

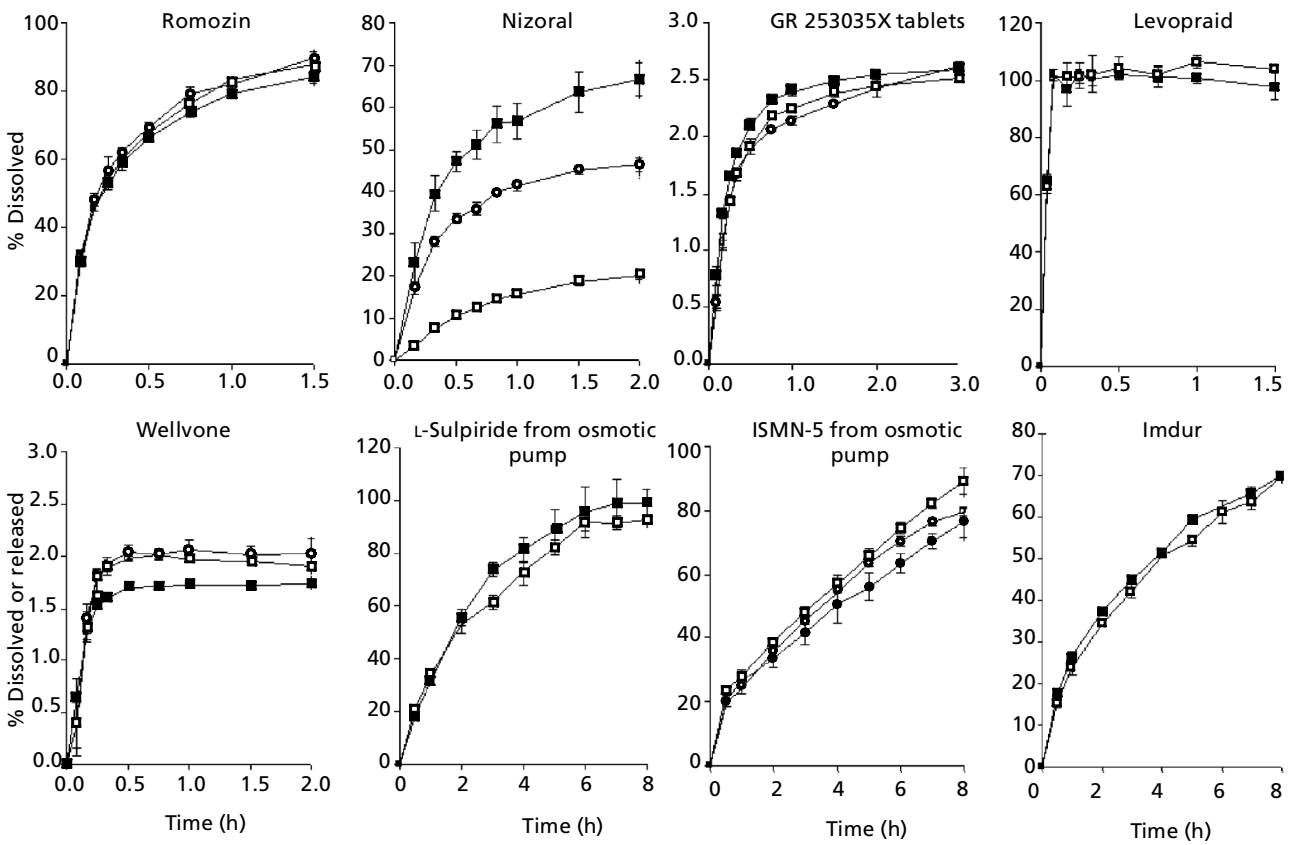

Figure 2 Mean ± s.d. cumulative % drug dissolved vs time profiles from Romozin, Nizoral, GR253035X tablets, Levopraid and Wellvone, and mean ± s.d. cumulative % released vs time profiles of L-sulpiride from an osmotic pump, ISMN from an osmotic pump and Imdur. ■, FeSSIF; ○, FeSSIF made with crude sodium taurocholate from ox bile with total bile acid concentration ~15 mM using the Sigma-Aldrich lot 048H0684; □, FeSSIF made with crude sodium taurocholate from ox bile with total bile acid concentration ~15 mM using the Fluka lot. With Romozin, the data were obtained in FeSSIFc. Please note that the y-axis scale may vary with the drug/dosage form under consideration.

concentration in FeSSIF is three times higher than in FaSSIF). The $f_{1,area}$ values indicate that, regardless of the lot, the use of crude sodium taurocholate from ox bile, also containing other bile salts at a total concentration equimolar to pure sodium taurocholate in FeSSIF, does not affect drug dissolution of Romozin, GR253035X tablets and Levopraid, the release of L-sulpiride and ISMN from osmotic pumps and the release of ISMN from Imdur ($0.03 \leqslant f_{1,area} \leqslant 0.15$). However, compared with data in FeSSIF, the use of FeSSIF containing taurocholic acid and other bile salts led to about 18% higher dissolution profile of atovaquone from Wellvone (regardless of the lot of the crude material), and to 74% (with the Sigma-Aldrich lot no. 048H0684) and 29% (with the Fluka lot) lower dissolution profiles of ketoconazole from Nizoral. The more complete dissolution of the highly lipophilic atovaquone in FeSSIF containing a mixture of tri- and di-hydroxy bile salts can be attributed to the fact that compared with tri-hydroxy bile salts (such as cholates) di-hydroxy bile salts are more discriminating with respect to hydrophobicity (Wiedmann & Kamel 2002). On the other hand, ketoconazole has been shown to be sensitive to the exact composition of bile salt micelles. For example, it has been shown that inclusion of oleic acid in pure taurocholate solutions above the critical micelle concentration (CMC) actually decreases the solubilization ratio (Poelma et al 1990, 1991; Wiedmann & Kamel 2002).

Substitution of partially hydrolysed soybean phosphatidylcholine for egg phosphatidylcholine

Figure 3 shows the cumulative data sets of four immediate-release tablets and three extended-release products in FaSSIF and in FeSSIF prepared with egg phosphatidylcholine and with partially hydrolysed soybean phosphatidylcholine. The $f_{1,area}$ values indicate that, apart from Wellvone in FeSSIF, the origin or purity of phosphatidylcholine does not affect the dissolution or the release process in either FaSSIF or FeSSIF ($0.01 \leqslant f_{1,area} \leqslant 0.11$). For Wellvone the dissolution profile in FeSSIF prepared with soybean phosphatidylcholine was 21% higher than in FeSSIF (Table 2), despite the slightly lower molar phospholipid concentration used (see Materials and Methods).

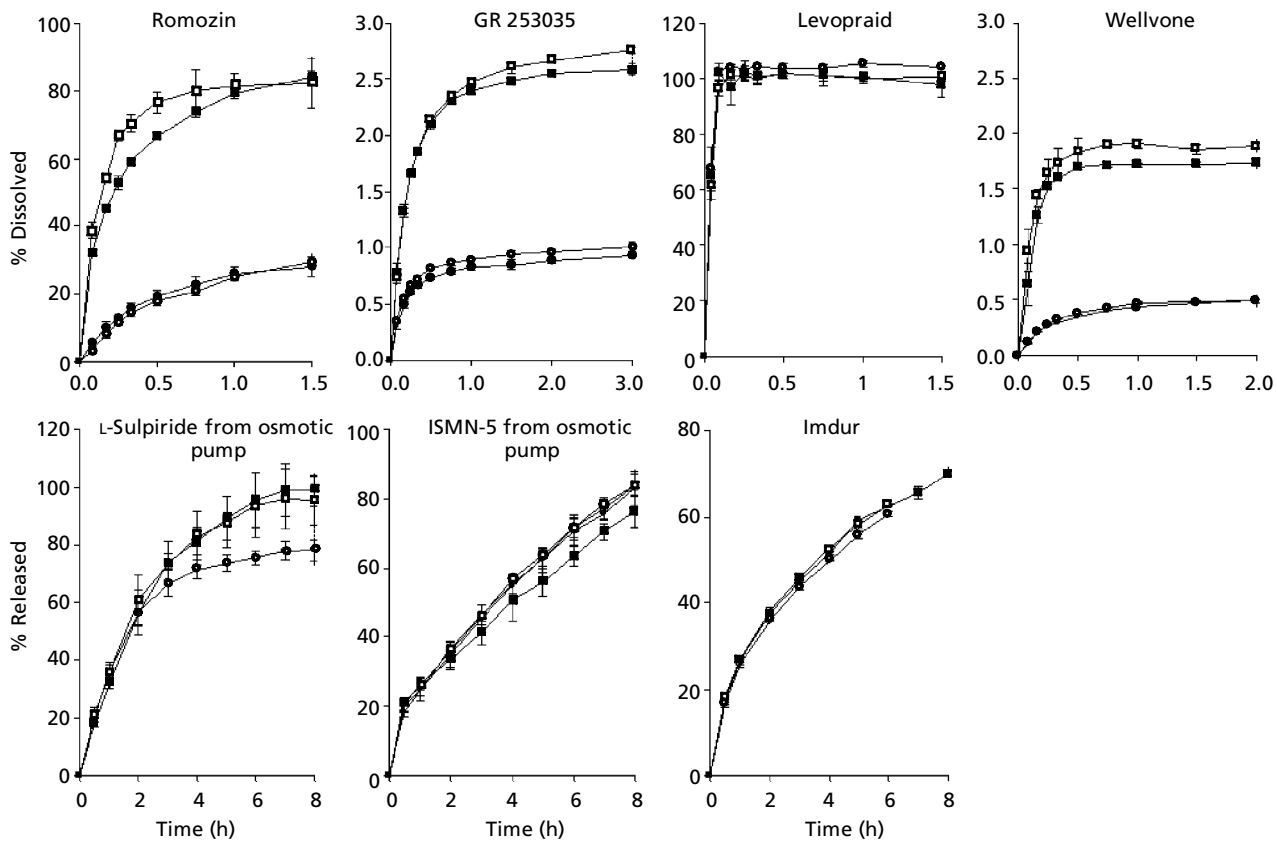

Figure 3 Mean ± s.d. cumulative % drug dissolved vs time profiles from Romozin, GR253035X tablets, Levopraid and Wellvone, and mean ± s.d. cumulative % released vs time profiles of L-sulpiride from an osmotic pump, ISMN from an osmotic pump and Imdur. ●, FaSSIF; ○, FaSSIF made with soybean, partially hydrolysed phosphatidylcholine; ■, FeSSIF; □, FeSSIF made with soybean, partially hydrolysed phosphatidylcholine. Please note that the y-axis scale may vary with the drug/dosage form under consideration.

Discussion

Osmolality-adjusting agents in biorelevant media

In the fasted small intestine, the principal cationic species is sodium, with typical concentrations of 100–140 mM (Banwell et al 1971; Davenport 1982; Lindahl et al 1997). Potassium concentrations are much lower in comparison, around 5 mM (Banwell et al 1971; Lindahl et al 1997). Therefore, at least in FaSSIF, it is more appropriate to use sodium-based osmotic agents and buffers than those containing potassium as the cation. Results in these studies, with two highly lipophilic weak acids and one highly soluble neutral compound, indicate that substituting sodium for potassium in standard buffer systems on an equimolar basis has no practical effect on the dissolution process.

Buffers used in FaSSIF and FeSSIF

As indicated in the first article proposing the use of biorelevant media (Dressman et al 1998), the principal buffer species in the fasted small intestine are the bicarbonates. However, media containing bicarbonates must be continuously sparged with carbon dioxide to maintain the desired pH, buffer capacity, ionic strength and osmolality. Further, external factors such as the air currents above the vessel, the rate of the reaction and the geometry of the vessel will all affect the partial pressure of carbon dioxide at the surface of the solution (Butler 1964), suggesting that the sparging procedure would have to be validated anew for each separate laboratory situation and each desired final concentration of carbon dioxide.

For the fed state, it is difficult to define the appropriate buffer system on a physiological basis. In this case it can be assumed that buffer species generated by food digestion (e.g. amino acids) play an important role in maintaining the pH value. It is highly doubtful that at pH values typical of the upper small intestine in the fed state (pH 5), the necessary buffer capacity could be achieved with bicarbonate buffer alone.

Practical considerations, therefore, lead to the use of non-physiologically relevant buffer species, which then raises the question of whether the buffer type influences the results. Theoretically, the type of buffer may affect the stability or the solubility product of the active compound and the performance characteristics of an extended-release product. Data from this study suggest that hydrolytic and oxidative reactions in FeSSIF are decreased when acetates are replaced by citrates. When stability of the drug is not an issue and immediate-release dosage forms are considered, the solubility product becomes of prime importance. For the dissolution of weak acids, the selection of buffer species is of less importance than the achievement of the appropriate pH and buffer capacity, because it is the buffer cation that will determine the solubility product of the weak acid anion and, consequently, any limitations to the contribution of the ionized form of the drug to the dissolution process. Although sodium is clearly the cation of choice (Table 2), this study shows that using potassium is not expected to affect the data. For weak bases, the question of whether the solubility product is different in the presence of bicarbonates than in the presence of non-physiologically relevant anions is of concern when $pK_a(s)$ of the dissolving compound is higher than about 5 (weak bases with $pK_a(s)$ lower than 5 are not ionised in FaSSIF, FeSSIF or in the small intestinal lumen) and, also, solubility product(s) are relatively low (e.g., L-sulpiride in Levopraid, although having a $pK_a > 5$, it dissolves completely within few minutes (Figure 2), and, therefore, buffer effects are of no practical importance). For neutral compounds, buffer effects on dissolution can be of interest only in cases of highly lipophilic compounds with extremely low solubility and relate to the salting in/out properties of the buffer anion. Finally, this study shows that the buffer selection may also be important for the performance of extended-release products with release mechanisms sensitive to the salting out properties of the buffer anion.

Type and purity of bile salt(s) in FeSSIF

Human bile contains predominantly di- and tri-hydroxy acids conjugated with glycine and taurine (Hofmann 1993). Although the nature of conjugation does not affect solubilization, the number of hydroxy groups can be an important factor in determining solubilization (Weidmann & Kamel 2002). However, in the study of bile salt solubilization, trihydroxy acids (and specifically taurocholates) have been utilized in the vast majority of cases because of the relative insensitivity of the aggregation number of the taurocholate micelle to changes in pH, ionic strength and temperature (Weidmann & Kamel 2002) and, also, because they are comparatively cheaper. For these reasons sodium taurocholate has also been favoured for biorelevant dissolution testing (e.g Nicolaides et al 1999) and, although solubility data in aspirated human intestinal fluid are very limited, it has been shown, for example, that danazol's solubility in fasting intestinal fluid is similar to its solubility in FaSSIF (Galia et al 1998; Pedersen et al 2000).

This study suggests that, in general, the specific composition of bile acids in FeSSIF and the presence of multiple bile salts are of minor importance to simulating drug dissolution or release in the small intestine. However, in two cases with highly lipophilic compounds (atovaquone and ketoconazole) the identity of bile salts or the purity of the material had a substantial effect on the dissolution profiles. Interestingly, based on previous correlations between the dissolution in FeSSIF and the in-vivo performance (Galia et al 1998; Nicolaides et al 2001), dissolution profiles of both atovaquone (from Wellvone) and ketoconazole (from Nizoral) in FeSSIF prepared with crude sodium taurocholate would tend to produce a better simulation of the in-vivo performance of these two drugs. However, from a practical point of view, the use of crude bile extract is problematic. For one of the crude sodium taurocholate lots, it was impossible to prepare FeSSIF. Although identification of good batches might be possible, validation of this procedure would be very

difficult. Another problem is that, although the manufacturers provide the approximate content of bile salts in the extract, further bile salts and other substances that may indirectly affect dissolution (e.g. via changes in ionic strength) are also present. To standardize the amount of bile salts using a crude product, the concentration of total bile salts in the product must, therefore, be determined by the user on a batch-to-batch basis. Thus, although the crude bile salt extracts are cheaper to purchase and tend to produce results that are, in some cases, closer to in-vivo than those obtained with pure bile salts, the need to standardize the amount of bile salt added to the medium, to validate this on a batch-to-batch basis and the unreliability in terms of being able to manufacture the medium must also be carefully weighed when making a decision about whether to use pure or crude bile salt products.

Type and purity of phosphatidylcholine in FaSSIF and FeSSIF

Phosphatidylcholine accounts for about 95% of the biliary phospholipids in man, with the remaining 5% being mostly phosphatidylethanolamine (Alvaro et al 1986). The major fatty acids of biliary phospholipids are palmitic, stearic, oleic, linoleic and arachidonic in amounts that are roughly similar to the amount of fatty acids in egg phosphatidylcholine (Angelico et al 1992; Hayes et al 1992; Hatsushika et al 1993; Hay et al 1993). This study suggests that the fatty acid composition of phosphatidylcholine is only likely to be important in drug dissolution when the drug is highly lipophilic (e.g. Figure 3, Wellvone). Literature data indicate that the molecular species of lecithins adsorbed onto filamentous cholesterol crystals (during cholesterol crystallization) are more saturated than in whole bile (Konikoff et al 1994). It is tempting, therefore, to speculate that the solubility of very highly lipophilic compounds will also be decreased when phospholipids with more saturated fatty acids are used (i.e. when egg phosphatidylcholine is used).

Conclusions

In the drive to better simulate dissolution in the gastrointestinal tract, practical considerations (cost, validation, ease of manufacture and analysis) should not be forgotten. Improvements in simulation range from simple and problem-free changes, like the substitution of sodium for potassium as the cation, to the use of crude bile extracts which, while more attractive in terms of simulating the mix of bile components in the gut, generate other problems in terms of batch-to-batch variation and manufacturability. The use of non-physiologically relevant buffers is inevitable, especially for simulation of the fed state in the small intestine. However, in certain cases, it is prudent to run an additional dissolution test in FeSSIF or FaSSIF containing alternative buffer species. Egg phosphatidylcholine can be substituted by partially hydrolysed soybean phosphatidylcholine in case the drug is not extremely lipophilic. Finally, further aspects, such as adding digestion products to the fed state medium, still have to be evaluated.

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