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Review

Characterising the behaviour of poorly water soluble drugs in the intestine: application of biorelevant media for solubility, dissolution and transport studies

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Abstract

Objectives Based on the knowledge of human intestinal fluids, compositions of biorelevant media and their impact on solubility, dissolution and permeability studies of poorly soluble drug compounds are discussed.

Key findings Human intestinal fluids show large variations with regard to composition and pH, which complicate the selection of biorelevant media. The influence of concentration and ratio of bile salts, phospholipids and hydrolysis products, such as monoglycerides and free fatty acids, in well characterised media, on the solubility, dissolution and permeability of a given drug provides valuable information on the behaviour of the drug in the intestine, thus enabling the prediction of the in-vivo absorption.

Summary This review discusses the implications of biorelevant media composition on the solubility, dissolution and permeability of poorly soluble drug compounds. Biorelevant media contain bile salts and phospholipids and when simulating the fed state also monoglycerides and free fatty acids. Solubility of some poorly soluble drugs increase independently of the type of surfactants included in the biorelevant media, while others have a higher solubility in monoglyceride- and fatty acid-containing media. This is independent of the log P (the octanol–water partition coefficient) of the drug. The use of biorelevant dissolution media improves the correlation to in-vivo data, compared with compendial media, and although the field of permeability studies is complex the use of biorelevant media in this setting shows promise with respect to a better prediction of absorption.

Keywords bile salt; biorelevant media; dissolution; intestinal permeability; poorly water soluble drugs

Introduction

When developing an oral delivery system for a poorly water soluble drug compound, it is important to consider the rate limiting step for the absorption of the particular drug. As described by the Biopharmaceutics Classification System (BCS), the limiting factor is usually the solubility, the dissolution rate or the permeability. The solubility in the gastrointestinal tract is a crucial parameter to consider, because only free drug molecules in solution will be available for absorption across the intestinal epithelium. According to the BCS a drug compound is highly soluble when the highest dose is soluble in 250 ml of aqueous buffer at all pH values between 1 and 7.5.[1] However, using pH as the only denominator does not define the actual situation in the gastrointestinal fluids. The BCS definition will consider an acid drug compound poorly soluble, even though the drug is soluble in the small intestine, where it will be absorbed. For BSC class II and IV compounds the aqueous solubility is often misleading and can cause an overestimation of the solubility problems encountered during the development of the drug, because the environment in the human intestine has a much higher solubilising capacity than pure water, or the buffers and compendial dissolution media proposed by the pharmacopoeias. During the last 10–15 years, much effort has been put into designing dissolution and solubility assessment media that emulate human physiological conditions: biorelevant media per definition.[2] These media are primarily intended for development purposes since their complexity often impairs their suitability for quality control. The presence of amphiphiles in the gastrointestinal tract greatly enhances the solubility of many poorly water soluble

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compounds, but another issue that should be considered is how the different amphiphiles affect solubility and how the drug partitions into the colloids, since this can affect the rate and extent of absorption.

Absorption is often assessed by in-vitro permeability models using cell cultures or excised tissue. Traditionally, transport media for permeability studies that are optimised to be compatible with the cells or tissue have been used for this purpose. However these media are very far from the actual composition of the gastrointestinal fluids. As a consequence the application of biorelevant media in transport studies has been examined. It is hypothesised that the use of biorelevant transport media will lead to a better understanding and predictability of drug permeability; however, it is important that the transport media are compatible with the cells or tissue.

To make media applicable for in-vitro solubility, dissolution and permeability testing, the complexity of such media should be minimised. For this reason it is often not possible to comply with all the gastrointestinal conditions when composing a biorelevant medium. The task, yet to be fully accomplished, therefore revolves around identifying the most important parameters as well as establishing how they individually and together affect the solubility, dissolution and permeability of poorly water soluble drug compounds.

This review focuses on intestinal biorelevant media. Though gastric conditions do play a significant role for the dissolution of formulations, it is the behaviour of the compounds in the intestinal environment that will be conclusive for the absorption, and therefore gastric media are not covered. The review will elaborate on the development of biorelevant media that simulate the composition of the intestinal fluids, as well as the experiences with these media when applied for solubility, dissolution and transport studies.

Gastrointestinal conditions

The composition and volume of the gastrointestinal fluids will be major determinants for the solubility, and thereby absorption, of a drug in the gastrointestinal tract. The gastrointestinal fluids can be characterised by physicochemical parameters such as surface tension, osmolality, viscosity, pH and content of bile salts, phospholipids and other amphiphiles. The presence of amphiphiles in the intestinal fluids plays a major role for the solubilisation of poorly soluble compounds. First of all they reduce the surface tension of the gastrointestinal fluids, thereby improving the initial wetting of the compound. More importantly, however, intestinal amphiphiles form mixed micelles, and possibly other colloid structures, that solubilise the compounds and thus increase the solubility. The mixed micelles composed of bile salts/phospholipids will facilitate diffusion of the drug from the lumen, across the mucus layer, reaching the intestinal wall. Adjacent to the enterocytes a pH gradient, or a yet unknown mechanism, is believed to cause a disintegration of the mixed micelles, thereby liberating and making the drug available for absorption.[3,4]

Recently, knowledge on composition of intestinal fluids has increased. Table 1 summarises available literature data on some of the important physicochemical parameters of human duodenal and jejunal fluids.[5–17]

As can be seen from Table 1, bile salt concentrations determined in duodenum and jejunum in the fasted state span from 1.5 to 5.9 mM, with most values around 3 mM. In the fed state values range from 0.5 to 24 mM, and with no representative value (median value 12 mM).

Phospholipids are present in the biliary secretions, but also enter the intestinal lumen as a constituent of many foodstuffs. Therefore, the concentration of phospholipids depends on the feeding state and on the type of food. Phospholipids are lipophilic surfactants and part of the mixed micelles, which are of great importance for the wetting and solubilisation of drugs. The ratio of bile salts to phospholipids varies by as much as a factor of 10: from 4.5 to 39 in the fasted state, and from 1.3 to 16 in the fed state. On this background, not just one bile salt/phospholipid ratio can be extracted to represent the physiological situation.

The large variations in bile salt and phospholipid levels, as well as their ratio, reflect first of all the different study procedures, in terms of different equipment, liquid intake, sampling sites and sampling time points, but underlying that is a large individual variation regarding biliary secretions.

In the fed state, ingested lipid and its digestion products contribute to the solubilisation of lipophilic compounds. Triglycerides from food are converted to monoglycerides and free fatty acids by gastric and pancreatic lipases. These species are solubilised by bile salt/phospholipid micelles to some extend and also form vesicular structures.[18,19] The presence of free fatty acid and monoglyceride is proven to solubilise many lipophilic compounds, and thus often contributes to the higher bioavailability of BCS class II compounds frequently seen in the fed state. Very few studies have analysed human aspirates for the content of these species, and levels have been reported of from 10 to 30 mM for free fatty acid and 2 to 6 mM for monoglyceride. In the two studies in Table 1 reporting the content of free fatty acid (FFA) and monoglycerides (MG) the ratio FFA/MG was approximately 6. In addition to the causes of variations in the gastrointestinal fluid composition mentioned above, the type of food ingested will also have impact on the levels of free fatty acids and monoglycerides.

From the intraluminal pH values shown in Table 1, it is clear that no striking difference between the fasted and fed state (mean values of 6.8 ± 0.4 compared with 6.2 ± 0.3) could be identified. The postprandial values are only slightly lower, and taking the variability of the values into account it could be argued whether a pH differentiation between fed and fasted state is relevant when composing biorelevant intestinal media. The relevance will depend on the intended application of the medium, e.g. solubility testing, dissolution testing or transport studies.

The influence of osmolality on the solubilisation of drugs is a factor that has been studied very little. From Table 1 it appears that duodenal values in the fasted state are hypotonic and jejunal values tend to be isotonic. In the fed state variation is much larger, probably due to variation in the type of meal and in the time span from meal ingestion to sampling. Although osmolality has been shown to affect the stability of liposomes and lipid vesicles, the effects on bile salt/phospholipid mixed micelles and other colloids present in the gastrointestinal tract, as well as a potential additional effect on drug solubility, remain to be illuminated.[20,21]

Table 1 Composition of human intestinal fluids

Region (number of replicates)	Total BS (mM)	Total PL (mM)	Ratio (BS/PL)	Total MG (mM)	Total FFA (mM)	Ratio (FFA/MG)	pH	Surface tension (mN/m)	Osmolality (mOsm/kg)
Fasted state									
D (7)[5]	5.9 ± 1.8	–	–	–	–	–	6.8	–	–
D (4)[6]	3.5 ± 1.8	0.1 ± 0.1	39	–	–	–	6.5 ± 0.5	–	–
D (15)[9]	2.6	–	–	–	–	–	6.2	32.3	178
D (12)[9]	2.82	–	–	–	–	–	6.7	33.6	197
D (6)[12]	2.6 ± 1.6	–	–	–	–	–	7.0 ± 0.4	–	137 ± 54
D (7)[10]	2.5	0.4	6	–	–	–	–	–	–
D (5)[7]	2.7	0.6	4.5	–	–	–	6.6	41.2	224
J (37)[8]	2.9 ± 2.9	–	–	–	–	–	7.1 ± 0.6	–	271 ± 15
J (10)[11]	1.5 ± 1.8	–	–	–	–	–	6.7 ± 0.9	33.7 ± 2.8	278 ± 16
J (6)[12]	3.5 ± 1.6	–	–	–	–	–	6.8 ± 0.4	–	200 ± 68
J (3)[13]	2 ± 0.2	0.2 ± 0.07	10	–	0.09	–	7.5	28 ± 1	–
J (6)[14]	–	–	6	–	–	–	–	–	–
Fed state									
D (7)[5]	13.4 ± 4.3	1.9 ± 0.4	9.6	–	–	–	6.4	–	–
D (5)[8]	14.5 ± 8.8	4.8 ± 1.8	3	–	–	–	–	–	–
D (6)[15]	9.3 ± 0.8	2.4 ± 0.35	3.9	–	–	–	5.7	–	–
D (12)[9]	11.8	4.31	2.7	5.95	39.4	6.6	6.5	27.8	416
D (8)[10]	24	1.5	16	–	–	–	–	–	–
D (5)[7]	3.6	1.8	2	–	–	–	5.9	35	285
D (5)[7]	5.2	1.2	4.3	–	–	–	6.1	35	278
J (3)[13]	8 ± 0.1	3 ± 0.3	2.7	2.2	13.2	6	6.1	27 ± 1	–
J (15)[9]	12	–	–	–	–	–	6.6	28	400
J (6)[14]	0.5–8.6	0.1–3.9	1–3	–	–	–	–	–	–
J (13)[16]	16.19 ± 1.51	–	–	–	–	–	–	–	–
J (16)[17]	15	–	–	–	–	–	–	–	–

D, duodenal fluids; J, jejunal fluids; BS, bile salts; PL, phospholipid; MG, monoglyceride; FFA, free fatty acids. Data represent mean ± SD.

As can be seen in Table 1, the surface tension in all studies is significantly reduced compared with water, and the range of the reported values was quite narrow. The values in the fed state are slightly lower than in the fasted state (31 vs 34 mOsm/kg), corresponding to higher levels of surfactants in the fed state.

Some other important parameters to consider when developing media simulating the gastrointestinal fluids are the volume and the viscosity, however very few studies have considered these factors.

The volume of the intestinal fluids is an important factor to consider when developing dissolution models. For class II compounds solubility is the rate limiting step in the absorption process and the permeability properties of such compounds are thought to assure that sink conditions are present. *In vitro*, the volume needed to provide sink conditions often by far exceeds physiological relevant volumes, or even volumes compatible with normal dissolution testing equipment (USP apparatus 2). In the fasted and fed state, intestinal volumes of 45–319 and 20–156 ml, respectively, have been determined by use of magnetic resonance imaging. Although these volumes are fully compatible with normal dissolution equipment, they may not be able to provide sink conditions *in vitro* because no drug is removed from the system.[22] This review will not enter into the technical discussion concerning practical volume-associated problems in dissolution testing. To some extent the Bio-Dis (USP apparatus 3) and flow through dissolution equipments (USP apparatus 4) accommodate these issues by creating settings where sink conditions are provided by other means than permeation.

Viscosity is a factor that is often disregarded when discussing biorelevant media. The viscosity of the gastric content varies depending on the feeding state, and greatly affect the gastric emptying.[23] This directly impacts the residence time of a drug compound in the ventricle and is, depending on the compound and formulation, important for the extent of dissolution and solubilisation occurring in the ventricle. Like volume, viscosity is a parameter that is of greatest importance in a setting of dissolution testing, where it exerts an important effect on the diffusivity of a compound and disintegration of the formulation.[24,25] From the Stokes– Einstein's equation (eqn 1) describing diffusivity, it appears that only small changes in viscosity will cause a considerable effect on the diffusivity, D.

$$D = \frac{kT}{6\pi\rho r} \quad (1)$$

where k is the Boltzman constant, T is the temperature, ρ is the viscosity and r the radius of the drug molecule.

Concerning intestinal fluids, dietary fibres are able to cause increased viscosity in the upper gastrointestinal tact,

and this has been shown to affect the absorption of some drugs.[25,26] However, the nutritional composition of normal meals only causes a modest increase in viscosity of upper intestinal fluids because of the hormonal response to high gastric fat and protein content, resulting in increased biliary and pancreatic secretions.[23,26]

In terms of solubility determination, the viscosity of the media is less important and has been shown not to affect the solubility of drug compounds.[25]

Biorelevant media

Taurocholate and glycocholate are the most abundant bile acids in human bile, together accounting for 35–65% of the total bile acid content.[6,12,13,27,28] They differ in their pK_a value (1.5 and 3.7, respectively), and in the pH range of the small intestine only taurocholate will be fully ionised. For this reason sodium taurocholate is most often used as a model bile salt in biorelevant media. Porcine and ox bile extracts have also been used and provide the advantage of containing a mixture of different bile acids, creating a situation that is more true to the physiology. However, the type of bile salts and the relative composition differ from that of humans and bile extracts exhibit great batch to batch variation.[29–31] Therefore the bile salt level and composition have to be determined before use of a new batch. Phosphatidylcholine (PC) is the most abundant phospholipid in bile secretions and has been included in biorelevant media from very early on.[2] Usually phospholipids from egg or soy bean are used; both contain primarily PC but have a different fatty acid composition, with egg phospholipid containing most saturated fatty acids.[32] What is often neglected is that PC is converted to lyso-PC in the lumen of the small intestine, and that the surface active properties of the two are different: lyso-PC and PC interact with bile salts to form micelles, however lyso-PC is able to form micelles by itself, while PC forms vesicles in aqueous media.[18]

In spite of their high relevance and huge importance for drug solubilisation, products of lipid digestion have been omitted from some of the most applied biorelevant media to date.

Table 2 summarises the media most extensively used in the existing literature, either as they appear in the table or with small modifications. Fasted-state simulated intestinal fluid (FaSSIF) and fed-state simulated intestinal fluid (FeSSIF) were first composed in 1998 by Galia *et al*.[2] They have recently been revised to incorporate new knowledge of the in-vivo conditions (FaSSIF-V2 and FeSSIF-V2, see Table 2).[33] The original media, FaSSIF, underwent only small changes. The phospholipid concentration was decreased, causing the bile salts/phospholipid (BS/PL) ratio to increase to 15. The buffering species were changed from phosphate to maleate and the osmolality decreased from 270 to 180 mOsm/kg, which is in agreement with the in-vivo data. In FeSSIF-V2 the buffering species was also changed to maleate, the BS/PL levels decreased and the BS/PL ratio changed from 4 to 5. Furthermore lipolysis products were added, but in sparse amounts compared with the in-vivo data, and the oleic acid/monoolein (OA/MO) ratio of 0.16 is not representative for the reported physiological values. Osmolality was decreased to a level matching the in-vivo data.

Table 3 contains a specification of the Copenhagen media in Table 2. Keeping the pH, BS/PL and OA/MO ratios constant, the impact of increasing either BS/PL or OA/MO levels could be systematically studied on each drug, creating a basis for the design of an optimal biorelevant medium for that particular drug, fitted to the purpose of the study as well. Furthermore, knowledge about how the different surfactants solubilise different types of compounds could be generated, and hopefully lead to a quality-by-design approach to the composing of biorelevant media and a scientific understanding of the behaviour of the drug.

Colloid phase characterisation

In fasted state media containing bile salt levels lower than 10 mM, and bile salts and phospholipids in a ratio of 4/1 or 5/1, particles with sizes between 50 and 100 nm have been identified by means of dynamic light scattering. The composition and structure of these particles have not been clarified, but it has been observed that when the BS/PL levels are increased to match the fed state the particle size decreases to approximately 5 nm. This has been confirmed by particle size measurements in studies of biorelevant media and by visualisation by cryogenic transmission electron microscopy (Cryo-TEM).[19,34–37] This indicates that the particles in the

Table 2 Composition of fasted and fed state simulated media

	FaSSIF	FaSSIF-V2	FeSSIF	FeSSIF-V2	Copenhagen fasted	Copenhagen fed
BS (mM)	3	3	15	10	2.5[a]	5–20[a]
PL (mM)	0.75	0.2	3.75	2	0.625	1.25–5
BS/PL	4	15	4	5	4	4
MO (mM)	–	–		5	–	0–10
OA (mM)	–	–		0.8	–	0–45
OA/MO				0.16		2–7.5
Buffer species	Phosphate	Maleate	Acetate	Maleate	Trizma maleate	Trizma maleate
pH	6.5	6.5	5	5.8	6.5	6.5
Osmolality (mOsm/kg)	270	180	635	390	270	Varying

BS, bile salts; PL, phospholipid; MG, monoglyceride; FFA, free fatty acids; MO, monoolein; OA, oleic acid; FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid. [a]Bile salt as either sodium taurocholate or crude porcine bile extract.

Table 3 The content of amphiphiles in the Copenhagen media

Oleic acid/ monoolein (mM)	Oleic acid/ monoolein ratio	Bile salt/phospholipid (mM)				
		2.5/0.625	5/1.25	10/2.5	15/3.75	20/5.0
0	–	0-0	1-0	2-0	3-0	4-0
5/2.5	2		1-A	2-A	3-A	4-A
15/2.5	6				3-A6	4-A6
10/5	2		1-B	2-B	3-B	4-B
30/5	6				3-B6	4-B6
15/7.5	2		1-C	2-C	3-C	4-C
45/7.5	6				3-C6	4-C6
20/10	2		1-D	2-D	3-D	4-D
20/3.33	6					4-D$^{1/6}$

fasted state media could also be mixed micelles, but larger due to the lower bile salt content. Bile salts are water soluble and a given concentration will always be in free monomeric form in the water phase, while the rest will form micelles with phospholipids. When the bile salt concentration is reduced, less bile salt will be able to form micelles with phospholipid, and the BS/PL micelles will then grow in size, since phospholipid is not able to make micelles, but forms monolayers in aqueous solution.[18] The addition of free fatty acids and monoglycerides to biorelevant media leads to the formation of vesicular structures, which are important for the solubilisation of lipophilic compounds in particular. How these structures influence the solubility and also the dissolution rate and diffusivity of drugs remains to be fully understood. Recent studies have suggested that it is a complex interplay of surfactant type, level, and ratio.[19,37,38] Figure 1 contains representative Cryo-TEM images of media 3-0, 3-C, 3-D and 4-D, visualising the structures present.

BS/PL mixed micelles should be able to solubilise both free fatty acids and monoglyceride to a certain extent, but the limit of solubilisation has not been determined. In medium 4-D$^{1/6}$ containing lyso-PC instead of PC and added 1 mM cholesterol, no vesicles were observed. Vesicles were observed in medium 3-C containing lyso-PC and 0.75 mM cholesterol.[38] This indicates that the monoolein concentration is important for the formation of vesicles. In another study, applying PC and not lyso-PC, vesicles were observed at lower OA/MO concentrations. This correlates with lyso-PC as a more efficient surfactant. Lyso-PC is a soluble amphiphile, which is able to form micelles, whereas PC is an insoluble swelling amphiphile forming monolayers in aqueous solution. The difference emerges from the split-off of one of the two fatty acid side chains on lecithin leaving only one left, resulting in a more hydrophilic molecule.[18] More studies are needed to identify features and relationships of

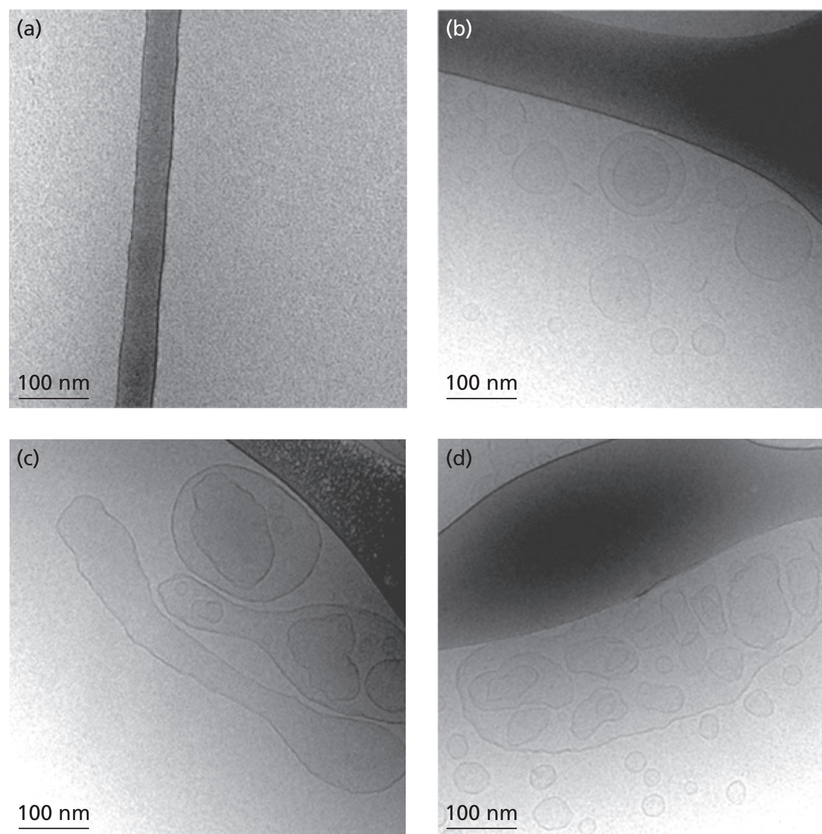

Figure 1 Cryogenic transmission electron microscopy images of biorelevant media. (a) Medium 3-0: micelles present as small dots. (b) Medium 3-C: vesicles and micelles co-exist. (c) Medium 3-D: elongated and larger vesicles are also present. (d) Medium 4-D: more bile salt and phospholipid leads to formation of smaller vesicles compared with (c) and more similar to (b). Reproduced with permission from Fatouros *et al*.[19]

the colloids possibly present in the intestinal lumen, leading to an understanding of solubilisation of drugs *in vivo*.

Biorelevant media for solubility studies

Only a few solubility studies have been performed in which the solubility in human aspirates has been directly compared with the solubility in biorelevant media simulating human intestinal fluids.[9,13,39] The findings are summarised in Table 4. Fasted and fed state media were compared with fasted and fed state human intestinal fluids (HIFs), accordingly.

Unless otherwise stated, the media were in accordance with the media in Table 2. As it appears from Table 4, the cases where the solubility in the simulated fluids deviated from the one in HIF comprise the vast majority. No consistency can be inferred with regard to what media provided the 'true' solubilising capacity, and certainly the most appropriate medium differs from compound to compound. What should also be noted is that the solubility of danazol and ketoconazole in HIF was analysed in two studies. The fasted state ketoconazole solubilities correlated; however, the fed state solubilities for both substances differed between the studies. This perfectly reflects the variation of the in-vivo data in Table 1. In addition to the studies in Table 4, solubility studies of a range of compounds have been conducted in biorelevant media, using the media presented

Table 4 Comparative studies of drug compound solubility in human intestinal fluids and different biorelevant media

Drug compound	Acid/base	log P	HIF solubility* (μg/ml)	Biorelevant medium[a]	Biorelevant medium solubility* (μg/ml)	Conclusion
Griseofulvin	Neutral	2	60	FeSSIF	30	Underestimation[13]
Nifedipine	Neutral	2	Fasted	FaSSIF	68 μM	Underestimation[39]
			130[¤] (μM)	FeSSIF	173 μM	Overestimation
			Fed	FeSSIF-V2	175 μM	Overestimation
			150[¤] (μM)			
Dipyridamole	Weak base	2.74	Fasted	FaSSIF	13	Underestimation[#][9]
	(pK_a: 5.7–6.4)		20	FaSSIF[m]	15	Underestimation[#]
			Fed	FaSSIF[cr]	17	Underestimation[#]
			167	FaSSIF[cr,m]	17.4	Underestimation[#]
				FeSSIF	232	Overestimation[#]
				FeSSIF[c]	194	Overestimation[#]
				FeSSIF[cr]	202	Overestimation[#]
				FeSSIF[cr,c]	170	Accordance[#]
Diazepam	Weak base	2.9	Fasted	FaSSIF	355 (μM)	Underestimation[39]
	(pKa: 3.4)		800[¤] (μM)	FeSSIF	909 (μM)	Underestimation
			Fed	FeSSIF-V2	950 (μM)	Underestimation
			1160 (μM)			
Ciclosporin	Neutral	3	250	FeSSIF	100	Underestimation[13]
Indometacin	Weak acid	3.4	Fasted	FaSSIF	1376 (μM)	Underestimation[39]
	(pK_a: 4.5)		2700 (μM)	FeSSIF	402 (μM)	Underestimation
			Fed	FeSSIF-V2	1000 (μM)	Accordance
			1020 (μM)			
Ketoconazole	Weak base	4.45	Fasted	FaSSIF	17	Underestimation[#][9]
	(pK_a: 2.94;		30	FaSSIF[m]	20	Underestimation[#]
	6.51)		Fed	FaSSIF[cr]	25	Accordance[#]
			912	FaSSIF[cr,m]	26	Accordance[#]
			Fasted	FeSSIF FeSSIF[c]	660	Underestimation[#]
			65[¤] (μM)	FeSSIF[cr]	520	Underestimation[#]
			Fed	FeSSIF[cr,c]	630	Underestimation[#]
			1140 (μM)	FaSSIF	565	Underestimation[#]
				FeSSIF	18.8 (μM)	Underestimation[39]
				FeSSIF-V2	1420 (μM)	Overestimation
					500 (μM)	Underestimation
Danazol	Neutral	4.5	Fed	FeSSIF	20	Underestimation[13]
			100	FaSSIF	16 (μM)	Underestimation[39]
			Fasted	FeSSIF	52 (μM)	Underestimation[39]
			44[¤] (μM)	FeSSIF-V2	85 (μM)	Accordance
			Fed			
			99 (μM)			
Felodipine	Neutral	4.5	420	FeSSIF	200	Underestimation

FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; HIF, human intestinal fluid; log P, the octanol–water partition coefficient. [a]See Table 2 for composition. [m]Medium contains maleates as buffering species. [c]Medium contains citrates as buffering species. [cr]Medium contains crude ox bile. [*]Data extracted from graphs. [#]According to reference's own statistics. [¤]Mean of values extracted from graphs.

in Tables 2 and 3 starting points. Those studies did not compare the solubility in HIF, but studied how different types and levels of surfactants impacted the solubility of drugs. Most studies concerned BS/PL levels, only few included monoglyceride and free fatty acid levels. Below, some of the findings regarding the impact of the different surfactants are outlined.

The solubility of danazol has been thoroughly studied in many different media with different levels of BS/PL and with or without lipolysis products. The solubilising effect on this compound is characteristic because the solubility increases linearly with the total molar concentration of surfactants in the medium, regardless of the relative content. This was demonstrated by Sunesen *et al*.[35] and recently by Kleberg *et al*.,[37] and the same behaviour has been shown for estradiol.[40] Comparing the solubility of danazol in HIF identified by Persson *et al*.[13] it could be deduced from those studies that the total amphiphile concentration (bile salt + phospholipid + free fatty acid + monoglyceride) should be 35–45 mM to match the solubility in HIF, regardless of the relative composition.[35,37] This concentration range exceeds that of FeSSIF and FeSSIF-V2, but is found in medium 2-D, 3-C, and 4-B. In an in-vitro–in-vivo correlation (IVIVC) study, however, the best IVIVC was obtained with a medium with an amphiphile concentration of almost 60 mM and consequently higher danazol solubility.[35] This example emphasises that solubility was one important parameter to approach, but not the only one determining the solubilisation, diffusivity and absorption in the gastrointestinal tract. In addition, a recent study comparing the solubility of five different compounds in human intestinal fluids at different time points after meal ingestion showed a large inter-individual variability and a great dependency on time.[39] This also adds to the complications faced when deciding the composition of biorelevant media.

Other compounds exhibited a quite different solubilisation pattern. The solubility of celecoxib was shown to be very sensitive to the level of phospholipid in a comparison of FaSSIF and FaSSIF-V2.[41] A high sensitivity to phospholipid concentration was also demonstrated for gemfibrozil, which was more sensitive to increased phospholipid levels than increased monoglyceride levels in terms of monoolein. The sensitivity of gemfibrozil solubility to increased bile salt levels was lower than for both phospholipid and lipolysis products.[42]

In another study, increasing BS/PL levels was shown to affect the solubility of seocalcitol more than increasing levels of lipolysis products, regardless of whether these were medium or long chained.[43] The opposite was observed to be the case for NNC-25-0926, a highly lipophilic (octanol – water partition coefficient, log P, 6.9) compound, where lipolysis products were shown to be more efficient in solubilising the compound than BS/PL.[44] None of these observations could be directly correlated to one physicochemical property of the drug compounds. Danazol and seocalcitol are both neutral compounds and have similar log P values (4.5 and 4.8, respectively), but they differ in their sensitivity to surfactant type.

However, as would be expected for lipophilic compounds like cinnarizine, fenofibrate and NNC-25-0926, lipolysis products have a major enhancing effect on solubility.

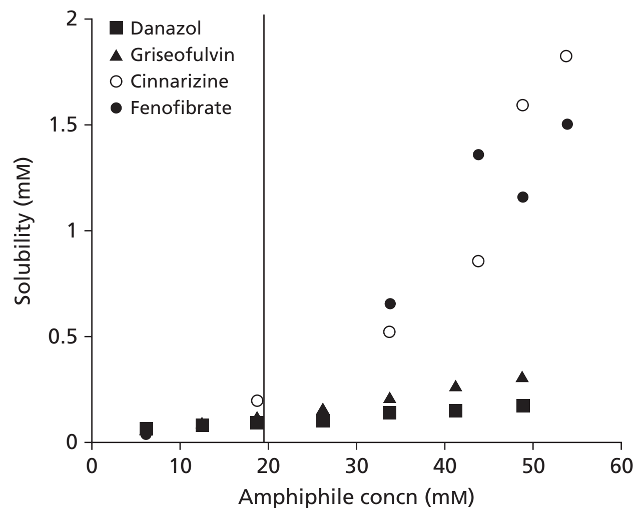

Figure 2 The impact of surfactants on the solubility on a selection of compounds. Vertical line indicates a change in surfactant type. Left side: increasing levels of bile salt/phospholipid media (1-0, 2-0, and 3-0). Right side: bile salt/phospholipid constant at 15 : 3.75 mM and increasing amounts of lipolysis products (oleic acid/monoolein 2/1).

Figure 2 illustrates how the solubility of some of the above mentioned drugs were affected by the type of surfactant used in the biorelevant media. Danazol was indifferent to the type of surfactant; the solubility of griseofulvin was slightly improved by addition of lipolysis products compared with BS/PL alone, and for fenofibrate and cinnarizine the addition of lipolysis products had a large impact on their solubilisation.

Biorelevant media for dissolution and in-vitro–in-vivo correlation testing

One major application of biorelevant media is in predictive dissolution methods for use in the development of optimal dosage forms. Three levels of IVIVC are defined. Level C describes a correlation between one time point on the dissolution curve and one pharmacokinetic parameter. Level B is a correlation between the mean dissolution time *in vitro* and either the mean residence time or mean dissolution time *in vivo*. Level A is a point-to-point correlation between the in-vitro dissolution profile and the pharmacokinetic profile of the drug.[45]

The use of biorelevant dissolution media renders the dissolution test closer to the in-vivo situation and therefore creates a basis for a better IVIVC. In agreement with this, the use of biorelevant dissolution media has proven to be a successful approach for many drug compounds.[35,41,44,46–48] Table 5 summarises the most recent dissolution studies where IVIVC level A or B has been established by use of biorelevant media.[35,41,44,46–48]

Many studies have used FaSSIF media to predict pharmacokinetic studies carried out in the fasted state. In a recent study using FaSSIF in both flow through (USP 4) and paddle dissolution (USP 2) equipment, a level A IVIVC was established for montelukast (a BCS class II compound) in the flow through equipment, whereas in the USP 2 incomplete

Table 5 Dissolution studies with biorelevant media where in-vitro–in-vivo correlation level A or B has been obtained

Compound	BCS class	Medium	Equipment	Data processing	IVIVC
Montelukast	II	USP buffer pH 6.8 Blank FaSSIF pH 6.5 **FaSSIF**	USP-2 **USP-4 pH gradient from 2.0–6.5–7.5–5.0**	Gastroplus Software using a P_{eff} estimated by ADMET Predictor software[47]	Level A
Glibenclamide	II	USP SIF pH 6.5 Blank FaSSIF pH 6.5 $FaSSIF_{HQ}$ **$FaSSIF_{LQ}$**	USP-2 **USP-2 pH gradient from 6.0–6.5–7.0–7.5–5.0**	Gastroplus software, using a P_{eff} estimated by Caco-2 studies[48]	Level B
Diclofenac sodium	II	USP buffer pH 6.8 **FaSSGF/FaSSIF-V2/ half FaSSIF-V2[a]/Blank FaSSIF pH 7.5 and SCoF**	USP-2 **USP-3 and USP-4 pH gradient from 1.6–6.5–7.0–7.5–5.8**	Deconvoluted plasma data (using WinNonlin Software)[46]	Level A
Celecoxib	II	SGF_{sp}/SIF_{sp} pH 6.8 FaSSGF/FaSSIF **FaSSGF/FaSSIF-V2**	**USP-2**	STELLA software with no permeability restrictions[41]	Level A
Danazol	II	**Modified Copenhagen media[b,c]**	**USP-4**	Deconvoluted plasma data (using GloboMax software)[35]	Level A
Danazol	II	Modified Copenhagen media[a]	**USP-4**	Deconvoluted plasma data (using GloboMax software)[35]	Level A
NNC-25-0926	IV	1-0, 3-A, **4-D**	**USP-2**	Deconvoluted plasma data (using GloboMax software)[44]	Level A
Diclofenac sodium	II	USP buffer pH 6.8 **FeSSGF/New FeSSIF[e]/half New FeSSIF[f]/Blank FeSSIF pH 7.5 and SCoF**	USP-2 **USP-3 and USP-4 pH gradient from 5.0–5.8–6.5–7.5–5.8**	Deconvoluted plasma data (using WinNonlin Software)[46]	Level A
Celecoxib	II	SGFsp/SIFsp pH 6.8 FeSSGF/FeSSIF **FeSSGF/FeSSIF-V2**	**USP-2**	STELLA software with no permeability restrictions[41]	Level A

The media and models providing the stated in-vitro–in-vivo correlation (IVIVC) are highlighted in bold type. [a]As FaSSIF-V2 but 1.5 mM, pH 7.0 and 270 mOsm/kg; [b]pH 6.8, bile salt 2.5 mM, phospholipid 0.5 mM; [c]pH 6.8, bile salt 6.3 mM, phospholipid 1.25 mM; [d]pH 5.5, bile salt 18.8 mM, phospholipid 3.75 mM, oleic acid 30 mM, monoolein 4 mM; [e]New FeSSIF: as FeSSIF-V2 but 7.5 mM bile salt; [f]As New FeSSIF but 3.0 mM bile salt, pH 6.5 and 270 mOsm/kg. BCS, Biopharmaceutics Classification System; FaSSGF, fasted-state simulated gastric fluid;[63] FaSSIF, fasted-state simulated intestinal fluid; FeSSGF, fed-state simulated gastric fluid;[30] FeSSIF, fed-state simulated intestinal fluid; OA/MO, oleic acid/monoolein; SCoF, simulated colon fluid.[64]

dissolution was observed in both compendial and biorelevant media.[47] This emphasises that the creation of sink conditions can be important for the achievement of IVIVC.

Using the flow through equipment, IVIVC level A was obtained for danazol in the modified fasted state Copenhagen media.[35] Demonstrating the important balance between flow and media composition in the USP 4, equally good IVIVC was obtained using low BS/PL levels combined with high flow rate (32 ml/min) and high BS/PL levels combined with a lower flow rate (8 ml/min).

Traditionally, dissolution data have been compared with the deconvoluted plasma profile of the drug in question and the IVIVC relation established only on this basis. New in-silico methods include software operating with more parameters, taking into account the physicochemical, permeability and ionisation properties, as well as gastric and intestinal volumes and transit times in the gastrointestinal tract. An important share of mathematical modelling is involved but such software has proven useful for the establishment of IVIVC in recent studies.

Comparing FaSSIF with FaSSIF containing low purity sodium taurocholate and phospholipid as dissolution media for glibenclamide (another BCS class II compound), IVIVC was obtained in USP 2 dissolution equipment, using a volume of 900 ml in the media with the low purity bile salt and phospholipid components. Those media were also the ones providing the highest solubility.[48] The dissolution data was combined with an effective permeability, obtained from Caco-2 permeability studies and used as input in the GastroPlus software to simulate the in-vivo plasma profile of the drug. In studies applying FaSSIF-V2, a level A IVIVC was obtained for modified release formulations of diclofenac and celecoxib.[41,46] The latter study also used in-silico modelling (STELLA software) to establish IVIVC in the media.

In the fed state IVIVC has been obtained by the addition of lipolysis products to the biorelevant media and by those means adjusting the properties of the media to fit the in-vivo profile of the drug. In the study by Sunesen *et al.*,[35] using USP 4, it was found that inclusion of free fatty acids and monoglycerides into the dissolution media was a prerequisite for obtaining IVIVC for danazol bioavailability in a fed state clinical study. In a dog study, the in-vivo performance of NNC 25-0926 in the fed state was better predicted by use of media 4-D compared with media 3-A.[44] Media 4-D contained higher levels of BS/PL, free fatty acid and monoglyceride, which reflected the high level of BS/PL in dogs and the fatty meal the dogs received.

Media 1-0, reflecting the fasted state, did not correlate well with the in-vivo performance.

Thus media modification has been shown to be another important way to simulate in-vivo drug dissolution in the fed state without involving in-silico modelling.[35,44]

Negative food effect on modified release tablets of diclofenac has been confirmed *in vitro* by use of modified versions of FeSSIF in USP 3 and USP 4. Different media were used in consecutive order, simulating different segments of the gastrointestinal tract, differentiated by pH and surfactant contents. USP 3 and USP 4 showed satisfactory IVIVC, established by Weibull parameters and numerical deconvolution.[46] Employing USP 2, a positive food effect of celecoxib modified release tablets was predicted by use of fasted state simulated gastric fluid (FeSSGF) and FeSSIF-V2 coupled with in-silico simulating technology (STELLA software). In this case, dissolution using FeSSGF and FeSSIF media alone could not predict the in-vivo situation, but the combination with simulation software enabled prediction.

In summary, more development work is needed to optimise the use of biorelevant media to ensure a fast and rational development of predictive in-vitro dissolution models.

Biorelevant media for studying drug transport

In-vitro models for intestinal drug absorption are usually based on cell cultures or excised tissue.[49]

Generally, in-vitro permeability models may be more susceptible to toxicity than in-vivo models due the inherent physiological discrepancies. The lack of some natural defence mechanisms can partly explain why e.g. bile salt and PC show a higher level of toxicity *in vitro*. This, to some extent makes it difficult to transfer in-vitro toxicity data to the in-vivo situation. The contact time between the enterocyte and noxious compound or metabolites may be prolonged *in vitro* as removal by the vascular blood and lymphatic system is not present. Lack of epithelial restitution by re-epithelialisation of villous tips after insult induced by digested food components happens within one hour postprandial *in vivo*.[50] Further, the Caco-2 cell culture model, which is used world-wide, lacks the protective mucus layer at the luminal cell surface.

Assessments of tissue compatibility with biorelevant media are summarised in Table 6.[6,51–57] The findings showed that Caco-2 cells were compatible with: firstly, fasted state biorelevant media containing up to 5 mM bile salts and up to 1.5 mM phospholipids (BS/PL ratio up to 4), pH in apical compartment ranging from 6.1 to 6.8; secondly, fed state biorelevant media without lipolysis products containing 15 mM bile salts and 3.75 or 7.5 mM phospholipid (BS/PL ratio 2–4), pH in apical compartment ranging from 5.0 to 6.5, where the majority of studies employed pH 6 or higher; and thirdly, fed state biorelevant media with lipolysis products containing 5 mM bile salt, 1.25 mM phospholipid (BS/PL ratio 4) and free fatty acids 0.5 mM, monoglycerides 0.25 mM (i.e. FFA/MG ratio 2), pH in apical compartment 6.1. However, excised rat ileum was not compatible with fasted state biorelevant media containing bile salt and phospholipid, 3 and 0.75 mM, respectively, nor fed state media containing 15 mM bile salts and 7.5 mM phospholipid.[56]

It is generally believed that the monomeric form of the drug compound is taken up by the enterocyte and that the colloids act as cargos facilitating diffusion of solubilised poorly soluble drugs from the lumen, across the mucus layer, to the enterocyte. Poorly soluble drugs may have different affinities to bile salt micelles, mixed micelles and aggregates, respectively; hence, different release rates of the drugs from the various colloids may be expected. Further, co-administration of lipids and solubilising excipients may enhance intraluminal drug concentration. The excipients may influence the absorption process by modulating membrane fluidisation and/or activity of membrane transport carriers and reducing free drug concentration by micellar encapsulation.

The amount of monomeric drug available for penetration into the enterocyte depends on the partitioning between drug solubilised in colloids and drug present as monomers. Accordingly, the chosen medium for in-vitro permeability studies will have a great impact on the acquired permeability. Conventional transport media used for in-vitro permeability studies are plain buffered saline solutions, supplemented with glucose or nutritional cell culture media, and with a fixed pH of 7.4. These conventional media have some limitations regarding permeability studies of poorly soluble drug compounds, as the media do not simulate the pre- and postprandial intestinal composition. This limits drug solubility and aggravates the risk of adsorption of lipophilic drugs to plastic device surfaces. Also, conventional media do not consider the drug partition in colloids, which plays an essential role for the absorption mechanism of such compounds.[3,4]

Table 7 summarises in-vitro permeability studies of poorly soluble drugs and potency of excipients using tissue compatible media as transport media.[6,51,53,54,56,58–60] A comparison of biorelevant media with conventional transport media showed a higher solubility of poorly soluble drugs in the biorelevant media, and either a decreased or increased flux/permeability using biorelevant media. Composition of the buffer used in the biorelevant media may need careful consideration, as the chosen pH of the media could influence the predictability of permeability of ionisable drugs and the concentration of inorganic ions could affect the stability of a prodrug.[52,61] A parabolic or sigmoid relation was obtained between in-vivo fraction absorbed in humans covering the whole range from low to high fraction absorbed and in-vitro permeability using Hank's balanced salt solution (HBSS), fasted state media or fed state media.[52,58,59] The use of HBSS and a fasted state biorelevant medium did not influence the overall predictability of a number of BCS class I–IV drugs, thus impact was shown on the permeability of effluxed drugs, solubility and permeability of low aqueous soluble drug, and improved recovery of lipophilic drugs (i.e. less adsorption to device).[58,59] Adsorption could be further defeated by adding 1% bovine serum albumin to the receptor compartment.[58] Use of fasted state biorelevant media has been shown to impact the effect of excipients; micelle encapsulation was proposed as causing a decreased potency of some absorption enhancers, whereas other enhancers showed either no impact or improved enhancement of paracellular transport.[60] Applying fasted state biorelevant media in a combined dissolution/Caco-2-permeation model showed in-vitro–in-vivo correlation for performance of six solid formulations containing a poorly soluble drug. Similar formulations were dosed orally to rats.[62]

Table 6 Overview of composition and tissue compatibility of biorelevant media

Model (number of replicates)	Control	Biorelevant media							
		Total BS (mM)	Total PL (mM)	Ratio (BS/PL)	Total MG (mM)	Total FFA (mM)	Ratio (FFA/MG)	pH (A/B)	Osmolality (mOsm/kg)
Fasted state									
Caco-2 (3–24)[54]	Leibovitz L-15[a]	**5**, 10, or 15	**1.25**, 2.5, or 3.75	4	0	0	0	6.1 ± 0.1/7.4	364–380
Caco-2 (4–18)[56]	HBSS	3	**0.75**	4	0	0	0	6.5/7.3	343
Caco-2 (8)[6]	HBSS	**1.5**	**0.375**	4	0	0	0	6.5/7.4	
Caco-2 (3–7)[55]	HBSS	8	0	0	0	0.5, 1	0	–/7.35	–
		10	0	0	0	1	0	–/7.35	
					0		0	–/7.35	
					0		0	–/7.35	
		10	0	0		0.5, 1			
		20	0	0		**1**			
Caco-2 (3)[53]	HBSS	**3**	**1.5**	2	0	0	0	6.5/-	–
Caco-2 (3–5)[57]	DPBS	10	0.5	20	0	0	0	–/7.4	
Caco-2 (<3)[52]	Phosphate in salt solution	**3**	**0.75**	4	0	0	0	6.5/7.4	270 ± 10
	HBSS	**3**	**0.75**	4	0	0	0	6.5/7.4	312 ± 0.6
Caco-2 (3)[51]	HBSS	**5**	**1.5**	3.3	0	0	0	6.8/7.4	
Ussing, rat ileum (5)[56]	KBR	3	0.75	4	0	0	0	6.5/7.4	343
Fed state									
Caco-2 (3–24)[54]	Leibovitz L-15[a]	**5**, 10, or 15	**1.25**, 2.5, or 3.75	4	**0.25**	**0.5**	2	6.1 ± 0.1/7.4	364–380
Caco-2 (4–18)[56]	HBSS	**15**	**7.5**	2	0	0	0	6.0/7.3	336
Caco-2 (3)[53]	HBSS	**15**	**7.5**	2	0	0	0	6.0/-	–
Caco-2 (3–5)[57]	DPBS	10	0		0	5 or 10% (w/v)		–/7.4	–
Caco-2 (<3)[52]	Acetic acid and salt solution	15	3.75	4	0	0	0	5.0/7.4	635 ± 10
	HBSS	**15**	**3.75**	4	0	0	0	5.0/7.4, 6.5/7.4	327 ± 1 326 ± 1
Caco-2 (3)[51]	HBSS	15	3.75	4	0	0	0	5.0/7.4	600
Ussing rat ileum (5)[56]	KBR	15	7.5	2	0	0	0	6.0/7.4	336

Biorelevant media contained bile salts, lipids and lipolysis products in control media. Concentrations highlighted in bold type were considered tissue compatible compared with control. [a]CO2-independent nutritional cell culture medium. –, Not stated; A, apical; B, basolateral; BS, bile salts; DPBS, Dulbecco's modified phosphate buffer; FFA, free fatty acids; HBSS, Hank's balanced salt solution; KBR, Krebs bicarbonate Ringer solution; MG, monoglyceride; PL, phospholipid. Data represent mean ± SD.

Tissue compatible biorelevant media allows work with physiological relevant drug concentrations in the presence of intestinal colloids acting as cargos and depots, thereby improving permeability prediction, as it is influenced by drug solubility, drug partition in colloids and the effects of components present in the media on (efflux)-transporters. Otherwise solubility may be underestimated and permeability may be either over- or underestimated.

Conclusions

When developing biorelevant media, many parameters should be considered to simulate the composition of the intestinal fluids. As appears from the data on human intestinal fluids in Table 1, there is a significant degree of variation in composition, which should be taken into account when these data are used as a starting point for the composition of biorelevant media for use as a tool in in-vitro studies.

Taking into consideration the variability of the data presented in Table 1, we argue that it is not reasonable to assume that one medium can model human intestinal fluid to an extent that makes it suitable for all purposes. We recommend that the effect of the individual component on solubility, dissolution, and transport are investigated, so that media can be composed to fit both the compound and the purpose in question.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Table 7 Overview of in-vitro models using biorelevant transport media to study permeability of poorly soluble compounds

Model (number of replicates)	Control[a]	Medium composition (mM)	Drug or excipient	Solubility enhancement	Permeability (% of control)[b]
Fasted state					
Caco-2 (11–21)[54]	Leibovitz's L-15	TC/PC or TC/lyso-PC (5/1.25)	Estradiol	Increased (2-fold)	Decreased (–32)
Caco-2 (4-18)[56]	HBSS	TC/PC (3/0.75)	Metoprolol	–	Decreased (–33)
Caco-2 (3)[6]	HBSS	TC/PC (1.5/0.375)	Amprenavir	–	Increased (60)
		Human intestinal fluid Bile salt/lipids (1.75/0.05)		Increased (above 25-fold)	Increased (62)
Caco-2 (3)[53]	HBSS	TC/PC (3/1.5)	*p*-Hydroxybenzoate esters	Increased	Increased (–)
Caco-2 (3)[51]	HBSS	TC/PC (5/1.5)	Theophylline	–	Unchanged
Caco-2 (3)[59]	HBSS[c]	TC/PC (3/0.75)[c]	19 drugs	Increased	Unchanged or decreased
Caco-2 (3)[60]	HBSS + 1% FaSSIF	TC/PC (3/0.75)	5 absorption enhancers	–	Potency increased, unchanged, or decreased
Caco-2 (2)[58]	HBSS	TC/PC (0.3/0.075)	35 drugs	–	Decreased
Fed state					
Caco-2 (11–21)[54]	Leibovitz's L-15	TC/PC or TC/lyso-PC (5/1.25) + OA/MO (0.5/0.25)	Estradiol	2	Decreased (–32)
Caco-2 (4–18)[56]	HBSS	TC/PC (15/0.75)	Metoprolol	–	Decreased (–75)
Caco-2 (3)[53]	HBSS	TC/PC (15/7.5)	*p*-Hydroxybenzoate esters	Increased	Decreased (–)

[a]See footnote to Table 6. [b]Negative value indicates decreased permeability. [c]Supplemented with 1% dimethyl sulfoxide. –, Not stated; HBSS, Hank's balanced salt solution; MO; monoolein; OA, oleic acid; PC, phosphatidylcholine; TC, Na-taurocholate.

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