



Application of simulated intestinal fluid on the phospholipid vesicle-based drug permeation assay

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ABSTRACT

The compatibility of fasted state simulated intestinal fluid (FaSSIF) in drug permeation studies employing the phospholipid vesicle-based permeation assay (PVPA) model was confirmed by a set of different integrity indicators. Neither calcein permeability nor electrical resistance were found significantly changed indicating unaffected barrier tightness. Furthermore, the release of phospholipid from the barriers in contact with FaSSIF was negligible, although sodium taurocholate disappeared from the donor – possibly due to transfer into the barrier. Visual examination of the barrier structure by confocal laser scanning microscopy (CLSM) revealed no changes. The model drugs, cimetidine, nadolol, ketoprofen and griseofulvin showed either slightly enhanced or unchanged permeability values in the presence of FaSSIF. This may be attributed to micellar encapsulation and/or slight changes in barrier characteristics. Particularly for poorly soluble drugs, FaSSIF appeared favourable in terms of markedly improved recovery. Moreover, utilisation of BSA in the receiver compartment seems to augment this beneficial effect on recovery rate. It is likely that this experimental set-up affords better sink conditions in the receiver phase, which results in higher fluxes. Overall, a combination of FaSSIF in the donor phase and BSA in the receiver phase facilitates improved experimental output.

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1. Introduction

Prediction of the absorptive properties of new drug entities has in recent years become a key component in the industrial drug development process. Modern drug discovery strategies provide a steadily increasing number of potential drug candidates boosting the development of high through-put screening tools for oral bioavailability prediction. Among these, non-cellular models based upon artificial membranes are especially valuable due to their good reproducibility and comparably low cost (e.g., PAMPA, PVPA) (Flaten et al., 2006b; Kansy et al., 1998). The phospholipid vesicle-based permeation assay (PVPA) developed for the screening of passive permeability, consists of a tight barrier of liposomes on a filter support, packed by use of centrifugation (Flaten et al., 2006a,b). It generates permeability data which correlate well with the fraction absorbed in humans following oral administration (Flaten et al., 2006a). More recently, it has been demonstrated to be compatible with a variety of co-solvents and surfactants, as well as at a broad range of pH values (Flaten et al., 2007, 2008).

At the same time, the occurrence of increasingly lipophilic and less water soluble drugs requested the development of alternative dissolution media. Various additives have been suggested as they facilitate the wetting of solids and the solubilisation of lipophilic drugs into micelles (Kimura et al., 1972; Kleberg et al., 2010b; Lehto et al., 2011; Persson et al., 2005; Schwebel et al., 2011). A general expectation is that such media mimic the *in vivo* dissolution process more closely than simple buffers. Among the simulated intestinal fluids (Galia et al., 1998; Kleberg et al., 2010a,b), the most widely used is the fasted state simulated intestinal fluid (FaSSIF) developed by Dressman and co-workers (Galia et al., 1998). Encompassing both bile salt and lipid components, and buffered to a pH of between 6.5 and 6.8, it closely resembles intestinal fluid in its fasted state. While originally applied as dissolution medium to simulate the *in vivo* dissolution behaviour of compounds, it has more recently been employed as transport medium in permeability investigations. Indeed, cumulative evidence indicates good applicability of FaSSIF with the Caco-2 model (Fossati et al., 2008; Ingels et al., 2002, 2004; Patel et al., 2006). Initial work by Ingels et al. (2002) illustrated that incubation of Caco-2 monolayers with FaSSIF did not adversely affect cell viability or barrier integrity. However, the influence of FaSSIF on drug permeability is controversial and widely discussed. Ingels et al. (2004) described that FaSSIF appeared to

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facilitate solubilisation and concomitantly improved flux of poorly water soluble drugs in suspension. However, it has been suggested that FaSSIF may affect active efflux mechanisms (i.e., Pgp). Studies revealed a Pgp inhibitory effect by sodium taurocholate, a bile salt component of FaSSIF (Brouwers et al., 2006; Ingels et al., 2002, 2004). On the other hand, more recent work by Fossati et al. (2008) found permeability to be unchanged or depressed.

In this study we sought to investigate the applicability of FaSSIF as a transport medium for use in the PVPA *in vitro* model. To our knowledge, this is the first study into the effect of FaSSIF on an *in vitro* permeability model in which the underlying transport mechanisms are exclusively passive. Integrity of the PVPA barrier in the presence of FaSSIF was evaluated by means of electrical resistance (ER) and calcein permeability, a hydrophilic marker. The release of phospholipid from the barriers was examined, and furthermore the barriers were visualised using confocal laser-scanning microscopy (CLSM). Notably, the bile salt taurocholate, which is a key component of FaSSIF, is known to interact with phospholipid bilayers (Nichols, 1986; Schubert et al., 1983). Indeed, numerous studies have explored this effect and the resultant alterations in structure (Schubert et al., 1986; Schubert and Schmidt, 1988). Thus, we also assessed potential binding of FaSSIF with the barrier's phospholipid bilayers by measuring the loss of sodium taurocholate from the donor phase. Collectively, our results indicate that FaSSIF displays good compatibility with the PVPA *in vitro* model. In light of this, we examined both the recovery rate and the permeability of four drug compounds (cimetidine, ketoprofen, nadolol, and griseofulvin) of varying physicochemical properties in the presence of FaSSIF. Finally, the effect of bovine serum albumin (BSA) in the receiver compartment on the recovery and permeability of the poorly soluble compound griseofulvin was explored.

2. Materials and methods

2.1. Materials

Egg phosphatidyl choline, Lipoid E-80, was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Clear Millicell® cell culture plates (24 well) and mixed cellulose ester filters (0.65 μ m pores) were purchased from Millipore (Copenhagen, Denmark). Costar transparent 96-well plates were purchased from Corning (Corning, NY, USA). FaSSIF was prepared by using SIF® instant powder (Phares, Muttenz, Switzerland) according to the instructions of the manufacturer, as well as FaSSIF buffer (FaB) (pH 6.5). The phosphate buffer (PBS) used in liposome preparation and in the receiver compartments in permeation experiments contained KH₂PO₄ 0.60 g, Na₂HPO₄ \times 12H₂O 6.40 g, NaCl 7.42 g (1000 ml) and was adjusted to pH 7.4. Calcein, cimetidine, ketoprofen, nadolol, griseofulvin, sodium taurocholate, bovine serum albumin (fraction V) (BSA) and all other chemicals were purchased from Sigma–Aldrich (Copenhagen, Denmark).

2.2. Preparation of phospholipid vesicle-based barriers

Phospholipid vesicle-based barriers were prepared as described previously (Flaten et al., 2006b). In brief, mixed cellulose ester filters (Millipore) were sealed by heat (150 °C, 30 s) on clear Millicell® plates (Millipore) using a custom-made sealing machine (IBR-Ingenieurbüro, Waldkirch, Germany). Liposomes were made of egg phosphatidylcholine Lipoid E-80 by film hydration with PBS and subsequent extrusion through polycarbonate filters (0.4 and 0.8 μ m). The liposomes were spun down successively on the filter inserts: first the smaller liposomes to allow them to enter the pores of the filter support and then the larger ones to layer

Table 1
Physico-chemical parameters for the model compounds.

Compound	MW ^a	pK _a ^b	log P ^b	Aqueous solubility
Cimetidine	252.34	6.80	0.40	5.00 ^c
Nadolol	309.40	9.39	0.71	8.33 ^c
Ketoprofen	254.28	4.60	3.12	0.051 ^c
Griseofulvin	352.77	–	2.18	0.030 ^d

^a Molecular weight in g/mol.

^b From Hansch et al. (1990).

^c From Thomas et al. (2008) in mg/ml (unbuffered).

^d From Mithani et al. (1996) in mg/ml.

on top. The inserts were frozen at –80 °C and thawed at 65 °C resulting in fusion of the liposomes so that tight barriers were obtained.

2.3. Preparation of sample solutions

All drug solutions were prepared in FaB and FaSSIF and were adjusted to pH 6.5. The griseofulvin solution was prepared by dilution of a DMSO stock solution with FaB or FaSSIF, where DMSO did not exceed 0.5%. The concentration of the hydrophilic marker calcein was 10 mM, while the concentrations of the model drugs used were 4.6 mM (ketoprofen), 8 mM (nadolol), 8 mM (cimetidine) and 0.1 mM (griseofulvin). The calcein concentration of 10 mM is appropriate to provide reliable permeability data (Flaten et al., 2008). The concentrations of the model drugs were chosen to be well below the saturation limits (at pH 6.5) and at the same time to yield reliably detectable receiver concentrations during permeation studies. For additional information regarding the model drugs, an overview of their physicochemical properties is provided in Table 1.

2.4. Confocal laser-scanning microscopy (CLSM)

The liposome barriers were prepared as described above, with the exception that 0.2 mol% of the egg phospholipid was displaced by the fluorophore Lissamine™ rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Rh-DHPE) (Kirjavainen et al., 1996). The incubations were performed as described above. Using a scalpel, the barriers and filters were gently removed from the inserts and placed on a microscope slide. The fluorophores were stabilised by the addition of Vectashield mounting media (Vector Laboratories, Peterborough, United Kingdom) and the barrier was covered with a cover slip. The sample was examined under a confocal laser-scanning microscope (CLSM; Zeiss LSM 510, Göttingen, Germany). Rh-DHPE was excited by use of a laser with a wavelength of 543 nm.

2.5. Phospholipid release assay

Phospholipids B – enzymatic colorimetric method was purchased from MTI Diagnostics (Idstein, Germany). The assay was performed using a modified setup that enables utilisation of 96-well plates (Grohganz et al., 2003). Briefly, the colouring reagent solution was prepared by adding 20 mL of buffer solution (containing 5 mM Tris buffer, 0.34 mM calcium chloride, and 20 mM phenol) to the dry colouring reagent (phospholipase D 0.44 U/ml, choline oxidase 2 U/ml, peroxidase 5.3 U/ml, 4-aminophenol 0.74 mM). Following 4 h incubation of the phospholipid vesicle-based barriers (donor phase) with FaB, FaSSIF, and Triton X-100 0.5% solution (positive control), samples were taken and diluted with Triton X-100 solution so that a final concentration of 1% was achieved. Then, 50 μ L of each sample was transferred into individual wells of a 96-well plate to which 250 μ L of colouring reagent was added. Standards were prepared by dissolving E-80 in PBS containing 1% Triton X-100. The 96-well plates were warmed to 37 °C and initially

shaken for 5 min. A blank consisting of FaSSIF alone was also used and subsequently subtracted from the absorbance values acquired for the FaSSIF samples. After 45 min incubation and completion of the reaction, quantification was performed by measuring the absorbance at $\lambda = 492$ nm. All samples were prepared and measured in triplicate.

2.6. Loss of sodium taurocholate

In order to assess any potential interaction of sodium taurocholate in the FaSSIF with the phospholipid vesicle-based barriers, the loss of sodium taurocholate in the donor phase was measured while the barriers were incubated with FaSSIF. In addition, a blank consisting of FaSSIF alone on a filter was carried out in a similar manner to that of the barrier samples. Samples from the donor phase were taken at different time points (1–4 h) and analysed by HPLC. The loss of sodium taurocholate in % (Loss TC) was then calculated according to the following equation: $\text{Loss TC} (\%) = (1 - (\text{Sample}/(\text{FaSSIF} - \text{Blank}))) \times 100$, where *Sample* is the concentration of the donor samples after incubation, *FaSSIF* is the concentration of sodium taurocholate in the pure FaSSIF, and *Blank* is the concentration of sodium taurocholate in the blank sample.

2.7. Permeation experiments

Before the permeation experiments, the phospholipid vesicle-based barriers were incubated with FaB for 1 h. The buffer in the donor compartment was then removed and replaced by sample solution. The receiver compartment contained PBS during the incubation and throughout the course of the permeation experiment. Over a period of 4 h, the inserts were moved to fresh wells containing PBS at pH 7.4 at certain time intervals to ensure sink conditions. The amount of drug in the receptor phase was then quantified (for details see Section 2.9). The cumulative amount of drug that had permeated through the barrier was plotted against the time giving the cumulative flux. When the flux reached steady state, meaning the slope was linear, the apparent permeability coefficient (P_{app}) was calculated according to the following equation: $P_{app} = dm/dt \times (1/A \times C_0)$, where *dm* is the cumulative amount of drug permeated by the time *dt*, *A* is the area of the insert used and *C₀* is the initial donor concentration. Steady state conditions of the fluxes ($r^2 \geq 0.99$) were achieved after 1.75 h. For calculation of the P_{app} , 4 points of the linear part of the flux curve were used in the case of cimetidine, nadolol, and griseofulvin and 5 for ketoprofen.

In the case of griseofulvin, additional permeation experiments were also performed in the presence of BSA (4%) in the receiver phase.

For all model compounds the mass balance i.e., the quantitative recovery upon completion of the experiment was calculated by dividing the amount found after the permeation experiment in the donor as well as receiver chamber by the initial input of compound in the donor compartment. The recovery rate was then expressed as percentage.

2.8. Electrical resistance measurements

The electrical resistance was measured after each permeation experiment using a Millicell-ERS (Millipore). The blank resistance (i.e., resistance of the pure filter hydrated in PBS) was subtracted from the total resistance to obtain the actual resistance of the barriers. Finally, these resistance values multiplied by the area of an insert (0.6 cm^2) gave the final electrical resistance (ER) in $\Omega \text{ cm}^2$.

Upon completion of the drug permeability experiments, it was not necessary to exclude any of the inserts for permeability evaluation since all exhibited satisfactory ER-values.

Table 2
HPLC parameters for the model compounds and sodium taurocholate.

Compound	Chromatographic conditions	Flow (ml/min)	λ (nm)
Cimetidine	Isocratic 80/20 Water ^a /methanol	1.0	229
Nadolol	0–6 min gradient 95/5 to 75/25 Water/acetonitrile ^a , thereafter isocratic	1.0	260
Ketoprofen	Isocratic 20/80 Water/methanol ^b	0.8	262
Griseofulvin	Isocratic 55/45 Water/acetonitrile ^b	1.0	291
Taurocholate	Isocratic 25/75 Water/methanol ^b	0.7	200

^a Mobile phase contained 0.05% triethylamine, adjusted to pH 3 with phosphoric acid.

^b Mobile phases contained 0.1% formic acid.

2.9. Analysis

Calcein was analysed by fluorescence spectroscopy using a Fluostar Omega, BMG Labtech GmbH, Offenburg, Germany (excitation 485 nm, emission 520 nm). The model compounds as well as taurocholate were analysed using a Waters 2695 HPLC with UV detection (Waters 2487 Dual λ Absorbance Detector). Separation was performed by an Acclaim 120 (C18, 5 μm particle size, 120 \AA , 4.6 mm \times 250 mm) column. Run times, mobile phases (isocratic in the case of ketoprofen, cimetidine, griseofulvin and taurocholate, and with gradient in the case of nadolol), flow and wavelengths are listed in Table 2. The lowest standard concentrations used for each substance were 1.2 μM (ketoprofen), 2.0 μM (nadolol), 0.8 μM (cimetidine), 0.05 μM (griseofulvin) and 5 μM (taurocholate), which in all cases were far above the quantification limits. All samples ranged well within the constraints of the lowest and highest standard, respectively. The standard curves yielded linear fits ($r^2 \geq 0.999$). The software used was Chromeleon 6.80 (Dionex, Hvidovre, Denmark).

The BSA containing samples were cleaned from BSA by adding two volumes of acetonitrile to one volume of sample as described by Hubatsch et al. (2007). As suggested, the samples were then vortexed and centrifuged (10 min, 15,000 $\times g$). The supernatant was immediately withdrawn and analysed as per the pure PBS samples.

2.10. Statistical analysis

For statistical analysis, Origin 6.0 (OriginLab Corporation, Northampton, MA, USA) was employed. A one-way analysis of variance (ANOVA) was performed where $p < 0.05$ was considered as significant.

3. Results and discussion

3.1. Integrity of the phospholipid vesicle-based permeation barriers in the presence of FaSSIF

3.1.1. Electrical resistance and calcein permeability

In order to evaluate whether or not FaSSIF affected barrier function of the PVPA model, two independent assessments of integrity were performed; measurement of electrical resistance (ER) and permeability of calcein, a hydrophilic model compound which exhibits relatively low permeability across lipid bilayers. In tandem, this approach has previously been shown to confirm the compatibility of the model in the presence of different co-solvents and surfactants (Fischer et al., 2011; Flaten et al., 2008; Kanzer et al., 2010). While a decrease in ER may depend on a variety of factors, including compromised barrier integrity, any sudden increase in

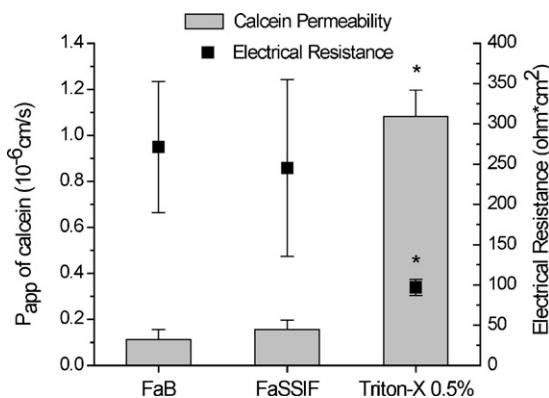


Fig. 1. Integrity of the PVPA model in the presence of FaB and FaSSIF in comparison with Triton X-100 0.5% (positive control): calcein permeability (bars) and ER (■). Values are given as mean \pm SD, $n = 12$. Significant differences are denoted by $^*p < 0.05$.

calcein permeability is assumed to exclusively reflect leakage of the barrier. In addition, the presence of barrier phospholipids in the donor phase, as a sign of partial dissolution of the barrier, has been shown to correlate with both diminished ER and increased calcein permeability (Fischer et al., 2011; Flaten et al., 2008).

ER and calcein permeability were measured in the presence of FaB and FaSSIF following incubation for 4 h (Fig. 1). In the case of FaSSIF, values for ER and permeability were slightly lower and higher, respectively, when compared to FaB, but the differences were not significant. Noteworthy, ER- and calcein permeability-values are somewhat different from earlier published data for the PVPA model (Flaten et al., 2006b, 2008). Most likely this can be ascribed to the fact that the filter area as well as the donor volume here are bigger than in the previously employed set-up. We have chosen larger inserts because the increased filter area results in higher flux per time and thus facilitates improved detection of drug(s), especially those which are poorly soluble. Overall, in our experimental set-up, it could be concluded that in terms of calcein permeability and ER, the tightness of the PVPA barriers in the presence of FaSSIF appeared to be maintained.

3.1.2. Barrier structure

To further confirm compatibility of the PVPA model with FaSSIF, we visualised the fluorescently labelled liposome barrier by CLSM, both prior to and after exposure to FaSSIF. Although not capable of identifying nano- and micro-size domains of lipid structure, it facilitated visualisation of potential defects or holes in the barrier. Rhodamine ethanamine is widely used in studies of membrane fusion, ion permeation and lipid transfer between vesicles. Importantly, it is not dependent on pH changes or molecular aggregation

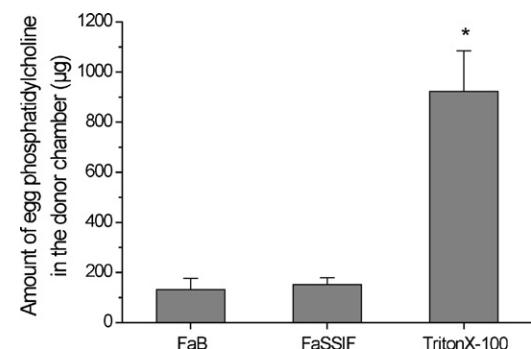


Fig. 3. Release of phosphatidylcholine from the PVPA barrier following incubation with FaB and FaSSIF in comparison to TritonX-100 0.5% (positive control). Values are given as mean \pm SD, $n = 9$. Significant differences are denoted by $^*p < 0.05$.

in the concentration used in this study (Massari et al., 1988). The results are depicted in Fig. 2. Z-stack images show that the barrier is composed of lipid-filled filter-pore structures with an additional layer of liposomes atop the filter. Crucially, in neither barriers incubated with buffer, nor those incubated with FaSSIF, were any larger aqueous channels penetrating through the filter observed. This further supports our assertion that FaSSIF elicits no obvious defects in phospholipid structure of the barrier or that it induces partial solubilisation of the barrier.

3.1.3. Release of phospholipid from the barriers

The aforementioned integrity studies, whereby calcein permeability and ER measurements were employed, showed compatibility with the model, although the variance was somewhat high. To further assess barrier integrity we quantified the release of phospholipid from the barriers using an enzymatic assay. An enhanced release of phospholipid from the barriers would be indicative of (partial) barrier disintegration. After 4 h of incubation with FaB, FaSSIF, and Triton X-100 0.5% (positive control), the amount of phospholipid measured in the donor phase was similar for both FaB and FaSSIF, while the positive control showed hugely elevated levels of PC in the donor (Fig. 3). Thus, in the presence of FaSSIF, the barriers released approximately the same amount of phospholipid as that observed following incubation with FaB. This finding complements our other observations regarding barrier integrity in the presence of FaSSIF, and verifies that the PVPA barriers remain intact and are not dissolved in contact with FaSSIF. It is worth mentioning that despite its aggressive surfactant nature, sodium taurocholate in the given combination with phosphatidylcholine (i.e., FaSSIF), appears to be suitably applicable in the PVPA model. This is in contrast to a number of other surfactants previously tested (Fischer et al., 2011).

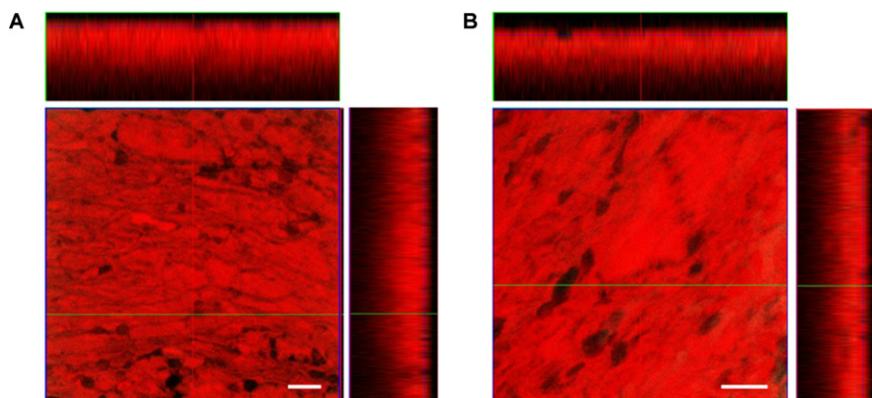


Fig. 2. CLSM Z-stack images of the PVPA barrier following incubation with phosphate buffer (PBS) (a) and FaSSIF (b). Scale bars are 20 μm .

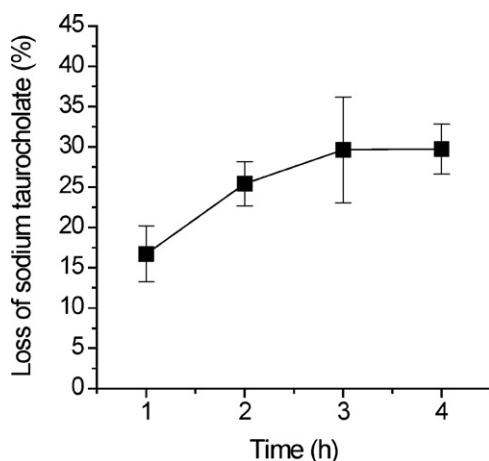


Fig. 4. Loss of sodium taurocholate in the donor phase versus time during incubation with FaSSIF. Values are given as mean \pm SD, $n=9$.

3.1.4. Loss of sodium taurocholate from FaSSIF

The composition of FaSSIF as used here, is derived from Galia et al. (1998), which specifies the content of sodium taurocholate (TC) and phosphatidylcholine (PC) in FaSSIF to be 3 mM and 0.75 mM, respectively. FaSSIF has been described to contain mixed TC/PC micelles (Galia et al., 1998; Schubert et al., 2011). The influence of bile salts on phospholipid bilayers or liposomes has earlier been investigated (Nichols, 1986; Schubert et al., 1983). Bile salts, at increasing mixing ratios, initially form pores within bilayers, then sequester smaller vesicles and finally convert bilayers into mixed micelles (Schubert et al., 1986; Schubert and Schmidt, 1988). Hence, it could be expected that the sodium taurocholate may interact in some way with the phospholipid bilayer structure of the PVPA barriers during permeation studies. Therefore, we have investigated the content of sodium taurocholate in the donor phase, while incubated with the barriers. Over a period of 4 h, the amount of sodium taurocholate in the donor compartment appeared to decrease, levelling off at a loss of $\sim 30\%$ after 4 h (following subtraction of the blank). The blank measurement confirmed that only negligible amounts of taurocholate in the FaSSIF ($\sim 2\text{--}4\%$) adsorbed to plastic surfaces. Thus, the loss of $\sim 30\%$ after 4 h indicates that TC is to a large extent bound to or incorporated within the PVPA barriers (Fig. 4). Evidently, there occurs a net transfer of taurocholate from the donor to the barrier, while the PC concentration in the donor stays constant. Despite this selective accumulation of taurocholate in the PVPA-barrier, there were only minor changes seen in barrier tightness, in terms of ER-decrease and calcein permeability-increase. From the experimental data, it cannot be completely ruled out whether the observed minimal increase in calcein permeability is connected with a formation of aqueous pores spanning the barrier. This is unlikely, however, since leakage or solubilisation of unilamellar vesicles has been described to occur first from a TC/lipid ratio of 0.15 or 0.3, respectively, onwards (Schubert et al., 1986). In the present study, a TC/lipid ratio of 0.05 is estimated under the assumption that accumulated TC is evenly distributed within the barrier and the barrier contains 10 mg PC/cm² (Flaten et al., 2006a). This correlates with earlier studies indicating that permeability of small hydrophilic molecules is first increased when TC-binding to PC-bilayers approaches solubilising concentrations (Albalak et al., 1996).

3.2. Permeation of model drugs in the presence of FaSSIF

The PVPA-permeability of four model compounds was determined using FaSSIF in the donor compartment as compared to FaB. The results are summarised in Figs. 5 and 6. For cimetidine and

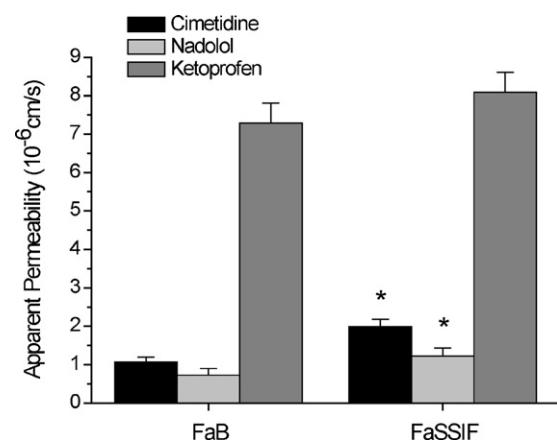


Fig. 5. Apparent permeability (P_{app}) of model drug compounds in FaB, and FaSSIF. Values are given as mean \pm SD, $n=12$ (cimetidine and nadolol), $n=16$ (ketoprofen). Significant differences are denoted by $*p < 0.05$.

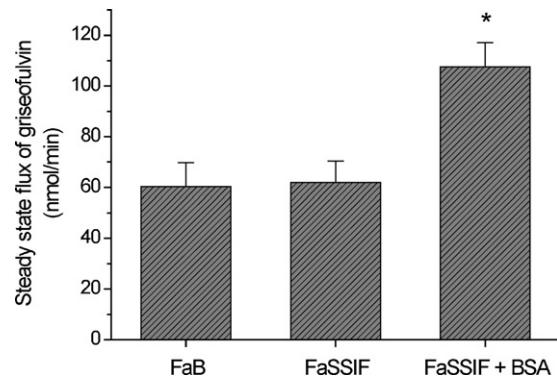


Fig. 6. Apparent steady state fluxes of griseofulvin in FaB and FaSSIF as well as FaSSIF in the donor and BSA (4%, w/v) in the receiver phase. Values are given as mean \pm SD, $n=12$. Significant differences are denoted by $*p < 0.05$.

nadolol, the apparent permeability was found to be augmented in FaSSIF as compared to buffer. A somewhat increased permeability was also observed in the case of ketoprofen, albeit not reaching significance. For the fourth model compound, griseofulvin, we have chosen to present flux data instead of P_{app} -values (Fig. 6). This is due to the poor recovery of the compound (see mass balance discussion in Section 3.3). The flux of griseofulvin was found unchanged in the presence of FaSSIF when compared to blank buffer solution (i.e., FaB). To our knowledge, this study is the first report on the influence of FaSSIF on permeation models, which exclusively describe passive permeation. In various Caco-2 permeability studies, the presence of FaSSIF in the apical compartment either reduced the permeability of passively transported drugs or showed no effect (Fossati et al., 2008; Ingels et al., 2002; Lind et al., 2007; Patel et al., 2006). Only a few reports have found an increase in permeability of model compounds in the presence of FaSSIF (Lakeram et al., 2008). Brouwers et al. (2006) and Ingels et al. (2004) postulated that the increase of drug permeability across Caco-2 monolayers may be a result of Pgp inhibition induced by taurocholate. Yet, in our case, a Pgp-influence can be excluded.

In a recent study with the PVPA model (Fischer et al., 2011), we have demonstrated that the passive permeability of ketoprofen and nadolol in the presence of poloxamer 188 is depressed in a surfactant concentration-dependent manner. We concluded that micelle-associated drug remains inaccessible for permeation. More recently, Miller et al. (2011) have postulated a solubility-permeability interplay in conjunction with micellar solubilisation. In the literature there are clear indications for

Table 3

Recovery rates (%) of the model compounds with different media in permeation studies. Values are given as mean \pm SD ($n=12$).

Medium ^a	Cimetidine	Ketoprofen	Nadolol	Griseofulvin
FaB	95 \pm 2	85 \pm 2	94 \pm 3	31 \pm 3
FaSSIF	92 \pm 2	87 \pm 3	91 \pm 2	46 \pm 2
FaSSIF + BSA _{receiver}	–	–	–	52 \pm 2

^a Medium in the donor phase. The receiver phase consisted of PBS pH 7.4 throughout, except for FaSSIF + BSA_{receiver} where 4% (w/v) BSA was added to the receiver phase.

interactions of three of the model drugs used here with either TC-micelles or TC/PC-micelles. Nadolol has previously shown an increased solubility in the presence of TC-micelles and to partition into mixed micelles (de Castro et al., 2001). In addition, Sheng et al. (2009) reported an enhanced dissolution rate for ketoprofen in FaSSIF. The solubility of griseofulvin was doubled in a 15 mM TC solution (Mithani et al., 1996) while a ten to twenty-fold increase in solubility was described for various SIF-media (Nielsen et al., 2001) as compared to buffer. Moreover, Kataoka et al. (2003) found the dissolution rate of griseofulvin in 5 mM TC enhanced. Nadolol, ketoprofen and griseofulvin were thus expected to be present in their micellar form to some extent, which according to our hypothesis, should result in decreased permeability values in the presence of FaSSIF. The unchanged or slightly increased permeabilities observed here may lead to the assumption that there is greater complexities underlying this interplay. For example, the incorporation of TC in PVPA-barriers may induce a slight change in barrier characteristics (below the threshold detectable by calcein and ER) in terms of small pores (Schubert et al., 1986) and/or altered partitioning of drugs (de Castro et al., 2001). de Castro et al. (2001) has reported increasing affinity of nadolol for mixed cholate/lecithin systems with increasing cholate content. In the case of griseofulvin, where micellar encapsulation of this highly lipophilic compound can be assumed, there was still no diminution in permeability observed. Concurrently, the higher recovery rates of griseofulvin in the presence of FaSSIF in the donor phase (see Table 3 and Section 3.3) indicate a steadily higher drug concentration in the donor compartment. Higher concentrations in the donor did, however, not affect or enhance the flux. Most probably, this is due to micellar encapsulation, i.e., the micellar encapsulated fraction of the drug is not available for drug permeation.

3.3. Effect of FaSSIF on recovery

Recovery can be defined as the quantity recovered in both the donor and receiver chambers upon completion of the experiment, and expressed as a percentage of that which was added at time zero. A satisfactory recovery is crucial to ensure appropriate predictive power of permeability studies. For example, a low recovery may reflect accumulation in the permeation barrier and/or adsorption to the experimental apparatus surface. In our opinion, any calculation of P_{app} will be compromised in such instances. In the case of cimetidine, ketoprofen and nadolol, recovery rates were quite high (i.e., >85%) and comparable for buffer and FaSSIF (Table 3). In notable contrast, the recovery rate calculated for griseofulvin – a compound of strong lipophilic character – was remarkably low in the presence of FaB (approx. 31%). In the presence of FaSSIF the recovery rate was enhanced to a level of 46%. Thus, FaSSIF exerts a beneficial effect in terms of its influence on drug adsorption onto the experimental device(s) and accumulation within the barrier. Collectively, this ensures that undesirable drug-loss from the donor compartment is minimised. Similar effects have been observed with the Caco-2 cell model (Fossati et al., 2008; Ingels et al., 2004).

3.4. Influence of basolaterally applied bovine serum albumin on the recovery and flux of griseofulvin in the presence of FaSSIF

BSA is frequently employed to avoid low mass balance due to adsorption to plastic devices (Hubatsch et al., 2007; Krishna et al., 2001; Yamashita et al., 2000). At the same time, it provides an improvement in sink conditions in the receiver phase (Hubatsch et al., 2007; Krishna et al., 2001). In light of this, we utilised BSA in the receiver compartment, and investigated the mass-balance and flux of griseofulvin in the presence of FaSSIF. The recovery rate was increased to ~52%, which translates to a 10% increase when compared to FaSSIF alone in the donor phase (~46%) (Table 3). Most probably, this can be attributed to avoidance of drug adsorption to the apparatus surface(s) in the receiver chamber. In addition, the presence of BSA also served to enhance the flux of griseofulvin (Fig. 5). It is likely that this can be ascribed to an improvement in sink conditions. Analogous effects in cellular-based models have previously been described (Fossati et al., 2008; Ingels et al., 2002).

4. Conclusion

The applicability of FaSSIF in drug permeation studies employing PVPA barriers was confirmed by different integrity studies. No significant alterations in calcein permeability or electrical resistance were observed. The release of phospholipid from the barriers was negligible ruling out partial dissolution. Furthermore, visual examination of the barrier structure by CLSM revealed no significant changes. Notably, sodium taurocholate in the FaSSIF interacted with the phospholipids of the barrier. Drug permeability-values were found slightly enhanced or unchanged in the presence of FaSSIF, which may be attributed to micellar encapsulation and/or slight changes in barrier characteristics due to TC-uptake. Especially for poorly soluble drugs, FaSSIF improved the performance of PVPA-permeability studies in terms of markedly enhanced recovery, while permeability values remained comparable to those observed in buffer. Moreover, utilisation of BSA in the receiver compartment appears to reduce unspecific adsorption to plastic surfaces and more easily affords sink conditions. At the same time, the somewhat higher cost and complexity associated with the use of FaSSIF in permeability studies must be considered. However, overall, our results using the PVPA model suggest that for poorly soluble drugs a combination of FaSSIF in the donor phase and BSA in the receiver phase permits superior experimental conditions.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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