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Research Paper

In-vitro permeability screening of melt extrudate formulations containing poorly water-soluble drug compounds using the phospholipid vesicle-based barrier

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Abstract

Objectives The phospholipid vesicle-based barrier has recently been introduced as an in-vitro permeation model mimicking gastro-epithelial barriers in terms of passive diffusion of drugs. The aim of this study was to investigate whether the phospholipid vesicle-based barrier was suitable for permeability screening of complex formulations such as solid dispersions.

Methods Solid dispersions containing the poorly water-soluble drugs HIV-PI 1 (log $P = 6.2$, molar mass = 628.80 g/mol) and HIV-PI 2 (log $P = 5.3$, molar mass = 720.95 g/mol), a hydrophilic polymer and different surfactants were tested with respect to their influence on integrity of the barrier in terms of electrical resistance and permeability for calcein. Furthermore, utilisation of a more biologically relevant medium, Hank's balanced salt solution supplemented with Mg^{2+}- and Ca^{2+}-ions (HBSS (Mg^{2+}, Ca^{2+})), has been tested.

Key findings Except for the polyoxyl 40 hydrogenated castor oil-containing solid dispersion, no influence on the phospholipid vesicle-based barrier could be observed from the tested samples. Presence of active pharmaceutical ingredients (APIs) in the solid dispersions led to the same results as the corresponding placebo results. First experiments analysing the passive diffusion of both APIs in HBSS (Mg^{2+}, Ca^{2+}), evaluated as suitable transport medium, have shown promising results regarding the suitability of the phospholipid vesicle-based barrier for investigation of solid dispersions.

Conclusions The study indicated that the phospholipid vesicle-based barrier was compatible with selected melt extrudate formulations. The model seemed capable to reveal different transport routes in comparison with Caco-2 cell permeability tests.

Keywords artificial membrane; melt extrudate; permeability; poorly water-soluble drug; surfactant

Introduction

The oral route is the preferred route of drug administration due to its cost efficiency, convenience and patient compliance. In recent years, in-vitro permeability screening has become an integral part of drug discovery regimes to pre-estimate oral absorption potential of new drug compounds. Cell culture based models such as the Caco-2 cells, originating from a human colon adenocarcinoma, and the Mardin-Darby canine kidney (MDCK) cell line are commonly used for investigation of passive and active transport properties of drug compounds.[1–3] Furthermore, noncellular models based on lipids, such as the parallel artificial membrane permeability assay (PAMPA) and immobilised artificial membranes (IAM), have successfully been proven to assess passive absorption of active pharmaceutical ingredients (APIs).[4,5]

Recently, a novel model, the phospholipid vesicle-based barrier, has been introduced. It has been shown to deliver a good correlation with in-vivo data for passive diffusion for a diverse test set of model drugs.[6] The model is based on a tight barrier of phospholipid vesicles, deposited on a filter support by centrifugation and partly fused by freeze–thaw

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cycling and solvent evaporation. Apparent permeability data obtained using the model were shown to be independent of the so-called unstirred water layer and a broad range of pH values was found compatible with the model making it a valuable, easy handling tool to mimic different sections of the intestinal tract.[7,8]

In recent years a major turnaround in drug discovery and development has been seen based on the fact that the vast majority of new drug candidates lacks sufficient water solubility and/or dissolution rate to achieve satisfying oral bioavailability.[9] A variety of advanced formulation strategies is employed to overcome limited drug solubility such as micronisation or lipid-based formulations.[10,11] One of the most promising formulation types to increase bioavailability of poorly water-soluble drugs is solid dispersions prepared by melt extrusion.[12] During melt extrusion the raw materials are heated, molten, mixed and cooled down.[13] Solid dispersions are defined as at least one active ingredient in a carrier and can be divided into subclasses such as solid solutions, eutectic mixtures, glassy solutions and suspensions.[14] Excipients such as surface active agents are implemented within solid dispersions to ease polymer processing, to stabilise the system and to achieve solubilisation of the poorly water-soluble drug compound.[15,16] Regarding dissolution it could be shown that drug compounds are released *in situ* as nanoparticles and thus should show enhanced dissolution rate due to the small particle size.[17,18]

Although evaluation of oral bioavailability and in-vitro permeability screening has been extended from single drug compounds to various types of formulations, the consequences are far from well understood.[19–21] A pitfall often underestimated is that formulation additives may not only influence solubility and dissolution rate of the drug, but also impair integrity and functionality of the barrier used. The Caco-2 model has been investigated for its compatibility with various surfactants.[22,23] The phospholipid vesicle-based barrier has been studied also for its suitability for selected surfactants and co-solvents. Some of the tested additives were shown to be compatible with the model in the whole concentration range or up to a certain concentration, while others were shown to impair the integrity of the barrier.[24]

In this study, solid dispersions containing poorly water-soluble APIs in a polymer matrix containing different non-ionic surfactants with a hydrophilic–lipophilic balance (HLB) range from 4 to 14 as complex formulations have been investigated for the first time with respect to their influence on the phospholipid vesicle-based barrier. Both APIs belong to the group of the human immunodeficiency virus (HIV) protease inhibitors with a log $P = 6.2/5.3$ and a molar mass = 628.80/720.95 g/mol (HIV-PI 1/HIV-PI 2). The single excipients were tested as well as both API-containing and placebo solid dispersions of varying composition. In addition to conventional phosphate buffer a more physiological relevant transport medium (Hank's balanced salt solution) was employed to facilitate comparison with cell-based permeability models. Selected solid dispersion formulations found compatible with the model in the first step were tested in terms of drug permeability. The results were compared with results obtained using the Caco-2 cell assay.

Materials and Methods

Materials

Egg-phosphatidylcholine (Lipoid E-80) was kindly provided by Lipoid, Ludwigshafen, Germany. Calcein, 5-carboxyfluorescein and Hank's balanced salts (HBSS) were obtained from Sigma-Aldrich Chemie GmbH, Munich, Germany. $MgSO_4 \times 7\ H_2O$, $CaCl_2 \times 2\ H_2O$, KH_2PO_4, $Na_2HPO_4 \times 12\ H_2O$ and NaCl were all of analytical grade and purchased from Merck Co & KG, Darmstadt, Germany. Caco-2 cells were received from American Cell Culture Collection, Rockville, USA. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), L-glutamine, nonessential amino acids, sodium pyruvate and penicillin/streptomycin (10 000 U/10 000 μg/ml) were obtained from Biochrom AG, Berlin, Germany. Collagen (rat) was purchased from Roche Diagnostics, Mannheim, Germany. Filter inserts and plates (Transwell clear, $d = 6.5$ mm and 12 mm with a 0.4 μm polycarbonate membrane) were obtained from Corning Inc., Corning, NY, USA. Mixed cellulose ester filters (0.65 μm) were purchased from Millipore, Billerica, MA, USA. Polymer and formulations were provided by SOLIQS, Abbott, GmbH & Co. KG, Ludwigshafen, Germany. Compositions of formulations are shown in Table 1. The total concentration of APIs was between 20 and 30% in the melt extrudates. The amount of surfactant ranged between 7 and 10%.

Methods

Melt extrusion

The melt extrudates were prepared applying hot-melt extrusion. According to Meltrex technology, a blend of raw

Table 1 Excipients of various compositions of formulation A1, A2, and A3 prepared by melt extrusion

Formulation	HIV-PI 1	HIV-PI 2	PVP/VA copolymer	Sorbitan monolaurate	Polyoxyl 40 hydrogenated castor oil	Propylene glycol laurate	Hydrophilic fumed silica
A1 API	x	x	x		x		x
A1 placebo			x		x		
A2 API	x	x	x	x			x
A2 placebo			x	x			
A3 API	x	x	x			x	x
A3 placebo			x			x	

API, active pharmaceutical ingredient; PVP/VA, vinylpyrrolidone/vinylacetate.

materials was filled into a con-rotating twin screw extruder at temperature ranges of 100–150°C.[13] The melt was calendered after extrusion and solid oblong shaped tablets of extruded material were obtained.

Wide angle X-ray scattering

To investigate the melt extrudates for crystalline parts of HIV-PI 1 and HIV-PI 2, analysis with wide angle X-ray scattering (WAXS) was performed. The patterns were recorded using a PANalytical X'Pert Pro MPD diffractometer (PANalytical, Almelo, The Netherlands) with a PIXcel detector, data collector and HighScore software. A Cu Kα radiation source at 40 kV voltage and 40 mA current from 5 to 27° 2θ in a continuous scanning mode were employed for measurements. A step width of 0.026° 2θ and a measurement time per step of 4000 s was used. The irradiated sample length was 20 mm. The sample was prepared by milling approximately 1.5 g extrudate with a ball mill (Pulverisette 23, Fritsch, Idar-Oberstein, Germany) at 50 Hz for 20 s. For measurements, a back-loading 27 powder diffraction sample holder (PANalytical) was used.

Sample preparation

HBSS was prepared according to the product information. This was supplemented with 3.98 mM $MgSO_4$ and 1.26 mM $CaCl_2$ to obtain HBSS (Mg^{2+}, Ca^{2+}). Phosphate buffer was used in the composition as described previously.[6] A piece of melt extrudate was dispersed in HBSS (Mg^{2+}, Ca^{2+}) by magnetic stirring for 1 h at 400 rev/min and 37°C. The concentrations of HIV-PI 1 and HIV-PI 2 were chosen to reflect pharmacologically relevant concentrations. In the case of placebo extrudates the amount of surfactant was calculated equal to the amount in extrudates containing APIs.

Osmolality

Osmolality was measured using a Semi-Micro osmometer (Type ML, Knauer GmbH, Berlin, Germany).

Phospholipid vesicle-based barrier

Preparation of the barrier

The phospholipid vesicle-based barrier was prepared as described previously and stored for up to two weeks at –80°C.[6] In brief, liposome dispersions, produced by applying the film hydration method, were extruded through filters with pore size 800 followed by 400 nm and only 800 nm, respectively.[25] Afterwards, the dispersions were spread on the filter inserts and fused with cellulose ester filters using centrifugation.[26] Smaller liposomes were added first, followed by the bigger ones. Freeze–thaw cycling was employed to fuse the liposomes to a tight barrier. Before carrying out experiments the frozen barrier was incubated for 30 min at 65°C, cooled to room temperature and equilibrated in transport buffer for 45 min.

Integrity/compatibility experiments

To test the influence of solid dispersions on the phospholipid vesicle-based barrier, it was incubated with the sample dispersion for 4.5 h. The electrical resistance was measured before and after the end of experiments using a STX-100M probe connected to a Millicell-ERS device (Millipore, Billerica, MA, USA). The resistance of the empty filter equal to 119 Ω was subtracted and the values were normalised to $\Omega \times cm^2$. Barriers with an electrical resistance between 450 and 850 $\Omega \times cm^2$ after the end of the experiments were regarded as intact. The change in electrical resistance was calculated as the ratio of change in electrical resistance as compared with the control. Calcein has been found to be a suitable marker for monitoring the tightness of the barrier and permeation studies were performed as follows: aqueous dispersions of melt extrudates and fluorescent marker were added to the barrier, the inserts were moved to new wells containing fresh transport buffer at every time point of sample withdrawal and the amount of permeated calcein was measured at 25°C using a Polarstar fluorometer (Fluorstar, BMG Labtech GmbH, Offenburg, Germany).[6] The excitation and emission wavelengths were set to 485 and 520 nm, respectively. Apparent permeability for calcein was calculated from the transport rate during steady state.

Permeability experiments

Permeability experiments of the APIs were performed as described for calcein. The amount of API was analysed using high performance liquid chromatography (HPLC) (see below).

Caco-2 cell assay

Culture conditions

Caco-2 cells were grown in DMEM supplemented with 10% FBS, 1% L-glutamine, nonessential amino acids, sodium pyruvate and penicillin/streptomycin mixture at 37°C in an atmosphere of 5% CO_2/95% air.[27] Cells were seeded at a density of 65 000 cells/cm^2 on collagen coated 0.4 μm polycarbonate filters. Medium was changed every other day and after 21 days of growing and differentiating cell monolayers with the passage number 27–29 were used.

Permeability experiments

Before the experiment the medium was removed, the cell monolayers were washed with the transport buffer HBSS (Mg^{2+}, Ca^{2+}) and were allowed to equilibrate for 30 min. The transepithelial electrical resistance (TEER) was measured with a STX-100M probe connected to the Millicell-ERS device and the transport buffer in the apical compartment was exchanged with sample solution. The plates were placed on a plate shaker (Titramax 101, Heidolph, Schwabach, Germany) at 37°C. The inserts were moved to new wells containing fresh transport buffer at every time point of sample withdrawal. At the end of the experiment the TEER was measured again, the monolayers were washed and the permeability for the paracellular maker 5-carboxyfluorescein was determined. The content of API was analysed using HPLC. Calculation of mass balance was performed to ensure that more than 90% of drug substance was recovered.

High performance liquid chromatography

HIV-PI 1 and HIV-PI 2 were separated using an HPLC 2695 separation system with a Symmetry – C18 column (3.5 μm, 4.6 × 75 mm, 100 Å) from Waters (Milford, MA, USA). The

injection volume was 50 μl and the flow rate was adjusted to 1.5 ml/min at 35°C. The eluent consisted of KH_2PO_4 buffer ($c = 30$ mmol) and acetonitrile in the ratio 50 / 50, v / v. Samples were detected using a UV detector at 215 nm (Waters, Milford, MA, USA).

Permeability calculation

Permeability for HIV-PI 1 and HIV-PI 2 was investigated by comparing the flux J (μg/cm²/h) at steady state of the mass transport (dc) per unit time related to the area (A) as expressed in equation 1:

$$J = \frac{dc}{dt} \times \frac{1}{A} \quad (1)$$

Permeability for calcein was calculated according equation 2:

$$P_{app} = \frac{dm}{dt} \times \frac{1}{c_0 \times A} \quad (2)$$

where dm/dt is the permeated mass over time, c_0 the initial concentration and A the area.

Data analysis

Statistical analysis was performed using GraphPad Prism, version 4.00 (GraphPad software, Inc., San Diego, CA, USA). Comparison of data was performed applying one-way analysis of variance. Dunnett's test was used to identify differences between treatment and control. $P < 0.05$ was considered as statistically significant.

Results and Discussion

Wide angle X-ray scattering

The solid state of the melt extrudates was investigated applying WAXS. The WAXS pattern did not show any evidence of crystalline structures (data not shown), which confirmed the absence of crystalline HIV-PI 1 and HIV-PI 2. Thus, it could be concluded that the extrudate was a true molecular dispersion of the APIs dissolved in the matrix.

Investigation of the suitability of a different transport buffer

The phospholipid vesicle-based barrier was developed using a conventional phosphate buffer as transport medium. To test if the barrier was suitable for other media, for example buffers more related to physiological conditions, HBSS (Mg^{2+}, Ca^{2+}) was chosen as the transport medium and evaluated. HBSS (Mg^{2+}, Ca^{2+}) is widely used in cell assays, for example the Caco-2 cell assay, and therefore the possibility of using the same buffer in comparable studies would eliminate any influence on sample properties.

First, the osmolality of both buffers was determined. The values did not show differences (osmolality phosphate buffer = 285 mosmol/kg ($n = 3$) and osmolality HBSS (Mg^{2+}, Ca^{2+}) = 289 mosmol/kg ($n = 3$)). Furthermore, the electrical resistance was measured over 5 h and showed the same behaviour for both buffers (data not shown). Permeability for calcein did not show any significant differences between the two transport media (P_{app} phosphate buffer = $0.078 \pm 0.024 \times 10^{-6}$ cm/s), P_{app} HBSS (Mg^{2+}, Ca^{2+}) = $0.056 \pm 0.026 \times 10^{-6}$ cm/s); $n = 8$–10; $P = 0.05$). Therefore, it was concluded that HBSS (Mg^{2+}, Ca^{2+}) could be an alternative to phosphate buffer and the following experiments were performed using HBSS (Mg^{2+}, Ca^{2+}).

Compatibility of the phospholipid vesicle-based barrier with melt extrudate formulations

Three prototypes of melt extrudates were chosen, all containing HIV-PI 1 and HIV-PI 2 in a vinylpyrrolidone/vinylacetate copolymer (PVP/VA copolymer) matrix but with different surfactants with HLB values of 14, 8.6 and 4, respectively (Table 1). The compatibility testing was performed by monitoring electrical resistance and calcein-permeability measurements (Table 2), in absence and presence of both API-containing and API-free (placebo) extrudate dispersions. At the point of addition of transport buffer the electrical resistance was low. Previously, small angle X-ray scattering studies have shown that the barriers were partially dehydrated after preparation and rehydrated within one hour incubation time.[8] The state of hydration is reflected in the change of electrical resistance and based on current measurements equilibration of the electrical resistance of the barrier took even longer. Thus, flux and permeability values were calculated in a time range where only slight changes of the electrical resistance could be observed (at 120–300 min).

Furthermore, the PVP/VA copolymer was tested alone (structure in Figure 1). Different types of polymers including charged and uncharged, hydrophilic and amphiphilic polymers have been described to interact with phospholipid bilayers. The effects ranged from coating of the phospholipid layer to leakage, fusion and reduced permeability.[28–31] In contrast,

Table 2 Apparent permeability values of calcein and ratio of electrical resistance in presence of various formulations

Sample	P_{app} ($\times 10^{-6}$ cm/s)[a]		Ratio electrical resistance (%)[b]	
Control	0.044	(0.016)	100.00	(22.73)
PVP/VA copolymer solution	0.040	(0.015)	115.42	(25.45)
A1 placebo	0.073*	(0.030)	75.77*	(19.96)
A2 placebo	0.045	(0.014)	111.54	(35.20)
A3 placebo	0.036	(0.015)	104.51	(21.26)
A1 API	0.093*	(0.032)	72.92*	(20.57)
A2 API	0.040	(0.015)	84.09	(32.96)
A3 API	0.056	(0.017)	86.80	(33.14)

[a]Apparent permeability (P_{app}) values expressed as means ± SD, $n = 8$–27, standard deviation shown in parentheses.

[b]Ratio of electrical resistance (%) expressed as means means ± SD, $n = 8$–27, standard deviation shown in parentheses.

*Values that were significantly ($P = 0.05$) different from the control HBSS (Mg^{2+}, Ca^{2+}). API, active pharmaceutical ingredient; PVP/VA, vinylpyrrolidone/vinylacetate.

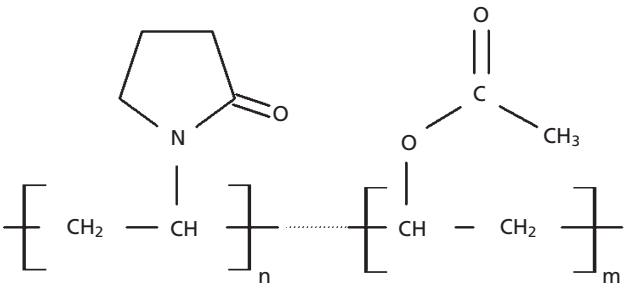

Figure 1 Chemical structure of vinylpyrrolidone/vinylacetate copolymer.

the PVP/VA copolymer used in this study did not show any significant effect, neither on the permeability for calcein nor on electrical resistance as compared with control. This may have been attributed to the hydrophilic characteristics of the polymer and absence of specific functional groups.[32] Thus, melt extrudates containing PVP/VA copolymer as matrix polymer were further investigated in terms of compatibility with the phospholipid vesicle-based barrier.

Compatibility testing of the various melt extrudates with the phospholipid vesicle-based barrier yielded mixed results: Both the API-free and the API-containing melt extrudate with polyoxyl 40 hydrogenated castor oil as surfactant (formulation A1 according to Table 1) led to a significant increase in permeability for calcein and at the same time significant decrease in electrical resistance. That was in agreement with earlier reports on polyoxyl 35 castor oil, which was reported to partly dissolve the barrier, as indicated by release of phospholipids.[24] This may point out that polyethylene glycol ethers in general are not compatible with the phospholipid vesicle-based barrier because they seemed to cause leaks within the barrier.

The other two melt extrudate prototypes did not induce significant changes in electrical resistance or calcein permeation, irrespective of whether they contained APIs. The sorbitan monolaurate-containing solid dispersion (formulation A2 according to Table 1) did not significantly affect the permeability for calcein or the progression of electrical resistance-values as compared with the control. This was in agreement with earlier observations, where only higher concentrations of surfactant than the one used here resulted in induced increases in permeability for calcein.[24] Sorbitan monolaurate in the form of melt extrudates seemed to show the same behaviour as the pure surfactant in solution and therefore it could be concluded that it was compatible with the phospholipid vesicle-based barrier, at least in the concentration used here.

Melt extrudates containing propylene glycol laurate (formulation A3 according to Table 1) with an HLB value of 4 did not lead to any change in permeability for calcein and the electrical resistance did not show any significant changes either. Thus, propylene glycol laurate was regarded as a suitable surfactant in terms of compatibility with the phospholipid vesicle-based barrier at least in the concentration studied here.

It was remarkable that API-containing melt extrudates behaved very similarly compared with the placebo extrudates. Thus, the presence of APIs within dispersions of melt extrudates did not appear to influence the integrity of the phospholipid vesicle-based barrier.

Permeation

The phospholipid vesicle-based barrier

The melt extrudate formulation containing sorbitan monolaurate (formulation A2 API according to Table 1) was chosen for permeability experiments. The permeability experiments showed a lag-phase of approximately 3.0 h, which was in agreement with earlier reports.[6] This lag phase was attributed to the fact that some time was needed to hydrate and saturate the barrier before steady-state flux was reached. The reason why we have presented flux-values, J, rather than apparent permeability values was because the calculation of the former was independent of the initial drug concentration. In cases where drugs are never fully dissolved, apparent permeability coefficients are misleading to our understanding because the donor concentration of dissolved drug is not exactly known. Hence, in further experiments it was assured that the same donor concentration was used.

When measuring flux of both HIV-PI 2 and HIV-PI 1 from the bi-extrudate in HBSS (Mg^{2+} Ca^{2+}) (Figure 2a and b) they ranged in the same magnitude. The fact that HIV-PI 2 (Figure 2a) and HIV-PI 1 (Figure 2b) fluxes were about the same was surprising because the bi-extrudate contained four times more HIV-PI 1 than HIV-PI 2. At the same time, HIV-PI 2 ($\log P = 5.3$, $c_{\text{solubility (25°C, pH 7, unbuffered water)}} = 0.37$ μg/ml (calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris.)) had a more than 3-fold higher solubility than HIV-PI 1 ($\log P = 6.2$, $c_{\text{solubility (25°C, pH 7, unbuffered water)}} = 0.11$ μg/ml). We surmised that the presence of surfactants may have affected the permeability for lipophilic drug to a higher extent than the more hydrophilic one due to the fact that the effective partition coefficient between the lipid barrier and the donor solution may have been lower for the more lipophilic drug. This could again have influence on the permeation of the more lipophilic drug in the presence of surfactants in the donor compartment to a higher extent.[33]

It has to be mentioned, however, that in all cases the concentrations of HIV-PI 2 and HIV-PI 1 in the acceptor phase were close to the quantification limit of the assay. The results presented here are meant to give a first insight into the permeation behaviour of poorly water-soluble drug compounds from solid dispersions. More advanced analytical approaches or a different experimental set-up will be needed to devise comparative permeation studies aiming at a performance ranking of different formulations.

Comparison with Caco-2 cell permeability

When investigating the permeability for HIV-PIs from the sorbitan monolaurate-containing melt extrudate (formulation A2) using the Caco-2 model, a difference between the steady-state flux of HIV-PI 1 (1.22 ± 0.04 $\mu g/h \times cm^2$) and HIV-PI 2 (0.52 ± 0.01 $\mu g/h \times cm^2$) was evident, i.e. HIV-PI 1 showed an approximate 2.3-fold higher flux than HIV-PI 2 (Figure 3). This indicated a difference between Caco-2 model derived

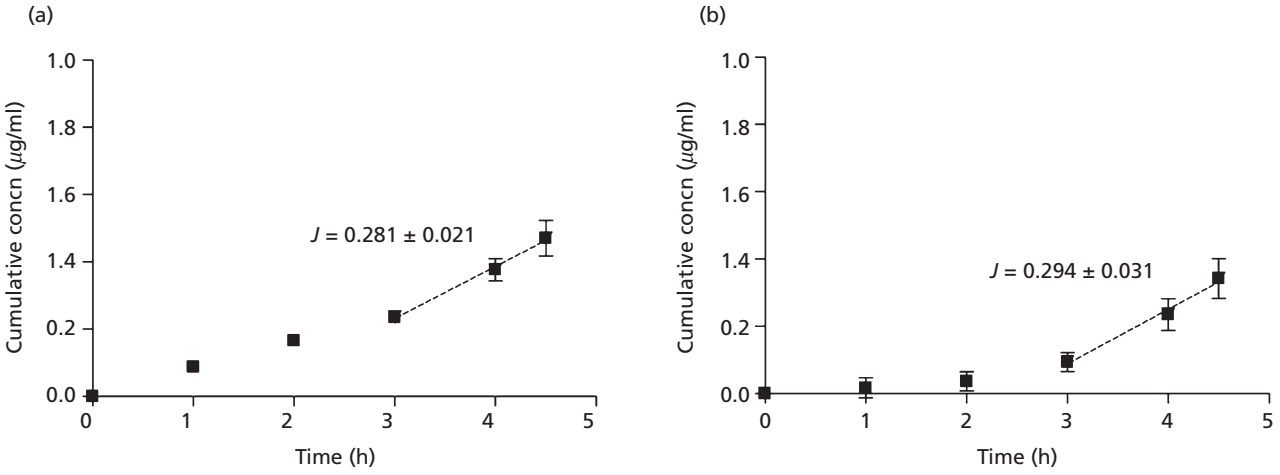

Figure 2 Permeation curve of HIV-PI 2 and HIV-PI 1 in A2 in HBSS (Mg^{2+}, Ca^{2+}). Permeation curve: cumulative concentration (μg/ml) over time (h). (a) HIV-PI 2 in A2 in HBSS (Mg^{2+}, Ca^{2+}). (b) HIV-PI 1 in A2 in HBSS (Mg^{2+}, Ca^{2+}). Flux J ($\mu g/(h \times cm^2)$) at steady state was calculated based on the slope. Values are mean ± SD, $n = 3$.

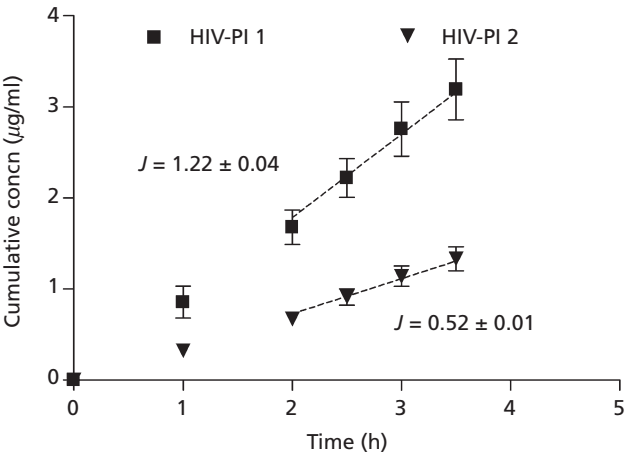

Figure 3 Permeation curve of HIV-PI 2 and HIV-PI 1 in A2 in HBSS (Mg^{2+}, Ca^{2+}) using the Caco-2 cell model. Permeation curve: cumulative concentration (μg/ml) over time (h). Flux J ($\mu g/(h \times cm^2)$) at steady state was calculated based on the slope. Values are mean ± SD, $n = 8$.

fluxes and phospholipid vesicle model based fluxes, which may have been related to the fact that the Caco-2 model measured both active and passive transport, while the phospholipid vesicle model exclusively determined passive transport, as known active transporters, such as the efflux pump P-glycoprotein (P-gp), belonging to the family of the ATP-binding cassette (ABC) transporters, are expressed in Caco-2 cells.[34] Various HIV protease inhibitors have been described to interact with P-gp as substrate, while the inhibitory effects were pronounced to different degrees for different compounds.[35–37] Therefore, it might be assumed that HIV-PI 2 interacted to a greater extent with the efflux pump and thus, the flux of HIV-PI 1 increased. Hence, the difference between the ratio of flux of HIV-PI 1 and HIV-PI 2 between the phospholipid vesicle-based barrier and the Caco-2 cell assay might have been related to the different affinity of the APIs to the active transporter P-gp. Although initial experimental evidence was available on the question as to whether the Caco-2 barrier may be looked at as fully compatible with the extrudates tested, further experiments are needed to put this on a firm basis. At the same time, a more thorough investigation including competitive inhibitors of the P-gp system and transport experiments in the opposite direction are required to check the presented hypothesis.

Conclusions

The phospholipid vesicle-based barrier has been demonstrated to be compatible with selected melt extrudate formulations. Neither the polymer alone nor the melt extrudate formulations containing the surfactants sorbitan monolaurate or propylene glycol laurate were found to influence electrical resistance or calcein permeation. This held true irrespective of whether API-containing melt extrudates or placebos were tested. First experiments on the permeability for HIV-PI 1 and HIV-PI 2 appeared to indicate that the flux of both APIs was in the same magnitude if a melt extrudate was tested that contained four times more HIV-PI 1 than HIV-PI 2. In comparison, the Caco-2 model yielded significantly higher flux values for HIV-PI 1 than for HIV-PI 2, a finding that requires further investigation. Clearly, the phospholipid vesicle-based barrier appeared to be suitable for investigating the passive transport of APIs from various complex formulations such as solid dispersions. It may therefore represent a useful extension of the permeability screen toolbox, especially in comparison with the Caco-2 model and in cases where a differentiation between passive and active transport is desired.

Declarations

Conflict of interest

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

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