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Dissolution/permeation with PermeaLoop™: Experience and IVIVC exemplified by dipyridamole enabling formulations

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ABSTRACT

It is our hypothesis that the presence of an absorptive sink for in-vitro dissolution experiments is decisive to predict extent and duration of super-saturation of low soluble drugs in formulations expected to increase oral absorption, often called enabling formulations. Combined dissolution-/permeation-testing may provide such absorptive sink. Commonly used in-vitro dissolution-/permeation tools have a limited interfacial area-to-donor-volume-ratio (A/V), far below the physiological one which is estimated for humans. In consequence, super-saturation is expected to be more pronounced and thus precipitation to occur more readily in these models as compared to the in-vivo situation. In the current study, a PermeaLoop™ prototype a of a novel in-vitro dissolution-/permeation-tool with a substantially larger A/V was employed to investigate the dissolution and permeation behaviour of model formulations of dipyridamole containing fumaric acid as modifier of the micro-environmental pH. After identifying the most suitable experimental conditions in terms of donor- and acceptor pH and composition, dose, flow-rate and sampling intervals, both the dissolution and the permeation were simultaneously assessed over time and the extent and duration of super-saturation monitored. The importance of biomimetic media in the donor was revealed not only in terms of increasing the dissolution but also the permeation. The formulations were ranked in terms of their performance (cumulative amount permeated). As a result the data generated by PermeaLoop experiments showed for the same formulations a superior correlation with in rat bioavailability data than obtained from a traditional side-by-side Dissolution-/Permeation-system with a Caco-2-cell membrane (D/P-system).

The insights into the effects of solubilisers and pH conditions gained in the present study contribute to an improved mechanistic understanding of dynamic dissolution/permeation behaviour of weakly basic drugs and their enabling formulations. Challenges with the current PermeaLoop prototype are still to be solved, as dispersed drug still tends to get stuck inside the system, but gained experiences are helpful for the improvement of the design.

1. Introduction

During the past two decades there has been a trend in drug discovery in terms of new chemical entities (NCE) being less and less water-soluble and approximately 90% of all NCE's in the current pharmaceutical pipelines are classified as to BCS II or IV (Ku and Dulin, 2012; Lipp R., 2013). In order to cope with these low solubility compounds, candidate-enabling strategies such as salts, cocrystals, co-amorphous systems, amorphous solid dispersions (ASD) and lipid-based formulations etc. are nowadays frequently considered in oral formulation development with the aim to increase the solubility of the poorly soluble compounds and thus (hopefully) also to enhance their absorption (Boyd et al., 2019).

Dissolution tests in single compartment apparatus have been utilised as a tool to predict the in-vivo absorption of formulations for over 50 years (Levy G. 1961). More recent studies suggest that single-chamber dissolution tests may fall short in predicting the in-vivo behaviour of super-saturating drug delivery systems (SSDDS). A study from 2012 by Bevernage and colleagues showed that coupling an absorptive chamber to the dissolution chamber for studying the poorly soluble drug loviride maintained the metastable super-saturated state for a significant period of time, considerably longer than when no

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List of abbreviations

A/*V* = Area/Volume (donor) ratio
ADME = Absorption, distribution, metabolism, excretion
API = Active pharmaceutical ingredient
ASB = acceptor sink buffer to the list
ASD = Amorphous solid dispersion
BCS = Biopharmaceutics classification system
D/P system = Dissolution/permeation system
FaSSIF = Fasted state simulated intestinal fluid
$FaSSIF_{mod100}$ = Fasted state simulated intestinal fluid, based on 100 mM phosphate buffer
$FaSSIF_{mod200}$ = Fasted state simulated intestinal fluid, based on 200 mM phosphate buffer
GIT = Gastrointestinal tract
IVIVC = In-vitro in-vivo correlation
LOD = Limit of detection
LOQ = Limit of quantification
NCE = New chemical entity
SSDDS = Super-saturating drug delivery systems
TPGS = D-α-Tocopherol polyethylene-glycol 1000 succinate

absorptive chamber was present (Bevernage et al., 2012). In 2017 Sironi and colleagues did a combined dissolution/permeation study comparing commercial micro- and nanoparticles of fenofibrate which revealed that even though the microparticles lead to a higher amount of apparently dissolved drug, the permeation of drug was higher for the nanoparticles as also is the case in vivo. This finding confirmed that a dynamic dissolution/permeation model is beneficial to predict the oral bioavailability of SSDDS (Sironi et al., 2017B). Both studies were carried out with conventional side-by-side cells which have an area/volume ratio $< 0.5\,cm^{-1}$, whereas the A/V in the intestinal tract of humans is suggested to be $1.9\,cm^{-1}$ to $2.3\,cm^{-1}$ (Mudie et al., 2012). It is hypothesised, that for some formulations using dynamic dissolution/permeation set-ups, the restricted permeation area will cause insufficient inhibition of precipitation due to the insufficient permeation of drug out of the donor chamber as compared to what is happening in-vivo (Sironi et al., 2018).

The API and formulations employed here have been selected from a previous study (Mizoguchi et al., 2018) which describes both in vitro dissolution-/permeation data generated with a conventional side-by-side-setup and Caco-2 as barrier and in vivo bioavailability data, which serves as a reference for the current work. To this end the current study represents an attempt to overcome the aforementioned limitations with respect to A/V-ratio: PermeaLoop is a novel prototype dissolution/permeation model with an A/V of $1.38\,cm^{-1}$, which is much closer to the in-vivo situation.

The PermeaLoop principle was first described by Sironi (Sironi et al., 2018). It consists of four main items, a donor reservoir, an acceptor reservoir, a peristaltic pump and a permeation cell. The drug formulation under investigation is dispersed in the aqueous donor medium and the donor dispersion is pumped from its reservoir into the permeation cell, where the dissolved fraction of the drug will be available to pass through a biomimetic barrier into the acceptor compartment. The acceptor medium is also pumped into the acceptor side of the permeation cell. The liquid passing through the permeation cell can either be transferred back into the respective reservoir for recirculation, or - in the case of the acceptor - it can be pumped out of the system so all acceptor buffer entering the system will be drug free to secure better sink-conditions. The permeation cells are designed in a spiral shape which ensures suitable hydrodynamics and mixing.

Dipyridamole is a weak base with a pKa of 6.4; it is readily soluble in acidic environment, but practically insoluble at neutral pH. The pH dependence of dipyridamole solubility is held responsible for significant inter-individual variabilities in its bioavailability (Zhou et al., 2005; Russell et al., 1994), due to low absorption in subjects with hypochlorhydria conditions which are often observed in infants, elderly adults and patients suffering from HIV or *Helicobacter pylori* infections (Champagne 1989; Lahner et al., 2009; Shelton et al., 1997). Studies have shown that granules containing an acid to decrease the microenvironmental pH were able to increase the absorption of dipyridamole in rats treated with proton pump inhibitors to the same level as observed in normal rats. Since granules with acid modifiers are straightforward to manufacture on an industrial scale, it appears to be promising choice for dipyridamole formulations (Taniguchi et al., 2012).

The aim of the method development studies was to investigate whether it is possible to establish conditions, under which a dynamic interplay between dissolution and permeation can be observed. To this end, and in contrast to conventional steady-state permeation experiments, where such interplay typically is not accomplished, a high flux of drug is desired. In the in-vivo situation, plasma protein-binding of the drug and high blood flow are expected to ensure that the drug-concentration on the blood side of the intestinal barrier at all times is virtually zero, i.e. there is a strong absorptive sink for drug permeating from the intestine to the blood. In-vitro dissolution/permeation set-ups are often failing to reach a similarly high flux of drug as it is the case in-vivo. We aimed at a PermeaLoop set-up with experimental conditions suitable to mimic the in-vivo situation, i.e. to achieve permeation rates of dipyridamole that enable a dynamic interplay between dissolution and permeation. For this purpose, different pH-conditions and the addition of solubilisers to the acceptor medium were investigated. Furthermore, fasted state simulated intestinal fluid (FaSSIF) vs phosphate-buffered dispersions in the donor and different doses of dipyridamole were tested and compared. The dissolution/permeation-behaviours of dipyridamole formulations containing pH modifiers were tested and compared with in-vivo data from rat studies and in-vitro data gained with an alternative dissolution-/permeation-model, namely side-by-side cells with Caco-2 cell monolayers as absorptive barrier, as described in Mizoguchi et al., 2018. Finally, in order to confirm the dynamic situation in the described experiments, it was tested if the drug would precipitate faster by replacing the biomimetic barrier with an impermeable barrier between the donor and acceptor compartments.

2. Materials and methods

2.1. Chemicals

Used during experiments in Denmark: Dipyridamole (purity ≥ 98%), sodium phosphate monobasic dihydrate, sodium phosphate dibasic dihydrate and citric acid were purchased from Sigma-Aldrich® Denmark ApS (Brøndby, Denmark). Methanol (HPLC grade), trifluoracetic acid, sodium chloride were purchased from VWR International LLC (Soeborg, Denmark). D-α-Tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) (NF grade) was a kind gift of Gustav Parmentier GmbH, Frankfurt, Germany). Hydroxypropyl-β-cyclodextrine was purchased from Roquette (Lestrem, France).

Used during experiments in Japan: Ethanol (HPLC grade), trifluoracetic acid, sodium phosphate monobasic dihydrate, sodium phosphate dibasic dihydrate, citric acid were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Dipyridamole (purity ≥ 98%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). D-α-Tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) (NF grade) was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). Mannitol 50 INT was purchased from Roquette GmbH (Frankfurt, Germany). Acceptor sink buffer (ASB) was purchased from

Pion Inc. (MA-Billerica, USA). Fumaric acid was purchased from Tate&Lyle PLC. (England). FaSSIF/FeSSIF/FaSSGF powder were purchased from Biorelevant (London, United Kingdom). Methanol was purchased from Fischer Pure Chemical Corporation (Osaka, Japan). Highly purified water was prepared in house using a Milli-Q® water purification system (Merck Millipore, Tokyo, Japan).

Analytical grade has been used for all chemicals unless stated otherwise..

2.2. Buffer preparation

50 and 100 mM phosphate buffer pH 7.4 were prepared by dissolving respectively 75 or 38 mM dibasic sodium phosphate and 25 or 12 mM monobasic sodium phosphate in purified water. The pH was measured by a 907 Titrando from Metrohm AG (Herisau, Switzerland) and adjusted to 7.4 with sodium hydroxide solution and/or hydrochloric acid.

50, 100 or 200 mM phosphate buffer pH 6.5 was prepared by dissolving 18, 36 or 72 mM dibasic sodium phosphate and 32, 64 or 128 mM monobasic sodium phosphate in purified water. The pH was adjusted to 6.5 with sodium hydroxide solution and/or hydrochloric acid.

100 mM acetate buffer pH 4.0 was prepared by dissolving 23 mM sodium acetate in purified water before addition of 77 mM acetic acid. The pH was measured and adjusted by addition of hydroxide solution and/or hydrochloric acid.

50 mM citrate-phosphate buffers pH 4.0 and 5.0 were prepared by dissolving 28 or 33 mM dibasic sodium phosphate and 22 or 16 mM citric acid in purified water. The pH was adjusted to 4.0, with sodium hydroxide solution and/or hydrochloric acid.

Solutions containing 0.2% or 2% (m/m) Vitamin E TPGS were prepared by dissolving Vitamin E TPGS in buffer by stirring overnight to ensure dissolution and equilibration. For modified FaSSIF solutions 224 mg FaSSIF/FeSSIF/FaSSGF powder (equal to 3 mM taurocholate and 0.75 mM lecithin) were dispersed in 100 mL of 100 mM ($FaSSIF_{mod100}$) or 200 mM ($FaSSIF_{mod200}$) phosphate buffer by stirring overnight.

2.3. Screening of the impact of solubilisers in the acceptor chamber on dipyridamole permeation using a 96-well plate

All bottom wells of a PermeaPad microtiter plate (PermeaPad®Plate, kind gift of InnoMe GmbH, Espelkamp, Germany) were filled with 350 µL of 2.5 µg/mL dipyridamole solution in phosphate buffer pH 6.5. The top compartments were filled with 200 µL each of either of the following acceptor media: (1) sink buffer (PION), (2) phosphate buffer pH 6.5, (3) phosphate buffer pH 6.5 with 0.2% vitamin E TPGS. The microtiter plate was incubated in a heating cabinet at 37 °C with shaking at ~ 120 rpm on a Mildmixer PR-36 from TAITEC (Nishikata, Japan) and samples were withdrawn from both, the top and bottom compartments after 1.5, 3, 4.5 and 6 h in triplicate, such that each well was used for sampling only once. All samples were diluted with MeOH and their dipyridamole-content quantified by HPLC.

2.4. PermeaLoop system

The PermeaLoop® system was custom-built and used as previously described (Sironi et al., 2018) with the following modifications (3rd generation of PermeaLoop® cells):Inlet- and outlet-tubing were connected straight to the system (to the spiral-shaped channel) instead of a 90 deg bend, which earlier was found to potentially cause clogging. The donor and acceptor tubings were TYGON E-LFL tubes from Cole Parmer GmBH (Wertheim, Germany) with inner diameters of 1.02 mm and 0.64 mm in the donor and acceptor respectively, and flowrates of the dispersions at 0.3 and 1 mL/min respectively. Both reservoirs were stirred at approx. 200 rpm. PermeaPad®, (kindly provided by InnoMe GmbH, Espelkamp, Germany), was employed as a biomimetic barrier in all studies except for two studies where aluminium foil was utilised. The whole system was incubated at 37 °C in a PVO-450 vaccuum oven from Eyela (Tokyo, Japan). The start volumes in the donor reservoirs were 22 mL, while the acceptor was pumped through the cells in flow-through-mode to ensure fresh buffer (void of drug) on the acceptor side thougout the whole permeation experiment. In order to minimize non-specific drug adsorption to the tubing material, previously used tubes were briefly rinsed with buffer at a flowrate of 3 mL/min for 10 min inbetween each permeation experiment.

2.5. Permeation experiments

Solutions of 50 µg/mL dipyridamole in 100 mM acetate buffer pH 4.0 or 50 mM phosphate-citrate buffer pH 4.0 or solutions of 5 µg/mL and 2.5 µg/mL dipyridamole in 50 mM phosphate-citrate buffer pH 5.0, respectively, were used on the donor side of PermeaLoop as described in 2.5. On the acceptor side 100 mM acetate buffer pH 4.0, 50 mM phosphate-citrate buffer pH 4.0, or 100 mM/50 mM phosphate buffer pH 7.4 was used, with or without addition of 2% Vitamin E TPGS. When the pre- warmed buffer solutions had been poured into the beakers, the pumps were started, indicated as the start of experiment. Samples were withdrawn from the acceptor beaker after 30, 60, 90, 120, 150, 180 and 240 min (and additionally at 300 and 360 min for the 6-hours experiment) and diluted with methanol. Before each experiment and when the final acceptor sample was taken, donor-samples were drawn in order determine mass balance. The pH values of all donor and acceptor solutions were measured before and after the experiments.

2.6. Preparation of dipyridamole granules and physical mixture

Granules were prepared by conventional wet granulation with mortar and pestle. The compositions of the formulations correspond to the formulations in Mizoguchi et al., 2018 in order to compare the results. A total mass of 15 g of Mannitol, dipyridamole and fumaric acid, according to Table 1, were added to a porcelain mortar and well premixed prior to the addition of 1.5 mL 5% hydroxypropyl cellulose (HPC)-solution in 70% (v/v) ethanol. The blend was then carefully mixed with the pestle while 70% ethanol solution was added till a snowball texture was obtained, followed by sieving through a 500 µm sieve. The sieved granules were then dried for 2 h at 60° in a vacuum drying oven, DP-23 from Yamato Scientific Co. Ltd. (Tokyo, Japan). After drying, the fine particle-fraction was removed using a 125 µm sieve. For the physical mixture (PM), the powders were thouroughly mixed in the mortar.

2.7. Solubility test

20 mg dipyridamole was suspended in 7.5 mL of 200 mM phosphate buffer (pH 6.5) and $FaSSIF_{mod100}$ with or without 360 µg/mL fumaric acid (equal to highest amount of fumaric acid released from formulations) in brown flasks with lid. The suspensions were incubated at 37 °C, and 150 rpm on a Julabo SW23 shaking water-bath (Buch & Holm A/S, Herlev, Denmark). Aliquot samples were withdrawn after 24 and 48 h. The brown flasks were thoroughly agitated for 5 s on a vortex mixer right before samples of 1 mL were transferred to 1.5 mL microcentrifuge tubes, and immediately centrifuged at 20817rcf and 37 °C on an

Table 1
Compositions of prepared granules and physical mixture.

API:acid ratio	1:0	1:0.5	1:1	1:2	1:1
Wet granulation?	yes	yes	yes	yes	no (PM)
Dipyridamole (mass,%)	29.85	29.85	29.85	29.85	29.85
Mannitol (mass,%)	69.65	54.75	39.8	9.95	39.8
Fumaric acid (mass,%)	0	14.90	29.85	59.7	29.85
HPC (mass,%)	0.5	0.5	0.5	0.5	0.5
Total (mass,%)	100	100	100	100	100

Eppendorf Centrifuge 5804R (Eppendorf Nordic A/S, Horsholm, Denmark) for 20 min. After spinning, the supernatants were filtered through a 0.2 µm pore-size Whatman Anotop™ 25 filters (Fisher Scientific, Roskilde, Denmark) and diluted with methanol and fresh buffer, such that the final sample contained 50% (v/v) methanol and 50% (v/v) phosphate buffer.

2.8. Combined dissolution/permeation studies of dipyridamole using PermeaLoop

Before the experiment an excess of pre-temperatured 0.2% vitamin E TPGS in phosphate buffer at pH 6.5 was added to the acceptor-reservior and coupled to the acceptor inlet, the acceptor side of the system was filled by letting the pump run for 4 min with a flowrate at 3 mL/min. Dipyridamole formulations equal to a total dose of 3.6 mg dipyridamole (1.8 and 0.9 mg in the respective experiments with reduced doses) was added to each donor reservoir. At the start of the experiment 22 mL donor medium (phosphate buffer 200 mM, $FaSSIF_{mod200}$, $FaSSIF_{mod100}$) were added to each donor reservoir. The pumps were started with a flowrate at 4 mL/min in the donor and 0.3 mL/min in the acceptor. After 4 min, the flowrate of the donor pump was decreased to 1 mL/min. After 15, 30, 60, 120, 240 and 360 min 500 µL samples were withdrawn from the donor reservoirs. A 50 µL-aliquot of each sample was diluted 1:1 with MeOH to determine the total (combined dispersed and dissolved) drug (HPLC). The rest of the sample was filtered with a 0.22 µm pore-size, non-sterile Millex durapore® syringe filter from Millipore (Darmstadt, Germany), and a 50 µL-aliquot of the filtrate was diluted 1:1 with MeOH to determine the dissolved amount of dipyridamole (HPLC). The acceptor samples were withdrawn from the collection beaker after 50, 65, 95, 125, 185, 245, 305 and 365 min. After each experiment the system was detached immediately to determine the drug content in/on the membranes by separating the support sheets and extracting in ethanol for 60 min.

2.9. Quantification by HPLC

All quantifications were carried out on reverse phase HPLC on an Alliance Waters 2695D, with a C18 column from Dionex (Thermo Fischer) at 50 °C with a flowrate at 1 mL/min or, alternatively, a 3 µm, 3.0 x 100 mm intersil ODS-3 HP column from GL Sciences Inc. (Tokyo, Japan) at 50 °C with a flowrate at 0.7 mL/min. The mobile phase consisted of 55% (48%) methanol and 45% (52%) of 0,1% (V/V) trifluoracetic acid in highly purified water. UV-detection was done at a wavelength of 284 nm (288 nm). A new calibration curve was made daily.

2.10. Data analysis

Two-tailed unpaired students *t*-test with equal variance were carried out in excel (Microsoft® Excel® 365, version 2002 build 12,527.20278). P-values $\leq$ 0.05 were considered significantly different.

3. Results and discussion

3.1. Method development

In this study, it has been prioritised to establish conditions in the acceptor compartment which ensure a high flux of drug rather than conditions which closely mimic the conditions observed in vivo, since this is considered most appropriate for performance-ranking of supersaturating formulations (Sironi et al., 2017A).

3.1.1. The influence of solubilisers in the acceptor compartment on drug solubility and permeation

To screen for ways to improve sink conditions, permeation studies with different solubilisers in the acceptor were performed in the 96-well plate set-up with the top and bottom well separated by PermeaPad®. In this study, the commercially available "acceptor sink buffer" (Pion inc.) was intended to be compared with phosphate buffer pH 6.5 with/without 0.2% TPGS as acceptor media. Due to a potential interference between dipyridamole and one of the solubilisers in the "acceptor sink buffer" (the composition of which is unknown to the public), it was not possible to quantify the peaks in these samples using HPLC. With 0.2% TPGS added to the acceptor a 1.86-fold increase in drug permeating after 6 h was observed (Fig. 1). One should have in mind that overall recovery of dipyridamole was low. Enhancing the drug permeating by addition of 0.2% TPGS in the acceptor was the best option found even though the permeability increase was lower than desired.

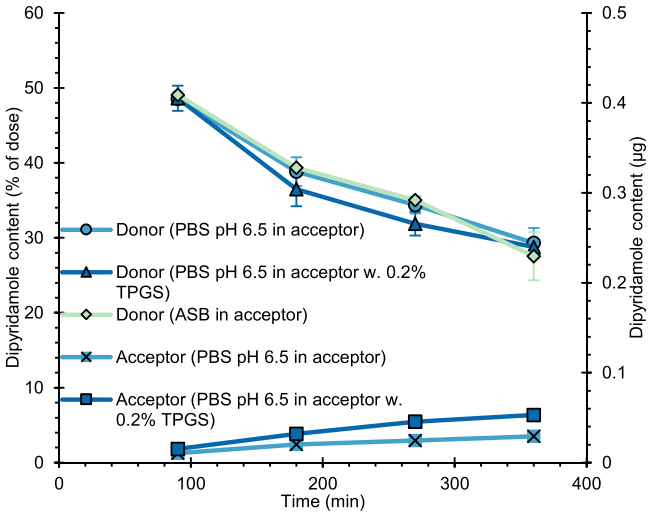

Fig. 1. Permeation experiment on of 96-well micro titre plate with PermeaPad. The experiments were carried out with phosphate buffer pH 6.5, containing 2.5 µg/mL dipyridamole in the donor and either phosphate buffer pH 6.5 with/without TPGS in the acceptor or acceptor sink buffer from Pion Inc. The plates were incubated for 6 h at 37 °C and with shaking at 300 rpm. Data are reported as mean ± SD (n = 3). The lines in the figure are a guide to the eye. .

3.1.2. The influence of pH on drug permeation

Since dipyridamole is a weakly basic compound, it was tested if lowering the pH in the acceptor, which causes an increased solubility of dipyridamole, would lead to enhanced permeation.

Initially an acetate-buffer was used as an acidic medium either in the donor only, or both, in donor and acceptor. A dramatic increase in the amount of dipyridamole permeated was observed. Due to the high fraction permeated, the cumulative curves also show a clear deviation from linearity indicating that the setup is leaving steady-state conditions (Fig. 2). However, checking pH values before and after the permeation experiment revealed, that with the acetate-buffer pH 4 in the donor and phosphate buffer pH 7.4 in the acceptor the pH-gradient was not maintained (Table 2). The only hypothetic explanation we could come up with, was, that acetate could have permeated and caused the pH-drift observed. This is surprising, since PermeaPad has earlier been described to show an excellent resistance against proton permeation (Di Cagno M and H.A., 2015). Therefore, in the next experiment, acetate-buffer pH 4 was replaced by phosphate-/citrate-buffer pH 4. This combination of buffer salts was chosen because in the pH-range we are working, there should always be a majority of charged species, which less prone to permeate.

In order to test our hypothesis, whether a pH-gradient between phosphate-buffer of pH 7.4 and phosphate-/citrate-buffer pH 4.0 could be maintained over a relevant period of incubation (6 h) a control experiment with the PermeaPad 96-well titre plate was carried out. The pH-values measured after incubation, for both compartments are shown in Table S1 (supplementary). Only negligible pH changes were

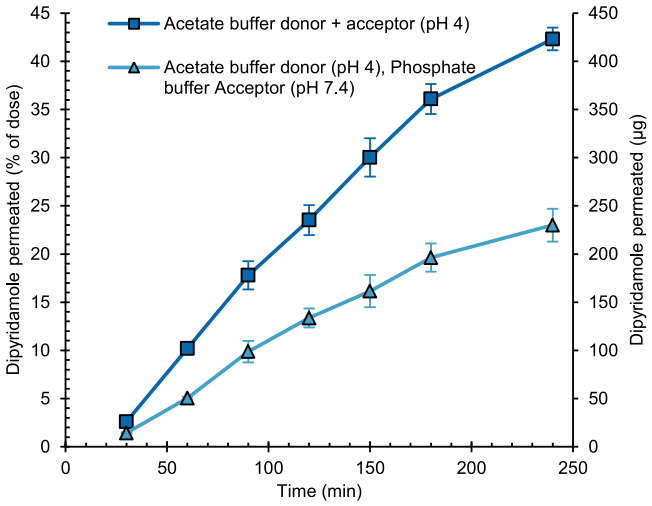

Fig. 2. Permeation experiment with dipyridamole solutions on PermeaLoop across PermeaPad. The experiments have been carried out with dipyridamole solutions with a starting concentration in the donor at 50 µg/mL at 37 °C. pH 4.0 medium was 100 mM acetate buffer and pH 7.4 medium 100 mM phosphate buffer. Data are reported as mean ± SD (n = 3). The lines in the figure are a guide to the eye.

observed after 6 h of incubation.

It was concluded that permeation studies with pH gradients should be feasible if phosphate and phosphate-/citrate buffers are used, since the observed pH changes, and inter-well variability was moderate only, i.e. PermeaPad can be considered fairly resistant to proton permeation using these buffer types.

Subsequently, a set of preliminary PermeaLoop experiments was done to further study the impact of pH and a solubilizing agent on the permeation of dipyridamole from solutions. In permeation studies with pH 4.0 buffer in the donor it was observed that a pH 4.0 in the acceptor caused 9.4-fold and 5.3-fold higher permeation (after 4 h) in comparison to acceptor buffer pH 7.4 and buffer pH 7.4 with 2% TPGS, respectively. There is no doubt that a low pH in the acceptor is useful for achieving high permeation of dipyridamole, and this study indicates that a low pH in the acceptor will be far more effective than the use of solubilisers in the acceptor (Fig. 3).

An ideal set-up the permeation of drug should lead to drug permeation high enough to cause a considerable decrease in the donor concentration. Therefore, in an attempt to achieve a non-steady state flux within reasonable time periods, a change from pH 4.0 to pH 5.0 in the donor and a lower drug-load were tested. The donor pH of 5.0 (instead of pH 4.0) caused a small increase in the fraction of the cumulative amount of drug permeated, but a linear flux was still observed within the first 4 h (donor concentration 5 µg/mL). An extension of the experiment time to 6 h while lowering the start concentration to 2.5 µg/mL triggered the curve to bend within the last two hours of the experiment. Since the permeation relative to the dose was the same for both doses, the permeation on this set-up was considered highly dependant on the dose in the donor. A bend off caused by decreasing donor concentration during an experiment takes time and 4 h might not be enough to observe the non-steady state flux.

The studies in Fig. 3 with a large pH gradient between the donor at pH 4.0 and the acceptors at pH 7.4 with and without 2% TPGS showed high relative standard deviations after four hours (29% and 35%, respectively). It is hypothesized that this variability is due to minor pH gradient differences between repeated experiments: A strong correlation between the final pH values in the donor and acceptor after 4 h and the fraction of dose permeated in 4 h was found in both studies with a steep pH-gradient (Fig. S2, supplement). Based on our observations, it is suggested to avoid steep pH-gradients when studying compounds with a pKa close to the pH-gradient, even when using the PermeaLoop system separated by the biomimetic barrier PermeaPad. Therefore, alternatives to a low pH in the acceptor for increasing permeation were further tested.

3.2. Dipyridamole formulations with pH modifiers

3.2.1. Dipyridamole-content of prepared granules and physical mixture

The dipyridamole content in the prepared granules was quantified using an HPLC assay. Results are shown in Table 3. The dipyridamole content in all formulations was considered acceptable for the combined dissolution/permeation studies.

Dipyridamole's thermodynamic solubility in the chosen donor media

Fig. 4 displays the thermodynamic solubility of dipyridamole in the selected donor media for the combined dissolution/permeation experiment. To account for the potential pH change coming from the fumaric acid within the granules were solubility experiments with 360 µg/mL fumaric acid added to the dissolution medium carried out, equal to the amount of acid added with the 1:2 granules. There were no significant differences observed between 24 h and 48 h results ($p > 0.05$). Addition of the fumaric acid in the 200 mM phosphate buffer and the FaSSIF-MOD100 increased the average solubility of dipyridamole by 5.0% and 8.8% respectively a very minor change, even though the increase was statistically significant ($p < 0.05$) in the FaSSIF-MOD100 medium. The effects of fumaric acid was in both cases rather low, confirming that the pH change of the bulk-solution from fumaric acid does not have major effects on the dipyridamole equilibrium solubility.

3.3. Combined dissolution/permeation experiments on PermeaLoop

3.3.1. Drug recovery during dissolution/permeation experiments on PermeaLoop

A set of combined dissolution/permeation experiments was carried out using the three granule formulations with different fumaric acid content (1.2, 1:1, 1:05 ratio), a physical mixture (1:1 ratio) and the drug alone (designated in Table 3 and Fig. 5 as 1:0 ratio). The concentration of drug dispersed, the concentration of drug dissolved, the concentration of drug permeated, and the amount of drug removed by sampling are plotted in Fig. 5 along with the recovery (sum of the aforementioned concentrations) as percentage of drug dose employed in each experiment. In the dissolution/permeation experiments with

Table 2
Buffer solutions used for permeation studies of dipyridamole on PermeaLoop®. pH values indicated the pH in donor and acceptor at the start (0 h) and end (4 h, 6 h) of experiments. pH values are shown as mean ± SD (n = 3). .

Donor buffer	pH_{0h}	pH_{4h}	Acceptor buffer	pH_{0h}	pH_{4h}
Acetate buffer	4.0	4.0	Acetate buffer	4.0	4.0
Acetate buffer	4.0	5.3	Phosphate buffer	7.4	5.5
Phosphate citrate buffer	4.0	4.5 ± 0.1	Phosphate buffer	7.4	7.1 ± 0.1
Phosphate citrate buffer	4.0	4.5 ± 0.1	2% Vitamin E TPGS in phosphate buffer	7.4	7.2 ± 0.1
Phosphate citrate buffer	4.1	4.0 ± 0.1	Phosphate citrate buffer	4.1	4.0
Phosphate citrate buffer	4.0	4.1	2% Vitamin E TPGS in phosphate citrate buffer	4.1	4.1
Phosphate citrate buffer (5 µg/mL)	5.0	4,9	Phosphate-citrate-buffer	4.0	4.1
Phosphate citrate buffer (2.5 µg/mL)	5.1	4.7	Phosphate citrate buffer	4.1	4.1 ± 0.1

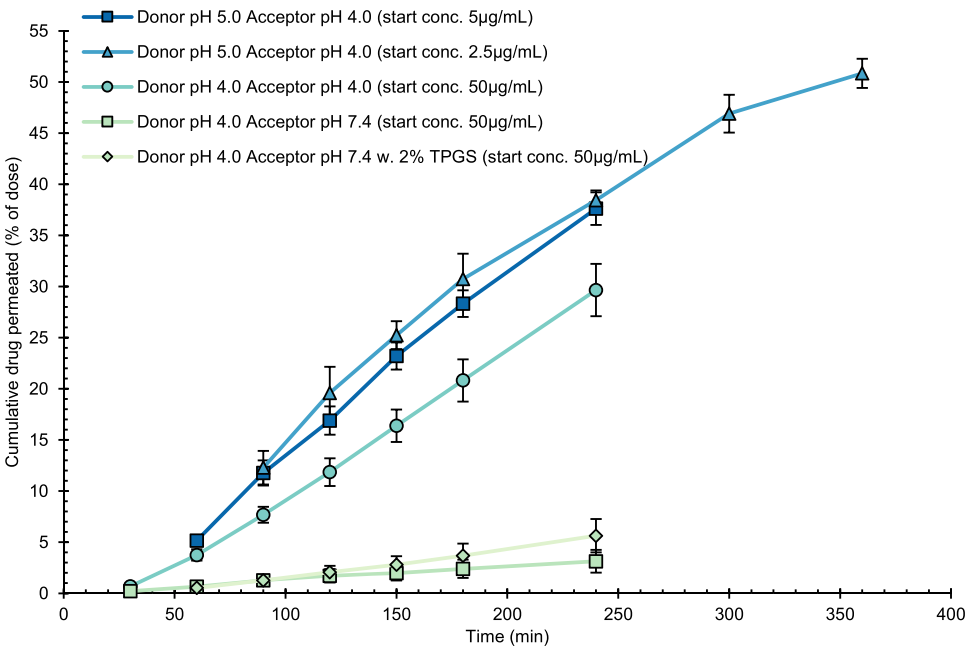

Fig. 3. Permeation experiment with dipyridamole solutions on PermeaLoop across PermeaPad. All experiments have been carried out at 37 °C. pH 4.0 and 5.0 media was 50 mM phosphate-citrate buffer and pH 7.4 medium 50 mM phosphate buffer. In all experiments with pH 4.0 in the donor were the dipyridamole start concentration in the donor 50 µg/mL. Data are reported as mean ± SD (n = 3). The lines in the figure are a guide to the eye.

200 mM phosphate buffer in the donor a high and continuously increasing loss of drug was observed, and the recovery was as little as 13% of the dose after 6 h (Fig. 5). When using FaSSIF as the donor medium, continuous drug loss over time was also observed (recovery below 50%), however, the recovery was higher as compared to plain buffer.

The poor recoveries of 10–25% for the respective doses are attributed to either sticking to the surface of the membrane or being dissolved in the lipid-layer of the barrier (quantitative data not shown). This was supported by its yellow colouration, which may be reasonable bearing the pronounced lipophilicity of dipyridamole in mind. Another reason for the observed poor recoveries may be non-specific adsorption, which appears rather unlikely to cause such high drug-loss since the used tubes were saturated with dipyridamole beforehand, and the formulations with the least dissolved drug had most drug percentage disappearing. Another and more probable reason is that undissolved drug was deposited within the lumen of tubes and permeation cells. This is confirmed by the observation that the dispersed drug disappeared within the first 1–2 h, and the formulations of the highest extent of dissolution also had the highest recovery. Furthermore, undissolved drug particles were observed to come out of the system when the flowrate of the pump was increased after experiments, even though there had not been found any dispersed drug particles in samples during the previous 4 h of the experiments. In Fig. 6 undissolved drug particles can be seen sticking to different parts of the dismantled system.

3.3.2. The dissolution/permeation behaviour of different doses on PermeaLoop

Subsequently, two attempts were made: (1) the dose was lowered and (2) the donor medium was changed in order to test if an increase in the concentration of dissolved drug in the donor would bring about a better performance in terms of the non-specific drug loss. A reduction of the dose from 1.8 and 3.6 mg, respectively , to 0.9 mg dipyridamole increases the relative amount of permeated drug (Fig. 7). However, the dissolution profiles show that at any of the doses, between 15 and 20% of the drug content dissolves at the start of the experiments and super-

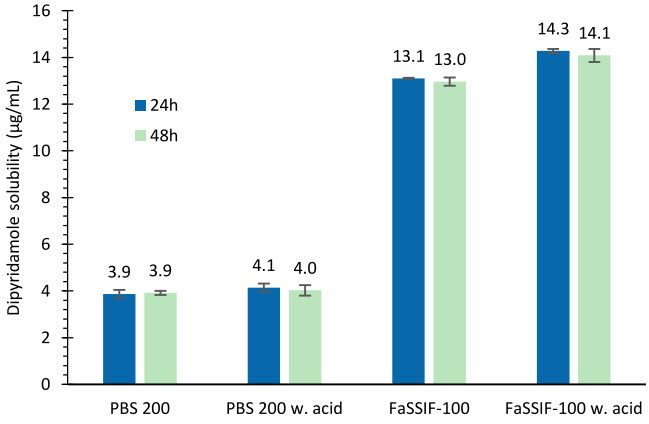

Fig. 4. Solubility of dipyridamole in 200 mM phosphate buffer and $FaSSIF_{MOD100}$ pH 6.5 with and without addition of 360 µg/mL fumaric acid. The experiment was carried out at 37 °C and 150 rpm in a shaking water bath. Samples were centrifuged at 20,000 rcf and filtered through a 0,2 µm Anotop filter before quantification on HPLC. All data are reported as mean ± SD (n = 3). No significant difference observed between samples after 48 and 96 h, $p \leq 0.05$. . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Dipyridamole content in prepared formulations. Data are shown as mean ± SD (n = 3).

Assay of granules Formulation	1:2 API: ACID granule	1:1 API: ACID granule	1:0.5 API: ACID granule	1:0 API: ACID granule	1:1 API: ACID physical mix
Proportion of expected dipyridamole content	91.2 ± 1.8%	97.7 ± 0.9%	98.1 ± 0.3%	102.2 ± 0.9%	99.4 ± 1.5%

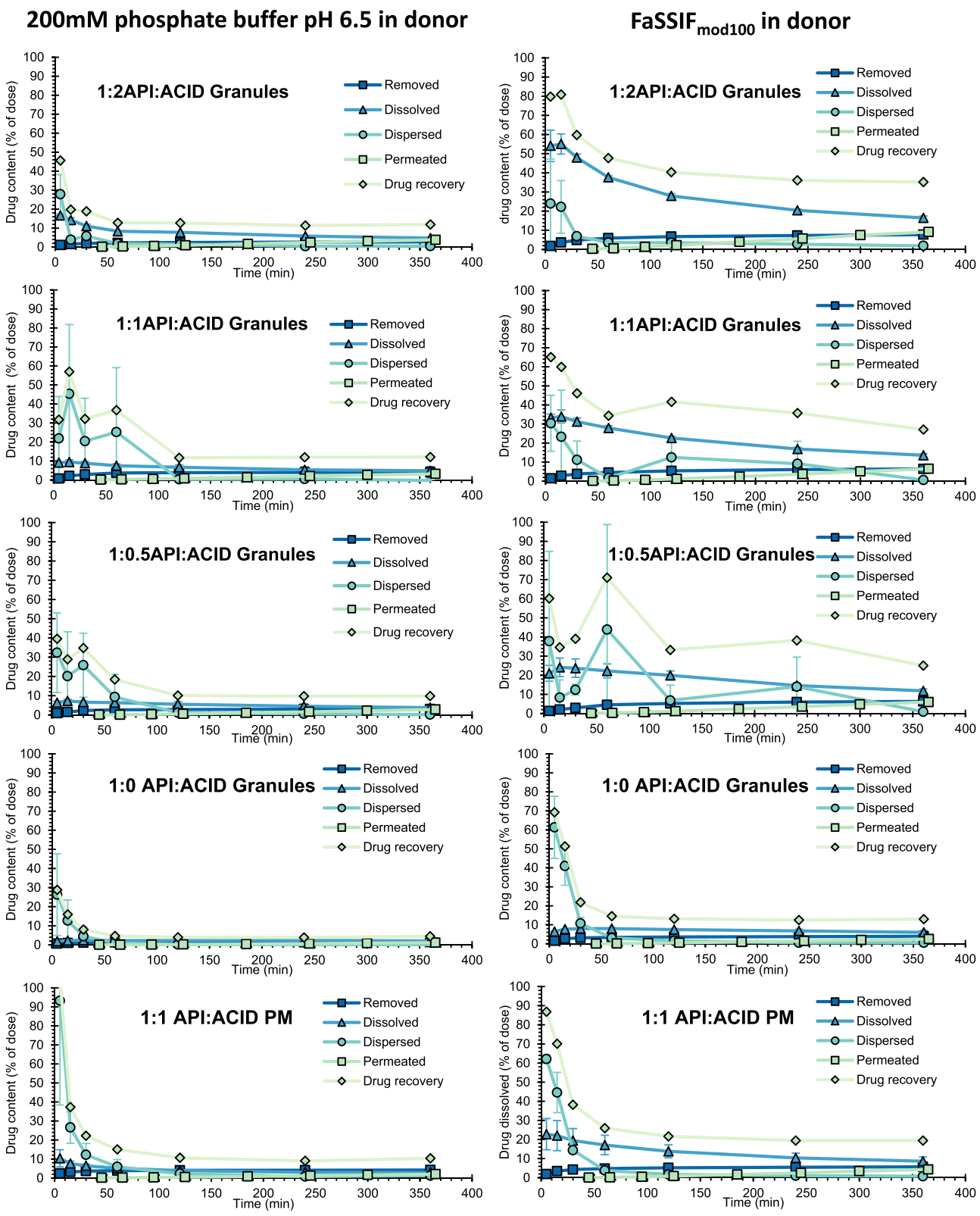

Fig. 5. Distribution of dipyridamole during dynamic dissolution/permeation experiments on PermeaLoop with phosphate buffer pH 6.5 with 0.2% TPGS in the acceptor. 1:0 API:ACID granules in phosphate buffer are every time plotted as mean (n = 2). For the rest are drug removed, dissolved, dispersed and permeated are reported as mean ± SD and drug recovery as mean (n = 3). The lines in the figure are a guide to the eye.

Fig. 6. Picture of separated parts of PermeaLoop right after the experiment with 1:2 API: ACID granules.

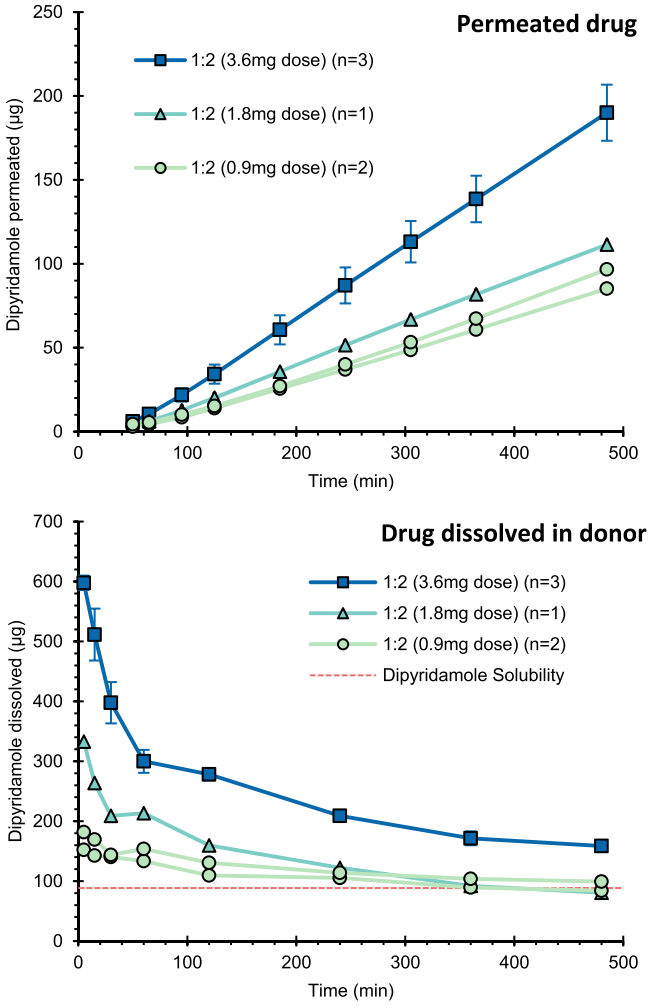

Fig. 7. Dissolution and permeation profiles of three different doses of 1:2 API:acid dipyridamole granules in dynamic dissolution/permeation experiments on PermeaLoop. 200 mM phosphate buffer pH 6.5 and 0.2% TPGS in 200 mM phosphate buffer pH 6.5 was used in the donor and acceptor respectively. The solubility line indicates the solubility of dipyridamole in 200 mM phosphate buffer with fumaric acid. 3.6 mg dose is plotted as mean ± SD and the other doses as singlets. The lines in the figure are a guide to the eye.

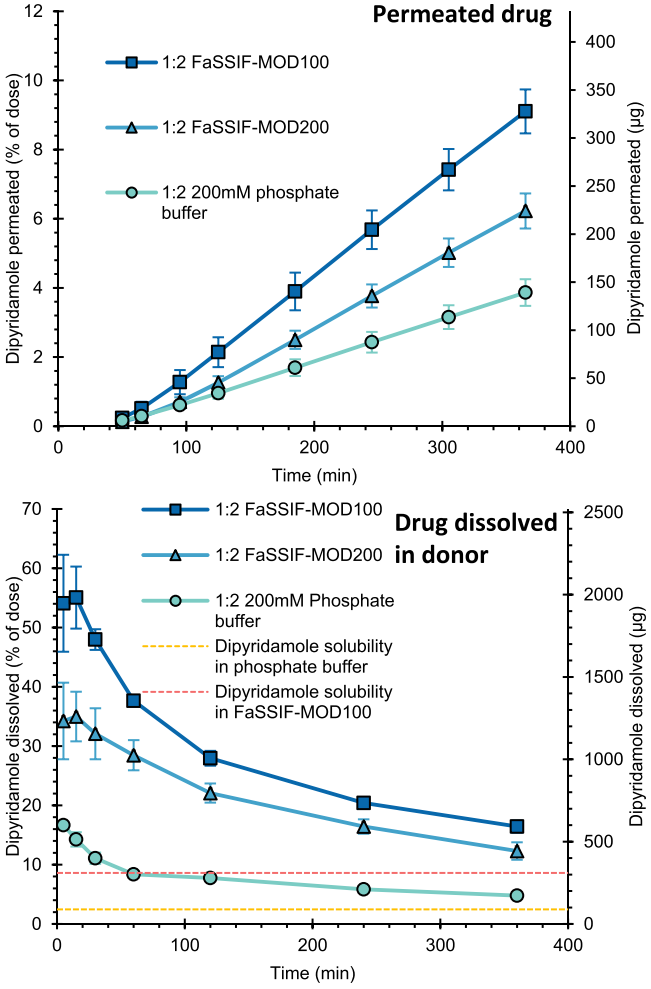

Fig. 8. Dissolution profiles and permeation profiles of 1:2 API:acid dipyridamole granules in dynamic dissolution/permeation experiments on PermeaLoop with various donor mediums. The acceptor medium was 0.2% TPGS in pH 6.5 phosphate buffer with a molarity corresponding to the donor. The solubility lines indicates the solubility of dipyridamole in 200 mM phosphate buffer and $FaSSIF_{MOD100}$ both with fumaric acid. All results were reported as mean ± SD (n = 3). The lines in the figure are a guide to the eye.

saturation is observed. The 1.8 mg dose and 3.6 mg dose precipitate and concentrations decrease to 5%, while the 0.9 mg dose maintains the dissolved amount of drug in the donor stabilised at around 10% of the dose with a mean concentration at 4.58 µg/mL (Fig. 7), which is below the saturation solubility of dipyridamole. The higher the donor concentration is, the larger amount of drug permeate. However, since the gap between saturated and super-saturated dissolved drug is smaller with the lower dose, is it considered less promising to use the lower dose for performance testing of dipyridamole formulations on PermeaLoop.

3.3.3. Influence of biomimetic dissolution medium and different buffer capacities in combined dissolution/permeation tests

The protocol for FaSSIF suggests using 30 mM phosphate buffer as the vehicle for preparation of FaSSIF (Biorelevant.com 2019 FaSSIF/FeSSIF/FaSSGF), however, the fumaric acid in the test formulations

would cause significant pH changes (quantitative data not shown). To adjust the buffer capacity for the 1:2 granules, FaSSIF powder was dissolved in 100 mM and 200 mM phosphate buffer, respectively. The experiments revealed that lowering the buffer capacity and use of FaSSIF increased both the dissolution and permeation (Fig. 8). The effects of decreased buffer capacity are probably due to slower neutralisation of the microenvironmental pH differences, whereas small pH differences in the bulk-solutions will have a minor impact on the dissolution and permeation processes of the dipyridamole formulations. A strong buffer avoids considerable pH changes of the bulk solution, but a strong buffer is not biomimetic since the buffers in-vivo are relatively weak (Hens et al., 2017; Vertzoni et al., 2019).

It is not surprising that the addition of FaSSIF increased the dissolution since it is common knowledge that bile salts are solubilisers. However, the fact that the permeation of dipyridamole also is enhanced in the presence of FaSSIF is more surprising since solubilised drug can't permeate (Buckley et al., 2013). Bile salts may inhibit precipitation, stabilise the super-saturations and thereby increase the permeation (Augustijns and Brewster, 2012). Because of these positive effects, the dissolution/permeation studies were carried out with FaSSIF in 100 mM phosphate buffer (FASSIF $_{mod\ 100}$)as a donor medium.

3.3.4. Monitoring dissolution and permeation profiles over the course of experiments

It was observed both in FaSSIF$_{mod100}$ and in phosphate buffer that all the formulations containing fumaric acid showed initial super-saturation the extents and durations of which correlated very well with fumaric acid content of the formulations, while the formulation without fumaric acid the concentration increased slightly for the first 30 min, from which on it stayed constant during the rest of the experiment (Fig. 9). These observations correlated with the expectation that the formulations containing fumaric acid will supersaturate and precipitate, while the formulation with the drug alone will reach the solubility and stay constant.

The permeation profiles show a lag-time until the 65 min sampling point for all formulations before a steady-state flux correlating with the dissolution profiles is observed for the rest of the experiments (Fig. 9), which is in contrast to the desired bend off of the curves in dynamic scenarios (Sironi et al., 2017A). The reason why a bend off of the curve was not observed in any of the curves is that only a maximum of 9.1 ± 0.6% of the dose permeated during 6 h for the 1:2 granules in FaSSIF$_{mod100}$, which is far less than the approx..50% that were necessary to observe a bend off with the solution experiments described above (Fig. 2), and the 53.7% dipyridamole absorption after 6 h in

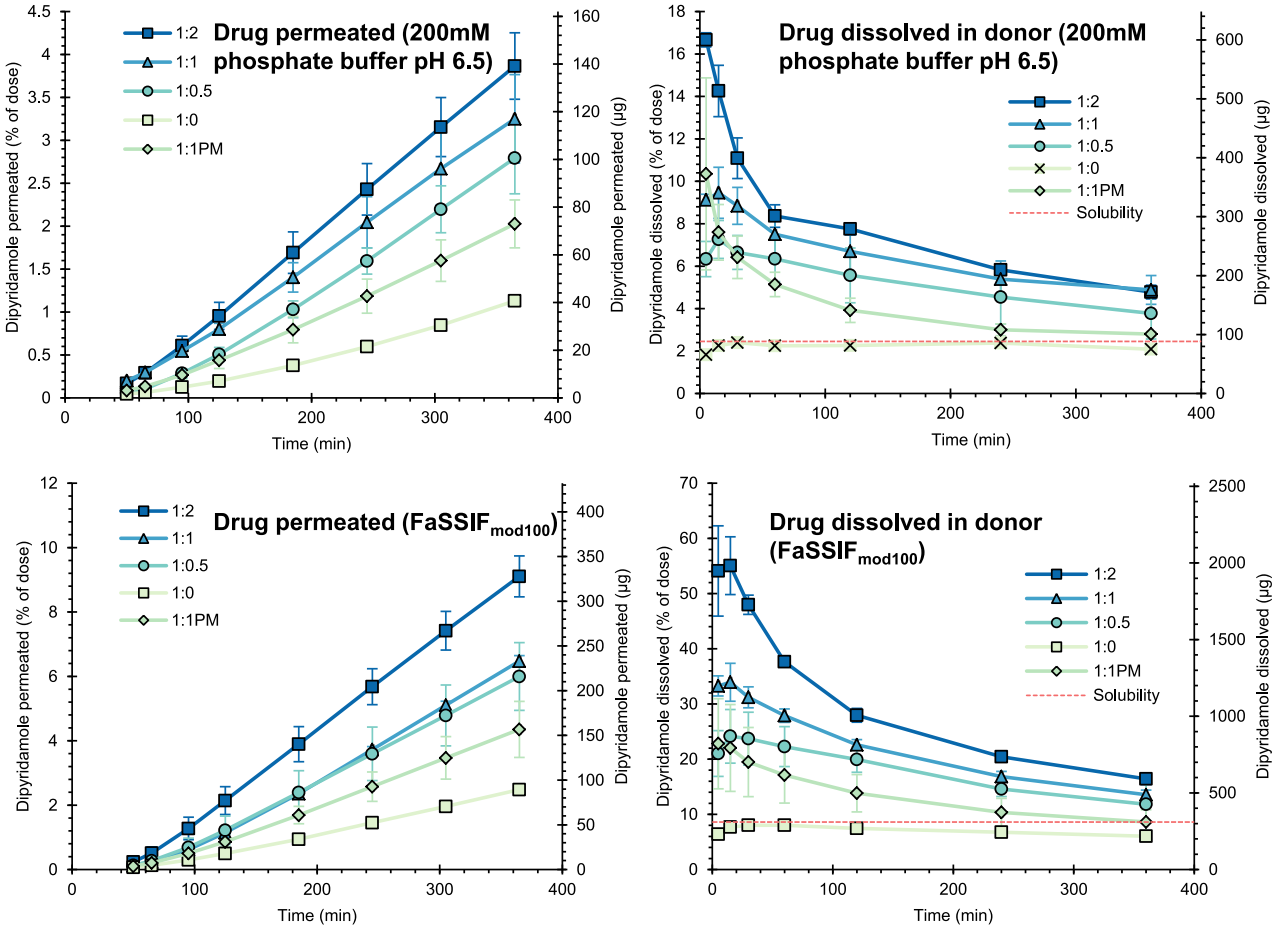

Fig. 9. Direct comparison of dissolution and permeation profiles of the five prepared dipyridamole formulations on PermeaLoop. Either 200 mM phosphate buffer pH 6.5 (A, B) or FaSSIF$_{mod100}$ pH 6.5 (C, D) were used in the donor, and 0.2% TPGS in pH 6.5 phosphate buffer with a molarity corresponding to the donor in the acceptors. The solubility lines indicates the solubility of dipyridamole in the corresponding buffer (200 mM phosphate buffer and FaSSIF$_{mod100}$) both with fumaric acid. All values are plotted as mean ± SD (n = 3) except 1:0API: ACID the formulation in phosphate buffer, which is plotted as mean (n = 2). The lines in the figure are a guide to the eye.

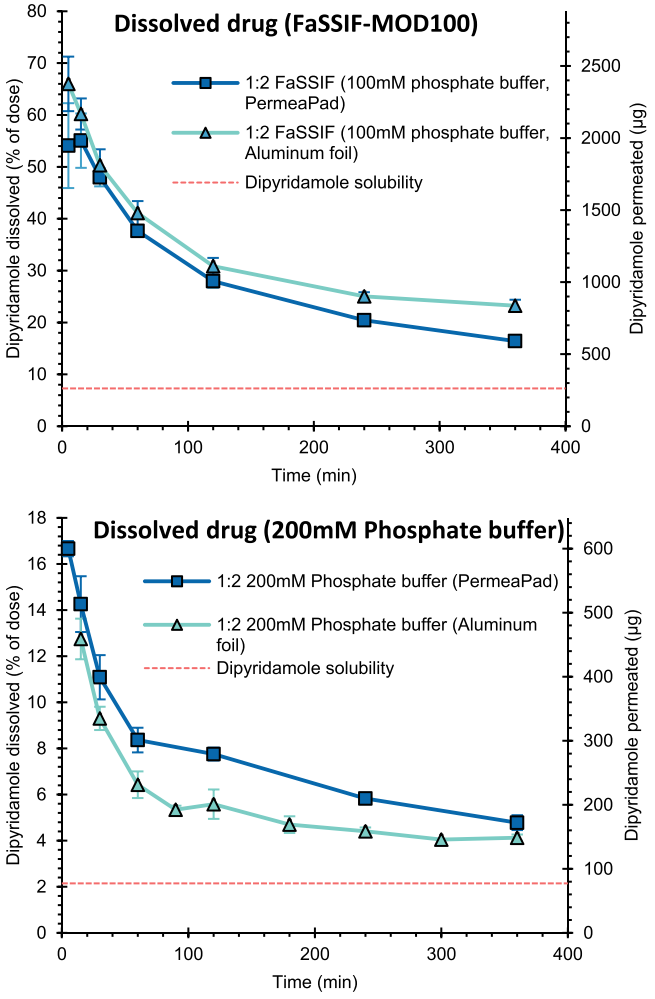

Fig. 10. Dissolution profiles of 1:2 dipyridamole:acid granules on PermeaLoop with separation of the donor and acceptor by either PermeaPad or aluminium foil. Either 200 mM phosphate buffer or FaSSIF$_{mod100}$ were used in the donor. The solubility lines indicates the solubility of dipyridamole in the corresponding buffer (200 mM phosphate buffer and FaSSIF$_{mod100}$) both with fumaric acid. Data are reported as mean ± SD.

human in-vivo studies with the commercial dipyridamole tablet Persantin® (Nielsen-Kudsk and Pedersen, 1979).

3.3.5. Drug dissolution vs. combined dissolution/permeation on Permealoop

The effect of the absorptive sink on the degree of supersaturation was tested by using the same experimental set-up, but impeding permeation. This was achieved by using aluminium foil to separate the donor and acceptor compartments. In FaSSIF$_{mod100}$ the super-saturations in the donor for the 1:2 granules was practically unchanged, decreasing with a slightly slower rate than if PermeaPad had been used (Fig. 10). The degree of precipitation in the two cases is considered to be equal, since the difference between the curves corresponds to the amount of drug leaving the donor by permeation. In contrast in phosphate buffer the initial supersaturation did precipitate faster in the experiment with aluminium foil compared to the similar experiment with PermeaPad (with absorptive sink). This indicates that the absorptive sink works as a precipitation inhibitor, but only if there is no redistribution into solubiliser micelles. Obviously FaSSIF$_{mod100}$ might work as a potent precipitation inhibitor (parachute effect) to overrule the absorptive sink effects.

In a similar study by Sironi and colleagues (Sironi et al., 2017b), the initial super-saturation of fenofibrate nanoparticles in side-by-side cells was observed only when the donor and acceptor were separated by a permeation barrier, in contrast to absence of super-saturation with aluminium foil. Various difference in the experimental set-up should be mentioned like the first sampling after 15 min.

3.3.6. In-vitro in-vivo correlation of Permealoop with rat studies in comparison to a more traditional D/P-system

The experiment with phosphate buffer in the donor on PermeaLoop (as by the 2 h samples) revealed the same performance ranking as the in-vivo study, and the same one with an earlier described the D/P system (Mizoguchi et al., 2018). The PermeaLoop experiment with FaSSIF$_{mod100}$ showed the same ranking if one disregards, that one of the replicates had an unexpected and almost 2-fold higher permeation than the other replicates (Fig. 11), which may be regarded as an outlier. Nevertheless, the PermeaLoop set-up with FaSSIF$_{mod100}$ in the donor at the final time-point provides more than two-fold higher permeation of the 1:2 granules compared to the other in-vitro tools, which also resulted in a superior IVIVC compared to the alternative set-ups. These results highly support the theory that more substantial removal of the drug by permeation is necessary to appropriately predict the in-vivo performance ranking of SSDDS with in-vitro tools.

4. Conclusion

Appropriate conditions for dynamic dissolution/permeation experiments for enabling formulations of dipyridamole with pH-modifying agents on PermeaLoop were identified. The method development process contributes to a better understanding of how micro- and macro-environmental pH and the presence of solubilisers affect the permeation of weakly basic drug compounds across a biomimetic barrier like PermeaPad.

Dynamic dissolution/permeation experiments were performed, and it was possible to monitor simultaneous dissolution and permeation processes resulting in a performance ranking of the tested formulations. The rankings obtained with PermeaLoop were comparable to those observed in-vivo and also compared to a traditional D/P system. However, with FaSSIF$_{mod100}$ in the donor, a two-fold increase in permeation from the high acid granules was observed, and the IVIVC was superior to that obtained with the D/P system under similar conditions.

While there is room for further optimisation, PermeaLoop appears promising as an in-vitro predictive tool for acid-modified enabling formulations of weak bases. This work yielded helpful insights for its further development. Aspects of optimization include to enhance the flux values in order to allow fully dynamic scenarios with a bend off of the permeation curve to induce the precipitation inhibition by permeation.

Challenges observed with the current PermeaLoop prototype and the model dipyridamole formulations were, that dispersed drug may get stuck inside the system. These observations are helpful for the improvement of the current design of the combined dissolution/permeation tool.

CRediT authorship contribution statement

Jonas Borregaard Eriksen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Roman**

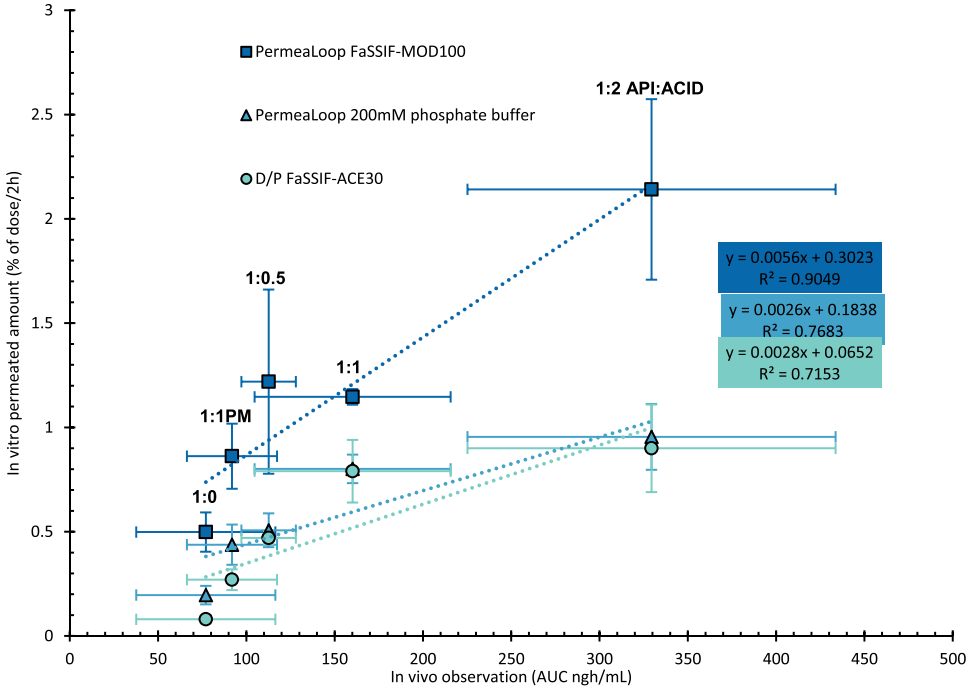

Fig. 11. Correlations between the permeated amount of dipyridamole with in-vitro tools and AUC observed from in-vivo rat-studies. The in-vivo data and D/P results are from Mizoguchi et al., 2018. All data are reported as Mean ± SD (In-vitro $n = 3$, in-vivo $n = 4$). The lines in the figure are a guide to the eye.

Messerschmid: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - original draft. **Mikkel Lund Andersen:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Koichi Wada:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft. **Annette Bauer-Brandl:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing - review & editing. **Martin Brandl:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft, Writing - review & editing.

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Roman Messerschmid and Koichi Wada are employees of Nippon Boehringer Ingelheim Co. Ltd. Annette Bauer-Brandl is the owner of the trade-mark PermeaLoop.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2020.105532.

References

Augustijns, P., Brewster, M.E., 2012. Supersaturating drug delivery systems: fast is not necessarily good enough,. J. Pharm. Sci. 101 (1), 7–9. https://doi.org/10.1002/jps.22750.

Bevernage, J., Brouwers, J., Annaert, P., Augustijns, P., 2012. Drug precipitation-permeation interplay: supersaturation in an absorptive environment. Eur. J. Pharm. Biopharm. 82 (2), 424–428. https://doi.org/10.1016/j.ejpb.2012.07.009.

Biorelevant.com, FaSSIF/FeSSIF/FaSSGF.https://biorelevant.com/fassif-fessif-fassgf/buy/(accessed December 27, 2019).

Boyd, B.J., Bergström, C.A.S., Vinarov, Z., Kuentz, M., Brouwers, J., Augustijns, P., Brandl, M., Bernkop-Schnürch, A., Shrestha, N., Préat, V., Müllertz, A., Bauer-Brandl, A., Jannin, V., 2019. Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems. Eur. J. Pharm. Sci. 137. https://doi.org/10.1016/j.ejps.2019.104967. art. No. 104967.

Buckley, S.T., Frank, K.J., Fricker, G., Brandl, M., 2013. Biopharmaceutical classification of poorly soluble drugs with respect to "enabling formulations" Eur. J. Pharm. Sci. 50 (1), 8–16. https://doi.org/10.1016/j.ejps.2013.04.002.

Champagne, E.T., 1989. Low gastric hydrochloric acid secretion and mineral bioavailability. Adv. Exp. Med. Biol. 249, 173–184.

Di Cagno M, Bibi, H.A., Bauer-Brandl, 2015. A New biomimetic barrier Permeapad™ for efficient investigation of passive permeability of drugs. Eur. J. Pharm. Sci. 73, 29–34. https://doi.org/10.1016/j.ejps.2015.03.019.

Hens, B., Tsume, Y., Bermejo, M., Paixao, P., Koenigsknecht, M.J., Baker, J.R., Hasler, W.L., Lionberger, R., Fan, J., Dickens, J., Shedden, K., Wen, B., Wysocki, J., Loebenberg, R., Lee, A., Frances, A., Amidon, G., Yu, A., Benninghoff, G., Salehi, N., Talattof, A., Sun, D., Amidon, G.L., 2017. Low Buffer Capacity and Alternating Motility along the Human Gastrointestinal Tract: implications for in Vivo Dissolution and Absorption of Ionizable Drugs. Mol. Pharm. 14 (12), 4281–4294. https://doi.org/10.1021/acs.molpharmaceut.7b00426.

Ku, M.S., Dulin, W., 2012. A biopharmaceutical classification-based Right-First-Time formulation approach to reduce human pharmacokinetic variability and project cycle time from First-In-Human to clinical Proof-Of-Concept. Pharm. Dev. Technol. 17 (3), 285–302. https://doi.org/10.3109/10837450.2010.535826.

Lahner, E., Annibale, B., Fave G., Delle, 2009. Systematic review: heliocobacter pylori infection and impaired drug absorption. Aliment. Pharmacol. Ther. 29 (4), 379–386. https://doi.org/10.1111/j.1365-2036.2008.03906.x.

Levy, G., 1961. Comparison of dissolution and absorption rates of different commercial aspirin tablets. J. Pharm. Sci. 50 (5), 388–392. https://doi.org/10.1002/jps.2600500503.

Lipp, R., 2013. The innovator pipeline: bioavailability challenges and advanced oral drug delivery opportunities. Am. Pharmaceutical Rev. 16 (3).

Mizoguchi, M., Kataoka, M., Yokoyama, K., Aihara, R., Wada, K., Yamashita, S., 2018. Application of an In Vitro Dissolution/Permeation System to Early Screening of Oral Formulations of Poorly Soluble, Weakly Basic Drugs Containing an Acidic pH-Modifier. J. Pharm. Sci. 107 (9), 2404–2410. https://doi.org/10.1016/j.xphs.2018.05.009.

Mudie, D.M., Shi, Y., Ping, H., Gao, P., Amidon, G.L., Amidon, G.E., 2012. Mechanisticanalysis of solute transport in an in vitro physiological two-phase dissolutionapparatu. Biopharm. Drug Dispos. 33, 378–402. http://dx.doi.org/10.1002/bdd.1803.

Nielsen-Kudsk, F., Pedersen, A.K., 1979. Pharmacokinetics of Dipyridamole. Acta Pharmacol. Toxicol. (Copenh) 44 (5), 391–399. https://doi.org/10.1111/j.1600-0773.1979.tb02350.x.

Russell, T.L., Berardi, R.R., Barnett, J.L., O'Sullivan, T.L., Wagner, J.G., Dressman, J.B., 1994. pH-Related Changes in the Absorption of Dipyridamole in the Elderly. Pharmaceutical Res.: An Official J. Am. Assoc. Pharmaceutical Scientists 11 (1), 136–143. https://doi.org/10.1023/A:1018918316253.

Shelton, M.J., Adams, J.M., Hewitt, R.G., Steinwandel, C., DeRemer, M., Cousins, S., Morse, G.D., 1997. Effects of spontaneous gastric hypoacidity on the pharmacokinetics of zidovudine and didanosine. Pharmacotherapy 17 (3), 438–444.

Sironi, D., Christensen, M., Rosenberg, J., Bauer-Brandl, A., Brandl, M., 2017a. Evaluation of a dynamic dissolution/permeation model: mutual influence of dissolution and barrier-flux under non-steady state conditions. Int. J. Pharm. 522 (1–2), 50–57. https://doi.org/10.1016/j.ijpharm.2017.03.002.

Sironi, D., Rosenberg, J., Bauer-Brandl, A., Brandl, M., 2017b. Dynamic dissolution-/permeation-testing of nano- and microparticle formulations of fenofibrate. Eur. J. Pharm. Sci. 96, 20–27. https://doi.org/10.1016/j.ejps.2016.09.001.

Sironi, D., Rosenberg, J., Bauer-Brandl, A., Brandl, M., 2018. PermeaLoop™ a novel in vitro tool for small-scale drug-dissolution/permeation studies. J. Pharm. Biomed. Anal 156, 247–251. https://doi.org/10.1016/j.jpba.2018.04.042.

Taniguchi, C., Inoue, R., Kawabata, Y., Yamashita, K., Wada, K., Yamauchi, Y., Yamada, S., Onoue, S., 2012. Novel formulations of dipyridamole with microenvironmental pH-modifiers for improved dissolution and bioavailability under hypochlorhydria. Int. J. Pharm. 434 (1–2), 148–154. https://doi.org/10.1016/j.ijpharm.2012.05.040., pp.

Vertzoni, M., Augustijns, P., Grimm, M., Koziolek, M., Lemmens, G., Parrott, N., Pentafragka, C., Reppas, C., Rubbens, J., Van Den Abeele, J., Vanuytsel, T., Weitschies, W., Wilson, C.G., 2019. Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption: an UNGAP review. Eur. J. Pharm. Sci. 134, 153–175. https://doi.org/10.1016/j.ejps.2019.04.013.

Zhou, R., Moench, P., Heran, C., Lu, X., Mathias, N., Faria, T.N., Wall, D.A., Hussain, M.A., Smith, R.L., Sun, D., 2005. pH-Dependent dissolution in Vitro and absorption in Vivo of weakly basic drugs: development of a canine model. Pharm. Res. 22 (2), 188–192. https://doi.org/10.1007/s11095-004-1185-3.