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PermeaLoop™, a novel in vitro tool for small-scale drug-dissolution/permeation studies

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Dissolution; Permeation; Amorphous solid dispersion; ABT-869; dynamic; non-steady state; non-sink

Graphical abstract

Highlights

- Novel dissolution/permeation setup with more biorelevant area-to-volume ratio.
- Permeation of a realistic fraction of the dose within physiological time frame feasible.
- Dynamic scenario with interdependent dissolution and permeation profiles.
- Large permeation area allows dissolution to be rate-limiting, similar to in vivo.
**Abstract**

Here we report first experiences with the novel in vitro dissolution/permeation setup PermeaLoop™. It was designed to overcome current limitations of in vivo predictive dissolution testing of enabling formulations, such as lack of relevant absorptive drag to allow for meaningful interplay between dissolution and permeation, as it is occurring in vivo. We propose a novel setup with a high area-to-volume ratio and report as a model case the dissolution/permeation behavior of an enabling formulation of the poorly soluble and poorly permeable drug ABT-869. Mini tablets consisting of an amorphous solid dispersion were tested at a downscaled clinically relevant dose. At room temperature, release was fast, and more than 35% of the employed dose permeated within 6 hours. In consequence, the amount in the donor decreased significantly. By contrast, only 9% of the employed dose was released when performing the experiment at 35 °C. Still, most of the released drug permeated into the acceptor (> 80%), and the permeation rate was release-dependent and vice versa, i.e. the scenario was highly dynamic. Hence, due to a sufficiently large permeation area the dissolution step became rate-limiting. Therefore, PermeaLoop™ is regarded a promising tool for evaluating enabling formulations.

1. **Introduction**

A large and increasing fraction of new drug compounds in the pipelines of pharmaceutical industry is poorly soluble and, thus, requires formulations that increase their inherently low bioavailability [1]. Amorphous solid dispersions (ASD) are an example of such enabling formulations and contain the active pharmaceutical ingredient (API) in the amorphous state dispersed in a polymeric matrix [2]. Additionally, surfactants may be present if required by the manufacturing process.

The dissolution of an ASD in aqueous medium is a complex process, which eventually leads to a supersaturated solution (with regard to molecularly dissolved drug) and which likely is the trigger for its enhanced oral bioavailability [3,4]. Moreover, colloidal structures such as surfactant-micelles and polymer-complexes may form and solubilize the drug [5,6]. Physiological bile salts and lipids may give rise to additional colloidal states [7]. However, there are indications that the permeation behavior of truly dissolved (i.e. molecularly dissolved) and apparently dissolved drug species (including solubilized drug) differs widely [8,9]. Such insights led to a sharpened awareness about our current biopharmaceutical toolbox lacking an extension with approaches suited to capture the transitions of API between the solid, the truly dissolved, and the solubilized state, which are expected to occur in a highly dynamic and interdependent scenario such as in vivo [10]. For example, a
relatively small degree of true supersaturation (i.e. slightly enhanced concentration of molecularly dissolved drug) might be sufficient for an increase of its bioavailability, provided that drug molecules are continuously removed by absorption and, thus, continuous release of new drug from the reservoir (undissolved drug, drug in colloidal structures) is possible. To this end, the combination of dissolution testing with simultaneous permeation processes appears to be a promising in vitro tool for selection and evaluation of enabling formulations in pre-formulation and early formulation development.

However, a major limitation of current dissolution/permeation testing is the restricted permeation area of the currently used designs. The physiological area-to-volume ratio is estimated to be between 1.9 cm\(^{-1}\) and 2.3 cm\(^{-1}\) [11]. In contrast, the area-to-volume ratio of most dissolution/permeation setups described in literature is less than 0.5 cm\(^{-1}\) [12] and, hence, these setups do not appropriately represent the balance between dissolution and absorption rates found in vivo. Accordingly, only a minor fraction of the released drug can permeate within physiological time frames, and thus the impact of permeation on the dissolution process is negligible. As a consequence, the evaluation of permeability is often based on comparison of steady state flux values, which does not fully capture the dynamic nature of the dissolution processes. Moreover, a small permeation area can make the permeation step rate-limiting even though, in vivo, the rate-limiting step would be dissolution from a certain formulation.

We therefore designed a combined dissolution/permeation setup, PermeaLoop™, which has a novel geometry providing a high area-to-volume ratio. We tested its potential to bring about the drug release and absorption processes of drug from an ASDs in a dynamic, i.e. interdependent, manner. For this purpose, an ASD of the poorly soluble (solubility: 0.03 µg/mL) and poorly permeable model compound ABT-869 (class IV according to the Biopharmaceutics Classification System (BCS)) was used as an example.

2. Materials and methods
2.1. Chemicals

ABT-869 (see Fig. 1 for molecule structure), placebo extrudate and mini tablets were provided by AbbVie GmbH & Co. KG. (Ludwigshafen, Germany). The mini tablets consisted of a milled and compressed ASD of ABT-869 which had been prepared by hot melt extrusion (see Table 1 for the composition). For a general review of the hot melt extrusion process; please consult [13]. Vitamin E TPGS NF grade (TPGS) was obtained from Isochem S.A.S. (Vert-le-Petit, France), and acetonitrile (ACN) and buffer salts were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Media

Phosphate-buffered saline (PBS) pH 6.5 was prepared according to a protocol by biorelevant.com (FaSSIF-V1, blank buffer) and contained 0.42 g L⁻¹ sodium hydroxide pellets, 3.95 g L⁻¹ monobasic sodium phosphate monohydrate and 6.19 g L⁻¹ sodium chloride. PBS pH 7.4 was prepared with the same protocol but was adjusted to pH 7.40 ± 0.05 with 1 N sodium hydroxide solution. 0.2 % (w/v) TPGS solution was prepared by dissolving TPGS in PBS pH 7.4 under stirring overnight. Ammonium formate buffer (25 mM, pH 5) for HPLC analysis was prepared by dissolving ammonium formate in highly purified water and adjusting the pH to 5.00 ± 0.05 with formic acid.

2.3. High Performance Liquid Chromatography (HPLC)

ABT-869 was quantified with an Agilent 1100 HPLC system (Agilent Technologies Deutschland GmbH, Böblingen, Germany) consisting of pump, degasser, autosampler, column oven and diode array detector. A Gemini® NX-C18 column (3 µm particle size, 150 mm x 3 mm, 110 Å pore size) (Phenomenex, Torrance, CA, USA) was used as column at a column temperature of 30°C and the flow
rate was 0.5 mL/min. ACN and ammonium formate (25 mM; pH 5) were used as eluents and a gradient was used: initially, the mobile phase consisted of 30 % ACN; the fraction of ACN was increased to 62.5 % over 15 minutes and then to 90 % over 3 minutes; it was then kept at 90 % for 2 minutes and finally the column was re-equilibrated for 6 minutes with 30 % ACN and 70 % ammonium formate. ABT-869 was detected at a wavelength of 270 nm after a run time of approx. 12.5 minutes. The injection volume was 60 µL.

All standards were prepared in ACN-water (50:50, v/v). The calibration curve was prepared in the range of 0.04 – 45 µg/mL. For quality control purposes, three different dilutions were prepared from a separate stock solution and injected twice (bias < 10 % and coefficient of variation < 8 % for the lower end of the calibration curve).

2.4. Combined dissolution/permeation studies using PermeaLoop™

The PermeaLoop™ setup consists of three parts: donor and acceptor reservoirs (beakers with internal diameter of 3 cm), peristaltic pump, and custom-made permeation cells (Fig. 2). A volume of 20 mL PBS pH 6.5 was used as dissolution medium. PBS was chosen as dissolution medium (instead of FaSSIF) in order to introduce no additional solubilizing agents than those already present in the formulation itself. As acceptor medium, a volume of 35 mL 0.2 % TPGS solution was used. TPGS was included in the acceptor medium for ensuring excess solubilization capacity of ABT-869 and, thus, sink conditions in the acceptor. Both compartments were stirred at 200 rpm with a rod-shaped stirbar (13 mm x 3 mm) on a multi-position magnetic stirrer (MIXdrive 6 MTP, 2mag AG, Munich, Germany). The media were continuously pumped through the permeation cells at a flow rate of 1 mL/min with a peristaltic pump (MCP Standard, Cole-Parmer GmbH, Wertheim, Germany). The custom-made permeation cells consist of three aluminum blocks in which a narrow, spiral-shaped channel has been milled out (Fig. 3). Between each cell, a hydrophilic cellulose hydrate membrane (Pütz GmbH, Taunusstein, Germany) was placed, leading to a donor compartment (middle cell) and
two acceptor compartments (top and bottom cell). The total permeation area was 27.64 cm², and hence, the ratio between the permeation area and the donor volume was 1.38 cm⁻¹. The cells were perfused with a co-current flow, and the media were re-circulated into the respective vessel.

Experiments were performed both at room temperature and at temperature close to body temperature. For experiments at 35 °C, the beakers were inserted into a custom-made thermostatted aluminum block, and thermostatted aluminum blocks were placed between the permeation cells. Additionally, the sandwich consisting of permeation cells and heating blocks was placed inside an insulation box made of polystyrene.

The system was rinsed with medium by starting the pump and the mounted membrane was hydrated at least 20 minutes prior to adding the tablet to the donor beaker. Samples were taken every 45 minutes from both the donor and the acceptor vessel; two additional samples (5, 20 minutes) were taken from the donor vessel. A volume of 200 µL was withdrawn from the donor vessel, of which 50 µL were diluted directly with 50 µL ACN in order to determine the combined amount of dispersed and apparently dissolved drug; the rest was centrifuged for 5 minutes at 21,000 rcf and 23°C or 35°C respectively (Centrifuge 5424R, Eppendorf AG, Hamburg, Germany). A volume of 100 µL was sampled from the supernatant and diluted with 100 µL ACN in order to determine the amount of apparently dissolved drug. From the acceptor, samples of 500 µL were taken and diluted with the same volume of ACN. While all samples withdrawn from the acceptor compartment were replaced with the same amount of fresh TPGS solution, the samples withdrawn from the donor were not replaced. All experiments were run over 6 hours and performed in triplicate.

The mini tablets investigated contained 780 µg ABT-869. One mini tablet in 20 mL donor volume is equivalent to a dose of 9.8 mg API in 250 mL of fluid, which matches a clinical dose of ABT-869 which is between 2.5 mg and 17.5 mg [14].

2.5. Solubilizing capacity of the extrudate formulation
The solubilizing capacity of the components of the extrudate formulation (sorbitan monolaurate and copovidone) was evaluated by dispersing approx. 30 mg placebo extrudate and 5 mg ABT-869 in 5 mL PBS. The dispersion was stirred at 450 rpm both at room temperature and at 35°C. After 6 h, 22 h and 48 h, a sample of 500 µL was taken and the amount of apparently dissolved drug was determined as described for the combined dissolution/permeation experiments (cf. 2.4). The experiment was performed in triplicate.

2.6. Data evaluation

For each time interval, the flux value of the specific interval was calculated as

\[ J = \frac{\Delta Q}{\Delta t \cdot A} \]

where \( \Delta Q \) is the amount of API permeated within the time interval, \( \Delta t \) is the time interval, and \( A \) is the permeation area.

For comparison of data sets, a paired, two-tailed Student's t-test was applied. A value of \( p \leq 0.05 \) was considered as significantly different.

3. Results and discussion

At room temperature, the mini tablet disintegrated quickly and formed a milky dispersion in the donor compartment. Within 45 minutes, the combined amount of dispersed and apparently dissolved drug in the donor reached its maximum (Fig. 4A). Subsequently, it continuously decreased (by 55 % after 6 hours). Likewise, the amount of apparently dissolved drug (determined after centrifugation) decreased continuously after the first 45 minutes, but to a lesser extent than the combined amount (decrease by 35 %). Still, the cumulative amount permeated into the acceptor
compartment increased initially in a close-to-linear manner over time and eventually exceeded the amount dispersed in the donor medium (after 6 hours).

The decrease of the dispersed amount in the donor can have two reasons: sedimentation of the formulation and (re-)dissolution as a consequence of permeation. Dissolution of an amorphous solid dispersion leads to a metastable state and consequently, precipitation of the drug is likely to occur. It has, however, been shown for this formulation that the dispersion is stable over several hours under comparable conditions (data not shown). Therefore, it is more likely that the disappearance of finely dispersed drug is caused by the continuous dissolution and permeation into the acceptor compartment. This hypothesis is supported by the fact that the sum of the dispersed/dissolved amount, the permeated amount and the amount removed from the donor by sampling, remained constant after 45 min.

The overall recovery of drug was 76 % ± 4 % after 6 hours (Fig. 4B), which is regarded acceptable for a very poorly soluble drug like ABT-869 (solubility = 0.03 µg/mL). In such a case, incomplete release from an amorphous solid dispersion is not unusual and can, for instance, be caused by fast recrystallization. Furthermore sedimentation of undissolved particles as a consequence of the moderate stirring rate cannot be excluded. In contrast, non-specific adsorption to the devices is unlikely to be the cause since ABT-869 had shown only moderate propensity to adsorb to plastic material in preliminary experiments (data not shown) and the rubber-tubing had been pre-saturated.

In total, 37 % ± 3 % of the employed dose were found permeated into the acceptor within 6 hours. This is a considerable amount, and to our knowledge, a similar permeated fraction has not yet been reported for combined dissolution/permeation testing of dosage forms with a clinically relevant dose (per donor volume). This corresponds to an accumulation of nearly 50 % of the total amount of drug recovered after 6 hours in the acceptor compartment, and thus, the permeation exerted a significant influence on the dissolution profile.
Finally, comparison of the donor concentration and the acceptor concentration reveals that the concentration in the acceptor exceeded the concentration of apparently dissolved drug in the donor compartment after 4 hours (Fig. 4C). Yet, flux values at 5.5 hours were only slightly lower than at 2 hours (2.0 ± 0.2 µg h\(^{-1}\) cm\(^{-2}\) vs. 1.6 ± 0.4 µg h\(^{-1}\) cm\(^{-2}\)). This is taken as an indication that the solubilization by TPGS in the acceptor is sufficient for ensuring a constant driving force and (close to) sink permeation of ABT-869. Moreover, it is evident that, under the chosen experimental conditions, the flux follows the donor profile and that dissolution/permeation occurs under dynamic, non-steady state conditions.

As opposed to the experiment at room temperature, the mini tablet hardly disintegrated at 35 °C and the donor medium remained clear. It appears that the solubilizing capacity of the extrudate formulation depends on the temperature: when dispersing ABT-869 and placebo extrudate in PBS, the concentration of apparently dissolved drug determined after 48 h (equilibrium) was higher at room temperature than at 35°C (18.1 ± 2.0 µg/mL vs. 9.9 ± 1.1 µg/mL; no significant difference between values measured after 22 h and after 48 h). The reason for this dependency on the temperature is currently under investigation and is out of scope of this short communication. Nevertheless, the experiment gives valuable insights into the usefulness of the chosen dissolution/permeation setup.

Since the mini tablet did not disintegrate, the amounts released from the mini tablet were small (Fig. 5A) and most of the drug remained undispersed (Fig. 5B). Still, significant amounts permeated into the acceptor compartment (8 % ± 3 % of the total dose), and after only two hours the amount in the acceptor compartment exceeded the combined amount of dispersed and dissolved drug in the donor compartment. Considering that the recovery after 6 hours was only 9 % ± 3 %, more than 80 % of the released drug had permeated into the acceptor compartment. Hence, in this case, the rate-limiting process obviously was dissolution – a phenomenon hardly ever observed with common dissolution/permeation setups due to the limited permeation area.
The dynamic nature of the permeation process becomes even more evident at 35 °C than at room temperature: at around 2 hours, the flux is highest and drops to approx. 13 % of the peak value within the following 4 hours (Fig. 5C). Surprisingly, the highest concentration observed at 35 °C dropped to 17 % of the highest concentration observed at room temperature, while the highest flux observed at 35 °C dropped only to 47 % of the highest flux observed at room temperature. This discrepancy might indicate that at room temperature, when the release rate is fast, the permeation might still be compromised by an insufficiently large permeation area. However, this might not hold true for BCS class II compounds which exhibit high permeability and which form a larger group than BCS IV compounds.

4. Conclusion

This proof-of-principle study has shown that PermeaLoop™ allows to overcome two major shortcomings of combined dissolution/permeation testing: firstly, an increase of the area-to-volume ratio to an extent where a substantial fraction of the employed dose permeates and where the permeation does affect the dissolution kinetics; and secondly, to simulate the physiological scenario, where dissolution can be rate-limiting (instead of permeation). In essence, PermeaLoop™ appears to provide a promising extension of our toolkit for the evaluation of enabling oral formulations of poorly soluble drugs. Further testing of additional formulations and comparison with their behavior in vivo will be needed.

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References


Figure captions

Fig. 1

Chemical structure of ABT-869 structure and physicochemical properties.
Fig. 2

Schematic drawing of the PermeaLoop™ setup (without heating blocks).
Fig. 3 (print version)

Schematic drawing of the PermeaLoop™ permeation cells.
Fig. 4 (print version)

A

B

C

Fig. 4
Dissolution/permeation experiment with ABT-869 mini tablets at room temperature. A: Amount of apparently dissolved drug (gray circles), drug determined as sum of dispersed and dissolved amount (hollow circles), drug removed from the donor by sampling (hollow diamonds), and permeated drug (hollow squares). The black triangles depict the total amount, which is the sum of all other curves except for the apparently dissolved amount. All data is reported as mean ± SD.

B: Distribution of the drug in the respective fraction (mean values).

C: Comparison of donor concentration (black circles) and acceptor concentration (hollow squares) and of the flux of each time interval (gray columns). All data is reported as mean ± SD.
Fig. 5 (print version)

A

B

C

Fig. 5
Dissolution/permeation experiment with ABT-869 mini tablets at 35 °C. A: Amount of apparently dissolved drug (gray circles), drug determined as sum of dispersed and dissolved amount (hollow circles), drug removed from the donor by sampling (hollow diamonds), and permeated drug (hollow squares). The black triangles depict the total amount, which is the sum of all other curves except for the apparently dissolved amount. All data is reported as mean ± SD.

B: Distribution of the drug in the respective fraction (mean values).

C: Comparison of donor concentration (black circles) and acceptor concentration (hollow squares) and of the flux of each time interval (gray columns). All data is reported as mean ± SD.
### Tables

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount [%]</th>
<th>ASD</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td>ABT-869</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sorbitan monolaurate</td>
<td>15</td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>Copovidone</td>
<td>69</td>
<td>81.2</td>
<td></td>
</tr>
<tr>
<td>Fumed silica</td>
<td>1</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

Composition of the amorphous solid dispersion used for the preparation of the mini tablets and of the placebo extrudate.