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Evaluation of a dynamic dissolution/permeation model: Mutual influence of dissolution and barrier-flux under non-steady state conditions

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ABSTRACT

Combined dissolution/permeation testing is gaining increasing attention as an *in vitro* tool for predictive performance ranking of enabling oral formulations. The current aim was to study how *in vitro* drug permeation evolves under conditions, where the donor concentration is changing (non-steady state). To this end, a model case was construed: compacts of pure crystalline hydrocortisone methanolate (HC·MeOH) of slow release rates were prepared, and their dissolution and permeation determined simultaneously in a side-by-side setup, separated by a biomimetic barrier (Permeapad®). This was compared to a corresponding setup for a suspension of micronized hydrocortisone (HC). The HC suspension showed constant dissolved HC concentration and constant flux across the barrier, representing the permeation-limited situation. For the HC·MeOH compacts, various dynamic scenarios were observed, where dissolution rate and flux influenced each other. Interestingly, for all the dynamic scenarios, the incremental flux values obtained correlated nicely with the corresponding actual donor concentrations. Furthermore, donor depletion was tested using a HC solution. The dynamic interplay between decrease in donor concentration (down to less than 10% of the initial concentration) and flux was studied. The experiences gained are discussed in terms of further developing combined dissolution/permeation setups.

1. Introduction

The permeability of a drug compound is one of the determinants in the Biopharmaceutics Classification System (BCS) (Amidon et al., 1995). The classification of the compound into one of the four classes is one of the requirements for obtaining a BCS-based biowaiver for the final drug product. The preferred way for determining whether a compound has a high or a low permeability are pharmacokinetic studies in humans (FDA, 2015). Yet, it is also possible to determine the permeability using an appropriately validated setup with cell monolayers and reference substances. Therefore, permeability is often determined *in vitro* during late drug discovery/early drug development phase. In order to facilitate data evaluation, permeation experiments at this stage are typically designed to meet sink conditions, both in the donor and acceptor compartment. More precisely, the concentration in the acceptor compartment is kept virtually zero so that a significant contribution of back-diffusion can be ruled out. General consensus is that the acceptor concentration should be less than 10% of the donor concentration (Buckley et al., 2012).

Especially for solutions of compounds with limited aqueous solubility, a minor decay in donor concentration during the experiment may occur. A decay by up to 10 % is commonly considered as compatible with the sink-definition (Artursson, 1990).

In case of sink conditions, the steady state flux (J) of a drug across a permeation barrier can be derived from the linear part of the curve obtained when plotting the cumulative permeated amount vs. time (dQ/dt) following normalization by the permeation area (A) (Eq. (1)).

$$J = \frac{1}{A} \cdot \frac{dQ}{dt} \quad (1)$$

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Since, according to Fick's first law of diffusion, the flux is dependent on the concentration gradient, the steady state flux can be normalized by the initial concentration (c_0) and be reported as apparent permeability (P_{app}) in order to allow comparison of permeability data obtained with different experimental setups (Eq. (2)).

$$P_{app} = \frac{J}{c_0} \quad (2)$$

In a traditional permeability experiment (aiming for steady state permeation), a small permeation area and a large acceptor and donor compartment are basically beneficial for maintaining both sink conditions and a constant donor concentration.

On the other hand, there are a number of permeation-approaches described in literature, where the drug in the donor compartment is not present in dissolved state, but in solid state or in the form of a drug formulation or dosage form. These experiments are not intended to have any regulatory relevance but rather to give mechanistic insights or to allow a performance ranking of different formulations. To this end, there have been approaches suggested, which combine dissolution and Caco-2 permeation testing. Polli et al. transferred samples from a pharmacopeial paddle dissolution test of various tablets at a distinct time point to the donor compartment of a Caco-2 permeation setup (Ginski and Polli, 1999), or continuously circulated medium between dissolution and donor compartment (Ginski et al., 1999). Lehr at al. combined a pharmacopeial flow-through dissolution cell with Caco-2 and tested immediate release dosage forms (Motz et al., 2007). Yamashita et al. used a classical side-by-side diffusion setup and introduced griseofulvin in solid state, corresponding to 1% of the clinical dose, to the 8 mL donor chamber and followed its dissolution and permeation across the Caco-2 barrier simultaneously (Kataoka et al., 2003). During a series of follow-up studies using the same setup, they evaluated a number of influence factors e.g. food intake (Kataoka et al., 2006, 2011). More recent approaches employ non-cellular biomimetic barriers for the same purpose in order to overcome the limitations of cellular screens in terms of sensitivity against salts, inactive ingredients and biomimetic media (Fischer et al., 2011, 2012; Gantzsch et al., 2014).

In contrast, another type of dissolution/permeation setups employs (non-biomimetic) dialysis membranes to separate the donor (dissolution) from the acceptor compartment. Lovering and Black already pointed out in their pioneering work (Lovering and Black, 1973) that hydrophilic polydimethylsiloxane dialysis membranes are useful to determine the permeable fraction during a dissolution experiment and may thus help to predict intestinal absorption for a series of similar drugs, provided the drug compound does not interact with the membrane. Amphiphilic weak bases and acids, however, tend to interact with biomimetic membranes in response to their pH-dependent change in lipophilicity, a feature which cannot be captured by simple dialysis screens (Bibi et al., 2016). In recent decades considerably more sophisticated dialysis-based dissolution/permeation models have been designed, like the TNO model of dynamic gastric and intestinal transit and absorption, which is being used for drug studies (Blanquet et al., 2004), yet with the aforementioned limitation.

Finally, there is third group of non-sink dissolution approaches, where the dissolution compartment is supplemented by an absorptive sink-compartment consisting of octanol or similar non-water-miscible organic solvents, also addressed as biphasic dissolution. For a comprehensive review over biphasic systems see (Phillips et al., 2012).

With the advent of a large fraction of poorly water-soluble drug compounds in the pipelines of pharmaceutical industry and the inherent need for enabling formulations (Ku and Dulin, 2012), these types of non-steady state permeation settings are gaining increasing attention. Combined dissolution/permeation testing allowed, for instance, to identify the different underlying mechanisms of the enhanced permeation rate of a micro- and a nanoparticle formulation of fenofibrate (Sironi et al., 2017). Furthermore, combined dissolution/permeation testing has proven to be useful for estimating the food effect and the effect of the dose strength (Kataoka et al., 2011). Introducing an absorptive compartment is also useful for predicting the bioavailability in cases where precipitation plays an important role (Bevernage et al., 2012; Frank et al., 2014).

When combining dissolution testing with permeation studies, the donor concentration is subject to continuous changes, eventually in excess of the aforementioned limits. The donor and acceptor profiles are dynamically interrelated, and complex profiles are, hence, to be expected. Non-steady state conditions are e.g. given when the dissolution of the drug progresses slowly and thus may be rate-limiting for the overall process, or in cases where depletion of the donor compartment occurs. In consequence, there is limited information on how the geometry of such a combined dissolution/permeation setup should look like in order to render it appropriate to predict the *in vivo* behavior of (enabling) formulations.

The aim of this study was thus to explore the capabilities and limitations of a side-by-side (Ussing chamber) dissolution/permeation setup, equipped with a biomimetic barrier (Permeapad®). During a previous study (Sironi et al., 2017), the dissolution rates of the drug formulations used were rather high, and therefore, steady state permeation (constant flux) was observed in all cases (after an initial lag phase), which is regarded inappropriate for drugs, the biopharmaceutical behavior of which is expected to be solubility-/dissolution-rate-compromised. In order to better see the interplay between dissolution and permeation, a simplified model case was construed, where the dissolution rate is comparably low. Moreover, the dissolved drug was exclusively present in the molecularly dissolved state, i.e. not associated with micelles or other solubility-enhancing supramolecular assemblies (Fischer et al., 2011; Flaten et al., 2008).

Hydrocortisone (HC) was chosen as poorly soluble model drug with high permeability (BCS class II), and in order to reduce the dissolution rate, large crystals of hydrocortisone methanolate (HC·MeOH) were prepared. HC·MeOH was compressed without the use of excipients to compacts, and in some cases the tablets were partially covered with hard paraffin to further reduce the surface exposed to dissolution medium.

2. Material and methods

2.1. Chemicals

Hydrocortisone (micronized, Ph. Eur. 8.0, lot 15021106) and hard paraffin (Ph. Eur. 8.0) were purchased from Caesar & Loretz GmbH (Hilden, Germany). Highly purified water was prepared in-house using a Milli-Q® water purification system (Merck Millipore, Darmstadt, Germany). Methanol, ethanol and buffer salts were purchased from Sigma-Aldrich ApS (Brøndby, Denmark).

2.2. Media

Phosphate-buffered saline (PBS) contained 1.73 g L^{-1} of sodium dihydrogen phosphate dihydrate and 4.92 g L^{-1} disodium hydrogen phosphate dodecahydrate in highly purified water. The pH was adjusted with sodium hydroxide to a value between 7.35 and 7.45; the osmolality was adjusted with sodium chloride to a value between 280 and 290 mOsmol kg^{-1}.