European Journal of Pharmaceutical Sciences 96 (2017) 154–163

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

A novel microdialysis-dissolution/permeation system for testing oral dosage forms: A proof-of-concept study

Sophia Yui Kau Fong, Jessie Poulsen, Martin Brandl, Annette Bauer-Brandl *

Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 14 September 2016
Accepted 15 September 2016
Available online 16 September 2016

Keywords:
Dissolution
Permeation
Microdialysis
Molecular solubility
Hydrocortisone

ABSTRACT

A novel microdialysis-dissolution/permeation (M-D/P) system was developed for the biopharmaceutical assessment of oral drug formulations. This system consists of a side-by-side diffusion chamber, a microdialysis unit fixed within the dissolution chamber for continuous sampling, and a biomimetic Permeapad® as the intestinal barrier. In the M-D/P system, the concentration of the molecularly dissolved drug (with MWCO <20 kDa) was measured over time in the dissolution compartment (representing the gastrointestinal tract) while the concentration of the permeated drug was measured in the acceptor compartment (representing the blood). The kinetics of both the dissolution process and the permeation process were simultaneously quantified under circumstances that mimic physiological conditions. For the current proof-of-concept study, hydrocortisone (HCS) in the form of slowly dissolving solvate crystals and buffer and the biorelevant fasted state simulated intestinal fluids (FaSSIF), were employed as the model drug and dissolution media, respectively. The applicability of the M-D/P system to dissolution and permeation profiling of HCS in buffer and in FaSSIF has been successfully demonstrated. Compared to the conventional direct sampling method (using filter of 0.1–0.45 μm), sampling by the M-D/P system exhibited distinct advantages, including (1) showing minimal disturbance of the permeation process, (2) differentiating "molecularly" dissolved drugs from "apparently" dissolved drugs during dissolution of HCS in FaSSIF, and (3) being less laborious and having better sampling temporal resolution. M-D/P system appeared to be a promising, simple and routine tool that allows for the researchers' intensive comprehension of the interplay of dissolution and permeation thus helping for better oral formulation screening and as an ultimate goal, for better dosage forms assessment.

1. Introduction

Introduced four decades ago, microdialysis is a technique originally developed for real-time measurement of free extracellular fluid levels of small molecules in living animals in neurological sciences. Its sporadic use within the *in vitro* field has been reported since 1990s, with dissolution test being one of its many applications. At the beginning (1994; by Shah et al., 1994), the application of microdialysis to dissolution sampling mainly aimed to provide an automated system that is less laborious compared to the conventional direct sampling method recommended by Pharmacopoeiae. By continuous sampling, microdialysis omits many steps such as filtration, collection and replenishment of medium that are necessary for the direct sampling method. Since then, modified microdialysis systems have been developed in an attempt to improve the analytical resolution (Fang et al., 1999; Nagai et al., 2009). Recently (2014), the merit of using microdialysis for *in vitro* dissolution test has been further appreciated by its potential to characterize supersaturation and precipitation of poorly soluble drugs (Shah et al., 2014). Compared to direct sampling, pulsatile microdialysis demonstrated superiority in correctly predicting the dissolution profiles of supersaturating formulations (Shah et al., 2014). Although the *in vitro* application of microdialysis to dissolution test is still in its infancy, increasing acceptance is expected to be gained for its high automation potential and its promise to characterize advanced oral formulations that have surging market. However, the majority of abovementioned dissolution tests utilized hand-made loop probes and/or large dissolution volumes (900–1000 mL). Both factors limit the practicability of applying microdialysis for high-throughput screening in industrial settings. A ready-made, reproducible, and down-scaled microdialysis system would be desirable and favorable for its application to routine dissolution testing. Another limitation of the current settings is that dissolution tests were commonly conducted in one compartment such that the dissolved drugs accumulated. In our opinion such approach appears less

Abbreviations: D/P system, dissolution/permeation system; DSC, differential scanning calorimetry; FaSSIF, fasted state simulated intestinal fluid; HCS, hydrocortisone; IS, internal standard; *Jss*, steady-state flux; LLOD, lower limit of detection; LLOQ, lower limit of quantification; M-D/P system, microdialysis-dissolution/permeation system; MWCO, molecular weight cut-off; PBS, phosphate buffer saline; QC, quality control; RSD, relative standard deviation; UHPLC-UV, Ultra-High-Performance Liquid Chromatography-Ultraviolet; XRD, X-ray powder diffraction.

* Corresponding author.
E-mail address: annette.bauer@sdu.dk (A. Bauer-Brandl).

http://dx.doi.org/10.1016/j.ejps.2016.09.018
0928-0987/

physiologically relevant since any dissolved drug *in vivo* is continuously absorbed into the body ("sink"). A dynamic model which takes absorption (permeation processes) into account will be of higher physiological relevance.

Indeed, an effective absorption of an orally administered drug is governed by both processes: dissolution within the gastrointestinal fluids and permeation across the intestinal epithelial barrier. The importance of simultaneously evaluating both parameters for oral dosage forms, especially enabling formulations for poorly soluble drugs, has been highlighted from ours and many others studies (Buckley et al., 2013; Fong et al., 2016; Dahan et al., 2010). A combined dissolution/permeation (D/P) *in vitro* model, firstly introduced by Ginski and Polli (1999) in 1999 and subsequently modified by many other researchers (Kataoka et al., 2003; Motz et al., 2007; Buch et al., 2009), is considered as the state-of-the-art approach for simultaneous measurement of dissolution and permeability of the tested drugs or dosage forms. The D/P system had been proven useful, regarding *in vitro-in vivo* correlation for potential bioavailability screening of new active pharmaceutical ingredients, screening of innovative formulation approaches, and investigating potential food effects on drug absorption (Motz et al., 2008). However, the majority of these models utilized Caco-2 cell monolayers as the intestinal barrier, which limits the wide use of these systems due to poor reproducibility of the results, high cost of each test, and long cell incubation time. More importantly, the choice of the dissolution/permeation medium is limited since the cells cannot withstand components such as fed-state gastrointestinal fluids and solubilizing excipients that are often used in formulations (*e.g.* surfactants/co-solvents) (Bibi et al., 2015). Non-cellular barriers such as parallel artificial membrane permeability assay (PAMPA), cellulose-based Cuprophan membrane, and phospholipid vesicle-based permeation assay (PVPA), are attractive alternatives for assessing passive, transcellular permeation and have been recently employed in the D/P systems (Gao, 2012; Gantzsch et al., 2014; Kataoka et al., 2014). Nevertheless, these barriers also lack functional stability against some of the surfactants and co-solvents and have other deficiencies in which the former two do not reflect biological membrane lipid composition and structures (*i.e.* less bio-mimetic); while the latter involves laborious preparation procedures and has a short shelf-life (up to 2 weeks) (Flaten et al., 2006). In an attempt to overcome the abovementioned limitations of the existing barriers, our group recently invented a bio-mimetic artificial barrier, Permeapad® (di Cagno & Bauer-Brandl, 2014), that has proven to be a powerful tool for a fast and reliable determination of drug passive permeation properties (di Cagno et al., 2015). It had been demonstrated to be compatible with a wide range of surfactants, co-solvents and biorelevant media in relevant concentrations (Bibi et al., 2015). The easy preparation procedures, mechanical flexibility and its long shelf-life (>1 year) also make Permeapad® a perfect tool for high-throughput permeation assay.

Put together the concepts of microdialysis and D/P systems, the current article introduces a novel microdialysis-dissolution/permeation (M-D/P) system for oral drug formulation testing. The unique features of the M-D/P are:

i) Combination of microdialysis sampling with D/P system
ii) A commercially available concentric microdialysis probe
iii) A biomimetic barrier Permeapad® which permits the use of a variety of dissolution/permeation media
iv) A down-scaled testing system with small volume of medium needed (6.4 mL) in the side-by-side diffusion chamber

In this M-D/P system, the concentration of the molecularly dissolved drug (with MWCO <20 kDa) was measured over time in the dissolution compartment (represents the gastrointestinal tract), as well as in the "sink" (the acceptor compartment that represents the blood). The kinetics of both the dissolution process and the permeation process were simultaneously quantified under circumstances that mimic physiological conditions. In the current proof-of-concept study, a model drug hydrocortisone (HCS) that has low aqueous solubility (0.28 mg/mL) and high permeability (Log *P* 1.61), was employed in the forms of micronized powder and slowly dissolving, large solvate crystals. For direct comparison, dissolution or dissolution-permeation of HCS using the same set-up without the microdialysis unit (*i.e.* conventional direct sampling followed by filtration) was conducted simultaneously or in parallel. Step-wise experiments were conducted to achieve the following objectives:

1) To characterize, optimize and validate the microdialysis probe for dissolution sampling of HCS
2) To demonstrate the applicability of M-D/P system to dissolution and permeation profiling of HCS
3) To apply the M-D/P system to dissolution and permeation study in fasted state simulated intestinal fluid (FaSSIF)

2. Materials and methods

2.1. Chemicals

Micronized HCS of European Pharmacopoeia 8.0 grade was purchased from Caelo (Germany). Diclofenac sodium used as an internal standard (IS) was purchased from Sigma-Aldrich (St. Louis, USA). SIF® Powder Origin for preparing FaSSIF media was obtained from biorelevant.com (Surrey, UK). Methanol (UPLC grade), formic acid, hydrochloric acid, sodium chloride, sodium hydroxide, sodium phosphate monobasic monohydrate, sodium di-hydrogen phosphate di-hydrate, and di-sodium hydrogen phosphate dodeca-hydrate, were purchased from Sigma-Aldrich (St. Louis, USA). Soy phosphatidylcholine (S-100) was a gift from Lipoid GmbH (Ludwigshafen, Germany). Purified water was supplied from Milli-Q® Millipore (Merck, Germany).

2.2. Preparation of medium

Phosphate buffer saline (PBS, pH 7.4), FaSSIF buffer (pH 6.5), and the biomimetic medium FaSSIF (pH 6.5), were media employed in the current dissolution and permeation studies. A 74 mM PBS was prepared by mixing 2.5% (w/v) sodium dihydrogen phosphate dihydrate solution with a 1.8% (w/v) di-sodium hydrogen phosphate dodecahydrate solution in a 1:4 ratio. The pH of the solution was adjusted to 7.4 by addition of sodium hydroxide, and osmolality was adjusted to 285 mOsm by adding sodium chloride and measured with Semi-Micro Osmometer K7400 (Herbert Knauer GmbH, Berlin, Germany). The prepared PBS was then filtered through a 0.1 μm filter paper using a vacuum pump apparatus (KNF Laboport®, Germany). FaSSIF buffer and FaSSIF medium were prepared in accordance with the procedures suggested by the manufacturer (biorelevant.com, UK). For the preparation of FaSSIF buffer, 0.42 g of sodium hydroxide, 6.19 g of sodium chloride, and 3.95 g of sodium phosphate monobasic monohydrate were dissolved in 1 L of purified water. The pH of the buffer was adjusted to 6.5 with 1 M of sodium hydroxide or hydrochloric acid. FaSSIF was prepared by dissolving 2.24 g of SIF powder in 1 L of FaSSIF buffer (pH 6.5). The freshly prepared FaSSIF was allowed to stand at room temperature for 2 h before use.

2.3. Preparation and structural analysis of HCS crystals

HCS in its micronized powder form and in crystal form were used for the current study. HCS powder was used as purchased while HCS crystals were prepared according to the method described by Chen et al. (2008). Briefly, HCS was dissolved in methanol at 50 °C under agitation to get a saturated solution. After filtration, the solvent methanol was allowed to evaporate gradually by using a pierced film cover at room temperature.