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In situ artificial membrane permeation assay under hydrodynamic control: Correlation between drug *in vitro* permeability and fraction absorbed in humans

Matěj Velický [a], Kin Y. Tam [b,*], Robert A.W. Dryfe [a,*]

[a] School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK
[b] AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

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ABSTRACT

The purpose of this study was to develop an *in vitro* permeation model that will predict the fraction of drugs absorbed in humans. A rotating-diffusion cell with two aqueous compartments, separated by a lipid-impregnated artificial membrane, was used to determine the permeability of drugs under conditions of controlled hydrodynamics. The measured effective permeability coefficient was modified to include the paracellular transport derived from a previously reported colorectal adenocarcinoma epithelial cell line (Caco-2) permeability study and the effects of unstirred water layer anticipated *in vivo*.

Permeability data were collected for 31 different marketed drugs with known absolute oral bioavailability and human hepatic clearance data. Literature bioavailability values were corrected for the first pass hepatic clearance thus obtaining the fraction absorbed from intestinal lumen (fraction absorbed), F_a, while assuming that the fraction escaping intestinal extraction, F_g, was approximately ~1. Permeability obtained under conditions of controlled hydrodynamics was compared with the permeability measured under unstirred conditions. It is shown that the optimized effective permeability correlates with the fraction absorbed. In contrast, permeability data obtained under unstirred conditions does not show a good correlation.

The *in vitro* permeation model developed in this study predicts the fraction absorbed of the selected drugs in humans within experimental uncertainty. It has been demonstrated that the correlation with the fraction absorbed is greatly improved using the permeability data obtained under controlled hydrodynamics with paracellular transport included in the model.

1. Introduction

Since the introduction of the parallel artificial membrane permeability assay (PAMPA) by Kansy et al. (1998), the technique has received considerable attention in the pharmaceutical industry. PAMPA offers a simple physicochemical measure of permeability for research compounds. The throughput is higher than other cell-based permeability assays (e.g. Caco-2, Madin-Darby Canine Kidney cell line), and it is relatively cheap to run (Artursson and Karlsson, 1991; Fade, 1998; Irvine et al., 1999). PAMPA is a potentially useful tool in early phase discovery projects where permeability is seen to be an issue for the chemical structures of interest. For instance, in combination with other *in vitro* assays, such as metabolic assessment, aqueous solubility, plasma protein binding, PAMPA could form part of the testing cascade to evaluate the suitability of research compounds.

Over the past decade, several enhanced versions of PAMPA have been developed by different researchers, including variations in membrane composition, solution composition, and hydrodynamics/stirring. The organic phase immobilized within the membrane has been varied from the original use of egg lecithin in *n*-dodecane (egg-PAMPA) to modified composition as dioleoyl phosphatidylcholine in dodecane (DOPC-PAMPA) (Avdeef et al., 2001), the complex double-sink method (DS-PAMPA) (Avdeef, 2003a,b; Bermejo et al., 2004), *n*-hexadecane alone (HDM-PAMPA) (Wohnsland and Faller, 2001) or the mixture of lipids dissolved in 1,7-octadiene to form a 'bio-mimetic' PAMPA (BM-PAMPA) (Sugano et al., 2001). Avdeef et al. introduced a modification of DS-PAMPA that employed stirring of the donor compartment (Avdeef et al., 2004). On the other hand, some PAMPA practitioners still prefer to adopt the original experimental design developed by Kansy et al. (1998), with their choice of membrane compositions in setting up their own PAMPA assays. It has been show recently that

* Corresponding authors. Tel.: +44 (0) 161 306 4522; fax: +44 (0) 161 275 4734 (R.A.W. Dryfe), tel.: +44 (0) 1625 230338 (K.Y. Tam).
E-mail addresses: kin.tam@astrazeneca.com (K.Y. Tam), robert.dryfe@manchester.ac.uk (R.A.W. Dryfe).

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Abbreviations and symbols

a	permeability function
A, B, C	permeation constants
BCS	biopharmaceutics classification system
BM-PAMPA	bio-mimetic PAMPA
$c_A(t)$	time-dependent acceptor concentration
c_B/c_P	total blood to total plasma drug concentration ratio
Caco-2	colorectal adenocarcinoma epithelial cell line
CL_h	first pass hepatic clearance
D_{aq}	aqueous diffusion coefficient
DOPC	dioleyol phosphatidylcholine
DOPC-PAMPA	dioleoyl phosphatidylcholine
PAMPA	DS-PAMPA double-sink PAMPA
e	elementary charge
egg-PAMPA	egg lecithin PAMPA
$E(\Delta_\varphi)$	electric potential drop function
$F_R(r_{HYD}/R_p)$	Renkin hydrodynamic sieving function
F	absolute bioavailability in humans
F_a	fraction absorbed from intestinal lumen
F_g	fraction escaping intestinal extraction
F_h	fraction escaping hepatic extraction
$\%F_a$	(percent) fraction absorbed from intestinal lumen
$f_{(-)}$, $f_{(+)}$	concentration fraction of an anionic, cationic form, respectively
$f_{(\pm/0)}$	concentration fraction of neutral/zwitterionic form
G, H	fitting constants
HDM-PAMPA	hexadecane PAMPA
HJP	human jejunal permeability
k	concentration function from permeability measurement
k_B	Boltzmann constant
K_d	generic distribution coefficient
K_d^A	membrane/acceptor distribution coefficient
K_d^D	membrane/donor distribution coefficient
K_d^{OCT}	octanol/water distribution coefficient
M	membrane area
M_r	molar mass
N_A	Avogadro constant
P_0	intrinsic permeability coefficient
PAMPA	parallel artificial membrane permeation assay
P_e	effective permeability coefficient
P_m	membrane permeability coefficient
P_p	paracellular permeability coefficient
PTFE	polytetrafluoroethylene
P_u	unstirred water layer permeability coefficient
PVDF	polyvinylidene fluoride
Q_h	hepatic blood flow
r_{HYD}	hydrodynamic radius
R_p	intercellular junction pore radius
S	transcellular/paracellular transport scaling factor
T	absolute temperature
t	time
UWL	unstirred water layer
V_A, V_D	acceptor, donor volume, respectively
α	hydrodynamic exponent
β	linear regression coefficient (hydrodynamic extrapolation)
δ	path length ratio
δ_u	unstirred water layer thickness
$\Delta\varphi$	electric potential drop at the channel surface
$\Delta\varphi$	electrical potential drop in intercellular junctions
ε/δ	porosity/path-length capacity factor
η	dynamic viscosity
$\kappa = e/k_BT$	constant
ν	kinematic viscosity
ω	angular velocity of stirring

the original design of PAMPA has some limitations with regard to the understanding of permeability and the correspondence with conditions found *in vivo* (Avdeef, 2003a, 2005; Youdim et al., 2003; Avdeef et al., 2004, 2007; Korjamo et al., 2009; Velický et al., 2010). In particular, the experiment is performed in a 'static' configuration where the permeating research compounds are allowed to diffuse through the PAMPA membrane, without any control of the hydrodynamics. For lipophilic molecules where the permeability is already close to the rate of diffusion across the unstirred water layer (UWL), the permeability data obtained from 'static' PAMPA conditions are of little use for ranking purposes. Another issue is that the PAMPA model does not account for any paracellular transport. This may potentially lead to an underestimation of permeability for some research compounds, especially at the early phase of drug discovery, where the size of the molecules tend to be smaller than the fully elaborated drug candidates.

In our previous study (Velický et al., 2010), we have developed a rotating-diffusion cell with two aqueous compartments, separated by a lipid-impregnated artificial membrane, for the determination of drug permeability under conditions of controlled hydrodynamics. With this novel experimental setup, we have addressed and resolved the three most neglected theoretical and experimental problems, namely: reproducible stirring (controlling the mass-transport regime), *in situ* permeability measurements (accurate determination of the permeability coefficient) and use of the appropriate analytical model (considering lag-time, two-way flux, pH-gradient and membrane retention). The term '*in situ*' in relation to the presented permeation assay reflects the fact that the detection of the drug molecule is carried out in real time inside the permeation cell. In the present study, we apply this advanced assay to a diverse set of marketed drugs with the explicit aim of establishing a correlation with literature bioavailability data and developing a tool to predict the fraction absorbed in humans that could be used as a tool in early drug discovery. Primarily, PAMPA is designed to mimic transcellular transport across human intestine epithelial cells. As a result, the low permeability response of small hydrophilic molecules ($M_r < 250$ g mol^{-1}) often does not correlate with their fraction absorbed due to the simultaneous paracellular route for their *in vivo* transport. Inspired by recent work from the laboratories of Sugano et al. (2002, 2004)) and Avdeef (2010), Avdeef and Tam (2010) and Tam et al. (2010) we report herein the incorporation of a paracellular component in our model based on the Renkin function (Renkin, 1954). The paracellular permeability model derived from a recent detailed analysis of the Caco-2 permeability data of Adson et al. was employed for this purpose (Avdeef, 2010). The measured effective permeability coefficient is corrected for the paracellular transport occurring *in vivo* thus expanding the range of drugs that can be properly ranked by this permeation assay.

As a part of this analysis, a novel approach to determine the optimal effective permeability as a function of UWL thickness is obtained, which can be converted to the corresponding stirring rate. The unstirred water layer, i.e. the aqueous layer adjacent to the membrane, where the flux of the solute is diffusion limited, is accurately controlled using the rotating-diffusion device (Molloy et al., 2008; Velický et al., 2010). Firstly, the effective permeability is measured for at least two stirring rates. Then, using the known analytical solution relating solute flux to applied stirring rate