Passive Lipoidal Diffusion and Carrier-Mediated Cell Uptake Are Both Important Mechanisms of Membrane Permeation in Drug Disposition

Dennis Smith,*  ‡ Per Artursson, ‡ Alex Avdeef,§ Li Di,∥ Gerhard F. Ecker,⊥ Bernard Faller,‡ J. Brian Houston,§ Manfred Kansy,¶ Edward H. Kerns,† Stefanie D. Krämer,○ Hans Lennernäs,∥ Han van de Waterbeemd,● Kiyohiko Sugano,△ and Bernard Testa▲

‡ The Maltings, Walmer, Kent, CT14 7AR, U.K.
§ Department of Pharmacy, Biomedical Centre, Uppsala University, S-752 63 Uppsala, Box 580, Sweden
∥ Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., Groton, Connecticut 06340, United States
⊥ Department of Medicinal Chemistry, University of Vienna, Althanstrasse, 141090 Wien, Austria
§ Novartis Institutes for Biomedical Research, WSJ-350.3.04, CH-4002 Basel, Switzerland
¶ School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, U.K.
△ The Non-Clinical Safety Department, F. Hoffmann-La Roche, CH-4070 Basel, Switzerland
♦ National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland 20850, United States
○ Institute of Pharmaceutical Sciences, ETH, Zürich, Switzerland
● 14 Rue de la Rasclose, 66690 Saint André, France
▲ Department of Pharmacy, University Hospital Lausanne, CH-1011 Lausanne, Switzerland

ABSTRACT: Recently, it has been proposed that drug permeation is essentially carrier-mediated only and that passive lipoidal diffusion is negligible. This opposes the prevailing hypothesis of drug permeation through biological membranes, which integrates the contribution of multiple permeation mechanisms, including both carrier-mediated and passive lipoidal diffusion, depending on the compound’s properties, membrane properties, and solution properties. The prevailing hypothesis of drug permeation continues to be successful for application and prediction in drug development. Proponents of the carrier-mediated only concept argue against passive lipoidal diffusion. However, the arguments are not supported by broad pharmaceutics literature. The carrier-mediated only concept lacks substantial supporting evidence and successful applications in drug development.

KEYWORDS: permeation, passive lipoidal diffusion, “carrier-mediated only” concept

INTRODUCTION

Absorption, distribution, and elimination of drug molecules and their metabolites from the point of dosing, throughout body tissues, into cells, exposure to biochemical targets, and routes of clearance involve crossing diverse tissue, cellular, and organelle membranes. The process of membrane crossing is broadly referred to as “permeation”, which includes mechanisms of passive lipoidal diffusion, carrier-mediated influx, carrier-mediated efflux, paracellular diffusion, aqueous boundary layer (mucus) diffusion, endocytosis, and others. Owing to the importance of permeation in drug absorption and disposition, these mechanisms have been and continue to be an active research area. The preponderance of the data on permeation has led to a widely accepted and applied prevailing drug permeation paradigm, which embodies the contribution of all the mechanisms. This paradigm is routinely applied with success in drug design to optimize efficacy and safety, predict human PK/PD, select dose, and plan dosing regimens.

This review discusses the prevailing permeation hypothesis, which we conclude is consistent with the experimental data.

Received: November 26, 2013
Revised: February 10, 2014
Accepted: April 11, 2014
Published: April 11, 2014
Molecular Pharmaceutics

Importantly, this review also addresses uncertainties among drug researchers that result from recent articles1−3 published by a group that assert the carrier-mediated only concept (CMOC) of drug permeation and attempt to invalidate passive lipoidal diffusion across biological membranes into cells and across cell layer membranes. We conclude that the CMOC lacks sufficient evidence. The CMOC is not a sound scientific principle and lacks experimental evidence. Carrier-mediated transport and passive lipoidal diffusion permeation mechanisms are complementary, their relative contribution depending on many physicochemical and biochemical factors (e.g., concentration gradient, lipophilicity and hydrogen-bonding capacity of the diffusing compound, transporter affinity, and $K_m$). The integrated system follows the laws of thermodynamics.

Discussion of divergent hypotheses, based on experimental data, is certainly desirable. Thus, recent articles5,6 have discussed the experimental evidence that both passive lipoidal diffusion and carrier-mediated transport permeation mechanisms affect the absorption and disposition of drugs. There is broad consensus in the pharmaceutics field on the existence, effects, and application of knowledge about both passive lipoidal diffusion and carrier-mediated transport. Clearly, drug researchers have, throughout the history of drug discovery, utilized physicochemical properties (lipophilicity, hydrogen-bonding capacity, molecular size, ionization/charge), which have been correlated to passive lipoidal diffusion permeation rate and not to carrier-mediated permeation rate, to enhance the success of drug delivery.7–23 In recent years, drug delivery researchers have also utilized knowledge about transporter uptake to enhance drug exposure to certain tissues. For example, liver specific transporters (OATP1B1 and 1B3) selectively increase liver concentration of their substrates, which minimize the exposure to peripheral tissue and reduce toxicity.24,25

Founded on our experience with detailed experimentation on drug permeation mechanisms, scientific literature evidence, and application of drug permeation mechanisms in drug research, we reiterate and support the roles of both passive lipoidal diffusion and carrier-mediated transport in the prevailing drug permeation theory. The purpose of this review is to provide guidance for drug researchers, so that potential drug candidates will not be overlooked by mistaken evaluations that ignore the important effects of passive lipoidal diffusion.

Prevailing Drug Permeation Philosophy Restated. The prevailing theory of drug permeation through biological membranes is as follows:

1. There are multiple permeation mechanisms by which drug molecules, in general, cross biological membranes and are absorbed and distributed throughout the body, including passive lipoidal diffusion, carrier-mediated influx, paracellular diffusion, aqueous boundary layer (mucus) diffusion, and transcytosis.

2. The rate with which a given compound crosses specific biological barriers (epithelium, endothelium) varies with (a) the compound’s properties (e.g., lipophilicity, pK, transporter parameters [K, and V_max]), (b) the membrane’s properties (e.g., composition, transporter function, tight junction characteristics, and membrane potential), and (c) solution properties (pH, pH gradient across membrane, and permeant concentration gradient across membrane).

This theory is based on results from drug disposition in pharmaceutical research and from fundamental investigations as discussed below.

Unifying Role of Lipoidal Permeability in Drug Disposition. Overview. Tissues of the body are constructed of cells. Cells are encapsulated by membranes made of phospholipid bilayers. Proteins are embedded in the cell membrane and function as sensors for the cell, maintain the intracellular homeostasis, or transport nutrients and other substances. Cells are joined, through junction proteins, in varying degrees of tightness.

Paracellular permeation occurs when drug molecules diffuse through the cell junctions. Examples of the role of paracellular permeation in defining drug permeation include the following:

1. For much of the vasculature of systemic circulation, the junctions are not tight and allow aqueous diffusion of even large drug molecules. Thus, drug molecules in circulation are rapidly exposed to tissue cell surfaces.

2. The capillary vasculature in the brain has very tight junctions between cells, forming the distinct blood–brain barrier (BBB), which greatly attenuates the diffusion of drug molecules to the surface of the CNS tissue cells.

3. The cells of the upper gastrointestinal tract are moderately tight and allow a portion of small drug molecules (typically polar and less than 300 Da) to pass through the junctions. These drugs can also reach cell surfaces for many tissues but will have restricted access to the CNS.

Paracellular diffusion does not account for most drug permeation processes, such as for larger molecules, exposure to intracellular drug targets, or penetration to CNS drug targets through the BBB. Experimental data also support two additional major permeation mechanisms: passive lipoidal diffusion through the phospholipid membrane and carrier-mediated transport through one of the transporter proteins. The theoretical framework and the experimental evidence for membrane permeability of drugs have been reviewed. Additional recent data from fluorine NMR studies on uptake of modified nucleosides (L-FMAU) into erythrocytes (biological systems that include transporters) provide clear indication of two different mechanism governing uptake of L-FMAU in erythrocytes: “facilitated transport via nucleoside transporter and nonfacilitated diffusion”.

Removal of drug and metabolite molecules from the body is by metabolism, renal clearance, or “biliary excretion”. Again, transport proteins may play an important role for some compounds. Passive lipoidal permeability has a central role in controlling these processes, as outlined below.

Passive lipoidal permeability is correlated positively with lipophilicity (e.g., as expressed by the log of the octanol–water partition, log P, or the apparent value at a given pH, often 7.4, log D) and negatively with the hydrogen-bonding capacity of a molecule (e.g., as expressed by the polar surface area, PSA, the solvatochromic parameters $\alpha$ (H-bond donor) and $\beta$ (H-bond acceptor), and the molecular hydrogen-bonding potentials, MHBPs).27–30 If a drug molecule has positive log D and low PSA, passive lipoidal permeability is high and is a large fraction of the total permeability, owing to the large surface area of the cell membrane. In this case, any transporter permeability is a low fraction of the total permeability, even if the compound has carrier-mediated transport, because it is limited by the carrier concentration and the $K_m$ (left diagram in Figure 1). If a drug
molecule has negative log $D$ and high PSA, passive lipoidal permeability is low and, if the molecule has carrier-mediated permeability, a significant portion of the total permeability may be carrier-mediated (right diagram in Figure 1). The schematics shown in Figure 1 illustrate the separation between drugs of high and low lipoidal permeability.

The evidence for lipoidal diffusion is provided by many experiments, and importantly the results are consistent for passage across any cell type, organ, or species and are not energy or concentration dependent.

**Evidence from in Vitro Monolayer Cell Systems.** Lipoidal diffusion is well exemplified by in vitro monolayer cell systems used to predict or rationalize drug absorption. These measure flux in an apical to basolateral (A→B) direction and vice versa. A highly passive lipoidally permeable drug will show the following:

- a comparatively high rate of apical to basolateral (A→B) flux of a group of compounds
- no effect of concentration on rate constant, up to the limit of solubility
- near identical A→B and B→A flux regardless of concentration
- lack of effect of inhibitors of known transport proteins
- similar rates of flux in cell lines selectively cultured for low and high transporter expression.

There is only small variation in in vitro permeation rates of drugs between various cell layer permeation experiments in which large variation exists in transporters. The same is true in permeation rates of drugs between Caco-2 (from human colon carcinoma) and MDCK (from canine kidney), where the following observations were made (Figure 2):

- There is a very close correlation between physicochemical systems (including PAMPA and liposomes) and cell based systems.
- It does not require energy.
- It happens in all organs, species, and cell types.

Typical MCDK and averaged Caco-2 results are illustrated in Table 1 for a series of beta adrenergic blockers. Permeation rate increases with increasing lipophilicity, as observed in many experiments, and clearly indicated for Caco-2 in Table 1, which is consistent with passive diffusion through the lipophilic core of the bilayer membrane. The high passive lipoidally permeable compounds (lipophilic, with low polar surface area such as bufarolol, alprenolol, and propranolol) show identical high rates of transport in either direction across the MDR1-MDCKII cells and are not influenced by the presence of a transporter inhibitor. Less passive lipoidally permeable compounds, such as labetalol, show different rates A→B and B→A (due to different heights of the energy barrier), and are markedly affected by the presence of the transporter inhibitor. The transport of atenolol, a low molecular weight drug of low lipophilicity, is based on paracellular permeation. The permeation rate is related to ionization constant $pK_a$ and pH according to the "pH-partition theory" and to intrinsic lipophilicity (log $P$), with permeation rate increasing in relation to increased concentration of a compound’s neutral species (to which log $P$ refers), which is the form that permeates the bilayer membrane while the charged form has very low permeability. log $D$ is a particularly useful term in explaining the interplay between intrinsic lipophilicity (log $P$) and $pK_a$. In Table 1, the drugs have similar $pK_a$ but the degree of ionization means that their log $D$ at pH 7.4 is approximately 2 log units lower than their intrinsic lipophilicity (log $P$). Other experiments show that permeation rate is well correlated between a biological membrane (e.g., Caco-2), an artificial membrane (e.g., PAMPA), and octanol/water partition coefficient.
Table 1. Unidirectional Permeability across MDR1-MDCKII of High Lipoidal Compounds Exemplified by Beta Adrenoceptor Antagonists, Compared with Caco-2 Permeability.35

<table>
<thead>
<tr>
<th>Drug</th>
<th>log P</th>
<th>log D(7.4)</th>
<th>PSA (Å²)</th>
<th>Papp,A−B</th>
<th>Papp,A−B</th>
<th>Efflux Ratio</th>
<th>Papp,A−B (plus GF120918)</th>
<th>efflux ratio (plus GF120918)</th>
<th>Caco-2 Papp,a/b</th>
<th>Rate Limit</th>
<th>Rate Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>propranolol</td>
<td>3.5</td>
<td>1.4</td>
<td>42</td>
<td>40</td>
<td>42</td>
<td>1.0</td>
<td>44</td>
<td>1.1</td>
<td>494</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
<tr>
<td>metoprolol</td>
<td>2.0</td>
<td>−0.2</td>
<td>51</td>
<td>30</td>
<td>36</td>
<td>1.2</td>
<td>37</td>
<td>1.3</td>
<td>231</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
<tr>
<td>alprenolol</td>
<td>3.0</td>
<td>0.9</td>
<td>42</td>
<td>46</td>
<td>47</td>
<td>1.0</td>
<td>43</td>
<td>0.9</td>
<td>99</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
<tr>
<td>oxprenolol</td>
<td>2.5</td>
<td>0.3</td>
<td>51</td>
<td>31</td>
<td>42</td>
<td>1.4</td>
<td>41</td>
<td>1.0</td>
<td>42</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
<tr>
<td>bufuralol</td>
<td>3.0</td>
<td>0.6</td>
<td>45</td>
<td>64</td>
<td>50</td>
<td>0.8</td>
<td>48</td>
<td>0.8</td>
<td>14</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
<tr>
<td>labelalol</td>
<td>1.3</td>
<td>1.1</td>
<td>96</td>
<td>4.1</td>
<td>36</td>
<td>8.8</td>
<td>7.4</td>
<td>1.8</td>
<td>31</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
<tr>
<td>atenolol</td>
<td>0.2</td>
<td>−1.9</td>
<td>85</td>
<td>0.3</td>
<td>0.3</td>
<td>1.0</td>
<td>0.2</td>
<td>0.7</td>
<td>0.7</td>
<td>6 cm/s</td>
<td>6 cm/s</td>
</tr>
</tbody>
</table>

*Permeability in units of 10⁻⁶ cm/s. bAverage measured value at pH 7.4, corrected for aqueous boundary layer and paracellular effects.36 cCalculated (Avdeef, unpublished).

Figure 3 is an example of the in vitro—in vivo correlation between the blood—brain barrier permeability and that of PAMPA based on 10% porcine brain lipid extract.39 Figure 4 shows that the uptake of drugs into human red blood cells significantly correlates with log P. Permeation rate also decreases as the hydrogen-bond donor and/or acceptor capacity of the molecule increases, consistent with breaking hydrogen bonds with water to diffuse into the lipophilic core of the membrane. Such stereoelectronic effects are expressed as the polar surface area (PSA) and derived parameters.38,42,43 The phenomenon is illustrated by comparison of bufuralol (low PSA, high passive lipoidal permeability compound) and labelalol (high PSA, lower permeability compound). This compound now shows the influence of transporters and has a B–A flux greater than its A–B.

Measurement of cell permeability can be surprisingly complicated. For example, very lipophilic molecules are often rate-limited in passage across a barrier that has little to do with the transport properties of biological cell membranes. Adjacent to the surface of any cell is a stagnant water layer, known as the "aqueous boundary layer" (ABL). Before molecules encounter the cell surface, they first need to traverse the ABL by passive aqueous diffusion, which is a relatively slow process, particularly when the assay solution is unstimred. For very lipophilic molecules, this process is often rate limiting and can obscure the contribution of faster carrier-mediated or lipoidal passive diffusion processes to the measured total permeability. In Table 1, the first five drugs (with log P ≥ 2) indicate essentially the same MDCK Papp (43 ± 10 × 10⁻⁶ cm/s) regardless of variation in log P or the transport directions. This should be expected for molecules of comparable size when ABL limits the rate of transport in both directions. Methods have been developed (e.g., summarized in ref 36) to deconvolute the contributions of various processes affecting transport, and it is possible to estimate the passive lipoidal permeability due to the cell membrane itself.

The last column in Table 1 lists Caco-2 permeability at pH 7.4 (averaged from numerous studies), after correction for ABL, paracellular, and filter porosity contributions. Evidently, Papp values do change substantially (14–494 × 10⁻⁶ cm/s) for the first five drugs, suggesting that the uncorrected MDCK permeability in the Table is ABL-limited. For ionizable drugs, carrier-mediated effects can be recognized, but it is necessary to perform the assay over a range of pH values (not just 7.4). Figure 5 shows such studies performed for the β-blockers.
propranolol, metoprolol, and atenolol. The intrinsic permeability values of the uncharged forms (pH > 10) of these molecules can reach enormous values: 28840, 14125, and 46 × 10^-6 cm/s, respectively. For very lipophilic molecules (e.g., propranolol, metoprolol), carrier-mediated transport is generally not as fast as suggested by a study of the transport of verapamil in the Caco-2 model. For the two lipophilic bases, the solid curves start to level off in acidic pH, very likely because of a carrier-mediated (CM) uptake process affecting the positively charged bases, with Papp = 29 and 2.8 × 10^-6 cm/s in the cases of propranolol and metoprolol, respectively. In the case of atenolol, the leveling off in acidic pH is most likely due to the paracellular pore permeability (Papp = 0.59 × 10^-6 cm/s).

**Evidence from in Vivo Investigations.** In vivo evidence is also fully supportive of passive lipoidal permeability being the major influence on drug disposition. Access to intracellular and intraorgan (CNS) targets shows, for a highly passive lipoidally permeable drug, identical concentrations in the aqueous phase between plasma (unbound free drug) and the intracellular or intraorgan water.

The CNS represents an important vascular/acellular barrier that is accessed in most cases by lipoidal diffusion and is amenable to quantitative structure–permeation relations.
0.2, 0.79, and 0.01 mL per gram of brain tissue, respectively. For intrabrain distribution by passive lipoidal diffusion, \( K_{pu,cell} \) can be simply calculated by taking into account the \( pK_a \) of the drug and the pH and volumes of the three brain compartments, as demonstrated elegantly. Examples of passive distribution are shown in Figure 7 by filled circles (87% of the drugs in the example) bounded by the two dashed lines. There appear to be net efflux processes (checkered circles, 5% of the drugs), driving compounds from brain cells into the brain interstitial fluid. Examples of net clearance from the interstitial fluid into the brain cells are shown by molecules represented by unfilled circles (8% of the drugs).47

The clearance of highly lipid-permeable drugs mirrors all the above. Even if the drug is a possible substrate of a transporter, clearance is invariably metabolic. This is evidenced by the following:

1. A normally negligible excretion or clearance of a lipophilic compound in urine. Even though the unbound drug is filtered at the glomerulus and could be concentrated in the renal tubule by transporter involvement, passive lipoidal diffusion means the drug is passively reabsorbed as the urine is concentrated, resulting in negligible urinary excretion of all highly lipoidally permeable drugs.

2. When urinary pH is altered, compelling proof of lipoidal permeability is shown for drugs with a positive log \( P \) value (i.e., whose neutral form is lipophilic) and a negative log \( D \) value (i.e., whose ionized form is hydrophilic).50–52 Thus, the basic drug amphetamine (log \( P \) 1.8, \( pK_a \) 9.94, TPSA 26 Å²) is poorly excreted (3–7%) in alkaline urine and markedly excreted (60%) in acidic urine. This behavior is due to the drug undergoing renal filtration, followed by passive reabsorption of the lipophilic neutral form, or lack of reabsorption of the hydrophilic cationic form. Conversely, acetylsalicylic acid (log \( P \) 0.90, \( pK_a \) 3.50, TPSA 64 Å²) shows greater excretion at a urinary pH of 7 (3%) compared to pH 5.5 (1%). Memantine, a basic drug (log \( P \) 2.1, \( pK_a \) 10.2, TPSA 26 Å²) has been examined at urinary pH values of 5 and pH 8 and shows a 7–10-fold higher renal clearance at acidic compared to basic urinary pH.53 At pH 5 unbound renal clearance values (6 mL/min/kg) exceed glomerular filtration rate (2 mL/min/kg), indicating some active tubular secretion in addition to glomerular filtration, illustrating how transport proteins can exist alongside passive diffusion processes. It is difficult to conceive of a system able to explain the effects of pH on urinary excretion and clearance other than passive lipoidal diffusion.

3. The rapid transfer of drugs in both directions across the sinusoidal membrane results in intracellular hepatocyte concentrations approximating unbound plasma concentrations. Although not direct evidence, this probably explains the utility of the well-stirred liver model and its robust use in in vitro to in vivo predictions of clearance for highly lipid permeable drugs.

Thus, for an oral, high lipoidal permeability drug, only during the absorption process will a concentration gradient exist in the body and free drug concentrations will be identical between plasma water, extracellular water, and total body water. The absence of concentration gradient indicates the passive diffusion nature of the process.

When a drug has moderate or low passive lipoidal permeability, behavior different from that outlined above occurs in a predictable manner. As the rate of passive lipoidal diffusion is lower, the influence of transporter proteins can become apparent. Transport gradients will now exist across the aqueous compartments of the body. The evidence for the passive diffusion of highly permeable drugs now is reflected in almost an inversion of all the statements above for drugs of moderate to low lipid permeability. These concepts allow the rational understanding of drug disposition based on compound characteristics.

Figure 7. Many CNS drugs distribute inside the brain by passive lipoidal diffusion. Examples are shown by filled circles (87% of the drugs in the example) bounded by the two dashed lines. There appear to be net efflux processes (checkered circles, 5% of the drugs), driving compounds from brain cells into the brain interstitial fluid. Examples of net clearance from the interstitial fluid into the brain cells are shown by molecules represented by unfilled circles (8% of the drugs).47

Considerable amounts of human data are available. This is exemplified by studying sedating (highly passive lipoidal permeable) and nonsedating (lower passive lipoidally permeable, influenced by efflux transporters) H1 receptor antagonists (antihistamines).49 Chorpheniramine, in contrast to the nonsedating antihistamine ebastine, when studied with PET scanning, had a \( K_a \) calculated from CNS receptor occupancy (7 nM) and unbound plasma concentrations matching that measured in in vitro receptor experiments (3–4 nM). The receptor occupancy of ebastine was much lower than its plasma concentration would suggest. Ebastine rapidly forms the zwitterionic (low passive lipoidal permeability) active metabolite cavedastine and is partially excluded from the brain due to this and the activity of efflux transporters.

The clearance of highly lipid-permeable drugs mirrors all the above. Even if the drug is a possible substrate of a transporter, clearance is invariably metabolic. This is evidenced by the following:

1. A normally negligible excretion or clearance of a lipophilic compound in urine. Even though the unbound drug is filtered at the glomerulus and could be concentrated in the renal tubule by transporter involvement, passive lipoidal diffusion means the drug is passively reabsorbed as the urine is concentrated, resulting in negligible urinary excretion of all highly lipoidally permeable drugs.

2. When urinary pH is altered, compelling proof of lipoidal permeability is shown for drugs with a positive log \( P \) value (i.e., whose neutral form is lipophilic) and a negative log \( D \) value (i.e., whose ionized form is hydrophilic).50–52 Thus, the basic drug amphetamine (log \( P \) 1.8, \( pK_a \) 9.94, TPSA 26 Å²) is poorly excreted (3–7%) in alkaline urine and markedly excreted (60%) in acidic urine. This behavior is due to the drug undergoing renal filtration, followed by passive reabsorption of the lipophilic neutral form, or lack of reabsorption of the hydrophilic cationic form. Conversely, acetylsalicylic acid (log \( P \) 0.90, \( pK_a \) 3.50, TPSA 64 Å²) shows greater excretion at a urinary pH of 7 (3%) compared to pH 5.5 (1%). Memantine, a basic drug (log \( P \) 2.1, \( pK_a \) 10.2, TPSA 26 Å²) has been examined at urinary pH values of 5 and pH 8 and shows a 7–10-fold higher renal clearance at acidic compared to basic urinary pH.53 At pH 5 unbound renal clearance values (6 mL/min/kg) exceed glomerular filtration rate (2 mL/min/kg), indicating some active tubular secretion in addition to glomerular filtration, illustrating how transport proteins can exist alongside passive diffusion processes. It is difficult to conceive of a system able to explain the effects of pH on urinary excretion and clearance other than passive lipoidal diffusion.

3. The rapid transfer of drugs in both directions across the sinusoidal membrane results in intracellular hepatocyte concentrations approximating unbound plasma concentrations. Although not direct evidence, this probably explains the utility of the well-stirred liver model and its robust use in in vitro to in vivo predictions of clearance for highly lipid permeable drugs.

Thus, for an oral, high lipoidal permeability drug, only during the absorption process will a concentration gradient exist in the body and free drug concentrations will be identical between plasma water, extracellular water, and total body water. The absence of concentration gradient indicates the passive diffusion nature of the process.

When a drug has moderate or low passive lipoidal permeability, behavior different from that outlined above occurs in a predictable manner. As the rate of passive lipoidal diffusion is lower, the influence of transporter proteins can become apparent. Transport gradients will now exist across the aqueous compartments of the body. The evidence for the passive diffusion of highly permeable drugs now is reflected in almost an inversion of all the statements above for drugs of moderate to low lipid permeability. These concepts allow the rational understanding of drug disposition based on compound characteristics.
Transdermal Medicines: A Case for Passive Permeation.

Skin was originally considered an essentially impermeable barrier whose function was restricted to protecting animal organisms from their environment. Since years, it is now known that the skin also represents a potential portal of entry by which many chemicals may gain access to the systemic circulation. This route of administration of systemically acting drugs provides strong evidence for the determining involvement of passive permeation processes in their transdermal permeation.

During skin absorption, drugs have to pass a complex multilayer structure before reaching the blood. Structurally, the skin can be viewed as a series of layers, the three major divisions being epidermis, dermis, and subcutis or hypodermis. The latter lies below the dermis and the vascular system, and is divided into the stratum corneum, papillary dermis, and reticular dermis. The former is a multilayered structure composed of keratinized cells, closely packed in a nonpolar lipid matrix composed of ceramides, cholesterol, and fatty acids, but largely devoid of phospholipids. In contrast and to the best of our knowledge, there is no influx transporter in the stratum corneum.

The transdermal administration of systemically acting topical medications relies on specific formulations (e.g., hydrogels, creams, ointments, patches, or disks), from which drugs exit transdermally active medicines to illustrate the variety of media and a brief recounting follows.

A Debate on the CMO or an Integrated System Defined by Lipoidal Diffusion. The major points made in support of the CMO are followed by a response. Readers will find greater detail in the articles. Arguments 1–13 are from Dobson and Kell; argument 14 is from Dobson et al.; argument 15 is from Kell et al.

1. Lipophilic cations are charged and cannot cross membranes owing to Born charging. Response: Drug molecule ions are in equilibrium with neutral nonionized drug molecules, which have much higher lipophilicity and much higher passive diffusion permeation rate. According to the pH-partition theory, permeation rate varies with solution pH and a compound’s pKa, such that an increasing ratio of nonionized/ionized forms correlates with increasing permeation rate. The term log D is a descriptor incorporating the effects of lipophilicity and ionization.

2. The mass ratio of protein/lipid in vivo (1/1 to 3/1) affects the transport properties of lipids. Artificial membranes do not model biological membranes, owing to the high protein content in vivo. Response: These ratios include the cytoplasmic and exoplasmic portions of membrane protein mass, not just the relevant transmembrane fraction. The lipid:protein molar ratio is estimated as 40:1, making lipid an important portion of the membrane exposed to drug molecules. A further refined consideration would take into account the

<table>
<thead>
<tr>
<th>drug</th>
<th>therapeutic class</th>
<th>major indication</th>
<th>formulation</th>
<th>MW</th>
<th>log P</th>
<th>pK_a</th>
</tr>
</thead>
<tbody>
<tr>
<td>diclofenac</td>
<td>NSAID</td>
<td>locomotor pain</td>
<td>hydrogel, cream</td>
<td>296.1</td>
<td>4.5</td>
<td>4.1</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>estrogenic agonist</td>
<td>hormone replacement therapy</td>
<td>gel, patch, disk</td>
<td>272.4</td>
<td>4.0</td>
<td>10.3</td>
</tr>
<tr>
<td>fentanyl</td>
<td>μ-opioid agonist</td>
<td>narcotic analgesia</td>
<td>patch</td>
<td>336.5</td>
<td>4.1</td>
<td>8.8</td>
</tr>
<tr>
<td>glycol salicylate</td>
<td>presumably a prodrug of salicylic acid</td>
<td>locomotor pain</td>
<td>hydrogel, cream</td>
<td>182.2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>nicotine</td>
<td>nicotinic agonist</td>
<td>nicotine withdrawal</td>
<td>disk, patch</td>
<td>162.2</td>
<td>1.2</td>
<td>8.8</td>
</tr>
<tr>
<td>nitroglycerine</td>
<td>vasodilator</td>
<td>angina pectoris</td>
<td>disk</td>
<td>227.1</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>scopolamine</td>
<td>muscarinic antagonist</td>
<td>motion sickness</td>
<td>disk</td>
<td>303.4</td>
<td>0.98</td>
<td>6.9</td>
</tr>
<tr>
<td>selegiline</td>
<td>MAO-B inhibitor</td>
<td>Parkinson’s disease</td>
<td>patch</td>
<td>187.3</td>
<td>2.7</td>
<td>8.7</td>
</tr>
<tr>
<td>testosterone</td>
<td>androgenic agonist</td>
<td>hormone replacement therapy</td>
<td>patch, hydrogel</td>
<td>288.4</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

Values (4–10). Indirect evidence obtained from the statistical analysis of in vivo and in vitro skin permeation studies have revealed excellent correlations with molecular descriptors such as molecular weight and volume, log P, log D, and H-bonding parameters.

Why Debate the Role of Passive Lipoidal Permeability? There are now authors who doubt the role of passive lipoidal diffusion in drug permeation. It is possible their view is colored by a growing interest in transporters as drugs become less lipid-permeable to conform to target active site characteristics (see above). Some of these authors state that carrier mediation is the only existing form of permeation, at the exclusion of lipoidal permeation (CMO concept). While healthy debate is warranted, the group most advocating the CMOC has made statements to discredit passive lipoidal diffusion and its foundational evidence. This is contrary to the conclusion of many, including the authors. Passive lipoidal diffusion exists alongside carrier-mediated transport, and the pathways are defined by physicochemical characteristics. The proponents of the CMOC state that “...evidence and reasoning we describe may be seen as circumstantial...”. The statements against passive lipoidal diffusion have been strongly refuted, and a brief recounting follows.

Table 2. Representative Transdermal Medicines

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic Class</th>
<th>Major Indication</th>
<th>Formulation</th>
<th>MW</th>
<th>log P</th>
<th>pH_Ka</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>Nonsteroidal Anti-Inflammatory Drug</td>
<td>Locomotor pain</td>
<td>Hydrogel, cream</td>
<td>296.1</td>
<td>4.5</td>
<td>4.1</td>
</tr>
<tr>
<td>17ß-Estradiol</td>
<td>Estrogenic Agonist</td>
<td>Hormone replacement therapy</td>
<td>Gel, patch, disk</td>
<td>272.4</td>
<td>4.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Mu-Opioid Agonist</td>
<td>Narcotic analgesia</td>
<td>Patch</td>
<td>336.5</td>
<td>4.1</td>
<td>8.8</td>
</tr>
<tr>
<td>Glycol Salicylate</td>
<td>Presumably a prodrug of salicylic acid</td>
<td>Locomotor pain</td>
<td>Hydrogel, cream</td>
<td>182.2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>Nicotinic Agonist</td>
<td>Nicotine withdrawal</td>
<td>Disk, patch</td>
<td>162.2</td>
<td>1.2</td>
<td>8.8</td>
</tr>
<tr>
<td>Nitroglycerine</td>
<td>Vasodilator</td>
<td>Angina pectoris</td>
<td>Disk</td>
<td>227.1</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Scopolamine</td>
<td>Muscarinic Antagonist</td>
<td>Motion sickness</td>
<td>Disk</td>
<td>303.4</td>
<td>0.98</td>
<td>6.9</td>
</tr>
<tr>
<td>Selegiline</td>
<td>MAO-B Inhibitor</td>
<td>Parkinson’s disease</td>
<td>Patch</td>
<td>187.3</td>
<td>2.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Androgenic Agonist</td>
<td>Hormone replacement therapy</td>
<td>Patch, hydrogel</td>
<td>288.4</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

Values (4–10). Indirect evidence obtained from the statistical analysis of in vivo and in vitro skin permeation studies have revealed excellent correlations with molecular descriptors such as molecular weight and volume, log P, log D, and H-bonding parameters.
relative cross-sectional area at the membrane surface of the 40 phospholipid molecules to one typical protein. The lipid surface area would still be significantly greater than that of the transporter protein.

3. Correlations of drug uptake with log \( P \) and Caco-2 permeation can be weak. **Response:** For drugs permeating predominantly by passive lipid diffusion, the apparent Caco-2 permeability coefficient, \( \text{P}_{\text{app}} \), can be (and often is) affected by the aqueous boundary layer, filter, paracellular, and lipidal (transcellular) permeability, as well as the solution pH, as illustrated by the examples in Figure 5. \( \log P \) cannot be directly compared to \( \log \text{P}_{\text{app}} \). The Caco-2 intrinsic permeability, \( \log \text{P}_o \), is the rational term to compare to \( \log P \). \( \text{P}_o \) is easy to deduce from \( \text{P}_{\text{app}} \) \( ^{58} \) but this is seldom done, which often leads to “weak” correlation, as “apple seeds are compared to whole watermelons.” Caco-2 cells from 10 different laboratories were compared in terms of transporter mRNA levels of 72 drug and nutrient transporters, and 17 other targets. It was concluded that “Caco-2 cells from different laboratories produce different results even when using standard protocols for transport studies. The differences may be due to transporter expression as shown for e.g. PepT1 and MDR1 which in turn is determined by the culture conditions. Although the majority of the laboratories used similar culture conditions, absolute expression of genes was variable indicating that even small differences in culture conditions have a significant impact on gene expression, although the overall expression patterns were similar.” \( ^{34} \) Therefore, it is not astonishing that results of Caco-2 cell based permeabilities, when correlated with octanol \( \log P/D \) values, sometimes show differences in correlations. This is mainly due to the origin and composition of the analyzed data set (ratio actively versus passively [lipoidal diffusion] transported compounds) and use of partition coefficients (\( \log P \)) or pH-dependent distribution coefficients (\( \log D \)). Interestingly, correlations of transport studies performed with different cell lines (e.g., Caco-2/MDCK) commonly used in absorption prediction, with presumably different transportexpression levels, often give excellent correlations, \( ^{32} \) further supporting the coexistence of active and passive transport in biological systems.

4. Transport across model artificial membranes is stated to occur via pore defects or dissolution in the lipid mixture that are not seen in vivo. **Response:** The studies cited are computational simulations (so-called molecular dynamics) of Na\(^+\) and Cl\(^-\) ion (non-drug-like) transport under unusual conditions. No convincing experimental evidence for the relevance of pores has been reported. Other experiments indicate the unimportance of pores. Membrane resistance excluding pore diffusion is usually determined by conductivity measurements. Otherwise function of, e.g., ion channels could not be determined. \( ^{5} \)

5. A dominant role for carrier-mediated transport (and against passive diffusion) is inferred from the hundreds of publications on drug transporters. **Response:** A large number of papers have been published in recent years on transporters. These result from the recent intense research on transporters. However, it is a logical fallacy and a sleight of hand to state that this is evidence of the rate and extent of dominance of carrier-mediated permeation over passive lipoidal diffusion. (An analogy would be to state that newspapers contain a predominance of articles about bad events (e.g., fires, wars, violence, accidents), therefore, bad events dominate good events in the world.) Thus, the large number of citations of publications on transporter research is misleading, because the research they report or review was not undertaken nor concluded by the publication authors as evidence that supports CMOC, as is implied (“There is considerable and increasing evidence that drugs get into cells more or less solely by hitchhiking on carriers normally used for the transport of nutrients and intermediary metabolites”). \( ^{3} \)

6. Selected small molecules, urea and glycerol, which cross BLM, permeate to some extent in vivo via transporters, except in yeast because glycerol is an osmolyte. **Response:** Urea and glycerol are more hydrophilic than typical drugs that permeate membranes, thus, they are not good models of permeants on which to support theories.

7. In liposomes the rate of transfer of nonelectrolytes depends on MW rather than \( \log P \). **Response:** Liposomes correlate well with the permeation behaviors usually observed in artificial and biological membranes. \( ^{18,36,72−74} \) Molecular weight is partly correlated with lipophilicity and hydrogen-bonding capacity, and as molecular weight increases in drugs normally so does hydrogen bonding. Molecular weight is therefore a hybrid term expected to show a relationship to lipoidal membrane permeation.

8. In vitro models of diffusion rates across membranes are not based on large sample numbers and validated with compounds not used in the method development. **Response:** This is out of date information. \( ^{36} \)

9. The flux across in vitro PAMPA membranes can be poor even when human absorption is good (e.g., cepalexin, ticarcilast). **Response:** This is out of date information and also is misleading. PAMPA membranes serve to model passive diffusion, whereas cepalexin and a number of other molecules are carrier-mediation transported, as extensively compiled. \( ^{30} \) The present authors claim that passive and active transport processes coexist. PAMPA has been described to only account for passive membrane permeation processes. Therefore, it is not astonishing that actively transported compounds like, e.g., cepalexin cannot be correctly predicted regarding human absorption by methods exclusively focusing on passive transport.

10. Activities of anesthetics were previously thought to be controlled by passive diffusion and correlated to \( \log P \), but are now thought to be protein-binding related. **Response:** There is common agreement that drug molecules and anesthetics might interact with proteins, but this is misleading in the context of the discussion, which is around drug transport and not mechanism of action. A recent publication on anesthetics has summarized thus: “The molecular mechanism of general anesthesia is still a controversial issue. Direct effect by linking of anesthetics to proteins and indirect action on the lipid membrane properties are the two hypotheses in conflict.” \( ^{75} \)

11. Many molecules (e.g., ethanol) have relatively specific receptors, so they may have similar protein binding (unidentified) that affects membrane permeation.
Accurate Understanding of Drug Permeation Mechanisms is Important for Drug Development Success. A major value of the prevailing permeation hypothesis is that it guides drug research and development, providing a reliable foundation for lead selection, candidate optimization, preclinical studies, and product development. Therefore, it is imperative that accurate permeation theories are implemented. Translation of a discovery therapeutic “hit” to a clinical candidate, in part, requires accurate prediction of drug exposure to the target, which is enabled, along with models of other drug properties (e.g., solubility, metabolism), by an accurate permeation model. The prevailing permeation hypothesis has facilitated success in drug design, interpretation of pharmacokinetics, and planning clinical dose and dosing regimens.

Permeability is a component of many in vivo pharmacokinetic processes in drug absorption and disposition, including the following examples in which permeation rate can be rate-determining:

- absorption: permeation across the gastrointestinal epithelial membranes to reach blood capillaries
- hepatic clearance: permeation across the sinusoidal and canicular hepatocyte cell membrane
- renal clearance: permeation across the nephron tubules
- CNS exposure: permeation across blood–brain barrier
- efficacy: permeation across the cell membrane for intracellular targets

Permeability can also affect in vitro cell-based assays in drug discovery because molecules must cross cell membranes to reach intracellular targets.

Many of the rates for the preceding permeability processes are measured using in vitro and in vivo protocols during drug discovery and development to observe the rates of permeation of an experimental compound in compound screening for lead optimization and in detailed characterization for preclinical studies predicting human pharmacokinetics and safety. The in silico, in vitro, and in vivo tools that have been developed using the prevailing drug permeation hypothesis provide reliable predictions of drug disposition in vitro, in preclinical species and in human. Drug researchers regularly apply these tools and have achieved notable progress and success in drug design and optimization.81

Major New Evidence Is Needed To Support the CMOC. There is insufficient evidence supporting CMOC to lead to its significant application in drug development, and the prevailing permeation hypothesis continues to be widely applied. A fresh collection of experiments initiated based on the unique claims of CMOC has not been reported. Commentaries on CMOC have mainly focused on reinterpretation of data from earlier experiments, not excluding elements of confirmation bias.82 Only one research paper83 with results from experiments that are based on CMOC found previously unknown linkage of carriers to permeability of selected compounds that are toxic to yeast. These carriers have not, to our knowledge, been validated and characterized with the type of rigor expected of any new target or transporter in drug research or for publication.

Since its first description in 2008, the CMOC has not been the basis for new advances in drug delivery and disposition. There have been no original research articles, attributed to a foundation of CMOC, in which carriers were demonstrated to completely explain the permeation of clinical drugs that were previously thought to permeate mainly by passive lipid membrane diffusion. Of the many in silico tools used widely in drug disposition prediction, CMOC has not been embraced for incorporation. Considerable experimental evidence and peer review concurrence would be needed to gain acceptance for CMOC, in accordance with the usual practices for permeation research. These include the following:

1. For the large number of drugs that are currently concluded to primarily permeate via passive diffusion, the CMOC should provide data that
   a. Identify the specific transporter(s), whether known or new, that transport each. Note that the CMOC postulates the existence of a large number of unidentified transporters to explain transport across any lipidoidal barrier. Specific evidence, however, is still missing.
   b. Account quantitatively for the known permeation rates and in vivo disposition effects. Indeed, the CMOC proponents note that many approved drugs interact with a transporter, but no data are provided to characterize the rate and extent of these interactions and effects on in vivo disposition. For each tissue to which the drug is known to have exposure, proof is needed that the purported transporter(s) are in sufficient concentration to produce the observed level of drug exposure.

Response: This is an assumption and generalization awaiting to be proven by experimental data, but which currently does not rule out transport by passive (lipoidal diffusion) mechanism.

12. Carrier-mediated drug uptake is observed where it has been studied. (Presumably this circumstance indicates that transporters will be found for all drugs.) Response: Carrier-mediated drug uptake may be observed, but it may not account for 100% of the transport. In Michaelis–Menten analysis, the nonsaturable term usually is related to the passive diffusion contribution.

13. Drugs can concentrate in specific tissues beyond the stoichiometry of internal binding sites. This phenomenon absolutely requires an active uptake process. Response: This can be due to pH gradients between intracellular and extracellular compartments as described for, e.g., basic amines and safety relevant lysosome accumulation (phospholipidosis).76

14. Biophysical forces in drug–lipid membrane interactions (e.g., lipophilicity, hydrogen bonding) are no different from drug–protein interaction. Thus, physicochemical properties and the rule of 5 need not be evidence of passive diffusion.77 Response: Of course biophysical forces apply to both carriers and bilayers. However, the physical property differences between the rate limiting barriers for a particular drug in carriers and bilayers can dictate the predominant route of transport.19 A second argument is that ligand–protein recognition is dominated by highly selective stereoelectronic features far more than by global (molecular) physicochemical properties.78–80

15. The notion of passive lipidoidal permeation is traced back to artificial membrane systems, which are not successful predictors of membrane permeation. Response: On the contrary, artificial membrane models have been successful predictors of passive lipidoidal permeation.11,14,17–20,36
Molecular Pharmaceutics

Review

For cases where the drug is known to permeate transcellularly (i.e., across both membranes, such as the gastrointestinal epithelium, blood–brain barrier, Caco-2), CMO-based investigations should provide evidence for both the apical and basolateral transporters for each drug and metabolites. Furthermore, where the drug is known to permeate transcellularly in both directions (i.e., apical-to-basolateral and basolateral-to-apical, such as in Caco-2), CMO proponents should identify the transporters in both apical and basolateral membranes that allow transport in both directions for that membrane.

2. Discover major new influx transporters whose substrates are lipophilic compounds.
   a. These carriers should recognize and transport compounds having a physicochemical profile (lipophilicity, MW, hydrogen-bonding capacity) currently linked to passive lipoidal diffusion. This demand is in line with the argument of CMO proponents that new, yet unidentified, transporters will transport lipophilic compounds that satisfy the rule of 5, rules 1 to 4.
   b. There is a clear need to clone the genes for the purported new transporters, express them, and study their characteristics in an in vitro cell line. These transporters should demonstrate the following common transporter characteristics, except in limited cases:
      i. Display saturation of permeation rate with increasing drug concentration, since it is far from sufficient to speculate, on behalf of CMO, that there are so many transporters for every compound that permeation rate does not saturate. Note however that a few transporters (e.g., PEPT1) that have very high capacity are limited cases that do not readily saturate.
      ii. Show inhibition of permeation rates by an inhibitor.
      iii. Show stereospecific differences in permeation rates.
   c. Exhibit variations of permeation rate with pH that are consistent with the pH-partition theory.
   d. Show that the transporters’ permeation rates follow the observed concentration gradient dependence.

3. Discover and characterize new high-flux transporters in Caco-2, MDCK, 2/4/1A, and other cell lines commonly used for in vitro cell layer permeation experiments. This demand is warranted by the speculations of CMO proponents that many new significant transporters will be discovered in these highly studied cell lines.

4. Discover a mechanism that stops or slows down passive lipoidal diffusion in biological membranes. This demand is justified by the fact that passive lipoidal diffusion has been demonstrated in liposomes in vitro, and these are a model of the observed passive lipoidal permeation of biological membranes, as discussed above.

CONCLUSION

Debates on important scientific points are necessary and valuable for advancing science. The current debate, in our view, has contained some unusual elements. First, we agree with the significant role of carrier-mediated transport in drug permeation, however, we disagree with the attempt to invalidate the well-established scientific process of passive lipoidal diffusion, which is done, seemingly, to support the new CMO concept. The arguments against passive lipoidal diffusion are frequently illogical, and they cherry-pick the data and overlook the large body of data that support the major contribution of passive lipoidal diffusion in drug permeation. Second, insufficient experiments have been planned, conducted, and reported that are based on the necessary outcomes of CMO, which would differentiate it from the prevailing permeation theory. Third, we do not think that CMO is widely accepted and supported, as has been implied by the statement, “There is burgeoning evidence for the carrier-mediated view of drug uptake...” The references that are listed in support of this view are reports of transporter research or literature reviews by authors who do not conclude that their research supports CMO. Until significant direct evidence is provided that supports CMO, drug researchers should withhold decisions based on CMO that could affect drug discovery and development.

Expansion of investigation on drug permeation is greatly encouraged. The prevailing drug permeation hypothesis, which incorporates several parallel active and passive permeation mechanisms (i.e., passive lipoidal diffusion, carrier-mediated influx, carrier-mediated efflux, paracellular diffusion, mucus resistance, endocytosis, transcytosis), whose rates are determined by the properties of the compound and the membrane, is consistent with the experimental evidence and provides successful predictions for drug discovery and development. CMO has not produced new evidence and predictions that warrant changing drug permeation theories, nor their application to drug research.

AUTHOR INFORMATION

Corresponding Author
*E-mail: professordennis@deltic50.freeserve.co.uk.

Present Address
© 21705 Mobley Farm Drive, Laytonsville, MD 20882, United States.

Notes
The authors declare no competing financial interest.

REFERENCES

(4) Kell, D. B.; Dobson, P. D.; Bilsland, E.; Oliver, S. G. The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we (need to) know and how we can do so. Drug Discovery Today 2013, 18, 218–239.


(21) Ahlin, G.; Hilgendorf, C.; Karlsson, J.; Al-Khalili Szigarty, C.; Uhlen, M.; Artursson, P. Endogenous gene and protein expression of drug-transporting proteins in cell lines routinely used in drug discovery programs. *Drug Metab. Dispos.* 2009, 37, 2275–2283 (also in Figure 2).


(23) Avdeef, A.; Tam, K. Y. How well can the Caco-2/MDCK models predict effective human jejunal permeability? *J. Med. Chem.* 2010, 53, 3566–3584. (also in Figure 2).


(27) Avdeef, A.; Artursson, P.; Neuhoff, S.; Lazarova, L.; Gräsji, J.; Tavelin, S. Caco-2 permeability of weakly basic drugs predicted with the double-sink PAMPA pKα flux method. *Eur. J. Pharm. Sci.* 2005, 24, 333–349 (also in Figure 5).

(28) Tsimin, O.; Tsimin, K.; Sun, N.; Avdeef, A. Pharmacocinetic selectivity of the BBB microenvironment governing passive diffusion – matching with a porcine brain lipid extract artificial membrane permeability model. *Pharm. Res.* 2011, 28, 337–363 (also in Figure 3).

(29) Dagenais, C.; Avdeef, A.; Tsimin, O.; Dudley, A.; Beliveau, R. P-glycoprotein deficient mouse in situ blood-brain barrier permeability and its prediction using an in confo PAMPA model. *Eur. J. Pharm. Sci.* 2009, 38, 121–137 (also in Figure 3).


Molecular Pharmaceutics

(65) www.drugbank.ca.