Mass Transfer Phenomena and Biological Membranes

Introduction

Mass transfer is the net movement of mass from one location to another in response to applied driving forces. Mass transfer is used by different scientific disciplines for different processes and mechanisms. It is an important phenomena in the pharmaceutical sciences; drug synthesis, preformulation investigations, dosage form design and manufacture and finally ADME (absorption, distribution, metabolism and excretion) studies. In nature, transport occurs in fluids through the combination of advection and diffusion. Diffusion occurs as a result of random thermal motion and is mass transfer due to a spatial gradient in chemical potential or simply, concentration. However the driving force in convective mass transport is the spatial gradient in pressure (Fleisher, 2000). On the other hand, there are other variables influencing mass transfer like electrical potential and temperature which are important in pharmaceutical sciences. In a complex system mass transfer may be driven by multiple driving forces. Mass transfer exists everywhere in nature and also in human body. In fact in the body, mass transport occurs across different types of cell membranes under different physiological conditions. This chapter is aimed at reviewing transport across biological membranes, with an emphasis on intestinal absorption, its model analysis and permeability prediction.

Transport across membranes

Biomembrane or biological membrane is a separating amphipathic layer that acts as a barrier within or around a cell. The membrane that retains the cell contents and separates the cell from surrounding medium is called plasma membrane. This membrane acts as a lipid bilayer permeability barrier in which the hydrocarbon tails are in the centre of the bilayer and the electrically charged or polar headgroups are in contact with watery or aqueous solutions. There are also protein molecules that are attached to or associated with the membrane of a cell. Generally cell membrane proteins are divided into integral (intrinsic) and peripheral (extrinsic) classes. Integral membrane proteins containing a sequence of hydrophobic group are permanently attached to the membrane while peripheral proteins are temporarily attached to the surface of the cell, either to the lipid bilayer or to integral proteins. Integral proteins are responsible for identification of the cell

for recognition by other cells and immunological behaviour, the initiation of intracellular responses to external molecules (like pituitary hormones, prostaglandins, gastric peptides,…), moving substances into and out of the cell (like ATPase,…). Concerning mass transport across a cell, there are a number of different mechanisms, a molecule may simply diffuses across, or be transported by a range of membrane proteins (Washington et al., 2000, Lee and Yang, 2001).

Passive transport

Lipophilic drug molecules with low molecular weight are usually passively diffuses across the epithelial cells. Diffusion process is driven by random molecular motion and continues until a dynamic equilibrium is reached. Passive mass transport is described by Fick,s law which states that the rate of diffusion across a membrane (R) in moles s-1 is proportional to the concentration difference on each side of the membrane:

$$R=(Dk/h).A.\Delta C \quad (1)$$

Where D is the diffusion coefficient of the drug in the membrane, k is the partition coefficient of the drug into the membrane, h is the membrane thickness, A is the area of membrane over which diffusion is occurring, and ΔC is the difference between concentrations on the outside and the inside of the membrane. However it should be noted that the concentration of drug in systemic blood circulation is negligible in comparison to the drug concentration at the absorption surface and the drug is swept away by the circulation. Therefore the driving force for absorption is enhanced by maintaining the large concentration gradient throughout the absorption process. The diffusion coefficient of a drug is mainly influenced by two important factors, solubility of the drug and its molecular weight. For a molecule to diffuse freely in a hydrophobic cell membrane it must be small in size, soluble in membrane and also in the aqueous extracellular systems. That means an intermediate value of partition coefficient is needed. On the other hand, it is necessary for a number of hydrophilic materials, to pass through the cell membranes by membrane proteins. These proteins allow their substrates to pass into the cell down a concentration gradient, and act like passive but selective pores. For example for glucose diffusion into the cell by hexose transporter system, no energy is expended and it occurs down a concentration gradient. This process is called non-active facilitated mass transport (Sinko, 2006, Washington et al., 2000).

Active transport

In the cell membrane there are a group of proteins that actively compile materials in cells against a concentration gradient. This process is driven by energy derived from cellular metabolism and is defined as primary active trasport. The best-studied systems of this type are the ATPase proteins that are particularly important in maintaining concentration gradients of small ions in cells. However this process is saturable and in the presence of extremely high substrate concentration, the carrier is fully applied and mass transport rate is limited. On the other hand cells often have to accumulate other substances like amino acids and carbohydrates at high concentrations for which conversion of chemical energy into electrostatic potential energy is needed. In this kind of active process, the transport of an ion is coupled to that of another molecule, so that moving an ion out of the membrane down the concentration gradient, a different molecule moves from lower to higher concentration.

Depending on the transport direction this secondary active process is called symport (same directions) or antiport (opposite directions). Important examples of this process are absoption of glucose and amino acids which are coupled to transporter conformational changes driven by transmucosal sodium gradients (Lee and Yang, 2001).

Endocytic processes

All the above-mentioned mass transport mechanisms are only feasible for small molecules, less than almost 500 Dalton. Larger objects such as particles and macromolecules are absorbed with low efficiency by a completely different mechanism. The process which is called cytosis or endocytosis is defied as extending the membrane and enveloping the object and can be divided into two types, pinocytosis and phagocytosis. Pinocytosis (cell drinking) occurs when dissolved solutes are internalized through binding to non-specific membrane receptors (adsorptive pinocytosis) or binding to specific membrane receptors (receptor-mediated pinocytosis). In some cases, following receptor-mediated pinocytosis the release of undegraded uptaken drug into the extracellular space bounded by the basolateral membrane is happened. This phenomenon called transytosis, represents an important pathway for absorption of proteins and peptides. On the other hand phagocytosis (cell eating) occurs when a particulate matter is taken inside a cell. Although phagocytic processes are finding applications in oral drug delivery and targeting, it is mainly carried out by the specialized cells of the mononuclear phagocyte systems or reticuloendothelial system and is not generally relevant to the transport of drugs across absorption barriers (Lee and Yang, 2001, Fleisher, 2000, Washington et al., 2000).

Pore transport

The aqueous channels which exist in cell membranes allow very small hydrophilic molecules such as urea, water and low molecular weight sugars to be transported into the cells. However because of the limited pore size (0.4 nm), this transcellular pathway is of minor importance for drug absorption (Fleisher, 2000, Lee and Yang, 2001).

Persorption

As epithelial cells are sloughed off at the tip of the villus, a gap in the membrane is temporarily created, allowing entry of materials that are not membrane permeable. This process has been termed persorption which is considered as a main way of entering starch grains, metallic ion particles and some of polymer particles into the blood.

Intestinal drug absorption

Interest has grown in using in vitro and in situ methods to predict in vivo absorption potential of a drug as early as possible, to determine the mechanism and rate of transport across the intestinal mucosa and to alert the formulator about the possible windows of absorption and other potential restrictions to the formulation approach. Single-pass intestinal perfusion (SPIP) model is one of the mostly used techniques employed in the study of intestinal absorption of compounds which provides a prediction of absorbed oral dose and intestinal permeability in human. In determination of the permeability of the intestinal wall by external perfusion techniques, several models have been proposed (Ho

and Higuchi, 1974, Winne, 1978, Winne, 1979, Amidon et al., 1980). In each model, assumptions must be made regarding the convection and diffusion conditions in the experimental system which affects the interpretation of the resulting permeabilities. In addition, the appropriateness of the assumptions in the models to the actual experimental situation must be determined. Mixing tank (MT) model or well mixed model has been previously used to describe the hydrodynamics within the human perfused jejunal segment based on a residence time distribution (Lennernas, 1997). This model has also been used in vitro to simulate gastrointestinal absorption to assess the effects of drug and system parameters on drug absorption (Dressman et al., 1984). However complete radial mixing (CRM) model was used to calculate the fraction dose absorbed and intestinal permeability of gabapentine in rats (Madan et al., 2005). Moreover these two models (MT and CRM) were utilized to develop a theoretical approach for estimation of fraction dose absorbed in human based on a macroscopic mass balance approach (MMBA) (Sinko et al., 1991). Although these models have been theoretically explained, their comparative suitability to be used for experimental data had not been reported. The comparison of proposed models will help to select the best model to establish a strong correlation between rat and human intestinal drug absorption potential. In this section three common models for mass transfer in single pass perfusion experiments (SPIP) will be compared using the rat data, we obtained in our lab. The resulting permeability values differ in each model, and their interpretation rests on the validity of the assumptions (valizadeh et al., 2008).

Mass transfer models

Three models are described that differ in their convection and diffusion assumptions (Fig 1).

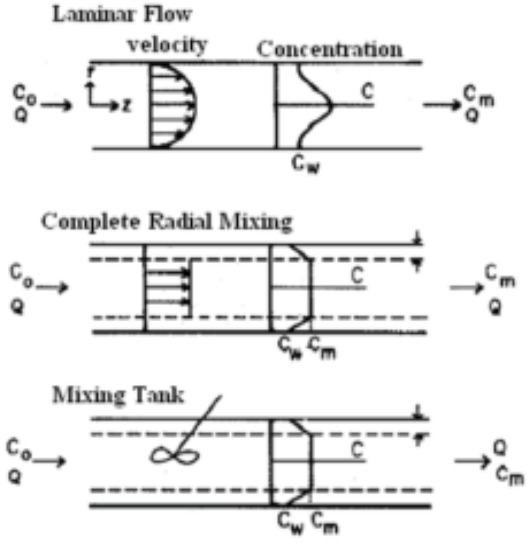

Fig. 1. Velocity and concentration profiles for the models. The concentration profiles are also a function of z except for mixing tank model (Amidon et al., 1980)

These models are the laminar flow, complete radial mixing (diffusion layer) for convective mass transport in a tube and the perfect mixing tank model. It is convenient to begin with the solute transport equation in cylindrical coordinates (Sinko et al., 1991, Elliott et al., 1980, Bird et al., 1960):

$$v_z^* \frac{\partial C}{\partial z^*} = Gz\left(\frac{1}{r^*}\frac{\partial}{\partial r^*} r^* \frac{\partial C}{\partial r^*}\right) \tag{2}$$

Where, Z* = Z / L, r* = r / R, $\upsilon_z^* = v_z$ / Vm, Gz = πDL/2Q , R = radius of the tube, L = length of the tube, Vm = maximum velocity, Q = perfusion flow rate
This relationship is subject to the first-order boundary condition at the wall:

$$\left.\frac{\partial C}{\partial r^*}\right|_{r^*=1} = -P_w^* C_w \tag{3}$$

where P_w^* = Pw R/D = the dimensionless wall permeability.
The main assumptions achieving Eq. 1 are: (a) the diffusivity and density are constant; (b) the solution is dilute so that the solvent convection is unperturbed by the solute; (c) the system is at steady state (∂C/∂t = 0); (d) the solvent flows only in the axial (z) direction; (e) the tube radius, R, is independent of Gz; and (f) axial diffusion is small compared to axial convection (Bird et al., 1960). The boundary condition (Eq. 2) is true for many models having a tube wall but does not describe a carrier transport of Michaelis-Menten process at the wall, except at low solute concentrations.

Complete radial mixing model

For this model the velocity profile as with the plug flow model is assumed to be constant. In addition, the concentration is assumed to be constant radially but not axially. That is, there is complete radial but not axial, mixing to give, uniform radial velocity and concentration profiles. With these assumptions, the solution is written as:

$$C_m/C_0 = \exp\left(-4 P_{eff}^* \; Gz\right) \tag{4}$$

where P_{eff}^* replaces P_w^* (Ho and Higuchi, 1974, Winne, 1978, Winne, 1979). Since no aqueous resistance is inc1uded in the model directly, the wall resistance is usually augmented with a film or diffusion layer resistance. That is, complete radial mixing occurs up to a thin region or film adjacent to the membrane. In this model the aqueous (luminal) resistance is confined to this region. Hence, the wall permeability includes an aqueous or luminal resistance term and can be written as:

$$P_{eff}^* = \frac{P_w^* P_a^*}{P_w^* + P_a^*} \tag{5}$$

where P_w^* is the true wall permeability and P_a^*, is the effective aqueous permeability. The aqueous permeability often is written as:

$$P_a = D/\delta \tag{6}$$

Or

$$P_a^* = R/\delta \tag{7}$$

where δ is the film thickness and represents an additional parameter that needs to be determined from the data to obtain P_w^*. For typical experiments, P_a^* or R/δ is an empirical parameter, since the assumed hydrodynamic conditions may not be realistic at the low Reynolds numbers. The complete radial mixing model also can be derived from a differential mass balance approach (Ho and Higuchi, 1974) and often is referred to as the diffusion layer model. The Calculated P_{eff}^* values for tested drugs and the corresponding plot are shown in Table 2 and Fig. 2 respectively.

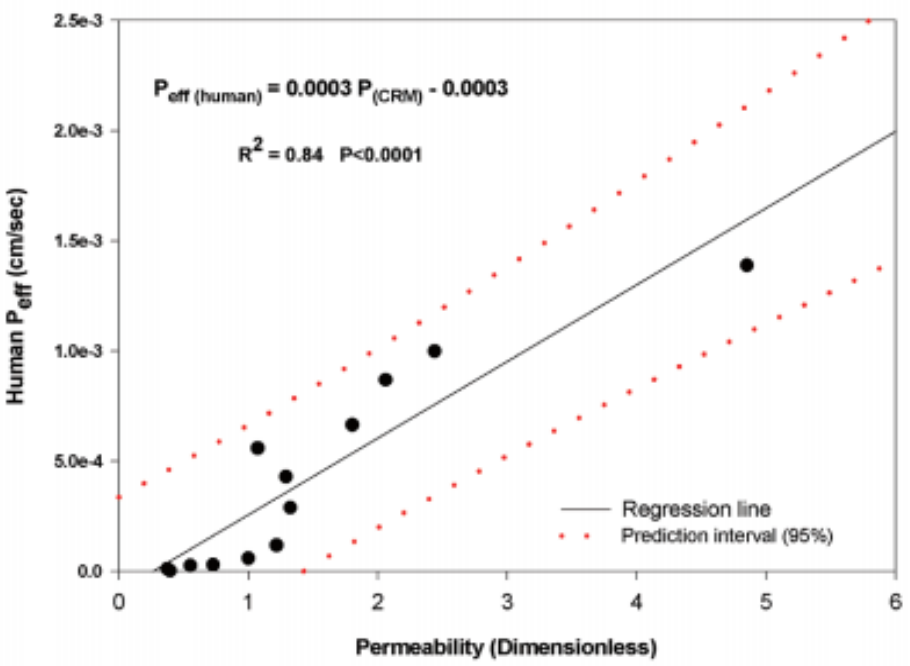

Fig. 2. Plot of dimensionless permeability values vs human P_{eff} values in complete radial mixing model

Laminar flow model

For flow of a newtonian fluid in a cylindrical tube, the exit concentration of a solute with a wall permeability Pw is given by (Amidon et al., 1980):

$$C_m/C_o = \sum_{n=1}^{\infty} M_n \exp(-\beta_n^2 G_z) \quad (8)$$

Where, Cm = "cup-mixing" outlet solute concentration from the perfused length of intestine,

$$Co = \text{inlet solute concentration; } Gz = \pi DL/2Q; \quad (9)$$

Gz is Graetz number, the ratio of the mean tube residence time to the time required for radial diffusional equilibration.

D = solute diffusivity in the perfusing fluid
L = length of the perfused section of intestine
Q = volumetric flow rate of perfusate = πR2(υ)
R = radius of perfused intestine
(υ) = mean flow velocity

Both the Mn and βn in Eq. 7 are functions of P_w^*, the dimensionless wall permeability,

$$P_w^* = \frac{P_w R}{D} \tag{10}$$

From the form of the solution it appears that Gz is the only independent variable and that the solution is an implicit function of P_w^*. Since P_w^* (or Pw) is the parameter of interest, Eq. 4 is not in a convenient form for its determination.
We now define:

$$\frac{1}{P_{eff}^*} = \frac{1}{P_w^*} + \frac{1}{P_{aq}^*} = \frac{1}{{}^{o}P_w^*} + \frac{1}{{}^{o}P_{aq}^*} \tag{11}$$

$$P_{eff}^* = \frac{\ln[(C_m/C_0)]_{exp}}{-4Gz} \tag{12}$$

$${}^{*}P_{aq}^* = \frac{\ln[(C_m/C_0)]_0}{-4Gz} \tag{13}$$

$$[(C_m/C_0)]_0 = \sum_{n=1}^{5} {}^{o}M_n \exp(-{}^{*}\beta_n^2 Gz) \tag{14}$$

where the superscript o denotes the sink condition (Graetz solution), the superscript * denotes dimensionless quantities [Eq. 8] and subscripts exp stands for experimental condition. The wall permeability is determined in the following manner: First the ${}^{*}P_{aq}^*$ is calculated using Eqs. 9 , 11, 14 and Table 1.

(n)	${}^{*}\beta_n$	${}^{*}M_n$
1	2.7043	0.81905
2	6.6790	0.09752
3	10.6734	0.03250
4	14.6711	0.01544
5	18.6699	0.00878

Table 1. Coefficients, ${}^{*}M_n$ and exponents, ${}^{*}\beta_n$ for the Graetz solution, equation (12), (sink conditions) (Elliott et al., 1980)

Then the P_{eff}^* is calculated from the experimental results using Eq. 8 and 11 at the third step the value of ${}^{*}P_w^*$ is found out from Eq. 10 and finally the value of ${}^{*}P_w^*$ is multiplied by the correction factor in Fig 3 to obtain P_w^*.

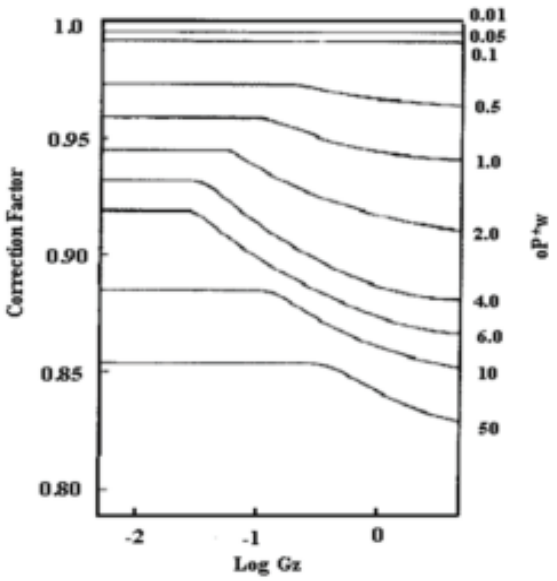

Fig. 3. Correction factor to obtain exact wall permeability (P_w^*) given the estimated wall Permeability (${}^*P_w^*$) and value of *Gz*. (Elliott et al., 1980)

All calculations were performed for our data in SPIP model. The Gz values were calculated based on equation 8, using the compound diffusivity, length of intestine and flow rate of perfusion which are shown in Table 2. The average value of Gz was found to be 3.34×10^{-2} ($\pm$ 8.6×10^{-3}). It seems that there are limitations for the use of laminar flow model in determination of the dimensionless wall permeability of highly permeable drugs. For instance a negative value of ibuprofen dimensionless wall permeability was obtained based on laminar flow model because of the high P^*_{eff} value of ibuprofen in comparison with its calculated P^*_{aq} sink value and as a result the drug was excluded from correlation plot. Table 2 also represents the obtained dimensionless rat gut wall permeabilities (P_w^*) for tested compounds. The plot of P_w^* versus the observed human intestinal permeability values is shown in Fig. 4.

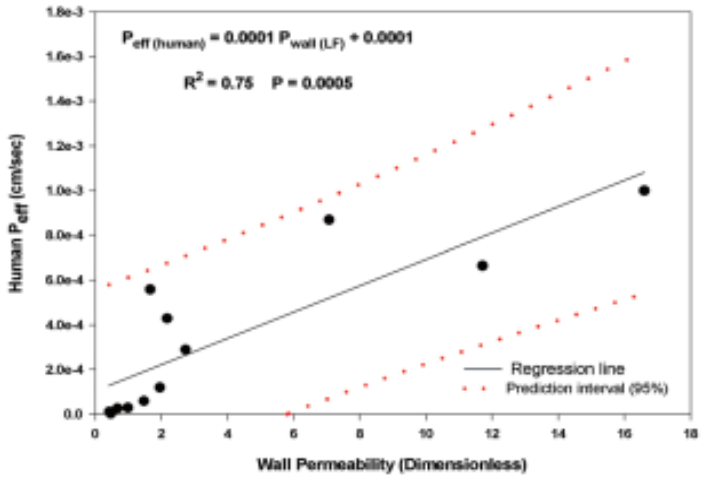

Fig. 4. Plot of dimensionless rat gut wall permeability values vs human Peff values in laminar flow model

Mixing tank model

This model takes the next step and assumes that both radial and axial mixing are complete. The aqueous resistance again is believed to be confined to a region (film) next to the membrane where only molecular diffusion occurs, and the rest of the contents are well mixed (perfect mixer). This model is described most easily by a mass balance on the system: (mass/time)inlet - (mass/time)outlet = (mass/time)absorbed or:

$$QC_0 - QC_m = (2\pi RL)(P'_{eff})C_m \tag{15}$$

where $2\pi RL$ is the area of the mass transfer surface (cylinder) of length L and radius R, P'_{eff} is the permeabilily or mass transfer coefficient of the surface, and C_m is the concentration in the tube (which is constant and equal to the outlet concentration by the perfect mixing assumption). From Eq. 15 it is obtained:

$$\frac{C_0 - C_m}{C_m} = P'_{eff}\frac{2\pi RL}{Q} \tag{16}$$

$$C_0/C_m = 1 + 4P'^{*}_{eff}Gz \tag{17}$$

As with the complete radial mixing model, P^{*}_{eff} contains additional parameter $P'^{*}_{a} = R/\delta'$ that must be estimated from the data, The P'^{*}_{a} and P'^{*}_{eff} values for the mixing tank model differ from those for the complete radial mixing model by nature of the different hydrodynamic assumptions (Amidon et al., 1980). While this model is not appropriate to most perfusion experiments, it is useful to compare its ability for correlation of mass transfer data with other models. As a matter of fact the P^{*}_{eff} for our data was calculated on the basis of assumptions of mixing tank model. The data and representative plot for this model are shown in Table 2 and Fig. 5 respectively (Valizadeh et al. 2008).

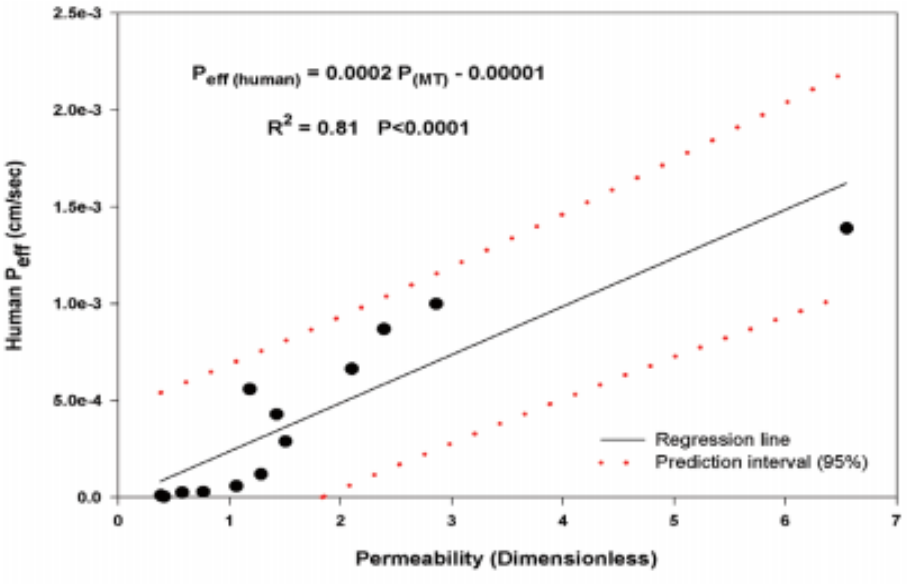

Fig. 5. Plot of dimensionless permeability values vs human P_{eff} values in mixing tank model

Compound	P^{*}_{wall} (±SD) (LF)	P^{*}_{eff} (±SD) (MT)	P^{*}_{eff} (±SD) (CRM)	Graetz no.	Rat no.	Diffusivity[a] ($\times 10^{-6}$ m^2/sec)
Atenolol	0.41± 0.00	0.38±0.00	0.37±0.00	3.53E-02	1	7.70
				3.46E-02	2	
				2.59E-02	3	
Cimetidine	1.46± 0.07	1.06±0.03	0.99±0.02	3.32E-02	1	8.70
				4.68E-02	2	
				3.98E-02	3	
Ranitidine	0.67± 0.32	0.57±0.25	0.55±0.02	2.99E-02	1	7.40
				3.16E-02	2	
				2.16E-02	3	
Antipyrine	1.65± 0.13	1.18±0.06	1.07±0.04	5.34E-02	1	9.92
				3.56E-02	2	
				4.45E-02	3	
Metoprolol	1.94± 1.35	1.28±0.62	1.21± 0.56	2.01E-02	1	4.98
				1.39E-02	2	
				1.68E-02	3	
Piroxicam	11.70 ± 14.4	2.09±1.18	1.80±0.92	2.84E-02	1	7.92
				3.56E-02	2	
				2.74E-02	3	
Propranolol	2.72± 1.8	1.50±0.61	1.32±0.48	3.46E-02	1	7.70
				3.98E-02	2	
				5.19E-02	3	
Carbamazepine	2.17± 0.35	1.42±0.14	1.29±0.12	3.71E-02	1	8.70
				3.94E-02	2	
				3.47E-02	3	
Furosemide	0.98± 0.69	0.76±0.47	0.72±0.44	2.92E-02	1	8.22
				2.36E-02	2	
				2.58E-02	3	
Hydrochlorothiazide	0.46± 0.26	0.41±0.22	0.39±0.21	4.07E-02	1	9.26
				4.24E-02	2	
				3.82E-02	3	
				4.15E-02	4	
Ibuprofen	-------	6.54±0.53	4.85±0.54	3.82E-02	1	7.40
				2.49E-02	2	
				2.76E-02	3	
Ketoprofen	7.07±3.97	2.38±0.52	2.06±0.40	3.40E-02	1	8.42
				3.02E-02	2	
				4.53E-02	3	
				2.72E-02	4	
Naproxen	16.59± 15.8	2.85±0.55	2.43±0.41	3.26E-02	1	8.55
				3.26E-02	2	
				2.92E-02	3	
				2.96E-02	4	

[a] Diffusivities were calculated using 2D structure of compounds applying he method proposed by Heyduk et al (Hayduk and Laudie, 1974)

Table 2. Dimensionless permeabilities determined based on three mass transfer models

The calculated dimensionless wall permeability values were in the range of 0.37 – 4.85, 0.38-6.54 and 0.41-16.59 for complete radial mixing, mixing tank and laminar flow models respectively. It is clear that drugs with different physicochemical properties belonging to all four biopharmaceutical classes were enrolled in the study. Atenolol a class III drug (high soluble-low permeable) showed lowest effective permeability value in all three investigated models. It is also shown that there is only a small difference in the calculated atenolol permeability coefficients in three models. However this variation becomes more salient for high permeable drugs; i.e. class I (high soluble-high permeable) and class II (low soluble-high permeable) drugs especially in term of permeability in laminar flow model. For instance the observed mean permeability values for naproxen, a class II drug, are 2.43, 2.85 and 16.59 in CRM, MT and LF models respectively. Therefore it seems that in comparison to other model laminar flow model provides larger values for highly permeable drugs in comparison to the other models. However the ranking order for intestinal absorption of tested drugs is almost the same in other evaluated models. In addition it seems that it would be possible to classify drugs correctly by the resulting values. Fig. 2, 4 and 5 demonstrate the obtained correlations for investigated models. It is seen that the plots of rat permeability versus human P_{eff} values, present rather high linear correlations with intercepts not markedly different from zero (R^2= 0.81, P <0.0001 for MT, R^2= 0.75, P =0.0005 for LF, R^2= 0.84, P <0.0001 for CRM). The permeabilities differ for the various models. The permeabilities resulting from application of the other models can be interpreted if it is assumed that the laminar flow permeability measures the wall permeability. The permeability values for the complete radial mixing model are lower than the laminar flow model since this model assumes radial mixing, which leads to lower estimated luminal (aqueous) resistance values and a higher estimated membrane resistance (lower permeability value). However, the usual interpretation of the complete radial mixing model recognizes that the permeability value includes an aqueous resistance. While the permeabilities in mixing tank model, which takes the final step in assuming both radial and axial mixing, were expected to be the lowest among all models, they were in the range between permeabilities in complete radial mixing and dimensionless wall permeabilities. Although theoretically laminar flow model has been established to a reasonable approximation in external perfusion studies, based on the results of correlations of this study, it seems the hydrodynamics in normal physiological situation clearly are more complex and need more investigation to choose from proposed models. Therefore it is concluded that all investigated models work relatively well for our data despite fundamentally different assumptions. The wall permeabilities fall in the order laminar flow > mixing tank > complete radial mixing. Based on obtained correlations it is also concluded that although laminar flow model provides the most direct measure of the intrinsic wall permeability, it has limitations for highly permeable drugs such as ibuprofen and the normal physiological hydrodynamics is more complex and finding real hydrodynamics require further investigations.