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Mass Transfer Phenomena and Biological Membranes

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1. 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.

2. 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).

2.1 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).

2.2 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).

2.3 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).

2.4 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).

2.5 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.

3. 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).

4. Mass transfer models

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

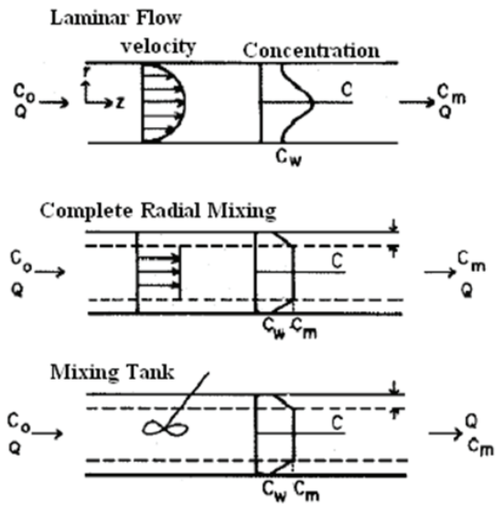

Fig. 1. Velocity and concentration profiles for the models. The concentration profiles are also a function of z except for mixing tank model (Amidon et al., 1980)

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):

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

Where, Z* = Z / L, r* = r / R, $\upsilon^*_z = \nu_z$ / 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.

4.1 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.

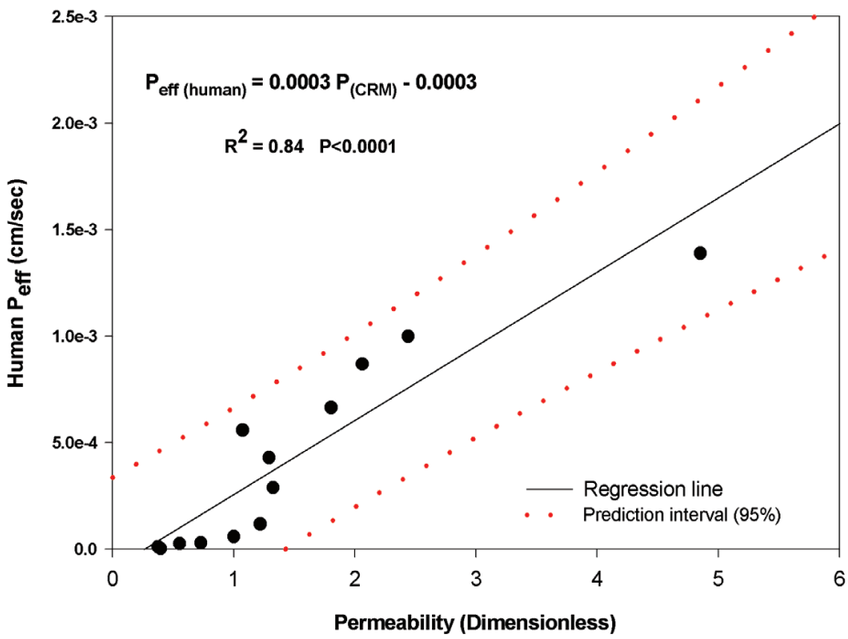

Fig. 2. Plot of dimensionless permeability values vs human P_{eff} values in complete radial mixing model

4.2 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:

$$\frac{1}{\mathrm{P}_{\mathrm{eff}}^*} = \frac{1}{\mathrm{P}_{\mathrm{w}}^*} + \frac{1}{\mathrm{P}_{\mathrm{aq}}^*} = \frac{1}{{}^{\circ}P_w^*} + \frac{1}{{}^{\circ}P_{aq}^*} \tag{11}$$

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

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

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

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 ${}^{\circ}P_{aq}^*$ is calculated using Eqs. 9 , 11, 14 and Table 1.

(n)	${}^{\circ}\beta_n$	${}^{\circ}M_n$
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, ${}^{\circ}M_n$ and exponents, ${}^{\circ}\beta_n$ 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 ${}^{\circ}P_w^*$ is found out from Eq. 10 and finally the value of ${}^{\circ}P_w^*$ is multiplied by the correction factor in Fig 3 to obtain P_w^*.

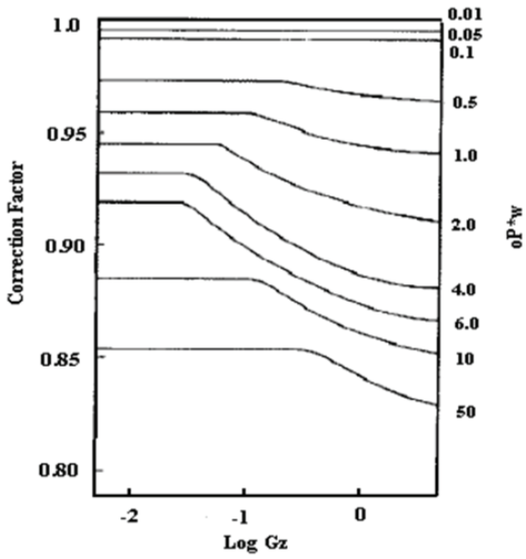

Fig. 3. Correction factor to obtain exact wall permeability (P_w^*) given the estimated wall Permeability ($^\circ P_w^*$) and value of *Gz*. (Elliott et al., 1980)

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.

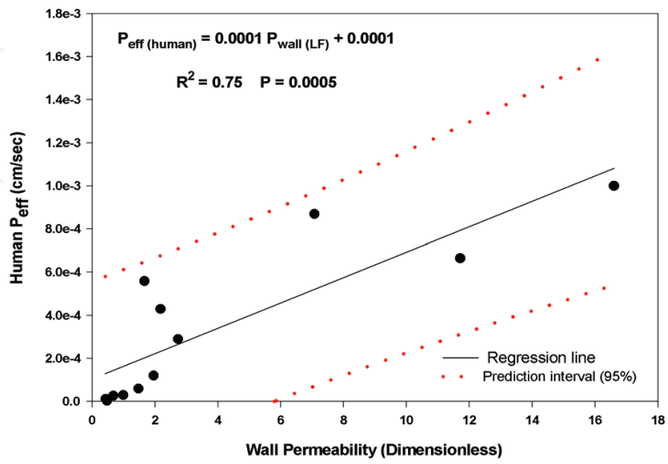

Fig. 4. Plot of dimensionless rat gut wall permeability values vs human Peff values in laminar flow model

4.3 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 2πRL 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).

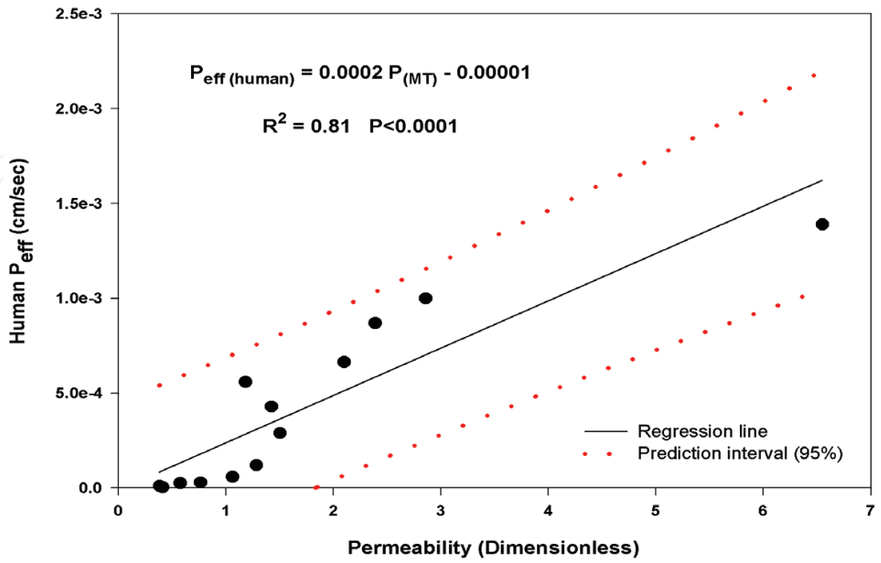

Fig. 5. Plot of dimensionless permeability values vs human P_{eff} values in mixing tank model

Compound	P^*_{wall} (±SD) (LF)	P^*_{eff} (±SD) (MT)	P^*_{eff} (±SD) (CRM)	Graetz no.	Rat no.	Diffusivity[a] (×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.

5. Prediction of human intestinal permeability using SPIP technique

Previous studies have shown that the extent of absorption in humans can be predicted from single-pass intestinal perfusion technique in rat (Salphati et al., 2001, Fagerholm et al., 1996), however, in this section (Zakeri-Milani et al., 2007) we compare the quantitative differences between permeabilities in human and rat models directly using a larger number of model

drugs with a broad range of physicochemical properties for both high and low permeability classes of drugs. In fact more poorly absorbed drugs (cimetidine and ranitidine) have been included in the present work and therefore it is likely that the obtained equations will give a more reliable prediction of the human intestinal permeability and fraction of dose absorbed than previously reported equations. Single-pass intestinal perfusion studies in rats were performed using established methods adapted from the literature. Briefly, rats were anaesthetized using an intra peritoneal injection of pentobarbital (60 mg/kg) and placed on a heated pad to keep normal body temperature. The small intestine was surgically exposed and 10 cm of jejunum was ligated for perfusion and cannulated with plastic tubing. The cannulated segment rinsed with saline (37^{o}C) and attached to the perfusion assembly which consisted of a syringe pump and a 60 ml syringe was connected to it. Care was taken to handle the small intestine gently and to minimize the surgery in order to maintain an intact blood supply. Blank perfusion buffer was infused for 10 min by a syringe pump followed by perfusion of compounds at a flow rate of 0.2 ml/min for 90 min. The perfusate was collected every 10 min in microtubes. The length of segment was measured following the last collection and finally the animal was euthanitized with a cardiac injection of saturated solution of KCl. Samples were frozen immediately and stored at -20^{o}C until analysis. Effective permeability (P_{eff}) (or better named practical permeability, since the effective area of segment is not considered in the calculation) was calculated using following equation (Eq.18) according to the parallel tube mode:

$$P_{eff} = -Q \ln(C_{out}/C_{in})/2\pi rl \quad (18)$$

In which C_{in} is the inlet concentration and C_{out} is the outlet concentration of compound which is corrected for volume change in segment using phenol red concentration in inlet and outlet tubing. Q is the flow rate (0.2 ml/min), r is the rat intestinal radius (0.18 cm) and l is the length of the segment. It has been demonstrated that in humans at a Qin of 2-3 ml/min, P_{eff} is membrane-controlled. In the rat model the Q_{in} is scaled to 0.2 ml/min, since the radius of the rat intestine is about 10 times less than that of human. In 1998 Chiou and Barve (Chiou and Barve, 1998) reported a great similarity in oral absorption (F_a) between rat and human; however they have used an in vivo method, quite different from in situ techniques, that can give an idea of the absorption from the entire GI tract, therefore the significance of rat jejunal permeability values for predicting the human F_a has not been tested in that report. In the present study the obtained P_{eff} values ranged between 2 ×10^{-4} cm/sec to 1. 6 ×10^{-5} cm/sec and showed a high correlation (R^2=0.93, P<0.0001) with human P_{eff} data for passively absorbed compounds (Fig 6) confirming the validity of our procedure. This correlation was weakened when the actively transported compounds (cephalexin and α methyl dopa) were added to the regression (R^2=0.87, P<0.0001).

The plot of predicted vs observed human P_{eff} values presents a high linear correlation with intercept not markedly different from zero (R^2= 0.93, P <0.0001) (Zakeri-Milani et al., 2007). According to previously reported equations by Salphati et al (Salphati et al., 2001) in the ileum and Fagerholm et al (Fagerholm et al., 1996) in the jejunal segment, the slopes for the same correlation between two models were 6.2 and 3.6 respectively. However based on our results for larger set of compounds including more low-permeable drugs the rat P_{eff} values were on average 11 times lower than those in human. The species differences and the differences in effective absorptive area might be the reasons for the lower permeability values in the rat model. In addition, any changes in the intestinal barrier function during the

surgery might be a main reason for obtaining different results in literature concerning intestinal permeability of drugs. A strong correlation was observed between rat permeability data and fraction of oral dose absorbed in human fitting to chapman type equation; $F_{a\ (human)} = 1 - e^{-38450 Peff\ (rat)}$ ($R^2 = 0.91$, $P<0.0001$) (Fig. 7).

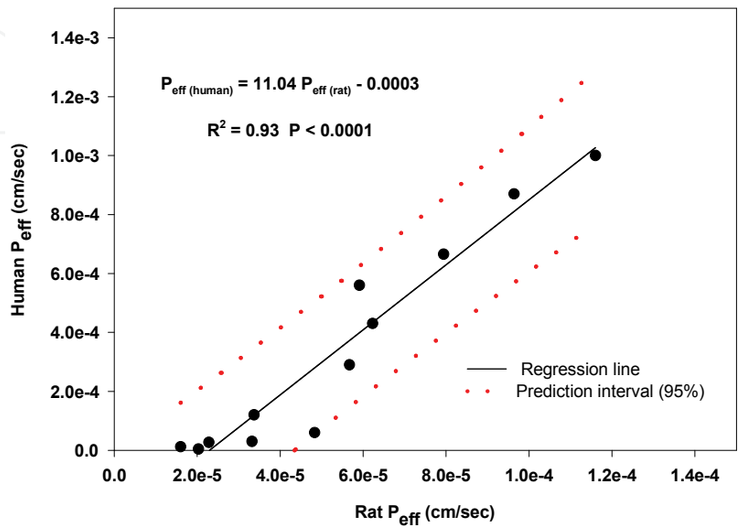

Fig. 6. Plot of P_{eff} rat vs P_{eff} human

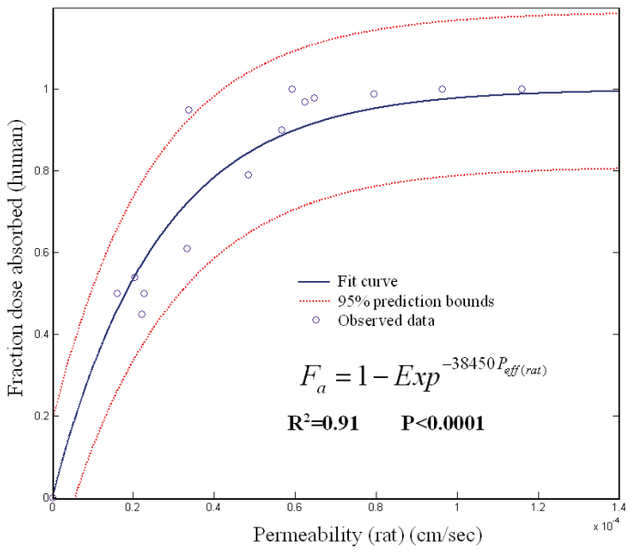

Fig. 7. Plot of rat P_{eff} vs human F_a

The same fitting using human intestinal permeability gives a lower correlation coefficient. The comparison of rat Peff and intestinal absorption in man (F_a) showed that rat P_{eff} values greater than 5.9×10^{-5} cm/sec corresponds to $F_a \approx 1$ while rat P_{eff} values smaller than 3.32×10^{-5} cm/sec corresponds to F_a values lower than 0.6. Corresponding estimates in human are >

0.2×10^{-4} cm/sec and $<0.03\times10^{-4}$ cm/sec, respectively. Moreover the predicted and observed human F_a (%) are linearly correlated ($R^2 = 0.92$, $P < 0.0001$). The rank order for P_{eff} values in rat was compared with those of human P_{eff} and F_a (Zakeri-Milani et al., 2007). The spearman rank correlation coefficients (rs) were found to be 0.96 and 0.91 respectively. Based on the obtained results, it is concluded that in situ perfusion technique in rat could be used as a reliable technique to predict human gastrointestinal absorption extent following oral administration of a drug. However, to render our observation more reliable, it seems that using larger number of compounds belonging to all four biopharmaceutical classes, i.e., different solubility and permeability properties (Lobenberg and Amidon, 2000) especially drugs with low permeability must be tested.

6. Biopharmaceutics classification system using rat P_{eff} as a surrogate for human P_{eff}

In 1995 Amidon et al. devised a biopharmacetics classification system (BCS) to classify drugs based on their aqueous solubility and intestinal permeability, two fundamental properties governing drug absorption (Amidon et al., 1995). This system divides active moieties into four classes: class I (high permeability, high solubility), class II (high permeability, low solubility), class III (low permeability, high solubility) and class IV (low permeability, low solubility). For highly permeable drugs the extent of fraction dose absorbed in human is considered to be more than 90% as defined by US Food and Drug Administration (FDA) (Lennernas and Abrahamsson, 2005, Zakeri-Milani et al., 2009a). The classification of drug solubility is based on the dimensionless dose number (D0) which is the ratio of drug concentration in the administered volume (250 ml) to the saturation solubility of the drug in water. If a drug has dose/solubility ratio less than 250 ml over the pH range from 1 to 7.5 it is classified as highly soluble drug compound (Kasim et al., 2004). BCS classification can help pharmaceutical companies to save a significant amount in development time and reduce costs. This classification provides a regulatory tool to substitute in vivo bioequivalence (BE) studies by in vitro dissolution tests. In fact for immediate-release (IR) solid oral dosage forms containing rapidly dissolving and easily permeating active ingredients bioequivalence studies may not be required because they act like a solution after oral administration. Therefore dissolution rate has a negligible impact on bioavailability of highly soluble and highly permeable (BCS Class I) drugs. As a result, various regulatory agencies including the United States Food and Drug Administration (FDA) now allow bioequivalence of formulations of BCS Class I drugs to be demonstrated by in vitro dissolution (often called a biowaiver) (Takagi et al., 2006) . Waivers for class III drugs have also been recommended (Blume and Schug, 1999, Yu et al., 2002) . Moreover BCS provides distinct rules for determining the rate-limiting factor in the gastrointestinal drug absorption process. As a result it could be helpful in the selection of candidate drugs for full development, prediction and clarification of food interactions, choice of formulation principle and the possibility of in vitro-in vivo correlation in the dissolution testing of solid formulations (Lennernas and Abrahamsson, 2005, Fleisher et al., 1999). Although permeability classification of drugs would be ideally based on human jejunal permeability data, such information is available for only a small number of drugs. Therefore in this section a new classification is presented which is based on a correlation between rat and human intestinal permeability values. However first the calculation of used parameters is explained.

7. Dose number calculation

Dose number is a criterion for solubility (Do) which is defined as the ratio of dose concentration to drug solubility. It is calculated as follows:

$$D_o = \frac{M / V_o}{C_s} \tag{19}$$

Where (C_s) is the solubility, (M) is the maximum dose strength, and (V_o) is the volume of water taken with the dose (generally set to be 250 mL). The values of solubility and maximum dose strength of tested compounds are listed in table 3 . Dose number would be as unity (Do = 1), when the maximum dose strength is soluble in 250 ml of water and the drug is in solution form throughout the GI tract. This criterion is extended to 0.5 for borderline classification, considering the average volume of fluid (500 ml) under fed conditions (Zakeri-Milani et al., 2009b).

8. Dissolution number calculation

Dissolution number refers to the time required for drug dissolution which is the ratio of the intestinal residence time to the dissolution time, which includes solubility (C_s), diffusivity (D), density (ρ), initial particle radius (r_0) of a compound and the intestinal transit time (T_{si}) (Zakeri-Milani et al., 2009b, Varma et al., 2004).

$$Dn = \left(\frac{3D}{r_0^{\,2}}\right)\left(\frac{C_s}{\rho}\right)\langle T_{si}\rangle = \frac{\langle T_{si}\rangle}{\langle T_{diss}\rangle} \tag{20}$$

where ρ and T_{si} are generally considered to be 1200 mg/cm^3 and 199 min respectively.

$$T_{diss} = \frac{\rho h r_0}{3DC_s} \tag{21}$$

9. Absorption number calculation

This is the ratio of permeability (P_{eff}) and the gut radius (R) times the residence time in the small intestine which can be written as ratio of residence time and absorption time (Zakeri-Milani et al., 2009b, Varma et al., 2004).

$$An = \frac{P_{eff}}{R} * \langle T_{si}\rangle = \frac{\langle T_{si}\rangle}{\langle T_{abs}\rangle} \tag{22}$$

For calculation the R value of 1.7 cm and the predicted human P_{eff} (based on rat P_{eff}) were used.

10. Absorption time calculation

This parameter is proportional to P_{eff} through the following equation (Zakeri-Milani et al., 2009b, Varma et al., 2004).

$$T_{abs} = \frac{R}{P_{eff}} \tag{23}$$

11. Absorbable dose calculation

Absorbable dose is the amount of drug that can be absorbed during the period of transit time, when the solution contacting the effective intestinal surface area for absorption is saturated with the drug (Zakeri-Milani et al., 2009b, Varma et al., 2004).

$$D_{abs} = P_{eff} C_s A < T_{si} > \tag{24}$$

In this equation A is the effective intestinal surface area for absorption. If the small intestine is assumed to be a cylindrical tube with a radius of about 1.5 cm and length of 350 cm, the available surface area and volume are 3297 cm^2 and 2473 ml, respectively. In reality, the actual volume is around 600 ml and the effective intestinal surface area is then estimated to be about 800 cm^2 assuming the same ratio. Drugs were classified to the BCS on the basis of dose number (Do) and rat jejunal permeability values, which are taken as indicative of fundamental properties of drug absorption, solubility and permeability. On the basis of the relationship between human and rat intestinal permeability (Zakeri-Milani et al., 2009a, Zakeri-Milani et al., 2007) , rat P_{eff} values greater than 5.09×10^{-5} cm/sec corresponds to F_a > 85 % while P^{eff} values smaller than 4.2×10^{-5} cm/sec corresponds to Fa values lower than 80 %. Therefore, as it can be seen in Fig 8 a cutoff for highly permeable drugs, $P_{eff\ rat} = 5.09\times10^{-5}$ cm/sec with a border line cutoff of 4.2×10^{-5} cm/sec can be set. Drugs with permeability in the range of 4.2-5.09e^{-5} cm/sec were considered as borderline drugs. The intersections of dashed lines drawn at the cutoff points for permeability and dose/solubility ratio divide the plane in Fig. 8 into four explicitly defined drug categories (I – IV) and a region of borderline.

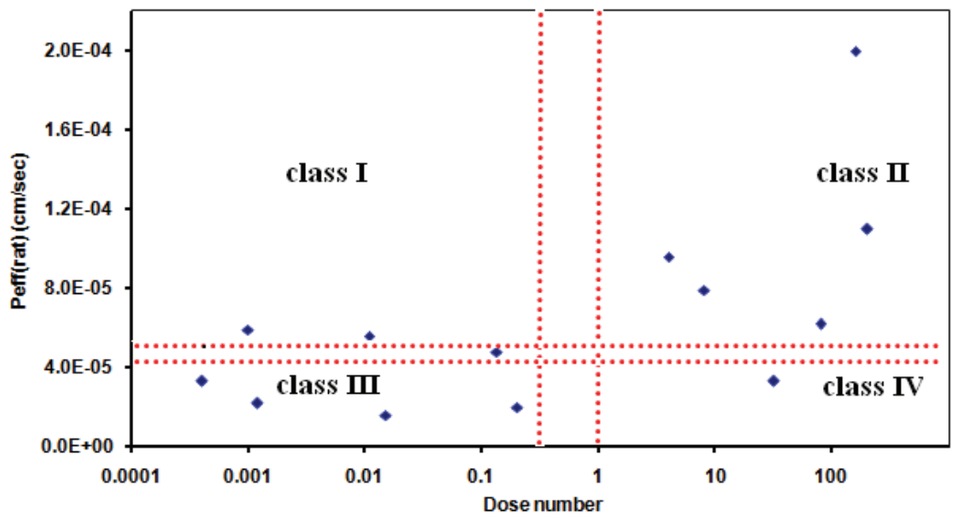

Fig. 8. Plot of Dose number vs rat P_{eff} values representing the four classes of tested compounds

The biopharmaceutical properties of a drug determine the pharmacokinetic characteristics as below:

Class I, Do <0.5, $P_{eff\ (rat)} > 5.09\times10^{-5}$ cm/sec
The drugs in this category are highly soluble and highly permeable and are ideal candidates for oral delivery. These drugs are characterized by the high An, high Dn and low Do, showing that they are in solution form throughout the intestine and is available for permeation. Therefore the rate of absorption of drugs in this class is controlled only by gastric emptying. Examples of this category include antipyrine and propranolol.
Class II, Do > 1, $P_{eff\ (rat)} > 5.09\times10^{-5}$ cm/sec
Class II drugs have high lipophilicity and therefore are highly permeable across the Gl membrane, primarily by passive transport. These drugs are characterized by mean absorption time less than mean dissolution time, and thus gastric emptying and GI transit are important determinants of drug absorption (Varma et al., 2004). These drugs are expected to have a dissolution-limited absorption and an IVIVC is expected (Lennernas and Abrahamsson, 2005). Low dissolution rate of these molecules limit the concentration at the site of absorption thereby leading to less passive diffusion. Therefore formulation plays an important role in the rate and extent of intestinal absorption of such drugs. Although there are methods to enhance the solubility of class II drugs (Valizadeh et al., 2004, Valizadeh et al., 2007), incorporation of polar groups into the chemical backbone, salt generation and prodrug approaches are the primary methods for improving deliverability during lead optimization.. This class includes drugs such as ketoprofen, naproxen, piroxicam and carbamazepine.
Class III, Do <0.5, $P_{eff\ (rat)} < 4.2\times10^{-5}$ cm/sec
The absorption of class III drugs is limited by their intestinal permeability and no IVIVC should be expected. These drugs are either having unfavorable physicochemical properties leading to less intrinsic permeability and/or are strong substrates to efflux transporters and/or gut wall metabolic enzymes (Varma et al., 2004). Therefore the rate and extent of intestinal absorption may be controlled by drug molecule properties and physiological factors rather than pharmaceutical formulation properties (Yu et al., 2002). They must possess optimum lipophilicity in order to permeate the lipophilic epithelial cell membranes lining the gastrointestinal tract. Thus for highly polar compounds, administration of less polar, more lipophilic prodrugs may improve absorption. Balance between the hydrophilicity and lipophilicity should be maintained during incorporation of lipophilic groups into the structure. Atenolol, hydrochlorothiazide and ranitidine are examples of drugs in this group.
Class IV, Do > 1, $P_{eff\ (rat)} < 4.2\times10^{-5}$ cm/sec
Low and variable absorption for these drugs is anticipated because of the combined limitation of solubility and permeability. Formulation may improve the bioavailability of these drugs. However they are compromised by their poor intestinal membrane permeability. These drugs are more likely susceptible to P-gp efflux and gut metabolism, as the concentration of the drug in the enterocytes at any given time will be less to saturate the transporter (Varma et al., 2004). Strategies to improve both solubility and permeability should be worked out for these molecules, which may not be an easy task. However, obtaining this type of quality information will certainly improve drug design and help in optimizing candidates with "brick-like" properties.
Borderline Class, 0.5 <Do <1 or $4.2\times10^{-5} < P_{eff\ (rat)} < 5.09\times10^{-5}$ cm/sec
In this region, bordered by the dashed lines of the four cutoff points, the predictions become more uncertain for drugs lying. Cimetidine which is supposed to be in class III, has been

classified in this region. All in all, 13 of 15 test drugs (87%) are correctly classified with respect to their rat Peff values, however, metoprolol, a drug with high permeability, was classified as a low permeability drug in the presented plot (False negative). Furthermore there are some more fundamental parameters describing oral drug absorption. These parameters include absorption number, dissolution number, absorption time and dissolution time (Varma et al., 2004). There is also an extra parameter named absorbable dose which was calculated to propose the absorption limiting steps in oral absorption of tested drugs. Three dimensionless parameters (Do, An and Dn) which were shown in Table 3 can be used to qualitative classification of drugs. The four BCS classes of drugs were defined as below on the basis of these three parameters. For easy comparison Table 3 was set in which the dimensionless parameters for each class of drugs were compared.

Compound	D_{abs} Calculated (mg)	T_{diss} calculated (min)	T_{abs} Calculated (min)	D_n Calculated	A_n calculated	D_o calculated	C_s (mg/ml)	Dose (mg)	Mean P_{eff} ($*10^5$ cm/s)
Antipyrine	3519359	0.01	76.5	11784.9	2.58	0.001	1000 [a]	250[a]	5.9 ± 0.2
Propranolol	109621	0.65	81.1	302.1	2.44	0.011	33 [a]	90 [a]	5.6 ± 2.0
Carbamazepine	38	1915.2	71.2	0.10	2.78	80.0	0.01 [a]	200 [a]	6.2 ± 0.6
Ibuprofen	150	2249.7	18.0	0.08	10.99	160.0	0.01 [a]	400 [a]	20 ± 2.2
Ketoprofen	328	395.8	41.1	0.50	4.81	4.0	0.05 [b]	50 [a]	9.6 ± 1.8
Naproxen	81	1947.3	33.1	0.10	5.98	200.0	0.01 [b]	500 [a]	11 ± 0.2
Piroxicam	26	4204.7	52.1	0.04	3.79	8.0	0.005 [a]	10 [a]	7.9 ± 4.0
Metoprolol	1448424	0.03	185.9	5917.4	1.06	0.0004	1000 [a]	100 [a]	3.3 ± 1.5
Furosemide	14	2025.8	190.6	0.09	1.03	32.0	0.01 [a]	80 [a]	3.3 ± 2.0
Cimetidine	15841	3.1	102.0	62.0	1.94	0.133	6 [c]	200 [a]	4.8 ± 0.1
Atenolol	196	0.81	36326.8	242.6	0.005	0.015	26.5 [a]	100 [a]	1.6 ± 0.02
Ranitidine	560303	0.02	480.6	8800.8	0.41	0.001	1000 [a]	300 [a]	2.2 ± 1.0
Hydrochlorothiazide	360	17.9	747.0	11.0	0.26	0.200	1 [a]	50 [a]	2.0 ± 1.0

Table 3. Dose, solubility and calculated oral drug absorption parameters for tested compounds (Zakeri-Milani et al., 2009b)

Class	Dimensionless parameters	Permeability	Solubility
I	A_n↑* D_n↑ D_o↓	High	High
II	A_n↑ D_n↓ D_o↑	High	Low
III	A_n↓ D_n↑ D_o↓	Low	High
IV	A_n↓ D_n↓ D_o↑	Low	Low

*symbols↓ and↑ represent low and high quantity for parameters

Table 4. Qualitative classification of drugs based on dimensionless parameters

Absorption limiting step	Examples	Comments	Condition
No limited	Antipyrine, Propranolol, Cimetidine	There is no limitation in drug absorption since all three parameters are in acceptable range.	T_{diss}< 50 min $P_{eff\ rat}$ > 4.2×10^{-5} D_{abs} >> Dose
Dissolution limited	Ketoprofen, Piroxicam	Although solubility itself imparts to poor dissolution, the dissolution here mainly refers to particle size. The absolute bioavailability increases with increasing dose.	T_{diss} > 199 min $P_{eff\ rat}$ > 4.2×10^{-5} D_{abs} >> Dose
Solubility limited	Ibuprofen, Carbamazepine, Naproxen	Solubility-limited absorption occurs mainly when a high dose saturates part of the gut. The absolute bioavailability does not increase with increasing dose.	T_{diss} > 199 min $P_{eff\ rat}$ > 4.2×10^{-5} D_{abs} < Dose
Permeability limited	Ranitidine, Atenolol, Metoprolol, Hydrochlorothiazide	This limiting step is considered for highly soluble drugs dosed in solutions: assume no precipitation occurs. The absolute bioavailability increases with increasing dose.	T_{diss}< 50 min $P_{eff\ rat}$ < 4.2×10^{-5} D_{abs} >> Dose
Dissolution-permeability-solubility-limited	Furosemide	Drug absorption is limited by all steps including solubility, permeability and dissolution	T_{diss} > 199 min $P_{eff\ rat}$ < 4.2×10^{-5} D_{abs} < Dose

Table 5. Absorption limiting steps and their corresponding conditions

This classification is in accordance with quantitative classification model which was given in the first part of current section, i.e. all compounds lie in the same class as did in quantitative classification. For example atenolol with a Do = 0.015 (low), An = 0.005 (low) and Dn = 242 (high) is classified in class III which is in agreement with above-mentioned QBCS. Again metoprolol with An of 1.06 lies in class III as it did before in quantitative model. However this is a false negative result, since it was known to have a high permeability belonging to class I. Another interesting aspect of using these dimensionless parameters is to determine the absorption limiting steps which was summarized as a framework in Table 5. As it was mentioned before, the mean small intestinal transit time was found to be 199 minutes with a standard deviation of 78 minutes (Yu, 1999, Zakeri-Milani et al., 2009b). This means that as a worst case, the small intestinal transit in some individuals may be only 43 minutes (mean

small intestinal transit time – 2 × standard deviation). The time of 50 minutes was used as a reference time of dissolution to determine if the dissolution is fast enough to permit complete dissolution in the small intestine (Yu, 1999). The $P_{eff\ (rat)}$ was set at 4.2×10^{-5} cm/sec which based on our correlations, corresponds to over 80% of dose absorbed. Table 3 provides distinguishing conditions under which each limiting case occurs. Considering these conditions, antipyrine and propranolol meet the criteria for no-limited absorption. All of these three drugs belong to class I. However cimetidine a drug which was false positive in our previous quantitative and qualitative classification lies in no-limited class again. On the other hand based on dissolution time, permeability and absorbable dose for furosemide, a drug of class IV, its absorption would be limited by all three parameters. Therefore it takes place in the last class of Table 5. Furthermore drugs with low permeability which have a high absorbable dose and low dissolution time such as ranitidine and hydrochlorothiazide (class III), are classified in permeability-limited category. Finally the drugs of remaining class of BCS (class II) are divided in two groups based on their relative values of dimensionless parameters. All of these drugs have high dissolution time (Table 3), but regarding the absorbable dose, their absorption could be dissolution or solubility-limited. For instance, piroxicam and ketoprofen lie in dissolution-limited class, while naproxen is placed in solubility-limited category. According to obtained results and proposed classification for drugs, it is concluded that drugs could be categorized correctly based on dose number and their P_{eff} values in rat model using SPIP technique. This classification enables us to remark defined characteristics for intestinal absorption of all four classes using suitable cutoff points for both dose number and rat effective intestinal permeability values. Therefore the classification of drugs using their intestinal permeability values in rats can help pharmaceutical companies to save a significant amount in development time and reduce costs. Moreover it could be as a regulatory tool to substitute in vivo bioequivalence (BE) studies by in vitro dissolution tests. However this work relies on only 13 compounds which their P_{eff} values in rat were measured and to confirm the proposed classification the larger data set is needed.

12. Biopharmaceutical classification of drugs using intrinsic dissolution rate (IDR) and rat intestinal permeability

The solubility and dissolution rate of active ingredients are of major importance in preformulation studies of pharmaceutical dosage forms (Valizadeh et al., 2007, Valizadeh et al., 2004, Barzegar-Jalali et al., 2006, Zakeri-Milani et al., 2009a). The formulation characteristics including shelf life, process behavior, and even the bioavailability are affected by physicochemical properties of drug molecules (Haleblian and McCrone, 1969). The intrinsic dissolution rate (IDR) has been used to characterize solid drugs for many years. For example it could be used to understand the relationship between the dissolution rate and crystalline form and also to study the effects of surfactants and pH on the solubilization of poorly soluble drugs (Amidon et al., 1982, Yu et al., 2004, Zakeri-Milani et al., 2009a). IDR is generally defined as the dissolution rate of a pure drug substance under the condition of constant surface area, agitation or stirring speed, pH and ionic strength of the dissolution medium. The true intrinsic dissolution rate may be better described as the rate of mass transfer from the solid surface to the liquid phase. The apparatus for intrinsic dissolution testing was originally developed by John Wood which enables the calculation of the dissolution rate per centimeter squared of the intrinsic ingredients of pharmaceutical

products (Levy and Gumtow, 1963, Nelson, 1958). It has been suggested that it might be feasible to use IDR to classify drugs instead of solubility (Yu et al., 2004). The reason is that, just like permeability, IDR is a rate phenomenon instead of an equilibrium phenomenon. Therefore it might correlate better with in vivo drug dissolution rate than solubility, although for drugs having either extremely high or low dose, discrepancies may exist between the solubility and IDR methods since dose is considered in the classification of solubility while intrinsic dissolution does not consider the effect of dose. In the present study the intrinsic dissolution rate and rat intestinal permeability (using SPIP technique) were measured for drugs with different physicochemical properties. The suitability of IDR-permeability for biopharmaceutical classification of drugs was evaluated.

13. Procedure of IDR measurement

A quantity of 100 mg of each drug was compressed at an average compression force of 7.84 MPa for 1 minute to make non-disintegrating compacts using die and punch with diameter of 6 mm. The surface area of the compacts was 0.2826 cm^2. The improved method of wood et al was used for disk dissolution studies (Wood et al., 1965). Compacts were placed in a molten beeswax-mold in such a way that only one face could be in contact with dissolution medium. Dissolution study was conducted using USP II dissolution apparatus using 900mL of phosphate buffer (pH=6.8) at temperature of 37°C ± 1°C as the dissolution media with paddle rotating at 100 rpm. Samples were collected through 0.45-µm syringe filters over a period of 8 hours for low-soluble and 20 minutes for highly soluble drugs. Sampling time intervals were 30 min and 2 min respectively. All studies were carried out in triplicate. Absorbances were determined in triplicate using a UV-Vis spectrophotometer at the maximum absorbance wavelength for each active tested. The cumulative amount dissolved per surface unit of the compact was plotted against time for each vessel. The slope of the linear region ($R^2 \geq 0.95$) was taken as intrinsic dissolution rate. IDR is easily calculated by

$$G = (dw/dt)(1/S) = DCs/h \tag{25}$$

where G is intrinsic dissolution rate ($mg/min/cm^2$); dw is the change in drug dissolved (mg); dt is the change in time (minutes); S is the surface area of the compact (cm^2); D is diffusion coefficient (cm^2/sec); Cs is solubility (mg/cm^3) and h is stagnant layer thickness (cm) (Zakeri-Milani et al., 2009a).

14. Solubility studies

Solubilities were determined in at least triplicates by equilibrating excess amount of drugs in phosphate buffer solutions (pH=6.8). The samples were kept in thermostated water bath at 37°C and shaked at a rate of 150 rpm for 24 hours. The absorbances of filtered and suitably diluted samples were measured with an UV-VIS spectrophotometer at the maximum absorbance wavelength for each active tested. The solubilities were calculated using calibration curves determined for each drug (Zakeri-Milani et al., 2009a). Current BCS guidance defines an API as "highly soluble" when the highest dose recommended is soluble in 250 mL or less of aqueous media over the pH range of 1.2 to 7.5 (Gupta et al., 2006). However the pH 6.8 is scientifically justified over pH 7.4 (Gupta et al., 2006). In order to set a condition for BCS classification of compounds and since small intestine is the major site for drug absorption, where the pH is about 6.8, IDR measurements were conducted in pH 6.8.

The presence of sink condition in dissolution medium during the experiment is upholded by comparison of the final concentration of drugs and their solubility in dissolution medium. Classification of tested drugs based on their intestinal permeability and IDR for human and rat is shown in Fig. 9 and Fig. 10 respectively. Drugs are scientifically identified based on their solubility and human intestinal permeability. Since human intestinal permeability could be predicted with precise using the rat effective permeability values, the same classification can be constructed utilizing the solubility and rat intestinal permeability values. IDR is a parameter which could be used easily to characterize the pure drug substance. The determination of this parameter allows labs to screen experimental drug formulations and to understand their behavior under different bio-physical conditions. Table 6 shows the obtained solubility and IDR values in the present work for tested drugs.

Compound	Drug class					IDR (mg cm^{-2} min^{-1})	Solubility (mg/l)	Wavelength (nm)
	Dissolution based	BDDCS	BCS	This work**	This work*			
Antipyrin	I	I	I	I	I	56.79	683271.6	243
Metoprolol	I	I	I	III	I	34.64	779580.8	274
Propranolol	II	I	I	I	I	16.596	71797.17	288
Verapamil	I	I	I	I	I	16.192	71602.64	274
Ketoprofen	II	I	I	II	II	0.6348	2121.80	261
Naproxen	II	II	II	II	II	0.388	1604.45	262
Carbamazepine	IV	II	II	II	II	0.0355	164.59	285
Ibuprofen	-	II	II	II	II	0.2844	1315.41	222
Piroxicam	-	II	II	II	II	0.0739	157.64	353
Atenolol	III	III	III	III	III	3.449	16868.14	224
Cimetidine	-	III	III	III	III	7.2	46276.68	219
Ranitidine	III	III	III	III	III	42.18	>1000000	228
Furosemide	IV	IV	IV	IV	IV	0.58	1464.42	277

* proposed class based on IDR and human intestinal permeability
**proposed class based on IDR and rat intestinal permeability

Table 6. Experimental wavelength, Solubility, intrinsic dissolution rate (IDR), and respective class of tested compounds using different approaches

The IDR results on tested drugs are in agreement with previously reported values (Yu et al., 2004). In the present study the obtained rat Peff values showed a high correlation (R^2=0.93, P<0.0001) with human Peff data for passively absorbed compounds confirming the validity of our procedure (Zakeri-Milani et al., 2007). It was found that a strong correlation was observed between rat permeability data and fraction of oral dose absorbed in human (R^2=

0.91, P<0.0001). The same correlation for human intestinal permeability data and fraction of oral dose absorbed gives a lower correlation coefficient (R^2= 0.81, P<0.0001). However according to obtained equations, the permeabilities of 0.0000509 and 0.000047 cm/sec in rat and human respectively corresponds to Fa=85% which are set as cut-off points for highly permeable drugs. On the other hand, IDR correlates with the BCS solubility classification with 1-2 mg/min/cm^2 as a class boundary. It is seen that antipyrin, ranitidine and metoprolol with IDRs of 56.79, 42.18 and 34.64 mg/cm^2/min respectively have the higher values in comparison to others whereas carbamazepine and piroxicam have the lowest intrinsic dissolution rate in the series (IDR=0.035 and 0.07 mg/cm^2/min respectively). This order is almost the same for solubility of mentioned drugs. However in the case of permeability this arrangement is not expected. The reason is that the investigated drugs belong to all four biopharmaceutical classes. That means a drug with high IDR value may belong to high or low permeability classes. In the present study passively absorbed drugs are classified based on their intrinsic dissolution rates and human intestinal permeability values (Zakeri-Milani et al., 2009a).. IDR was expected to correlate more closely with in vivo dissolution dynamics of drug than solubility. Therefore it could be used to correct assignment of a drug to a specific BCS class. This classification is presented in Fig 9 and Fig 10 (Zakeri-Milani et al., 2009a).

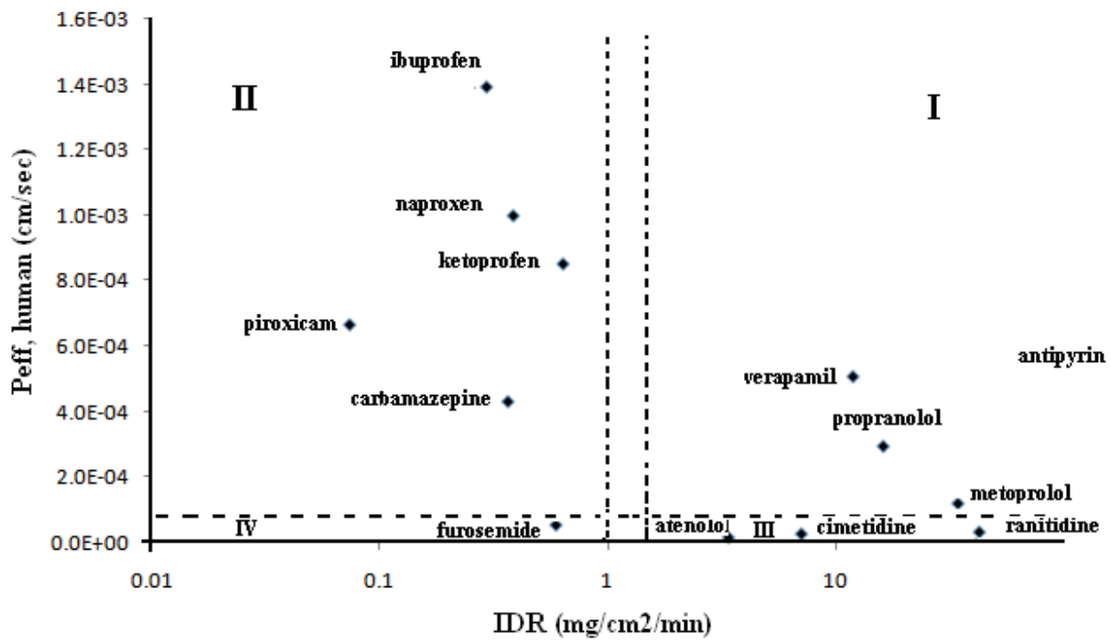

Fig. 9. Classification of tested drugs based on their human intestinal permeability and IDR

Based on this classification, drugs are placed in four explicitly defined categories (I-IV) which are made by intersections of dashed lines drawn at the cutoff points for permeability and IDR. These classes are characterized as below:

Class I: $P_{eff,rat} > 5\times 10^{-5}$ (cm/sec) or $P_{eff,human} > 4.7\times 10^{-5}$ (cm/sec) , IDR > 2 (mg/min/cm^2)

Examples of the compounds of this category include propranolol, metoprolol, verapamil and antipyrin which exhibit a high dissolution and absorption. However according to intestinal permeability estimates in rat, metoprolol is assigned in class III.

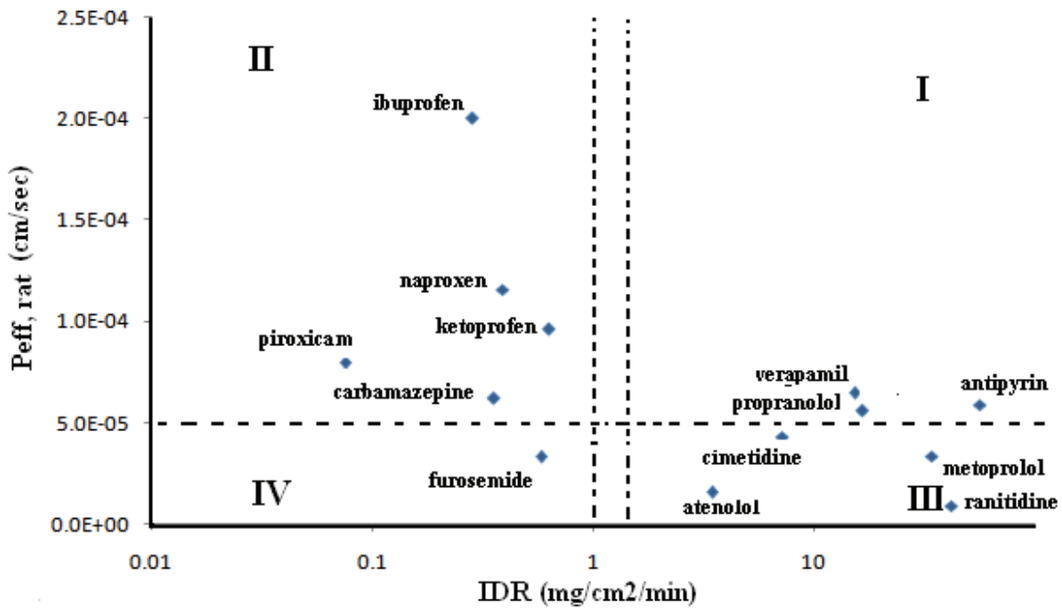

Fig. 10. Classification of tested drugs based on their rat intestinal permeability and IDR

Class II: $P_{eff,rat} > 5\times 10^{-5}$ (cm/sec) or $P_{eff,human} > 4.7\times 10^{-5}$ (cm/sec), IDR < 1 (mg/min/cm^2)
Drugs like ketoprofen, naproxen, piroxicam, ibuprofen and carbamazepine are included in this category. Class II drugs have a high absorption but a low dissolution therefore absorption is limited primarily by drug dissolution in the gastrointestinal tract (Amidon et al., 1995).
Class III: $P_{eff,rat} < 5\times 10^{-5}$ (cm/sec) or $P_{eff,human} < 4.7\times 10^{-5}$ (cm/sec) , IDR > 2 (mg/min/cm^2)
Class III drugs, have high dissolution and low absorption. In vivo permeability is rate limiting step for drug absorption . Examples are atenolol, ranitidine and cimetidine.
Class IV: $P_{eff,rat} < 5\times$ 10-5 (cm/sec) or $P_{eff,human} < 4.7\times 10^{-5}$ (cm/sec) , IDR < 1 (mg/min/cm^2)

Furosemide is an example of drugs of this category which exhibit a lot of problems for effective oral administration. From the obtained results it is provided that the presented classification based on IDR and human intestinal permeability of drugs is in high agreement with previously introduced classification and most of the compounds are placed in correct categories they belong to (Amidon et al., 1995). Although using the rat intestinal permeability values instead of human intestinal permeability, metoprolol was almost misclassified, considering non-feasibility of using human in intestinal perfusion studies, which is the major difficulty in assigning drugs to BCS classes, it may be suggested that determined intestinal permeability of drugs in rats could be used as a criterion for biopharmaceutical classification of compounds. On the other hand, it was proposed that a biopharmaceutics drug disposition classification system (BDDCS) based on extent of drug metabolism could provide an alternative simple method to assign drugs in class I for a waiver of in vivo bioequivalence studies (Takagi et al., 2006, Benet et al., 2008, Wu and Benet, 2005). According to this classification highly metabolized drugs exhibit high permeability. Therefore a drug is considered to be class I if it is highly soluble and highly metabolized. However this definition excludes drugs that have high absorption but are excreted unchanged in to bile and urine (Takagi et al., 2006). Comparison of our results with

BDDCS classification (≥50% being defined as extensive metabolism) of drugs (Wu and Benet, 2005) shows high agreement (92% and 85% using human and rat intestinal permeability respectively) in classification of tested compounds (Table 6). Another classification system namely dissolution-based classification was developed by Papadopoulou et al (Papadopoulou et al., 2008) using mean intestinal transit time (MITT), mean dissolution time (MDT) and mean absorption time (MAT). The comparison of this classification with our results is also shown in Table 6. However in dissolution-based classification propranolol and carbamazepine are classifies as class II and class IV drugs respectively which are expected to be assigned in class I and II respectively as was shown in other classifications in Table 6. It seems that the presented classification could be used to waive in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms which allows pharmaceutical companies to forego clinical bioequivalence studies, if their drug product meets the required specification. However at the time being, our attempt is to introduce some thermodynamic parameters as a surrogate for permeability measurements.

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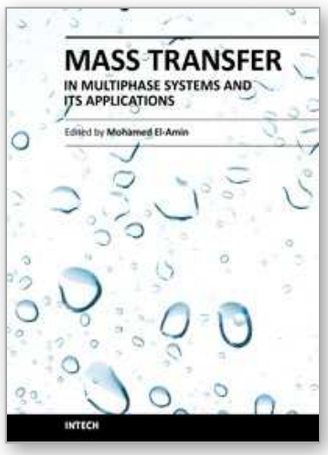

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