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°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°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:

$$P_{eff} = -Q \ln(C_{out}/C_{in})/2\pi r l \qquad (18)$$

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; $F_{a\,(human)} = 1 - e^{-38450 P_{eff\,(rat)}}$ ($R^2 = 0.91$, $P<0.0001$) (Fig. 7).

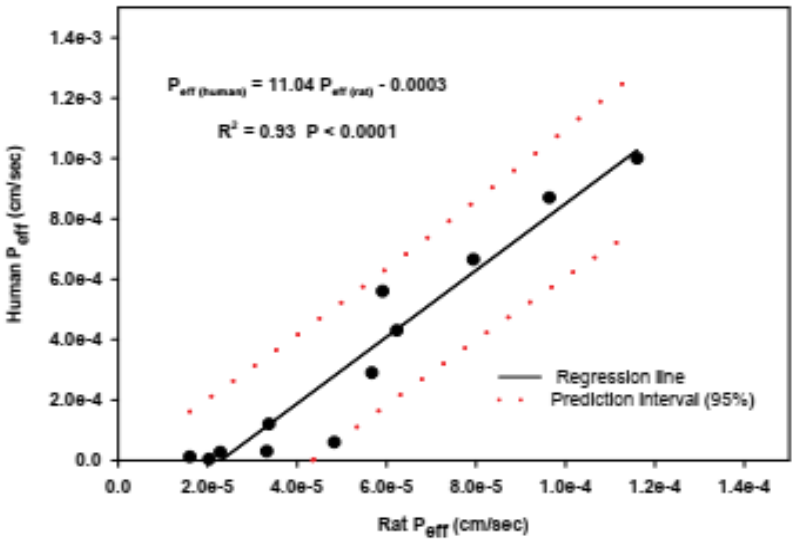

Fig. 6. Plot of P_{eff} rat vs P_{eff} human

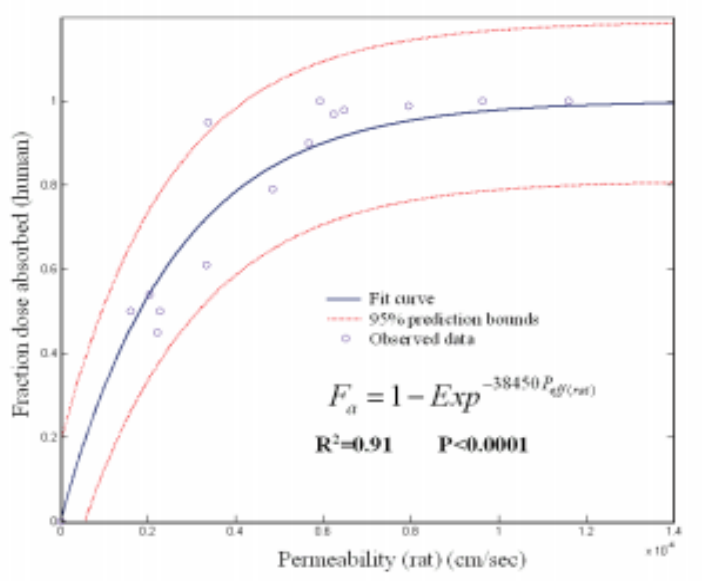

Fig. 7. Plot of rat P_{eff} vs human F_a

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.

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.

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).

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.

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

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.

Absorption time calculation

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

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

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.

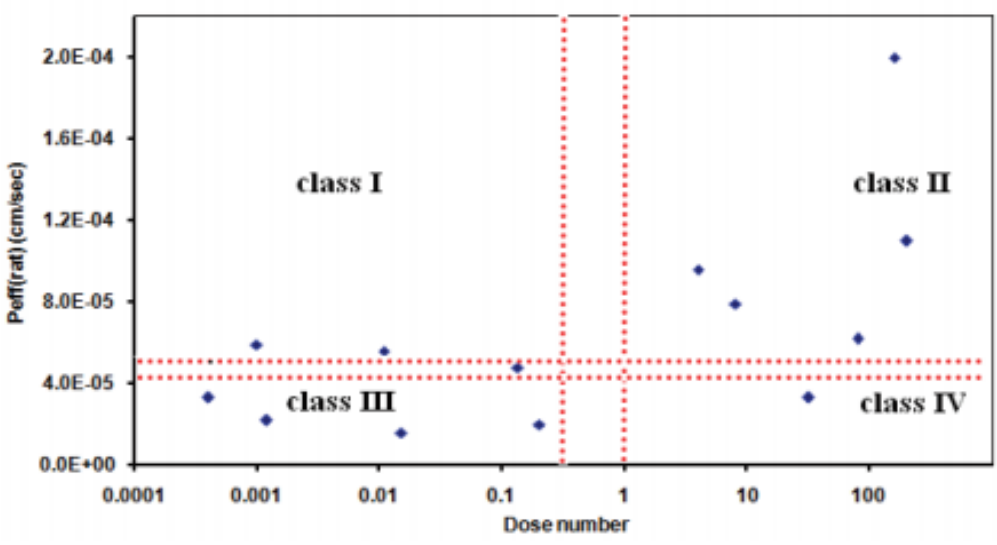

Fig. 8. Plot of Dose number vs rat P_{eff} values representing the four classes of tested compounds

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 GI 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	$A_n\uparrow$* $D_n\uparrow$ $D_o\downarrow$	High	High
II	$A_n\uparrow$ $D_n\downarrow$ $D_o\uparrow$	High	Low
III	$A_n\downarrow$ $D_n\uparrow$ $D_o\downarrow$	Low	High
IV	$A_n\downarrow$ $D_n\downarrow$ $D_o\uparrow$	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.

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.