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).

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).

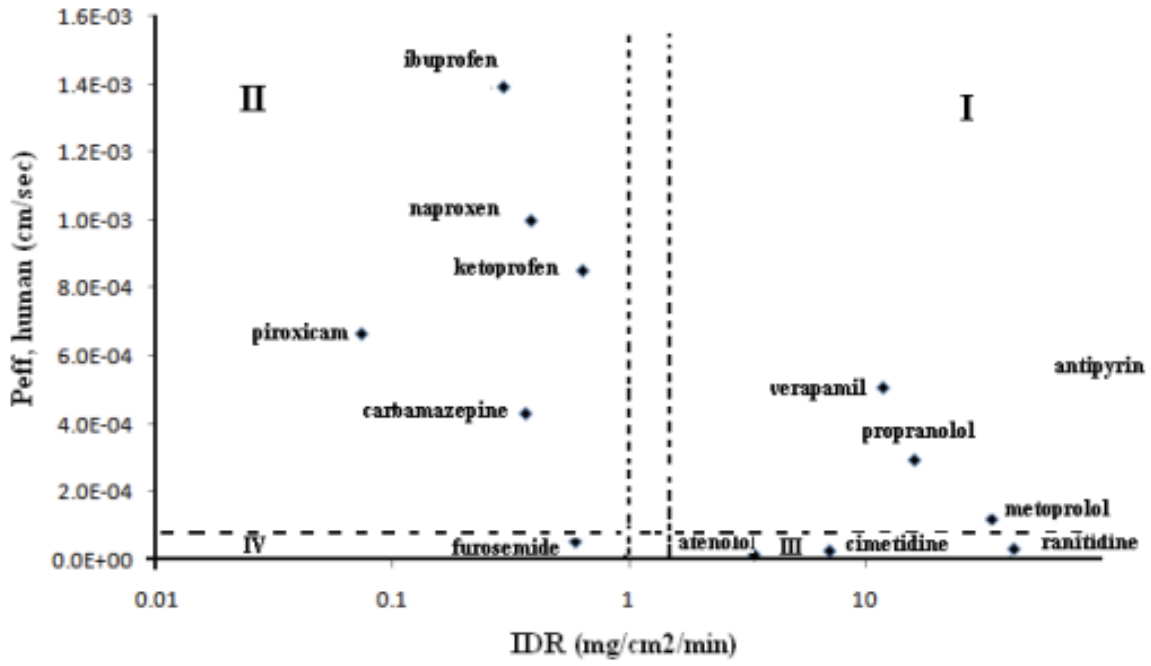

Fig. 9. Classification of tested drugs based on their human intestinal permeability and IDR

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.

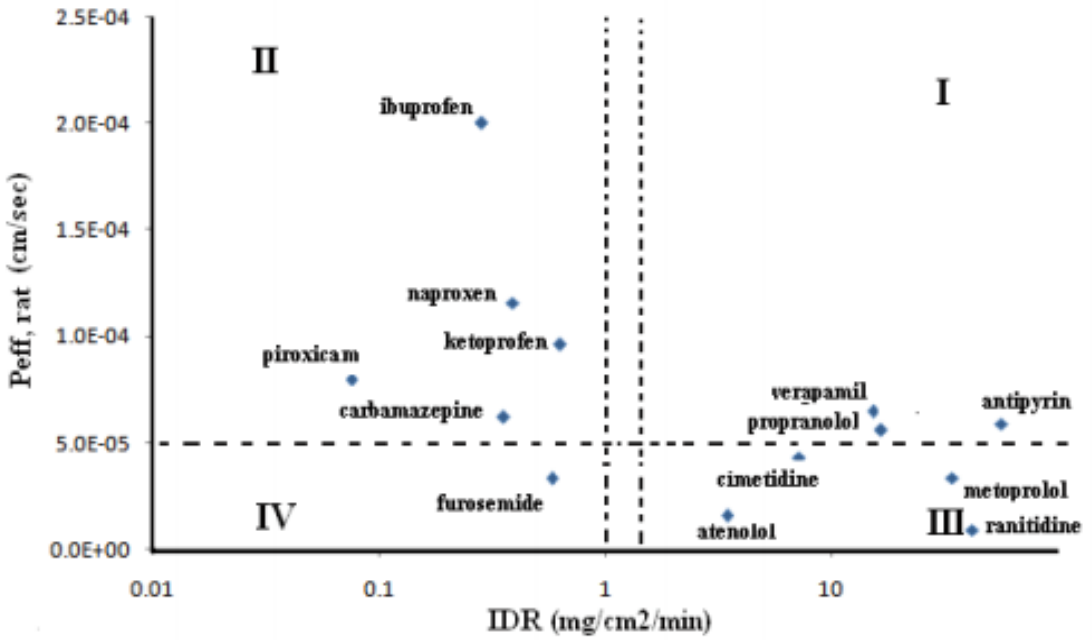

Fig. 10. Classification of tested drugs based on their rat intestinal permeability and IDR

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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