Crystal-liquid Fugacity Ratio as a Surrogate Parameter for Intestinal Permeability

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ABSTRACT - Background: We assessed the feasibility of using crystal-liquid fugacity ratio (CLFR) as an alternative parameter for intestinal permeability in the biopharmaceutical classification (BCS) of passively absorbed drugs. **Methods:** Dose number, fraction of dose absorbed, intestinal permeability, and intrinsic dissolution rate were used as the input parameters. CLFR was determined using thermodynamic parameters i.e., melting point, molar fusion enthalpy, and entropy of drug molecules obtained using differential scanning calorimetry. **Results:** The CLFR values were in the range of 0.06-41.76 mole percent. There was a close relationship between CLFR and in vivo intestinal permeability ($r > 0.8$). CLFR values of greater than 2 mole percent corresponded to complete intestinal absorption. Applying CLFR versus dose number or intrinsic dissolution rate, more than 92% of tested drugs were correctly classified with respect to the reported classification system on the basis of human intestinal permeability and solubility. **Conclusion:** This investigation revealed that the CLFR might be an appropriate parameter for quantitative biopharmaceutical classification. This could be attributed to the fact that CLFR could be a measure of solubility of compounds in lipid bilayer which was found in this study to be directly proportional to the intestinal permeability of compounds. This classification enables researchers to define characteristics for intestinal absorption of all four BCS drug classes using suitable cutoff points for both intrinsic dissolution rate and crystal-liquid fugacity ratio. Therefore, it may be used as a surrogate for permeability studies.

INTRODUCTION

Oral administration is the easiest and most common method for drug delivery. Good oral bioavailability is required for drug candidates in pharmaceutical discovery and development pipelines. Prediction of drug absorption is therefore very important in drug development (1-3). In 1995 Amidon and colleagues introduced a biopharmaceutics classification system (BCS) to classify drugs based on their aqueous solubility and intestinal permeability, two fundamental properties governing drug absorption and the main driving forces of an orally administered drug to get bioavailable (4-8). In the past, It has been observed that drug candidates often failed for reasons of poor pharmacokinetics (9, 10). To avoid the high risk associated with such failures the prediction of in vivo pharmacokinetics from in vitro data is recognized as a highly desirable technique. Physicochemical surrogates, which can be measured in vitro, would allow to estimate important oral properties like intestinal permeability, P_{eff}. Thus far, only a few experimental in vitro methods based on artificial membrane models (11-14) and partition coefficient (15) have been established for predicting drug permeation capabilities (16). The crystal-liquid fugacity ratio (CLFR), also sometimes called the ideal solubility is dependent on the crystalinity of the solute (10, 17). It reflects the tendency of a substance to prefer one phase (liquid, solid, or gas) over another (17, 18) and can be literally defined as the tendency to flee or escape the one to other phase.

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At a fixed temperature and pressure, a homogeneous substance will have a different fugacity for each phase. The phase with the lowest fugacity will be the most favorable, and will have the lowest Gibbs free energy. CLFR is an important determinant of the solubility of a substance in lipid bilayers, and reflects the solubility of the drug in octanol (18), representing the lipid bilayer of a cell membrane. For this reason, its calculation could be of importance in determining the tendency of compounds to dissolve or penetrate into biological membranes. On the other hand, the distribution and fate of organic compounds in the body is influenced by the compounds propensity to favor, or disfavor, water or the biological lipoid phase (19). The behavior of pharmaceuticals in the gastro-intestinal tract is largely controlled by their relative tendencies to partition into water and organic phases e.g. membranes. Accordingly, the key descriptors of these tendencies are solubility in water and octanol–water partition coefficient (K_{OW}) (20). These quantities are, thus, routinely required when assessing the likely fate of existing or new chemicals.

To the best of our knowledge the utility of CLFR in the BCS classification has not been studied, and it has not been used as a predictive tool for assessing the capability of a molecule to get orally absorbed. Considering the above mentioned facts and given that CLFR of a compound is one of the first and reliably measurable properties, we propose that it could be advantageously used to guide the screening process in selecting lead compounds. CLFR is a product of molecular weight, melting point as well as the heat of fusion, and it reflects the solubility of the compound in octanol and lipid bilayers. The purpose of the present study was to correlate CLFR to intestinal permeability and to evaluate the suitability of using CLFR as a surrogate for intestinal permeability for BCS. Only passively transported drugs were evaluated in this study. Compounds known to be extensively metabolized in the gut were excluded too.

MATERIALS AND METHODS:

Materials

Naproxen, atenolol, metoprolol, propranolol, and ibuprofen were provided from Shasun (Shasun Chemical & Drugs LTD, India). Ketoprofen and antipyrine were from Sigma (Sigma, Canada) and Hoechst (Hoechst, Germany) respectively. Furosemide and carbamazepine were provided from Fls (Fls, Italy). Ranitidine and cimetidine were obtained from Uquifa (Uquifa, Spain) and piroxicam was from Ciba-Geigy (Barcelona, Spain). All other compounds were gifted by Zahravi (Tabriz, Iran) and Daroupakhs (Tehran, Iran) pharmaceutical companies. All compounds were of USP grade and were in the same chemical forms that usually are used in oral formulations.

Calorimetric studies

The calorimetric measurements were performed using differential scanning calorimeter (Shimadzu - DSC-60, Kyoto, Japan). The data were recorded and analyzed using TA-60 software. A sample of 5 mg of all tested compounds was accurately weighed in an aluminum pan. Five mg Al_2O_3 served as the reference in a separate pan. The reference and sample were heated at 20 °C per minute over the 30-400 °C range. Melting point (T_m) was determined using endothermic peak on the thermogram. Enthalpy of fusion (ΔH_f) or specific melting heat which is defined as the amount of energy required to overcome intermolecular forces to convert one mole of a solid to a liquid, was calculated from the area under the melting endothermic peak of the obtained DSC thermograms. The increase in degree of disorder in the transition from the organized solid to the disorganized structure of its liquid form or entropy of fusion (ΔS_f) is related to the melting point and the heat of fusion. ΔS_f in (J/°K mol) was calculated using the following equation:

$$\Delta S_f = \Delta H_f / T_m \qquad \text{Eq.1}$$

Crystal-liquid fugacity ratio determination

CLFR is the ratio of the thermodynamic fugacities of a substance as a crystal compared to that of a liquid at the same pressure and temperature. CLFR for tested compounds in mole fraction were estimated by equation 2 (18):

$$Ln\ CLFR = \frac{-\Delta H_f\,(T_m - T)}{R T_m T} + \left(\frac{\Delta C_{pm}}{R}\right)\left[\frac{T_m - T}{T} - \ln\left(\frac{T_m}{T}\right)\right] \qquad \text{Eq. 2}$$

Where ΔH_f is the molar enthalpy of fusion of the pure solute, T_m is the absolute melting point, T is the absolute solution temperature (310.15 Kelvin, the body temperature), R is the gas constant (1.987 Cal/mol °K), and ΔC_{pm} is the difference between the molar heat capacity of the solid form and the molar heat capacity of the hypothetical supercooled liquid form, both at the solution temperature (18). Using the Gibbs relationship at the phase transition, $\Delta H_f / T_m$

can be substituted by entropy of fusion (ΔS_f). Based on the assumption (18, 21-24) that either ΔC_{pm} and/or the difference between the terms in the bracket are nearly zero for most compounds, Eq.2 can be reduced to (18):

$$Ln\ CLFR = \frac{-\Delta S_f\ (T_m - T)}{RT} \qquad \text{Eq. 3}$$

Measurement of rat intestinal permeability coefficients

The study was reviewed and approved by the local ethical review board of Tabriz University of Medical Sciences in Iran and conducted in conformity with the international guidelines. In all animal studies "Guide to the care and use of experimental animals" by Canadian Council on Animal Care, was followed (25).

The anesthesia and surgery were performed in accordance with a previously validated in situ intestinal perfusion method in rats (26). Detailed procedure, analytical methods and also permeability coefficient calculation were previously published (27-29). Briefly, male Wistar rats (250-300 g; age, 7-9 weeks; n=4 for all tested drugs shown in Table 1) were maintained on 12 h light- dark cycle and fasted 12-18 h before experiment. On the day of the experiment a single pass constant flow (0.2 ml/min) of drug containing perfusate (PBS pH=7.2, 37ºC) was established through the ligated rat jejunal segment and the outlet samples were collected every 10 min in micro tubes up to 90 min and stored at -20ºC until analysis. Finally the animal was euthanatized with a cardiac injection of saturated solution of KCl.

Permeability values were calculated using following equation according to the parallel tube model (28, 30):

$$P_{\text{eff}} = \frac{-Q \ln(\frac{C_{out}}{C_{in}})}{2\pi rl} \quad \text{Eq.4}$$

Where C_{in} and C_{out} are the inlet and corrected outlet concentrations of compound respectively. 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 intestinal segment (31). Absorption or secretion of water was calculated by differences between inlet and outlet concentrations of non-absorbable marker, phenol red.

Fraction of a dose absorbed and Dose Number

Values for the fraction of a dose absorbed in humans (Fa%) were obtained from references (32-40). When the Fa% value was reported as a range, the mid-value of the range was used.

Dose Number values were taken from the work published by Kasim and coworkers (41).

Intrinsic dissolution rate (IDR) measurement

Non-disintegrating compacts using 100 mg of each compound were prepared to determine IDR (compression force was 7.84 MPa, hold for 1 min using die and punch with a diameter of 6 mm). Compacts were placed in a molten beeswax-mold in such a way that only one face could be in contact with the dissolution medium. Dissolution study was conducted using USP II dissolution apparatus (100 rpm) using 900 mL of phosphate buffer (pH = 6.8, 37 ± 1 ˚C). Drug concentration analysis was performed spectrophotometrically (UV160, Shimadzu, Kyoto, Japan) at the maximum absorbance wavelength for each active tested sample. IDR (mg/min/cm^2) was calculated by equation 5;

$$IDR = \frac{dw}{S.dt} \qquad \text{Eq. 5}$$

where *dw* is the change in drug dissolved; *dt* is the change in time; S is the surface area of the compact (0.2826 cm^2) (29).

STATISTICAL ANALYSIS

The linear regression analysis was performed by using Excel 2010, and nonlinear regression was performed by using MATLAB (version R2013a, MathWorks Inc., Natick, MA, USA). The regression coefficients were obtained by least-squares regression analysis. For each regression, the following descriptive information is provided: number of observations used in the analysis (n) and correlation coefficient (r). The absolute average error (AAE) for each calculation was determined by

$$\text{AAE} = \frac{1}{n}\sum_{i=1}^{n}|A_i - F_i|$$

where A_i is the actual value and F_i is the forecast value.

RESULTS and DISCUSSION

Table 1 and 2 provide experimental melting point, enthalpy, entropy and CLFR for tested compounds. The table also presents the respective determined rat

intestinal permeability ($P_{eff,rat}$), the reported human intestinal permeability ($P_{eff,human}$) and fraction dose absorbed in human (F_a). In addition, the reported biopharmaceutical classes of each compound based on different approaches are listed. Experimentally derived human intestinal permeability and F_a data were taken from literature which is cited in the Table 1. For the drugs shown in Table 1, the melting point ranged between 83.39 and 298.34 °C for ibuprofen and furosemide, respectively, whereas hydrochlorothiazide and ibuprofen (with lowest and highest human effective intestinal permeability) exhibited the lowest (0.4) and highest (39.6) CLFR (mole percent) values, respectively. Figure 1 shows the correlation between CLFR and jejunal Peff in humans and rats. As it is evident from the obtained results the proportionality exists only at higher values of CLFR. Unfortunately only a limited number of the intestinal permeability data exist. To obtain a reliable correlation between CLFR and intestinal permeability, greater number of experimental data is required. Therefore, the possibility of a positive correlation between CLFR and Fa was assessed. To do so, thermodynamic properties of a greater number of drugs were measured to obtain their CLFR. Table 2 provides experimental melting point, fusion enthalpy, entropy and CLFR for tested compounds together with their human fraction dose absorbed (Fa). Compounds with lower enthalpy of fusion and melting point showed higher absorption capability. This result is in agreement with observation of Chu and Yalkowsky who reported that low melting point compounds will be better absorbed than high melting point compounds (42). The obtained results (Figure 2) indicate that there is a sigmoidal (chapman type) dependency between CLFR and Fa. When the CLFR is less than 2 (mole percent) the fraction dose absorbed is exponentially dependent on the CLFR. i.e a very small change in CLFR corresponds with a drastic change in Fa. Compounds with CLFR greater than 2 are almost completely absorbed. A similar trend has been reported for intestinal permeability versus Fa, in which an intestinal effective permeability greater than 2×10^{-4} cm/sec will result in complete absorption (4, 6, 43). The average absolute error of the model for the prediction of Fa (AAE) was 11.60 % (Table 2, Figure 2, n=47).

BCS Classification

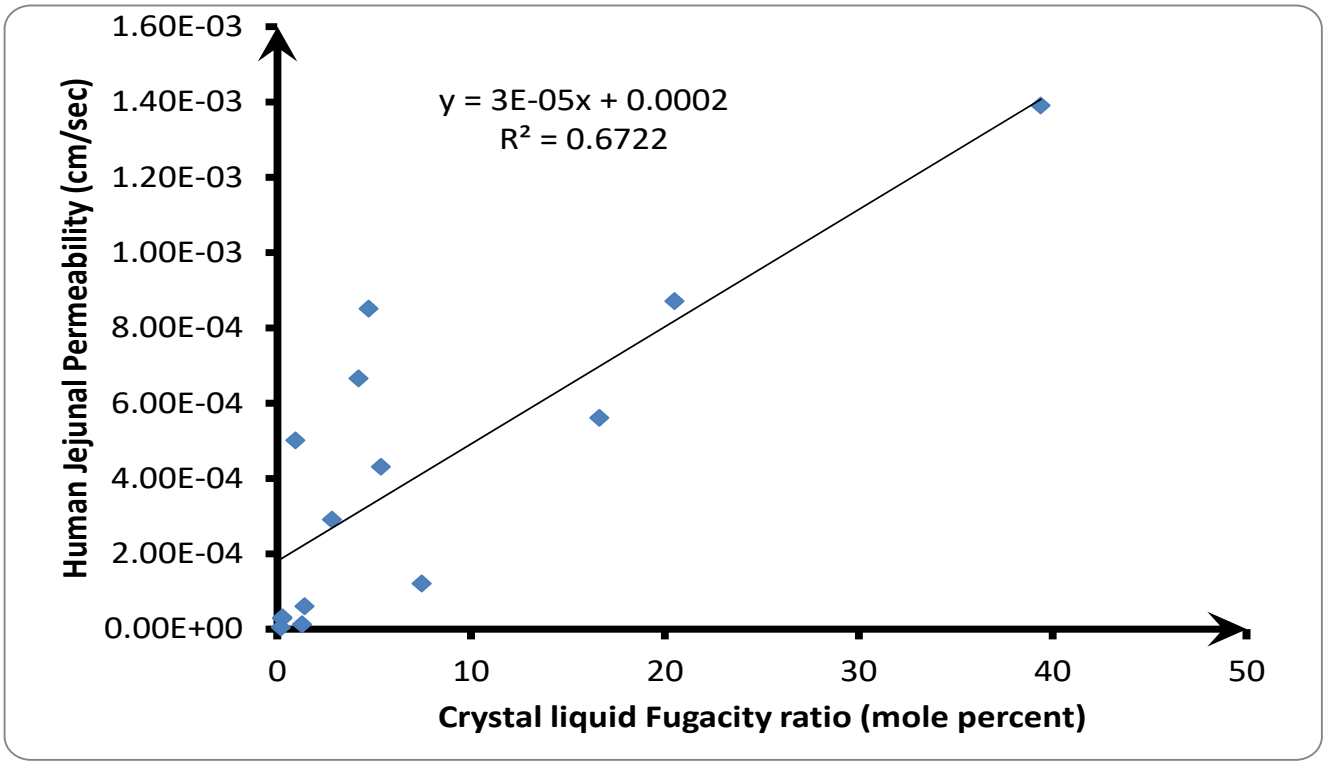
Drugs are classified based on their solubility and human intestinal permeability (4, 44-46). The BCS consists of four drug categories: class I (highly soluble and highly permeable), class II (low soluble and highly permeable), class III (highly soluble and low permeable) and class IV (low soluble and low permeable). Previously, we introduced the rat intestinal permeability as a parameter to classify drugs (47). Considering the non-feasibility of using human intestinal perfusion studies routinely, which measures the true human P_{eff}, drugs were historically classified on the basis of their dose number and rat jejunal permeability or cell culture estimates (47-51). In another classification approach, introduced by us (29) intrinsic dissolution rate (IDR) and human or rat intestinal permeability values were used. However in the present work, CLFR is applied as a reliable physical property for screening and selection of lead compounds instead of permeability. The proposed classification system is based on CLFR-dose number (D_o) or CLFR-intrinsic dissolution rate (IDR) (Figures 3 and 4, respectively). These parameters could be easily and rapidly acquired by in vitro measurements and no in vivo setup is required. 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 cut-off points for CLFR and dose number or IDR. These classes, which are in agreement with the conventional BCS are characterized as below:

Category I: Dose number < 1, CLFR (mole percent) > 2, IDR ($mg/cm^2/min$) > 1

These drugs are highly soluble and highly permeable. They are expected to have low crystal lattice energy and to be in solution form throughout the intestine available for permeation. Therefore, the rate of absorption of drugs in this class is controlled only by gastric emptying (47). Examples of compounds of this category include antipyrine, propranolol, verapamil and metoprolol. However based on CLFR as a main parameter for classification, verapamil is assigned to class III.

Category II: Dose number > 1, CLFR (mole percent) > 2 , IDR ($mg/cm^2/min$) < 1

Here the dissolution rate is the governing parameter controlling bioavailability of class II drugs. These drugs have low crystal lattice energy and higher tendency to partition into lipid bilayers. These compounds are characterized by mean absorption time less than mean dissolution time. Hence gastric emptying and gastrointestinal transit are important determinants of drug absorption. Therefore, formulation plays an important role in the rate and extent of intestinal absorption of such drugs and there are several methods to enhance the solubility of class II drugs (3, 4, 44, 46). Drugs like ketoprofen,



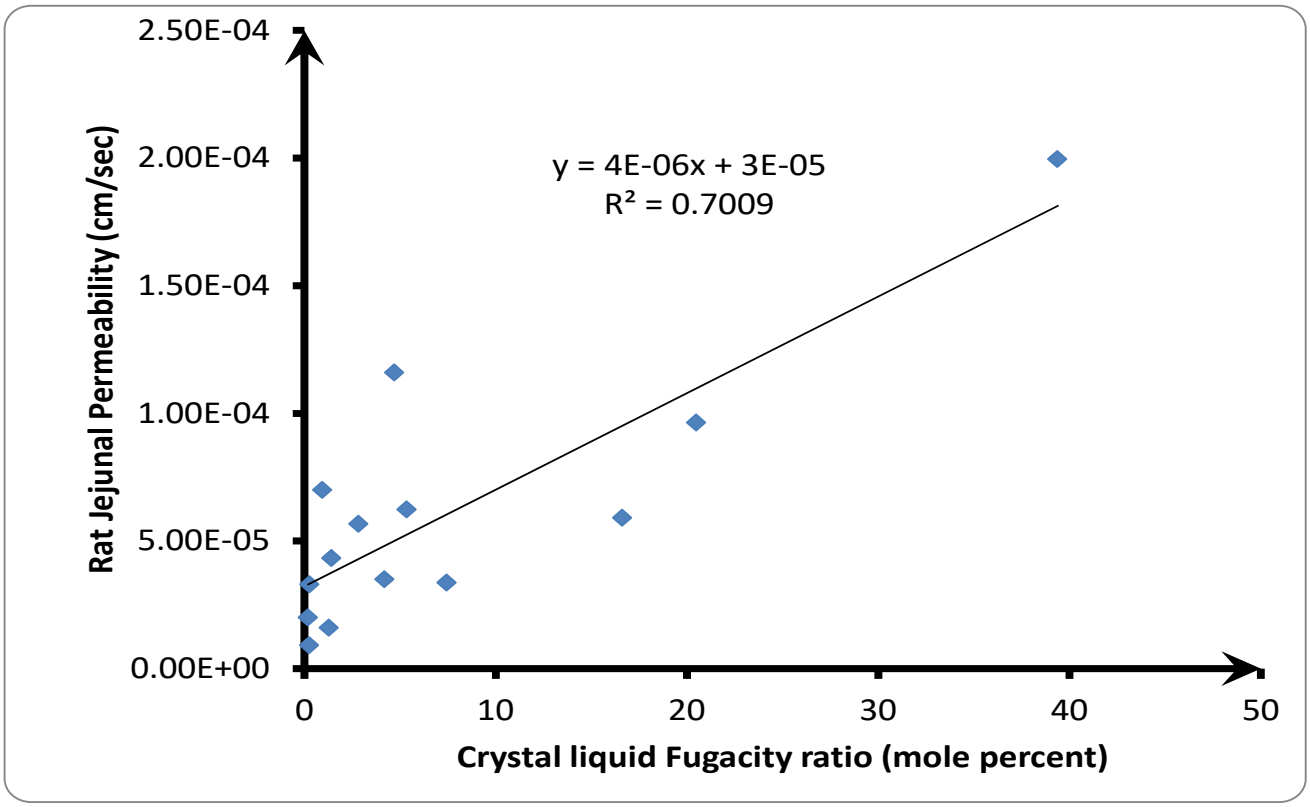


Figure 1. Correlation between crystal liquid fugacity ratio and human (top) or rat (bottom) jejunal permeability. Two clusters is seen in both graphs. At high CLFR values a linear relationship may exist, however, at lower CLFR values there is no correlation.

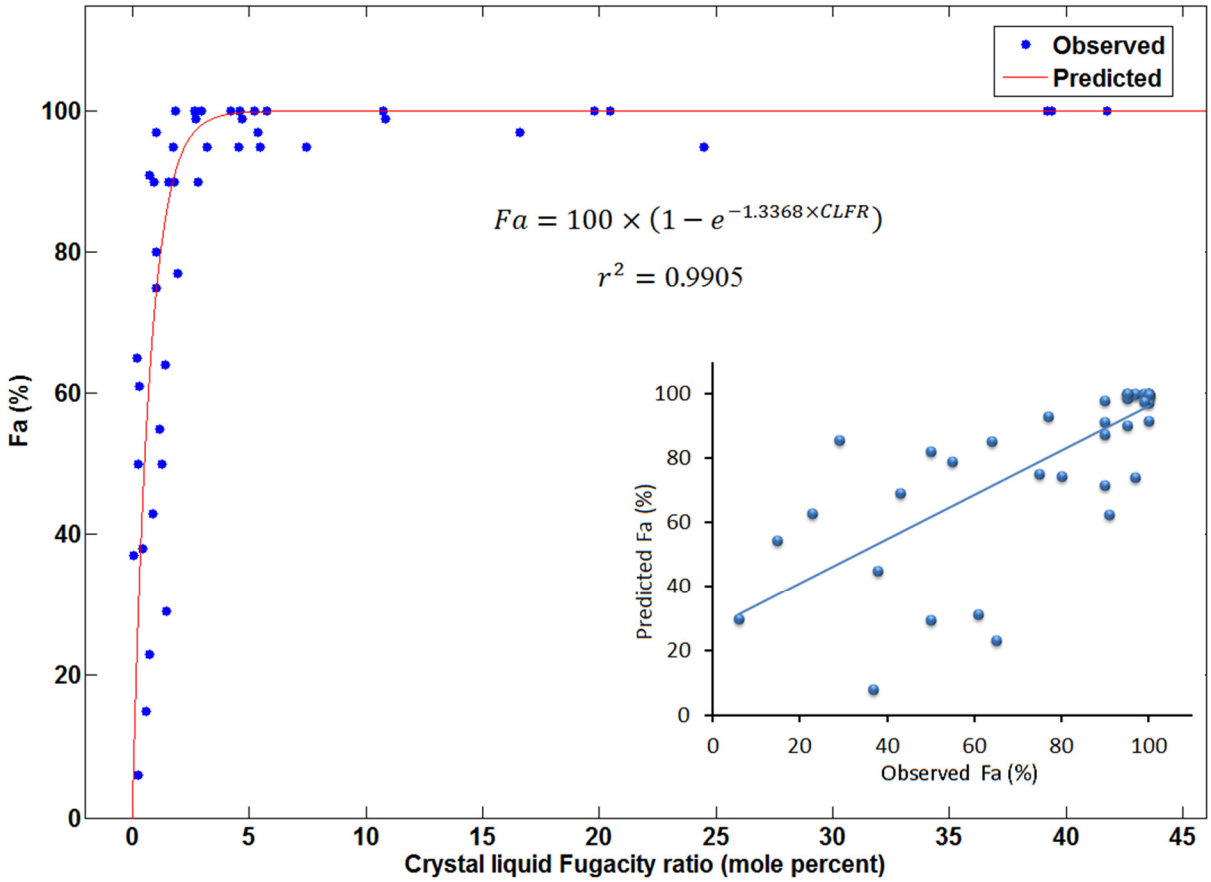


Figure 2. The relationship between crystal liquid fugacity ratio (CLFR) and fraction dose absorbed in human (Fa). The insert depicts the correlation between observed vs predicted Fa based on the obtained equation (n=47).

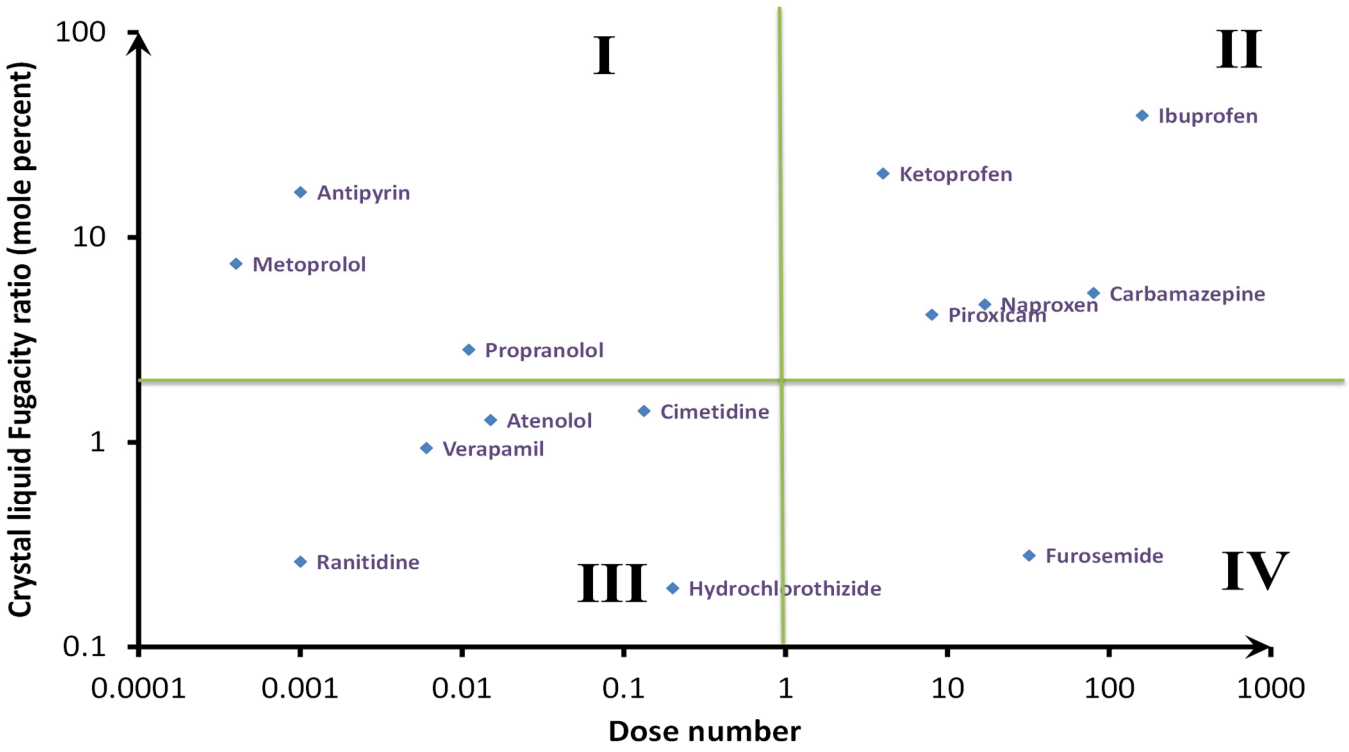


Figure 3. Classification of tested drugs based on their crystal-liquid fugacity ratio and dose number

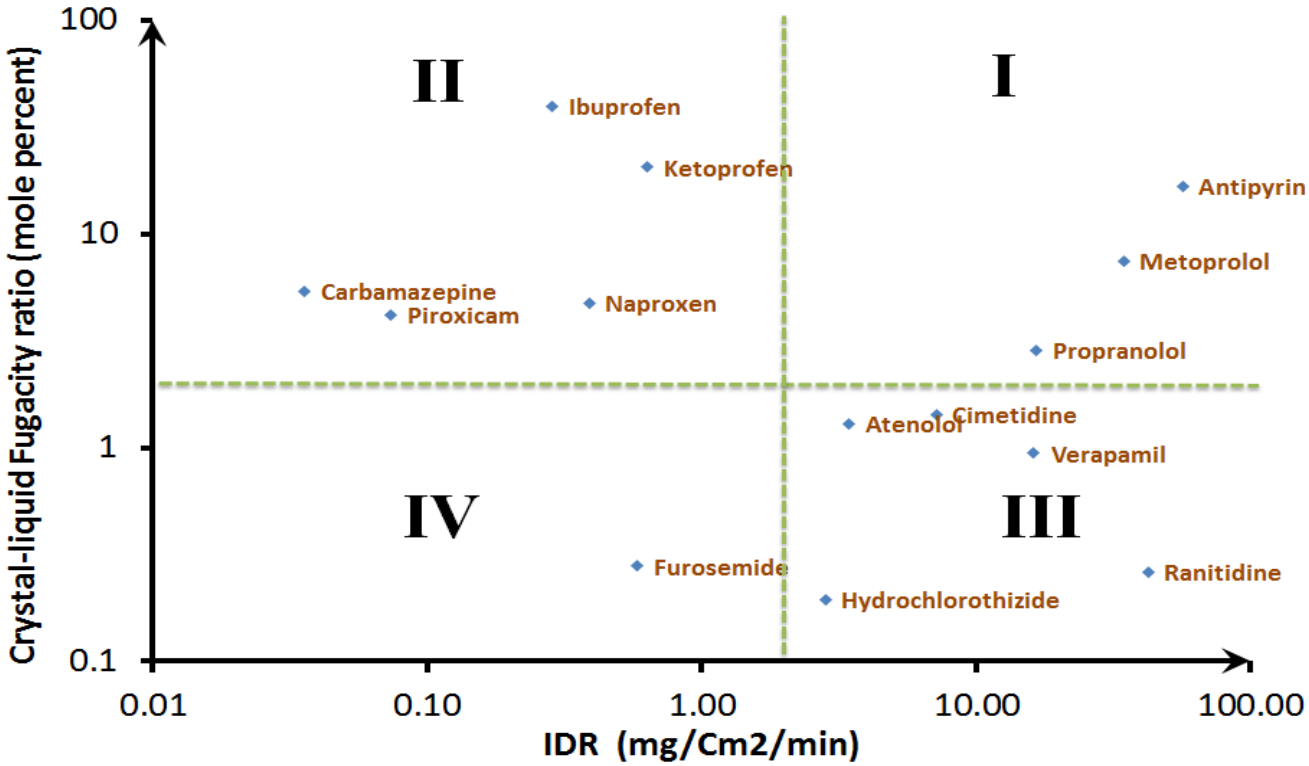


Figure 4. Classification of tested drugs based on their crystal-liquid fugacity ratio and intrinsic dissolution rate (IDR).

naproxen, piroxicam, ibuprofen and carbamazepine are included in this category.

Category III: Dose number < 1, CLFR (mole percent) < 2 , IDR (mg/cm^2/min) > 1
Class III drugs exhibit high crystal lattice energy, indicating low dissolution in the lipid bilayer of cell membrane. Therefore the absorption is limited by their intestinal permeability. In fact the rate and extent of intestinal absorption may be controlled by drug molecule properties and physiological factors rather than pharmaceutical formulation properties. Atenolol, cimetidine, and ranitidine are examples of drugs in this group.

Category IV: Dose number > 1, CLFR (mole percent) < 2 , IDR (mg/cm^2/min) < 1
Furosemide is an example of drugs in this category which exhibit a lot of problems for effective oral administration because of the combined limitation of solubility and permeability. These drugs exhibit high crystal lattice energy. Therefore strategies to improve their solubility and also permeability need to be addressed, which may not be an easy task (4, 47).

As the results indicate, the presented categorization based on CLFR and dose number/IDR is in high agreement with previously introduced classifications, and most of compounds are assigned to their correct BCS class (Table 1 and 2). On the other hand, considering the biopharmaceutics drug disposition classification system (BDDCS) (29, 45, 52-54) which gives scientists a roadmap for predicting a drug's disposition, metabolism and drug-drug interaction characteristics with little additional data, again the classification is in high agreement with the presented classification (Table 1). In addition there is another classification system developed by Papadopoulou et al which is based on mean intestinal transit time, mean dissolution time, and mean absorption time. The comparison of our results with this dissolution-based classification is also provided in Table 1.

CONCLUSION

This study reports, for the first time, the experimental fusion enthalpy and entropy together with CLFR of 47 drugs. CLFR can be used for the biopharmaceutical classification of compounds based on only in vitro measurements, however this method is limited to passively transported drugs. In this system, the CLFR acts as a surrogate for intestinal permeability which allows classifying drug compounds to predict their in vivo performance. The cutoff value of 2 mole percent is suggested for

CLFR, below which there is an exponential dependency of Fa on CLFR. Compounds with CLFR values of greater than 2 are almost completely absorbed. This new surrogate for human permeability can be used as first screening step before compounds are undergoing more costly and labor intensive screening methods such as low throughput in vivo animal models or human intestinal absorption studies. However, a larger number of compounds belonging to all four biopharmaceutical classes, need to be added to this data base.

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Table1. Experimental melting point, enthalpy, entropy, crystal-liquid fugacity ratio (CLFR), rat intestinal permeability ($P_{eff,rat}$), Human intestinal permeability ($P_{eff,human}$), Fraction dose absorbed in human (F_a) and respective class of tested compounds using different approaches

Compound	Melting point (°C)	Fusion Enthalpy (Cal/mol)	Fusion Entropy (Cal/mol °K)	CLFR (Mole Percent)	IDR [a] (mg cm^{-2} min^{-1})	Dose no[b]	[c] $P_{eff,rat}$ ($\times10^5$ cm/s)	[d] $P_{eff,human}$ ($\times10^4$ cm/s)	[e] Human F_a	Drug class						
										This work		Based on IDR and $P_{eff(human)}$[a]	Based on IDR and $P_{eff(rat)}$[a]	BCS[a]	BDDCS[a]	Dissolution based[a]
										*	**					
Antipyrin	116.62	5414.39	13.89	16.626	56.79	0.001	5.9	5.60	97	I	I	I	I	I	I	I
Metoprolol tartrate	128.93	6999.25	17.41	7.457	34.64	0.0004	3.3	1.34	95	I	I	I	III	I	I	I
Propranolol HCl	168.57	7372.78	16.69	2.837	16.596	0.011	5.6	2.91	90	I	I	I	I	I	I	II
Verapamil HCl	146.81	11003.33	26.20	0.940	16.192	0.006	7	5.00	90	III	III	I	I	I	I	I
Ketoprofen	99.47	5827.28	15.64	20.499	0.6348	4	9.6	8.70	100	II	II	II	II	II	I	II
Naproxen	159.28	6657.74	15.40	4.717	0.388	17	11	8.50	99	II	II	II	II	II	II	II
Carbamazepine	199.23	5253.56	11.12	5.357	0.0355	80	6.2	4.30	97	II	II	II	II	II	II	IV
Ibuprofen	83.39	4414.39	12.38	39.387	0.2844	160	20	13.90	100	II	II	II	II	II	II	-
Piroxicam	205.45	5551.42	11.60	4.202	0.0739	8	7.9	6.65	99	II	II	II	II	II	II	-
Atenolol	158.17	9551.88	22.14	1.287	3.449	0.015	1.6	0.20	50	III	III	III	III	III	III	III
Cimetidine	145.79	10090.96	24.09	1.425	7.2	0.133	4.8	0.26	64	III	III	III	III	III	III	-
Ranitidine HCl	151.26	13627.35	32.11	0.260	42.18	0.001	2.2	0.27	50	III	III	III	III	III	III	III
Furosemide	298.34	7930.05	13.88	0.279	0.58	32	3.3	0.05	61	IV	IV	IV	IV	IV	IV	IV
Hydrochlorothiazide	272.66	8920.90	16.34	0.193	2.83	0.2	2	0.04	65	III	III	III	III	III	-	III

*Based on IDR and CLFR

** Based on Dose no and CLFR

a, b, c: taken from ref (29), (41, 55), and (28), respectively

d: $P_{eff,human}$ values taken from reference (54) and (56).

e: Fraction dose absorbed (Fa) data taken from Reference: (28-30, 32, 39-41, 51, 54, 56-59)

Table 2. Experimental melting point, enthalpy, crystal-liquid fugacity ratio (CLFR), fraction dose absorbed in human (F_a) and respective calculated Fa together with Fa prediction absolute error (AE). The average absolute error (AAE) was 11.60 %. The conventional as well as obtained BCS class using the proposed model is included.

Compound	M.W	Melting Point (°C)	Enthalpy (Cal/mol)	Entropy (Cal/mol°K)	CLFR (Mole Percent %)	Dose no	Observed Fa (%)	Calculated Fa (%)	AE (%)	Classification	
										BCS	This
Acetaminophen	151.16	175.85	4427.0	9.9	**10.850**	0.20	**99**	100.00	1.00	I	I
Acyclovir	225.2	262.28	6216.0	11.6	**1.436**	0.08	**29**	85.33	56.33	III	III
Amantadine HCl	187.71	317.59	1215.8	2.1	**39.185**	0.01	**100**	100.00	0.00	I	I
Antipyrine	188.23	116.62	5414.4	13.9	**16.623**	0.001	**97**	100.00	3.00	I	I
Atenolol	266.34	158.17	9551.9	22.1	**1.286**	0.02	**50**	82.08	32.08	III	III
Azithromycin	785	149.33	17205.6	40.7	**0.060**	0.06	**37**	7.71	29.29	III	III
Caffeine	194.193	239.22	4611.7	9.0	**5.219**	0.01	**100**	99.91	0.09	I	I
Carbamazepine	236.27	199.23	5253.6	11.1	**5.355**	80.00	**97**	99.92	2.92	II	II
Chlordiazepoxide	299.75	246.1	6106.3	11.8	**1.851**	0.05	**100**	91.58	8.42	I	III
Chlorphenamine maleate	390.9	137.24	10519.9	25.6	**1.547**	0.001	**90**	87.36	2.64	I	III
Cimetidine	252.34	145.79	10091.0	24.1	**1.425**	0.133	**64**	85.12	21.12	III	III
Clarithromycin	748	229.97	7168.6	14.2	**1.155**	1.00	**55**	78.65	23.65	IV	IV
Clonazepam	315.71	251.18	6056.0	11.5	**1.807**	0.08	**90**	91.07	1.07	II	III
Diazepam	284.75	136.74	5654.8	13.8	**10.727**	0.70	**100**	100.00	0.00	I	I
Dipyridamole	504.6	173.94	6207.1	13.9	**4.575**	43.00	**95**	99.78	4.78	II	II
Divalproex sodium	308.2	107.23	2915.6	7.7	**41.756**	1.54	**100**	100.00	0.00	II	II
Famotidine	337.45	167.39	11282.0	25.6	**0.444**	0.16	**38**	44.76	6.76	III	III
Fenfluramine	267.7	180.66	6863.8	15.1	**2.945**	0.19	**100**	98.05	1.95	I	I
Fingolimod	307.471	113.86	4366.8	11.3	**24.488**	0.29	**95**	100.00	5.00	II	I
Fluconazole	306.27	145.08	6922.9	16.6	**5.489**	0.80	**95**	99.93	4.93	I	I
Furosemide	330.7	298.34	7930.1	13.9	**0.279**	32.00	**61**	31.13	29.87	IV	IV
Glibenclamide	494	180.98	7648.4	16.8	**1.956**	6.00	**77**	92.68	15.68	II	II
Griseofulvin	352.8	221.52	7830.8	15.8	**0.875**	10.00	**43**	68.95	25.95	II	IV
Hydrochlorothiazide	297.7	272.66	8920.9	16.3	**0.193**	0.20	**65**	22.74	42.26	III	III
Hydrocortisone acetate	404.5	228.72	7939.1	15.8	**0.730**	0.20	**91**	62.31	28.69	I	III
Ibuprofen	206.28	83.39	4414.4	12.4	**39.383**	160.00	**100**	100.00	0.00	II	II
Imipramine HCl	316.9	179.69	5580.9	12.3	**5.767**	16.48	**100**	99.96	0.04	II	II
Indomethacin	357.79	169.57	6343.6	14.3	**4.588**	80.00	**100**	99.78	0.22	II	II
Ketoconazole	531.438	152.74	13478.3	31.6	**0.263**	116.00	**6**	29.64	23.64	II	IV
Ketoprofen	254.28	99.47	5827.3	15.6	**20.495**	4.00	**100**	100.00	0.00	II	II
Mefenamic acid	241.29	238.12	5395.6	10.6	**3.195**	13.00	**95**	98.60	3.60	II	II
Metoprolol tartrate	684.8	128.93	6999.2	17.4	**7.455**	0.0004	**95**	100.00	5.00	I	I
Metronidazole	171.2	169.28	6347.9	14.3	**4.600**	0.20	**100**	99.79	0.21	I	I
Naproxen	230.26	159.28	6657.7	15.4	**4.715**	17.00	**99**	99.82	0.82	II	II
Nifedipine	346.33	181.07	7050.1	15.5	**2.657**	13.00	**100**	97.13	2.87	II	II
Olanzapine	312.4	198.37	8227.9	17.4	**1.037**	16.00	**75**	75.00	0.00	-	IV
Pentoxifylline	278.3	108.61	5323.6	13.9	**19.788**	0.01	**100**	100.00	0.00	I	I
Piroxicam	331.3	205.45	5551.4	11.6	**4.201**	8.00	**100**	99.64	0.36	II	II
Prednisolon	360.4	248.8	5477.8	10.5	**2.715**	0.05	**99**	97.35	1.65	I	I
Propranolol HCl	295.8	168.57	7372.8	16.7	**2.836**	0.01	**90**	97.74	7.74	I	I
Ranitidine HCl	350.9	151.26	13627.3	32.1	**0.260**	0.001	**50**	29.36	20.64	III	III
Sirolimus	914.2	191.19	9544.9	20.6	**0.585**	4.62	**15**	54.25	39.25	IV	IV
Theophylline	180.2	275.99	5741.1	10.5	**1.736**	0.30	**95**	90.18	4.82	I	III
Thiamine HCl	337.3	258.93	6787.8	12.8	**1.012**	0.07	**80**	74.15	5.85	III	III
Triamcinolone acetonide	434.5	281.17	6878.8	12.4	**0.733**	0.10	**23**	62.46	39.46	I	III
Trimethoprim	290.3	205.91	8039.2	16.8	**1.007**	0.5	**97**	73.98	23.02	III	III
Verapamil HCl	491.1	146.81	11003.3	26.2	**0.939**	0.006	**90**	71.50	18.50	I	III

Fa% (the fraction of a dose absorbed in humans) values were obtained from previously reported values (32-40). When the Fa% value was reported as a range, the mid-value of the range was used. Reference for dose numbers were taken from (41, 55). Conventional BCS class taken from (60-70).