

PARTITIONING BASED PHYSICOCHEMICAL MODELS FOR ASSESSING INTESTINAL PERMEABILITY AND ABSORPTION OF DRUGS

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ABSTRACT

Oral administration of drugs is highly preferred for almost all human beings than any other route of drug delivery except during some health challenges. Therefore, permeability assessment of drugs across intestinal membrane is essential in the early stages of drug discovery for time and cost reasons. Animals, including humans, have been used for decades as *in vivo* models for determining intestinal drug permeability and absorption. However, *in vivo* models are very invasive, time-consuming, and not cost-effective methods. Numerous *in vitro* models have been used to screen drug permeability and absorption through intestinal membranes. In this article partitioning based physicochemical models that can predict a compound/drug permeability potential across intestinal membrane will be elaborated upon.

Keywords: Partitioning based physicochemical models, *In vitro* models, Intestinal permeability and absorption

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INTRODUCTION

The therapeutic efficacy of a given drug is dictated by its pharmacodynamic activity and pharmacokinetic properties such as its access to the site of activity [1]. The oral route is preferred for most systemic acting drugs. Therefore, in the drug discovery process, once sufficient pharmacological activity has been attained, the next crucial need for a candidate drug is to achieve acceptable bioavailability after oral administration [2]. The ability of drugs to cross biological membranes is based upon several physicochemical properties that must be examined to help establish pre-formulation parameters before dosage form development. Various *in vivo* and *in vitro* models that are descriptive of drug absorption have been used for such purposes. *In vivo* models based on using animals or humans have been used for a long time. However, these approaches have been criticized, either related to their efficiency as a suitable model for high throughput screening or their ethical reasons. *In vitro* models have been used parallel to *in vivo* methods and have gained wide acceptance as alternatives to human or animal models. Many *in vitro* models that relate the physicochemical properties of a drug candidate to its ability to cross intestinal membranes have been described. When a molecule is present in the gastrointestinal (GI) lumen, it must be transported across the GI membrane to reach systemic circulation. Drug passage across a membrane or permeability (P_m) is a function of the following formula [3]:

$$P_m = D_m K_m / L$$

With D_m is the membrane diffusion coefficient of the drug, K_m is the membrane partition coefficient and L is the membrane thickness. Since L is a constant for a given membrane, D_m and K_m are important in the evaluation of drug permeability. Hence, D_m is a measure of the rate by which a drug moves through a membrane, K_m is an important criterion in evaluating drug permeability and subsequent absorption. Evaluating *in vivo* partitioning is a difficult process, therefore, *in vitro* methods that measure K_m employing models that simulate drug crossing biological membranes have been developed [3]. Three major partitioning based physicochemical systems have been used for evaluating drug permeability through intestinal membranes including lipophilicity measurements using simple organic/aqueous solvent partitioning [4], chromatographic partitioning and partitioning into liposomes [5]. These models along with other partitioning-based physicochemical systems such as parallel artificial membrane permeation assay [6], absorption

potential [7] and some other physicochemical descriptors are presented in this study.

Partitioning based physicochemical models

Organic/aqueous solvent partitioning

The partitioning of a drug between organic/aqueous solvent mixture parallels well with its biological activity since the pioneering work of Meyer [8] and Overton [9]. Their studies indicated that biological activities of drugs correlate with its oil/water partition coefficients. Since then, the logarithm of the partitioning between nonpolar and polar solvents ($\log P$) of a compound has been used as a lipophilicity index to indicate the degree of systemic availability and in some cases, activity of the drug. In essence, $\log P$ has become an accepted model for lipophilicity evaluation in quantitative structure-activity relationships [10-12]. In such studies, the polar phase is always water while many organic solvents are utilized as the nonpolar part, such as octanol, chloroform, hexanes, and others, with octanol as the most common choice [11]. Garst and Wilson [13] indicated that there is nothing distinctive about biological correlations using octanol/water mixture as opposed to other non-polar solvent/water systems. El Tayar *et al.* [14] suggested the use of four non-polar solvents for modeling biological membranes. These solvents are octanol, chloroform, cyclohexane, and propylene glycol dipelargonate. They found that $\log P$ values measured in these different solvents show differences mainly due to hydrogen bonding effects. Octanol can donate and accept hydrogen bonds whereas cyclohexane is not capable of doing so. Propylene glycol dipelargonate can only accept hydrogen bond. Among them octanol is common and chosen as a simple model of phospholipid membrane [15]. However, it was reported that nonpolar/polar partitioning systems are good models only when the polar group interactions between the solute and the phospholipid bilayer are minimal or absent [16]. In other words, partitioning through such systems correlates well with drug partitioning into fluid membranes for hydrophobic compounds; however, for polar compounds, the correlations are not satisfactory [17]. Because of such drawbacks of using some nonpolar solvents, Hansch and Dunn [10] suggested that octanol is a rational solvent because it models polar molecule interactions between solutes and membranes. Therefore, it is not surprising that $\log P$ determined using other solvents rather than octanol exhibits poor correlation since these solvents do not model the subtle polar and non-polar molecule interactions between solutes and membranes as octanol does [10]. It is well established that drug crosses intestinal membrane by the

following pathways: 1) passive transport, 2) active transport, and 3) others of minor importance in the overall absorption processes such as endocytosis. Simple passive diffusion is the main absorption pathway for most drugs and is based on the lipid solubility of the drug, or normally. Although it is recognized that there is a direct correlation between lipophilicity and log P, it should be considered that the relationship between log P and bioavailability after oral administration is not always direct as exhibited in fig. 1 [18]. The study indicated that compounds having log P values outside the range of -1 to 4 may have poor intestinal permeability and absorption. In general terms, drugs showing log P values close to 2 are predicted to be well

absorbed in humans [19]. Determination of log P using nonpolar/polar solvent system is based on dissolving the compound under investigation in one solvent and then shaking both solvents together until equilibrium is achieved, followed by measuring the equilibrium concentrations of the compound. The model has the advantages of simplicity and applicability for a wide range of log P values.

However, it is time and chemical-consuming and requires pure materials to be used. In addition, a possible instability of the investigated compound in the solvent system and emulsion formation may hamper the separation and analysis processes.

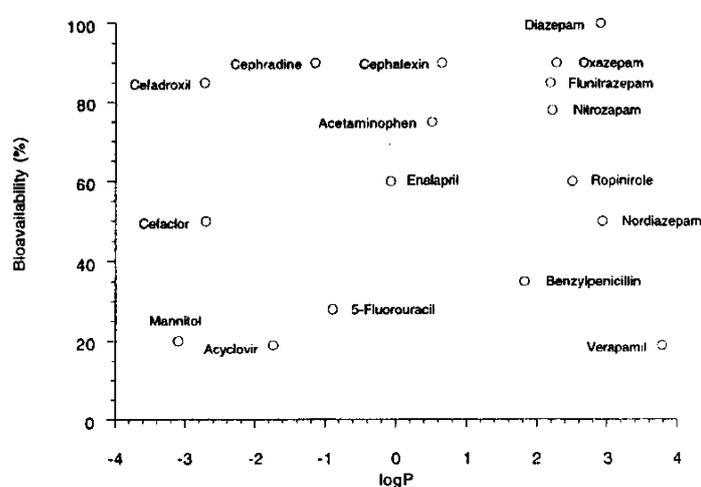


Fig. 1: Relationship between bioavailability after oral administration in humans and Log P for a set of 17 drugs [18]

Liposomes partitioning

Liposomes are spherical or multilayered spherical vesicles made by the self-assembly of diacyl-chain phospholipids (lipid bilayer) in aqueous medium [20]. The bilayer phospholipid membrane has a hydrophobic tail and a hydrophilic head [21] that drive the formation of an amphiphilic structure. Liposomes can be made from both natural and synthetic phospholipids [22]. Ever since the discovery of liposomes by Bangham *et al.* [23], the system has been used in drug delivery, pharmaceutical carrier/delivery system [24, 25], partitioning-QSAR of molecules [26], and solute partitioning as a model of biological membranes [27]. A similar system to liposomes is noisome drug delivery which is mainly made up of non-ionic surfactants [28]. Katz and Diamond [29] first demonstrated and determined that a drug partition coefficient through the phospholipid bilayer of liposomes into their aqueous cavities was like the partition coefficients measured using membranes.

The reason for using partitioning into liposomes to predict permeability and drug absorption is that they can model both polar and nonpolar solute-membrane binding interactions [29] given the natural chemical similarity of liposomes and biological membranes. Following the preparation of liposomes, the drug under investigation is added to the medium containing the liposome vesicles for partitioning study until equilibration is attained. The amount entrapped into the vesicles of the liposomes is then analyzed by quantitation. Different procedures can be used in the partitioning study of compounds through liposomes, for example, equilibrium dialysis [30], pH-titration method [31], immobilization in the pores of gel beads by avidin-biotin binding [32] or freeze-thawing technique [33], and the partitioning coefficients determined from retention data. Due to the chemical structural similarity of liposomes to the endogenous membrane, the system is highly acceptable as an *in vitro* model for studying drug partitioning, and hence, permeability gives genuine reliable data. Nevertheless, the model is costly, time-consuming, and not suitable for high throughput screening, and the stability of the system also raises some drawbacks.

Chromatographic partitioning

Screening of intestinal permeability of compounds has been investigated using chromatographic systems as models that simulate solute partitioning in endogenous membranes [34]. They include paper chromatography [35] and thin layer chromatography (TLC) [35, 36] using packing impregnated with nonpolar phase such as octanol or silicon. The partition parameter, R_m , determined from reserved phase-TLC is defined [35] according to the following equation:

$$R_m = \log \left(\frac{1}{R_f} - 1 \right)$$

R_f is the retention factor, which is used as a lipophilicity index that correlates well with the biological activity of drugs [36]. R_f is also used to calculate the log P of compounds using the following equations [35, 37]:

$$\log P = \log K + \log \left(\frac{1}{R_f} - 1 \right)$$

$$\log P = b R_m + a$$

Where K , b and a are constants for a given system. At the beginning of using chromatographic partitioning to study lipophilicity or to determine log P of solutes, TLC was preferred for the following reasons: 1) simple to use, 2) reproducible, 3) does not need instrument quantitative analysis, 4) small quantity of the solute is used, and 5) high purity of the compound is not necessary [38]. TLC also has expanded the range of log P values that could be determined [39]. However, after the discovery of high performance liquid chromatography (HPLC), partitioning on reserve-phase HPLC has been investigated as a means for determining lipophilicity of compounds based on their retention on solid stationary phase as first established by McCall [40] and Henry *et al.* [41]. The established retention parameters were retention time expressed by a term, k' and retention volume expressed by a term, V_R which are defined respectively as:

$$k' = \frac{(t_r - t_0)}{t_0}$$

$$V_R = [(t_r - t_0)(\text{flow rate})]$$

With t_r as the retention time of the compound and t_0 is the retention time of the solvent front. The logarithm of retention time, $\log k'$, and retention volume, $\log V_R$, are used as lipophilicity indices that linearly correlate with $\log P$, R_m and biological availability and activity of compounds [41]. Normally, the stationary phase used in HPLC studies to determine these parameters are columns packed with porous silica gel bonded chemically with octadecyl (ODS) chain [40, 41]. HPLC also inherits the most serious limitation of the octanol/aqueous system in that it lacks structural similarities to biological membrane [17]. Unlike octanol which contains a polar OH group and a nonpolar hydrocarbon chain, ODS only contains hydrocarbon chain [17]. In the early nineties, phospholipid covalently bonded to silica gel-packed columns namely, immobilized artificial membranes (IAM) were developed by Pidgeon and co-workers [42]. IAM columns are solid phases typically used as a chromatographic stationary phase, monolayers of phospholipid

molecules covalently bonded to the surface of silica particles [43]. IAM surface emulates the lipid surface in liposomes and cell membranes [43, 44]. These columns were first used to purify membrane proteins [45], immobilize enzymes [46], obtain enzyme-ligand binding constants for drugs [46] and to study hydrophobicity of drugs by other groups [47]. Afterwards, IAM columns were prepared from a mixture of phospholipid and phosphatidylcholine (PC) and gained the abbreviation IAM. PC, which are mixed lipid-liposome columns [17]. Drug interaction with the column surface simulates the interaction between the drug and liposomes as shown in fig. 2-A and B. Like HPLC, partitioning or binding of solutes to IAM. PC is used to predict permeability based on the retention time parameters [17, 48] expressed as a capacity factor, k'_{IAM} and as depicted in fig. 2-C.

$$k'_{IAM} = [(t_r - t_0)/t_0]$$

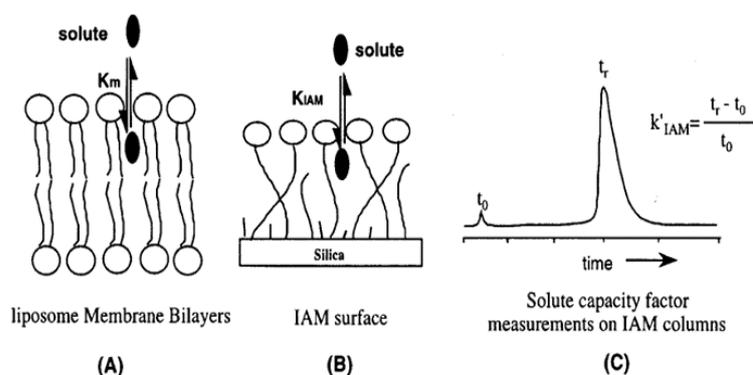


Fig. 2: Parts (A) and (B) show the similarity of molecule interaction with liposome membrane and IAM surface, and (C) shows measuring of the capacity factor from retention time on IAM columns [49]

The logarithm of the capacity factor, $\log k'_{IAM}$, is used to evaluate the lipophilicity of molecules and predict their absorption. Moreover, it was found to significantly correlate with the $\log P$, R_m , $\log k'$ [49], and the membrane partitioning coefficients into liposomes [27]. It is also reported that $\log k'_{IAM}$ correlates well with logarithm of the apparent permeability coefficients through caco-2 cells [50], and passive permeability coefficients through everted gut sacs [51]. IAM. PC columns are also used to predict drug permeability in skin tissues [50, 52]. These studies show that IAM. PC columns can be used to predict permeability through various biological membranes due to structure similarity with natural membranes [17]. These columns are made from phospholipid analogs, which simulate the main part of the cellular membrane that encounters drugs before absorption [48]. Based on that fact, IAM. PC columns are considered a good model for predicting drugs' intestinal permeability for their simplicity and suitability for high throughput screening.

Parallel artificial membrane permeation assay (PAMPA)

The technique design is based on a 96-well microtiter plate technology as originally developed by Kansy *et al.* [53]. The wells are filled with a buffer and then covered, in a sort of sandwich construction, with a hydrophobic microtiter filter plate pre-impregnated with a solution of phospholipid dissolved in an inert organic solvent. A solution of the compound under investigation is applied on the top of the filter plate and the flux into the buffer is measured spectrophotometrically against reference solution. The plot of flux data obtained from PAMPA vs. % of human absorption of a diverse set of molecules showed similarity to a plot of permeability data in Caco-2 cells vs. % of human absorption for the same set [54]. The technique was modified a little to closely mimic the intestinal membrane by Sugano *et al.* [55]. They modified the composition of the lipid solution used in the original method by changing the chain length of the organic solvent which resulted in the addition of a negative charge to the membrane. In general, the PAMPA technique is simple, less grueling, and suitable for high throughput screening.

Absorption potential

Dressman *et al.* [56] presented the absorption potential (AP) index that can be used to estimate the intestinal permeability of compounds. The index utilizes some physicochemical characteristics of the compound under investigation such as its $\log P$, fraction of the compound unionized at pH 6.5 (F_{un}), and the aqueous solubility of the unionized species (S_{un}) as follows:

$$AP = \log [P \cdot F_{un} (S_{un} V_L / X_0)]$$

With V_L is the luminal volume (approximately 250 ml) and X_0 is the dose of the compound. The index estimates the permeability of compounds passively transported only as the other models. A good correlation was found between AP and the fractions absorbed in humans for 7 diverse well-established drugs as shown in fig. 3 [57]. However, to validate such a model it needs to be applied to a large set of compounds so that it can be widely applicable.

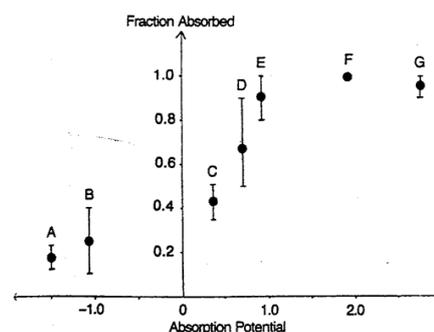


Fig. 3: Relationship between absorption potential and fraction absorbed of (A) acyclovir, (B) chlorothiazide solution, (C) griseofulvin, (D) hydrochlorothiazide, (E) phenytoin, (F) prednisolone, and (G) digoxin [57]

Other physicochemical descriptors

In addition to the introduced models to predict intestinal permeability and absorption, other physicochemical properties of compounds, for example, molecular weight (MW), aqueous solubility, ionization constant, and hydrogen bonding ability are also important descriptors. MW is one of the four physicochemical parameters that constitute Lipinski's rule of 5 [58]. MW of 500 is indicated as a limit. Beyond it, permeability after oral administration is more likely to decrease. In a deconstructed analysis study [59], it was reported that highly absorbed drugs (>80%) have MW less than 500. Despite the lipid nature of the biological membranes and the need for the drug to partition and permeate through them, aqueous solubility is an important parameter in the absorption process. Furthermore, partitioning through the aqueous part of the membrane or the interstitial fluid is also necessary for the completion of the absorption course. Hence, the aqueous solubility of compounds is of value in the overall permeability process. Therefore, poorly soluble drugs will not only show a slow rate of partitioning from the membrane to the extracellular fluid but also protein binding characteristics in the extracellular submucosal tissues which may influence drug permeability [60]. It is reported that aqueous solubility of less than 100 µg/ml indicates poor dissolution, which limits intestinal absorption [61].

In summary, the manuscript explored the prediction of intestinal permeability for drugs using *in vitro* models. The topic is relevant to current research trends. The content discussed one of four steps needed to determine the ability of a drug to cross the intestinal membrane. These four steps start with using *in silico* models, partitioning-based physicochemical models, direct measurement-based models that employ laborious work of using animal/human cells or tissues and finally the bioavailability studies in animals or humans. The importance of the presented models in this study is that they can be used as a guide to follow during drug development to decide whether a candidate moves to the next complicated techniques to investigate its ability to cross intestinal membranes before formulation studies are initiated.

CONCLUSION

Advanced biotechnological and combinatorial chemistry have made a breakthrough in syntheses of compounds. As a result, the synthesizing of lead candidates has not become the major obstacle in the drug discovery platform. Usually, failure of a drug candidate to reach the market has been reported to happen very often during the development process because of bioavailability issues. For that reason, it is essential to use effective *in vitro* models that can predict intestinal permeability of compounds in a timely and cost-effective manner. Permeability prediction based on the partitioning of candidates as a function of their molecular physicochemical properties is of great value in testing their ability to cross intestinal membranes. Nevertheless, the main shortcoming of these models is that they lack the architectural resemblance to the intestinal membrane. However, they simplify the complicated gastrointestinal absorption process. In addition, they are mainly assessing the permeability of compounds that are passively transported; therefore, they underrate compounds transported by active mechanisms. The importance of these techniques is that they can be used as primary screening tools to assess losers or lead compounds.

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AUTHORS CONTRIBUTIONS

All authors contributed equally to the study

CONFLICT OF INTERESTS

The authors declare no competing interest

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