

## Research Article



## Studying the Relationship between Diffusion and Absorption of the Oral Dosage Forms locally Manufactured Containing Macrolids and $\beta$ -Lactam groups using Schulman cell

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### ABSTRACT

The aim of the present study was to develop a model that can predict gastrointestinal absorption for a diverse range of drugs using the immobilized artificial membrane retention properties and a selected diffusion cell (Schulman type); the proposed method was employed to evaluate the apparent permeability of a set of 12 structurally diverse drugs having different solubility and permeability properties. An excellent linear correlation ( $R^2=0.92$ ) was obtained between the developed cell apparent permeability and human absorption data. A comparison of permeation data of some drugs obtained using retention properties of the immobilized artificial membrane (IAM) and the corresponding HIV values in humans further confirmed the proposed permeation method as predictor of the oral absorption of passively absorbed drugs. Hoping to give insight in our country about the importance of such a tool to predict some important drug properties before bioequivalence studies.

**Keywords:** Permeability, Diffusion, Absorption, IAM column.

### INTRODUCTION

Oral drug absorption is one of the main factors governing the bioavailability of drugs. The absorption of drugs from the gastrointestinal tract is largely controlled by: dissolution rate and solubility, which determine how fast a drug reaches a maximum concentration in the luminal intestinal fluid; and intestinal permeability, which relates to the rate at which dissolved drug will cross the intestinal wall to reach the portal blood circulation. The determination of the dissolution, solubility, and permeability properties of drugs can thus provide information about their absorption.<sup>(1)</sup>

In this study, we evaluate the diffusion of some selected antibiotics, first we designed and developed *in vitro* diffusion chamber system using isolated rabbit intestinal membrane to evaluate the diffusion rate of the selected drugs compared by the apparent permeability coefficient.

In the other hand Membranes of the gastro intestinal tract which are biologic barriers that selectively inhibit the passage of drug molecules and are composed primarily of a bimolecular lipid matrix, containing mostly cholesterol and phospholipids. Absorption from the gastro intestinal tract, as well as penetration of other membrane barriers may be passive or active. Passive transport is governed by physicochemical properties whereas active transport involves specific binding of a molecule to a binding site on a transport protein. Immobilized artificial membrane (IAM) chromatography has recently gained acceptance as a chromatographic method for the estimation of the membrane permeability of drugs. IAMs are chromatographic surfaces prepared by covalently immobilizing cell membrane phospholipids to solid surfaces at monolayer densities. Since phosphatidylcholine (PC) is the major phospholipid found

in cell membranes, IAM surfaces prepared from PC analogs mimic the phospholipids environment found in the cell membrane. They simulate the hydrophobic and hydrophilic contribution of drug-membrane partitioning and can be used to as a fast screening column for predicting drug absorption.<sup>2</sup>

### MATERIALS AND METHODS

#### Materials

Raw materials from local pharmaceuticals companies (Metoprolol, Ampicillin, Cefaclor, Ciprofloxacin, Furocemid, ibuprofen, cefexime, carbamazepine, Ketorolac. Krebs Ringer buffer (KRB) Consist of Glucose-D, Cefalexine Working Standard (Sigma Aldrich, Lot & Filling code 1382532, 52918177).

Krebs Ringer (KRB) buffer<sup>4</sup>: consist of glucose-D (scharlau chemi spain 7093:batch 0345), sodium chloride (BDH laboratory supplier, lotvk 20858832GPR), Anhydrous magnesium chloride (scharlau chemi spain: batch 0995), potassium chloride (Merck lot TA 638535920), Sodium phosphate (Merck 64271).

Phosphate buffer according to USP30-NF25

Solvent for HPLC: Acetonitrile - Methanol- water(Merck).

#### Methods

Diffusion Cell (Shullman Type)

The diffusion cell is consist of water bath, pump, thermometer, two chambers.<sup>3,4</sup>

#### Tissue preparation

Male rabbits weighing 250–280 g were used for all diffusion studies. Prior to each experiment, the rabbits were fasted overnight (12–18 h) with free access to water Two to three minutes after death, A piece of rumen wall



was taken from the ventral sac. The pieces were first carefully cleaned by immersion in a buffer solution. The epithelium was then stripped from the muscle layer and the isolated epithelium was taken (within 15–20 min) to the laboratory in a buffer solution kept at 37°C, cut into squares and mounted between the two halves of an diffusion chamber to give an exposed serosal area of 1.13 cm<sup>2</sup>. Edge damage was minimized by rings of rubber on both sides of the tissue. During preparation and transport, the buffer solution was gassed with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. The mounted tissues were bathed on each side with krebs-ringer buffer solution by using a gas-lift system and were gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> at 37°C,<sup>5</sup> after an initial equilibration period of 20 min Drug solution (10 ml) was added to the donor side, and the same volume of drug free buffer was added to the opposite side. The temperature of cells was maintained at 37 °C with a water bath, and solutions from both sides of cells were circulated with a gas lift (95% O<sub>2</sub>, 5% CO<sub>2</sub>). During the transport studies, 0.1 ml aliquots were taken from the receiver side at predetermined time intervals up to 120 min, and immediately replaced by an equal volume of buffer solution.

Samples were taken with intervals (30, 45, 90, 120 min) and the assays done using UV Spectrophotometer.

Drug concentrations in the receiving chamber samples were determined, and the cumulative amount of drug permeating the intestinal was calculated and plotted vs time for each experiment. Permeation rates were calculated and normalized for surface area to give the drug flux. The results are expressed as permeability coefficients, there were generally four or more experiments for each group. The results are expressed as the mean ± S.E. Statistical comparisons were made using t-tests.<sup>11</sup>

Apparent permeability coefficient (P<sub>app</sub>) was calculated as the following Equation:

$$P_{app} = \frac{dc}{dt} * \frac{V}{A} * \frac{1}{C}$$

where P<sub>app</sub> is the apparent parameter of permeability (cm/s), the permeability flux (dc/dt) is the slope of linear portion of cumulative transport amount to time at the steady state (pmol/min); area (A) is the area of diffusion chamber for transport (1.13 cm<sup>2</sup>); and C<sub>0</sub> is the drug concentration before transport.<sup>6,7,8,10,15</sup>

### Immobilized membrane chromatography<sup>11</sup>

The retention (capacity factor, *k*) of 6 drugs with low to high intestinal absorption in humans was determined by reverse-phase HPLC (Integrator, JMBS for scientists

SCL-10ASP Shimadzu System Controller UV/VIS Detector, Shimadzu SPD-10AV

Shimadzu liquid chromatography pump LC-10AV  
Shimadzu Degasser JU-14A

Auto Sampler SIL-10AD VP) using an IAM.PC.DD2 column (prepared with Phosphatidyl Choline analogs, 12mM, 300Å, 15 cm × 4.6mm)

(Regis Technology, Morton Grove, IL, USA) with an eluent of acetonitrile 0.1M phosphate buffer at pH 7.5 (0/100-45/55, v/v). The mobile phase was filtered

(0.45 μm, Milford, MA, USA) and degassed in an ultrasonic bath prior to use. All experiments in this study were carried out at a room temperature (25 ± 2 °C) using the flow rate of 1.0 ml/min. The ratio of acetonitrile–phosphate buffer solution was 15:85 and the detection wavelength varied for individual drugs (UV wavelength was 210–278 nm).

### Capacity factor

The capacity factor (*k*) was calculated by the following equation:

$$K = \frac{t_R - t_0}{t_0}$$

where *t<sub>r</sub>* is retention time of the drug and *t<sub>0</sub>* is column void volume time of a non-retained compound or reference (citric acid).<sup>9,13,14</sup>

## RESULTS AND DISCUSSION

The flux and the Intestinal permeation of some selected drugs that ranged between low, moderate and high intestinal absorption as a reference drugs were calculated and validated our tool.

There were a good correlation between the apparent permeability and the predicted intestinal absorption.

The same conditions were applied on ciprofloxacin, cefaclor and cefexime which classified as poor permeability and a special drug absorption mechanisms, a poor correlation were predicted with the β-lactam antibiotics (Cefaclor, Ampicillin, cefexim), and with ciprofloxacin which have a poor solubility and poor permeability according to biopharmaceutical classification system (class IV) and several mechanisms contribute their absorption as the p-glycoprotein that expressed in intestinal brush border and reducing the oral bioavailability.<sup>11,12</sup>

As the pharmaceutical classification system metoprolol considered class I, so metoprolol used as a reference in every experiment and to predict the membrane's integrity.

### Method validation

Three deferent drugs were chosen acidic, basic and neutral (Ketorolac, sodium diclofenac and carbamazepine) to study IAM chromatographic method validation

The retention time reproducibility were calculated and the coefficient of variation inter-day and intra-day were <2. and the average and the standard deviation were calculated.



**Table 1:** The apparent permeability for the selected drugs compared with their observed and predicted intestinal absorption.

Compound	Papp cm/sec	Observed IA%	Predicted IA%	<i>In vitro</i> Permeability class <sup>9</sup>
Metoprolol	1.17×10 <sup>-4</sup>	95	88.53	High
Ranitidine	1.795×10 <sup>-5</sup>	50	59.01	Low
Verpamil	1.47×10 <sup>-4</sup>	61	65.99	High
Furocemide	5.9×10 <sup>-5</sup>	91	86.32	Low
Ibuprofen	2.2×10 <sup>-4</sup>	100	97.41	High
Cefalexin	1.179×10 <sup>-4</sup>	98	68.71	High
Clarythromycine	5.89×10 <sup>-5</sup>	100		High
Correlation factor		R=0.8685	R=0.917	

**Table 2:** The apparent permeability for the selected drugs and the β-lactam antibiotics compared with their observed and \*predicted intestinal absorption

Compound	Papp cm/sec	Observed IA%	Predicted IA%
Metoprolol	1.17×10 <sup>-4</sup>	95	88.53
Ciprofloxacin	2.166×10 <sup>-4</sup>	67	84.4
*Cefaclor	1.278×10 <sup>-4</sup>	50	
Ampicillin	2.258×10 <sup>-4</sup>	67	62
Ranitidine	1.795×10 <sup>-5</sup>	50	59.01
Verpamil	1.47×10 <sup>-4</sup>	61	65.99
Furucimde	5.9×10 <sup>-5</sup>	91	86.32
Ibuprofen	2.2×10 <sup>-4</sup>	100	97.41
Cefalexin	1.179×10 <sup>-4</sup>	98	68.71
*Cefexime	1.47×10 <sup>-4</sup>	50	
*Clarythromycine	5.89×10 <sup>-5</sup>	100	

\* no reference data founded about their predicted IA

**Table 3:** Capacity factor, log capacity factor and the observed intestinal absorption

Compound	K'	Log k'	HIV%
Clarithromycine	5.99	0.777	100
Cefexime	3.73	0.5717	50
Diclofenac	6.67	0.824	100
Cefaclore	4.77	0.678	50
Ketorolac	7.01	0.8457	95
Carbamazepine	5.98	0.77	100
Correlation factor	R =0.89	R=0.88	

**Table 4:** Retention time for the comparable compounds

Compound (N=3)	Tr (min) in the day (Mean ± SD)	Tr (min) several days (Mean ± SD)
Sodium diclofenac	10.55±0.01	10.57±0.01
Carbamazepine	8.675±0.004	8.56±0.01
Ketorolac	4.55±0.008	4.43±0.049

The good correlation obtained from the previous study lead to a result that the evaluation of intestinal permeability *in vitro* is relatively easier, and in the case of segmental absorption studies avoids complicated surgery and maintenance of surgically prepared animals, it is

more rapid and has the potential to reduce animal usage since a number of variables can be examined in each experiment; it provides insights into the mechanisms (e.g., carrier-mediated versus passive) routes (e.g., transcellular versus paracellular), and is analytically more

simple because compounds are being analyzed in an aqueous buffer solution as opposed to whole blood or plasma.<sup>1</sup>

### CONCLUSION

Each *in vitro* method has its distinct advantages and drawbacks. Based on the specific goal, one or more of these methods can be used as a screening tool for selecting compounds during the drug discovery process. The successful application of *in vitro* models to predict drug absorption across the intestinal mucosa depends on how closely the *in vitro* model mimics the characteristics of the *in vivo* intestinal epithelium. Although it is very difficult to develop a single *in vitro* system that can simulate all the conditions existing in the human intestine, various *in vitro* systems are used routinely as decision-making tools in early drug discovery. So it is important to keep working on this kind of studies to improve our drug researches.

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### REFERENCES

- R.D. Schoenwald, J. Keith Guillory, L.E. Matheson, E.L. Parrott, D.R. Flanagan, D.E. Wurster, P. Veng-Pedersen and D.J.W. Gran. Developing solid oral dosage forms (pharmaceutical theory and practice), 1<sup>th</sup> ed, London copyright© Elsevier Inc, 2009, 289.
- Klára V, Application of high-performance liquid chromatography based measurements of lipophilicity to model biological distribution, Journal of Chromatography A, 1037, 2004, 299–31.
- Albert L, Corwin H, David E, Partition coefficient and their uses, V 71, N 6 Dec 1971.
- Stephan A. M, Michael Bur, Ulrich F. S, Claus-Michael L, Instrumented In Vitro Approaches to Assess Epithelial Permeability of Drugs from Pharmaceutical Formulations, Chapter 19.
- Bernd S, Ina R, Ernst P, Gerhard B, In vitro studies on calcium absorption from the gastrointestinal tract in small ruminants, J Comp Physiol B, 167, 1997, 43-51.
- Ronald T. Borcard T, Philip L. Smith, Glynn W, Models for assessing drug absorption and metabolism, Pharmaceutical biotechnology, 8, 12, 14-30.
- Jennifer D, and Johann W G, Pharmaceutical dissolution testing. ©Taylor & Francis Group, 756, 2005, 129-170.
- Hiroshi S, Christine G, Bruce J, The secretory intestinal transport of some beta- lactam antibiotics and anionic compounds; mechanism contributing to poor oral absorption, 278(1), 1996, 206.
- Yen T.E, Agatonovic-Kustrin S, Evans A.M, Nation R.L, Ryand J, Prediction of drug absorption based on immobilized artificial membrane (IAM) chromatography separation and calculated molecular descriptors, Journal of Pharmaceutical and Biomedical Analysis, 38, 2005, 472–478.
- Yulian L, SHEN Q, Hidemasa K, Naoki O, Takuya, Xuehua J, Akira Y, Effects of Labrasol and Other Pharmaceutical Excipients on the Intestinal Transport and Absorption of Rhodamine 123, a P-Glycoprotein Substrate, in Rats, Biol. Pharm. Bull. 30(7), 2007, 1301–1307.
- Robert M. R, Real World Drug Discovery a Chemist's Guide to Biotech and ©Pharmaceutical Research, 361, 357, 2008, 353-360.
- Luckner p, brandsch M, interaction of 31 β-lactam antibiotics with the H<sup>+</sup>/ Peptide symporter PEPT2: analysis of affinity constants and comparison with PEPT1, European Journal of Pharmaceutics and Biopharmaceutics, 59, 2005, 17–24.
- Ivan K, Jelena P, Irena H, Marija T-G, Peter L, Justification of Biowaiver for Carbamazepine, a Low Soluble High Permeable Compound, in Solid Dosage Forms Based on IVIVC and Gastrointestinal Simulation. Mol. Pharmaceutics, 2009, 6 (1), 40-47.
- Vrakas D, Hadjipavlou-Litina D, Tsantili-Kakoulidou A, Retention of substituted coumarins using immobilized artificial membrane (IAM) chromatography: A comparative study with n-octanol partitioning and reversed-phase HPLC and TLC, J Pharma, 39(5), 2005, 908-13.
- Testa B, Van de Waterbeemd H, Folkers G, Guy R, Pharmacokinetic optimization in drug research, biological, physicochemical, and computational strategies, 1<sup>th</sup> ed©verlag Helvetica chimica Acta, 2001, 202.

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