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# Magnesia–zirconia based mimetic biomembrane chromatography for predicting human drug absorption

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**Abstract**

In this paper, a novel mimetic biomembrane chromatography stationary phase of magnesia–zirconia composite matrix were prepared with the Lewis acid–base interaction between phosphatidylcholine's residue phosphonate group and Lewis acid sites of magnesia–zirconia composite; the retention factors of a chemically diverse set of drugs on the new stationary phase were determined; the drugs log $K_{mbm}$ values were correlationed with the absorbed fraction of drugs orally administered in humans (%$F_a$) and a hyperbolic relationship was obtained. Meanwhile, the relationship between the log $K_{mbm}$ values and hydrophobic parameters (log $P_{oct}$ and log $D_{oct}$) were discussed. The usefulness of the new column for predicting oral drug absorption in humans is demonstrated by comparing this model with IAM, ILC and BMC models. Results show that the log $K_{mbm}$ values have good relationship with log $K_W^{IAM}$, log $K_{BMC}$ and have moderate to fair relationship with log $K_s$ determined on four different ILC column (EPL, PC, PC-PE, PC-PS). Therefore, the log $K_{mbm}$ values can provide key information about the transport properties of drugs and this chromatographic model may be applicable for prediction of drug uptake through epithelial cell membranes during the drug discovery process.


*Keywords:* Magnesia–zirconia composite; Oral drug absorption; Mimetic biomembrane stationary phase

## 1. Introduction

Today with the development of combinatorial chemistry, thousands of drugs that have potential biological activity are synthesized. Whether the drug candidates suitable for clinical development depend on their pharmacokinetic and pharmacodynamic properties, in the early stage of drug discovery, the traditional pharmacokinetic studies are very expensive, time consuming and usually require the use of experimentation animals. For ethical and/or economical reasons, a great deal efforts is currently being made to develop in vitro systems to avoid or reduce the use of experimentation animals and provide primary information abort the capability of new compounds in the first steps of drug development [1].

Regardless of the route of exposure, drug absorption is a requirement for a substance to be capable of producing a pharmacological effect; therefore, in the screening process for development of new drugs is very important to estimate drug passage across the cell membrane along with the possibility of decomposition by drug-metabolizing enzymes. Drugs are absorbed through epithelial cell layers by diffusion across the lipid bilayer of the cell membranes by transport via membrane proteins (transcellular pathway), through the paracellular pathway, or by transcytosis. In order to predict oral drug absorption, several in vitro methods had been developed: QSAR models [2–7], Caco-2 cell monolayers [8–15], parallel artificial membrane permeation assay [16,17], immobilized artificial membrane (IAM) chromatography [18–22], immobilized liposome chromatography [23–28], micellar chromatography [29–31], biopartitioning micellar chromatography [32–34], surface plasmon resonance (SPR) biosensors [35].

Hydrophobicity is an important factor during the process of drug absorption and transcellular transport. The hydrophobicity of a solute, measured as its partition coefficient between octanol

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and water (log *P*) [7], has been commonly used to predict its transmembrane permeability, but good correlations were found only when homologous series of compounds used.

Most of the in vitro studies examining drug uptake and transport in the intestinal epithelium have utilized different anatomical structures as everted sacs, brush border membrane vesicles, isolated cells and intestinal rings. More recent works have focused on Caco-2 cells, a colorectal adenocarcinoma cell line of human origin, as a model for studying intestinal transport. The use of Caco-2 cell monolayers has gained in popularity as an in vitro human absorption surrogate. Moreover, the Caco-2 cell monolayers are generally accepted as a primary absorption screening tool in several pharmaceutical companies [1]. However, the lack of standardization and time consuming in cell culturing and high implementation cost limit its use as a high-throughput tool.

Chromatographic models to predict drug absorption are commonly experimental simplicity, low cost, accuracy and high-throughput. Immobilized artificial membranes (IAMs) are solid phase membrane mimetics whereby cell membrane phospholipid molecules are covalently bonded to silica particles at high molecular surface densities. Pidgeon et al. [20] used an $^{\text{ether}}$IAM.PC$^{\text{C10/C3}}$ column to predict drug absorption of 11 structurally cephalosporin analogs ($r^2 = 0.89$). A linear correlation was obtained between the permeability coefficients through Caco-2 cells measured by Artursson et al. [8] and the retention factors obtained at pH 7.4 for 11 unrelated drugs ($r^2 = 0.58$). This relationship was slightly improved when corrections for the size of molecules were made (log $k_{\text{IAM}}$/MW) ($r^2 = 0.73$).

Immobilized liposome chromatography (ILC) uses stationary phases where liposomes are steric, hydrophobic, electrostatic or covalently immobilized into gel beads. Beigi et al. [25] used an egg phosphatidylcholine (EPC) liposome column to predict the drug absorption of 12 unrelated drugs. A hyperbolic relationship between oral absorption in humans (%$F_a$) and the specific capacity factors (log $K_s$) were obtained. Öterberg et al. [52] used four different liposome columns (EPL, PC, PC-PE, PC-PS) to investigate the effects of lipid composition on drug partitioning, and evaluate the effect of electric charge of the drugs. A similar relationship between log $K_s$ values and fraction absorbed in humans as to surface plasmon resonance signals representing drug–liposome interaction was obtained [35]. Recently, Liu et al. [28] immobilized unilameller liposomes in the pores of gel beads by avidin–biotin binding. The membrane partition coefficients values (log $K_{\text{LM}}$) of 29 structurally diverse drugs also correlated well with that obtained using surface plasmon resonance (SPR) biosensor [35].

Another chromatographic approach to predict oral drug absorption is biopartitioning micellar chromatography (BMC). Molero-Monfort et al. [34] studied the correlation between the logarithm of retention factors in BMC and reported oral drug absorption values for a heterogeneous set of 74 compounds.

Zirconia and zirconia-containing mixed oxides have received considerable attention as a stationary phase for HPLC over the last decades due to their remarkable mechanical, chemical and thermal stability [36–38]. It is well known that zirconia exhibits a great affinity for inorganic and organic phosphate because of strong Lewis acid–base interaction [39]. One kind of zirconia-containing mixed oxide, magnesia–zirconia composites, has been proved to have a more appropriate surface area, pore size distribution and pore structure, and greater affinity for phosphonates compared to bare $ZrO_2$ in our previous work [40,41].

In previous work, the matrix for the immobilization of liposomes was mainly soft gel particles, the large pore size is helpful for the immobilization of liposomes and the good biocompatibility established a solid base for the wonderful performance of drugs screening, but at the same time, the ILC stationary phase paid a lot price in the stability and mechanic strength. In this paper, the magnesia–zirconia composite was used as the matrix for the immobilization of phospholipids bilayers, a new mimetic biomembrane stationary phase were prepared. The possibility of the mimetic biomembrane chromatography as an in vitro system to predict passive drug absorption is studied. Regression models for the prediction of passive drug absorption were obtained and the correlations with other well established models were evaluated.

## 2. Experimental

### 2.1. Chemicals

Magnesia–zirconia ($ZrO_2$–MgO) composite was made in the laboratory [36] with a mean particle diameter of 50 m. The surface area of the packing, which was determined by the nitrogen adsorption procedure, is 110.19 $m^2\ g^{-1}$. Phosphatidylcholine of purity >95% was home made with fresh eggs according to the method denoted in Ref. [42]. Water used in this work was redistilled.

Atenolol, timolol, metoprolol, propanolol, bisoprolol, benzocaine, bupivacaine, procaine, lidocaine, tetracaine, tramadol, antipyrine, piroxicam, acetaminophen, acetphenetidine, primidone, hydrocorticosterone, ketoprofen, cephalexin, diphenhydramine, ranitidine, theophylline, caffeine, barbitalum, ketoprofen, diazepam, phenytoin, cimetidine, acyclovir and acetanilide were provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

### 2.2. Instrumental and measurement

The high-performance liquid chromatography (HPLC) apparatus was consisted of a LC-6A pump (Shimadzu, Tokyo, Japan), an SPD-10A ultraviolet (UV) detector (Shimadzu) and a Rheodyne 7725i injector with 0.7 ml sample loop (Cotati, CA, USA). The column used for HPLC was a 50 mm × 46 mm stainless-steel column. The mobile phases were Tris–HCl buffers (pH 7.4) containing 0.05 M NaCl solutions, before use they were filtered through a G-3 sintered glass funnel and degassed in an ultrasonic bath for 5 min under reduced pressure. The detection wavelength was set at the best absorption wavelength of solutes and in all cases, the mobile phase flow rate was set at 1 ml/min. The chromatographic data was acquired by chromatography working station TL-9900. The pH values of the mobile phase solutions were measured with a Delta 320-S pH

Meter from Mettler Toledo Instruments (Shanghai, China). The retention data determined in this study were averages of at least triplicate determinations.

### 2.3. Preparation of mimetic membrane stationary phase

The mimetic membrane stationary phase was prepared according to the procedure reported elsewhere [43]. Briefly, about 220 mg phosphatidylcholine dissolved in 50 ml of methanol was placed in a round-bottomed flask. Then magnesia–zirconia composite (2.0 g) was added, and the flask was placed in a rotary evaporator. The methanol was removed under reduced pressure at room temperature, and when the magnesia–zirconia composite seemed to be dry the flask was allowed to stand under reduced pressure about 1 h. In this way, a thin layer of magnesia–zirconia composite was formed on the inside wall of the flask.

All mimetic membrane stationary phase were transferred to a 100 ml glass bottle, and 0.01 M Tris–buffer solution (pH 7.4) containing 0.01 M $CaCl_2$ were added. The suspension was kept quiet for 3 h and then processed in an ultrasonic bath for 10 min, and then 0.01 M Tris–buffer solution (pH 7.4) was added to remove unimmobilized phosphatidylcholine for five times. The slurry was then packed into the stainless-steel column.

### 2.4. Capacity factor $K_{mbm}$

For normalization of the results obtained on gel beds with different amounts of phospholipids, and for elimination of the dead volume of the system, a capacity factor, $K_{\mathrm{mbm}}$, for a drug was calculated by use of the equation:

$$K_{\mathrm{mbm}} = \frac{(V_{\mathrm{r}} - V_0)}{A}$$

where $V_{\mathrm{r}}$ is the retention volume of the drug, $V_0$ is the retention of acetone (whereby the dead volume of the system is eliminated) and $A$ is the amount of immobilized phospholipids which was calculated by the phosphorus amount according to the method Bartlett described in Ref. [44].

### 2.5. Software and data processing

Excel 2000 from Microsoft Office and Origin 6.1 software were used to perform the statistical analysis of the regressions.

## 3. Results and discussions

### 3.1. Preparation of Magnesia–zirconia based mimetic biomembrane (ZMBMB) stationary phase

A phospholipid is defined as a molecule with a polar headgroup (containing a phosphate group) and a double-chained hydrophobic tail. Phospholipids aggregate to form a bilayer where two lipid monolayers combine to form a two-dimensional sheet. Phospholipid bilayers are fundamental to the structure of all biological membrane and they have been used to study the physic behavior of biological membranes and membrane-bound macromolecules after absorbed on a solid support [45,46].

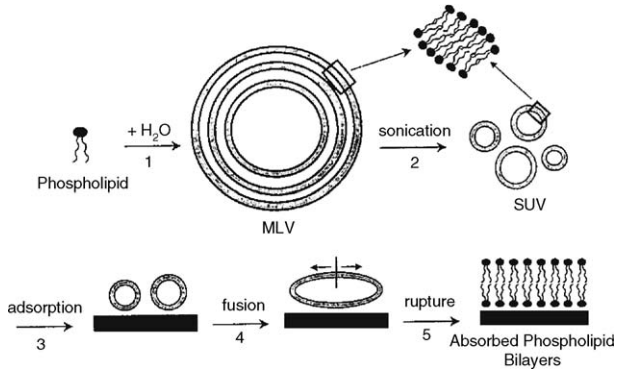


Fig. 1. The possible process of the immobilization of phospholipid bilayers on the surface of magnesia–zirconia composite to prepare mimetic biomembrane stationary phase (described as Ref. [49]).

Fig. 1 shows the possible scheme identifying the steps in the forming of phospholipid bilayers on the surface of magnesia–zirconia composite. When the gel beads packed with phosphatidylcholine are suspended in Tris–buffer solution, multilamellar vesicles (MLVs) will be formed for bilayers swell and self-close. After sonication processing, the MLVs should be turned in small unilamellar vesicles (SUVs), the generating SUVs will be gradually fused and ruptured with the existing of fusogenic agent (e.g. $C_a^+$) [47,48], thus leading to the formation of bilayers. The strong Lewis acid–base interactions between phosphatidylcholine's polar headgroup and Lewis acid sites of magnesia–zirconia composite caused the incorporation of phosphatidylcholine on the surface of magnesia–zirconia composite, which resulted in the immobilization of phospholipid bilayers, and simultaneously guarantee the good stability performance of mimetic biomembrane stationary phase.

### 3.2. The stability of the ZMBMB stationary phase

The stability of the **ZMBMB** stationary phase was investigated by determining the retention factors of procaine and metoprolol after elution of the column with various volumes of the mobile phase (0.01 M Tris–HCl buffer at pH 7.4 containing 0.05 M NaCl). Fig. 2A shows the dependence of $k'$ on the volume of the mobile phase eluted during HPLC, as can be seen that the decrease in retention factor of procaine was only 3.54% while metoprolol was only 5.11% with elution volumes; this result indicates that the **ZMBMB** stationary phase has good stability in the environment of physiological pH due to strong Lewis acid–base interactions between phospholipids and solid phase which played an important role in the stability of the **ZMBMB** stationary phase.

### 3.3. The drugs retention factors reproducibility on the ZMBMB stationary phase

In order to evaluate the reproducibility of the **ZMBMB** column, the adjusted retention factors ($k' = (t_{\mathrm{r}} - t_0)/t_0$) of three β-adrenolytic drugs and three local anesthetics were determined

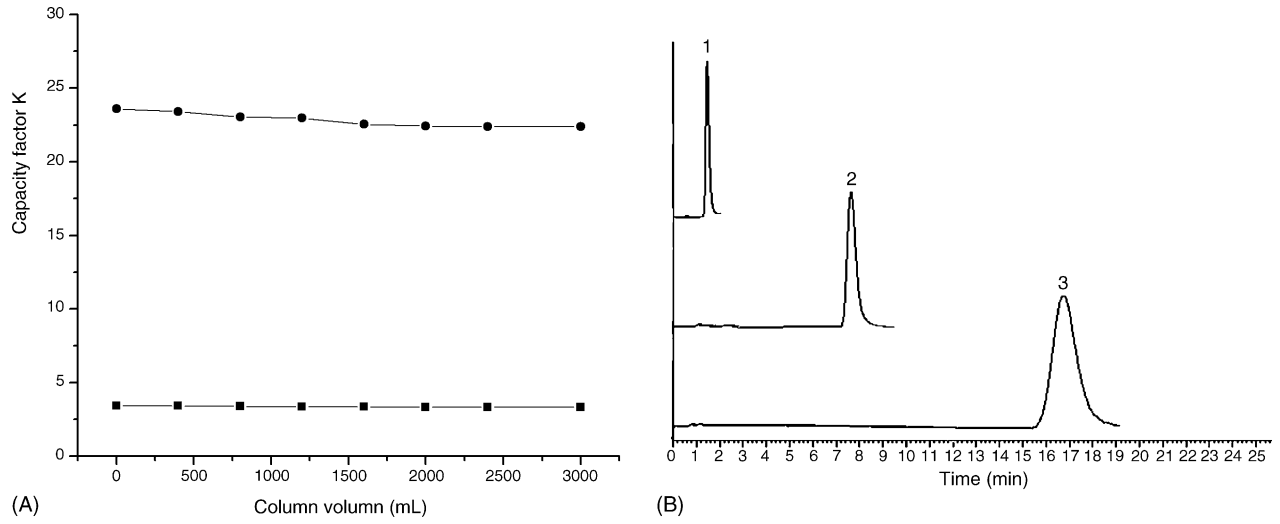


Fig. 2. (A) The stability of magnesia–zirconia composite based mimetic biomembrane stationary phase for the elution. Column, 50 mm × 4.6 mm; mobile phase, Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl; flow rate, 1 ml/min; solutes: (■) procaine, (●) metoprolol. (B) The chromatograms of three drugs on the ZMBMB stationary phase. Column, 50 mm × 4.6 mm; mobile phase, Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl; flow rate, 1 ml/min; solutes: (1) atenolol, (2) procaine, (3) metoprolol.

for five times, the 0.01 M Tris–HCl buffer solution (pH 7.4) containing 0.15 M NaCl was used as mobile phase. In this experiment, the retention time of diluted acetone aqueous solution was used as dead time ($t_0$ = 0.683 min). Table 1 shows the adjusted retention factors of six drugs and statistical analysis of variation, as can be seen from Table 1 that the **ZMBMB** column shows excellent reproducibility for the retention of these drugs, and the variations for every drugs are all less than 1%, among which the largest variation that of lidocaine was only 0.91%. It is indicated that drugs retention behavior was dominated by the drugs–membrane interactions, and other factors have little effect on the drugs retention. So, the retention factor of drugs can be used as a parameter characterizing the interactions between drugs and the immobilized phosphalipid bilayers membrane. Fig. 2B shows the chromatograms of atenolol, procaine and metoprolol.

### 3.4. Potential of ZMBMB stationary phase for predicting oral drug absorption

Oral drug delivery is the preferred route of drug administration. The major absorption barrier to orally administered drugs is the intestinal mucosa, where drugs are generally absorbed by passive diffusion. Oral drug absorption in humans is an important index to evaluate compounds in the early stage of drug discovery. Chromatographic technique is a convenient model for predicting oral drug absorption, among which the IAM, ILC, BMC system are well recognized model. In this experiment, the usefulness of **ZMBMB** stationary phase for predicting oral drug absorption in humans is evaluated; for this purpose, the retention factors ($K_{mbm}$) of 18 chemically different drugs on the **ZMBMB** stationary phase were determined with Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl as mobile phase, the model drugs were chosen to cover a wide range of absorption after oral administration (16–100%) and the reliable bibliographic absorption data were available. Table 2 listed their properties and $\%F_a$ values (percentage of the absorbed fraction after oral administration in humans).

Fig. 3 shows the correlation between the retention data ($\log K_{mbm}$) obtained on the **ZMBMB** stationary phase and the fraction absorption in humans ($\%F_a$), as can be observed from Fig. 3 that the absorption data increased dramatically with $\log K_{mbm}$ when $\log K_{mbm}$ varied from 0 to 0.8. These drugs show low permeability and high variability in the rate and extent

Table 1
The adjusted retention factors' ($k'$) reproducibility of three β-adrenolytic drugs and three local anesthetics on magnesia–zirconia based mimetic biomembrane stationary phase

| Drugs | $k'_1$ | $k'_2$ | $k'_3$ | $k'_4$ | $k'_5$ | Ave.$k'$ | S.D. | R.S.D. (%) |
|---|---|---|---|---|---|---|---|---|
| Atenolol | 0.610 | 0.608 | 0.609 | 0.611 | 0.611 | 0.610 | 0.0013 | 0.21 |
| Timolol | 1.754 | 1.747 | 1.750 | 1.755 | 1.746 | 1.750 | 0.0040 | 0.23 |
| Metoprolol | 23.317 | 23.631 | 23.593 | 23.672 | 23.735 | 23.590 | 0.1612 | 0.68 |
| Procaine | 3.416 | 3.428 | 3.431 | 3.413 | 3.421 | 3.422 | 0.0077 | 0.23 |
| Lidocaine | 10.311 | 10.532 | 10.313 | 10.450 | 10.378 | 10.397 | 0.0947 | 0.91 |
| Bupivacaine | 46.787 | 47.385 | 46.892 | 47.073 | 47.614 | 47.150 | 0.3445 | 0.73 |

The following conditions were used: Tris–HCl buffer solution (pH 7.4) containing 0.05 M NaCl was used as mobile phase; the isocratic eluent was used at a flow rate of 1 ml/min; the analyte detection was at 220 and 254 nm; diluted acetone aqueous solution was used as the void ($t_0$ = 0.683 min) marker. R.S.D. (%) [= (S.D./Ave) × 100] denotes the percentage relative standard deviation.

Table 2
Experimentally determined log $K_{mbm}$ values and compilation of related physicochemical data obtained from the literature

| No. | Solutes | Log $K_{mbm}$ | $\%F_a$ | Log $P_{oct}$ | Log $D_{oct}$ | Log $k_w^{IAM}$ | Log $K_s$ (EPL) | Log $K_s$ (PC) | Log $K_s$ (PC–PE) | Log $K_s$ (PC–PS) | Log $K_{BMC}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Mannitol | 0.38 | 16[f] | −3.1[d] | | | | | | | −0.7[h] |
| 2 | Acyclovir | 0.42 | 20[e] | −1.56[d] | | | | | | | −0.15[h] |
| 3 | Ranitidine | 0.46 | 50[g] | 0.27[b] | −0.59[b] | | | | | | |
| 4 | Atenolol | 0.49 | 54[a] | 0.14[a] | −1.61[a] | | 0.24[a] | 0.37[a] | 0.65[a] | 0.55[a] | −0.4[h] |
| 5 | Cemitidine | 0.57 | 60[c] | 0.21[b] | 0.11[b] | | | | | | 0.28[h] |
| 6 | Theophylline | 0.6 | 90–100[a] | −0.02[a] | −0.02[a] | | 0.45[a] | 0.54[a] | 0.82[a] | 0.62[a] | |
| 7 | Acetaminophen | 0.64 | 80[c] | 0.49[b] | 1.82[b] | | | | | | |
| 8 | Antipyrine | 0.69 | 97[a] | 0.38[a] | 0.38[a] | | 0.31[a] | 0.39[a] | 0.65[a] | 0.51[a] | |
| 9 | Cephalexin | 0.81 | 95[a] | 0.65[a] | | | −0.12[a] | −0.11[a] | 0.27[a] | 0.01[a] | |
| 10 | Timolol | 1.1 | 90[c] | 1.83[d] | | | | | | | |
| 11 | Procaine | 1.39 | | 2.14[a] | 0.42[a] | 0.39[a] | 0.69[a] | 0.85[a] | 1.08[a] | 0.97[a] | |
| 12 | Ketoprofen | 1.66 | >90[a] | 3.12[a] | −0.25[a] | 1.12[a] | 1.17[a] | 1.16[a] | 1.25[a] | 1.05[a] | 0.98[h] |
| 13 | Piroxicam | 1.8 | ∼100[a] | 3[a] | −0.05[a] | | 1.61[a] | 1.6[a] | 1.87[a] | 1.59[a] | |
| 14 | Lidocaine | 1.88 | | 2.34[a] | 1.65[a] | 0.75[a] | 1.01[a] | 1.14[a] | 1.07[a] | 1.23[a] | 0.83[h] |
| 15 | Tramadol | 1.99 | | 2.31[a] | | | 0.78[a] | 1.05[a] | 0[a] | 1.12[a] | |
| 16 | Metoprolol | 2.23 | 102[a] | 1.95[a] | −0.26[a] | | 1.02[a] | 1.25[a] | 1.1[a] | 1.48[a] | 0.36[h] |
| 17 | Diazepam | 2.43 | 97[a] | 2.8[a] | 2.8[a] | | 2.58[a] | 2.68[a] | 2.59[a] | 2.77[a] | 1.56[h] |
| 18 | Bupivacaine | 2.53 | | 3.45[a] | 2.59[a] | 1.45[a] | 1.49[a] | 1.69[a] | 1.55[a] | 1.79[a] | |
| 19 | Hydrocortisone | 2.6 | 89[a] | 1.61[a] | 1.61[a] | | 1.8[a] | 1.96[a] | 1.97[a] | 1.97[a] | 1.17[h] |
| 20 | Phenytoin | 2.6 | >90[a] | 2.47[a] | 2.47[a] | | 2.54[a] | 2.66[a] | 2.59[a] | 2.72[a] | 1.42[h] |
| 21 | Tetracaine | 3.41 | | 3.51[a] | 2.3[a] | 1.75[a] | 2.17[a] | 2.39[a] | 2.26[a] | 2.51[a] | |
| 22 | Propanolol | 3.97 | 90[a] | 3.28[a] | 1.07[a] | 1.81[a] | 2.7[a] | 3.01[a] | 2.73[a] | 3.19[a] | 1.23[h] |

Abbreviations: PC, phosphatidylcholine; PC–PS, phosphatidylcholine–phosphatidylserine; EPL, egg phospholipids; PC–PE, phosphatidylcholine–phosphatidylethanolamine; log $P_{oct}$, log(1-octanol–water partition coefficient of neutral form); log $D_{oct}$, log(1-octanol–water partition coefficient at pH 7.4); log $K_s$, logarithm of the liposome chromatography capacity factor($K_s$).

[a] Cited from Ref. [27].
[b] Cited from Ref. [52].
[c] Cited from Ref. [28].
[d] Cited from Ref. [53].
[e] Cited from Ref. [54].
[f] Cited from Ref. [8].
[g] Cited from Ref. [55].
[h] Logarithm of the $K_{BMC}$ values cited from Ref. [34].

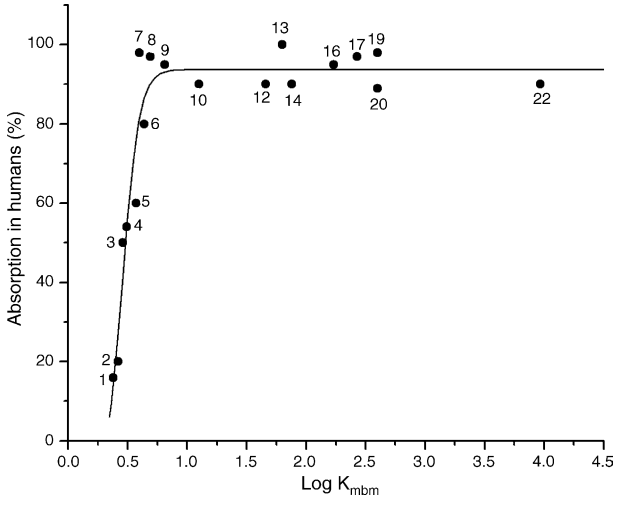


Fig. 3. Relationship between log $K_{mbm}$ on the ZMBMB stationary phase and the fraction absorption in humans ($\%F_a$). Column, 50 mm × 4.6 mm; mobile phase, Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl; flow rate, 1 ml/min; solutes: (1) mannitol, (2) acyclovir, (3) ranitidine, (4) atenolol, (5) cimetidine, (6) theophylline, (7) acetaminophen, (8) antipyrine, (9) cephalexin, (10) timolol, (12) ketoprofen, (13) piroxicam, (14) lidocaine, (16) metoprolol, (17) diazepam, (19) hydrocortisone, (20) phenytoin, (22) propanolol.

of absorption, drugs in this class could be poorly absorbed and therefore encounter significant problems for effective oral delivery. On the other hand, those drugs with greater retention (log $K_{mbm} > 0.8$) seem to have high permeability and are rapidly and completely absorbed with extents of absorption 90%. This tendency was similar with the BMC model. It is indicated that the log $K_{mbm}$ values obtained on the **ZMBMB** column can be used as an evaluation parameter for drugs fraction absorption in humans, and this model may be used for the estimation of drug partitioning and drug absorption through cell membranes.

### *3.5. Relationship between octanol–water partitioning (log $P_{oct}$ and log $D_{oct}$) and drug retention on ZMBMB stationary phase*

Hydrophobicity is a fundamental physicochemical property of drugs which represents the affinity of a molecule for a hydrophobic environment, commonly determined by the partitioning of the molecule in a biphasic systems, either liquid–liquid (e.g. partitioning in octanol–water) or liquid–solid (e.g. retention on reverse phase chromatographic columns). The partitioning coefficient, *P*, is a constant and refers to a single molecular species, whereas the distribution coefficient, *D*, refers to the apparent partition coefficient, which varies with pH when

ionized compounds are considered. The octanol–water system ($\log P_{oct}$) has become the benchmark solvent system mainly because of the availability of a large database of experimentally determined values; so, it is of much interest to compare the retention data of mimetic biomembrane chromatography with octanol–water partitioning.

In this experiment, the capacity factors of 14 diverse set of drugs on the **ZMBMB** stationary phase were determined with 0.01 M Tris–HCl buffer containing 0.05 M NaCl as mobile phase. The $\log P_{oct}$ of these drugs varied from −1.56 to 3.51 and covered most typical drugs; the drugs with too large $\log P_{oct}$ values and the negatively charged drugs were excluded from the determination. For the drugs with too large $\log P_{oct}$ values, the retention time were too long and their peaks broadened seriously, which will leading to the difficulty for chromatographic characterization and large error for statistical analysis; meanwhile, for the negatively charged drugs, the existence of Lewis acid–base interactions with residue Lewis acid center of the magnesia–zirconia composite will contribute to the attention of these drugs; therefore, the retention factors of drugs ($\log K_{mbm}$) cannot represent the drugs–biomembrane interactions. Fig. 4A shows the relationship between the retention factors of drugs ($\log K_{mbm)}$ and the $\log P_{oct}$ values, as can be seen from Fig. 4A that a moderate relationship was obtained between $\log K_{mbm}$ versus $\log P_{oct}$ ($r = 0.791$), which indicate that the hydrophobic interactions were the key retention mechanism between drugs and the ZMBMB stationary phase. Fig. 4B shows the relationship between the retention factors of drugs ($\log K_{mbm)}$ and the $\log D_{oct}$ values, weaker relationship ($r = 0.656$) was obtained according to the analyzing results. The reason for the difference between the two relationships may be the existence of electrostatic interactions between drugs and the **ZMBMB** stationary phase. Under the experimental condition, some compounds partially ionized and existed not in neutral form; on the other hand, as we know, phospholipids is a kind of zwitterionic surfactant, the electrostatic interactions will play an important role between drugs and the phospholipid bilayers; therefore, the hydrophobic interactions and electrostatic interactions were the main retention mechanism of the the **ZMBMB** stationary phase.

$$\log P_{oct} = 0.942(\pm 0.210)\ \log K_{mbm} + 0.253(\pm 0.472)$$
$$n = 14; \quad r = 0.791; \quad s = 0.774; \quad P < 0.001 \qquad (1)$$

$$\log D_{oct} = 0.857(\pm 0.284)\ \log K_{mbm} - 0.795(\pm 0.639)$$
$$n = 14; \quad r = 0.656; \quad s = 1.040; \quad P < 0.025 \qquad (2)$$

### *3.6. Relationship between drug retention on ZMBMB stationary phase and IAM column*

Immobilized artificial membrane (IAM) chromatography stationary phase was prepared by covalently immobilizing monolayers of cell membrane phospholipids to silica particles at high molecular surface densities. The phospholipids monolayers structurally resembles the ordered array of the membranous hydrocarbon chains; therefore, IAMs can mimic the lipid environment of a fluid cell membrane, and it has been a well-known system for charactering drug–membrane interactions and high throughout method for drugs screening.

In this experiment, the comparison was made between **ZMBMB** stationary phase and IAM column. The relationships between **ZMBMB** stationary phase ($\log K_{mbm)}$ and IAM column ($\log k_w^{IAM}$) was analyzed with six drugs as probes. The retention values ($\log k_w^{IAM}$) obtained on an IAM column were taken from the Ref. [50,51]. The statistical results show that a good relationship is obtained between $\log K_{mbm}$ and $\log k_w^{IAM}$; the linear

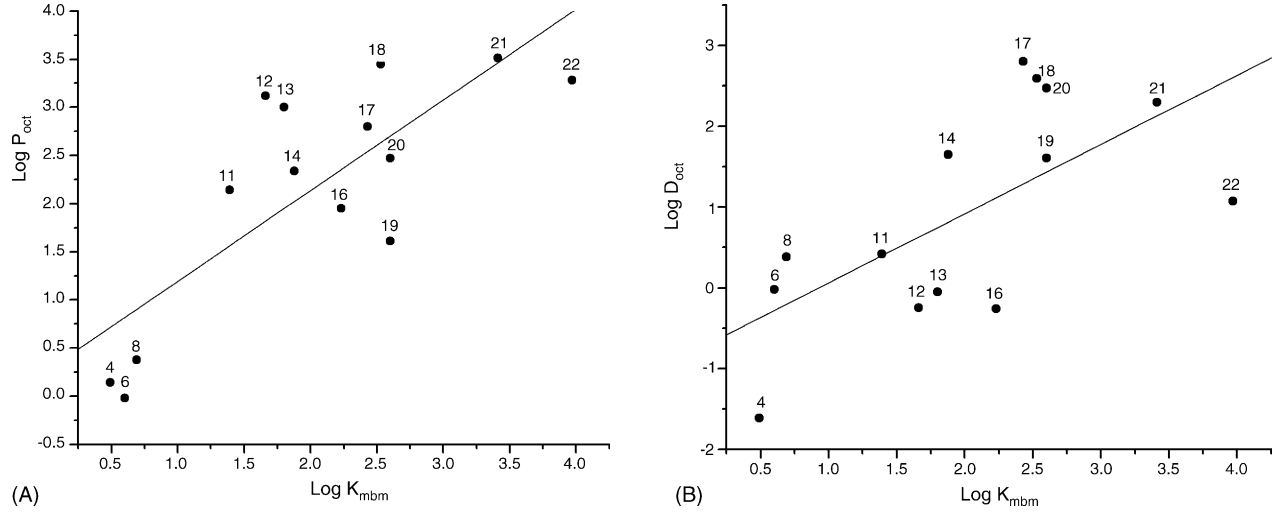


Fig. 4. (A) Relationship between $\log K_{mbm}$ and $\log P_{oct}$ for 14 diversity of drugs. Mobile phase, Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl; flow rate, 1 ml/min; solutes: (4) atenolol, (6) theophylline, (8) antipyrine, (11) procaine, (12) ketoprofen, (13) piroxicam, (14) lidocaine, (16) metoprolol, (17) diazepam, (18) bupivacaine, (19) hydrocortisone, (20) phenytoin, (21) tetracaine, (22) propanolol. (B) Relationship between $\log K_{mbm}$ and $\log D_{oct}$ for 20 diversity of drugs. Mobile phase, Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl; flow rate, 1 ml/min; solutes: (4) atenolol, (6) theophylline,(8) antipyrine, (11) procaine, (12) ketoprofen, (13) piroxicam, (14) lidocaine, (16) metoprolol, (17) diazepam, (18) bupivacaine, (19) hydrocortisone, (20) phenytoin, (21) tetracaine, (22) propanolol.

relationship is described in Eq. (3):

$$\log K_{\mathrm{mbm}} = 1.656(\pm 0.378)\log k_{\mathrm{w}}^{\mathrm{IAM}} + 0.466(\pm 0.498)$$
$$n = 6; \quad r = 0.910; \quad s = 0.478; \quad P < 0.05 \qquad (3)$$

According to the statistical analysis results, the $\log K_{\mathrm{mbm}}$ values showed good relationship ($r = 0.910$) with the $\log k_{\mathrm{w}}^{\mathrm{IAM}}$ at the 95% confidence level, which indicates that the **ZMBMB** stationary phase showed similar prediction ability of drugs absorption with IAM columns.

### 3.7. Relationship between drug retention on ZMBMB stationary phase and ILC (EPL, PC, PC-PE, PC-PS) column

Immobilized liposome chromatography (ILC) is a recently innovated method for rapid and precise analysis of drug–membrane interactions [27], have reported series of compounds partitioning on four different original liposome columns [52]. In this experiment, the relationships between the retention of 16 drugs ($\log K_{\mathrm{mbm}}$) and $\log K_{\mathrm{s}}$ (EPL, PC, PC-PE, PC-PS) were investigated. Fig. 5A–D shows the correlations between $\log K_{\mathrm{mbm}}$ and $\log K_{\mathrm{s}}$ (EPL, PC, PC-PE, PC-PS). The results of the statistical comparisons between the $\log K_{\mathrm{mbm}}$ and $\log K_{\mathrm{s}}$ (EPL, PC, PC-PE, PC-PS) are shown in Eqs. (4)–(7):

$$\log K_{\mathrm{s}}(\mathrm{EPL}) = 0.811(\pm 0.123)\log K_{\mathrm{mbm}} - 0.303(\pm 0.269)$$
$$n = 15; \quad r = 0.877; \quad s = 0.477; \quad P < 0.0001 \qquad (4)$$

$$\log K_{\mathrm{s}}(\mathrm{PC}) = 0.845(\pm 0.103)\log K_{\mathrm{mbm}} - 0.201(\pm 0.225)$$
$$n = 15; \quad r = 0.915; \quad s = 0.400; \quad P < 0.0001 \qquad (5)$$

$$\log K_{\mathrm{s}}(\mathrm{PC-PE}) = 0.666(\pm 0.106)\log K_{\mathrm{mbm}} + 0.206(\pm 0.232)$$
$$n = 15; \quad r = 0.867; \quad s = 0.411; \quad P < 0.0001 \qquad (6)$$

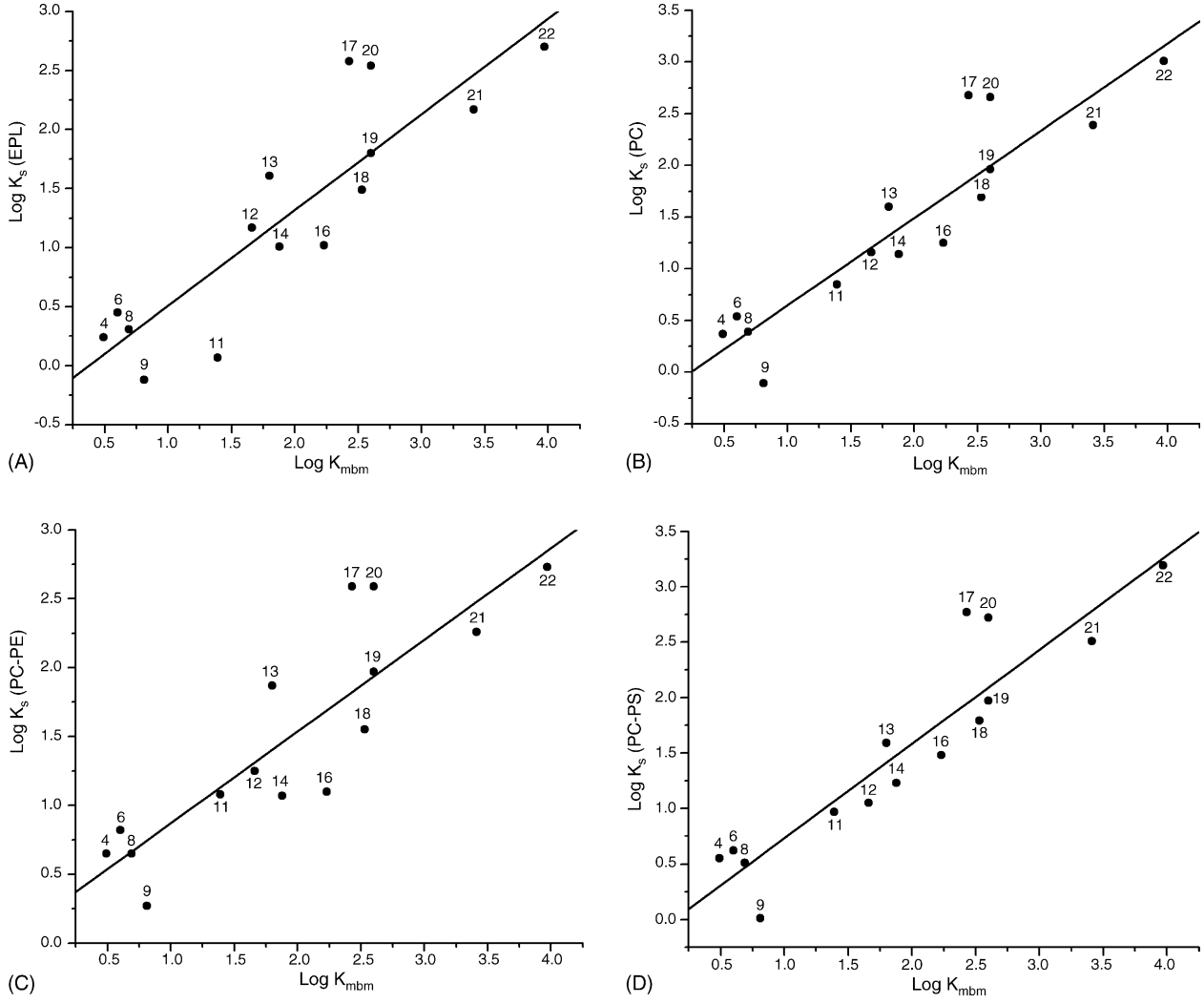


Fig. 5. (A–D) Relationship between the $\log K_{\mathrm{mbm}}$ and $\log K_{\mathrm{s}}$ (EPL, PC, PC-PE, PC-PS) for 15 diversity of drugs. Column, 50 mm × 4.6 mm; mobile phase, Tris–HCl buffer (pH 7.4) containing 0.05 M NaCl; flow rate, 1 ml/min; solutes: (4) atenolol, (6) theophylline, (8) antipyrine, (9) cephalexin, (11) procaine, (12) ketoprofen, (13) piroxicam, (14) lidocaine, (16) metoprolol, (17) diazepam, (18) bupivacaine, (19) hydrocortisone, (20) phenytoin, (21) tetracaine, (22) propanolol.

$$\log K_s(\mathrm{PC-PS}) = 0.850(\pm 0.100)\log K_{mbm} - 0.117(\pm 0.216)$$
$$n = 15; \quad r = 0.922; \quad s = 0.383; \quad P < 0.0001 \qquad (7)$$

According to the statistical results, the $\log K_{mbm}$ values showed moderate-to-fair relationships with $\log K_s$ values of four different original liposome columns, among which the $\log K_{mbm}$ showed the best relationship with the $\log K_s$ (PC-PS) values ($r = 0.922$), whereas the weakest with the $\log K_s$ (PC-PE) values ($r = 0.867$), although the two liposome columns were all prepared by phospholipids containing phosphatidylcholine. The existence of phosphatidylethanolamine (PE) and phosphatidylserine (PS) may change the polarity and electric charge on the outside of liposome bilayers, as illustrated by öterberg et al. in Ref. [52], the $\log K_s$ can be expressed with the following equation:

$$\log K_s = aV - \Lambda + E$$

where $a$ is a constant, $V$ is the molar volume and $\Lambda$ accounts for the polarity of the molecule including H-bonding capacity, $E$ is a term reflecting the electrostatic interactions of the drug molecules with the phospholipid head group region. The introduction of phosphatidylethanolamine (PE) and phosphatidylserine (PS) will lead to the difference of item $E$, which will affect the interactions between solutes and diverse liposome chromatography stationary phases. In this experiment, phosphatidylcholine was the material preparing **ZMBMB** stationary phase; therefore, the relationship between $\log K_{mbm}$ with the $\log K_s$ of liposome containing phosphatidylcholine was comparatively higher, whereas lower with the $\log K_s$ of liposome containing phosphatidylethanolamine. It was obviously that the $\log K_{mbm}$ values of **ZMBMB** column showed strong relationships with the $\log K_s$ of different liposome columns, although the linear relationship was a little different. With the comparison between **ZMBMB** column and different liposome columns, it was indicated that the **ZMBMB** stationary phase has similar retention mechanism and ability of describing and predicting oral drug absorption with ILC stationary phase.

### 3.8. Relationship between log $K_{mbm}$ and log $K_{BMC}$

Biopartitioning micellar chromatography (BMC) was another in vitro chromatographic method to predict oral drug absorption. It was constituted by a $C_{18}$ reversed stationary phase and a polyoxyethylene (23) lauryl ether (Brij35) micellar mobile phase. The retention data obtained in this system under adequate experimental conditions are helpful in describing the biological behavior of different kinds of drugs [34]. The comparisons between BMC system and other well recognized natural systems such as red cell membrane lipid liposomes (MLs), human red cell membranes vesicles (vesicles), native membranes of adsorbed red cells (ghosts) and egg phospholipids liposomes (EPLs) were demonstrated. Excellent linear correlations were obtained in all cases (($r^2 \geq 096$) indicating that the BMC system mimics adequately the relative importance of drugs interactions with biomembranes; so, it is of great interest to make a comparison between **ZMBMB** column and BMC system.

In this experiment, the relationships between $\log K_{mbm}$ and $\log K_{BMC}$ was investigated with the retention data of 11 drugs in Table 2. Results showed that moderate relationship ($r = 0.835$) was obtained between the two models; however, the correlation coefficients were lower than those of MLs, vesicales, ghosts and EPLs mentioned in Ref. [56].

## 4. Concluding remarks

The application of zirconia and zirconia-containing mixed oxides as matrix in HPLC has received considerable attention owing to their remarkable mechanical, chemical and thermal stability. It is a new attempt to prepare ZMBMB stationary phase using magnesia–zirconia composite. The strong Lewis acid–base interactions between Lewis acid sites on the surface of magnesia–zirconia composite and phospholipids' polar headgroup contribute to the immobilization of phospholipid bilayers, and is helpful for the stability of the ZMBMB stationary phase. In our experiment, excellent stability was found with this stationary phase for 1 month chromatographic runs. The phospholipids loss was under 5%; moreover, the $\log K_{mbm}$ values for experimental drugs showed highly reproducible. The potential of the ZMBMB column for preliminary drug screening and in vitro prediction of fraction absorption in humans ($\%F_a$) was evaluated by the correlation analysis between the $\log K_{mbm}$ values with $\%F_a$, and the drugs $\%F_a$ values were found varied with $\log K_{mbm}$ values with an hyperbolic relationship. The linear regression analysis was made for the comparison between the mimetic biomembrane column and commercial IAM column with six drugs. Results showed that there was good relationship between these two columns. On the other hand, comparisons have been made between the $\log K_{mbm}$ values and the portioning coefficients of different liposome columns prepared with EPL, PC, PC-PE, PC-PS, moderate to fair relationships were obtained, comparatively, the difference of rectilinear relationships may be caused by the variation of the polarity and electric charge on the outside of phospholipds bilayers resulting from the diversity of phospholipid composition. In addition, moderate linear relationship was found between the $\log K_{mbm}$ values and the retention data of BMC system ($\log K_{BMC}$).

In summary, $\log K_{mbm}$ value, the retention parameter of drugs on the **ZMBMB** stationary phase can be used to predict drug absorption at the early stage of the drug discovery process, and the mimetic biomembrane column can be developed to model for preliminary drug screening and for predicting of fraction absorption in humans ($\%F_a$) through transcellular passive transport route. Owing to the column's easily preparation, the $\log K_{mbm}$ value's conveniently determination and excellent reproducibility, this model can be used associatively with other chromatographic models such as IAM, ILC, MLC and BMC for screening drugs passive absorption.

## 5. Nomenclature

$\%F_a$ percentage of absorbed fraction of drug diffusivity of a hypothetical molecule

| | |
|---|---|
| $\log D$ | logarithm of the partition coefficient in the system octanol–water at a given pH |
| $\log P$ | logarithm of the partition coefficient in the system octanol–water |
| ZMBMB | magnesia–zirconia based mimetic biomembrane stationary phase |
| MBM | mimetic biomembrane |
| $K_{mbm}$ | specific capacity factors measured in the magnesia–zirconia based mimetic biomembrane stationary phase |
| PC | phosphatidylcholine |
| PC–PS | phosphatidylcholine–phosphatidylserine |
| PC–PE | phosphatidylcholine–phosphatidylethanolamine |
| EPL | egg phospholipid |
| ILC | immobilized liposome chromatography |
| IAM | immobilized artificial membrane chromatography |
| BMC | biopartitioning micellar chromatography |
| SUVs | small unilamellar vesicles |
| MLVs | multilamerllar vesicles |

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