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Study on interaction between drug and membrane by using liposome coated zirconia–magnesia chromatography

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Abstract

The interactions between drug molecules and membrane were studied using the new chromatography stationary phase of liposome coated zirconia–magnesia. log K_s(ZrO_2–MgO) on this new chromatography for some drugs, compared with that on liposome coated silica chromatography and other reported data, fair correlations were observed between them when excluding effect of special adsorption. log K_s(ZrO_2–MgO) values for barbitalum, diazepam, benzene, benzocaine and toluene correlated well with corresponding values on liposome coated silica chromatography ($R = 0.99778$, $P < 0.001$; $R = 0.98229$, $P < 0.003$; $R = 0.9985$, $P < 0.0001$; $R = 0.99925$, $P < 0.0001$, pH value of mobile phase at pH 7.4, 7.0, 6.4 and 5.4, respectively). They also correlated well with the literature data on immobilized artificial membrane chromatography ($R = 0.99999$, $P < 0.004$ at pH 7.4) and liposome chromatography ($R = 0.99994$, $P < 0.008$) for procaine, lidocaine and bupivacaine. Liposome coated zirconia–magnesia chromatography can thus be used for studying drug–membrane interaction and prediction of drug absorption as another liposome chromatography method.

Keywords: Liposome chromatography; Drug absorption; Zirconia; Phosphatidylcholine

1. Introduction

With the continuous variation of nosogenic bacterium and viruses, leading to drug-resistance for the existing drugs, it urges pharmaceutical scientists to ceaselessly find new drugs to treat them. Nowadays, there are two main ways to look for new drugs: screening from combinatorial chemistry data and natural products [1,2]. However, these ways are all faced to how to screen active substances from complex compounds. Hence, finding high-efficient drug-screening methods become an urgent problem need to be solved. At the present time, there are only two drug-screening methods from the broadest perspective: conventional activity assays and cell membrane transport properties predictions [3]. Because of conventional activity assays having some drawbacks, i.e., long time consumption, high cost and low efficiency, it is unfit as a high-efficient screening method. Cell membrane transport properties can be easily predicted by physico-chemical methods using modern analytical instruments. Consequently, the latter becomes a usually used method to screen drugs.

Because drug transport through tissues mainly by transcelluar pathway, and it is also a prerequisite step to transport across the target cell membrane for the drug to elicit activity [3], prediction of cell membrane transport properties is certainly considered as a primary drug-screening method. Nowadays, there are many methods to predict cell membrane transport properties, i.e., octanol–water partitioning [4–6], hydrophobilicity measurements [7–9], liposome partitioning [10,11], etc., some of which can be easily performed by chromatography [12–15]. Among these methods, immobilized liposome chromatography is a simple and accurate method because liposome has similar lipid bilayer structure and fluid characteristic of real cell membrane [16]. Immobi-

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lized liposome chromatography of drugs for model analysis of drug–membrane interactions was reviewed by Lundahl and Beigi [17].

Soft gel and silica are two matrices usually used in liposome chromatography. These matrices have their own virtues and drawbacks. The soft gel matrix has good biocompatibility and can be easily modified chemically owing to having many hydroxyl groups, but the range of particle size is wide and the mechanic strength is weak resulting from large pore size. Silica matrix has some advantages such as the structure of pore and surface easy to be controlled, good chemical stability and chemical reaction easy to be done on surface. However, the range of pH for use is narrow (usually pH 2–8), and the peak of basic solute has serious tailing on silica matrix owing to special adsorption. Compared with above two matrices, zirconia is a new developing chromatography stationary phase. It has some advantages such as strong mechanic strength, wide use range of pH, what's more, there are some Lewis base sites on its surface and can be modified easily by Lewis acid–basic action [18–20]. Carr and co-workers have done much work on this new matrix and have been successfully applied it for commercial use [21–25].

A new immobilized liposome chromatography with zirconia–magnesia as matrix was prepared and chromatographic properties were also evaluated in our study [26]. Due to Lewis acid–base interaction between phosphate group of liposome and surface of zirconia, the stability of liposome coated zirconia–magnesia chromatography was better than that of silica. Thus, the probability of liposome chromatography for commercial use to screen drugs becomes great. In this paper, we want to further investigate the properties of this new chromatography for studying drug–membrane interaction.

2. Experimental

2.1. Materials and reagents

Zirconia–magnesia particles (5–7 μm, pore size 20–25 nm) and silica particles (5 μm, pore size 20–25 nm) were home-made. Soybean phosphatidylcholine was obtained from soybean degummed oil residues by using column chromatography (purity > 95%) [27].

Tris(hydroxymethyl)aminomethane, hydrochloric acid, benzene and toluene were of analytical grade, purchased from Shanghai Medicine Co. (Shanghai, China).

Pilocarpine, benzocaine, bupivacaine, procaine, lidocaine, barbitalum, ketoprofen, diazepam were provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.2. Apparatus

The analytical HPLC apparatus was provided by Dalian Elite Analytical Apparatus Ltd. Co., which was consisted of a P200II pump (Elite, Dalian, China), an UV200II ultraviolet (UV) detector (Elite, Dalian, China) and a Rheodyne 7725i injector with 20 μL sample loop (Cotati, California, USA). The chromatographic data was acquired by chromatography working station Echrom98 (Elite, Dalian, China). The HPLC analyses were performed on 50 mm × 4.6 mm column packed with home-made liposome chromatography packings. The mobile phases were Tris–HCl buffers with different NaCl concentrations and pH values, and the flow-rate was set at 1 mL/min.

KS-300D ultrasonic cleaner (Ningbo, China) and Delta320-S pH detector (Mettler-Toledo, Shanghai) were purchased from Hubei Sci. and Edu. Instruments Co. (Wuhan, China).

2.3. Preparation of liposome chromatography

Two immobilized liposome chromatography, liposome coated zirconia–magnesia and silica, were prepared by using coating method. The preparation process referred to the reported method [28,26].

2.4. Comparison of properties of two kinds of immobilized liposome chromatography columns

Retention behaviors of some solutes on liposome coated zirconia–magnesia and silica chromatography were compared by changing pH value and salt concentration of mobile phase.

2.5. Comparison with reported data determined by other methods

Retention of some selected drugs on liposome coated zirconia–magnesia was determined. By comparing with the reported data for evaluating drug–membrane interaction, the prediction ability of this new chromatography for screening new drugs was also investigated.

3. Results and discussion

3.1. Comparison of properties of two kinds of immobilized liposome chromatography columns

The amounts of immobilized liposome were different in various columns. Retention of solute was correlation with the amount of liposome in a column. Hence, it was necessary to eliminate this affection when comparing the properties of different liposome chromatography columns. In order to eliminate this effect, a new adjusted capacity factor (K_s) was introduced to investigate the chromatographic properties [29]:

$$K_s = \frac{V_r - V_0}{A}$$

where V_r is the retention volume of drug, V_0 the dead volume of system and determined by KNO_3, and A is the amount of immobilized liposome in column, which was determined in Ref. [26].

Barbitalum, diazepam, benzene, benzocaine, toluene and ketoprofen were used to investigate the properties of two immobilized liposome chromatography columns by changing pH value and salt concentration of mobile phase. The relations of logarithm of K_s on two kinds of liposome chromatography columns under different chromatography conditions are shown in Figs. 1A–E and 2A–D.

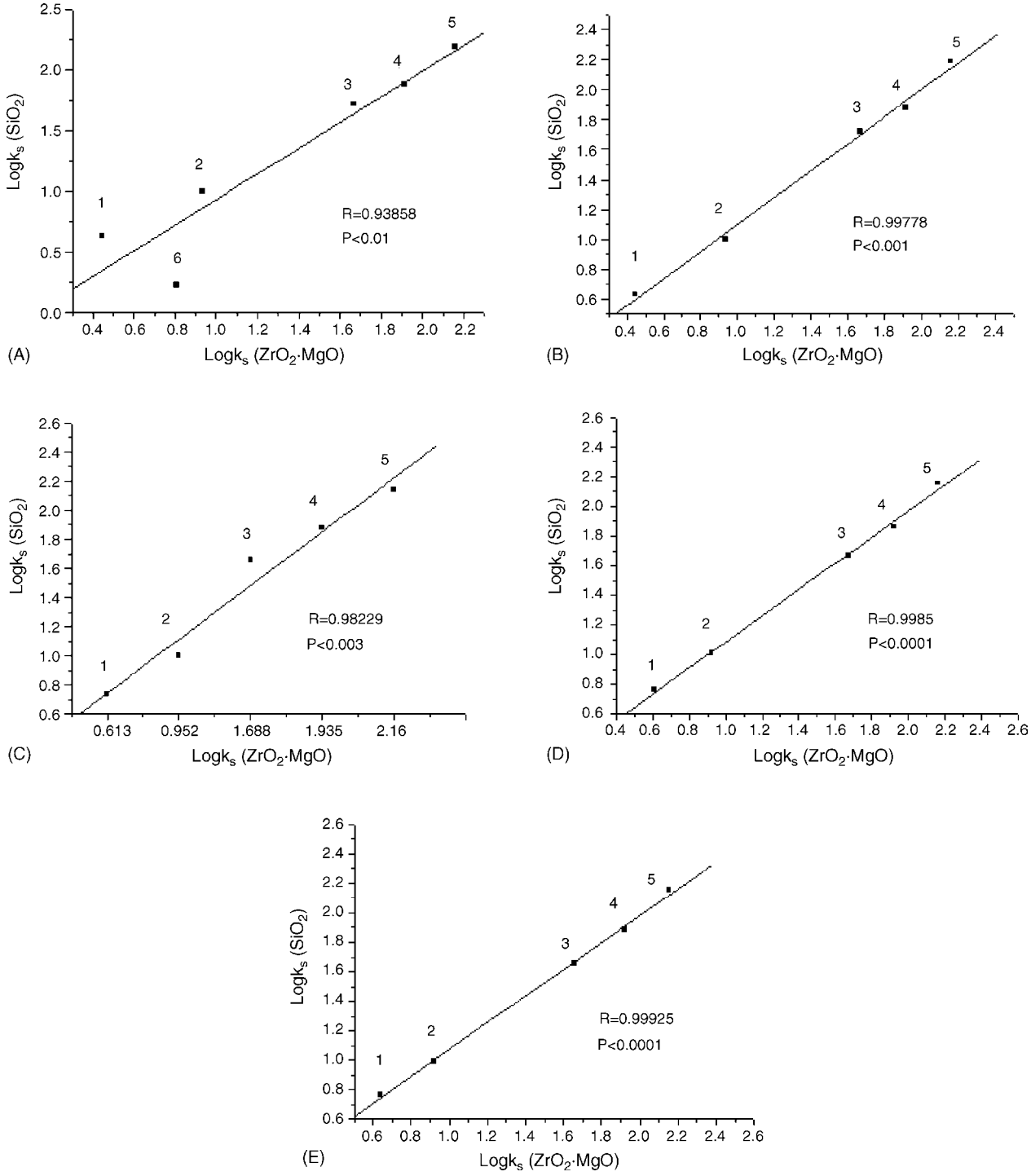

Fig. 1. Relationships between log K_s(ZrO_2–MgO) and log K_s(SiO_2) for barbitalum (1), diazepam (2), benzene (3), benzocaine (4), and toluene (5), ketoprofen (6). Column, 50 mm × 4.6 mm (packing, ZrO_2–MgO–liposome and SiO_2–liposome); mobile phase, Tris–HCl buffer at pH: (A) and (B) 7.4, (C) 7.0, (D) 6.4, (E) 5.4; flow rate: 1 mL/min.

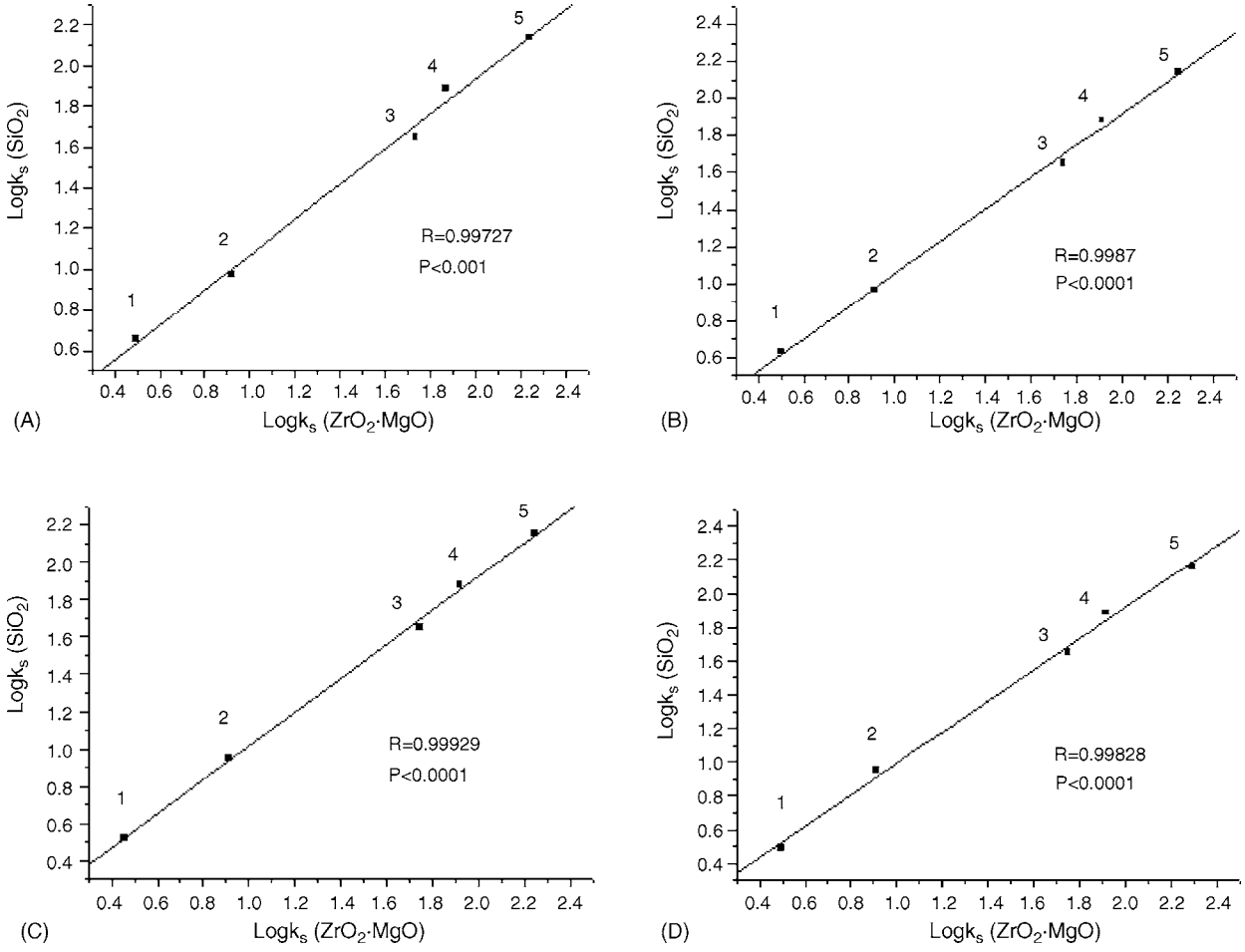

Fig. 2. Relationships between log K_s(ZrO_2–MgO) and log K_s(SiO_2) for barbitalum (1), diazepam (2), benzene (3), benzocaine (4), and toluene (5). Column, 50 mm × 4.6 mm (packing, ZrO_2–MgO–liposome and SiO_2–liposome); mobile phase, Tris–HCl buffer at pH 7.4 with (A) 25 mM, (B) 50 mM, (C) 100 mM, (D) 150 mM NaCl; flow rate: 1 mL/min.

A moderate rectilinear correlation ($R = 0.93858$, $P < 0.01$) was observed between log K_S values for barbitalum, diazepam, benzene, benzocaine, toluene and ketoprofen on the liposome coated zirconia–magnesia and silica chromatography (see Fig. 1A). However, the rectilinear correlation was improved greatly ($R = 0.99778$, $P < 0.001$) after removing ketoprofen (see Fig. 1B). This phenomenon was result from that ketoprofen was acid solute that had special adsorption on the surface of zirconia owing to Lewis acid–base interaction. What kinds of solutes were fit to be analyzed on liposome coated zirconia–magnesia or silica was studied in our previous work [26]. This result was agreed with that.

There were good rectilinear correlations between two kinds of liposome chromatography for barbitalum, diazepam, benzene, benzocaine and toluene under the condition of different pH value of mobile phase. The correlation coefficients were 0.99778 ($P < 0.001$), 0.98229 ($P < 0.003$), 0.9985 ($P < 0.0001$) and 0.99925 ($P < 0.0001$) corresponding to the pH value of mobile phase being pH 7.4, 7.0, 6.4 and 5.4, respectively (see Fig. 1B–E).

Fig. 2A–D illustrated that there were also good rectilinear correlations between two kinds of liposome chromatography for above five solutes when the NaCl concentration of mobile phase changing from 25 to 150 mM. The correlation coefficients were all more than 0.99 ($P < 0.01$).

From above analyses, we could conclude that the chromatographic behaviors of solutes on liposome coated zirconia–magnesia and silica chromatography column were consistent when excluding the effect of special adsorption between solutes and surface of matrices.

3.2. Comparison with reported data determined by other methods

Amongst the drug screening methods depending on physico-chemical parameters, the frequently used methods were determining octanol–water partition coefficient (P_{oct} and D_{oct}), capacity factor (K_s) on liposome chromatography and capacity factor (K^{IAM}) on immobilized artificial membrane chromatography, etc. The parameters of many drugs for evaluating drug–membrane interaction were deter-

Table 1
The physicochemical data of some local anesthetics obtained from literature [30]

	Procaine	Lidocaine	Bupivacaine
$\log P_{oct}$	2.14	2.34	3.45
$\log D_{oct}$	0.42	1.65	2.59
$\log K_w^{IAM}$	0.39	0.75	1.45
$\log K_s$(PC)	0.85	1.14	1.69
$\log K_s$(PC–PS)	0.97	1.23	1.79
$\log K_s$(EPL)	0.69	1.01	1.49

PC: phosphatidylcholine; EPL: egg phospholipids; PC–PS: phosphatidylcholine–phosphatidylserine; $\log P_{oct}$: log(1-octanol–water partition coefficient of neutral form); $\log D_{oct}$: log(1-octanol–water partition coefficient at pH 7.4); $\log K_w^{IAM}$: logarithm of IAM chromatographic retention factor extrapolated to 100% aqueous phase (eluent: mixtures of acetonitrile and 0.1 M phosphate buffer saline pH 7.4); $\log K_s$: logarithm of the liposome chromatography capacity factor (K_s).

mined by above methods and reported. In order to investigate whether new liposome coated zirconia–magnesia chromatography could be used for screening drug or not, some local anesthetics, procaine, lidocaine and bupivacaine, were selected as solutes to evaluate the properties of it comparing with other reported methods.

The reported parameters of procaine, lidocaine and bupivacaine are listed in Table 1. The relationship between logarithm of retention factor, $\log K_s(ZrO_2–MgO)$, on liposome coated zirconia–magnesia chromatography and the reported data are shown in Eqs. (1)–(6):

$$\log K_s(\text{EPL}) = -0.028 + 0.959 \log K_s(ZrO_2–MgO), \quad R = 0.99734, \quad P < 0.05 \tag{1}$$

$$\log P_{oct} = 0.761 + 1.653 \log K_s(ZrO_2–MgO), \quad R = 0.98133, \quad P < 0.2 \tag{2}$$

$$\log D_{oct} = -1.301 + 2.507 \log K_s(ZrO_2–MgO), \quad R = 0.96513, \quad P < 0.2 \tag{3}$$

$$\log K_w^{IAM} = -0.601 + 1.287 \log K_s(ZrO_2–MgO), \quad R = 0.99999, \quad P < 0.004 \tag{4}$$

$$\log K_s(\text{PC}) = 0.0673 + 1.019 \log K_s(ZrO_2–MgO), \quad R = 0.99994, \quad P < 0.008 \tag{5}$$

$$\log K_s(\text{PC–PS}) = 0.191 + 1.000 \log K_s(ZrO_2–MgO), \quad R = 0.99979, \quad P < 0.02 \tag{6}$$

There were good rectilinear correlations between $\log K_s(ZrO_2–MgO)$ and $\log K_w^{IAM}$, $\log K_s$(PC) ($R > 0.99$, $P < 0.01$) (see Eqs. (4) and (5)). Moderate rectilinear correlations were also observed between $\log K_s(ZrO_2–MgO)$ and $\log K_s$(EPL), $\log K_s$(PC–PS) ($R > 0.99$, $P < 0.05$) (see Eqs. (1) and (6)). However, the line relationships were bad with $\log P_{oct}$ and $\log D_{oct}$ ($R < 0.99$, $P < 0.2$) (see Eqs. (2) and (3)). In $\log K_w^{IAM}$ and $\log K_s$(PC), the artificial membrane and liposome were made from phosphatidylcholine and that was similar to our new stationary phase. Hence, the retention behaviors were similar for these three methods. Although there were all liposome chromatography for $\log K_s$(EPL), $\log K_s$(PC–PS) and our new column, EPL was a mixed phospholipids composed of phophatidylcholine, phosphatidylethanolamine and other lipids, and PC–PS was a mixture of phosphatidylcholine and phosphatidylserine. Different compositions of liposome led to diversity of polar group and electric charge on the outside of liposome bilayer, which resulted in interactions between solutes and various liposome chromatography stationary phases were different. Consequently, the line relationships between $\log K_s(ZrO_2–MgO)$ and $\log K_s$(EPL), $\log K_s$(PC–PS) were not perfect. $\log P_{oct}$ and $\log D_{oct}$ were parameters for evaluating hydrophobic capability of drugs. Bad line relationships between $\log K_s(ZrO_2–MgO)$ and them illustrated that liposome chromatography was not pure reverse-phase chromatography mode. This result was also obtained in our previous work [26]. Lundahl and co-workers had been compared $\log K^{IAM}$ and $\log K_s$(PC) with $\log P_{oct}$ for some drug molecules, and obtained similar result as above [30]. Compared with other methods, liposome chromatography had similar structure as real cell membrane and can be used to investigate hydrophobic and electrostatic interactions between drug and membrane. Hence, the determining result for screening drug would approach truth more.

4. Conclusions

There was consistent between liposome coated zirconia–magnesia chromatography and other liposome or immobilized artificial membrane chromatography for studying drug–membrane interaction. It was illustrated that this new liposome chromatography could be used for screening drugs. Owing to existing Lewis acid–base interaction between acid solutes and zirconia, the new liposome chromatography was unfit to be used for screening acidic active substances. However, it could be used for screening basic solutes, which was unfit to be done on liposome chromatography column using silica as matrix. Consequently, Two kinds of liposome chromatography with zirconia and silica as matrices were perfectly complementary. Although liposome coated zirconia–magnesia chromatography provided another useful tool for screening drugs, it needed to be further studied.

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