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The QRAR model study of β-lactam antibiotics by capillary coated with cell membrane

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Abstract

With the accelerating development of new drugs, there is a great need for rapid and simple screening technologies. In this paper, a new in vitro method, capillary coated with cell membrane, was presented for drug screening based on the real biomembrane–drugs interaction, in which the cell membrane was applied to chromatography as pseudo-stationary phase directly. As the cell membrane was coated on the bare-fused capillary via sol–gel technology in our present work, it will be shown to be superior to other pseudo-stationary phases mimicking biological environment. Meanwhile, the quantitative retention–activity relationships (QRAR) model of β-lactam antibiotics was studied through investigating the effect of the membrane coating amount and pH of running buffer on the retention behaviors of the drugs and the logarithm of octanol–water partition coefficient (log *P*) at pH 7.4 and pH 6.5 were also obtained for comparison. The results showed that the capillary coated with cell membrane could suit the study of QRAR model.

Keywords: Cell membrane; Capillary; β-Lactam antibiotics; QRAR

1. Introduction

In recent years, micellar liquid chromatography (MLC) and micellar electrokinetic chromatography (MEKC) have attracted considerable attention as an in vitro model to predict the pharmacological and pharmacokinetic properties of drugs in the early stage of the drug discovery phase. Medina-Hernandez and co-workers developed a Brij35 MLC system as biopartitioning micellar chromatography (BMC) [1]. The BMC system was used to predict different pharmacological behaviors of drugs such as toxicity [2,3], absorption [4], bioconcentration [5] and blood–brain barrier [6]. Recently, BMC was used to evaluate quantitative structure–property relationships for pesticides [7]. Yang and co-workers developed a dodecyl dimethyl betaine (BS-12) MLC system for predicting protein–drug binding [8]. The results showed that MLC was a better model to predict some pharmacological activities. The usefulness of MEKC in establishing good pharmacodynamic models could be attributed to the following: The retention of a drug in the MEKC system is mainly governed by its hydrophobic, electronic properties, and to a lesser extent, by its steric properties. Previous studies in our laboratory have described and estimated pharmacological parameters of cardiovascular system drugs by the capability of biopartitioning micellar chromatography [9].

The log *P* value, defined as the logarithm of the partition coefficient between *n*-octanol and water, is an important parameter to judge a molecule's drug likeness [10]. Meanwhile, it has been widely utilized as an important parameter in quantitative structure–activity relationship (QSAR) analysis such as Hansch approach. QSARs are employed as scientifically credible tools for predicting the acute toxicity of chemicals when few empirical data are available [11]. Some studies have been performed on the relationship between toxicity and chemical structure for several compounds [12]. Several QSARs which predict toxicity values for Daphnia, green algae, and fish [13] have been developed. And many QSARs estimate Vibrio fischeri

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toxicity for specific groups of compounds using molecular and physicochemical descriptors [14,15].

It is well known that the cellular membrane plays an important role in medicine transporting. Phospholipids, which are composed of biomembrane, have been covalently immobilized to silica propyl amide particles (immobilized artificial membrane chromatography) [16]. Immobilized liposome, proteoliposomes and biomembrane vesicles have been proposed as stationary phases for chromatographic analysis of membrane–solute interaction [17]. Biochromatography with immobilized protein stationary phase has been applied to probe the interaction between the group of compounds in Traditional Chinese Medicines (TCMs) and the proteins [18,19].

As there should be some active biomacromolecule in the biomembrane, which is totally different from the synthesized membrane, more attention has been paid to immobilization of biomembrane or whole cells for chromatography in the recent years [20]. Modern pharmacological studies have shown that combining cell membrane with some receptors or channels is the first step of drug action. Therefore, the ability of a drug to interact with cell membranes is very important for the behavior of the drug in the organism.

In this paper, a novel capillary electrophoresis system by coating with cell membrane was developed as an in vitro model to predict the physicochemical parameter. As the cell membrane can provide more interacted site to those drugs having similar chemical structure, the capillary coated with cell membrane can simulate the human body circumstance more substantially than others. Apart from the effect of running buffer pH and cell membrane coating amount on the retention factors were investigated, the retention data of β-lactam antibiotics obtained in this membrane system were compared with the logarithm of octanol–water partition coefficient (log P), the correlation between the retention factors (k) and the Eigenvalue (log P) of β-lactam antibiotics drugs was also investigated. Finally, quantitative retention–activity relationships (QRAR) model of β-lactam antibiotics was established, the statistical analysis done and predictive features of the QRAR model were studied.

2. Materials and methods

2.1. Chemicals and materials

Bare-fused-silica capillaries (150 μm i.d., total length is 41 cm and the effective length is 33 cm) were obtained from Yong Nian Optical Factory (Hebei Province, China). Tetramethyl orthosilicate (TMOS) and glycidochloropropyl methyl trimethoxy silane (HG-560) were obtained from Hangzhou Guibao Chemical Co. Ltd (Jiangsu Province, China). The blood of healthy volunteer with added liquemine was provided by Hebei University Hospital. Running buffer was prepared with disodium hydrogenphosphate and sodium dihydrogenphosphate (Tianjin Beilian Chemical Co. Ltd, Tianjin, China). The running buffer and the solution injected into the chromatograph system were filtered through 0.45 μm nylon membrane respectively. Cloxacillin, ampicillin, azlocillin, amoxicillin, piperacillin, penicillin, cefradine, cefuroxime, cefotaxime, ceftazidime, cefoperazone, cefazolin, cefpiramide, ceftriaxone and cefepime were obtained from Institute of Medicament (Hebei Province, Shijiazhuang, China). Micellar phases were prepared by aqueous solutions of sodium dodecyl sulfate, Huazhen Reagent, Tianjin, China. Micellar eluent pH was adjusted with 0.02 mol/L phosphate buffer, which was prepared with disodium hydrogenphosphate and potassium dihydrogenphosphate (analytical reagent, Beijing, China). And all the compounds were prepared at the concentration of 1.0 g/L. All the other reagents were of analytical-reagent grade.

2.2. Instrumentation and measurements

The experiment was performed on a Waters Quanta 4000 capillary electrophoresis system (Milford, MA, USA) with a built 0–30 kV high voltage power supply, a fixed wavelength UV detector near the column end and a forced-air cooling system. Data processing was carried out with a JASCO LC-1500 chromatography workstation. Centrifuge equipment was Anke TGL-16G-A High Speed Refrigerated Centrifuges.

Bare-fused-silica capillary (150 μm i.d.) and the cell membrane capillary that had the same length were used as the analytical column. The detection wavelength was 254 nm, and the applied voltage was 7 kV. Samples were injected into the capillary by gravitational force for 5 s and the sensitivity of the detector was 0.005 AUFS. All the assays were carried out at 25.0 °C. Data collection and processing was treated by CKChrom Chromatography Data System. SPSS 13.0 for windows program was used to perform the statistical analysis of the regressions.

2.3. Preparation of the capillary column coated with cell membrane

The column preparation involved the following steps: (1) pretreatment of the capillary: the fused-silica capillary was flushed with sodium hydroxide (1.0 mol/L) for 1h and then with distilled water for 15 min. Then the capillary was kept under a flow of nitrogen overnight. (2) Preparation of the cell membrane: (1) 1 mL blood and 9 mL physiologic saline were mixed and then transferred to refrigerated centrifuge and centrifuged at 1000 rpm for 20 min. Precipitation made of red cells was collected. (2) The precipitation was mixed with 10 mL deionized water for swelling the red blood cell. The mixture was centrifuged at 8000 rpm for 20 min. The precipitation made of cell membrane was washed three times with deionized water. Then the precipitation of cell membrane was diluted five-fold, eight-fold, ten-fold, fifteen-fold, and stored in the refrigerator at 4 °C. (3) Coating procedure: the sol–gel solution, which was prepared by mixing TMOS, HG-560, ethanol and hydrochloric acid of 0.03 mol/L (5:6:3:1.5, V/V), was stirred for 6 h at room temperature and then coated onto the inner surface of capillary. This sol–gel solution was allowed to react with the inner surface of capillary for 15–20 min. Then the excess unreacted solution was flushed out under the nitrogen flow. The coated capillary was dried overnight under nitrogen flow [21]. For the membrane coated capillary, the cell membrane suspensions of

different amount were injected into the pretreated capillary and allowed to react with the inner surface of capillary for 1 h, then the excess suspension was flushed out under nitrogen flow for 10 min and the membrane coated capillary was dried overnight under nitrogen flow.

2.4. Formulary and data process

The retention factor (k) of β-lactam antibiotics was estimated according to an approach described in ref. [22]:

$$k = \left(\frac{t_R}{t_{R(REF)}}\right)(1 + k_{REF}) - 1, \quad (1)$$

The t_R is the experimental retention time of the β-lactam antibiotics assayed and $t_{R(REF)}$ is the experimental retention time of a reference compound (Acetanilide) injected during the working session. The use of this approach provided retention factor estimations that are more reliable and easier to obtain than the classical estimations based on the measurement of the dead time, reducing the impact of changing the column and running buffer, among other experimental factors, on the k estimations.

3. Results and discussion

As cell membrane permeability plays an important role in drug absorption, different models were used to examine drug uptake and transport in the intestinal epithelium. The cell membrane was composed of lipoids, proteins and polysaccharides. It has been widely accepted that lipophilicity and hydrophobicity are the key factors in oral drug absorption. The degree of protein binding is an important parameter in the evaluation of the pharmacological and pharmacokinetic properties of potential drugs, which greatly influences absorption, distribution, metabolism and excretion properties of typical drugs. Considering the potential of cell membrane with bioactivity in vitro, we focused on investigating the change of the k value affected by pH value and the cell membrane coating amount. Then the relationships between the logarithm of k and the logarithm of octanol–water partition coefficient (log P) were established.

3.1. How the cell membrane binds to the capillary

The phosphatidylethanolamine was contained in the cell membrane bound to the treated capillary via covalence binding (Fig. 1), because of the existence of amino-group in cell membrane. It was shown that the amino-group could stick to the sol–gel with the group of epoxy at room temperature. And there were other forces such as electrostatic interaction, hydrophobicity interaction, space interaction and affinity binding contributing to cell membrane coating.

3.2. Effect of biomembrane on the retention behaviors of drugs

The activity of biomembrane could be explained not only by the bilayer phospholipid but also by the biomacromolecule, which was made of peripheral proteins and integral proteins. Those membrane proteins contain acetylcholine esterase, folic acid acceptor, ATP synthase, sodium potassium ATPase and so on. Red blood cell membranes were electrostatically adsorbed on "Celite" or DEAE-cellulose for chromatographic analyses of D-glucose binding to the then unidentified sugar transporter in 1966 [23,24]. Based on the cell membrane activity, Dong developed a method for screening potential active components from TCMs by using biomembrane extraction and high performance liquid chromatography [25]. It is obvious that the cell membranes keep their activity in vitro.

As a novel pseudo-stationary phase applied to capillary, the effect of cell membrane on the retention behavior of β-lactam antibiotics was investigated. The pH of running buffer was adjusted with 0.02 mol/L phosphate buffer (pH 7.4). UV detector wavelength was 254 nm and applied voltage was 7 kV.

3.2.1. Comparison of the retention behaviors of different columns

The performances of the cell membrane column, the sol–gel column without cell membrane and the bare-fused capillary column were studied. Under the same experimental conditions, the retention behaviors of the drugs on these columns were investigated. The results were listed in Table 1.

The results above showed that the retention times of the drugs on the cell membrane column were much longer than that on bare-fused column and the sol–gel column. It could be explained that the biomacromolecule with activity in the cell membrane coating interacted with the tested drugs.

Because of the similar chemical structures and proximal pK_a value, no much change had ever been found for the retention times of the tested drugs on the bare-fused capillary column. But different results were obtained on the sol–gel column and the cell membrane column. Coating sol–gel may result in the change of EOF, which led to the change of retention behavior of drugs, but the differences were not very obvious. However, for the cell membrane column, great changes of the retention times of these drugs were observed in comparison with the bare-fused column. It indicated that the retention behavior of tested drugs was affected not only by EOF but also by the cell membrane coated on the capillary that interacted with the tested drugs. The reason was that the biomacromolecule with activity in the cell membrane interacted with the tested drugs, which implied that the cell membrane had been successfully coated on the capillary via sol–gel technology. Fig. 2 showed the different retention behaviors of ceftazidime on the bare-fused capillary, the capillary sol–gel and the capillary coating cell membrane.

3.2.2. Effect of cell membrane coating amount on the retention behavior of drugs

Fig. 3 showed the effect of the different cell membrane amount on the retention behaviors of β-lactam antibiotics. As it is hard to describe the cell membrane with molarity, the cell membrane amount was described as the cell membrane diluted obtained from 1 mL blood.

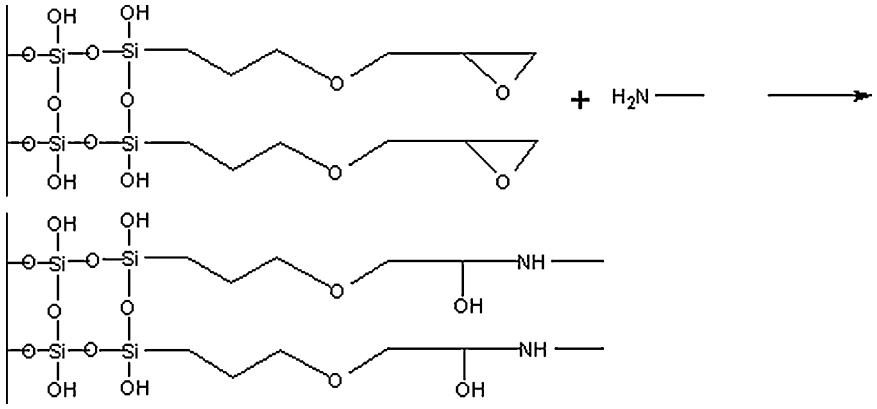

Fig. 1. The mechanism of cell membrane bound to the inner surface of a capillary.

Table 1
Comparison of retention times of the coated column and the bare-fused capillary ($n = 6$)

Drugs	*a* (min)	R.S.D.%	*b* (min)	R.S.D.%	*c* (min)	R.S.D.%
Cloxacillin	4.967	0.5	9.158	1.3	33.225	1.5
Azlocillin	4.508	0.4	7.458	1.4	23.917	1.4
Amoxicillin	4.417	0.7	7.583	1.1	16.650	1.6
Piperacillin	4.733	0.4	8.925	1.4	18.875	1.2
Ceftazidime	4.475	0.3	8.408	1.7	44.925	1.9
Cefazolin	4.750	0.2	10.558	0.9	26.900	1.5
Cefpiramide	4.375	0.6	8.925	1.5	20.267	2.1
Cefoperazone	4.350	0.4	7.783	1.3	19.317	0.8
Cefepime	4.550	0.6	7.717	1.1	21.055	1.4

a, The retention times on the bare-fused capillary; *b*, the retention times on the capillary coating sol–gel; *c*, the retention times on the capillary coating cell membrane.

As shown in Fig. 3, with the membrane amount increasing, the retention behaviors of tested drugs experienced a big change. Low membrane amount such as at a ten-fold dilution leads to shorter retention time. And the higher is the membrane coating amount, the longer is the retention time. The reason may be that the more the cell membrane coated on the capillary, the more was the interacting site provided. With the increase in the amount of cell membrane coated, the interaction between the coating and tested drugs strengthens largely. But when the cell membrane amount was higher than diluted five-fold, the retention behavior did not change obviously any more. So the cell membrane amount diluted five-fold was selected as the tested coating amount in the following experiments.

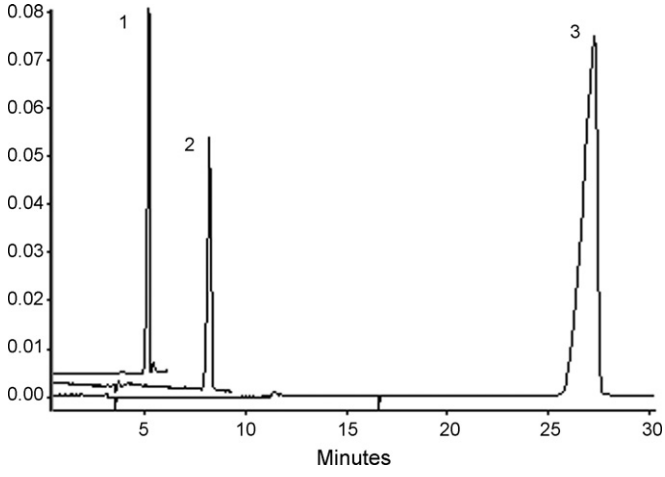

Fig. 2. The retention behavior of ceftazidime on different column: (1) the bare-fused capillary; (2) the capillary coated by sol–gel; (3) the capillary coating cell membrane with sol–gel.

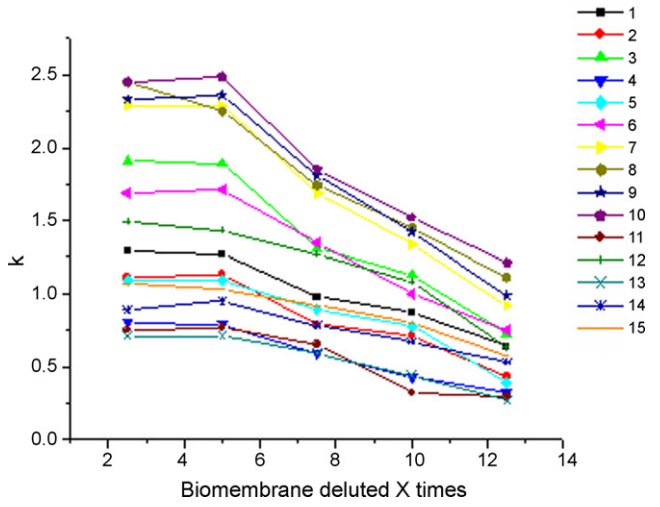

Fig. 3. Effect of cell membrane concentration on the retention factors of β-lactam antibiotics in membrane capillary: (1) cloxacillin; (2) ampicillin; (3) azlocillin; (4) amoxicillin; (5) piperacillin; (6) penicillin; (7) cefradine; (8) cefuroxime; (9) cefotaxime; (10) ceftazidime; (11) cefoperazone; (12) cefazolin; (13) cefpiramide; (14) ceftriaxone; (15) cefepime.

3.3. Effect of pH on the retention behaviors of drugs in capillary coated with cell membrane

To investigate the effect of the pH value of running buffer, the retention factors of drugs on the membrane coating capillary were obtained at several pH values ranging from 6.0 to 8.0. Fig. 4 shows the effect of pH on the retention behaviors of some representative drugs.

It can be seen from Fig. 4 that the retention times of β-lactam antibiotics changed with pH value of the running buffer and EOF. Because the pK_a values that range from 2.4 to 2.7 of those β-lactam antibiotics were similar to each other, the ionization states of those drugs were similar to each other at the experiment condition, which would result in the similar retention times (Table 1) for these drugs. However, due to the influence of the cell membrane, although the drugs have similar chemical structure, retention behaviors are quite different. Besides, at pH 7.4, most of the drugs have the strongest interaction with cell membrane than that at other pH values (Fig. 4).

The above results indicated that at the pH level of human body, the cell membrane with the biggest biology activeness played an important role in interacting with the tested drugs. Since the structure of active molecule was affected by the pH value of running buffer, it leads to the change of the function of the active molecules. That may affect the interaction between the stationary phase and the tested drugs in turn. The results also showed that pH has great effect on the retention factors and similar trends appeared for drugs with similar structures, which is quite different from BMC models [9]. Because the pH was adjusted to 7.4 to obtain experimental conditions as close as possible to physiological ones, and the pH 6.5 was considered as the average pH of the small intestine, so the retention time of drugs in pH 6.5 and pH 7.4 were chosen to establish the model in this experiment.

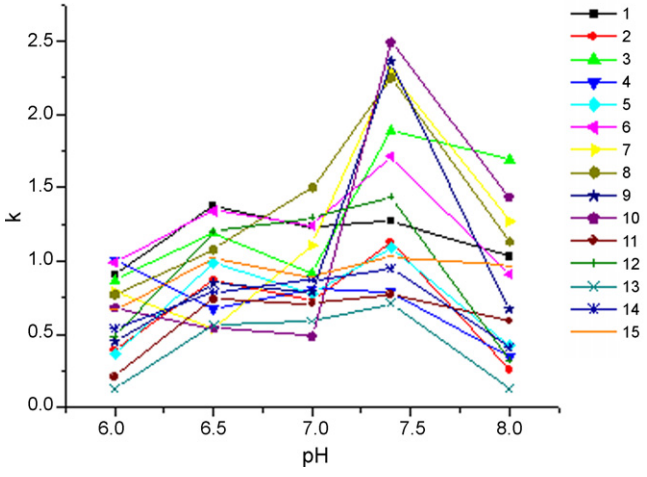

Fig. 4. Effect of the pH on the retention factors of β-lactam antibiotics in membrane capillary: (1) cloxacillin; (2) ampicillin; (3) azlocillin; (4) amoxicillin; (5) piperacillin; (6) penicillin; (7) cefradine; (8) cefuroxime; (9) cefotaxime; (10) ceftazidime; (11) cefoperazone; (12) cefazolin; (13) cefpiramide; (14) ceftriaxone; (15) cefepime.

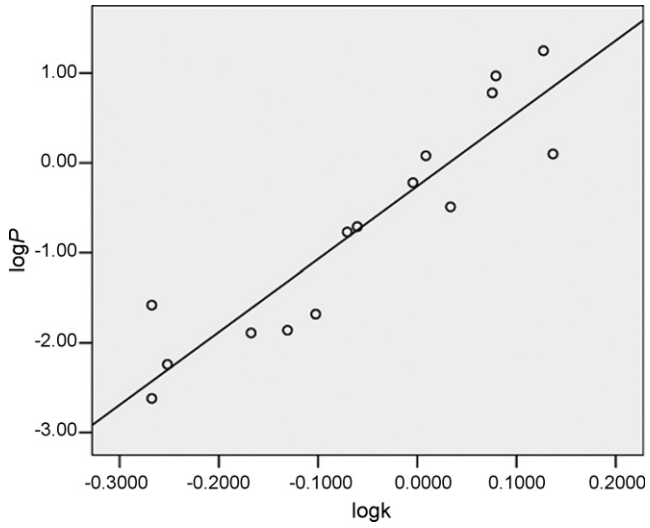

Fig. 5. Relationship between retention data on the capillary coating membrane at pH 6.5 and the log *P* of β-lactam antibiotics.

3.4. Performance of the column coating cell membrane

The cell membrane capillary via sol–gel technology has being working continuously for 15d online. Good repeatability and resolution were obtained. But bad resolution and ugly shapes of the peaks were obtained on the membrane capillary beyond 15d.

Coating the bare-fused capillary with cell membrane but without sol–gel, the retention times of β-lactam antibiotics were also longer than that of on the bare-fused capillary. But the peak shapes of β-lactam antibiotics were very poor. The repeatability of the separation was bad and resolution became worse in the second run. The reason may be that the cell membrane was not stably coated on the wall of the capillary.

To evaluate this coated column, six 150 μm × 41 cm i.d. capillaries coated by cell membrane were prepared under identical conditions and used to test column-to-column reproducibility. The R.S.D. values were 1.9% for column efficiency (*N*), 1.7% for retention factor (*k*). And each sample continuously injected six times to test run-to-run reproducibility. The R.S.D. values were 1.7% for column efficiency (*N*), 1.4% for retention factor (*k*). To test the stability of membrane column, day-to-day reproducibility was carried out. The R.S.D. value was 2.4% for column efficiency (*N*), 2.6% for retention factor (*k*). These values indicated that the reproducibility was excellent for this coated column and the membrane column offered good stability.

3.5. Establishment of QRAR model

According to the above results, the quantitative relationship was established between the retention factors on the capillary coated with cell membrane and the log *P* for β-lactam antibiotics at pH 6.5 and pH 7.4, respectively. Table 2 showed the log *P* value [26] of β-lactam antibiotics. The experimental data of *k* versus log *P* were given in Figs. 5 and 6.

The correlation equations were:

Table 2
log *P* value of β-lactam antibiotics

Sample	Cloxacillin	Ampicillin	Amoxicillin	Azlocillin	Piperacillin
log *P*	0.10	−0.71	−1.89	0.78	0.22
Sample	Cefotaxime	Cefuroxime	Benzylpenicillin	Ceftazidime	Cefazolin
log *P*	−0.77	−0.49	1.25	−2.62	0.97
Sample	Cefpiramide	Cefoperazone	Cefradine	Ceftriaxone	Cefepime
log *P*	−2.24	−1.86	−1.58	−1.68	0.08

At pH 6.5:

$$\log P = 8.1(\log k) - 0.26 \quad n = 15, R = 0.92 \tag{2}$$

At pH 7.4:

$$\log P = -127.99(\log k)^3 + 8.84(\log k)^2 + 12.67(\log k) - 0.87 \quad n = 15, R = 0.94 \tag{3}$$

where n is the number of drugs and R is the correlation coefficient.

Table 3 contains the statistical analysis and the predictive features of the QRAR models obtained. Since the *P*-values for QRAR models are less than 0.05, there is a statistically significant relationship between the retention of β-lactam antibiotics on the capillary coated with cell membrane and log *P* at the 95% confidence level. And while the running buffer at pH 6.5, a linear relationship was obtained. But at pH 7.4, a non-linear relationship was obtained.

The pH value of running buffer influences not only the ionization states of β-lactam antibiotics but also the structure of proteins and polysaccharide of cell membrane, which affect the interaction between cell membrane and tested drugs as more than half of membrane proteins were the potential target site of drugs [27]. Bohr effect explained the H^+ concentration influences on the structure of haemoglobin, which leads to the change of function [28]. It indicated that pH value of running buffer might affect the biofunction of membrane proteins. The activity of many proteins and polysaccharides may be reduced at pH 6.5, that lead to the linear relationship between the logarithm of retention times and the logarithm of octanol–water partition coefficient (log *P*).

log *P*, which is the dominating independent parameter in the field of aquatic toxicity, can also contribute to the uncertainty of the QSAR models. Experimental values are difficult to obtain. The membrane–water partition was more realistic than octanol–water partition in describing absorption process of human body. The linear relationship between the retention times of β-lactam antibiotics and log *P* showed that the establishing of QRAR could be a simple and convenient method to predict the log *P* value of new β-lactam antibiotics. And the non-linear relationship may be more realistic than the other QRAR model in describing the pharmacodynamical parameter in human body.

3.6. Comparison with QRAR models of MEKC

In order to compare with other QRAR model, the QRAR model of β-lactam antibiotics was established by MEKC. This study was achieved under the condition of micellar phase of 0.02 mol/L SDS, 0.02 mol/L phosphate solutions. In order to reproduce the osmotic pressure of biological fluids, 5% dimethylcarbinol was added to the micellar mobile phase. And the relationship (Fig. 7) between log *k* and log *P* was established.

Comparing Fig. 5 with Fig. 7, it is obvious that both of the models can give good linear relationships between log *k* and

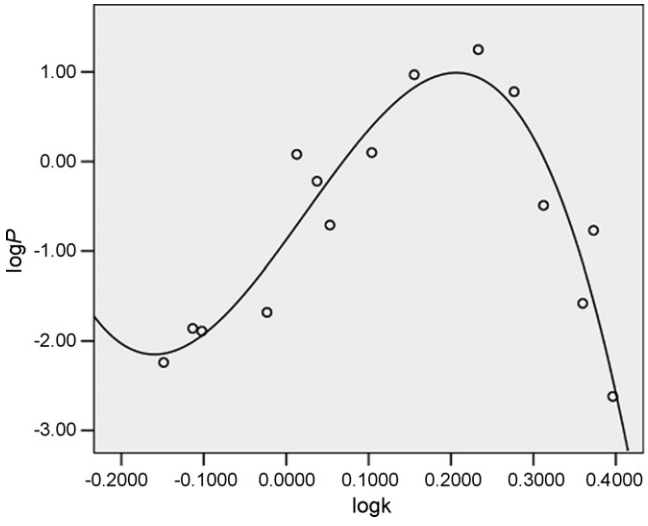

Fig. 6. Relationship between retention data on the capillary coating membrane at pH 7.4 and the log *P* of β-lactam antibiotics.

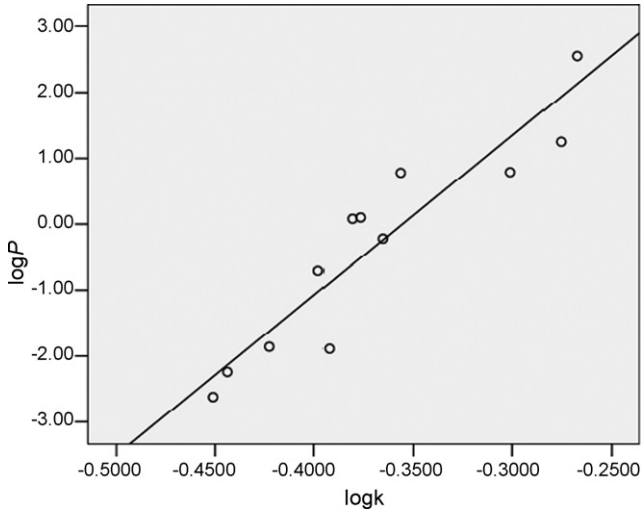

Fig. 7. The relationship between log *k* and log *P* at pH 6.5, running buffer: 0.02mol/L SDS.

Table 3
Statistical analysis and predictive features of the QRAR models

Model	Unstandardized coefficients		*T*	*R*	S.E.	*F*	Sig
	B	S.E.					
1	−0.259	0.138	−1.877	0.921	0.491	73.651	0.0004
	8.113	0.945	8.582				
2	−0.921	0.390	−2.363	0.936	0.656	25.699	0.0002
	1.524	1.756	0.868				

Statistically significant: 95% confidence interval for coefficients estimates; *B*: deflection regression; S.E.: standard error of the estimate; *T*: *T*-test; *R*: correlation coefficients; *F*: *F*-ratio.

log *P* at pH 6.5, but the micelle system and the cell membrane are quite different at pH 7.4. It means that the micelle system and the cell membrane at pH 6.5 without the bioactivity or less bioactivity were good at describing the process of octanol–water partition, but the bioactivity of biomacromolecule in cell membrane cannot be reflected in those models. Though a good linear relationship of log *k*–log *P* was obtained, it could not describe the absorption of drugs to the cell membrane exactly in the human body. As an in vitro method to describe pharmacological behaviors of drugs, the approach of coating cell membrane was not a perfect method, but more realistic than other methods in mimicking human body environment. The approach coating cell membrane provided a simple and more realistic method.

4. Conclusion

The approach proposed in this paper, involving QRAR could be a useful and rapid tool to estimate the log *P* value of a set of drugs. The retention behavior of compounds on the membrane capillary, which depends on biomacromolecule with activity, is capable of describing the absorption process in human body. This approach may be very useful in the development of new drugs studying pharmacology.

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