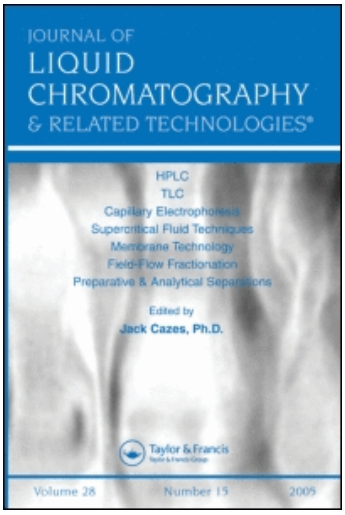

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Mixed-Model of Affinity and Hydrophobic Interaction for Drug Retention in Cell Membrane Chromatography

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Abstract: According to the stoichiometric displacement concept and some reasonable assumptions for the surface property and the interaction between the cell membrane stationary phase (CMSP) and drugs, a mixed model of affinity and hydrophobic interaction for drug retention on the CMSP is reported. An equation expressing the "mixed mechanism" was tested by five kinds of calcium antagonists and a rabbit myocardium CMSP with three kinds of different mobile phases. The experimental results basically coincide with that of the theoretical expectation.

Keywords: Cell membrane stationary phase, Cell membrane chromatography, Stoichiometric displacement model, Retention mechanism, Calcium antagonist

INTRODUCTION

There are various models for describing the retention characteristics of solutes in liquid chromatography (LC). For example, solubility parameter models,[1] solvatochromic models,[2–4] and solvophobic models[5,6] are suitable for that of reversed-phase liquid chromatography (RPLC). In addition, Snyder[7] also reported a retention model which is applied in liquid-solid absorption chromatography. These models can reflect the basic property and the rule of the solute retention in an LC system, resulting in an insight into the

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chromatographic separation of solutes. An intensive understanding should lead to promoting these chromatographic methods for wide applications. The authors have first prepared a cell membrane stationary phase (CMSP) and established a cell membrane chromatography (CMC) to be a chromatographic system of bionics.[8,9] The stationary phase in this kind of chromatographic system has both the characteristics of cell membrane bio-activity and chromatographic separation.

Due to different interactions among components in the CMC system, solute retention in the CMC should have some special and complex properties. If a model of solute retention is derived only by one kind of interaction force between solute and stationary phase, it would be hard to explain the mechanism of drug retention on the CMSP in this study. The stoichiometric displacement theory for retention (SDT-R) of solutes[10,11] was derived by means of five kinds of thermodynamic equilibria representing each kind of interaction occurring in RPLC should be used for the most of LC, except size exclusion chromatography (SEC).[12,13] In addition, a mixed-mechanism of ion-exchange and hydrophobic interaction, originally derived for protein retention, also derived by the SDT-R being suitable either for ion-exchange (IEC) or hydrophobic interaction chromatography (IEC), was reported.[14,15] In this study, the SDT-R would be expected to be suitable for describing the complex interactions between the drug and the cell membrane or the membrane receptor on the CMSP. The experimental results indicated that the drug retention in the CMC mathematically follows this direction. However, from the point of view of physical meaning, the retention mechanism of drugs in the CMC is a mixed-mechanism of affinity and hydrophobic interaction, but not ion-exchange and hydrophobic interaction. The influences of salts and their concentrations in an aqueous salt solution on the drug retention at a given temperature (37°C) were also investigated.

EXPERIMENTAL

Instrumentation

A Waters high performance liquid chromatographic system (Milford, MA, USA) was employed in this study. A Hermel ZK401 high speed refrigerated centrifuge (Berthold, Hermel AG, Gosheim, Germany), an LKB-2219 circulating water bath (Bromma, Sweden), a CS-20 supersonic cleaner (Shimadzu, Kyoto, Japan), and an EA940 IonAnalyzer (Orion Research Incorporated, Cambridge MA, USA) were also used.

Chemicals

Verapamil hydrochloride (VP), diltiazem hydrochloride (DT), nimodipine (NM), and nitrendipine (NT) were bought from RBI (Natick, MA, USA).

Nifedipine (NF) was obtained from Sigma (St. Louis, MO, USA). Ammonium sulphate ($(NH_4)_2SO_4$), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), sodium dihydrogen phosphate (NaH_2PO_4), sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4), and ammonia water ($NH_3 \cdot H_2O$) are of analytical grade. Deionized water is of HPLC grade. All solvents were filtrated through a 0.45 μm membrane filter.

Chromatographic Conditions

The cardiac muscle CMSP column (50 mm × 2.0 mm I.D.) was prepared according to the literature.[8,9] The detection wavelength, flow rate, and column temperature were selected at 236 nm, 0.5 mL · min^{-1}, and 37°C, respectively. The dead volume (V_0) of the column was determined by a solvent with no retention. The following three mobile phases were used in this study: (1) 50 mmol · L^{-1} ammonium sulphate buffer (pH 7.4); (2) 50 mmol · L^{-1} ammonium phosphate buffer (pH 7.4); (3) sodium phosphate buffer (pH 7.4). The concentration of water ($[W]_i$) in mobile phases (1)–(3) was calculated according to the following equation:

$$[W]_i = \frac{[(d_s - d_w - M)S/N + d_s] \times 1000}{18} (\text{mol} \cdot \text{L}^{-1}) \quad (1)$$

Where, d_s is the densities of 3 mol · L^{-1} $(NH_4)_2SO_4$ ($d_s = 1.198$), 2.0 mol · L^{-1} $(NH_4)_2HPO_4$ ($d_s = 1.134$), and 1.0 mol · L^{-1} Na_2HPO_4 ($d_s = 1.071$) in water solutions, respectively, d_w is the water density ($d_w = 1$), M denotes the amounts of 3.0 mol · L^{-1} $(NH_4)_2SO_4$ (M = 0.3964 g · mL^{-1}), 2.0 mol · L^{-1} $(NH_4)_2HPO_4$ (M = 0.2641 g · mL^{-1}) and 1.0 mol · L^{-1} Na_2HPO_4 (M = 0.1420 g · mL^{-1}) in 1 mL salt water solution (g mL^{-1}). S represents the concentration of salt in mobile phase measured, the number 18 is the molecular weight of water, and N is the moles of the salt when its density is d_s.

RESULTS AND DISCUSSION

Aqueous Ammonium Sulphate Solution

When the rabbit cardiac muscle cell membrane column was used, the capacity factors of CaAs, verapamil, diltiazem, niffedipine, nitrendipine, and nimodipine were measured under different concentrations of ammonium sulphate of 0.01 to 1.6 mol · L^{-1}. The resultant curves of the k' vs. C appears as a "U" shape shown in Figure 1. In the $k' \sim C$ curves, the k's of verapamil and diltiazem show a monotonous reduction with the increases in the salt concentration in the low salt concentration region (0.01 ∼ 0.5 mol · L^{-1}), while that indicates a monotonous increase in the high salt concentration region (0.5 ∼ 1.6 mol · L^{-1}). However, the changes in k's of nifedipine, nitrendipine,

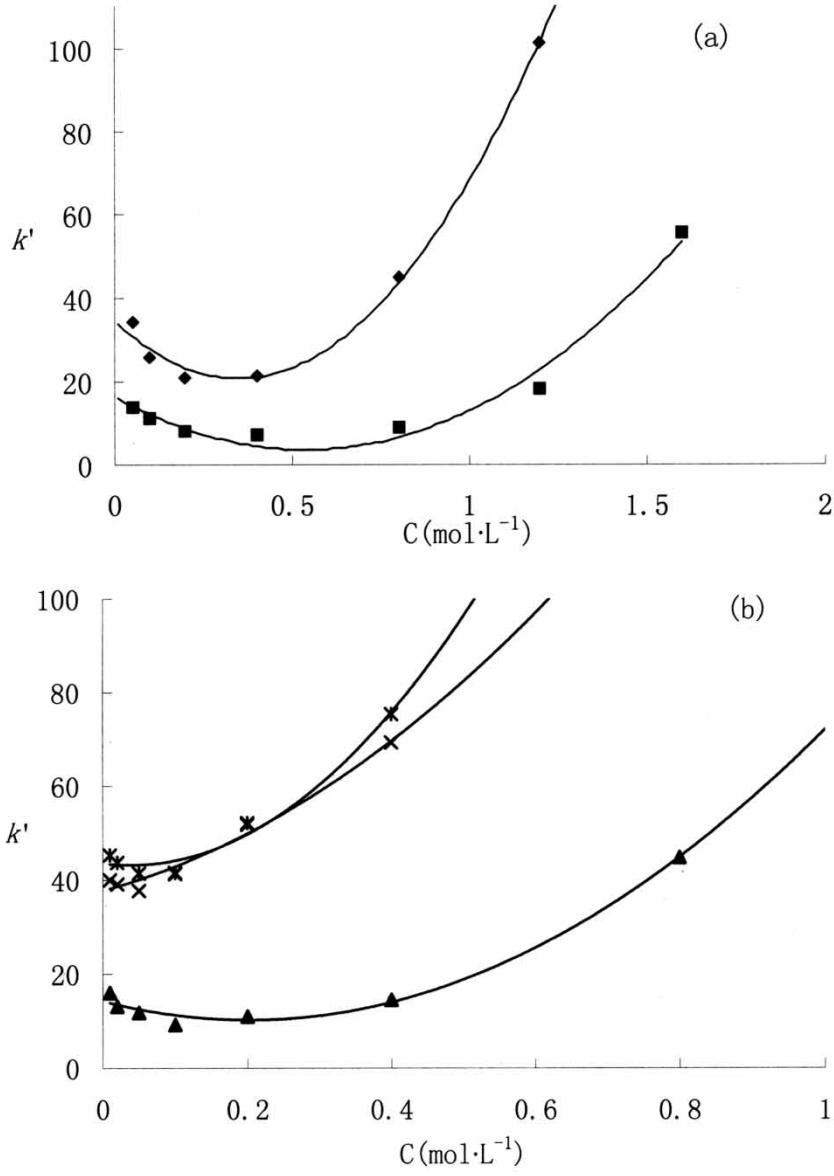

Figure 1. Effect of the concentration of $(NH_4)_2SO_4$ (C) on the drug retention molecule. Drugs: (a) verapamil (◆), diltiazem (■), (b) nimodipine (✳), nitrendipine (×) and nifedipine (▲). Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.), Mobile phase: $0.01 \sim 1.6\,mol \cdot L^{-1}$ ammonium sulphate buffer (pH 7.4), Flow rate: $0.5\,mL \cdot min^{-1}$, Detection: 236 nm, Temperature: 37°C.

and nimodipine were found to be different. The region in the low salt concentration appears more narrow ($0.01 \sim 0.1\,\text{mol}\cdot\text{L}^{-1}$) and the increase in k's shows low salt concentration. But there still is a clear increase with the salt concentration ($0.1 \sim 0.8\,\text{mol}\cdot\text{L}^{-1}$).

The elution curves with 'U' shapes were reported to be a double-mechanism of ion-exchange and hydrophobic interaction for protein retention in either IEC, or HIC.[14,15] Thus, a double- or mixed-mechanism for drug retention must exist. The first question that should be answered, i.e., does the equation describe the elution curve of the "U" shape fit? That is shown in Figure 1. To answer this question, we must understand the equation and each physical meaning.

Aqueous Ammonium Phosphate Solution

Figure 2 shows the plot of k' vs. C of CaAs in the concentration range of ammonium phosphate of 0.01 to $1.5\,\text{mol}\cdot\text{L}^{-1}$, under the same condition as those of CMSP in Figure 1. As shown in Figure 2, the k's of verapamil and diltiazem appear to have a decreasing tendency with the salt concentration increasing in the low salt concentration region, while that of a continuous increase in the high salt concentration region. However, the k's of nitrendipine, nimodipine, and nifedipine have a continuously monotonous increase, and almost do not exist at a lowest point in their k' vs. c curves. The chromatographic behavior of nitrendipine appears almost the same as that of nimodipine, with only a little difference.

Aqueous Sodium Phosphate Solution

Owing to the small solubility of sodium phosphate in water, the k' of CaAs measured is only from a very low salt concentration region of 0.01 to $0.03\,\text{mol}\cdot\text{L}^{-1}$, under the same condition as those of CMSP pointed above. But from the plot of k' vs. the concentration of disodium hydrogen phosphate C shown in Figure 3, the changeable tendency of the drug retention can still be seen. The k' values of verapamil and diltiazem show a monotonous decrease with the increases in the concentration of disodium hydrogen phosphate solution, while that of nitrendipine, nimodipine, and nifedipine appears as a linear increase. The average and standard deviations from that are still very small.

Imitation of the Elution Curve with "U" Shape by Equation

The CMSP prepared by the active cell membrane has the following characteristics: (1) to maintain the basic feature and enzymatic activity of the cell membrane; (2) drug receptor (membrane protein) on it basically maintains

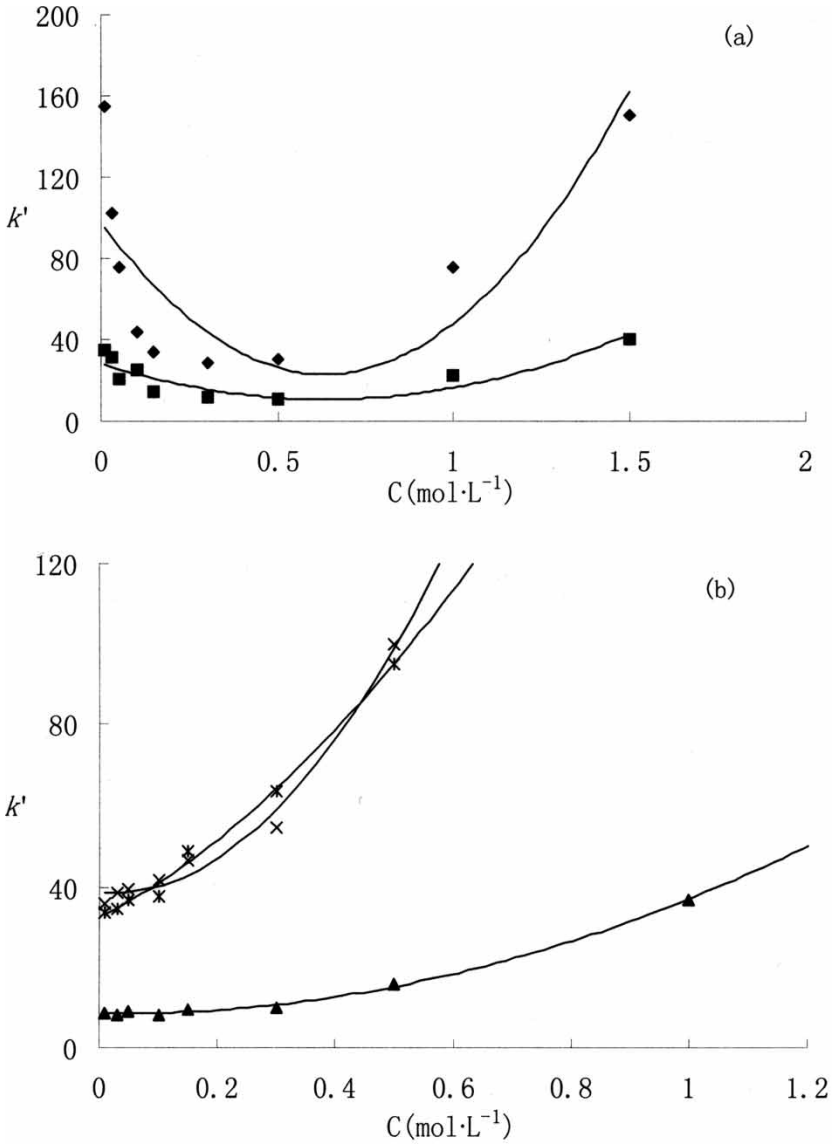

Figure 2. Effect of the concentration of $(NH_4)_2HPO_4$ (C) on the drug retention. Drugs: (a) verapamil (◆), diltiazem (■), (b) nitrendipine (×), nimodipine (✳) and nifedipine (▲). Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.). Mobile phase: $0.01 \sim 1.5\,mol \cdot L^{-1}$ ammonium phosphate buffer (pH 7.4), Flow rate: $0.5\,mL \cdot min^{-1}$, Detection: 236 nm, Temperature: 37°C.

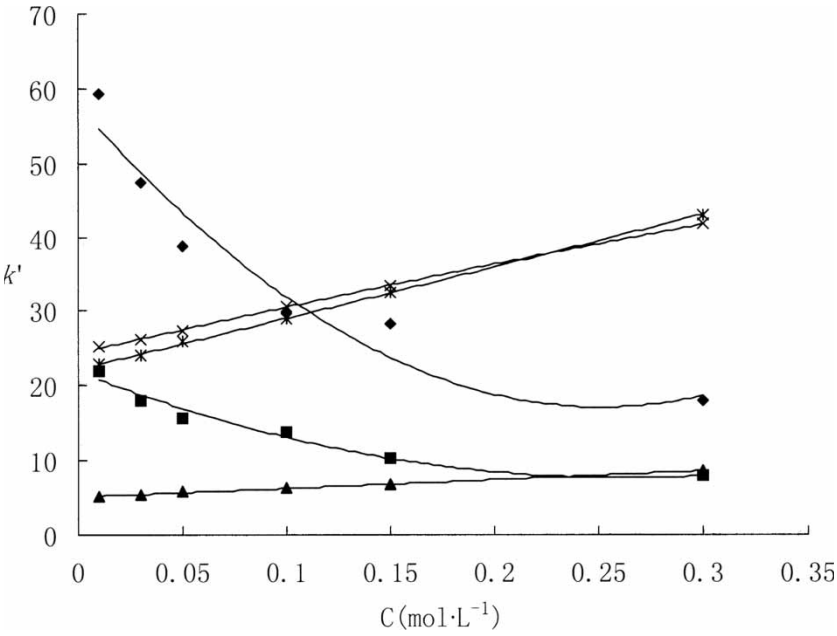

Figure 3. Effect of the concentration of Na_2HPO_4 (C) on the drug retention. Drugs: nimodipine (✳), nitrendipine (×), verapamil (◆), diltiazem (■) and nifedipine (▲). Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.). Mobile phase: $0.01 \sim 0.03\,mol \cdot L^{-1}$ sodium phosphate buffer (pH 7.4), Flow rate: $0.5\,mL \cdot min^{-1}$, Detection: 236 nm, Temperature: 37°C.

its original stereochemical structure and its environment; (3) to be strongly polar in heterogeneous distribution on the surface; (4) to have a hydrophobic layer formed by the lipid linkage and a hydrophobic cavity formed by the membrane protein.

To describe the interactions between drug molecules and cell membranes on the CMSP, it is first important to understand the type of the interactions among components in CMC. As is well known, a cell membrane is a lipid-protein assembly, and can be referred as a phospholipid bilayer. Thus, some membrane proteins (receptors) can go through the whole width of the bilayer. The lipid-protein membranes are amphipathic, with hydrophobic functions associating with each other in the interior of the membrane, and the hydrophilic function on its outside binding polar molecules. But, a specific affinity between drug molecules and membrane protein should exist when the cell membrane on the CMSP still keeps its enzymatic activity. Therefore, the identification and magnitude of the interactions between them is very difficult to acertain.

Wei et al.[17] presented an equation to describe a curve of "U" shape for a mixed mechanism of ion exchange and hydrophobic interaction of proteins. This equation should also be suitable for elucidating drug retention in CMC in this study, and is shown as:

$$\log k' = \{\log K_5 + \log \varphi + n(r + r')\log K_e\} - nr\log\{1 + K_e(m - q + 1)[W]\} - q\log[W] - (n^2 r' + q')\log[S] \quad (2)$$

where K_5 is a thermodynamic equilibrium constant for a drug displacing displacer when the solvated drug is adsorbed by the solvated CMSP. K_e and m are the parameters relating to the CMC system used. The terms of nr and nr' are the moles of water and salt molecules, which are released from the CMSP at the interface between the CMSP and the drug molecules, respectively. The q and q' corresponding to nr and nr' represent the moles of water and salt molecules released from the drug molecules at the interface between the CMSP and the drug molecules, respectively. φ is the column phase ratio. $[W]$ and $[S]$ are water and salt molar concentration. Equation (2) can be also represented as:[17]

$$\log k' = B_0 + B_1\log[W] + B_2[W] + B_3[W]^2 + B_4\log[S] \quad (3)$$

where:

$$B_0 = \log K_5 + \log \varphi + n(r + r')\log K_e \quad (4)$$

$$B_1 = -q(5)$$

$$B_2 = nr(m - q + 1)K_e \quad (6)$$

$$B_3 = 1/2nr(m - q + 1)^2 K_e^2 (7)$$

$$B_4 = -(n^2 r' + q') \quad (8)$$

According to the physical meanings of nr, q, q', and r', the B_1, B_2, and B_3 in Eqs. (5–7) above would be the contribution of hydrophobic interaction to the drug retention, while the B_4 in Eq. (3) would be that of salt in the mobile phase to drug retention.

By using a multivariate linear regression analysis, the experimental data fit, Eq. (3), each set of parameters ($B_0 - B_4$) obtained is listed in Tables 1 and 2. It indicates that the theoretical expectation fits the experimental result well, when ammonium sulphate is the salt in the mobile phase. The average and standard deviations of the results are less than 0.04 and 0.05, respectively.

The retention rule of CaAs in the aqueous ammonium phosphate solution generally is similar to that obtained from the aqueous ammonium sulphate solution and can be also expressed by Eq. (3). The predicted retention log k'_p and experimental log k'_e of the CaAs really coincides well with only very

Table 1. Parameters (Eq. (7)) and deviations for five kinds of drugs in the CMSP

	Parameters in Eq. (7)						
Drugs	B(0)	B(1)	B(2)	B(3)	B(4)	*AD*	*SD*
Verapamil	14.58	−4.18	8.89×10^{-1}	3.79×10^{-3}	−0.733	0.0113	0.0146
Diltiazem	−10.78	−1.48	7.76×10^{-1}	9.59×10^{-3}	−0.763	0.0375	0.0462
Nifedipine	2.82	8.13	1.42×10^{-1}	2.79×10^{-3}	−0.357	0.0223	0.0280
Nitrendipine	−5.12	15.29	3.63×10^{-1}	2.66×10^{-5}	−0.056	0.0100	0.0136
Nimodipine	−5.91	18.11	4.36×10^{-1}	1.79×10^{-5}	−0.125	0.0074	0.0097

Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.). Mobile phase: 0.01–1.6 mol · L^{-1} ammonium sulphate buffer (pH 7.4), Flow rate: 0.5 mL · min^{-1}, Detection: 236 nm, Temperature: 37°C. *AD* = average deviation, *SD* = standard deviation.

Table 2. Retention values, prediced values and deviation for five of the drugs in the CMSP

Drugs		Concentration of $(NH_4)_2SO_4$ ($mol \cdot L^{-1}$)							
		0.01	0.02	0.05	0.1	0.2	0.4	0.8	1.2
Verapamil	$\log k'_e$			2.00	1.65	1.33	1.31	1.41	1.54
	$\log k'_p$			2.01	1.64	1.35	1.30	1.39	1.54
	d			−0.01	0.01	−0.02	0.01	0.02	0
Diltiazem	$\log k'_e$			1.26	0.94	0.85	0.91	1.04	1.14
	$\log k'_p$			1.21	1.01	0.86	0.88	1	1.18
	d			0.05	−0.07	−0.01	0.03	0.04	−0.04
Nifedipine	$\log k'_e$	1.65	1.16	1.04	0.96	1.07	1.12	1.20	
	$\log k'_p$	1.64	1.20	1.02	1.00	1.02	1.12	1.22	
	d	0.01	−0.04	0.02	−0.04	0.05	0	−0.02	
Nitrendipine	$\log k'_e$	1.84	1.71	1.62	1.58	1.59	1.60		
	$\log k'_p$	1.85	1.69	1.62	1.59	1.59	1.60		
	d	−0.01	0.02	0	−0.01	0	0		
Nimodipine	$\log k'_e$	1.88	1.72	1.62	1.62	1.64			
	$\log k'_p$	1.88	1.70	1.63	1.61	1.66			
	d	0	0.02	−0.01	0.01	−0.02			

$\log k'_e$ and $\log k'_p$ represent the experimental value and the predictive value, respectively. d is deviation in logarithm ($d = \log k'_e - \log k'_p$).

Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.). Mobile phase: $0.01 \sim 1.6\ mol \cdot L^{-1}$ ammonium sulphate buffer (pH 7.4), Flow rate: $0.5\ mL \cdot min^{-1}$, Detection: 236 nm, Temperature: 37°C.

Table 3. Retention values, predicted values and deviations for five of the drugs in the CMSP

		Concentration of $(NH_4)_2HPO_4$ mol · L^{-1}								
Drugs		0.01	0.03	0.05	0.1	0.15	0.3	0.5	1.0	1.5
Verapamil	log k'_e	2.19	2.01	1.88	1.64	1.54	1.45	1.48	1.88	2.18
	log k'_p	2.29	1.94	1.79	1.61	1.54	1.49	1.56	1.86	2.16
	d	−0.10	0.07	−0.09	0.03	0	−0.04	−0.08	0.02	0.02
Diltiazem	log k'_e	1.54	1.49	1.31	1.21	1.14	1.06	1.01	1.35	1.60
	log k'_p	1.61	1.39	1.30	1.20	1.13	1.07	1.09	1.30	1.61
	d	−0.07	0.10	0.01	0.01	0.01	−0.01	−0.08	0.05	−0.01
Nifedipine	log k'_e	0.93	0.90	0.91	0.92	0.98	0.99	1.19	1.57	
	log k'_p	0.95	0.90	0.92	0.90	0.93	1.04	1.20	1.55	
	d	−0.02	0	−0.01	0.02	0.05	−0.05	−0.01	0.02	
Nitrendipine	log k'_e	1.55	1.58	1.60	1.62	1.67	1.74	2.00		
	log k'_p	1.57	1.57	1.58	1.62	1.66	1.79	1.97		
	d	−0.02	0.01	0.02	0	0.01	−0.05	0.03		
Nimodipine	log k'_e	1.52	1.54	1.57	1.58	1.69	1.80	1.98		
	log k'_p	1.52	1.54	1.56	1.61	1.66	1.80	1.98		
	d	0	0	0.01	−0.03	0.03	0	0		

Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.), Mobile phase: 0.1 ∼ 1.5 mol · L^{-1} ammonium phosphate buffer (pH 7.4), Flow rate: 0.5 mL · min^{-1}, Detection: 236 nm, Temperature: 37°C. d = deviation (log k'_e − log k'_p).

Table 4. Parameters (Eq. (7)) and deviations for five of the drugs in the CMSP

	Parameters in Eq. (7)						
Drugs	B(0)	B(1)	B(2)	B(3)	B(4)	*AD*	*SD*
Verapamil	−5.11	−1.45	5.09×10^{-2}	2.00×10^{-3}	-2.18×10^{-1}	0.0143	0.0186
Diltiazem	−6.97	−1.00	5.23×10^{-2}	2.09×10^{-3}	-1.65×10^{-1}	0.0139	0.0200
Nifedipine	7.01	1.17	-4.35×10^{-2}	-1.94×10^{-3}	2.15×10^{-2}	0.0022	0.0033
Nitrendipine	6.80	1.77	-4.41×10^{-2}	-1.94×10^{-3}	2.83×10^{-2}	0.0051	0.0058
Nimodipine	7.74	2.53	-5.98×10^{-2}	-2.40×10^{-3}	3.32×10^{-2}	0.0032	0.0043

Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.), Mobile phase: $0.01 \sim 0.03\ \text{mol} \cdot \text{L}^{-1}$ sodium hosphate buffer (pH 7.4), Flow rate: $0.5\ \text{mL} \cdot \text{min}^{-1}$, Detection: 236 nm, Temperature: 37°C. *AD* = average deviation, *SD* = standard deviation.

Table 5. Retention values, predicted values and deviation for five of the drugs in the CMSP

Drugs		Concentration of Na_2HPO_4 mol · L^{-1}					
		0.01	0.03	0.05	0.1	0.15	0.3
Verapamil	log k'_e	1.77	1.68	1.59	1.47	1.45	1.25
	log k'_p	1.78	1.66	1.60	1.50	1.42	1.27
	d	−0.10	0.02	−0.01	−0.03	0.03	−0.02
Diltiazem	log k'_e	1.34	1.26	1.19	1.14	1.02	0.89
	log k'_p	1.34	1.25	1.20	1.11	1.50	0.89
	d	0	0.01	−0.01	0.03	−0.03	0
Nifedipine	log k'_e	0.72	0.73	0.76	0.79	0.83	0.93
	log k'_p	0.71	0.74	0.76	0.79	0.83	0.93
	d	0.01	−0.01	0	0	0	0
Nitrendipine	log k'_e	1.40	1.42	1.44	1.49	1.52	1.62
	log k'_p	1.40	1.42	1.44	1.48	1.52	1.62
	d	0	0	0	0.01	0	0
Nimodipine	log k'_e	1.36	1.38	1.41	1.46	1.51	1.63
	log k'_p	1.36	1.39	1.41	1.46	1.51	1.64
	d	0	−0.01	0	0	0	−0.01

Column: The cardiac muscle CMSP column (50 mm × 2 mm, i.d.), Mobile phase: 0.01 ∼ 0.03 mol · L^{-1} sodium phosphate buffer (pH 7.4), Flow rate: 0.5 mL · min^{-1}, Detection: 236 nm, Temperature: 37°C. d = deviation (log k'_e − log k'_p).

small deviations (Table 3). The results indicate that, with Eq. (2), the retention of CaAs in this mobile phase can be predicted.

In addition, as shown in Tables 4 and 5, the comparison of the predictive log k'_p with experimental log k'_e of CaAs indicates that Eq. (3) is still suitable for describing the retention of CaAs in an aqueous solution of disodium hydrogen phosphate, even though its concentration range is very narrow.

Contribution of Water and Salt to the Drug Retention

The released water *nr* from the interface between the surfaces of the CMSP and these drugs can be obtained by the following Eq. (9) when these drug molecules interact with the stationary phase.

$$\mathrm{nr} = \mathrm{B}_2^2/2\mathrm{B}_3 \tag{9}$$

$$\mathrm{B}_2(\mathrm{nr}(\mathrm{m}-\mathrm{q}+1)\mathrm{K}_\mathrm{e}) \quad \text{and} \quad \mathrm{B}_3(1/2\mathrm{nr}(\mathrm{m}-\mathrm{q}+1)^2\mathrm{K}_\mathrm{e}^2)$$

are in Eqs. (6) and (7), respectively. B_1 in Eq. (5) is the released water moles q from the surface of drug molecules. So the parameters obtained by Eqs. (5)

Table 6. Comparison of parameters for the drugs in three kinds of mobile phase system

	$(NH_4)_2SO_4$			$(NH_4)_2HPO_4$			Na_2HPO_4		
Drug	nγ	Q	B(4)	nγ	q	B(4)	nγ	q	B(4)
Verapamil	1.0	4.2	0.73	0.47	78.9	0.81	0.6	1.5	0.22
Diltiazem	31.4	1.5	0.76	155.5	60.1	0.48	0.7	1.0	0.17
Nifedipine	3.6	8.1	0.36	39.9	31.1	0.14	0.5	1.2	0.02
Nitrendipine	2478	15.3	0.06	1.0	7.01	0.04	0.5	1.8	0.03
Nimodipine	5332	18.1	0.13	1.7	3.3	0.07	0.8	2.5	0.03

nr = B(2)2/2B(3) in Eq. (13), q = B(1) in Eq. (9).

and (9) can reflect the contributions of water in the mobile phase to the retention of drugs. The term $B_4(n^2r' + q')$ in Eq. (8) is that of salt in the mobile phase to the drug retention. The nr, q, and B_4 in the three kinds of mobile phases employed in this study were listed in Table 6. The results show that the influence of water on the drug retention is very complicated. So, it is still very hard to explain the contribution of water to the drug retention in the CMC only with nr and q values. But, there is a significant tendency of the B_4 value in the three kinds of mobile phases. The B_4 values of verapamil and diltiazem are totally more than that of nifedipine, nitrendipine, and nimodipine. The results indicate that salt would make the drugs display an ionic property to contribute to the drug affinity to the cardiac muscle CMSP. The basic property or ionic property of verapamil and diltiazem hydrochlorides is also stronger than those of nitrendipine, nimodipine, and nifedipine hydrochlorides. This fact can be employed to explain the reason why the curves for the plot of k' vs. C in the region of the low salt concentration, for the latter did not significantly decrease with salt concentration. This also coincides with the magnitude of B_4 values.

Mixed-Mechanism of Affinity and Hydrophobicity

From the changeable tendency of k' values with the salt concentration in the mobile phase and that of B_4 values with the type of salt pointed out above, the drug retention in the CMC system was mainly caused by two kinds of interactions, affinity and hydrophobic interactions; in the higher salt concentration region, a hydrophobic force (push force from the mobile phase to drug molecules) and the London force between the non-polar heads of drug molecules and the non-polar region on the CMSP. In the lower salt concentration region, however, if the drug retention appears to decrease with the salt concentration increase, there would be two kinds of mechanism, affinity or ion exchange, or a mixture of the both. Because the retention in the

affinity[18] and ion exchange chromatography[19–21] would decrease with the salt concentration increase in the mobile phase employed. It is clear that, because the effect of the retention of the five CaAs owing to pH values of the three kinds of mobile phases are small, the contribution of drug retention from the ion exchange mechanism may be very small. The retention should be mainly dominated by the affinity between drug molecules and membrane receptor on the cardiac muscle CMSP. However, it is really difficult to confirm the retention dominated by affinity interaction in the low salt concentration region by the usual manner, because affinity chromatography is always limited to the retention of macromolecules as solutes in HPLC.[18] So far, there has not been any method to follow. However, from the stand point of molecular mechanism, the interaction between biopolymers as solute and the stationary phase with ligands of small molecules would be the same interaction as that of a stationary phase with ligands of biopolymer and small solutes. It would be reasonable that the interaction between drug molecules and the CMSP can be attributed to an affinity mechanism.

As mentioned above, the retention of CaAs is mainly dominated by the affinity and hydrophobic interactions in the CMC system. Thus, the total interactions between a solute and stationary phase would be compensated between the decreases in the retention due to hydrophobic interaction and increases in that are due to affinity force as salt concentration increases, and *vice versa.* If the affinity and hydrophobic interactions in CMC simultaneously contribute to the drug retention, the elution curve would appear as a "U" shape. In this study, the k' vs. C curves of the five CaAs on the cardiac muscle CMSP in the 3 kinds of mobile phases indicate that the retentions of verapamil and diltiazem would be dominated by both affinity and hydrophobic interactions, while those of nitrendipine, nimodipine, and nifedipine would be mainly dominated by hydrophobic interaction. This experimental result was found to correlate with Borchard's pharmacological conclusion[22] that the contractility of the normal myocardium (cardiac muscle) may be depressed by calcium antagonists in the sequence verapamil > diltiazem > nifedipine > DHP (dihydropyridine) derivatives; calcium antagonists of the DHP type are more potent vasodilators than drugs of the verapamil type or diltiazem.

Characteristics of the CMC System

Owing to the characteristics of CMSP, containing a membrane receptor and having a bio-activity,[8,9] the drug retention mechanism cannot be simply compared with that of either ion exchange, or hydrophobic interaction chromatography of the elution curve having also a "U" shape. Especially, in the low salt concentration region, the affinity mechanism would become a dominatant factor, if a special interaction between the drug molecules and its membrane receptor exists. In the high salt concentration region of the elution curve of "U" shape, the hydrophobic mechanism would be the main one only if nonselective interactions exist between the drug molecules and the cell

membrane and/or other membrane proteins. The special affinity in the CMC system is a really comprehensive effect, closely relating to the membrane receptor and the three-dimensional structures of drug molecules. If the type and kind of these affinity forces are confirmed further, other methods of cell membrane chromatography are still needed. The character of the CMSP as a chromatographic stationary phase having bioactivity can still be employed to recognize and separate enantiomers of drug, and have a extremely strong retention for some drug molecules.[23,24]

Though the characteristics of the CMSP have been described by the phenomenology method, at present, it still cannot confirm the contributions of each interaction to the drug retention alone. In other words, the retention mechanism of the drug on the CMSP is different from any of the other chromatographic methods known at present. However, the double mechanism of affinity and hydrophobicity still needs further proof.

CONCLUSIONS

The retention model of a drug in cell membrane chromatography (CMC) appears as a specific property of "mixed mechanism" of affinity and hydrophobic interactions. The stoichiometric displacement theory for retention (SDT-R) has been employed to express the "double mechanism" by an equation.

According to the experimental testing, the retentions of five calcium antagonists (CaAs) are mainly dominated by the specific affinity in low salt concentration region of its elution curve, while it is mainly controlled by hydrophobic interaction, non-specifically, in the high salt concentration region. The retentions of the five calcium antagonists (CaAs) on the cardiac muscle CMSP in three kinds of mobile phases were found to fit the double mechanism mentioned above, but with different retention behaviors. The special affinity of verapamil and diltiazem on the cardiac muscle CMSP correlates well with their pharmacological effects.

Although the "mixed mechanism" of the CMC system is similar to that of usual affinity chromatography, ion exchange chromatography, and hydrophobic interaction chromatography, some significant differences still exist, owing to the CMSP with a special bioactivity. The model of double mechanism still needs further testing.

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