Suitability of Steric Mass-action Model for Ion-exchange Equilibrium of Micromolecule*

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Abstract The steric mass-action (SMA) model has been widely reported in the literature for ion-exchange and metal-affinity interaction adsorption equilibrium of biomacromolecules. In this paper, the usefulness of SMA model is analyzed for describing micromolecule ion-exchange equilibrium onto cation exchangers, CM Sephadex C-25 and Streamline SP. Batch adsorption experiments with ephedrine hydrochloride as a model adsorbate are carried out to determine the model parameters, that is, steric factor, characteristic charge and equilibrium constant. The result shows that the SMA model parameters of micromolecule cannot be obtained using the nonlinear least-square fitting method as protein's due to the remarkable difference between the molecular mass and dimension of micromolecule and protein. It is considered that the small size of the adsorbates dealt with in this study justifies the neglect of steric hindrances arising from adsorbate bulkiness. Thus, the three-parameter SMA model is reduced to two-parameter one (*i.e.*, steric factor is equal to zero) for describing micromolecule ion-exchange equilibrium. It is found that the equilibrium constant for CM Sephadex C-25 increases with increasing ionic strength, while the equilibrium constant for Streamline SP shows an opposite trend. This is probably due to the remarkable difference between the physical properties of the two adsorbents. Then, the relationship between the equilibrium constant and ionic strength is described by an expression. The computer simulations show that, the theoretical model with the correlation is promising in the prediction of micromolecule adsorption decrease with increasing ionic strength in a wide range of salt concentration.
Keywords micromolecule, ion-exchange equilibrium, steric mass-action model, chromatography

1 INTRODUCTION

With better understanding of traditional Chinese medicines and natural products, researchers have become increasingly interested in studying the active natural products as medicines[1−3] or as food additives[4]. Since pharmacologically active compounds in herbal plants usually are in low concentrations, a great deal of research has been done to develop more effective and selective extraction methods for recovery of these compounds from the raw materials[5]. High-performance ion-exchange chromatography, which has been widely employed for the recovery and purification of biomolecules such as proteins[6,7], both in the laboratory and in the production plant, has attracted great interest in recent researchers due to their versatility and relatively low price[8]. It is well known that the gradient elution by increasing ionic strength is widely adopted in the ion-exchange chromatography[9,10]. Therefore, to better understand and optimize a chromatographic operation by computer simulations, it is necessary to take into account the influence of ionic strength on the ion-exchange equilibrium. However, it is found that the most frequently used adsorption models, that is, the Langmuir model and its various modified forms[11,12], cannot directly reflect the influence of ionic strength on ion-exchange equilibrium, which is not adequate for predicting biomolecule adsorption behavior as a function of ionic strength[13]. The reason may be due to the assumption of adsorption reaction between an adsorbate and an adsorption site in the Langmuir model. Over the past decade or two, a double-layer model has been presented and developed[14−16]. In the model, it is assumed that no adsorption of adsorbates occurs at the ion exchanger surface but that the adsorbate concentration is increased in the electric double layer of the ion exchanger surface, which incorporates influences of ionic strength and adsorbate charge. To date, however, the theoretical model has not been applied to practical chromatographic separation unless some theoretical analyses due to the retention model based on the solution of the linearised or nonlinearised Poisson-Boltzmann equation[15,16].

In the 1990s, Brooks and Cramer[13] presented the steric mass-action (SMA) model to describe linear or nonlinear adsorption of proteins in ion-exchange chro-

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matographic systems on the basis of the combination of the stoichiometric displacement model[17,18] with the concept of macromolecule steric shielding[19]. In the model, it is assumed that the adsorption is an exchange reaction between a free protein and a certain number of bound ions, which explicitly accounts for the influence of ionic strength on ion-exchange equilibrium. To date, the SMA formalism incorporating with appropriate transport equations has been widely used to describe protein nonlinear chromatographic behavior in various elution processes such as isocratic[20], step gradient[10,21] and linear gradient[9,22]. However, it should be noted that the molecular mass and dimension of micromolecules such as traditional Chinese medicines and natural products, are much smaller than those of proteins. The SMA model may not be directly used to predict micromolecule nonlinear equilibrium behavior because the SMA model is constructed on the basis of the biomacromolecule ion-exchange equilibrium.

Therefore, in the present work, for describing the effect of salt concentration on micromolecule ion-exchange equilibrium, we attempt to improve the SMA model study on the basis of the results reported in literature[10,13,23]. Static batch adsorption of ephedrine hydrochloride as a model adsorbate onto cation exchangers, CM Sephadex C-25 and Streamline SP, is carried out to investigate the validity and usefulness of the theoretical models.

2 MATERIALS AND METHODS
2.1 Materials
Ephedrine hydrochloride (purity 99.2%) was provided by Neimenggu Chifeng Pharmaceutical Factory (Chifeng, China). Cation exchangers, CM Sephadex C-25 and Streamline SP, were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). All other reagents are of analytical grade.

2.2 Adsorption equilibrium
Adsorption equilibrium experiments of ephedrine hydrochloride on the cation exchangers were performed using the stirred batch adsorption method[24] in $10\,mmol\cdot L^{-1}$ acetate buffer (pH 4.92) and $10\,mmol\cdot L^{-1}$ glycine-NaOH buffer (pH 10.01), respectively, with different NaCl concentrations up to $200\,mmol\cdot L^{-1}$ at $25°C$. The procedure is described as follows. A known amount of the cation exchanger preequilibrated in the buffer was added to each of the flasks containing known volume of buffered ephedrine hydrochloride solution with different concentrations $(0.2—10\,mg\cdot ml^{-1})$. The flasks were shaken for 10 h in a shaking water bath at $25°C$, which is confirmed by the results of adsorption equilibrium studies to be sufficient to reach adsorption equilibrium under all the

conditions studied. The ion exchanger was then allowed to settle and the supernatant was filtered before determining the equilibrium ephedrine hydrochloride concentration with a Model Ultrospec 2100 UV-VIS spectrophotometer at 256 nm. The amount of ephedrine hydrochloride adsorbed to the cation exchanger was then calculated by mass balance.

2.3 Analysis and measurements
The ion-exchange capacities of CM Sephadex C-25 and Streamline SP were determined using acid-base titration method as well described in literature[25,26]. The wet densities of the mediums were measured by a pycnometer at $25°C$.

3 ADSORPTION MODEL
In the present work, single-component equilibrium with ephedrine hydrochloride as a model adsorbate is studied to examine the usefulness of the SMA model for describing micromolecule ion-exchange equilibrium. The SMA model is constructed on the basis of the following assumptions[13,23]: (1) The multipointed nature of adsorbate binding can be described by characteristic charge, ν[27]; (2) The binding of adsorbates cause the steric hindrance of salt counterions bound to the ion exchanger; (3) The model parameters are constant and independent of salt counterion concentration during the adsorption process; (4) The effect of salt concentration on the intermolecular interactions of adsorbate can be neglected. Thus, the stoichiometric exchange of the adsorbate and exchangeable salt counterions can be written by[13,23]

$$c + \frac{\nu}{n}\bar{q}_s \longleftrightarrow q + \frac{\nu}{n}c_s \qquad (1)$$
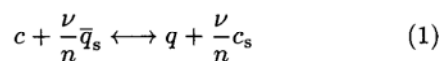
The equilibrium constant K_a for the ion-exchange process is defined as

$$K_a = \left(\frac{q}{c}\right)\left(\frac{c_s}{\bar{q}_s}\right)^{\nu/n} \qquad (2)$$

As elsewhere deduced[13], the adsorption isotherm is obtained as

$$c = \left(\frac{q}{K_a}\right)\left[\frac{nc_s}{A - (\nu + n\sigma_a)q}\right]^{\nu/n} \qquad (3)$$

Eq. (3) shows that the adsorption model involves three parameters (ν, K_a and σ_a) for each solute.

4 RESULTS AND DISCUSSION
4.1 Determination of model parameters
The ion-exchange capacities of the adsorbents, CM Sephadex C-25 and Streamline SP, were measured to be $588.7\,mmol\cdot L^{-1}$ and $237.0\,mmol\cdot L^{-1}$, respectively. For acetate or glycine-NaOH buffers with difference NaCl concentrations, valences of salt counterions are one, that is, $n = 1$.

It is well known that, for protein ion-exchange system, the SMA model parameters can be estimated by nonlinear least-square fitting to batch adsorption equilibrium data[10,23], or by plotting $\lg(q/c)$ vs. $\lg c_s$[13]. The model parameters thus obtained are commonly regarded as constant and independent of salt concentration[10,13]. However, it is found that the micromolecule model parameters cannot be obtained using the same methods as protein's due to the remarkable difference between the molecular mass and dimension of micromolecules and proteins. The molecular mass and dimension of proteins are usually larger than those of micromolecules by orders of magnitude. Therefore, it is deduced that the traditional assumption that the binding of adsorbates cause the steric hindrance of salt counterions bound to the ion exchanger may not be correct for micromolecules such as ephedrine hydrochloride with molecular mass of 202.3.

Therefore, in the next work, we take $\sigma_a = 0$ for ephedrine hydrochloride ion-exchange system, that is, the binding of the micromolecule cannot cause the steric hindrance of salt counterions bound to the ion exchanger. Thus, Eq. (3) is transformed into Eq. (4)

$$c = \left(\frac{q}{K_a}\right)\left(\frac{nc_s}{A - \nu q}\right)^{\nu/n} \quad (4)$$

Eq. (4) shows that the three-parameter SMA model is reduced to two-parameter stoichiometric model for describing micromolecule ion-exchange equilibrium.

Then, Eq. (5) is obtained according to electroneutrality on the stationary phase

$$A = nq_s + \nu q \quad (5)$$

Under overadsoption condition (i.e., $c \to \infty$, $q_s \approx 0$), the following equation is obtained from Eq. (5)

$$q_m = \frac{A}{\nu} \quad (6)$$

The characteristic charges determined using Eq. (6) are summarized in Table 1. Then, the ion-exchange

equilibrium constant can be calculated by nonlinear least-square fitting to experimental equilibrium data with NaCl concentrations of 0, 10, 20, 50 and 100 mmol·L^{-1} as elsewhere described[10,23]. The equilibrium constant thus obtained is a constant and independent of salt concentration. It is here written as $K_{a,1}$, and listed in Table 1. We find that the calculated isotherms from the theoretical model using the values of ν and $K_{a,1}$ can not fit the experimental curves (data not shown) due to the following reasons: (1) Studies on the adsorption of proteins on adsorbent have showed that the equilibrium constant between the protein and the adsorbent in different regions of ionic strength is not constant[28]; (2) In the present model, the equilibrium constant as a lumped parameter including the nonideal influences such as ionic strength effect on the intermolecular interactions of adsorbate may result in change of the value of equilibrium constant with changing salt concentration[23].

Therefore, in the present work, with the value of ν, the equilibrium constant K_a is estimated by matching the mathematical model with each salt concentration experiment data, and summarized in Table 1. Figs. 1 and 2 show the comparison between the adsorption model simulations using the set of model parameters listed in Table 1 (i.e., ν and K_a) and the ion-exchange equilibrium data. As can be seen, the theoretical predictions with the independently determined model parameters agree well with the experimental results in the range of investigated salt concentrations, which indicate the presented model is useful in the prediction of micromolecule adsorption decrease with increasing ionic strength.

From Table 1, we find that the value of ν increases obviously with decreasing buffer pH value from 10.01 to 4.92, while the K_a decreases rapidly. The main reason for significant decrease of K_a may be due to progressively decrease of the degree of ionization of the cation exchanger groups as the pH value decreases in the pH value range studied[29], thus resulting in decrease of electrostatic force between adsorbent and
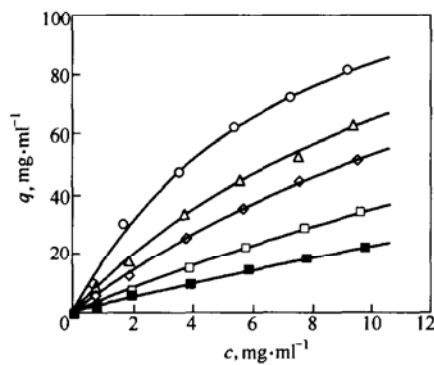
Table 1   Theoretical model parameters obtained from batch adsorption methods ($\sigma_a = 0$)

Buffer	pH	c_s, mmol·L^{-1}	CM Sephadex C-25			Streamline SP		
			ν	$K_{a,1}$	K_a	ν	$K_{a,1}$	K_a
acetate	4.92	10			0.28			1.90
		20			0.30			1.11
		30	1.250	0.33	0.31	0.868	1.32	0.66
		60			0.35			0.34
		110			0.37			0.23
glycine-NaOH	10.01	10			0.53			4.68
		20			0.57			2.50
		30	0.881	0.58	0.59	0.475	3.18	1.64
		60			0.59			1.02
		110			0.60			0.53

ephedrine hydrochloride. The linear increase of ν with decreasing q_m (*i.e.*, decreasing buffer pH value) is thus easily understandable if one recalls the definition for ν [Eq. (6)]. Furthermore, we also find from Table 1 that the changes of model parameters for Streamline SP system are greater than those for CM Sephadex C-25 system in the investigated pH range (ν increases by 42%, K_a decreases by about 40% for CM Sephadex C-25; ν increases by 83%, K_a decreases by greater than 55% for Streamline SP). These results may be caused by the fact that cation exchanger, Streamline SP, with larger particle diameter (100—300 μm) and smaller specific surface area, is fabricated by coating cross-linked agarose to fine crystalline quartz for expanded bed chromatography according to the manufacturer, which may result in the adsorption characteristics of the adsorbent are more sensitive to pH value change than the other.
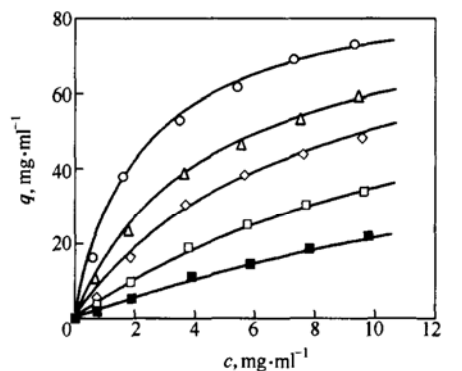
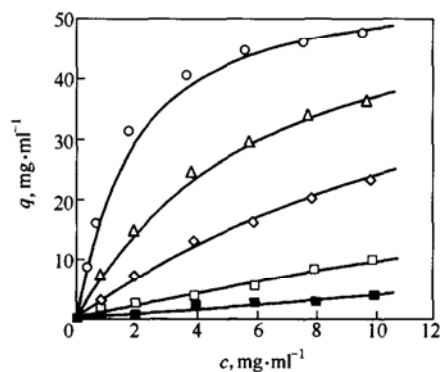
(a) acetate pH 4.92

(b) glycine-NaOH buffers pH 10.01

Figure 1   Adsorption isotherms of ephedrine hydrochloride onto CM Sephadex C-25 with different NaCl concentrations
(Solid lines are calculated from the theoretical model using the set of model parameters, that is ν and K_a, listed in Table 1)
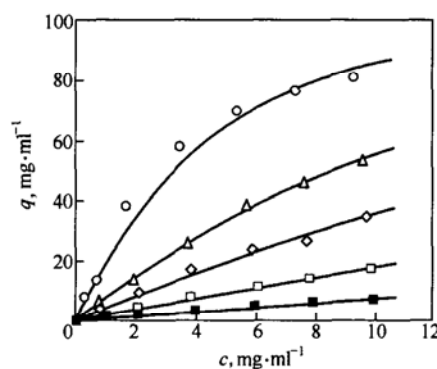c_s, mmol·L^{-1}: ○ 10; △ 20; ◇ 30; □ 60; ■ 110

4.2   Dependence of ion-exchange equilibrium constant on ionic strength

Figures 1 and 2 show that the ephedrine hydrochloride adsorption density decreases rapidly with increasing ionic strength as observed in protein ion-exchange

systems[9,23]. Table 1 shows that the value of K_a for CM Sephadex C-25 increases with increasing ionic strength, while K_a for Streamline SP show an opposite trend. This is probably due to, as above-mentioned, in the mathematical model, equilibrium constant as a lumped parameter including many factor effects (*e.g.*, ion-exchange capacity and pore structure of ion exchanger), resulting in no general trends detected for ion exchangers with different physical properties.

(a) acetate pH 4.92

(b) glycine-NaOH buffers pH 10.01

Figure 2   Adsorption isotherms of ephedrine hydrochloride onto Streamline SP with different NaCl concentrations
c_s, mmol·L^{-1}: ○ 10; △ 20; ◇ 30; □ 60; ■ 110

On the basis of the analyzing the data listed in Table 1, it is found that the relationship between the equilibrium constant and the salt concentration can approximately be expressed by a simple correlation [see Fig. (3)]

$$\ln(K_a) = a + b\ln(c_s) \qquad (7)$$

where a and b are constant (a and b in acetate buffer are -1.594 and 0.130 for CM Sephadex C-25, 2.768 and -0.918 for Streamline SP; in glycine-NaOH buffer are -0.706 and 0.044 for CM Sephadex C-25, 3.584 and -0.891 for Streamline SP, respectively). This equation is found to regress the experimental data with a correlation coefficient of above 0.92 (Fig. 3). For the present systems dealing with micromolecule,

we consider that the sign of the slopes of the correlated straight lines may depend on the factors such as ion-exchange capacity and pore structure of ion exchanger (e.g., pore diameter and specific surface area) and so on.
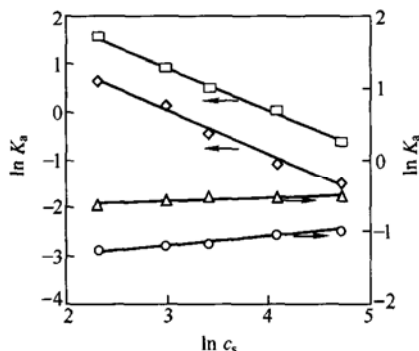

Figure 3   The relationship between the equilibrium constant (K_a) and salt concentration (c_s)
O pH 4.92 acetate buffer, CM Sephadex C-25;
△ pH 10.01 glycine-NaOH buffer, CM Sephadex C-25;
◇ pH 4.92 acetate buffer, Streamline SP;
□ pH 10.01 glycine-NaOH buffer, Streamline SP. The arrows indicate the ordinates for the corresponding data

4.3   Prediction of adsorption isotherms

Using the ν determined by Eq. (6) and the K_a calculated from Eq. (7), the adsorption equilibrium isotherms at given salt concentration can be predicted. In order to investigate the validity and usefulness of the theoretical models, we further perform adsorption experiments under conditions different from those shown in Figs. 1 and 2. It is found that the theoretical predictions using the ν listed in Table 1 and the K_a calculated from the linear correlations shown in Fig. 3 [Eq. (7)] agree well with the experimental results at each salt concentration (see Fig. 4). The standard deviation of all points on the figure is estimated at 1.02. This deviation of the model predictions may be due to the fact that the correlation of the K_a as functions of the ionic strength is approximate, as shown in Fig. 3.

At present, many articles have been published on the ion-exchange SMA model of protein, however, little study of the suitability of SMA model for describing micromolecule ion-exchange equilibrium has been reported in literature. Chen et al.[23] reported that the values of ν, K_a and σ_a for adsorption of bovine serum albumin (BSA) onto an anion exchanger, DEAE Spherodex M, with an ion-exchange capacity of 53.0 mmol·L^{-1} in pH 5.26 acetate buffer are 3.0, 47 and 13.7, respectively. The adsorption equilibrium of BSA onto a strong anion exchanger, Q-Sepharose FF, with an ion-exchange capacity of 200 mmol·L^{-1} was investigated by Bosma and Wesselingh[30]. In their study, the values of ν and σ_a for BSA in pH 7.8 Tris-HCl buffer are 4.3 and 142, respectively. Gallant et al.[9] found that

the values of ν, K_a and σ_a for adsorption of lysozyme onto a strong cation exchange column (SCX) (sulfopropyl, 54 mm×5 mm I.D.) with a column capacity of 525 mmol·L^{-1} in pH 6.0 phosphate buffer are 6.14, 0.211 and 0, respectively. Combining the results listed in Table 1, it can be seen that there is obviously difference in model parameters for different ion-exchange systems. The ν for micromolecule is much smaller than those for proteins, which is reasonable if considering remarkable difference in ion-exchange capacities of adsorbents and molecular mass and dimensions of adsorbates.
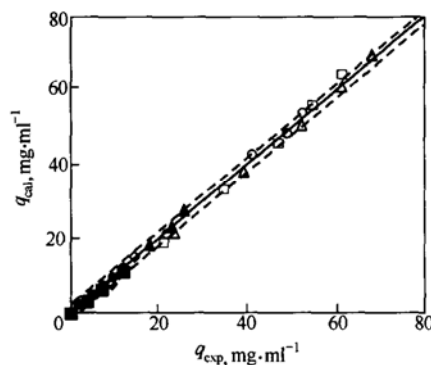

Figure 4   Parity plot of measured and calculated adsorbent-phase ephedrine hydrochloride concentrations from the theoretical model
O $c_s = 25$ mmol·L^{-1}, pH 4.92 acetate buffer, CM Sephadex C-25;
● $c_s = 210$ mmol·L^{-1}, pH 4.92 acetate buffer, CM Sephadex C-25;
△ $c_s = 15$ mmol·L^{-1}, pH 10.01 glycine-NaOH buffer, CM Sephadex C-25;
▲ $c_s = 80$ mmol·L^{-1}, pH 10.01 glycine-NaOH buffer, CM Sephadex C-25; ◇ $c_s = 45$ mmol·L^{-1}, pH 4.92 acetate buffer, Streamline SP;
◆ $c_s = 160$ mmol·L^{-1}, pH 4.92 acetate buffer, Streamline SP; □ $c_s = 16$ mmol·L^{-1}, pH 10.01 glycine-NaOH buffer, Streamline SP;
■ $c_s = 80$ mmol·L^{-1}, pH 10.01 glycine-NaOH buffer, Streamline SP; The dashed lines represent $q_{cal} = q_{exp} \pm 2SD$

5   CONCLUSIONS

In this article, the small size of the adsorbates dealt with in this study justifies the neglect of steric hindrances arising from adsorbate bulkiness. The three-parameter SMA model is reduced to two-parameter one for describing the effect of salt concentration on micromolecule ion-exchange equilibrium. The results show that the theoretical model with the ν determined by Eq. (6) and the K_a calculated from Eq. (7) can predict micromolecule adsorption decrease with increasing ionic strength in a wide range of salt concentration. It is considered that the model incorporating with appropriate transport equations would be useful in the practical optimization of micromolecule ion-exchange chromatography.

NOMENCLATURE

A	ion-exchange capacity (monovalent salt counterions), $mmol \cdot L^{-1}$
a, b	constants in Eq. (7)
c	ephedrine hydrochloride concentration in bulk phase, $mg \cdot ml^{-1}$
c_s	salt counterion concentration in bulk phase, $mmol \cdot L^{-1}$
K_a	equilibrium constant for SMA model
n	value of valence of salt counterions
q	adsorbed ephedrine hydrochloride density, $mg \cdot ml^{-1}$
q_m	saturation adsorption capacity of ephedrine hydrochloride, $mg \cdot ml^{-1}$
q_s	stationary phase salt concentration, $mmol \cdot L^{-1}$
$\bar{q}_s$	stationary phase salt concentration (sterically nonhindered), $mmol \cdot L^{-1}$
ν	characteristic charge
σ_a	adsorbate steric factor

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