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Modeling of adsorption in hydrophobic interaction chromatography systems using a preferential interaction quadratic isotherm

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Abstract

A preferential interaction quadratic isotherm model for hydrophobic interaction chromatographic systems is presented in this paper. In this isotherm, the nonlinear effect of salt on the capacity factor is described using the preferential interaction model developed by Perkins et al. [J. Chromatogr. A, 766 (1997) 1]. This is then coupled with a quadratic nonlinear isotherm to describe nonlinear adsorption behavior at high solute concentrations. The resulting preferential interaction quadratic isotherm is examined for its ability to describe solute adsorption behavior under both linear and nonlinear conditions over a wide range of salt concentrations in HIC systems. The results indicate that this isotherm is well suited for predicting nonlinear adsorption behavior in HIC systems for both proteins and low-molecular mass HIC displacers.

Keywords: Hydrophobic interaction chromatography; Adsorption isotherms; Salt effects; Retention; Mathematical modelling

1. Introduction

Hydrophobic interaction chromatography (HIC) is widely employed in the biotechnology industry for protein purification [1–5]. The selectivity in this mode of chromatography derives from the hydrophobic interactions between the hydrophobic resin and non-polar hydrophobic patches on the solute surfaces [1,6]. Adsorption in HIC systems has been shown to be an entropically driven process under linear adsorption conditions [7]. The presence of salt changes the system entropy by affecting the water structure around the protein and stationary phase ligands. Further, salts will have varying effects on solute binding in HIC systems depending on their ranking in the Hofmeister series [8–12]. At present, there are two theories to explain salt effects in HIC systems: the solvophobic theory and the preferential interaction theory. The solvophobic theory is based on the association and solvation of the participating species [13]. This model is based on the assumption that the variation of retention time with salt concentration is proportional to the molal surface tension increment of the salt. However, this is not valid for salts that interact strongly with the proteins. On the other hand, the preferential interaction theory is based on the interaction of salt and proteins [12,14–16] and is valid for a wide range of salt concentrations [17]. The interaction between the protein and salt is quantified by the preferential interaction coefficient (PIC), which can be estimated via densitometric methods [8].

It is well known that a $\log k'$ versus salt concentration plot in HIC systems can exhibit nonlinear behavior at low salt concentrations. This poses a

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challenge in predicting protein nonlinear retention under a wide range of salt concentrations. Melander and co-workers [2,13] first proposed an adsorption model that considered salt effects in HIC systems. However, their model was not valid for a wide range of salt concentrations (e.g., low salt concentrations) [18,19]. Perkins et al. [17] have derived a capacity factor relationship with salt concentration based on the preferential interaction analysis. Geng et al. [20] proposed a stoichiometric displacement retention model for protein adsorption in HIC. In their approach, water was taken as the displacing agent and salt as a diluent or promoter that affected both water molality and protein conformations. However, their study was not able to address non-linear retention behavior in HIC systems. Staby and Mollerup [19] have proposed a model for solute retention prediction based on the Debye–Huckel theory. Good agreement between theory and experiment was achieved for lysozyme retention under various ammonium sulfate concentrations and pH on four HIC perfusion media. In the current work we will employ the model proposed by Perkins et al. for modeling linear and non-linear retention behavior in HIC systems at low loading conditions.

In RPLC systems, the isotherm is most commonly described using partition or adsorption models [21]. In the partition model a solute-sized cavity is formed in the stationary phase, the solute then transfers from the mobile phase to the cavity formed in the stationary phase, and finally the cavity remaining in the mobile phase is closed [22]. In the adsorption model, the solute transfers from the mobile phase to the solid–liquid interface. In the process, it displaces the adsorbed solvent molecules on the interface [23]. Kaczmarski et al. [24] have successfully used a mixed retention mechanism (adsorption/partition) to describe the sorption on C_8 and C_{18} columns.

In order to model nonlinear adsorption in HIC systems, it is necessary to account for the effects of salt in these nonlinear systems. Antia and Horváth [25] employed an exponentially modified Langmuir (EML) isotherm for RPLC and HIC systems. In this nonlinear model, the salt contribution to the capacity factor is described by an exponential relationship that accounts for only linear solute retention behavior. Antia and Horváth [26] further proposed the use of an ideal adsorption solution (IAS) model for HIC systems. Although the IAS model has several advantages, this isotherm model requires determination of the adsorption isotherms of all the components, including modulator (e.g., salt for HIC; organic solvent for RPLC), which is a daunting task.

While these various modeling approaches have their merits, to date there is no model that can accurately predict both nonlinear retention behavior as well as the nonlinear adsorption behavior of proteins under a wide range of salt concentrations.

In the current paper, the preferential interaction model developed by Perkins et al. [17] is coupled with a quadratic nonlinear isotherm to describe nonlinear adsorption behavior at high solute concentrations. The resulting preferential interaction quadratic isotherm is examined for its ability to describe protein and displacer adsorption behavior under both linear and nonlinear conditions over a wide range of salt concentrations in HIC systems.

2. Theory

2.1. Preferential interaction theory

The interaction between the salt and protein can be quantified using the preferential interaction coefficient (PIC), which is defined as [8]:

$$\xi^{m}_{3,2} \equiv \left(\frac{\partial m_3}{\partial m_2}\right)_{T,\mu_1,\mu_3} \tag{1}$$

where, m is molal concentration. The subscripts 1, 2 and 3 refer to the solvent, protein and salt, respectively. Perkins et al. [17] applied the two-domain model of Timasheff's theory [9,14,27] to HIC systems and obtained the following relationships:

For non-electrolyte:

$$\ln k' = c - \frac{n\Delta\nu_1}{m_1 g} m_3 + \frac{(\Delta\nu_+ + \Delta\nu_-)}{g} \ln(m_3) \tag{2}$$

For electrolyte:

$$\ln k' = c + \left[\frac{(\Delta b_+ + \Delta b_-)}{g} - \frac{n\Delta\nu_1}{m_1 g}\right] m_3 \tag{3}$$

where $\nu_i = b_i m_3$, and Δb is the stoichiometrically weighted change in the ion binding coefficients; $g = (\partial \ln m_3 / \partial \ln a_{\pm})_{T,P}$; a is the activity of ions. For

a given HIC system, n and g are constant. In this paper we will employ this approach for the development of a non-linear HIC adsorption model.

2.2. Preferential interaction quadratic isotherm

The polynomial Langmuir isotherm model [6,28–32] derived from statistical thermodynamics has the following expression:

$$Q_i = \frac{a_{i,1}C_i + a_{i,2}C_i^2 + a_{i,3}C_i^3 + \cdots}{1 + \sum_{j=1}^{N_c} b_{i,1}C_i + b_{i,2}C_i^2 + b_{i,3}C_i^3 + \cdots} \quad (4)$$

$$k_i' = \phi C^{\infty} b_i \quad (5)$$

where ϕ denotes the phase ratio; C_i and Q_i are solute concentration on mobile phase and stationary phase for component i; C^{∞} denotes adsorption saturation capacity. N_c denotes number of component.

In practice, the second-order isotherm, or quadratic isotherm, is often employed [6,28–32]. In this paper we will combine the preferential interaction model with the quadratic isotherm to develop a non-linear HIC isotherm model. The resulting model is given as the following:

$$Q_i = \frac{k_i'(a_i \cdot C_i + d_i C_i^2)}{1 + \sum_{j=1}^{N_c} k'(b_j C_j + c_j C_j^2)} \quad (6)$$

$$\ln k_i' = \alpha_i + \beta_i \cdot C_{\text{salt}} + \gamma_i \cdot \ln(C_{\text{salt}}) \quad (7)$$

where i and j denote the solutes; C_i and Q_i denote concentrations in the liquid and solid-phases, respectively; k' is the capacity factor; N_c is the number of component; and C_{salt} is the salt (modulator) concentration. α, β and γ are the retention parameters that are determined from isocratic experiments under linear adsorption conditions. a, b, c and d are the isotherm parameters that are obtained by fitting the isotherms under a variety of conditions. As will be shown in this paper, the preferential interaction quadratic (PIQ) model is capable of predicting solute adsorption behavior under both linear and nonlinear conditions over a wide range of salt concentrations.

The assumptions for the PIQ isotherm models are as follows:

(1) Both solution and stationary phase are thermodynamically ideal systems; therefore we can use concentrations instead of activities.

(2) Competitive binding in HIC systems can be described solely by the preferential interaction equilibrium.

(3) Equilibrium parameters in the PIQ models of proteins and small molecules are all independent of solute and salt concentrations.

(4) There is no aggregation or tertiary structural changes of the proteins.

(5) A constant saturation capacity is employed for each protein isotherm.

3. Experimental

3.1. Materials

Lysozyme (hen egg white) and lectin (*Arachis hypogaea*, peanut) were purchased from Sigma (St. Louis, MO, USA). Big Chap was purchased from Calbiochem (La Jolla, CA, USA). This low-molecular mass displacer [33] is a non-ionic detergent analog of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxypropane sulfonate (CHAPSO) and is shown in Fig. 1. Phenyl 650M bulk resin (consists of phenyl ligands on a polymethacrylate polymer backbone, average particle size is 65 μm) was obtained from Tosoh Biosep (Montgomeryville, PA, USA). Size-exclusion chromatography (SEC) column, G3000SWXL (300×7.8 mm I.D.) and a guard column (40×6 mm I.D.) were purchased from Tosoh Biosep. Sodium phosphate (monobasic), sodium phosphate (dibasic),

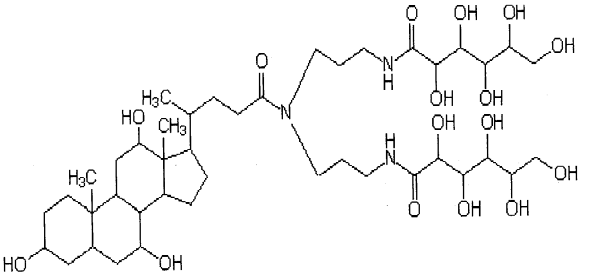

Fig. 1. Chemical structure of big chap [*N*,*N*-bis-(3-D-gluconamidopropyl)cholamine].

ammonium sulfate, sodium nitrate and sodium chloride were purchased from Sigma.

3.2. Apparatus

Frontal chromatography was carried out using a Model 590 HPLC pump (Waters, Milford, MA, USA) connected to the chromatographic columns via a Model C10W 10-port valve (Valco, Houston, TX, USA). Data acquisition and processing were carried out using a Strawberry Tree system (Sunnyvale, CA, USA). The column effluent was monitored using a model 484 UV–Vis absorbance detector (Waters). Fractions of the column effluent were collected using an LKB 2212 HeliFrac fraction collector (LKB, Sweden).

Analysis of the fractions from the frontal experiments was carried out using a chromatographic system (Waters) consisting of a 600E Multisolvent Delivery System, a PDA 996 photodiode array detector and a 712 WISP auto sampler with a cooling module.

3.3. Procedures

3.3.1. Determination of PIC parameters

The PIC parameters were determined from retention time data obtained under isocratic conditions. Experiments were carried out at different ammonium sulfate concentrations, ranging from 200 to 1200 m*M* in 100 m*M* phosphate buffer, pH 7.0. Capacity factors (k') for the solutes were determined and the data was used to determine the retention parameters of Eq. (7). All experiments were carried out at room temperature.

3.3.2. Frontal experiments to obtain adsorption isotherm of proteins and displacer

A phenyl 650M column (90×5 mm I.D.) column was initially equilibrated with the carrier buffer, 100 m*M* phosphate, pH 7.0, containing various concentrations of ammonium sulfate (700, 1000, 1100 and 1350 m*M*) and then sequentially perfused with the protein and displacer solutions dissolved in the same salt concentration buffer. The column effluent was monitored at 215 nm, flow-rate was 0.2 ml/min (61 cm/h) and all experiments were carried out at room temperature.

3.3.3. Proteins and displacer analysis by SEC

The effluent fractions from the frontal experiments were analyzed by size exclusion chromatography using a G3000SWXL column from Tosoh Biosep. Proteins were monitored at 280 nm and displacer was monitored at 215 nm. The mobile phase for the SEC analysis was 190 m*M* sodium phosphate, pH 7.6, with 200 m*M* sodium chloride. The flow-rate was 1 ml/min.

4. Results and discussion

In this paper, we have extended the preferential interaction analysis to a nonlinear isotherm model for HIC systems that can predict both protein and small molecule adsorption behavior over a wide range of salt and solute concentrations.

4.1. Variation of capacity factor with salt concentration

Isocratic experiments were carried out with two model proteins (lysozyme and lectin, 40 μg each) and a HIC displacer (big chap, 40 μmol) at various concentrations of the kosmotropic salt ammonium sulfate. The $\ln k'$ versus $\ln C_{salt}$ plots for the proteins lysozyme, lectin and the surfactant big chap are presented in Figs. 2–4, respectively. As seen in the figures, the non-linear retention behavior of these solutes are well described from the preferential

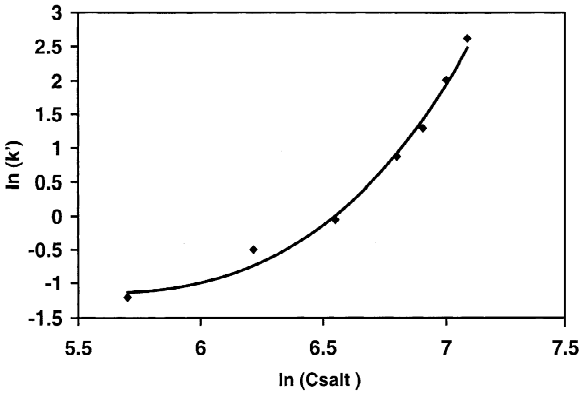

Fig. 2. PIC-based retention fitting (lysozyme). (—) Calculated from Eq. (7); (♦) experiment data.

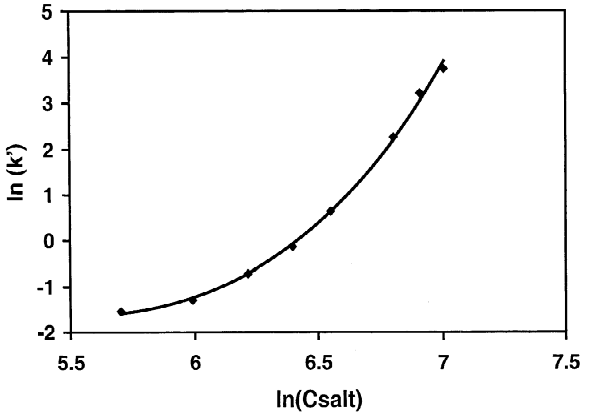

Fig. 3. PIC-based retention fitting (lectin). (—) Calculated from Eq. (7); (♦) experiment data.

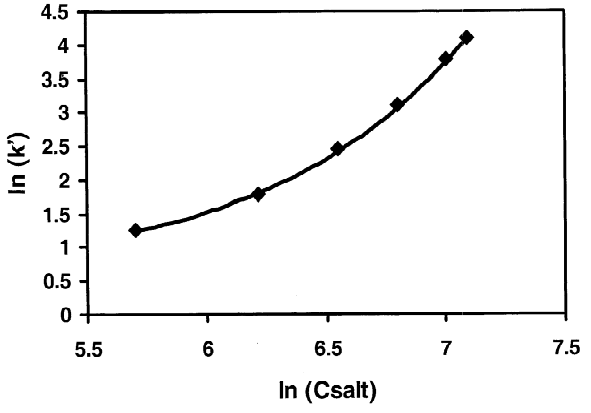

Fig. 4. PIC-based retention fitting (big chap). (—) Calculated from Eq. (7); (♦) experiment data.

interaction retention model, Eq. (7), proposed by Perkins et al. [17].

The parameters α, β and γ obtained by fitting the experimental data to Eq. (7) are given in Table 1. The β and γ values represent the linear and nonlinear regions, respectively, of the $\ln k'$ plot. As seen in the table, the β values are positive for both proteins. Since $\beta = -n\Delta\nu_1/m_1 g$, a positive value of β indicates that $\Delta\nu_1$ is negative, which implies that water molecules are released from the protein and ligand surface during the adsorption process. Lysozyme has a lower β value than that for lectin. This is expected since the β value of the $\ln k'$ versus $\ln C_{\text{salt}}$ plot is proportional to the number of water molecules released during binding in HIC systems ($\beta = -n\Delta\nu_1/m_1 g$). The released water also corresponds to the contact surface area of the solute [17], which is expected to be higher for lectin (M_r 51 000) than for lysozyme (M_r 14 300). The γ value represents the release or salt ions during the adsorption process. As seen the table, the γ values are negative for all three solutes, indicating that the local ion concentration around the adsorbed protein is lower than the bulk ion concentration. Again, this is in agreement with the results of Perkins et al. [17].

Table 1
Summary of the parameters for retention prediction

	Lysozyme	Lectin	Big chap
α	8.16	10.20	2.20
β	0.0071	0.011	0.0037
γ	−2.00	−2.66	−0.36

All parameters are obtained by fitting Eq. (7): $\ln k' = \alpha + \beta C_{\text{salt}} + \gamma \ln(C_{\text{salt}})$.

4.2. Single component isotherm

As described in the theory section, we have proposed a modified nonlinear isotherm for describing adsorption in HIC systems over a wide range of salt and solute concentrations. For a single solute, Eq. (6) reduces to:

$$Q_i = \frac{k_I'(a_i C_i + d_i C_i^2)}{1 + k'(b_i C_i + c_i C_i^2)} \tag{8}$$

where $\ln k_i = \alpha_i + \beta_i \cdot C_{\text{salt}} + \gamma_i \cdot \ln(C_{\text{salt}})$.

Under linear adsorption conditions the isotherm simplifies to:

$$Q_i = k_i' a_i C_i = K_i \cdot C_i \tag{9}$$

where K_i is adsorption equilibrium constant for component i. The initial slope of the isotherm at a given salt concentration is constant and is defined by $k'a_i$. Under overloaded conditions [e.g., $C_i \to \infty$, $C_i^2 \gg C_i \to \infty$, and $1 \ll k_i'(b_i C_i + c_i C_i^2)$], the isotherm becomes:

$$Q_i = \frac{d_i}{c_i} \tag{10}$$

Frontal chromatography was carried out to determine the adsorption isotherms for the three model solutes at 0.7, 1 or 1.1 M and 1.3 M ammonium

Table 2
Summary of the parameters for preferential interaction quadratic (PIQ) isotherm model

	Lysozyme	Lectin	Big chap
a	1.43	0.37	0.89
b	0.31	0.13	0.0065
c	1.04	0.23	−2e-04
d	5.21	2.03	−0.021
K (at 1 *M* salt)	6.06	6.24	57.08

All parameters are optimized using CONSTR optimization function in Matlab.

sulfate. The PIQ parameters were optimized using the CONSTR optimization functions in Matlab [34,35] and the corresponding values are presented in Table 2. In addition, the equilibrium constant (K) at 1 M salt is also provided. As expected, the value of K corresponds to the affinity of these solutes under linear binding conditions. The experimental isotherms as well as the PIQ isotherm model results obtained from Eqs. (7) and (8) are presented in Figs. 5–7. In order to further validate the PIQ model, model predictions were compared with experimental

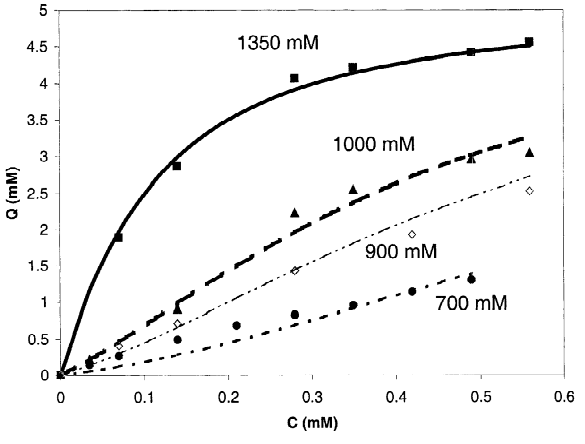

Fig. 5. Comparison between the PIQ isotherm model and experimental data for lysozyme. Lines denote the PIQ model fitting results calculated from Eq. (8); (—) 1350 mM; (- - -) 1000 mM; (···) 700 mM. Dash-dot line denotes the PIQ model predicted data at 900 mM salt concentration; (– · · –· ·) 900 mM. Synbols denote the experimental data: (■) 1350 mM, (▲) 1000 mM, (●) 700 mM, (◇) 900 mM. Experimental data at 700, 1000 and 1350 mM salt were used to generate the PIQ isotherm model. PIQ model prediction result was shown at 900 mM. C and Q denote the protein concentration on mobile phase and stationary phase, respectively. The solute binding concentrations in stationary phase (Q) is calculated based on the mass of dry solid-phase only.

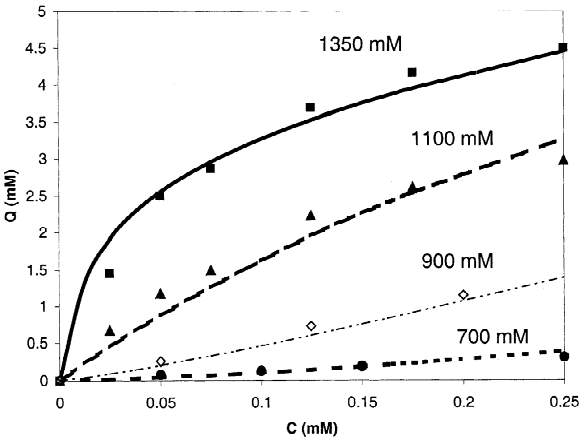

Fig. 6. Comparison between the PIQ isotherm model and experimental data for lectin. Lines denote the PIQ model fitting results calculated from Eq. (8): (—) 1350 mM; (- - -) 1100 mM; (···) 700 mM. Dash-dot line denotes the PIQ model predicted data at 900 mM salt concentration; (– · · –· ·) 900 mM. Marks denote the experimental data: (■) 1350 mM, (▲) 1100 mM, (●) 700 mM, (◇) 900 mM. Experimental data at 700, 1100 and 1350 mM salt were used to generate the PIQ isotherm model. PIQ model prediction result was shown at 900 mM. C and Q denote the protein concentration on mobile phase and stationary phase, respectively. The solute binding concentrations in stationary phase (Q) is calculated based on the mass of dry solid-phase only.

data for all three solutes at a particular salt concentration. Experimental data used for evaluating the predictive power of this isotherm were lysozyme and lectin at 900 mM salt and big chap at 1000 mM salt (note: the experimental data for predictions were not used in generating the parameters of the PIQ model). The PIQ predictions are shown as the dash-dot lines. The experimental data is shown using the diamond marks. From Figs. 5–7 it can be seen that the PIQ isotherm model can satisfactorily predict experimental data under a wide range of salt concentrations. These results indicate that the PIQ model can indeed successfully predict the protein and small molecule isotherm data over a wide range of ammonium sulfate and solute concentrations. To our knowledge, this is the first report in the literature of a non-linear isotherm for HIC systems that can describe this range of conditions.

5. Conclusions

This paper presents a multicomponent isotherm for

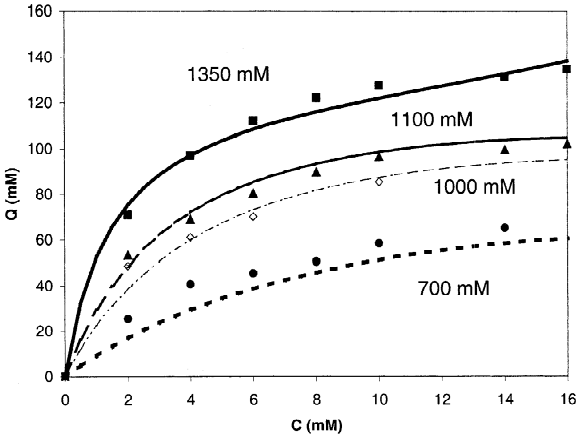

Fig. 7. Comparison between the PIQ isotherm model and experimental data for big chap. Lines denote the PIQ model fitting results calculated from Eq. (8): (—) 1350 m*M*; (- - -) 1100 m*M*; (···) 700 m*M*. Dash-dot line denotes the PIQ model predicted data at 1000 m*M* salt concentration; (– · · – · ·) 1000 m*M*. Marks denote the experimental data: (■) 1350 m*M*, (▲) 1100 m*M*, (●) 700 m*M*, (◇) 1000 m*M*. Experimental data at 700, 1100 and 1350 m*M* salt were used to generate the PIQ isotherm model. PIQ model prediction result was shown at 1000 m*M*. *C* and *Q* denote the protein concentration on mobile phase and stationary phase, respectively. The solute binding concentrations in stationary phase (*Q*) is calculated based on the mass of dry solid-phase only.

hydrophobic interaction chromatographic systems based on the preferential interaction analysis. The proposed preferential interaction quadratic (PIQ) isotherm model offers the advantage of predicting the solute behavior under both linear and nonlinear conditions over a wide range of salt concentrations. Future investigations will focus on the characterization of HIC resins and the development of a general rate model combined with the proposed isotherm model. The effect of different types of salt (kosmotropes, chaotropes and neutral salt) on solute binding in HIC systems is under investigation and will be the subject of a future report.

6. Nomenclature

a, b, c, d	isotherm parameters in Eq. (8)
C_i	liquid phase concentration (mg/ml)
C_{salt}	salt concentration in liquid phase (mg/ml)
C^{∞}	adsorption saturation capacity
K	adsorption equilibrium constant
k'	capacity factor
n	salt ion constant
N_c	number of component
Q_i	solid-phase concentration (mg/ml)

Greek

α, β, γ	parameters in Eq. (7)
ϕ	phase ratio
ν_1	moles of water molecule released during the binding process
$\Delta\nu_{+/-}$	stoichiometrically weighted change in ion binding

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