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Thermodynamic modeling of protein retention in mixed-mode chromatography: An extended model for isocratic and dual gradient elution chromatography

Yi Feng Lee [a], Heiner Graalfs [b], Christian Frech [a,*]

[a] Institute of Biochemistry, University of Applied Sciences Mannheim, Mannheim, Germany
[b] Merck KGaA, Darmstadt, Germany

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ABSTRACT

An extended model is developed to describe protein retention in mixed-mode chromatography based on thermodynamic principles. Special features are the incorporation of pH dependence of the ionic interaction on a mixed-mode resin and the addition of a water term into the model which enables one to describe the total number of water molecules released at the hydrophobic interfaces upon protein-ligand binding. Examples are presented on how to determine the model parameters using isocratic elution chromatography. Four mixed-mode anion-exchanger prototype resins with different surface chemistries and ligand densities were tested using isocratic elution of two monoclonal antibodies at different pH values (7–10) and encompassed a wide range of NaCl concentrations (0–5 M). U-shape mixed-mode retention curves were observed for all four resins. By taking into account of the deprotonation and protonation of the weak cationic functional groups in these mixed-mode anion-exchanger prototype resins, conditions which favor protein-ligand binding via mixed-mode strong cationic ligands as well as conditions which favor protein-ligand binding via both mixed-mode strong cationic ligands and non-hydrophobic weak cationic ligands were identified. The changes in the retention curves with pH, salt, protein, and ligand can be described very well by the extended model using meaningful thermodynamic parameters like Gibbs energy, number of ionic and hydrophobic interactions, total number of released water molecules as well as modulator interaction constant. Furthermore, the fitted model parameters based on isocratic elution data can also be used to predict protein retention in dual salt-pH gradient elution chromatography.

1. Introduction

In the recent decade there is an increasing demand towards the development of a downstream process (DSP) for recombinant monoclonal antibody (mAb) purification with higher process efficiency but lower production cost [1–5]. One outcome of such innovations is the development of mixed-mode resin which incorporates multiple types of interactions like hydrogen bonding, hydrophobic interaction and electrostatic interaction [6–9]. A well-known advantage of mixed-mode resin is its salt-tolerant characteristic which is attributed to its multimodal ligands [10,11]. This allows the capture of proteins directly from salt-containing feed streams without the need of pre-dilution or diafiltration step thus saving buffer consumptions, process time, and ultimately operational costs [1,12]. The coexistence of multiple modes of interactions also leads to increased selectivity of the mixed-mode resin over a broad range of ionic strengths and pH values [1,9,13,14]. While some groups have reported enhanced aggregates removal in post protein A pool using mixed-mode chromatography [15–19], others have suggested to fully replace protein A capture step with mixed-mode resin [20,21].

Although many studies have been conducted to elucidate protein-ligand binding mechanism in mixed-mode chromatography [22–24], the design and optimization of a purification process using this type of resin are still based on trial and error which require substantial amounts of experimental works [25,26]. To accelerate the design of an efficient, robust and economical purification process, rational approaches aided by in-silico simulations should be adopted [27–30]. By developing mathematical models using thermodynamic principles, protein retention under various process conditions can be predicted using minimal sets of empirical data [27,28,31–34].

* Corresponding author at: Institute of Biochemistry, University of Applied Sciences Mannheim, Paul-Wittsack-Str. 10, 68163 Mannheim, Germany.
E-mail address: c.frech@hs-mannheim.de (C. Frech).

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Melander et al. [35,36] described both electrostatic and hydrophobic interactions on weak and strong ion exchange media. They combined Manning's counter-ion condensation theory and Sinanoglu's solvophobic theory to describe the effects of salt on the elution behavior in ion exchange and mixed mode chromatography [37,38]. Mollerup et al. [27,28,32,33] introduced a general thermodynamic framework for protein adsorption in ion exchange chromatography (IEC) and hydrophobic interaction chromatography (HIC) based on the activity coefficients of protein and salt in the mobile and stationary phases. It can be employed to assess the influences of ligand density and protein load on protein adsorptions over a wide range of salt concentrations [25,27,28,32,33,38]. Deitcher et al. [25] modified Mollerup's model by inserting solvent molecules as standalone species in the adsorption process to enable the determination of the number of water molecules released upon protein-ligand binding in HIC. Nevertheless, Deitcher's model does not elucidate the "salting in" effects at very low salt concentrations [25]. Nfor et al. [39] on the other hand, extended Mollerup's model and adapted it to study the effects of salt concentration, pH and protein on mixed-mode media by using high throughput isotherm measurement techniques. Yamamoto's [40–42] linear gradient elution (LGE) model is often used to describe protein retention using single salt or pH gradient in IEC. In our previous paper [43], we have demonstrated that Yamamoto's [40–42] LGE model can be used to describe protein retention in cation mixed-mode chromatography but it is constrained to low salt concentration where hydrophobic interaction is assumed to be negligible.

In this work, an extended thermodynamic model is introduced to describe protein retention in mixed-mode chromatography using isocratic elution experiments. This extended model is based on previous platforms suggested by Deitcher et al. [25], Melander et al. [36], Mollerup et al. [27,28,32,33], and Nfor et al. [39]. The advantage of this extended model is its ability to describe mixed-mode protein retention over a wide range of salt concentrations and it can be used on mixed-mode resins with different surface chemistries and ligand densities. Furthermore, the number of water molecules released at the hydrophobic interfaces is also integrated into the extended model to describe the influence of surface contact area between the protein and the ligand on the protein-ligand binding. The effects of pH, salt, protein, and ligand on the mixed-mode retention behavior are investigated based on various meaningful thermodynamic model parameters. Finally, the fitted model parameters from the isocratic elution data are transferred into Yamamoto's [40–42] LGE model to predict retention behavior in dual salt-pH gradient elution chromatography.

2. Theoretical consideration

2.1. Isocratic elution

The fundamental of our mixed-mode model is based on the framework demonstrated by Deitcher et al. [25], Melander et al. [36], Mollerup et al. [27,28,32,33], and Nfor et al. [39]. Our mixed-mode interaction stoichiometry is assumed to be as follows:

$$P + \nu SL^{\pm} + nLW_{\xi} \Leftrightarrow PL_{\nu}^{\pm}L_n + \nu S + n\xi W \tag{1}$$

in which the protein P can interact with ν numbers of ionic ligands $L^{\pm}$ and simultaneously bind with n numbers of hydrophobic ligands L, exchanging with counter-ions S and ξ numbers of solvent molecules (water) W. The thermodynamic equilibrium constant K_E can be defined using the activities of the participating species as [39]:

$$K_E = \left(\frac{a_{PL_{\nu}^{\pm}L_n}}{a_P}\right)\left(\frac{a_S}{a_{SL^{\pm}}}\right)^{\nu}\left(\frac{1}{a_{LW_{\xi}}}\right)^{n} {a_W}^{n\xi} \tag{2}$$

where the activity of the solvent molecule (water) a_W is included as an extension to Nfor's model [39].

Adopting similar mathematical treatments as Nfor et al. [39] (see Appendix A for the details of the derivations), the inclusion of the term ${a_W}^{n\xi}$ into Eq. (2) will result in a modified expression of the distribution coefficient or initial slope of the mixed-mode isotherm A_i, which becomes:

$$\ln A_i = -\frac{\Delta\hat{G}_P^0}{RT} + \nu\left(\frac{\Delta G_S^0}{RT} + \ln\frac{\Lambda_{IEC}}{z_s}\right) + \frac{n\left(\xi\hat{\mu}_W^0 - \hat{\mu}_L^0\right)}{RT}$$
$$+ n\ln\left(\frac{\Lambda_{HIC}}{c}\right) - \nu\ln c_s + \left(K_S - n\xi\rho\right)c_s \tag{3}$$

where the change in Gibbs energy of association of protein and counter-ion are depicted as $\Delta\hat{G}_P^0$ and ΔG_S^0, respectively, the universal gas constant is R, the absolute temperature is T, the molar concentration of the solution per pore volume is c, the counter-ion concentration in the mobile phase is c_s, the salt interaction constant is K_S, the hydrophobic ligand density is Λ_{HIC}, the ionic ligand density is Λ_{IEC}, the standard state chemical potentials of the water and ligand are $\hat{\mu}_W^0$ and $\hat{\mu}_L^0$, respectively, the empirical correlation constant between salt and water activity is ρ (=−0.034 for sodium chloride) [44], and the charge of the counter-ion is z_s.

2.2. Linear gradient elution (LGE)

Carta and Jungbauer [45] had adapted Yamamoto's LGE model [41,42] to describe gradient elution using a mobile phase modifier (M) which they had regarded as a component competing with the feed mixture during the adsorption process. Under the assumptions that both modifier and feed mixture depict linear adsorption isotherms and that the adsorption of the former is independent of the concentration of the latter, they [45] had presented a general relationship as follows:

$$\frac{\beta L}{\upsilon} = \int_{c_{M,init}}^{c_{M_i,elu}} \frac{1}{k'_i(c_M) - k'_M}\,dc_M \tag{4}$$

where the gradient slope is β, the column length is L, the interstitial velocity is υ, the initial modifier concentration is $c_{M,init}$, the modifier concentration at which the protein species i elutes is $c_{M_i,elu}$, and the retention factors of the modifier M and of the protein species i are k'_M and $k'_i(c_M)$, respectively. By expressing k'_i in terms of elution volume in isocratic conditions at the peak modifier concentration (see Appendix B for the details of the derivations) will result in a correlation between the normalized gradient slope $G_{salt,pH}\Phi$ and the distribution coefficient A_i as:

$$\frac{dG_{salt}\Phi}{dc_{s,elu}} = \frac{dG_{pH}\Phi}{dpH_{elu}} = \frac{1}{K_{d,i}A_i\left(c_{s,elu}, pH_{elu}\right) + K_{d,i} - 1}$$
$$= \left[K_{d,i}\cdot\left(\exp\left(-\frac{\Delta\hat{G}_p^0}{RT} + \nu\frac{\Delta G_s^0}{RT}\right)\cdot {\Lambda_{IEC}}^{\nu}\cdot {c_{s,elu}}^{-\nu}\cdot\exp\left(\frac{n\left(\xi\hat{\mu}_W^0 - \hat{\mu}_L^0\right)}{RT}\right)\cdot\left(\frac{\Lambda_{HIC}}{c}\right)^{n}\cdot\exp\left(\left(K_s - n\xi\rho\right)\cdot c_{s,elu}\right)\right) + K_{d,i} - 1\right]^{-1} \tag{5}$$

where salt and pH are used as the modifier, respectively. The elution salt concentration and elution pH of the protein are $c_{s,elu}$ and