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Thermodynamic description of peptide adsorption on mixed-mode resins

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ABSTRACT

In this work the adsorption of tri-peptides on a mixed-mode resin was studied using isocratic pulse response experiments. Various salt concentration, temperature and pH combinations were used to measure retention times of several tri-peptides. The experiments were evaluated according to an extension of the stoichiometric displacement model and the steric mass action model of protein–ligand binding. The application of this model in the understanding of mixed mode adsorption process is discussed. A unique set of meaningful thermodynamic parameters was obtained for each resin-peptide-temperature and resin-peptide-pH combination. Finally it was shown that these thermodynamic parameters can be used in defining quantitative relationships within the framework of extra thermodynamic relationships.

1. Introduction

Fundamental understanding of the binding behaviour of biomolecules such as proteins, polypeptides or amino acids at liquid–solid interfaces in chromatographic systems from a mechanistic point of view will help designing these systems better. Cramer et al. [1] used quantitative structure–property relationships (QSPR) models to understand protein retention in ion-exchange systems. Kaliszan et al. [2] used QSPR models that use linear free energy relationships (LFER) to understand reversed phase liquid chromatography (RPLC) adsorption behaviour. Hearn et al. [3] used van't Hoff analysis to understand both ion-exchange (IEX) and hydrophobic interaction chromatography (HIC) systems. Mollerup [4] developed a theoretical thermodynamic framework to study retention of proteins and peptides in IEX and HIC which addressed mobile phase salt concentration, ligand density and protein loading effects.

Mixed mode chromatography (MMC) with two orthogonal modes of interactions to effect separation has several advantages over single mode chromatography, like a better selectivity [5] compared to a conventional IEX chromatography or HIC. Moreover MMC can be used for direct capture [6] of product from unadjusted feed streams thus avoiding the use of any pretreatment techniques. In spite of these advantages there is not much understanding on the mechanisms underlying mixed mode adsorption. So there is a need to get fundamental insight into the mechanistic understanding of mixed-mode chromatography in order to optimally use mixed-mode chromatography in industry. The combination of fundamental knowledge on binding of peptides at liquid–solid surfaces with an experimental approach to obtain thermodynamic parameters of binding of peptides on a mixed-mode resin, can be a valuable part of understanding the complex interactions with these novel ligands. Nfor et al. [7] have done work in this direction using protein adsorption on mixed-mode resins. This work uses smaller peptides with known sequences to gain more insight into the complex binding phenomenon occurring on mixed-mode resins. If the thermodynamic parameters can be coupled to linear or non-linear energy relationships associated with the binding of peptides with mixed-mode ligands, it can be a valuable tool in understanding the complex mechanisms underlying mixed mode interaction.

In this work we define a thermodynamic framework for the analysis of peptide adsorption in MMC and discuss how thermodynamic parameters of adsorption can be determined using this framework. Furthermore we describe the interaction of the different peptide sequences with this mixed-mode resin using linear free energy relationships (LFER) and group molecular

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parameters (GMP) relations to see if any such relationships exist for the different sequences chosen in this work. These relations might give insight into the important physicochemical factors that influence binding equilibrium, steric, charge and hydrophobic effects in mixed-mode systems. The ability to predict isotherm parameters from the structure of adsorbed molecules will lead to a better understanding and a more optimal use of mixed-mode chromatography.

2. Theory and thermodynamic model

Different thermodynamic frameworks were developed to mechanistically understand the interaction of various chromatography modes, but most of them are based on one type of interaction. For example, the Steric Mass Action (SMA) formalism of Brooks and Cramer [8] describes complex behaviour in ion exchange chromatography and Van't Hoff analysis is used to determine the enthalpy change from retention data in a small temperature range which is used for HIC modelling [3,9,10]. Mollerup used fundamental thermodynamic principles of ligand-binding principles and derived adsorption isotherms for both ion-exchange and hydrophobic interaction chromatography [4].

For the mixed mode mechanism studied in the present case the following stoichiometry is used:

$$P + \nu\mathrm{SL} \rightleftharpoons PL_\nu + \nu S \tag{1}$$

where ν is the stoichiometric coefficient of the salt counter-ion (the number of IEX ligands), SL, associated to a peptide unit, P and L, associated to the same peptide unit.

This stoichiometry model is similar to Steric Mass Action formalism but takes besides the electrostatic interactions also hydrophobic interactions into account by the non-ideality term as given in the definition of equilibrium constant:

$$K_{eq} = \frac{a_{PL_\nu}}{a_p}\left(\frac{a_s}{a_{SL}}\right)^\nu = \left(\frac{q_p\gamma_{PL_\nu}}{C_p\gamma_p}\right)\left(\frac{C_s\gamma_s}{q_s\gamma_{SL}}\right)^\nu \cong \left(\frac{q_p}{C_p}\right)\left(\frac{C_s}{q_s}\right)^\nu\left(\frac{1}{\gamma_p}\right) \tag{2}$$

All activity coefficients except γ_p can be presumed unity or constant [4]. Following this assumption, the right hand side of Eq. (2) is obtained. In order to normalize the activity coefficient, it is recommended to work with asymmetric activity coefficients ($\tilde{\gamma}$) by using the relationship $\tilde{\gamma}_\iota = \gamma_i/\gamma_i^\infty$ in which γ_i^∞ represents the activity coefficient of the peptide at infinite dilution. The activity coefficient at infinite dilution is a constant that can be lumped with the K_{eq} in $\tilde{K}_{\mathrm{eq}}$.

The HIC interactions are captured by the non-ideality term $\tilde{\gamma}_p$. An isotherm relation for the above stoichiometry can be derived [4,7] and is of the form:

$$\frac{q_{p,i}}{C_{p,i}} = A_i\left(1 - \sum_{j=1}^{m}\frac{q_{p,j}}{q_{p,\mathrm{MM},j}^{\max}}\right)^\nu \tag{3}$$

The initial slope A_i of the isotherm Eq. (3) can be obtained by applying the limiting conditions as $q_p \to 0$ is given as:

$$A_i = \lim_{q_p\to 0}\left(\frac{q_p}{C_p}\right) = \Lambda^{\nu_i}(z_sC_s)^{-\nu_i}\tilde{K}_{\mathrm{eq},i}\tilde{\gamma}_{p,i} \tag{4}$$

Λ is the ligand density, $\tilde{K}_{\mathrm{eq},i}$ is the thermodynamic equilibrium constant, C_s is the liquid phase salt concentration and $\tilde{\gamma}_{p,i}$ is the asymmetric activity coefficient of the peptide in solution. Furthermore $\nu = z_p/z_s$ is the stoichiometric coefficient of the salt counter ion where z_p is the effective binding charge of the peptide and z_s is the charge on the salt counter-ion. This initial slope expression can be incorporated into a linear retention model [11,12] equation to give:

$$V_{R,i} - V_{\mathrm{sec},i} = V_c(1-\varepsilon_b)\varepsilon_p K_{d,i}A_i \tag{5}$$

$$\ln\left(\frac{V_{R,i}-V_{\mathrm{sec}}}{V_c}\right) = \ln(1-\varepsilon_b)\varepsilon_p K_{d,i} + \nu_i\ln\left(\frac{\Lambda}{C_sz_s}\right) + \ln\tilde{\gamma}_{p,i} + \ln\tilde{K}_{\mathrm{eq},i} \tag{6}$$

$\tilde{\gamma}_{p,i}$ can be rewritten to $\tilde{\gamma}_{p,i} = e^{K_sC_s+K_pC_{p,i}}$ based on the equilibrium of the salt and the peptide, but at low peptide concentration $C_p = 0$ which states that $\tilde{\gamma}_{p,i} = K_sC_s$ yielding:

$$\ln\left(\frac{V_{R,i}-V_{\mathrm{sec}}}{V_c}\right) = \ln(1-\varepsilon_b)\varepsilon_p K_{d,i} + \nu_i\ln\left(\frac{\Lambda}{C_sz_s}\right) + K_sC_s + \ln\tilde{K}_{\mathrm{eq},i} \tag{7}$$

In case the retention of peptides at various temperatures are measured the standard enthalpy change of adsorption (ΔH^0) can be determined as the temperature derivative of ΔG via the van't Hoff equation.

$$\frac{\partial\ln\tilde{K}_{\mathrm{eq}}}{\partial(1/T)} = \frac{\partial\left(\frac{\Delta G^0_{\mathrm{exc}}}{RT}\right)}{\partial(1/T)} = -\frac{\Delta H^0}{R} \tag{8}$$

$\tilde{K}_{\mathrm{eq},i}$ determines the direction of equilibrium for the adsorption reaction (Eq. (1)). When $\tilde{K}_{\mathrm{eq},i}$ has a value above one the direction of the reaction is towards the right hand side and below one this reaction is directed to the left hand side, i.e. towards the reactants. With the $\tilde{K}_{\mathrm{eq},i}$ the free energy of exchange for adsorption can be calculated using:

$$\Delta G^0_{\mathrm{exc}} = -RT\ln\tilde{K}_{\mathrm{eq}} \tag{9}$$

Furthermore, for a reversible process the standard Gibbs free energy is given by:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{10}$$

Where ΔH^0 and ΔS^0 are the standard enthalpy and entropy changes of the process respectively. Eq. (10) is finally used to calculate the entropy change during adsorption.

2.1. Estimation of model parameters

When using Eq. (7) to fit the three parameters ν_i, K_s and K_{eq} against experimental retention volume (V_r) values, an almost linear correlation between ν_i and K_s is found. Therefore, additional experimental information was included in the fitting procedure, being the salt concentration at minimum retention volume. Theoretically the following equation holds:

$$C_{s,\min} = \frac{\nu_i}{K_s} \tag{11}$$

Eq. (11) can be derived using Eq. (7) and taking the derivative of V_r to C_s being zero, i.e. $\partial V_r/\partial C_s = 0$. Subsequently substituting Eq. (11) in Eq. (7) yields the final equation used for regressing the parameters:

$$\ln\left(\frac{V_{R,i}-V_{\mathrm{sec}}}{V_c}\right) = \ln(1-\varepsilon_b)\varepsilon_p K_{d,i} + C_{s,\min}K_s\ln\left(\frac{\Lambda}{c_sz_s}\right) + K_sC_s + \ln\tilde{K}_{\mathrm{eq},i} \tag{12}$$

The unknown thermodynamic parameters in Eq. (12) namely $\tilde{K}_{\mathrm{eq},i}$ and K_s were determined by weighted nonlinear regression