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Water on hydrophobic surfaces: Mechanistic modeling of hydrophobic interaction chromatography

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ABSTRACT

Mechanistic models are successfully used for protein purification process development as shown for ion-exchange column chromatography (IEX). Modeling and simulation of hydrophobic interaction chromatography (HIC) in the column mode has been seldom reported. As a combination of these two techniques is often encountered in biopharmaceutical purification steps, accurate modeling of protein adsorption in HIC is a core issue for applying holistic model-based process development, especially in the light of the Quality by Design (QbD) approach.

In this work, a new mechanistic isotherm model for HIC is derived by consideration of an equilibrium between well-ordered water molecules and bulk-like ordered water molecules on the hydrophobic surfaces of protein and ligand.

The model's capability of describing column chromatography experiments is demonstrated with glucose oxidase, bovine serum albumin (BSA), and lysozyme on Capto™ Phenyl (high sub) as model system. After model calibration from chromatograms of bind-and-elute experiments, results were validated with batch isotherms and prediction of further gradient elution chromatograms.

1. Introduction

Biologics, especially therapeutic proteins, count among the fastest-growing market segments in the current pharmaceutical industry. To cope with the growing number of drug candidates, downstream process development (DSP) faces the challenge of set-up processes for new molecules with limited sample volume within a short time frame. For purification of therapeutic proteins, especially monoclonal antibodies (mAbs), preparative chromatography is the core technology to deliver a highly pure product [1].

Hydrophobic interaction chromatography (HIC) is a frequently used method for intermediate and polishing purposes in DSP [2–4]. HIC is well-known for its remarkable capability to remove aggregates and other non-polar contaminants [4–6]. To date, HIC process development commonly relies on rules of thumb [7] or high-throughput experimentation [8]. Alternative methodologies based on the three-dimensional structure of the protein and its surface hydrophobicity were examined by Mahn and Asenjo [9].

To meet the demands of the Quality by Design approach (QbD) [10–12], a high degree of process understanding is required that can be demonstrated by model building and simulations. At the same time, the sample volume needed for model calibration is expected to be much less than the amount needed for screening with the Design-of-Experiments (DoE) approach. To create a chromatography model, the fluid dynamical principle of mobile phase mass transfer through the chromatography column as well as the thermodynamical principles of protein–ligand interaction [13] must be described. The mass transfer within a chromatography column is well-studied. Several variants of equilibrium and transport-dispersive models are widely accepted [14]. Model-based process development was demonstrated several times for ion-exchange chromatography (IEX) [15,16] with the steric-mass action (SMA) isotherm [17]. The SMA isotherm describes the salt-protein relationship based on the electrostatic equilibrium theory and displacement effect, such that the salt-dependent protein–ligand interaction is directly incorporated. In the case of HIC, this derivation cannot be applied. The challenge of HIC modeling lies in accurately describing the more complex salt-dependent protein–ligand interaction.

The very first theoretical framework for modeling salt effects in hydrophobic interaction chromatography was derived by Melander

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and Horvath [18], facilitating experimental design and result interpretation. Staby and Mollerup investigated the thermodynamical nature of HIC and presented a mechanistic isotherm [19,20]. The salt dependence of protein adsorption was described with an exponential term containing protein activity as well as salt activity coefficients. Perkins et al. applied the preferential interaction model to examine the salt dependence of the HIC capacity factors [21]. Chen et al. also used the preferential interaction model to determine the water molecules released upon salt-related protein binding [22]. Haimer et al. applied an extended Langmuir kinetic derived by Lundstrom to model the spreading phenomenon resulting from injection of a pure protein sample [23,24]. In addition, Jungbauer et al. introduced a modified salt-dependent Langmuir isotherm for linear and nonlinear conditions [25]. Water molecules were first included as participants in the adsorption process by Deitcher et al. [26]. Their isotherm described the salt-dependent release of water molecules from the contact area of protein and ligand. Chen and Sun proposed to consider a dehydrated state of the protein, caused by the hydration effect of salt ions [27]. Mirani and Rahimpour extended this two-state isotherm with the consideration of activities instead of concentrations [28].

The formation of a hydrophobic protein–ligand interface is thought to be driven by the hydrophobic effect, involving a reorganization of the water structure on the hydrophobic surfaces of protein and ligand [29]. Frank and Evans assumed a well-ordered ice-like formation of water around hydrophobic groups [30]. Later, a clathrate-like structure of water on hydrophobic surfaces was proposed [31]. In 2014, Shiraga et al. carried out terahertz spectroscopy to study the water structure on hydrophobic surfaces of biomolecules and found water molecules around the hydrophobic groups to be more ordered [32].

The present manuscript was enabled by the aforementioned contributions. The new insights into the water structure on hydrophobic surfaces were taken into account and a new mechanistic isotherm was derived for HIC. Keeping the requirements of model-based HIC process development in mind, several assumptions and simplifications were made to facilitate practical applicability. Glucose oxidase, bovine serum albumin (BSA), and lysozyme were chosen as model proteins. This set of proteins covers very different hydrophobic properties and a wide range of sizes, being 160 kDa, 66 kDa, and 14.6 kDa. Gradient elution as well as high-throughput batch experimentation was carried out to prove the predictability of the new isotherm beyond the calibration space.

2. Theory

2.1. Derivation of a HIC isotherm considering the water structure

The adsorption mechanism in HIC has been proposed to be an equilibrium between a protein molecule P with stoichiometric number n of ligands L and the protein–ligand complex P_{Ln} [20,21]:

$$P + nL \longleftarrow P_{Ln} \tag{1}$$

This proposition does not explicitly describe the salt dependence in the case of HIC. Later, Deitcher et al. proposed water to be included as a product W with stoichiometric number ξ by assuming a release of water molecules at the protein–ligand contact area [26]:

$$P + nL \longleftarrow P_{Ln} + n\xi W \tag{2}$$

A water displacement process originally proposed by Geng et al. [33] was used as theoretical foundation. The salt was included as a participant by considering the thermodynamic activity of water in electrolyte solutions. They applied a linear empirical model, which describes the natural logarithm of water activity to be equal to the product of salt concentration and a constant factor [26].

Meanwhile, thermodynamical studies have shown that hydrogen bonds between water molecules on hydrophobic surfaces are disrupted to create a cavity. As a counterreaction, water molecules form well-ordered structures, such that a thermodynamically favored state is achieved [34–36]. Upon adsorption, these well-ordered water molecules are thought to be reorienting in a bulk-like structure. This so-called hydrophobic hydration phenomenon is added to the general consideration of the adsorption mechanism according to Eqs. (1) and (2) to formulate the new adsorption isotherm.

The hydrophobic surfaces of protein P and ligand L are thought to be stabilized by well-ordered water molecules. The protein–ligand complex P_{Ln} is assumed to be bound with n binding sites, which are stabilized by β bulk-like water molecules W_B each:

$$P + nL \longleftarrow P_{Ln} + n\beta W_B \tag{3}$$

Under consideration of Gibbs free energy and following the approach presented by Mollerup [20], the equilibrium contant K is derived:

$$K \cong a_{W_B}^{n\beta} \frac{q}{c_p c_L^n} \tag{4}$$

Here, q and c_p are the concentrations of bound protein and protein in solution, respectively. c_L depicts the concentration of ligands available for binding and a_{W_B} the activity of the bulk-like water molecules. In the next step, parameterizations for a_{W_B}, c_L and β have to be found.

According to stoichiometric considerations, the number of water molecules involved is linearly correlated to the protein concentration in the stationary phase q. Thus, a linear correlation is proposed to substitute a_{W_B}:

$$a_{W_B}^{n\beta} \cong \nu q^{n\beta} \tag{5}$$

Here, the stoichiometric constant ν is assumed to be independent of the salt concentration.

In Eq. (6), the free ligand concentration c_L is defined as a function of total capacity Λ, hydrophobic binding sites n, and steric hindrance factor s similarly to the SMA model and formulation by Mollerup for HIC [17,20].

$$c_L^n = (\Lambda - (n+s)q)^n \tag{6}$$

Inserting Eqs. (5) and (6) into Eq. (4) and collecting all constants on the left-hand side, we obtain the following isotherm equation:

$$K = \frac{\nu q^{1+n\beta}}{c_p(\Lambda - (n+s)q)^n} \tag{7}$$

$$\overset{\frac{\Lambda^n}{\nu}}{\Leftrightarrow}: k_{eq} = \frac{q^{1+n\beta}}{c_p(1-(q/q_{\max}))^n} \tag{8}$$

with the saturation capacity $q_{max} = \Lambda/(n+s)$.

In the final step, the salt dependency of the bulk-like ordered water molecules β is modeled. To this, β and the hydration number of the salt ions h are assumed to be reciprocal. As the salt ions attract water molecules to form a hydration shell, the ionic hydration number h is described with high reliability by an exponential relation as [37]:

$$h = h_0 \exp(-kc_s) \tag{9}$$

with h_0 being the ionic hydration number at infinite dilution and k the constant that describes the change of hydration number with increasing ionic concentration c_s. Thus, the salt-dependence of the model parameter β can be described by the exponential term:

$$\beta = \beta_0 \exp(\beta_1 c_s) \tag{10}$$

This completes the derivation of the equilibrium formulation of the isotherm model.