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A modified Poisson-Boltzmann equation applied to protein adsorption

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ABSTRACT

Ion-exchange chromatography has been widely used as a standard process in purification and analysis of protein, based on the electrostatic interaction between the protein and the stationary phase. Through the years, several approaches are used to improve the thermodynamic description of colloidal particle-surface interaction systems, however there are still a lot of gaps specifically when describing the behavior of protein adsorption. Here, we present an improved methodology for predicting the adsorption equilibrium constant by solving the modified Poisson-Boltzmann (PB) equation in bispherical coordinates. By including dispersion interactions between ions and protein, and between ions and surface, the modified PB equation used can describe the Hofmeister effects. We solve the modified Poisson-Boltzmann equation to calculate the protein-surface potential of mean force, treated as spherical colloid-plate system, as a function of process variables. From the potential of mean force, the Henry constants of adsorption, for different proteins and surfaces, are calculated as a function of pH, salt concentration, salt type, and temperature. The obtained Henry constants are compared with experimental data for several isotherms showing excellent agreement. We have also performed a sensitivity analysis to verify the behavior of different kind of salts and the Hofmeister effects.

1. Introduction

Protein adsorption is the key phenomenon of countless biological processes as well of many protein separation and purification technologies. It is mainly governed by electrostatic interactions between the protein surface and the adsorbate. Because of that, it is crucial to have a good description of the electrostatic system when modeling this phenomenon. One of the first attempts to model the behavior of colloidal systems came from the Derjaguin-Landau-Verwey-Overbeek theory (DLVO) that considers an electrostatic double layer formed on the surroundings of a charged surface [1] which could be described by a linearized form of the Poisson-Boltzmann (PB) equation [2]. Based on the Hamaker [3] contribution, Verwey [2] improved the PB approach by combining the attractive London-van der Waals potential with electrostatic interactions. For protein adsorption, Ståhlberg et al. [4] applied the DLVO theory to study the chromatography of proteins using an analitical equation combining the effect of coulombic and van der Waals interactions. Results come from this contribution [4] are improved due to the link between the parameter of protein retention and the forces related to protein-support interaction.

Another approach often used to model protein adsorption is the Sterical Mass-Action method (SMA) [5–7]. SMA applies a stoichiometric binding theory and couples, in a set of correlation parameters, all the electrostatic and equilibrium information of the system. In this case they do not consider important effects like non-electrostatic (NES) and co-ion effects [5], leading to a poor accuracy at higher salt concentrations or pH values close to the protein isoelectric point (pI) [7]. The same happens to all the models based on the classical DLVO theory because it does not take into account the non-electrostatic effects between ions and protein. Even though NES effects can be neglected at low ionic strength (0.01 M), they cannot be ignored when modeling highly concentrated electrolyte solutions or multivalent ions. This directly impacts on the need for improvement of the colloid theory for biological applications [8]. Most of the time, their impact follows directly the Hofmeister series. To be able to predict this kind of behavior it is essential to consider dispersion forces in the model [9].

Another way to improve the theoretical description of protein adsorption was suggested by Roth and Lenhoff [10] which takes into

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Nomenclature

Symbols

$B_{\pm P}$	Ion-protein dispersion constant (-)
$B_{\pm S}$	Ion-support dispersion constant (−)
B_+	Cation dispersion constant (−)
B_-	Anion dispersion constant (−)
c	Concentration of the colloid (mol m^{-3})
c_{anion}	Concentration of the anions (mol L^{-1})
c_{cation}	Concentration of the cations (mol L^{-1})
c_{H^+}	Concentration of H^+ ions (mol L^{-1})
c_0	Bulk concentration of the colloid (mol m^{-3})
d	Half distance between the center of the sphere and the support (m)
e	Elementary charge of the electron (1.602·10^{-19} C)
F	Faraday constant (C mol^{-1})
f	Nondimensional force (−)
I	Ionic strength (mol L^{-1})
h	Distance between the protein surface and the stationary surface (m)
H	Hamaker constants (J)
K	Dimensional Henry constant (m)
Ka	Effective dissociation constant (mol L^{-1})
Ks	Effective association constant (mol L^{-1})
q	Surface excess concentration (mol m^{-2})
r_1	Distance between the ion and the colloid (m)
r_2	Distance between the ion and the planar surface (m)
r_{ion}	Ion radius (m)
r_w	Protein hydration-layer thickness (m)
R_{sphere}	Protein radius (m)
T	Absolute temperature (298.15 K)
$U_{\pm}$	Ion-protein and ion-adsorbent van der Waals interaction (−)
W	Potential of the mean force of sphere-plate interaction (J)
W^{PB}	Electrostatic contribution to the potential of mean force (J)
W^{hs}	Hard-sphere contribution to the potential of mean force (J)
W^{Ham}	Potential Hamaker contribution to the potential of mean force (J)
x,z	Cartesian coordinates

Greek symbols

γ	Activity coefficient of the salt (−)
β	Parameter related with the protein radius and η_0(−)
ε_0	Vacuum permittivity (8.854·10^{-12} F m^{-1})
ε_R	Dielectric constant of the medium (80 for water)
η,θ	Independent variables of the bispherical coordinate system
ρ	Density of ionizable surface group (mol m^{-2})
k_B	Boltzmann constant (1.3806·10^{-23} J K^{-1})
k_D	Inverse Debye length (m^{-1})
$\sigma_{NH_3^+}$	Charge density of amino groups (C m^{-2})
σ_{His^+}	Charge density of histidine groups (C m^{-2})
σ_{COO^-}	Charge density of carboxyl groups (C m^{-2})
$\sigma_{SO_3{}^-}$	Charge density of sulfonated groups (C m^{-2})
σ_1	Colloid charge density (C m^{-2})
σ_2	Stationary phase charge density (C m^{-2})
$\sigma_{\pm}$	Charge density of the amino acid groups (C m^{-2})
ψ	Dimensionless electrostatic potential $\left(e\varphi/k_BT\right)$

account the three-dimensional configuration of the protein, using information of the mesh conformation of lysozyme while interacting with a stationary surface. This approach, though, still used the linearized form of the Poisson-Boltzmann equation. Considering Hamaker interactions, the result from Roth and Lenhoff [10] showed a good agreement with experimental data for lysozyme at small ionic strength, as expected, but not for a wide range of ionic strength.

When applying the Poisson-Boltzmann equation to describe spherical colloids it is important to define the better coordinate system in which this equation is applied. This helps to improve the calculation of the interaction between two colloids or colloid-surface particles in contrast with using a planar geometry together with the Derjaguin approximation to resemble spherical-planar geometry [11–13]. The PB equation in Bispherical Coordinates was chosen by Lima et al. [14] as a better approach that provides a good computational time, discretization scheme and accuracy. These bispherical coordinates and numerical procedure were able to calculate the osmotic second virial coefficient as a function of ionic strength considering the interaction between two charged colloids (globular proteins). The article also reports the application of the Hamaker constant and analyses the effect of the kind of salt arising due to non-electrostatic interactions.

An equilibrium model was reported by Ståhlberg et al. [15] showing the relationship between the electrostatic contribution to the retention factor (relative to adsorption equilibrium constant for a diluted system, i.e. Henry constant) for ion-exchange chromatography (IEC) of proteins.

In order to predict the influence of protein adsorption, here we calculate the Henry constant of a protein modeled as a charged colloidal particle to be adsorbed on an ion-exchange adsorbent using the modified PB equation in bispherical coordinates. We consider the dispersion interactions between ions and protein and between ions and adsorbent surface, and the Hamaker potential between protein and adsorbent surface. With this approach, we can predict the Henry constant as a function of pH, ionic strength, ionic specificity, and temperature.

2. Electrostatic model for different coordinate systems

To describe the adsorptive behavior of proteins in chromatographic columns, including the electrostatic interactions between charged surfaces, it is necessary to establish a model capable to describe the retention of protein such as reported in [11–13]. The model is developed by combining the Poisson-Boltzmann equation and the Langmuir adsorption model, considering that the surfaces, both protein and support, contain homogeneous distributed charge density that are dependent on pH and ionic strength.

Using the equilibrium condition for the chemical potential of protein at the dilute system, we obtain [16]:

$$c = c_0 \exp\left[-\frac{W(I, pH, h)}{k_B T}\right] \tag{1}$$

where c is the concentration of colloid (protein) at h, c_0 is the bulk concentration of colloid, h is the distance between the protein surface and the surface of the stationary phase, I is the ionic strength of the solution, W is the free energy of interaction between the colloid and the adsorbate, k_B is the Boltzmann constant, and T is the absolute temperature (here 298.15 K).

Knowing the concentration of protein at different distance h, the surface excess concentration can be obtained by:

$$q(pH, I) = \int_0^\infty (c - c_0)\, dh \tag{2}$$

where q is the surface excess concentration of the protein for given desired pH and ionic strength, I.