

Ion-Specific Potential of Mean Force Between two Aqueous Proteins

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Abstract

There is a considerable effort in the literature trying to calculate the mean force between globular proteins (and colloidal particles). To this purpose, we used here the ion-specific Poisson-Boltzmann (PB) equation that presents good results of ionic concentration profiles around a macroion, especially for salt solutions containing monovalent ions. The ion-specific PB equation includes not only electrostatic interactions but also dispersion potentials, originated from polarizabilities of ions and proteins. This enables us to predict ion-specific properties of colloidal systems. Results are in agreement with the experimental observed Hofmeister series. The main contribution of this paper is the use of a differential approach to calculate the mean force between aqueous proteins and colloidal particles instead of the classical quadrature approach. The integral expressions needed to calculate the mean force, potential of mean force and second virial coefficients have been avoided using this new numerical procedure. These integrals were transformed in a set of first order partial differential equations solved simultaneously with the ion-specific PB equation. Resulted expressions were written in bispherical coordinates, and then numerically solved through finite volume method. This simultaneous approach presents more accuracy in the calculation of the mean force in comparison with the classical approach, where the potential profile is obtained by solving the PB equation, and mean force is calculated afterwards. Important thermodynamic properties are obtained from the mean force (and consequently, from the potential of mean force), e.g., osmotic second virial coefficients and phase diagrams. These thermodynamic properties are related to protein aggregation, essential in biotechnology and pharmaceutical industries.

Keywords: Poisson-Boltzmann, Hofmeister effects, protein aggregation

1. Introduction

The mean force between charged surfaces in an aqueous electrolyte solution is a central preoccupation of colloid science and biotechnology. Especially, protein aggregation and precipitation – governed by the force acting between the particles – are crucial in medicine and biotechnology industry.

Forces between charged surfaces in electrolyte solutions have been found to be highly ion specific [1-6]. The reason for this ion specificity, as pointed out by Ninham and co-workers [1], is to a large extent due to previously neglected ion-specific nonelectrostatic (NES) potentials acting between ions and between ions and charged interfaces.[1,5,6] The original DLVO [1] theory fails to predict any such ion specificity. However, when ion-specific NES forces are treated at the same nonlinear level as the electrostatic forces, the origin of ion-specific effects finally comes into sight [7].

As indicated by Moon *et al.* [8], salt-induced protein precipitation is commonly used to purify aqueous proteins. Protein solubility is governed by many factors including pH, surface charge distribution, size, salt type, and salt concentration [9].

More than 100 years ago, Hofmeister showed that the concentration of a salt required to precipitate a protein solution depends strongly on the choice of the salt [10]. According to Ninham *et al.* [1,2,6], contributions that lead to ion specificity depend on ion size, effective polarization of ions, and solvation near interfaces with varying water concentration. For a recent overview of some of these effects, see ref 4.

Using Monte Carlo simulations, Tavares and co-workers [5,11,12] showed that nonelectrostatic (NES) potentials play an important role in protein-protein interactions in salt solutions. Lima *et al.* (2007b) [9] solved the modified Poisson-Boltzmann equation to determine the protein-protein interaction under varying conditions such as pH, salt concentration, and ion type. Without adjustable parameters, results obtained were in excellent agreement with experiment for osmotic second virial coefficients. The model used took into account that the protein surface is covered with acidic and basic charged groups that can be described with the charge regulation model, as shown in Ninham and Parsegian [14]. This same model is used here, but with a different numerical technique to calculate the integrals in bispherical coordinates, as described in Section 3.

Hoskins and Levine (1956) [14] made the first attempt to solve the non-linear Poisson–Boltzmann (PB) equation for identical spheres. More accurate calculations have been obtained over the years since then, using similar techniques [15–17].

A more recent contribution was the calculation of double layer forces between identical spherical particles using a spline collocation scheme [18]. In 1996, Stankovich and Carnie [19] employed similar methods to study double layer interactions between a sphere and a plate, geometry relevant to atomic force microscope measurements.

In the present paper, a modified version of the PB equation is solved using finite volume method in bispherical geometry [7,9,20]. This modified version considers non-electrostatic potentials together with electrostatic potential in the nonlinear PB theory. Lima *et al.* 2007c [20] solved this PB equation for two spheres using finite volume method. Once the electrostatic potential profile was obtained, the double layer force between the spheres (colloidal particles) was calculated solving an integral in bispherical coordinates. Here this integral is converted in a differential equation that is solved using finite volume discretization scheme. The mean forces between two colloidal particles and between two aqueous globular proteins predicted here are compared with those obtained using conventional integration method (9, 20).

2. Ion-Specific Poisson-Boltzmann Equation in Bispherical Coordinates

Lima et al. (2007b,c) have recently presented results for the interaction between two charged spherical colloidal particles and two globular proteins by solving the Poisson-Boltzmann equation in bispherical coordinates using a finite-volume method [9, 20]. The protein charges are assumed to be smeared out on the surface.

Including ion-protein dispersion potentials, the non-linear Poisson-Boltzmann equation in bispherical coordinates is

$$\nabla^2 \psi = \frac{1}{2} [\exp(\psi - U_-) - \exp(-\psi - U_+)], \quad (1)$$

where ∇^2 is the Laplacian operator expressed in bispherical coordinates [18-21]; $\psi = \phi e z_{ion} / (k_B T)$ is the electrostatic potential in nondimensional form, where ϕ is the potential, e is the electric charge, z_{ion} is the charge of ion i , k_B is the Boltzmann constant and T is the absolute temperature. U_+ and U_- are the dispersion van der Waals interactions between a cation (+) and both proteins and between an anion (-) and both proteins, respectively. These NES potentials are obtained from Lifshitz theory [5,9,20].

The ion-specific Poisson-Boltzmann equation is solved using the finite-volume method described in Ref. 13. Once the nondimensional electrostatic potential is calculated, we use the expression for the double-layer nondimensional force [19,20]:

$$f(\eta) = 2\pi \int_0^\pi \left\{ \left[\frac{a^2 (\cosh(\psi) - 1)}{(\cosh(\eta) - \cos(\theta))^2} + \frac{1}{2} \left(\left(\frac{\partial \psi}{\partial \theta} \right)^2 - \left(\frac{\partial \psi}{\partial \eta} \right)^2 \right) \right] \right. \\ \left. \times \left[1 - \cosh(\eta) \cos(\theta) \right] + \frac{\partial \psi}{\partial \theta} \frac{\partial \psi}{\partial \eta} \sinh(\eta) \sin(\theta) \right\} \frac{\sin(\theta) d\theta}{\cosh(\eta) - \cos(\theta)}. \quad (2)$$

Here, (η, θ) are bispherical coordinates and $a = \kappa \sigma \sinh(\eta_0) / 2$, where κ is the inverse of the Debye screening length, σ is the particle diameter and η_0 is the value of η at the surface of the sphere. The nondimensional electrostatic force f is evaluated at the surface $\eta = 0$. This plane was chosen to give good accuracy [19].

From the double-layer force calculated using Eq.(2), we obtain the corresponding double-layer contribution to the potential of mean force W^{PB} [9]:

$$\frac{W^{PB}}{k_B T} = \frac{\epsilon \epsilon_0 k_B T \sigma}{e^2} \int_\infty^{R/\sigma} f \cdot d \left(\frac{R}{\sigma} \right). \quad (3)$$

The total potential of mean force W is

$$W = W^{PB} + W^{Ham} + W^{hs}, \quad (4)$$

where W^{hs} is the hard-sphere contribution, and W^{Ham} is the Hamaker dispersion interaction, i.e., the direct potential between two protein particles due to non-electrostatic interactions both calculated according to ref. 9.

In Eq. (4), W is the free energy required to bring two proteins initially separated by an infinite distance to the center-to-center distance R . Using the potential of mean force W , we calculate the osmotic second virial coefficient [9] from

$$B = \frac{N_A}{2M^2} \int_0^\infty (1 - e^{-W(R)/k_B T}) 4\pi R^2 dR, \quad (5)$$

where N_A is Avogadro's number and M is the molecular weight of the protein.

3. Numerical Method

In this paper we use finite volume method to solve the modified Poisson-Boltzmann equation (Eq.(1)). The same method is used to calculate the integral in Eq. (2). This procedure requires Eq. (2) to be written as a differential equation as follows:

$$\frac{\partial F(\theta)}{\partial \theta} = 2\pi \left\{ \left[\frac{a^2 (\cosh(\psi) - 1)}{(\cosh(\eta) - \cos(\theta))^2} + \frac{1}{2} \left(\left(\frac{\partial \psi}{\partial \theta} \right)^2 - \left(\frac{\partial \psi}{\partial \eta} \right)^2 \right) \right] \right. \\ \left. \times [1 - \cosh(\eta) \cos(\theta)] + \frac{\partial \psi}{\partial \theta} \frac{\partial \psi}{\partial \eta} \sinh(\eta) \sin(\theta) \right\} \frac{\sin(\theta)}{\cosh(\eta) - \cos(\theta)}, \quad (6)$$

where $\eta = 0$ and $F(0) = 0$. The value of the derivatives are calculated numerically from the solution of the modified Poisson-Boltzmann equation for each value of θ .

The value of the force f given in Eq.(2) is obtained evaluating the function F at the end of the interval:

$$f = F(\pi). \quad (7)$$

Eq. (6) is integrated simultaneously with Eq.(1). Once we use the same discretization method to solve both equations, results given by Eq. (7) are expected to be more accurate than those using conventional integration methods.

More details about finite volume method are given elsewhere [20].

4. Results

In this section we present some results obtained from the simultaneous integration of Poisson-Boltzmann equation and Eq.(6), using finite volume method, as described in Section 3. All the results are in agreement with previous ones obtained from the solution

PB equation using finite volume method and subsequent integration using Simpson Method to calculate the force [7,9,20].

In Fig. 1a we reproduce the results reported by Lima *et al.* (2007c) [20]. In this figure we show the force between two similar spherical colloidal particles with nondimensional radius $\kappa r_s = 6.57$ ($r_s = 20\text{\AA}$) as a function of the closest approach distance κh between the particles. The surface charge densities are $\sigma_1 = -0.03\text{C}/\text{m}^2$ and $\sigma_2 = 0.01\text{C}/\text{m}^2$. Four different 0.1 M salt solutions are considered: non-polarizable ions (NP), NaCl, NaBr and NaI. The ions radii are $r_i = 2\text{\AA}$. We can see that the results obtained from finite volume method (symbols) are exactly the same as those reported by Lima *et al.* (2007c), using Simpson method (lines).

Fig. 1b shows the function F obtained from the solution of Eq.(6) as function of θ for the same electrolytes considered in Fig. 1a. These curves correspond to a fixed closest distance $\kappa h = 1$. Because of the initial condition, all the curves begin in $F(0) = 0$. The values of $F(\pi)$ corresponds to the nondimensional double layer force f between the particles at $\kappa h = 1$.

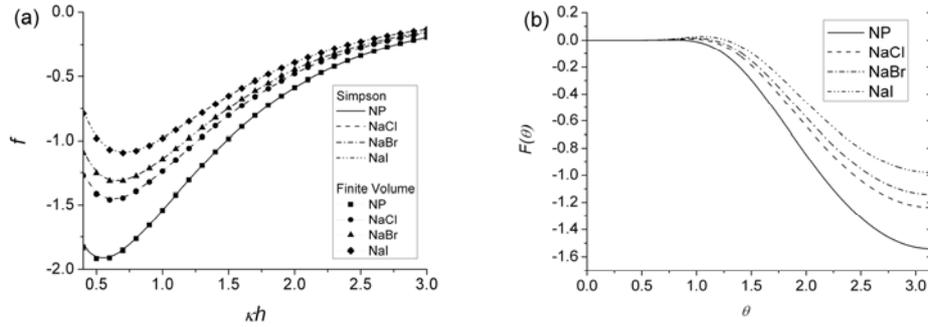


Fig.1. (a) Nondimensional force f between two spheres with charge densities $\sigma_1 = -0.03\text{C}/\text{m}^2$ and $\sigma_2 = 0.01\text{C}/\text{m}^2$ interacting in different 0.1 molar salt solutions. We consider four cases: non-polarizable ions (solid line, square), NaCl (dashed line, circle), NaBr (dash-dotted line, triangle), and NaI (dash-double-dotted line, diamond). Lines correspond to Simpson method [20] and symbols correspond to finite volume method. (b) Function $F(\theta)$ (see Eq.(6)) for a closest distance is $\kappa h = 1$. The value of $F(\pi)$ corresponds to the force f .

The same technique described in Section 3 can be used to calculate the integrals in Eq.(3) and Eq.(5). Using this approach we compute the potential of mean force and second virial coefficient as a function of the salt concentration for different pHs. Fig. 2 illustrates this approach for lysozyme protein at $\text{pH} = 12$. This pH is above the isoelectric point of the protein ($\text{pI} \approx 11.2$).

We observe in Fig. 2 that, at this pH, the less polarizable the anion, the more attractive the force. This happens because above pI protein surfaces are negatively charged. Therefore, when the polarizability of the anion increases, there is an increase in the concentration of co-ions near the surfaces due to dispersion (attractive) interactions between ions and surfaces. This higher concentration of “undesirable” anions gives rise to a repulsive contribution to the double layer force. An opposite series was reported by Lima *et al.* (2007b) [9], for lysozyme at $\text{pH} = 4.5$ (below pI), as expected according to results reported by Hofmeister’s and other works on ion specificity.

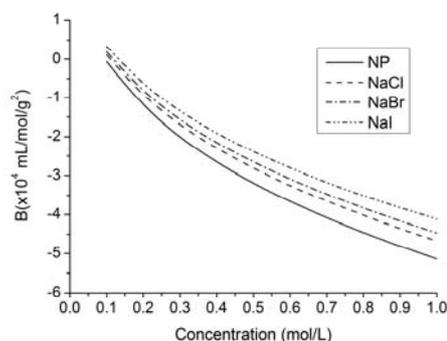


Fig.2. Second virial coefficient for aqueous lysozyme at pH 12 and at 298 K as a function of salt concentration. Lines are as in Fig.1.

5. Conclusions

Double layer force, potential of mean force and second virial coefficients were successfully calculated for colloidal particles and globular proteins. The technique proposed to calculate integrals using the same discretization method as for the Poisson-Boltzmann equation revealed to be efficient and consistent. Results presented have reproduced previous ion specific effects and are in agreement with Hofmeister effects reported in the literature.

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