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The Interaction Potential Functions in an Electrolyte Protein Solution

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Abstract: Recent developments in equations of state for molecular fluids have demonstrated the feasibility of using the hard-sphere equation to describe the effects of repulsive forces in simple fluids. By including a suitable term for attractive forces, most conveniently a uniform background potential, the properties of bio-macromolecular interaction can be roughly calculated. However, the choice of the potential used in perturbed hard-sphere chain (PHSC) theory for describing the attractions between macromolecules is rather complicated. For hard-sphere chains, the prediction accuracy from each model strongly depends on the choice of potential function.

Keywords: equation of state, hard sphere, potential function.

Introduction

The latest theoretical investigations have aimed at developing microscopic analytical theories for chain fluids based on simple molecular potential models and at applying such theories to real systems. This approach has the advantage that model parameters derived from these theories possess physical significance and hence may be estimated with greater confidence than those derived from empirical correlations.[1] In these theoretical treatments, the chain molecule is assumed to be composed of a series of freely-jointed segments that interact with other segments through a spherically symmetric potential. Two adjacent segments in a chain molecule are allowed to rotate freely around each other, and are only subjected to the constraint that the bond length between them is held fixed. In the case where the segment-segment interaction is of the hard-sphere type, analytical results for the equation of state and molecular correlation functions for the freely jointed hard chains were derived based on the thermodynamic perturbation theory of intermolecular potential.[2-5] The analytical equation of state (EOS) is consisted of hard sphere fluid as the reference system and a perturbation including the protein-protein overall potential of mean force (PMF).

A modeling of the thermodynamic properties and phase equilibria of macromolecular chain fluids and their mixtures is of practical relevance to biological processing industries. Several molecular-thermodynamic models are developed to describe salt-induced protein precipitation.[6-8] Grigsby *et al.*[9] and Park *et al.*[10] proposed a model for the correlation and prediction of the CPT as a function of salt type and salt concentration to investigate the protein-solution interactions in cloud-point temperature (CPT) measurements. The CPT also provides a guide to the net attractive interactions with valuable information to describe the properties of aqueous protein solutions, which are related qualitatively to the crystallization temperatures. They calculated strength of protein-protein interactions based on the RPA theory in conjunction with a square-well potential as a function of solution conditions for all salts studied.[11,12]

Some of these theoretical approaches have been extended the purely repulsive hard-sphere system to sphere interacting through potentials involving a hard-core repulsion plus a short-range attraction.[13,14] For example, Kim *et al.*[15] was able to capture the essential thermodynamic behavior of chain fluids correctly and provide a convenient theoretical framework for the further development of microscopic equation of state models for chain fluids involving continuous potentials, such as the generalized Lennard-Jones (GLJ) potential. The new pair potential-energy function was proposed to explain the interaction between protein and salt molecules. The energy parameter is then obtained from the experimental CPT data for lysozyme/salt solutions.

CPT in aqueous salt solutions has been studied to investigate self-association which is originated from protein-protein and protein-salt interactions. The experimental results show that as salt concentration rises the effective protein-protein interactions are more attractive. However, it is also not clear whether these interactions are related to solvation forces between proteins or possibly to specific short-range forces that stabilize protein crystals, such as van der Waals (vdW) contacts, salt bridges, or hydrogen bonds.[16,17] Chang *et al.*[18] reported that a molecular-thermodynamic framework for protein-protein and protein-salt interactions by highly concentrated inorganic salt. They proposed a new square well

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protein-protein effective two-body potential that includes hydrogen bonding forces and hydrophobic forces between protein molecules and ion binding interactions between the protein surface and salt ions. The attractive potential energy is correlated with experimental CPT data of lysozyme in aqueous electrolyte solutions at pH 4.0 and 7.0.

In this study, we study the capability of producing perturbed hard-sphere chain (PHSC) equations of state from several selected models that are developed for hare-spheres, by proposing several types of potential energy. We then compare to the effective interaction energy from each of potential models.

Model Development

Protein interactions can be described quantitatively by a two-body PMF. However, the generating protein phase behavior from the intermolecular potential between proteins, salts, and water is impossible without adopting physically realistic simplifying assumptions. Grigsby *et al.*[9] employed a square-well potential of mean force to describe the interactions of two lysozyme molecules in an electrolyte solution:

$$W(r)=\begin{cases}\infty & r<\sigma\\ -\varepsilon & \sigma_p<r<\sigma_p+\delta\\ 0 & \sigma_p+\delta<r\end{cases} \tag{1}$$

where r is the center to center distance between proteins, σ_p is the protein diameter, ε and δ are the depth and width of the square-well, respectively. Grisby *et al.* assumed only specific ions except Na^+ and Cl^- bind to protein surface because Na^+ is a marginal kosmotropic ion and Cl^- is a marginal chaotropic ion. Therefore, NaCl was selected as a reference salt, and for all salts except NaCl, a change in the square-well potential was introduced to account for these specific ion effects and is given by

$$W(r)=-[\varepsilon_{NaCl}(I)+\varepsilon_{sp}(I)] \tag{2}$$

where I is the ionic strength. However, it is well-known that the surface chemistry of protein, which is related to the water structure making or water structure breaking ability of the salt, needs to be incorporated into models for the effective PMF.

To generate the expression for the competition of attractive and repulsive interaction between molecules affected by a salt ion binding, Chang *et al.*[18] redefined the square-well potential as follows:

$$W(r)=\begin{cases}\infty & r<\sigma_p\\ -f & \sigma_p<r<\sigma_p+\delta\\ 0 & \sigma_p+\delta<r\end{cases} \tag{3}$$

The free energy parameter, f, includes the specific interaction parameter and the entropy effect generated by the specific interaction. To explain competition between specific and nonspecific interactions, f is defined as

$$f=\varepsilon_0+\varepsilon_{NaCl}-kT\ln[\theta+(1-\theta)\exp(\delta\varepsilon_{sp}/kT)] \tag{4}$$

where ε, ε_{NaCl}, and $\delta\varepsilon_{sp}$ are potentials between proteins in salt free solution, NaCl solution and specific ion solution, respectively. The third term represents the entropic unfavorable contribution with respect to f. In other words, it represents the repulsive force contribution, which is resulted by the specific ion biding, that deviations from the reference including hydration forces and water, with respect to attractive force, f, structuring of the solution. If the surfaces of all proteins are assumed to be uniform, θ is regarded by the hydrophobic surface fraction of protein. Therefore, θ of these interactions is specific and 1 - θ is nonspecific.

Kim *et al.*[15] proposed a GLJ potential function that can cover various interaction ranges, which is adjusted by a temperature-dependent parameter. It can be expressed as follows:

$$W(r)=\begin{cases}\infty & (0<r<\sigma)\\ -\varepsilon & (\sigma<r<2\sigma/A(\tilde{T}))\\ \dfrac{\varepsilon}{1-A(\tilde{T})\left(\dfrac{r}{\sigma}\right)} & (2\sigma/A(\tilde{T})<r<\lambda\sigma)\\ 0 & (r>\lambda\sigma)\end{cases} \tag{5}$$

where, σ is the collision diameter, ε is the minimum potential energy, $\tilde{T}=kT/\varepsilon$ is the reduced temperature with Boltzman constant k, and $A(\tilde{T})$ is the temperature-dependent parameter that is determined from the computer simulation data for the compressibility factor. The calculated value of $A(\tilde{T})$ against the reduced temperature to simply correlate with simulation data is suggested:

$$A(\tilde{T})=\frac{1.761-1.579\tilde{T}}{1-\tilde{T}} \tag{6}$$

To determine protein interactions from experimental CPT data in binary aqueous solutions, these models was based on the Random Phase Approximation (RPA) theory to generate expressions for the pressure and chemical potential. The equation for pressure P is:

$$\frac{P}{\rho kT}=\left(\frac{p}{\rho kT}\right)_{ref}+\left(\frac{\rho U}{2kT}\right)_{per} \tag{7}$$

where, ρ is the protein number density, T is the CPT, and U is the perturbation energy per unit density that accounts for interactions between proteins including charge-charge, van der Waals and hydration interactions. The perturbation

energy is related to the potential of mean force as given:

$$U = 4\pi \int W(r) r^2 dr \tag{8}$$

Kim *et al.*[19] reported the overall effective two-body potential between two different protein molecules is given by the sum of four potentials.

$$W_{ij}^{overall}(r) = W_{ij}^{elec}(r) + W_{ij}^{disp}(r) + W_{ij}^{osmotic}(r) + W_{ij}^{specific}(r) \tag{9}$$

where, $W_{ij}^{elec}(r)$ is the electric double-layer-repulsion potential, $W_{ij}^{disp}(r)$ is the dispersion potential of Hamaker, $W_{ij}^{osmotic}(r)$ is an attractive interaction due to the excluded-volume effect of the salt ions, and $W_{ij}^{specific}(r)$ is an attractive potential between proteins representing any specific chemical effects such as hydrophobic interactions.

In the mean time, Chang *et al.*[20] derived analytical expressions for an equation of state, which is based on the generalized van der Waals partition function. The attractive term in the context of the effective potential of mean force is perturbed by the statistical mechanical perturbation theory. The precipitation behaviors with various conditions such as the ionic strength and the protein shape, which plays a significant role in the protein precipitation behavior, are studied. For an aqueous protein solution, Park *et al.*[10] employed a statistical thermodynamic model based on the same fundamental ideas that is the generalized van der Waals partition function. The partition function Q depends on temperature T, system volume V and number of protein molecules N. The generalized van der Waals partition function for a hard sphere fluid is given:

$$Q(T,V,N) = \frac{1}{N!}\left(\frac{V}{\Lambda^3}\right)^N \left(\frac{V_f}{V}\right)^N \left[\exp\left(\frac{-E_o}{2k_BT}\right)\right]^N (q_{r,v})^N \tag{10}$$

where, Λ is the de Broglie wavelength and the free volume V_f is the volume available to the center of mass of a molecule. E_o is the intermolecular potential energy of one molecule due to the attractive forces from all other molecules. The contribution per molecule from rotational and vibrational degree of freedom, q, is a unit (= 1) for a perfect r, v sphere molecule. For large molecules, $q_{r,v}$ depends significantly on density, when the molecules deviate from the spherical shape. $\bar{E}_0 = \tau_{ps} E_0$, which is the intermolecular potential energy of a non-spherical protein. Interacting types of protein molecules affect the intermolecular potential energy between non-spherical proteins. The fact is found in literature that the interacting types between non-spherical molecules affect the intermolecular potential energy. Therefore, τ_{ps} reflects the rotational and vibrational contributions per a protein molecule that are affected by the presence of non-spherical proteins. The difference between $\bar{E}_0$ and E originated from non-sphericality is mainly due to the additional volume of a non-spherical molecule.

$$\tau_{ps} = \left(1 + \frac{\delta v_{add}}{v_{sph}}\right) \approx \left(\prod_{j=1}^{n} \frac{\sigma_{pi}}{\sigma_p^n}\right)^{\frac{1}{n}} \approx \left(\frac{\sigma_{px}\sigma_{py}\sigma_{pz}}{\sigma_p^3}\right)^{\frac{1}{3}} \tag{11}$$

Equation of state of PHSC theory is following:

$$\frac{P}{\rho kT} = 1 + \frac{\tau_{ps}(4\eta - 2\eta^2)}{(1-\eta)^3} + \frac{\tau_{ps}^2 \rho U}{2kT} \tag{12}$$

A thermodynamic model to describe the salt-induced protein precipitation with effective potentials is developed based on a statistical mechanical perturbation theory and the reference term. At liquid-liquid phase separation of onset of clouding for the protein precipitation, the volume of dense phase is negligibly small compared to total volume, the protein concentration in supernatant phase is equal to the initial protein concentration. At two phase equilibria, the interaction energy is calculated from the classical equilibrium conditions.

Results and Discussion

In general, protein interactions are known to be governed by many factors, such as pH, surface hydrophobicity, surface-charge distribution, salt-type, and salt concentration.[21-23] The energy parameter ε for salts is obtained with various ionic strength I for pH 4.0 and 7.0 at the given CPT by Grigsby *et al.*[9] and Park *et al.*[10] The calculated ε for corresponding conditions are shown in Figures. For simplicity, to estimate of energy parameter ε, those are assumed to have linear, parabolic shapes and quadratic functions of ionic strength. Figures represents the obtained values for ε plotted against ionic strength at different pH for NaCl, NH_4Cl, $(NH_4)_2SO_4$ respectively. The lines are calculated by the correlating equations given in previous sections. In Figure 1, the calculated

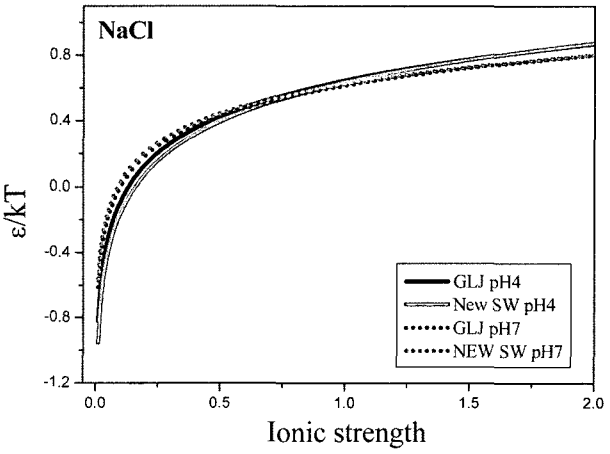

Figure 1. The calculated values of ε for NaCl plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by correlating equations given with the solid lines at pH 4 and doted lines at pH 7, black lines with GLJ function and gray lines with a new SW.

values of ε for NaCl plotted against ionic strength, and the lines calculated from the correlating equations are given to the solid at pH 4 and doted lines at pH 7, black lines with GLJ function and gray lines with a new SW. For NaCl, two potential functions are represented similarly. In Figure 2, Park *et al.* used to thermooptical analysis (TOA) technique that overcomes many defects of the light scattering method[9] which is most commonly used for liquid-liquid phase separation data, and that provides a simple, rapid and reliable method with small amount sample to determine CPT. The calculated values of ε for NaCl plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by modified PHSC model that takes into account the shape of proteins to interpret the salt's effects. In Figure 3, the calculated values of ε for NH_4Cl plotted against ionic strength at different pH, and the lines are calculated by the correlating equations given. In Figure 4, the values of ε for NH_4Cl are calculated with potential function of shape factor from experimental data by Park *et al.* at the same solution conditions. At a fixed ionic strength, deviations of the CPT for a salt relative to that for NaCl were attributed to specific ion interactions with lysozyme from eq. (2). Figures 5 and 6 for $(NH_4)SO_4$ show the calculated values of ε plotted against ionic strength at pH 4.0 and 7.0 by the correlating equations given with each of potential functions.

To investigate a more efficient isolation or separation method of protein solutions, Grisby *et al.* and Park *et al.* were

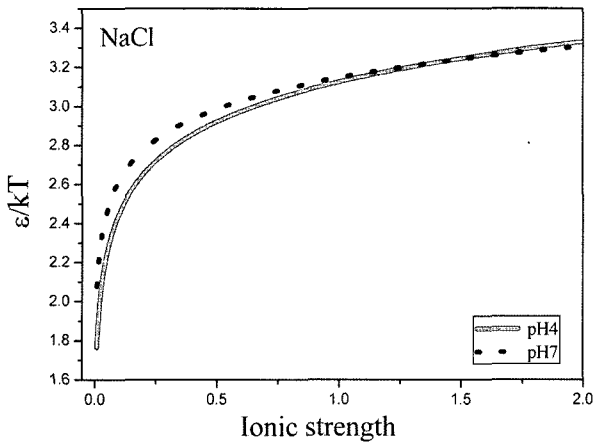

Figure 2. The calculated values of ε for NaCl plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by correlating equations given with the solid lines at pH 4 and doted lines at pH 7, these lines with potential function of shape factor. The data reported by Park *et al.*[10]

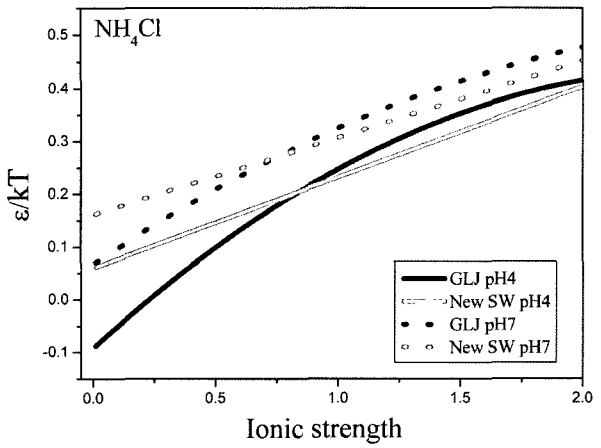

Figure 3. The calculated values of ε for NH_4Cl plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by correlating equations given with the solid lines at pH 4 and doted lines at pH 7, black lines with GLJ function and gray lines with a new SW.

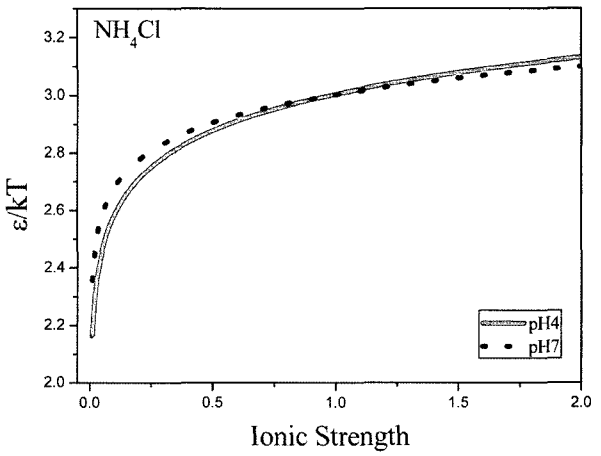

Figure 4. The calculated values of ε for NH_4Cl plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by correlating equations given with the solid lines at pH 4 and doted lines at pH 7, these lines with potential function of shape factor. The data reported by Park *et al.*[10]

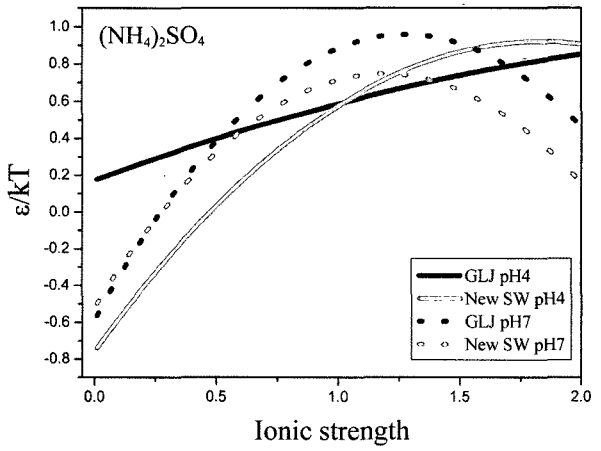

Figure 5. The calculated values of ε for $(NH_4)_2SO_4$ plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by correlating equations given with the solid lines at pH 4 and doted lines at pH 7, black lines with GLJ function and gray lines with a new SW.

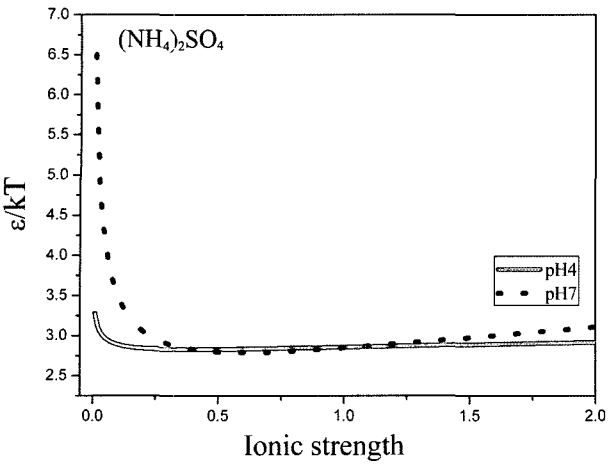

Figure 6. The calculated values of ε for $(NH_4)_2SO_4$ plotted against ionic strength at pH 4.0 and 7.0. The lines are calculated by correlating equations given with the solid lines at pH 4 and doted lines at pH 7, these lines with potential function of shape factor. The data reported by Park *et al.*[10]

based on predicting the solution conditions, including the CPT as a function of protein concentration, salt type, salt concentration and pH, that favor selective precipitation of the target protein. Protein precipitation and crystallization due to non-covalent forces including Coulombic, van der Waals and hydrophobic forces governing protein interactions are fundamental procedures to recover and characterize all proteins of in application fields. Therefore, these interaction forces remained obscurely for complex bio-macromolecules are understood on the level of small molecules with an effective potential function as previously stated.

Conclusions

Protein interactions with salts, other protein molecules, and surfaces govern the physical properties are investigated from potential functions. The potential functions have been developed in the statistical mechanical identification of the protein-protein potential, essentially with free energy including specific interactions over all solvent configurations. We reviewed several well-known analytical potential function based on RPA theory. To investigate the macromolecular interaction of an asymmetric binary mixture of hard sphere, several approximations are taken as an effective potential function which spherical molecules interact via intermolecular forces in aqueous electrolyte solutions. The significance and simplicity of these proposed potential functions will be sampled mainly for protein-protein interactions as the determinant of solution thermodynamic properties and phase behavior.

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