Model for a mixture of macroions, counterions, and co-ions in a waterlike fluid

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(Received 7 April 2014; published 25 July 2014)

We propose an integral equation theory for a mixture of macroions, counterions, and co-ions in a waterlike fluid in which all the components are accounted for explicitly. The macroions can carry positive and negative surface charges simultaneously, mimicking in this way the situation occurring in protein solutions. To solve this complex model numerically, we utilize the associative mean spherical approximation, developed earlier for low-molecular-mass charge-symmetric electrolyte solutions. To illustrate the potential of this approach, we present numerical results for various experimental conditions. Among the measurable properties we choose to calculate the osmotic coefficient, a quantity that reflects the stability of the solution. We show that the osmotic coefficient depends not only on the magnitude of the net charge on the macroion but also on its sign, as well as on the nature of the low-molecular-mass electrolyte present. These specific ion effects are the consequence of differences in hydration between the ions in solution and charged groups on the macroion.

DOI: 10.1103/PhysRevE.90.012308 PACS number(s): 64.75.Bc, 82.60.Lf, 82.70.−y

I. INTRODUCTION

Highly asymmetric electrolytes such as colloids, surfactant micelles, and globular proteins are classical objects of experimental and theoretical research. Due to the different length scales of interactions between the various species (macroions are typically of the size of several hundred angstroms, while solvation effects take place at distances of a few angstroms), these systems represent a challenge for theoretical modeling. Different approximative models have been applied to allow (see, for example, Ref. [1]) comparison with experimental results.

The stability of these systems, a problem of significant practical importance, is most often considered in the framework of the Derjaguin-Landau-Verwey-Overbeek (DLVO) [2,3] theory. In this approach the solution is treated as an effective one-component fluid, where the low-molecular-mass electrolyte and solvent (water) are pictured as a continuum, modifying the interactions between macroions. The theory, originally developed for lyophobic sols, found its application also in protein solutions. Perhaps the most recent contributions in this direction are due to Pellicane and co-workers [4,5]. These authors were successful in rationalizing the small-angle neutron-scattering results obtained under a variety of experimental conditions. A somewhat different approach, but similar in spirit, has been suggested by Valadez-Perez *et al.* [6]. While DLVO-based studies are important, in principle they cannot satisfactorily answer the question why different $+1$: -1 electrolytes influence the stability of proteins differently (see, for example, [7–11] and the references therein).

Recently we applied a Hamiltonian type of approach to analyze the osmotic properties of alkali halides in water [12]. We proposed an equitable model of solution, where the molecules representing water were treated on an equal footing with the ions. The molecule modeling water was pictured as a hard sphere with four off-center square-well sites. These sites served either to bind another water molecule or to solvate the ions. The ions were imagined as charged hard spheres with their crystal size diameters, but with the ability to bind water through discrete attractive sites distributed on the surface. The two basic assumptions of the study were that (i) the strength of the ion-site–water attraction is inversely proportional to the size of the ion and (ii) the number of available attractive sites on an ion is proportional to its surface area. In short, small ions bind water molecules strongly, but fewer of them. Large ions bind water weakly, but many of them. The model [12] was able to reproduce correctly the nontrivial ionic size dependence of the osmotic coefficient found experimentally for alkali-metal-halide solutions in water.

The success of this concept [12] prompted us to go one step further. In this approach we generalize the theoretical approach mentioned above, introducing an extra component: the charged macroion. We examine aqueous solutions of macroions in a mixture with low-molecular-mass salts. The principal components in the model solution are macroions (modeling a protein or the surfactant micelle), solvent molecules mimicking water [12], and a $+1$:-1 electrolyte, represented as charged hard spheres with their crystal radius size.

We model the macroions as uncharged hard-sphere macroparticles with a certain number of small positively and/or negatively charged hard spheres attached to their surface, mimicking solvent-exposed charged groups (charged amino acid residues) on a protein. They are attached to the macroparticles due to the infinitely strong attraction between the sticky sites located on the macroparticle surface and those on the residue-forming ions.

The net charge of such a macroion is equal to the difference between the positively and negatively charged small hard spheres bound to the macroparticle. We can prepare macroions with negative, positive, or zero net charge, while the system as

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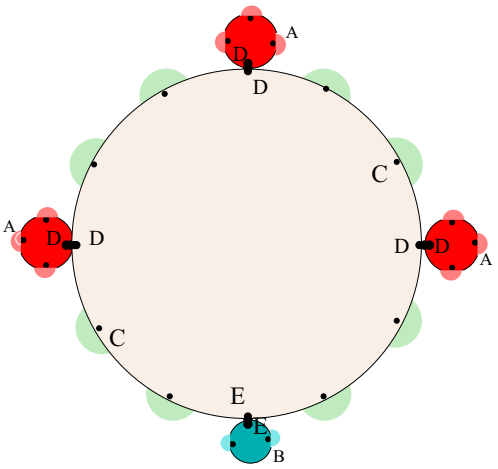

FIG. 1. (Color online) Model of a macroion with three positively charged residues $n_{p_D} = 3$ (small red spheres) and one negatively charged residue $n_{p_E} = 1$ (small blue sphere) attached to the macroparticle (large sphere) due to the infinitely strong interaction between sticky sites of type D on the macroparticle and the positively charged residue-forming ions and between the E-type sticky sites on the macroparticle and the negative residue-forming ions. Light green and light red semispheres denote the range of the square-well site-site interaction between the C-type sites, which belong to two different macroparticles, and between A and B types of sites, which are positioned on the positively and negatively charged species in the system, respectively.

a whole is electroneutral in all cases. The model (schematically shown in Fig. 1) is not new; in Refs. [13,14] similar models were used to represent a lysozyme. The macroions and small ions are distributed in a collection of waterlike molecules, modeled as in our previous paper [12]. We focus on calculation of the osmotic properties in such mixtures. Namely, the osmotic pressure is a measure of the stability of a macroion solution (see, for example, [15,16]). Particularly important is the correlation between the osmotic second virial coefficient and the solubility of proteins. Experimental data suggest that proteins crystallize when the second virial coefficients assume slightly negative values [17–23]. The origin of this important correlation has been investigated by Rosenbaum *et al.* [23]. This is perhaps the first integral equation study of osmotic pressure, treating all the components in the aqueous macroion-electrolyte solution on an equal footing.

II. MODELING MACROIONS IN AQUEOUS ELECTROLYTE SOLUTION

For the sake of calculation we discriminate eight species: model water molecules denoted w, monovalent anions a and cations c, uncharged macroparticles p, with attached positive r^+ and negative r^- hard-sphere residues, and the corresponding negatively and positively charged counterions c^- and c^+, respectively. The species are modeled as charged or uncharged hard spheres of size σ_i with n_{i_L} square-well (or sticky) sites placed randomly at a distance d_i from the corresponding hard-sphere center. Here the subscripts i and L denote the particle species and the type of site, respectively.

Sites of type A and B are located on charged species and account for the ion-water (note that our water model has no dipole), charged residue-water, and possible ion-ion, ion-residue, and residue-residue association effects. The A-type site plays the role of the positive charge and the B-type site the negative charge. Water molecules have sites of both type: two A sites representing hydrogens and two B sites representing the lone-pair electrons.

In addition to either A or B types of sites, each residue ion has one of either D (for positively charged) or E (for negatively charged) type of sticky site located on the surface. Sites of the same types are also positioned on the macroion surface, i.e., it has n_{p_D} and n_{p_E} sites of D and E type, respectively. Owing to these sites, macroions with n_{p_D} positive and n_{p_E} negative charges are formed (see Fig. 1). In addition, each model macroion possesses n_{p_C} square-well sites of C type, which are used to model the van der Waals (vdW) interaction between protein molecules.

III. PAIR POTENTIAL AND MODEL PARAMETERS

The pair potential $U_{ij}(12)$ used here can be represented as a sum of the hard-sphere term $U_{ij}^{(HS)}(r)$, the Coulomb term $U_{ij}^{(C)}(r)$, and the term describing association $U_{i_M j_L}^{(as)}(12)$:

$$U_{ij}(12) = U_{ij}^{(HS)}(r) + U_{ij}^{(C)}(r) + \sum_{ML} U_{i_M j_L}^{(as)}(12), \qquad (1)$$

where M and L assume values A,B,C,D,E and the labels 1 and 2 denote positions and orientations of the two particles. Here

$$\beta U_{ij}^{(C)}(r) = L_B \frac{z_i z_j}{r}, \qquad (2)$$

where $\beta = 1/k_B T$, k_B is the Boltzmann constant, T is the absolute temperature, $L_B = \beta e_0^2/4\pi\epsilon_0\epsilon$, $z_i e_0$ and $z_j e_0$ are the charges of the particles (e_0 is the elementary charge), ϵ_0 is the permittivity of a vacuum, and

$$U_{i_M j_L}^{(as)}(x) = \begin{cases} \epsilon_{i_M j_L} \Delta_{i_M j_L}, & x \leqslant w_{i_M j_L} \\ 0, & x > w_{i_M j_L}, \end{cases} \qquad (3)$$

where the subscripts M and L take the values A, B, and C. Further, x is the distance between the square-well sites and $\epsilon_{i_M j_L}$ and $w_{i_M j_L}$ are the square-well depth and width, respectively. In addition, we assume that the interaction between the sites representing charges takes place only between different types of sites, while the interaction between the protein vdW sites occurs only between themselves. These rules are enforced by the function $\Delta_{i_\alpha j_\beta}$,

$$\Delta_{i_\alpha j_\beta} = (1-\delta_{ip})(1-\delta_{jp})(\delta_{\alpha A}\delta_{\beta B} + \delta_{\alpha B}\delta_{\beta A}) + \delta_{ip}\delta_{jp}\delta_{\alpha C}\delta_{\beta C}, \qquad (4)$$

where $\alpha,\beta = 0,A,B,C$. Here $\alpha,\beta = 0$ denote the nonbonded states of the particles.

The system is studied in the limit of the infinitely strong sticky interaction, acting between the sites of D type, positioned on a macroparticle and positively charged ions forming residues. The same holds true for sites of E type on the macroparticle and on negatively charged (residue-forming) ions. Thus, for the corresponding Mayer functions

we have

$$\bar{f}_{p_D r_D^+}(r) = K_{p_D r_D^+}\delta(r-\sigma_{pr^+}),$$
$$\bar{f}_{p_E r_E^-}(r) = K_{p_E r_E^-}\delta(r-\sigma_{pr^+}) \quad (5)$$

with $K_{p_D r_D^+}, K_{p_E r_E^-} \to \infty$. In summary, due to the sites of types D and E, model protein molecules with n_{p_D} positive and n_{p_E} negative charges are formed. Here

$$\bar{f}_{i_\alpha j_\beta}(r) = \left\langle f^{(as)}_{i_M j_L}(12)\right\rangle_{\Omega_1\Omega_2}, \quad (6)$$

where $f^{(as)}_{i_M j_L}(12)$ is the Mayer function for the associative potential acting between the sites L and M of particles of the types i and j and $\langle\cdots\rangle_{\Omega_1\Omega_2}$ denotes the orientational averages over the angles Ω_1 and Ω_2 of particles 1 and 2, respectively.

As in our previous study [12], we assume that the depth of the site-site square-well potential, acting between charged species, is inversely proportional to the sum of their diameters:

$$\epsilon_{i_A j_B} = \frac{2k_{ij}}{\sigma_i+\sigma_j}. \quad (7)$$

The depth of the charged species-water square-well site-site potential is inversely proportional to the hard-sphere size of the corresponding ion or residue

$$\epsilon_{w_A i_B} = \frac{k_{w-}}{\sigma_i}, \quad \epsilon_{w_B i_A} = \frac{k_{w+}}{\sigma_w+\sigma_i}, \quad (8)$$

and the number of square-well sites on the ions and residues is proportional to the square of their hard-sphere sizes

$$n_{i_L} = s_\pm \sigma_i^2. \quad (9)$$

In the present study the model parameters are chosen to be the same as in our previous paper [12]. The only exception is the proportionality coefficient k_{ij} in Eq. (7) for charged species-residue associative interaction. To account for the reduction of the dielectric constant in the vicinity of the protein, we assume a six times larger value for the depth of the corresponding square-well associative potential, i.e., while for $i,j \neq r^\pm$, $k_{ij} = -675$ K [12], for $i = a,c,r^\pm,c^\pm$ we have $k_{ir^\pm} = -4050$ K. Other model parameters remain unchanged. For the square-well potential depth ϵ_{ww}, width w_{ww}, and site displacement d_w of the water molecules we have $\epsilon_{ww} = -1625$ K, $w_{ww} = 0.679\sigma_w$, $d_w = 0.279\sigma_w$, and $\sigma_w = 3.099$ Å. Instead of the square-well width and displacement, similarly as before [12], we use the so-called bonding volume V_{ij} as an input parameter for the other site-site associative interactions. This quantity represents the volume available for bonding and we assume it to be the same for all interacting species, i.e., $V_{ij} = V_{ww}$, with V_{ww} obtained from Eq. (14). Finally, for the proportionality coefficients in Eqs. (8) and (9) we have $k_{w+} = -5050$ K Å, $k_{w-} = -3200$ K Å, and $s_\pm = 0.6921$ Å^{-2}.

IV. ASSOCIATIVE MEAN SPHERICAL APPROXIMATION

The thermodynamic properties of the model under consideration are calculated using the associative mean spherical approximation (AMSA) (or its extension, the polymer MSA) approach developed earlier [24–28]. The theory is composed of the multidensity Ornstein-Zernike (OZ) equation, MSA-type closure conditions, and the relation between the density of the particles in different bonding states (the statistical-mechanical analog of the mass action law). Taking into account the symmetry of the OZ equation, which arises as a consequence of the equivalence of the square-well sites, we have

$$\hat{\mathbf{h}}_{ij}(k) = \hat{\mathbf{c}}_{ij}(k) + \sum_l \rho_l \hat{\mathbf{c}}_{il}(k)\boldsymbol{\alpha}_l\hat{\mathbf{h}}_{lj}(k). \quad (10)$$

Here ρ_i is the number density of the particles of species i and α_i, $\hat{\mathbf{h}}_{ij}(k)$, and $\hat{\mathbf{c}}_{ij}(k)$ are matrices with the elements

$$\boldsymbol{\alpha}_i = \begin{pmatrix} 1 & n_{i_L} & n_{i_M} & \cdots \\ n_{i_L} & n_{i_L}(n_{i_L}-1) & n_{i_L}n_{i_M} & \cdots \\ n_{i_M} & n_{i_M}n_{i_L} & n_{i_M}(n_{i_M}-1) & \cdots \\ \vdots & \vdots & \vdots & \ddots \end{pmatrix},$$

$$\hat{\mathbf{c}}_{ij}(k) = \begin{pmatrix} \hat{c}_{i_0 j_0} & \hat{c}_{i_0 j_L} & \hat{c}_{i_0 j_M} & \cdots \\ \hat{c}_{i_L j_0} & \hat{c}_{i_L j_L} & \hat{c}_{i_L w_M} & \cdots \\ \hat{c}_{i_M j_0} & \hat{c}_{i_M j_L} & \hat{c}_{i_M j_M} & \cdots \end{pmatrix},$$

where L and M assume the values A,B,C,D,E, depending on which type of site is assigned to the particles of a given type. The dimensionality of these matrices is defined by the number of different site types that are associated with particles of a given species. For example, the dimensionality of the matrices α_w and $\hat{\mathbf{c}}_{wp}(k)$ are 3×3 and 3×4, respectively, since water has two types of sites A and B and protein has three types of sites C, D, and E. The elements of the matrices $\hat{\mathbf{h}}_{ij}(k)$ and $\hat{\mathbf{c}}_{ij}(k)$ are Fourier transforms of the partial correlation functions $h_{i_\alpha j_\beta}(r)$ and $c_{i_\alpha j_\beta}(r)$ $(\alpha,\beta = 0,A,\ldots,E)$, describing the correlation between the particles in different bonding states. Here $\alpha = 0$ denotes the nonbonded state and $\alpha = L$ the state with the site L bonded. The total pair correlation function $h_{ij}(r)$ is equal to the sum of the partial correlation functions $h_{i_\alpha j_\beta}(r)$ over all bonding states, i.e.,

$$h_{ij}(r) = \sum_{\alpha\beta=0} h_{i_\alpha j_\beta}(r). \quad (11)$$

The AMSA closure relation is

$$\mathbf{c}_{ij}(r) = -\mathbf{E}_{ij}\beta U^{(C)}_{ij}(r) + \frac{\delta(r-\sigma_{ij})}{2\pi\sigma_{ij}}\mathbf{t}_{ij},$$
$$r \geqslant \sigma_{ij} = \tfrac{1}{2}(\sigma_i+\sigma_j) \quad (12)$$
$$\mathbf{h}_{ij}(r) = -\mathbf{E}_{ij}, \quad r < \sigma_{ij},$$

where $[\mathbf{E}_{ij}]_{\alpha\beta} = E_{i_\alpha j_\beta} = \delta_{\alpha 0}\delta_{\beta 0}$, σ_i is the diameter of species i,

$$t_{i_\alpha j_\beta} = X_{i_\alpha}X_{j_\beta}K_{i_\alpha j_\beta}g_{i_0 j_0} + \frac{\delta_{\alpha\beta}}{2\rho_p}\left(\frac{\delta_{\alpha D}}{n_{p_D}\sigma_{pr^+}}\Delta_{ij;pr^+} + \frac{\delta_{\alpha E}}{n_{p_E}\sigma_{pr^-}}\Delta_{ij;pr^-}\right), \quad (13)$$

$g_{i_0 j_0}$ is the contact value of the partial distribution function $g_{i_0 j_0}(r) = h_{i_0 j_0}(r)+1$, $\Delta_{ij;kl} = (\delta_{ik}\delta_{jl}+\delta_{il}\delta_{jk})$, and

$$K_{i_\alpha j_\beta} = \frac{2\pi}{\sigma_{ij}}(1-\delta_{\alpha 0})(1-\delta_{\beta 0})\int \bar{f}^{(as)}_{i_M j_L}(r)r^2 dr = (e^{-\beta\epsilon_{ij}}-1)V_{ij}\Delta_{i_\alpha j_\beta}. \quad (14)$$

Here V_{ij} is the volume available for bonding and X_{i_M} denotes the fraction of particles not bonded at a site M. The latter quantity follows from the relation [26,28]

$$2X_{i_M}\sum_j \rho_j\sigma_{ij}\sum_L K_{i_M j_L} g_{00}^{ij} X_{j_L} + X_{i_M} - 1 = 0. \qquad (15)$$

The OZ equation (10), the AMSA closure conditions (12), and the density relation (15) form a closed set of equations. The solution of this set of equations was derived earlier [26] and we refer the reader to our previous paper for more details. The Helmholtz free energy, pressure, and chemical potentials are calculated numerically using thermodynamic integration, with subsequent differentiation of the resulting free energy with respect to volume and number of particles of each species. The resulting thermodynamic properties are given with respect to the reference values. The reference system is represented by the "ions" stripped of charges, i.e., for $z_i = 0$ and its properties evaluated by the thermodynamic perturbation theory [28–31].

In the present version of the theory we combine the MSA-type closure for the long-range Coulomb interaction and the ideal network approximation [28,32] to account for the association between particles. In the ideal network approximation it is assumed that the partial correlation functions $c_{i_\alpha j_\beta}(r)$ and $h_{i_\alpha j_\beta}(r)$ describing the correlation between particles with more than one site bonded (with either α and/or β denoting the set of more than one attractive site) are small and can be neglected. Thus the right-hand side of the closure conditions (12) and the relation between the densities (15) include the contact values of the partial distribution function between nonbonded particles $g_{i_0 j_0}$ only. As a result, the pair distribution function $g_{pp}(r)$ between macroions is not sensitive to their charge and for highly charged macroions the repulsive contribution is underestimated. As a consequence, the predicted values for the fraction of the protein particles X_{p_C} not bonded on site C are too small since the value of $g_{p_0 p_0}$ is too large.

We choose to correct this drawback of the theory at the level of the reference system assuming Debye-Hückel repulsion between macroions. According to the thermodynamic perturbation theory used to describe the reference system, the Helmholtz free energy of the model in excess of its hard-sphere value is expressed in terms of the fractions of particles $X_{i_L}^{(\mathrm{ref})}$ not bonded on the site L [28–31]. These fractions satisfy the same set of equations used to calculate the values X_{i_L} (15) for the original system, i.e., Eq. (15). The only difference is that in the corresponding equation for the reference system contact values $g_{i_0 j_0}^{(\mathrm{ref})}$ are substituted by hard-sphere contact values $g_{ij}^{(HS)}$. To account for the protein charge effects we assume Debye-Hückel repulsion between macroparticles and suggest the following approximation for the corresponding contact value:

$$g_{p_0 p_0}^{(\mathrm{ref})} = g_{pp}^{(HS)} \exp(-\beta U_{DH}), \qquad (16)$$

which is then used in the equation for $X_{p_C}^{(\mathrm{ref})}$. Here

$$\beta U_{DH} = \frac{U_0 L_B}{\sigma_p}\left(\frac{n_{p_D} z_{r^+} + n_{p_E} z_{r^-}}{1+\kappa\sigma_p/2}\right)^2, \qquad (17)$$

$\kappa^2 = 4\pi L_B \sum_i \rho_i z_i^2$, and U_0 (chosen arbitrarily) is 0.15. For zero value of the protein charge, i.e., $n_{p_D} = n_{p_E}$, $U_{DH} = 0$ and $g_{p_0 p_0}^{(\mathrm{ref})} = g_{pp}^{(HS)}$.

The results are not very sensitive to the particular choice of U_0; in broad limits of U_0 values the theory qualitatively correctly reproduces the ion specific effects. The value of U_0 may depend on the type of the protein studied.

V. RESULTS

The quantity of interest is the osmotic coefficient ϕ, defined as

$$\phi = \frac{\Pi}{\rho k_B T} = 1 + B_2\rho + \cdots, \qquad (18)$$

where Π is the osmotic pressure, ρ the protein number concentration, and B_2 the second virial coefficient. Osmotic pressure is determined [12] by considering the equilibrium distribution of water molecules and low-molecular-mass electrolyte between the aqueous electrolyte solution and protein-water-electrolyte mixture. The two subsystems are assumed to be separated by a membrane permeable to water and small ions, but not to protein molecules. At equilibrium the activity coefficients of water and low-molecular-mass electrolyte must be equal on both sides of the membrane. Under such conditions, the pressure difference between the two subsystems is equal to the osmotic pressure Π (also called Donnan pressure). Note again that activity coefficients are calculated by differentiation of the free energy with respect to the number of water molecules or the particular ionic species.

To illustrate the potential of the approach we calculated ϕ for the solution where the hard-sphere diameter of the model protein, attached charged groups, and counterions are $\sigma_p =$ 34 Å, $\sigma_{r^+} = 4.57$ Å and $\sigma_{r^-} = 1.6$ Å, and $\sigma_{c^-} = \sigma_a$ and $\sigma_{c^+} = \sigma_c$, respectively. The water molecules and electrolyte ions are modeled as in our previous study [12], i.e., water is represented by a hard sphere of size $\sigma_w = 3.099$ Å with four off-center square-well sites and ions by charged hard spheres with sticky sites to bind water molecules and other ions. The hard-sphere sizes of anions and cations in solution are chosen to be equal to their crystal sizes [12]. For Li, Cs, Cl, and I ions this is 1.2, 3.38, 3.62, and 4.32 Å, respectively. Using these values of $\sigma_\pm$ and corresponding values of the proportionality coefficients $k_{w\pm}$ and k_{ij}, the depth of the square-well site-site potential (3), $\epsilon_{i_A j_B}$, $\epsilon_{w_a i_B}$, and $\epsilon_{w_B i_A}$ can be calculated from Eqs. (7) and (8).

In Figs. 2–4 we present the results for ϕ of the model macroion in aqueous electrolyte solutions of LiI, LiCl, CsI, and CsCl at salt concentrations of $c = 0.1$ and $c = 0.4$ mol/dm^3. Macroions with three different net charges are studied: (i) +16:−1 ($n_{p_D} = 16$, $n_{p_E} = 1$), (ii) +1:−16 ($n_{p_D} = 1$, $n_{p_E} = 16$), and (iii) +16:−16 ($n_{p_D} = n_{p_E} = 16$). Thus, in the first case the protein net charge is positive, while in the second and third cases it is negative and zero, respectively. In all cases we assume that number of the macroparticle sites responsible for the vdW attraction is $n_{p_C} = 40$ and the depth of the corresponding square-well site-site potential is $\epsilon_{pp} =$ −1180 K. Note that the numbers of positive and negative charges on the model macroion, as well as the number of vdW sites and the depth of their square-well potential, are chosen arbitrarily. Numerical results are presented in Figs. 2–4 merely

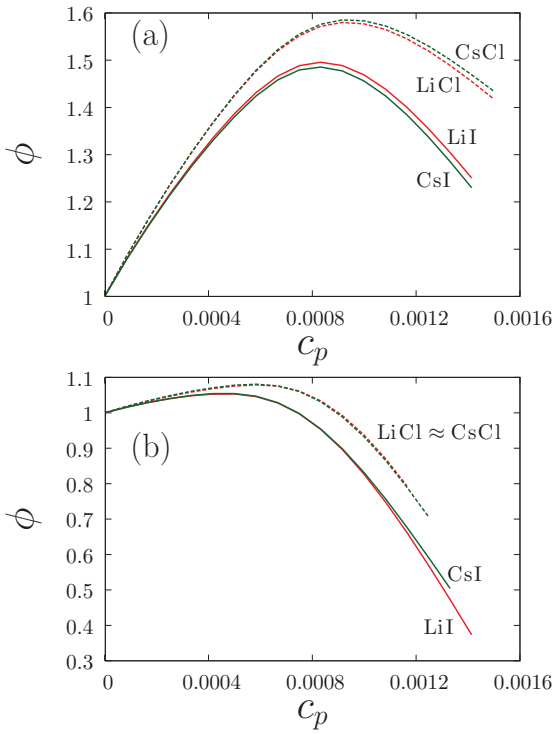

FIG. 2. (Color online) Osmotic coefficient ϕ as a function of protein concentration for the protein model with 16 positive residues and one negative residue: +16:−1. The solid red line denotes LiI and the dashed red line LiCl; the solid green line denotes CsI and the dashed green line CsCl. The cations (a) $c = 0.1$ mol/dm^3 and (b) $c = 0.4$ mol/dm^3. Note that on the scale of the figure the curves for LiCl and CsCl in (b) coincide.

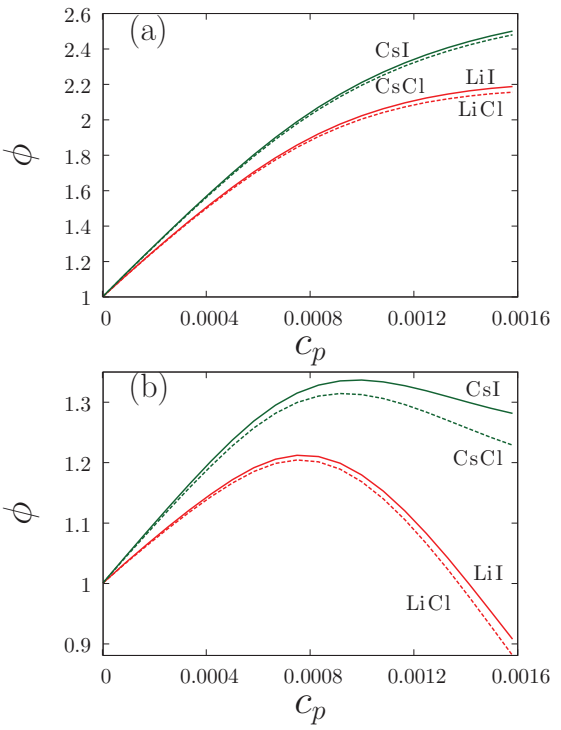

FIG. 3. (Color online) Osmotic coefficient ϕ as a function of protein concentration for a model protein with one positive and 16 negative residues: +1:−16. The color code and other parameters are the same as in Fig. 2.

to illustrate the potential of the proposed approach. Here the results for LiI are shown by solid red lines, for LiCl by dashed red lines, for CsI by solid green lines, and for CsCl present in the system by dashed green lines.

It is clear from Fig. 2 (upper curve), where the +16:−1 macroion is examined, that the slope of the osmotic coefficient curves is less steep in the case of higher concentration of added low-molecular-mass electrolyte. This is due to stronger electrostatic screening in the latter case, the effect of which is correctly predicted by the classical DLVO theory. The influence of the nature of salt can be observed in the same figure. The difference between the LiI (red) and LiCl (green) curves is clearly visible; notice that anions are counterions in this case. The effect of the nature of co-ion is small under these conditions. The difference between the two salts gets smaller at higher concentration of added electrolyte (see lower panel of Fig. 2).

Next we discuss Fig. 3. Within the DLVO theory the results should be identical to those in Fig. 2; however, we see that this is not the case. The reason is that, as for real proteins, negative charges on the model macroion (mimicking carboxylic groups) are differently solvated than the positive ones. As expected, the nature of the cation is more important here; the results for CsI (green) are different from those of LiI (red). In Fig. 4 we present the results for the model macroion with net charge zero (iso-ionic point) +16:−16. The slope is negative here, indicating possible precipitation under such conditions. This

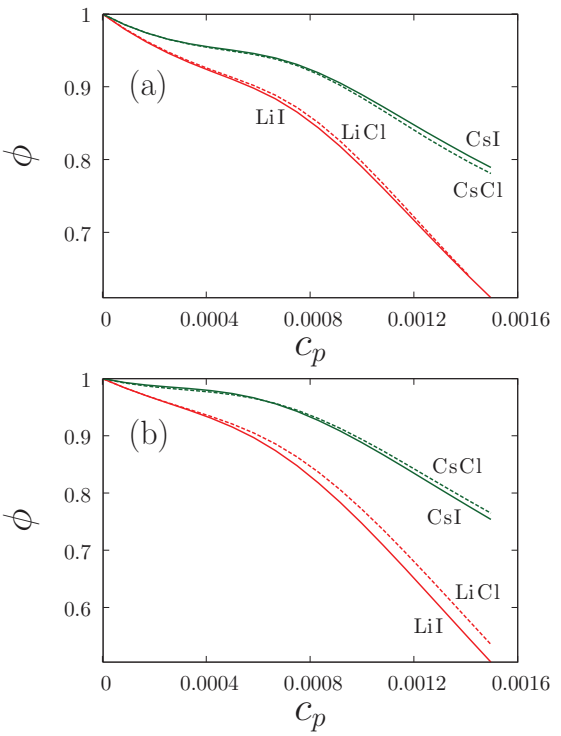

FIG. 4. (Color online) Osmotic coefficient ϕ as a function of protein concentration for a model protein with 16 positive and 16 negative residues: +16:−16. The color code and other parameters are the same as in Fig. 2.

TABLE I. Reduced second virial coefficient B_2^* for +16:−1 macroions (top), +1:−16 macroions (middle), and +16:−16 macroions (bottom) in the presence of $0.1M$ and $0.4M$ concentrations of added electrolyte.

c/M	LiI	LiCl	CsI	CsCl
		+16:−1		
0.1	19.5	20.8	19.3	20.9
0.4	4.0	4.4	4.0	4.6
		+1:−16		
0.1	27.4	27.2	29.9	29.6
0.4	7.9	7.7	10.3	9.9
		+16:−16		
0.1	−5.3	−5.2	−3.9	−3.8
0.4	−4.1	−4.1	−2.0	−2.2

result is predicted by the DLVO theory as being a consequence of the zero net charge on the protein.

To put the results presented in Figs. 2–4 on a more quantitative basis we calculated the second virial coefficient B_2 [Eq. (18)], reflecting binary interaction between macroions. This quantity, which can be determined experimentally, is of special importance for protein solutions [17–23]. George and Wilson [17] pointed out that in order to grow well defined crystals the second virial coefficient must be negative in a narrow range of values termed the crystallization slot.

The reduced quantity presented here is defined as $B_2^* = B_2/B_2^{(HS)}$, where $B_2^{(HS)} = (2/3)\pi\sigma^3$, with σ the diameter of the protein modeled. Notice that the values of B_2^* smaller than unity signal attractive interaction among particles. The results collected in Table I confirm qualitative conclusions inferred from Figs. 2–4. Strongly positive B_2^* values suggest stability of such solutions and are (see the case of the +16:−1 solution) the consequence of strong electrostatic repulsion.

Here we wish to focus on the salt specific effects, which cannot realistically be accounted for by the theories that do not include solvent explicitly. As seen from Table I the second virial coefficient of the model +16:−1 macroion solution is higher in the presence of chloride than iodide anions. This is consistent with experimental results for solubility of aqueous lysozyme [11] solutions, measured in the presence of various salts. The experimental results [11] at $p\mathrm{H} = 4.0$, where the net charge on the protein is +8, indicated that anions decrease the lysozyme solubility in the order $F^- < Cl^- < Br^-$ (the inverse Hofmeister series).

Rosenbaum *et al.* [23] report the B_2^* values for lysozyme-salt mixtures at $p\mathrm{H} = 4.6$, $T = 298$ K, and ionic strength $0.35M$. At this pH the protein should have net charge [33] around +10. The static light scattering results for B_2^* listed in Table I of Ref. [23] are −0.9 for lysozyme-NaCl and −0.6 for lysozyme-KCl mixtures. Our calculations, without any adjustment of parameters, yield values $B_2^* = -0.65$ and −0.22 under such conditions. The errors in experimental determination are around ±0.2.

On the other hand, for +1:−16 macroions (negative net charge) the B_2 values are higher in the case of the cesium salts than in the presence of lithium salts. In both cases an increase of the low-molecular-mass electrolyte content decreases the ion specific effects. The nature of co-ions plays a less important role here. For the net charge zero, the second virial coefficient is negative, indicating marginal stability of the model solution under such conditions. A low value of B_2^* is a consequence of the absence of the Coulomb repulsion between the model macroions. The result is in qualitative agreement with experimental data, which indicate that proteins are most easy to precipitate around their iso-ionic points.

VI. CONCLUSION

The DLVO theory treats an aqueous electrolyte solution as a structureless fluid that merely modifies the interaction between charged macroparticles. All the properties of this complex solvent are subsumed in the Debye screening length, which depends on the ionic strength (concentration in the case of a +1:−1 electrolyte) and also on the dielectric constant of the solvent and the temperature. Neither cations and anions nor solvent molecules are included explicitly in such calculations. Accordingly the macroion-macroion interaction, besides the central symmetric van der Waals forces, is characterized only by the net charge of the macroparticle, and not by the value of the positive and negative charges, separately. For such models, there is no obvious way of accommodating the solvation effects, which yield the ion specific results. The latter effects are local; they occur on contact of the surface charge with the solvent and ionic atmosphere and by putting the charges in the center we lose them. In other words, while the so-called colloidal models are very useful for describing the macroion-macroion correlation in scattering experiments, they are not subtle enough to explain differences in the solubility of proteins in the presence of different +1:−1 electrolytes.

The approach presented herein, though still approximate in its treatment of solvent, allows systematic investigation of how the nature of charged groups on the macroion and electrolyte in solution affects the stability of the system. A more detailed study of the effect of different low-molecular-weight electrolytes on the osmotic second virial coefficient B_2, which is recognized as an important parameter related to protein crystallization, is beyond the scope of the present paper.

A limitation of the present approach is associated with the fact that the waterlike molecules representing solvent interact only via the short-range interaction. In other words, there is no dipole or any higher moment associated with our model solvent. This may frustrate an accurate determination of thermal properties such as enthalpy of dilution and/or mixing.

We can summarize this work as follows. (i) The osmotic coefficient is lower for solutions with higher content of added electrolyte. (ii) The model protein with a zero net charge has a negative slope of the osmotic coefficient at low concentrations and is accordingly less stable than a macroion solutions with a nonzero net charge. These results merely confirm the findings obtained by the DLVO approach. (iii) Effects of the electrolyte type are clearly visible. As expected, the nature of the counterion (with respect to the net charge) is generally more important than the nature of the co-ion. Our calculations are consistent with experimental data for solubility of lysozyme in the presence of different salts. A semiquantitative agreement with static light scattering data for B_2 is demonstrated. (iv) It

is not only the net charge that plays a role; as seen from the results, our model discriminates between the +16:−1 and the +1:−16 case. In summary, the proposed theoretical approach possesses enough flexibility to model realistic situations in protein solutions.

Note added. Recently, we became aware of a work by Bernard *et al.* [34]. These authors used a similar theoretical approach, based on Wertheim's theory, but they examined a different model of solution than ours. The most notable difference from our work is in their treatment of water as continuous dielectric.

ACKNOWLEDGMENTS

This study was supported by the Slovenian Research Agency fund (ARRS) through Program No. P1-0201, ARRS Project No. J1-4148, and NIH (U.S.) research Grant No. GM063592.

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