Acta Chim. Slov. **1998**, *45*(2), pp. 199-208

Received 8. 5. 1998

ANALYSIS OF OSMOTIC PRESSURE DATA FOR AQUEOUS PROTEIN SOLUTIONS VIA A ONE - COMPONENT MODEL [1]

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Abstract

A modification of the one-component model of protein solutions is presented that accounts for the self-association of protein molecules in solution. In addition to the usual screened Coulomb interaction the protein molecules can form dimers, but no higher clusters are allowed. Essentially, we treat the solution as a mixture of hard spheres and hard dumb-bells characterized by some effective diameter. A simple variational approach is proposed to relate the effective diameter to the parameters of the solution under investigation, i.e. the real diameter of the protein, its charge and concentration. The new method is used to analyse the reported data for the osmotic pressure of three different proteins with various degrees of self-association. The method, which requires little numerical work, seems to be able to explain the osmotic pressure behaviour of protein solutions in terms of a single parameter, i.e. the fraction of dimers in solution.

Introduction

Professor Savo Lapanje in the Preface to his monograph on protein denaturation [1] wrote: "the physicochemical aspects of protein denaturation represent the basis

[1] Dedicated to the memory of Professor Savo Lapanje

for understanding all other aspects of this important phenomenon". In other words, knowledge of the protein-protein and protein-solvent interactions is a prerequisite for understanding of their properties in solution. It appears, however, to be very difficult to build a consistent microscopic picture of these complex systems. The structure and thermodynamics of protein solutions will result from a subtle balance between the protein-protein interaction, protein-electrolyte and protein-solvent interaction, including the influence of the electrolyte-solvent and solvent-solvent interaction. It is clear that a general theory for these ternary systems, based on Hamiltonian models, cannot be expected soon. Yet, there is need to study the sources of nonideality in protein solutions and to interpret the experimental results in the light of molecular theories. One such attempt is presented in this paper.

Among experimental techniques used to identify the principal interactions in macromolecular solutions, measurements of the osmotic pressure play very important role (see, for example [2], [3], [4], [5]). The first measurements of the osmotic pressure in aqueous protein solution were conducted as early as 1899 [6] and since that time osmometry has become an important tool for characterization of proteins in solution. Very recently, some of us have applied this experimental technique to study the association of human serum albumin (HSA) in aqueous solutions in mixtures with phosphate buffer [5]. These measurements indicate strong deviations from ideality, the osmotic pressure being influenced by factors like pH, the concentration of added electrolyte and protein concentration. The osmotic pressure measurements [5] were complemented by an X-ray study of the same system and the protein association was identified as the principal source of the nonideality. So far, only the experimental results were presented and no theoretical predictions were compared with our experimental data for HSA [5].

Traditionally, osmotic pressure data are interpreted in terms of the second virial coefficient, B_2. This automatically limits the theoretical analysis to solutions which are very dilute with respect to the protein component. A more satisfying approach is to include higher virial coefficients through a suitable integral equation theory. In some cases [7], good agreement between the experiment and theory can be obtained. Unfortunately, integral equation theories, based on the concentration expansion (the first term of this expansion is proportional to B_2) are not well suited for systems with a strong attractive interaction [8]. For the strongly nonideal systems studied here, where the osmotic coefficient does not approach unity even in a dilute regime, the classical integral equation theories become inapplicable. For example, the hypernetted chain approximation, an otherwise successful theory for charged solutions, does

not yield convergent results under conditions where strong attractive forces yield to partial association of ions. In the last decade, however, statistical-mechanical theories were developed which permit us to study model solutions, where the particles form dimers or even higher clusters [9].

Very recently, Kalyuzhny and Vlachy [10] have proposed a theoretical model in which the protein molecules, in addition to the usual Coulomb forces, interact via a short-range directional attractive force. Our approach is based on the theory developed by Wertheim [9] for treating associated systems. Parameters of the short-range interaction are chosen to result in formation of dimers. This model provides a basis to quantify the effects of macroion association on factors such as protein and electrolyte concentration, charge and size of the protein and others. The major disadvantage of the proposed theory is that it requires a solution of the integral equations for the multicomponent system and it is therefore less applicable for daily analysis of experimental results.

In the present paper we propose a much simpler approach to analyse osmotic pressure data in protein solutions: the method is an extension of the one-component model and should apply equally well to both associated and nonassociated systems. In the proposed theory the solution is treated as a mixture of dimers (pairs of protein molecules) and monomers. The particles forming the mixture are assumed to have some "effective" size which differs from their "real" (molecular) size. This way we can use a known formula to obtain the osmotic pressure for a hard sphere - hard dumb-bell mixture [11]. The effective size of the particles reflects the interactions between the protein molecules in solution; it is obtained using the variational principle [12], taking into account the "real" size of the protein, its charge, the concentration of added electrolyte and other parameters which characterize the solution under investigation.

Theoretical Part

Ternary solutions containing protein, a simple electrolyte and water are too complicated for a complete description on the molecular level. Fortunately, many experimental properties of globular proteins can be explained using a simple one-component model wherein a pseudo- solvent (simple electrolyte and water) modifies the interactions between the protein molecules. In this article we present an extension of this approach to account for the possible self-association of the protein molecules in solution.

The aqueous solution of a globular protein is represented as a two-component mix-

ture of charged hard spheres (monomers) and charged hard sphere diatomics (dumb-bells), mimicking the dimers. The effect of added electrolyte is modelled by the screened Coulomb potential acting between each pair of monomer units. Thus, the total pair potential $U(r)$ between the diatomic sites, the diatomic site and the charged hard sphere and between the charged hard spheres is of the following form:

$$U(r) = U_h(r) + U_Y(r) \tag{1}$$

where $U_h(r)$ is the hard-sphere potential, and

$$U_Y(r) = \frac{A}{r}\exp(-\kappa r), \tag{2}$$

where

$$A = \frac{z_p^2 L_B \exp(\kappa\sigma)}{(1+\kappa\sigma/2)} \tag{3}$$

In Eqs. (2,3) κ is the Debye-Hückel screening parameter defined as

$$\kappa^2 = 4\pi L_B N_A c_s \tag{4}$$

where $e_0 z_p$ is the macroion charge, σ is the hard-sphere (molecular) diameter, c_s is the molar electrolyte concentration and N_A is the Avogadro number. Further, L_B is the Bjerrum length given by

$$L_B = \frac{e_0^2}{4\pi\epsilon_0\epsilon_r k_B T}, \tag{5}$$

where $\epsilon_0\epsilon_r$ is the permittivity of the solution, k_B Boltzmann's constant and T the absolute temperature.

To calculate the thermodynamic properties of the model system described above we utilize a simple thermodynamic perturbation theory and the Boublik equation of state for a mixture of hard convex bodies [11]. First, the original system interacting via the potential given by Eq. (1) is replaced by a reference system represented by the two-component mixture of hard spheres and hard dumb-bells (representing dimers) of some "effective" diameter. The effective hard-sphere diameter σ_{ef} for the dumb-bell sites and hard spheres, reflecting the screened Coulomb interaction, is then determined using the condition proposed by Lado [12]:

$$4\pi\int_0^\infty r^2 dr[e^{-\beta U(r)} - e^{-\beta U_h(\sigma_{ef},r)}]\frac{\partial y_h(\sigma_{ef},r)}{\partial\sigma_{ef}} = 0 \tag{6}$$

In this calculation the dumb-bell elongation L is $L = \sigma$. An alternative is to choose the value of L in such a way that the dumb-bell excluded volume is the same as of

original hard spheres. The choice of L, however, does not affects σ_{ef} significantly. In order to evaluate integral in Eq. (6) we need to know y_h; the so-called cavity distribution function for the hard-sphere system [13]. The hard-sphere cavity distribution function $y_h(\sigma_{ef}, r)$ was calculated using the Henderson-Grundke prescription [14]. It is important to stress that condition given by Eq. (6) enforces thermodynamic consistency; the energy and the virial route to osmotic pressure yield exactly the same result [12].

In the last step, Boublik's equation for the osmotic coefficient [11] is applied in the form:

$$\Phi = \frac{\beta P}{N k_B T} = \frac{1}{1-v} + \frac{2 r_c s}{\rho(1+x)(1-v)^2} + \frac{2 q s^2 (1 - v/3)}{3\rho(1+x)(1-v)^3}, \tag{7}$$

where

$$r_c = \frac{1}{4}\rho(1+x)\sigma_{ef}\left[\frac{1}{2}(1-x)(1+\frac{1}{2}\sigma) + x\right], \tag{8}$$

$$s = \frac{1}{2}\rho(1+x)\pi\sigma_{ef}^2\left[\frac{1}{2}(1-x)(1+\sigma) + x\right], \tag{9}$$

$$v = \frac{\pi}{12}\rho(1+x)\sigma_{ef}^3\left[\frac{1}{2}(1-x)(1+\frac{3}{2}\sigma - \frac{1}{2}\sigma^3) + x\right], \tag{10}$$

$$q = \frac{1}{8}(1+x)\rho\sigma_{ef}^2\left[(1+\frac{1}{2}\sigma)^2 + x\right]. \tag{11}$$

In Eqs. (7-11) x is the fraction of the hard spheres, ρ is the total number density of the monomeric units (stoichiometric number concentration of protein) in the system, defined by

$$\rho = \rho_0 + 2 * \rho_d \tag{12}$$

In the last equation (12) ρ_0 is the number density of hard spheres and ρ_d the number density of dumb-bells. One important advantage of the perturbation theory described above is that it requires very little numerical work in comparison with the theories based on the integral equation approach [10]. Equation (6) was solved numerically using Newton - Raphson method.

Analysis of experimental data

The theory outlined in the previous section yields results for the osmotic coefficient (or osmotic pressure) which can be compared with the experimental values. The computational procedure is the following. First, we calculate the interaction potential given by Eq. (1) using the "real" (molecular) parameters of the system of

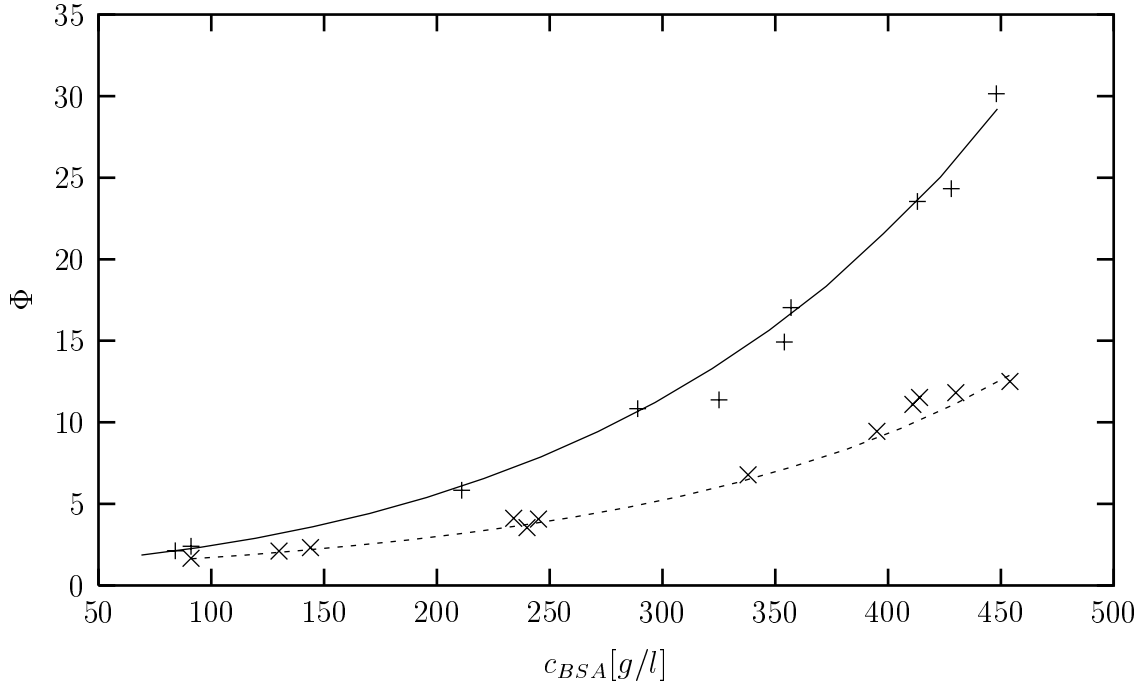

Figure 1: Osmotic coefficient $\Phi = \Pi/\Pi_{id}$ as a function of BSA concentration [3]. Theoretical predictions at pH 7.3 are represented by the solid line and at pH 5.4 by the dashed line; experimental data at pH 7.3 (+++) and at pH 5.4 (xxx). Protein charges are -20 and -9 at pH 7.3 and 5.4, respectively.

interest. This information is used as input to Eq. (6) which determines the effective dimensions of the hard sphere - hard dumb-bells mixture. Once the effective diameter for the model mixture is known the osmotic coefficient can be determined from Eq. (7). The calculated osmotic coefficient (or pressure) can be fitted to experimental results to obtain the fraction of dimers in the solution. The results for three different proteins in solution are presented below.

First we apply our analysis to the osmotic pressure measurements of bovine serum albumin (BSA) in 0.15 M sodium chloride. These results are shown in Fig. 1, where the osmotic coefficient is given as a function of the protein concentration. The lines in figures are 'eye best fit'. The experimental data are from ref. [3]. The protein molecular weight used in the calculation of osmotic coefficient is 69,000 g/mol. Under these conditions (for other parameters see the caption to the figure) no self-association of protein molecules is detected - the fraction of dimers giving good agreement with experiment is zero. Note, however, that these results apply to a very high concentration of BSA molecules. In Fig. 2 we present the results for the human serum albumin (HSA) solutions recently studied by some of us [5]. Again the osmotic coefficient is analysed as a function of the protein concentration

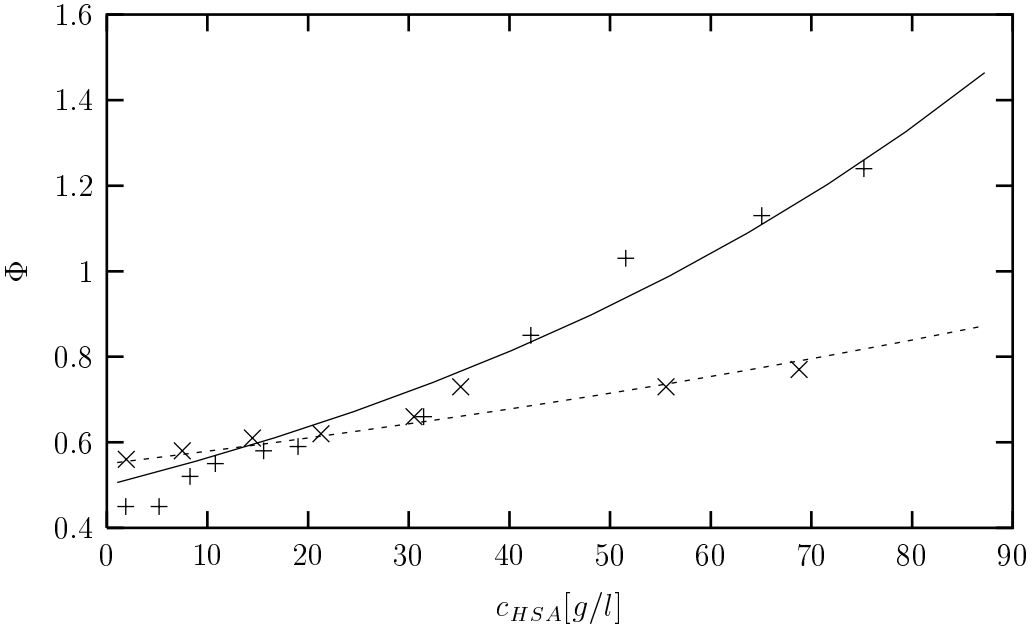

Figure 2: Osmotic coefficient as a function of the HSA concentration [5]. Theoretical predictions at pH 8.0 are represented by the solid line and at pH 5.4 by the dashed line; experimental data at pH 8.0 (+++) and at pH 5.4 (xxx). The number of negative charges on the HSA molecule are 22 and 0 at pH 8.0 and 5.4, respectively.

and for two different pH values. The concentration of added phosphate buffer was 0.1 M. The protein molecular weight used in this calculation was 66,700 g/mol and the charges on the protein [17] are given in caption to this figure. In this case the strong nonideality in solution can be explained by the formation of dimers. A reasonably good agreement between calculation and experiment is obtained when the fraction of dimers is equal to 1 (full dimerization) and 0.9, for pH values equal to 8.0 and 5.4, respectively. Appreciable association in dilute solutions of HSA has been confirmed by an X-ray scattering study [5].

The third figure (Fig. 3) presents the osmotic pressure results for moderately concentrated BSA solutions at two different pH values (for experimental details see [15], [16]). The number of (negative) charges on the protein molecule is 21 and 17 at pH 7.3 and 6.9, respectively. The ionic strength of the added simple electrolyte is 0.1 M. The fraction of dimers used as input in these calculations was 0.3. Equally good agreement between theory and experiment was also obtained for pH = 8.0, but these results are not shown here. Diammeter of both HSA and BSA molecules used in calculations is 6.0 nm.

In the final example studied in this paper we present the results for α-chymotrypsin solutions. In Fig. 4 the osmotic pressure is plotted as a function of protein concen-

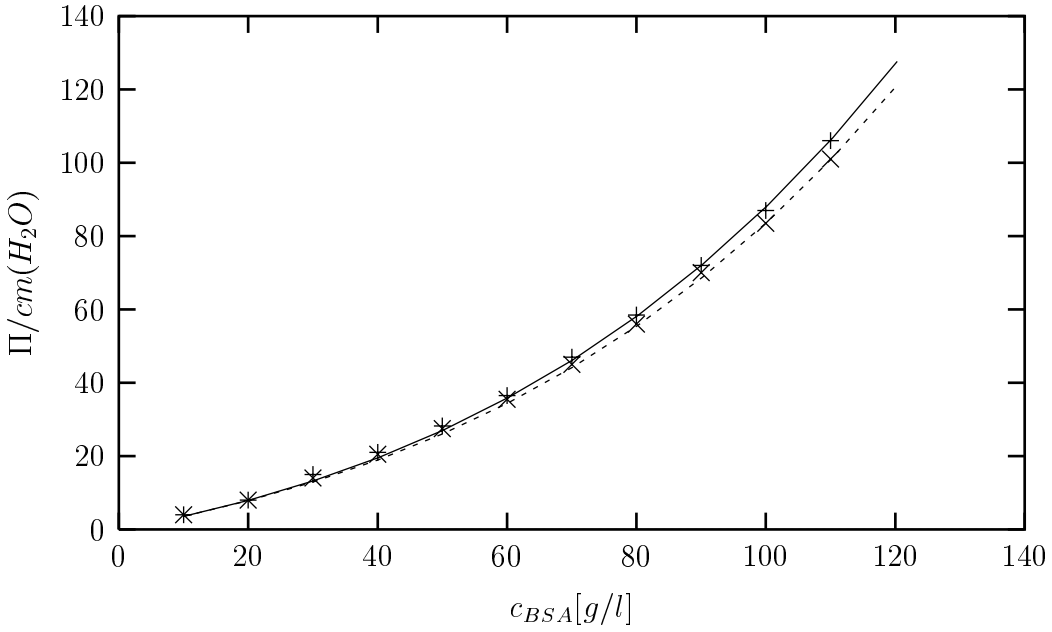

Figure 3: Osmotic pressure Π as a function of BSA concentration [15], [16]. Theoretical predictions at pH 7.3 are represented by the solid line and at pH 6.9 by the dashed line; experimental data at pH 7.3 (+++) and at pH 6.9 (xxx).

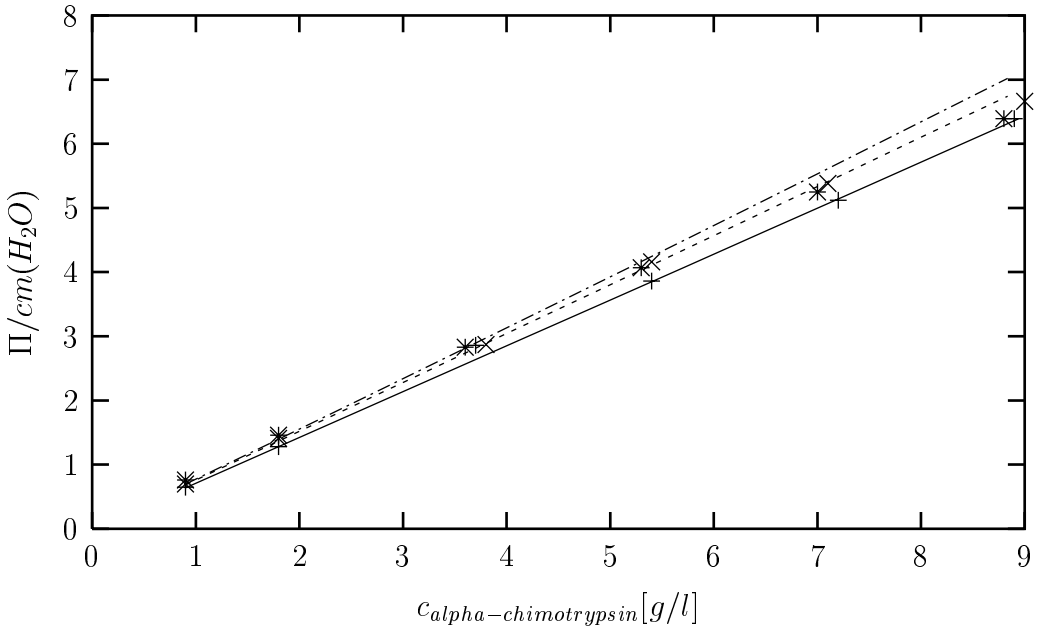

Figure 4: Osmotic pressure Π as a function of α-chymotrypsin concentration [4]. Theoretical predictions at pH 8.25 are represented by the solid line, at pH 6 by the dashed line and at pH 4 by the dashed-dotted line. Experimental data at pH 8.25 are denoted by (+++), at pH 6 by (xxx) and at pH 4 by (***).

tration for three different values of pH. Experimental values are taken from ref. [4]; the ionic strength of the added simple electrolyte is 0.3 M in this example and the number of charges on the protein for the three pH values is 0 (pH=8.25), 3 (pH=6.0) and 10 (pH=4.0). The protein diammeter is 2.17 nm. The fraction of dimers used to calculate the lines in Fig. 4 are 0.48 (pH=8.25), 0.38 (pH=6), and 0.35 (pH=4).

Discussion

The nonideality of protein solutions may originate from several sources; the association between protein molecules is one of them. It is known that this association plays an important role in the control of enzyme activity. To know the degree of protein self-association and the factors which control this phenomenon is therefore important. Among experimental methods, membrane osmometry is a traditional method of measuring the nonideality of protein solutions.

In an attempt to explain the osmotic pressure data of aqueous solutions of globular proteins, we propose a simple perturbation theory. The idea was to develop a theoretical model that predicts how factors such as protein size and charge (pH), salt concentration and protein aggregation affect the osmotic pressure. The perturbation theory presented in this paper has two advantages: i) it does not require massive numerical work as do integral equation theories, and, ii) it applies equally well to associated and non-associated systems of molecules. The analysis of experimental data for three different proteins in a broad range of protein concentration, (varying pH and concentration of added simple electrolyte) is presented in the previous section. The results indicate that we can fit the experimentally obtained osmotic pressure by adjusting the fraction of dimers in solution. In this way, the fraction of dimers in solution can be determined. With this respect, the theory proposed above is positioned somewhere between the molecular theories based on Hamiltonian models [10] and fully empirical methods [16] of data analysis. At this level of the theory only formation of dimers is considered. At higher degrees of dimerization (e.g. the system presented in Fig. 2), the contribution of higher clusters may become appreciable, what essentially limits applications of the theory only to situations where the degree of pairing is low. It is not impossible, however, to extend the calculation to treat trimers and higher clusters or, for example, to account for the interpenetration of the protein molecules. We hope to present some of these developments in the near future.

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Povzetek

Članek obravnava razširitev enokomponentnega modela raztopine globularnih proteinov v vodi. Novi model omogoča študij asociiranih sistemov, kjer molekule ali ioni tvorijo pare. Poleg običajnega, zaradi prisotnosti elektrolita zasenčenega coulombskega potenciala delujejo med proteinskimi molekulami tudi kratkosežne sile, ki vodijo do nastanka parov. Gruče, ki vsebujejo tri ali večmolekul, smo v tem delu prezrli. Raztopino smo obravnavali kot mešanico, ki jo sestavljajo toge kroglice (monomer) in pa v par povezane toge kroglice (dimer). Za takšen primer je bila izpeljana enačba stanja, ki je dana v analitični obliki. Velikost togih kroglic, ki smo jo uporabili v računu, ne ustreza molekularnim dimenzijam proteina, ampak že odraža tudi njihovo interakcijo s topilom in dodanim elektrolitom. Razširjeni model omogoča analizo termodinamičnih količin pri neasociiranih kot tudi pri asociiranih raztopinah in zahteva razmeroma malo numeričnega dela. Uporabnost predlaganega modela smo prikazali z analizo izmerjenih osmoznih tlakov v raztopinah treh različnih proteinov in sicer v širokem območju koncentracij in pH vrednosti. Kot rezultat podajamo delež asociiranih molekul v teh raztopinah.