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Prediction of collective diffusion coefficient of bovine serum albumin in aqueous electrolyte solution with hard-core two-Yukawa potential

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A new method to predict concentration dependence of collective diffusion coefficient of bovine serum albumin (BSA) in aqueous electrolyte solution is developed based on the generalized Stokes–Einstein equation which relates the diffusion coefficient to the osmotic pressure. The concentration dependence of osmotic pressure is evaluated using the solution of the mean spherical approximation for the two-Yukawa model fluid. The two empirical correlations of sedimentation coefficient are tested in this work. One is for a disordered suspension of hard spheres, and another is for an ordered suspension of hard spheres. The concentration dependence of the collective diffusion coefficient of BSA under different solution conditions, such as pH and ionic strength is predicted. From the comparison between the predicted and experimental values we found that the sedimentation coefficient for the disordered suspension of hard spheres is more suitable for the prediction of the collective diffusion coefficients of charged BSA in aqueous electrolyte solution. The theoretical predictions from the hard-core two-Yukawa model coupled with the sedimentation coefficient for a suspension of hard spheres are in good agreement with available experimental data, while the hard sphere model is unable to describe the behavior of diffusion due to its neglect of the double-layer repulsive charge–charge interaction between BSA molecules.

Introduction

The collective diffusion coefficients of charged protein in aqueous electrolyte solutions are required for many biological processes, such as membrane filtration, separation or sedimentation. For example, in therapeutics and diagnostics, in order to prevent disease, an important pathological event is the separation of a biological solution into coexisting protein-rich and protein-poor phases;[1] In biochemical reactions, the degree of separation, purity and yield of a particular protein is influenced to a considerable extent by the properties of the media, and the diffusion coefficient plays a significant role in these properties. Moreover, it is crucial to have some knowledge of the concentration dependence of the collective diffusion coefficient for a wide range of volume fraction, because the biological fluids in such process are concentrated solutions.[2] However, the collective diffusion coefficient is studied much less than it is needed, due to the difficulty both in theory and experiment.

Experiment on the determination of diffusion coefficients of globular proteins in aqueous solution is usually carried out using dynamic light scattering (DLS). This method has been used by Placidi and Cannistraro,[3] and Meechai *et al.*[4] to measure the collective diffusion coefficient of BSA under isothermal condition as a function of protein concentration and in the presence of glycerol. However, due to the opacity of the concentrated suspension and of multiple light scattering effects, it fails at high volume fraction. Besides DLS, capillary methods, porous barrier (membranes) methods or Gouy interferometric method may be used,[5] but all of them are limited to dilute solutions. For example, a capillary method based on a principle of hydrodynamic stability was used by Anderson *et al.*[5] to determine the mutual diffusion coefficient of BSA as a function of concentration in aqueous solutions of potassium chloride.

In theory, the pioneering work done by Einstein[6] used the diffusion coefficient to describe the Brownian motion of an isolated particle at infinite dilution solution. After that, the friction coefficient of the particle which determines the behavior of the diffusion coefficient at infinite dilute solution is investigated by several researchers.[7] For example, Booth[7] developed a consistent theory for the friction coefficient by macroscopic hydrodynamics, which does not agree with experimental data. Schurr[8] proposed a theory considering both the electric and the hydrodynamic forces. Although the results from Schurr's theory agree well with the experimental data, the theory is not consistent. At low volume fraction, it is relatively a little more complex to derive the expressions for the diffusion coefficient. Batchelor[9] and Felderhof[10] separately developed a generalization of Einstein theory, which combined hydrodynamics with hard sphere interactions by the statistical methods. They obtained the first order correction of the collective diffusion coefficient with respect to the volume fraction of the particles where the charge of the proteins is not included. Petsev and Denkov[11] further modified this theory by considering some well defined quantities such as particle radius, charge, surface potential *etc*. At intermediate volume fractions (up to 0.2), the combined effects of electrostatic repulsion and hydrodynamic interactions become important. By determining the electrostatic repulsion from the thermodynamically consistent Rogers–Young scheme,[12] Genz and Klein[13] studied various trends for the collective diffusion coefficients of charged spheres. By using a dynamic mode-coupling theory of diffusion in binary fluid mixtures, a relation between collective diffusion coefficient and viscosity in suspensions was obtained and can be used to correlate the experimental data.[14]

An alternative way to predict the collective diffusion coefficients of charged globular proteins in aqueous electrolyte solutions is to use the generalized Stokes–Einstein equation. In this method, the sedimentation coefficient and the osmotic

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pressure must be known in advance. Many theoretical works have been done to determine the sedimentation coefficient of colloidal suspensions. There are two empirical correlations available for the sedimentation coefficient of hard sphere colloidal suspensions: one is used for Brownian hard spheres and another was proposed by Happel and Brenner[15] using a cell model for ordered suspensions of sphere. The osmotic pressure can be calculated by the integral equation, Poisson-Boltzman (PB) cell model[16] or the free-solvent model.[17] The integral equation may be based on the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory[18] or Yukawa potentials,[19–22] using the hypernetted chain approximation or mean spherical approximation (MSA). The advantage of MSA is that it can give an analytical solution and an explicit equation of state (EOS).[19] In the PB cell model,[16] the osmotic pressure includes the contributions from electrostatic interactions, London–van Der Waals forces and configurational entropy. The electrostatic interactions are accounted for by a Wigner–Seitz cell approach involving a numerical solution of the nonlinear PB equation. The free-solvent model was developed by Yousef *et al.*,[17] in which hydration and salt binding are assumed to be dominant factors for non-ideality and the average solute–solvent and microion–solute interactions are considered.

The purpose of this work is to establish a predictive method for the concentration dependence of the collective diffusion coefficient as a function of the main physicochemical conditions. We investigate the collective diffusion coefficient of bovine serum albumin (BSA) in aqueous electrolyte solution by means of the generalized Stokes–Einstein equation, where the required concentration dependence of the osmotic pressure is described by the MSA solution of the hard-core two-Yukawa potential.

Theory

The generalized Stokes–Einstein equation[15] relates the collective diffusion coefficient of proteins with the osmotic pressure and the sedimentation coefficient for the whole range of volume fractions, and for any interparticle potential. Its basis is that the driving force underlying diffusion is thermodynamic in origin. In the dispersion there exists a thermodynamic force acting on the particles. Combining the thermodynamic force, the flux of particles, Fick's law and the expression for the mobility, one can obtain the generalized Stokes–Einstein equation.[15] It can be written as

$$D_c(\phi) = D_0 K(\phi)\left[\frac{4\pi R_p^3}{3k_B T}\frac{\partial \Pi(\phi)}{\partial \phi}\right] \qquad (1)$$

where D_0 is the collective diffusion coefficient at infinite dilution, $K(\phi)$ is the sedimentation coefficient, R_p is the radius of the globular protein, k_B is the Boltzmann constant, T is the absolute temperature, $\Pi(\phi)$ is the osmotic pressure and ϕ is the volume fraction of protein. To employ eqn. (1) to calculate the collective diffusion coefficient, we need the knowledge of the concentration dependence of osmotic pressure and the sedimentation coefficient.

Osmotic pressure for BSA solution

In this paper the charged BSA-electrolyte aqueous solution is considered as a pseudo one-component system, and water molecules are treated as a continuous medium with dielectric constant D. The interactions between charged BSA macromolecules are modeled as a hard-core two-Yukawa potential, *i.e.*,

$$u(r) = \begin{cases} \infty & r < \sigma \\ u^{dis}(r) + u^{cc}(r) & r \geq \sigma \end{cases} \qquad (2)$$

where $u^{dis}(r)$ and $u^{cc}(r)$ represent, respectively, the dispersion interaction and the double-layer repulsive charge–charge interaction between charged BSA molecules. The dispersion interaction can be expressed as a Yukawa potential

$$u^{dis}(r) = -\frac{\varepsilon \exp[-\lambda(r/\sigma - 1)]}{r/\sigma} \quad (r > \sigma) \qquad (3)$$

where ε is the dispersion energy parameter, σ is the hard-sphere diameter of a BSA molecule, and λ is the range parameter. Because the Lennard-Jones potential is the best potential for the dispersion interaction and when $\lambda = 1.8$, the Yukawa potential yields results comparable with those obtained from Lennard-Jones potential,[23] we adopt $\lambda = 1.8$ in this work. The value of ε is taken from Lin *et al.*[20] and the hard-sphere diameter of a BSA molecule is the hydrodynamic particle diameter taken from Anderson *et al.*[5] and they have values of $\varepsilon/k_B = 91.3$ K and $\sigma = 7.20$ nm, respectively.

The double-layer repulsive charge–charge interaction between BSA molecules can be also described using the Yukawa potential

$$u^{cc}(r) = \frac{z_p^2 e^2 \exp[-\kappa(r-\sigma)]}{Dr(1+\kappa\sigma/2)^2} (r > \sigma) \qquad (4)$$

where z_p is the BSA charge number, e is the charge of an electron, and κ is the Debye screening parameter, which is determined by

$$\kappa^2 = \sum_i \frac{\rho_i e^2 z_i^2}{D k_B T} \qquad (5)$$

where ρ_i and z_i are the number density and the valence of microion i, respectively. Eqn. (4) was deduced from the classical DLVO theory. Comparing eqn. (4) with eqn. (3), we can obtain the Yukawa parameters for the double-layer repulsive charge–charge interaction between BSA molecules.

$$\varepsilon' = -\frac{z_p^2 e^2}{\sigma D(1+\kappa\sigma/2)^2} \text{ and } \lambda' = \kappa\sigma$$

Using McMillan-Mayer solution theory, the osmotic compressibility factor Z of aqueous BSA-electrolyte solutions can be decomposed according to the interaction potential between BSA molecules

$$Z = \frac{\Pi}{\rho_p k_B T} = Z^{Donnan} + Z^{hs} + Z^{dis} + Z^{cc} \qquad (6)$$

where ρ_p is the number density of BSA molecules. The superscripts *Donnan*, *hs*, *dis*, and *cc*, represent the contributions of the Donnan effect, hard sphere repulsion, attractive dispersion, and double-layer repulsive charge–charge interactions, respectively.

In BSA-electrolyte solution, to maintain electro-neutrality and equilibrium, the concentrations of micro ions on both sides of a membrane will be unequal, which may bring additional osmotic pressure and is called the Donnan effect. The contribution of Donnan effect to osmotic compressibility factor Z^{Donnan} can be expressed as

$$Z^{Donnan} = (\rho_+^{in} + \rho_-^{in} - \rho_+^{out} - \rho_-^{out})/\rho_p \qquad (7)$$

where ρ_p, ρ_+ and ρ_- are the number densities of protein, micro cation, and micro anion, respectively. In eqn. (7) the superscripts *in* and *out* represent the protein side and the microion side of the membrane, respectively.

The equations of electro-neutrality and the equal ionic concentration products on both sides of the membrane can be expressed as

$$z_p\rho_p + z_+\rho_+^{in} + z_-\rho_-^{in} = 0 \qquad (8)$$

$$z_+\rho_+^{out} + z_-\rho_-^{out} = 0 \qquad (9)$$

$$(\rho_+^{in})^{|z-|}\,(\rho_-^{in})^{z+} = (\rho_+^{out})^{|z-|}\,(\rho_-^{out})^{z+} \qquad (10)$$

where z_p, z_+ and z_- are the charge numbers of protein, micro cation, and micro anion, respectively.

The hard sphere repulsion contribution Z^{hs} can be obtained from the Carnahan–Starling equation

$$Z^{hs} = \frac{1 + \phi + \phi^2 - \phi^3}{(1 - \phi)^3} \tag{11}$$

where ϕ is the packing fraction, *i.e.*,

$$\phi = \pi\rho_p\sigma^3/6 \tag{12}$$

Here σ is the hard-sphere diameter of a BSA molecule, which has a value of 7.20 nm.[23] If we know the protein concentration C_p, then the volume fraction can be calculated from $\phi = C_p v\sigma^3/\sigma^3_{\mathrm{dry}}$, where v and $\sigma_{\mathrm{dry}}(= 2.69\ \mathrm{nm})$[16] are the specific volume and dry protein particle diameter, respectively.

The dispersion and the double-layer repulsive charge–charge interaction contributions to the osmotic compressibility factor Z can be obtained by solving Ornstein–Zernike (OZ) integral equation with proper closures. There are many closure relationships available, but only the hypernetted-chain (HNC), Rogers–Young closure[12] and the mean spherical approximation are more popular. Although the former two closures are more accurate, they should be solved numerically using Fourier transforms and this limits their applications. The mean spherical approximation (MSA) is the most widely used closure because an analytical solution is available for this approximation. There are two methods to establish the two-Yukawa equation of state based on MSA. One is to sum the infinite order expansion of the Duh and Mier-y-Teran EOS[19] twice directly. Another is based on Baxter–Wertheim factorization of the OZ equation for one-component and the *w*-Yukawa case by using MSA. The former is simpler in mathematics, while the latter is much stricter in theory. For simplicity, the former is adopted in this work to calculate the osmotic pressure of BSA-electrolyte aqueous solution. By differentiating excess Helmholtz free energy with respect to the volume fraction ϕ, the dispersion and the double-layer repulsive charge–charge interaction contributions to the compressibility factor Z can be expressed as

$$\begin{aligned} Z^{dis} + Z^{cc} = &-\phi\frac{\beta\varepsilon}{\phi_0}\left[\frac{\partial\alpha_0}{\partial\phi} - \frac{\alpha_0\partial\phi_0}{\phi_0\partial\phi}\right] \\ &+ \frac{\lambda^3}{6\phi}\left[F(x) - F(y) - (x - y)\frac{\mathrm{d}F(y)}{\mathrm{d}y}\right] \\ &- \frac{\lambda^3}{6}\left\{\frac{\partial x}{\partial\phi}\left[\frac{\mathrm{d}F(x)}{\mathrm{d}x} - \frac{\mathrm{d}F(y)}{\mathrm{d}y}\right] - \frac{\partial y}{\partial\phi}(x - y)\frac{\mathrm{d}^2F(y)}{\mathrm{d}y^2}\right\} \end{aligned} \tag{13}$$

where

$$F(x) = -\frac{1}{4}\ln(1 - 2x) - 2\ln(1 - x) - \frac{3}{2}x - \frac{1}{1 - x} + 1 \tag{14}$$

$$x = (1 + \lambda\psi)w\varepsilon/(\lambda^2 k_{\mathrm{B}}T) \tag{15}$$

$$y = w\psi\varepsilon/(\lambda k_{\mathrm{B}}T) \tag{16}$$

$$\alpha_0 = L(\lambda)/[\lambda(1 - \phi)]^2 \tag{17}$$

$$\phi_0 = [\exp(-\lambda)L(\lambda) + S(\lambda)]/[\lambda^3(1 - \phi)^2] \tag{18}$$

$$w = 6\phi/\phi_0^2 \tag{19}$$

$$\psi = \frac{\lambda^2(1 - \phi)^2(1 - e^{-\lambda}) - 12\phi(1 - \phi)\left[1 - \frac{\lambda}{2} - \left(1 + \frac{\lambda}{2}\right)e^{-\lambda}\right]}{e^{-\lambda}L(\lambda) + S(\lambda)} \tag{20}$$

$$L(\lambda) = 12\phi[(1 + \phi/2)\lambda + 1 + 2\phi] \tag{21}$$

$$S(\lambda) = (1 - \phi)^2\lambda^3 + 6\phi(1 - \phi)\lambda^2 + 18\phi^2\lambda - 12\phi(1 + 2\phi) \tag{22}$$

Sedimentation coefficient

Sedimentation coefficient is a key quantity for the prediction of mutual diffusion coefficient for colloidal particles. The sedimentation of charged spheres is affected by the influence of colloidal force on hydrodynamic interactions. It can be enhanced by attractive colloidal interaction and slowed down by repulsive interaction at a given volume fraction. For electrostatically stabilized BSA, if electrolyte concentration in solution is not high (as the case in this study), the interaction between two BSA molecules is repulsive and the sedimentation coefficient always decreases as the volume fraction increased.

For a disordered suspension of hard spheres, the following equation gives an accurate sedimentation coefficient[15]

$$K(\phi) = (1 - \phi)^{6.55} \tag{23}$$

Another analytical expression for the sedimentation coefficient was developed by Happel and Brenner[15] based on a cell model for ordered suspensions of spheres. It is given by

$$K(\phi) = \frac{1 - \frac{3}{2}(\phi)^{1/3} + \frac{3}{2}(\phi)^{5/3} - \phi^2}{1 + \frac{2}{3}(\phi)^{5/3}} \tag{24}$$

In this paper both expressions are used to verify which one is more suitable for the prediction of the mutual diffusion coefficient for BSA when coupled with eqn. (6) *via* the generalized Stokes–Einstein equation.

Results and discussion

The collective diffusion coefficient of BSA in aqueous electrolyte solution is investigated at selected values of temperature, pH, ionic strength, and BSA concentration. The values of the parameters used in the calculation of the collective diffusion coefficient are collected and listed in Table 1. It should be pointed out that in Table 1, the net charges of BSA at pH 7.4, $I = 0.15\ \mathrm{mol\ L^{-1}}$ and pH 4.7, $I = 0.1\ \mathrm{mol\ L^{-1}}$ are approximated as −20.4 and +4.5, respectively, according to the work of Vilker *et al.*[24] Since the net charge of BSA at pH 7.4 and $I =$ 1.5 mol $\mathrm{L^{-1}}$ is not available in the literature, its value is determined from the reduced light-scattering intensity data[4] at the same condition.

Even though we know from the work of Lin *et al.*[20] that the hard-core two-Yukawa model with MSA closure is able to predict the osmotic pressure of aqueous BSA solution with good accuracy at various values of pH and ionic strength, we need to check the performance of the hard-core two-Yukawa model for the derivative of osmotic pressure with respect to the volume fraction since the collective diffusion coefficient is strongly dependent on $\partial\Pi(\phi)/\partial\phi$. In Fig. 1, we compare

Table 1 Parameters used to evaluate collective mutual diffusion coefficient for aqueous BSA solution

Parameter	Value	Ref.
M_p	66 210 g $\mathrm{mol^{-1}}$	4
R_p	3.60 nm	5
v	0.734 $\mathrm{cm^3\ g^{-1}}$	16
λ	1.8	20
$\varepsilon/k_{\mathrm{B}}$	91.3 K	20
z_p	−20.4 at pH = 7.4	24
	+4.5 at pH = 4.7	24
	−15.1 at pH = 6.5, $I = 0.1\ \mathrm{mol\ L^{-1}}$	5
	−6.3 at pH = 6.5, $I = 0.001\ \mathrm{mol\ L^{-1}}$	5
	−45 at pH = 7.4, $I = 1.5\ \mathrm{mol\ L^{-1}}$	4

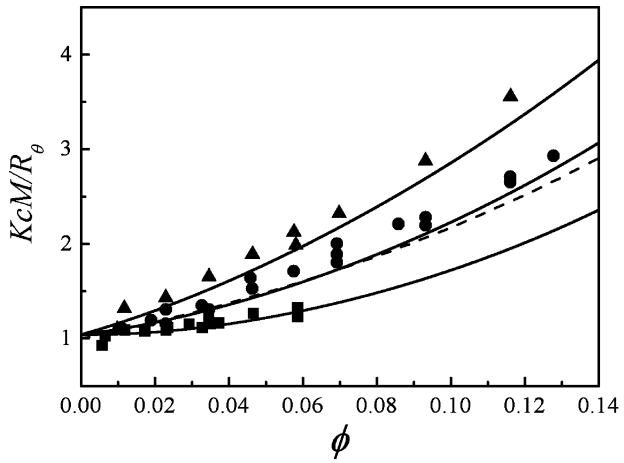

Fig. 1 Reduced light-scattering intensities for BSA solutions at $T = 295.15$ K in the form $KcM/\Delta R_\theta = (M/RT)\mathrm{d}\Pi/\mathrm{d}c$, where c is the protein concentration. The solid squares, solid triangles and solid circles represent experimental data[4] at pH 4.7 and $I = 0.1$ mol L^{-1}, pH 7.4 and $I = 0.15$ mol L^{-1}, and pH 7.4 and $I = 1.5$ mol L^{-1}, respectively. The solid lines are predicted results from the hard-core two-Yukawa model and the dash line is the normalized hard-sphere virial expansion:[4] $(M/RT)\mathrm{d}\Pi/\mathrm{d}c = 1 + 8\phi + 30\phi^3 + 72\phi^3$.

$\partial\Pi(\phi)/\partial\phi$ calculated from the hard-core two-Yukawa model with those from static light scattering experiments. The results from the normalized hard-sphere virial expansion[4] are also plotted in Fig. 1. As can be seen from Fig. 1, the values of $\partial\Pi(\phi)/\partial\phi$ are dependent on BSA concentration, pH and ionic strength. The two-Yukawa model with MSA closure predicted the behavior of $\partial\Pi(\phi)/\partial\phi$ accurately over a wide range of volume fraction, while the hard-sphere virial expansion is unable to predict the pH and ionic dependence of $\partial\Pi(\phi)/\partial\phi$ due to its neglect of intermolecular electrostatic interactions.

In Figs. 2–6 we predict the collective diffusion coefficients of aqueous BSA solutions using the hard-core two-Yukawa model coupled with the two sedimentation coefficient equations. In order to decide which one is more accurate in the prediction of the collective diffusion coefficients, the experimental data available in the literature are also included in Figs. 2–6. It should be pointed out that the experimental diffusion coefficients in Figs. 2,3,6 and 7 are determined using the dynamic light scattering method,[4] and those in Figs. 4 and 5 are measured using a simple capillary[5] method based on a principle

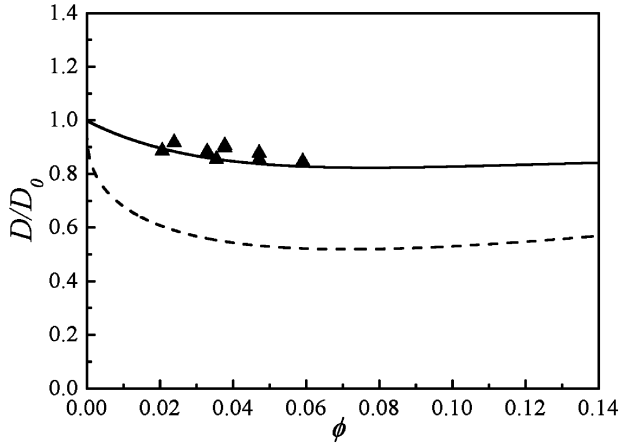

Fig. 2 Reduced collective diffusion coefficient of BSA in aqueous NaOAc solutions at pH 4.7, $I = 0.1$ mol L^{-1} and $T = 295.15$ K. The solid triangles represent the dynamic light scattering experimental data taken from Meechai *et al.*,[4] the solid curve is calculated from the hard-core two-Yukawa model coupled with the sedimentation coefficient eqn. (23) for the disordered suspension of hard spheres, and the dashed curve is calculated from the two-Yukawa model coupled with that for the ordered suspension of hard spheres (eqn. (24)).

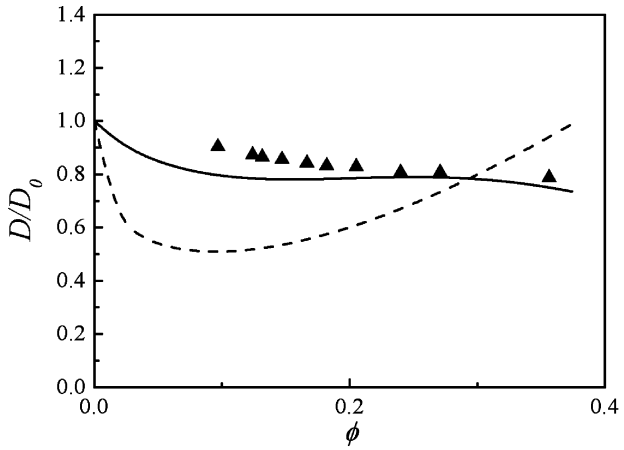

Fig. 3 Reduced collective diffusion coefficient of BSA in aqueous NaCl solutions with pH 4.7, $I = 0.1$ mol L^{-1}, and $T = 293.15$ K. The solid triangles represent the dynamic light scattering experimental data taken from Placidi and Cannistraro.[3] The curves have same meanings as in Fig. 2.

of hydrodynamic stability and the quasi-elastic light scattering spectroscopy,[25] respectively. From these figures one can see that the two-Yukawa model coupled with sedimentation coefficient eqn. (23) of the disordered suspension of hard spheres gives satisfactory predictions when compared with the corresponding experimental data at different values of pH, ionic strength and protein concentration. When coupled with sedimentation coefficient eqn. (24) of the ordered suspension of hard spheres, the theory substantially underestimates the collective diffusion coefficients of aqueous BSA at low to moderate protein concentrations and overestimates them at high volume fractions. The average relative deviations of correlations for collective diffusion coefficient are 7.96% and 33.5% when sedimentation coefficient correlations for the disordered (eqn. (23)) and ordered hard-sphere suspension (eqn. (24)) are used, respectively.

At lower value of pH (see Figs. 2 and 3), the collective diffusion coefficient decreases with protein concentration monotonously at ionic strength $I = 0.1$ mol L^{-1}. But at pH 7 and $I = 0.1$ or 0.15 mol L^{-1}, there is a maximum on the curve of reduced collective diffusion coefficient as a function of volume fraction, as shown in Figs. 4–6. The pH has a great effect on the curve shape of reduced collective diffusion coefficient as a function of volume fraction because pH regulates the

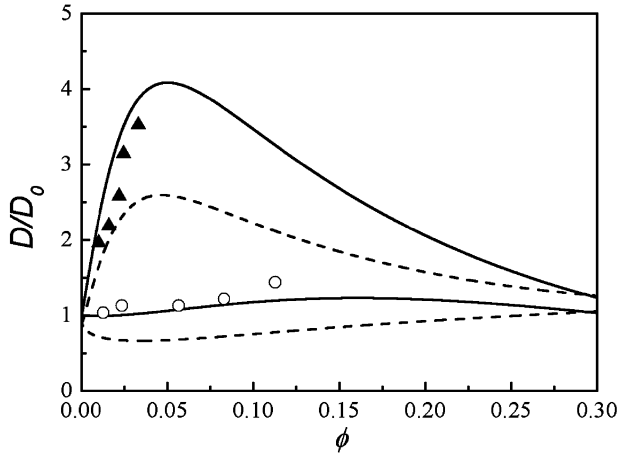

Fig. 4 Reduced collective diffusion coefficient of BSA in aqueous KCl solutions at pH 6.5 and $T = 277.15$ K. The solid triangles and opened circles represent the experimental data measured by Anderson *et al.*[5] using a simple capillary method based on a principle of hydrodynamic stability at $I = 0.001$ and 0.1 mol L^{-1}, respectively. The curves have same meanings as in Fig. 2.

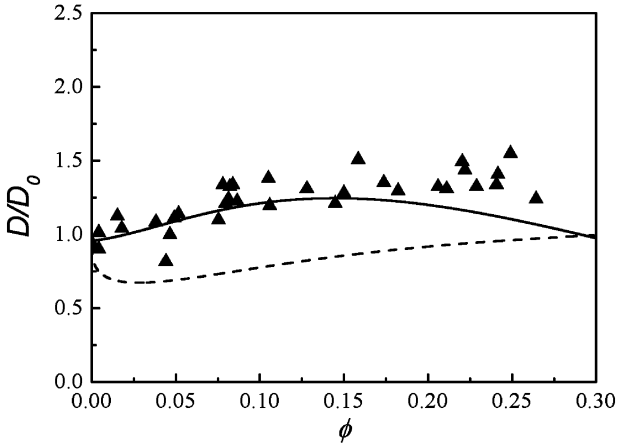

Fig. 5 Reduced collective diffusion coefficient of BSA in aqueous KCl solutions at pH 7, $I = 0.15$ mol L^{-1} and $T = 298.15$ K. The solid triangles represent the experimental data measured by Phillies *et al.*[25] using the quasi-elastic light scattering spectroscopy. The curves have same meanings as in Fig. 2.

BSA charge and thus the magnitude of the Coulomb interaction, which is further screened by the ions in solution. Fig. 4 indicates that the maximum on the curve becomes more pronounced at lower salt concentration. The difference between the volume fraction dependence of the collective diffusion coefficient at different ionic strengths reflects the influence of thermodynamic interaction on D_c. The hard-core two-Yukawa model coupled with the sedimentation coefficient eqn. (23) for the disordered suspension of hard spheres reproduces these differences quite well owe to its good description of the thermodynamic behavior of aqueous BSA solution. Compared to the Poisson–Boltzmann cell model,[16] the advantage of the present method is that it has an analytical expression for the collective diffusion coefficient and this makes calculation much easier. The only shortage of the present method is that we should know the net charge number on the protein at a specific solution condition in advance.

In Fig. 7, we compare the results from the hard-core two-Yukawa model with experimental data under three different solution conditions. Also plotted in Fig. 7 are the results from the hard sphere expression found by Al-Naafi and Selim,[26] which accurately describe their experimental data on the collective diffusion coefficients of hydrophobic silica macro-spheres:

$$\frac{D_c}{D_0} = K(\phi)\frac{[(1+2\phi)^2 + (\phi-4)\phi^3]}{(1-\phi)^4} \qquad (25)$$

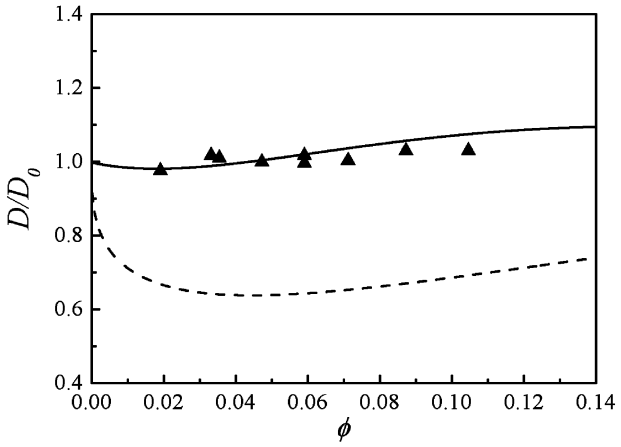

Fig. 6 Reduce collective diffusion coefficient of BSA in aqueous NaOAc solutions at pH 7.4, $I = 1.5$ mol L^{-1} and $T = 295.15$ K. The solid triangles represent the dynamic light scattering experimental data taken from Meechai *et al.*[4] The curves have same meanings as in Fig. 2.

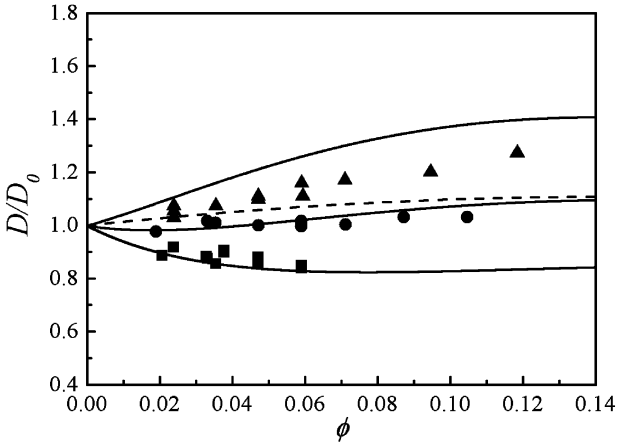

Fig. 7 Reduced diffusion coefficient of BSA in aqueous NaOAc solutions at $T = 295.15$ K. The solid triangles, solid circles and solid squares represent the dynamic light scattering experimental data[4] at pH 7.4 and $I = 0.15$ mol L^{-1}, pH 7.4 and $I = 1.5$ mol L^{-1}, and pH 4.7 and $I = 0.1$ mol L^{-1}, respectively. The solid curves are calculated from the hard-core two-Yukawa model coupled with the sedimentation coefficient equation for the disordered suspension of hard-spheres (eqn. (23)) and the dash curve is calculated from hard sphere model (eqn. (25)).

where $K(\phi)$ is calculated from eqn. (23). It is quite clear in Fig. 7 that the reduced collective diffusion coefficients of BSA in the three electrolyte solutions exhibit different trends as the volume fraction increases, and the agreement between theoretical predictions from hard-core two-Yukawa model coupled with eqn. (23) and experimental data is good. The experimental D_c/D_0 values deviate widely from the hard-sphere predictions from eqn. (25). The hard-sphere calculations can only semi-quantitatively describes the collective diffusion coefficient data at $I = 1.5$ mol L^{-1}, where the BSA predominantly screened by the small ions. The discrepancies at the two ionic strengths not high enough reflect that there surely exists a double-layer repulsive charge–charge interaction between BSA molecules. From Figs. 2–7, one can see that the present method, which completely neglects the influence of electrostatic interaction on sedimentation coefficient, works well over a wide range of charge and ionic strengths. This demonstrates that the two-Yukawa model is suitable to describe $\partial\Pi(\phi)/\partial\phi$ as a function of volume fraction and the electrostatic interactions has little effect on sedimentation coefficient of BSA in electrolyte solutions.

Conclusions

We have proposed a new method to predict collective diffusion coefficient of bovine serum albumin (BSA) in aqueous electrolyte solution based on the generalized Stokes–Einstein equation. The hard-core two-Yukawa model with mean spherical approximation is introduced to evaluate the influence of thermodynamic interactions on collective diffusion coefficient. Both sedimentation coefficient equations for the disordered and ordered suspensions of hard spheres are used to calculate the hydrodynamic effect. From the comparison with available experimental data we conclude that the hard-core two-Yukawa model coupled with the sedimentation coefficient equation for a disordered suspension of hard spheres (eqn. (23)) is a good theory to predict the collective diffusion coefficient of BSA in electrolyte solutions. In comparison, the hard sphere model fails due to its neglect of the double-layer repulsive charge–charge interaction between BSA molecules.

It is interesting to point out that using the proposed method we can determine how the diffusion coefficient varies with protein concentration from solution properties such as pH and ionic strength. This has interesting practical implications. For example, to increase an ultrafiltration membrane throughput, one may increase the diffusion coefficient of the protein

being filtered, and this can be achieved by a reduction in solution ionic strength according to the calculated results from the present method. It is expected that the present method can be used to predict collective diffusion coefficient of other globular proteins in electrolyte solutions equally well.

Appendix

List of symbols

c	Protein concentration/mol L^{-1}
C_p	Concentration of protein dispersion/g cm^{-3}
D	Dielectric constant of solvent
D_c	Collective diffusion coefficient/m^2 s^{-1}
D_0	Diffusion coefficient at infinite dilution/m^2 s^{-1}
e	Charge of an electron = $1.602\ 19 \times 10^{-19}$ C
I	Ionic strength, mol L^{-1}
$K(\phi)$	Sedimentation coefficient
k_B	Boltzmann constant = $1.380\ 62 \times 10^{-23}$ J K^{-1}
M	Molecular mass/g mol^{-1}
R_p	Hydrodynamic radius of globular protein/nm
r	Distance between two interacting particles/nm
T	Temperature/K
$u(r)$	Interaction potential/J
v	Specific volume/cm^3 g^{-1}
Z	Osmotic compressibility factor
z	Charge number
Greek letters	
ε	Energy parameter/J
ϕ	Volume fraction of protein = $\pi\rho_p\sigma^3/6$
κ	Debye screening parameter/nm^{-1}
λ	Range parameter
Π	Osmotic pressure/Pa
ρ	Number density/nm^{-3}
σ	Hard-sphere diameter of a protein = $2R_p$
σ_{dry}	Dry protein particle diameter/nm
Subscripts	
c	Collective
i	Microion i
p	Protein
+	Micro cation
—	Micro anion
Superscripts	
cc	Charge–charge interaction
dis	Dispersion interaction
Donnan	Donnan effect
in	Protein side of a membrane
out	Microion side of a membrane

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