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Effect of Lysozyme Proteins on the Mutual-Diffusion Coefficient of Sodium Chloride in Water

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ABSTRACT: We measured accurate NaCl main-term diffusion coefficients in aqueous lysozyme solutions at 25 °C and pH 4.5 using the Gosting diffusiometer operated in its Rayleigh interferometric optical mode. The dependence of this diffusion coefficient on lysozyme concentration was examined using the obstruction-effect theory. Agreement between experimental results and theory is achieved if lysozyme proteins are treated as hydrated spheres with a hydration number of 240, a value that is comparable with those reported in literature for this protein. Electrostatic interactions and common-ion effects due to lysozyme net charge at pH 4.5 do not contribute significantly to the behavior of the NaCl diffusion coefficient within our experimental range of salt concentrations [(0.25 to 0.90) mol·dm^{-3}].

INTRODUCTION

In a binary liquid mixture, mutual diffusion coefficients characterize the motion of solute and solvent molecules relative to each other in the presence of their concentration gradients. For a binary solution, the diffusion coefficient, D, can be defined by Fick's first law:

$$-J = D\nabla C \quad (1)$$

where J is the molar flux of the solute and C its molar concentration.[1,2] When macromolecules or colloidal particles are added to the system, the mutual diffusion coefficient of the initial binary fluid is expected to decrease due to obstruction effects.[3−6] According to theory, the mutual diffusion coefficient, D', of the interstitial binary fluid at low concentrations of spherical macroparticles is given by the power series expansion:[3]

$$\frac{D'}{D} = 1 - 1.5\phi + \ldots \quad (2)$$

where ϕ is the volume fraction of the macroparticles. In eq 2, higher order terms in ϕ may need to be included if macroparticle−macroparticle net interactions become important. While there are several theoretical reports on the effect of obstruction on mutual diffusion coefficients,[3−6] accurate experimental investigations on this phenomenon are virtually absent to our knowledge. These studies are important for a wide range of phenomena, including the effect of proteins and other biomacromolecules on the diffusion of small molecules and ions inside living systems.[7]

Mutual diffusion coefficients are typically measured by dynamic light scattering[8−10] and macroscopic-gradient techniques such as the diaphragm cell,[1,2] Taylor dispersion,[11,12] and interferometric methods (Gouy and Rayleigh).[13−16] However, dynamic light scattering cannot be applied to low-molecular weight components (e.g., inorganic salts) because of their poor light scattering power and the fast relaxation of their concentration fluctuations in solution. Thus, the determination of D' is limited to macroscopic-gradient techniques.

Another complication for the experimental determination of D' is related to the multicomponent nature of these systems. Indeed, for a ternary mixture, Fick's first law relates the fluxes of the two solute components not only to their own concentration gradients (main diffusion) but also to the concentration gradient of the other solute component (cross diffusion). Thus, four diffusion coefficients (two main terms and two cross terms) are required to accurately characterize diffusion processes in a ternary mixture. Within this framework, D' can be identified as the main-term diffusion coefficient related to the low molecular-weight solute.[14]

In general, the error associated with measured ternary diffusion coefficients is larger than that associated with binary data.[13] This implies that a successful characterization of D'/D as a function of ϕ requires high-precision measurements, especially at low ϕ. Here, we report an experimental characterization of the main-term diffusion coefficient, D', of sodium chloride in water as a function of lysozyme concentration at four constant salt concentrations, 25 °C, and pH 4.5. Our diffusion data were determined using the high-precision Gosting diffusiometer operated in its Rayleigh interferometric mode.[14,15] The accuracy of the measured diffusion coefficients is known to be ∼0.1 % for binary systems and superior compared to other macroscopic-gradient techniques such as Taylor dispersion and diaphragm cell.[1,2,13] This study complements our previous report on the main-term diffusion coefficient of lysozyme obtained in the same experimental conditions and future reports on the corresponding cross-term diffusion coefficients.[15]

Lysozyme is a stable protein, which is commercially available at high purity. Lysozyme is regarded as a model protein, and its aqueous mixtures at pH 4.5 have been extensively investigated for crystal-growth studies.[17] At this pH value, lysozyme is positively charged.[18,19]

EXPERIMENTAL SECTION

Materials and Solution Preparation. Hen egg-white lysozyme (14,307 g·mol^{-1}), recrystallized six times and lyophilized,

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Table 1. Values of NaCl Main-Term Diffusion Coefficients[a], D'

C_{NaCl}	C_{LYS}	D'		
mol·dm^{-3}	10^{-3} mol·dm^{-3}	10^{-9} m^2·s^{-1}	ϕ	D'/D
0.250	0.300	1.466 ± 0.001	0.00306	0.9941
0.250	0.450	1.461 ± 0.001	0.00459	0.9907
0.250	0.600	1.459 ± 0.001	0.00612	0.9894
0.250	0.700	1.455 ± 0.002	0.00714	0.9867
0.250	1.000	1.446 ± 0.001	0.01020	0.9806
0.250	1.500	1.429 ± 0.001	0.01530	0.9691
0.250	2.500	1.393 ± 0.001	0.02550	0.9447
0.500	0.300	1.463 ± 0.001	0.00306	0.9936
0.500	0.450	1.457 ± 0.001	0.00459	0.9895
0.500	0.600	1.455 ± 0.001	0.00612	0.9882
0.500	1.000	1.441 ± 0.001	0.01020	0.9786
0.500	1.500	1.425 ± 0.001	0.01530	0.9678
0.500	2.500	1.391 ± 0.002	0.02550	0.9446
0.650	0.300	1.463 ± 0.001	0.00306	0.9922
0.650	0.450	1.459 ± 0.001	0.00459	0.9895
0.650	0.600	1.455 ± 0.001	0.00612	0.9868
0.650	0.800	1.447 ± 0.001	0.00816	0.9813
0.650	1.000	1.443 ± 0.002	0.01020	0.9786
0.650	1.200	1.435 ± 0.001	0.01224	0.9732
0.650	1.500	1.424 ± 0.002	0.01530	0.9657
0.900	0.300	1.472 ± 0.001	0.00306	0.9947
0.900	0.450	1.462 ± 0.001	0.00459	0.9879
0.900	0.600	1.461 ± 0.001	0.00612	0.9872
0.900	1.000	1.446 ± 0.001	0.01020	0.9770

[a] C_{NaCl} = molar concentrations of NaCl. C_{LYS} = molar concentrations of lysozyme. D' = main-term diffusion coefficients for NaCl in the ternary lysozyme chloride + NaCl + H_2O system. ϕ = volume fractions of lysozyme. D = diffusion coefficients for NaCl in the binary NaCl + H_2O system at the interstitial concentration.

was purchased from Seikagaku America and used without further purification. Deionized water was distilled and then passed through a four-stage Millipore filter system to provide high-purity water for all of the experiments. Mallinckrodt AR NaCl was dried by heating at 450 °C for about seven hours and used without further purification. The purity of the NaCl was listed as 99.9 % by the supplier. Its molecular mass was taken to be 58.443 g·mol^{-1}. Mallinckrodt reagent HCl (~12 M) was diluted to about 0.063 mol·dm^{-3} (pH ~1.2) and used to adjust the pH of solutions to 4.50. Measurements of pH were made using a Corning model 130 pH meter with an Orion model 8102 combination ROSS pH electrode. All solutions were prepared by weight using a Mettler-Toledo AT400 analytical balance. Molar concentrations were obtained from the density of solutions. All density measurements were made with a Mettler-Paar DMA40 density meter. More experimental details on solution preparation and density measurements are reported in ref 14.

Rayleigh Interferometry. All macroscopic-gradient diffusion measurements were made with the high-precision Gosting diffusiometer operated in its Rayleigh interferometric optical mode. By performing experiments with different initial conditions, the four diffusion coefficients describing a ternary system are obtained. Details on Rayleigh interferometry and the Gosting diffusiometer can be found in refs 13 and 14.

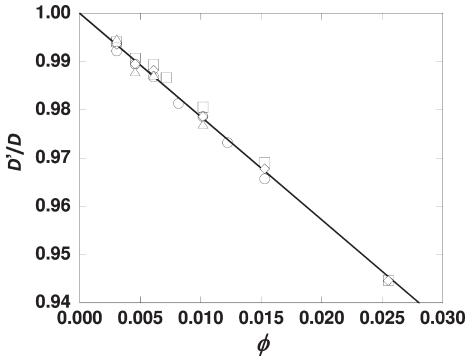

Figure 1. Ratios, D'/D, of the NaCl ternary diffusion coefficient to the corresponding binary values as a function of lysozyme volume fraction, ϕ, and at constant NaCl concentrations: C_{NaCl} = □, 0.25 mol·dm^{-3} M; ◇, 0.50 mol·dm^{-3} M; ○, 0.65 mol·dm^{-3} M and △, 0.90 mol·dm^{-3} M. The solid line is a linear fit through all experimental data using eq 3.

RESULTS AND DISCUSSION

Our diffusion measurements were used to determine D', the main-term diffusion coefficient describing the flux of NaCl due to its own concentration gradient. In Table 1, we report our results on D' as a function of protein concentration, C_{LYS}, and for four NaCl concentrations: C_{NaCl} = (0.25, 0.50, 0.65, and 0.90) mol·dm^{-3}. The corresponding lysozyme volume fractions, ϕ, were calculated using $\phi = C_{LYS}\overline{V}_{LYS}$, where $\overline{V}_{LYS}$ = 10.2 dm^3·mol^{-1} is the known[14] partial molar volume of lysozyme. Here, we have assumed that $\overline{V}_{LYS}$ coincides with the intrinsic molar volume of lysozyme. The calculated values of protein volume fraction were lower than 3 % in all cases. The effect of protein concentration on D' can be describe by considering the ratio, D'/D, where D is the NaCl diffusion coefficient of the binary NaCl−H_2O interstitial fluid. For any given composition (C_{NaCl}, C_{LYS}) in Table 1, the corresponding value of D was calculated after fitting available binary diffusion data[20] using the following equation:

$$D = D^0[1 + a_1(C'_{NaCl}/C^0)^{0.33} + a_2(C'_{NaCl}/C^0) + a_3(C'_{NaCl}/C^0)^{1.5} + a_4(C'_{NaCl}/C^0)^2 + a_5(C'_{NaCl}/C^0)^{2.8}]$$

where $C^0 \equiv 1$ mol·dm^{-3}, $D^0 = 1.610\cdot10^{-9}$ m^2·s^{-1}, a_1 = 0.28813070, a_2 = 0.24955352, a_3 = 0.09683006, a_4 = −0.20658596, a_5 = −0.00810490, and $C'_{NaCl} = C_{NaCl}/(1-\phi)$ is the salt concentration of the interstitial fluid. It is important to note that the value of D at C'_{NaCl} does not significantly differ from that calculated at C_{NaCl} due to both the low experimental values of ϕ and the weak dependence of D on salt concentration observed within our experimental concentration range.

The behavior of D'/D as a function of protein volume fraction at all four NaCl concentrations is illustrated in Figure 1. As we can see in this figure, D'/D linearly decreases as ϕ increases. This indicates that the effect of protein−protein interactions on D' can be neglected. Furthermore, the observed decrease of D'/D appears to be essentially independent of salt concentration. Thus we have fitted all our experimental data in Table 1 using the equation:

$$\frac{D'}{D} = 1 - K\phi \qquad (3)$$

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Table 2. Values for the Fitting Parameter, K

C_{NaCl} / mol·dm^{-3}	K
0.25	2.078 ± 0.049
0.50	2.142 ± 0.024
0.65	2.211 ± 0.030
0.90	2.235 ± 0.113

We have obtained $K = 2.139 \pm 0.024$, where the error represents the standard deviation obtained from the least-squares procedure. This value is found to be 43 % larger than the value of 1.5 shown in eq 2.

A possible explanation for the observed discrepancy is that the protein hydration shell contributes to the obstruction. Thus the actual protein volume fraction should be calculated using not $\overline{V}_{LYS}$ but the molar volume of the hydrated lysozyme: $\overline{V}_{LYS} + \nu_{H_2O} V_{H_2O}$, where ν_{H_2O} is the protein hydration number and we have assumed that the molar volume of water in the protein hydration shell is the same as that in bulk water $V_{H_2O} = 0.01807$ dm^3·mol^{-1}. Hence, by combining eq 2 with eq 3, we obtain:

$$K = 1.5 \frac{\overline{V}_{LYS} + \nu_{H_2O} V_{H_2O}}{\overline{V}_{LYS}} \quad (4)$$

Equation 4 allows us to extract the hydration number of $\nu_{H_2O} = 240 \pm 10$ for the lysozyme. Since the solvent-accessible area for this protein is 67.1 nm^2,[21] this value of ν_{H_2O} corresponds to an average thickness of the hydration layer of 0.11 nm.

Our estimated hydration number is somewhat larger than the values of 193 and 162 obtained from NMR titrations[22] and volumetric data,[23] respectively. However, it is in good agreement with the value of 255 and 260 obtained from high-resolution X-ray diffraction of lysozyme crystals[24] and infrared spectroscopy,[25] respectively. On the other hand, it is lower than the values of 292 and 310 obtained from Monte Carlo simulations[26] and calorimetric measurements on lysozyme powders,[27] respectively. Thus, our value of ν_{H_2O} falls within the range of hydration numbers reported in literature.

The lysozyme hydration number can be also estimated from our previously reported lysozyme main-term diffusion coefficients.[15] These data were used to calculate the equivalent hydrodynamic radius of 1.86 nm by applying the Stokes−Einstein equation.[15] Assuming a spherical shape, we found that the corresponding hydration volume is 16.2 dm^3·mol^{-1}, thereby yielding a hydration number of 332. A more accurate estimate can be made if the lysozyme is regarded as a prolate ellispsoid with an axial ratio of 0.667. This value was estimated from crystallographic data (Brookhaven protein database structure 2LYZ).[28] By applying the Stokes−Einstein equation for prolate ellipsoids,[2] we calculate the hydration volume of 15.5 dm^3·mol^{-1}. This yields the lower hydration number of 293. Both estimates are higher than the value obtained from eq 3.

It is important to remark that the lysozyme is positively charged at pH 4.5.[18,19] This implies that electrostatic interactions occur between the lysozyme, Cl^-, and Na^+. Furthermore, Cl^- should be regarded as a common ion between the two solute components. Clearly, these aspects are not taken into account by eq 2. However, it is important to observe that their contribution to the behavior of D'/D is expected to become less important as the NaCl concentration increases. Hence, we examined K as a function of $1/C_{NaCl}$ by applying eq 3 to individual salt concentrations. We report the values of K extracted from the least-squares procedure in Table 2. The corresponding behavior of K as a function of $1/C_{NaCl}$ is illustrated in Figure 2. As we can see in this figure, K slightly decreases as $1/C_{NaCl}$ increases. By applying a weighed linear fit through the experimental K values, we obtain 2.265 ± 0.050 at $1/C_{NaCl} = 0$. This value is 5.9 % larger than that reported above based on all experimental data. Since changes in K are found to be small, it is difficult to unambiguously assign them to the electrolyte nature of our system. Regardless, we can conclude that ionic effects are not significant for our relatively high experimental C_{NaCl} values.

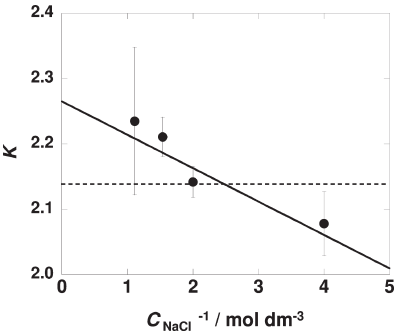

Figure 2. Fitting parameter, K, as a function of $1/C_{NaCl}$. The solid line is a weighed linear fit through the experimental K values (intercept, 2.265 ± 0.050; slope, -0.051 ± 0.023 mol·dm^{-3}). The horizontal dashed line represents the value of $K = 2.139$ obtained using all D'/D data in Table 1.

CONCLUSIONS

We have examined the effect of lysozyme concentration on the main-term diffusion coefficient, D', of sodium chloride in water. Agreement between experimental results and obstruction-effect theory (eq 2) is achieved if proteins are treated as hydrated spheres with a hydration number of 240. Electrostatic interactions and the common-ion effect do not contribute significantly to the behavior of D'/D.

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