Measurements of Multicomponent Diffusion Coefficients for Lysozyme Chloride in Water and Aqueous Na$_2$SO$_4$

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Abstract

This paper presents a diffusion experimental study for ternary lysozyme-Na$_2$SO$_4$-water system, from moderate precipitant concentrations into the supersaturated region and provides a complete set of four diffusion coefficients. These data are important in order to provide accurate models of protein diffusion with applications in growth of protein crystals for X-ray diffraction studies. All three-component mutual-diffusion experiments reported here were performed by Rayleigh interferometry at pH= 4.5, T= 25° C and at a mean lysozyme concentration (average of top and bottom solution concentrations) of 0.6 mM (8.6 mg/mL). Four experiments, with different combinations of protein and Na$_2$SO$_4$ concentration differences, were performed at each of five mean Na$_2$SO$_4$ concentrations (0.1, 0.25, 0.5, 0.65 and 0.8 M), for a total of 20 experiments. In addition, we have measured dynamic light-scattering diffusion coefficients of the ternary system lysozyme chloride-Na$_2$SO$_4$-water.

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Motivation

The diffusion of protein is one of the fundamental processes occurring in biological systems, and it is also an important step in the crystallization mechanism. Obtaining protein crystal of good structural quality is often the main issue for three dimensional atomic resolution structure studies of biomacromolecules. Crystallization is an intrinsically non-equilibrium process, and concentration gradients will occur around the crystal. The protein crystallizes, reducing its concentration at the moving face of the growing crystal and creating a protein gradient between the bulk solution and the crystal. This gradient in turn causes multicomponent diffusive transport of protein and precipitant. Diffusion in protein crystal growth inevitably occurs under conditions for which no species has an uniform concentration raising the issue of multicomponent diffusion.

Crystallization experiments conducted under microgravity conditions have yielded protein crystals that provided diffraction data of significantly higher resolution than the best crystal of these proteins grown under normal conditions [1]. The difference between microgravity and normal gravity is the magnitude of the buoyancy forces. Commonly, this difference is assigned to some consequences of the reduction of buoyancy driven convection in the microgravity conditions. With convection, the lysozyme concentration in the bulk solution is more uniform [2] and the probability for nucleation of parasitic crystals is strongly reduced.

The complete description of an \( n \)-solute system requires an \( n \times n \) matrix of diffusion coefficients relating the flux of each solute component to the gradients of all solute components [3]. The importance of other species on protein diffusion follows from the one-dimensional flux relations [3]:

\[
- J = \sum_{j=1}^{n} (D_{ij}) v \frac{\partial C_j}{\partial x} \quad i = 1, \ldots, n
\]  

in which the cross-term diffusion coefficients (off-diagonal elements \( D_{ij}, i \neq j \)) can be positive or negative. In ternary systems \( n = 2 \), our case, the one-dimensional flux relations could be written as:

\[
- J_1 = (D_{11}) v \frac{\partial C_1}{\partial x} + (D_{12}) v \frac{\partial C_2}{\partial x} 
\]

\[
- J_2 = (D_{21}) v \frac{\partial C_1}{\partial x} + (D_{22}) v \frac{\partial C_2}{\partial x}
\]
where $J_1$ and $J_2$ - the protein flux and respectively salt flux, $(D_{11})_v$ and $(D_{22})_v$ - the main-term diffusion coefficients relating to the flux of component to its own concentration gradient, and $(D_{12})_v$ and $(D_{21})_v$ - the cross-term diffusion coefficients relating the flux of each component to the gradient of the other. For some systems [4, 5], a cross-terms $(D_{ij})_v$ can have considerably larger magnitude than the main-terms $(D_{ii})_v$, as our measurements show for the Lys-Na$_2$SO$_4$-Water system described here and in agreement with the results about Lys-NaCl-Water system [6]. The index $v$ from the diffusion coefficients shows that the experiment were done under the assumption the volume change on mixing and changes in concentrations across the diffusion boundary were small. Consequently, with a good approximation, the measured diffusion coefficients may be considered to be for the volume-fixed reference frame [7] defined by:

$$\sum_{i=0}^n J_i \bar{V}_i = 0 \quad (4)$$

where $\bar{V}_i$ is the partial molar volume of the $i$th species, and the subscript 0 denotes the solvent.

The importance of multicomponent diffusion has been recognized in the crystal growth community [8, 9] and a crystal growth model has properly accounted for multicomponent diffusive transport in lysozyme chloride-NaCl-water system [6, 10]. The experimental multicomponent diffusion coefficients are essential for accurate modeling of protein transport, especially in view of the very large cross-term coefficient $(D_{21})_v$ reported here. Moreover, the concentration of supporting electrolyte dependence of all the diffusion coefficients should be important for supersaturation region and also for its directly contribution to the protein flux.

Experimental section

All the experimental work was performed at Texas Christian University, in the Chemistry Department.

Materials. All the materials, solution preparation procedures, apparatus and density measurement procedures are described in the work [6]. We used a hen egg-white lysozyme, recrystallized six times purchased from Seikagaku
America.
The molecular mass of the lysozyme solute, $M_1$, was taken as 14307 g/mol, and this value \[1\] was used to calculate all concentrations after correction for the moisture and chloride content. Buoyancy corrections were made with the commonly used lysozyme crystal density \[12, 13, 14\] of 1.305 g/cm$^3$.

The molecular mass of water, $M_o$, was taken as 18.015 g/cm$^3$ and the molecular mass of Na$_2$SO$_4$, $M_2$, was taken as 142.037 g/mol.

Mallinckrodt reagent HCl ($\sim$ 12 M) was diluted by half with pure water and distilled at the constant boiling composition. This resulting HCl solution ($\sim$ 6 M) was then diluted (pH 1.2) and used to adjust the pH of solution.

**Preparation of Solutions.** All solutions were prepared by mass with appropriate buoyancy corrections. All weighings were performed with a Mettler Toledo AT400 electrobalance. Since the as-received lysozyme powder was very hygroscopic, all manipulations in which water absorption might be critical were performed in a dry glove box. Stock solutions of lysozyme were made by adding as-received protein to a pre-weighted bottle that had contained dry box air, capping the bottle, and reweighing to get the weight and thus mass of lysozyme. Water was added to dissolve the lysozyme, and the solution was weighed. An accurate density measurement was made and used to obtain the molarity of the stock solution.

For ternary experiments, precise masses of Na$_2$SO$_4$ were added to flasks containing previously weighed quantities of lysozyme stock solutions. These solutions were mixed and diluted to within 10 cm$^3$ of the final volume. The pH was adjusted, and the solutions were diluted to their final mass.

**Measurements of pH.** The pH measurements were made using a Corning model 130 pH meter with an Orion model 8102 combination ROSS pH electrode. The meter was calibrated with standard pH 7 and pH 4 buffers and checked against a pH 5 standard buffer.

**Density Measurements.** All density measurements were made with a Mettler-Paar DMA40 density meter, with an RS-232 output to an Apple II+. By time averaging the output, a precision of 0.00001 g/cm$^3$ or better could be achieved. The temperature of the vibrating tube in the density meter was controlled with water from a large well-regulated water bath whose temperature was $25.00 \pm 0.01 ^\circ C$. 

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Free-Diffusion Measurements. For binary Na$_2$SO$_4$-water and ternary Lys-Na$_2$SO$_4$-Water we performed measurements for free-diffusion using the high-precision Gosting diffusiometer [15, 16, 17] operated in its Rayleigh interferometric optical mode. The procedure for measuring binary ($D_2$)$_v$ and ternary diffusion coefficients ($D_{ij}$)$_v$ were described in detail in the work [6]. In order to measure the four diffusion coefficients of the system, the experiments must be performed with at least two different concentration differences at each combination of mean concentration [15, 18, 19]. For ternary experiments, for each pair of mean concentrations, two measurements were performed with $\alpha_1 = 0$ and the two with $\alpha_1 = 0.8$ ($\alpha_i$ - the refractive index fraction due to the $i$th solute [6]).

In order to make the data analysis of the free-diffusion experiments we used the Fick’s second law:

$$\frac{\partial C_i}{\partial t} = \sum_{j=1}^{2} D_{ij} \frac{\partial^2 C_j}{\partial x^2} \quad i = 1, 2$$

(5)

for two solutes. We made the assumption that the concentration differences of the solutes across the initial boundary are small enough and the diffusion coefficients are constant [20]. Also the volume changes on mixing were negligible, thus all the measured diffusion coefficients are given relative to the volume-fixed frame of reference defined by equation (4).

Dynamic Light-Scattering Diffusion Coefficients. We measured the dynamic light-scattering diffusion coefficients $D_{DLS}$ for samples from all ternary experiments and for 0.1, 0.25, 0.5, 0.65 and 0.8 M mean concentrations of Na$_2$SO$_4$.

Measurements were made using a Protein Solution DynaPro-801 TC molecular sizing instrument with a fixed scattering angle of 90° and the procedure was described in the work [6]. This apparatus allowed us to calculate also the eigenvalues $\lambda_1$ and $\lambda_2$ of the matrix of diffusion coefficients, the $D_{DLS}$ predicted by Leaist’s theory [21], and to compare with ours and also with interferometric value ($D_{11}$)$_v$.

The viscosity for ternary lys-Na$_2$SO$_4$-water system was also measured using an Ostwald viscosimeter.
Results

Ternary diffusion experiments were performed on the lysozyme chloride-Na$_2$SO$_4$-water system at pH= 4.5 and T=25$^0$ C. To obtain the four ternary diffusion coefficients we performed four experiments at the same mean concentrations but with different values of $\Delta C_i$ for the solutes. There were two experiments with $\Delta C_1 = 0$ and $\Delta C_2 \neq 0$ and two with $\Delta C_1 \neq 0$ and $\Delta C_2 = 0$ at each mean Na$_2$SO$_4$ concentration of 0.1, 0.25, 0.5, 0.65 and 0.8 M. The interferometric data for the diffusion coefficients $(D_{11})_v$, $(D_{22})_v$, $(D_{12})_v$ and $(D_{21})_v$ are reported in the Tables 1,2,3.

Partial molar volumes values, $\bar{V}_1$, $\bar{V}_2$ and $\bar{V}_o$, were calculated for each component using eqs A-7 ($q = 2$) and 5 in [22] and reported in the Tables 1,2,3.

Values of mean density $\bar{d}$ and $H_i = (\partial d/\partial C_i)_{T,p,C_j,j\neq i}$ in the Table 1 were calculated using densities of all eight solutions from each experiment set. Densities were assumed to be linear in solute concentrations respecting the equation $[6]$

$$d = \bar{d} + H_1(C_1 - \bar{C}_1) + H_2(C_2 - \bar{C}_2)$$

where $\bar{C}_1$ and $\bar{C}_2$ are the averages of the mean concentrations for all four experiments in a series.

Dynamic light-scattering experiments provided the diffusion coefficients $D_{DLS}$ for each Na$_2$SO$_4$ mean concentrations and also the eigenvalues $\lambda_i$ ($i = 1, 2$). We could make an direct comparison to the interferometric values $(D_{11})_v$, the smallest eigenvalues of the matrix of diffusion coefficients $\lambda_1$ which are reported in the Table 1,2,3 and the $D_{DLS}$ data. The values for $D_{DLS}$ are approximately 4% higher than $(D_{11})_v$, and approximately 5% higher than the smallest eigenvalue $\lambda_1$ of the matrix of diffusion coefficients.

The viscosity values for ternary solution are reported also in the Tables 1,2,3 at each mean value of salt concentrations.

The diffusion coefficients dependence on $C_2$ mean values are shown in the Fig.1.

From Fig. 1 we could see also the coefficient $(D_{11})_v$, due to the gradient of lysozyme, corrected from viscosity. The values for main-term $(D_{11})_v$ are 13% smaller than the values for the same coefficient but using the lys-NaCl-water system, at the same concentration [6]. The cross-term $(D_{21})_v$ for the flux of Na$_2$SO$_4$ caused by the gradient of lysozyme chloride increases as the Na$_2$SO$_4$
concentration increases. The values for \((D_{21})_v\) are 25% smaller than the values for the same coefficient using the NaCl as salt [6]. At 0.8 M \(\text{Na}_2\text{SO}_4\), this term becomes 14 times larger than the \(\text{Na}_2\text{SO}_4\) main-term diffusion coefficient \((D_{22})_v\). The cross-term \((D_{12})_v\) for the flux of lysozyme caused by the gradient of \(\text{Na}_2\text{SO}_4\) is small in comparison with all the other diffusion coefficients as it was expected, and 35% smaller than \((D_{12})_v\) in the case of lys-NaCl-water [6]. Fig. 1 shows us also that the ternary main-term \((D_{22})_v\), for the flux of \(\text{Na}_2\text{SO}_4\) caused by the own gradient of concentration, is smaller with 1.5% than the binary coefficient \((D_2)_v\), over the entire composition range, and 40% smaller than the \((D_{22})_v\) for ternary lys-NaCl-water [6], for the same range of concentration.

**Conclusions** We reported the complete set of multicomponent diffusion coefficients for ternary lys-\(\text{Na}_2\text{SO}_4\)-water system at concentrations high enough to be relevant to crystallization studies. We also made a comparison with the reported diffusion data about lys-NaCl-water [6] in order to understand the influences of the type of salt in the diffusion process and, implicitly in the crystallization process.

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Table 1

|                  | C1 (mM)  | C2 (M)   |
|------------------|----------|----------|
| d (g cm⁻³)       | 1.012189 | 1.030671 |
| H1 (10² g mol⁻¹) | 4.286    | 1.149    |
| H2 (10² g mol⁻¹) | 0.12500  | 0.12230  |
| V1 (cm³ mol⁻¹)   | 10050    | 10182    |
| V2 (cm³ mol⁻¹)   | 17.090   | 19.780   |
| V₀ (cm³ mol⁻¹)   | 18.067   | 18.058   |
| D_DLS (10⁻⁹ m² s⁻¹) | 0.11970 | 0.11230  |
| λ1 (10⁻⁹ m² s⁻¹) | 0.11700  | 0.10840  |
| (D₁₁)ᵥ (10⁻⁹ m² s⁻¹) | 0.1169± 0.0001 | 0.1090± 0.0001 |
| (D₁₂)ᵥ (10⁻⁹ m² s⁻¹) | 0.000013± 0.000001 | 0.000108± 0.000001 |
| (D₂₁)ᵥ (10⁻⁹ m² s⁻¹) | 2.49± 0.01 | 4.14± 0.01 |
| (D₂₂)ᵥ (10⁻⁹ m² s⁻¹) | 0.9661± 0.0001 | 0.8826± 0.0001 |
| η (cp)           | 1.042395 | 1.110034 |
### Table 2

|                  | Table 2                  |                  |
|------------------|--------------------------|------------------|
| $C_1$(mM)        | 0.6000                   | 0.6000           |
| $C_2$(M)         | 0.5000                   | 0.6500           |
| $\bar{d}$(gcm$^{-3}$) | 1.060538                | 1.078032         |
| $H_1$($10^3$gmol$^{-1}$) | 4.104                  | 4.049            |
| $H_2$($10^3$gmol$^{-1}$) | 0.11733                 | 0.11610          |
| $\bar{V}_1$(cm$^3$mol$^{-1}$) | 10209                   | 10257            |
| $\bar{V}_2$(cm$^3$mol$^{-1}$) | 24.720                  | 25.930           |
| $\bar{V}_o$(cm$^3$mol$^{-1}$) | 18.026                  | 18.013           |
| $D_{DLS}$($10^{-9}$m$^2$s$^{-1}$) | 0.10102                 | 0.09430          |
| $\lambda_1$($10^{-9}$m$^2$s$^{-1}$) | 0.09566                 | 0.08775          |
| $(D_{11})_v$($10^{-9}$m$^2$s$^{-1}$) | 0.0969± 0.0001          | 0.0894± 0.0001   |
| $(D_{12})_v$($10^{-9}$m$^2$s$^{-1}$) | 0.000132± 0.000001      | 0.000134± 0.000001 |
| $(D_{21})_v$($10^{-9}$m$^2$s$^{-1}$) | 6.75± 0.01              | 8.26 ± 0.01      |
| $(D_{22})_v$($10^{-9}$m$^2$s$^{-1}$) | 0.7791 ± 0.0001         | 0.7294 ± 0.0001  |
| $\eta$(cp)      | 1.236427                 | 1.322355         |

### Table 3

|                  | Table 3                  |
|------------------|--------------------------|
| $C_1$(mM)        | 0.6000                   |
| $C_2$(M)         | 0.8000                   |
| $\bar{d}$(gcm$^{-3}$) | 1.095745                |
| $H_1$($10^3$gmol$^{-1}$) | 3.954                  |
| $H_2$($10^3$gmol$^{-1}$) | 0.11502                 |
| $\bar{V}_1$(cm$^3$mol$^{-1}$) | 10341                  |
| $\bar{V}_2$(cm$^3$mol$^{-1}$) | 26.980                 |
| $\bar{V}_o$(cm$^3$mol$^{-1}$) | 17.993                 |
| $D_{DLS}$($10^{-9}$m$^2$s$^{-1}$) | 0.08683                 |
| $\lambda_1$($10^{-9}$m$^2$s$^{-1}$) | 0.08021                 |
| $(D_{11})_v$($10^{-9}$m$^2$s$^{-1}$) | 0.0822± 0.0001          |
| $(D_{12})_v$($10^{-9}$m$^2$s$^{-1}$) | 0.000130± 0.000001      |
| $(D_{21})_v$($10^{-9}$m$^2$s$^{-1}$) | 9.50± 0.01              |
| $(D_{22})_v$($10^{-9}$m$^2$s$^{-1}$) | 0.6900± 0.0001          |
| $\eta$(cp)      | 1.418206                 |