Extraction of Thermodynamic Data from Ternary Diffusion Coefficients of Lysozyme Chloride in Water and Aqueous Na$_2$SO$_4$

Daniela Buzatu, Emil Petrescu, Florin D. Buzatu, John G. Albright

Abstract

This paper presents, for ternary lysozyme-Na$_2$SO$_4$-water system, the thermodynamic data extracted from the measured values of four ternary diffusion coefficients and the Onsager reciprocal relations. The calculation for derivatives of solute chemical potentials with respect to solute molar concentrations was made using the method presented in [1]. This method is applicable to systems in which the molar concentration of one solute is very small compared to that of the other, like in our case. The approach is illustrated for the lysozyme chloride-Na$_2$SO$_4$-water system at 25$^\circ$ C, pH 4.5 and at 0.6 mM (8.6 mg/mL) lysozyme chloride and 0.1, 0.25, 0.5, 0.65, and 0.8 M Na$_2$SO$_4$ concentrations. The calculated solute chemical potential derivatives were used to compute the protein cation charge approximately. We also compute the diffusion Onsager coefficients ($L_{ij}$) for each composition at pH 4.5.
Motivation

In order to determine the protein structure through X-ray diffraction, high quality crystals are required. The protein crystallization process usually occurs in aqueous solution that contain salts as precipitant agents. The physical and chemical properties of these solutions affect drastically the nucleation and crystal growth processes. Protein aggregation depends on protein-protein and protein-precipitant interactions in the solution. In these interactions, the effective charge of the protein plays an important role. From titration experiments only the stoichiometric value of the protein charge can be determine, but it does not take into account the presence of ions that may bind on the macromolecules and change their net charge as already shown by diffusion experiments.

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. 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.

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. The importance of other species on protein diffusion follows from the one-dimensional flux relations:

\[
- J = \sum_{j=1}^{n} (D_{ij})_{v} \partial C_{j}/\partial x \quad i = 1, ..., n
\]  

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

\[
- J_1 = (D_{11})_{v} \frac{\partial C_1}{\partial x} + (D_{12})_{v} \frac{\partial C_2}{\partial x}
\]
\[-J_2 = (D_{21})_v \frac{\partial C_1}{\partial x} + (D_{22})_v \frac{\partial C_2}{\partial x}\]  

(3)

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.

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 \[^5\] defined by:

$$\sum_{i=0}^{n} J_i \bar{V}_i = 0$$  

(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 \[^6, 7\] and a crystal growth model has properly accounted for multicomponent diffusive transport in lysozyme chloride-NaCl-water system \[^8, 1\]. 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.

The use of ternary diffusion coefficients to determine binding coefficients and other thermodynamic data is very well established \[^9, 10\] . For our ternary system, the molar concentration of one solute is very small compared to that of the other, and also small enough that an inverse concentration dependence dominates certain activity coefficient derivatives. For such systems, using the Onsager reciprocal relations (ORR), along with precision measurements of ternary diffusion coefficients from our earlier paper \[^11\], we determined concentration derivatives of the chemical potentials ($\mu_{ij} \equiv \partial \mu_i / \partial C_j$) of two solutes with respect to one solute molar concentrations.

In our ternary experiments, the molarity of lysozyme chloride is small compared to that of Na$_2$SO$_4$. Thus, the self-derivative for lysozyme chloride, $\mu_{11}$, is dominated by its concentration term. The self-derivative $\mu_{22}$ for Na$_2$SO$_4$
is essentially that of the binary with minor correction. In order to obtain the molarity cross-derivatives ($\mu_{12}$ and $\mu_{21}$ which are unequal [12]) we have to use two additional equations: (1) equality of the molality cross-derivatives [13]:

$$\frac{\partial \mu_1}{\partial m_2} = \frac{\partial \mu_2}{\partial m_1}$$

(5)

where $m_i$ is the molality of solute $i$, and (2) the ORR equation:

$$\mu_{11}(D_{12})_o - \mu_{12}(D_{21})_o = \mu_{22}(D_{21})_o - \mu_{21}(D_{22})_o$$

(6)

relating the four molarity derivatives and the ternary diffusion coefficients in a solvent-fixed reference frame ($D_{ij}$)$_o$ [14, 15]. This method [1] yields an estimate of lysozyme charge and a functional approximation to the change of the chemical potential of lysozyme chloride with Na$_2$SO$_4$ concentration. This, together with the diffusion coefficients, will permit the modeling of protein crystallization processes.

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 [8]. 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 [16] 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 [17, 18, 19] 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 Π+. 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±0.01 °C.

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 [20] [21] [22] 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 [8]. 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 [20] [23] [24]. 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 [8]).
In order to make the data analysis of the free-diffusion experiments we used the Fick’s second law:

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

(7)

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 [25]. 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). Also we could made a comparison with the dynamic light scattering [27] results from our previous paper [11].

Results

Ternary diffusion experiments were performed on the lysozyme chloride-Na$_2$SO$_4$-water system at pH=$4.5$ and $T=25^\circ$ C. The four ternary diffusion coefficients obtained, $(D_{11})_v, (D_{22})_v, (D_{12})_v$ and $(D_{21})_v$ were published in our earlier paper [11] for a 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 [14] 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 Tables 1,2,3 were calculated using densities of all eight solutions from each experiment set. Densities were assumed to be linear in solute concentrations respecting the equation [8]:

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

(8)

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

Use of irreversible thermodynamics and diffusion data to calculate chemical potential derivatives. For our ternary system lysozyme
chloride-Na₂SO₄, the molar concentration of a macromolecule and the supporting electrolyte are small and large, respectively. So, in this case it is possible to estimate the chemical potential derivatives \( \mu_{11} \) and \( \mu_{22} \). The molality cross-derivative relation, eq.(5), comes from classical thermodynamics. From eq. (5), an expression can be derived relating the four molality partial derivatives \( \mu_{ij} \). The eq.(6), the ternary ORR of irreversible thermodynamics, relates the four \( (D_{ij})_o \) values of diffusion coefficients in a solvent-fixed reference frame and the four \( \mu_{ij} \) values [14 15].

Fundamental equations. The analysis of chemical potential derivatives was made in terms of quantities referred to a solvent-fixed reference frame, identify by a subscript 0, with the diffusion Onsager coefficients denoted by \( (L_{ij})_o \). The solvent-fixed \( (D_{ij})_o \) values shown in the Tables 1,2,3 were obtained from experimental volume-fixed \( (D_{ij})_v \) values by standard equations involving the \( \bar{V}_i \) [1, 18, 19].

The diffusion Onsager coefficients \( (L_{ij})_o \) and the solvent-fixed \( (D_{ij})_o \) diffusion coefficients are related (in matrix form) by:

\[
\begin{bmatrix}
(D_{11})_o & (D_{12})_o \\
(D_{21})_o & (D_{22})_o
\end{bmatrix} =
\begin{bmatrix}
(L_{11})_o \mu_{11} + (L_{12})_o \mu_{21} & (L_{11})_o \mu_{12} + (L_{12})_o \mu_{22} \\
(L_{21})_o \mu_{11} + (L_{22})_o \mu_{21} & (L_{21})_o \mu_{21} + (L_{22})_o \mu_{22}
\end{bmatrix}
\]

(9)

The inverse relation is:

\[
\begin{bmatrix}
(L_{11})_o & (L_{12})_o \\
(L_{21})_o & (L_{22})_o
\end{bmatrix} = \frac{1}{\mu_{11} \mu_{22} - \mu_{12} \mu_{21}} \times
\begin{bmatrix}
\mu_{22}(D_{11})_o - \mu_{21}(D_{12})_o & \mu_{11}(D_{12})_o - \mu_{12}(D_{11})_o \\
\mu_{22}(D_{21})_o - \mu_{21}(D_{22})_o & \mu_{11}(D_{22})_o - \mu_{12}(D_{21})_o
\end{bmatrix}
\]

(10)

Since the ORR, \( (L_{12})_o = (L_{21})_o \) [14 15] apply to the solvent-fixed frame, eq. (10) yields eq. (6).

Also, from eq. (5) for cross-derivative molality and the relations between \( C_i \) and \( m_i \), we can show that [11]:

\[
\frac{1}{C_o M_o} \frac{\partial \mu_1}{\partial m_2} = \mu_{12}(1 - C_2 \bar{V}_2) - \mu_{11}C_1 \bar{V}_2 = \mu_{21}(1 - C_1 \bar{V}_1) - \mu_{22}C_2 \bar{V}_1 = \frac{1}{C_o M_o} \frac{\partial \mu_2}{\partial m_1}
\]

(11)
where $M_o$ is the molecular mass of water.

In order to calculate the $\mu_{ij}$ molarity cross-derivatives, we took into account their general thermodynamics expressions in terms of volume concentrations and the corresponding mean ionic activity coefficients $y_i$ for volume concentrations [26], and also we assumed that lysozyme chloride has stoichiometry $\text{LyCl}_{zp}$. Thus, the $\mu_{ij}$ for our case could be written in a matrix form as:

$$
\begin{bmatrix}
\mu_{11} & \mu_{12} \\
\mu_{21} & \mu_{22}
\end{bmatrix} = RT \times \begin{bmatrix}
\frac{1}{C_1} + \frac{z_p^2}{z_p/2C_1 + C_2} + (z_p + 1)\frac{\partial \ln y_1}{\partial C_1} & \frac{z_p}{z_p/2C_1 + C_2} + (z_p + 1)\frac{\partial \ln y_1}{\partial C_2} \\
\frac{1}{C_2} + \frac{1}{z_p/2C_1 + C_2} + 2\frac{\partial \ln y_2}{\partial C_1} & \frac{1}{z_p/2C_1 + C_2} + 2\frac{\partial \ln y_2}{\partial C_2}
\end{bmatrix}
$$

where the quantity $z_p/2C_1 + C_2$ is equivalent to the total normality $N$ of our ternary solution.

Using the eq. (12) we computed the partial derivatives of chemical potentials, $\mu_{ij}$, for the case in which the molarity of at least one component is very low. From eq. (12) we calculated the $\mu_{11}$ and $\mu_{22}$ taking into account the terms which are dominant for our case. In order to calculate the other two derivatives chemical potentials, $\mu_{12}$ and $\mu_{21}$ we use the eqs. (6) and (11) and the four $(D_{ij})_o$:

$$
\mu_{12} = \left\{\mu_{11}[C_1V_2(D_{22})_o - (1 - C_1V_1)(D_{12})_o] - \mu_{22}[C_2V_1(D_{22})_o - (1 - C_2V_2)(D_{21})_o]\right/\{(1 - C_2V_2)(D_{22})_o - (1 - C_1V_1)(D_{11})_o\}
$$

$$
\mu_{21} = \left\{\mu_{11}[C_1V_2(D_{11})_o - (1 - C_2V_2)(D_{12})_o] - \mu_{22}[C_2V_1(D_{11})_o - (1 - C_2V_2)(D_{21})_o]\right/\{(1 - C_2V_2)(D_{22})_o - (1 - C_1V_1)(D_{11})_o\}
$$

All the values for derivative chemical potentials $\mu_{ij}$ are reported in the Tables 1,2,3 for each salt concentration.

Using the eqs. (10), (12), (13) and (14) we calculated the thermodynamics transport coefficients $(L_{ij})_o$ for pH 4.5 and $C_1 = 0.60$ mM, which are reported also in the Tables 1,2,3.

**Calculation of lysozyme chloride charge using the values for $\mu_{12}$ and $\mu_{21}$ is described in [1].** Looking into eq. (12), the expressions for $\mu_{12}$ and $\mu_{21}$ suggest that we can obtain the lysozyme chloride charge multiplying $\mu_{12}/RT$.
and \( \mu_{21}/RT \) by \( z_p/2C_1 + C_2 \) and we’ll receive the following dependence:

\[
Y_{12} = (z_p/2C_1 + C_2) \frac{\mu_{12}}{RT} = z_p + (z_p/2C_1 + C_2)(z_p + 1) \frac{\partial \ln y_1}{\partial C_2}
\] (15)

\[
Y_{21} = (z_p/2C_1 + C_2) \frac{\mu_{21}}{RT} = z_p + 2(z_p/2C_1 + C_2) \frac{\partial \ln y_2}{\partial C_1}
\] (16)

The dependence of \( Y_{21} \) and \( Y_{21} \) on \( (z_p/2C_1 + C_2) \) is shown in the Fig.1. From the graph we could estimate the protein charge, \( z_p \) and to compare it with the value obtain from thermodynamics data from [II]. At pH 4.5, the average value of \( z_p \) obtained from eqs. (15) and (16) is \( z_p = 3.29 \), in comparison with the value obtained, using the same approach, for lysozyme-NaCl-Water system, \( z_p = 8.9 \). This data were obtained from thermodynamics data for ternary system, which were in turn obtained in part from transport data.

**Conclusions** We reported the complete set of multicomponent diffusion coefficients for ternary lys-Na_2SO_4-water system at concentrations high enough to be relevant to crystallization studies, in the volume-fixed frame, \((D_{ij})_v\) and in the solvent-fixed frame, \((D_{ij})_o\). Also we calculated the derivatives chemical potentials \( \mu_{ij} \) for our ternary system and after that we estimated the lysozyme chloride charge, \( z_p \), from irreversible thermodynamics and diffusion data. We also reported the four thermodynamics transport coefficients \((L_{ij})_o\) at pH 4.5 and \( C_1 = 0.60 \) mM.

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|                  | \(\bar{C}_1\) (mM) | \(\bar{C}_2\) (M) | \(\bar{d} \text{ (gcm}^{-3}\) | \(H_1 \text{ (10}^3\text{g mol}^{-1}\) | \(H_2 \text{ (10}^3\text{g mol}^{-1}\) | \(\bar{V}_1 \text{ (cm}^3\text{mol}^{-1}\) | \(\bar{V}_2 \text{ (cm}^3\text{mol}^{-1}\) | \(\bar{V}_o \text{ (cm}^3\text{mol}^{-1}\) | \((D_{11})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | \((D_{12})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | \((D_{21})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | \((D_{22})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | \(\mu_{11}/RT\text{ (M}^{-1}\) | \(\mu_{12}/RT\text{ (M}^{-1}\) | \(\mu_{21}/RT\text{ (M}^{-1}\) | \(\mu_{22}/RT\text{ (M}^{-1}\) | \(RT(L_{11})_o \text{ (10}^{-9}\text{Mm}^2\text{s}^{-1}\) | \(RT(L_{12})_o \text{ (10}^{-9}\text{Mm}^2\text{s}^{-1}\) | \(RT(L_{22})_o \text{ (10}^{-9}\text{Mm}^2\text{s}^{-1}\) |
|-----------------|--------------------|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| \(\bar{C}_1\) (mM) | 0.6000             | 0.6000            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\bar{C}_2\) (M)  | 0.1000             | 0.2500            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\bar{d} \text{ (gcm}^{-3}\) | 1.012189           | 1.030671          |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(H_1 \text{ (10}^3\text{g mol}^{-1}\) | 4.286              | 1.149             |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(H_2 \text{ (10}^3\text{g mol}^{-1}\) | 0.12500            | 0.12230           |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\bar{V}_1 \text{ (cm}^3\text{mol}^{-1}\) | 10050              | 10182             |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\bar{V}_2 \text{ (cm}^3\text{mol}^{-1}\) | 17.090             | 19.780            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\bar{V}_o \text{ (cm}^3\text{mol}^{-1}\) | 18.067             | 18.058            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{11})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | 0.1169 ± 0.0001    | 0.1090 ± 0.0001  |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{12})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | -0.000013 ± 0.000001 | 0.000108 ± 0.000001 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{21})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | 2.49 ± 0.01        | 4.14 ± 0.01       |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{22})_v \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | 0.9661 ± 0.0001    | 0.8826 ± 0.0001  |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{11})_o \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | 0.1176              | 0.1097            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{12})_o \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | -0.0000031         | 0.0001193         |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{21})_o \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | 2.6                | 4.5               |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \((D_{22})_o \text{ (10}^{-9}\text{m}^2\text{s}^{-1}\) | 0.968              | 0.887             |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\mu_{11}/RT\text{ (M}^{-1}\) | 1787               | 1715              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\mu_{12}/RT\text{ (M}^{-1}\) | 41.9               | 22.5              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\mu_{21}/RT\text{ (M}^{-1}\) | 64.2               | 43.3              |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(\mu_{22}/RT\text{ (M}^{-1}\) | 21.894             | 8.131             |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(RT(L_{11})_o \text{ (10}^{-9}\text{Mm}^2\text{s}^{-1}\) | 0.0000707          | 0.0000684         |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(RT(L_{12})_o \text{ (10}^{-9}\text{Mm}^2\text{s}^{-1}\) | -0.0001354         | -0.0017452        |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| \(RT(L_{22})_o \text{ (10}^{-9}\text{Mm}^2\text{s}^{-1}\) | 0.044487           | 0.1094762         |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |

Table 1
|                | \(C_1\) (mM) | \(C_2\) (M) | \(\bar{d}\) (g cm\(^{-3}\)) | \(H_1(10^3\text{g mol}^{-1})\) | \(H_2(10^3\text{g mol}^{-1})\) | \(\bar{V}_1(\text{cm}^3\text{mol}^{-1})\) | \(\bar{V}_2(\text{cm}^3\text{mol}^{-1})\) | \(\bar{V}_0(\text{cm}^3\text{mol}^{-1})\) | \((D_{11})_{\nu}(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{12})_{\nu}(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{21})_{\nu}(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{22})_{\nu}(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{11})_o(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{12})_o(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{21})_o(10^{-9}\text{m}^2\text{s}^{-1})\) | \((D_{22})_o(10^{-9}\text{m}^2\text{s}^{-1})\) | \(\mu_{11}/RT(M^{-1})\) | \(\mu_{12}/RT(M^{-1})\) | \(\mu_{21}/RT(M^{-1})\) | \(\mu_{22}/RT(M^{-1})\) | \(RT(L_{11})_o(10^{-9}\text{M}m^2\text{s}^{-1})\) | \(RT(L_{12})_o(10^{-9}\text{M}m^2\text{s}^{-1})\) | \(RT(L_{22})_o(10^{-9}\text{M}m^2\text{s}^{-1})\) |
|----------------|-------------|-------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                | 0.6000      | 0.6000      | 1.060538                   | 4.104                       | 0.11733                     | 10209                       | 24.720                      | 18.026                      | 0.969±0.0001                | 0.000132±0.000001           | 6.75±0.01                   | 0.7791±0.0001               | 0.0977                      | 0.0001444                   | 7.3                         | 0.790                       |
|                | 0.6500      | 0.6500      | 1.078032                   | 4.049                       | 0.11610                     | 10257                       | 25.930                      | 18.013                      | 0.0894±0.0001               | 0.000134±0.000001           | 8.26±0.01                   | 0.7294±0.0001               | 0.0902                      | 0.0001475                   | 9.0                         | 0.742                       |
|                | 1691        | 1685        |                            |                            |                            |                            |                            |                            | 1691                       | 1685                       |                            |                            |                            |                            |                            |                            |                            |
|                | 17.7        | 17.7        |                            |                            |                            |                            |                            |                            | 17                         | 17                         |                            |                            |                            |                            |                            |                            |                            |
|                | 36.8        | 35.6        |                            |                            |                            |                            |                            |                            | 36.8                       | 35.6                       |                            |                            |                            |                            |                            |                            |                            |
|                | 3.755       | 2.811       |                            |                            |                            |                            |                            |                            | 3.755                      | 2.811                      |                            |                            |                            |                            |                            |                            |                            |
|                | 0.0000634   | 0.0000601   |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
|                | -0.0002606  | -0.0003110  |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
|                | 0.2117597   | 0.31738664  |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |                            |
|                         | Value                          |
|-------------------------|--------------------------------|
| $C_1$ (mM)              | 0.6000                         |
| $C_2$ (M)               | 0.8000                         |
| $d$ ($g cm^{-3}$)       | 1.095745                       |
| $H_1$ ($10^3$ gmol$^{-1}$) | 3.954                        |
| $H_2$ ($10^3$ gmol$^{-1}$) | 0.11502                     |
| $V_1$ ($cm^3$ mol$^{-1}$) | 10341                        |
| $V_2$ ($cm^3$ mol$^{-1}$) | 26.980                       |
| $V_o$ ($cm^3$ mol$^{-1}$) | 17.993                       |
| $(D_{11})_v$ ($10^{-9} m^2 s^{-1}$) | 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               |
| $(D_{11})_o$ ($10^{-9} m^2 s^{-1}$) | 0.0829                      |
| $(D_{12})_o$ ($10^{-9} m^2 s^{-1}$) | 0.0001418                     |
| $(D_{21})_o$ ($10^{-9} m^2 s^{-1}$) | 10.4                       |
| $(D_{22})_o$ ($10^{-9} m^2 s^{-1}$) | 0.706                       |
| $\mu_{11}/RT$ (M$^{-1}$)  | 1682                          |
| $\mu_{12}/RT$ (M$^{-1}$)  | 16.1                          |
| $\mu_{21}/RT$ (M$^{-1}$)  | 34.5                          |
| $\mu_{22}/RT$ (M$^{-1}$)  | 2.241                         |
| $RT(L_{11})_o$ ($10^{-9} Mm^2 s^{-1}$) | 0.1808868                   |
| $RT(L_{12})_o$ ($10^{-9} Mm^2 s^{-1}$) | -0.000341                    |
| $RT(L_{22})_o$ ($10^{-9} Mm^2 s^{-1}$) | 0.3173864                    |