Conductivity and Viscosity Measurements for Binary Lysozyme Chloride Aqueous Solution and Ternary Lysozyme-Salt-Water Solution

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Abstract

We use the conductimetric method, adequate to electrolytes, to determine the lysozyme charge in lys-water and ternary lys-salt-water systems. We measured also the viscosities for the above binary and ternary systems in the same conditions at pH= 4.5 and T= 298 K, measurements that allow us to see any effect of viscosity on cations mobilities and implicitly on the lysozyme charge. The method is illustrated for the lysozyme chloride aqueous solution system at 25°C, using the data reported here for pH= 4.5 at 0.15, 0.6, 0.8, 1., 1.5, 2., 2.5, 3., 3.5 mM (mg/mL) lysozyme chloride concentrations. The method was also applied to ternary lys-salt-water systems in the same conditions at pH= 4.5 and T= 25°C. Ternary conductivities are reported for a mean concentration 0.6 mM of lysozyme chloride in all systems and a mean concentration 0.01, 0.025, 0.05, 0.1, 0.175, 0.2, 0.5, 0.7, 0.9, 1.2, 1.3 and 1.4 M for NaCl; 0.005, 0.01, 0.05, 0.1, 0.175, 0.2, 0.5, 0.7, 0.9, 1.2, 1.3, 1.4 and 1.5 M for KCl; 0.005, 0.01, 0.05, 0.1, 0.175, 0.2, 0.5, 0.7, 0.9, 1.2, 1.3, 1.4, 1.5, 1.6 and 1.7 M for NH₄Cl.
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 [1] 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 [2].

A more direct way to estimate the protein charge for different salts concentrations is based on conductimetric experiments. The conductivity [16, 17] of the protein-salt aqueous systems is function of the mobility and of the ionic charge species present in solution and it seems to be very sensitive to charge changes of the protein. These changes are influenced by modifications in pH, salt concentrations and the type of the used salt.

Theoretical Background

Assuming that the lysozyme chloride is an electrolyte with the chemical formula Lys$^{z_p}$Cl$^{-z_p}$, the conductivity for a ternary system Lys$^{z_p}$Cl$^{-z_p}$-Salt-Water can be expressed as:

$$\sigma_{sol} = c_p F(z_p u_p + z_p u_-) + c_{salt} F(u_+ + u_-)$$ (1)

where $c_p$ is the protein concentration, $F= 96484 \text{ C/mol}$ is the Faraday constant, $u_p$, $u_-$ and $u_+$ are the mobilities of the protein complex ion, the negative and positive ion of the used salt in the solution, respectively. Using the Stoke’s law and the definition of the ion mobility, we can express the ion mobility in terms of the viscosity of the medium and of the radius $r_i$ of the ion in the solution (by considering the ion a hard sphere). We assume also the radius of the ions doesn’t change with the salt concentration at very low protein concentrations (0.6 mM in our case).

Using the Einstein’s equation for mobility of protein complex ion at infinite
dilution

\[ u_p^\infty = \frac{F^2 D_p^\infty}{RT} \]

where \( R = 8310 \text{ J/mol K} \) is the universal gas constant and \( D_p^\infty = 0.132 \times 10^{-9} \text{ m}^2/\text{s} \) is the infinite dilution tracer diffusion coefficient for the lysozyme [2], and Stokes-Eistein’s equation

\[ D_p^\infty = \frac{kT}{6\pi \eta_W r_p} \]

where \( k \)-Boltzmann’s constant, and \( \eta_W = 0.8904 \text{ cp} \) is the water viscosity at \( T= 298 \text{ K} \), we can write the conductivity of the ternary solution as:

\[ \sigma_{sol} = c_p \Lambda_p + c_{salt} \Lambda_{salt} \]

where

\[ \Lambda_p = z_p u_p + z_p u_- = z_p^2 \lambda_p + z_p \lambda_- \]

\[ \Lambda_{salt} = u_+ + u_- = \lambda_+ + \lambda_- \]

are the conductances for lysozyme chloride (protein) and respectively the conductance for used salt. The specific conductance of the positive/negative ions \( \lambda_\pm \) and of the protein ion can be determine from:

\[ \lambda_\pm = \frac{\eta_{salt}}{\eta_{sol}} t_\pm \Lambda_\infty \]

\[ \lambda_p = \frac{F^2 \eta_{water}}{RT} \frac{\eta_{water}}{\eta_{sol}} D_p^\infty \]

where \( t_\pm \) is the transference number of the positive/negative ions of the used salt in the binary solution [3], \( \eta_{sol} \) is the viscosity of the ternary solution, \( \eta_{salt} \) is the viscosity of the binary salt solution, and \( \Lambda_{salt}^\infty \) is the molar conductance of the salt solution at infinite dilution.

From the Eqs. (5)-(8), we obtain the following expression for \( \sigma_{sol} \):

\[ \sigma_{sol} = c_p \left( z_p^2 \frac{F^2 \eta_{water}}{RT} \frac{\eta_{water}}{\eta_{sol}} D_p^\infty + z_p \frac{\eta_{salt}}{\eta_{sol}} t_- \right) + c_{salt} \frac{\eta_{salt}}{\eta_{sol}} \Lambda_{salt}^\infty - c_p \left( z_p - z_p^o \right) \frac{\eta_{salt}}{\eta_{sol}} t_+ \Lambda_{salt}^\infty \]
where \( z_p^0 = 6.7 \) is the lysozyme charge at infinite dilution \[2\].
The Eq. (9) shows the strong dependence of the \( \sigma_{sol} \) on the protein charge, \( z_p \).

**Experimental section**

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

**Materials.** The materials, solution preparation, density and pH measurements are all described in the Ref. [2]. We used a hen egg-white lysozyme, recrystallized six times, purchased from Seikagaku America. The choice of the supplier was guided by the work of Rosenberger and co-workers [4, 5, 6] who reported detailed analysis of commercial HEWL products.
The molecular mass of the isoelectric lysozyme solute, \( M_{lys} \), was taken as 14307 g/mol [7], and this value 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 [8, 9, 10] of 1.305 g/cm\(^3\).
Deionized water was distilled and then passed through a four-stage Millipore filter system to provide high-purity water for all the experiments. The molecular mass of water, \( M_{water} \), was taken as 18.015 g/cm\(^3\). Mallinckrodt reagent HCl (\( \sim 12 \) M) was diluted by half with pure water and distilled at the constant boiling composition. The resulting HCl solution (\( \sim 6 \) M) was then diluted to about 0.063 M (pH 1.2) and used to adjust the pH of the solution.
Following the work of Rard [11], Mallinckrodt AR NaCl and KCl were dried by heating at 450\(^\circ\) C for 7 h, and used without further purification. Mallinckrodt AR NH\(_4\)Cl was dried heating it at 70\(^\circ\) C for 7 h under vacuum. The purity of the salts were listed as 99.9% by the supplier. The molecular mass for NaCl, KCl and NH\(_4\)Cl, \( M_{salt} \), were taken to be respectively 58.443 g/mol, 74.55 g/mol and 53.49 g/mol, and their crystal density [12] as 2.165 g/cm\(^3\), 1.984 g/cm\(^3\) and 1.527 g/cm\(^3\) for buoyancy corrections.

**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 binary experiments, the solutions were first diluted to within 10 cm$^3$ of the final volumes with pure water. From 1 to 3 mL of the dilute HCl was added to adjust the pH to the desired value, any residual solution on the pH electrode was washed back into the solutions, and the dilutions were completed by mass. The densities and final pH values of these solutions were measured and the final concentrations were calculated.

For ternary experiments, precise masses of NaCl, KCl and NH$_4$Cl 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 dilute to their final mass.

**pH Measurements.** The pH measurements were done 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. It was assumed that the pH values remaind valid at higher NaCl, KCl and NH$_4$Cl concentrations. After four or five experiments, the electrode was soaked in 5% NaClO for 10 min, and then the internal reference solution was replaced with fresh solution.

**Density Measurements.** All density measurements were performed with a Mettler-Paar DMA40 density meter, with an RS-232 output to a 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± 0.01 °C.

**Conductivity and Viscosity Measurements.** We performed measurements on conductivity for ternary systems: Lys-NaCl-Water, Lys-KCl-Water and Lys-NH$_4$Cl-Water, and for binary Lys-Water using a pair of capillarity cells characterized by the length (l) and cross section (S), and 8 silver-chloride electrodes; 2 current carrying electrodes, 2 bridge electrodes between cells and 4 probe electrodes.
The DC-current intensity (I) and the drop tension (U) were determined using an AMEL-Instruments galvanostat for very low current intensity (µA) and a Hewlett Packard multimeter which allowed us to measure very high resistance (MΩ) corresponding to very low concentration of the studied solutions (mM). The internal resistance of multimeter was adjusted to a value bigger than 10 GΩ. Thus, the measurements of the drop tensions on systems with a resistance of 10 MΩ are not affected by significant errors.

In order to determine the geometrical factor of the cells, we calibrated them with KCl (very high purity) solution for a large range of the concentration (0.5 mM up to 1.5 M).

The binary and ternary protein solutions, and the binary salt-water systems were prepared as we described above, at the same pH=4.5; conductivities and viscosities were measured at T= 25°C with a very good accuracy.

Ternary conductivities are reported for a mean concentration 0.6 mM of lysozyme chloride in all systems and a mean concentration 0.01, 0.025, 0.05, 0.1, 0.175, 0.2, 0.5, 0.7, 0.9, 1.2, 1.3 and 1.4 M for NaCl; 0.005, 0.01, 0.05, 0.1, 0.175, 0.2, 0.5, 0.7, 0.9, 1.2, 1.3, 1.4 and 1.5 M for KCl; 0.005, 0.01, 0.05, 0.1, 0.175, 0.2, 0.5, 0.7, 1.2, 1.3, 1.4, 1.5, 1.6 and 1.7 M for NH4Cl.

Binary conductivities for Lysozyme chloride-Water are reported for a mean concentration 0.15, 0.6, 0.8, 1., 1.5, 2., 2.5, 3., 3.5 mM of lysozyme chloride. Binary conductivities for Salt-Water are reported for the same concentrations used in the ternary solutions.

For the same binary and ternary systems we performed measurements of viscosity using an Ostwald viscosimeter.

Results

**Binary lysozyme-water conductivity.** Conductivities were determined from Eq. (10) the in the concentration range from 0.15 mM to 3.5 mM using the experimental data for DC-current intensity (I), the drop tension (U) on the cells and the diameter of the all conductivity cells \(d = 2\) mm:

\[
\sigma_p = \frac{I \cdot l}{S \cdot U}
\]

(10)

After that we made the correction due to the geometrical factor. The obtained values are plotted in Fig.1, where the fitting curve can be expressed by the following formula:

\[
\sigma_p = (0.0040 \pm 0.0006) + c_p[(90.71 \pm 1.39) - (371.7 \pm 23.1)\sqrt{c_p}]
\]

(11)
with $\chi^2 = 4.6 \times 10^{-6}$. Using the fitting Eq. (11), we expressed the conductance $\Lambda_p$ as:

$$\Lambda_p = (90.71 \pm 1.39) - (371.7 \pm 23.1) \sqrt{c_p}$$  \hspace{1cm} (12)

The first term in Eq. (12) represents the lysozyme chloride conductance at infinite dilution ($c_p \to 0$), $\Lambda^\infty_p = 0.091 \text{Sm}^2/\text{mol}$. Taking into account the Eq. (9), we can write the lysozyme chloride conductance at infinite dilution as:

$$\Lambda^\infty_p = \frac{F^2}{RT} \left( ((z^o)_p)^2 D^\infty_p + z^o_p D^\infty_{Cl} \right)$$  \hspace{1cm} (13)

where $D^\infty_p = 0.132 \times 10^{-9} \text{m}^2/\text{s}$ is the infinite dilution tracer diffusion coefficient for the lysozyme \[2\] and $D^\infty_{Cl} = 2.03 \times 10^{-9} \text{m}^2/\text{s}$ is the infinite dilution tracer diffusion coefficient for chloride ion obtained from the limiting ionic conductances \[13\].

Using the Eq. (13) and the data from conductimetric experiments, we calculated the lysozyme charge, $z^o_p = 7.9$. This value can be compare with the lysozyme charge calculated from extrapolated limiting diffusion coefficients, in binary lysozyme-water diffusion experiments \[2\], $z^o_p = 6.7$ at pH= 4.5 and $T= 298 \text{K}$.

**Ternary lys-salt-water conductivity.** Using the same Eq. (10) for ternary lys-salt-water solution, we determined the conductivities for Lys-NaCl-Water, Lys-KCl-Water and Lys-NH$_4$-Water. For these three ternary systems, we plotted in Fig. 2 the experimental data for the difference $\sigma_{sol} - \sigma_{salt}$ as function of salt concentration $c_{salt}$. The used fitting curves are given bellow:

$$\sigma_{lys-NaCl-w} - \sigma_{NaCl} = (0.0450 \pm 0.0038) - (0.1625 \pm 0.0053)c_{NaCl}$$  \hspace{1cm} (14)

$$\sigma_{lys-KCl-w} - \sigma_{KCl} = (0.0281 \pm 0.0053) - (0.1174 \pm 0.0066)c_{KCl}$$  \hspace{1cm} (15)

$$\sigma_{lys-NH4Cl-w} - \sigma_{NH4Cl} = (0.0401 \pm 0.0052) - (0.1215 \pm 0.0055)c_{NH4Cl}$$  \hspace{1cm} (16)

In the Fig. 3 we plotted the experimental data measured for binary NaCl-Water, KCl-Water and NH$_4$-Water viscosities. Below are given the fitting curves:

$$\eta_{NaCl} = 0.8904 + (0.0736 \pm 0.0041)c_{NaCl}$$  
$$+ (0.0043 \pm 0.0089)c^2_{NaCl} + (0.0043 \pm 0.0089)c^3_{NaCl}$$  \hspace{1cm} (17)
\[ \eta_{KCl} = 0.8904 - (0.0025 \pm 0.0035)c_{KCl} \\
- (0.0054 \pm 0.0069)c_{KCl}^2 + (0.0055 \pm 0.0033)c_{KCl}^3 \] (18)

\[ \eta_{NH_4Cl} = 0.8904 - (0.0098 \pm 0.0037)c_{NH_4Cl} \\
+ (0.0045 \pm 0.0064)c_{NH_4Cl}^2 - (0.0009 \pm 0.0027)c_{NH_4Cl}^3 \] (19)

In Fig. 4 is plotted the ratio \( \eta_{salt}/\eta_{sol} \) and the lysozyme effect on viscosity can be observed. For \( c_{salt} > 0.05 \text{ M} \) the lysozyme has a constant effect on all the three salts.

Using the Eq. (9), we calculated also the lysozyme charge dependence on salt concentrations; the results are shown in Table 1.

The propagation of errors was done assuming an error \( \delta \sigma = 0.01 \text{ S/m} \) estimated by the least square on raw data. We do not consider any systematic error associated to the model or to the binary salt conductivities.

In the Fig. 5 are plotted the values obtained for lysozyme charge versus the salt concentration. The graph shows a higher dependence of the lysozyme charge on KCl and NH\(_4\)Cl salt concentrations than in the NaCl case; it follows \( z_p \) depends on type of salt.

This behavior may be connected with the dependence of the mobility ratio Lys/Cation (\( u_p/u_+ \)) on the salt concentrations as it is shown in Fig. 6.
Fig. 2

Fig. 3
Fig. 4

Fig. 5
The lysozyme charge values in the case when the salt concentration goes to zero in ternary systems, are: $z_p = 7.12$ for ternary Lys-NaCl-Water solution, $z_p < 6.42$ for ternary Lys-KCl-Water solution and $z_p = 5.95$ for ternary Lys-NH$_4$Cl-Water solution. We can compare the value of $z_p$ obtained for Lys-NaCl-Water by conductimetric method with the value $z_p = 8.9$ obtained from thermodynamic data applied to precision ternary diffusion data for the same system \[14\].

**Conclusions**

In the present paper we calculated the protein charge by using, for the first time in our knowledge, a conductimetric method to binary lysozyme-water, and ternary lys-salt-water systems for different salts, at different salt concentrations. We used also the experimental values of viscosities for the same binary and ternary systems at pH= 4.5 and T= 298 K. We determined the protein charge $z_p$ from Eq. (9) where we introduced all the experimental

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**Table 1**

| $c_{NaCl}$ (M) | $z_p$ | $\delta z_p$ | $c_{KCl}$ (M) | $z_p$ | $\delta z_p$ | $c_{NH_4Cl}$ (M) | $z_p$ | $\delta z_p$ |
|---------------|-------|-------------|---------------|-------|-------------|--------------------|-------|-------------|
| 0             | 7.12  | 1.8         | 0.05          | 6.42  | 2.6         | 0                  | 5.96  | 2.8         |
| 0.01          | 7.65  | 1.7         | 0.1           | 8.62  | 1.9         | 0.005              | 6.57  | 2.5         |
| 0.025         | 8.07  | 1.6         | 0.175         | 11.15 | 1.5         | 0.01               | 7.02  | 2.4         |
| 0.05          | 8.64  | 1.6         | 0.2           | 11.6  | 1.5         | 0.05               | 8.98  | 1.9         |
| 0.1           | 9.5   | 1.5         | 0.5           | 17.1  | 1           | 0.1                | 10.6  | 1.6         |
| 0.175         | 10.5  | 1.3         | 0.7           | 19.8  | 0.9         | 0.175              | 12.5  | 1.3         |
| 0.2           | 10.8  | 1.3         | 0.9           | 22    | 0.8         | 0.2                | 13.1  | 1.3         |
| 0.5           | 13.3  | 1.1         | 1.2           | 24.9  | 0.7         | 0.5                | 18.1  | 0.9         |
| 0.7           | 14.4  | 1.1         | 1.3           | 25.8  | 0.7         | 0.7                | 20.5  | 0.8         |
| 0.9           | 15.1  | 1.1         | 1.4           | 26.6  | 0.6         | 0.9                | 22.5  | 0.8         |
| 1.2           | 15.9  | 1           | 1.5           | 27.4  | 0.6         | 1.2                | 25.2  | 0.7         |
| 1.3           | 16.1  | 1           | 1             | 1.3   | 26          | 0.7                |
| 1.4           | 16.3  | 1           | 1             | 1.4   | 26.8        | 0.6                |
|               |       |             |               | 1.5   | 27.4        | 0.6                |
|               |       |             |               | 1.6   | 28.2        | 0.6                |
|               |       |             |               | 1.7   | 28.8        | 0.6                |
data. From binary lys-water conductivities data we obtained a value of $z_p = 7.9$ for lysozyme charge at infinite dilution, instead of $z_p = 6.7$ received from extrapolated limiting diffusion coefficients for aqueous lysozyme chloride [2] at pH = 4.5 and T = 298 K (these limiting values of the diffusion coefficients were obtained by extrapolating the diffusion coefficients measured at low, but nonzero, concentrations of protein). Our value $z_p$ should be compared with $z_p = 3.9$ obtained by a Harned-type analysis [15], in which the charge in the Debye-Hückel limiting law was adjusted to match the concentration dependence of the diffusion data [2].

From ternary lys-salt-water systems conductivities data we received the following values: $z_p = 7.12$ from Lys-NaCl-Water, $z_p < 6.42$ from Lys-KCl-Water, and $z_p = 5.95$ from Lys-HN$_4$Cl-Water and we can compare with $z_p = 8.9$ obtained at higher lysozyme concentrations from thermodynamic data for Lys-NaCl-Water system [14] at pH = 4.5 and T = 298 K.

The obtained experimental data for binary lys-water, salt-water and ternary lys-salt-water viscosities, allowed us to calculate the mobilities of positive ions and to compare the dependence of the ratio lys/cation mobility ($u_p/u_+$) of the salt concentrations and to presume its influence on the protein charge dependence for all three ternary systems (Fig. 5 and Fig. 6).
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References

[1] Tanford, C; Wagner, M.L. J.Am.Chem.Soc.1954, Vol. 76, 3331-3336

[2] Albright, J.G. and colab. J.Am.Chem.Soc., 1999 Vol. 121, 3256-3266

[3] Spiro, M., Determination of transference numbers in A. Weisenberg (ed), Interscience Publishers, Inc., New York, 1960

[4] Muschl, M.; Rosenberger, F. J.Chem.Phys.1997, 107, 1953-1962

[5] Thomas, B.R.; Vekilov, P.G.; Rosenberger, F. Acta Crystallogr.Sect.D 1996,52, 776-784

[6] Rosenberger, F. J. Cryst. Growth 1996, 166, 40-54

[7] Canfield, R.E. J.Biol.Chem. 1963, 238, 2698-2707

[8] Palmer, K.J.; Ballantyne, M.; Galvin, J.A. J.Am.Chem.Soc. 1948, 70, 906-908

[9] Tanford, C. Physical Chemistry of Macromolecules; John Wiley&Sons: New York, NY 1961

[10] Westbrook, E.M. In Methods in Enzymology; Wyckoff,H.W., Hirs,C.H.W., Timasheff,S.N., Eds.; Academic Press: New Yprk, NY 1985,Vol. 114, 187-196

[11] Rard,J.A. J.Chem.Thermodyn. 1996, 28, 83-110

[12] Weast,R.C. CRC Handbook of Chemistry and Physics, 57th ed.; CRC Press: Cleveland, OH 1975
[13] Robinson, R.A.; Stokes, R.H. *Electrolyte Solutions*, 2nd ed.; Butterworths: London, 1970

[14] Annunziata, O. and colab. *J. Am. Soc.* 2000, 122, 5916-5928

[15] Harned, H.S. *Proc. Natl. Acad. Sci. USA* 1954, 40, 551-556

[16] Țolea, T; Tănase, I.Gh.; Luca, C. *Revista de chimie* 2002, 53, 754-757

[17] Țolea, T; Tănase, I.Gh.; Luca, C. *Revista de chimie* 2003, 54, 172-177