Analysis of the Dielectric Relaxation of a Gelatin Solution

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The behavior of the dielectric properties of gelatin in the frequency range from 10¹ Hz to 10¹⁰ Hz was investigated and compared with that of the globule protein, bovine serum albumin (BSA), desalted gelatin and BSA being used. Dielectric relaxation was observed for both the gelatin and BSA solutions. The relaxation data were fitted well by the Cole-Cole equation; the Cole-Cole parameter (β) and the relaxation time (τ) were obtained. For the BSA solutions, τ was proportional to the solution viscosity (η) at 40°C and 25°C, and the values of β at 40°C were similar to those at 25°C. For gelatin solution, τ was proportional to η at 40°C, but was not proportional to η at 25°C. In addition, the values of β at 25°C were smaller than those at 40°C. These results indicate that the rotation of gelatin and/or polarization of submolecular groups in the coil state greatly contributed to the dielectric relaxation at 40°C; on the other hand, the formation of cross-linking junctions consisting of helix structures would have affected the dielectric relaxation at 25°C.

Key words: dielectric relaxation; relaxation time; gelatin; viscosity

The dielectric constant, ε, reflects the number of the electric dipole moments in a system. ¹) If the orientation of the electric dipole moments is slower than that of the electric fields, the dipole moments cannot be observed. Therefore, the dielectric constant decreases with increasing frequency, this phenomenon being the so-called dielectric relaxation. ²) The dielectric relaxation gives us useful information on the internal structure of a material. ²) However, the relationship between the dielectric relaxation and the internal structure of foods has not been fully investigated.

The dielectric relaxation for such globule protein solutions as bovine serum albumin (BSA) and cytochrome C ³-⁵) in the region 10¹ to 10¹⁰ Hz is ascribed to the orientational polarization resulting from the overall rotation of the protein molecules. On the other hand, the dielectric relaxation for such linear polyelectrolyte solutions as polyglutamate, ⁶) sodium polystyrene sulfonate, ⁷, ⁸) and polyacrylic acid ⁹) shows two kinds of relaxation process: one involves low-frequency relaxation around kHz, and the other is high-frequency relaxation around MHz. The low-frequency relaxation of linear polyelectrolyte solutions is considered to reflect such phenomena as fluctuation of the polymer ¹⁰) and fluctuation of bound counterions along the polyelectrolytes. ¹¹, ¹²) High-frequency relaxation around MHz is ascribed to fluctuation of the counterions that are loosely bound to the polyelectrolytes. Ikeda et al. ¹³, ¹⁴) have investigated the dielectric properties of the typical ionic food polysaccharides, alginates and κ-carrageenan, in the frequency range from 10¹ Hz to 10¹⁰ Hz. They showed that high-frequency relaxation of the polysaccharides could be ascribed to fluctuation of the counterions loosely bound to the polyelectrolytes. They evaluated the crossover concentration from the dilute to semi-dilute region for polysaccharide solutions. In addition, they evaluated the fluctuation length and the concentration of counterions bound to the polyelectrolytes in solutions and gels. ¹³-¹⁵)

Gelatin is a fibrous protein produced from collagen. Due to the remarkable mechanical properties of gels and to the natural biological origin of gelatin, gels are used in such fields as pharmacology, food, cosmetics, photography, glue, and composite materials. To make good use of gelatin, it is necessary to quantitatively examine the dynamics of the molecule. Frick and Jacobson ¹⁶) have investigated the dielectric relaxation of gelatin solutions in the frequency range from 2 kHz to 65.5 MHz. They showed that the frequency dependence of the dielectric constant fitted an empirical power-law equation, although the physical meaning of the parameter involved was ambiguous. The gelatin they used contained 3.0 × 10⁻⁴ kg/kg of sample metal ions. Generally speaking, metal ions in a polyelectrolyte solution cause dielectric relaxation; moreover, the counterions cause electrode polarization, especially at low frequency. ¹⁷, ¹⁸) In addition, the dielectric measurements in their study were carried out at a gelatin concentration of more than 1.0 × 10⁻² kg/kg of solution. At such a high concentration, the gelatin molecules would be entangled with each other. ¹⁹-²²) In order to clearly understand the dynamic behavior of gelation molecules by the dielectric method, a study needs to be carried out at a much lower concentration.

In this present study, the behavior of the dielectric properties of desalted gelatin in the frequency range from 10¹ Hz to 10¹⁰ Hz was investigated and compared with that of the globule protein, BSA.

Materials and Methods

Preparation of the platinum platinized electrodes. With ionic materials, electrode polarization, which occurs at the interface between the electrode and the sample, creates an error in dielectric measurements, especial-
ly in the low-frequency range.\textsuperscript{17,18} In this study, to reduce electrode polarization, platinum platedized electrodes (hereafter referred to as Pt-Pt electrodes) were prepared and used.\textsuperscript{23}

Two platinum electrodes (44 mm in diameter and 1 mm in thickness) were boiled in nitric acid (Kanto Chemical) for 30 minutes and then washed in water. The electrodes were inserted into a solution containing 1\% hydrogen hexachloroplatinate (IV) hexahydrate (Kanto Chemical) and 0.01\% lead (II) acetate trihydrate (Kanto Chemical). By passing a direct current through the solution for 5 minutes at a current density of 50 mA/cm\(^2\) of the electrode surface, platinum was deposited on the cathode. The current direction was changed at intervals of one minute to deposit on both platinum electrodes. The electrodes covered with platinum black were washed in water and then inserted into a solution of 0.1 M sulfuric acid (Kanto Chemical). A direct current was then passed through the solution for 20 minutes, the current direction being changed at intervals of one minute. The resulting Pt-Pt electrodes were then sufficiently washed in water.

\textbf{Preparation of the desalted samples.} Bovine serum albumin (BSA; Sigma Chemical) was dissolved in water and then passed through a large excess of H-form cation-exchange resin (Dowex 50W-X8) and OH-form anion-exchange resin (Amberlite IRA-400). The resulting solution was freeze-dried. A desalted BSA solution was prepared by mixing the freeze-dried sample and a selected amount of water.

Gelatin (Nitta Gelatin; type B: from calf bone, 205 Bloom) was suspended in water, and allowed to swell for 10 min at room temperature. The sample was then dissolved at 60°C by applying a magnetic stirrer for an hour. Since a gelatin solution cannot be passed through an ion-exchange column due to gelatin, salt was removed by electrodialysis with a Micro Acilizer S1 (Asahi Chemical Industry Co.) at room temperature. After being freeze-dried, the deionized gelatin was suspended in water and allowed to swell for 10 min at room temperature. The sample was then dissolved at 60°C, and thereafter, the sample temperature was reduced and maintained at 40°C or 25°C for 20 h.

The water used for preparation and in the experiments was Millipore-purified (resistivity of 18 MW cm) after distillation.

\textbf{Dielectric measurements.} The capacitance was measured in the frequency range from 10\(^0\) Hz to 10\(^7\) Hz with LCR meters (Hewlett-Packard Japan; 4284A and 4285A) equipped with a parallel plate type of cell (Hewlett-Packard Japan; 16452A) to which the specially prepared Pt-Pt electrodes were attached. The dielectric constant, \(\varepsilon\), was calculated by Eq. (1):

\[ C = \varepsilon C_1 + C_s \]  

(1)

where \(C\) is the capacitance; \(C_1\), the cell constant; and \(C_s\), the stray capacitance. Cell constant \(C_1\) and stray capacitance \(C_s\) were determined by using several standard liquids. All measurements were performed at 25°C and 40°C.

\textbf{Analysis of the dielectric relaxation.} The dielectric relaxation data were analyzed by the following equation proposed by Cole and Cole:\textsuperscript{24}

\[ \varepsilon = \varepsilon_\infty + \frac{1}{2} \Delta \varepsilon \left( 1 - \frac{\sinh \beta x}{\cosh \beta x + \cos \frac{\beta}{2} \pi} \right) \]  

(2)

where \(\varepsilon_\infty\) is the high-frequency limit of \(\varepsilon\); \(\Delta \varepsilon\), the dielectric increment; \(\beta\), the Cole-Cole parameter (0<\(\beta\)<1); \(x = \ln(2n\tau)\); \(f\), the frequency; and \(\tau\), the relaxation time. Cole-Cole parameter \(\beta\) is smaller for a broader distribution of relaxation time;\textsuperscript{24} moreover, it is well-known that \(\beta < 1\) when two or more relaxation processes exist in the system.\textsuperscript{24-26}

By fitting the dielectric relaxation data to Eq. (2), parameters \(\beta\), \(\Delta \varepsilon\) and \(\tau\) were obtained.

\textbf{Viscosity measurements.} Relaxation time \(\tau\) for orientation polarization of the dipole moment in a solution is considered to be influenced by the viscosity of the system.\textsuperscript{21-23} Therefore, viscosity \(\eta\) for solutions of BSA and gelatin was measured, using an Ostwald-type viscometer (Kusano Scientific Instrument Mfg. Co.) or a Cannon-Fenske-type viscometer (Sibata Scientific Technology). All measurements were performed at 25°C and 40°C. The value of \(\eta\) enabled the specific viscosity, \(\eta_p\) (\(= (\eta - \eta_0)/\eta_0\); \(\eta\) is the solution viscosity, and \(\eta_0\) is the solvent viscosity) to be calculated as an index of the interaction between protein molecules (note that \(\eta_p\) is proportional to the polymer concentration for a dilute solution).

\textbf{Results} Figure 1 shows examples of the frequency dependence of dielectric constant \(\varepsilon\) for the BSA solutions, the dielectric relaxation being observed. The solid curves in Fig. 1 represent the Cole-Cole equation (Eq. (2)) fitted to the data. All the experimental data on dielectric relaxation are in good agreement with the best-fit curves using Eq. (2).

Dielectric increment \(\Delta \varepsilon\), relaxation time \(\tau\), Cole-Cole parameter \(\beta\), and specific viscosity \(\eta_p\) for the BSA solutions are plotted as a function of BSA concentration in Fig. 2. As shown in Fig. 2(a), \(\Delta \varepsilon\) is proportional to the concentration, probably because the number of the dipole moment in a BSA solution increased with increasing number of BSA molecules. The values of \(\tau\) (Fig. 2(b)) increased with increasing BSA concentration, and those of \(\beta\) (Fig. 2(c)) were from 0.6 to 0.8. As shown in Fig. 2(d), the values of \(\eta_p\) at 25°C and 40°C similarly increased with increasing BSA concentration.

Figure 3 represents the dependence of relaxation time \(\tau\) on BSA solution viscosity \(\eta\). Parameter \(\tau\) is proportional to \(\eta\).

Figure 4 shows examples of the frequency dependence of dielectric constant \(\varepsilon\) for a gelatin solution. The dielectric relaxation was observed, all the experimental data fitting well with Eq. (2).
Fig. 1. Frequency Dependence of Dielectric Constant $\varepsilon$ for BSA Solutions at (a) 40°C and (b) 25°C.
BSA concentration: ○ 0.10 kg/kg of solution; ● 0.05 kg/kg of solution; □ 0.02 kg/kg of solution. The solid curves are the best fit by the Cole-Cole equation, Eq. (2).

Fig. 2. Concentration Dependence of (a) Dielectric Increment $\Delta \varepsilon$, (b) Relaxation Time $\tau$, (c) Cole-Cole Parameter $\beta$, and (d) Specific Viscosity $\eta_s$ for BSA Solutions.
Temperature: ○ 25°C; ● 40°C.
In Fig. 5, the concentration dependence of $\Delta \varepsilon$ (a), $\alpha$ (b), $\beta$ (c), and $\eta_\infty$ (d) for a gelatin solution is presented. As shown in Fig. 5(a), $\Delta \varepsilon$ at 40°C is proportional to the gelatin concentration, while the value of $\Delta \varepsilon$ at 25°C suddenly increases at a concentration above 0.005 kg/kg of solution. In Fig. 5(b), the value of $\alpha$ at 40°C increases slightly with increasing gelatin concentration, while that of $\alpha$ at 25°C increases by about two orders of magnitude. The value of $\beta$ (Fig. 5(c)) is almost constant at 40°C, while the value of $\beta$ at 25°C decreases with increasing gelatin concentration, suggesting that there were more relaxation processes at 25°C than those at 40°C. In Fig. 5(d), the value of $\eta_\infty$ increases more sharply at 25°C than at 40°C with increasing gelatin concentration.

Figure 6 presents the dependence of $\tau$ on gelatin solution viscosity $\eta$. Relaxation time $\tau$ at 40°C is proportional to $\eta$, while the value of $\tau$ at 25°C suddenly increases with a viscosity of more than 1 mPa·s.

**Discussion**

It has been reported that the dielectric relaxation at below 100 MHz of a globular protein solution can be ascribed to orientational polarization. Relaxation time $\tau$ for overall rotation of the spherical molecule in a viscous medium is described by the Debye theory as

$$\tau = \frac{4\pi R^3}{k_B T} \eta_\infty$$

where $R$ is the radius of the spherical particle; $k_B$, the Boltzmann constant; $T$, the absolute temperature; and $\eta_\infty$, the viscosity of the solvent. This equation is based on the assumption that the friction of a spherical particle is proportional to solvent viscosity $\eta_\infty$. Hendrickx et al. have analyzed a BSA solution (0.02 to 0.04 kg/kg of solution) from 20 kHz to 10 MHz; they showed that $\tau$ was proportional to solvent viscosity $\eta$. They adjusted the viscosity of the system by adding sucrose and used the sucrose solution as the "solvent." On the other hand, as can be seen in Fig. 3 of the present study, $\tau$ was proportional to solution viscosity $\eta$. In our present study, such additives as sucrose were not used. Therefore, BSA that was added enhanced the system viscosity in the present study, while in the work of Hendrickx et al., sucrose that was added is considered to have mainly contributed to the system viscosity. If interaction between BSA molecules is not negligible (especially at a high concentration), the friction of each molecule would be proportional to $\eta$. In this respect, the solvent viscosity $\eta_\infty$ in Eq. (3) should be replaced by solution viscosity $\eta$ as follows:
Fig. 5. Concentration Dependence of (a) Dielectric Increment $\Delta e$, (b) Relaxation Time $\tau$, (c) Cole-Cole Parameter $\beta$, and (d) Specific Viscosity $\eta_s$ for Gelatin Solutions.
Temperature: $\circ$ 25°C; $\bullet$ 40°C.

Fig. 6. Viscosity Dependence of Relaxation Time for Gelatin Solutions at (a) 40°C and (b) 25°C.
To confirm the validity of Eq. (4), the value of $R$ for a BSA molecule will be estimated by using this equation. As shown in Fig. 3, the slope of the $\tau$ vs. $\eta$ plot at 40°C is $2.78 \times 10^{-4} \text{m}^2/\text{N}$. By substituting the following values into Eq. (4), $k_B=1.380 \times 10^{-23} \text{J/K}$ and $T=313.15 \text{K}$, the value of $R$ was calculated to be 4.5 nm. This value of $R$ is similar to the literature value\(^\text{27}\) (long axis, 7.5 nm; short axis, 2.0 nm). Consequently, the friction of the BSA molecule is proportional to solution viscosity $\eta$ in our system; the behavior of $\tau$ can thus be explained by Eq. (4).

As can be seen from Fig. 4, the experimental data for the dielectric relaxation of a gelatin solution can be fitted well by the Cole-Cole equation. Fricke and Jacobson\(^\text{16}\) have shown that the frequency dependence of the dielectric constant for a gelatin solution could not be fitted by the Cole-Cole equation, but by a power-law equation. In their study, the measurements were performed at concentrations above 0.01 kg/kg of solution at 21°C. In addition, they used gelatin powder without conducting any deionization, while desalted gelatin was used in the present study. These differences in concentration and the method for sample preparation would have caused the different results between the two studies.

For the solutions of BSA, a globule protein, the concentration dependence of $\beta$ at both 25°C and 40°C (in Fig. 2(c)) was similar. On the other hand, the values of $\beta$ at 25°C were smaller than those at 40°C (in Fig. 5(c)). As already explained, $\beta \ll 1$ when two or more relaxation processes exist in a system.\(^\text{24-26}\) Part of the gelatin chains are in the helix conformation at 25°C, while the chains are in the coil conformation at 40°C.\(^\text{19-22}\) Such formation of a helix structure would make the number of relaxation processes increase and the values of $\beta$ small.

As shown in Fig. 3, $\tau$ of the BSA solution was proportional to $\eta$ at both 25°C and 40°C. Relaxation time $\tau$ of the gelatin solution was also proportional to $\eta$ at 40°C (Fig. 6(a)). In general, relaxation time $\tau$ for the orientation polarization due to polymer chain motion such as overall rotation and fluctuation of end-to-end distance is proportional to viscosity.\(^\text{10,28-40}\) As already stated, the gelatin chains are in the coil conformation above 40°C.\(^\text{24-26}\) The rotation of gelatin and/or polarization of submolecular groups in the coil state would thus greatly contribute to the dielectric relaxation of a gelatin solution at 40°C.

As shown in Fig. 6(b), $\tau$ was not proportional to $\eta$ at 25°C and increased suddenly above 0.005 kg/kg of solution. Pezon et al.\(^\text{29}\) have reported that overlap concentration $c^*$ for a gelatin solution at 50°C was about 0.005 kg/kg of solution by light and small-angle neutron scattering measurements. Djabourov et al.\(^\text{19}\) have observed that a gelatin solution was gelatinized at 0.005 kg/kg of solution by being kept at room temperature for two weeks. In our present study, the value of $\eta_p$ at 25°C increased sharply above 0.005 kg/kg of solution, indicating that the value of $c^*$ for our gelatin solution was about 0.005 kg/kg of solution. In addition, helix growth induces chain association and three-dimensional network formation.\(^\text{19}\) The formation of cross-linking junctions which consist of the helix structure would thus determine the behavior of $\tau$ at 25°C. Above a concentration of 0.005 kg/kg of solution, the value of $\beta$ decreased (Fig. 5(c)); the formation of junction zones would make the number of relaxation processes increase.

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### References

Dielectric Relaxation of a Gelatin Solution


