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트립토판 수용액의 마이크로파 유전 특성분석

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Microwave Dielectric Characterization of Aqueous Solutions of Tryptophan

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요 약. 여러 농도 및 온도에서 트립토판수용액의 유전이완이 논문에 기술되었다. 반사모드에서 시간영역측정학을 사용해 10 Mhz - 20 GHz 주파수 영역에서 복소수인 반사계수 $\rho^*(\omega)$를 측정하였다. 이로부터 쌍일차 검정방법을 사용해 복소수인 유전율스팩트럼 $\varepsilon^*(\omega)$을 얻었다. 유전율스팩트럼을 비선형 최소제곱법 피팅을 통해 유전변수인 정유전율 및 이완시간을 얻었다. 이완시간 값으로부터 열역학변수들을 얻었다. 유전이완변수 값들은 트립토판 몰농도가 증가함에 따라서 증가하였다.

주제어: 시간영역반사학, 트립토판, 유전이완

ABSTRACT. This paper describes dielectric relaxation in aqueous solutions of tryptophan at different temperatures and concentrations. Time Domain Reflectometry in reflection mode has been used to measure the complex reflection coefficient $\rho^*(\omega)$ in the frequency range of 10 MHz to 20 GHz. Bilinear calibration method has been used to obtain complex permittivity spectra $\varepsilon^*(\omega)$ from the complex reflection coefficient. Dielectric parameters i.e. static permittivity and relaxation time were obtained from the complex permittivity spectra using nonlinear least squares fit method. From the values of relaxation time, thermodynamic parameters were obtained. The values of dielectric relaxation parameters increase with an increase in molar concentration of Tryptophan.

Keywords: Time Domain Reflectometry, Tryptophan, Dielectric Relaxation

INTRODUCTION

Aqueous solutions are fundamentally involved in the chemical processes of life and all of biological chemistry takes place in an aqueous environment. Aqueous solutions are perplexing since they adopt some of the eccentric behavior of water. The eccentricities of aqueous solutions are closely tied to the structure and dynamics of the liquid. Amino acids are the basic building blocks of proteins and are considered to be the model compound of proteins. In view of the role of water in the structural and functional properties of macromolecules and their interactions, much attentions has been paid to the

properties of water in aqueous biomolecular system.[1-37] Several theoretical studies on environmental effects on the molecular structure of amino acids have been performed.[2-8] There are various investigations reporting structure, stability and solvent effect on amino acid.[2,6-23] The dielectric relaxation in aqueous solutions of some amino acids has also been reported earlier.[24-37] However, to the best of our knowledge, the dielectric relaxation in aqueous solutions of tryptophan has not been studied so far. The objective of the present paper is to report the dielectric properties of aqueous solutions of Tryptophan over the frequency range of 10 MHz to 20 GHz using Time Domain Reflectometry (TDR) technique at various temperatures from 25°C to 40°C and different concentration. This paper is structured as follows : The next section gives the experimental details. Results are discussed in section III. Conclusions are inferred in section IV.

EXPERIMENTAL

Tryptophan in powder form was commercially obtained from Spectrochem, India and used without further purification. Distilled and deionized water was used to prepare solutions. Considering solubility at 25°C and molecular weight of Tryptophan, 10 ml stock solution was prepared. The solutions for five different molar concentrations of Tryptophan were prepared at room temperature.

The basic TDR setup consists of broadband sampling oscilloscope, TDR module, coaxial transmission line and sample cell. Hewlett Packard's HP 54750A sampling oscilloscope with 20 GHz bandwidth and TDR module HP 54754A with step generator and sampling head is used. A 200 mV step pulse with 40 ps rise time and 250 KHz repetition rate passes through flexible coaxial 50 Ω line, of 1 meter length, to SMA (standard military application) sample cell. The SMA sampling cell has 50 Ω input impedance and 1.35 mm effective pin length. All measurements were carried out in open load condition. Sampling oscilloscope monitors changes in step pulse after reflection from sample cell. Reflected pulse without sample $R_i(t)$ and with sample $R_x(t)$ were recorded in time window of 5 ns and digitized in 1024 points. The temperature of sample was maintained at desired value, within accuracy limit of ±1°C, by circulating constant temperature water through heat insulating jacket surrounding sample cell.

The step pulses recorded without sample $R_i(t)$ and with sample $R_x(t)$ are subtracted and added to get

$$p(t) = [R_i(t) - R_x(t)] \quad (1)$$

$$q(t) = [R_i(t) + R_x(t)] \quad (2)$$

Reflection coefficient spectra $\rho^*(\omega)$ over the frequency range of 10 MHz to 20 GHz is obtained as

$$\rho^*(\omega) = \frac{c}{j\omega d}\frac{p(\omega)}{q(\omega)} \quad (3)$$

where $p(\omega)$ and $q(\omega)$ are Fourier transforms of $p(t)$ and $q(t)$ obtained using summation and Samulon methods respectively. c is velocity of light, ω is angular frequency and d is effective pin length (1.35 mm). The complex permittivity spectra $\varepsilon^*(\omega)$ is obtained from complex reflection coefficient $\rho^*(\omega)$ by using bilinear calibration method.[38]

RESULTS AND DISCUSSIONS

The general form of the relaxation model is given by the Havriliak-Negami equation[39]

$$\varepsilon^*(\omega) = \varepsilon_\infty - \frac{(\varepsilon_0 - \varepsilon_\infty)}{[1 + (j\omega\tau)^{(1-\alpha)}]^\beta} \quad (4)$$

where ε_0 is the static permittivity, ε_∞ is the permittivity at high frequency, τ is the relaxation time, α and β are the empirical parameters for the distribution of relaxation times with values between 0 and 1. The Havriliak-Negami equation includes three relaxation models as limiting forms. The Debye model (α=0 and β=1) implies a single relaxation time while the Cole-Cole ($0 \le \alpha \le 1$ and β=1) and Cole-Davidson (α=0 and $0 \le \varepsilon \le 1$) models both suggest a distribution of relaxation times. The magnitudes of α and β indicate the width of the distribution. The aqueous solutions of Tryptophan at all molar concentration of Tryptophan in this study display Debye type dispersion. Therefore, here α=0 and β=1 and the experimental values of $\varepsilon^*(\omega)$ were fit-

ted to the Debye equation.

$$\varepsilon^*(\omega) = \varepsilon_\infty - \frac{\varepsilon_0 - \varepsilon_\infty}{(1 + j\omega\tau)} \tag{5}$$

As the frequency range of dielectric study in present work is from 10 MHz to 20 GHZ, the value of (ε_∞) is just a fitting parameter. This value does not correspond to real value of permittivity which one gets after completion of dispersion processes related to vibrational and electronic motion in liquid. It is found reasonably satisfactory procedure to keep value of (ε_∞) fix. The value of (ε_∞) is kept fix as 4.0 for all the systems studied here. A non-linear least squares fit method was used to determine the values of dielectric parameters as follows.

1) First estimate the initial values of fit parameters ε_0, ε_∞, τ
2) Compute theoretical values of ε_i^* at frequencies ω_i from Eq. (5).
3) Compute the value of χ

$$\chi = \sum_{i=1}^{N} \left| \varepsilon_i^{*exp} - \varepsilon_i \right|^2 \tag{6}$$

where N is number of frequencies. There were 220 frequencies points in the frequency range of 10 MHz to 20 GHz.

4) Determine the values of $\delta\varepsilon_0$, $\delta\varepsilon_\infty$, $\delta\tau$ by using the following matrix expression,

$$\begin{pmatrix} \varepsilon^{*exp}(\omega_1) - \varepsilon^*(\omega_1) \\ \vdots \\ \varepsilon^{*exp}(\omega_i) - \varepsilon^*(\omega_i) \\ \vdots \\ \varepsilon^{*exp}(\omega_N) - \varepsilon^*(\omega_N) \end{pmatrix} = \begin{pmatrix} \frac{\partial\varepsilon^*(\omega_1)}{\partial\varepsilon_0} & \frac{\partial\varepsilon^*(\omega_1)}{\partial\tau} & \frac{\partial\varepsilon^*(\omega_1)}{\partial\varepsilon_\infty} \\ \cdot & \cdot & \cdot \\ \frac{\partial\varepsilon^*(\omega_i)}{\partial\varepsilon_0} & \frac{\partial\varepsilon^*(\omega_i)}{\partial\tau} & \frac{\partial\varepsilon^*(\omega_i)}{\partial\varepsilon_\infty} \\ \cdot & \cdot & \cdot \\ \frac{\partial\varepsilon^*(\omega_N)}{\partial\varepsilon_0} & \frac{\partial\varepsilon^*(\omega_N)}{\partial\tau} & \frac{\partial\varepsilon^*(\omega_N)}{\partial\varepsilon_\infty} \end{pmatrix} \begin{pmatrix} \delta\varepsilon_0 \\ \vdots \\ \delta\tau \\ \vdots \\ \delta\varepsilon_\infty \end{pmatrix} \tag{7}$$

The values of derivatives (Jacobian) in Eq. (7) are evaluated exactly by using Eq. (5).

5) Compute new values of ε'_0, ε'_∞ and τ' by using the values of $\delta\varepsilon_0$, $\delta\varepsilon_\infty$ $\delta\tau$ by using the expressions:

$$\begin{aligned} \varepsilon'_0 &= \varepsilon_0 + \delta\varepsilon_0 A_1 \\ \varepsilon'_\infty &= \varepsilon_\infty + \delta\varepsilon_\infty A_2 \\ \tau' &= \tau' + \delta\tau A_3 \end{aligned} \tag{8}$$

where A_1, A_2 and A_3 are constants with their values less than one. Their values are chosen such that the new values of χ' should be less than χ, where χ' is calculated value of χ from Eq. (6) by taking new

Table 1. Dielectric relaxation parameters for aqueous solutions of Tryptophan at different temperatures and molar concentration of tryptophan. The number in bracket indicates error. For e.g. 80.08(2) means 80.08±0.02.

Molar Concentration of Tryptophan	ε_0	τ (ps)	ε_0	τ (ps)
	25°C		30°C	
0	79.11(0)	10.27(0)	76.00(0)	8.24(0)
0.01	80.08(2)	11.55(2)	77.16(2)	10.26(2)
0.02	81.61(3)	12.80(3)	78.92(1)	11.10(3)
0.03	82.14(1)	13.60(1)	79.25(3)	12.62(2)
0.04	83.21(2)	15.33(2)	80.09(1)	14.64(1)
0.05	84.33(2)	16.62(2)	82.48(2)	16.22(2)
	35°C		40°C	
0	75.10(0)	7.69(0)	71.50(0)	7.21(0)
0.01	76.57(2)	8.34(2)	70.85(2)	7.85(4)
0.02	78.96(3)	9.86(1)	71.94(3)	7.79(2)
0.03	78.49(1)	10.84(2)	73.24(1)	10.4(2)
0.04	79.24(1)	12.66(3)	73.65(1)	11.15(1)
0.05	80.64(2)	14.60(2)	75.19(3)	12.72(1)

values of parameters from Eq. (8).

6) One may use iterative process by taking these new values of ε'_0, ε'_∞ and τ' as new estimated values and repeat the process from step 2. One may continue this iterative process till one gets value of $(\chi'-\chi)$ less than some desired limit.

The temperature dependent dielectric relaxation parameters for aqueous solutions of Tryptophan are tabulated in *Table* 1. For the aqueous solutions of Tryptophan, the static permittivity and relaxation time both increase with an increase in molar concentrations of Tryptophan and decrease with an increase in temperature. The strong electrostatic interactions between Tryptophan dipolar ions and the water molecules can be expected since the dipole moment for the Tryptophan dipolar ion is much greater than that of ordinary polar molecules. Kirkwood[40] described such an amino acid dipolar ion as a superpolar molecule surrounded by an intense electrostatic field. It is expected that the permittivity of aqueous solutions of Tryptophan should be large due to large dipole moment of Tryptophan. This is confirmed by present work where Tryptophan produces remarkable permittivity increment in aqueous solution. Similar trend of increase in static permittivity values with an increase in molar concentration of amino acid was observed earlier for aqueous solutions of isoleucine,[34] glycine,[32] valine,[34] alanine,[35] and phenylalanine.[35] These studies also reported only one relaxation peak and the dielectric spectra were well fitted with the Debye model. The decrease in values of static permittivity and relaxation time with an increase in temperature in this study may be due to decrease in an orientational correlation of dipole moments with an increase in temperature.

On comparing the values of relaxation time for aqueous solutions of Tryptophan at different molar concentration of Tryptophan with that for pure water, it is observed that former are larger than that for the latter. The values of relaxation time are increased with an increase in molar concentration of Tryptophan than that for pure water. It indicates the shifting of the absorption peak (f_{max}) to lower frequency with increase in molar concentration of

Table 2. Thermodynamic parameters for aqueous solutions of Tryptophan

Molar concentration of Tryptophan	Molar entropy (ΔH^*) in kJ/ mole	Molar enthalpy (ΔS^*) J/mole K
0	15.06(3)	0.24
0.01	18.53(2)	0.25
0.02	24.49(6)	0.27
0.03	27.13(6)	0.28
0.04	32.38(3)	0.29
0.05	26.10(2)	0.27

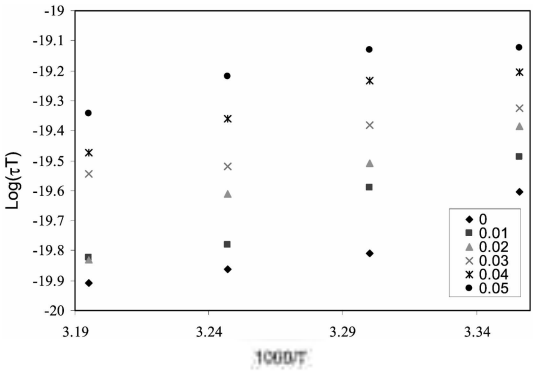

Fig. 1. Eyring rate plot for different molar concentration of Tryptophan.

Tryptophan. It can be said that in aqueous solution of Tryptophan, water forms clatharate like (cage like) hydration region with enhanced relaxation times around the solute. The higher values of relaxation may be due to strong solute-solvent interaction and seem to reflect the formation of hydrogen bond.[34,35] As the temperature increases the dielectric parameters are decreased. It indicates that the orientational motions of dipolar water molecules are hindered by interactions with neighbors in particular by hydrogen bonding.

The enthalpy of activation is defined as the barrier to be surmounted in order to achieve the group dipole reorientation in the relaxation processes whereas the entropy of a system is a measure of its orderly nature. The molar enthalpy of activation ΔH^* and molar entropy of activation ΔS^* at different concentrations are also determined using the Eyring rate equation[41]

$$\tau = (h/kT) \exp [(\Delta H^* - T\Delta S^*)/RT)] \qquad (9)$$

Here, h is the Planck constant, k is the Boltzmann constant, T is the absolute temperature, τ is the relaxation time and R is the gas constant. The molar enthalpy of activation and molar entropy of activation are listed in *Table* 2. *Fig.* 1 shows the Eyring rate plot for different molar concentration of Tryptophan. The temperature dependence of the relaxation times follows the Arrhenius behavior. The values of molar enthalpy of activation (ΔH*) for all molar concentration of Tryptophan are positive indicating endothermic interactions. An increase in values of ΔH* for all molar concentration of Tryptophan than that for the pure water indicates that the dipoles require more energy in order to attain equilibrium with applied field and hence the ΔH* values increase with an increase in molar concentration of Tryptophan. It also indicates that the enthalpy depends upon the local environment of the molecule. The positive values of molar entropy of activation (ΔS*) for all molar concentrations of Tryptophan indicate that the activated state is less ordered than normal state.

CONCLUSIONS

We have studied the dielectric relaxation in aqueous solutions of Tryptophan. The values of the static permittivity and relaxation time for aqueous solutions of Tryptophan increase with an increase in molar concentration of Tryptophan. The large dipole moment of such biomolecule should, according to the theory of Debye, result in large permittivity values for these solutions. This is confirmed by the present work. The values of molar enthalpy of activation and molar entropy of activation indicate strong hydrogen bonding and endothermic interactions. The solvent structure is modified by the solute.

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