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# Calculation of Dynamic Properties of Drug-Added Aqueous Solutions with $T_1$ and $T_2$ Relaxation Times

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**Abstract**

The development of modern spectroscopic methods has facilitated and accelerated structure analysis. The NMR method is the most popular way to perform structural analysis of compounds with very complex structures. $D_2O$ is a solvent that is frequently used in NMR analysis of both chemical molecules and many biological molecules such as drugs, proteins, and enzymes. In this paper, the study of residual water in proton drug-added protein solutions was carried out via NMR relaxation. The spin-lattice ($T_1$) and the spin-spin relaxation ($T_2$) times of residual water in drug-added protein solutions were studied depending on temperature by Avance Bruker 400 MHz $^1$H-NMR Spectrometer, and activation energies ($E_a$) and rotational correlation times ($\tau_0$ and $\tau_c$) have been determined for $T_1$ and $T_2$ relaxation times.

***Keywords:*** $D_2O$; Proton NMR; Relaxation times; Activation energy; Flurouacil.

## İlaç Katkılı Sulu Çözeltilerin Dinamik Özelliklerinin $T_1$ ve $T_2$ Rölaksasyon Zamanları ile Hesaplanması

**Öz**

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Modern spektroskopik yöntemlerin gelişimi, yapı analizini kolaylaştırmış ve hızlandırmıştır. NMR yöntemi, çok karmaşık yapılara sahip bileşiklerin yapısal analizini gerçekleştirmenin en popüler yoludur. $D_2O$, hem kimyasal moleküllerin hem de ilaçlar, proteinler, enzimler gibi birçok biyolojik molekülün NMR analizinde sıklıkla kullanılan bir çözücüdür. Bu çalışmada, ilaç katkılı protein çözeltilerinde residual su çalışması, proton NMR rölaksasyonu yoluyla gerçekleştirilmiştir. Avance Bruker 400 MHz $^1$H-NMR Spektrometresi ile ilaç katkılı protein çözeltilerinde residual suyun spin-örgü ($T_1$) ve spin-spin rölaksasyon ($T_2$) süreleri sıcaklığa bağlı olarak ölçülmüş, $T_1$ ve $T_2$ rölaksasyon süreleri için aktivasyon enerjileri ($E_a$) ile $\tau_0$ ve $\tau_c$ dönme korelasyon zamanları belirlenmiştir.

***Anahtar Kelimeler*:** $D_2O$; Proton NMR; Rölaksasyon zamanları; Aktivasyon enerjisi; Flurouacil.

## 1. Introduction

The contribution of magnetic resonance to science and technology is one of the greatest discoveries of the century in the field of spectroscopy. Nuclear magnetic resonance technique (NMR) is a spectroscopic technique commonly used by researchers in many different fields to reveal the structures of chemicals, especially organic molecules, in liquid or solid form. After successful experiments by Purcell, Pound, Torrey at Harvard and Bloch, Hansen, Packard at Stanford in 1946, the field of application of magnetic resonance developed rapidly. The theoretical and technical development of this phenomenon, whose basic formulation is summarized by Abragam [1], is still ongoing.

The application of NMR relaxation times to understand the moving mechanisms of molecules in aqueous solutions has become an indispensable and frequently used method. The relaxation rates of water molecules are influenced by the local magnetic interactions between the nuclei of the water and the rate of molecular motion and proton exchange [2]. There are two mechanisms for the relaxation rates of water molecules: spin-lattice and spin-spin.

The relaxation time $T_1$, which defines the return to equilibrium in the magnetic field direction, is also known as spin-lattice relaxation or longitudinal relaxation and can be measured from the magnetization creation along the applied static magnetic field. $T_1$ relaxation can also be measured by the decay of a signal that creates a rotating magnetic field around the resonance frequency perpendicular to the static magnetic field. $T_1$ longitudinal or spin-lattice relaxation may be more sensitive to intramolecular mobility on flexible substrates, as it is associated with general rotational of the molecule in solution. The standard method for measuring $T_1$ is known as reversal recovery.

The relaxation time $T_2$, which describes the decay of induced magnetization perpendicular to the applied magnetic field, is also called spin-spin relaxation or transverse relaxation. It is known that the observed spectral line width is associated with the $T_2$ relaxation time, that is, spin-spin relaxation, and is also affected by magnetic inhomogeneity. The relaxation time $T_2$ is used to determine the decay rate of the magnetization in the xy plane. $T_1$ and $T_2$ relaxation times are long in rapidly rotating and small molecules such as free water. As in the case of proteins, as molecular motion slows down, $T_2$ relaxation gets shorter while $T_1$ increases. As a result, $T_2$ relaxation is faster than $T_1$ relaxation, and however, the $T_1$ relaxation time always takes a value longer than or equal to $T_2$ [3].

The rotational correlation time ($\tau_c$), used in molecular-scale viscosity measurements and protein characterization, refers to the average time it takes for a molecule to rotate one radian, and its value in solutions is on the order of picoseconds and is known to be 1.7 ps for water [4]. The rotational correlation time can be determined for a molecule by $T_1$ and $T_2$ relaxation times and can be measured by methods such as microwave, dielectric, and nuclear magnetic resonance NMR spectroscopy [5, 6].

The most widely known mechanism is the magnetic dipole-dipole interaction. In this interaction, the mechanism between a nucleus and another nucleus or the magnetic moment of another environment such as the electron, atom, ion, or molecule is considered. Besides magnetic dipole-dipole interaction, there are different mechanisms for relaxation such as Chemical shift anisotropy (CSA) relaxation, Spin rotation (SR) relaxation mechanism, and quadrupole relaxation mechanism. In addition to these mechanisms, molecular reorientation or tumbling and the electrostatic interaction between nuclei can also lead to spin transitions and relaxation.

By analyzing aqueous solutions with NMR relaxation techniques, important data about rotational motion, molecular interactions and structure can be obtained. It was mentioned before that $T_2$ is smaller than $T_1$. The $T_1$ and $T_2$ curves overlap in the region called the extreme narrowing limit ($\omega^2 \tau_c^2 << 1$) which includes small rotational correlation times [7-14]. The expression “extreme narrowing” describes rapid molecular rotation on the order of picoseconds and simplifies the equations of rotational correlation [15].

Using proton resonance, the water dynamics has been extensively investigated for low frequencies by NMR relaxation methods, and it has been thought that the use of proton resonance is a major disadvantage [2-7]. However, several biological molecules, especially

proteins and enzymes have been studied in $D_2O$ to decrease the water in the environment and increase the effectiveness of the bound water on the protein [16-25].

$D_2O$ is one of the most important and widely preferred solvents used in NMR studies of chemical molecules. The standard water ($H_2O$) ratio in this solvent, which is tried to be purified but cannot be 100% pure, contains some residual hydrogen, which is called residual water.

The hydrogen-deuterium exchange reaction (H-D or H/D Exchange) between residual water molecules and $D_2O$ is written as follows.

$$H_2O + D_2O \Leftrightarrow 2HDO$$

This expression indicates that the residual water in $D_2O$ is in the HDO form. Therefore, it is very important to investigate HDO relaxation for information about the relaxation mechanism in drug-protein containing solutions.

Since the residual water in $D_2O$ is in the form of HDO, investigation of HDO relaxation is valuable for understanding the mechanism of relaxation in several protein solutions. [26-30]. To easily calculate the input parameters of the chemical change-exchange formula, the proton relaxation of residual water must be known.

In this paper, to investigate residual water in drug-added protein solutions, proton NMR relaxation methods have been used. $T_1$ and the $T_2$ relaxation times of residual water in drug-added protein solutions were studied depending on temperature by Avance Bruker 400 MHz 1H-NMR Spectrometer, and activation energies ($E_a$) and rotational correlation times ($\tau_0$ and $\tau_c$) have been calculated for $T_1$ and $T_2$ relaxation times.

## 2. Theoretical Background

$T_1$ relaxation time is also called spin-lattice or longitudinal relaxation time. This relaxation time is a measure of the energy transfer from the core spin system to neighboring molecules or lattices. This relaxation takes place in the z-axis and causes the Boltzmann equilibrium to change.

The states of a dipole pair in a water molecule exposed to the external field $B_0$ applied in the z direction are as follows.

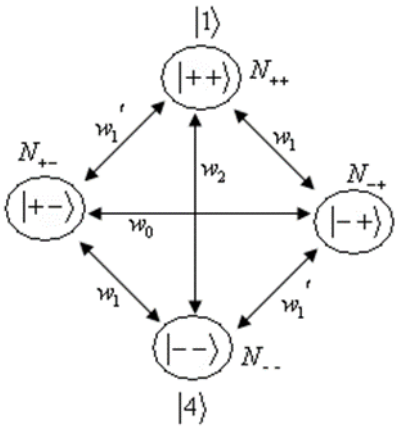


**Figure 1**: States of a dipole pair in a water molecule exposed to an external field in the z-direction

Figure 1 showing the eigenstates of $I_z$ with respect to a spin pair are valid for all pairs in the example. Therefore, the population of each condition is shown above. If an RF field is applied to the water sample, there will be transitions between the above levels and saturation will occur. In other words, the magnetization in the z direction lies in the y direction. After the RF is cut off, the dipolar hamiltonian, which occurs due to the interaction of a couple among themselves, leads to transitions between states and eventually reaches the Boltzmann equilibrium. The dipolar hamiltonian leading to transitions between states can be written as;

$$H_D(t) = \left[I_z^{(1)}I_z^{(2)} - \tfrac{1}{4}(I_+^{(1)}I_-^{(2)} + I_-^{(1)}I_+^{(2)})\right]F_0(t) + [I_-^{(1)}I_z^{(2)} + I_z^{(1)}I_-^{(2)}]F_1(t) + [I_+^{(1)}I_z^{(2)} + I_z^{(1)}I_+^{(2)}]F_{-1}(t) + [I_-^{(1)}I_-^{(2)}]F_2(t) + [I_+^{(1)}I_+^{(2)}]F_{-2}(t) \quad (1)$$

Here, the terms in square brackets denote the operators, while the terms abbreviated as F denote the time-dependent parts of the hamiltonian. It is known that the F terms expressing the time-dependent parts of the Hamiltonian are written as follows.

$$F_0(t) = \mathrm{d}\,(1 - 3cos^2\theta) \qquad d = \frac{\mu_0\ \gamma_1\gamma_2\hbar^2}{4\pi b^3}$$

$$F_1(t) = -\tfrac{3}{2}d\ \sin\theta\cos\theta\exp\ (i\phi) \qquad F_{-1}(t) = F_1(t)^* \quad (2)$$

$$F_2(t) = -\frac{3}{4}d\ \sin^2\theta\exp\ (\ 2i\phi) \qquad F_{-2}(t) = F_2(t)^*$$

To examine the transitions between the $|4\rangle$ and $|1\rangle$ states shown in Figure 1, it will be useful to start with Perturbation theory by considering the perturbation calculation between paired spins that Solomon's work in the past [13]. Accordingly, the transition probability between states $|4\rangle$ and $|1\rangle$can be written as,

$$a_{41}=\frac{1}{i\hbar}\int_0^t\langle 1|H'(t')|4\rangle\exp\left[i(\omega_1+\omega_2)t'\right]dt' \quad (3)$$

Continuing the quantum mechanical calculations and considering the $R_{22}(\tau)$ autocorrelation function $w_{41}$ transition probability per unit time is written as,

$$w_2=\frac{1}{\hbar^2}\int_{-t}^{t}R_{22}(\tau)\,exp[-i(\omega_1+\omega_2)\tau]\,d\tau=\frac{2}{\hbar^2}\langle|F_2(t)|^2\rangle\frac{\tau_c}{1+(\omega_1+\omega_2)^2\tau_c^2}=\frac{1}{\hbar^2}J_2(\omega_1) \quad (4)$$

As can be seen from Eqn. (4), only $F_2$ is included in the process. That's why $w_{41}$ is called $w_2$. If the transitions between other levels are calculated in a similar way, the following equations are obtained.

$$w_0=\frac{1}{8\,\hbar^2}\,\langle|F_0(t)|^2\rangle\,\frac{\tau_c}{1+(\omega_1-\omega_2)^2\,\tau_c^2} \quad (5)$$

$$w_1=\frac{1}{2\,\hbar^2}\,\langle|F_1(t)|^2\rangle\,\frac{\tau_c}{1+\omega_1{}^2\,\tau_c^2} \quad (6)$$

$$w_1{}'=\frac{1}{2\,\hbar^2}\,\langle|F_1(t)|^2\rangle\,\frac{\tau_c}{1+\omega_2{}^2\,\tau_c^2} \quad (7)$$

The importance of dipolar interaction in NMR relaxation has been proven in the past work of Bloembergen, Purcell and Pound [14]. By substituting these equations in $\frac{1}{T_1}=2\,(w_1+w_2)$ and $\frac{1}{T_1}=w_0+2\,(w_1+w_2)$ written for the relaxation time $T_1$ in similar spin and different spin states, $\frac{1}{T_1}$ relaxation rates are obtained for similar spin and different spin states, respectively.

$$\frac{1}{T_1}=\frac{6}{20}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\gamma^4\,\hbar^2}{b^6}\left(\frac{\tau_c}{1+\omega^2\,\tau_c^2}+\frac{4\tau_c}{1+4\,\omega^2\,\tau_c^2}\right) \quad (8)$$

$$\frac{1}{T_1}=\frac{1}{10}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\gamma_1^2\,\gamma_2^2\,\hbar^2}{b^6}\left(\frac{\tau_c}{1+(\omega_1-\omega_2)^2\,\tau_c^2}+\frac{3\,\tau_c}{1+\omega_2{}^2\,\tau_c^2}+\frac{6\tau_c}{1+(\omega_1+\omega_2)^2\,\tau_c^2}\right) \quad (9)$$

It is known that after an RF pulse is applied to a system, the magnetization lies in the y direction and this magnetization goes to zero by dephasing, and the process of this magnetization to zero is equal to the $T_2$ time. Therefore, $T_2$ is also called dephasing time. The same process followed to calculate the $T_1$ time in a dipole pair sample in a water molecule under the external field effect can also be followed to calculate the $T_2$ time. For similar spins and different spins, the equations for $T_2$ are written as follows, respectively.

$$\frac{1}{T_2}=2\,(u_1+u_2) \quad (10)$$

$$\frac{1}{T_2} = (u_0 + 2\ u_1 + u_2) \tag{11}$$

Considering Fig.1, the diagram showing all states and the transition probabilities between these states, by using the transition probabilities $u_0$, $u_1$ and $u_2$between states calculated for similar spins and different spins; $\frac{1}{T_2}$ relaxation rate for the two different situations are respectively written as follows.

$$\frac{1}{T_2} = \ 2\ (u_1 + u_2)\ = \frac{3}{20}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma^4\ \hbar^2}{b^6}\left(\ 3\ \tau_c\ + \frac{5\ \tau_c}{1+\omega^2\ \tau_c^2}\ + \frac{2\tau_c}{1+4\ \omega^2\ \tau_c^2}\right) \tag{12}$$

$$\frac{1}{T_2} = u_0 + 2\ u_1 + u_2 = \frac{1}{20}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_1^2\ \gamma_2^2\ \hbar^2}{b^6}\left(\begin{matrix}4\ \tau_c + \frac{\tau_c}{1+(\omega_1-\omega_2)^2\ \tau_c^2}\ + \frac{3\ \tau_c}{1+{\omega_1}^2\ \tau_c^2} \\ + \frac{6\ \tau_c}{1+{\omega_2}^2\ \tau_c^2} + \frac{6\tau_c}{1+(\omega_1+\omega_2)^2\ \tau_c^2}\end{matrix}\right) \tag{13}$$

Considering Eqns. (9) and (13), $T_1$ and $T_2$ relaxation rates because of dipolar interaction between hydrogen (H) and deuterium (D) were determined by Solomon-Bloembergen.

$$\frac{1}{T_1} = \frac{1}{10}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_D^2\ \gamma_H^2\ \hbar^2}{b^6}\left(\frac{\tau_c}{1+(\omega_D-\omega_H)^2\ \tau_c^2}\ + \frac{3\ \tau_c}{1+{\omega_D}^2\ \tau_c^2}\ + \frac{6\tau_c}{1+(\omega_D+\omega_H)^2\ \tau_c^2}\right) \tag{14}$$

$$\frac{1}{T_2} = \frac{1}{20}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_D^2\ \gamma_H^2\ \hbar^2}{b^6}\left(\begin{matrix}4\ \tau_c + \frac{\tau_c}{1+(\omega_D-\omega_H)^2\ \tau_c^2}\ + \frac{3\ \tau_c}{1+{\omega_D}^2\ \tau_c^2}\ + \frac{6\ \tau_c}{1+{\omega_H}^2\ \tau_c^2} \\ + \frac{6\tau_c}{1+(\omega_D+\omega_H)^2\ \tau_c^2}\end{matrix}\right) \tag{15}$$

In Eqns. (14) and (15), $\gamma_D$ and $\gamma_H$ are the deuteron and proton gyromagnetic ratio, respectively, b is the distance between the H and D spins, $\hbar$ is Planck's constant divided by 2π, $\tau_c$ is rotational correlation time, $\omega_H$ and $\omega_D$ are Larmor frequencies of proton and deuteron, respectively and $\mu$ is the magnetic permeability.

The rotational correlation time $\tau_c$ in Eqns. (14) and (15) is given by the Arrhenius equation as follows.

$$\tau_c = \tau_0\ exp(E_a/RT) \tag{16}$$

Here, $E_a$ is the activation energy for molecular motions, R is the gas constant, and T is the temperature, $\tau_0$ and $\tau_c$ are rotational correlation time constant and rotational correlation time, respectively.

It is known that the $T_1$ and $T_2$ times in Eqns. (14) and (15) are equal to each other in the case of extreme narrowing, expressed as $\omega^2 \tau_c^2 << 1$. In this case, Eqns. (14) and (15) turn into Eqn. (17).

$$\frac{1}{T_1} = \frac{1}{T_2} = \left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_D^2 \gamma_H^2 \hbar^2}{b^6}\right) \tau_c \tag{17}$$

Using Eqns. (16) and (17), Eqn. (18) is obtained.

$$\frac{1}{T_1} = \frac{1}{T_2} = \left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_D^2 \gamma_H^2 \hbar^2 \tau_0}{b^6}\right) exp(E_a/RT) \tag{18}$$

Here, assuming that $A = \left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_D^2 \gamma_H^2 \hbar^2 \tau_0}{b^6}\right)$ the Eqn. (18) is written as follows.

$$\frac{1}{T_1} = \frac{1}{T_2} = A \ exp(E_a/RT) \tag{19}$$

$$ln\, T_1 = ln\, T_2 = ln\frac{1}{A} - \left(\frac{E_a}{R}\right)\left(\frac{1}{T}\right) \tag{20}$$

According to Eqn. (20), it is seen that there is a linear dependence between $ln\, T_1$ and $\left(\frac{1}{T}\right)$ and it also has a slope with a negative sign.

**3. Experimental**

The most commonly used deuterated solvent in NMR experiments is $D_2O$. All chemicals used in this study were obtained from the Sigma-Aldrich catalog, together with the high purity $D_2O$ (99.9%) used as the solvent in this study.

Albumin, the most abundant protein in human serum, is a monomeric structure with a molecular weight of 66 kD, consisting of three helical segments, each divided into two subregions, and has many ligand-binding abilities [31]. The most important factor affecting the distribution of intravenous drugs is the binding affinity of that drug to albumin in human blood serum, and therefore, the interaction between the drug and albumin must be optimal for the drug to reach the target organ and provide an effective treatment.

5-Fluorouracil, which is the active ingredient of the drug named Biosyn, is a fluorinated pyrimidine derivative and is a chemotherapy drug belonging to the group of antimetabolites.

Spin-Lattice Relaxation Times ($T_1$) and Spin-Spin Relaxation Times ($T_2$) were measured with a 400 MHz Bruker NMR Spectrometer. Measurements of Spin-Lattice Relaxation Times were performed with the Inversion Recovery technique using (180-τ-90) pulse steps.

Measurements of Spin-Spin Relaxation Times were carried out using CPMG (Carr-Purcell-Meiboom-Gill) technique with (90-τ-180-τ) pulse steps. After the solutions were prepared, they were placed in 5 mm NMR tubes to make NMR measurements. The delay times τ required for each measurement were chosen to match each peak observed in the spectrum. Temperature dependence measurements were made at 25, 30, 35, 40, 45 $^0$C values by increasing the sample temperature by 5 $^0$C.

## 4. Results and Discussion

The $^1$H- NMR spectrum of FU solution for relaxation time measurements is given in Fig. 2. As seen in Fig. 2, the peak that appears as a single narrow line is the HDO peak at 4.7044 ppm and a doublet peak at 7.5978 ppm and 7.5845 ppm. The peak of the CH molecule seen in the chemical formula of FU was split in half due to the neighboring HN molecule and formed a doublet. The distance between the peaks of this doublet gives the coupling constant (j).

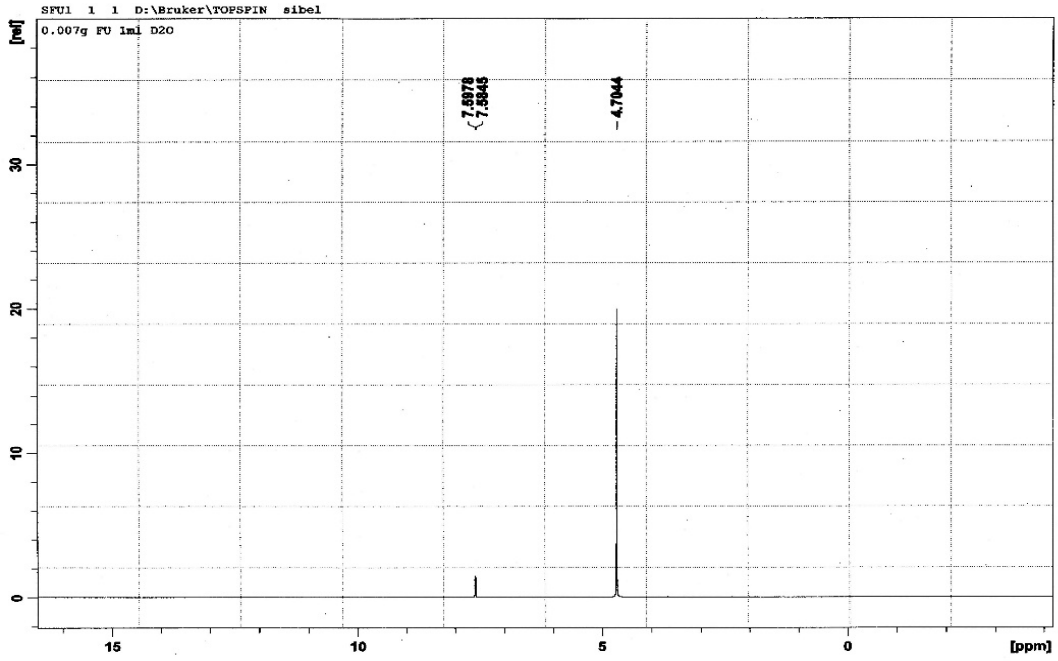


**Figure 2:** $^1$H- NMR spectrum of FU in $D_2O$

$T_1$ and $T_2$ relaxation times graphs are shown in Fig. 3 a and b, respectively.

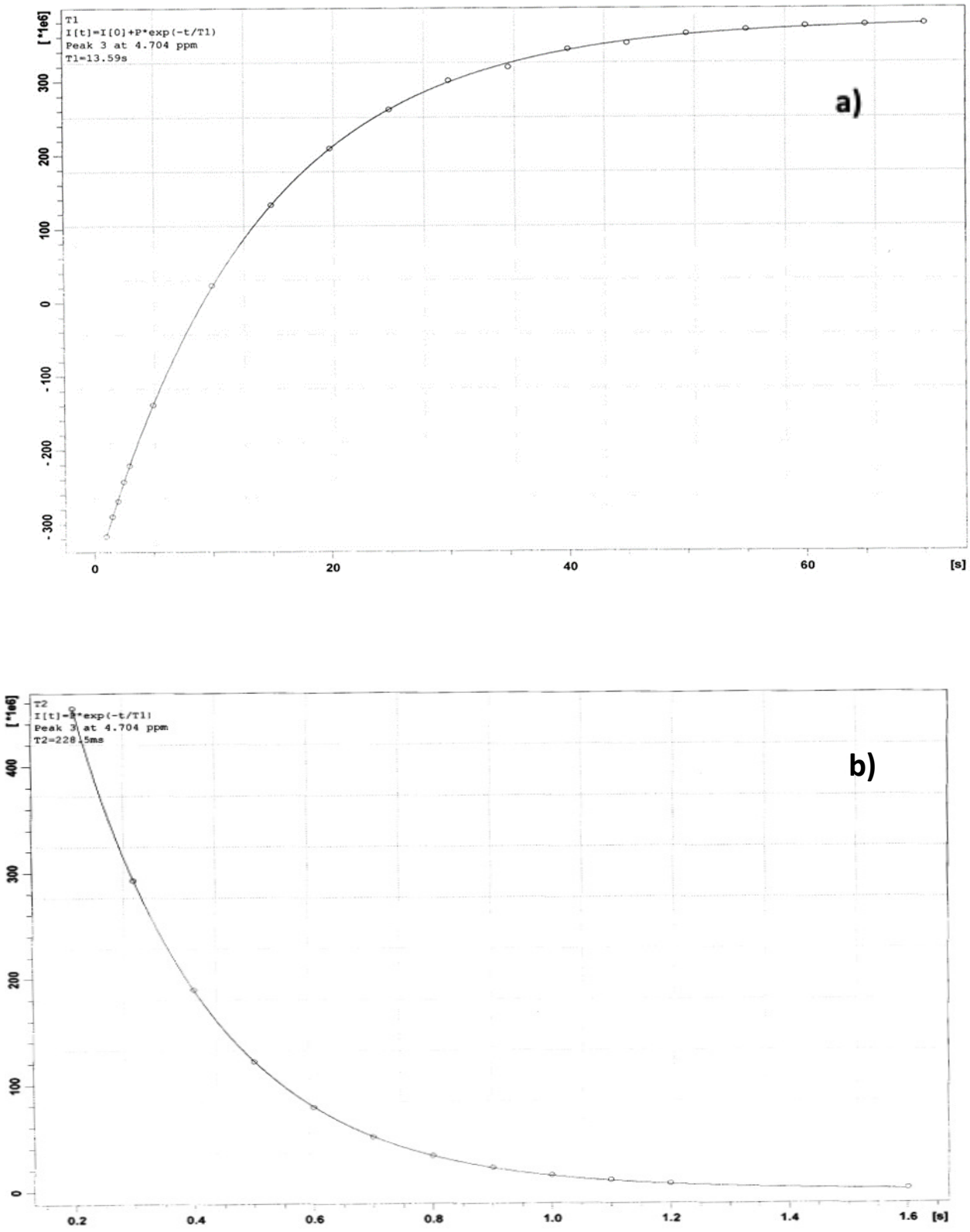


**Figure 3: a)** Inversion Recovery Spin-Lattice Relaxation Times ($T_1$), **b)** Spin Echo Spin-Spin Relaxation Times ($T_2$)

The measured $T_1$ and $T_2$ relaxation time values of HDO and doublet peaks in the spectrum shown in Fig. 2 for different temperatures are given in Table 1 and Table 2, respectively.

**Table 1:** $T_1$ and $T_2$ relaxation times of HDO peak in Fig. 2

| T (K) | $T_1$ (s) | $T_2$ (ms) |
|---|---|---|
| 298 | 7.815 | 118.6 |
| 303 | 7.991 | 111.0 |
| 308 | 8.486 | 109.8 |
| 313 | 8.885 | 108.6 |
| 318 | 8.994 | 107.3 |

**Table 2:** $T_1$ and $T_2$ relaxation times of Doublet peak in Fig. 2

| T (K) | $T_1$ (s) | $T_2$ (ms) |
|---|---|---|
| 298 | 1.728 | 114.2 |
| 303 | 2.063 | 147.4 |
| 308 | 2.297 | 193.4 |
| 313 | 2.573 | 261.4 |
| 318 | 2.591 | 340.1 |

As seen in Table 1 and Table 2, $T_1$ and $T_2$ values of Doublet peak and $T_1$ values of HDO peak increase depending on temperature, but $T_2$ values of HDO peak decrease depending on temperature. In addition, the case of $T_1 >> T_2$ in both tables draws attention. This clearly shows that the dynamics of the water molecule are complex and that intramolecular movements are also possible in the system [8, 12].

Temperature dependence graphs of ln $T_1$ and ln $T_2$ values for both peaks in the spectrum, shown in Fig. 4 and Fig. 5, respectively, were created to find the activation energies and rotational correlation times.

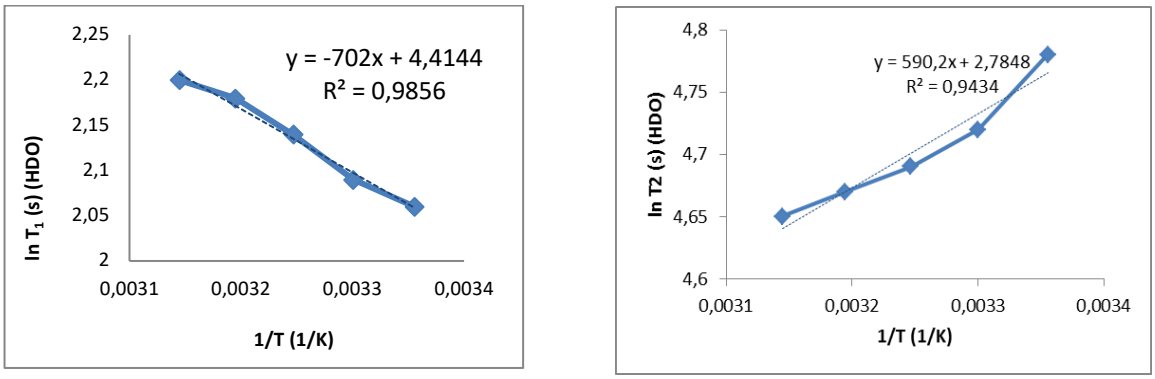


**Figure 4:** (**a**) ln $T_1$ and (**b**) ln $T_2$ vs. reciprocal temperature (1/T) for HDO peak measured at 400 MHz

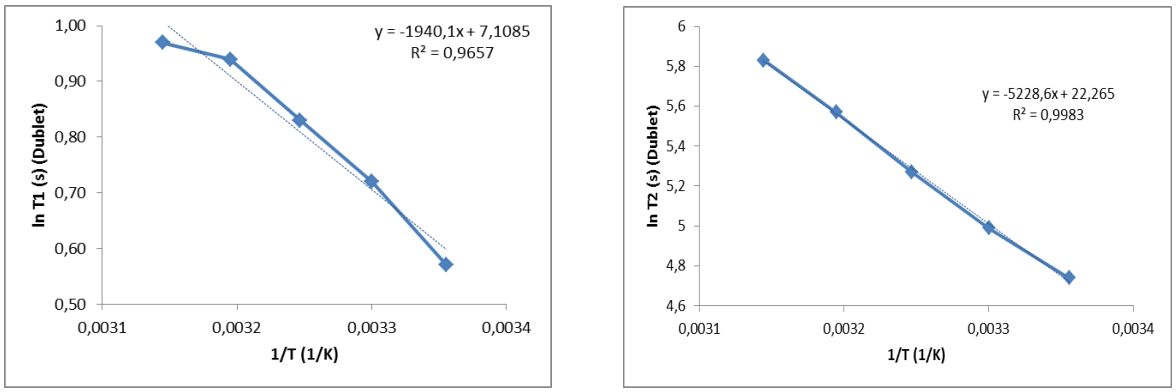


**Figure 5:** (**a**) ln $T_1$ and (**b**) ln $T_2$ vs. reciprocal temperature (1/T) for Doublet peak measured at 400 MHz

For the Doublet peak, both $\ln T_1$ and $\ln T_2$ decreased linearly with increasing 1/T. For the HDO peak, while $\ln T_1$ decreased linearly with increasing 1/T, $\ln T_2$ increased linearly. Therefore, in aqueous solutions, the $T_1$ and $T_2$ times are considered to be under high mobility. According to Eqn. (17), $T_1$ and $T_2$ relaxation times are inversely proportional to the rotational correlation time for molecular motion; According to Eqn. (19), it seems that $T_1$ and $T_2$ decrease as the temperature decreases.

In this case, the minimum $T_1$ relaxation appears to occur on the high-temperature side and $\tau_c$ provides the value $\omega\,\tau_c \ll 1$ of the correlation time characterizing the reorientation of the core. This approach should be applicable to some water molecules, or at least part of their dynamics. [11, 32, 37]. The intermolecular proton exchange affects the correlation time, and the spin-spin coupling causes the magnitude difference between $T_1$ and $T_2$ [32-36].

For the HDO peak, starting from the graph of $T_1$ and $T_2$ versus 1/T shown in Fig. 4, the following equations can be written, respectively.

$$\ln T_1 = -702\ (1/T) + 4.4144 \qquad R^2 = 0.9856 \qquad (21)$$

$$\ln T_2 = 590.2\ (1/T) + 2.7848 \qquad R^2 = 0.9434 \qquad (22)$$

Similarly, for the doublet peak, starting from the graph of of $T_1$ and $T_2$ versus 1/T shown in Fig. 5, the following equations can be written, respectively.

$$\ln T_1 = -1940.1\ (1/T) + 7.1085 \qquad R^2 = 0.9657 \tag{23}$$

$$\ln T_2 = -5228.6\ (1/T) + 22.265 \qquad R^2 = 0.9983 \tag{24}$$

According to Eqn. (20), activation energies ($E_a$) were calculated from the slopes of the curves in Fig. 4 and Fig. 5. Eqns. (16) and (17) were used to calculate the rotational correlation times ($\tau_0$, $\tau_c$). Ea and $\tau_0$, $\tau_c$ values for HDO peak and Doublet peak are given in Table 3 and Table 4, respectively, at 25 ºC.

**Table 3**: $E_a$, and $\tau_0$, $\tau_c$ values of HDO peak at 25 ºC

| | $T_1$ | $T_2$ |
|---|---|---|
| $E_a$ (kcal.mol$^{-1}$) | 1.39 | 1.17 |
| $\tau_0$ (s) | $1.88\times10^{-11}$ | $1.038\times10^{-9}$ |
| $\tau_c$ (s) | $11.38\times10^{-11}$ | $7.49\times10^{-9}$ |

**Table 4:** $E_a$, and $\tau_0$, $\tau_c$ values of Doublet peak at 25 ºC

| | $T_1$ | $T_2$ |
|---|---|---|
| $E_a$ (kcal.mol$^{-1}$) | 3.85 | 10.39 |
| $\tau_0$ (s) | $8.697\times10^{-10}$ | $1.864\times10^{-16}$ |
| $\tau_c$ (s) | $5.15\times10^{-10}$ | $7.78\times10^{-9}$ |

## 5. Conclusion

For the HDO peak, the rotational correlation time and activation energy values were found as 1.14 ps, 1.39 kcal.mol$^{-1}$ for $T_1$ and 7.5 ns, 1.17 kcal.mol$^{-1}$ for $T_2$, respectively. For the Doublet peak, the rotational correlation time and activation energy values were found as 0.05 ps 3.85 kcal.mol$^{-1}$ for $T_1$ and 7.8 ns, 10.39 kcal.mol$^{-1}$ for $T_2$, respectively. As a result of the very fast molecular motion, the small $\tau_c$ value for $T_1$ was calculated and this result confirms the extreme narrowing condition mentioned above. However, the value of $\tau_c$ calculated for $T_2$ conforms to condition $\omega\tau_c > 1$. The reason for this situation can be considered as the effect of intermolecular proton exchange on shortening the correlation time and the spin-spin coupling that causes the difference in magnitude between $T_1$ and $T_2$. The calculated activation energies are compatible with the rotational activation energy of water given in the literature and are associated with the isotropic rotational motion of unbound water molecules. Consequently, the effective process for the spin-spin relaxation mechanism in an aqueous solution may be

chemical exchange and dipolar coupling between protons and deuterons, while spin-lattice relaxation may be caused by the molecular tumbling reorientation process. This indicates that two different molecular species exist in aqueous solutions with respect to reorientation dynamics.

**References**

[1] Abragam, A., *The Principles of Nuclear Magnetism*. Oxford: Clarendon Press, 32 ed., 599p. 1961.

[2] Mathur, R., Vré, D., *The NMR studies of water in biological systems*, Progress in Biophysics and Molecular Biology, 35, 103-134, 1980.

[3] Traficante, D.D., *Relaxation. Can $T_2$ be longer than $T_1$?*, Concepts in Magnetic Resonance, 3, 171-177, 1991.

[4] Lankhorst, D., Schriever, J., Leyte, J.C., *Determination of the Rotational Correlation Time of Water by Proton NMR Relaxation in $H_2{}^{17}O$ and Some Related Results*, Berichte der Bunsengesellschaft für physikalische Chemie, 86(3), 215–221, 1982.

[5] Bulla, I., Törmälä, P., Lindberg, J.J., *Spin Probe Studies on the Dynamic Structure of Dimethyl Sulfoxide-Water Mixtures*, Acta Chemica Scandinavica A, 29, 89–92, 1975.

[6] Reich, H.J. (2017). 8.1 Relaxation in NMR Spectroscopy

[7] Marchi, M., Sterpone, F., Ceccarelli, M., *Water Rotational Relaxation and Diffusion in Hydrated Lysozyme*, Journal of the American Chemical Society, 124(23), 6787–6791, 2002.

[8] Fung, B.M., Mcgaughy, T.W., *Study of Spin-Lattice and Spin-Spin Relaxation Times of $^1H$, $^2H$, and $^{17}O$ in Muscle Water*, Biophysical Journal, 28, 293- 304, 1979.

[9] Kiihne, S., Bryant, R.G., *Protein-bound water molecule counting by resolution of (1)H spin-lattice relaxation mechanisms*, Biophysical Journal, 78(4), 2163-2169, 2000.

[10] Kivelson, D., *Rotational correlation times for small molecules in liquids*, In: Dorfmüller, T., Pecora, R. Eds., *Rotational Dynamics of Small and Macromolecules. Lecture Notes in Physics*, 293. Springer, Berlin, Heidelberg, 1987.

[11] Cope, F.W., *Nuclear Magnetic Resonance Evidence using $D_2O$ for Structured Water in Muscle and Brain*, Biophysical Journal, 9, 303-319, 1969.

[12] Woessner, D.E., Pettegrew, J.W. eds., *Relaxation Theory with Applications to Biological Studies*. In: NMR: *Principles and Applications to Biomedical Research*, Springer, New York, 618p. 1990.

[13] Solomon, I., *Relaxation Processes in a System of Two Spins*, Physical Review, 99(2), 559-565, 1955.

[14] Bloembergen, N., Purcell, E.M., Pound, R.V., *Relaxation Effects in Nuclear Magnetic Resonance Absorption*, Physical Review, 73(7), 679-712, 1948.

[15] Güven, M., Köylü, M.Z., *Dynamic Investigation of β-Hydroxy Amide Derivatives by 400 MHz $^1H$-NMR*, International Journal of Science and Research, 7(10), 1130-1133, 2018.

[16] Bryant, R.G., Korb, J.P., *Nuclear magnetic resonance and spin relaxation in biological systems*, Magnetic Resonance Imaging, 23(2), 167-173, 2005.

[17] Van-Quynh, A., Willson, S., Bryant R.G., *Protein Reorientation and Bound Water Molecules Measured by $^{1}H$ Magnetic Spin-Lattice Relaxation*, Biophysical Journal, 84(1), 558-563, 2003.

[18] Halle, B., *Protein hydration dynamics in solution: a critical survey*, Philosophical Transactions of the Royal Society B, 359 (81448), 1207-1224, 2004.

[19] Venu, K., Denisov, V.P., Halle, B., *Water $^{1}H$ Magnetic Relaxation Dispersion in Protein Solutions. A Quantitative Assessment of Internal Hydration, Proton Exchange, and Cross-Relaxation*, Journal of the American Chemical Society, 119(13), 3122–3134, 1997.

[20] Tadimalla, S., Momot, K.I., *Effect of partial $H_2O$-$D_2O$ replacement on the anisotropy of transverse proton spin relaxation in bovine articular cartilage*, PLoS One, 9(12), 2014.

[21] Gultekin, D.H., Gore, J.C., *Temperature dependence of nuclear magnetization and relaxation*, Journal of Magnetic Resonance, 172(1), 133- 141, 2005.

[22] Singer, P.M., Asthagiri, D., Chapman, W.G., Hirasaki, G.J., *Molecular dynamics simulations of NMR relaxation and diffusion of bulk hydrocarbons and water*, Journal of Magnetic Resonance, 277, 15-24, 2017.

[23] Zhang, L., Bouguet-Bonnet, S., Buck, M., *Combining NMR and molecular dynamics studies for insights into the allostery of small GTPase-protein interactions*, Methods in Molecular Biology, 796, 235-59, 2012.

[24] Korb, J.P., Bryant, R.G., *Magnetic Field Dependence of Proton Spin-Lattice Relaxation Times*, Magnetic Resonance in Medicine, 48, 21–26, 2002.

[25] Noskov, S.Y., Kiselev, M., Kolker, A.M., *Role of bound water in protein-ligand association processes*, Biophysics, 55(1), 29-34, 2010.

[26] Rezus, Y.L.A., Bakker, H.J., *On the orientational relaxation of HDO in liquid water*, The Journal of Chemical Physics, 123, 114502, 2005.

[27] Lawrence, C.P., Skinner, J.L., *Vibrational spectroscopy of HOD in liquid $D_2O$. III. Spectral diffusion, and hydrogen-bonding and rotational Dynamics*, The Journal of Chemical Physics, 118, 264, 2003.

[28] Duplan, J.C., Mahi, L., Brunet, J.L., *NMR determination of the equilibrium constant for the liquid $H_2O$–$D_2O$ mixture*, Chemical Physics Letters, 413, 400-403, 2005.

[29] Thuduppathy, G.R., Hill, R.B., *Applications of NMR spin relaxation methods for measuring biological motions*, Methods in Enzymology, 384, 243-64, 2004.

[30] Gallot, G., Lascoux, N., Gale, G.M., Leicknam, J-Cl., Bratos, S., Pommeret, S., *Non-monotonic decay of transient infrared absorption in dilute HDO/$D_2O$ solutions*, Chemical Physics Letters, 341, 535-539, 2001.

[31] Korunur, S., Zengin, B., Yilmaz, A., *Investigation of relaxation times in 5-fluorouracil and human serum albumin mixtures*, Turkish Journal of Biochemistry, 44(4), 524-529, 2019.

[32] Reuhl, M., Vogel, M., *Temperature-dependent dynamics at protein-solvent interfaces*, The Journal of Chemical Physics, 157(7), 074705, 2022.

[33] Halle, B., Denisov, V.P., *A new view of water dynamics in immobilized proteins,* Biophysical Journal, 69(1), 242-9, 1995.

[34] Goddard, Y.A., Korb, J.P., Bryant R.G., *Water molecule contributions to proton spin-lattice relaxation in rotationally immobilized proteins*, Journal of Magnetic Resonance, 199(1), 68-74, 2009.

[35] Charlier, C., Cousin, S.F., Ferrage, F., *Protein dynamics from nuclear magnetic relaxation, Chemical Society Reviews*, Royal Society of Chemistry, 45(9), 2410-2422, 2016.

[36] Mallamace, F., Corsaro, C., Mallamace, D., Baglioni, P., Stanley, H.E., Chen S.H., *A possible role of water in the protein folding process*, The Journal of Physical Chemistry B, 115(48), 14280-94, 2011.

[37] Köylü, M.Z., *Temperature Dependences of $T_1$ and $T_2$ of Residual Water in $D_2O$ Determined at 400 MHz $^1$H-NMR*, International Journal of Science and Research, 7(10), 40-43, 2018.