

## Concentration-Dependent Proton Magnetic Cross Relaxation in Aqueous Polyoxyethylene Solutions

B. Benko, V. Buljan, and S. Vuk-Pavlović\*

Macromolecular Biophysics Laboratory, Institute of Immunology, Rockefellerova 2, 41000 Zagreb, Yugoslavia  
(Received September 7, 1979)

Longitudinal proton magnetic relaxation rates were measured in aqueous solutions of polyoxyethylene (POE) 400 as a function of the polymer concentration. At all concentrations the plot of reduced magnetization vs. pulse separation in the  $180^\circ-t-90^\circ$  sequence yields a nonlinear line which is easily separated into two relaxation processes. Both relaxation rates increase faster with the concentration above 50% POE than below. Above this concentration, the fractions of  $z$  magnetization ascribed to each of the two relaxation phases deviate from the values expected from the chemical composition of the solution. This demonstrates the transfer of magnetization between these phases, i.e., the cross-relaxation phenomenon. The relaxation rates of POE and water protons in the absence of cross relaxation, the cross-relaxation rates, and the normalized intensities of the relaxation components for each of the relaxation phases were computed according to Edzes and Samulski after modifications required by the specificities of the POE-water system. According to the present data, along with those from literature, it is concluded that polymer ordering within the aqueous solution takes place above 50% POE, providing thus a structural basis for the apparent cross-relaxation phenomenon.

Early proton magnetic resonance studies of aqueous solutions of polyoxyethylenes (POE) revealed that the spin-lattice magnetic relaxation rate of water protons was significantly enhanced when POE was introduced as a solute.<sup>1</sup> Using the saturation recovery method, Liu and Ullman<sup>1</sup> were able to measure separately the values of the relaxation times  $T_1$  of the polymer and water protons. They noted that polymer protons relaxed more efficiently than water protons in solutions of about 10% POE and elaborated the underlying physical mechanisms of proton magnetic relaxation.

In this paper we present the results of a systematic proton magnetic relaxation study of POE concentrated solutions in water. Using the pulsed nuclear magnetic resonance technique, we confirmed the existence of two proton relaxation rates in POE solutions. The analysis of the concentration dependence of the relaxation parameters in these solutions demonstrated that magnetic cross relaxation between water and polymer protons becomes prominent above 50% POE. We were able to apply the cross-relaxation model<sup>2-4</sup> and to solve the unknown parameters without assumptions. One of the computed parameters could also be obtained experimentally and the close agreement between the computed and experimental data verifies the applicability of the model. The magnetic cross relaxation detected above 50% polymer indicates the concentration dependent ordering in the solution, a conclusion in agreement both with the NMR results and with those of other techniques.<sup>5</sup>

### Experimental Section

**Materials and Methods.** Polyoxyethylene of molecular weight 400 (Fisher Scientific) was a gift of Dr. K. C. Ing-ham, Blood Research Laboratories, American National

Red Cross, Bethesda, Md. It was used without further purification.

Polyoxyethylene was dissolved in 10 mM phosphate-1 mM ethylenediaminetetraacetic acid, pH 7.0, in  $^1\text{H}_2\text{O}$  or  $^2\text{H}_2\text{O}$  (99.9%; Prochem, B.O.C.) and the concentration was expressed as weight percents. POE concentrations in  $^2\text{H}_2\text{O}$  were expressed as  $m_{\text{POE}}/[m_{\text{POE}} + m_{^2\text{H}_2\text{O}}\rho_{^1\text{H}_2\text{O}}/\rho_{^2\text{H}_2\text{O}}]$  (here  $m$  represents the mass and  $\rho$  the density of the respective component), in order to make data commensurate with those in  $^1\text{H}_2\text{O}$ .

The proton longitudinal (spin-lattice) magnetic relaxation times,  $T_1$ , were measured by the  $180^\circ-t-90^\circ$  pulse sequence at 24 MHz, using a pulsed NMR apparatus with a digital readout (Jožef Stefan Institute, Ljubljana, Yugoslavia) equipped with a homonuclear pulsed field-frequency lock. The values of the amplitude of the free-induction decay were read 200  $\mu\text{s}$  following the  $90^\circ$  pulse. A Bruker BE40A11 high-resolution magnet was used. The temperature of the samples was kept at  $20 \pm 0.5^\circ\text{C}$ .

For each  $T_1$  measurement at least 30 data points (different delays between 180 and  $90^\circ$  pulses) were taken. A graph of the logarithms of reduced magnetization  $m = -[M(t) - M_\infty]/2M_\infty$  as a function of  $t$  was then constructed ( $M_\infty$  and  $M(t)$  denote the values of the projection of macroscopic magnetization on the  $z$  axis at equilibrium and at time  $t$ , respectively, measured as the amplitudes of the free-induction decay following the  $90^\circ$  pulse). Relaxation parameters were evaluated graphically and by a computer program written for a Hewlett-Packard HP 9820A computer.

### Theory

Due to the large difference between  $T_2^*$  values of water protons and protons of the solute in some hydrated mac-

romolecular systems a biphasic free-induction decay is observed.<sup>3,4</sup> This fact enables a convenient separate measurement of the two  $T_1$  values of the system.<sup>3,4</sup> In case in such a two-phase system magnetic cross relaxation takes place, each of the phases relaxes with two relaxation rates,  $R_1^+$  and  $R_1^-$ . For both phases the larger and the smaller apparent relaxation rates are identical,  $R_{1i}^+ = R_{1j}^+ = R_1^+$ , and  $R_{1i}^- = R_{1j}^- = R_1^-$ . However, normalized intensities of the two relaxation components for each of the phases relaxing with  $R_1^+$  and  $R_1^-$  are different, i.e.,  $c_i^+ \neq c_j^+$  and  $c_i^- \neq c_j^-$  (ref 3 and 4).

If the  $180^\circ$  pulse (in a  $180^\circ$ - $t$ - $90^\circ$  pulse sequence) is much shorter than the shorter of the  $T_2^*$ 's, the system is described by the following set of equations:<sup>3,4</sup>

$$[M_{ij\infty} - M_{ij}(t)] / (2M_{ij\infty}) = c_{ij}^+ \exp(-R_1^+ t) + c_{ij}^- \exp(-R_1^- t) \quad (1)$$

$$2R_1^\pm = R_{1i} + R_{1j} + k_i + k_j \pm [(R_{1i} - R_{1j} + k_i - k_j)^2 + 4k_i k_j]^{1/2} \quad (2)$$

$$R_{1ij} = c_{ij}^+ R_1^+ + c_{ij}^- R_1^- \quad (3)$$

$$c_{ij}^+ + c_{ij}^- = 1 \quad (4)$$

$$p_i k_i = p_j k_j \quad (5)$$

$M_\infty$  and  $M(t)$  indicate the magnitudes of the  $z$  components of macroscopic magnetization at equilibrium and time  $t$ , respectively.  $R_1$  is the relaxation rate in the absence of cross relaxation. Symbols  $k_i$  and  $k_j$  represent the cross-relaxation rate (i.e., the rate of the  $z$ -magnetization transfer) from the phase  $i$  to  $j$  and vice versa, respectively. Proton fractions in each of the phases are denoted by  $p_i$  and  $p_j$ .

In systems where the difference between  $T_2^*$ 's is not large enough to result in an apparent biphasic free-induction decay (as is the present case), the plot of the logarithms of reduced magnetization vs. pulse separation may yield a nonlinear line indicating two relaxation processes. In such experiments the sum of  $z$  magnetizations of the two phases is measured, so that  $M_\infty = M_{i\infty} + M_{j\infty}$  and  $M(t) = M_i(t) + M_j(t)$ , leading to the following expression:

$$[M_\infty - M(t)] / (2M_\infty) = M^+ \exp(-R_1^+ t) + M^- \exp(-R_1^- t) \quad (1a)$$

where

$$M^+ = (M_{i\infty} c_i^+ + M_{j\infty} c_j^+) / M_\infty = p_i c_i^+ + p_j c_j^+ \quad (1b)$$

$$M^- = (M_{i\infty} c_i^- + M_{j\infty} c_j^-) / M_\infty = p_i c_i^- + p_j c_j^-$$

In the case where large differences between  $T_2^*$  values are apparent eq 1-5), the values of  $c_{ij}^\pm$  are obtained directly.<sup>3,4</sup> In the latter case these values can be computed from eq 1b.

## Results

In all the measurements the  $180^\circ$  pulse duration,  $t_p \approx 50 \mu\text{s}$ , was much shorter than the single observed  $T_2^* \approx 5 \text{ ms}$ . For all the concentrations of POE 400 in  $^1\text{H}_2\text{O}$ , the plot of the logarithms of reduced magnetization,  $m = [M_\infty - M(t)] / (2M_\infty)$ , vs. pulse separation,  $t$ , in the  $180^\circ$ - $t$ - $90^\circ$  sequence, yields a nonlinear line which is easily separated into two components, each representing one  $R_1$ . The correlation coefficients of the least-squares fits for each of the component lines were always larger than 0.99. If the line with the smaller slope is extrapolated to  $t = 0$ , the fractions of the total apparent magnetization ascribed to each of the  $T_1$  values,  $M^+$  and  $M^-$ , can be determined.

The results of the concentration dependencies of the two apparent relaxation rates,  $R_1^+$  and  $R_1^-$ , are presented in

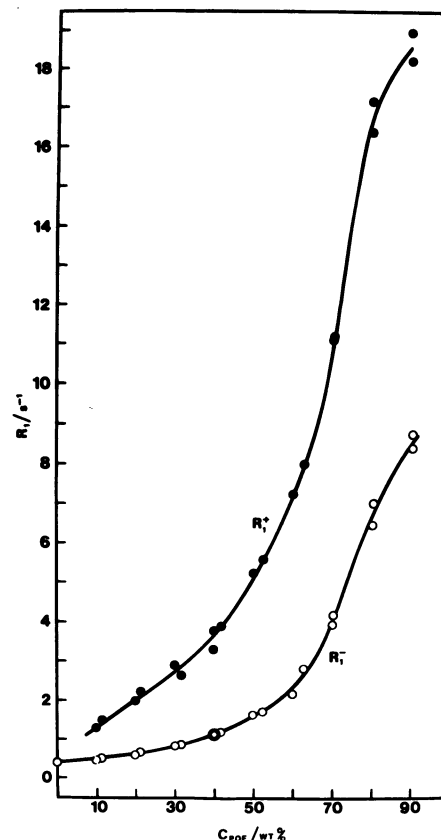


Figure 1. Concentration dependence of the slow ( $R_1^-$ ) and fast ( $R_1^+$ ) longitudinal relaxation rates in POE 400 aqueous solutions.

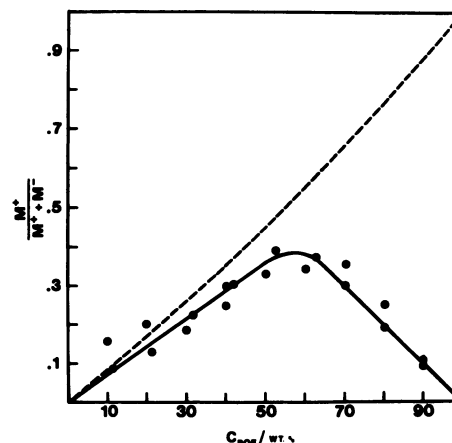


Figure 2. Concentration dependence of the fraction of  $z$  magnetization ascribed to  $R_1^+$ . Magnetization components  $M^+$  and  $M^-$  are defined by eq 1b.

Figure 1. It can be noted that  $R_1^+$  and  $R_1^-$  are present in the whole concentration range (except with pure water and pure polymer) and that both these parameters increase with increasing concentration. The coexistence of  $R_1^+$  and  $R_1^-$  indicates that the two components of the system (methylene protons being one component and water with hydroxyl protons of the polymer the other one) are not in thermodynamic equilibrium with each other. In Figure 2 the concentration dependence of the fraction of the total apparent equilibrium magnetization ascribed to  $R_1^+$ ,  $M^+ / (M^+ + M^-)$ , is presented. Examination of Figures 1 and 2 shows that increments of  $R_1^+$  and  $R_1^-$  are larger above 50% polymer than below. Furthermore, at this concentration  $M^+ / (M^+ + M^-)$  begins to show a marked deviation from the values expected from the chemical composition of the solution (dashed line in Figure 2). This

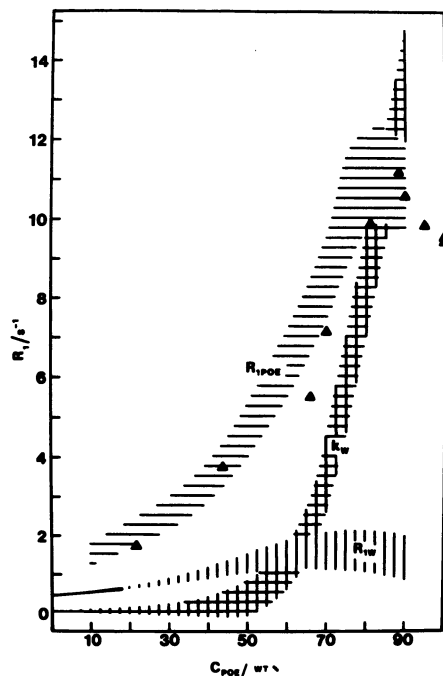


Figure 3. Concentration dependence of the computed relaxation parameters  $R_{1W}$ ,  $R_{1POE}$ , and  $k_W$ . The shaded areas denote the standard deviations of the computed parameters. The triangles denote  $R_1$  of POE methylene protons measured in  $^2\text{H}_2\text{O}$  solutions.

latter fact, showing the existence of a magnetization transfer between protons of the polymer and water protons, led us to apply the model of magnetic cross relaxation<sup>3,4</sup> for the interpretation of our data.

The relaxation parameters in Figures 1 and 2 were obtained by fitting experimental data according to eq 1a. After having inserted  $R_1^+$ ,  $R_1^-$ , and  $M^+$ , eq 1b and 2-5 yield direct solutions for the remaining parameters  $R_{1W}$ ,  $R_{1POE}$ ,  $c_W^{\pm}$ ,  $c_{POE}^{\pm}$ ,  $k_W$ , and  $k_{POE}$ . These computed parameters are displayed in Figures 3 and 4 ( $k_{POE}$ ,  $c_W^-$ , and  $c_{POE}^-$  are not shown for clarity of graphs and can be easily derived from eq 4 and 5).

The parameters shown in Figures 3 and 4 are computed from the data in Figures 1 and 2 without taking into account that exchangeable hydroxyl polymer protons may be magnetically equivalent to solvent protons.<sup>3,4</sup> If the appropriate correction is made, only relaxation parameters of the solvent are affected. At 90% polymer, where correction is the largest,  $R_{1W}$  would increase from  $1.4 \pm 0.6$  to  $2.6 \pm 1.1 \text{ s}^{-1}$ ,  $k_W$  would be diminished by 13%, and  $c_W^+$  would increase (become less negative) by 17%. These corrections, made under the assumption that all the hydroxyl protons belong to the solvent, are the maximal possible and they are smaller at lower POE concentrations. The value of  $R_{1POE}$  is increased by less than 2%. The magnitudes of all these corrections show that hydroxyl protons of POE 400, even if all of them participate in the cross-relaxation phenomenon,<sup>3,4</sup> do not affect the overall picture derived without these corrections.

We have also measured the concentration dependence of  $R_1$  in solutions of POE 400 in  $^2\text{H}_2\text{O}$ . Since the proton-deuteron dipole-dipole interaction is only 1/16 of the corresponding interaction between two protons,<sup>6</sup> no significant cross relaxation between polymer protons and water deuterons takes place. Therefore, if we neglect possible kinetic and structural consequences of water-heavy water substitution, the magnitude of polymer proton  $R_1$  in  $^2\text{H}_2\text{O}$  should correspond to  $R_{1POE}$ . The close similarity of the computed  $R_{1POE}$  and the measured  $R_1$  of POE in  $^2\text{H}_2\text{O}$  (triangles in Figure 3) independently substantiate

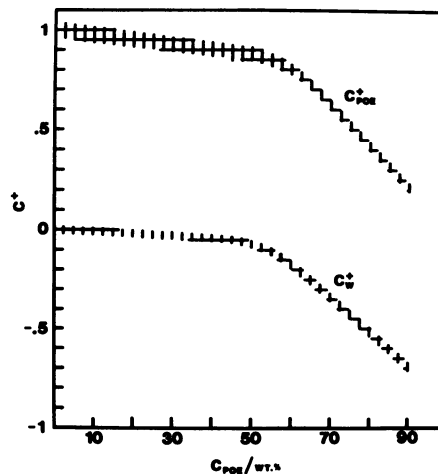


Figure 4. Concentration dependencies of the normalized intensities of the fast relaxation components of water protons,  $c_W^+$ , and protons of the polymer,  $c_{POE}^+$ .

the applicability of the model and the preciseness of the fit.

## Discussion

The cross-relaxation mechanism between water and the solute has been recently modeled with experimental data obtained with hydrated biomacromolecules.<sup>3,4,7,8</sup> Here we have demonstrated that cross relaxation becomes apparent only above a threshold concentration of about 50% polymer. This fact indicates that at 50% POE 400 a change of modes of molecular motion in solutions, relative to lower concentrations, takes place. This notion parallels infrared and high-resolution NMR data<sup>5</sup> which also demonstrate deviations from linearity in concentration dependence studies of POE 400 and POE 600. These spectroscopic data are interpreted as a significant degree of polymer ordering above 50% POE in an aqueous environment.<sup>5</sup>

According to the model we accepted in this paper,<sup>3,4,8</sup> for the existence of magnetic cross relaxation between solvent and macromolecular protons, there are two basic requirements to be fulfilled by the system. First, it must consist of components which are mutually not at thermodynamic equilibrium. Each of the components has efficient mechanisms for equilibration of its own temperature. This requirement is fulfilled for aqueous POE 400 solutions in the whole concentration range, as evidenced by the coexistence of  $R_1^+$  and  $R_1^-$ . The second requirement is the existence of a contact through which magnetization can be communicated between the components. This contact must persist within the system for a time longer than the reciprocal cross-relaxation rate. It can be speculated that cross relaxation, which becomes prominent above 50% POE, is related to the relative polymer ordering for which ample experimental evidence exists.<sup>5</sup>

It is noteworthy that both  $R_{1POE}$  and  $R_1$  of POE in  $^2\text{H}_2\text{O}$  decrease with the concentration, increasing from 90% solution to the pure polymer. Again, this is in complete agreement with spectroscopic data showing a higher polymer ordering in concentrated aqueous solutions than in the melt.<sup>5</sup>

*Acknowledgment.* S.V.-P. thanks Dr. K. C. Ingham for his generous gift of polyoxyethylene.

## References and Notes

- (1) K.-J. Liu and R. Ullman, *J. Chem. Phys.*, **48**, 1158-1168 (1968).
- (2) A. Kalk and H. J. C. Berendsen, *J. Magn. Reson.*, **24**, 343-366 (1976).
- (3) H. T. Edzes and E. T. Samulski, *Nature (London)*, **265**, 521-523 (1977).

- (4) H. T. Edzes and E. T. Samulski, *J. Magn. Reson.*, **31**, 207-229 (1978).
- (5) K.-J. Liu and J. L. Parsons, *Macromolecules*, **2**, 529-533 (1969).
- (6) A. Abragam, "The Principles of Nuclear Magnetism", Clarendon Press, Oxford, 1961, Chapters 5 and 8.
- (7) M. Eisenstadt and M. E. Fabry, *J. Magn. Reson.*, **29**, 591-597 (1978).
- (8) S. H. Koenig, R. G. Bryant, K. Hallenga, and G. S. Jacob, *Biochemistry*, **17**, 4348-4358 (1978).
- (9) This work has been taken from the thesis to be submitted by V.B. to the University of Zagreb in partial fulfillment of the requirements for the M.Sc. degree in Structural Biophysics.