

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

## Dynamic Electrical Birefringence Studies of Poly- $\gamma$ -benzyl-L-glutamate<sup>1</sup>

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The dynamic electrical birefringence of two samples of different molecular weight poly- $\gamma$ -benzyl-L-glutamate in ethylene dichloride has been studied. A specific Kerr coefficient ( $B/c$ ) of 0.057 cm.<sup>4</sup>/volt g. for  $M_w = 84,000$  and 0.31 cm.<sup>4</sup>/volt g. for  $M_w = 350,000$  was obtained. Both permanent and induced dipole moments contributed to the orientation of the molecules in the electric field. A rotary diffusion coefficient ( $\Theta_{20,w}$ ) at infinite dilution of  $9.8 \times 10^2$  sec.<sup>-1</sup> was obtained for the high molecular weight sample. This value gives a length of  $2.7 \times 10^3$  Å. for a rigid rod or ellipsoid.

### Introduction

Poly- $\gamma$ -benzyl-L-glutamate (PBLG) should be an excellent model substance for dynamic electrical birefringence measurements. Doty, *et al.*,<sup>3</sup> have shown that in certain solvents this polypeptide exists in a wide range of molecular weights as unassociated rigid rods with the  $\alpha$ -helix<sup>4</sup> configuration. Besides its known structure, PBLG has special advantages for electrical birefringence studies because it is soluble in non-conducting solvents and is uncharged. The interpretation of previous electrical birefringence experiments on charged molecules in water<sup>5-9</sup> is complicated by ion atmosphere<sup>5,9</sup> and proton migration<sup>10-11</sup> effects which are absent in these non-conducting systems. Furthermore, the polarizability ellipsoid of a helix must be a figure of revolution and the dipole moment must be along the symmetry axis (except for end effects). This simplifies the equations for the dynamic birefringence<sup>11</sup> to those given by Benoit.<sup>6</sup>

The orientation of a rigid molecule in a time dependent parallel electric field can be characterized by three parameters: the dipole moment  $\mu$ , the polarizability  $\alpha$ , and the rotary diffusion coefficient  $\Theta$ . The decay of the orientation after application of a square electrical pulse gives a direct measure of  $\Theta$ . Unlike the values obtained from hydrodynamic experiments such as flow birefringence<sup>12</sup> and viscosity,<sup>13</sup> this  $\Theta$  does not depend upon explicit assumptions about the shape, for an asymmetric molecule. Values of the dipole moment  $\mu$  and the electrical ( $\alpha_E$ ) and optical ( $\alpha_O$ ) polarizabilities cannot in general be separated, however, using only electrical birefringence measurements. Either the optical factor must be obtained from flow birefringence or depolarization of

scattered light experiments, or dielectric dispersion measurements must be made to obtain  $\mu$  and  $\alpha_E$ .

In the present study the dynamic electrical birefringence of two PBLG samples of different molecular weight has been measured. A rotary diffusion coefficient for the high molecular weight sample was obtained, but the exact nature of the orienting electrical couple must await further information.

### Experimental

The poly- $\gamma$ -benzyl-L-glutamate was obtained through the courtesy of Dr. E. R. Blout and Dr. P. Doty. The two samples were: RK 1149-B with a weight average molecular weight ( $M_w$ ) of 84,000 and RK 1263,  $M_w = 350,000$ . Ethylene dichloride (EDC), Union Carbide and Carbon technical grade, was dried with Drierite, then distilled through a 30 plate column at atmospheric pressure. The concentrations of the two stock solutions were determined by dry weight.

The electrical birefringence apparatus has been described before.<sup>7,8</sup> A Teflon cell was used, however, instead of the glass cell described previously. The cell, machined from Teflon rod, has threaded ends for standard polarimeter end plates. Gold electrodes,  $0.079 \times 0.476 \times 8.86$  cm., rest on the bottom of the cell and are held 0.249 cm. apart against the sides of the cell by Teflon spacers. Electrical connection to the electrodes is made by platinum wires. The volume of solution necessary to fill the cell is about 2.5 ml.

The performance of the apparatus was checked using nitrobenzene (J. T. Baker, purified grade). A Kerr coefficient ( $B = \Delta n/\lambda E^2$ ) of  $4.24 \times 10^{-6}$  cm./volt<sup>2</sup> was obtained for nitrobenzene at the average effective wave length of 510 m $\mu$ . This is in satisfactory agreement with previously obtained values at 546 m $\mu$  after correction for the wave length difference. The instrumental relaxation time was also obtained from the nitrobenzene experiments; the molecular relaxation time is of course negligible. A value of 13.7  $\mu$ sec., in excellent agreement with that obtained by Billick,<sup>8</sup> resulted.

All measurements were made at room temperature; an average value of 298°K. was used in calculations.

The methods for analyzing the primary data have been described previously.<sup>7</sup> It was not necessary to correct the observed rotary diffusion coefficient for the instrumental relaxation time as it was negligible compared to the relaxation time of the high molecular weight PBLG. To calculate the rotary diffusion coefficients for the molecules in water at 20°, the viscosity of the solvent ( $\eta = 0.0078$  poise)<sup>14</sup> at 25° was used.

### Results

The Kerr coefficients and rotary diffusion coefficients for solutions of PBLG in EDC are given in Table I; the Kerr coefficients are plotted *vs.* concentration in Fig. 1.

For the high molecular weight sample the birefringence was studied as a function of field strength

(14) A. Weissberger, E. T. Proskauer, J. A. Riddick and E. E. Toops, Jr., "Organic Solvents," Interscience Publishers, Inc., New York, N. Y., 1955.

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(2) Public Health Service Fellow of the National Cancer Institute at Yale University.

(3) P. Doty, J. H. Bradbury and A. M. Holtzer, *THIS JOURNAL*, **78**, 947 (1956).

(4) L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci.*, **37**, 205 (1951).

(5) C. T. O'Konski and B. H. Zimm, *Science*, **111**, 113 (1950).

(6) H. Benoit, *Ann. Phys.*, **6**, 561 (1951).

(7) I. Tinoco, Jr., *THIS JOURNAL*, **77**, 3476 (1955).

(8) I. H. Billick and J. D. Ferry, *ibid.*, **78**, 933 (1956).

(9) C. T. O'Konski and A. J. Haltner, *ibid.*, **78**, 3604 (1956).

(10) J. G. Kirkwood and J. B. Shumaker, *Proc. Natl. Acad. Sci.*, **38**, 855 (1952).

(11) I. Tinoco, Jr., *THIS JOURNAL*, **77**, 4486 (1955).

(12) H. A. Scheraga, J. T. Edsall and J. O. Gadd, *J. Chem. Phys.*, **19**, 1101 (1951).

(13) H. A. Scheraga, *ibid.*, **23**, 1526 (1955).

over the range 170–1200 practical<sup>15</sup> volts per cm. The Kerr coefficient was found to be independent of field strength. The number of measurements made at different voltages is given in parentheses to the right of the average value of the Kerr coefficient which is shown in the table.

The slopes of the plots in Fig. 1 give specific Kerr coefficients of  $B/c = 0.31 \text{ cm.}^4 \text{ volt}^2 \text{ g.}$  for  $M_w = 350,000$  and  $B/c = 0.057 \text{ cm.}^4 \text{ volt}^2 \text{ g.}$  for  $M_w = 84,000$ .

The curves for the rise of the birefringence of the high molecular weight sample on application of a rectangular electrical pulse were not symmetrical to the decay curves. The shape of the curves showed that both permanent and induced dipole orientation were important.

TABLE I

KERR COEFFICIENTS AND ROTARY DIFFUSION COEFFICIENTS FOR POLY- $\gamma$ -BENZYL-L-GLUTAMATE				
Concn. (g./l.)	Mol. wt. = 350,000		Mol. wt. = 84,000	
	$B \times 10^4$ (cm./volt <sup>2</sup> )	$\theta_{20,w} \times 10^{-2}$ (sec. <sup>-1</sup> )	Concn. (g./l.)	$B \times 10^4$ (cm./volt <sup>2</sup> )
3.75	10.9(7)	2.8	14.80	9.2
1.35	4.2(10)	7.0	7.25	3.4
0.50	1.5(7)	8.2	2.05	1.1
0.15	0.52(4)	10.1	1.10	0.66
(0)		(9.8)		

### Discussion

**Kerr Coefficients.**—The specific Kerr coefficient for a molecule with a polarizability ellipsoid of revolution and permanent dipole  $\mu_1$  along the symmetry axis is related to molecular parameters by the equation<sup>11</sup>

$$B/c = (2\pi/15n_0\rho\lambda_0)(g_1 - g_2)(p_1 + q)$$

The quantities  $n_0$ ,  $\rho$ ,  $\lambda_0$  are the refractive index of the solvent, the density of the solute, and the vacuum wave length of the light, respectively. The anisotropy of the optical polarizability per unit volume of particle is  $(g_1 - g_2)$ . The electrical orientation parameters are  $p_1 = (\mu_1/kT)^2$  and  $q = v(g_1^0 - g_2^0)/kT$ . The permanent dipole moment is  $\mu_1$ , and the anisotropy of the electrical polarizability is  $(g_1^0 - g_2^0)$ .

Although the factors cannot be untangled from the Kerr data alone, we can deduce the following facts. From the definitions of the molecular parameters we see that for different length rods, e.g., different molecular weights of PBLG: (1)  $(g_1 - g_2)$  is independent of molecular weight for long rods, (2)  $p_1$  is proportional to the square of the molecular weight, (3)  $q$  is proportional to the molecular weight. Therefore, for the two samples studied, if the permanent dipole is the principal orienting factor, then

$$(B/c)_{M_1}/(B/c)_{M_2} = 17.4$$

or, if the induced dipole is primarily responsible

$$(B/c)_{M_1}/(B/c)_{M_2} = 4.2.$$

Experimentally we find  $(B/c)_{M_1}/(B/c)_{M_2} = 5.4$ ; thus indicating that, although both terms are effective, induced dipole orientation is the more im-

(15) Unless designated otherwise voltages are given in ESU volts (1 ESU = 300 practical volts).

portant. For example, if we take  $p_1 = q/2$  for the high molecular weight sample,  $(B/c)_{M_1}/(B/c)_{M_2} = 5.6$ .

The orienting torque acting on a rigid ellipsoid of revolution of dielectric constant  $\epsilon$  in a solvent of dielectric constant  $\epsilon_0$  can be calculated from an equation derived by Maxwell.<sup>6</sup> Using this equation a value of  $q$  can be calculated for PBLG in EDC if the helices are approximated by ellipsoids of the same length and volume. This  $q$  is nearly independent of shape for axial ratios greater than 10, if  $\epsilon$  is finite.

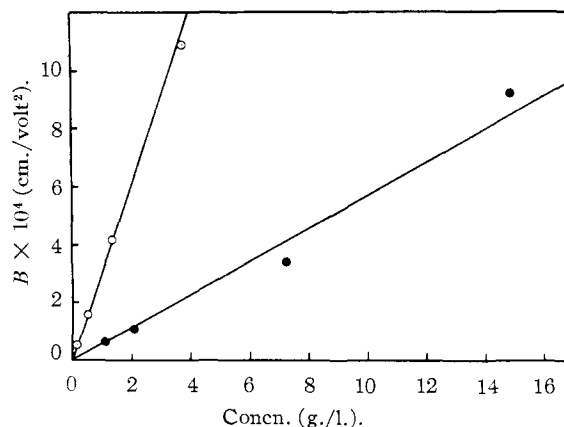


Fig. 1.—The Kerr coefficient of poly- $\gamma$ -benzyl-L-glutamate in ethylene dichloride vs. concentration. The open circles are for  $M_w = 350,000$ ; the closed circles are for  $M_w = 84,000$ .

For the calculation a dielectric constant of 2.5 for PBLG was used. This value is based on the assumption that the electrical polarizability of PBLG is completely electronic and thus  $\epsilon$  is equal to the square of the refractive index obtained from Doty's  $(dn/dc)$  measurements.<sup>3</sup> The other parameters used were:  $\epsilon_0 = 10.36$ ,<sup>14</sup>  $\rho = 1.32$ .<sup>3</sup> The shape of the rise of the birefringence curves and the molecular weight dependence of the Kerr coefficients were consistent with values of  $p_1$  and  $q$  of the same order of magnitude for the high molecular weight sample; therefore,  $p_1$  can be set equal to  $q$ , and the dipole moment  $\mu_1$  and optical factor  $(g_1 - g_2)$  also can be estimated. For the calculation of  $(g_1 - g_2)$  the following data were used:  $n_0 = 1.443$ ,<sup>14</sup>  $\lambda_0 = 510 \text{ m}\mu$ . The values thus obtained for the 350,000 molecular weight sample were:  $q = 4.1 \times 10^{-6} \text{ cm.}^3 \text{ erg}$ ,  $\mu_1 \cong 80 \text{ debye}$ ,  $(g_1 - g_2) \cong 8.9$ . The value of  $(g_1 - g_2)$  is very large compared to values found for many proteins in water solution.<sup>16</sup> Furthermore, a value of  $\mu_1 = 7,200 D$  would be estimated for a polyglycine  $\alpha$ -helix of the same length.<sup>17</sup> Because of these discrepancies we should examine the assumptions in

(16) R. Cerf and H. A. Scheraga, *Chem. Revs.*, **51**, 185 (1952).

(17) Schellman<sup>18</sup> gives  $\mu_1 \cong (7.2 + 4.5)n$  debye for a polyglycine  $\alpha$ -helix where  $n$  is the degree of polymerization. The first term is the contribution to the dipole moment from a charged carboxyl group at one end of the molecule and a charged amino group at the other end. The second term is the contribution from each NH and C=O group in the helix. Obviously, only this second term is present for our system.

(18) J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, Serie chim.*, **29**, 254 (1955).

our calculation. The critical assumption is that the electrical polarizability of PBLG is purely electronic, *i.e.*,  $\epsilon = 2.5$ . If instead we consider that the dipolar ester side chains can orient in the electric field and thus give rise to a very large atomic polarizability, the value of  $\epsilon$  could be much larger than  $\epsilon_0$ , the dielectric constant of the solvent. To calculate an upper limit for  $\mu_1$  we shall let  $\epsilon \rightarrow \infty$ . The value of  $q$  then approaches a limit depending on the axial ratio of the ellipsoid. For an axial ratio of 100,  $q$  becomes  $1.9 \times 10^{-2}$  cm.<sup>3</sup>/erg,  $\mu_1 \cong 5,400 D$ ,  $(g_1 - g_2) \cong 1.9 \times 10^{-3}$ .

By measurements of dielectric dispersion and flow birefringence,  $\mu_1$  and  $(g_1 - g_2)$  may be determined independently.

**Rotary Diffusion Coefficients.**—The rotary diffusion coefficients given in Table I are seen to be highly concentration dependent, thus indicating a great deal of interaction among the long dipolar rods. Only a part of this apparent dependence could be removed by using the viscosity of the solution instead of that of the solvent for correcting to water at 20°.

From the infinite dilution value of  $\Theta_{20,w}$  a length can be calculated for the molecule. Using either a rod<sup>9</sup> or ellipsoidal<sup>6</sup> model one obtains a length of  $2.7 \pm 0.1 \times 10^3$  Å. for the 350,000 molecular weight

PBLG. This value may be compared with a light scattering length of about  $2.0 \times 10^3$  Å. obtained by Doty, *et al.*,<sup>3</sup> and a calculated  $\alpha$ -helix length of  $2.4 \times 10^3$  Å. (length = 1.5 times degree of polymerization).<sup>4</sup> Doty's light scattering lengths agreed well with the  $\alpha$ -helix configuration for the lower molecular weights; for the high molecular weight he attributed the disagreement to a slight flexibility in the longer rods. This flexibility might lower the rotational diffusion of the molecules and cause an apparent longer length. However, O'Konski<sup>9</sup> found a rotational diffusion length for tobacco mosaic virus significantly larger than electron microscope values, thus suggesting that rod-like models for these molecules may not be adequate.

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NOTE ADDED IN PROOF.—Professor Doty has kindly supplied a value of  $(g_1 - g_2)$  for PBLG in EDC of  $4.1 \times 10^{-3}$  from flow birefringence experiments of J. T. Yang and P. Doty. If one assumes  $p_1 = q$ , this value leads to  $\mu_1 = 3,900 D$ . for  $M_w = 350,000$ .

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