Electric Birefringence of Macromolecular Suspensions

Electric birefringence is useful for measuring electric, optical, and hydrodynamic properties of macromolecular suspensions. In this technique the particles of the dispersed phase are oriented to an aligned state by an applied electric field and thus form a system which is optically birefringent. Measurement of the time dependence of the birefringence for an applied square wave pulse leads to the determination of optical and electric susceptibilities and the rotatory diffusion constant of the suspended particles. This technique also gives a test of monodispersity and presents a means of studying the distribution and interaction of polymeric species that might be present. Benoit of Strasbourg and O'Konski of Berkeley have worked out the mathematics of electric birefringence for suspended rigid particles by extending the diffusion equation to achieve a relation between birefringence and the applied electric field. Application of electric birefringence to dissolved collagen determines its hydrodynamic parameters, gives information on the distribution of aggregates, and measures its permanent and induced dipole moments.

Electric birefringence as applied to macromolecules in suspension takes advantage of their electric dipolar nature through their tendency to become aligned with an electric field to form a birefringent uniaxial crystal. A square wave pulsed field is generally employed, and after it is created the rotatory diffusion of each macromolecule is in accordance with its overall dipole moment, the geometry of the particle, and its coefficient of friction. This orientation process is carried out in a polarized light beam whose intensity at the exit of the optical path will vary with the rate of alignment of the suspended macromolecules. By means of a photomultiplier and cathode ray oscilloscope, the time dependence of the macromolecular alignment can be observed. The birefringence is expressed as the difference between the indices of refraction of the macromolecular suspension parallel to and perpendicular to the electric field. This value can therefore be either positive or negative.

A schematic diagram of the apparatus has already been published by Kahn and Witnauer (1969). To minimize heating, electrolysis, and electrophoresis of the sample, it is customary to work with single pulses or short bursts rather than with pulse trains of indefinite length. From these considerations it becomes obvious that the oscilloscope must be able to display two traces simultaneously and must also incorporate a data storage system so that a single pulse can be held on display for long periods of time. All electronic circuits used in this type of work must be adequately compensated for high- and low-frequency losses and also for phase shift so that good fidelity of the trace will be achieved.

At the time of writing, no ready-made complete apparatus for electric birefringence measurements is available on the market, and both optical path and much of the electronic circuitry must be designed and constructed before a research program can be started.

The result of a typical observation on calfskin corium collagen dissolved in citrate buffer in the acid pH range and held below the gelatinization temperature is shown in Figure 1. (Throughout this paper, this type of collagen preparation will be used as a model macromolecular system.) The upper trace of the oscillogram is the square wave field applied to the suspension, and the lower trace is its birefringent response as it varies with time. After generation of the electric field, there occurs a brief induction period followed by a logarithmic buildup of birefringence to a steady state. When the field is quenched, there is a rapid exponential decay of birefringence as the aligned particles return to random spatial orientations. From these data it is possible to determine the rotatory diffusion constant, dipole moment, and electric and optic susceptibilities of the suspended particles, and to study polydispersity.

Electric birefringence was first observed by Kerr (1875, 1880), who worked with pure liquids and solutions of low molecular weight compounds. This phenomenon was given the name of Kerr effect. Kerr further noted that when magnitude of electric birefringence is plotted *vs.* the square of the electric field, the graph is linear for low field strength. This is known as the Kerr law, and the range over which it is valid is the Kerr region. These considerations led to a characteristic value called the Kerr constant, which is given by

$$B = \frac{\Delta n}{\lambda E^2} = \frac{\delta}{2\pi l E^2}$$

where Δn is the observed birefringence given as the difference in the indices of refraction of the material parallel to and perpendicular to the electric field, λ is the wavelength of the light employed, and E is the electric field. In the right-hand term, the birefringence is expressed in angular terms by δ, and l is the length of the light path through the material under study.

An alternate characteristic constant, K, has been proposed by Debye (1925) and is given by

$$K = \frac{\Delta n}{nE^2} = \frac{B\lambda}{n}$$

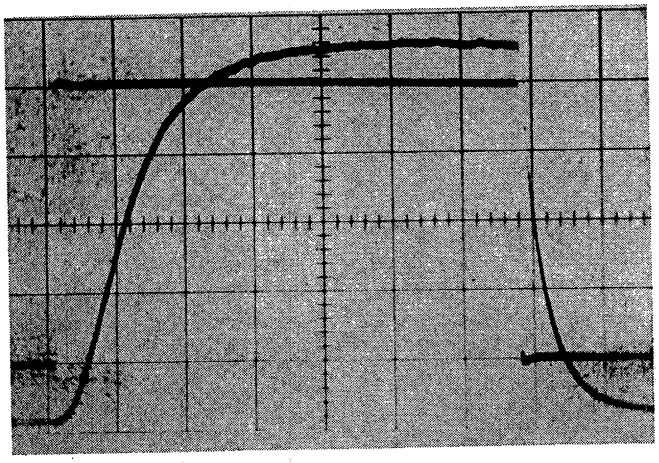

Figure 1. Electric birefringence pattern for calfskin corium collagen dissolved in citrate buffer at 0.10% concentration, pH 4.25, and ionic strength of 0.04; the field strength is 3660 V/cm and the sweep is 0.500 msec/cm

where n is the index of refraction of the system when no electric field is present. Specific constants can be obtained by dividing B or K by the concentration of the macromolecular suspension.

Curves of electric birefringence at the steady state as a function of the square of the electric field and of collagen concentration are shown in Figure 2. At low values of field there is evidence of a Kerr region which increases in extent as the collagen concentration is increased.

The basic development of the mathematical relations between electric birefringence and the characteristics of macromolecules in suspension was done by Peterlin and Stuart (1939), who considered a suspension of ellipsoids of revolution having a net dipole moment parallel to the principal hydrodynamic axis. They started with the fundamental relations

$$1 + 4\pi G/E = n^2 = \epsilon \tag{3}$$

and

$$G/E = \chi \tag{4}$$

where G is polarization, ϵ is dielectric constant, and χ is polarizability. Applying these fundamentals to the angular distribution function of suspended particles in an electric field as given by

$$dN = Nf(\theta,t)\, 2\pi \sin\theta\, d\theta \tag{5}$$

where N is the number of particles whose principal hydrodynamic axis is oriented at an angle of θ with the electric field, and t is the time the electric field has been active, yielded the equation

$$\Delta n = 2\pi C\,(g_1 - g_2)\,\Phi/n \tag{6}$$

where C is concentration and $(g_1 - g_2)$ is the optical anisotropy of the suspended particles. The orientation factor, Φ, is a function of the angular distribution function, θ, as given by

$$\Phi = {}^1/_2\,(3\,\langle\cos^2\theta\rangle - 1) \tag{7}$$

O'Konski *et al.* (1959) applied these concepts to the saturated birefringent state. Under this condition, the angular distribution function can be expressed in terms of energy by

$$f(\theta) = \frac{e^{-\frac{U}{kT}}}{\int_0^\pi e^{-\frac{U}{kT}}\, 2\pi \sin\theta\, d\theta} \tag{8}$$

where k is the Boltzmann constant. The potential energy of dipole interaction, U, is due to the sum of the permanent and induced dipole moments whose respective energies are given by

$$U_p = -\mu E \cos\theta \tag{9}$$

$$U_i = -{}^1/_2(\alpha_1 - \alpha_2)E^2\cos^2\theta \tag{10}$$

Here α_1, α_2 are the excess polarizabilities of the suspended particles over the solvent in directions parallel to and perpendicular to the principal hydrodynamic axis, and μ is the permanent dipole moment, assuming that the field within each particle is identical with the field immediately external to it. When this assumption does not hold, the value of μ, as used here, can be obtained by multiplying the actual dipole moment by a suitable correction factor. Combining these equations led to an expression for the orientation distribution based on two-dimensionless numbers, β and γ, which are related to the permanent and induced dipole moments, respectively. The orientation distribution at saturation is then given by

$$\Phi(\beta,\gamma) = \frac{3}{4\gamma} \times \left[\frac{e^{\frac{\beta^2}{4\gamma}+\gamma}\left\{\sqrt{\gamma}\,(e^\beta + e^{-\beta}) - \frac{\beta}{2\sqrt{\gamma}}\,(e^\beta - e^{-\beta})\right\}}{E\left(\frac{\beta}{2\sqrt{\gamma}} + \sqrt{\gamma}\right) - E\left(\frac{\beta}{2\sqrt{\gamma}} - \sqrt{\gamma}\right)} + \frac{\beta^2}{2\gamma} - 1 \right] - \frac{1}{2} \tag{11}$$

with

$$\beta = \mu E/kT \tag{12}$$

and

$$\gamma = \frac{(\alpha_1 - \alpha_2)E^2}{2kT} \tag{13}$$

This leads to the relationships

$$\frac{15\Phi(\beta,\gamma)}{\beta^2 + 2\gamma} = \frac{\Delta n/E^2}{(\Delta n/E^2)_{E\to 0}} \tag{14}$$

and

$$15\Phi(\beta,\gamma) = (\beta^2 + 2\gamma)_{E\to 0} \tag{15}$$

and a method for determining β and γ. Equations 14 and 15 can be made numerically identical with the relation between $(\Delta n/E^2)/(\Delta n/E^2)_{E\to 0}$ and E^2 by using the proper scale factor which, at this stage, is unknown. A method for overcoming this difficulty has been proposed by Yoshioka and O'Konski (1966) and Yoshioka and Watanabe (1969). A family of curves of values of $15\Phi\,(\beta,\gamma)/(\beta^2 + 2\gamma)$ as ordinates and values of $\ln\,(\beta^2 + 2\gamma)$ as abscissae is plotted for a series of values of $\beta^2/2\gamma$ to form a reference base. The experimental data are plotted on transparent paper as $(\Delta n/E^2)/(\Delta n/E^2)_{E\to 0}$ *vs.* $\ln E^2$, using the same vertical scale as in the previous plot. The two graphs are superimposed and moved horizontally relative to each other until the experimental curve coincides with one of the theoretical traces. This matching technique is analogous to multiplying one set of abscissae by a constant and

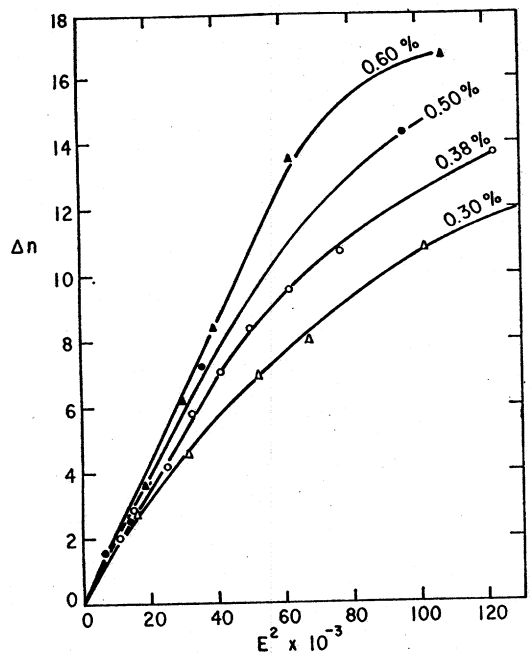

Figure 2. Graph of electric birefringence of calfskin corium collagen *vs.* the square of the field strength at pH 4.22 and ionic strength of 0.04

gives the proper relation between experimental and theoretical parameters. There is thus available sufficient data to calculate β and γ for the experimental case, and from these values μ and $(\alpha_1 - \alpha_2)$ can be obtained *via* eq 12 and 13. The induced dipole, ψ, is given by

$$\psi = (\alpha_1 - \alpha_2) E \tag{16}$$

Figure 3 illustrates an example of this application of the O'Konski *et al.* (1959) mathematical development. A family of curves obtained by plotting $15\Phi(\beta,\gamma)/(\beta^2 + 2\gamma)$ *vs.* $(B^2 + 2\gamma)$ has superimposed on it a set of points corresponding to the values of $(\Delta n/E^2)/(\Delta n/E^2)_{E\to 0}$ *vs.* ln E^2 obtained for collagen at 0.38% concentration, pH 4.22, and ionic strength of approximately 0.05. The scale of ln E^2 values has been shifted horizontally until the experimental points have shown coincidence with one of the theoretical curves, in this case at $\beta^2 = 16\gamma$. Carrying out the calculations shows that collagen in solution under these conditions has a dipole

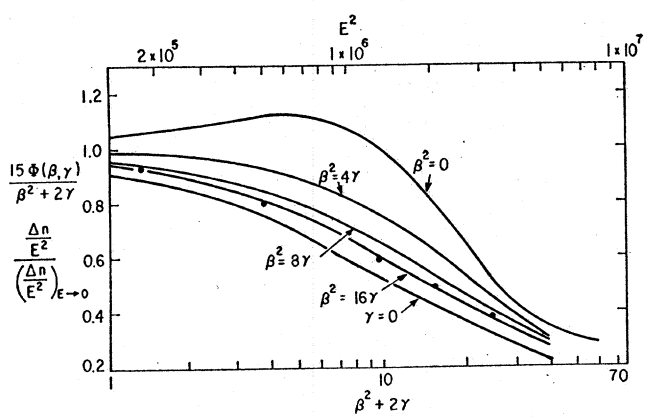

Figure 3. Experimental points plotted as $\frac{\Delta n}{E^2} \Big/ \left(\frac{\Delta n}{E^2}\right)_{E\to 0}$ *vs.* E^2 and superimposed on a family of curves of $15\Phi(\beta,\gamma)/(\beta^2 + 2\gamma)$ *vs.* $(\beta^2 + 2\gamma)$ with the scales of abscissae offset

moment of 31,000 D and a polarizability of 3.1×10^{-15} cm³.

Working with solutions of rat tail tendon collagen in dilute acetic acid, Yoshioka and O'Konski (1966) observed corresponding values of 15,000 D and 2.7×10^{-15} cm³, respectively.

Benoit (1951) studied the buildup phase of the electric birefringence curve by combining the rotatory diffusion equation with an orientation distribution function for the suspended macromolecules expressed as a series of Legendre polynomials. His result, which gives the electric birefringence at any point on the buildup curve for low values of field (say, within the Kerr region), is

$$\frac{\Delta n}{\Delta n_\infty} = 1 - \frac{3r}{2(r+1)} e^{-2Dt} + \frac{r-2}{2(r+1)} e^{-6Dt} \tag{17}$$

where Δn_∞ is the birefringence at saturation, and

$$r = P/Q \tag{18}$$

with

$$P = \mu^2/k^2T^2 \tag{19}$$

$$Q = (\alpha_1 - \alpha_2)/kT \tag{20}$$

and D is the rotatory diffusion constant, which can be determined from the electric birefringence decay curve.

It is seen from these relations that P is a measure of permanent dipole moment, and Q relates to the induced dipole moment. Also, it is seen by combining eq 12 and 13 with 18, 19, and 20 that $r = \beta^2/2\gamma$.

The decay portion of the electric birefringence pattern has been treated by O'Konski and Zimm (1950) and Benoit (1951). This is simpler than the buildup phase because, with the electric field terminated, only hydrodynamic considerations affect the motion of the suspended particles. Under this condition the expression for birefringence is

$$\Delta n = \Delta n_0 e^{-6Dt} \tag{21}$$

where Δn_0 is the electric birefringence at the time the square wave field drops to zero, and t is the decay time beyond this point.

If the macromolecular suspension under study is monodisperse, it becomes apparent that a plot of ln n/n_0 *vs.* t will be a straight line with a negative slope of $6D$. If the macromolecular suspension, however, is polydisperse, each species present will act in accordance with its rotatory diffusion constant and its concentration to give a composite curve expressed by

$$\Delta n = \Sigma \Delta n_{0i} e^{-6D_i t} \tag{22}$$

where the subscript i identifies each macromolecular species present, and the semi-log plot will be the sum of a number of straight lines and will appear as a curved line. This, therefore, becomes a test for monodispersity and a means of determining the rotatory diffusion constant of a monodisperse system. The rotatory diffusion constant leads to the dimensions of an ellipsoid of revolution *via* Perrin's equation (1934, 1936) and to the dimensions of a rigid cylinder *via* Burgers' equation (1938).

Figure 4 shows a semi-logarithmic plot of electric birefringence decay curves for collagen at pH 3.4, ionic strength 0.04, and a series of concentrations by Kahn and Witnauer (1969). It is seen that at collagen concentrations above 0.233%, the lines are curved, indicating either polydispersity or interaction between the suspended particles. From this

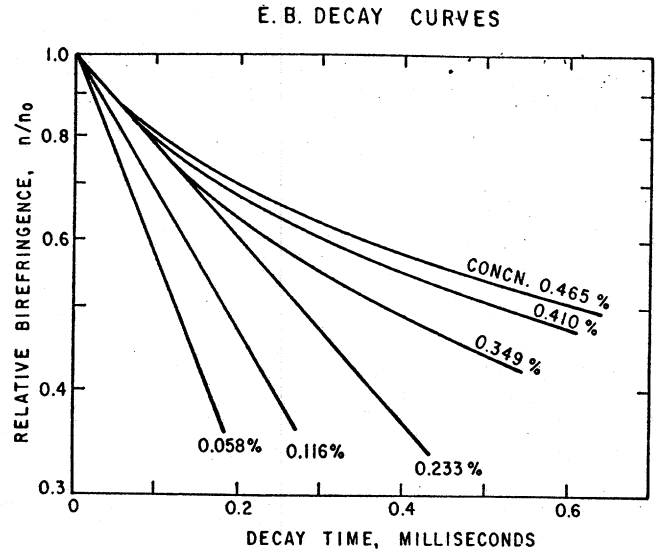

Figure 4. Graph of relative birefringence *vs.* electric birefringence decay time for collagen at pH 3.4 and ionic strength of 0.04. Reprinted with permission of: Kahn and Witnauer, *J. Amer. Leather Chem. Ass.* 64, 12 (1969)

concentration down to the lowest of 0.058%, the data appear to give straight lines whose slopes increase as collagen concentration decreases. Determining the rotatory diffusion constants from the curves at the lower concentrations and plotting these values *vs.* collagen concentration gives a graph which, when extrapolated to zero collagen concentration, yields a limiting rotatory diffusion constant of 1000 sec^{-1}. The collagen molecule in solution is known to be a rigid rod 3000 Å long and 14 Å in diameter, and this value of rotatory diffusion constant is in agreement with these dimensions when it is applied to Burgers' formula.

The techniques described here can be extended so as to study the aggregation and electric charge patterns of macromolecules in suspension and also interactions between them by observing their electric birefringence behavior under a variety of solution environments obtained by varying parameters such as solute concentration, pH, ionic strength, and dielectric constant of the solvent.

In addition to collagen reported by Kahn and Witnauer (1969) and Yoshioka and O'Konski (1966), protein systems that have been studied by electric birefringence include: fibrinogen, investigated by Haschemeyer and Tinoco (1962), Holcomb and Tinoco (1963), and Tinoco (1955); actin by Kobayasi *et al.* (1964); tropomyosin by Asai (1961); bovine serum albumin by Moser *et al.* (1966) and Riddiford and Jennings (1966). Gamma globulin, ovalbumin, and β-lactoglobulin were also investigated by Riddiford and Jennings (1967).

LITERATURE CITED

Asai, H., *J. Biochem. (Tokyo)* **50**, 182 (1961).
Benoit, H., *Ann. Phys.* **6**, 561 (1951).
Burgers, J. M., *Verh. Kon. Ned. Acad. Wetensch. Afdel. Natuurk. Sect. I* **16**(4), 113 (1938).
Debye, P., in "Handbuch der Radiologie," Vol. **6**, Akad. Verlagsges, Leipzig, 1925, p 597.
Haschemeyer, A. E. V., Tinoco, I., Jr., *Biochemistry* **1**, 996 (1962).
Holcomb, D. N., Tinoco, I., Jr., *J. Phys. Chem.* **67**, 2691 (1963).
Kahn, L. D., Witnauer, L. P., *J. Amer. Leather Chem. Ass.* **64**, 12 (1969).
Kerr, J., *Phil. Mag. Ser. 4* **50**, 337, 446 (1875).
Kerr, J., *Phil. Mag. Ser. 5* **9**, 157 (1880).
Kobayasi, S., Asai, H., Oosawa, F., *Biochem. Biophys. Acta* **88**, 528 (1964).
Moser, P., Squire, P. G., O'Konski, C. T., *J. Phys. Chem.* **70**, 744 (1966).
O'Konski, C. T., Zimm, B. N., *Science* **111**, 113 (1950).
O'Konski, C. T., Yoshioka, K., Orttung, W. H., *J. Phys. Chem.* **63**, 1558 (1959).
Perrin, F., *J. Phys. Radium* **5**, 497 (1934).
Perrin, F., *J. Phys. Radium* **7**, 1 (1936).
Peterlin, A., Stuart, H. A., *Z. Phys.* **112**, 129 (1939).
Riddiford, C. L., Jennings, B. R., *Biopolymers* **5**, 757 (1967).
Riddiford, C. L., Jennings, B. R., *J. Amer. Chem. Soc.* **88**, 4359 (1966).
Tinoco, I., Jr., *J. Amer. Chem. Soc.* **77**, 3476 (1955).
Yoshioka, K., O'Konski, C. T., *Biopolymers* **4**, 499 (1966).
Yoshioka, K., Watanabe, H., in "Physical Principles and Techniques of Protein Chemistry," S. J. Leach, Ed., Part A, Academic Press, New York, N.Y., 1969, p 335

Received for review October 14, 1970. Accepted April 16, 1971. Presented at Symposium on Characterization of Proteins, Division of Agricultural and Food Chemistry, 160th ACS Meeting, Chicago, Ill., September 1970.