the induced polarization of acepleiadylene. The results are listed in Table I. Since the P_∞ and R values are identical within our experimental error, it may be assumed that the R value for acepleiadylene represents the total induced polarization. This assumption is supported by the value of a_∞ from Table II in spite of the fact that the sodium-D line is close to an absorption band of acepleiadylene (λ = 555 mμ), a condition which might lead to an abnormal refraction.

Table II

Slopes of Dielectric Constant and Loss, and Relaxation Times of Acepleiadylene in Benzene Solution at 30°

λ, cm.	α' ± 0.01	α'' ± 0.001	$\tau \times 10^{12}$, sec.
1.25	2.42	0.097	20.2
3.22	2.52	.140	19.2
10.0	2.63	.114	23.2
25.0	2.69	.058	21.2
50.0	2.69	.029	19.7
	a_∞ = 2.37	Av.	20.7 ± 1.2

The results of the measurements at microwave frequencies are listed in Table II. While Debye behavior remains a possibility, in view of the uncertainty cited for a', the most probable values of a' and a'' indicate α = 0.09 and τ = 20.7 ± 1.2 $\mu\mu$sec. Assuming Debye relaxation (α = 0), τ = 24.3 ± 2.1 $\mu\mu$sec. which may be considered a maximum value.

Discussion

The most probable value for the dipole moment of acepleiadylene appears to be 0.49×10^{-18}. This was obtained by comparing the value of 0.47×10^{-18} calculated from a_∞ with the value of 0.51×10^{-18} calculated from γ', both by the Halverstadt-Kumler method.[16] The moment is about 50% of the value predicted by Pullman, *et al.*,[4] indicating the approximate character of the molecular orbital calculations. The inadequacy of the calculation may possibly be ascribed to steric factors. The dipole moment of azulene has been found[17] to be 1.0×10^{-18}. Since the distance between the seven- and five-membered rings is smaller than in acepleiadylene, the separation of a greater quantity of charge is indicated in azulene. The distorting influence of the relatively rigid naphthalene nucleus in acepleiadylene apparently affects the ease of displacement of charge from the seven- to the five-membered ring as a result of the geometrical requirements of the acepleiadylene ring system.

The relaxation time of the acepleiadylene molecule in benzene solution is in reasonable agreement with values obtained for other planar aromatic compounds of similar size and shape. Fischer[18] has reported values, at 23°, of 17.7, 15.7 and 19.2 ($\times 10^{-12}$ sec.) for α-bromonaphthalene, α-chloronaphthalene and 1-chloroanthraquinone, respectively. That these are of the same order of magnitude is to be expected since the longest dimension of acepleiadylene is comparable to that of anthraquinone. A more detailed comparison is not warranted because of complications introduced by the position of the dipole axis relative to the major axes of the molecule and by the possibility of a distribution of relaxation times.

Acknowledgment.—The authors are indebted to Professor V. Boekelheide of the University of Rochester for his extensive work in synthesizing a quantity of acepleiadylene sufficient for dielectric characterization.

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Princeton, New Jersey

[Contribution from the Department of Chemistry, University of California, Berkeley]

Electric Properties of Macromolecules. I. A Study of Electric Polarization in Polyelectrolyte Solutions by Means of Electric Birefringence[1]

By C. T. O'Konski and A. J. Haltner[2]

Received April 9, 1957

An investigation was made of electric birefringence in dilute solutions of a homogeneous preparation of the rod-like Tobacco Mosaic Virus (TMV). Variables studied included TMV and electrolyte concentrations, *p*H, field strength and frequency of the polarizing field, and the behavior in various transient fields. Some new experimental methods are described. The sign and magnitude of the intrinsic Kerr constant, its frequency dependence and the transient behavior show that orientation is produced by an induced polarization about 50 times greater than expected from electronic and atomic effects, and that the permanent dipole coupling is negligible. It is proposed that the large electric anisotropy arises because of polarizability of the ion atmosphere. The phenomena are interpreted with the aid of calculations on models incorporating a surface conductivity related to the relatively high local concentration of counterions at low ionic strengths. Results are in order-of-magnitude agreement with theoretical expectations on this basis. They cannot be explained with Jacobson's proposal of an ice-like hydration shell. Relaxation properties are qualitatively accounted for with the proposed model. No evidence could be found in the experimentally accessible *p*H region for a large contribution from the proton polarization of Kirkwood and Shumaker, which can be incorporated as an additional term in the surface conductivity. The significance of the counterion polarization in interpretation of the dielectric properties of proteins and other polyelectrolytes is discussed.

Introduction

Although a large number of investigations of the dielectric properties of macromolecular solutions have been reported in the last two decades, interpretations of the experimental results for polyelectrolytes such as the proteins and nucleic acids have been seriously limited by the inadequate understanding of the mechanisms by which electric polarization is produced in such systems. Aside

[1] This work is based upon the Thesis submitted by A. J. Haltner for the Ph.D. degree in Chemistry, January, 1955. Presented at the 131st meeting of the American Chemical Society in Miami, April, 1957.

[2] National Science Foundation Fellow, 1953–1954.

from spurious electrode polarization phenomena, there are a number of sources of electric polarization, among them the familiar electronic, atomic and orientation polarizations occurring in insulating systems. In addition, when considering a polyelectrolyte, polarization mechanisms involving ion transport may be important. One of these is called Maxwell–Wagner polarization,[3-5] which has its origin in the accumulation of charge carriers at interfaces between volume elements of different electrical properties when a heterogeneous system is placed under electric stress. This phenomenon is of importance in suspensions of semiconductors in insulating media,[6] and may be significant when the solute is an insulator and the solvent is a conductor. Another type of polarization is associated with production of an asymmetry of the ion atmosphere by an external field. Such asymmetry has been considered in theories of the dielectric constant and the conductivity of solutions of simple electrolytes.[7-10] A number of investigators have suggested that this may be of importance in aqueous colloids[11,12] and in solutions of polyelectrolyte macromolecules.[13-16] A third phenomenon which has been considered is proton transfer. This has been proposed to account for the electric properties of certain hydrogen bonded substances,[17-19] and has been postulated as a mechanism which may be responsible for the dielectric increments of aqueous proteins.[20]

The dielectric increments of some polyelectrolyte solutions have been discussed with reference to equations describing Maxwell–Wagner polarization.[14] In another proposal,[21] an effort was made to revive the idea of long-range solvation effects[22] to account for high dielectric increments and intrinsic viscosities in aqueous solutions of polyelectrolytes. Clearly, diverse phenomena may contribute to the dielectric properties, so interpretation in terms of any one polarization mechanism may not be valid. Definitive criteria for evaluation of the various mechanisms have not been established.

It appeared that important information regarding the relative importance of the various electric polarization mechanisms would be obtained from appropriately designed electric birefringence experiments. The combination[13] of transient polarizing fields, an optical system which permits direct observation of the sign as well as the magnitude of the birefringence, and oscillographic photography, provides a powerful tool for such studies. A previous investigation[23] of a number of preparations of Tobacco Mosaic Virus (TMV) indicated that solutions of this macromolecular species would be suitable for model studies of a large rigid polyelectrolyte. The relatively low rotational diffusion constant of TMV places the critical frequency for dispersion of the macromolecular orientation polarization well below the critical frequency for relaxation of a charge distribution in dilute electrolyte solutions, and probably below that for the relaxation of the ion atmosphere of a large polyelectrolyte macromolecule, which has been estimated recently.[15] The birefringence observed in TMV solutions clearly is due to macromolecular orientation, and is not directly related to the various polarization effects produced in the solvent, except in so far as these contribute to the production of an orienting torque upon the macromolecule. The transient response of the macromolecular system can give information regarding polarization mechanisms. For these reasons, electric birefringence appeared particularly effective as an aid in understanding the origin of electric polarization in polyelectrolyte systems.

In this study, experiments were conducted in various types of polarizing fields to establish the general nature of the orienting mechanism, and in solutions of varying conductivity and *p*H to determine some of its specific features. The results are compared with predictions from available theory, some new concepts and calculations are presented, and some proposals are made regarding the extension of experimental investigations and of the theory.

Experimental

Materials.—The Tobacco Mosaic Virus preparation employed throughout this work was TMV (I), which was shown to be monodisperse in an earlier study.[23] All buffers were Clarks and Lubs solutions prepared with analytical reagents and freshly boiled distilled water, employing potassium salts throughout. They were stored at about 0° and checked periodically by looking visually for the Tyndall effect accompanying bacterial contamination. The stock virus solution, also stored near 0°, was remarkably stable. It gave no evidence of bacterial contamination and maintained constant transient electric birefringence properties in the course of experimental studies from April, 1952, to November, 1954. Glassware was cleaned with hot dilute nitric acid, to avoid possible denaturation or adsorption of the virus on surfaces treated with dichromate solutions.

Apparatus.—The Kerr cell, the optical system, and the oscillographic methods developed for this work have been described.[23] The analyzer angle was typically 3° from the crossed position. The limiting time constant of the detection system was that of the photomultiplier load resistor and the capacitance (*ca.* 20 $\mu\mu$fd.) associated with the photomultiplier anode and the input circuit of the cathode follower amplifier. The load resistor was generally 10^5 ohms, so the input time constant was 2 μsec., which was negligible compared with the observed relaxation time

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(9) L. Onsager, *ibid.*, **28**, 277 (1927).

(10) H. Falkenhagen, M. Leist and G. Kelbg, *Ann. Physik*, [6] **11**, 51 (1952).

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(14) H. M. Dintzis, J. L. Oncley and R. M. Fuoss, *Proc. Natl. Acad. Sci.*, **40**, 62 (1954).

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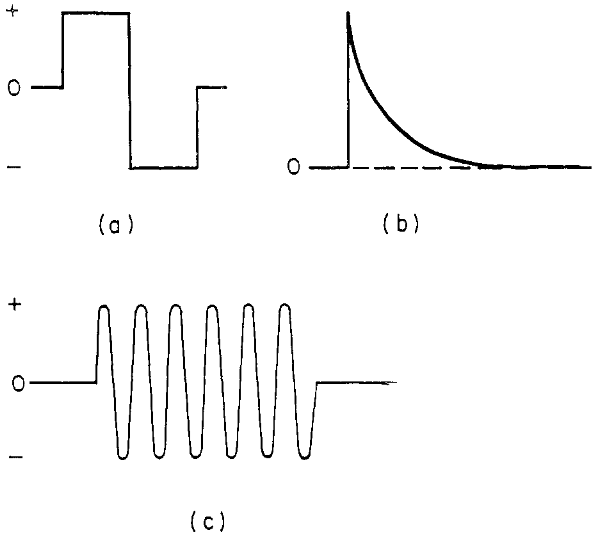
(21) B. Jacobson, THIS JOURNAL, **77**, 2919 (1955).

(22) See, *e.g.*, S. Glasstone, "Textbook of Physical Chemistry," Second edition, D. Van Nostrand, Co. New York, N. Y., 1940, p. 1264.

(23) C. T. O'Konski and A. J. Haltner, THIS JOURNAL, **78**, 3604 (1956).

(500 μsec.). The stray light constant[24] of the system was around 2×10^{-4}.

Wave Forms.—In addition to the square pulses applied in the previous studies,[13,23] several new types of wave forms were used. These may be described as (a) reversing square pulse, (b) exponentially decaying pulse, and (c) pulsed sine wave, and they are illustrated schematically in Fig. 1. The reversing square pulse was employed to permit deductions, from the oscillographically observed birefringence transient, regarding the relative importance of permanent dipole moments and induced polarization effects. It was produced by properly phasing the output signals of two Tektronix[25] type 161 pulse generators, operated from a single type 162 wave form generator. The last was triggered by means of flash contacts on the oscillograph camera.



(a) (b)

(c)

Fig. 1.—Curves of field intensity *vs.* time illustrating wave forms employed in this research: (a) reversing square pulse; (b) exponentially decaying pulse; (c) pulsed sine wave.

The pulsed sine wave train (c) was employed in studies of the electrical dispersion of the Kerr effect. Compared with a continuous sine wave, it provides the advantages that heating in the specimen under test may be kept to a minimum, and buildup and decay transients may be observed. It was produced at audiofrequencies by means of a sine-wave generator and an electronic switch which was actuated by means of the square pulse generator employed previously,[23] and at radio frequencies by means of a 100 watt radio transmitter[26] which was gated by rectangular pulses at the power amplifier stage. Peak-to-peak output voltages of 200 to 1500 volts were obtained at radio frequencies, depending upon the frequency and load resistance.

The exponentially decaying pulse (b) was investigated because it is probably the easiest wave form to generate for transient birefringence experiments. In the simplest case it was produced by connecting a condenser across a voltage source and then across the Kerr cell, or the cell and a known resistor in parallel, either manually or by means of a switch. Alternatively, by discharging the condenser through a hydrogen thyratron in series with the Kerr cell, pulses of over a thousand volts amplitude and with rise times around 0.2 μsec. and decay constants as short as a few μsec. were produced readily. Mechanical switching was not entirely reliable at short time constants (<100 μsec.), apparently because of intermittent arcing which occurred at the points of contact when the connections were made mechanically, but this simple arrangement was useful for the relaxation time encountered here.

Equations and Computations; Calibration Procedure.—The electric birefringence, Δn, of a fluid is conventionally defined

$$\Delta n = n_{\parallel} - n_{\perp} \tag{1}$$

where $n_{\parallel}$ and $n_{\perp}$ are the refractive indices for linearly polarized components of electromagnetic radiation with electric vectors parallel and perpendicular, respectively, to the direction of the applied field. If the wave length of the radiation *in vacuo* is λ_0, the corresponding difference of optical path length, in wave lengths, is $l(n_{\parallel} - n_{\perp})/\lambda_0$, where l is the length of the path in the birefringent medium. Accordingly, the optical retardation δ, in radians, of the parallel component with respect to the perpendicular component is given by

$$\delta = 2\pi l(n_{\parallel} - n_{\perp})/\lambda_0 \tag{2}$$

It has been shown for many cases that the Kerr law[27] holds, *viz.*

$$\Delta n = KnE^2 \tag{3}$$

where K is a constant depending upon the system. In macromolecular solutions for which this relation applies, and in which the solvent contributes negligibly to the birefringence, the Kerr constant will be a function of the concentration, c, of the macromolecular component, becoming linear in c at sufficiently low values. Therefore it is useful[28] to define a specific Kerr constant

$$K_{\text{sp}} = K/c = \delta\lambda_0/2\pi lnE^2c \tag{4}$$

Similarly, in analogy to the definition of intrinsic viscosity, we may define an intrinsic Kerr constant

$$[K] = \lim_{c \to 0} K_{\text{sp}} \tag{5}$$

Sometimes another quantity, $B = \Delta n/\lambda_0 E^2$, is employed.[29] This "constant" will vary strongly with wave length, even when Δn is essentially independent of λ_0. In many cases of interest, the optical dispersions are small because studies are most generally carried out in the spectral region where neither solute nor solvent absorb strongly; therefore K is a more appropriate quantity.

The equation derived earlier[30] for the optical system is

$$\Delta I_\delta/I_0 = [(2 \sin \alpha + \delta \cos \alpha)^2/(4 + \delta^2)] - \sin^2 \alpha \tag{6}$$

Here ΔI_δ is the change in light intensity at the photomultiplier for an optical retardation δ. The angle α specifies the rotation of the analyzer from the crossed position,[13] and is taken positive when the optical configuration is such that a positive birefringence or δ produces a positive ΔI_δ. I_0 is the light intensity which would reach the multiplier if the analyzer were set in the same direction as the polarized beam emerging from the cell. I_0 and α were determined by appropriate measurements[23] with solution in the cell, to take into account possible effects of absorption, refractive index or small optical rotations.

Values of δ were computed from eq. 6, making allowance for the stray light in the system.[23] Time and amplitude calibration procedures, the method of calibrating the optical system and the method of determining $\Delta I_\delta/I_0$ were described previously.[23] The optical retardation, δ, could be determined to ±2% for values of 2×10^{-3} radian or greater, and to ±10% for $\delta = 10^{-4}$ radian.

Results

In this investigation, emphasis was placed upon the measurement of the absolute values and sign of the birefringence, to facilitate comparison of the results with theory. All measurements were carried out with the Kerr cell thermostated at 25.0 ± 0.1°. In each experiment, the applied fields were measured with a calibrated oscilloscope and with solution in the cell, so conditions were identical to those under which the birefringence was observed. The tem-

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[24] Unless otherwise specified, the terminology is that employed in ref. 23.

[25] Tektronix, Inc., Portland, Oregon.

[26] Bendix aircraft transmitter, type TA-12C.

[27] J. Kerr, *Phil. Mag.*, **50**, 337, 446 (1875); **8**, 85, 229 (1879); **9**, 157 (1880); **13**, 153, 248 (1882).

[28] A. Peterlin and H. A. Stuart, "Handbuch und Jahrbuch der Chemischen Physik," Vol. 8, Sect. 1B, Becker and Erler, Leipzig, 1943. Edward Bros., Inc., Ann Arbor, Mich., 1948.

[29] See, *e.g.*, C. G. LeFevre and R. J. W. LeFevre, *Rev. Pure Applied Chem.*, **5**, 261 (1955).

[30] This equation contained a misprint in ref. 13, as pointed out in footnote 38 of ref. 23.

perature rise from joule heating during any applied pulse was negligible.

Relaxation Time.—Values of the relaxation time,[13] $\tau = 1/6D$, were determined at concentrations of TMV (I) from 2.2×10^{-3} to 1.0 mg./cc. and were reported previously.[23] They indicate that the solutions behave ideally with respect to dynamical behavior at 1 mg./cc. or less, with τ = 0.50 msec. The significance and interpretation of the rotational diffusion constant has been discussed[23] in relation to other studies.

Birefringence *vs.* Field Strength in Dilute Solutions.—At each of the concentrations for which the relaxation times were measured, the steady-state birefringence was determined for various values of field strength. The duration of the square polarizing pulse was around 3 msec. The phosphate buffer (*p*H 7.0) was 1.5×10^{-4} *M* in total phosphate, allowing for the low concentration of buffer from the stock virus solution. The TMV (I) stock was found to contain 3.3×10^{-3} *M* buffer by conductance measurements. To permit calculation of electric field strengths from pulse amplitudes the electrode separation was measured with a micrometer eyepiece. The spacing was 2.50 mm., constant within 0.05 mm.

Results are presented in Fig. 2, where the straight lines represent Kerr law behavior. They indicate that the Kerr law is obeyed from 2×10^{-3} to 1 mg./cc. within the experimental precision, and that electrode polarization effects are negligible. Values of $10^7 \delta/E^2$ in radians volts^{-2} cm.^{2} were 10.7, 5.3, 1.8, 3.2×10^{-1}, and 3.2×10^{-2} at 1.00, 0.50, 0.13, 1.7×10^{-2}, and 2.2×10^{-3} mg./cc., respectively. The virus concentrations were not accurately known at the lower concentrations, primarily because of cumulative dilution errors. Variations of $10^7\ \delta/E^2c$ below 0.5 mg./cc. were considered to be within the experimental errors. Accordingly, the best value of δ/E^2c at 1 mg./cc. or less is 1.07×10^{-6} radians volts^{-2} cm.^{2}g.$^{-1}$l. with a probable accuracy of 0.05×10^{-6}. Independent values computed from the earlier preliminary results[13] at the same buffer concentration are in satisfactory agreement.

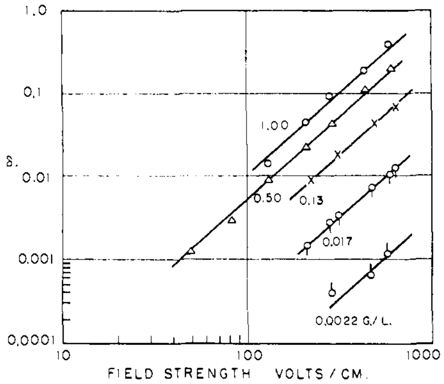

Fig. 2.—Birefringence *vs.* field strength for dilute solutions of TMV (I) at *p*H 7.0 in 1.5×10^{-4} *M* phosphate buffer. Numbers are virus concentrations, in g./liter.

Birefringence Build-up.—It was of interest to determine precisely the shape of the build-up curve with a square pulse since calculations[31,32] based upon the model of Peterlin and Stuart[28] indicate that under certain conditions the build-up and decay curves are not symmetrical. A plot of δ *vs.* time for the virus at 0.50 mg./cc. and *p*H 7.0 phosphate buffer is presented in Fig. 3. The data were fitted with a straight line with most weight given to points at intermediate values as those points are least affected by errors in the measurement of time, birefringence, and δ_s, the steady-state birefringence. Within the experimental precision (*ca.* 1%), the growth follows a first-order rate law, with a time constant of 0.48 ± 0.02 msec. This is to be compared with the time constant for the decay,[23] 0.50 ± 0.02 msec.

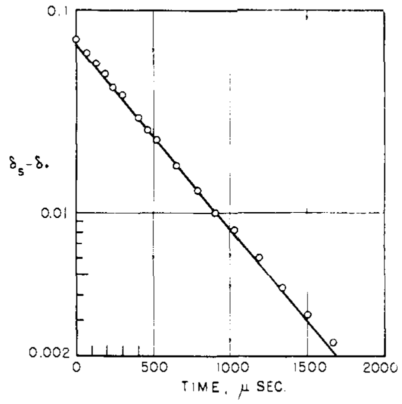

Fig. 3.—Birefringence build-up curve: δ_s is the steady-state birefringence attained after 3 msec. with a rectangular pulse, and δ is the value at any time measured from the onset of the polarizing field.

Birefringence in a Rapidly Reversed Field.—In earlier studies in this Laboratory, some experiments with square wave fields over a range of frequencies had led to the conclusion that the permanent dipole moment character of the electrical orientation was negligible in TMV.[13] It seemed desirable to repeat this observation by means of a single pulse experiment, taking advantage of the greater sensitivity of the present apparatus in an attempt to detect the transient in the birefringence which would occur upon reversal of the polarizing field if a permanent dipole moment contributes significantly to the electrical orientation of TMV. The wave form shown in Fig. 4a was applied to a solution containing 0.5 mg./cc. of TMV (I) in phosphate buffer at *p*H 7. The duration of the negative part of the pulse was around 4 msec., which was sufficient to achieve essentially the steady-state birefringence signal, as shown in Fig. 4b. Upon reversal of the field, which occurred within 5 μsec., no transient significantly greater than the random noise could be observed. Several visual observations were made with the same result. This confirmed the earlier result, and leads to the conclusion that the orienting torque in TMV solutions is a result of an induced polarization which can be reversed, in periods short compared to the relaxation time, by reversing the applied field. Thus, the torque remains unchanged upon field reversal

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[31] H. Benoit, *Ann. phys.*, **6**, 561 (1951).

[32] I. Tinoco, THIS JOURNAL, **77**, 4486 (1955).

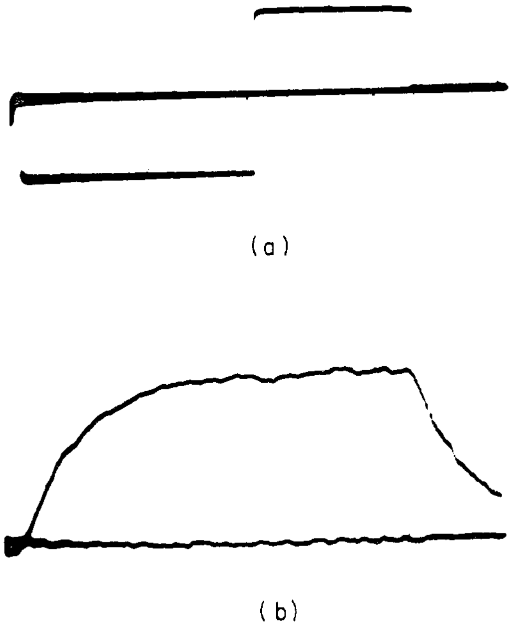

Fig. 4.—Effect of rapid reversal of the polarizing field. (a) Applied wave form. The middle line corresponds to zero field intensity. (b) Birefringence signal, indicating no appreciable change of the steady-state macromolecular orientation upon reversing the field. Sweep, 0.90 msec./scale division.

in contrast with the well-known case of permanent dipole orientation.

Transient Birefringence in an Exponentially Decaying Field.—It was shown above that the transient birefringence of dilute solutions of TMV follows a first-order rate law, which may be written

$$d\delta/dt = k(\delta_s - \delta) \qquad (7)$$

where δ is the optical retardation at time t, δ_s is the steady-state value of the retardation for an applied field E, and k is the rate constant, equal to $1/\tau$ or $6D$. In certain instances when the applied wave form is not square, it is possible to solve the differential equation. A simple and useful example is furnished by the case of an exponentially decaying field produced, *e.g.*, by the discharge of a condenser, C, through a resistor, R

$$E = 0, t < 0$$
$$E = E_0 \exp(-t/RC), t \geq 0 \qquad (8)$$

where E is the field strength at time t, and E_0 is the field strength at $t = 0$. The Kerr law may be written

$$\delta_s = AE^2, A = 2\pi nK/\lambda_0 \qquad (9)$$

Inserting in (9)

$$d\delta/dt + \delta/\tau = AE^2_0 \exp(-2t/RC)/\tau \qquad (10)$$

for which we find the solution

$$\delta = AE^2_0[\exp(-t/\tau) - \exp(-2t/RC)]/[(2\tau/RC) - 1] \qquad (11)$$

Since this is indeterminate for $RC = 2\tau$, the differential equation was solved separately for this special case. The result is

$$\delta = AE^2_0\, t \exp(-t/\tau)/\tau \qquad (12)$$

The optical retardation rises to a maximum

$$\delta_{\max} = AE^2_0/e \qquad (13)$$

when $t = \tau = RC/2$. The initial slope of the buildup is

$$(d\delta/dt)_{t=0} = AE^2_0/\tau \qquad (14)$$

If RC is chosen several times smaller than τ, then for large values of t, $\exp(-2t/RC)$ will be negligible compared to $\exp(-t/\tau)$ in eq. 11, so it reduces to

$$\delta \simeq AE^2_0 \exp(-t/\tau)/(2\tau/RC - 1) \qquad (15)$$

Under these conditions a plot of log δ *vs.* t is linear providing only a single relaxation time is present. In this way, τ can be determined. The product AE^2_0 can likewise be obtained and RC can be measured in an auxiliary experiment. Thus, it is possible to calculate the entire birefringence curve.

Figure 5 shows an exponential voltage pulse obtained with the thyratron circuit and the transient birefringence signal which resulted when this pulse was applied to a solution of TMV (I) containing 0.50 mg. of virus per cc. The measured time constant for the condenser discharge was 101 μsec. For times greater than 400 μsec. the birefringence followed an exponentially decaying curve with a relaxation time of 0.48 ± 0.02 msec. From points in this region of the decay curve the value of $(AE^2_0)/[(2\tau/RC) - 1]$ was found to be 0.32, which corresponds to $\delta/E^2c = 1.08 \times 10^{-6}$. The birefringence curve was calculated from eq. 11 using these parameters, and the result is shown in Fig. 6. The experimental points agree satisfactorily with the calculated curve except in the region near the birefringence peak where the deviations are outside

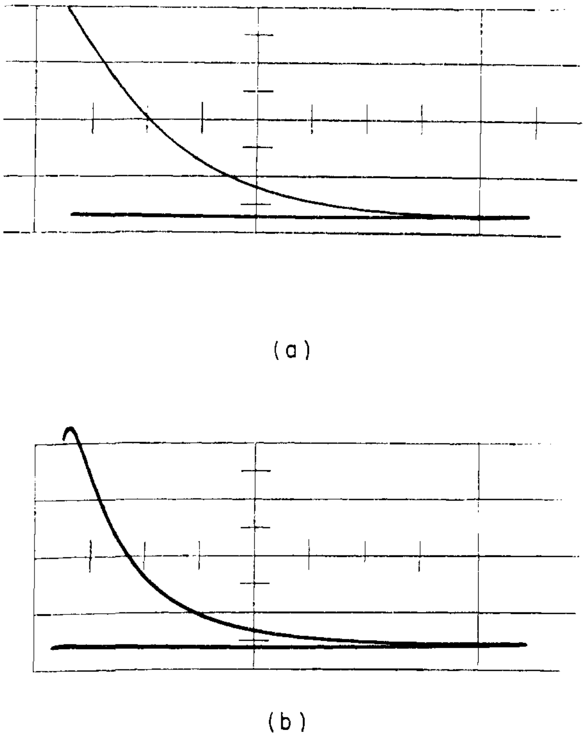

Fig. 5.—Transient birefringence in an exponentially decaying field. (a) Applied pulse; RC = 101 μsec. Sensitivity, 160 v./cm.; sweep, 50 μsec./cm.; (b) Signal for TMV (I), 0.50 g./liter, pH 7.0, κ = 18.5 μmho/cm. Sensitivity, 0.32 v./cm.; sweep, 0.30 msec./cm. Divisions on the grid are cm.

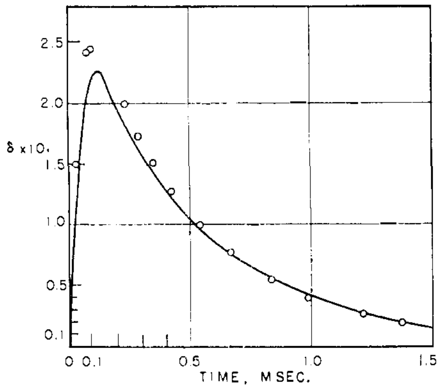

Fig. 6.—Birefringence plot for Fig. 5. The points are experimental values and the solid curve is computed from eq. 11.

of the experimental error, possibly because of some saturation. The initial field E_0, was over 2 kv./cm.

Birefringence in Pulsed Sine Wave Fields.—Another case of interest is that of a voltage pulse consisting of several oscillations of a sine wave. For this case the applied field is given by

$$E = 0, t < 0$$
$$E = E_0 \sin \omega t, t \geq 0 \qquad (16)$$

where E is the instantaneous value of the field at the time t, E_0 is the maximum amplitude of the oscillating field with angular frequency ω. The differential equation describing the behavior of the birefringence, assuming buildup and decay following the same first-order rate law, is

$$\frac{1}{\omega}\frac{d\delta}{dt} + \frac{1}{\omega\tau}\delta = \frac{AE^2_0}{\omega\tau}\sin^2 \omega t \qquad (17)$$

Equation 17 is solved easily by standard methods. The complete solution is

$$\delta = \frac{AE^2_0}{1 + 4\omega^2\tau^2}\left[\frac{1}{\omega\tau}\sin^2 \omega t - 2 \cos \omega t \sin \omega t + 2\omega\tau(1 - e^{t/\tau})\right] \qquad (18)$$

For $t >> \tau$, eq. 18 reduces to the steady-state solution

$$\delta = \frac{AE^2_0}{1 + 4\omega^2\tau^2}\left[\frac{1}{\omega\tau}\sin^2\omega t - 2 \cos \omega t \sin \omega t + 2\omega\tau\right] \qquad (19)$$

From eq. 19 the average value of δ is

$$\delta_0 = AE^2_0/2 = A(E_{\text{rms}})^2 \qquad (20)$$

Equation 19 can be put in the form

$$\delta = \delta_0\left[1 \pm \frac{\cos(2\omega t - \phi)}{(1 + 4\omega^2\tau^2)^{1/2}}\right] \qquad (21)$$

where

$$\tan \phi = 2\omega\tau \qquad (22)$$

In the steady state the birefringence consists of two components, one of which is constant in time and the other of which alternates with twice the frequency of the applied field and differs in phase from the applied field by the angle ϕ. In the limit of high frequencies, ϕ approaches 90°, and the second term of the right-hand member of eq. 21 approaches zero. Therefore, at sufficiently high frequencies a steady birefringence is obtained with magnitude given by eq. 20. The extreme values of δ, δ_M, are given by

$$\delta_M = \delta_0\left[1 \pm \frac{1}{(1 + 4\omega^2\tau^2)^{1/2}}\right] \qquad (23)$$

If A, E_0 and δ_M are measured, ϕ can be calculated for each frequency. According to eq. 22, a plot of $\tan \phi$ *vs.* frequency should be linear with a slope of $4\pi\tau$.

Equation 21 is identical in form with an equation given by Peterlin and Stuart[28] and used by Benoit.[33] In the notation of Peterlin and Stuart, $\tau_1 = 2\tau$, $\tan \delta_1 = \tan \phi$ and $\bar{K}_1/\delta = K/\delta_0$. The derivation given by Peterlin and Stuart applied strictly only to gases since the Lorentz internal field was used in calculating the torque on the molecules. In deriving eq. 21, no specific assumption has been made concerning the nature of the orienting torque. It has been sufficient to assume only that the transient behavior of the birefringence follows a first-order rate law. In general, A may be frequency dependent if there are dispersion phenomena other than the one associated with dipolar reorientation. By expanding the cosine term of eq. 21, it can be seen that the alternating portion of the birefringence is a superposition of a pure cosine term and a pure sine term. Zimm[34] has measured the relative values of both the sine and cosine components as a function of frequency of a continuous a.c. field in a solution of TMV. The preparation he used was aggregated, and he found his results consistent with the presence of two species having relaxation times of 0.3 and 1.9 μsec.

Figure 7 is a plot of minimum and maximum values of the steady-state birefringence in a solution of TMV (I) containing 0.5 g./l. *vs.* the log of the frequency. The solid curves are calculated from eq. 23 assuming that $\tau = 0.50$ msec. The experimental points fall reasonably well on the theoretical curves. However, to obtain this agreement it was necessary to assume a value of 6.5×10^{-7} for A. The value of A found for long pulses was $5.4 \pm 0.2 \times 10^{-7}$. The discrepancy probably occurred because of some distortion of the audio frequency sine waves. The wave forms used here had slightly flattened maxima, and this would lead to a greater

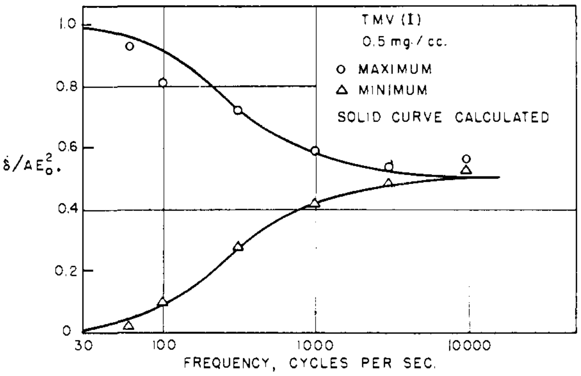

Fig. 7.—Birefringence minima and maxima *vs.* frequency in audio frequency sine wave fields for TMV (I), 0.50 g./liter, *p*H 7.0. See text for definitions of A and E_0.

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[33] H. Benoit, *J. chim. phys.*, **49**, 517 (1952).

[34] B. H. Zimm, private communication.

birefringence than for pure sine waves having the same peak-to-peak values. To account for the difference in *A* the r.m.s. values would have had to be approximately 10% greater than that calculated using the measured peak-to-peak voltage and the assumption of sine wave shape. In view of the results obtained with the condenser discharge, it is also possible that a slight deviation from eq. 23 may be occurring.

Figure 8 is a plot of tan ϕ *vs.* frequency, obtained from the extreme values, δ_M, by application of eq. 22 and 23. The experimental points fall reasonably well on a straight line through the origin. From the slope, a value of $\tau = 0.52$ millisecond was obtained. Since the experimental errors in this determination were considerable, this result is in satisfactory agreement with the more accurate value of 0.50 msec. obtained previously.[23] This result may be considered an independent check on the value for the relaxation time in TMV(I).

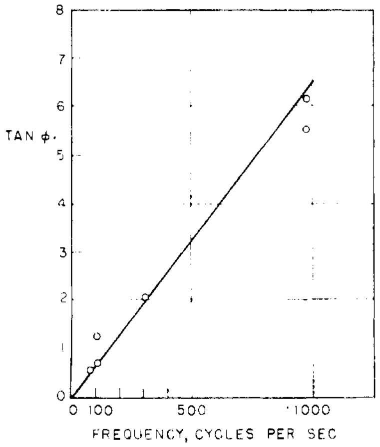

Fig. 8.—Phase angle *vs.* frequency for TMV (1), 0.50 g./liter, *p*H 7.0.

Effect of Added Electrolyte on the Steady-state Birefringence.—O'Konski and Zimm[13] discovered that the magnitude of the electric birefringence in solutions of TMV was decreased by increasing the concentration of dilute buffer. Further investigation was undertaken here to determine whether this would occur with a simple electrolyte, and to establish the effect more quantitatively. Solutions of TMV containing 1.00 mg. of TMV (I) per cc. buffered at *p*H 7.0 with 1.9×10^{-4} *M* phosphate buffer were diluted to 0.50 mg. of virus per cc. by solutions containing 1.0×10^{-4} *M* buffer and varying concentrations of KCl. The magnitude of the electric birefringence of these solutions was measured as a function of field strength. The results for one set of experiments are plotted in Fig. 9. Additional results were obtained using a plastic cell. The electrode separation and the cell constant for conductivity for that cell were not very accurately known. The data of Fig. 9 are more accurate since they were obtained with the glass cell,[23] the constants of which were known to ±2%. The Kerr law was obeyed over an approximately 10-fold range of electrolyte concentration. As the concentration increased, the ratio of δ/E^2 steadily decreased and changed by nearly a factor of two. A plot of log δ/E^2 *vs.* $\log_{10}$ of the specific conductivity, κ, is given in Fig. 10. The five points fall reasonably well on a straight line with slope -0.28 ± 0.03. Accordingly, the data can be correlated by the relation

$$\delta/E^2 = 27 \times 10^{-9}\, \kappa^{-0.28} \qquad (24)$$

where κ is in ohm^{-1} cm.$^{-1}$, *E* in volts cm.$^{-1}$, and δ in radians cm.$^{-1}$. Other points obtained in measurements with the plastic cell also fell fairly well on a straight line with the same slope as that found above.

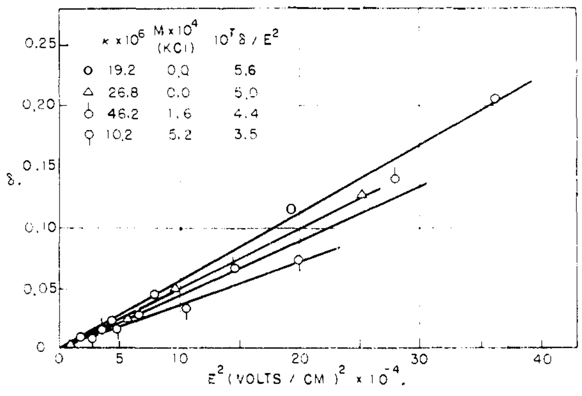

Fig. 9.—Effect of KCl upon the steady-state birefringence at constant buffer concentration for TMV (I) at 0.50 g./liter and *p*H 7.0.

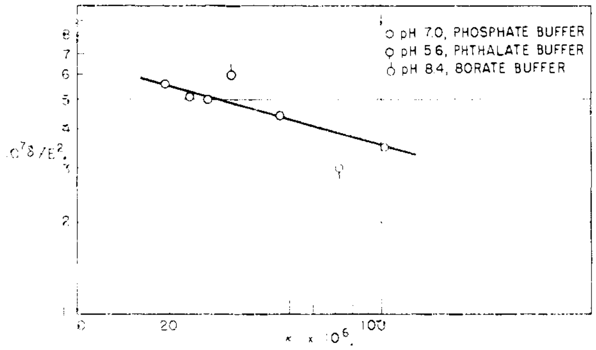

Fig. 10.—Variation of the birefringence with conductivity and *p*H for TMV (I) at 0.50 g./liter.

Effect of *p*H on the Steady-state Birefringence.—Since the TMV molecule contains a large number of ionizable acidic and basic groups, it might be anticipated that the *p*H of the solution would be an important variable determining the magnitude of the electric birefringence. Besides the runs at *p*H 7.0 in 1.5×10^{-4} *M* phosphate buffer, experiments were carried out at *p*H 5.6 ± 0.2 in approximately 5×10^{-4} molar phthalate buffer and at *p*H 8.4 ± 0.2 in 1.2×10^{-4} *M* borate buffer. These solutions were obtained by dialyzing solutions of 0.50 mg. per cc. TMV against four changes of the buffers for several days at 0°. The results are plotted in Fig. 11. The birefringence is found to obey the Kerr law at the *p*H's investigated. The values of δ/E^2 were determined and are plotted against specific conductivity in Fig. 10. The point at *p*H 5.6 is found to lie below the line representing the points determined at *p*H 7.0 under similar conditions, but the point at *p*H 8.4 lies above this curve. The variations are well outside of the probable experimental error.

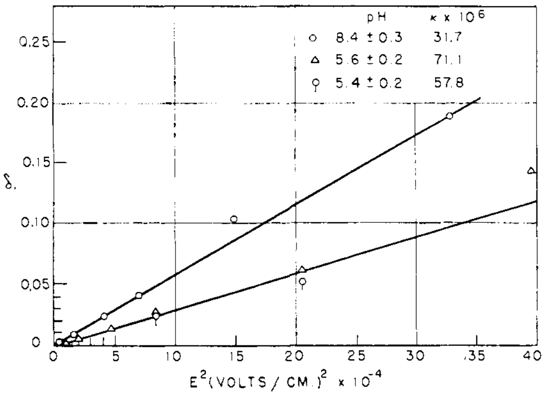

Fig. 11.—Variation of the birefringence with pH for TMV (I) at 0.50 g./liter.

In all but one of these experiments at various pH's and KCl concentrations the transient behavior of the birefringence was unchanged and was characterized by a single relaxation time of 0.50 msec. The single exception was in the experiments at pH 5.6. Here the major portion of the decay curve indicated a relaxation time of 0.50 msec., but there was evidence of the presence of a small amount of a polymerized species having a much longer relaxation time of approximately 20–30 msec. The steady-state birefringence due to this component was estimated to be approximately 5% of the total steady-state birefringence in the solution. It is possible that aggregation of some of the TMV may have occurred at this pH, and as a consequence the value of δ/E^2 for the monomer should be slightly larger than shown in Fig. 10.

Electrical Dispersion of the Birefringence at Radio Frequencies.—Experiments described above and others reported earlier[13,33] have shown that the magnitude of the electric birefringence in TMV does not change when the frequency of the applied field is increased to 10 to 20 kc. Here, these experiments have been extended to cover the region from 10 kc. to 5 mc. Pulses of radio frequency were applied to the cell from the Bendix transmitter and the magnitude of the birefringence was observed as a function of the magnitude of the *rf* field and of the frequency. In Fig. 12, some representative plots are shown of the log of the steady state birefringence *versus* the log of the r.m.s. field strength. It can be seen that the Kerr law is applicable at radio frequencies. Accordingly, the ratio δ/E^2_{rms} was used to compare results at various frequencies, in accordance with eq. 22 above.

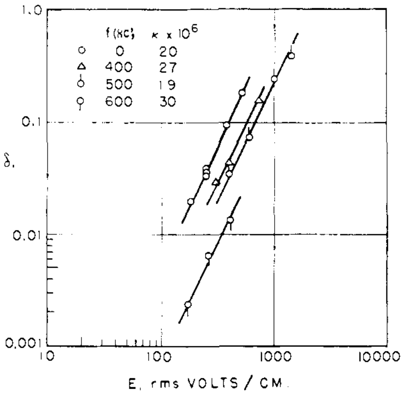

Fig. 12.—Birefringence *vs.* field strength at various frequencies for TMV (I) at 0.50 g./liter, pH 7.0.

The results at various KCl concentrations and 1.5×10^{-4} M phosphate buffer of pH 7.0 are shown in Fig. 13. In Fig. 14, the results at pH 8.4 and 5.4 are presented with the series at pH 7.0 of nearest conductivity. In five of the six runs shown there was no evidence of dispersion in the electric birefringence until the frequency of the applied field exceeded 10 kc., which is in agreement with the previous square wave experiments[13] and the results reported by Benoit.[33] The single exception is the solution run at pH 8.4 where there is evidence that the beginning of the dispersion region lies between 3 and 10 kc. It can be seen that $\delta/(E_{\text{rms}})^2$ for a given solution decreases rapidly above about 30 kc., and at 5 mc. its value is less than 1% of the value found for d.c. fields.

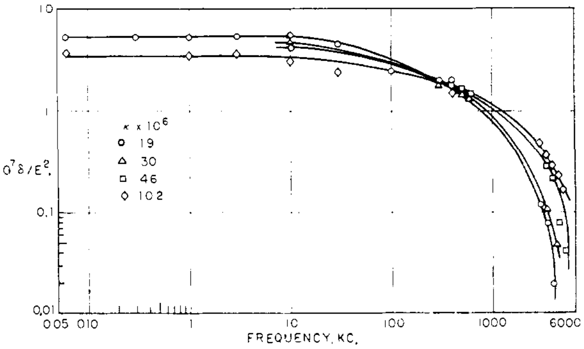

Fig. 13.—Log birefringence *vs.* frequency for TMV (I) at 0.50 g./liter, pH 7.0, various concentrations of KCl.

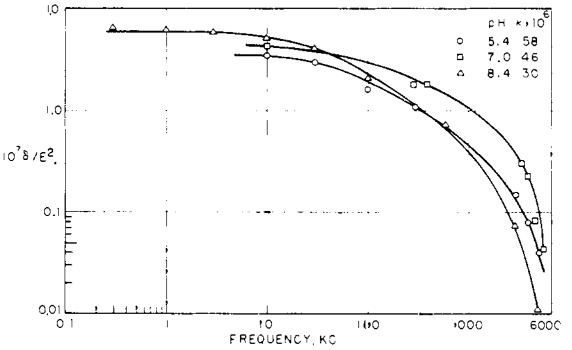

Fig. 14.—Log birefringence *vs.* frequency for TMV (I) at 0.50 g./liter, pH 5.4, 7.0, and 8.4. Solvent conductivities were not equal.

The dispersions observed are rather broad, extending over a frequency range of at least 2.5 decades. There appears to be no tendency for the birefringence to level off at the highest frequencies employed. The magnitudes and shapes of the curves are quite sensitive to conductivity and pH. The results at pH 7.0 show that $\delta/(E_{\text{rms}})^2$ decreases less rapidly with frequency as the conductivity of the solution is increased. At low frequencies the values of $\delta/(E_{\text{rms}})^2$ decrease as conductivity increases; but above 300 kc. the trend is reversed, and

$\delta/(E_{\text{rms}})^2$ increases as conductivity increases. Somewhat analogously to the case of dielectric dispersion, a mid-value frequency, ν_{m}, may be arbitrarily defined as the frequency at which the value of $\delta/(E_{\text{rms}})^2$ has fallen to $^1/_2$ of its value in low frequency fields. The values of ν_{m} for the various runs are given in Table I.

TABLE I

MID-VALUE FREQUENCIES IN TMV SOLUTIONS AT 0.50 MG. PER CC. TMV (I)

*p*H	TMV (I) K, ohm^{-1} cm.$^{-1}$	ν_{m} (mc.)
7.0	19	0.16
7.0	30	.22
7.0	46	.30
7.0	102	.35
8.4	30	.065
5.4	58	.13

At *p*H 7.0 the values of ν_{m} increase regularly with conductance. At *p*H 5.4 and 8.4, it can be seen that the values of ν_{m} are considerably lower than those for solutions of comparable conductivity at *p*H 7.0.

Behavior in More Concentrated Solutions.—The complex behavior of the transient electric birefringence in more concentrated solutions, which was observed previously,[13] has been substantiated by additional more quantitative experiments.[1] An analysis of the results leads to the tentative conclusion that a part of the behavior at 2.5 and 5.0 mg./cc. can be explained by the reversible formation of macromolecular complexes, with indications that side by side dimers may be present. The strong dependence of the specific birefringence and the relaxation behavior upon concentration suggests that transient electric birefringence may prove useful in studies of macromolecular interactions. Interpretations are complicated by the appearance of a birefringence component of negative sign in the more concentrated solutions, and because the Kerr law is not obeyed. These more involved results will be presented separately in the future.

Discussion

Intrinsic Kerr Constant.—The value of δ/E^2c reported above can be used to compute $[K]$, the intrinsic Kerr constant. Since a distribution of wave lengths is involved in the optical system, the observed δ will be a weighted mean over the region of spectral response. The response of the optical system is determined by the spectral distribution of the tungsten source and the spectral sensitivity of the photomultiplier. Since neither the virus nor the solvent absorb light significantly in the region of interest, the variation with wave length λ_0 of $[K]$ should be small. However, for a given Δn, δ depends upon λ_0, as shown by eq. 2. Accordingly, the spectral response curve of the optical system, which is the product of the spectral response $S(\lambda_0)$ of the 1P21 multiplier and the spectral emission $E'(\lambda_0)$ of the tungsten source[35] should be multiplied by $1/\lambda_0$ to obtain the weighted response curve. Then a value of λ_0 may be selected for the computation of K_{sp} from eq. 4. The resulting curve, SE'/λ_0 *vs.* λ_0, was roughly symmetrical, with a maximum at 5500 Å., a half-width of 1240 Å. centered at 5320 Å., and an area divided equally at 5350 Å. Accordingly, λ_0 (effective) = 5.4×10^{-5} cm. Taking $\delta/E^2c = 1.07 \times 10^{-6}$ for low concentrations (see Results), l = 1.00 cm., n = 1.33, we obtain $[K] = 6.9 \times 10^{-12}$ (in c.g.s., except E (volts/cm.) and c (g./liter)). Taking the specific volume of TMV as 0.73 cc./g.,[36] expressing c as volume fraction, and converting units, we obtain $[K] = 8.5 \times 10^{-4}$ c.g.s. for TMV in 1.5×10^{-4} *M* phosphate buffer of *p*H 7.0 at 25.0°.

Mode of Orientation in Dilute Solutions.—Since the electric birefringence is positive, and flow birefringence is also positive,[37] it can be concluded that the effect of the electric field in dilute solutions is to align the rods along the direction of the field, as suggested from some earlier results.[13] The two principal refractive indices of TMV are practically equal, and greater than the refractive index of water[38]; hence this is primarily a case of form birefringence.

Comparison with a Theory Based upon Dipolar Orientation and Distortion Polarization.—The mode of orientation is qualitatively consistent with interaction of the TMV with an applied field through a permanent electric moment directed along the long axis of the macromolecule, or with production of an orienting torque by distortion polarization, but there are additional observations which rule out both mechanisms as principal sources of the Kerr effect in aqueous TMV solutions. In previous experiments[13] in this Laboratory with square waves of varying frequency, it was observed that no dispersion of the birefringence could be detected as the frequency was varied from less than to greater than $1/\tau$, which led to the conclusion that the obvious possibility of a permanent dipole moment in the direction of the long axis could not account for the birefringence. Similar experiments with sine waves later confirmed the absence of a dispersion at frequencies around $1/\tau$. It may therefore be concluded that an electric polarization mechanism which is rapid compared to rotational diffusion about the short axis of the TMV rods is responsible for the effect.

The experiments with a rapidly reversed field, illustrated in Fig. 4, and the proximity of the higher frequency values to the mean of the low frequency extremes in the sine wave experiments of Fig. 7 are separate confirmations that an electric dipole moment interaction is not predominant. That a permanent moment across the symmetry axis is not the primary cause is shown by the sign of the birefringence. If a dipole moment along the long axis were important, a visible transient would occur upon field reversal, and the mean δ would fall significantly in Fig. 7.

The fact that the buildup of the birefringence is accurately symmetrical to the decay is illustrated in Fig. 3. A similar qualitative observation was reported by Benoit.[31] However, his preparation was polydisperse, and it has been shown subsequently[32] that symmetrical curves can be obtained for highly

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[35] RCA Tube Handbook.

[36] M. A Lauffer, THIS JOURNAL, **66**, 1188 (1944). For a summary of various measurements see p. 3608 of ref. 23.

[37] M. A. Lauffer, *J. Phys. Chem.*, **42**, 935 (1938).

[38] J. B. Dounet, *J. Polymer Sci.*, **12**, 53 (1954).

elongated molecules for orientation by a dipole moment perpendicular to the long axis. But again, this is not consistent with the observed mode of orientation. Therefore, the symmetry of the transient for the monodisperse preparation studied here supports the conclusion that permanent dipole moment interactions do not produce the orientation.

It is well known that when a dielectric ellipsoid is suspended in a dielectric medium in which a static uniform field is established, the internal field, though uniform, will not in general be parallel to the applied field, and there will be a torque on the ellipsoid tending to orient it with its longest axis along the field.[39] The mode of preferred orientation is the same whether the dielectric constant of the ellipsoid is greater or less than that of the medium. This result follows from a consideration of the entire polarization energy of the system as a function of the macromolecular orientation in calculating the orienting torque. The substantial changes in the electrostatic energy of the solvent which occur as the components of the applied field are altered by macromolecular orientation are an important aspect of the problem. A similar result applies in the case of a dielectric cylinder.[40] Thus, although the distortion polarization of the macromolecule may be lower, as a result of a lower dielectric constant, than the distortion polarization of an equal and similarly oriented specimen of solvent, polarization of this type is consistent with the observed tendency to orient along the field. It is therefore of interest to compare the magnitude of the low-frequency Kerr constant with calculations based upon a model incorporating the contribution of distortion polarization. Because both the electronic and atomic displacements which contribute to distortion polarization are very rapid (of the order of optical and infrared frequencies, respectively) compared to the orientational relaxation time, this type of polarization would be consistent with the observed absence of a dispersion of electric birefringence in the audio frequency region.

A theory of the Kerr effect in suspensions which is probably applicable to insulating solutions of rigid un-ionized macromolecules has been proposed by Peterlin and Stuart,[28] and has been extended by Benoit[31] and Tinoco.[32] In those treatments, orientation is considered to arise because of permanent electric moments and distortion polarization. The macromolecular model for the general case is an ellipsoid of principal axes a_1, a_2, a_3, along which the refractive indices are n_1, n_2, n_3, the dielectric constants are ϵ_1, ϵ_2, ϵ_3, and the electric moment components are μ_1, μ_2, μ_3. For time dependent calculations, the rotational diffusion constants are D_1, D_2 and D_3 about the respective axes. The solvent is treated as an insulating isotropic continuum of refractive index n and dielectric constant ϵ. Expressions were obtained[28] for the low frequency Kerr constants for a suspension of ellipsoids, and of ellipsoids of revolution ($a_2 = a_3$, $n_2 = n_3$, $\epsilon_2 = \epsilon_3$), for the case where the dipole moment is zero. Similar expressions, and equations for the buildup and the decay of birefringence in square pulsed fields, were obtained for ellipsoids of revolution with dipole moment μ_1 along the symmetry axis,[31] and for ellipsoids of revolution with dipole moment components[32] μ_1 and $\mu_2 = \mu_3$.

Peterlin and Stuart find, for the case where the particles are ellipsoids of revolution and orientation is produced by distortion polarization, that

$$K_{\text{sp}} = \Delta n/cnE^2 = \frac{2\pi v}{15n^2kT}(g_1 - g_2)(g_{e1} - g_{e2}) \quad (25)$$

where v is the volume of a suspended particle. Their units are c.g.s., and c is the concentration of the macromolecular phase expressed as volume fraction. The quantity $(g_1 - g_2)$ is an optical anisotropy factor given by

$$g_1 - g_2 = \frac{4\pi(n_1^2 - n_2^2) - [(n^2_1 - n^2)(n_2^2 - n^2)/n^2](L_1 - L_2)}{\{4\pi + [(n^2_1 - n^2)/n^2]L_1\}\{4\pi + [(n^2_2 - n^2)/n^2]L_2\}} \quad (26)$$

where n_1 and n_2 are the principal indexes of refraction for the particles, and L_1 and L_2 are elliptical integrals dependent only on the axial ratio ($p = a_1/a_2$) for the particles. The quantity $(g_{e1} - g_{e2})$ of eq. 25 is an electrical factor identical in form with eq. 26, but with ϵ_1 and ϵ_2 substituted for n^2_1 and n^2_2, respectively. The quantities ϵ_1 and ϵ_2 are the principal dielectric constants for the particle.

For TMV, $n_2 \cong n_1 = 1.57$.[37] The value of ϵ is not known, but, from Maxwell's equation, $\epsilon = n^2$ at optical frequencies. At low frequencies ϵ is generally somewhat greater than n^2 even in the absence of orientation polarization because of small contributions to the dielectric constant from atomic polarization. In view of the optical properties of TMV, it is reasonable to take $\epsilon_1 = \epsilon_2 = 3$. This is with the assumption that the dipoles of the protein and nucleic acid comprising TMV are rigidly fixed in macromolecular structure. However, the exact value chosen is not critical, because of the unusually high dielectric constant of water (78.5 at 25°[41]). The volume of a TMV molecule in solution is 6.0×10^{-17} cc. This is calculated from a circular cylinder 150 Å. in diameter and 3410 Å. in length.[23] Actually, eq. 25 applies only to ellipsoidal shapes, but for a particle as anisometric as TMV this approximation should not be serious. If the axial ratio of TMV is taken as 3410/150 = 22.7,[23] the values of L_1 and L_2, computed from equations given by Peterlin and Stuart,[28] are 0.068 and 6.184, respectively. The value of $[K]$ from eq. 25 is found to be 1.6×10^{-5}. This can be compared with the experimental value, $[K] = 8.5 \times 10^{-4}$, given above. Evidently, a polarization mechanism about 50 times more important than distortion polarization is operating in this system. Although there have been earlier speculations[11-13] that other polarization mechanisms may be important, this is the first study, so far as we are aware, in which the experimentally observed birefringence has been quantitatively compared with the predictions of a theory based upon distortion and orientation polarization phenomena, and found to be almost two orders of magnitude greater than expected from those effects.

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[39] J. A. Stratton, "Electromagnetic Theory," Chap. III, McGraw-Hill Book Co., Inc., New York, N. Y., 1941.

[40] W. R. Smythe, "Static and Dynamic Electricity," Chap. 4, McGraw-Hill Book Co., Inc., New York, N. Y., 1950.

[41] A. A. Maryott and E. R. Smith, Circular 514, Natl. Bur. Standards, Aug. 10, 1951.

Equation 25 was derived on the assumption that the electric field due to a light wave is uniform over the space occupied by a molecule. For this to be true, the dimensions of the molecule must be small compared with the wave length of light. For TMV the diameter is small compared to the wave length of light, but the length is not. Therefore, in the complete treatment of the problem[42] a small correction to the optical factor of eq. 25 is expected. Neglect of this correction should not introduce serious error, particularly since the TMV particles have an index of refraction fairly close to that of water.

To demonstrate the validity of this assumption, the refractive index increment, dn/d**C** for dilute solutions of TMV can be calculated using the relationship[43] for a suspension of spheres. This equation is the same for the dielectric constant of a mixture,[43] except for the introduction of the Maxwell expression, $\epsilon = n^2$. In eq. 27, n is the index of refraction of the solution, n_2 the index of refraction of the dispersed phase, and n_1 is the index of refraction of the solvent; c is the volume fraction of the dispersed phase.

$$\frac{n^2 - n^2{}_1}{3n^2} = c\,\frac{n^2{}_2 - n^2{}_1}{n^2{}_2 + 2n^2} \qquad (27)$$

Inserting numerical values and differentiating, we obtain

$$\mathrm{d}n/\mathrm{d}c = 2.07/(4n + 8.70/n^3) \qquad (28)$$

In dilute solutions $n = 1.33$ and $\mathrm{d}\mathbf{C} = 1.37\,\mathrm{d}c$ where **C** is the concentration of TMV in grams per cc. Then dn/d**C** is found to be 0.168. This is excellent agreement with the experimental value of 0.169 reported by Oster, Doty and Zimm.[44] The long dimension of TMV does not appear to cause complications in the index of refraction of solutions. The optical problem, therefore, cannot account for any significant part of the difference between the experimental value of $[K]$ and the value calculated from eq. 25.

If distortion polarization were the primary cause of the electric orientation of the macromolecules, no variation of the Kerr constant with frequency of the applied field would be expected until one approaches infrared or optical frequencies. However, the experimental results (Figs. 14 and 15) with TMV indicate that the magnitude of the electric birefringence decreases very rapidly when the frequency of the applied field is increased beyond 30 kc. This dispersion evidently is associated with the electric polarization mechanism responsible for the extremely large intrinsic Kerr constant in this system.

It is concluded that neither dipolar orientation nor distortion polarization can account for the electric birefringence observed in solutions of TMV. Dipolar orientation is ruled out by the above deductions involving the mode of orientation, the absence of a dispersion in the audio frequency region and the absence of a transient upon field reversal, and by the shape of the buildup curve. The magnitude of the birefringence in TMV is much greater than that computed theoretically for distortion polarization. The dispersion of the electric birefringence in TMV at radio frequencies is incompatible with an orienting torque arising from distortion polarization, and a saturation effect has been reported[31] at much lower field strengths than anticipated on the latter basis. Therefore, it is necessary to seek other orienting mechanisms to account for the observed phenomena.

Interpretation in Terms of Ion Atmosphere Polarization.—A theory of the frequency dependent dielectric constant and conductivity of simple electrolytes was introduced by Debye and Falkenhagen,[7] and recently has been extended to spherical ions of finite diameter by Falkenhagen, Leist and Kelbg.[10] It has been shown that the theory is in agreement with experimental results for the conductivity of some 1:1 electrolytes to concentrations of the order of 1 M when the finite size of the ions is taken into account.[10] The problem of the dielectric constant, which is complicated by the ion–solvent interactions, has been discussed recently.[16] Presumably, the extended theory would be applicable to polyelectrolytes if the polyion could be represented adequately as a charged sphere of uniform surface charge density. Since orientation of anisometric macromolecules by an electric field is of primary interest here, this model is clearly inadequate. However, the processes described by the fundamental equations of the theory occur in the present case. Accordingly, the concept of the production of a time-average electric asymmetry, or electric polarization of the ion atmosphere, by an external field, is useful in the present discussion. This phenomenon has been qualitatively discussed in connection with electric birefringence studies by Errera, Overbeek and Sack,[11] on V_2O_5 sols, by Mueller and Sakmann[45] and Norton[46] on bentonite sols, and by O'Konski and Zimm[13] on TMV solutions. No quantitative formulation regarding either the magnitude of the electric birefringence or its frequency dependence was then available.

O'Konski[15] has suggested that when the ion atmosphere is thin compared to particle dimensions, the effect of its polarization upon dielectric properties may be treated on the basis of models which incorporate a surface conductivity,[47] λ, to represent the effects of the high local concentration of mobile counterions. A calculation of the dielectric relaxation time τ has been made[15] for a dilute system of spheres of radius a and dielectric constant ϵ_2, in a medium of dielectric constant ϵ_1, with the simplifying assumption that the bulk conductivities may be neglected. The resulting equation, in c.g.s. units, is

$$\tau = (\epsilon_2 + 2\epsilon_1)a/8\pi\lambda \qquad (29)$$

It was pointed out that there will be more than one relaxation time for anisometric particles. For a rod-like macromolecule, two principal relaxation times are expected. They may be designated τ_l for relaxation of longitudinal ion atmosphere polarization, and τ_t for relaxation of transverse

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(42) F. Möglich, *Ann. Physik*, [4] **83**, 609 (1927).

(43) C. J. F. Böttcher, "Theory of Dielectric Polarization," Elsevier, Houston, Texas, 1952; D. Polder and J. H. Van Santen, *Physica*, **12**, 257 (1946).

(44) G. Oster, P. M. Doty and B. H. Zimm, This Journal, **69**, 1193 (1947).

(45) H. Mueller and B. W. Sackmann, *Phys. Rev.*, **56**, 615 (1939).

(46) F. J. Norton, *ibid.*, **55**, 668 (1939).

(47) J. Th. G. Overbeek, "Colloid Science," Ch. 5, Vol. 1, Ed. by H. R. Kruyt, Elsevier Publishing Co., Houston, Texas, 1952.

polarization. Further considerations of this model now suggest that for the present case, τ_t will be of the order of τ for a sphere of radius equal to that of the rod, whereas τ_l will be greater by a factor of the order of the square of the axial ratio. With an estimate for λ, we may then make order-of-magnitude estimates for τ_t and τ_l, to decide whether the electrical dispersion of the birefringence reported above is in the frequency range expected for the ion atmosphere polarization phenomenon.

To obtain an order-of-magnitude estimate of the surface conductivity component, λ_c, to be associated with the counterions, let us introduce some simplifying approximations. Consider that the macromolecular charge is distributed uniformly over the surface of a cylinder circumscribing the macromolecule, and that the counterions all have tangential mobilities equal to their infinite dilution values. If the main contribution to λ_c is from a surface layer of depth less than the radius of the rod, then λ_c may be estimated by considering that essentially all of the counterions are in this layer. This leads immediately to the expression

$$\lambda_c = \Lambda_c \, \sigma/Ne \tag{30}$$

where λ_c is the surface conductance in ohms^{-1}, Λ_c is the equivalent conductance of the counterion, in ohms^{-1} cm.^{2} equiv.$^{-1}$, N is Avogadro's number, σ is the surface charge density and e is the electronic charge, in the same units. For use of λ_c in eq. 29, multiplication by the factor 9×10^{11} converts to e.s.u.

The macromolecular charge may be computed from titration data for TMV, which have been reported by Welsh.[43] The values for one of his fresh preparations, TMV 6, were converted to units of electronic charge per macromolecule and are shown in Fig. 15. For the macromolecular weight, 5×10^7 was taken.[49] Maximum values of the macromolecular charge as a function of *p*H may be computed from the known amino acid composition,[50] *pK* values for the ionizable groups,[51] and the known isoelectric point.[52] Two curves (not shown) were calculated using the upper and lower extremes of the ranges of *pK* values. They showed inflections around *p*H 4–5, and a broad plateau around *p*H 7. The macromolecular charge at *p*H 7 was about ten times greater than the experimental value of Fig. 15. This is probably because of inaccessibility of most of the ionizable groups, which may be hydrogen bonded within the folded protein structure. Some difference in shape of the curves indicated that the calculated curves were probably an oversimplification, since the contribution to the free energy from the macromolecular charge was not considered. The experimental values probably give the better estimate of macromolecular charge.

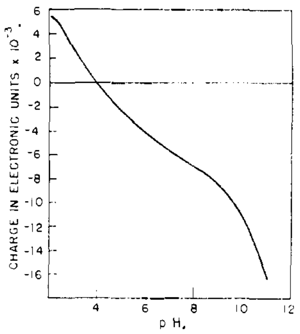

Fig. 15.—Charge *vs.* *p*H for TMV, computed from titration data of R. S. Welsh.[48]

Taking, from Fig. 15 at *p*H 7, a charge of 6×10^3 e, $\Lambda_c = 73.5$ (for K^+), and 1.5×10^{-10} A^2 for the area of the macromolecule, $\lambda_c = 4.9 \times 10^{-9}$ ohm^{-1} $= 4.4 \times 10^3$ statohm^{-1}, from eq. 30. This leads to longitudinal and transverse relaxation times of the order of 5×10^{-7} and 10^{-9} sec.$^{-1}$, respectively. Since the electrical anisotropy will be due primarily to a large longitudinal polarizability, dispersion of the birefringence would be expected to occur as the frequency approaches the corresponding critical frequency, which is $1/2\pi\tau_l$, or around 300 kc. Another dispersion of the Maxwell–Wagner type is expected in about the same region, as a result of the conductivity in the solvent. Some dispersion should persist until the transverse ion atmosphere polarization cannot follow the field, or to around $(1/2\pi\tau_t)$ or 10^8 c.p.s. It can be seen that these expectations are roughly in accord with the experimental results of Figs. 13 and 14. At much higher frequencies, the ion atmosphere polarization would be expected to become relatively unimportant, and the specific Kerr constant should approach the value calculated on the basis of Peterlin and Stuart's equation. At the highest frequency employed here, 5 mc., the observed specific Kerr constants were sometimes considerably larger than the value computed on the basis of distortion polarization, although lower, and even negative values can be expected. Evidently the ion atmosphere polarization is still an important effect at this frequency.

On the basis of a model which incorporates the effect of the counterion atmosphere in terms of a surface conductivity, the specific Kerr constant is expected to increase with increasing macromolecular charge, at constant solvent conductivity. Accordingly, the increases of δ/E^2 with increasing *p*H, which are illustrated in Fig. 10, can be explained. Also, as found at *p*H 7.0, the specific Kerr constant is expected to decrease with increasing electrolyte concentration because the surface conductivity becomes relatively less important in determining the intensity of the electric fields, and thus the polarization energy.

We may calculate a maximum value of the electric anisotropy factor on this model by considering the macromolecule to be an infinitely better conductor than the surrounding medium. In the electrostatic problem, this is equivalent to an infinite dielectric constant.[53] Then from eq. 26 we may find

$$g_{e_1} - g_{e_2} = \epsilon(L_2 - L_1)/L_1L_2 \tag{31}$$

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[48] R. S. Welsh, *J. Gen. Physiol.*, **39**, 437 (1956).

[49] R. C. Williams, R. C. Backus and R. L. Steere, THIS JOURNAL, **73**, 2062 (1951).

[50] C. A. Knight, *J. Biol. Chem.*, **171**, 297 (1947).

[51] R. A. Alberty in H. Neurath and K. Bailey, "The Proteins," Vol. I, Part A, Chap. 6, Academic Press, New York, N. Y., 1953.

[52] G. Oster, *J. Gen. Physiol.*, **31**, 89 (1947).

[53] J. A. Stratton, "Electromagnetic Theory," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 213. For similar examples, see C. T. O'Konski and H. C. Thacher, Jr., *J. Phys. Chem.*, **57**, 955 (1953), and C. T. O'Konski and F. E. Harris, *ibid.*, **61**, 1172 (1957).

For $L_1 << L_2$, or very anisometric prolate ellipsoids of revolution, this is approximately equal to ϵ/L_1. Inserting values as given above into the equations, we obtain $K = 34 \times 10^{-4}$, which may be compared with the experimental value, 8.5×10^{-4}. It is seen that the calculated value is of the right order of magnitude, and greater than the experimental result, which is expected because the conductivity of the medium has been ignored. This is to be contrasted with the calculation above for distortion polarization, which gives much too small a value, even if ϵ (protein) is considered unity.

It can be seen from Fig. 13 that there is a significant change in the breadth of the dispersion with concentration of added electrolyte. The low frequency Kerr constants are higher at lower electrolyte concentrations, but the high frequency values are lower. The curves all cross at about 300 kc. The trend of the low frequency values, illustrated in Fig. 10, can be attributed primarily to increased conductivity of the solvent, which makes the effect of the counterion atmosphere, or λ_c, relatively smaller. This explanation is along the lines of a treatment of the corresponding problem of the dielectric properties of a suspension of polyelectrolyte spheres.[54] One possible reason for the broadening of the dispersion with increased electrolyte concentration is the corresponding change of thickness of the ion atmosphere. At the lowest electrolyte concentrations employed, the ion atmosphere thickness, which may be taken as $1/\kappa'$, where κ' is the Debye-Hückel parameter, is of the order of the radius of the TMV rod. Accordingly, the counterion conductivity cannot be treated strictly in terms of a two-dimensional conductivity on the rod surface. It seems clear that the gradient in concentration of the counterions will produce a continuously varying conductivity near the surface of the rod. This will result in a distribution of transverse relaxation times, with the longer components becoming relatively more important as the ion atmosphere thickness becomes greater. The corresponding effect upon the longitudinal relaxation time is relatively much less important because the ion atmosphere is thin compared to the length of the rod. Therefore, the ratio of τ_L to the central transverse relaxation time will become greater with increasing electrolyte concentration, which would correspond to the broader dispersion region observed. A shift of the critical frequencies to higher values is also expected as a result of the increased conductivity of the solvent.

On the basis of the simplified picture employed above, it would be expected that as the macromolecular charge increases, at constant ionic strength and solvent conductivity, the ion atmosphere relaxation time should decrease, because of the increased concentration of counterions (*cf.*, eq. 29 and 30). Since increasing the *p*H increases the charge, (*cf.*, Fig. 15), one may expect corresponding changes in the high frequency behavior. Adopting ν_m as defined above as a crude criterion of the inverse longitudinal relaxation time, it can be seen from Table I that the trend is in the direction expected in going from *p*H 5.6 to 7.0, but is strongly the reverse in going from *p*H 7.0 to 8.4. Interpretation of the latter observation is difficult, because of the different modes of relaxation, but the magnitude of the change in ν_m raises a question as to whether the simplified calculation of λ_c is qualitatively adequate. A change in buffer composition was required to change the *p*H, so there is a possibility that specific effects of the buffer are responsible. Binding of the borate ion at *p*H 8.4 would increase the negative charge on the macromolecule, and presumably increase λ_c, which would be in the wrong direction to explain the discrepancy. Lack of binding for this buffer, with appreciable binding of phosphate (*p*H 7.0) and acid phthalate (*p*H 5.6) ions would be in the right direction, but with this explanation, the trend from *p*H 5.6 to 7.0 would be fortuitous. There is no reason to believe that binding will be important at the low buffer concentrations employed. At *p*H 5.6, the protons in the counterion atmosphere, which have been ignored thus far, might contribute significantly to λ_c because of their relatively high mobility. Similarly, a contribution from OH^- might be expected at the high *p*H. However, the concentrations of buffer cations in the solvent are greater by factors which outweigh the mobility ratios, and from the Poisson–Boltzmann equation, one expects the same in the ion atmosphere. An alternative explanation in terms of proton contributions on the basis of the mechanism proposed by Kirkwood and Shumaker[20] is considered improbable, for reasons given under "Other Polarization Mechanisms" below.

An explanation for the decrease in critical frequency at *p*H 8.4, in terms of ion atmosphere polarization might seem possible if we consider that the tangential mobilities of the counterions are decreased above a critical macromolecular charge. This might occur as a result of an increase in the effective viscosity of water in the immediate vicinity of the macromolecular surface. This is qualitatively reasonable in view of the intense fields in the vicinity of a polyelectrolyte,[55] and in view of the increased dielectric relaxation times of water in concentrated solutions of simple electrolytes.[16] But if this were the proper explanation, one would not expect the increase of the low frequency specific Kerr constant observed in going from *p*H 7.0 to 8.4 at constant conductivity (Fig. 10). Further characterization of the effect of *p*H on the dispersion, preferably at constant conductivity and ionic strength, would be of interest.

Hydrodynamic Orientation.—The possibility of the production of an orienting torque by the hydrodynamic forces accompanying electrophoresis has received some consideration.[12,13,56] Heller[12] suggested that on this mechanism, the magnitude of the birefringence would be proportional to the first power of the electric field, which is contrary to the experimental results in dilute solutions. It has been pointed out that this explanation is improbable because of the tendency of anisometric bodies to hydrodynamically orient with long axes across

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[54] C. T. O'Konski and J. J. Hermans, in preparation for publication.

[55] See, for example, the calculations of R. M. Fuoss, *J. Polymer Sci.*, **12**, 199 (1954).

[56] K. J. Mysels, *J. Chem. Phys.*, **21**, 201 (1953).

the direction of motion.[18] The TMV monomers appear to be cylindrically symmetrical rods or helixes, and no evidence of permanent moment could be found, so there appears to be no basis for orientation of the type discussed by Mysels.[56]

Other Polarization Mechanisms.—The proposal of Kirkwood and Shumaker[20] is that the dielectric increments and the dielectric dispersion of protein solutions may be explained by the migration of protons among the acid and basic sites on the protein. When sites of a given type are nearly occupied or nearly vacant, the contribution of those sites to electric polarization is expected on a statistical basis to be less than when they are half-filled, where the effect would reach a maximum value. If electrostatic interaction between the protons is neglected, it is expected that a series of maxima, corresponding to the pK values of the various amino acids, would be observed in an electric polarization *vs.* pH curve. Within the stated approximation, a similar effect might be expected in the Kerr constant *vs.* pH curve. However, the behavior is strongly influenced by the electrostatic interaction, as was shown by Kirkwood and Shumaker[20] in calculations for ovalbumin. Similar computations are not available for TMV, so we shall make use of a qualitative argument regarding the expected trend of the proton polarization with pH. The occupancy of protons on basic sites is affected by the polarizing electric field and the hydrogen ion activity because they both influence the free energy. Therefore in regions where a small increment of pH causes relatively large changes in occupancy (or macromolecular charge), an electric field should produce large changes in the proton configuration. On this basis, and with reference to Fig. 15, one expects a shallow minimum in the proton polarization around pH 7–8. The observed trend of the experimental results of Fig. 10 (compared at equal conductivities) is quite different, and is qualitatively in accord with expectations on the counterion polarization mechanism outlined above.

Kirkwood and Shumaker do not discuss the details of the process by which protons move from one site to another. Unless the sites are unexpectedly close, migration probably would involve transfer of a proton to the solvent, followed by drift in the electric field and subsequent acquisition by an acceptor site. Then the problem of the rate of charge transport becomes analogous to the well known case of a two-step consecutive chemical reaction. The favorable situation for proton polarization will be the one in which proton transfer from sites to solvent, and *vice versa*, is very rapid. Then the rate of charge transfer by protons in the ion atmosphere becomes limiting. This may be estimated relative to the counterion contribution by application of eq. 30 to both species, employing the Poisson–Boltzmann equation and the known ionic concentrations in the solvent. Calculations show that for the consecutive step process with TMV, proton migration would be smaller than the contribution from K^+ at 10^{-4} M if the pH is between 5 and 9. At lower pH values proton transfer becomes important; at higher pH values the OH^- contribution could exceed the K^+ contribution. By the same type of calculation, both H^+ and OH^- contributions become relatively more important as the concentrations of other species are reduced. From this point of view it appears that the proton polarization mechanism should be regarded as a special case of the more general ion transport phenomenon, unless a parallel process involving direct transfer of protons along a chain of consecutive sites on the macromolecule turns out to be important. No evidence could be found that this is so.

The dielectric properties of a long-chain polyelectrolyte have been studied by Dintzis, Oncley and Fuoss[14] who discussed them as a Maxwell–Wagner polarization phenomenon with the aid of the equations of Sillars[6] for a suspension of conducting ellipsoids. They also stated: "Whether the observed increments in dielectric constants are primarily due to a Maxwell–Wagner dispersion or primarily a Debye–Falkenhagen effect, or quite possibly a combination of the two, cannot be decided on the basis of the facts available at present." There is no apparent reason for assigning a relatively high volume conductivity to the material comprising the polyion, but it does seem appropriate to regard the solution in the immediate vicinity of a polyelectrolyte as being a better conductor than the solvent. In simple examples, the conductivity may vary continuously with distance from the macromolecule, asymptotically approaching the bulk conductivity of the solution at large distances. When the conductivity of the solvent is not excessive, this will give rise to a type of Maxwell–Wagner polarization. The Debye–Falkenhagen treatment for simple electrolytes involves a similar physical situation. In polyelectrolytes, the dielectric properties will resemble in some respects a system exhibiting Maxwell–Wagner polarization, and in others, a simple electrolyte.

A connection has been established between equations obtained for the dielectric properties of a suspension of spheres with surface conductance, and the equations for Maxwell–Wagner polarization involving a volume conductivity.[54] The equations for the complex dielectric constant of a suspension of conducting spheres with surface conductivity λ are of the same form as those for conducting spheres alone, with a term $(2\lambda/a)$ added to the bulk conductivity in the latter instance. Thus, interpretations of ion atmosphere polarization with the aid of equations for Maxwell–Wagner polarization should be useful, providing it is recognized that the calculated conductivities of the spheres may arise partially or entirely from a surface contribution. With anisometric particles, the equivalent volume conductivity may be anisotropic. When the ion atmosphere is not thin compared to macromolecular dimensions, a more general treatment would be required.

Jacobson[21] recently reviewed some of the results on the dielectric properties of solutions of proteins and nucleic acids and pointed out several inconsistencies between the explanations based upon orientation polarization and experimental findings. He proposed qualitatively that the dielectric properties be explained in terms of crystallization of water to form an ice-like hydration shell, stable at room temperature, but having dielectric properties similar to ice near 0°. To test this proposal, calculations were made with the aid of eq. 26, assuming that the solvated region about TMV is a cylindrical shell, and considering the entire aggregate to have an effective dielectric constant equal to that of ice at 0°.[57] Even with a shell 75 Å. thick, *i.e.*, the radius of the TMV rod, the electrical factor is too small by a factor of about 350 on this picture. With a similar shell of dielectric constant 154, the discrepancy is a factor around 40 in the same direction. Larger dielectric constants or solvation shells appear highly improbable. Accordingly, the solvation hypothesis cannot reasonably account for the large electrical anisotropy factor ($g_{e1} - g_{e2} = 2.8 \times 10^2$ in 1.5×10^{-4} *M* phosphate, *p*H 7.0) found in this research.

The possibility of the transfer of protons in the hydrogen bonds of the protein structure has been discussed by Wirtz.[19] A coöperative phenomenon involving displacement of many protons in a chain could lead to very high dielectric constants which would be qualitatively consistent with the high electrical anisotropy factor. Such phenomena are apparently involved in the KH_2PO_4 type ferroelectrics.[58] Short strong O–H. . .O or N–H. . .O linkages would presumably be required for such effects. That a short O–H. . .O linkage is not a sufficient requirement was indicated by a dielectric study of some bicarbonates.[59] $NaHCO_3$ has an O–H. . .O distance equal to KH_2PO_4, but the dielectric constant is low. Increases of the low frequency dielectric constants with increasing humidity in powdered samples were explained as an effect of surface conductivity. If coöperative proton shifts in the proteins produced the very large dielectric constants accompanying the ferroelectric state, the characteristic property of hysteresis would be expected,[60] but neither hysteresis nor anomalously large dielectric constants have been reported for solid proteins.[61]

General Consequences of Ion Atmosphere Polarization.—With the above interpretation of the large electric anisotropy factor, a number of conclusions may be drawn concerning the dielectric and electroöptic properties of solutions of polyelectrolytes, *e.g.:* (a) the finite rate of polarization of the ion atmosphere will give rise to a dielectric dispersion. (b) When the macromolecules are highly asymmetric, the dispersions will be broad. (c) Because of the close similarity to Maxwell–Wagner polarization, large dielectric increments can be found, even when the permanent dipole moment contributions are negligible. (d) For spherical polyions, such as the globular proteins, the low frequency dielectric increments in dilute electrolyte solutions can approach those of a dilute suspension of metallic spheres of equivalent volume, $\Delta\epsilon = 3\epsilon_1 c$, where ϵ_1 is the dielectric constant of the solvent and *c* is the volume fraction of conducting spheres. It is significant that this is the order of magnitude of the dielectric increments observed for some globular proteins.[62]

Interpretation of the dielectric increments of aqueous solutions of proteins and nucleic acids in terms of permanent dipole moments, by application of equations valid for insulating systems,[62] might already have led to incorrect deductions because of neglect of the polarization attributable to the counterions. Isoelectric proteins will in general contain charged centers, so localized ion atmospheres will exist even when the net macromolecular charge and the dipole moment are zero. Accordingly, it is the view of these authors that permanent dipole moments of polyelectrolyte macromolecules computed from dielectric increments are of questionable significance, and may be entirely fictitious in some cases. Similarly, the interpretation of dielectric relaxation times solely in terms of dipolar reorientation can give erroneous results for rotational diffusion constants, and invalid conclusions regarding macromolecular size and shape. This is clearly indicated by studies of aqueous solutions of *Helix pomatia* hemocyanin, which exhibit dielectric relaxation frequencies over thirty times greater than expected from macromolecular reorientation.[63] The dielectric relaxation in solutions of the protein zein[64] also could not be satisfactorily explained on the basis of macromolecular reorientation, but the discrepancies were not unequivocally established. It was found by Allgén and Roswall[65] that the dielectric properties of aqueous solutions of carboxymethylcellulose (CMC) were very similar to those of biological polyelectrolytes such as desoxyribonucleic acid, nucleohistone and hyaluronic acid, although "the electric structure of CMC is unipolar rather than dipolar." This is not surprising if the polarization is interpreted as arising from the counterion atmosphere as discussed above. Similarly the large Kerr effects in colloidal suspensions, such as the clays and V_2O_5, are explicable.

The importance of the ion atmosphere in the electric polarization of solutions of polyelectrolytes presents a formidable complication in the interpretation of their dielectric data. Clearly, the independent determination of rotational diffusion constants from transient electric birefringence studies will be helpful in this respect. A re-examination of existing dielectric data and the extension of theories of electric polarization to conducting systems are evidently desirable. Problems involving electric polarization and orientation in polyelectro-

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(57) The effective polarizability of a sphere of low dielectric constant surrounded by a shell of high dielectric constant is near that of a sphere of the high dielectric constant material of radius equal to the larger radius of the shell. See, *e.g.*, H. C. van de Hulst, "Scattering of Light by Spherical Particles," John Wiley and Sons, Inc., New York, N. Y., 1957.

(58) For a review see E. T. Jaynes, "Ferroelectricity," Princeton University Press, Princeton, N. J., 1953.

(59) C. T. O'Konski, This Journal, **73**, 5093 (1951).

(60) See, *e.g.*, W. G. Cady, "Piezoelectricity," McGraw-Hill Book Co., Inc., New York, N. Y., 1946; W. F. Mason, "Piezoelectric Crystals and their Application to Ultrasonics," D. Van Nostrand Co., New York, N. Y., 1950.

(61) S. T. Bayley, *Trans. Faraday Soc.*, **47**, 509 (1951).

(62) J. L. Oncley, *J. Phys. Chem.*, **44**, 1103 (1940); J. L. Oncley, J. D. Ferry and J. Shack, *Ann. N. Y. Acad. Sci.*, **40**, 371 (1941).

(63) See B. Jacobson and M. Wenner, *Biochim. et Biophys. Acta*, **13**, 577 (1954). The macromolecular reorientation rate for the same preparation has been confirmed in this Laboratory by transient electric birefringence.

(64) J. L. Oncley, C. C. Jensen and P. M. Gross, Jr., *J. Phys. Colloid Chem.*, **53**, 162 (1949).

(65) L. G. Allgén and S. Roswall, *J. Polymer Sci.*, **12**, 229 (1954).

lyte systems, and the extension of the transient electric birefringence technique to smaller macromolecules, such as polypeptides of molecular weight *ca.* 10^4–10^5, are currently receiving attention in this Laboratory.

Berkeley, Cal.

[Contribution from the Department of Chemistry, Illinois Institute of Technology]

Carbon-14 Kinetic Isotope Effects in Nucleophilic Substitution Reactions[1]

By Myron L. Bender and Donald F. Hoeg[2]

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The carbon-14 kinetic isotope effects in the nucleophilic substitution reactions of methyl iodide-C^{14} with triethylamine, pyridine, hydroxide ion and silver ion have been investigated. The kinetic isotope effects (k_{12}/k_{14}) found in these reactions were 1.10, 1.14, 1.09 and 1.09, respectively. The surprisingly large kinetic isotope effects in these S_N2 reactions are of the same magnitude as many effects noted in reactions proceeding only with bond rupture. A comparison has been made between the observed kinetic isotope effects and those calculated using an equation of Bigeleisen and Wolfsberg for three-center reactions. It appears that the magnitude of the kinetic isotope effect is of only limited use in differentiating between two-center and three-center reactions in nucleophilic substitution (S_N1 and S_N2 reactions).

Introduction

It has long been recognized[3] and amply demonstrated by experiment[4-6] that substitution of one isotope of an element for another in a bond undergoing rupture or formation in the rate-controlling step of a reaction influences the rate of the reaction. This kinetic isotope effect is particularly striking in the case of the substitution of deuterium for hydrogen and has been utilized to good advantage in the elucidation of the mechanism of many organic reactions.[4,6] Although the kinetic isotope effect produced by the substitution of carbon-14 for carbon-12 is relatively small in magnitude, a large number of cases have been observed where such kinetic isotope effects occur.[7-9]

Recently theoretical equations have been presented for the prediction of the magnitude of the kinetic isotope effects[10] in two-center and three-center[11] reactions. These descriptions fit the type of covalency changes that are associated with the dual mechanisms of nucleophilic substitution reactions, S_N1 and S_N2.[12] It was therefore decided to investigate the carbon-14 kinetic isotope effects in S_N1 and S_N2 reactions, which Hammett has described as the most important in organic chemistry,[13] in order to test the validity of the theoretical equations, in order to determine whether such kinetic isotope effects could serve as a criterion of mechanism and whether the kinetic isotope effects would provide information for a more detailed understanding of the transition states of the S_N1 and S_N2 reactions. Carbon-14 kinetic isotope effects are reported here for three reactions of methyl iodide-C^{14} which fall into the S_N2 category, those with triethylamine, pyridine and hydroxide ion and for one reaction which falls into a borderline category, that with silver nitrate in aqueous ethanol.

Experimental

Materials.—Matheson, Coleman and Bell reagent grade methyl iodide was fractionally distilled at atmospheric pressure in a 14 in. column of glass helices, b.p. 42.5°, n^{21}_{D} 1.5295 (lit.[14] b.p. 42.4°, n^{21}_{D} 1.5293). Methyl iodide-C^{14}, purchased from Nuclear Instrument and Chemical Corp., was diluted with unlabeled methyl iodide. The methyl iodide-C^{14} was stored in an amber-colored glass-stoppered bottle at 0° in the dark. The refractive index of the methyl iodide-C^{14} remained unchanged for seven months, indicating negligible decomposition due to self-radiolysis.[15] Eastman Kodak Co. white label triethylamine was fractionally distilled, b.p. 87.5°, n^{20}_{D} 1.4003 (lit.[16] b.p. 89.4°, n^{20}_{D} 1.4003). Fisher reagent grade pyridine was distilled from sodium hydroxide pellets, b.p. 113–113.5° (lit.[17] b.p. 115°). Benzene was purified by fractional distillation, b.p. 80°. Commercial dioxane was purified according to the method of Fieser.[18] The purified anhydrous dioxane was freed of peroxides by passage over a column of activated alumina under a nitrogen atmosphere and used immediately in the kinetic studies. β-Naphthol was recrystallized twice from water; m.p. 121–122°.

Kinetics of the Substitution Reactions.—The kinetics of the alkylation of triethylamine with methyl iodide were measured in benzene solution at 25.05 ± 0.02°. After appropriate times of reaction, the contents of the reaction flasks were washed into excess hydrochloric acid with ethanol, which was used to disperse the benzene–water interface. The excess hydrochloric acid was then titrated with standard sodium hydroxide using lacmoid as indicator. No difference was observed in measurements made with ordinary methyl iodide and methyl iodide-C^{14}.

The kinetics of the displacement reaction of hydroxide ion with methyl iodide-C^{14} were measured in 50% dioxane–

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