may be employed as a useful approximate equation. As for an application of equation 79, reference is made to Oth and Desreux's paper.

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Characterization of the Monomer and Dimer of Tobacco Mosaic Virus by Transient Electric Birefringence[1]

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The rotational diffusion constant of the monomer unit of tobacco mosaic virus in dilute aqueous solution was found to be 333 sec.$^{-1}$ from transient electric birefringence measurements of a number of preparations. This value corresponds to a length of 3416 ± 50 Å., which is significantly greater than 2980 ± 10 Å., reported in the most recent electron microscope study. Assuming a compact circular rod, 3416 Å. long, and employing the X-ray diameter and the measured specific volume, the molecular weight is 50 × 10^6, in agreement with the result of an independent precise method. The new value for the length is discussed with reference to data from other types of measurements. Evidence was obtained for the existence of an end-to-end dimer in all but one of the preparations. Because the constant for rotational diffusion of an elongated macromolecule about its short axis depends most critically upon the length, transient electric birefringence is an especially sensitive method for measuring the lengths of rigid macromolecules. Improved experimental methods are described.

Introduction

As the physical characteristics of the tobacco mosaic virus (TMV) have been studied intensively by many different methods, it is an excellent material for model studies of the phenomenon of electric birefringence. According to prevailing ideas[3-8] on the subject, the pure crystalline virus probably consists of uniform rod-shaped macromolecules which become dispersed as independent units in dilute aqueous solutions. For quantitative measurements of the magnitude of the electric birefringence in such solutions, it is necessary to determine if a given preparation contains a single well-defined species, and to find the conditions under which a solution will behave ideally, that is, to ascertain the dilution required for the macromolecules to respond as individual kinetic units to the stress produced by an applied electric field.

In the course of investigations of the orienting mechanisms in electric birefringence, several TMV preparations were studied by the transient technique. In four of the more homogeneous preparations, there was found predominantly a species with a rotational diffusion constant of 333 sec.$^{-1}$, corresponding to the monomeric rods. In three of the four preparations there was another species of rotational diffusion constant around 56 sec.$^{-1}$, which suggests an end-to-end rigid dimer. Because the rotational diffusion constant is a sensitive function of the length of a rod-shaped macromolecule, relatively precise values of length can be calculated. The monomer length, 3416 Å., is significantly greater than the recent value of Williams and Steere[9] obtained by electron microscopy. When a molecular weight is computed from our value of the length the X-ray diameter of the rod and the partial specific volume, assuming a circular compact cross-section, one obtains 50 × 10^6, in agreement with the value of Williams, Backus and Steere,[10] obtained by a direct weighing and particle counting technique. Accordingly, the concept of a hexagonal cross-section, introduced[10] to produce consistency between certain length, density, molecular weight and lattice spacing measurements on the crystalline virus, is not supported by this research.

The occurrence of end-to-end dimers may have biological implications, and is of interest in the characterization of the macromolecules by other techniques, such as flow birefringence and light scattering, which do not easily permit clear resolution of the components. For these reasons, a detailed account of the results on these four preparations is presented here. Investigations concerning the nature of the orienting mechanism in electric fields, and the effects of mutual interactions between the macromolecules observed in somewhat more concentrated solutions, will be reported later.

The Transient Electric Birefringence Phenomenon.—When a fluid is subjected to electric stress, polar or electrically anisotropic molecules interact with the local electric field and the general result is that the molecular orientations are no longer random. Then, if the molecules are optically anisotropic, the fluid becomes doubly refracting.[11]

(1) Presented before the Section on Chemistry at the 121st meeting of the American Association for the Advancement of Science, Berkeley, California, Dec. 26, 1954. This paper is based upon the thesis submitted by Arthur J. Haltner in January, 1955, in partial fulfillment of the requirements for the Ph.D. in Chemistry.

(2) National Science Foundation Predoctoral Fellow, 1953–1954.

(3) W. M. Stanley, *Handbuch Virusforschung*, **1**, 477 (1938).

(4) M. A. Lauffer, *J. Biol. Chem.*, **151**, 627 (1943).

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(6) T. Sigurgeirsson and W. M. Stanley, *Phytopathology*, **37**, 26 (1947).

(7) W. N. Takahashi and T. E. Rawlins, *ibid.*, **39**, 672 (1949).

(8) H. K. Schachman, THIS JOURNAL, **73**, 4808 (1951).

(9) R. C. Williams and R. L. Steere, *ibid.*, **73**, 2057 (1951).

(10) R. C. Williams, R. C. Backus and R. L. Steere, *ibid.*, **73**, 2062 (1951).

(11) M. Born, "Optik," J. Springer, Berlin, 1933; Edwards Bros., Ann Arbor, Mich., 1943.

This phenomnon is called electric birefringence, or the Kerr electro-optic effect, after the discoverer.[12] It has formed the basis of many investigations of molecular structure by measurements in the gaseous state, for which the theory is relatively complete.[13]

Birefringence phenomena of various types were discussed by Peterlin and Stuart,[14] with particular reference to suspensions and to solutions of macromolecules. Electric birefringence has been studied in organic solutions,[15] and the phenomenon has been discussed in connection with the calculated[16] and measured[17] distortion of liquid droplets by an electric field.

The variation of the magnitude of the Kerr effect with the frequency of the applied electric field—the dispersion—has been investigated in several systems.[18-20] Recently, it was shown by O'Konski and Zimm[21] that the rotational diffusion constants of macromolecules and colloidal particles could be determined from the kinetics of the field-free decay of the birefringence produced by pulsed electric fields. From a general consideration of the relaxation of an orientational anisotropy by free rotational diffusion, the relation between the relaxation time, τ, and the rotational diffusion constant, D, was established, *viz.*, $\tau = {}^1/_6 D$. Special assumptions regarding the orienting mechanism were not required. The decay of the birefringence in the dilute aqueous TMV solutions fitted a single relaxation time, which was estimated to be 0.6 millisecond (msec.). The observation that electrolytes suppressed the magnitude of the birefringence led to the suggestion that ionic atmosphere polarization played a significant role in the orientation phenomenon. This was in accord with a similar suggestion for V_2O_5 sols.[20] The lack of a dispersion in square-wave fields of periods comparable to the relaxation time confirmed the suggestion that a permanent dipole moment was not the principal reason for orientation in the electric field. In the same study, an improved optical method for photoelectric studies was introduced.

In parallel studies, Benoit[22,23] utilized similar pulse techniques in conjunction with a standard optical system. He studied a preparation of TMV, of sodium thymonucleate, and some V_2O_5 sols. In each case a distribution of relaxation times was observed. In the case of TMV, the results were consistent with the behavior expected on the basis of a previous flow birefringence study[24] of the same preparation, but independent values for the rotational diffusion constants were not obtained.

Benoit made calculations[22,23] for the transient buildup of the birefringence by applying the theory of Peterlin and Stuart.[14] This treats the macromolecular solution as a suspension of ellipsoidal bodies, each with a permanent dipole moment and a given static dielectric constant, in a medium of another static dielectric constant. The effects of charge carriers in the surrounding medium, of a charge on the macromolecule, and of the attendant ionic atmosphere, are entirely neglected. His calculations have been recently summarized and extended by Tinoco.[25] However, the theoretical magnitude of the birefringence in TMV was not computed or compared to the experimental result. Furthermore, the existence of the electrolyte effect,[21] mentioned above, was not recognized or explained. Therefore any conclusions from those studies must be regarded as tentative.

The result, $D = {}^1/_6 \tau$, was also obtained by Benoit.[23] Thus, there is agreement that useful information regarding rotational diffusion constants can be obtained from the transient field-free decay of electric birefringence. The method has been applied to bentonite suspensions,[26] solutions of fibrinogen,[27] hemocyanin,[28] and aggregated TMV.[29]

In this investigation, measurements of the birefringence transients in TMV solutions were made with approximately an order of magnitude greater accuracy than previous studies by extensive improvement of apparatus and techniques.

Experimental

Apparatus.—The electric birefringence transients were produced by isolated rectangular pulses. The optical method of O'Konski and Zimm[21] was used, with the analyzer 3 or 4° from the crossed position. Signals were detected with a multiplier phototube, and were recorded by means of oscillographic photography. For this research, the equipment was modified so that only one rectangular pulse was required for each experiment. A schematic diagram of the apparatus is shown in Fig. 1. Synchronization of the camera with the pulsing equipment was achieved as follows. When the camera shutter was opened, a circuit involving the flash contacts triggered an oscilloscope and a trace corresponding to zero birefringence was presented on the screen. After an electronically controlled time delay, short compared to the period of time the shutter remained open, a thyratron was fired. The resulting signal triggered the Kerr cell pulse generator and initiated a second sweep, during which the birefringence signal was displayed. Subsequently the camera shutter closed. In this way, both base line and birefringence signal were recorded on the same photographic frame. A typical birefringence transient is shown in Fig. 2.

The rectangular voltage pulses were generated by specially designed electronic units, and were constant to $\pm 1\%$, adjustable stepwise from 0 to 160 volts, and continuously variable in duration from 50 μsec. (microseconds) to 100 msec. The 10 to 90% rise and decay times were 1 μsec.

For studies of biological materials, the electro-optic cell should require a small volume of material, be closed off from the atmosphere, and be relatively easy to clean. In addition there should be little or no strain birefringence in the

(12) J. Kerr, *Phil. Mag.*, **50**, 337 (1875).

(13) See, for example, H. A. Stuart, "Molekülstruktur," J. Springer, Berlin, 193 .

(14) A. Peterlin and H. A. Stuart, "Doppelbrechung Insbesondere Künstliche Doppelbrechung," Becker and Erler, Leipzig, 1943.

(15) C. G. LeFevre and L. J. W. LeFevre, *J. Chem. Soc.*, 4041 (1953); 1577 (1954).

(16) C. T. O'Konski and H. C. Thacher, Jr., *J. Phys. Chem.*, **57**, 955 (1953).

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(19) D. W. Kitchen and H. Mueller, *Phys. Rev.*, **32**, 979 (1928).

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

(23) H. Benoit, *J. chim. phys.*, **48**, 612 (1951).

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

(26) A. Kahn and D. R. Lewis, *J. Phys. Chem.*, **58**, 801 (1954).

(27) I. Tinoco and J. D. Ferry, THIS JOURNAL, **76**, 5573 (1954); I. Tinoco, *ibid.*, **77**, 3476 (1955).

(28) A. J. Haltner, Ph.D. Thesis, University of California, Berkeley, 1955.

(29) C. T. O'Konski and J. Applequist, to be published.

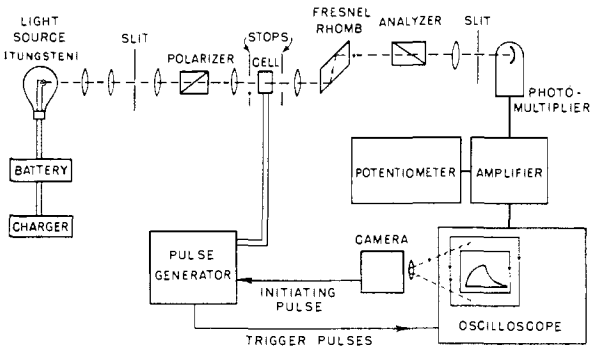
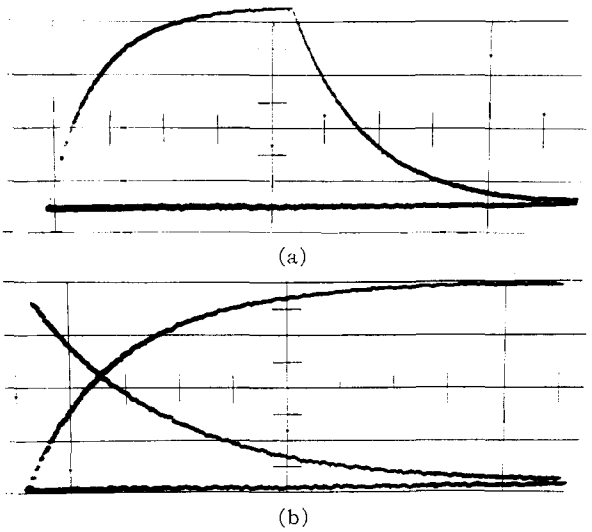


Fig. 1.—Schematic diagram of transient electric birefringence apparatus.

windows, and the cell should be mechanically stable with respect to the rest of the optical system. It should have a minimum capacity and conductance to facilitate coupling to the electronic pulsing system, consistent with good sensitivity. After considerable experimentation with various plastic, glass and metal combinations, we have found the cell shown in Fig. 3 about as easy as any to construct and the best of several models—optically, electrically and chemically.



(a)

(b)

Fig. 2.—Typical oscillograms of birefringence transients, TMV (I). The ordinate is proportional to light intensity at the multiplier, and the abscissa to time. (a) The mode of recording is as described in the text. (b) A similar transient, recorded in three sections by expanding the time scale and providing an additional triggering pulse at the instant of decay of the applied field. More accurate measurements of both buildup and decay are possible this way.

The container for the liquid was a Corex spectrophotometer cell,[30] A of Fig. 3, with a path length of 1.00 cm. The cell cap B was bakelite and the electrode support C was Teflon. The cap, electrode support and electrodes D constituted a precision machined assembly, removable from the top. The small hole E served as an air vent as the assembly was replaced subsequent to filling the cell. The electrodes were brass, electroplated with gold over a heavy silver deposit. They were 9.45 × 9.84 × 3.75 mm. The lower part of the cell fitted into a thermostated metal jacket, which was cut away to allow passage of the light beam.

The optical system[21] was chosen for its high signal-to-noise ratio, and the fact that it results in a polarized output. This means that signals corresponding to negative and

(30) Available from Beckman Instruments, Inc., Fullerton, Calif.

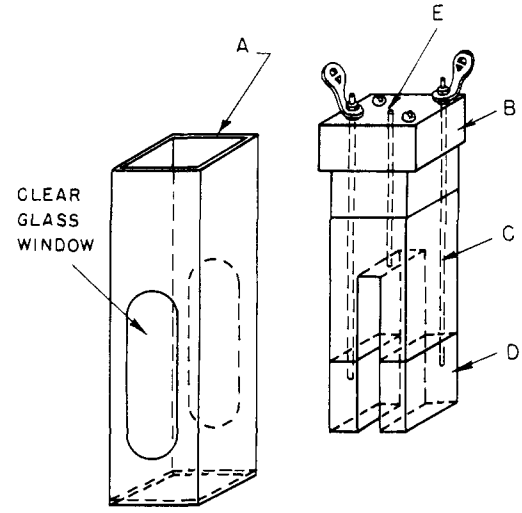


Fig. 3.—Construction of the Kerr cell.

positive birefringence appear with opposite signs, in contrast to the situation in the usual crossed polarizer and analyzer arrangement, where one obtains positive signals for both types of birefringence. Here, Glan-Thompson polarizing prisms[31] were used, and a Fresnel rhomb[32] was substituted for the quarter-wave retardation plate. The analyzer was supported in a theodolite mount[33] fitted with a scale for measuring the angular position to ±0.01°. All lenses were coated achromats.[34] Images of the source appeared at the rectangular entrance slit, which was 1.3 × 3.5 mm., at the center of the cell, in the 2.5 mm. space between the electrodes, and at the exit slit. The arrangement reduced stray light[35] and kept the light beam through the prisms parallel within a half-angle of 1°, well within the 5° required[31] for complete polarization by the prisms. To increase light intensity and sensitivity the light beam through the cell was made converging, with an extreme ray making an angle of 12.5° with the normal ray. This leads to a deviation in path length of the extreme ray from the mean ray of only 1%.

The detection apparatus employed a 1P21 photomultiplier, a direct coupled preamplifier, and a Tektronix type 513-D oscilloscope.[36] The preamplifier was like the one employed in a light scattering instrument which has been described.[37] The limiting time constant in the detecting system was 10^{-7} sec.

Procedures.—After each pulse the cell connections were reversed by means of a switch, to minimize the effects of electrophoresis and electrode polarization. In all experiments, signals were measured with respect to the zero signal oscilloscope trace provided by the arrangement described above. Oscilloscope deflection sensitivity was calibrated with a standardized voltage source, and the time base was calibrated by photographing a sine wave of known frequency, supplied from a standard frequency meter. Measurements of the signals and time were made on photographic enlargements of the 35 mm. negatives of the signal, the standardizing signals, and the sine wave of known frequency. Under favorable conditions signals could be measured to 1 or 2%. Experiments showed that within the range of operation, no detectable signals were obtained with water and buffer

(31) W. Haller in A. Weissberger, "Phys. Methods of Org. Chem.," Interscience Pub., New York, N. Y., 1949, Part II, Ch. 23. Manufactured by the Karl Lambrecht Crystal Optics Co., Chicago, Ill. Aperture 15 × 15 mm., length 60 mm.

(32) F. A. Jenkins and H. E. White, "Fundamentals of Optics," McGraw-Hill, Inc., New York, N. Y., 1950, 2nd Edition, Ch. 28.

(33) Manufactured by the David White Co., Milwaukee, Wis.

(34) Edmund Scientific Co., Barrington, N. J., Stock No. 6166 54 mm. f. l., 29 mm. dia.

(35) F. T. Gucker, Jr., and C. T. O'Konski, *J. Colloid Sci.*, **4**, 541 (1949).

(36) Tektronix, Inc., Portland, Oregon.

(37) C. T. O'Konski and G. J. Doyle, *Anal. Chem.*, **27**, 694 (1955); C. T. O'Konski, M. D. Bitron and W. I. Higuchi, to be published.

solutions in the cell, so background corrections were not required.

The equation for the optical system is equation 2 of reference (21). I_0 is the light flux which would reach the phototube if the analyzer were rotated to pass all of the polarized light, with solution in the cell. The determination of the voltage signal, V_0, corresponding to I_0, was carried out in the following way: After appropriately adjusting the optical elements, the analyzer was rotated from the crossed position through an angle r, and the corresponding signal, $\Delta V'$, was measured potentiometrically, employing the d.c. amplifier (Fig. 1) as a null indicator. Then V_0 was computed by means of the equation

$$\Delta V' = V_0 \sin^2 r \qquad (1)$$

The response of the system was linear, so that

$$\Delta I/I_0 = \Delta V/V_0 \qquad (2)$$

Optical retardations, δ, in radians/cm., were computed from the corrected[38] equation 2 of the earlier reference.[21] It should be noted that all values of δ represent a mean over the spectral band width of the optical system, of around 4000–5500 Å. A check of the over-all performance of the system was made by plotting $\Delta V'$ as a function of r, and comparing with the theoretical curve.

In preliminary experiments the birefringence decay curves were obtained under conditions such that oscilloscope deflections were proportional to optical retardations. Then a rapid and approximate analysis of the relaxation characteristics of the various preparations was made by determining τ_0, τ_1, ... τ_i, the apparent relaxation times, which we define arbitrarily as follows: The first, τ_0, is the relaxation time determined from the initial slope of the birefringence decay curve. It is conveniently obtained by constructing a tangent to the decay curve at zero time, defined as the instant of removal of the field. Then τ_0 is the time between removal of the field and the point of intersection of the tangent with the zero birefringence axis. The other values, τ_i are given by

$$\tau_i = t_i/i$$

where $i = 1, 2, \ldots\ldots$, and t_i is the time required for the birefringence to decay to $\exp(-i)$ of its initial steady-state value. For a single relaxation process, characterized by the relation

$$\delta = \delta_0 \exp(-t/\tau) \qquad (3)$$

it is readily shown that $\tau_0 = \tau_1 = \tau$ for all i. Here δ_0 is the retardation at zero time. The rotational diffusion constant is

$$D = 1/6\tau \qquad (4)$$

The advantage of this method of analyzing the results is its speed; the disadvantage is its limited accuracy, at best around 5 or 10%.

Materials.—The TMV samples were prepared by the method of Stanley[39] and were kept at 0° in dilute phosphate buffer ($\sim 10^{-3} M$) at pH 7. TMV (I) was isolated by Dr. W. F. Harrington in the Biochemistry and Virus Laboratory on April 15, 1952, and was supplied at a concentration of 37.5 mg./cc. TMV (II) was prepared in the laboratory of Professor H. K. Schachman on Jan. 22, 1954, and the stock solution contained 40 mg./cc. TMV (III) and (IV) were prepared by the Biorad Laboratories[40] on April 14, 1954, and February 13, 1953, and were stored at 8 and 1 mg./cc., respectively.

Results

All data were obtained at relatively low concentrations (0.5 mg./cc. TMV or less) where the solutions display a positive birefringence, which corresponds to orientation of the rod-like macromolecules along the electric field direction.[21] In all experiments the temperature was 25.0 ± 0.1°, taking into account the joule heating and the thermal lag in the system.

(38) This equation contained a misprint, kindly called to our attention by A. Kahn. The denominator should be $(4 + \delta^2)$ instead of $(4 + \delta)^2$.

(39) W. M. Stanley, THIS JOURNAL, **64**, 1804 (1942).

(40) 800 Delaware Street, Berkeley, California.

In Table I, the results are shown for TMV (I) and (IV). It is readily seen that, since $\tau_0 = \tau_1 = \tau_2 = \tau_3$, TMV (I) displayed a single relaxation time. In the other cases, τ_i increases with i. This is the trend predicted when the solution contains non-interacting species of two or more relaxation times. For such a system, it is evident that the optical retardation will be the sum over the individual species, that is

$$\delta = \sum_j \delta_j = \sum \delta_{0j} \exp(-t/\tau_j) \qquad (5)$$

Here δ_{0j} is the value of the retardation produced by component j at the instant of removal of the field and τ_j is the relaxation time for that component.

TABLE I

RELAXATION CHARACTERISTICS OF THE TOBACCO MOSAIC VIRUS PREPARATIONS

Preparation	Relaxation time in msec. τ_0	τ_1	τ_2	τ_3
I	0.54 ± 0.05	0.53 ± 0.03	0.53 ± 0.05	0.54 ± 0.10
II	0.57	0.57	0.60	0.69
III	.76	.86	1.80	..
IV	.54	.64	0.78	1.12

More accurate analyses of the relaxation properties were achieved by the use of plots of log δ *vs.* time, illustrated in Figs. 4 and 5 for preparations I and III, respectively. From the slope of the curve in Fig. 4, $\tau = 0.50$ msec., which yields $D = 333$ sec.$^{-1}$. The experimental results of Fig. 5 (circles) were adequately explained on the basis of two relaxation times. The longer value, 3.0 msec., or $D = 56 \pm 5$ sec.$^{-1}$, was determined from the slope of the curve at $t >> 0.5$ msec. The dashed line represents the contribution to δ from this species, and the remainder turned out to be linear on the log δ *vs.* t plot with a slope which yielded $\tau = 0.50$ msec., or $D = 333$ sec.$^{-1}$. Similar results were obtained on preparations II and IV, with different contributions from the slower component. All the above measurements were at 0.5 mg. TMV/cc.

Experiments were conducted to determine whether or not the rotational diffusion constant was concentration dependent up to 0.5 mg./cc. Results obtained at pH 7 in $1.5 \times 10^{-4} M$ phosphate buffer at various concentrations of TMV (I) are presented in Table II. Each value of τ was determined from a plot of log δ *vs.* t in the manner outlined above. Within the experimental limits, τ was independent of concentration, and no evidence for a second relaxation time could be found. Additional experiments over an approximately ten-fold range of ionic strength indicated that τ was independent of the thickness of the ionic atmosphere.

TABLE II

RELAXATION TIME OF TMV (I) *versus* CONCENTRATION

Concn. (mg./cc.)	τ (msec.)	Concn. (mg./cc.)	τ (msec.)
0.50	0.50 ± 0.01	0.017	0.51 ± 0.03
.50	.48 ± .02	.0022	.44 ± .10
.13	.51 ± .02	.0022	.54 ± .10

The best value of τ was 0.50 msec., to an accuracy estimated to be better than 0.02 msec. The same result was obtained from plots of the building up of the birefringence, which, like the decay, was shown

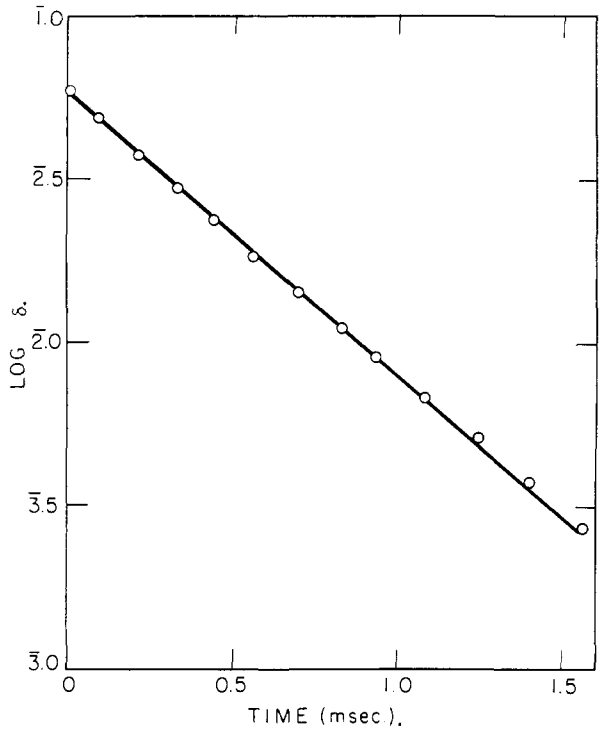


Fig. 4.—Log of optical retardation, δ, *vs.* time, for TMV (I), 0.5 mg./cc.; pure monomer.

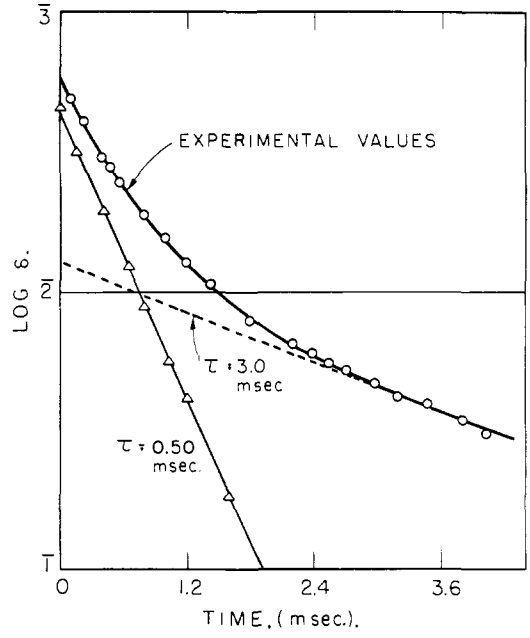


Fig. 5.—Log δ *vs.* time, for TMV (III), 0.5 mg./cc.; the experimental curve is a composite of two relaxation times; monomer plus end-to-end dimer.

to follow precisely a first-order rate law. Values obtained at the higher concentrations were given more weight because of the better signal/noise ratio.

Investigation by means of the electron microscope, and studies of solution viscosity, light scattering, flow birefringence and X-ray diffraction are clearly in agreement that the monomer unit of TMV is a relatively rigid rod, 150 Å. in diameter and about 3000 Å. long.[9,10,41-45] Accordingly, computations were made from the hydrodynamic equation given by Burgers[46] relating the dimensions of a circular rod to its rotational diffusion constant in a viscous medium

$$D = 3kT\{-0.80 + \ln(2a/b)\}/8\pi\eta a^3 \qquad (6)$$

where $2a$ is the length of the rod, $2b$ is the diameter, η is the viscosity of the medium (here, 8.94×10^{-3} poise) and other symbols have their usual meanings.

Since the largest rotational diffusion constant observed in any dilute solution was 333 sec.$^{-1}$, the only reasonable interpretation is that it corresponds to the end over end rotational diffusion of non-interacting monomeric rods. Taking the rod diameter, $2b$ = 150 Å. from the X-ray studies of Bernal and Fankuchen,[45] and D = 333 ± 13 sec.$^{-1}$, the calculated length is 3416 ± 50 Å. Because of the approximately inverse third power dependence of D upon 2a, which can be seen from equation 6, the fractional error in length is smaller by a factor of around three, than is the corresponding figure for D.

The specific volume of TMV has been determined by a number of authors, employing different techniques. Concordance is found between the values of Bawden and Pirie,[47] who obtained 0.73 cc./g. by two methods, and those of Lauffer,[42] who re-investigated the subject in view of conflicting data. As studies by Schachman and Lauffer[44] indicate little hydration of the rods, the density of the TMV in solution may be taken as 1.37 g./cc. Then the calculated molecular weight, assuming a circular rod 150 Å. in diameter and 3416 Å. long, is 49.8×10^6. This is in excellent agreement with the recent values 49.2 and 49.6×10^6 obtained by Williams, Backus and Steere.[10]

The value D = 56 ± 5 sec.$^{-1}$ corresponds very closely to that computed for an end-to-end rigid dimer. Thus, taking $2b$ = 150 Å., in equation 6, one obtains $2a$ = 6610 ± 200 Å., which is twice the length of the monomer, within the experimental accuracy.

Discussion

The results clearly indicate only two species in all of the dilute solutions of TMV; one the monomeric macromolecule, and the other most probably a relatively rigid end-to-end dimer. If there were any distributions of lengths about those two, they would have to be extremely narrow to give the relatively precise fits to the experimental results illustrated in Figs. 4 and 5. This supports the point of view that the common virus is a distinct macromolecular species.[3-8]

A light scattering and electron microscope study[43]

(41) W. M. Stanley and T. F. Anderson, *J. Biol. Chem.*, **139**, 325 (1941).
(42) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).
(43) G. Oster, P. M. Doty and B. H. Zimm, *ibid.*, **69**, 1193 (1947).
(44) H. K. Schachman and M. A. Lauffer, *ibid.*, **71**, 536 (1949).
(45) J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, **25**, 111, 149 (1941).
(46) J. M. Burgers, "Verhandel. Koninkl. Ned. Akad. Wetenschap. Afdeel. Natuurk.," Sec. 1, Dell XVI, No. 4, 113, 1938.
(47) F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc.* (*London*), **B123**, 274 (1937).

of TMV led to a length of 2700 ± 200 Å.,[48] whereas recent electron microscope measurements by improved techniques gave 2980 ± 10 Å. The difference between the latter value and the length reported in this research appears to be well outside the limits of experimental precision.

Schachman and Lauffer[44] reported from ultracentrifugation studies that the hydrodynamic unit of TMV may be considered equivalent to a rigid rod, with a 7 Å. shell of immobile water. Assuming, therefore, an effective diameter of 164 Å., one computes from equation 6, $2a$ = 3370 Å. Employing, instead of a diameter value, the axial ratios a/b = 17.3[43] and 20.3,[42] obtained from viscosity measurements, one calculates $2a$ = 3310 and 3370 Å., respectively. An axial ratio of 17.3 would require a diameter of 191 Å., which appears unlikely, and suggests that the sample employed in the light scattering study[43] might have been partially degraded. Because the rotational diffusion constant depends most critically upon the length, the observed spread of length values is relatively narrow, and reasonable variations in the effective diameter or axial ratio cannot remove the variance with previous results.

Donnet[49] reported D = 260 sec.$^{-1}$, within approximately 20%, from flow birefringence measurements on a TMV preparation which was polydisperse as indicated by electron micrographs. Because of the rather large experimental uncertainty, and the fact that the presence of end-to-end dimers in his preparation would reduce the value of D, this result is not considered to be in contradiction with D = 333 ± 7 sec.$^{-1}$, obtained for the monomer in this research. In a transient electric birefringence study, Benoit[22] reported that the birefringence decay curves of TMV could not be fit by a single relaxation time, which indicated a non-homogeneous system. He chose to fit his results with five relaxation times, and assumed that they would be in the same ratios, and give rise to electric birefringence contributions in the same ratios, as were reported from the flow birefringence studies.[23] He concluded that one of the resulting diffusion constants was 550 ± 55 sec.$^{-1}$, which corresponds to a length of 2800 ± 100 Å. Because the accuracy of the measurement of a given relaxation time is seriously limited by the existence of other roughly equal relaxation times, and because the assumption involved in his interpretation seems arbitrary, the significance of this result is questionable.

The electron microscope length of 2980 Å., considered with the partial specific volume and the X-ray spacing of the dry crystal, led to the suggestion[10] that the TMV rod has a hexagonal cross-section, so as to permit packing without voids in crystals. Such a hexagonal rod would have a rotational friction coefficient slightly less than that of the circumscribing cylinder (173.2 Å. diameter) of equal length. Taking $2b$ = 173.2 Å. in equation 6, one computes $2a$ = 3350 Å., which may be regarded as a lower limit. Thus, the assumption of a hexagonal shape cannot materially reduce the computed length.

Recent structure studies[50,51] suggest that the virus molecule is a helix, with wedge-shaped folded protein units arranged around a helical core, presumably the ribose nucleic acid. The results indicate a non-integral number of protein units per turn of the helix. With a structure of this sort, it appears unlikely that the periphery of the rod would be characterized by plane faces.

The electric birefringence and electron microscope results are not strictly comparable since in the electron microscope observations are made upon vacuum dried materials. In its natural state the virus is in hydration equilibrium with the juices of the living plant, where the thermodynamic activity of water is practically that of the pure solvent. Under these conditions it is not unlikely that some water is held by the virus, possibly between or within the polar protein units of the structure, as suggested by the X-ray work.[51] Upon dehydration in the electron microscope, a decrease in length may occur. Thus it appears that the agreement between our macromolecular weight, and that reported by Williams, Backus and Steere[10] may be fortuitous, as the calculation was made with the assumption that the length of the circular rod *in the crystal* is equal to 3416 Å. If the dry length is taken as 2980 Å., and the external shape as that of a smooth cylinder, the calculated molecular weight is 43 × 10^6. These computations involve the assumption that the dry virus is compact internally; that is, that there are no internal voids which become filled with water upon hydration, but they do not preclude the possibility that water is taken up internally in such a manner that the gross volume of the macromolecule in solution is increased by an amount equal to the volume of water absorbed. However, this possibility appears to be excluded by the early X-ray studies,[45] in which it was observed that the intramolecular spacings were substantially independent of degree of hydration.

The X-ray studies, and independent observations of the birefringence of TMV crystals by Franklin[51] strongly suggest that water is taken up in voids between the macromolecules upon hydration of the crystal. When the air-dried virus was strongly dried at room temperature there was a loss in weight of 10%. Although this is more water than would be required to fill voids equivalent in volume to those between smooth cylindrical rods in a hexagonal array, the excess would not be enough to account for an increase in length from 2980 to 3416 Å. If one accepts the latter value for the hydrated macromolecule, it follows that the length of the dry macromolecule is greater than 2980 Å. Since the 2980 Å. seemed to be based upon an accepted diameter of 2590 Å. for the standard latex particles employed in calibrating the electron microscope, a search was made of the available literature concerning measurement of the latex particle diameter. It was found that the agreement between a number of

(48) It has been pointed out (B. H. Zimm, private communication) that the estimated uncertainty of ±200 Å. was probably too optimistic, as a result of instrumental difficulties uncovered subsequent to the light scattering measurements.[43] See also B. H. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948), esp. last paragraph, p. 1114.

(49) J. B. Donnet, *J. chim. phys.*, **50**, 377 (1953).

(50) J. D. Watson, *Biochim. Biophys. Acta*, **13**, 10 (1954).

(51) R. E. Franklin, *Nature (London)*, **175**, 379 (1955).

independent investigators[52,53] who employed the electron microscope was very good, leading to a maximum variation of ±70 Å., and a probable variation considerably smaller. The possibility of a small systematic error cannot be completely excluded,[54] and indeed is suggested by the light scattering measurements of Dandliker[55] and by some recent X-ray diffraction measurements[56] in which long wave length X-rays were employed. Both methods give a somewhat larger diameter, ranging from 2700–2800 Å. However, this is not enough to account for the difference between 3416 and 2980 Å. Furthermore, since the latex particles serve only as intermediate standards in the electron microscope work, a reproducible error in their size would not influence the electron microscope value[9] for the legnth of TMV.[57] Thus, the 14% difference in computed lengths cannot be discounted by invoking reasonable experimental errors, or hydration effects, or combinations of the two.

We next consider the probable magnitude of the error introduced by use of an hydrodynamic equation for molecular properties. It has been recognized that such applications are of limited accuracy, for example, in considering the rotational diffusion of a solution of polar molecules in a solvent consisting of molecules of comparable size.[58,59] Here, however, we are concerned with a huge macromolecule in a solvent of very small molecules. One therefore expects that the hydrodynamic assumption of a continuous fluid medium would require corrections not exceeding a few solvent molecule diameters. Such numbers would clearly be too small to explain the discrepancy of over 400 Å. in the lengths. It appears more likely that end effects, not treated in detail by Burgers,[46] could give rise to corrections of the right order of magnitude. The possible need for an appreciable correction for a rotational relaxation effect associated with the counterion atmosphere, similar to the translational effects in cataphoresis,[60] has been tentatively ruled out in view of the experiments which indicated that D was independent of the ionic strength. This leaves the problem of the discrepancy unresolved.

In the absence of detailed knowledge of the orienting mechanism, it is not possible at this time to calculate the percentages of dimer in the solutions, but the preparations may be arranged in order of increasing dimer, that is, I (no dimer), II, IV, III, from the results in Table I. This sequence does not correlate with the concentration of the stock solution, or the age of the preparation. Furthermore, separate measurements on I over a period of two years always yielded a single relaxation time. Since all solutions were run at the same concentration, the dimer concentration cannot be attributed to a simple monomer–dimer equilibrium situation; on the contrary, it must be attributed to uncontrolled variables in the process of preparation, or to an intrinsic but variable property of the biological system.

(52) R. C. Backus and R. C. Williams, *J. Appl. Phys.*, **19**, 1186 (1949); **20**, 224 (1949).

(53) C. H. Gerould, *ibid.*, **21**, 183 (1950).

(54) See, for example, S. F. Kern and R. A. Kern, *J. Appl. Phys.*, **21**, 705 (1950).

(55) W. B. Dandliker, THIS JOURNAL, **72**, 5110 (1950).

(56) B. Henke and J. W. M. DuMond, *Phys. Rev.*, **89**, 1300 (1953); *Chem. Eng. News*, **32**, 2272 (1954).

(57) It was pointed out by R. C. Williams (private communication) that it is the latex particle diameter as determined in the electron microscope which is most relevant to the present discussion.

(58) N. E. Hill, *Proc. Phys. Soc.*, **67B**, 149 (1954).

(59) J. Ph. Poley, *Appl. Sci. Res.*, **B4**, 337 (1955).

(60) J. J. Hermans, *Trans. Faraday Soc.*, **36**, 133 (1940).

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Triarylboron Anions. II. Tri-β-methylnaphthylboron[1a]

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Tri-β-methylnaphthylboron reacts readily with sodium in tetrahydrofuran solution to form a green monosodium salt, NaTMeNB, which further reacts with sodium to form the dark brown disodium salt, Na_2TMeNB. The magnetic susceptibility of NaTMeNB has been measured in tetrahydrofuran, ether and two benzene–tetrahydrofuran mixtures by the Gouy method and compared with that of sodium trimesitylboron, NaTMB; their difference in behavior is discussed in terms of the steric requirements of the aryl groups as well as the relative electron affinities of the parent boron compounds. The electron affinity of TMeNB is higher than that of TMB by at least 3.5 kcal./mole, as shown by spectrophotometric studies of the electron transfer reaction NaTMB + TMeNB = NaTMeNB + TMB and its reverse reaction. The extent of the reaction NaTMB + NaTMeNB = Na_2TMeNB + TMB studied by susceptibility measurements also provides a rough measure of the relative electron affinities of TMB and $TMeNB^-$.

It has been demonstrated in previous papers[2,3] that sodium triphenylboron is diamagnetic in ether or tetrahydrofuran solution owing to the association of ion pairs into ion clusters by coulombic forces and that the replacement of phenyl groups in sodium triphenylboron by bulky mesityl groups causes the ion clusters to dissociate in tetrahydrofuran solution with the formation of a free radical. In non-polar solvents, such as benzene or cyclohexane, however, the coulombic interaction between ion pairs causes sodium trimesitylboron, NaTMB, to polymerize. Aside from the dielectric constant of the solvent, it appears that the steric factor is of great importance in the formation of monomeric sodium triarylboron. As a continua-

(1) (a) Taken in part from the Ph.D. dissertation of T. J. Weismann, Duquesne University, 1956. Presented before the Division of Physical and Inorganic Chemistry at the 129th A.C.S. Meeting, Dallas, Texas. April, 1956. (b) Westinghouse Research Laboratories, Pittsburgh, Pa. (c) Gulf Research & Development Co., Pittsburgh, Pa.

(2) T. L. Chu, THIS JOURNAL, **75**, 1730 (1953).

(3) T. L. Chu and T. J. Weismann, *ibid.*, **78**, 23 (1956).