

For interpretation of experiments 4, 2 and 19 especially, it should be noted that experimental errors are of the order of  $10^{16}$  molecules.

TABLE III  
APPROXIMATE UNSENSITIZED QUANTUM YIELDS

Expt.	$p_{CO_2}$	Source	$\lambda$ (m $\mu$ )	Time ( $\times 10^{10}$ )	$\bar{n}$ ( $\times 10^{17}$ )	$q'$ ( $\times 10^{17}$ )	$\phi'$
4	235	Zn	253	142	2.46	1.05	0.0023
2	264	Hg	254	117	0.528	4.78	.009
10	241	Al	186, etc.	139	37.1	104	.036
12	241	Al	186, etc.	132	15.2	57.7	.026
14	23.5	Al	186, etc.	130	22.0	102	.022
15	23.5	Al	186, etc.	130	4.31	141	.0029
19	1.7	Al	186, etc.	141	1.79	146	.0012

All the experiments undertaken by us are now accounted for except 3, 6 and 16 which were lost through breakage of the apparatus.

We intend to continue work in the field opened up by this investigation. We suspect that other reactions can be attributed to mercury atoms excited by radiation of  $\lambda 1849 \text{ \AA}$ . rather than  $\lambda 2537 \text{ \AA}$ ., and intend, without delay, to examine certain of these from this standpoint.

## Summary

Purified carbon dioxide freed from water vapor at  $-78^\circ$  and saturated with mercury vapor is unaffected by resonance radiation of  $\lambda 2537 \text{ \AA}$ . provided that radiation of  $\lambda 1849 \text{ \AA}$ . is entirely excluded by suitable filters. It is rapidly decomposed, however, when radiation of  $\lambda 1849$  is admitted.

The quantum yield of the decomposition sensitized at  $\lambda 1849$  is at least as great as 0.8 and very possibly could become unity if certain suggested corrections could be applied.

Assuming that mercury vapor is not at all excited by the radiation of the aluminum spark at  $\lambda 1860 \text{ \AA}$ . the unsensitized quantum yield estimated with the help of Kreuzler's absorption data is of the order of unity.

We intend, without delay, to determine the relative importance of radiations of  $\lambda 2537$  and  $1849 \text{ \AA}$ ., respectively, in certain reactions sensitized by mercury vapor.

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## The Dielectric Behavior of Solutions of the Protein Zein<sup>1</sup>

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There are now available several methods which are useful in the determination of the size or distribution of size of macromolecules. Less well developed are the methods for the study of the shapes of such molecules. Several attempts have been made to make available for certain classes of compounds these kinds of information by a study of the frequency variation of dielectric constant for dilute solutions containing such molecules, since from the dispersion of dielectric constant of their solutions there may be calculated, among other things, the relaxation time or times of the solute. As is now known these constants are related to the size and shape of the molecule. In so far as we now know, protein systems always show the anomalous dispersion. Also, due to the achievements of Svedberg and his associates with proteins, it was considered desirable to work with a member of this class of compounds since com-

parisons with sedimentation behavior can then be made.

In order to subject the dielectric constant methods to test in this way, it is desirable, at least for the early work, to select for observation a protein system having extremely low electrical conductivity. For the purpose we have chosen, as did Wyman<sup>2</sup> before us, studies of the plant protein zein prepared from the seeds of maize or from corn gluten in 72% aqueous ethyl alcohol.

Since the application of the Debye theory to the anomalous dispersion of dielectric constant of macromolecular solutions has been given in detail in previous publications,<sup>3-5</sup> it will be necessary to give only a brief outline here. The dielectric constant,  $\epsilon$ , of a solution can be said to consist of a real and an imaginary part

$$\epsilon = \epsilon' - i\epsilon'' \quad (1)$$

(2) Wyman, *J. Biol. Chem.*, **90**, 443 (1931).

(3) Debye, "Polar Molecules," Chemical Catalog Co., New York, N. Y., 1929.

(4) Williams, *Trans. Faraday Soc.*, **30**, 723 (1934).

(5) Oncley, *THIS JOURNAL*, **60**, 1115 (1938).

(1) More complete details of this work are to be found in the thesis of Myron A. Elliott submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree in June, 1938.

where  $\epsilon'$  is a measure of the capacitance or out-of-phase current and  $\epsilon''$  is a measure of the dielectric loss or in-phase current. From dipole theory

$$\epsilon' = \epsilon_{\infty} + \frac{\epsilon_0 - \epsilon_{\infty}}{1 + \left(\frac{\epsilon_0 + 2}{\epsilon_{\infty} + 2}\right)^2 \omega^2 \tau^2} \quad (2)$$

and

$$\epsilon'' = \frac{\epsilon_0 - \epsilon_{\infty}}{1 + \left(\frac{\epsilon_0 + 2}{\epsilon_{\infty} + 2}\right)^2 \omega^2 \tau^2} \left(\frac{\epsilon_0 + 2}{\epsilon_{\infty} + 2}\right) \omega \tau \quad (3)$$

where  $\epsilon_0$  is the static dielectric constant;  $\epsilon_{\infty}$  is the corresponding optical quantity;  $\omega = 2\pi\nu$ ; and  $\tau$  is the time of relaxation or time constant. In the derivation of these equations use was made of the Clausius and Mosotti relation between the polarization and the dielectric constant

$$p = (\epsilon - 1)/(\epsilon + 2) \quad (4)$$

where  $p$  is the polarization per cubic cm. Such a treatment is satisfactory for gases and in general for non-polar solvents but it is not designed for systems which make use of polar solvents. Wyman<sup>6</sup> has established empirically that dielectric constant is a linear function of polarization in polar solvents of dielectric constant greater than 20 or 25. That is

$$\epsilon = a + bp \quad (5)$$

where  $a$  and  $b$  are constants. He has pointed out that if this linear relation is substituted for the Clausius and Mosotti expression in the Debye derivation, one obtains expressions for  $\epsilon'$  and  $\epsilon''$  for use with systems which involve polar solvents. The equations are

$$\epsilon' = \epsilon_0 + \frac{\epsilon_0 - \epsilon_{\infty}}{1 + \omega^2 \tau^2} \quad (6)$$

$$\epsilon'' = \frac{\epsilon_0 - \epsilon_{\infty}}{1 + \omega^2 \tau^2} \omega \tau \quad (7)$$

It is seen that these equations differ but little from equations (2) and (3) since in solvents of high dielectric constant the fraction  $(\epsilon_0 + 2)/(\epsilon_{\infty} + 2)$  is always fairly close to unity.

The time of relaxation,  $\tau$ , is, by definition

$$\tau = \zeta/2kT \quad (8)$$

where  $\zeta$  is the inner friction constant for rotation of the particle in question and  $kT$  has its usual meaning. For a spherical particle Stokes has shown that

$$\zeta = 8\pi\eta a^3 \quad (9)$$

where  $a$  is the radius of the sphere and  $\eta$  is the viscosity of the solvent. If  $v$  is the partial specific volume of the particle,  $M$  the molecular weight, and  $N$  the Avogadro number

(6) Wyman, *Chem. Rev.*, **19**, 213 (1936).

$$Mv = \frac{4}{3} \pi a^3 N \quad (10)$$

or, combining equations 8, 9, and 10, we have

$$M = \frac{1}{3} \tau_{\text{sph}} \frac{RT}{\eta v} \quad (11)$$

The subscript "sph" is added to  $\tau$  to denote that the molecule being considered has spherical form. Since most molecules are not spherical in form, it is necessary to convert the experimentally determined time of relaxation to that of a sphere of equal volume. With the aid of the work of Perrin<sup>7</sup> this can be done readily for a molecule with the shape of an ellipsoid of revolution.

Let us consider an elongated ellipsoid of revolution. If we designate by  $\tau_1$  the time of relaxation for rotation around the short axis (end over end rotation), by  $\tau_2$  the time of relaxation for rotation around the long axis, and by  $\tau_{\text{sph}}$  the corresponding quantity for a sphere of the same volume as the ellipsoid, we have, according to Perrin

$$\frac{\tau_1}{\tau_{\text{sph}}} = \frac{2}{3} \frac{1 - \rho^4}{\left[ (2 - \rho^2) \frac{\rho^2}{\sqrt{1 - \rho^2}} \ln \frac{1 + \sqrt{1 - \rho^2}}{\rho} \right] - \rho^2} \quad (12)$$

$$\frac{\tau_2}{\tau_{\text{sph}}} = \frac{4}{3} \frac{1 - \rho^4}{\left[ (1 - 2\rho^2) \frac{\rho^2}{\sqrt{1 - \rho^2}} \ln \frac{1 + \sqrt{1 - \rho^2}}{\rho} \right] + 1} \quad (13)$$

Here  $a$  represents the length of the major axis (axis of revolution) and  $b$  the length of the minor axis, also  $\rho = b/a$ .

In a later paper Perrin<sup>8</sup> gives equations for the linear diffusion of an ellipsoid of revolution. For the elongated ellipsoid ( $\rho < 1$ ) again we find

$$\frac{D}{D_{\text{sph}}} = \frac{\rho^{3/2}}{\sqrt{1 - \rho^2}} \ln \frac{1 + \sqrt{1 - \rho^2}}{\rho} \quad (14)$$

where  $D$  is the diffusion constant of the ellipsoid and  $D_{\text{sph}}$  the diffusion constant of a sphere of equivalent volume. Thus, dielectric constant-frequency data may give information about diffusion constants. The variations of  $\tau_1/\tau_{\text{sph}}$ ,  $\tau_2/\tau_{\text{sph}}$  and  $D/D_{\text{sph}}$  with  $\rho$  are given in graphical form in the original articles. The Perrin equations for the flat plate-like ellipsoid ( $\rho > 1$ ) have been omitted here.

### Experimental

If a quantitative calculation of the size and shape of a rigid macromolecule is to be made from dielectric constant data, a sufficiently large frequency range must be covered to include the entire region of noticeable dispersion, and it

(7) Perrin, *J. phys.*, **5**, 497 (1934).

(8) Perrin, *ibid.*, **7**, 1 (1936).

is in this respect that our work represents a considerable extension of the earlier work of Wyman<sup>2</sup> and of one of us<sup>9</sup> with this system. The apparatus used in these experiments covered the frequency range from  $2.5 \times 10^4$  to  $3 \times 10^7$  cycles per second ( $\lambda = 12,000$  to  $10$  m.). A radio-frequency bridge gave frequencies  $2.5 \times 10^4$  to  $1.5 \times 10^6$  cycles per second ( $\lambda = 12,000$  to  $200$  m.) and a special resonance method was used for the interval  $1.5 \times 10^6$  to  $30 \times 10^6$  cycles per second ( $\lambda = 200$  to  $10$  m.). The bridge described in a recent laboratory manual<sup>10</sup> was of a standard capacity type with the three arms fixed and the fourth containing the dielectric cell, General Radio precision condenser, and electrolyte resistance in parallel. The circuit is shown in Fig. 1. The cell used was of the variable capacity type designed by Albright<sup>11</sup> and was used in the manner originally described by Williams and Krchma.<sup>12</sup>

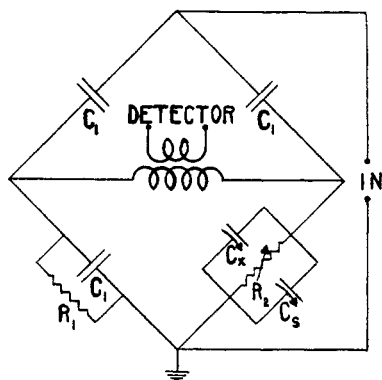


Fig. 1.—Radio-frequency bridge:  $C_1 = 1000 \mu\text{mf}$  fixed mica condenser;  $C_x =$  dielectric cell;  $C_s = 1500 \mu\text{mf}$  precision condenser;  $R_1 =$  fixed resistor (500 to 4000 ohms);  $R_2 =$  electrolyte resistor.

The resonance apparatus was built around twin capacitance cells, built in the fashion suggested by Fig. 2. Each container held 25 cc. of solution and was constructed of monel metal and grounded to act as a shield. The electrodes were formed from midget variable air condensers with all but three of the plates removed. The electrodes were plated with nickel. During the measurements these plates were covered by a standard amount of the solution. The twin cells will be called I and II and are indicated by  $C_1$  and  $C_{II}$  in Fig. 3. They were placed in parallel with a plug-in inductance  $L_1$  and midget variable condenser  $C_0$ . These cells were mounted very close together on a brass plate and thermostated. The electrical leads connecting them were brass bars about 2 cm. long and all external connections to these bars were made at a point halfway between the cells. This was done so that the inductance of a circuit through them was the same for the cell I path as for the cell II path.

In an experiment, cell I was filled with the solution being

studied and cell II was filled with pure solvent of the same d. c. conductivity. The high frequency conductivity of solution and solvent will differ to give a small error. The rotor of cell I was moved between two fixed points A and B as shown in Fig. 2. In order to keep the system in resonance with the oscillator frequency the rotor of cell II had to be moved in the opposite direction to that of cell I so as to change the capacity of that cell II by exactly the amount that the capacity of cell I was changed. A mirror was mounted on the rotor of cell II. With the aid of telescopes and a scale the amount of movement of the cell II rotor could be measured exactly. The cell was used as the precision condenser. It was necessary for the bearings in both cells to be close fitting. The dielectric constant of the solution and the pure solvent could in this way be accurately balanced against each other at frequencies from 1.5 to 30 megacycles. The apparatus was calibrated by putting pure solvent in both cells. This gave the small corrections necessary in the region of 20 to 30 megacycles. With a low dielectric constant solvent such as benzene the system would resonate

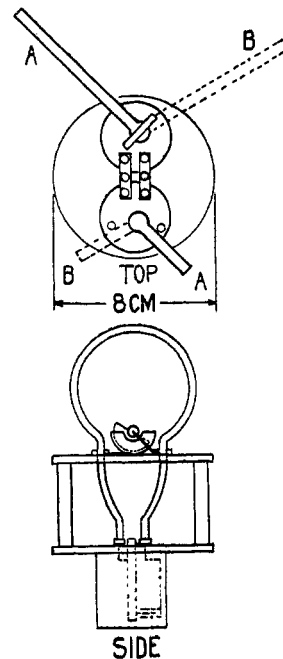


Fig. 2.—Twin capacity cell for solvent and solution.

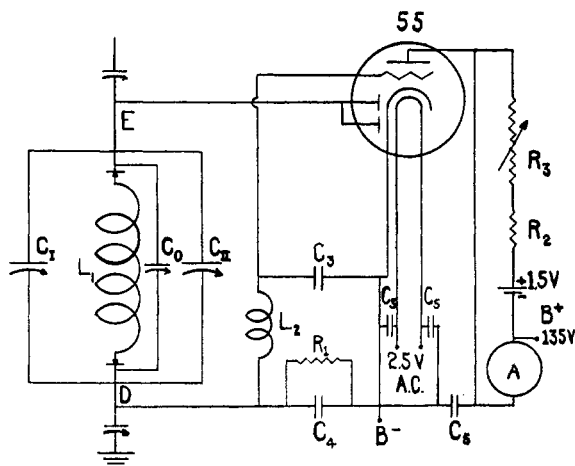


Fig. 3.—Resonance detector circuit:  $C_1 =$  solution cell;  $C_{II} =$  pure solvent cell;  $C_0 =$  midget variable condenser;  $C_3 = 500 \mu\text{mf}$  fixed mica condenser;  $C_4 = 250 \mu\text{mf}$  fixed mica condenser;  $C_5 = 0.01 \mu\text{mf}$  fixed mica condenser;  $R_1 = 500,000$  ohm fixed resistor;  $R_2 = 100$  ohm fixed resistor;  $R_3 = 40,000$  ohm variable resistor;  $L_1 =$  inductance;  $A =$  Rawson multimeter;  $L_2 =$  radio frequency choke.

(9) Watson, Arrhenius and Williams, *Nature*, **137**, 322 (1936).

(10) Daniels, Mathews and Williams, "Experimental Physical Chemistry," 2d ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1934.

(11) Albright, *THIS JOURNAL*, **59**, 2098 (1937).

(12) Williams and Krchma, *ibid.*, **48**, 1888 (1926).

at frequencies as high as 50 megacycles and accurate measurements could be made at that frequency.

The small air condenser  $C_0$  was built so that its rotor could be moved quickly and accurately between two fixed but adjustable points. This was done so that the relatively flat top of the resonance curve could be bracketed and advantage taken of the steep slope of the curve at either side of the resonance point. This was also a necessary part of the apparatus if the true resonance point was to be obtained for solutions having appreciable conductance.

The scheme of connections for the resonance detector circuit, originally worked out by Mr. L. C. Krchma, formerly of this Laboratory, is shown by Fig. 3. An alternating current voltage across points D and E will indirectly tend to cut off the main plate current in the vacuum tube. The power from the oscillator is adjusted so that the plate current at the resonance point is only a few microamperes. This detector is very sensitive to changes in the alternating current voltage across the cell. A 1.5-volt dry cell is inserted in such a manner that the minimum in the plate current at the resonance point is changed into a maximum.

**Preparation of Zein<sup>13</sup>**

The zein was prepared from corn gluten which had been dried at temperatures not higher than 35°. After being ground to a fine powder the gluten was extracted three times for twelve hours with absolute methyl alcohol, one part gluten to three parts methyl alcohol by weight. After the last filtration the gluten was extracted with 70% aqueous ethyl alcohol for eighteen hours. The extraction with methyl alcohol removed most of the fat and coloring matter and the more soluble protein fraction. The latter, which will be called zein I, comprised about 15% of the total protein. The main portion of the zein, called zein II, was dissolved in the extraction with 70% ethyl alcohol. The zein II fraction was electrodyalyzed as far as practical against its solvent and then it was precipitated by pouring the solution into three times its volume of diethyl ether. After the protein had settled out the ether was decanted and the protein allowed to dry in the form of a thick film on the bottom of the flask. This film was found to keep indefinitely and was always completely soluble in 70% aqueous alcohol. Experiments with zein that was not precipitated in ether but only electrodyalyzed showed that the precipitation did not change the electrical properties of the protein.

**Results**

A large number of complete dispersion experiments were made with several and various zein preparations. As representative of experiments with zein I, zein II, and whole zein in aqueous

(13) The corn gluten used in the preparation of the zein was supplied with the compliments of the A. E. Staley Mfg. Co., Decatur, Illinois, and the Corn Products Refining Company, Argo, Illinois.

alcoholic solvent, we present the data which have been collected to form Table I. These data are

TABLE I  
DIELECTRIC CONSTANT MEASUREMENTS WITH ZEIN SOLUTIONS AT 25°<sup>a</sup>

Solvent, 72% aqueous ethyl alcohol;  $\epsilon_{solv.} = 36.59$ ;  $\eta_{solv.} = 0.0198$ .

Curve Fig. 4 Zein II. $c = 3.63$ g./l. $\log \nu$ $\Delta C/\Delta C_0$		Curve Fig. 5 Whole zein. $c = 16.3$ g./l. $\log \nu$ $\Delta C/\Delta C_0$		Curve Fig. 6 Zein I. $c = 3.17$ g./l. $\log \nu$ $\Delta C/\Delta C_0$	
4.384	1.0466	4.438	1.260	4.384	1.0494
4.428	1.0466	4.618	1.255	4.428	1.0490
4.545	1.0467	4.743	1.248	4.545	1.0490
4.846	1.0460	4.920	1.232	4.846	1.0484
5.147	1.0419	5.220	1.194	5.147	1.0439
5.448	1.0336	5.446	1.152	5.448	1.0370
5.749	1.0239	5.709	1.108	5.749	1.0293
5.925	1.0193	5.874	1.085	5.925	1.0250
6.050	1.0163	6.132	1.056	6.128	1.0205
6.128	1.0143	6.488	1.029	6.484	1.0155
6.484	1.0086	6.748	1.018	6.739	1.0131
6.896	1.0035	6.898	1.011	6.896	1.0114
7.255	1.0028	7.094	1.004	7.255	1.0090
7.423	1.0024	7.284	0.998	7.423	1.0078

<sup>a</sup> In order to obtain the dielectric constant of a solution at frequency  $\nu$  it is necessary to multiply  $\epsilon_{solv.}$  by the ratio  $\Delta C/\Delta C_0$ . This ratio is determined directly from observations of capacity differences in condensers containing solution and solvent at the balance points.

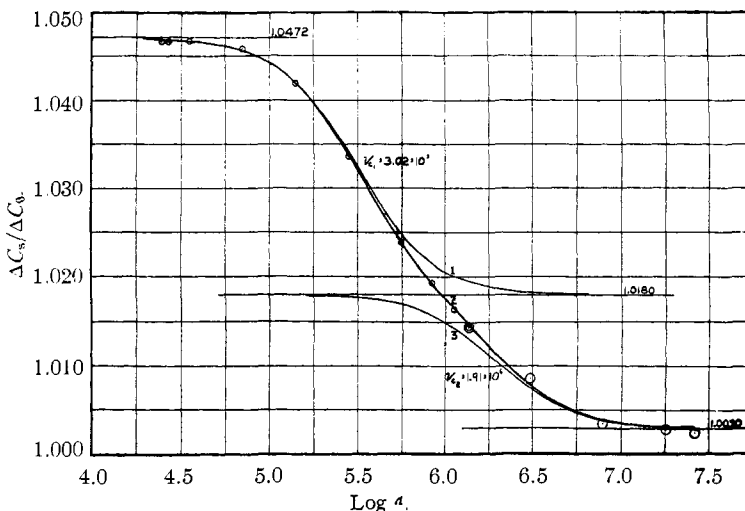


Fig. 4.—Dielectric constant ratio vs. log frequency curve for zein II solution.

utilized in turn to prepare Figs. 4, 5, and 6. If Fig. 4 (zein II) is examined it is found impossible to obtain a theoretical curve from the Debye theory that will fit these experimental points if the molecules of zein are assumed to be spherical in form and to have a single  $\tau$  value. If, however, the molecules are assumed to be elongated ellipsoids of revolution, then, from the Perrin modification of the dipole theory two times of relaxa-

tion may be expected. Thus, on the basis of this assumption, the experimental dielectric constant-frequency curve is to be considered a composite of two simple theoretical curves. For the fraction we have designated as zein II it is possible

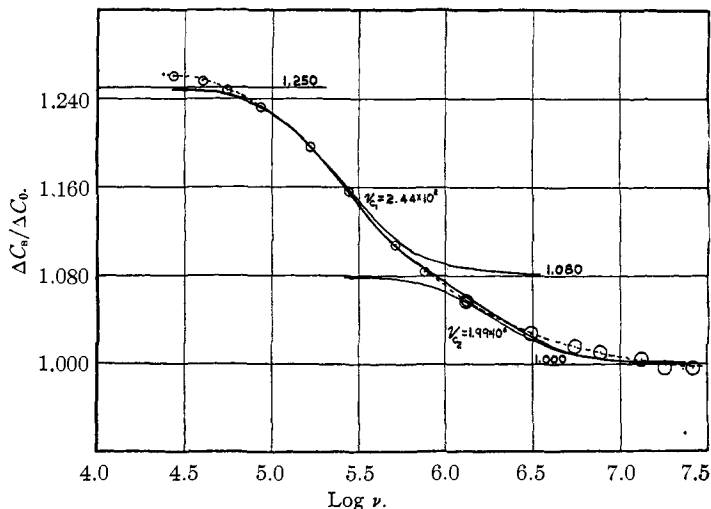


Fig. 5.—Dielectric constant ratio vs. log frequency curve for unfractionated zein.

to resolve the experimental curve into two such theoretical curves. Thus, in Fig. 4, the long heavy curve is an exact geometrical sum of the two simple theoretical curves. The time constants used to describe the theoretical curves (1 and 2) were adjusted to make the composite curve fit the experimental data as well as possible. The unusually good agreement between the calculated curve and experimental data is very interesting and unlikely to be accidental, and the position of this curve is reasonably sensitive to changes in the time constants.

It is true a similar result could be obtained if the protein consisted of spherical molecules of two sizes, one sphere having about seven times the volume of the other. However, sedimentation velocity studies with the Svedberg ultracentrifuge show that zein molecules have high dissymmetry factors and that two such distinct size groups of molecules are not present.<sup>9</sup>

The excellent agreement between the theoretical composite curve and the experimental points shown in Fig. 4 might lead one to believe that any set of data of this general type could be fitted with the proper combination of two Debye

dispersion curves, given the proper values for the constants. That this is not the case can be seen from data with unfractionated or whole zein. Thus, the dotted curve in Fig. 5 is a line drawn through the experimental points and the long solid curve is the best theoretical composite curve that could be calculated by using two  $\tau$  values. The deviation at the high frequency end of the curve is probably due to some smaller molecules being mixed with the main zein fraction while the deviation at the low frequency end may be due to interference between the zein molecules because of the relatively high concentration (16.3 g./l.) at which this particular experiment was made. Experimental data for one of the experiments with zein I are shown in Fig. 6. The solid curve represents one of the best possible combinations of theoretical curves that could be obtained by using two times of relaxation. The main portion of the molecules in zein I appears to have the same electrical behavior as

the molecules in zein II. That there are also present smaller particles in zein I is indicated by the marked deviation at the high frequency end of the curve.

Values for the dielectric increments and other

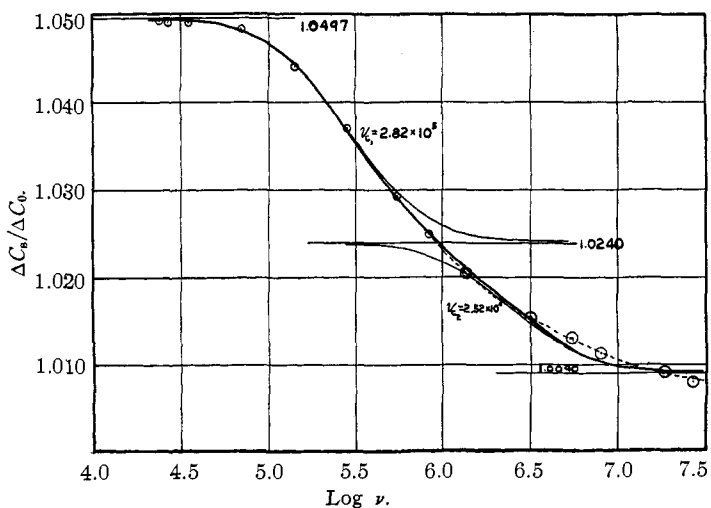


Fig. 6.—Dielectric constant ratio vs. log frequency curve for zein I solution.

related quantities given in Table II are calculated from the difference between the dielectric constant of the solution at the low frequency end of the curve and that at the high frequency end.

Ordinarily  $\Delta C_s/\Delta C_0$  will be less than one at the high frequency end of the dispersion. In Figs. 4, 5, and 6 slight variations in the composition of the mixed solvent have caused a slight upward displacement of the curve as a whole.

TABLE II

CONSTANTS USED IN OBTAINING THEORETICAL DISPERSION CURVES AND OTHER PERTINENT DATA EVALUATED FROM EXPERIMENTAL WORK

Zein II. $c = 3.63$ g./l.					
$(r_1)_0 = 1.0472$	$\tau_1 = 52.6 \times 10^{-8}$	$\tau_1^0 = 23.8 \times 10^{-8}$			
$(r_1)_\infty = 1.0180$	$\tau_2 = 8.3 \times 10^{-8}$	$\tau_2^0 = 3.8 \times 10^{-8}$			
$(r_2)_0 = 1.0180$	$\tau_{av} = 31.6 \times 10^{-8}$	$\tau_{sph}^0 = 3.0 \times 10^{-8}$			
$(r_2)_\infty = 1.0030$	$\rho = 1/7$	$\tau_{av}^0 = 14.3 \times 10^{-8}$			
$\vartheta_1 = 11,100$	$\Delta_1 = 0.30$	$\mu_1 = 310 \times 10^{-18}$			
$\vartheta_2 = 5,700$	$\Delta_2 = 0.15$	$\mu_2 = 220 \times 10^{-18}$			
$\vartheta_{av} = 16,800$	$\Delta_{av} = 0.45$	$\mu_{av} = 380 \times 10^{-18}$			
Whole Zein. $c = 16.3$ g./l.					
$(r_{av})_0 = 1.264$	$\tau_{av} = 34.2 \times 10^{-8}$	$\mu_{av} = 430 \times 10^{-18}$			
$(r_{av})_\infty = 1.000$	$\tau_{av}^0 = 18.5 \times 10^{-8}$	$\Delta_{av} = 0.59$			
Zein I. $c = 3.17$ g./l.					
$(r_{av})_0 = 1.0497$	$\tau_{av} = 29.6 \times 10^{-8}$	$\mu_{av} = 380$			
$(r_{av})_\infty = 1.0090$	$\tau_{av} = 13.4 \times 10^{-8}$	$\Delta_{av} = 0.46$			

$\rho$  is the ratio of minor to major axis of the molecule as calculated from  $\tau_1$  and  $\tau_2$  (Perrin).

$\eta$  is the viscosity of the pure solvent.

$\epsilon$  is the dielectric constant of the pure solvent.

$(r_1)_0$  is the ratio  $\Delta C_s/\Delta C_0$  at top of  $\tau_1$  curve (same as  $\epsilon_0$  when multiplied by  $\epsilon_{soln.}$ ).

$(r_1)_\infty$  is the ratio at the bottom of the  $\tau_1$  curve.

$(r_2)_0$  and  $(r_2)_\infty$  are ratios at top and bottom of  $\tau_2$  curve.

$(r_{av})_0$  and  $(r_{av})_\infty$  are upper and lower limits of total curve.

$\tau^0$  is the  $\tau$  value corrected to the basis of a rotation in pure water at 25°. This correction is made so that time of relaxation data in solvents of different viscosities may be compared directly.

$\mu$  is the electric moment of the molecule estimated from the dielectric increment in the manner described by Onley.<sup>5</sup>

$\vartheta$  is the dielectric increment ( $\Delta\epsilon/\Delta c$ ) where  $c$  is in moles/liter.

$\Delta$  is the dielectric increment ( $\Delta\epsilon/\Delta c$ ) where  $c$  is in grams/liter.

$$\tau_{av} = 1/2\pi\nu_c.$$

Less complete, but nevertheless very suggestive, dielectric constant-frequency data were obtained for solutions of zein I, zein II, and whole zein at temperatures from 0.8 to 50°. It has not as yet been possible to make a unique resolution of the zein II curves at temperatures above 25° so that in discussing the data taken at 35 and 50° it is necessary to introduce an empirical average time of relaxation,  $\tau_{av}$ . This time of relaxation quantity is calculated from the experimental data alone and is not the result of curve fitting. By definition

$$\tau_{av} = 1/2\pi\nu_c \tag{15}$$

where  $\nu_c$  is the frequency at which

$$\epsilon' = (\epsilon_0 + \epsilon_\infty)/2$$

The  $\epsilon_0$  and  $\epsilon_\infty$  correspond to the  $(r_{av})_0$  and  $(r_{av})_\infty$  used in Table II. The  $\tau_{av}$  values of the same molecule suspended in media of various viscosities should be proportional to the viscosity of the medium. However, the  $\tau_{av}$  values of different kinds of molecules are not proportional to the molecular weight unless each of these molecules gives a dispersion curve of exactly the same shape. The  $\tau_{av}$  values obtained for whole zein, zein I, and zein II at various temperatures are given in Table III.

TABLE III

AVERAGE TIME OF RELAXATION VALUES FOR ZEIN AT VARIOUS TEMPERATURES

Concn. g./l.	Temp., °C.	Viscosity of solvent	$\tau_{av} \times 10^8$	$\tau_{av}^0 \times 10^8$
Whole Zein				
16.3	25	0.0198	40.9	18.5
13.1	25	.0198	40	18
4.3	25	.0198	45	20
			Average 18.8	
Zein I				
1.81	25	0.0198	34	15
3.17	25	.0198	30	14
2.80	50	.0105	16	14
2.67	35	.0153	23	13
2.31	0.8	.0455	78	15
			Average 14.2	
Zein II				
1.69	25	0.0198	40	18
1.72	25	.0198	33	15
3.63	25	.0198	32	14
4.30	50	.0105	17	15
3.10	50	.0105	18	15
2.51	35	.0153	28	16
3.10	35	.0153	28	16
3.50	0.8	.0455	76	15
3.84	24.4	.0206	42	18
3.84	36.0	.0148	30	18
3.84	50.2	.0100	21	19
			Average 16.2	

The constancy of the  $\tau_{av}^0$  values over more than a four-fold change in viscosity is good evidence for the reliability of the experimental data. It also indicates that there is no appreciable amount of dissociation or aggregation of the zein molecules as the temperature is varied over the interval investigated. The data at 25 and 0.8° for zein II solutions could all be resolved to give unique values of  $\tau_1$ ,  $\tau_2$ , and  $\tau_{sph}$ . The results (corrected for viscosity) are given in Table IV.

The actual data for solutions of zein II at concentrations 3.50 and 3.60 g./l. are somewhat more

TABLE IV  
TIME OF RELAXATION VALUES FOR ZEIN II

Concn. g./l.	Temp., °C.	$\tau_1^0 \times 10^8$	$\tau_2^0 \times 10^8$	$\tau_{\text{sph}}^0$
1.69	25	27	4.8	3.8
1.72	25	27	4.5	3.6
3.60	25	22.4	3.8	3.0
3.50	0.8	20	3.5	2.8
	Average	24.1	4.15	3.3

reliable than those at the lower concentrations 1.69 and 1.72 g./l. as these latter values were near the concentration limit at which the apparatus would give useful data. Centrifuge and diffusion measurements indicate the main fraction of zein (which corresponds roughly to zein II) to be homogeneous and have a molecular weight of about 40,000. If  $\tau_1^0$  and  $\tau_2^0$  are known, then, with the aid of Perrin's deductions, it is possible to obtain a value for  $\tau_{\text{sph}}^0$ , the time of relaxation of a sphere having the same volume as the ellipsoid. For zein II,  $\tau_{\text{sph}}^0 = 3.0 \times 10^{-8}$  sec. when corrected to the viscosity of water at 25°. From equation 9

$$M = 38,000$$

if  $v = 0.73$ ,  $\eta = 0.00894$ , and  $T = 298$ . The ratio of the minor to the major axis,  $b/a$ , is also obtained from Perrin's equations to be

$$\rho = b/a = 1/7$$

From the volume of the molecule the minor axis is found to approximate 23 Å. and the major axis 160 Å. in length.

### Discussion

It has been found possible for the first time to analyze the complete dispersion curve for a single molecular species to reveal relaxation times characteristic of rotation about the different axes. The zein molecules studied in this work have been assumed to have the form of elongated ellipsoids of revolution. It is true Ferry and Oncley<sup>14</sup> have found it possible to represent their pseudoglobulin (normal horse serum) curve by two dispersion terms but this interpretation is not to be confused with the one given here since the attempt was made to associate the two times of relaxation with two different molecular units of weight 175,000 and 400,000 which were assumed to be present. In other words, the question of molecular asymmetry is not involved here. It is our belief that the dielectric constant measurements in this case were not made over a sufficient frequency interval to enable these investigators to determine uniquely

(14) Ferry and Oncley, *THIS JOURNAL*, **60**, 1123 (1938).

two time constants which would be characteristic of the rotations of the pseudoglobulin unit or units considered as ellipsoids of revolution.

It is found as well that the theory as developed here does permit calculation of the molecular weight of the zein and it can be expected it will be successful in the cases of other proteins when the proper experimental data are made available. The molecular weight of zein obtained by the generally accepted sedimentation-diffusion and osmotic pressure methods is very close to 40,000.

This dielectric behavior is to be contrasted with that of certain other polymeric molecules in solution which do not give a dispersion of dielectric constant because of an orientation or orientations as rigid body but do give contributions to the polarization resulting from orientations in the electrical field of recurring units which make up the solute molecules. In the case of the  $\omega$ -hydroxydecanoic acid esters, it has been found possible to give a quantitative account of the observed electric moment of the molecules by a proper summation of the effects of the individual polar groups contained in the molecule.<sup>15</sup>

Data of the type described above are of value to protein chemistry because they make it appear that proteins may possess an element of rigidity in their structure not found in other macromolecules. The nature of the grouping to cause such rigidity is still obscure.

The results of the experimental work and calculations to form Table III are of considerable value. As pointed out they are good evidence for the reliability of the experimental data, but more than that they demonstrate the essential correctness of the manner in which the ordinary coefficient of viscosity of the solvent has been introduced into the formulas for the calculation of times of relaxation and molecular weight and they attest the adequacy of the use of correction factors to reduce times of relaxation to common solvent and temperature (*i. e.*, water at 25°) for comparisons.

The constancy of the reduced times of relaxation for temperatures 0, 25, 35, and 50° appears to demonstrate the absence of any temperature dissociation of zein over this interval. Thus the data show conclusively that the discrepancy between the values of the time constant for zein originally presented by Wyman and those given

(15) Bridgman and Williams, *ibid.*, **59**, 1579 (1937); Wyman *ibid.*, **60**, 328 (1938).

in this report is not to be explained by any dissociation of zein at temperature 50°. The two sets of data illustrate the necessity of conducting the experiments over a very wide frequency range.

By using recent theoretical developments of Onsager and Wyman, the measurements are interpreted to indicate a dipole moment for the zein molecule of approximately 400D. Of course there is a considerable uncertainty in this calculation but it is believed to be a better evaluation than the much lower value, approximately 50D, obtained by the direct application of the classical Debye theory.

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### Summary

1. A combination of the Debye dipole theory and the Perrin study of the hydrodynamics of an ellipsoid of revolution is applied to experimental data involving the protein zein in aqueous alcoholic solution.

2. A resonance apparatus for making precision dielectric constant measurements with conducting solutions in the frequency range of 1.5 to 40 megacycles is described briefly. The principal feature of the method is the high accuracy of relative values at different frequencies.

3. The experimental dispersion of dielectric constant data with dilute zein solutions in the frequency range 25,000 to 30,000,000 cycles is accounted for quantitatively by theoretical equations if the molecules are treated as ellipsoids of revolution. The values 38,000 for the molecular weight and  $1/7$  for the ratio of minor to major axis of the zein molecule are obtained directly from these data.

4. It has been possible to analyze the dispersion of dielectric constant curve for zein in solution by considering the molecule to have the form of an ellipsoid of revolution to give relaxation times characteristic of rotation about its different axes.

5. There is no dielectric constant evidence of a temperature dissociation of zein molecules in solution between 0 and 50°.

MADISON, WISCONSIN

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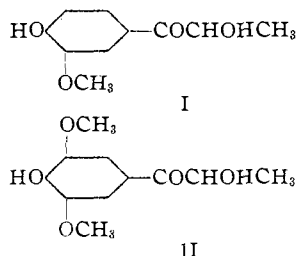
[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY]

## Studies on Lignin and Related Compounds. XXXVII. The Structure of Lignin and the Nature of Plant Synthesis

BY HAROLD HIBBERT

The isolation, in considerable yield, of  $\alpha$ -ethoxypropiovanillone by "ethanolysis" from spruce and of this, together with  $\alpha$ -ethoxypropiosyringone, from maple wood<sup>1</sup> would seem to be of marked importance not only in regard to the elucidation of the structure of lignin, but also with respect to the mechanism of plant synthesis.

The evidence indicates that these products are formed either from the corresponding free hydroxy compounds I and II



(1) (a) Cramer, Hunter and Hibbert, *THIS JOURNAL*, **61**, 509 (1939); (b) Hunter, Cramer and Hibbert, *ibid.*, **61**, 516 (1939).

or from relatively simple building units, and that "extracted" lignins are, at least in very large part, condensation polymers of I and II or their dismutation forms XV, XVI and XVII, XVIII of these. This view is in harmony with the ease<sup>2</sup> with which an "extracted" lignin, isolated from oak wood, under very mild conditions, may readily be converted into simpler building units similar to those already described.<sup>1</sup>

In the case of maple wood,<sup>1b</sup> the total amount of I and II isolated in the form of their  $\alpha$ -ethoxy derivatives was 12.4 g., based on a weight of 100 g. of lignin (Klason) in the original wood. The "ethanolysis" products of maple contain, in addition to the distillable phenolic substances, also aldehydic, acidic and neutral substances, to the extent of 5.4, 1.6, 2.1 g., respectively. In other words, some 21.5 g. of distillable derivatives was obtained from maple wood containing 100 g. of lignin (Klason).

(2) Peniston, McCarthy and Hibbert, *ibid.*, **61**, 530 (1939).